Investigation of the potential of calcareous grassland vegetation for green roof application in the UK

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Abstract

For the last few decades, the interest in green roofs has been developing quickly because of the benefits they have on the modern urban environment situation. Recently, of the range of native habitats in the UK appropriate for green roof application, calcareous grassland plant communities have been given much attention for use on green roofs because of the similarity that these habitats have to green roof conditions. However, there has been very little or no research into how locally-characteristic habitats can be recreated on green roofs, and this has led to a lack of specific recommendations for native plant communities or assemblages on green roofs in the UK. Thus, this thesis investigates the feasibility of calcareous grassland vegetation for green roof application in the UK. This study is comprised of four sections; (i) A review of calcareous grassland types, ecology and characteristics, (ii) A discussion of the restoration ecology of calcareous grasslands, (iii) An investigation into substrates for supporting calcareous grasslands on green roofs, (iv) Experimental investigation of plant selections and communities for calcareous grasslands on green roofs.

The substrates investigation consisted of testing mixtures containing Limestone, LECA (Light Expanded Clay Aggregate), Brick rubbles with organic matter and loam in five composition rates. *Leucanthemum vulgare*, *Briza media*, and *Prunella vulgaris* were selected as indicator species. All the substrates met the minimum requirements that conform to FLL standards (the German guidelines for green roofs, Society of Landscape Development and Landscape Design), except for LECA and Limestone substrate types that tended not to meet the minimum moisture content (20%). Most of the substrates supported high seedling survival. In general, Limestone substrate types and a 60:20:20 (mineral material: loam: organic matter) composition rate tended to produce high seedling emergence and growth across all of the species, while LECA and Brick rubble substrate types, and 100:0:0 composition rate did in the opposite. The most successful substrate was a Limestone substrate type with 60:20:20 composition rate that had relatively good balance of moisture content and air filled porosity, and supported high seedling emergence, survival and growth across all of the species.

To investigate plant selection and plant communities for calcareous grasslands on green roofs, seventeen forb species were planted to investigate the environmental tolerances of

a range of species and to explore patterns of plant growth and flowering performance at the community and individual species level. Deeper substrate depth, the Limestone-based substrate, supplementary watering, and fertiliser addition tended to support significantly higher plant abundance, growth, structural characteristics, and flowering performance of the plant community. Some of individual species, however, showed different responses. Watering was an important factor regarding plant establishment and growth, especially with substrates of shallower depth. A 50 mm deep substrate is not suitable for satisfactory plant growth without additional watering; Supplemental watering produced statistically similar plant growth in the shallower substrate to that of the deeper substrate without it. The minimum substrate depth should be at least 100 mm to support good growth of the species on a green roof. Most species did not show significant difference in plant growth and performance between 100 mm and 200 mm depth. All the species in the Limestone-based substrate had a higher abundance rate, and the Limestone-based substrate produced significantly greater plant growth than the Zinco substrate. Additional fertiliser resulted in greater plant abundance and growth but there was a tendency for plant growth to be very vigorous. *Hypochaeris radicata* and *Leucanthemum vulgare* showed the greatest abundance under drought conditions in the shallow substrate depth. Overall, *C. glomerata* and *H. nummularium* across all treatments in a standard commercial green roof substrate without fertiliser addition, and *P. officinarum* and *P. veris* across all treatments including the additional fertiliser treatment were not effective green roof plants under the given conditions of the experiment. Across all experimental treatments except for the additional fertiliser treatment, the one legume in the experiment (*Lotus corniculatus*) tended to dominate over the 2-year period.

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Chapter 1. Introduction

1.1 Introduction

As our cities gradually grow and become more complex, the demands and awareness of the public, as well as ecologists and conservationists, regarding nature conservation and urban environment have been constantly increasing. From these demands and awareness, the necessity of creation and conservation of green spaces or inner city nature areas have been constantly emphasised for improving the urban environment and quality of human life (Rohde and Kendle, 1997; Johnston, 1990). Furthermore, from the last century onwards, international environmental agreements or political agendas on climate change have greatly influenced decision-making of environmental policies of individual countries. For example, the Rio Earth summit which concluded in 1992 led the UK government to produce the UK Biodiversity Action Plan (BAP) in 1994. Consequently, the BAP has promoted Local Biodiversity Action Plans (LBAPs) that aim to conserve and enhance local species and habitats (Burton, 2001).

Many professionals, such as conservationists, ecologists, and architects, have begun to recognise green roof systems as alternative places for promoting wildlife, biodiversity, or natural habitat in built developments, and encouraged them to achieve policies for nature conservation and climate change (Dunnett and Kingsbury, 2008; Brenneisen, 2003; Grant et al., 2003; Gedge, 2003). The green roof systems can be mainly categorised into two types, which are intensive green roofs and extensive green roofs. Intensive green roofs are characterised by intensive management, thick substrate depths (more than 200 mm), supporting “the whole range of vegetation types, from trees and shrubs through to herbaceous planting and lawns” (Dunnett and Kingsbury, 2008, p.5). These green roofs can be so called as ‘roof gardens’ (Johnston and Newton, 1993, p.53). However, installing intensive green roofs on existing buildings is structurally limited because the load-bearing capacity of building should be enough to withstand the heavy loads of intensive green roof (Dunnett and Kingsbury, 2008, p.92). While on the other, extensive green roofs are designed to pursue minimal maintenance and irrigation, and install on existing buildings with little or no additional load-bearing structure. Due to

Table 1.1. Benefits of specifying a green roof (Source from: Dunnett and Kingsbury, 2008; Kadas, 2006; Grant et al., 2003).

• Provision of wildlife habitat and replacement of lost habitat through development
• Attenuation of storm-water runoff
• Improvement of runoff quality
• Absorption of air pollutants and carbon sequestration
• Reduction in the 'urban heat island' effect
• Reduction of noise pollution
• Energy efficiency for cooling
• Creation of amenity and visual aesthetic value
• Increase of roof life

the loading capacity of rooftops, extensive green roofs use thin (less than 200 mm) depth, lightweight and free-draining substrates. However, extensive green roofs are limited to supporting a wide range of vegetation types due to the shallow substrate depth, a minimal requirement of maintenance and irrigation, and harsh roof environments (Dunnett and Kingsbury, 2008).

For the extensive green roof systems, in urban areas, roof space could offer large areas of potential habitats compared to the limited areas of green space at ground level (Grant et al., 2003; Johnston and Newton, 1993; Dunnett personal communication); for example, the Greater London Authority (GLA) (2001) reported that buildings with flat rooftop cover 24,000 hectares or 16 % of Great London. In Seoul Metropolitan City in South Korea, flat rooftops cover about 25,359 hectares, which is approximately 70 % of the Seoul Metropolitan City. It has been estimated that over 20,000 hectares of the existing roofs could be vegetated (Seoul development institute, 2000). In the last few decades, green roof systems on existing buildings with little or no additional load bearing structure received the most attention as they are low maintenance, have little or no need for irrigation and use thin (less than 200 mm) depth lightweight and free-draining substrates. Its application has been constantly increasing in urban areas due to their numerous benefits to urban environments and quality of human life as shown in table 1.1 (Dunnett and Kingsbury, 2008; Grant et al., 2003; Johnston and Newton, 1993). Consequently, it has become increasingly prevalent that many local governments around the world have been making policies and programmes to promote green roofs on existing or new developments for ameliorating urban environmental problems (ref. Appendix 1).

Table 1.2. Benefit of the use of wider range of plant species (Source from: Dunnett et al., 2008a; Nagase, 2008; Lee and Koshimiz, 2007; Dunnett, 2004b; Grant et al., 2003; Tan, et al., 2003).

• Enhancement of biodiversity and wildlife value	- supporting large number of plant species - more attractive to wildlife; longer flowering period providing nectar and pollen resources to invertebrate - plant structural diversity promoting invertebrate richness
• Maximisation of green roof performance	- different contribution of temperature reduction - water conservation and runoff
• Enhancement of aesthetic and visual quality	- different and long flowering time, and visual and structural diversity from diverse species mixture - the public preference to mixed planting - wide range of planting options for green roof
• Reduction of management for green roof	- reducing irrigation needs in green roof using plant communities occurring dry habitats

For many years, however, the same type of vegetations such as Sedum species or turf, especially sedum vegetated roof greening has been promoted widely by commercial green roof companies around the world. Most research has tended to focus on the performance of green roofs based on Sedums and other succulents (e.g. Getter and Rowe, 2008; Durhman et al., 2007; Carter and Rasmussen; 2006; Denardo et al., 2005). Such mono-cultural planting can be very susceptible to environmental hazards such as diseases or stress periods, and this type of green roof shows a lack of diverse aesthetic pleasure (dull and uninteresting), ecological or biodiversity value, and local and regional identity (Baumann, 2006; MacDonagh et al., 2006; Dunnett and Kingsbury, 2008; Dunnett personal communication). Moreover, it is becoming more widely recognised that the use of wide range of plant species is more beneficial to the environmental performance, biodiversity of the green roof, and aesthetic and amenity for the public (Table 1.2.).

For a more ecological and sustainable rooftop environment, trials have begun to apply maximise benefits for both people and wildlife through using a diverse range of plant species (Dunnett and Basilio, 2008; Dunnett and Kingsbury, 2008; Dunnett, 2006; Dunnett, 2004b).

1.2 Plant selection and communities from native habitats for roof greening vegetation

Green roofs, which are more economic and widely applied, have a highly limited use of vegetation species due to the harsh environments of building rooftops and shallow substrate depth (less than 200 mm). To get a wider range of plant species as sources of plant species, and to avoid unpredictable problems to ecosystems that would be caused by the introduced species, and to meet the requirements of ecological and sustainable green roofs, some researchers have begun to explore native plants habitats for green roofs. This approach of using native plant community to green roof planting design can maximise wildlife habitat value and other ecological functions, and also enhance a sense of regional identity (Dunnett and Kingsbury, 2008; Coffman, 2007; Dunnett, 2006; Lundholm, 2006; MacDonagh et al., 2006; Kephart, 2005). Contrary to succulents from the Crassulaceae or stonecrop family that are most commonly used for green roofs, the native plants have evolved and adapted to the local climate and conditions over a long period of time and consequently, have stress-tolerance to the hazards (White and Snodgrass, 2003; Johnston and Newton, 1993). In plant ecology, natural habitats on infertile soils support a large number of plant species because stresses caused by the infertile environments limit establishment and growth of the vigorous aggressive species and give slow-growing and less competitive species more chance to establish. Thereby, a much greater diversity of plant species can co-exist (Grime, 2002). This species-richness does not only support many rare species, including many visually attractive flora species, but also a greater diversity of invertebrates (Keymer and Leach, 1990; Mclean, 1990). For instance, in most parts of north-western Europe the natural plant communities in which the greatest diversity of flora and fauna, especially invertebrate species, occur are found in habitats that contain shallow alkaline soils (Willems, 1990).

Therefore, appropriate natural plant habitats, or plant communities that include regional native species, may survive as green roofs when the green roof environments are similar to the harsh growing conditions of the native plant communities. The overlap of both conditions suggests that the native habitat could be ideal for extensive green roof. These points could allow green roofs to have fewer maintenance requirements such as less or no irrigation, and to have high value for biodiversity and aesthetics (Lundholm, 2006;

Dunnett and Kingsbury, 2008). For example, the soil profile of limestone has similarities to that of the extensive green roofs, which is thin, free-drainage, and low nutrient, and they tend to be much more species-rich than other grasslands on acidic substrate, and may contain over 50 species per $1m^2$ (Rorison, 1990). In sensitive rural areas, the use of green roofs that support native habitat may also aid integration of new buildings into the surrounding landscape (Dunnett, personal communication). However, the use of native plant species or communities on green roofs is a rather challenging concept because different regions have different climatic features (macro and micro) (MacDonagh et al., 2006; Kephart, 2005). Moreover, information pertaining to appropriate plant species selection, management (irrigation, feeding, or weeding, etc), and growing media for recreating natural plant communities on green roofs of each region are relatively limited.

There has been some research into the appropriate native species in order to apply natural plant communities for use on green roofs in other regions. In Nashville, Tennessee, the U.S., 15 species of plants, the Tennessee coneflower and Purple Prairie grass listed in the Endangered Species Act, were tested on a rooftop at the Neuhoff Meat Packing Plant. The plants belong to the threatened cedar glade plant community. All these plants had successfully established and grown on the rooftop (Shriner, 2003). In San Francisco, California, for successful living roof projects, a green roof demonstration and trial programme was developed to focus on plant selection, irrigation use and frequency, and to enable the design team to develop construction methodologies and identify technical assembly challenges. Twenty-four plant species occurring on uplifted impervious sand stone, overlain by shallow podsol formations in local intact grassland and coastal bluff plant communities were tested. The selected plant materials from local indigenous stock consisted of grasses, sedges, forbs, herbaceous and creeping perennials, and annuals. Several species were associated with local fauna (insects and birds), some of them on a rare and endangered species list. All twenty-four species survived without supplemental winter irrigation from September to May. Of the twenty-four plants, coverage of four perennial plants, *Fragaria chiloensis*, *Prunella vulgaris*, *Armeria maritima spp. californica*, *Sedum spathulifolium*, reached 70 % for three months (Kephart, 2005). Monterusso et al. (2005) evaluated eighteen taxa of Michigan native plants over three years for drought tolerance, growth, and survival

during both establishment and overwintering, and visual appearance. Of the eighteen plants, four species showed suitable growth and establishment under non-irrigation conditions. In New England and Canada, projects have been investigating if native coastal plants were better than exotic species used in extensive and semi-intensive plantings, and to evaluate native plants' competition in mini-habitats and a combination of substrates for suitable environment for the growth of native species. Subalpine, upland, coastal barren and wetland plants were examined with variables, sunlight and irrigation regimes, different substrate types and depths, and fabric related to water retention capacity. Sixty herbaceous and woody plants are now being examined additionally (Licht and Lundholm, 2006). In Seattle, Washington, native plant survival and vigour on extensive green roof with 650 m^2 size at the Woodland Park Zoo was conducted to evaluate performance and feasibility of using native plant species in the Pacific Northwest region in order to contribute to the enhancement of green roof ecological function and aesthetic potential. The green roof has four distinct planting zones which consist of *Arctostaphylos uva-ursi* 'Massachusetts' and *Allium cernuum*, *Lupinus polyphyllus* and *Fragaria chiloensis*, *Gaultheria shallon* and *Polystichum munitum*, and *Sisyrinchium douglasii* and *Arctostaphylos uva-ursi* 'Massachusetts' (Martin, 2007).

1.3 Natural plant communities on green roofs: case study

In practice, locally-natural plant communities have been applied dominantly for the plant-selection of green roofs in Northern and central Europe, and North America (Baumann and Tausendpfund, 2008; Dunnett and Kingsbury, 2008; MacDonagh, et al., 2006; Hauth and Liptan, 2003). This section explores green roofs that were established by local plant or plant communities originating from native habitats of their regions in order to achieve ecological and sustainable rooftop environments.

1.3.1 Switzerland

In Switzerland, implementation and construction of modern green roof systems mostly focus on recreating a wildlife habitat places for biodiversity, especially fauna species

(insects and birds), as mitigation of the losses due to urban development environment. This green roof system is defined as 'Brown roof'. Dunnett and Kingsbury (2008) states that 'Brown roof' is:

> "a term that has been developed to describe roofs that use 'urban substrates' such as brick rubble, crushed concrete, sands, gravels and subsoils (often derived from the development site of the new building) (p.8)"

The main planting design concept for green roofs in the country, the canton of Basel especially, is to encourage spontaneous colonisation with vegetation by use of local top soils including seeds reservoir and urban substrate materials, and to apply specific local seed mixes combining with Sedum spp. and dry grassland species, for wildlife and habitats of local natural surroundings (Brenneisen, 2003).

1.3.1.1 Chicken barn, Asphof, Rothenfluh, the Canton of Basel-Land

Figure 1.1. The green roof at Chicken barn, Asphof, Rothenfluh, blending the planting with the surrounding landscape (Photos by author taken on Sep 2005).

The green roof on Chicken barn was installed to provide temperature and ventilation control for the barn and to integrate it with the surrounding landscape. The roof was constructed with 15 cm of China reed, a very light and water storing ground layer, and topped by 5cm topsoil from the former orchard on site. *Phacelia*, a fast-growing species, were sown at first planting in order to improve the soil and prevent erosion. Mown grass from a dry meadow is spread on top of this to promote the establishment of a local dry meadow on the roof (Brenneisen, 2004 and 2005b).

1.3.1.2 Klinikum 2(Clinical centre 2) of the Cantonal Hospital of Basel

Figure 1.2. The green roof at the Klinikum 2 of the Cantonal hospital of Basel with substrate stimulating river terrace conditions (left: photo by Stephan Brenneisen, picture from Brenneisen 2006; right: picture from greenroofs.com).

The green roof was built in accordance with the city's new guidelines on green roofs and urban biodiversity. The substrate consisted of regional soils for near-natural dry meadows, sands, and a sand-gravel-loam mixture to create river bank conditions, and the depth were 6, 12, and 20 cm. The roof was seeded with a mixture of native annual and perennial herbs, including tall grasses, sedums, and the 'Basel mix' of seeds (Brenneisen, 2006).

1.3.1.3 Rossetti Research Building-Basel Hospital, Basel

Figure 1.3. The green roof at Rossetti Research Building-Basel Hospital, Basel (Photos by Stephan Brenneisen; pictures from Earth pledge, 2005).

The green roof was designed to reduce the building's impact on the ecosystem of the nearby Rhine riverbanks. The substrate consisted of a sandy to loamy gravel local soil

of various depths, ranging from 7 cm to small hills of 40 cm approximately. For planting on the roof a mix of local grassland plants was applied in minimum in order to lead natural colonisation of native species. The Seed mixture included the following 27 species: *Achillea millefolium, Campanula rotundifolia, Cerastium spp., Chrysanthemum leucanthemum, Clinopodium vulgare, Crepis capillaris, Dianthus carthusianorum, Echium vulgare, Erigeron annuus, Euphrasia roskoviena, Globularia* spp.*, Hieracium pilosella, Lactuca serriola, Leontodon hispidus, Medicago lupulina, Melilotus albus, M. indicus, Papaver rhoeas, Petrorhagia saxifraga, Plantago lanceolata, Potentilla argentea, Prunella vulgaris, Rosmarinus officinalis, Salvia pratensis, Scabiosa columbaria, S. acre, S. reflexum, S. sexangulare* (Earth pledge, 2005, p.96).

1.3.2 United Kingdom

Several projects associated with semi-natural grasslands on green roofs were completed in the UK. For example, following a green roof introduced by Grant (2006), semi-natural plant communities were established on a roof from wildflower seed mixtures supplied by a commercial company engaged in wildflowers. Dunnett and Kingsbury (2008) state that the use of seed mixture provides "great advantages in adaptability to a variety of environmental conditions as well as visual attractiveness" (p.179).

1.3.2.1 11 Shaw's Cottages in South London

Figure 1.4. The green roof at 11 Shaw's Cottages (Photos by Gary Grant; pictures from Grant, 2006).

For promoting biological diversity, the green roof that was constructed on a private residence in 1993 used a chalk and subsoil mixture, loamy topsoil, and gravel as substrate material, and native wildflower seed mixtures designed for alkaline, neutral, and acid soils (Emorsgate EM6, EM5, and EM7, respectively) were applied in the substrates with various depths, ranging from 50 mm to 100 mm. In addition, cornfield annual mixtures (Emorsgate EC1) was used to provide a show of colour in the first growing season, and *Sedum acre* and *S. reflexum* were applied later (Grant 2006, p.52-53; wildseed.co.uk for species lists).

Similarly to Switzerland, many 'brown roofs' have been recently installed, in London especially, to create habitat for key species associated with London Biodiversity Action Plans (Gedge and Kadas, 2004); the key species are vertebrate fauna, especially the Black redstart (*Phoenicurus ochruros*) which is a bird that breeds rarely in London and relies on old vacant lots and brown land (Gedge, 2003). For instance, the Laban Dance Centre, located in the Thames corridor within inner London constructed in 2002, was the first brown roof compensating for the loss of brownfield sites in inner city of London for the rare Black redstarts. The planting on the green roof was designed to rely on natural colonisation by vegetation, and then to apply a local wildflower seed mix (Earth Pledge, 2005, p.94). In Nagase's study (2008), however, it is described that "it seems that people prefer more flowering green roofs in the UK" (p.23). Furthermore, as a model of future-oriented green roofs, consideration has begun regarding green roofs incorporating both biodiversity and aesthetic appearance (Dunnett, 2006). The project at Sharrow School in Sheffield is good example of green roof associated with both approaches.

1.3.2.2 The green roof on the new Sharrow School, Sheffield, United Kingdom

This green roof is the first roof in the UK to be awarded LNR (Local Nature Reserve) Status by Natural England (Green Roofs Today, 2009). The accessible and visible roof on the school is a wildlife habitat green roof, playing a role as an urban nature reserve and for educational opportunities to the pupils. The green roof is designed with mounds and valleys, with a small wetland area, and area of birch forest. Vegetation types and

Figure 1.5. The green roof on Sharrow Primary School; spontaneous plants and annual meadow (Photos by author taken on Oct 2008).

habitats on the roof consist of urban post-industrial brownfield, diverse limestone grassland, spontaneously colonising areas, wildflower meadow, and colourful annual meadow. The planting was established through a combination of planting, seedling and natural colonisation on 100 mm to 500 mm deep substrate (Dunnett and Basilio, 2008).

1.3.3 United States

In recent years, concerns, research, and construction of green roofs have been growing in North America. Appropriate plant species lists for the present green roof systems have been mainly developed by Northern European countries, especially Germany. However, it would be hard to directly adapt European plant lists to the North America context because of different climatic conditions across North America from northern Europe (Snodgrass and Snodgrass 2006, p.12). For this reason, necessities for the research regarding the application of native plants or habitat templates, and green roof construction approaches have been increasing in North America. The following are examples associated with using native plants for green roofs in the United States.

Some green roofs with native plant communities, like green roof cases in Switzerland, have been installed in the United States. The following case study introduced by MacDonagh et al. (2006), is a green roof that has been planted by indigenous plant species originating from Minnesota's bedrock bluff prairie, which occurs on bluffs along the Mississippi and its tributaries in Southeast Minnesota and also the St. Croix

River occasionally. The bluff prairie supports the richness of native prairie grasses and forbs (over 25 species per 30 X 30 feet) and is found on steep south and west facing slopes, thin, very free-draining soil cover bedrock, ranging from 0 cm to 125 cm soil depth. While native plant communities were used for the green roofs like the Switzerland cases, design concept of the green roofs also has a strong aesthetical consideration, in contrast to the Switzerland's green roof cases creating mainly wildlife habitats. Similarly, another two cases are representative of locally appropriate plant community-based green roofs in California, which were established by a methodological approach, developed by Kephart (2005), to recreate semi-natural grasslands on buildings. The plants used for the green roofs were cultivated from coastal bluff plant communities of the region.

1.3.3.1 Phillips Eco-Enterprise Centre, Minneapolis, Minnesota

Figure 1.6. The green roof at the Phillips Eco-Enterprise Centre (Pictures from greenroof.com).

This extensive green roof was established to provide both demonstration and research on the benefits of green roofs related to reduction in storm water runoff, increasing lifespan of roofing membrane, and temperature above the roof, and to monitor establishment and survival of plants on it (MacDonagh et al., 2006). The green roof is accessible to building tenants and visitors to the building and used as a place for hosting receptions, meetings, or a relaxing space for individuals (Dunnett and Kingsbury 2008, p.180; MacDonagh et al., 2006). The planting design targets both close-up viewing as well as quick glances from a distance because the roofs are accessible and clearly visible to passengers on an adjacent elevated light rail transit line. 18 Minnesota's

bedrock bluff prairies were planted in 6 to 15 cm deep substrate and 11 traditional European green roof plants (Sedums) were planted in swale-like depressions with 6 cm deep substrate, oriented to the four points of the compass. Limestone outcroppings were placed on the compass simulating bedrock bluff prairie habitat.

1.3.3.2 The California Academy of Sciences, San Francisco, California.

Figure 1.7. The green roof at the California Academy of Sciences (Pictures from greenroof.com and calacademy.org).

The green roof on the rooftop of the museum has been developed to educate the public in the sustainability and ecological elements of the roof as one of the museum exhibits. The green roof has targeted eight goals, which include aesthetic integration of the natural landform, local flora, and habitats of the site, and energy savings through dropping interior temperature, reduction of storm-water runoff, sound attenuation, and urban heat island effect, water efficiency by use of reclaimed water, demonstration of the roof as an educational exhibit, and restoration and reconnection of wildlife habitat. To meet those goals, 197,000 square feet of the green roof has been installed at the museum with seven undulating, steeply sloped domed structures, mimicking the topography of San Francisco and the hills of nearby Twin Peaks (Hasan, 2009; Kephart, 2005). 1.7 million Native plants consisting of nine plant species, *Fragaria chiloensis*, *Prunella vulgaris*, *Armeria maritima ssp. californica*, and *Sedum spathulifolium* for four perennial species, and *Layia platyglossa*, *Lasthenia californica*, *Lupinus nanus*, *Eschscholzia californica*, and *Plantogo erecta* for five annual wildflowers, were specially chosen to flourish in Golden Gate Park's climate (calacademy.org). In order to install the plants on extreme dips and slopes, BioTray® vegetation container made from

rapidly renewable coconut coir fibres, was used, which is a biodegradable, reinforced, modular propagation tray. This tray was designed to have water retention for the plants and to hold the growing medium in place during plant establishment (Hasan, 2009).

1.3.3.3 The Gap headquarters, 901 Cherry, San Francisco, California

Figure 1.8. The green roof at the GAP headquarters, 901 Cherry (Pictures from greenroof.com).

The green roof at the Gap's 901 building was installed in order for the building project to meet the fundamental concept, which is it should have no adverse effect on the surrounding landscape from a bird's perspective due to the act of construction, and the notion of "creating a great place to work" at a wide range of scales. The green roof provides extraordinary thermal and acoustic insulation, consequently contributing to increased energy savings, storm-water retention, local habitat, and amelioration of the local microclimate (Burke 2003, p.228-229). The design concept included 69,000 square feet of green roof with an undulating roofline echoing the surrounding landscape covered in native grasses and prairies, reproducing the local coastal savannah ecosystem. The planting has been designed to change with the seasons as the native grasses flower and ripen, to integrate with the surrounding environment, and to extend the distribution of the natural plant community into an urban setting (Dunnett and Kingsbury 2008, p.44; Pledge 2005, p.12-13; Burke 2003, p.231). The plants were established in 15cm deep substrate and species included Idaho Fescue, Nodding Needlegrass, Blue-eyed grass, California bent grass, Tidy tips, Checkerbloom, Indian Paint Brush, and Woolly sunflower (Burke, 2003, p. 231).

1.4 Aims of the study

Of the range of native habitats in the UK appropriate for green roof application, calcareous grasslands (grasslands occurring over limestone or chalk substrates) are ideal candidates – they naturally occur on very thin, low nutrient and free draining soils. They occur naturally in most temperate regions of the world. Moreover, they generally support very species-rich vegetation that may contain over 50 species per $1m^2$ (Rorison, 1990, p.21). In the UK they are distributed predominantly in England. As native habitats, calcareous grasslands, which are also distributed in Sheffield district including the Peak district, have been considered as very important native habitats throughout continental Europe and the UK as they are species rich grassland associated with many rare and threatened fauna and flora species (UK Biodiversity group, 1998; Hillier et al, 1990). Calcareous grasslands are discussed in detail throughout the literature reviews in Chapter 2.

The value of using native habitats on green roofs to promote biodiversity, aesthetic value, and regional distinctiveness has received great attention recently, and recreating habitats at ground level has been greatly practiced and studied. However, the research into recreating these semi-natural plant communities on green roofs is relatively rare, and this has led to a lack of specific recommendations for native plant communities or assemblages on green roofs in the UK.

Therefore, the work described in this thesis considers the application of calcareous grassland vegetations to the green roof context. This provides a context for detailed experimental works which concentrate on locally-occurring calcareous grassland types. In order to meet this research gap, this thesis is comprised of four main work areas as listed below.

1. Calcareous grassland types, ecology and characteristics.
2. Restoration ecology of calcareous grasslands.
3. Substrate for calcareous grasslands on green roofs.
4. Plant selections and communities for calcareous grasslands on green roofs.

The study described here has a number of aims:

1. To identify calcareous grassland types, ecology and characteristics.
2. To review restoration ecology of calcareous grasslands.
3. To identify substrate and substrate characteristics in that support the target plant community.
4. To investigate suitable individual native species of calcareous grassland or other native habitats for use on green roofs in the UK.
5. To investigate key factors that can have an effect on plant establishment and growth success.

Chapter 2 Calcareous grassland

2.1 Introduction

As stated in Chapter 1, calcareous grasslands have received much attention as an ideal candidate in the UK as an appropriate native habitats or vegetations for planting on a green roof system (English Nature, 2006; Dunnett and Kingsbury, 2008). This is because the characteristics of soil on the habitat, typically thin, light, and dry (Tansley, 1965, p.98), are similar to that of green roof systems, and calcareous grasslands, which are the most species-rich plant communities, can offer an abundant source of native plant species.

Calcareous grasslands are considered as basic grassland communities occurring on soils with an alkaline reaction, developed in chalk or limestone bedrock outcrops. They are generally distributed in Britain and north-western Europe: “from western France to the present German-Polish border and from southern Scandinavia to the Alps and Pyrenees” (Willems, 1990, p.3). Limestones are sedimentary rocks, which are largely composed of calcium carbonate ($CaCO_3$) in most forms of calcite. The chalk is a very pure form of limestone, containing from 90 to 99 % of calcium carbonate (Smith, 1980, p.5; Tansley, 1965, p.525). The calcite of limestone and chalk is formed mainly by the depositions in water of marine organisms that secrete lime. The chalk accumulated at a slow rate during the Upper Cretaceous (*ca*. 100 million years before present). Opening the Palaeocene of the Tertiary Era (about sixty-five million years ago), the chalk seabed began to rise above water level (Smith, 1980, p.17). In the present, the deposition of white chalk can be found in England, Belgium, northern France, Denmark, Germany, Poland, Russia, parts of the Middle East, the Gulf Coast of the U.S.A., and Western Australia (Smith, 1980, p.2).

Calcareous grassland is sub-climax or biotic plagioclimax community stabilised by human activities conducted since the Neolithic period, such as sheep grazing for agriculture (Tansley, 1965, p.487). The influences of human activities have prevented natural succession towards final climatic climax or deflection towards a different climax. For this reason, these grasslands are also regarded as anthropogenic and semi-natural

plant community (Poschlod and WallisDeVries, 2002, p.361; Tansley, 1965, p.225).

This chapter aims to describe calcareous grasslands through literature reviews in order to meet the research objectives and questions described below.

2.1.1 Research Objectives

1. To identify calcareous grassland types, ecology and characteristics
2. To review restoration ecology of calcareous grasslands

2.1.2 Research Questions

1. What are calcareous grasslands?
2. Over what rock types do they occur?
3. What are the characteristics of typical natural soils of calcareous grasslands?
4. What are the typical plant communities that comprise calcareous grasslands?
5. What are the differences of typical plant communities between regional distributions?
6. What are the differences between the UK calcareous grasslands and other calcareous grassland types in Europe and other continents?

2.2 Historical context of calcareous grassland

Historically, since the last glaciations before the Neolithic Age, calcareous grasslands did not exist but were scarce, small, and isolated on calcareous outcrops or hilly domes in the Jurassic mountainous regions (Butaye, et al., 2005, p.111; Poschlod and WallisDeVries, 2002, p.362), which were also called “Steppenheide” habitats (according to Gradmann,1950; cited in Poschlod and WallisDeVries, 2002, p.362).

The present calcareous grasslands originated in the Neolithic period (*ca*. 7000 years before present) after man felled the primeval deciduous forests which occurred on moderately dry, basic soils in the hilly regions (Butaye et al., 2005, p.111; Willems, 1990, p.3). The process of forest clearance provided grass and herb species originating

from natural open habitats in southern and south-eastern Europe with opportunities to assemble and form species-rich grasslands in north-western Europe on calcareous outcrops or on steep slopes where forest development was prevented (Butaye, et al., 2005, p.111; Willems, 1990, p.3).

These grasslands have been established and developed by different types of land-use practices such as husbandry or the arable field-pasture farming system from the Bronze Age to the early Middle Ages, or the three-field-rotation system from the end of the early Middle Ages until the beginning of the twentieth century (Poschlod and WallisDeVries, 2002, p.365).

During the Bronze Age (*ca*. 1800 – 700 BC), grazing of forest by livestock created new habitats for calcareous grassland species preventing natural succession to forests. These grasslands began to spread throughout Central Europe by moving at a small scale during the Roman Empire (Poschlod and WallisDeVries, 2002, p.363). From the fifteenth to twentieth century when large sheep flock migration and transhumance occurred, calcareous grasslands were largely widespread across Europe (Poschlod and WallisDeVries, 2002, p.365).

Calcareous grasslands have been a significant source of food for domestic livestock for several centuries and the traditional agricultural practices maintained these grasslands to be well-balanced and species–rich (Willems, 1990, p.3). From the end of the nineteenth century until the 1970s, however, calcareous grasslands declined tremendously in both number and area due to intensification of agriculture, especially by the application of artificial fertilisers, and abandonment of traditional agricultural practices followed by afforestation, resulting in cessation of grazing by livestock (Poschlod and WallisDeVries, 2002, p.368). In the case of Britain, from the 12th to the 19th century chalk grasslands played an important role for wool trade. However, during the Napoleonic war (1792-1815), the areas were extensively ploughed to produce cereal crops and during both the First and Second World Wars, many areas of the grasslands were converted further into arable for the cultivation of crops under compulsory ‘Cultivation Orders’ (Keymer and Leach, 1990, p.12). Thereafter, the grasslands were improved and encouraged for producing arable crops by subsidisation of agriculture such as grants, modern machinery,

and artificial fertilisers, etc. (Keymer and Leach, 1990, p.12). Hereby, the amounts and the quality of the remaining calcareous grassland areas across Europe have constantly decreased (WallisDeVries et al., 2002; Willems, 1990).

The remaining areas of calcareous grasslands have been strongly fragmented into isolated patches as a result of habitat destruction and degradation (Butaye, et al., 2005, p.111; Poschlod and WallisDeVries, 2002, p.368; WallisDeVries et al., 2002, p.266). The fragmentation of the grasslands is leading to extinction of species and reduction of gene pools (WallisDeVries, et al., 2002, p.266; Zschokke, et al., 2000, p.559). Furthermore, extensification and eutrophication through aerial deposition have resulted in diminishment of habitat quality of the remaining grasslands by encroachment of dominant grasses, such as often *Bromus erectus* or *Brachypodium pinnatum* (Willems et al., 1993, p.; Willems, 1990, p.8; Bobbink and Willems, 1987).

2.3 Types and distributions of calcareous grassland in Britain

For Britain, fourteen calcareous grassland types have been identified in the National Vegetation Classification (NVC) as shown in Table 2.1 (Rodwell, 1990, p.30). Rodwell (1990) has summarised that these fourteen types have been classified by:

> "the composition and structure of the vegetation types, their relation to habitat factors, the zonations and successions in which they are found and their affinities with other kinds of British and Continental plant communities" (p.29).

The NVC has been divided into the lowlands group and the uplands group, or the south-eastern grasslands and the north-western grasslands as the two major floristic distinctions (The UK Biodiversity group, 1999 and 1998; Rodwell, 1990). The floristics and distribution of the communities are determined by variations in climate, particularly differences in precipitation and temperature, soils and management (Rodwell, 1990, p.29).

Keymer and Leach (1990) defined calcareous grassland as native habitat which occurs on (Figure 2.1):

Table 2.1. The fourteen types of calcareous grassland in the National Vegetation Classification (Source from: UK Biodiversity Group, 1999 and 1998; Rodwell, 1990, p.30).

		Plant community	Description and soil type	Location
Lowland calcareous grasslands; the south-eastern	CG1	*Festuca ovina-Carlina vulgaris* grassland	Local on summer-parched protorenzinas over rocky slopes of harder limestone	among the south-western coastal fringe
	CG2	*Festuca ovina-Avenula pratensis* grassland	Widespread as major plagioclimax pasture on rendzinas over drift-free limestones	in warm and dry south-east
	CG3	*Bromus erectus* grassland	Widespread through the warm and dry south-eat as intermediates between CG2 scrub where grazing has been relaxed or where arable has been abandoned	
	CG4	*Brachypodium pinnatum* grassland		
	CG5	*Bromus erectus-Brachypodium pinnatum* Grassland		
	CG6	*Festuca rubra-Avenula pubescens* grassland		
	CG7	*Festuca ovina-Thymus-Hieracium pilosella* Grassland	Local on impoverished and often disturbed rendzinas	in more continental eastern England
Upland calcareous grasslands; the north-western	CG8	*Sesleria albicans-Scabiosa columbaria* grassland	Distinctive local plagioclimax on rendzinas and brown calcareous earth over Durham Magnesian Limestone	in Durham
	CG9	*Sesleria albicans - Galium sterneri* grassland	Widespread plagioclimax pasture on rendzinas and brown calcareous earth on north Pennine Carboniferous limestone	in Cumbria
				in the Pennines
	CG10	*Festuca ovina- Agrostis capillaris-Thymus praecox* Grassland	Widespread but local as plagioclimax pasture of flushed brown earth on lime-rich rocks and superficial	throughout sub-montane north and west
	CG11	*Festuca ovina-Agrostis capillaris-Alchemilla alpine* grassland	Local plagioclimax grass-heath of flushed brown earth on lime-rich rocks and superficials	at higher altitudes in Scottish Highlands
	CG12	*Festuca ovina-Alchemilla alpina-Silene acaulis dwarf-herb community*	Local montane community of immature moderately base-rich mull soils subject to some snow-lie, frost heave and solifluction	in Scottish Highlands
	CG13	*Dryas octopetala-Carex flacca* grass-heath	Local plagioclimax grass-heath on rendzinas and rock	in oceanic north-west Scotland
	CG14	*Dryas octopetala-Silene acaulis* ledge community	Local montane community of ungrazed ledges and crag of lime-rich rocks	in Scottish Highlands

"soils derived from rocks rich in calcium carbonate, principally the limestone which – from south-east to north-west – comprise the Cretaceous chalk and the Jurassic and Carboniferous Limestones" (p.11).

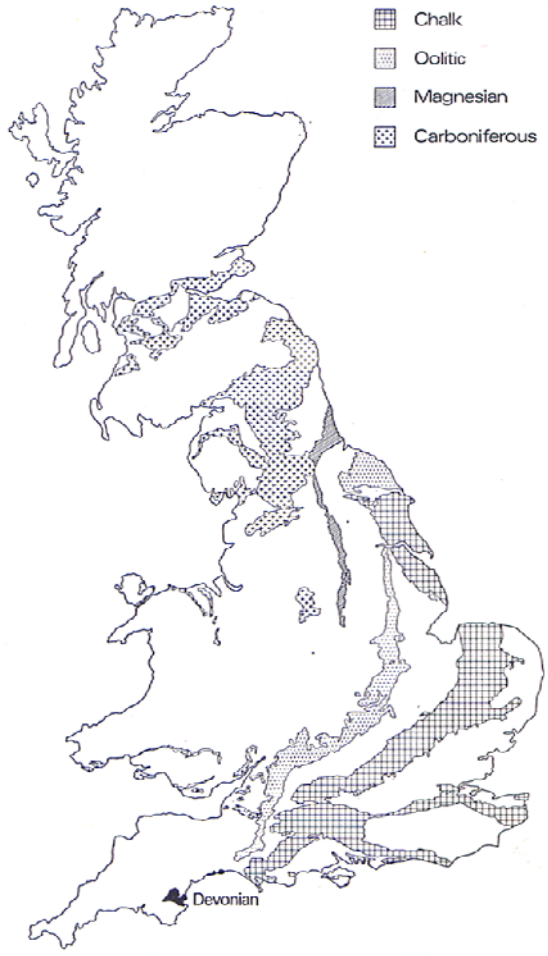


Figure 2.1.The distribution of the major calcareous rock formations in Britain (Adapted from: Duffey et al., 1974, p. 44)

In geographical position, these two blocks are located along an approximate line from Durham to the Mendips via Derbyshire and the edge of Wales. The important factors of this divide are precipitation and temperature, which determine the floristics and distribution of the communities (Rodwell, 1990, p.29). Calcareous soils have typically high water-holding and water-releasing capacity, are drought resistant, with pH between 7 and 8, and form shallow rendzina that occur on relatively steep slopes (Rorison, 1990, p.21; Tansley, 1965, p.94). Due to this pH range, calcareous grasslands have a number of species and compositions distinct from neutral or acid grasslands (Tansley, 1965, p.98).

The lowlands types defined as CG1 to CG9 in the NVC are found in the comparatively warmer and drier climatic conditions of southern and eastern England. However, CG8 and CG9 communities characterised by *Sesleria albicans* do not fall neatly into either of the two distinct communities. CG8 occur in Durham. The CG9 lowland types occur in the more clement conditions on the Craven and Morecambe Bay Carboniferous limestone in Cumbria, although they occur also in colder and wetter localities in uplands through the Pennines. The lowland communities are comprised of two sub-communities according to high and low grazing level. The three short-sward communities associated with heavy grazing are the CG2 community that is regarded as

typical chalk grassland and is a widespread community of the south-eastern grasslands, CG1 around south-west coastal fringe, and CG7 over the Chalk of East Anglia. CG3, CG4, CG5, and CG6 are tussocky grassland associated with low levels of grazing (UK Biodiversity group, 1998; Rodwell, 1990).

Five upland calcareous communities, CG10 to CG14, are distributed in areas of wetter climate through the uplands of Wales, northern England and Scotland. Carboniferous Limestone in the uplands is the most widely distributed and locally extensive calcareous rock in north and south Wales, the North Pennines, and Northern Ireland. The upland group is comprised of three sub-communities, which are CG10 associated with heavy grazing, CG11 and CG12 characterised by the presence of *Alchemilla alpina*, and CG13 and CG14 by *Dryas octopetala*. CG10 is a widespread short-sward community of upland communities (UK Biodiversity group, 1999; Rodwell, 1990).

Sheffield is located on the boundary between highland and lowland Britain. The geological formation and constituent rock types around Sheffield region are Carboniferous Limestone, Millstone Grit, Coal Measures, Magnesian Limestone, and Bunter Sandstone as shown in figure 2.2. (Lloyd et al., 1971). Two grassland types in terms of calcareous grassland, Carboniferous Limestone and Magnesian Limestone grassland, occur over Carboniferous Limestone and Magnesian Limestone rock. Each of the two grassland types has sub communities as shown in table 2.2. (Lloyd, 1972).

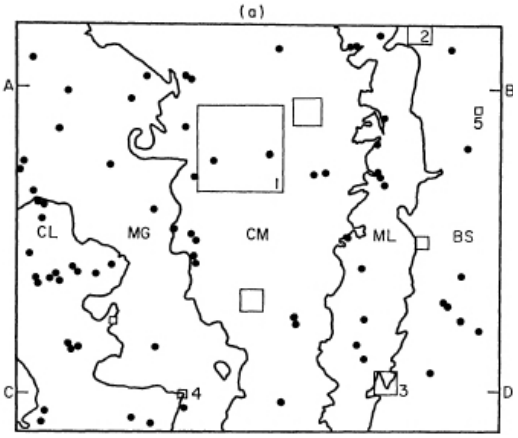


Figure 2.2. The distribution of geological strata. 1, Sheffield; 2, Doncaster; 3, Mansfield; 4, Matlock; 5, Bawtry. CL, Carboniferous Limestone; MG, Millstone Grit; CM, Coal Measures; ML, Magnesian Limestone; BS, Bunter Sandstone. (Adapted from Lloyd et al., 1971, p. 864).

Table 2.2. Grassland types of the Sheffield region (Source from: Lloyd, 1972).

Magnesian Limestone grassland	• Species-poor Magnesian Limestone grassland • Species-rich *Brachypodium* grassland • Cattle pasture with little *Brachypodium* • Pasture with much *Brachypodium* • Species-rich *Zerna* grassland
Carboniferous Limestone grassland	• Specie-rich A*rrhenatherum* grassland • Tall-herb communities • Open A*rrhenatherum* communities • Grassland of stabilized limestone screes
	• Species-poor *Festuca ovina* grassland • Species-rich *Festuca ovina* grassland • Species-rich Carboniferous Limestone grassland

2.4 Other calcareous grassland types in different regions

Calcareous grasslands are plant communities based on shallow calcium rich soils formed by limestone bedrock or rocks rich in calcium carbonate, which had been deposited by marine organisms for millions of years, and the floristic compositions and structures of the grassland are determined and stabilised by biotic and abiotic factors (Keymer and Leach, 1990; Rodwell, 1990; Smith, 1980). The similar edaphic conditions have not only often contributed to northern-west Europe including Britain, but also the rest of the world. Further to this, similar biotic and abiotic factors enabled similar plant communities to occur broadly around the world with different names. This section attempts to explore and identify other calcareous grassland types in Europe and other continents.

2.4.1 Alvar

Alvars are calcareous grassland communities based on a limestone plain with various thin deposits of soils (both calcareous and siliceous), generally ranging between 1 cm to 20 cm deep, originating from bedrocks which forms a plateau that consists mainly of Ordovician or Silurian limestone material or monolithic limestone rock (Rosén, 2006, p.387; Pärtel et al., 1999, p.561). Alvars are distributed mainly in the Baltic region of northern Europe; on the islands of Öland (the largest alvar area in the world covering

25,500 hectares in the southern part of the island) and Gotland in Sweden (Rosén, 2006, p.387), and Saaremaa, Hiiumaa and Muhu in Estonia, and in parts of coastal zone of the Estonian mainland (Pärtel et al., 1999, p.561), but small alvar areas are also found on the Swedish mainland in Västergötland, in Ahvenamaa, Finland, and in the St. Petersburg district, Russia (Pärtel et al., 1999, p.561). In the British Isles, alvars are known as limestone pavement and pavement barren and occur in Cumbria and the Yorkshire Dales of northern England and Counties Clare and Galway of western Ireland (Vincent, 1995, p.265; Ivimey-Cook, 1965, p.437). In North America, similar types of limestone areas are also known as ‘alvar’ or ‘stone prairie’ (Dunnett and Kingsbury, 2008, p.117; Rosén, 2006, p.387). Approximately 85 % of alvar sites of North America occur around the Great Lakes region in Michigan, New York and Ohio in the USA, in Ontario and Quebec in Canada, and more than 90 % of the alvar areas of the Great Lakes region are found in southern Ontario (Catling and Brownell, 1995, p.143).

Alvars comprise a wide range of vascular plants, bryophytes, mosses, and lichens, including species more commonly found in other types of dry or semi-dry grasslands, due to variation in soil types, soil depth, pH, moisture condition, type of grazing animals and grazing intensity (Rosén, 2006). Over 45 vascular plant species in the island of Öland and Gotland and 347 in the Great Lakes region were recorded (Rosén, 2006, p.390; Catling and Brownell, 1995, p.143). In the seven sites of the alvar habitat on the Bruce peninsula in southern Ontario, Canada, 180 vascular plant species, 50 bryophyte species, 53 lichen species, and 50 algal taxa, for a total of 333 species were recorded (Schaefer and Larson, 1997, p.800). The shallow soils are very susceptible to seasonal drought and flood caused by wind and direct solar irradiation, and heavy rain during summer. The extreme conditions and environmental factors limit establishment and growth of trees and shrubs, and support distinctive alvar plant communities (Rosén, 1995). Alvar plant communities are characterised by the vast extent, species richness in flora including endemic and rare species, diversity of phytogeographical elements and the large populations of many species, resulting in a variety of communities (Rosén, 2006, p.389). According to Rosén (2006, p.391) and Pärtel et al. (1999), seven alvar communities are classified in the island of Öland and in Estonia. There are also seven types of alvar communities found in the Great Lakes region (Catling and Brownell, 1995, p.143).

2.4.2 Steppe

Steppe is vast temperate grassland of Eurasia that extends over the lower region of the Danube in eastern Austria and in a broad belt over south and south-east European and Central Asian Russia, stretching east to the Altai and south to the Transbaykal and Manchurian plains (Steppe, 2008). Allaby (2010g) defines that "the term 'steppe' strictly refers to the temperate grassland of Eurasia" (p. 311). However, the term is also applied to an area with steppe-like vegetation which occurs outside Eurasia, which is "Puszta" for arid grassland of Hungary, "Prairies" for the United States, "Pampas" for South America, and the high "veld" for South Africa (Steppe, 2008), and the semi-arid regions on the fringe of the hot deserts (e.g. on the southern fringe of the Sahara (the Sahel zone), in parts of Namibia, and in south-western Australia) (Allaby, 2010g).

The Eurasia steppe dominantly comprises drought-resistant perennial grasses (species of the genera; *Agropyron*, *Cleistogenes*, *Festuca*, *Helictotrichon*, *Koeleria*, and *Stipa*) and forb species (*Allium* and *Filifolium*) (Lavrenko and Karamysheva, 1993, p.4). Many types of steppe occur typically on chernozemes and chestnut soils, which are characterised by deep and humus rich including high exchangeable cations (calcium and magnesium) (Allaby, 2010b; Lavrenko and Karamysheva, 1993, p.4). Although soil is very fertile, climate and edaphic factors, such as aridity, extremes of daily and seasonal temperature ranges, limited soil depth for rooting, high salinity, or a combination of these factors, prevent establishment and growth of woody plants in addition to natural disturbances such as floods, fires and herbivores (WallisDeVries et al., 1996, p.111).

Species composition within the steppe region varies from east to west and north to south, according to the rate of precipitation. The number and distribution of forb species decrease with increase of aridity of climate (Lavrenko and Karamysheva, 1993, p.4). In the case of the rhizomatous grasses and sedges, in the Black sea to Kazakhstan steppes of the western part of the Eurasia the dominant species are *Agrostis vinealis* (= *A. syreistschikowii*), *Bromopsis* (= *Bromus*) *inermis*, *Bromopsis* (= *Zerna*) *riparia*, *Elytrigia repens*, *E. trichophora* and *Poa angustifolia*, whereas in the steppes of Transbaykal and Mongolia of the eastern part, *Leymus chinensis* prevail (Lavrenko and

Table 2.3. Four zonal types of steppe (Source from: Allaby, 2010c; Lavrenko and Karamysheva, 1993, p.5).

(1) Meadow steppe	- Forest-steppe: adjacent to the forest to the north. - Various sod-forming grasses and subordinate tussock grasses. - Broad-leaved flowering herbs. - In semi-humid climate.
(2) True or typical steppe	- Bunch-grass steppes with many forbs, in semi-arid climate. - Bunch-grass steppes with few forbs, in arid climatic.
(3) Semi-desert steppe	- Desertified bunch-grass and dwarf half-shrub-bunch-grass in very arid climate
(4) Desert dwarf half-shrub-bunch-grass steppe	- Occurrence in hyper-arid climate

Karamysheva, 1993, p.4). The steppe consists of four vegetation zones through north to south with increasing aridity of climate (the highest rainfall in the north), which are meadow steppe, true or typical steppe, semi-desert steppe, and desert dwarf half-shrub-bunch-grass steppe (Table 2.3) (Lavrenko and Karamysheva, 1993, p.5).

2.4.3 Prairie

Prairie is major temperature grassland of North America occurring mostly on a vast area, ‘the Great Plains’. The bedrock of the Great Plains is composed of limestone and dolomite containing high levels of magnesium (Prairie, 2003). The grassland extends from south part of Manitoba, Saskatchewan, and Alberta in Canada to the Mexican border and from the eastern flank of the Rocky Mountains through Iowa, Illinois, and Indiana to west Ohio, which includes all of the Great Plains (Prairie, 2010 and 2008). The prairie usually includes a combination of variety of xeromorphic grasses, herbaceous broad-leaved annuals and perennials (Allaby, 2010e), and supports very high species richness and diversity; for example, in the Kansas region only, over 800 species of non-woody flowering plants, 150 grass species and, 200 woody species have been recorded (Mason, No date).

Three main types of prairies are classified based mainly on the rate of precipitation, which is short-grass prairie in the west of 381 mm to 635 mm annual precipitation,

mixed- or medium-grass prairie in the 508 mm to 762 mm rainfall zone, and tall-grass prairie in the east in the 762 mm to 1016 mm precipitation zone (Oregon State University, 2000; Tomanek, 1995). According to Oregon State University (2000) and Tomanek (1995), the short-grass prairie (Grama-Buffalograss Prairie) occurs in semi-arid climate zone that lies from central Nebraska westward to the Rocky Mountains and from Texas to Saskatchewan. Dominated species include *Bouteloua gracilis* (Blue grama) and *Buchloe dactyloides* (Buffalograss). The heights of the species reach *ca*. 15 cm to 46 cm. The medium or mixed-grass prairie (Bluestem-Grama Prairie) is a mixture of tall, medium, and short grasses and dominated by *Schizachyrium scoparium* (Little bluestem), *Bouteloua curtipendula* (Side oats grama), and dropseed (*Sporobolus* spp.). These species are from *ca*. 91 cm to 152 cm in height. For the tall-grass prairie (Bluestem Prairie), *Andropogon gerardii* (Big bluestem) and *Schizachyrium scoparium* (Little bluestem), *Panicum virgatum* (Switchgrass), and *Sorghastrum nutans* (Indiangrass), with ranges from *ca*. 183 cm to 244 cm in height, and in wet lands within this zone, cordgrass (*Stipa* spp.) and reed grass (*Phragmites* spp.) are the dominant species. Soils of the zone are rich and fertile due to managed and natural fires preventing forest development.

There are also some different prairie land-types that developed on shallow rocky soils derived from calcareous substrates, which are called ‘limestone cedar glade (true open cedar glade) and xeric limestone prairie’. Limestone cedar glades occur in nine physiographic regions in Kentucky, Tennessee, Alabama, Georgia, and Virginia of the south-eastern United States (Baskin and Baskin, 2003, p.101). Xeric limestone prairie is distributed in six physiographic regions within tall-grass prairie region such as Pennsylvania, Illinois, Indiana, Ohio, Oklahoma, Arkansas, Missouri, and Wisconsin (Lawless et al., 2006, p.238).

2.4.4. Limestone cedar glade

The nine physiographic regions of distribution of the limestone cedar glades are Outer Bluegrass, Kentucky Karst Plain, Central Basin, Western Valley, Tennessee Valley and Little Mountain, Moulton Valley, Sequatchie Valley, Ridge and Valley (Baskin and

Baskin, 2003, p. 103). The cedar glades are herbaceous edaphic climax communities characteristically dominated by non-woody species (C_4 summer annual grasses). They occur on shallow soils (less than 25 cm) in open areas with nearly flat to gently sloping topography of calcareous rock in the form of pavement, gravel, and flagstone. The bedrock of the cedar glades, according to physiogeographic regions, consists of different limestone or dolomite (Ordovician, Silurian, or Mississippian in geologic period) (Baskin et al., 2007, p.318). For example, cedar glades in the Moulton Valley in Alabama are on Bangor Limestone (upper Mississippian), Ordovician limestone for the Ridge and Valley of east Tennessee, and Silurian limestone for the Western Valley of Tennessee (Baskin et al., 2007, p.306). The cedar glades are developed on Mollisols (subgroup, Lithic Rendolls) soil derived from the bedrocks. In summer, the soils of the limestone cedar glades are generally characterised by extremes in soil moisture content ranging from saturation to severe drought due to high irradiance and subsequent high soil temperature (Baskin and Baskin, 2003, p.101).

According to investigation of Baskin and Baskin (2003), limestone cedar glades are dominated by "C_4 summer annual grasses, C_3 winter annuals, summer annuals, and perennial herbaceous dicotyledons, cryptogams, or some combination of these" (p.101). The cryptogams include mosses, primarily *Pleurochaete squarrosa* (Brid.) Lindb., a nitrogen-fixing cyanobacterium (*Nostoc commune* Vauch.), and crustose, foliose, and fruticose lichens (Baskin and Baskin, 2003, p. 101). The representative dominant grass species in the cedar glades are *Schizachyrium scoparium* (Little bluestem), *Sorghastrum nutans* (Indiangrass), and *Andropogon gerardii* (Big bluestem) (Baskin et al., 1994, p.245). These glades support high diversity and species richness, which include 448 native and 96 non-native taxa (Baskin and Baskin, 2003, p.105). The largest families that are recorded are the Asteraceae (67 native, 19 non-native species), Cyperaceae (30, 0), Fabaceae (23, 12), and Poaceae (49, 13), with the six largest genera which are *Aster* (10), *Carex* (14), *Euphorbia* (8), *Hypericum* (11), *Leavenworthia* (8), and *Panicum* (15 taxa including *Dichanthelium*) (Baskin et al., 2007, p.318; Baskin and Baskin, 2003, p.105). 21 taxa are endemic/near-endemic species to the southeastern cedar glades including: *Astragalus bibullatus, Astragalus tennesseensis, Dalea foliosa, Dalea gattingeri, Delphinium alabamicum, Echinacea tennesseensis, Forestiera ligustrina, Grindelia lanceolata, Isoetes butleri, Leavenworthia crassa, Leavenworthia exigua* var.

laciniata, Leavenworthia exigua var. *lutea, Leavenworthia stylosa, Lobelia appendiculata* var. *gattinergi, Onosmodium molle* var. *molle, Orbexilum stipulatum, Oxalis priceae* subsp. *priceae, Pediomelum subacaule, Phacelia dubia* var. *interior, Talinum calcaricum, Viola egglestonii* (Baskin and Baskin, 2003)

The cedar glades in the Southeast are highly stable due to the support of the edaphic climax conditions, and the lack of anthropogenic disturbances such as fire, grazing, etc. While xeric limestone prairie (Baskin, et al., 1994) in the Ozarks and in the mid-western United States are "secondary successional plant communities" with moderately high stability by anthropogenic disturbances such as fire, cultivation or pasturing, grazing (Baskin et al., 2007, p.319; Baskin and Baskin, 2003, p.103). In other words, if disturbances were withdrawn the areas would process succession to a redcedar and/or hardwood forest (Baskin and Baskin, 2003, p.103).

2.4.5 Xeric limestone prairie

Xeric limestone prairies are herbaceous plant communities that occur on open, non-forested areas with gently rolling to steep slope covered by rocky calcareous soils with shallow to moderate depth (less than 10 cm to 100 cm; common depth is less than 60 cm.) derived from bedrock of limestone, dolomite, and calcareous shale, primarily Cambrian, Ordovician, Silurian, and Mississippian. The bed rocks are formed in gravel, talus, and/or flagstone (Baskin et al., 2007, p.318; Lawless et al., 2006, p.238). The prairie grasslands are spread in six physiographic regions on various bedrock systems in eastern United States from Missouri and Pennsylvania south to Arkansas and Georgia: (1) Ozark Plateaus on Mississippian and Ordovician limestone, and Cambrian and Ordovician dolomite; (2) Central Lowlands on Mississippian and Ordovician limestones and shale; (3) Interior Low Plateaus on Mississippian, Silurian, and Ordovician limestones, shale, and dolomites; (4) Appalachian Plateaus on Pennsylvanian limestone; (5) Ridge and Valley on Devonian, Silurian, Cambrian, and Ordovician limestone, and Ordovician and Cambrian dolomite; (6) Coastal Plain on Tertiary (Eocene) limestone (Lawless, et al., 2006, p.241-244).

The soils of xeric limestone prairies are composed mostly of Alfisols, and others including Ultisols, Moolisols, Inceptisols, and Vertisols (Baskin et al., 2007, p.318). Fine-textured clay loams and silty clay loams are dominant soil types (Lawless et al., 2006, p.241). Soil types ranged from 6.0 to 8.0 pH values, and low in fertility and soil phosphorus levels. During the growing season, the soil moisture is susceptible to "saturated conditions in early spring and xeric conditions in summer and autumn" (Lawless, et al., 2006, p.241). The prairie grasslands occur mainly on moderate to steep slopes (Baskin et al., 2007, p.318). Xeric limestone prairies are generally stabilised by anthropogenic disturbance, especially fires, but also the topographic conditions and soil characteristics affect the distribution and the species composition of xeric limestone prairies. Laughlin and Uhl (2003) reported that "in Pennsylvania, xeric limestone prairies are restricted to limestone soils on south-southwest facing slopes in the valleys of the Ridge and Valley" (p. 310). Kucera and Martin (1957, p.289) also found that soil depth was a determinant factor affecting species distribution of the grassland and transition zones between the grassland and forest areas because the soil moisture availability and root penetration was affected by soil depth.

Xeric limestone prairies are typically dominated by C_4 perennial grasses including *Schizachyrium scoparium*, *Bouteloua curtipendula*, *Andropogon gerardii*, *Sorghastrum nutans*, and *Sporobolus clandestinus*, and also in some sites, by C_3 perennial herbs and *Carex* spp. and *Fimbristylis* spp. In some sites with extremely shallow soils (less than 10 cm), they are typified by local dominance of the C_4 summer annual grasses, *Sporobolus vaginiflorus* and *S. neglectus* (Lawless, et al., 2006, p.244). Xeric limestone prairies have highest taxonomic richness in flora with four dominant families, Asteraceae, Poaceae, Fabaceae, and Cyperaceae (Baskin et al., 2007, p.318). In a study of 10 study sites in Pennsylvania, Laughlin and Uhl (2003) found that "native species richness of xeric limestone prairies ranged from 20 to 57 species per site" and "a total 126 native taxa representing 40 families including 80 forbs (74 perennials and 6 annuals/ biennials), 3 ferns, and 23 woody plants" (p. 307). 13 species are considered as endemic and/or near endemic species to xeric limestone prairies of eastern United States, which is mainly restricted to Ozark Plateaus (Arkansas and Missouri) and Ridge and Valley (West Virginia, Virginia, and Alabama) (Baskin et al., 2007). In Ozark Plateaus in Missouri and Arkansas, *Delphinium treleasei*, *Echinacea paradoxa* var. *paradoxa*,

Scutellaria bushii, and *Valerianella ozarkan*a are restricted; in Ridge and valley of Alabama, *Castilleja kraliana*, *Coreopsis grandiflora* var. *inclinata*, *Dalea cahaba*, *Erigeron strigosus* var. *dolomiticola*, *Liatris oligocephala*, *Onosmodium decipiens*, *Silphium glutinosum*, and *Spigelia gentianoides*; in Ridge and Valley of West Virginia and Virginia, *Monarda fistulosa* subsp, *brevis*. (Lawless et al., 2006, p.257).

2.5 The distinctive features of calcareous grasslands

2.5.1 Natural succession and plagioclimax

Succession is a sequential process of ecological change in communities that develops in an area from the initial colonization until stable mature climax communities are attained (Morris, 1974, p.74). The climax community or climactic vegetation is a community that has reached the final or stable stage that is environmentally balanced (Allaby, 2010a). Morris (1974) defined that "the sequential communities which form the succession are collectively known as a sere and individually as seral stage" (p.74). Two kinds of succession in sere community are generally recognised, primary succession (prisere) and secondary succession (subsere) (Morris, 1974, p.74).

Primary succession takes place on an area that has not been originally completely vegetated, for example, first colonisation on an area covered by a flow of lava. Secondary succession arises on an area where a previously seral or climax community was once established but has then been destroyed by fire, flood, grazing, etc. (e.g. succession after forest destruction by wild fire) (Allaby, 2010f and 2010h; Morris, 1974, p.74; Tansley, 1965, p.219-221). Succession is mainly caused by two factors, nutrients changes in the soil with eutrophication as the vegetation grows (autogenic succession) and external environmental influences, such as changing climate (allogenic succession) (Morris, 1974, p.74 and p.76). According to Clement (1916), the ecological process of succession involves six basic phases: nudation, migration, ecesis, competition, reaction, and stabilisation (Table. 2.4).

Morris (1974) stated that "a short-lived seral stage in the succession to forest climax

Table 2.4. Clements' theory of succession (Source from: Allaby, 2010i; Clements, 1916).

1. Nudation	The new development of plant succession initiates at a bare site by a major environmental disturbance (e.g. a volcanic eruption).
2. Migration	In plant succession, specifically the arrival of migration propagules (migrules) at a newly denuded area.
3. Ecesis	It involves establishment and initial growth of migrating plant species.
4. Competition	As vegetation become well established, grew, and spread, various species began to compete for space, light, and nutrients. This phase is called competition.
5. Reaction	During this phase autogenic changes affect the habitat resulting in replacement of one plant community by another.
6. Stabilisation	Reaction phase leads to development of a climax community.

was a community having some of the characteristics of grassland" (p. 74). For example, after clearance of woodlands, the site may be dominated by grasses, taller herbs, and small shrubs for some time. However, if absence of disturbing factors is continued, the community would rapidly change back to a form of woodlands. In Britain and Western Europe, human activities, especially for agriculture, following forest clearance in Neolithic and historic time, play a main role to prevent calcareous grassland proceeding back to the original climax community (forest) (Poschlod and WallisDeVries, 2002; Tansley, 1965). For this reason, calcareous grassland is regarded as sub-climax or biotic plagioclimax vegetation, which refers to a stable vegetation community arising from a succession that has been deflected as a result of continuous human activities (anthropogenic), such as grazing, cutting, or burning, etc., and does not reach its final climatic climax (Figure 2.3) (Allaby, 2010d; Tansley, 1965, p.225-226). Morris (1974) also noted that "Grassland is neither a seral stage, nor a climatic climax, it is a deflected climax or plagioclimax" (p.75). The community would not have occurred without human intervention

Figure 2.3. Deflected succession-plasiosere and plagioclimax (Adapted from: Tansley, 1965, p. 226).

(Allaby, 2010d; Tansley, 1965, p.225-226). In consequence, human activities can be considered not as disturbance but as one component of environmental factors. This is a reason that they are also called “semi-natural grassland” (Poschlod and WallisDeVries, 2002, p.361). Tansley (1965) stated that “the constant intervention of man does not stop succession altogether but rather deflects it into a new course” (p. 225). Although if the constant human intervention is removed, succession to the original climax community is no longer possible (Allaby, 2010d; Tansley, 1965, p.225-226). For example, chalk grassland, which is a stable typical community of southern England so long as pasturing continues, would not proceed to the original climax community (beech forest), as it were, if pasturing were ceased. It is because that the grassland is different from any stage in the chalk prisere leading ultimately to beech forest (Tansley, 1965, p.225).

2.5.2 Species richness and biological diversity

2.5.2.1. Flora

Calcareous grasslands support very high biological diversity (e.g. mixture composition of grasses, sedges and mosses which exceed 50 species per m^2, Rorison, 1990, p.21) and are associated with many rare flora species. In the case of chalk grasslands, which are one of the most species-rich plant communities at scales between 0.001 and 10 m^2, have averagely exceeding 30 to 40 species per m^2 (Willems et al., 1993, p.203). The values of biological diversity of these habitats can be illustrated by the facts mentioned in many previous studies. Willems (1990) described that in Europe calcareous grassland communities embrace “approximately 700 plant species, including 200 bryophytes and lichens. Roughly one-third of this number of species is restricted to this habitat” (p.6). According to another studies for the Netherlands (Willems, 1987; Kuyper and Schreurs, 1984; cited in Willems, 1990, p.6), in the 20 sites of calcareous grasslands with vary size from 0.2 to 4.0 ha (20 ha in total), approximately 200 vascular plant species that is occupied about 15 % of the indigenous flora of the country, 120 bryophytes and lichens, have been recorded. In Germany, 488 of about 3200 flora species and 205 endangered plant species confined to calcareous grasslands was reported (Korneck et al., 1998; cited in WallisDeVries, 2002, p.266). In Britain, 26 to 65 species including mosses on 20

areas of chalk grassland were found (Tansley, 1965, p.528). 77 rare species were associated with calcareous grassland and 50 of the species were restricted to the grassland (Keymer and Leach, 1990, p.12). According to Grime (1990), the high diversity and species-richness of calcareous grassland and co-existence of various species in small area are affected by several factors related to conditions of infertile soil, animal grazing (e.g. by cattle, sheep, or rabbits, etc) and small sized plants. The mechanism promoting floristic diversity and richness in calcareous grasslands is further reviewed below.

As stated in the above, calcareous grassland hosts not only high diversity of phanerogam species, but also a large number of bryophyte and lichen species. During (1990) stated “bryophytes contribute significantly to the species diversity and phytomass of calcareous grassland” (p. 35). Al-Mufti et al. (1977, p.759) stated that bryophytes grow mainly in autumn and spring and have the highest biomass in winter. During (1990, p.37) suggested that bryophytes might have a role in calcareous grassland as nutrient source to enhance seedling growth and survival of vascular plants in dense bryophyte patches that arise from the dead part of bryophyte in early summer, when they are inactive.

In a quantitative study of the bryophytes of chalk grassland, Watson (1960) found that two important factors determined the composition and structure of bryophyte patches. One was aspect and inclination of the slope. This was related to different heat load of north- and south-facing slope affecting water economy. The other was the ‘turf character’, relating to height, floristic composition and density of the turf cover. A well-developed bryophyte layer occurred in intermediate turf dense and 8 – 20 cm in turf height (Watson, 1960, p.408).

2.5.2.2 Fauna

Calcareous grasslands are also considered to be an important habitat for many fauna species, especially for invertebrates and thus important for conservation. Many invertebrate groups were found on calcareous grasslands, such as butterflies,

grasshoppers, wild bees, owl flies, beetles, cicadas, bugs, spiders, and land snails, etc. (WallisDeVries, et al, 2002, p.267; Van Swaay, 2002, p.315; Willems, 1990, p.6). For example, Van Swaay (2002, p.316) found that of all 576 native butterfly species recorded in Europe, 274 (48%) occurred on calcareous grasslands. The number of species is more than on alpine and subalpine grassland as with their 261 species. 44 of these 274 butterfly species (16%) are European endemics. 37 (52%) of the 71 threatened species in Europe can be found on calcareous grasslands. According to Kratochwil (1983), Mabelis (1983), and Lefeber (1975) cited in Willems (1990, p.6), in southern Germany, no less than 131 bee species (Hymenoptera: Apoidea) and 56 species of butterflies (Lepidotera) was recorded only on small sized calcareous grassland (0.4 ha). Only on single 4 ha of the Dutch chalk grasslands 22 different ant species (Hymenoptera: Formicidae) and 174 bee and wasp species (Hymenoptera: Aculeata) were found.

The abundance of the invertebrate fauna is influenced by management regime (grazing) and vegetation structures of calcareous grassland. Morris (1990, p.129) described that the abundance, species-richness and diversity of some invertebrate groups (e.g. Auchenorhyncha and Hepteroptera) correlated with vegetation height. Butterflies, grasshoppers, a few leaf hopper, and some snails are encouraged by the hot and dry microclimate due to the shortness of the vegetation, resulting from intensive grazing. Brown et al. (1990, p.81) also found that management (grazing regimes) for calcareous grasslands directly affected the species richness and abundance of invertebrates. In the study, autumn grazing improved the density of individuals and species richness of Heteroptera, while intensive and spring grazing reduced them. For this reason, integrated management approach has been emphasized for high floral and faunal diversity (WallisDeVries, et al., 2002; Jones-Walters, 1990).

2.5.3 Mechanisms promoting floristic diversity in calcareous grassland

Calcareous grasslands have high floristic richness and diversity. This species-richness and diversity of calcareous grassland are mainly related with mineral nutrient stress and with grazing by animals. These conditions prevent invasions and establishments of

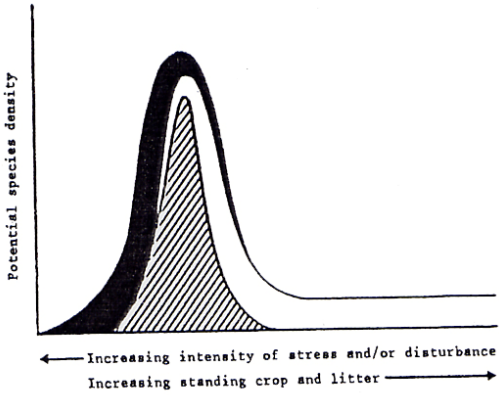


Figure 1. Model describing the impact of a gradient of increasing stress and/or disturbance upon the potential species density in herbaceous vegetation.
❑, potential dominants; ■, species or ecotypes highly adapted to the prevailing form(s) of stress or disturbance; ❑, species which are neither potential dominants, nor highly adapted to stress or disturbance. Reproduced from Grime (1973a) with the permission of MacMillan (Journals) Ltd., London.

Figure 2.4. The humped-back model (Adapted from: Grime, 1990, p. 52.)

vigorous plant species that can dominate the vegetation (Grime, 1990, p.51). According to Grime (1990, p.51), the humped-back model presents the relationship between species diversity and disturbance factors (Figure 2.4) and the model supports the relationship in other communities (e.g. intertidal algae, molluscs and coral reefs) as well as herbaceous vegetation. The model describes that unfavourable growing condition at the extreme left of the diagram reduces severely and/or destruct frequently total plant biomass and species-richness of the vegetation. On the contrary, moving to the right, the growing condition allows greater biomass accumulation and the density of species rises to a maximum in the central 'hump'. However, to the extreme right, species-richness is declined by dominance of robust species. The humped-back model for grassland management mainly implicates that high species-richness is unlikely to occur in condition with high productivity and/or low rates of biomass removal (Grime, 1990, p.51).

One of the reasons that many ancient calcareous pastures have high species-richness is species recruitment through the time and the richness depends upon structural and dynamic features which promote species co-existence (Grime, 1990, p.52). The mechanisms of co-existence include mineral nutrient stress, plant morphology, small phenotypes, slow dynamics, niche-differentiation related to "small-scale spatial variation in turf environment" (Grime, 1990, p.54), and mycorrhizal network connection between neighbouring species (Grime, 1990, p.51).

In addition to the suppression of dominance by intensity of stress and/or disturbance promoting diversity, Grime (1990) summarised about the mechanisms of co-existence of large numbers of individuals of different perennial species within small turf area

'dense packing of species' as the below:

> "Dense packing of species is related to the compact morphology of plants which have been attuned by natural selection over many generations to conditions of chronic mineral nutrient stress. The anatomy and small size of these plants is conducive to efficient internal recycling of captured mineral nutrients and is accentuated by close grazing which may result in exceedingly small phenotypes. The influence of mineral nutrient stress upon diversity is not confined to miniaturisation and dense species-packing; low productivity also dictates slow vegetation dynamics which in turn reduce the intensity of competitive interaction, delay the rate at which bare soil in vegetation gaps is recolonised and thus extend the opportunity for seedling establishment " (p. 51).

Another factor is related to the effect of vesicular-arbuscular (V-A) mycorrhizas which "achieves high levels of infection in the roots of many of the vascular plants associated with ancient species-rich grasslands" (Grime, 2002, p.286). In an experiment on relationship between floristic diversity and VA mycorrhizal fungi, Grime et al. (1987) showed that "mycorrhizas increased diversity markedly by raising the biomass of the subordinate species relative to that of the canopy dominant" (p.420). In an experiment on relationship between soil chemical factors and species diversity, Janssens et al. (1998) found that high species richness (more than 20 species per 100 m^2) occurred in low extractable phosphorus (less than 5 mg per 100 g of dry soil) and at rate between 15 and 20 mg per 100 g extractable potassium, whereas other factors, pH, organic matter, total nitrogen and calcium, did not affect significantly plant diversity. Grime (2002) suggested that: "The mechanistic basis for the correlation between low phosphorus and high diversity may also involve vesicular-arbuscular (V-A) mycorrhizas" (p. 286) and that it may be due to "facilitation of phosphorus uptake by inoculated plants with VA mycorrhizas as well as the export of assimilate from 'source species' (canopy dominants) to 'sink species' (understorey subordinates) through a common mycelia network" (p. 286).

2.5.4 Typical floristic composition of calcareous grasslands

Typical chalk grassland is mainly encountered on the relatively steep slopes of the escarpments and valley sides and on the summits of the narrower ridges where typical chalk soil (rendzina) is occupied. The edaphic factor, especially for soil pH which exceeds 7, is one of main factors affecting floristic composition of calcareous grassland, although stronger factor is grazing (Tansley, 1965, p.525). The alkaline soil condition is maintained by calcium carbonate supplying from the progressive solution of the underlying limestone rock through the agency of percolating rainwater. The soil condition enables the grassland to be relatively stable, as to prevent invasions of woody plant species (Tansley, 1965, p.525). These factors affecting botanical characteristics of calcareous grassland are discussed below.

In the UK, 26 to 65 species were recorded on 20 areas of chalk grasslands and about 20 of the commonest species were reported on all of them (Tansley, 1965, p.528). The dominant species were varied mainly according to the differences of grazing regime and water conditions. The typical flora species of chalk grassland are shown in Table 2.5.

Table 2.5. Typical flora species of 62 typical chalk grasslands in Sussex, Hampshire, Wiltshire, Berkshire and Oxfordshire (Source from: Tansley, 1965, p.530-532).

Occurrence*	Grasses	Other species	
More than 80 %	*Avena pratensis* *Briza media* *Festuca ovina* *Festuca rubra* *Koeleria cristata*	*Carex diversicolor (flacca)* *Cirsium acaule* *Leontodon hispidus* *Linum catharticum* *Lotus corniculatus*	*Pimpinella saxifraga* *Plantago lanceolata* *Poterium sanguisorba* *Scabiosa columbaria* *Thymus serpyllum*
80 % to 60 %	*Avena pubescens*	*Achillea millefolium* *Asperula cynanchica* *Galium verum*	*Ranunculus bulbosus* *Trifolium pratense* *Brachythecium purum*
60% to 40 %	*Anthoxanthum odoratum* *Bromus erectus* *Dactylis glomerata* *Trisetum flavescens*	*Bellis perennis* *Campanula rotundifolia* *Carex caryophyllea* *Carlina vulgaris* *Centaurea nemoralis* *Cerastium vulgatum* *Chrysanthemum leucanthemum* *Euphrasia nemorosa* *Filipendula hexapetala* *Hieracium pilosella*	*Medicago lupulina* *Phyteuma orbiculare* *Plantago media* *Polygala vulgaris* *Primula veris* *Prunella vulgaris* *Camptothecium lutescens* *Hylocomium squarrosum* *H. triquetrum*

*Occurrence indicates percentage areas out of 62 typical chalk grassland areas which the following species occurred in.

The species lists represent the constant species occurring in more than 40 % of 62 typical chalk grassland areas in Sussex, Hampshire, Wiltshire, Berkshire and Oxfordshire (Tansley, 1965, p.530).

2.5.5 Factors affecting botanical characteristics of calcareous grassland

Climate, soil, and human activities (e.g. animal grazing for agricultural) are main factors affecting species diversity and richness of the communities (Grime, 1990), and controlling the occurrence and composition of plant communities of calcareous grasslands (Rorison, 1990; Wells, 1974; Tansley, 1965). These factors have often interlocking relationship.

2.5.5.1 Edaphic factor

Calcareous grasslands develop directly on soils that are derived from the basic geological formations of chalk and limestone. The characteristics of the soil are primarily determined by the nature of the underlying rocks, but not by climate. The soil is the so called “rendzina” (Tansley, 1965, p.93). Rendzina is formed by weathering surface of the chalk or limestone rocks occurs typically on fairly steep south-facing slopes with various thickness, ranging from very thin layer to a depth of about 30 cm (rarely more than 30 to 40 cm thick; 20 to 25 cm is common depth) (Tansley, 1965, p.94). The very young chalk soils tend to be extremely shallow and greyish white, while the soil on steep northern chalk slopes are deeper and extremely rich in black colloidal humus (Tansley, 1965, p.94). The soil contains high amount of free calcium carbonate ($CaCO_3$) due to the nature of the parent rocks, and together with HCO_3^- and CO_2^-, the chemical element controls a soil reaction buffer at neutrality or slightly above, resulting that the pH value of soil surface (5.0 to 7.5 cm) exceeds 7 (Bache, 1984, p.393; Tansley, 1965, p.525). The soil pH is maintained by a continual supply of calcium carbonate from the underlying rocks through a process of water evaporation, and this prevents acidification of the soil. Rendzina is a permanently immature soil because of the soil pH, and condition of steep slope where the soils occurred and shallow depth of the soil

covering on the slope which make the percolating water quickly drain away and rapidly dry (Tansley, 1965, p.94). Thus, the soil is generally characterised by low level of available N, P, and K to plants, although total amounts of nitrogen and phosphorus are high because they exist in organic forms (Rorison, 1990, p.21).

The predominance of calcium carbonate affects nutrient balance and availability through pH, and in consequence, this influences plant composition and structure, and thus is a main factor in developing different plant communities (Rorison, 1990, p.22-23). Plant growth in soils with high pH decreases through effect on availability of nutrient to plants, amounts of nutrients held in soils, toxicities, and microorganisms, and different plant species have different optimum pH ranges (Handreck and Black, 2005, p.91). According to Rorison (1990, p.22), under the nutritional deficiency and imbalance of calcareous soils, plants have been evolved through optimisation of a phenology that extends their growing season, a physiology which allows flexibility of specific absorption rates, and a morphology which ensures the growth of fine roots and root hairs. Rorison (1990) described that “these species have been classified as calcicoles because soil Ca was considered the primary determinant of the plant’s distribution” (p. 22). Indeed, several studies have shown that level of calcium concentration influences growth and establishment of plants (Jefferies and Willis, 1964), and calcicole species are inhibited on acidic soils, along with indirect effects (competition or other nutrient elements, e.g. Nitrate), whereas calcifuge species are the opposite (Rorison and Robinson, 1984; Tansley, 1917).

2.5.5.2 Climatic factor

Development and survival of vegetations of calcareous grasslands are affected by interaction of environmental factors, between topography of calcareous landscapes, the physical and chemical nature of the soils, and temperature and rainfall, not least the chemical and physical nature of the soils (Rorison, 1990, p.26). Rodwell (1990) stated:

> “Variations in climate, particularly differences in precipitation and temperature, are of prime importance in determining the floristics and distribution of the communities, both by direct effects on the plants

themselves and also through process of soil development "(p. 29).

As in earlier section, in the UK floristic patterns are generally recognised as two blocks and separated by temperature and rainfall (1000 mm isohyets or 160 wet days per year) (Rodwell, 1990, p.29). One of the patterns is the south-eastern grasslands on the chalk soil region labelled warm and dry, and the other is the north-western grasslands on the predominant limestone soils of the North and West to be cool and wet (Rodwell, 1990). However, there are also many local variations in the floristics of the communities due to the topography (slope and aspect) on soil and microclimate (e.g. 14 communities in NVC) (Bennie et al., 2006; Rodwell, 1990; Rorison, 1990). Perring (1959) showed that slope and aspect influenced strongly the floristic composition of chalk grassland at many sites in Britain. Higher fluctuation in soil temperature is experienced on south-facing slope in summer due to higher solar radiation index, compared to north-facing slope, and the fluctuation reduces markedly soil moisture content (Rorison et al., 1986). A recent study by Bennie et al. (2006) investigated an influence of slope and aspect on long-term vegetation changes in British chalk grasslands and they found that steeper angles of slope and south-facing aspects had lower soil moisture contents (10% - 20 % lower values) higher solar radiation index than north-facing slopes during the summers of 2001-03. Lower total extractable phosphorus in July was also measured, and steeper and south-facing slopes maintained much more stable floristic composition of chalk grassland due to phosphorus and/or water limitation. Soil moisture contained in shallow rendzina soils, is susceptible to angle of slope and direction of aspect, and the moisture stress suppresses invasion and establishment by dominant plant species and resets plant distributions (Buckland et al., 1997).

2.5.5.3 Biotic factors

The biotic factor of grazing plays a major role in determining floristic composition of the grassland and/or stabilising it against processing toward the other plant formations as natural succession, and this may have by far stronger influence than the climatic and edaphic factors (Tansley, 1965, p.493). As from the earlier historical reviews section, grazing has been considered a crucial factor for grassland to be created and stabilised

after destruction of primeval forest. Also Tansley (1965, p.493) exemplified that in Wales, grasslands on which even soils remain highly fertile through effect of continuous grazing. In general, grazing adequately prevents growth of more vigorous species, and high productivity due to the increase of nutrient availability in soil through removal of the dead parts of vegetations obstructing fresh growth, while encouraging fresh young shoots to be produced and thus good turf is formed (Tansley, 1965, p.489).

The floristic composition of the calcareous grassland is often determined by grazing animals, and sensitive to changes in grassland management. Tansley (1965, p.180) instanced that heaths, *Calluna* and especially *Erica cinerea*, increased on the deeper and less purely calcareous soil of the South Down pastures in Britain when the pastures was grazed by cattle alone, while the species were not able to establish there under continuous sheep grazing. Kahmen et al. (2002) studied changes in plant species composition and response of functional traits (life form, life cycle, growth form, runners, lateral spread, fecundity, seed mass, germination season) during 25 years according to five management treatments, continuing sheep grazing, mowing, mulching, burning, and unmanaged treatment. They found that during 25 years of management, different management treatment resulted in different development of floristic composition, and that under both burning and unmanaged treatment the floristic composition and functional trait composition was considerably changed due to advanced succession and the establishment of woody species, while under mulching and mowing treatment preserved the trait composition that had been established, and the floristic composition resembled that under the grazing treatment.

2.6 Restoration ecology of calcareous grassland

Key factors that should be taken into account for successful restoration/recreation of calcareous grasslands can be recognised as soil characteristics, and selection of appropriate species and seed mixes and their establishment, and management. In terms of basic principles of recreation/restoration of grassland, the ideal soil characteristics necessary to create a diverse grassland sward have been stated as: correct pH, weed free, uncompacted and containing organic matter, good drainage, and poor nutrient status; the

ideal substrate is subsoil (Watson and Hack, 2000).

Semi-natural grasslands are divided into three groups (neutral, acid, and calcareous), depending on the local soil type and the geographical location of the site. Therefore, a species and mixture should always reflect the species and proportion occurring in natural grassland. Watson and Hack (2000) have recommended that for selection of correct species and mixes, it is best to use the National Vegetation Classification but that "any species mix based on an NVC type will not instantly create an example of that NVC grassland" (p.87). It is essential to manage new grassland through usually mowing, not just in the early stages but every year. Otherwise, new grassland will have failed by invasions of undesirable species (Gilbert and Anderson, 1998; Ash et al., 1992, p.13).

2.6.1 Soil

The most important chemical factors of soil are nitrogen (N), phosphate (P), and pH. The pH of the soil is the major factor determining grassland types that can be established. The fertility of soil is decided by the level of nitrogen (N) and phosphate (P) (Ash et al., 1992, p.17). The pH affects soil fertility. In extreme acid and alkaline soil, such soil can be ameliorated using sulphur or bracken litter soil for alkaline conditions, and by adding crushed limestone for the acid (Gilbert and Anderson, 1998). Too high a level of fertility soil make difficult to sustain diverse sward grasslands. Problems with fertile soil are usually associated with high P and not necessarily with high N. High levels of phosphorus militate against development of a species-rich grassland because it encourages one or two species to dominate and also mycorrhizal species. Nitrate-nitrogen tends to release from decaying organic matter within a year or two and hence if phosphorus is low, high level of nitrogen is not a factor in preventing biodiverse sward development (Gilbert and Anderson, 1998). However, where nitrogen level is too low, or due to this nitrogen release over long term, it may suffer from nitrogen deficiency. It is necessary to reduce levels of available phosphorus through one of four ways as below (Nicholas Pearson associate, 2004, p.9; Gilbert and Anderson, 1998):

1. Topsoil removal to expose low-nutrient subsoil or chalk; it is recommended method for creating chalk grassland,
2. Topsoil burial by deep ploughing,
3. Topsoil dilution by mixing with chalk, or nutrient stripping by cropping,
4. The use of the hemiparasite yellow rattle, *Rhinanthus minor* to reduce sward vigour.

2.6.2 Selection of correct species and mixture

The selection of species and mixture must agree with soil conditions on site and the geographical location of the site as closely as possible. The key factors to consider include substrate, soil fertility, soil pH, soil moisture regime, soil texture, aspect, previous land use, and bio-geographical location (Crofts and Jefferson, 1999; Ash et al., 1992, p.28). When choosing seed mixtures the following guidelines have been proposed as shown in Table 2.6.

Table 2.6. Seed mix design guideline (Source from: Watson and Hack, 2000; Ash et al., 1992).

Species to use	• No more than 20 species • Compile the species mix in the same proportions as found naturally; usually Grass and forb, 80:20. 50:50 grass: forb mix for a richer, more colourful sward (Wells, 1990) • native to the British isles; not known to be invasive or very competitive; and, common and widespread
Species availability	• Avoid rare species • Do not use species outside their natural range, and species known to fail in seed mixtures.
Soil pH	• The characteristic of selected species should be suitable with the pH of the site.
Relative abundances	• Recreate the characteristic abundances of the species as possible • Sowing seed or plants mixed by weight of seed or plants per m^2 in sward • Some species interact; two aggressive grasses may counterbalance each other and help to prevent each other from becoming dominant.
Longevity	• Annual species require and appreciable amount of bare ground for the next generation of seeds to germinate.
Structure	• A low or prostrate growth species will not persist in a sward that is left uncut for most of the summer. Species selection depends upon the aftercare management regime. • Grazed grassland should contain prostrate and small species. • For hay should include taller species

Successful establishment requires medium tilth, and effective distribution from manual broadcasting on small sites or through hand-held seed fiddle. The best season to sow new grassland is late summer or early autumn, August and September, due to frost in too late season, and drought damages and high competition against weeds and grasses in spring (Crofts and Jefferson, 1999; Gilbert and Anderson ,1998).

2.6.3 Management

Management for restoration of calcareous grassland is important for several reasons:

1. Sustainment to develop a species-rich grassland
2. Prevention of colonisation by invasive species
3. Promotion of desirable invertebrate fauna community

Two experiments carried out by Hutchings and Booth (1996a and 1996b) have concluded that chalk grassland species tend to be short-lived and need management including grazing or mowing to prevent more weedy species invading and eliminating the chalk grassland species, and that "sowing suitable species and implementing a management regime will maximize the chances of establishing chalk grassland species on ex-arable sites" (p.1189).

To maintain conservation interest the grassland must be managed by mowing, grazing or a combination of both (Crofts and Jefferson, 1999). Most calcareous grassland has been maintained by traditionally grazing using livestock such as sheep, cattle or rabbits. Rotational grazing can encourage invertebrate species to complete their life cycle on ungrazed sward, or to allow particular annual plants to flower and set seed depending on where a particular sward height or structure is required at a particular time (Bacon, 1990, p.125). Cutting, where use of livestock is not a practicable option, is not a traditional management technique. However, it has been used to maintain high quality old chalk grasslands or newly created grassland (Crofts and Jefferson, 1999). The main objective of long-term maintenance by mowing or grazing of chalk grassland is to prevent colonisation or domination by other species such as scrub or tall grasses, and to encourage a more varied species-rich sward.

Chapter 3. Substrates for calcareous grassland vegetations on green roofs: Effect of substrate type on seedling emergence, survival and initial growth of potential species for extensive green roofs in the UK.

3.1 Introduction

In order to achieve successful green roof, vegetations on a green roof have to establish and grow well over the long term. There may be many factors in rooftop conditions that affect plant performance. The most critical factors are the selection of suitable plants to rooftop environments and growing medium or substrate that are able to support plant growth. Due to loading capacity of rooftops, green roof substrates, especially for an extensive (80 to 150 kg/m^2) and semi-extensive system, should be of shallow depth between 20 mm and 150 mm and lightweight (Dunnett and Kingsbury, 2008, p.5 and p.94). Moreover, in extensive and semi-extensive green roof systems minimal maintenance and irrigation is pursued (Dunnett and Kingsbury, 2008, p.188; Johnston and Newton, 1993, p.70). As a result of these restrictions, the substrate has to include at least six characteristics: free-draining properties and aerated pore space, moisture holding capacity, plant anchorage properties, nutrient holding capacity, resistance to decomposition, and lightweight (Friedrich, 2005, p.2; Beattie and Berghage, 2004, p.2). The ideal substrate comprises 30 to 40 % solid and, 60 to 70 % pore volume. This composition creates a growing medium that has the ideal balance between water holding capacity and aerated pore space, 35 to 45 % and 15 to 25 % respectively (Friedrich, 2005, p.3; Beattie and Berghage, 2004, p.3; Handreck and Black, 2005, p.59).

Miller (2003, p.2) suggested that granular mineral materials could achieve these requirements. The majority of green roofs have also used these materials for substrate. The mineral materials used for green roof substrates have been typically divided into three groups, natural, artificial, and recycled or waste materials (Dunnett and Kingsbury, 2008, p.111). However, it is desirable to incorporate other mineral materials or organic matter to improve water holding capacity and nutrient retention. For example, LECA is very lightweight and has some moisture and nutrient storage capability (Dunnett and

Table 3.1. Comparison of organic matter characteristics (Source from: Friedrich, 2005; Scrivens, 2004).

Organic materials	Advantages	Disadvantages	Description
Composted organics	Preferred source of green roof media High nutrient and High microbial count Recycling value	Nitrogen deficiency Detrimental to plant growth	
Peat moss	High water holding capacity Difficult to dry	Low in nutrients and microbial population Difficult to wet back Oxidisation at 15 to 20% per annum	pH 3.3 to 3.5
Sphagnum moss peat	High porosity High water retention	Low inorganic content Low pH	Spongy fibrous texture Source from peat bogs and sphagnum moss
Sedge peat	More nutrients Higher CEC per unit weight than sphagnum peat	More humidified and decomposed Lower porosity Less durable structure Difficulty to re wet from dry	Source from sedges and reeds
Bark Soft wood bark	Slow decomposition Good drainage High CEC	Low nutrient Low pH Nitrogen deficiency Generation of toxic compounds Oxidisation at 10% per annum	Good source for the organic amendment in green roof media Particle size: less than 12mm No usage as the main bulk constituent of a growing media Sources from larch and pine in Britain, and Douglas, red and white fir in North America
Hardwood bark		Full decomposition Robbing the plants of nitrogen	Acid pH 3.5 to 6.5

Kingsbury, 2008, p.112). However, in the measurement undertaken for moisture content of substrates in this study (Ref. Appendix 2), LECA with 4 mm to 8 mm particle size in l litre pot had very low moisture content with 2.1% at 29.3°C, RH 43%, and completely

dried out by the next day with 0.0 % at 22.3°C, RH 57%. Therefore, it is suggested that LECA must be mixed with other materials and would have to be used as a soil amendment.

Organic matter plays a role in water retention improvement, root growth facilitation, and also as a nutrient supplier (Scrivens, 2004, p.232). However, because organics decompose over a long or short period depending on climate, the volume of substrate including organics can be depleted over time through degradation, and create slime that impedes the water flow in the medium. This could cause plant health problems if the water stays over the long term and increases the structural load. Therefore, it has been recommended that the volume of organics in the growing medium for extensive roofs does not exceed the range of 10 to 20 % (Friedrich, 2005, p.5). Advantages and disadvantages of organic matters have been summarised in Table 3.1.

The selection of substrate and the depth depends on the characteristics of the vegetation type that is desired. For example, crushed concrete, brick, chalk mixture, or local soil has been used for green roofs to mimic conditions of their target habitat (Gedge, 2005; Brenneisen, 2003). In order to achieve a substrate that supports calcareous grassland vegetation, the substrate should have similar characteristics of calcareous soil, which are free drainage, high water holding capacity, low nutrient level, and of pH 6.5-8.5.

3.2 Research aim, objectives and questions

Although there are a few studies (e.g. Molineux et al., 2009) for alternative green roof growing media or guidelines (e.g. FLL, 2004; the German guidelines for green roofs, Society of Landscape Development and Landscape Design) on substrates of extensive green roof systems, relatively little has been researched on alternative substrates supporting specific vegetation. The aim of this study is to investigate the nature of the potential substrates and to identify successful substrates that are possible to properly support calcareous grassland vegetations. The research objectives and questions of the study are listed as follows.

3.2.1 Research objectives

i) To identify substrate and substrate characteristics that support the target plant community.

ii) To investigate the effect of a range of substrates on emergence, survival, and growth (aboveground biomass) of species

3.2.2 Research questions

i) What materials can be the optimum alternative growing media instead of the original soil of calcareous grassland habitats?

ii) What is the optimum mineral aggregate and substrate composition for calcareous grassland species?

iii) How does each alternative substrate affect the seedling emergence, survival, and growth?

iv) What are the characteristics of the growing media? - pH, Water holding capacity, Air filled porosity, Bulk density.

3.3 Materials and Methods

3.3.1 Experiment 1

A series of procedure was undertaken in the laboratory to characterise the substrate used in the study. For this study, twenty nine substrate types were applied across the below two experiments, which were to investigate the effect of a range of substrates on seeding performance of species. For physical and chemical characteristics of the substrates, bulk density, moisture content, air-filled porosity, pH, and EC were determined for each substrate type. The physical and chemical characteristics of each substrate type are summarised in Table 3.2.

3.3.1.1 Bulk density

For the measurement, a container made from a vinyl coated hard paper box, with 70 mm by 70 mm width and 200 mm length as shown figure 3.1, was used. The container was filled with one litre of each substrate type. The bulk density of each substrate type was calculated for dry and then recalculated after saturation (Handreck and Black, 2005; Hitchmough et al., 2001). Each substrate had five containers for replicate and one measurement was made for each substrate in each replicate container, for a total of five measurements.

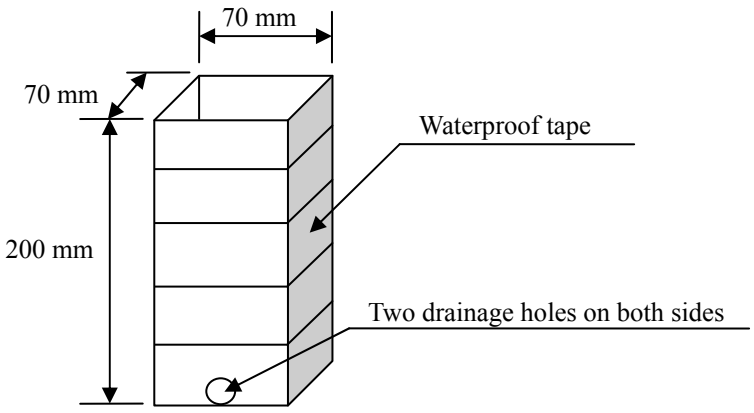


Figure 3.1. Container for measurement of air filled porosity

Table 3.2. Physical and chemical characteristics of the twenty-nine substrates used in the experiments.

	Bulk density (Dry) (Mg/m3)	Bulk density after saturation (Mg/m3)	Increase in bulk density (%)	Air filled porosity (%)	Moisture content[1] (%)	pH	EC (dS/m)
Limestone 1 100:0:0	1.52	1.58	3.53	42.26	6.90	8.04	0.27
Limestone 1 90:0:10	1.50	1.59	5.70	45.07	6.97	8.33	0.31
Limestone 1 80:0:20	1.45	1.53	5.86	41.54	7.71	7.93	0.43
Limestone 1 70:20:10	1.46	1.57	7.84	34.72	11.36	8.02	0.45
Limestone 1 60:20:20	1.40	1.58	12.87	26.05	8.68	7.97	0.54
Limestone 2 100:0:0	1.67	1.94	16.49	18.28	24.77	8.22	0.27
Limestone 2 90:0:10	1.59	1.84	15.83	16.50	28.98	7.94	0.30
Limestone 2 80:0:20	1.56	1.85	18.56	12.53	28.07	7.96	0.48
Limestone 2 70:20:10	1.56	1.89	21.36	11.26	29.40	7.84	0.35
Limestone 2 60:20:20	1.43	1.80	26.20	12.00	22.97	8.03	0.51
Limestone 3 100:0:0	1.82	2.00	10.12	19.17	16.74	8.05	0.25
Limestone 3 90:0:10	1.77	1.99	12.60	17.39	19.45	8.17	0.25
Limestone 3 80: 0:20	1.71	1.94	13.41	15.04	17.00	7.85	0.37
Limestone 3 70:20:10	1.70	1.94	14.24	11.58	22.91	7.80	0.47
Limestone 3 60:20:20	1.57	1.84	16.90	13.04	15.31	7.87	0.44
LECA 100:0:0	0.36	0.44	24.02	54.38	2.06	7.72	0.32
LECA 90:0:10	0.38	0.46	21.01	49.39	2.11	7.76	0.33
LECA 80:0:20	0.41	0.51	22.75	47.03	3.62	7.87	0.47
LECA 70:20:10	0.45	0.71	57.12	38.10	7.19	7.42	0.57
LECA 60:20:20	0.44	0.59	33.57	41.13	10.24	7.48	0.82
Brick A 80:0:20	1.25	1.47	17.50	38.99	20.04	7.94	0.93
Brick A 60:20:20	1.38	1.68	22.10	21.10	31.74	7.87	1.22
Brick B 80:0:20	1.22	1.49	22.10	32.48	20.40	7.90	1.25
Brick B 60:20:20	1.36	1.70	25.50	18.90	32.23	7.85	1.43
Brick A+B 80:0:20	1.28	1.52	19.37	36.54	20.75	7.82	1.26
Brick A+B 60:20:20	1.36	1.70	24.65	22.09	30.43	7.79	1.36
John Innes	1.14	1.63	43.66	10.30	28.02	6.61	0.91
Z. Semi-extensive	1.00	1.39	39.83	15.20	24.63	7.34	1.90*
Z. Sedum	1.16	1.46	26.13	27.44	22.23	7.50	1.94*

[1] Moisture reading on 29/08/2006 at 29.3 °C, RH: 43%.

* including three and two vales with over range value (range of Primo 5 EC meter is 0 to 1999 µS/cm (1.999 dS/m) in Zinco semi-extensive and Zinco sedum substrate respectively.

3.3.1.2 Air filled porosity

Air filled porosity was calculated using the protocol of Handreck and Black (2005) and Hitchmough (personal communication) and by a formula: final weight (g) of water drained/volume of substrate (cm^3) X 100 (%) (Hitchmough, personal communication). As with the above measurement, the container (figure 3.1) was used to calculate the air filled porosity of each substrate type. One measurement was made for each substrate in each replicate container. Each substrate type had five replicate to give a mean value.

3.3.1.3 Moisture content

For measurement of moisture content of the substrates, the substrates were filled in one litre black plastic pots with 130 mm below the lip (Hitchmough et al., 2001, p.291). Each substrate was tested in three replicates, for a total of 87 pots. Irrigation was carried out on one occasion only on the first day. During the course of the measurement (19 days), five points were chosen for each substrate in each replicate pot at two days interval in order to investigate changes of moisture content of the substrates as the time passed (Ref. Appendix 2), using moisture meter (HH2 moisture meter and SM200 moisture sensor, Delta-T Devices, Cambridge, England). Fifteen readings in total were taken for each substrate.

3.3.1.4 pH and EC values

The measurement of pH and EC of each substrate were taken by protocol of 'pour-through technique for mix pots' (Handreck and Black, 2005). There were five replications for each substrate. As with the measurement of air-filled porosity, one reading was made in each replicate pot, for a total five readings in each substrate to get the mean value for each substrate, using HANNA HI99104 pH meter and PRIMO 5 conductivity stick meter (Leighton Buzzard, UK).

3.3.2 Experiment 2

In the winter of 2005, an experiment for this study was undertaken in the green house of the experimental garden at the University of Sheffield in Broomhill. Experiment 2 was to answer the above questions, and focused on germination studies in different kinds of substrates. As an indicator species, *Leucanthemum vulgare* was used. The species was chosen for this Experiment 2 on the basis of their origin from dry habitats and its high possibility of adaption to green roof environment as it is drought tolerant, have shallow roots and low growing forms, and germinate readily. The seeds were obtained in May

2005 from Emorsgate Wild Seeds, Norfolk, UK. Characteristics of the species are summarised in Table 3.3.

Two kinds of Limestone granules (Trucal 6 and 12), LECA, two commercial green roof substrates (Zinco Semi-extensive and Zinco Sedum), and John Innes No.1 were used as a control. The Limestone substrates had three Limestone ranges in terms of particle size, which are Trucal 6, Trucal 12, and 50% Trucal 6: 50% Trucal 12 mix. The three Limestone ranges and LECA (Light expanded clay granules) with 4 mm to 8 mm particle size were mixed with loam and green waste compost for organic matter as the substrate in Table 3.4. The green waste compost was obtained from Heeley city farm in Sheffield.

For Experiment 2, twenty-three substrate types were applied. One litre black plastic pots with 130 mm diameter were filled with the substrates to 10 mm below lip (Hitchmough

Table 3.3. Ecological and other characteristics of species used in this study (Adapted from; Blamey et al., 2003; Brickell, 2003; Grime et al., 2007).

Species	Plant type	Geographical distribution	Habitat	Established strategy	Height (mm)	Flowering season
Briza media	Grass	Britain, Europe, temperate Asia.	Calcareous grassy places.	Intermediate between stress-tolerator and C-S-R.	to 500	June to Aug.
Leucanthemum vulgare	Forb	Britain, Europe to N. Scandinavia, temperate Asia and various region with cultivation	Rocky alpine slopes, moist – meadows, grassland, and waste land.	Intermediate between competitive-ruderal and C-S-R.	to 700 mm	June and July
Prunella vulgaris	Forb	Britain, Europe, temperate Asia, N Africa, N America and Australia.	Meadows, pastures, limestone quarry spoil, arable fields	C-S-R.	< 100 mm	June to September

Table 3.4. Mineral materials and substrate mixes by volume.

Mineral materials	Mineral : loam : organic
Limestone 1: Trucal 12*	100 : 0: 0
Limestone 2: Trucal 6*	90 : 0 :10
Limestone 3: Mixture of Trucal 6 and 12 in 50:50 %	80 : 0 : 20
LECA: 8mm to 4mm	70 : 20 :10
	60 : 20 : 20

***** Trucal products are dried, crushed and screened several times to produce a range of close graded materials. *Trucal 6: 100% passing through 3.35mm sieve aperture, 99.9%; 2.36mm, 98%; 1.70mm, 88%; 1.18mm. *Trucal 12: 100%; 8.00mm, 99%; 6.30mm, 80%; 5.00mm, 26%; 4.00mm, 4%; 3.35mm. The percentage passing is the amount of material that goes through a particular sieve. In the case of TRUCAL 12 there will be 26% passing through the 4mm sieve with 74% retained above 4mm (Source from: TRUCAL Product data of Tarmac Company, 2005).

et al., 2001, p.291). Each pot was sown with 50 seeds of *Leucanthemum vulgare*, and replicated ten times. 230 pots for the experiment in total were used in this experiment. The pots were distributed at random in the green house and periodically moved to different places. The experiment was lit for 16 hours a day. Sowing seeds was completed on 18th December 2005. During the course of the experiment, the pots were watered until fully saturated twice a week from day 1 through day 26, to make sure that germination occurred and once a week from day 27 until it was terminated on day 66 (21 February 2006).

The emergence of *Leucanthemum vulgare* was judged to have occurred when two cotyledons had fully emerged from the seed coat (Hitchmough et al., 2001, p.293). The total number of seedling emergence present in each pot was assessed at weekly intervals from the first recorded emergence on 25 December 2005. On day 28 (22 March 2006) from the final watering day of Experiment 2, all seedlings above ground level were harvested from each pot to provide dry weight data. The total number of seedling emergence of each pot was counted when they were harvested. The weight, foliage height, spread and number of leaves of each species from each substrate was recorded and sorted by species and substrate type.

3.3.3 Experiment 3

For this study, an experiment was carried out again to answer the above research questions including urban waste substrates such as brick rubble. In the summer of 2006, Experiment 3 was set up in the same experimental garden as Experiment 2. In addition, the mean maximum percentage of seedling of *Leucanthemum vulgare* for Experiment 2 was recorded with less than 10 % across all ranges of substrate (Table 3.11). It was considered that the mean emergence might be too low to obtain an accurate conclusion. From the result of Experiment 2, two composition rates were chosen again, which were 80:0:20 and 60:20:20. Brick rubble was obtained from a demolition site and screened into three grades through 8 mm, 4 mm, and 2 mm sieve prior to use, which are between 8 mm and 4 mm, 4 mm and 2 mm, and less than 2 mm in particle size. Each of the three brick rubble ranges was mixed with less than 2 mm particle sized Brick C instead of loam and the green waste compost as shown in Table 3.5. Three Limestone substrates with the 80:0:20 and 60:20:20 composition rates, the same as Experiment 2 mixes, the two commercial green roof substrates, and John Innes No.1 as a control were employed again.

As the indicator species, one species of forb and grass, *Prunella vulgaris* and *Briza media*, was used. These species were chosen for Experiment 3 on the basis of the same reason as Experiment 2. The seeds were obtained in February 2006 from Emorsgate Wild Seeds, Norfolk, UK. The species selected for Experiment 3 are listed and their characteristics are summarised in Table 3.3.

Table 3.5. The particle size of brick rubble and substrate mixes by volume.

Brick materials	Brick : Brick C : organic
Brick A: 8mm to 4mm	80 : 0 : 20
Brick B: 4mm to 2mm	60 : 20 : 20
Brick A+B: 50:50% mix	
Brick C: less than 2mm	

For Experiment 3, fifteen substrate types were employed. The substrates were filled in the same pots and in the same way as in Experiment 2. Each pot was sown with 50 seeds of one species only. Each substrate was replicated three times for each species of Experiment 3. 90 pots in total were used in this experiment. As with Experiment 2, the pots were randomly placed in the green house and periodically moved to different places. Sowing the seeds was completed on 5th June 2006. During the course of the experiment, the pots were watered until fully saturated every other day to protect the seedlings from severe drought until final watering on 52 day (26 July 2006).

The emergence of forbs was judged to have occurred when two cotyledons had fully emerged from the seed coat. Grass emergence was recorded on production of the first true leaf (Hitchmough et al., 2001, p.293). The total number of seedling emergence present in each pot was assessed at weekly intervals from the first recorded emergence on 12 June 2006. On day 14 (9 August 2006) from the final watering day of this experiment, all seedlings above ground level were harvested from each pot to provide dry weight data. The total seedling emergence of each pot was counted when they were harvested. The weight, foliage height, spread and number of leaves of each species from each substrate was recorded and sorted by species and substrate type.

3.3.4 Statistical analysis

An Anderson-Darling test is undertaken to test whether a set of data follows a normal distribution or not. Where the test indicated that data was substantially non-normal, and this could not be adequately improved by transformation, analysis was undertaken using non-parametric statistics. The Mann-Whitney *U*-test was used in lieu of *t*-test for paired comparisons and Kruskal-Wallis tests for one way ANOVA. Statistical tests were undertaken using MINTAP Release 14. Correlation tests were used to assess association between variables using Spearman rank correlation test, which was undertaken using SPSS version 12.

3.4 Results

3.4.1 Experiment 1

3.4.1.1 Bulk density

Figure 3.2 shows that substrate type significantly affected bulk density at dry ($P < 0.001$) and saturated conditions ($P < 0.001$) of means of all composition rates. At both dry and saturated, bulk density of substrate type was significantly the highest for the Limestone 3 (1.71 Mg/m^3; 1.94 Mg/m^3), and the lowest for the LECA substrate type (0.41 Mg/m^3; 0.54 Mg/m^3). Mann-Whitney *U*-test also revealed that all Limestone substrate types were significantly higher at both bulk density conditions than the other substrate types, LECA, Brick rubble substrate types, the two Zinco substrates, and John Innes.

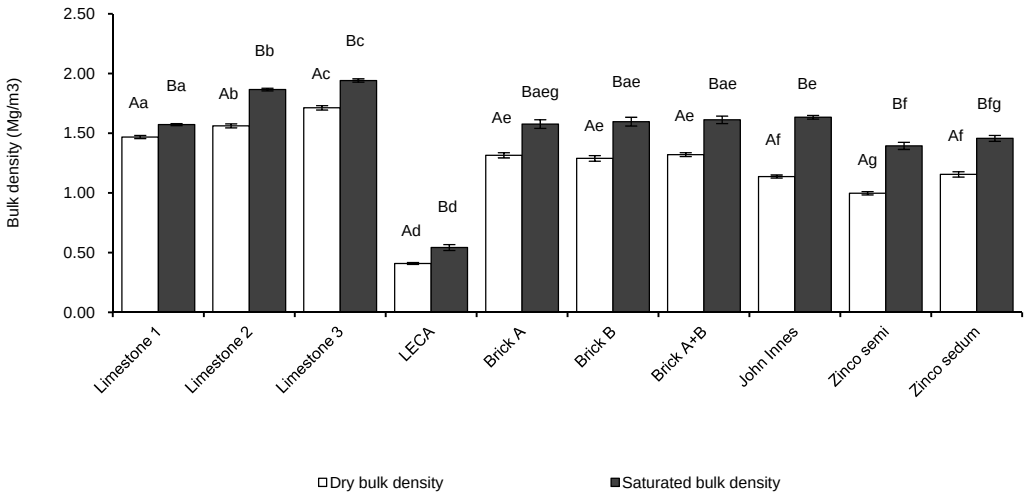


Figure 3.2. Mean bulk density (Mg/m^3), dry and saturated in response to substrate type across all composition rates.
Different capital letters indicate significant difference at $p = 0.05$ (Mann-Whitney *U*-test) between dry and saturated bulk density for the same substrate type. Different lower-case letters indicate significant difference at $p = 0.05$ (Mann-Whitney *U*-test after Kruskal-Wallis test) between substrate types for the same bulk density. Error bars represent standard error.

Table 3.6. Mean bulk density in dry and bulk density after saturation in response to substrate.

	Bulk density (Dry) (Mg/m3)			Bulk density after saturation (Mg/m3)			Increase in bulk density (%)		*P*-value[1]
	Mean	S.E		Mean	S.E.		Mean	S.E	
Limestone 1 100:0:0	1.52	0.015	a	1.58	0.014	b	3.57	1.384	0.0459
Limestone 1 90:0:10	1.50	0.017	a	1.59	0.014	b	5.71	0.329	0.0273
Limestone 1 80:0:20	1.45	0.010	a	1.53	0.011	b	5.98	0.754	0.0117
Limestone 1 70:20:10	1.46	0.026	a	1.57	0.009	b	7.99	2.161	0.0119
Limestone 1 60:20:20	1.40	0.035	a	1.58	0.032	b	13.14	3.577	0.0117
Limestone 2 100:0:0	1.67	0.019	a	1.94	0.018	b	16.50	0.346	0.0122
Limestone 2 90:0:10	1.59	0.024	a	1.84	0.016	b	15.94	2.201	0.0119
Limestone 2 80:0:20	1.56	0.003	a	1.85	0.011	b	18.56	0.766	0.0114
Limestone 2 70:20:10	1.56	0.008	a	1.89	0.011	b	21.37	0.845	0.0114
Limestone 2 60:20:20	1.43	0.012	a	1.80	0.003	b	26.23	1.056	0.0117
Limestone 3 100:0:0	1.82	0.017	a	2.00	0.017	b	10.18	1.767	0.0119
Limestone 3 90:0:10	1.77	0.009	a	1.99	0.010	b	12.60	0.071	0.0117
Limestone 3 80: 0:20	1.71	0.008	a	1.94	0.013	b	13.41	0.344	0.0114
Limestone 3 70:20:10	1.70	0.020	a	1.94	0.022	b	14.26	1.071	0.0117
Limestone 3 60:20:20	1.57	0.015	a	1.84	0.019	b	16.93	1.373	0.0122
LECA 100:0:0	0.36	0.001	a	0.44	0.002	b	24.02	0.563	0.0075
LECA 90:0:10	0.38	0.005	a	0.46	0.011	b	20.96	1.413	0.0114
LECA 80:0:20	0.41	0.012	a	0.51	0.028	b	22.82	5.895	0.0114
LECA 70:20:10	0.45	0.010	a	0.71	0.064	b	57.85	16.276	0.0122
LECA 60:20:20	0.44	0.014	a	0.59	0.023	b	33.57	2.924	0.0117
Brick A 80:0:20	1.25	0.009	a	1.47	0.016	b	17.49	0.543	0.0117
Brick A 60:20:20	1.38	0.012	a	1.68	0.018	b	22.09	0.377	0.0117
Brick B 80:0:20	1.22	0.006	a	1.49	0.007	b	22.10	0.215	0.0114
Brick B 60:20:20	1.36	0.015	a	1.70	0.022	b	25.49	0.251	0.0117
Brick A+B 80:0:20	1.28	0.007	a	1.52	0.007	b	19.37	0.278	0.0114
Brick A+B 60:20:20	1.36	0.011	a	1.70	0.023	b	24.64	1.165	0.0122
John Innes	1.14	0.013	a	1.63	0.016	b	43.67	0.748	0.0122
Z. Semi-extensive	1.00	0.014	a	1.39	0.030	b	39.82	2.216	0.0117
Z. Sedum	1.16	0.022	a	1.46	0.025	b	26.19	1.744	0.0119
P – value[2]	< 0.001			< 0.001			< 0.001		

[1] Within rows values followed by different letter are significantly different at $p = 0.05$ (Mann-Whitney *U*-test) between dry and saturated particle density for the same substrate.
[2] Significant differences at $p = 0.05$ between substrates (Kruskal-Wallis test) within the same column.

For individual substrates, the Kruskal-Wallis test on data shown in Table 3.6 also revealed significant differences between substrates at both dry ($P < 0.001$) and saturated ($P < 0.001$) bulk density. Across all substrates, the highest and the lowest value of both dry and saturated bulk density were observed in Limestone 3 and LECA with 100:0:0 composition rate respectively.

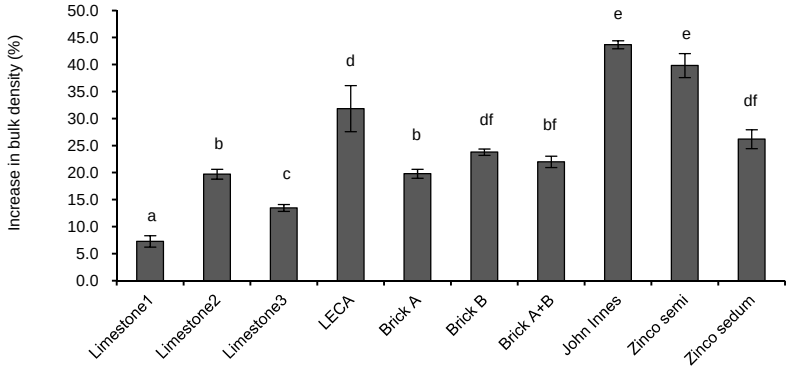


Figure 3.3. Mean increase percentage of bulk density in response to saturation across all composition rates. Letters above bars indicate statistically different at $p = 0.05$ (Mann-Whitney *U*-test after Kruskal-Wallis test).

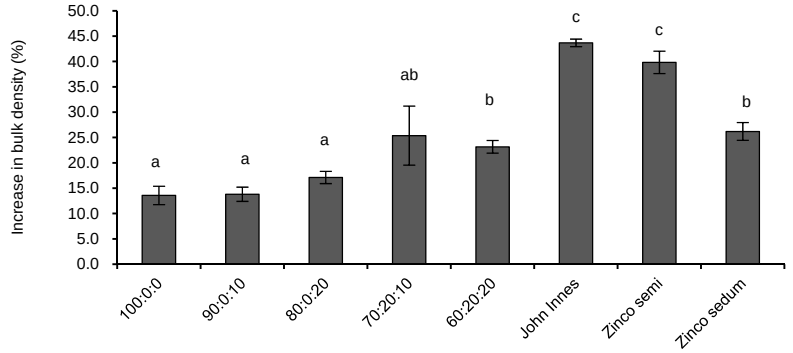


Figure 3.4. Mean increase percentage of bulk density in response to saturation across all substrate types. Letters above bars indicate statistically different at $p = 0.05$ (Mann-Whitney *U*-test after Kruskal-Wallis test).

As shown in Table 3.6, all substrates showed significant higher bulk density in response to saturation than at dry condition. Kruskal-Wallis test on data in Figure 3.3 and 3.4 shows that substrate type and composition rate significantly affected the increase of bulk density in response to saturation ($P < 0.001$ respectively). Limestone 1 substrate type showed the smallest increase of bulk density, and Zinco semi-extensive substrate the greatest (same as John Innes), followed by the LECA substrate type. 60:20:20 composition rate had statistically higher increase of saturated bulk density than substrates with 100:0:0, 90:0:10, and 80:0:20 composition rate. 70:20:10 composition rate was intermediate, and not statistically different to either the former or the later composition rate group (Figure 3.4).

3.4.1.2 Air filled porosity

Figures 3.5 and 3.6 show that air filled porosity of substrate was significantly affected by substrate type ($P < 0.001$) and composition rate ($P < 0.001$). LECA substrate type had the largest air filled porosity, followed secondly by Limestone 1, the third and fourth group are Brick rubbles (A, B, and A+B) and Limestone 2 and 3, and lastly the smallest for John Inness. There was no significant difference in air filled porosity

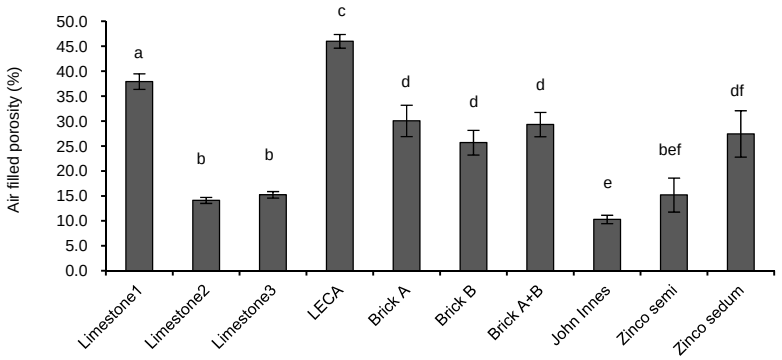


Figure 3.5. Mean air filled porosity (%) in response to substrate type across all composition rates. Letters above bars indicate significant difference at $p = 0.05$ (Mann-Whitney *U*-test after Kruskal-Wallis test).

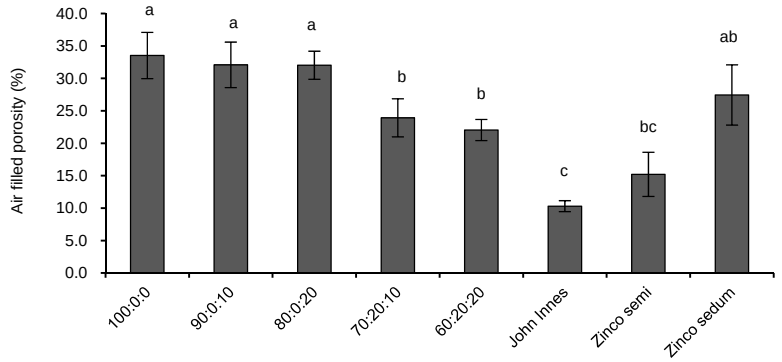


Figure 3.6. Mean air filled porosity (%) in response to composition rate across all substrate types. Letters above bars indicate significant difference at $p = 0.05$ (Mann-Whitney *U*-test after Kruskal-Wallis test).

between brick rubble substrate types (A, B, and A+B), Limestone 2 and 3, Zinco semi-extensive and John Innes, and Zinco semi-extensive and sedum substrate. Limestone 2 and 3 had statistically the same air filled porosity with Zinco semi-extensive and Brick rubbles for Zinco sedum. Mann-Whitney *U*-test on data in Figure 3.6 showed that 100:0:0, 90:0:10, and 80:0:20 composition rate had significantly higher air filled porosity than 70:20:10 and 60:20:20 composition rate (Figure 3.6). No significant differences were found in air filled porosity between the former composition rates and Zinco sedum substrate, and the later composition rates and the two Zinco substrates.

Table 3.7. Mean air filled porosity (%) in response to substrate type and composition rate.

		Composition rate					
Substrate type		100:0:0	90:0:10	80:0:20	70:20:10	60:20:20	*P* - value [1]
Limestone 1	Mean	42.26 Aa	45.07 Aa	41.54 ABae	34.72 Ba	26.05 Ca	0.001
	S.E	1.265	0.859	2.582	1.603	1.661	
Limestone 2	Mean	18.28 Ab	16.50 Ab	12.53 Bb	11.26 Cb	12.00 BCb	0.000
	S.E	0.770	0.622	0.321	0.231	0.157	
Limestone 3	Mean	19.17 Ab	17.39 Ab	15.04 Bc	11.58 Cb	13.04 BCbd	0.001
	S.E	1.070	0.438	0.322	0.284	1.147	
LECA	Mean	54.38 Ac	49.39 Bc	47.03 BCa	38.10 Ca	41.13 Cc	0.001
	S.E	1.153	1.018	2.213	1.786	2.017	
Brick A	Mean			38.99 Aae		21.10 Bae	0.0122*
	S.E			1.400		1.573	
Brick B	Mean			32.48 Adf		18.90 Bde	0.0122*
	S.E			0.972		1.864	
Brick A+B	Mean			36.54 Ae		22.09 Bae	0.0122*
	S.E			0.257		0.757	
John Innes	Mean	10.30 d	10.30 d	10.30 b	10.30 b	10.30 b	
	S.E	0.843	0.843	0.843	0.843	0.843	
Zinco semi-extensive	Mean	15.20 bd	15.20 bd	15.20 bcf	15.20 bc	15.20 abe	
	S.E	3.407	3.407	3.407	3.407	3.407	
Zinco sedum	Mean	27.44 ab	27.44 b	27.44 ef	27.44 ac	27.44 ace	
	S.E	4.643	4.643	4.643	4.643	4.643	
P - value [2]		< 0.001	< 0.001	0.001	< 0.001	< 0.001	

Different capital letters indicate significant difference at $p = 0.05$ (Mann-Whitney *U*-test after Kruskal-Wallis test[1]) between composition rates for the same substrate.
Different lower-case letters indicate significant difference at $p = 0.05$ (Mann-Whitney U-test after Kruskal-Wallis test[2]) between substrates for the same composition rate.
*Significant differences at $p = 0.05$(Mann-Whitney U-test) between composition rates for the same Brick rubble substrate.

For individual substrates, however, Mann-Whitney *U*-test on data in Table 3.7 showed that different responses were observed in a few substrates. In 100:0:0 and 90:0:10 composition rate, Limestone 2 and 3 substrates were statistically the same as the two Zinco substrates. There was also no significant difference in 100:0:0 between Zinco sedum and Limestone 1 substrate. In 80:0:20 composition rate, air filled porosity of Limestone 3 substrate was significantly higher than Limestone 2 substrate. Brick rubble B type substrate was significantly lower than both A and A+B type substrate, and the same with the two Zinco substrates. In 70:20:10, no significant differences were found between Limestone 1, LECA, and Zinco sedum. Limestone 2 and 3 were not significantly different from John Innes and Zinco semi-extensive. In 60:20:20, the experimental substrates showed no significant difference from at least one of John Innes and/or the two commercial green roof substrates. In the case of Limestone 1 substrate, no significant difference was revealed in comparison with Zinco semi-extensive and the sedum substrate.

The Spearman rank test revealed air filled porosity had significantly negative correlation with bulk density at dry condition (r_s = - 0.534, $P < 0.01$).

3.4.1.3 Moisture content

Kruskal-Wallis test on data shown in Figures 3.7 and 3.8 revealed differences ($P <$ 0.001 respectively) between substrates in terms of moisture content across all composition rates and substrate types. Mann-Whitney *U*-test shows that Limestone 2, the three Brick rubble types, John Innes, and Zinco semi-extensive substrate had the greatest moisture content significantly, but no significant differences were revealed between these substrates. LECA substrate type had the lowest value (Figure 3.7). The second place was the Zinco sedum substrate and the third and fourth was Limestone 3 and 1 substrate type respectively. Comparing the composition rates across all substrates (Figure 3.8), the highest moisture content occurred significantly in 60:20:20 composition rate and the lowest in 100:0:0 composition rate. 60:20:20 composition rate was statistically the same as John Innes and the two Zinco substrates.

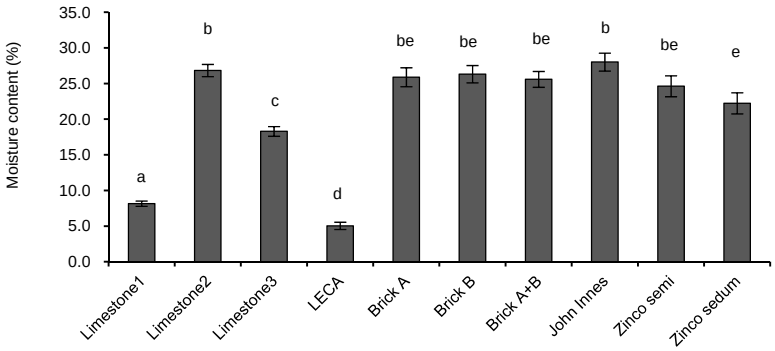


Figure 3.7. Mean moisture content (%) in response to substrate type across all composition rates. Letters above bars indicate significant difference at $p = 0.05$ (Mann-Whitney *U*-test after Kruskal-Wallis test).

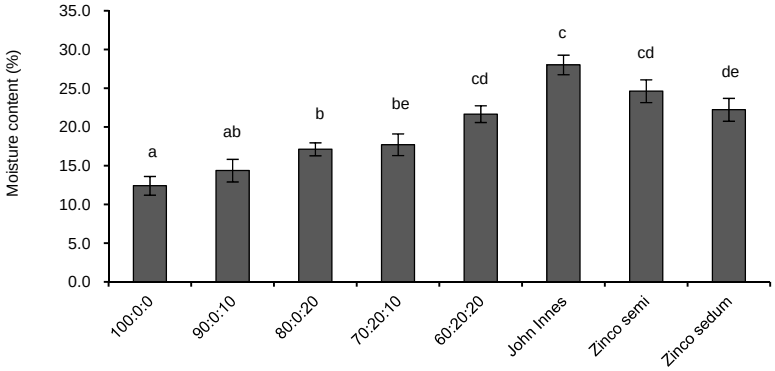


Figure 3.8. Mean moisture content (%) in response to composition rate across all substrate types. Letters above bars indicate significant difference at $p = 0.05$ (Mann-Whitney *U*-test after Kruskal-Wallis test).

However, as shown in Table 3.8, the Limestone substrate types show a different response to moisture content. The highest moisture content was observed in 70:20:10 composition rate for all Limestone substrate types (1, 2, and 3) and the lowest in 100:0:0 for Limestone 1 and in 60:20:20 for Limestone 2 and 3 substrate, which were statistically the same with the substrate types with 100:0:0 composition. In 90:0:10 and 70:20:10 composition rate, Limestone 3 substrate had statistically the same moisture content with Zinco semi-extensive and/or Zinco sedum substrate. In 60:20:20

Table 3.8. Mean moisture content (%) in response to substrate type and composition rate.

		Composition rate					
Substrate type		100:0:0	90:0:10	80:0:20	70:20:10	60:20:20	*P* - value [1]
Limestone 1	Mean	6.09 Aa	6.97 ABa	7.71 Ba	11.36 Ca	8.68 Ba	< 0.001
	S.E	0.203	0.621	0.642	1.019	0.685	
Limestone 2	Mean	24.77 Ab	28.98 BCb	28.07 BCb	29.40 Bb	22.97 ACbf	0.011
	S.E	0.677	1.219	1.337	2.668	2.508	
Limestone 3	Mean	16.74 Ac	19.45 ABc	17.00 Ac	22.91 Bc	15.37 Ac	0.005
	S.E	1.141	1.435	1.205	1.072	1.948	
LECA	Mean	2.06 Ad	2.11 Ad	3.62 Ad	7.19 Bd	10.24 Ba	< 0.001
	S.E	0.209	0.385	0.729	1.064	1.173	
Brick A	Mean			20.04 Ace		31.74 Bd	< 0.001*
	S.E			0.796		1.348	
Brick B	Mean			20.40 Ace		32.23 Bd	< 0.001*
	S.E			0.695		0.822	
Brick A+B	Mean			20.75 Ae		30.43 Bde	< 0.001*
	S.E			0.820		1.052	
John Innes	Mean	28.02 e	28.02 be	28.02 b	28.02 be	28.02 be	
	S.E	1.262	1.262	1.262	1.262	1.262	
Zinco semi-extensive	Mean	24.63 be	24.63 ef	24.63 bf	24.63 ce	24.63 bf	
	S.E	1.466	1.466	1.466	1.466	1.466	
Zinco sedum	Mean	22.23 b	22.23 cf	22.23 ef	22.23 c	22.23 f	
	S.E	1.479	1.479	1.479	1.479	1.479	
P - value [2]		< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	

Different capital letters indicate significant difference at $p = 0.05$ (Mann-Whitney *U*-test after Kruskal-Wallis test[1]) between composition rates for the same substrate.
Different lower-case letters indicate significant difference at $p = 0.05$ (Mann-Whitney *U*-test after Kruskal-Wallis test[2]) between substrates for the same composition rate.
*Significant differences at $p = 0.05$(Mann-Whitney *U*-test) between composition rates for the same Brick rubble substrate.

composition rate, the three Brick rubble types had significantly higher values than the two Zinco substrates. No significant difference was revealed in 60:20:20 composition rate between Limestone 1 and LECA substrate.

A Spearman rank correlation test found water holding capacity had significantly negative correlation with air filled porosity ($r_s = - 0.730$, $P < 0.001$).

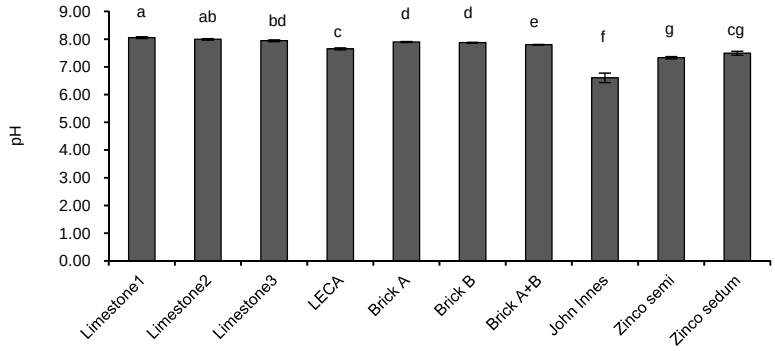


Figure 3.9. Mean pH in response to substrate type across all composition rates. Letters above bars indicate significant difference at $p = 0.05$ (Mann-Whitney *U*-test after Kruskal-Wallis test).

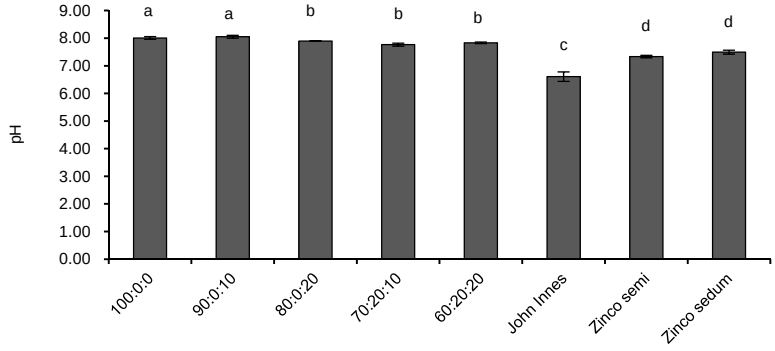


Figure 3.10. Mean pH in response to composition rate across all substrate types. Letters above bars indicate significant difference at $p = 0.05$ (Mann-Whitney *U*-test after Kruskal-Wallis test).

3.4.1.4 pH

Figures 3.9 and 3.10 show that the pH varied significantly between the ten substrate types and the five composition rates for mean of all composition rates and all substrate types ($P < 0.001$ respectively). The lowest mean values were for John Innes (pH 6.61) showing slight acid, followed by substrate types showing weak alkaline, which were Zinco semi-extensive (pH 7.34), Zinco sedum (pH 7.50), LECA (pH 7.65), Brick rubble A+B (pH 7.80), Brick rubble B (pH 7.88), Brick rubble A (pH 7.90), and Limestone 3 (pH 7.95). The highest mean values were for Limestone 1 (pH 8.06) and 2 (pH 8.00)

Table 3.9. Mean pH in response to substrate type and composition rate.

		Composition rate					
Substrate type		100:0:0	90:0:10	80:0:20	70:20:10	60:20:20	*P* - value [1]
Limestone 1	Mean	8.04 ACab	8.33 Ba	7.93 Aabc	8.02 Ca	7.97 ACa	0.008
	S.E	0.090	0.027	0.042	0.004	0.027	
Limestone 2	Mean	8.22 Aa	7.94 Bb	7.96 Ba	7.84 Cb	8.03 Da	< 0.001
	S.E	0.042	0.018	0.018	0.030	0.011	
Limestone 3	Mean	8.05 Ab	8.17 Ac	7.85 Babc	7.80 Bb	7.87 Bb	0.002
	S.E	0.044	0.024	0.037	0.069	0.021	
LECA	Mean	7.72 Ac	7.76 ABd	7.87 Bbc	7.42 Cc	7.48 Cc	0.000
	S.E	0.029	0.014	0.036	0.021	0.012	
Brick A	Mean			7.94 Aab		7.87 Ab	0.0586*
	S.E			0.019		0.024	
Brick B	Mean			7.90 Aabc		7.85 Ab	0.1161*
	S.E			0.019		0.020	
Brick A+B	Mean			7.82 Ac		7.79 Ad	0.3961*
	S.E			0.023		0.013	
John Innes	Mean	6.61 d	6.61 e	6.61 d	6.61 d	6.61 e	
	S.E	0.172	0.172	0.172	0.172	0.172	
Zinco semi-extensive	Mean	7.34 e	7.34 f	7.34 e	7.34 c	7.34 c	
	S.E	0.044	0.044	0.044	0.044	0.044	
Zinco sedum	Mean	7.50 e	7.50 f	7.50 e	7.50 c	7.50 c	
	S.E	0.070	0.070	0.070	0.070	0.070	
P - value [2]		< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	

Different capital letters indicate significant difference at $p = 0.05$ (Mann-Whitney *U*-test after Kruskal-Wallis test[1]) between composition rates for the same substrate.
Different lower-case letters indicate significant difference at $p = 0.05$ (Mann-Whitney *U*-test after Kruskal-Wallis test[2]) between substrates for the same composition rate.
*Significant differences at $p = 0.05$(Mann-Whitney *U*-test) between composition rates for the same Brick rubble substrate.

showing moderate alkaline in the terms by Handreck and Black (2005, p.20) and for individual substrates, it was Limestone 1 with 90:0:10 recorded in pH 8.33. In comparison of composition rates for mean of all substrates (Figure 3.10), 100:0:0 and 90:0:10 composition rate had significantly higher pH value showing moderate alkaline with pH 8.01 and pH 8.05 respectively than did 80:0:20, 70:20:10 and 60:20:20 composition rate showing weak alkaline with pH 7.90, pH 7.77, and pH 7.83 respectively. For individual substrates, across LECA substrate types, 80:0:20 composition rate revealed significantly the highest pH value amongst all of the composition rates. LECA substrate with 70:20:10 and 60:20:20 composition rate were

statistically the same as the two Zinco commercial substrates, while most of the substrate had significantly higher pH than the two Zinco commercial substrates (Table 3.9).

3.4.1.5 Electrical Conductivity (EC)

Kruskal-Wallis test on data shown in Figure 3.11 and 3.12 revealed differences ($P < 0.001$ respectively) between substrates in terms of EC across all composition rates and substrate types. As shown in Figure 3.11, Zinco sedum and semi-extensive substrate were significantly the highest EC with 1.936 dS/m and 1.904 dS/m respectively,

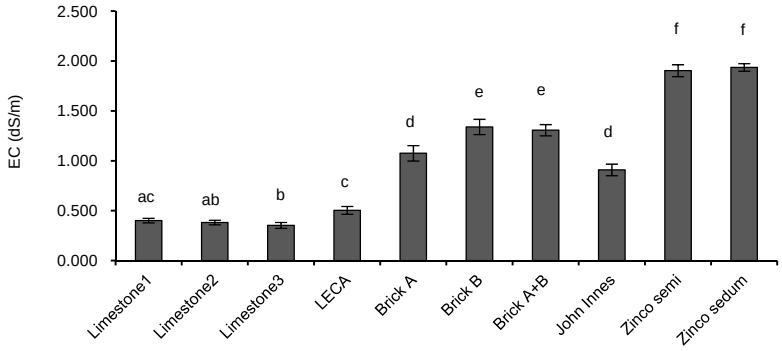


Figure 3.11. Mean EC (dS/m) in response to substrate type across all composition rates. Letters above bars indicate significant difference at $p = 0.05$ (Mann-Whitney *U*-test after Kruskal-Wallis test).

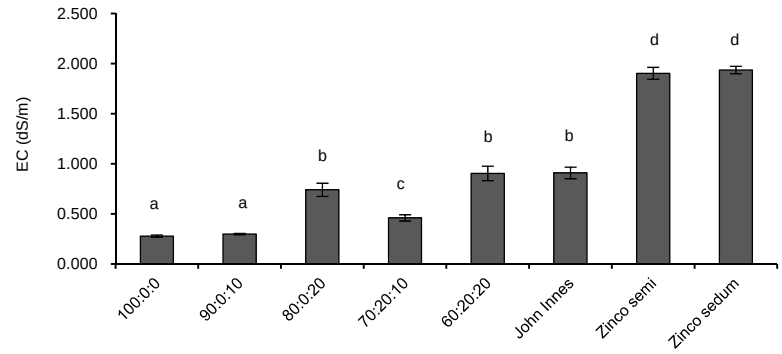


Figure 3.12. Mean EC (dS/m) in response to composition rate across all substrate types. Letters above bars indicate significant difference at $p = 0.05$ (Mann-Whitney *U*-test after Kruskal-Wallis test).

followed by Brick rubble B (1.340 dS/m) and A+B (1.307 dS/m). After that the third and fourth substrates were Brick rubble A (1.076 dS/m) and John Innes (0.909 dS/m), and LECA (0.505 dS/m) and Limestone 1 (0.402 dS/m), and lastly the lowest was Limestone 2 and 3 with 0.383 dS/m and 0.353 dS/m respectively. Mann-Whitney *U*-test on data in Figure 3.12 shows that EC of 60:20:20 and 80:0:20 composition rate was significantly higher than 100:0:0 and 90:0:10 composition rate. EC of 70:20:10 composition rate was intermediate, and statistically different to either the former or the later composition rate.

Table 3.10 shows that there were some different responses for EC of individual substrate in response to substrate type and composition rate. For example, there were no

Table 3.10. Mean EC (dS/m) in response to substrate type and composition rate.

		Composition rate					
Substrate type		100:0:0	90:0:10	80:0:20	70:20:10	60:20:20	*P* - value [1]
Limestone 1	Mean	0.272 Aa	0.308 Aa	0.433 Ba	0.453 Ba	0.542 Ba	0.000
	S.E	0.013	0.005	0.044	0.017	0.031	
Limestone 2	Mean	0.270 ACab	0.298 Aab	0.478 Ba	0.353 Cb	0.514 Ba	0.001
	S.E	0.038	0.010	0.014	0.004	0.051	
Limestone 3	Mean	0.247 Aa	0.253 Ac	0.367 Ba	0.465 Babcd	0.435 Ba	0.001
	S.E	0.003	0.003	0.054	0.107	0.042	
LECA	Mean	0.324 Ab	0.334 Ab	0.475 Ba	0.569 Bc	0.821 Cb	0.000
	S.E	0.006	0.007	0.041	0.029	0.041	
Brick A	Mean			0.929 Ab		1.223 Ac	0.0947*
	S.E			0.068		0.106	
Brick B	Mean			1.247 Ac		1.433 Ac	0.2963*
	S.E			0.091		0.118	
Brick A+B	Mean			1.256 Ac		1.359 Ac	0.5309*
	S.E			0.074		0.087	
John Innes	Mean	0.909 c	0.909 d	0.909 b	0.909 d	0.909 b	
	S.E	0.058	0.058	0.058	0.058	0.058	
Zinco semi-extensive	Mean	1.904 d	1.904 e	1.904 d	1.904 e	1.904 d	
	S.E	0.060	0.060	0.060	0.060	0.060	
Zinco sedum	Mean	1.936 d	1.936 e	1.936 d	1.936 e	1.936 d	
	S.E	0.037	0.037	0.037	0.037	0.037	
P - value [2]		< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	

Different capital letters indicate significant difference at $p = 0.05$ (Mann-Whitney *U*-test after Kruskal-Wallis test[1]) between composition rates for the same substrate.
Different lower-case letters indicate significant difference at $p = 0.05$ (Mann-Whitney *U*-test after Kruskal-Wallis test[2]) between substrates for the same composition rate.
*Significant differences at $p = 0.05$(Mann-Whitney *U*-test) between composition rates for the same Brick rubble substrate.

significant differences in 80:0:20 composition rate between Limestone 1, 2, 3, and LECA substrate type. There were also no significant differences in 60:20:20 composition rate between Limestone substrates and Brick rubble substrates for the same substrate type. Most of substrates had no significant differences between 80:0:20 and 60:20:20, while across LECA substrate types, 60:20:20 composition rate had significantly the highest EC value. Three substrates, which were Limestone 3 with 70:20:10, LECA with 60:20:20, and Brick rubble A with 80:0:20, have statistically similar EC to John Innes.

Spearman rank test of the data shows that EC had a significantly negative correlation with pH (r_s = - 0.583, $P < 0.001$).

3.4.2. Experiment 2

3.4.2.1 Seedling emergence

The mean maximum percentage of seedling emergence of *Leucanthemum vulgare* for Experiment 2 was recorded with less than 10 % across all ranges of substrate (Table 3.11). However, the statistical analysis between all ranges of substrate indicated valid significant differences.

Figures 3.13 and 3.14 show that the maximum percentage emergence of *Leucanthemum vulgare* was significantly affected by substrate type and composition rate ($P < 0.001$ respectively). The highest maximum percentage emergence occurred in Limestone 2 (5.92 %) and 3 (4.64 %), whose substrate types were not significantly different from one another, and in 60:20:20 composition rate (5.85 %). The substrates and composition rate had statistically higher emergence than the two Zinco substrates, and statistically the same value with John Innes. Kruskal-Wallis test on data shown in Table 3.11 revealed maximum percentage emergence was significantly different between substrate types for each of the same composition rate. In Limestone 1 and LECA substrate type, the composition rates had a significant effect on emergence, while in Limestone 2 (P = 0.242) and Limestone 3 (P = 0.517) substrate type, the composition rates did not affect

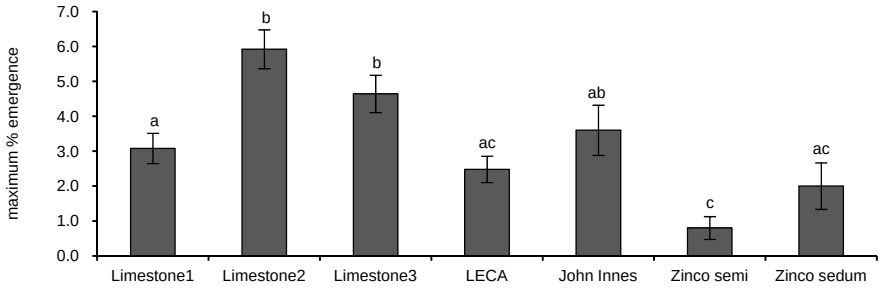


Figure 3.13. Mean maximum percentage emergence of *Leucanthemum vulgare* in response to substrate type across all composition rates. Letters above bars indicate significant difference at $p = 0.05$ (Mann-Whitney *U*-test after Kruskal-Wallis test).

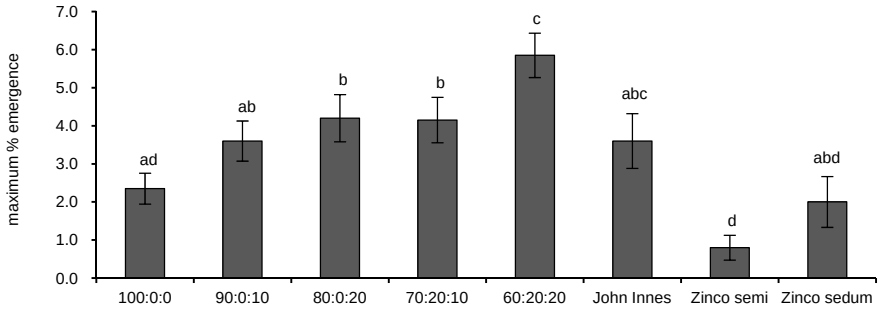


Figure 3.14. Mean maximum percentage emergence of *Leucanthemum vulgare* in response to composition rate across substrate types. Letters above bars indicate significant difference at $p = 0.05$ (Mann-Whitney *U*-test after Kruskal-Wallis test).

Table 3.11. Mean maximum percentage emergence of *Leucanthemum vulgare* in response to substrate type and composition rate.

		Composition rate					
Substrate type		100:0:0	90:0:10	80:0:20	70:20:10	60:20:20	*P* - value [1]
Limestone 1	Mean	1.80 Aac	1.40 Aa	3.80 ABa	3.20 ABac	5.20 Ba	0.024
	S.E	0.629	0.600	1.209	0.952	0.952	
Limestone 2	Mean	4.00 Aa	5.20 Ab	7.60 Ab	5.60 Aa	7.20 Aa	0.242
	S.E	0.943	0.904	1.392	1.360	1.405	
Limestone 3	Mean	3.40 Aa	5.40 Abc	3.20 Aa	5.60 Aac	5.60 Aa	0.517
	S.E	0.733	1.267	0.998	1.454	1.360	
LECA	Mean	0.20 Abd	2.40 Bacd	2.20 Bac	2.20 Babc	5.40 Ca	0.001
	S.E	0.200	0.833	0.629	0.554	0.945	
John Innes	Mean	3.60 a	3.60 bde	3.60 a	3.60 ac	3.60 ac	
	S.E	0.718	0.718	0.718	0.718	0.718	
Zinco semi-extensive	Mean	0.80 cde	0.80 a	0.80 c	0.80 bd	0.80 b	
	S.E	0.327	0.327	0.327	0.327	0.327	
Zinco sedum	Mean	2.00 ae	2.00 ae	2.00 ac	2.00 cd	2.00 bc	
	S.E	0.667	0.667	0.667	0.667	0.667	
P - value [2]		< 0.001	0.001	0.001	0.012	< 0.001	

Different capital letters indicate significant difference at $p = 0.05$ (Mann-Whitney *U*-test after Kruskal-Wallis test[1]) between composition rates for the same substrate.
Different lower-case letters indicate significant difference at $p = 0.05$ (Mann-Whitney *U*-test after Kruskal-Wallis test[2]) between substrates for the same composition rate.

emergence.

The maximum percentage emergence of *Leucanthemum vulgare* had a significantly negative correlation (Spearman rank correlation test) with air filled porosity (r_s = -0.666, $P < 0.001$), and a positive correlation with moisture content (r_s = 0.572, P = 0.004).

3.4.2.2 Seedling survival

For seedling survival of *Leucanthemum vulgare*, in Experiment 2, and *Briza media* and *Prunella vulgaris*, in Experiment 3, the total number of survived seedlings in each pot prior to the biomass harvest in March 2006 for Experiment 2 and in August 2006 for Experiment 3 was converted to a percentage of the maximum number of emerged seedling recorded.

Table 3.12. Mean survival of *Leucanthemum vulgare* in response to substrate type and composition rate, as a percentage of maximum emergence.

		Composition rate					
Substrate type		100:0:0	90:0:10	80:0:20	70:20:10	60:20:20	*P* - value [1]
Limestone 1	Mean	60.0 Ab	50.0 Aa	78.3 Aa	57.5 Aa	88.5 Aa	0.527
	S.E	16.33	16.67	13.16	15.83	6.15	
Limestone 2	Mean	82.5 Ab	86.3 Aa	87.1 Aa	67.2 Aa	89.9 Aa	0.410
	S.E	10.57	6.01	7.77	10.70	10.90	
Limestone 3	Mean	70.0 Ab	78.6 Aa	70.0 Aa	76.3 Aa	92.0 Aa	0.688
	S.E	13.33	11.14	15.28	12.94	18.18	
LECA	Mean	0.0 Aa	80.0 Ba	60.0 Ba	70.0 Ba	72.3 Ba	0.007
	S.E	0.00	28.09	16.33	20.00	13.60	
John Innes	Mean	76.4 b	76.4 a	76.4 a	76.4 a	76.4 a	
	S.E	18.63	18.63	18.63	18.63	18.63	
Zinco semi-extensive	Mean	50.0 b	50.0 a	50.0 a	50.0 a	50.0 a	
	S.E	22.36	22.36	22.36	22.36	22.36	
Zinco sedum	Mean	66.7 b	66.7 a	66.7 a	66.7 a	66.7 a	
	S.E	16.67	16.67	16.67	16.67	16.67	
P - value [2]		0.009	0.688	0.778	0.910	0.451	

Different capital letters indicate significant difference at p = 0.05 (Mann-Whitney *U*-test after Kruskal-Wallis test[1]) between composition rates for the same substrate.
Different lower-case letters indicate significant difference at p = 0.05 (Mann-Whitney *U*-test after Kruskal-Wallis test[2]) between substrates for the same composition rate.

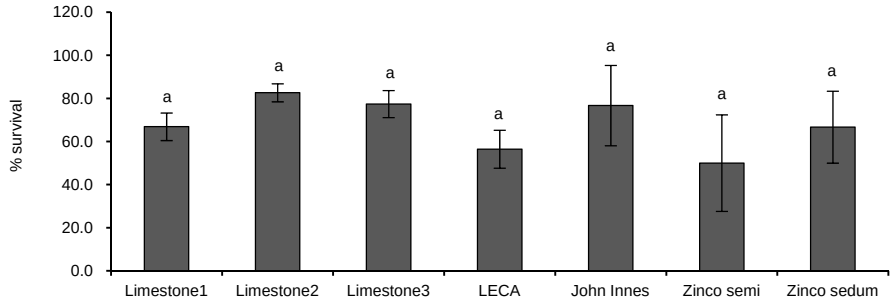


Figure 3.15. Mean survival of *Leucanthemum vulgare* in March 2006 in response to substrate type across all composition rates, as a percentage of maximum emergence. Letters above bars indicate significant difference at $p = 0.05$ (Kruskal-Wallis test).

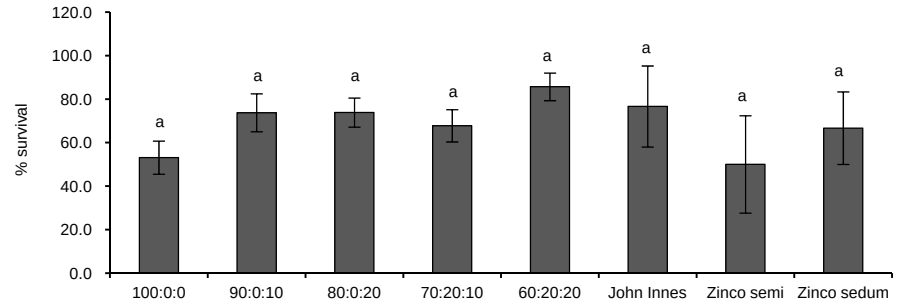


Figure 3.16. Mean survival of *Leucanthemum vulgare* in March 2006 in response to composition rate across all substrate types, as a percentage of maximum emergence. Letters above bars indicate significant difference at $p = 0.05$ (Kruskal-Wallis test).

Kruskal-Wallis test on data shown in Table 3.12, and in Figures 3.15 and 3.16 found that the substrate type and composition rate did not significantly affect seedling survival of *Leucanthemum vulgare*, except for LECA with 100:0:0 composition rate where no seedlings survived (Table 3.12). All other substrates had higher survival rates of over 50.0 %.

A Spearman's rank correlation test on survival of *Leucanthemum vulgare* also revealed that there were no significant correlations between seedling survival and substrate characteristics (moisture content; $r_s = 0.354$, $P = 0.0972$, pH; $r_s = 0.193$, $P = 0.377$, EC; $r_s = 0.000$, $P = 0.9982$), while it had a significantly negative correlation with air filled porosity ($r_s = - 0.441$, $P = 0.035$).

3.4.2.3 Seedling growth

Mean dry weights (aboveground biomass) of seedling of *Leucanthemum vulgare* differed considerably across the seven substrate types and the five composition rates (Figures 3.17 and 3.18). Kruskal-Wallis test on data shown in Figures 3.17 and 3.18 found that substrate type had a relatively weak but significant effect ($P = 0.011$) on seedling dry weight of *Leucanthemum vulgare* compared to composition rate ($P < 0.001$). No significant differences were shown in comparisons between the three Limestone substrate types (1, 2, and 3), LECA, and John Innes (Figure 3.17). However, the above substrate types, except for John Innes, had significantly higher dry weight than Zinco semi-extensive substrate. Figure 3.18 shows that the highest dry weight was significantly associated with 60:20:20 composition rate and the lowest dry weights with 100:0:0 composition rate.

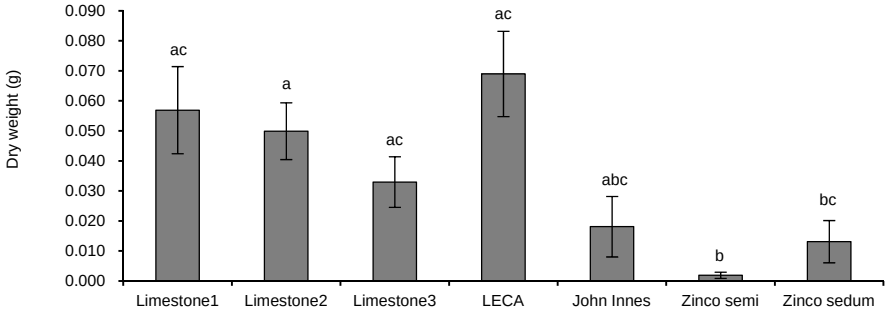


Figure 3.17. Mean dry weight (g) of *Leucanthemum vulgare* in response to substrate type across all composition rates. Letters above bars indicate significant difference at $p = 0.05$ (Mann-Whitney *U*-test after Kruskal-Wallis test).

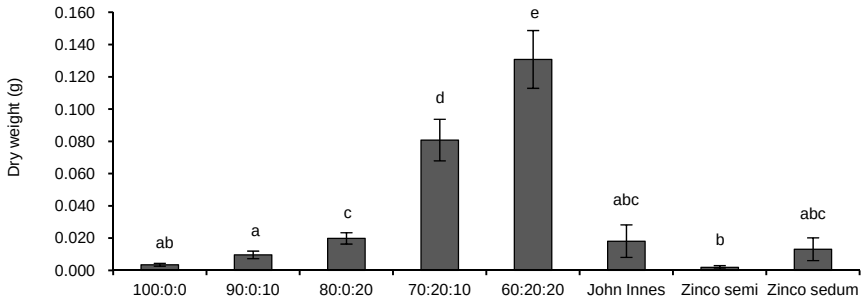


Figure 3.18. Mean dry weight (g) of *Leucanthemum vulgare* in response to composition rate across all substrate types. Letters above bars indicate significant difference at $p = 0.05$ (Mann-Whitney *U*-test after Kruskal-Wallis test).

Table 3.13. Mean dry weight (g) of *Leucanthemum vulgare* in response to substrate type and composition rate.

		Composition rate					
Substrate type		100:0:0	90:0:10	80:0:20	70:20:10	60:20:20	*P* - value [1]
Limestone 1	Mean	0.002 Aa	0.008 ABa	0.027 BCac	0.056 ACabc	0.193 Da	0.000
	S.E	0.0007	0.0046	0.0103	0.0191	0.0497	
Limestone 2	Mean	0.006 Aa	0.008 Aa	0.023 Ba	0.082 Ca	0.131 Cab	0.000
	S.E	0.0022	0.0021	0.0054	0.0175	0.0272	
Limestone 3	Mean	0.004 Aa	0.012 ABa	0.013 Aac	0.058 Bab	0.078 ABbc	0.033
	S.E	0.0012	0.0037	0.0045	0.0211	0.0310	
LECA	Mean	0.000 —	0.011 ABa	0.017 Aabc	0.127 BCab	0.121 Cab	0.009
	S.E	0.0000	0.0074	0.0066	0.0373	0.0254	
John Innes	Mean	0.018 a	0.018 a	0.018 abc	0.018 bc	0.018 cd	
	S.E	0.0101	0.0101	0.0101	0.0101	0.0101	
Zinco semi-extensive	Mean	0.002 a	0.002 a	0.002 b	0.002 c	0.002 d	
	S.E	0.0010	0.0010	0.0010	0.0010	0.0010	
Zinco sedum	Mean	0.013 a	0.013 a	0.013 bc	0.013 bc	0.013 cd	
	S.E	0.0070	0.0070	0.0070	0.0070	0.0070	
P - value [2]		0.171	0.131	0.032	0.006	< 0.001	

Different capital letters indicate significant difference at $p = 0.05$ (Mann-Whitney *U*-test after Kruskal-Wallis test[1]) between composition rates for the same substrate.
Different lower-case letters indicate significant difference at $p = 0.05$ (Mann-Whitney *U*-test after Kruskal-Wallis test[2]) between substrates for the same composition rate.

Kruskal-Wallis test on data in Table 3.13 revealed no significant differences between substrates at 100:0:0 ($P = 0.171$) and 90:0:10 ($P = 0.131$) composition rate. At the other composition rates, the three Limestone types and LECA had also statistically no differences from one another, with the exception of significant difference ($P = 0.0376$) between Limestone 1 and 3 substrate with 60:20:20 composition rate. The highest dry weight of the species occurred in Limestone 1 with 60:20:20 composition rate (0.193 g) and the lowest dry weight in Limestone 1 with 100:0:0 composition rate and Zinco semi-extensive substrate (0.002 g, respectively), with the exception of LECA with 100:0:0 composition rate which was recorded with no survived seedling.

A Spearman's rank correlation test on mean dry weight of the species in all substrates found that dry weight had significantly positive correlation with EC ($r_s = 0.543$, $P = 0.0074$).

3.4.3 Experiment 3

Mean maximum percentage emergence ($P = 0.6195$) and survival ($P = 0.7906$) as mean of all substrates did not significantly differ in comparison of *Briza media* with *Prunella vulgaris* (Ref. Appendix 5).

3.4.3.1 Seedling emergence

Kruskal-Wallis test on data in Table 3.14 indicates that substrates had a significant effect on maximum percentage of *Briza media* ($P = 0.003$) and *Prunella vulgaris* ($P = 0.015$). Figures 3.19 and 3.20 revealed that emergence of the two species was significantly affected by substrate type ($P = 0.003$ for *Briza media*; $P = 0.002$ for *Prunella vulgaris*) rather than composition rate as shown in Figure 3.21 ($P = 0.8472$ for *Briza media*; $P = 0.4285$ for *Prunella vulgaris*). Of the experimental substrates, for *Briza media*, the highest emergence occurred statistically in Limestone 3, which was not significantly different from Limestone 2 and John Innes. The lowest emergence occurred in

Table 3.14. Mean maximum percentage seedling emergence of *Briza media* and *Prunella vulgaris* in response to substrate.

	Maximum % emergence			
	Briza media		*Prunella vulgaris*	
	Mean	S.E.	Mean	S.E.
Limestone1 80:0:20	3.33	3.333	50.00	5.033
Limestone1 60:20:20	66.00	5.033	74.67	12.875
Limestone2 80:0:20	62.67	20.987	59.33	4.372
Limestone2 60:20:20	78.67	6.360	84.67	3.528
Limestone3 80:0:20	90.00	4.163	82.67	6.360
Limestone3 60:20:20	78.00	2.000	94.67	1.333
Brick A 80:0:20	58.00	6.110	33.33	24.395
Brick A 60:20:20	35.33	17.975	27.33	9.615
Brick B 80:0:20	50.00	4.163	56.00	12.702
Brick B 60:20:20	44.00	5.033	38.00	16.000
Brick A+B 80:0:20	75.33	8.110	47.33	14.530
Brick A+B 60:20:20	46.67	5.696	40.00	20.000
John Innes	84.67	9.262	91.33	7.688
Z. Semi	42.00	4.619	58.67	1.333
Z. Sedum	33.33	6.360	47.33	2.906
*P-value**	0.003		0.015	

* Significant differences between substrates (Kruskal-Wallis test) within the same column.

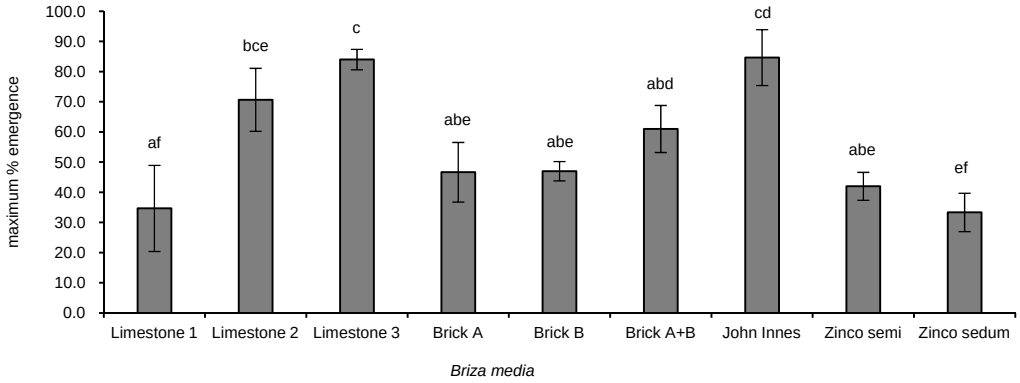


Figure 3.19. Mean maximum percentage emergence of *Briza media* in response to substrate type across composition rates. Letters above bars indicate significant difference at $p = 0.05$ (Mann-Whitney *U*-test after Kruskal-Wallis test)

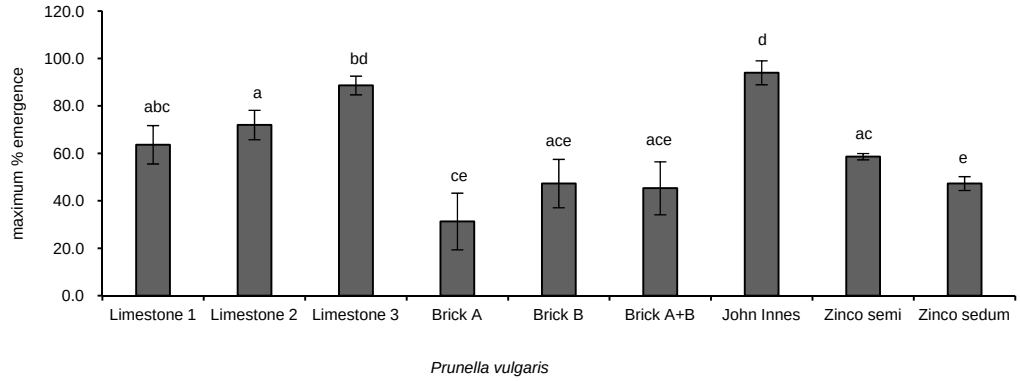


Figure 3.20. Mean maximum percentage of *Prunella vulgaris* in response to substrate type across composition rates. Letters above bars indicate significant difference at $p = 0.05$ (Mann-Whitney *U*-test after Kruskal-Wallis test)

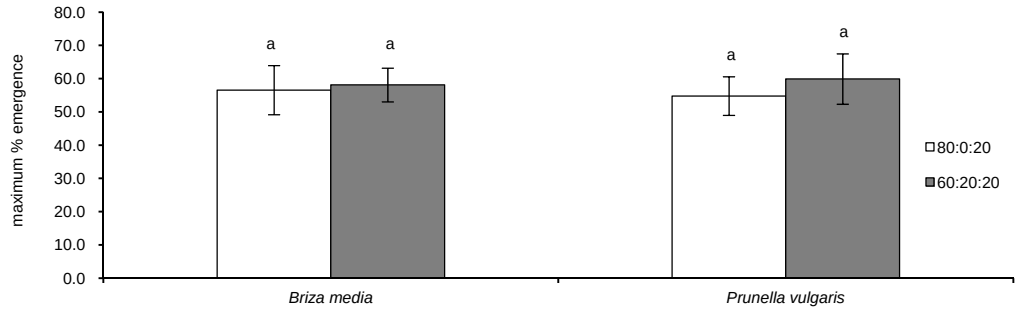


Figure 3.21. Mean maximum percentage emergence of *Briza* media and *Prunella vulgaris* in response to composition rate across all substrate types. Letters above bars indicate significant difference at $p = 0.05$ (Mann-Whitney *U*-test) for the same species.

Limestone 1, which was statistically same with the three Brick rubble substrate types and the two Zinco substrates. For *Prunella vulgaris*, Limestone 3 substrate type had the highest emergence, which was not statistically different from Limestone 1 and John Innes. Brick rubble A substrate type had the lowest emergence, which was not statistically different from Limestone 1, Brick rubble B and A+B, John Innes, and the two Zinco commercial substrates.

Percentage emergence had significantly negative correlation with air filled porosity for *Briza media* (r_s = - 0.561, P = 0.0297) and *Prunella vulgaris* (r_s = - 0.643, P = 0.009654), while it correlated with EC for *Prunella vulgaris* (r_s = - 0.568, P = 0.0271).

3.4.3.2 Seedling survival

Figures 3.22, 3.23, and 3.24 show that substrate type and composition rate did not affect seedling survival of *Briza media* (P = 0.384 and P = 0.1074 respectively) and *Prunella vulgaris* (P = 0.055 and P = 0.1255 respectively). Kruskal-Wallis test on data in Table 3.15 also indicates that survival of the two species was not significantly affected by

Table 3.15. Mean survival of *Briza media* and *Prunella vulgaris* in response to substrate, as a percentage of maximum emergence.

	% survival			
	Briza media		*Prunella vulgaris*	
	Mean	S.E.	Mean	S.E.
Limestone1 80:0:20	26.67	26.667	98.10	4.635
Limestone1 60:20:20	100.00	0.000	98.64	6.419
Limestone2 80:0:20	100.00	0.000	100.00	0.000
Limestone2 60:20:20	100.00	0.000	100.00	0.000
Limestone3 80:0:20	100.00	0.000	100.00	0.000
Limestone3 60:20:20	100.00	0.000	100.00	0.000
Brick A 80:0:20	92.69	9.380	100.00	0.000
Brick A 60:20:20	86.07	11.433	100.00	0.000
Brick B 80:0:20	89.50	1.965	73.55	7.887
Brick B 60:20:20	100.00	0.000	100.00	0.000
Brick A+B 80:0:20	100.00	0.000	83.62	17.054
Brick A+B 60:20:20	100.00	0.000	100.00	0.000
John Innes	99.02	0.980	100.00	0.000
Z. Semi	100.00	0.000	100.00	0.000
Z. Sedum	99.82	4.284	70.10	5.002
*P-value**	0.092		0.076	

* Significant differences between substrates (Kruskal-Wallis test) within the same column.

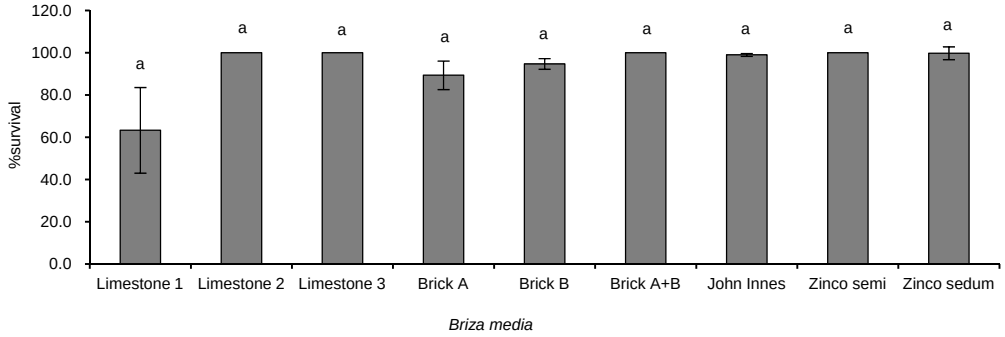


Figure 3.22. Mean survival of *Briza media* in August 2006 in response to substrate type across all composition rates, as a percentage of maximum emergence. Letters above bars indicate significant difference at $p = 0.05$ (Mann-Whitney *U*-test after Kruskal-Wallis test)

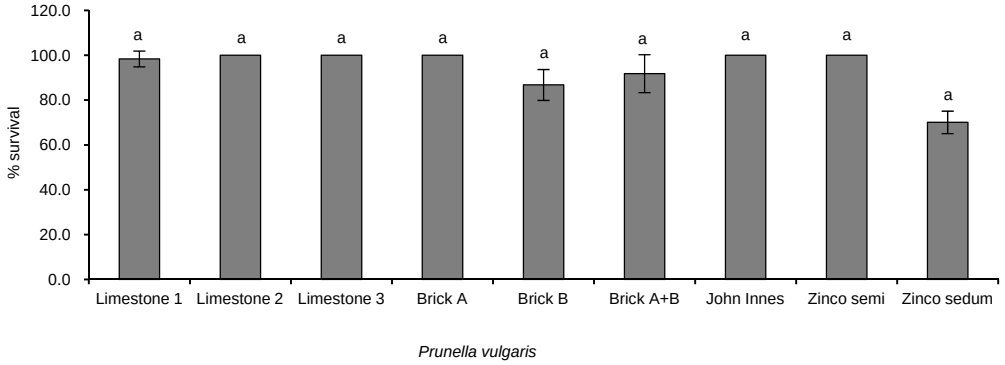


Figure 3.23. Mean survival of *Prunella vulgaris* in August 2006 in response to substrate type across all composition rates, as a percentage of maximum emergence. Letters above bars indicate significant difference at $p = 0.05$ (Mann-Whitney *U*-test after Kruskal-Wallis test)

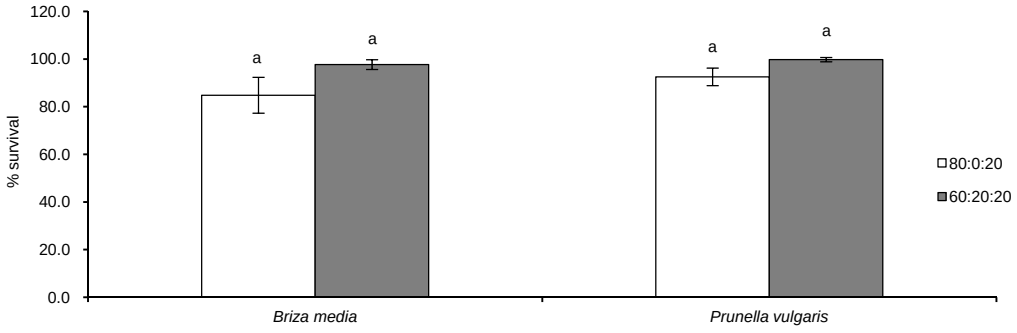


Figure 3.24. Mean survival of *Briza* media and *Prunella vulgaris* in August 2006 in response to composition rate across all substrate types, as a percentage of maximum emergence. Letters above bars indicate significant difference at $p = 0.05$ (Mann-Whitney *U*-test) for the same species.

substrate (P = 0.092 for *Briza media*; P = 0.076 for *Prunella vulgaris*). All substrate had higher survival rates with 50.0% over, except for *Briza media* in Limestone 1 with 80:0:20 composition rate which was recorded with 26.67 % survival rate.

Spearman's rank correlation test revealed no significant correlation between seedling survival of *Briza media* and substrate characteristics (air filled porosity; r_s = - 0.471, P = 0.0761, moisture content; r_s = 0.141, P = 0.6162, pH; r_s = - 0.046, P = 0.8696, EC; r_s = 0.024, P = 0.9319). However, survival of *Prunella vulgaris* was correlated significantly with air filled porosity (r_s = - 0.666, P = 0.006772), while there were no significant correlation with moisture content (r_s = 0.438, P = 0.1025), pH (r_s = 0.002, P = 0.994), and EC (r_s = - 0.238, P = 0.3927).

3.4.3.3 Seedling growth

Substrates had a highly significant effect on seedling dry weight of *Briza media* (P < 0.001) and *Prunella vulgaris* (P = 0.001) (Table 3.16). For *Briza media*, substrate type (P = 0.010) and composition rate (P = 0.0354) significantly affected dry weight (Figures

Table 3.16. Mean dry weight (g) of *Briza media* and *Prunella vulgaris* in response to substrate.

	Dry weight (g)			
	Briza media		*Prunella vulgaris*	
	Mean	S.E.	Mean	S.E.
Limestone1 80:0:20	0.005	0.0055	0.604	0.0613
Limestone1 60:20:20	0.622	0.0589	0.963	0.0416
Limestone2 80:0:20	0.210	0.0687	0.445	0.0258
Limestone2 60:20:20	0.522	0.0124	0.927	0.0054
Limestone3 80:0:20	0.301	0.0374	0.612	0.0958
Limestone3 60:20:20	0.480	0.0300	1.147	0.1129
Brick A 80:0:20	0.126	0.0357	0.142	0.0900
Brick A 60:20:20	0.066	0.0304	0.295	0.0497
Brick B 80:0:20	0.095	0.0227	0.481	0.0578
Brick B 60:20:20	0.190	0.0364	0.501	0.1359
Brick A+B 80:0:20	0.266	0.0840	0.494	0.2051
Brick A+B 60:20:20	0.109	0.0210	0.275	0.0583
John Innes	0.661	0.0668	2.825	0.1501
Z. Semi	0.160	0.0274	0.967	0.2078
Z. Sedum	0.092	0.0209	0.625	0.0039
*P-value**	< 0.001		0.001	

* Significant differences between substrates (Kruskal-Wallis test) within the same column.

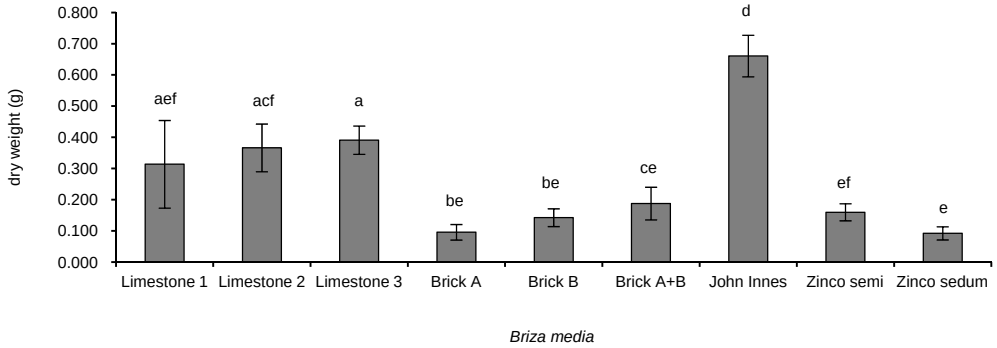


Figure 3.25. Mean dry weight (g) of *Briza media* in response to substrate type across all composition rates. Letters above bars indicate significant difference at $p = 0.05$ (Mann-Whitney *U*-test after Kruskal-Wallis test)

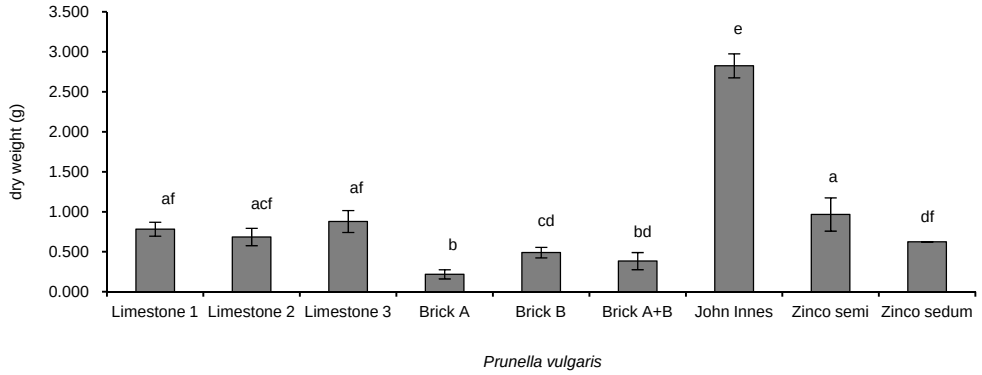


Figure 3.26. Mean dry weight (g) of *Prunella vulgaris* in response to substrate type across all composition rates. Letters above bars indicate significant difference at $p = 0.05$ (Mann-Whitney *U*-test after Kruskal-Wallis test)

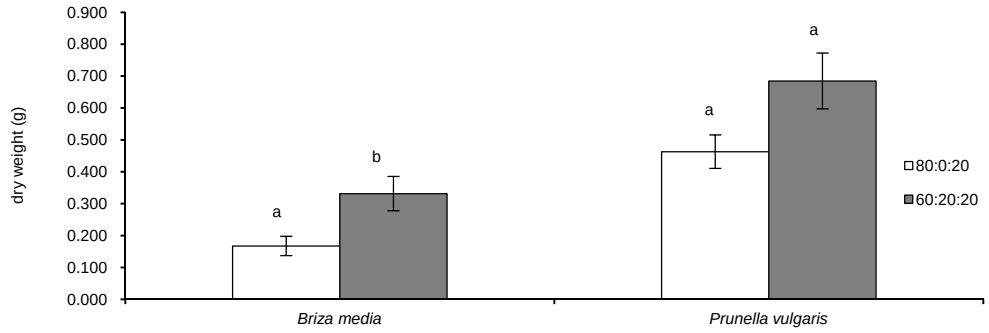


Figure 3.27. Mean dry weight (g) of *Briza* media and *Prunella vulgaris* in response to composition rate across all substrate types. Letters above bars indicate significant difference at $p = 0.05$ (Mann-Whitney *U*-test) for the same species.

3.25 and 3.27). For *Prunella vulgaris*, significant difference ($P < 0.001$) was revealed only between substrate types (Figures 3.26 and 3.27). The highest dry weight values for both species were most frequently associated with Limestone substrate types and the lowest dry weight with Brick rubble substrate types.

Spearman rank correlation test found that dry weight of *Briza media* had significantly negative correlation with air filled porosity ($r_s = - 0.643$, $P = 0.010$).

3.5 Discussion

3.5.1 Substrate characterisation

Substrate component materials and composition rate of the materials had a significant effect on physical and chemical characteristics of substrate, which were bulk density, increase of bulk density in response to saturation, air filled capacity, moisture content, pH, and EC.

3.5.1.1 Physical characteristics

The substrates used in this study revealed a wide range of bulk density between substrates, which were from 0.36 Mg/m^3 to 1.82 Mg/m^3 at dry condition and 0.44 Mg/m^3 to 2.00 Mg/m^3 at saturated condition (Table 3.5). All of the substrates could be classified as lightweight substrates for green roof. According to the study by Molineux, et al. (2009) on recycled materials for use as green roof growing media, lightweight aggregate was required to encounter the set limits of particle density at dry condition ≤ 2.00 Mg/m^3 and loose bulk density ≤ 1.20 Mg/m^3 (which were not measured in the current study). In comparison with the commercial green roof substrates at both dry and saturated bulk density, higher bulk density was most associated with Limestone substrate types and Brick rubble substrate types, and lower bulk density with LECA substrate types. All of the substrates increased significantly in bulk density when the substrates were fully saturated (Table 3.6). On the contrary, the higher increase of bulk density after saturation was nearly always associated with LECA and lower with Limestone and Brick rubble substrate types. In comparison with Limestone 1 and Brick rubble A substrate type that had similar particle size range from 8 mm to 4 mm (Figure 3.3), this trend was also observed. This may be due to typical nature of the main aggregate materials. For example, according to Dunnett and Kingsbury (2008, p.111), LECA has lightweight nature, produces large amounts of pore space because of the size of the mineral, and absorbs water because of the porous nature of the material. In addition to this, the amounts of fine particle materials (loam and organic matter)

included in substrate had a significant influence on bulk density and on the increase of bulk density after saturation. The results showed that, as the proportion of loam and organic matter included in substrate increased, bulk density decreased across all ranges of Limestone substrate especially (Table 3.5) as the bulk density after saturation increased with proportion of loam and organic matter (Figure 3.4). In a study of influence of organic amendments (sphagnum peat and food waste compost) on changes in bulk density and macro-porosity in compacted soils (sandy loam and clay loam), Rivenshield and Bassuk (2007) found that addition of organic matter to soil reduced bulk density and increased macroporosity. Increased loam and organic matter may be the main factor that caused higher increase rates of bulk density after saturation. Handreck and Black (2005, p.67) stated that smaller pores in growing media easily attract water and hold more firmly than larger pores due to 'capillary action'.

Moisture content varied between substrates, ranging from 2.06 % to 32.23 % (Table 3.5). FLL guideline (2004, p.50) recommended more than 20 % "water-storage capacity" of substrate as a minimum. This indicates that some of the substrates did not meet the minimum requirement, which were all ranges of LECA and Limestone 1 and 3 substrate, except for Limestone 3 with 70:20:10. The substrate with very low water holding capacity may inhibit successful plant growth. Moisture content tended to increase significantly as proportion of organic matter and loam content increased (Figure 3.8). The similar tendency was also observed in the study by Nagase (2008) on the relationship between the percentage of organic matter of substrate and moisture content in it. This result could be supported by Handreck and Black (2005, p.23), who stated that organic matter and loam improve soil structure and therefore make the soil highly efficient to absorb and retain higher amounts of water.

For plant growth, waterlogging makes the roots unable to get oxygen (Handreck and Black, 2005, p.79). Thus, one of the important considerations is that substrate should be free-draining to ensure air is available in the growing medium (Dunnett and Kingsbury, 2008, p.110). The result revealed that air filled porosity of all of the substrates ranged from 10.30 % in John Innes to 53.38 % in LECA with 100:0:0 composition rate, thus all substrates fall within the limits set out by FLL guidelines (2004, p.50), which is no less than 10 % at fully saturated condition. However, Dunnett and Kingsbury (2008, p.110)

stated that less than 15.0 % air-filled pore space of substrate in the long-term will result in poor plant growth. This suggests that substrates recorded with significantly lower than 15.0 % air filled porosity, which were Limestone 3 with 70:20:10 and 60:20:20 composition rate, and Limestone 2 with 80:0:20, 70:20:10, and 60:20:20 composition rate, may result in waterlogged substrate.

The finding of a significant negative association between bulk density at dry condition and increase of bulk density after saturation, bulk density at dry condition and air filled porosity, and air filled porosity and moisture content may indicate that particle size of materials composing of substrate influence the physical properties of it. The results align with Handreck and Black (2005) who stated "physical properties depend on the shape, size, and density of individual particles" (p. 137). In this study, high moisture content and low air filled porosity was nearly always associated with substrates containing relatively large proportion of smaller particles and conversely, low moisture content and high air filled porosity was associated with substrates containing large proportion of larger particles. According to Handreck and Black (2005, p.137) and Spomer (1983, p.77), this is because large pores formed by many large particles tend to give much air and facilitate drainage, while small pores formed by many small particles tend to hold water much firmly and impede drainage.

3.5.1.2 Chemical characteristics

The pH analysis of the substrates used in this study has revealed that all of the experimental substrates ranged from weak alkaline (pH 7.42 in LECA 70:20:10 substrate) to moderate alkaline (pH 8.33 in Limestone 1 90:0:10) in nature, which all fall within the limits (pH 6.0 to 8.5) outlined by FLL guidelines (2004, p.50). This suggests that all of the experimental substrates would be suitable to species originating from calcareous grassland in terms of pH. The pH was slightly reduced as amount of organic matter and loam content increased. Only 10 % addition of organic matter, however, did not make any significant change to pH. The similar result was also reported in the study by Molineux, et al. (2009), who found that pH was reduced by average of 2.71 units with 15 % and 25 % addition of organics to Red Brick, Clay

pellets, and Carbon 8 Pellets aggregate. This may be because of the chemical reaction of elements contained in organic matter. Handreck and Black (2005, p.20 and p.22) described that organic matter mainly contains carbon together with oxygen, hydrogen, nitrogen, and sulphur, thus addition of the organic matter increases the concentration of hydrogen ions in growing media, leading substrate to decrease pH.

The result showed a wide range of EC values between the experimental substrates, which were from 0.25 dS/m in Limestone 3 100:0:0 to 1.43 dS/m in Brick rubble B 60:20:20, which can be converted to 160.0 mg/L and 915.2 mg/L in TDS (total dissolved solids) (Handreck and Black, 2005, p.299). All of the substrates meet the limits outlined by FLL guidelines (2004, p.51), which is not to exceed 2.5 g/L for intensive greening and 3.5 g/L for extensive greening. The substrates also showed significantly lower EC values than the two commercial Zinco substrates, which were Zinco semi-extensive (1.90 dS/m) and Zinco sedum substrate (1.94 dS/m) (Table 3.10). High EC values were most frequently associated with Brick rubble substrates, and substrates with 80:0:20 and 60:20:20 composition rate (Figure 3.11 and 3.12). This is presumably because higher amounts of organic matter and brick rubble contain a higher concentration of elements, which contribute to salinity of water in the substrates. According to Handreck and Black (2005, p.298), the salinity of water in growing media depends on the amount of salts dissolved in it, which comes from the medium itself, from irrigation water and fertilisers. The water in growing media also contains ions from fertiliser salts and soil particles, which are mainly potassium, ammonium, nitrate, and phosphate, in addition to ions dissolved in natural water. The concentration of the main ions will depend on the amounts contributed by growing media components, and the amounts added in fertilisers.

3.5.2 Effect of substrate on seedling performance

Under the experimental conditions in this study, substrate component materials and the composition rate of the materials also had a significant influence on seedling emergence and growth, as well as physical and chemical properties of the substrates.

Table 3.17. Summary of performance of individual species in response to substrate.

	Seedling emergence			Seedling survival			Seedling growth		
	Leucanthemum vulgare	*Briza media*	*Prunella vulgaris*	*Leucanthemum vulgare*	*Briza media*	*Prunella vulgaris*	*Leucanthemum vulgare*	*Briza media*	*Prunella vulgaris*
L1 (100:0:0)	Low			High			Low		
L1 (90:0:10)	Low			High			Low		
L1 (80:0:20)	Medium	Low	Medium	High	Low	High	Low	Low	Medium
L1 (70:20:10)	Medium			High			Medium		
L1 (60:20:20)	High	Medium	High	High	High	High	High	High	High
L2 (100:0:0)	Medium			High			Low		
L2 (90:0:10)	High			High			Low		
L2 (80:0:20)	High	Medium	Medium	High	High	High	Low	Medium	Low
L2 (70:20:10)	High			High			Medium		
L2 (60:20:20)	High	High	High	High	High	High	High	High	High
L3 (100:0:0)	Medium			High			Low		
L3 (90:0:10)	High			High			Low		
L3 (80:0:20)	Medium	High	High	High	High	High	Low	Medium	Medium
L3 (70:20:10)	High			High			Low		
L3 (60:20:20)	High	High	High	High	High	High	Medium	High	High
LE (100:0:0)	Low			-			Low		
LE (90:0:10)	Low			High			Low		
LE (80:0:20)	Low			High			Low		
LE (70:20:10)	Low			High			High		
LE (60:20:20)	High			High			High		
Brick A 80:0:20		Medium	Low		High	High		Low	Low
Brick A 60:20:20		Low	Low		High	High		Low	Low
Brick B 80:0:20		Medium	Medium		High	High		Low	Low
Brick B 60:20:20		Medium	Low		High	High		Medium	Medium
Brick A+B 80:0:20		High	Medium		High	High		Medium	Low
Brick A+B 60:20:20		Medium	Medium		High	High		Low	Low
John Innes	Medium	High	High	High	High	High	Low	High	High
Zinco semi	Low	Medium	Medium	High	High	High	Low	Medium	High
Zinco sedum	Low	Low	Medium	High	High	High	Low	Low	Medium

For *Leucanthemum vulgare*; Emergence: High ≥5.0%, 5.0% > Medium ≥3.0%, Low < 3.0%, Survival: High ≥ 50.0 %, Low < 50 %, Dry weight: High ≥ 0.1 g, 0.1 g > Medium ≥ 0.05 g, Low < 0.05 g.
For *Briza media*; Emergence: High ≥ 70 %, 70 % > Medium ≥ 40 %, Low < 40%, Survival: High ≥ 50%, Low < 50 %, Dry weight: High ≥ 0.4 g, 0.4 g > Medium ≥ 0.15 g, Low < 0.15 g.
For *Prunella vulgaris*; Emergence: High ≥ 70 %, 70 % > Medium ≥ 40 %, Low < 40%, Survival: High ≥ 50%, Low < 50 %, Dry weight: High ≥ 0.9 g, 0.9 g > Medium ≥ 0.5 g, Low < 0.5 g.
L 1, L2, and L3: Limestone 1, 2, and 3substrate type; LE: LECA; Zinco semi: Zinco semi-intensive substrate; Zinco sedum: Zinco sedum substrate.

3.5.2.1 Seedling emergence

The result showed that *Leucanthemum vulgare* was recorded with very low germination rates (less than 10.0 %) across all of the substrates. This might indicate that the green house environmental conditions, temperature requirement especially, were not suitable for germination of the species. Grime et al. (2007, p.400) described that this species achieved 50 % of the maximum percentage germination attained at the temperature regime, ranging from 9 °C to 31 °C, the lower and upper limits, and that "in the Asteraceae, wide germination range is due mainly to the high germinability maintained at elevated temperature" (p. 51). In the study by Roberts (1986) of seed persistence in soil and seasonal emergence of 70 species from different habitat, it was found that *Leucanthemum vulgare* showed the highest germination in an April field sowing, while being very low in a January and February, and no emergence in a December field sowing.

From the results in terms of mean maximum percentage of emergence, three groups can be identified: substrate with low (< 3.0 %), medium (3.0 to 5.0 %), and high (≥ 5.0 %) for *Leucanthemum vulgare*, with low (< 40 %), medium (40.0 to 70.0 %), and high (≥ 70.0 %) emergence for both of *Briza media* and *Prunella vulgaris* (Table 3.17). Substrates that produced seedling emergence above medium range inclusive across the species were: Limestone substrates, excluding Limestone 1 with 100:0:0 and 90:0:10, and 80:0:20 for *Briza media*, Brick rubble substrates with 80:0:20, except for Brick rubble A for *Prunella vulgaris*, and LECA substrate with 60:20:20. In contrast, substrates that showed low seedling emergence were: all of the Brick rubble substrates with 60:20:20, LECA substrates excluding it with 60:20:20, and Limestone 1 substrate with 100:0:0 and 90:0:10. Generally, light, moisture, and temperature are considered as the most important environmental factors to control seed germination (Grime et al., 1981). Assuming the same environmental conditions in terms of light and temperature, this was presumably because of the different moisture content of the substrates due to the different physical properties of them. In addition to this, in the current study the findings of a significant positive association between seedling emergence of *Leucanthemum vulgare* and moisture content, and negative between seedling emergence of all of the species and air filled porosity, might indicate that the physical properties of

substrate are one of the important factors in seed germination. This might also suggest that under adequate water supplying environment, the species are sensitive to aeration availability of substrate at germination. This trend was frequently observed with low seedling emergence in substrates with large proportion of large particle size, which were Limestone 1, LECA, and Brick rubble A substrate type with the largest particle size ranging from 8 mm to 4 mm, and vice versa. This might be due to the coarseness of substrate, also affecting the physical properties of substrate. In the study by Fuller (1987) on seedling establishment of beach shingle species (*Festuca rubra* and *Silene maritima*) on shingle beaches, it was found that growing medium with greater proportion of fine shingle (smaller than 10 mm) in mixture showed significantly higher water retention and germination pattern than it did with greater proportion of coarse shingle (larger than 10 mm). Addition of humus also exhibited a similar pattern for water retention and germination rate.

Despite this, all of the Brick rubble substrates showed high moisture content and low emergence occurred in Brick rubble substrates with 60:20:20 composition, while high or medium emergence nearly always occurred in Brick rubble substrates with 80:0:20, with the exception of Brick rubble A substrate for *Prunella vulgaris*. The reason might be related to water transfer efficiency (which was not measured in the current study) rather than quantity of water, and soil compaction. Hitchmough et al. (2001) studied seedling performances of native forbs and grasses, and non-native forbs species in response to urban waste substrates, which were brick rubble, sand, and brick rubble with sand and subsoil with sand in 1 to 1 respectively. They discussed that "seed germination depends on soil moisture content but also on water flow at a micro-scale" (p. 303), on the basis of the result, which showed that in most species the greatest emergence tended to occur in sand with the greatest water transfer efficiency. Correlation analysis also revealed significant association between seedling emergence and water transfer efficiency for the substrates. In the case of Brick rubble substrate, a similar tendency was observed in the study by Hitchmough et al. (2001), which showed the highest water holding capacity, the lowest water transfer efficiency, and drastic reduction of surface permeability in brick rubble substrate, amongst substrates used in the study. They also discussed that:

"low water transfer efficiency and high resistance to root penetration

following repeated precipitation-irrigation events are factors that potentially restricted emergence of some species in brick rubble substrates" (p. 303).

Mullins (1991) described that cycles of drying and wetting of substrate, resulting from rainfall or irrigation, "allow aggregates to pack more closely together because of the ability of the smaller particles to fit into the spaces between larger particles" (p.96), consequently this might lead to increased soil resistance to root growth. In the current study, in terms of soil resistance to root penetration, it was observed that some seedlings often failed to penetrate, particularly in brick rubble substrates. This might be because that 20 % of brick rubble particles with smaller than 2.0 mm size included in the 60:20:20 composition rate accelerates the packing effect on the Brick rubble substrates, resulting in reduction of water transfer efficiency of the substrate and increase of resistance to root penetration.

3.5.2.2 Seedling survival

All of the substrates exhibited high seedling survival (more than 50% of maximum seedling emergence), except for Limestone 1 substrate with 80:0:20 composition rate for *Briza media*, presumably because of high water supply through recurrent irrigation. In the case of *Prunella vulgaris*, correlation analysis revealed significant associations between seedling survival and air filled porosity. Hitchmough et al. (2001) found that substrates that were more root penetrable and had higher moisture availability tended to have high seedling survival, in spite of the species origin from dry and infertile habitats, and that seedling survival for native forbs was significantly correlated with water transfer efficiency values of the substrates.

3.5.2.3 Seedling growth

As with the seedling emergence, substrates can be also classified into three groups, those which produced low seedling growth (< 0.05 g for *Leucanthemum vulgare*; < 0.15 g for *Briza media*; < 0.5 g for *Prunella vulgaris*), medium (0.05 to 0.1 g; 0.15 to 0.4 g;

0.5 to 0.9 g respectively), and high (≥ 0.1 g; ≥ 0.4 g; ≥ 0.9 g respectively) (Table 3.16). The results show that each species exhibited relatively different response to substrates. For *Leucanthemum vulgare*, seedling growth was more affected by composition rate, compared to the influence of substrate type. On the evidence of the experiment, no significant difference was shown in dry weight between the experimental substrates (Limestone 1, 2, and 3, and LECA substrate type) (Figure 3.17). Seedling growth increased with proportion of organic matter and loam content. Consequently in substrates with 60:20:20 composition rate, growth was greatest and poorest in substrates with 100:0:0 (Figure 3.18 and Table 3.16). This may be a result of increased nutrient availability and improved substrate structure as the organic matter and loam content increased, leading to adequate air filled porosity and moisture content. This result could be supported by a previous study. In the study by Nagase (2008) who carried out an experiment to investigate plant growth of 4 species (bulb, forb, grass, and shrub species) in response to the addition of organic matter to a substrate without organic matter. Organic matter was added in proportion of 10 %, 25 %, and 50 %, under two watering regimes, which were every 5 days for wet and every 15 days for dry regime. The addition of organic matter resulted in about 4%, 14%, 29%, and 54% in total respectively. The result revealed that in the wet regime, substrate that had a higher proportion of organic matter exhibited higher plant growth. According to the study by Birch (1958) on the effect of soil drying on humus decomposition and nitrogen availability, as organic matter is decomposed mainly due to a process of repeated cycles of air-drying and rewetting, essential elements for plant growth, such as nitrogen, phosphorus, and trace elements, are mineralised. Kendle and Sherman (2004) described that “any soil that is low in organic matter will exhibit nitrogen deficiency” (p.53).

Conversely, for *Briza media* and *Prunella vulgaris*, substrate type had much effect on seedling growth, although growth in substrates with 60:20:20 composition rate mostly tended to be higher than in substrates with 80:0:20, regardless of statistically significant responses. In Experiment 2 for *Leucanthemum vulgare*, substrate with 60:20:20 composition rate produced significantly higher growth than substrate with 80:0:20 did, while in Experiment 3 it did not do so for the other two species. For Experiment 3, this might be due to same proportion of organic matter was included in the substrates for the species, and indicate that loam content in the Limestone substrates does not have direct

influence on seedling growth for the species. In the case of brick rubble substrate, the two composition rates substantially had the same ratio of inorganic to organic content of the substrates because the 60:20:20 composition rate for the brick rubble substrates was a blend of organic matter and brick rubble particles less than 2.0 mm size to the aggregates, instead of loam component.

The result showed that Limestone substrates tended to produce higher seedling growth for the three species than brick rubble substrates, including the two Zinco commercial substrates. As with seedling emergence, this might be explained by soil compaction of the substrate for the species, leading to poor water use, restricted nutrient uptake, lack of oxygen, accumulation of carbon dioxide, or root impedance (Kendle and Sherman, 2004). Hitchmough et al. (2001) reported, "poor growth in the brick rubble substrates with seedlings often displaying reddish basal leaves indicative of nitrogen deficiency" (p. 304).

One of other potential reasons may be related to salinity of the substrates, although Spearman rank test did not reveal significant correlation between seedling growth and EC value. Nektarios et al. (2004) compared four different roof garden substrates and their impact on plant growth during one-year experimental period. The four substrates were: sandy loam soil (S), sandy loam soil with urea formaldehyde resin foam (S:F – 60%:40%), sandy loam soil with peat and perlite (S:P:Per – 50%:30%:20%), and peat with urea formaldehyde resin foam (P:F – 60%:40%). They found that P:F substrate could not support sufficient plant growth due to high salt accumulation, which was recorded with the highest EC value amongst the substrates, ranging from 2.18 dS/m for initial to 6.22 dS/m for final measurement. Handreck and Black (2005) described that "a low level of salinity from plant nutrients is essential to plant growth (p. 298), but as salinity increases, plant grow more slowly and are stunted more severely" (p. 302).

From the results, it was shown that overall, the Limestone 2 substrate type and 60:20:20 composition rate tended to be relatively well-balanced between moisture content and air filled porosity amongst the experimental substrates, and to have higher moisture content and pH value, but lower air filled porosity and EC values than the two Zinco commercial green roof substrates. Of the Limestone 2 substrate type, the substrate with

60:20:20 composition rate gave additional benefits to the emergence and initial growth of the species over the other experimental substrates, including the two Zinco commercial substrates. The Limestone 2 substrate with 60:20:20 composition rate supported higher seedling emergence and growth across all of the species, while the other substrates showed different responses of seedling emergence and growth for different species with tendency of exhibiting lower values compared to the Limestone substrate. This may indicate that the Limestone-based substrate could be more advantageous for various calcareous grassland species compared to the other substrate types, especially the two Zinco commercial substrates. This may be related to the habitat characteristics of the species itself. The result could be supported by the finding of Jefferies and Willis (1964), which was that successful plant establishment and growth of species tend to be closely related to soil conditions with similar characteristics to the soils of natural habitats which the species occurred.

Chapter 4. Plant Selections for calcareous grasslands on green roofs in the UK: Effect of substrate depth, irrigation, substrate type, and fertiliser on establishment and performance of plant community

4.1. Introduction

For many years, sedum green roofs have been broadly used by commercial green roof companies because of the ability that sedums and other succulent have, for high drought tolerance, efficient water use, and high survivorship under rooftop conditions. Moreover, they are easy to establish and perform well at shallow substrate depth (less than 50 mm) on green roofs (Nagase and Thuring, 2006; Durhman et al., 2007; Monterusso et al., 2005). Success of sedum green roofs implies that in order to recreate successful native habitats on green roofs, it is extremely important to select appropriate plants of native species that suit the green roof conditions. Although successful planting is positioned as the centre of successful green roofs, the majority of researches have dealt with environmental performances of green roof in terms of rainwater runoff, thermal efficiency, or mitigation of urban heat island effect. Even vegetation related-studies tend to focus on survival or growth of Sedums or other succulents. Although not exhaustive, existing guides suggest a wide range of potential plant lists for green roof systems (e.g. Dunnett and Kingsbury, 2008; Tan and Sia, 2008; Snodgrass and Snodgrass, 2006; Johnston and Newton, 1993). Furthermore, some of them are based on experience in different geographic or climatic regions. There has been virtually no tried and tested plant list for recreating specific native plant communities on green roof systems in the UK.

In general, the most considerable factors in plant selection for green roofs are the macro and microclimate, and the ability to tolerate growth in shallow substrate. In order for the plants chosen for a specific location to survive and thrive, they must withstand local climate extremes and harsh microclimate on roofs (Dunnett and Kingsbury, 2008, p.127; Getter and Rowe, 2006, p.1280; White and Snodgrass, 2003, p.2). One of advantages of using native plants for green roofs is that they are already evolved and adapted to the local climate and conditions over a long period of time, and have stress-tolerance

Table 4.1. General living condition on the roof (source: Dunnett and Kingsbury, 2008; Martin and Hinckley, 2007; Hitchmough, 2006; Köhler, 2004a; Boivin, et al., 2001; Johnston and Newton, 1993; Köhler, 1990)

Wind	Higher speed than at ground level Complexity: - swirls and eddies - Low speed in the central area of roof but high in the corner and edges; recommendation of use of heavy materials (ex. Gravels and slabs) - Desiccation of vegetation and substrate - Physical damage to plants	
Temperature	Air	- General temperature on the roof is higher than the ground due to no shelter from sun and the heat from interior of the building. - Higher in winter, and lower degree in summer
	Soil	- Fluctuation between very low and very high values in the thin substrate - lower and higher than that on the ground level in winter and summer
Moisture	Due to thin, free-draining substrate for growing media and increased exposure to sunny and windy condition - limitation of water retention; easy and quick to experience drought condition - rapid fluctuation between saturation and drought	

against the local climate extremes (Lundholm, 2006, p.88; Dunnett and Kingsbury, 2004, p.3). However, inner city roof environments have very different microclimates from the ground level as they are much more severe for plants growth (Table 4.1). Most of all, green roofs are more subject to drought condition due to the thin and free-draining substrate, high temperature, and exposure condition to sun and wind. Therefore, leaving aside the issues of climate, the first considerable factor is environmental severity of green roof systems, related to the depth of growing substrate and availability of irrigation and/or nutrients (Dunnett and Kingsbury, 2008, p.134; Getter and Rowe, 2006, p.1280).

A second factor is associated with the aesthetics of green roofs. Accessibility and visibility of the roof can be criteria to determine the appropriate characteristics of planting and substrate depth of the green roof systems (Dunnett and Kingsbury, 2008, p.136). As the degree of accessibility and/or visibility of roofs increases so does the degree of complexity of the vegetation that could have more aesthetical and seasonal interest (Table 4.2). Where roofs are accessible and/or visible, the visual appearance of the planting on the roofs is an important factor to provide aesthetically pleasing environment and benefits to human well-being (Lee and Koshimizu, 2007; Dunnett and

Table 4.2. The relationship between substrate depth and the visibility and accessibility of a roof in determining the appropriate character of planting. These are general indications only and assume minimal additional irrigation and temperate climate (Adapted from: Dunnett and Kingsbury, 2008, p.133)

	Accessibility/ visibility of the roof			
Depth	Inaccessible/invisible	Inaccessible/visible from a far distance	Inaccessible/visible from a close distance	Accessible
0-5 cm (0-2 in)	Simple sedum/moss communities	Simple sedum/moss communities	Simple sedum/moss communities	Simple sedum/moss communities
5-10 cm (2-4 in)		Dry meadow communities, low-growing drought-tolerant perennials, grasses and alpines, small bulbs	Dry meadow communities, low-growing drought-tolerant perennials, grasses and alpines, small bulbs	Dry meadow communities, low-growing drought-tolerant perennials, grasses and alpines, small bulbs
10-20 cm (4-8 in)			Semi-extensive mixtures of low to medium dry habitat perennials, grasses and annuals; small shrubs: lawn, turf, grass	Semi-extensive mixtures of low to medium dry habitat perennials, grasses and annuals; small shrubs: lawn, turf, grass
20-50cm (8-20 in)				Medium shrubs, edible plants, generalist perennials and grasses
50+ cm (20+ in)				Small deciduous trees and conifers

Kingsbury, 2008; Ulrich 1984 and 1979). A study by Dunnett (2006) regarding green roofs for biodiversity in Basel Switzerland, which have been greened by spontaneous colonisation of native species only, concluded that:

> "a uniform approach to supporting biodiversity on green roofs fails to take account of aesthetic factors and public preferences. Simple 'cues to care', and increasing the flowering component of vegetation to maximize colourful effects, can increase acceptance in visible and accessible locations." (p.5).

Jorgensen (2004, p.295) stated that urban planting could never be truly sustainable as a planting if the public cannot accept nature-like vegetation.

The structural characteristics of plants can have influence on the aesthetic appearance of

the vegetation layer of a green roof (Dunnett et al., 2008b; Dunnett and Kingsbury, 2008, p.130). Indeed, monocultural planting, such as simple sedum green roofs, can have less aesthetic value because it is dull and has a monotonous effect for much of the year due to similar flowering period, short display time, and a simple structural diversity (Dunnett and Kingsbury, 2008, p.148). In contrast, using mixtures of diverse species for the planting of a green roof can enhance the visual quality because the mixtures have a high possibility to have various forms, structures, and flowering times of individual species (Dunnett and Kingsbury, 2008, p.149). Thus, it is necessary to consider phenological study of species for using the mixtures. In a study by Dunnett (2004b), it is stated that:

> "the phenology of a species, i.e. its growth pattern through the growing season can be a crucial factor in creating compatible mixtures of species that have a long season of display" (p.109).

Moreover, using various structural characteristics of plants can have economic benefit to the installation of a green roof, as well as its visual quality. According to a study by White and Snodgrass (2003) it is stated that:

> "Rate of growth and ultimate shoot height are important plant selection criteria because the fewer plants needed to fill a given roof area the less expensive it costs to install the green roof" (p.171).

Therefore, it is important to be aware of growth characteristics of individual species such as plant coverage, height, phenological growth pattern and flowering season to create aesthetic and seasonal interesting green roofs (Nagase, 2008).

Although calcareous grassland vegetations are ideal candidates for green roof application due to their habitat characteristics, that they occur on, very thin, low nutrient and free draining soils, and have drought tolerance, in practice, not all the vegetations are likely to be suitable for green roof environment. Research into plant selection for native habitats and the dynamics of the species on green roofs has been relatively rare, and this has led to a lack of specific recommendations for native plant communities or assemblages on green roofs in the UK (Choi and Dunnett, 2008, p.2). It is, thus, important to screen and evaluate candidate plants under various environment stresses of

green roofs, related to substrate depths, different growing media or substrate composition, and availability of irrigation and/or nutrients.

4.1.1 Major impacts on plant establishment and performance in green roof

4.1.1.1 Substrate depth

Substrate depth is a major environmental stress to plant establishment and growth on green roofs. Many researches showed that substrate depth influenced survival, coverage, diversity, size, and flowering performance of plants on green roof systems. Deeper substrate depths tended to promote greater survival, growth and flowering performance than shallower depths (Getter and Rowe, 2008; Dunnett et al., 2008b; Durhman, et al., 2007; Dunnett and Nolan, 2004). However, according to individual species, the various patterns of its survival, growth and performance in response to different substrate depths were revealed. For instance, deeper substrate depths can make some species prolong the length of flowering display (Dunnett and Nolan, 2004). A study by Dunnett (2004a) showed that some species were not suitable for green roofs at deeper substrate depth compared with their growth at shallower depth. The research also revealed that some species, such as *Armeria maritima* 'Alba', *Eryngium bourgatii*, *Festuca scoparia*, and *Gaura lindheimeri*, during the initial 2 years growing season showed better survival at shallower substrate rather than deeper substrate. Researches carried out at Michigan State University also found that several species such as *Sedum sarmentosum* and *S. stefco* were possible to be grown where an extreme shallower depth was needed (Getter and Rowe, 2008; Durhman, et al., 2007). However, in a long-term research (Dunnett et al., 2008b) based on an experiment (Dunnett and Nolan, 2004) revealed that after the 2 years initial growing season, survival patterns of the species that had been better at the lower substrate depth tended to exhibit dramatic decline at the shallower depth compared to the deeper substrate depth. Some species have different cold tolerance depending on substrate depths. Shallower substrates give less survival chance to plants over winter because they are more subjective to low temperature and high fluctuation of temperature, thus plants in the shallower substrates are susceptible to more freezing injuries (Boivin et al., 2001). These researches can suggest what minimum depth of

substrate species should have for its successful establishment, growth and performance on green roofs.

4.1.1.2 Irrigation

Moisture availability is the most important factor to plant growth and performance on green roofs as in natural ecosystems. Ultimately, appropriate growing media and substrate depths for green roofs have a close relation to their water supplying availability to plant. For example, Rowe et al. (2006) reported that water availability was a more important factor than applying fertiliser in promoting the survival of natives; however, at low fertiliser level survival of the species increased. Hitchmough et al. (2001) found that seedling survival and establishment was positively associated with soil moisture content and water transfer efficiency values for substrates. VanWoert et al. (2005) also reported that deeper substrate depths had more moisture holding capacity than shallower depths. Modern green roof systems tend to aim for minimising regular or prolonged irrigation and feeding. However, it would not be feasible or practical to have no irregular or limited irrigation even in order to meet certain circumstances, for instance, green roofs for mainly aesthetical purposes, in unpredicted or particular dry periods, or reduction of fire hazard in very arid climates (Dunnett and Kingsbury, 2008, p.97). Supplementary irrigation could be beneficial to widening the range of potential native species, or to supporting diverse plant communities (Monterusso et al., 2005). Several studies showed that during the establishment period, especially in the first year, application of limited additional watering could benefit plant performance and the long-term persistence of plantings (Choi and Dunnett, 2008; Nagase and Thuring, 2006; Monterusso, et al., 2005; Dunnett and Nolan, 2004).

4.1.1.3 Substrate type

Plant establishment and performance in different kinds of substrates tend to exhibit different responses caused by physical and chemical characteristics of each substrate (Emilsson and Rolf, 2005; Hitchmough, et al., 2001). Nektarios et al. (2004) compared

four roof garden substrates including sandy loam soil (S), sandy loam soil amended formaldehyde resin foam in 60:40% (S: F), sandy loam soil amended with peat and perlite in 50:30:20% (S: P: Per), and peat amended with urea formaldehyde resin foam in 60:40% (P: F), to evaluate the effects of the substrates on plant growth of *Lantana camara* species. The result showed that S and S: F exhibited a faster shoot elongation rate, higher production of shoot and flowering number compared to the other substrates. However, in flowering performance, S: P: Per substrate indicated different flowering production rate increasing in August and November with S and S: F substrate increasing in June. This research suggested that although both the sandy loam soil and S: F substrate were the best for plant growth of *Lantana camara*, S: F substrate can be more desirable than the sandy loam soil due to its lighter weight. Furthermore, successful plant establishment and growth of species tend to be closely related to soil conditions with similar characteristics to the soils of natural habitats which the species occurred (Jefferies and Willis, 1964). Tansley (1965) stated that some species, which are so-called "exclusive species" (p.533), for example, *Campanula glomerata* and *Helianthemum nummularium*, are "confined or nearly confined to chalk grassland" (p.533). In the study by Tansley (1917), who carried out an experiment to evaluate the competition between *Galium saxatile* (calcifuge species) and *G. sylvestre* (calcicole species) on a calcareous soil, a non-calcareous and clayey reddish yellow garden loam, a strong acid peat, and a natural sandy loam, the results showed that *G. sylvestre* germinated mostly and grew vigorously on calcareous soil, while *G. saxatile* exhibited the opposite tendency.

4.1.1.4 Fertiliser

As noted in Chapter 3, growing substrates for green roof systems should contain low levels of organic matter because of decomposition of organics, resulting in substrate shrinkage, and discharge of nutrients into the storm water runoff (Friedrich, 2005, p.5). Thereby, vegetations on such green roof substrates could be subject to deficiency of available nutrient. The FLL guide recommended that fertiliser should be applied with 5g N/m^2 for extensive greening sites during the first years, by use of NPK slow-release fertiliser capsules (FLL, 2004, p.59). Several studies showed that in the case of some

species such as sedum species, a minimal amount of fertiliser is necessary to improve plant survival and growth, especially during the establishment period, and to maintain healthy plant growth (Retzlaff et al., 2009; Rowe et al., 2006; Emilsson, 2004; Kircher, 2004). A study by Turkington et al. (1998) showed that application of fertiliser could also increase overall growth of components of plant community in abundance. Rowe et al. (2006) found that some native herbaceous perennial species showed differing responses to fertiliser application. Three US native taxa, *Aster laevis* L. *Koeleria macrantha* Regel, and *Solidago speciosa* L., which were not fertilised, survived in greater number, but produced the least amount of growth. The nutrient-deficient characteristics of calcareous soils are one of the main factors which contribute to calcareous grassland to be species-rich (Rorison, 1990). However, in the study by Buckland and Grime (2000), who examined the effect of trophic structure and soil fertility (low, moderate, and high levels) on the development of plant communities comprised 16 native grasses and 32 native forbs of fertile and infertile habitats, the result showed that soils of moderate fertility promoted frequency of flowering and shoot biomass compared to those of low fertility, and had statistically the same species richness and number of individual plants to low soil fertility showing the highest values at both of the species richness and the number of individual plants among the fertility levels. Kirkham at al. (2008) also found that inorganic fertiliser applications had less detrimental effects on species richness of plant communities (MG3 and MG5 unimproved and semi-improved meadows) compared to organic fertiliser applications. The plant communities with the inorganic fertiliser application showed a higher species richness than that of the plant communities with the non-fertiliser application. However, excessive use of fertiliser or conventional fertilisers can increase the risk of the nutrient runoff as the high proportion of organics in growing substrate, or can change vegetation composition as more competitive species increase in abundance (Retzlaff et al., 2009; Kirkham et al., 2008; Emilsson et al., 2007; Turkington et al., 1998).

4.2 Research aim, objectives and questions

The aim of the experiment described in this chapter was to investigate the environmental tolerances of a range of species typical of dry, free draining calcareous

grasslands, using a standardised plant-screening methodology, and to explore patterns of plant growth and flowering performance at the community and individual species level. Specific objectives and questions of the study described in this chapter were as follows:

4.2.1 Research objectives

i) To investigate suitable individual native species of calcareous grassland for use on green roof in the UK.
ii) To investigate key factors that can have an effect on plant establishment and growth success.
iii) To investigate whether specific calcareous substrates were a requirement for establishment of calcareous grassland species.
iv) To investigate changes of plant growth and flowering performance at different environmental aspects (substrate depths, irrigation and fertiliser regimes, and substrate types) over time.

An important aspect of the work was to determine which species were able to tolerate the highly stressful conditions typical of many extensive green roofs, and to determine optimal design criteria for successful establishment and performance of calcareous grassland species.

4.2.2 Research questions

i) What depth of substrate can support successful establish, growth and performance of the plant community with and without supplementary irrigation?
ii) Is supplementary irrigation necessary in order for the plant community to establish successfully?
iii) Does fertiliser application or limestone-based substrate influence plant community development?
iv) How does plant community perform in different substrate depths, irrigation regimes, substrate types, and fertiliser treatment play over time?

4.3 Materials and Methods

This study included the following variables that were used to test environmental tolerances: irrigation regime (without and with supplemental watering to test drought tolerance), substrate depth (50mm, 100mm, and 200mm, to test tolerance of thin substrate depths), substrate type (Limestone-based substrate and a Zinco commercial green roof substrate, to test whether a calcareous substrate was necessary for successful growth), and nutrient regime (to test growth without additional nutrients).

The experiment was set up in spring of 2007 on a flat rooftop of a nine-story Sheffield University building in the city centre of Sheffield UK (52˚22'N, 1˚29'W, sea level; 120m). This site was open, surrounded by a parapet of 1200 mm height on the three sides, with an additional tenth storey on the other one side (Figure 4.1.). Monthly temperature (°C) and total rainfall (mm) in 2007 and 2008 the experiment were undertaken and the 30-year average (1971 to 2000) in Sheffield is given in Figure.4.2. According to climatic information given in Hitchmough et al. (2001) and Dunnett (2004a), Sheffield is relatively cooler in summer but also warmer in winter than other cities in continental Europe, with regular rainfall spreading over the year, although overall rainfall was lower. Temperature in Sheffield over the 2-year period of experiment was similar to the 30-year average with relatively warmer winter and spring, and cooler summer in 2007.

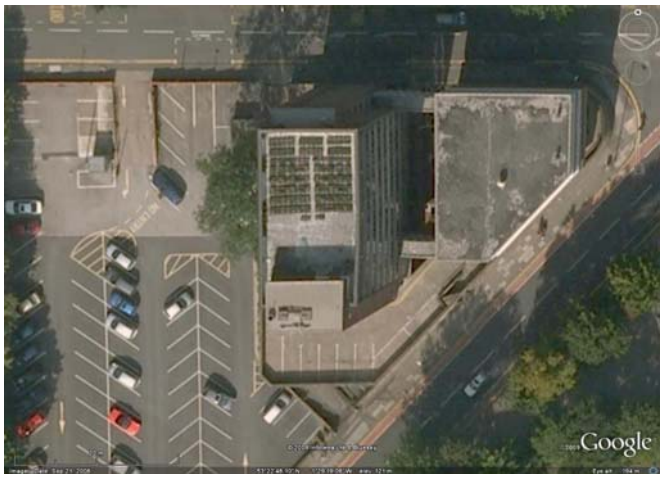


Figure 4.1. The experimental site (Source from: Google Earth).

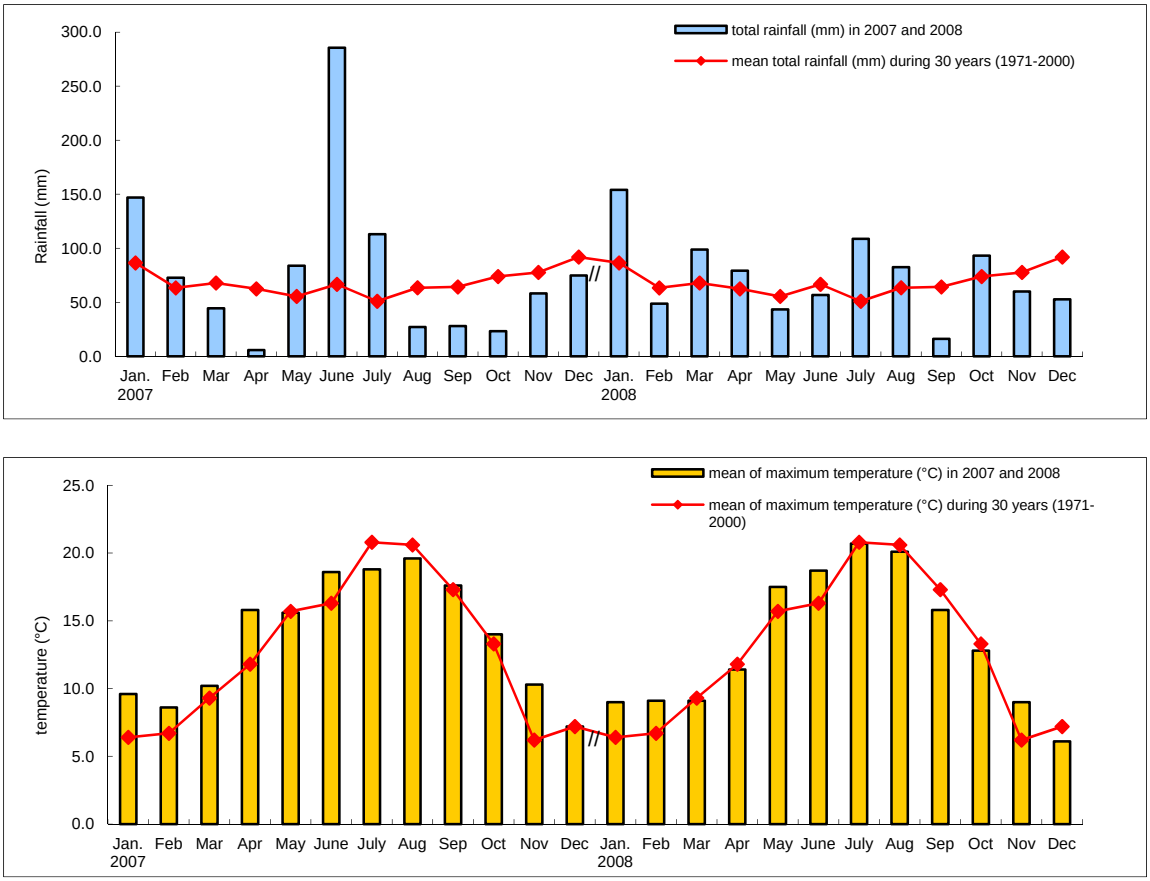


Figure 4.2. Monthly temperature and rainfall from 2007 to 2008 with the 30 year average (Source from: UK Meteorological office).

However, compared to the 30-year average rainfall, Sheffield in 2007 was extremely wet in early summer and drier during late summer to autumn. In the second year, it was relatively wetter until August and drier during the rest months with an extremely dry September.

The Zinco commercial substrate (based on crushed recycled brick and organic content) manufactured by Zinco (a German green roof company) for green roof systems, and a limestone-based substrate were used as growing media. The Zinco substrate was a mixture of their commercial 'Sedum carpet' and 'Semi-extensive' substrate in a 1 to 1 ratio. This is because the sedum carpet substrate contains little organic matter and would not be suitable for the species used in the experiment, while the semi-extensive contains too much. The product data of the two substrates are summarised in Table 4.3.

Table 4.3. Product data of the sedum carpet and roof garden substrate used for Zinco substrate*.

	Sedum carpet substrate	Semi-extensive substrate (Heather with lavender)
Granules of < 0.063mmØ	≤ 7 %	≤ 15 %
Granules of < 4mmØ	≥ 25 %	
Organic content	< 40 g/l	< 90 g/l
Salt content	< 1.5 g/l	< 1.5 g/l
Porosity	63 %	64 %
pH value	6.5 – 8.0	6.5 – 8.0
Dry weight	1120 kg/m^3	1000 kg/m^3
Saturated weight	1400 kg/m^3	1500 kg/m^3
Maximum water capacity	30 %	50 %
Air content at saturation	38 %	22 %
Water permeability	≥ 0.1 cm/s	≥ 0.064 cm/s

* Source from: Alumasc product data sheet (2011 and 2006).

Table 4.4. Physical and chemical properties of the mixed substrate of the two Zinco substrates (Zinco) and limestone-based substrates (Limestone).

	Bulk density at dry condition	Bulk density after saturation	Increase in bulk density (%)	Air filled porosity (%)	Water holding capacity [1] (%)	pH	EC (dS/m)
Zinco	1.02Aa	1.26Ab	23.47	33.52A	26.6A	7.79A	1.90A
Lime	1.43Ba	1.80Bb	26.20	12.00B	29.8A	8.03B	0.51B

[1] data from measurement on 30th Aug. 2007 (Mean temperature (temp.): 15.4˚C; maximum temp.: 19.6˚C; minimum temp.: 12.7˚C; source from: Sheffield weather page), after 2 days of watering to the both substrates

*Different capital letters indicate significant difference at p=0.05 (Mann-Whitney U-test) between substrate types for the same property. Different lower-case letters indicate significant at p=0.05 (Mann-Whitney U-test) between dry and saturated particle density for the same substrate.

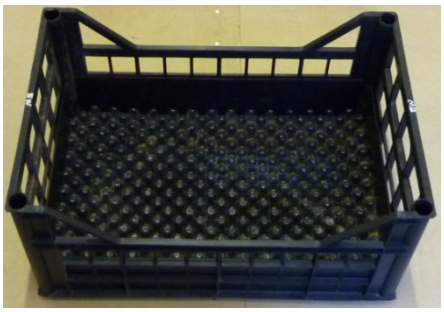
Zinco substrate was used for all treatments with the exception of the limestone substrate treatment. The limestone-based substrate consisted of 60 % limestone (less than 3.35 mm particle size), 20 % loam and 20 % organic matter. Green waste compost used as organic matter was obtained from Heely city farm in Sheffield. Physical and chemical analyses for the both substrates used in the study are given in Table 4.4.

Rigid plastic open sided stacking trays were filled with 50 mm, 100 mm and 200 mm of growing media for the substrate depth treatment, and 100 mm for other treatments. The build-up of a tray with 600 mm X 400 mm X 240 mm in outer size consisted of a commercial green roof drainage layer of 25 mm depth (Zinco Floradrain® FD25-E) on

the bottom, which is a lightweight and manufactured from thermoformed recycled polyethylene (Alumasc exterior building product, 2006), geotextile membrane over this as a filter layer preventing small particles from the growing media, which may cause obstruction of drainage layer, and plastic sheets as walls, which were placed around the edges to contain the growing medium, with 100 mm height for 50 mm substrate depth, of 150 mm height for 100 mm depth, and 240 mm height for 200 mm depth, (Figures 4.3 and 4.4). However, water could escape at the side when the substrate is fully saturated because gaps occurring between plastic sheets and a tray could not be completely sealed.

1. Empty tray

2. Tray with plastic sheets



3. Drainage layer

4. Filter layer (geotextile)

5. A completed tray with growing medium

Figure 4.3. Overview of order of building up a tray.

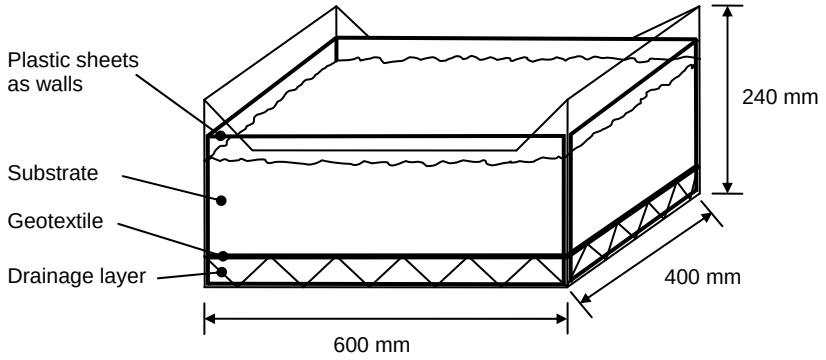


Figure 4.4. Components of a tray.

107 trays were used in total for the experiment and allocated as shown in figure 4.5. The experiment was divided into four main treatment blocks by 600 mm wide cross paths, and there was a 400 mm wide walkway between every two rows of trays to enable measurement of plants. Each row consisted of eight trays. Within each treatment block, trays with each treatment were randomly allocated to the same row. Substrate depth treatments without supplementary irrigation were separated by the paths from the other treatments to avoid any influence of watering. The treatments without supplemental irrigation received no additional watering over the 2-year period of the experiment, relying only on natural rainfall. Before starting irrigation regime, during two weeks after planting (from 22nd May 2007 to 4th June 2007), however, all treatments had four times the supplemental watering for initial establishment. Prior to watering, moisture contents of each treatment with supplemental irrigation were measured using a moisture meter (HH2 moisture meter and SM200 moisture sensor, Delta-T Devices, Cambridge, England). All the treatments were measured at the same time for the moisture contents when rainfall had not been significantly recorded and any dried surface of the substrates was observed. Nine trays per each depth with watering treatment and six trays each in the other two treatments (substrate type treatment and fertiliser treatment) were randomly chosen and measured from at least four points from each tray. In a study by Oomes and Elberse (1976), they found that in the field three ranges of soil water contents have been frequently observed, which were 23-28 %, 15-21 %, or 8-12 %. Hereby, for assuming minimum irrigation in the current study, only when the mean value of moisture content of the substrate showed less than 12% inclusive, supplemental

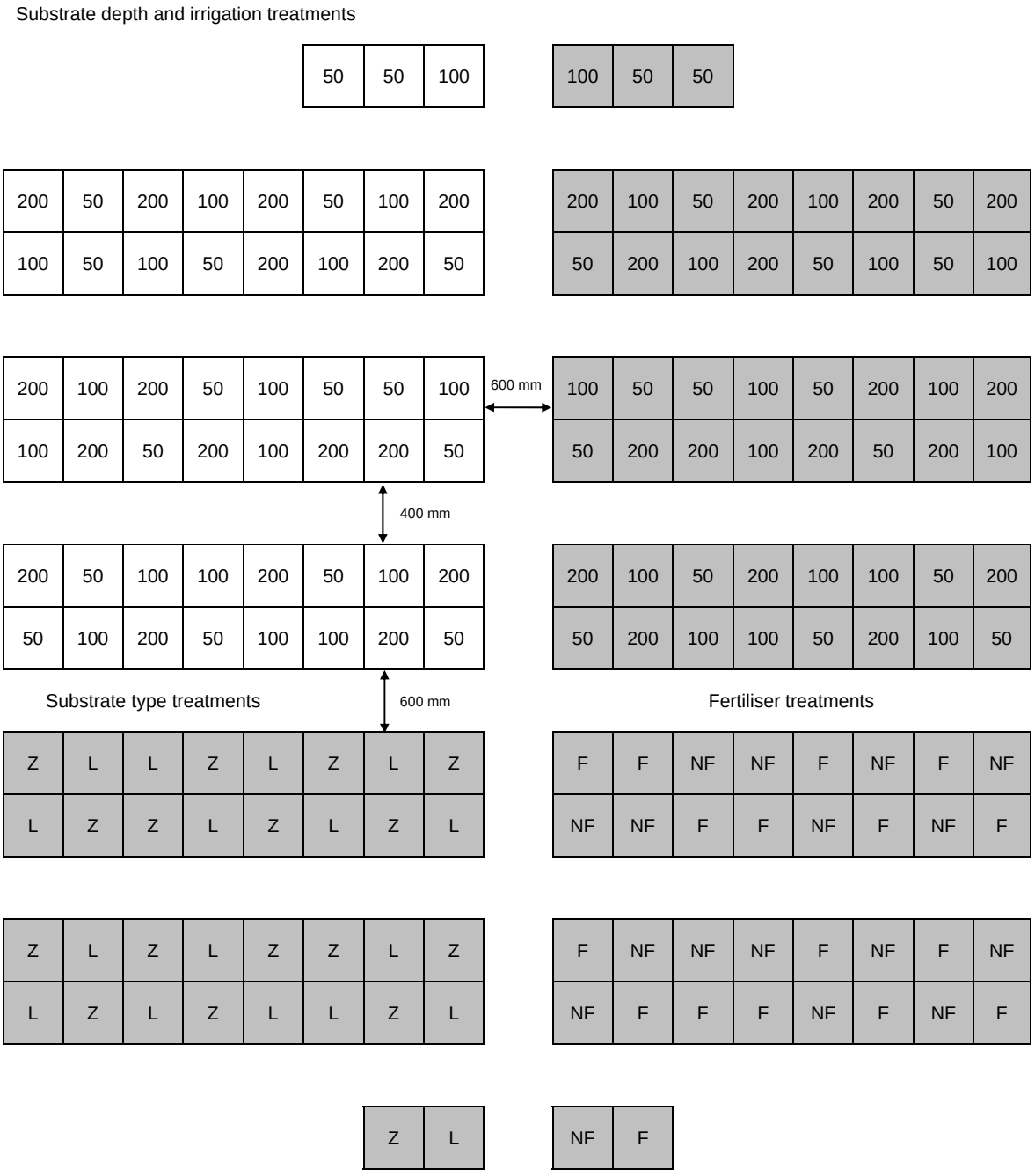


Figure 4.5. The arrangement of trays for each treatment. 50, 50 mm substrate depth; 100, 100 mm depth; 200, 200mm depth; Z, Zinco substrate treatment; L, Limestone-based substrate treatment; NF, Non-fertilised treatment; F, Fertilised treatment; ☐, Non-irrigated treatment; ▭, Irrigated treatment.

watering was applied by using a handheld hose with a fine spray. The trays were watered until the substrates had been fully saturated and the water from the trays was drained off. Figure 4.6 shows the mean of moisture content of substrate and

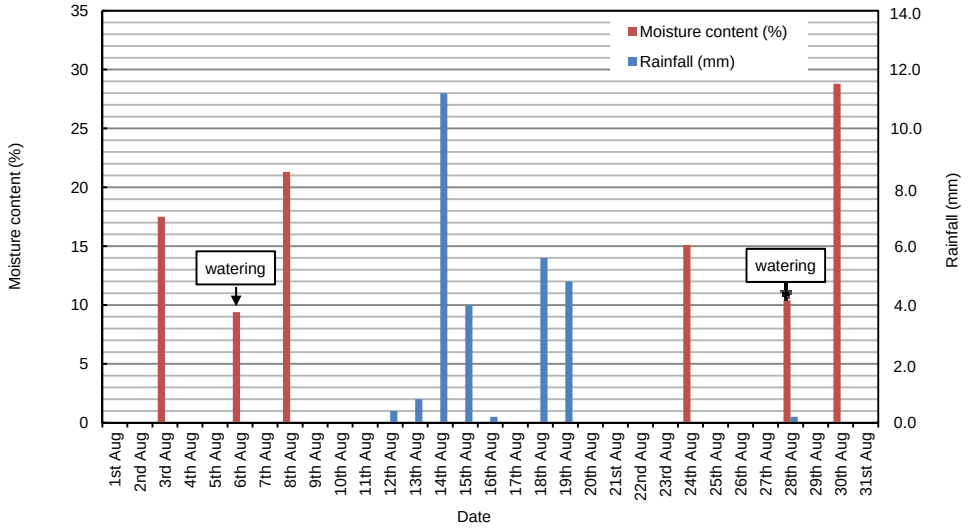


Figure 4.6. Mean moisture content (%) in the 100 mm substrate depth treatment and daily rainfall in Sheffield over August 2007 (Source from: Sheffield weather page).

supplemental watering applications in the 100 mm substrate depth treatment and the daily rainfall amounts over August 2007, the driest period. From 5th June 2007 to 30th September 2007, the supplemental irrigations were carried out on different occasions for each treatment; eight occasions for 50 mm substrate depth, seven for fertiliser treatment, six for 100 mm, 200 mm, non-fertiliser, and Zinco substrate treatment, and five for Limestone-based substrate (Ref. Appendix 8). For the second growing season, one occasion of irrigation was applied to all treatment with supplemental irrigation in May 2008.

Seventeen forbs species originating from calcareous grassland habitats were employed for these experiments: *Achillea millefolium, Agrimonia eupatoria, Campanula glomerata, Clinopodium vulgare, Galium verum, Helianthemum nummularium, Hypochaeris radicata, Knautia arvensis, Leontodon autumnalis, Leucanthemum vulgare, Linaria vulgaris, Origanum vulgare, Pilosella aurantiaca, Pilosella officinarum, Primula veris,* and *Scabiosa columbaria*. These forb species were selected on the basis of their origin from dry habitats and its high possibility of adaption to green roof environment as it is drought tolerant, have shallow roots and low growing forms. The characteristics of the species used in the experiment are summarised in Table 4.5. Each tray was planted with nine individuals and one individual of each species was allocated

Table 4.5. Ecology and characteristics of species used in this study (Adapted from; Blamey et al., 2003; Brickell, 2003; Grime et al., 2007)

Species	Geographical distribution	Habitat	Established strategy [1]	Height [2] (mm)	Flowering season	Initial size of plug plant (Height x Width; mm)[3]
Achillea millefolium	Britain, Europe, Asia, introduced in N. America, Australia and New Zealand	Dry grassland and waste places	C-S-R	Short/medium (100 to 600), erect and little-branched to 800	June to Aug.	34 × 39
Agrimonia eupatoria	Britain, Europe, and Asia.	Grassy habitat	C-S-R	Medium (300-600) to 1000	June to Aug.	73 × 79
Campanula glomerata	Britain, Europe (particularly in S. Europe), Turkey, and Asia.	Grassland on lime	Stress-tolerator	Low/short(less than 100 to 300) to 800	June to Oct.	8 × 29
Clinopodium vulgare	Britain, Europe	Dry grassy and bushy habitats, especially on lime	Intermediate between stress-tolerant competitor and C-S-R	Short/medium (100 to 600) to 750	July to Sept.	39 ×45
Galium verum	Britain, Europe, New Zealand, and N. America	Dry grassland on lime and open rocky habitats	Intermediate between stress-tolerant competitor and C-S-R	Often sprawling short/medium (100 to 600) to 1000	July and Aug.	33 × 40
Helianthemum nummularium	Britain (the S and SE of England), Europe except the extreme Northern part	Grassland on chalk and limestone, on acid soils in Scotland.	Stress-tolerator	Stems prostrate, to 500	June and July	24 × 42
Hypochaeris radicata	Britain, Europe except the NE region, and temperate zone	Short turf or sparsely grassy habitat	C-S-R	Short/medium (100 to 600)	June to Sept.	23 × 48
Knautia arvensis	Britain, Europe, Caucasus, Iran to D. Asia, and Russia (Siberia)	Limestone grassland, scrub, woodland, rough grassy and waste place.	C-S-R	Medium/tall (300 to over 600) to 1000	July to Sept.	25 × 58
Leontodon autumnalis	Britain, Europe, N. and W. Asia, NW Africa, and N. American	Short turf or sparsely grassy habitat, and damp grassland sometimes	Intermediate between ruderal and C-S-R	Short (100 to 300) to 600	June to Oct.	28 × 54
Leucanthemum vulgare	Britain, Europe to N. Scandinavia, temperate Asia and various region with cultivation	Rocky alpine slopes, moist – meadows, grassland, and waste land	Intermediate between competitive-ruderal and C-S-R	Medium (300 to 600) to 750	June and July	44 × 77
Linaria vulgaris	Britain, Europe except for the extreme N, the Mediterranean region, W. Asia, and N. America	Dry grassy habitat, wayside, waste place	Intermediate between competitive-ruderal and C-S-R	Short/medium (100 to 600) to 800	July to Oct.	40 × 29
Lotus corniculatus	Britain, Europe except for the extreme N, Asia, N. and E. Africa	Dry and rocky habitats	Intermediate between stress-tolerator and C-S-R	Prostrate or short/medium (100 to 600)	June and July	33 × 68

(*Continued next page*.)

Table 4.5. (*Continued*)

Species	Geographical distribution	Habitat	Established strategy [1]	Height [2] (mm)	Flowering season	Initial size of plug plant (Height x Width; mm) [3]
Origanum vulgare	Britain, Europe, N. and W. Asia, and N. America	Dry grassland on lime	Intermediate between stress-tolerant competitor and C-S-R	Short/medium downy (100 to 600)	July to Sept.	34 × 40
Pilosella aurantiaca	Britain, Europe, Asia, and North Africa.	Grassy habitats	Intermediate between ruderal and C-S-R	Short/medium (100 to 600)	June to Sept.	17 × 47
Pilosella officinarum	Britain (except Shetland), temperate and sub-Arctic Europe, W. Asia, an N. America	Dry grassy and heathy habitats	Intermediate between stress-tolerator and C-S-R	Low/short (less than 100 to 300)	May to Aug.	14 × 46
Primula veris	Britain, Europe (except the extreme Northern part and much of Mediterranean), and temperate Asia	Grassland on chalk, lime or limy clay	Intermediate between stress-tolerator and C-S-R	Low/medium (less than 100 to 600)	April and May	17 × 34
Scabiosa columbaria	Britain (except most of Scotland), Europe (except the extreme Northern part and Ireland), W. Asia, and N. Africa.	Dry meadows and rocky habitat	Intermediate between stress-tolerator and stress-tolerant ruderal	To 700	July and Aug.	17 × 55

[1] Data on established strategy from Grime *et al* (2007)
[2] Maximum typical height of the plant
[3] Mean height and width of plug plant measured in 25th May 2007

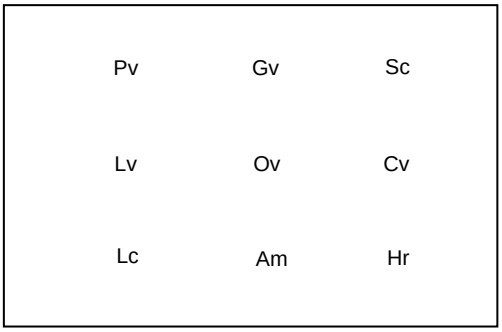


Figure 4.7. The arrangement of actual planting in a tray of the limestone-based substrate treatment. The species are: Am, *Achillea millefolium*; Cv, *Clinopodium vulgare*; Gv, *Galium verum*; Hr, *Hypochaeris radicata*; Lc, *Lotus corniculatus*; Lv, *Leucanthemum vulgare*; Ov, *Origanum vulgare*; Pv, *Primula veris*; Sc, *Scabiosa columbaria*.

to each tray, resulting in nine individuals of each species per treatment (Figure 4.7). Each species occurred in nine trays, resulting in a total of nine individuals for replication. All the plants were plug grown and were obtained in May 2007 from Really Wildflowers (Dorset, UK). The initial size (height × width) of plug plant is shown in Table 4.5. Planting was completed on 22nd May 2007. For the fertiliser treatment,

Osmocote ®Exact® standard, a slow release fertiliser (NPK fertiliser containing Magnesium with trace elements; N-P-K ratio, 15-9-9), was only applied in 22nd June 2007 after planting at a rate of 96g per tray (4-5 kg/m^3).

The height, number of leaves, shoots or stems, inflorescences, and coverage of each individual plant were recorded monthly from June to October 2007. From April to May in 2008, one measurement of the above characteristics of each individual plant was taken again. Any spontaneous species were identified and the numbers were recorded. After recording, these species were removed at this time. At the end of October 2007, all plants above ground were harvested and again at the end of October 2008, sorted by species and tray number where the individual was, and dried to provide dry-weight.

Statistical analysis

An Anderson-Darling test was undertaken to test whether a set of data follows a normal distribution or not. Where the test indicated that data was substantially non-normal, and this could not be adequately improved by transformation, analysis was undertaken using non-parametric statistics. The Mann-Whitney *U*-test was used in lieu of *t*-test for paired comparisons and Kruskal-Wallis tests for one way ANOVA. Statistical tests were undertaken using MINTAP Release 14.

4.4 Results

4.4.1 Abundance of the plant community

The total number of shoots in each tray was counted monthly over the 2 years of the experiments. The total number of survived shoots of each tray was converted to percentages as a percentage of the original number in each tray in June 2007. For statistical analysis, the mean total number of survived shoots per tray was assessed to investigate differences between original numbers of shoots in June 2007 and each month for the same environmental factor and between same environmental factors (substrate depths, substrate types, and fertiliser applications) for the same month using Kruskal-Wallis test and after that Mann-Whitney *U*-test were carried out to find where differences were (Dytham, 2003).

4.4.1.1. Effect of substrate depth and supplementary watering

For the two watering regimes comparison in the same substrate depths, significant differences were revealed at all substrate depths. The watered substrate depths were significantly higher in abundance [Figure 4.8 (a)]. In the non-watering treatment, the mean total percentage of survived shoots per month was significantly the highest at 200 mm substrate depth and the lowest at 50 mm substrate depth. Abundance at 100 mm depth was intermediate, and statistically different from both 50 mm and 200 mm depth. In the watering treatment, the 100 mm and 200 mm deep substrate had significantly higher abundance than 50 mm deep substrate. However, there was no significant difference between 100 mm and 200 mm depth.

Figures 4.8 (b) and (c) show the mean total percentage of survived shoots per tray at 50 mm, 100 mm, and 200 mm substrate depth in non-watering and watering treatment over the 2-years of the experiment, and shows that there were significant differences in abundance between months and substrate depths. Under the non-watering condition [Figure 4.8 (b)], abundance at 100 mm and 200 mm substrate depths had a significantly increasing pattern by September 2007, and then a declining pattern back to original

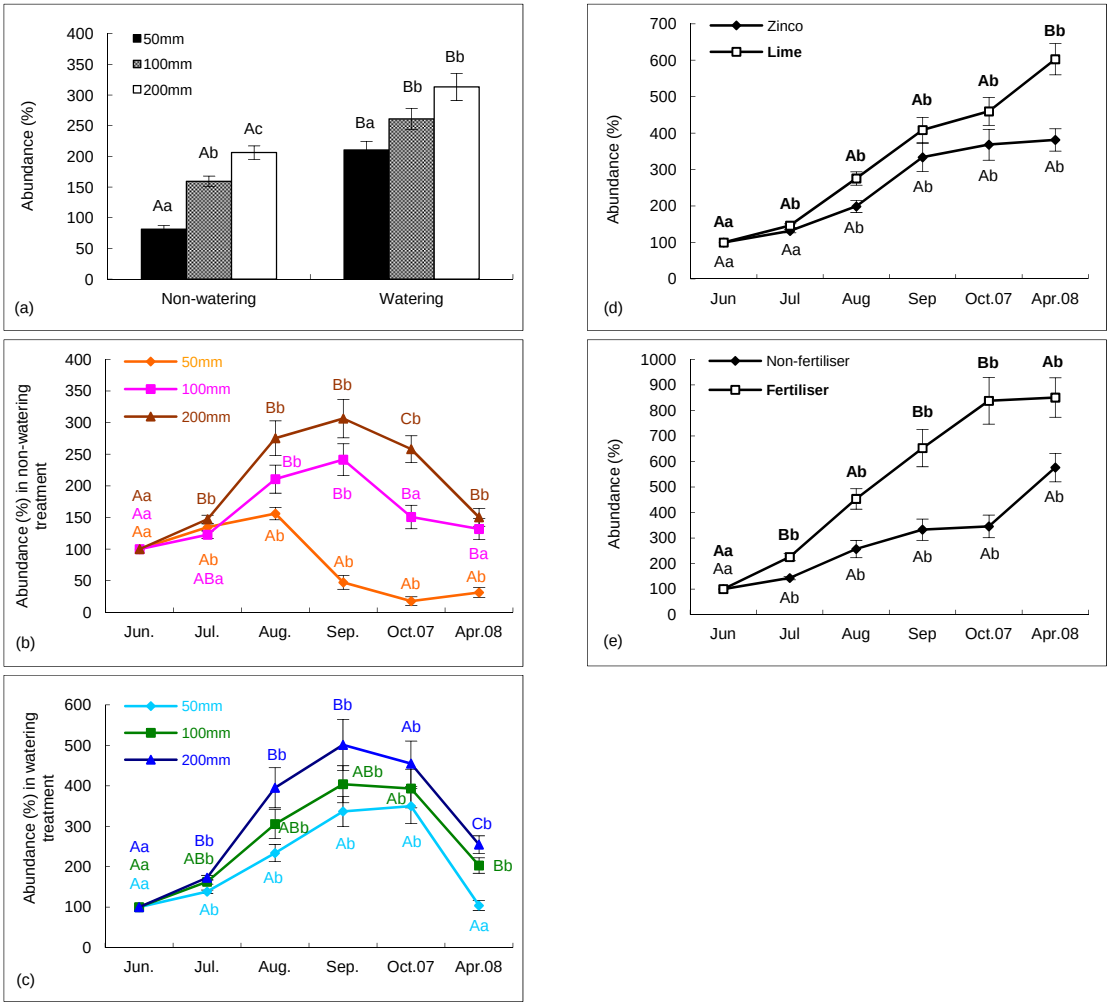


Figure 4.8. Mean total abundance (%) of shoot per tray (n = 17) in response to substrate depth; in non-watering and non-watering treatment across the period (a); in non-watering treatment (b); in watering treatment (c); substrate types (d), and fertiliser application (e).
For figure (a), different capital letters indicate significant difference between watering treatments for the same substrate depth. Different lower-case letters indicate significant difference between substrate depths for the same watering treatment.
For figures (b), (c), (d), and (e), different capital letters indicate significant difference between substrate depths, substrate types, and fertiliser applications for the same month. Different lower-case letters indicate significant difference between the initial survival in June 2007 and each month for the same substrate depth, substrate type, and fertiliser application. Error bars represent standard error.

numbers, whereas it had a significant decreasing pattern below the original number after August 2007 at 50 mm substrate depth. In April 2008 it was significantly higher ($P < 0.05$) for 200 mm substrate depth, and statistically the same ($P = 0.5969$) for 100 mm substrate depth although it increased in numbers compared to their original numbers, whilst at 50 mm depth it significantly decreased ($P < 0.001$) to less than 50 % of the original numbers. Abundance at 100 mm and 200 mm substrate depth in each month, apart from June, and July 2007 for 100 mm depth, was significantly higher than those at

50 mm substrate depth, however there were not significant differences in abundance between 100 mm and 200 mm depth except for October, 2007. Abundance was significantly greater at 100 mm and 200 mm depth in September 2007 and on 50 mm depth in August 2007.

In contrast with the abundance with the non-watering treatment, under the watering treatment, all substrate depths had similar abundance patterns over the 2-year period. Abundance of shoots significantly increased by September at 100 mm and 200 mm depth, October 2007 at 50 mm depth compared to their original numbers, and then it decreased back to their original numbers. In April 2008, abundance was significantly higher at 100 mm and 200 mm than their original numbers, or maintenance of original numbers at 50 mm substrate depth. Abundance was significantly greater on 200 mm substrate depth in each month except for June and October 2007 than on 50mm depth, with 100 mm substrate depth intermediate.

4.4.1.2 Effect of substrate type

The mean total percentage of survived shoots per tray in the Zinco commercial substrate ($P < 0.001$) and limestone-based substrate ($P < 0.001$) showed dramatically increasing patterns every month by the second year compared to their original numbers, with the exception of the Zinco commercial substrate in July 2007, as shown in figure 4.8 (d). However, there were not significant differences in abundance between the two substrates in the first year, except in the second year ($P = 0.0494$).

4.4.1.3 Effect of fertiliser application

Figure 4.8 (e) shows that abundance of shoots increased significantly in numbers for non-fertiliser ($P < 0.001$) and fertiliser treatment ($P < 0.001$) as compared to their original numbers every month over the 2 year period. Abundance was also significantly higher on the fertiliser treatment over the period except for August 2007 ($P = 0.0790$) and April 2008 ($P = 0.1018$).

4.4.2 Growth of the plant community

For assessing growth of the plant community, dry weights of all shoots were sorted by tray numbers to get the total dry weight (g) of shoots of each tray. In order to cancel out variations in the initial size of planting stock, the total dry weight (g) of shoots of each tray was converted to percentage as a percentage of the mean total dry weight of shoots per tray in the 100 mm depth in 2007 [Figure 4.9 (a)] and in 2008 [Figure 4.9 (b)], and for changes in biomass between 2007 and 2008, as a percentage of its total dry weight in 2007 [figure 4.9. (c)], and for the dry weight for the substrate type and fertiliser treatments in 2007 and 2008, as a percentage of the mean total dry weight of shoots per tray in Zinco substrate in 2007 [Figure 4.10 (a)] and in non-fertiliser treatment in 2007 [Figure 4.10 (b)] respectively. For statistical analysis, Kruskal-Wallis test was used to investigate the significant differences between the data set and after that Mann-Whitney *U*-test was carried out to find where the differences were (Dytham, 2003).

4.4.2.1 Effect of substrate depth and supplementary watering

There were significant differences in the mean total percentage of dry weight of shoots per tray between substrate depths and the two watering regimes in both growing seasons

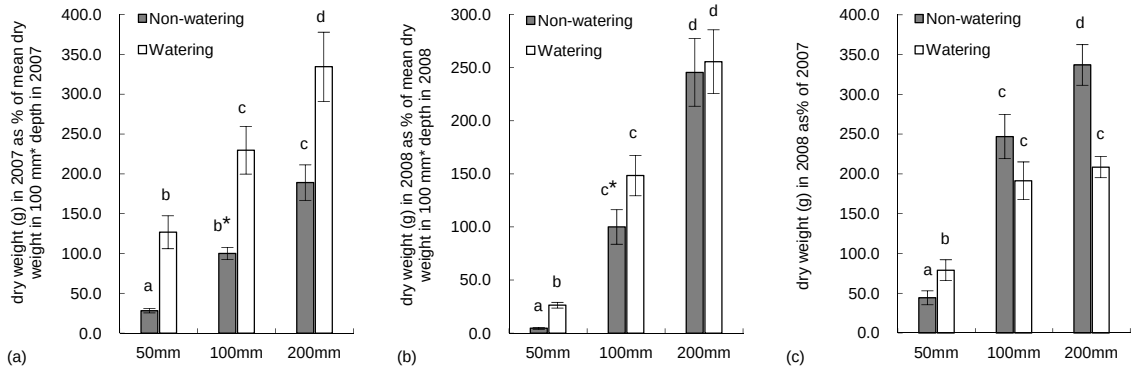


Figure 4.9. The mean total percentage of dry weight (g) of shoots per tray (n=17) in 2007 (a) and 2008 (b) as a percentage of the mean total dry weight per tray (n=17) in 100 mm depth* in 2007 (a) and in 2008 (b), and dry weight in 2008 (c) as a percentage of dry weight in 2007. The same letters are not statistically different at $p = 0.05$ (Mann-Whitney *U*-test). Error bars represent standard error.

[Figures 4.9 (a) and (b)]. In both watering treatments, as compared to growth of plant communities in 100 mm depth, 200 mm substrate depth showed significantly higher growth, whereas 50 mm depth had significantly lower growth in both growing seasons. In 2007, all substrate depth with watering treatment had significantly higher growth than without watering treatment, while in 2008, only the 50 mm depth showed significant difference. Figure 4.9 (c) shows that growth at 100 mm and 200 mm depth in 2008 with both watering treatments increased compared to it at the two depths in 2007, whilst for 50 mm depth it decreased. 200 mm depth without watering treatment showed the highest percentage increase in dry weight.

4.4.2.2 Effect of substrate type

In 2007, the Limestone-based substrate showed a significantly higher growth of plant communities compared to the Zinco substrate (P = 0.0043). In 2008, however, no significant difference (P = 0.1579) was revealed between the two substrate types [Figure 4.10 (a)]. Compared to the growth of plant communities in 2007, the Zinco substrate increased significantly (P = 0.0072), whilst the Limestone-based substrate decreased significantly (P =0.0252).

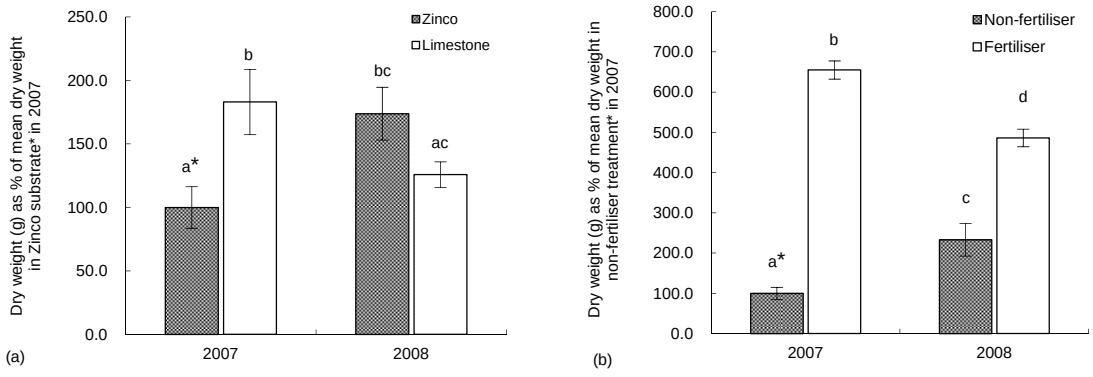


Figure 4.10. The mean total percentage of dry weight (g) of shoots per tray (n = 17) as a percentage of the mean total dry weight in Zinco substrate in 2007* (a) and in non-fertiliser treatment in 2007* (b). The same letters are not statistically different at p = 0.05 (Mann-Whitney U-test). Error bars represent standard error.

4.4.2.3 Effect of fertiliser application

In both growing seasons, plant communities with fertiliser treatment showed significantly greater ($P < 0.001$) increase in above ground biomass compared to those with non-fertiliser treatment [Figure 4.10 (b)]. During the two growing seasons, plant communities without fertiliser application increased significantly in above ground biomass ($P = 0.0131$) compared to the first growing season, while those with fertiliser application decreased significantly.

4.4.3 Structural characteristics of plant community

4.4.3.1 Effect of substrate depth and supplementary watering

(i) Height

For the two watering regimes comparison in the same substrate depths, significant differences were found in the mean height per tray at 50 mm and 100 mm depths, but not for 200 mm depth ($P = 0.6685$). The watered substrate depths were significantly higher in height [Figure 4.11 (a)]. In the non-watering treatment, height was significantly the highest at 200 mm depth and the lowest at 50 mm substrate depth. Height at 100 mm depth was intermediate, and statistically different from both 50 mm and 200 mm depths. In the watering treatment, the 100 mm and 200 mm deep substrate had significantly higher height than 50 mm deep substrate. However, there was no significant difference between 100 mm and 200 mm depths.

The mean height of plants per tray showed significant differences between months and substrate depths in the two watering regimes [Figures 4.11 (b) and (c)]. Under both watering treatment conditions, height at all substrate depths increased significantly into August 2007; however by the second year, it decreased significantly. Plants in 100 mm and 200 mm substrate depth maintained significantly taller height than the original height over the period in the two watering treatments. The exception is the watered

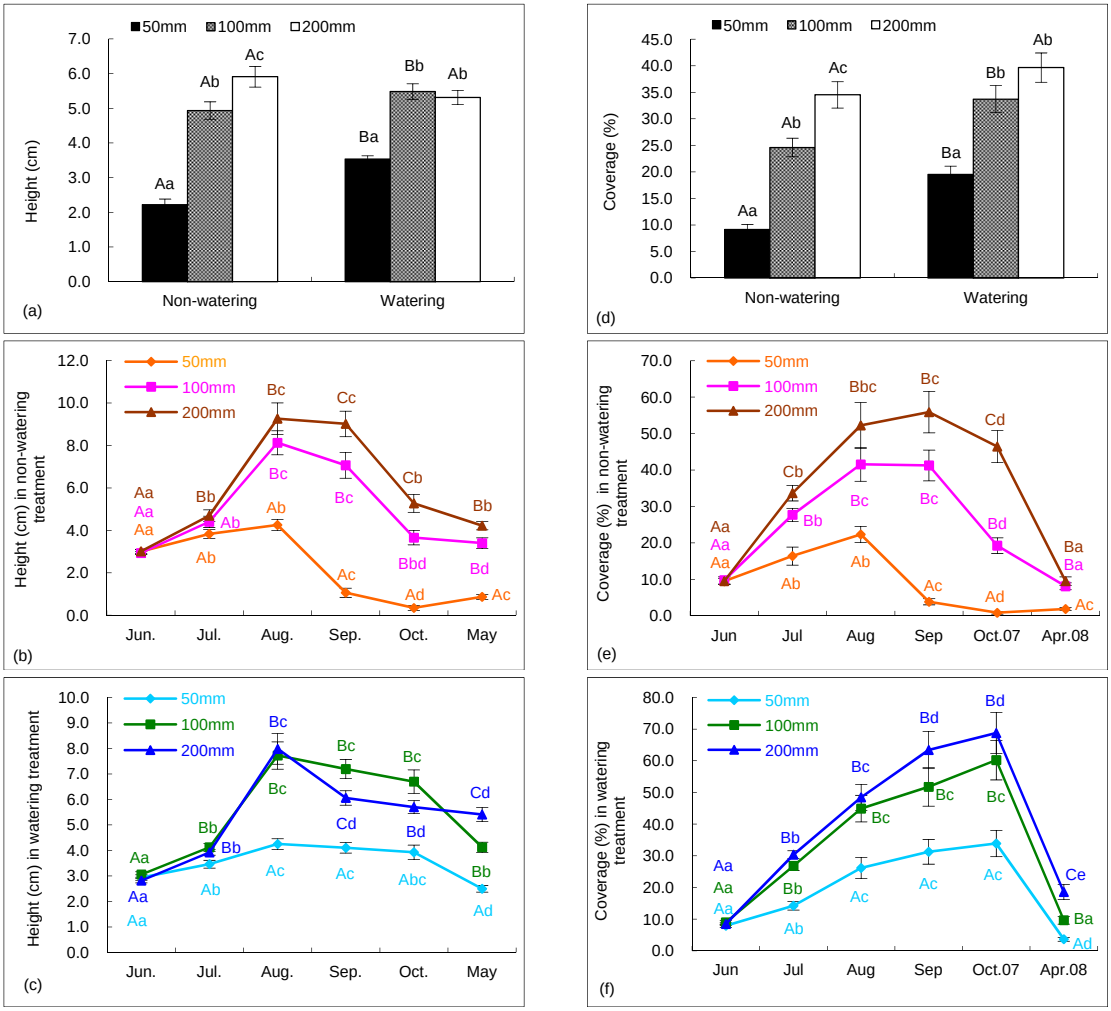


Figure 4.11. Mean height (cm) and total coverage (%) of plant per tray in response to substrate depth in non-watering and watering treatment across the period (a) and (d); in non-watering treatment (b) and (e); in watering treatment (c) and (f).
For figures (a) and (d), different capital letters indicate significant difference between watering treatments for the same substrate depth. Different lower-case letters indicate significant difference between substrate depths for the same watering treatment.
For figures (b), (c), (e), and (f), different capital letters indicate significant difference between substrate depths for the same month. Different lower-case letters indicate significant difference between months for the same substrate depth. Error bars represent standard error.

50 mm depth maintaining taller height for the first year, but in the second year decreasing significantly.

(ii) Coverage

Coverage across all substrate depths in watering treatment (31.0 %) was significantly higher ($P < 0.0001$) than in non-watering treatment (22.8 %). Plants in 50 mm and 100 mm depth with watering treatment showed significant higher coverage compared to the two substrate depths without watering treatment, however, there was no significant difference ($P = 0.1645$) in the mean coverage at 200 mm depth between the both watering treatments. Coverage was significantly greater ($P < 0.001$) on 200 mm depth with non-watering treatment, whereas in watering treatment there was no significant difference in coverage between 100 mm and 200 mm substrate depth ($P = 0.1052$).

Under non-watering treatment conditions, the mean total coverage of plants in 100 mm and 200 mm depth showed a significant increasing pattern into August and September 2007 respectively, and then declined significantly back to the initial coverage. On the other hand, at 50 mm depth the mean total coverage increased significantly into August 2007 and decreased dramatically to less than the initial coverage [Figure 4.11 (e)]. In contrast to non-watering treatment conditions, under watering treatment condition, coverage at all substrate depths showed significant increasing pattern into October 2007. However, in the second year of the experiment the plants maintained their initial coverage for 100 mm depth or had significantly higher coverage for 200 mm depth than the initial, whist 50 mm substrate depth had significantly lower coverage [Figure 4.11 (f)].

4.4.3.2 Effect of substrate type

(i) Height

Figure 4.12 (a) shows that there were significant differences in height between substrate types and months over the 2-years period of the experiment. Two substrate types had significant increasing pattern in height into September 2007 at the Zinco substrate and into October 2007 at the limestone substrate. Two substrate types maintained significantly taller height than their initial height throughout the period. The limestone

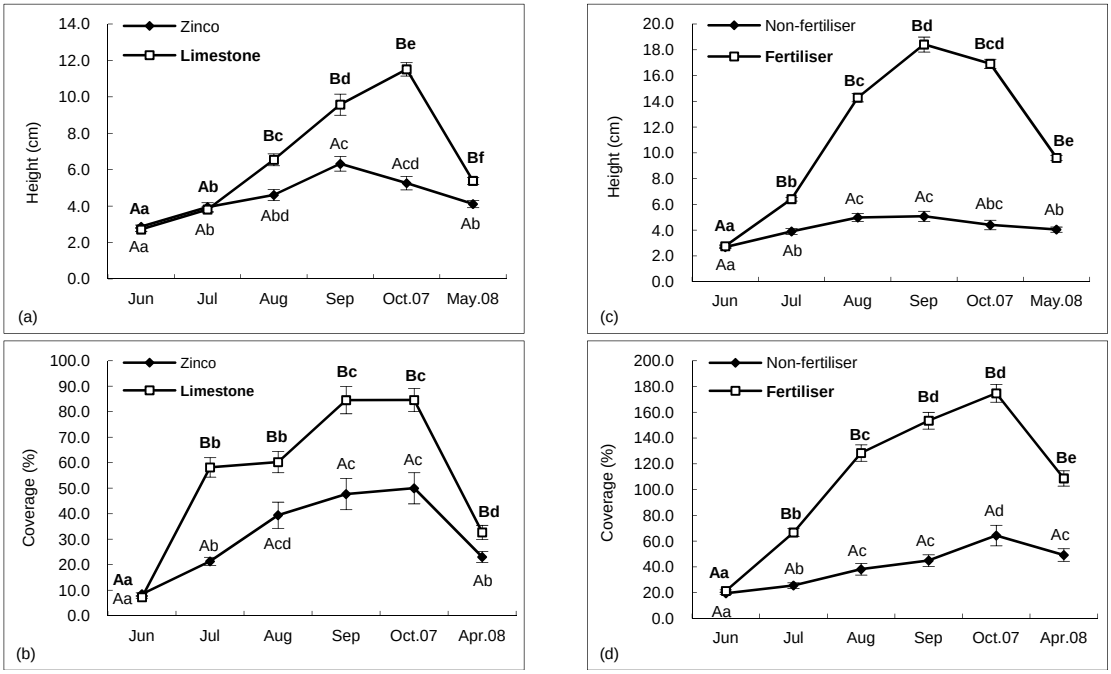


Figure 4.12. Mean height (cm), and total coverage (%) of plant per tray in response to substrate type (a) and (b); and fertiliser application (c) and (d).
Different capital letters indicate significant difference between substrate types and fertiliser applications for the same month. Different lower-case letters indicate significant difference between months for the same substrate type and fertiliser application. Error bars represent standard error.

substrate showed significantly higher value ($P < 0.001$) in height than the Zinco substrate throughout the left period after June ($P = 0.2192$) and July 2007 ($P = 0.6778$).

(ii) Coverage

Significant differences in coverage were found between the two substrate types and months [Figure 4.12 (b)]. Coverage was significantly the greatest in October 2007 at both substrate types. Two substrate types exhibited significantly higher coverage than the initial coverage over the entire 2-years growing season. Coverage was significantly higher every month for the limestone substrate than the Zinco substrate, apart from June 2007 ($P = 0.0786$).

4.4.3.3 Effect of fertiliser

(i) Height

The mean height of plants per tray with and without fertiliser application is shown in Figure 4.12 (c). Plants with fertiliser application were highly significantly ($P < 0.001$) taller than the plants with non-fertiliser application every month over the 2-years period, except in June 2007 ($P = 0.9035$). The growing pattern in fertiliser treatment showed largely significant increase, while slowly increasing in non-fertiliser treatment. Height in both treatments reached a peak in September 2007.

(ii) Coverage

Similar to the height growing pattern, plants with fertiliser treatment had rapid growth in coverage, with slow growth for plants without fertiliser treatment [Figure 4.12 (d)]. Until October 2007, the mean total coverage of plant with and without fertiliser treatment increased significantly. From August 2007, plants with fertiliser treatment exhibited mostly over 100% in coverage. Every month over the 2-years period, except in June 2007 ($P = 0.1682$), coverage in fertiliser treatment was significantly higher than in non-fertiliser treatment.

4.4.4 Flowering performance at community level

During the 2-year period of the experiment, surveys for flowering performance at community level was carried out every month on the mean total number of inflorescences per tray in each treatment; three substrate depths, two watering regimes, two substrate types, and fertiliser applications.

4.4.4.1 Effect of substrate depth and supplementary watering

The mean total number of inflorescences per tray across all substrate depths was not significantly different ($P = 0.1198$) between the non-watering (10.5) and the watering treatments (12.9). In 50 mm deep substrate, however, the mean total number of inflorescences produced by the watered plants was significantly higher than the non-watered plants ($P = 0.0039$). Kruskal-Wallis test revealed substrate depth had a significant effect on the number of inflorescences in the non-watering ($P < 0.001$) and watering treatments ($P = 0.009$). In both watering treatments, flowering performance at 100 mm and 200 mm depth was significantly higher than the 50 mm depth. There was no significant difference in number of inflorescences between 100 mm and 200 mm depth [Figure 4.13 (a)].

Figure 4.13 (b) shows that, under non-watering treatment conditions, inflorescences were first recorded in the all depth during first week of July. In the 50 mm depth treatment, flowering appeared until September, whilst in 100 mm and 200 mm depth it appeared until October 2007. At 50 mm and 100 mm depth, the number of inflorescences reached a peak in August, whereas at 200 mm depth it occurred in September although not significantly different from the number of inflorescences produced in August. In July, there was no significant difference in the number of inflorescences between all depths. However, from August to October, the number of inflorescences at 100 mm and 200 mm was significantly higher than 50 mm depth.

Under watering treatment conditions, at all depths the first flowering was recorded a month later compared to non-watering treatment [Figure 4.13 (c)]. Inflorescences at all substrate depths were present until October 2007. The number of inflorescences at 100 mm and 200 mm depth increased significantly until September, whilst at 50 mm depth it increased until August. During the flowering season, in September only significant difference was revealed in the number of inflorescences between substrate depths. At 100 mm and 200 mm depth the value were significantly higher than 50 mm depth, in the non-watering treatment.

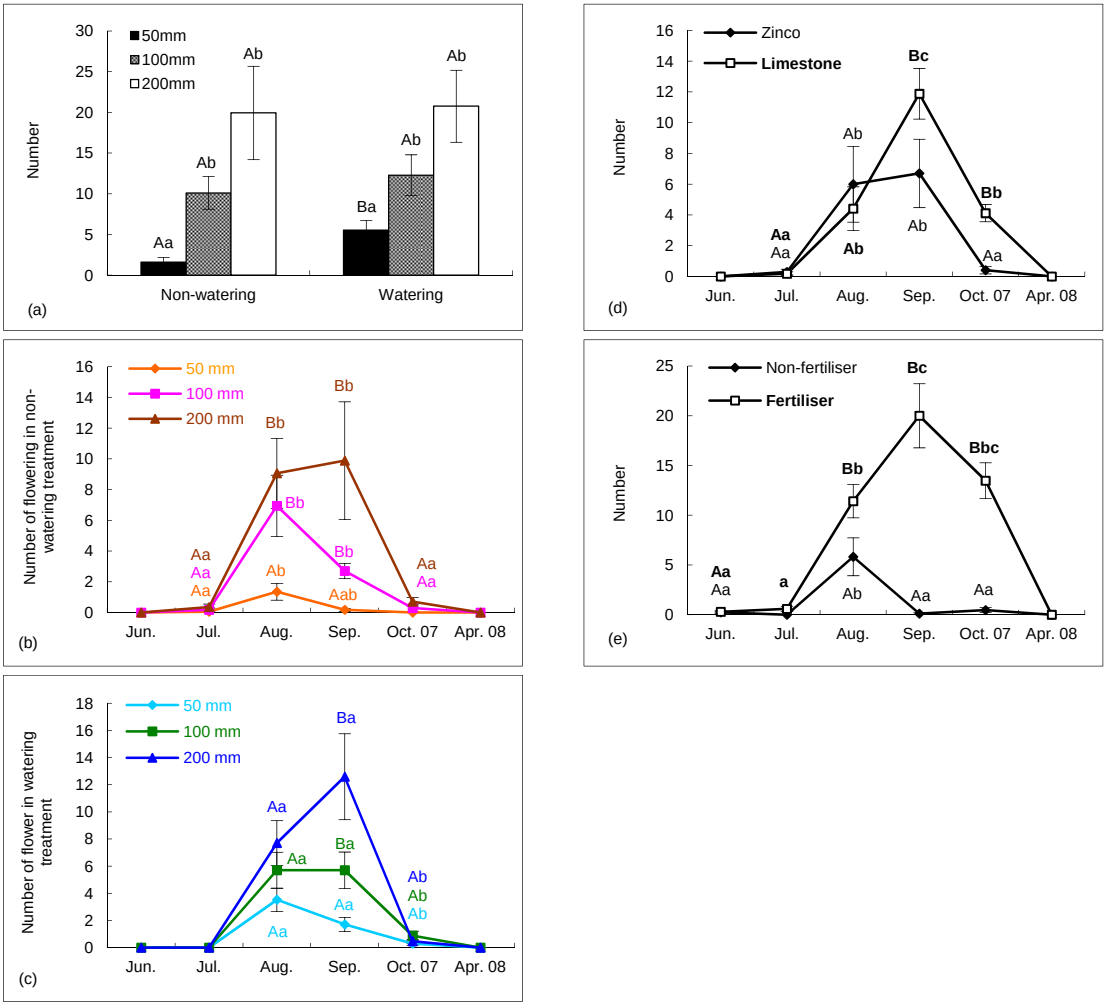


Figure 4.13. Mean total number of inflorescences per tray in response to substrate depth in non-watering and watering treatment across the period (a); in non-watering treatment (b); in watering treatment (c), substrate type (d), and fertiliser application (e).
For figure (a), different capital letters indicate significant difference between watering treatments for the same substrate depth. Different lower-case letters indicate significant difference between substrate depths for the same watering treatment. Error bars represent standard error.
For figures (b), (c), (d), and (e), different capital letters indicate significant difference between substrate depths, substrate types, and fertiliser applications for the same month. Different lower-case letters indicate significant difference between months for the same treatment. Error bars represent standard error.

4.4.4.2 Effect of substrate type

The mean total number of inflorescences in the both substrate types over time is shown in Figure 4.13 (d). Although the mean total number of inflorescences per tray across period was significantly higher (P = 0.0088) in the limestone-based substrate (20.6) than the Zinco substrate (13.4), different responses were observed each month between the substrates. No inflorescences were observed in the either substrates during June

2007 and April 2008. Flowering was present in the both substrates from July to October 2007. In July and August, there were no significant differences in the mean total number of inflorescences per tray between the both substrates. The largest number of inflorescences was shown in September 2007 in both substrates. After September, the number decreased significantly. During September and October, the total number of inflorescences produced by plants in the limestone-based substrate was significantly higher than that in the Zinco substrate. The Zinco substrate showed significantly most inflorescences during August to September, whilst the limestone-based substrate did from August to October [Figure 4.13 (d)].

4.4.4.3 Effect of fertiliser application

Figure 4.13 (e) shows change of the mean total number of inflorescences produced by plants with fertiliser application and without it per tray over time. The mean total number of inflorescences per tray across period produced by the fertilised plants (45.8) was greatly larger ($P < 0.0001$) than the one by the non-fertilised plants (6.6). No inflorescences were present in the non-fertiliser treatment during June, July 2007, and April 2008, although some were observed in the fertiliser treatment during July. Inflorescences in the non-fertiliser treatment were present from August to October 2007, with peak numbers in August, by September it declined significantly into near zero, and then increased slightly again during autumn (September to October). In the fertiliser treatment, inflorescences were present from July to October, reached a peak in September, and then decreased until October although not statistically different with the peak number. During the flowering period, the mean total number of inflorescence produced by plants with fertiliser application was significantly higher than that of plants without it.

4.5 Discussion

4.5.1 Plant community

4.5.1.1 Effect of substrate depth

Under the experimental conditions in this study, substrate depth had a significant effect on abundance, growth, height, coverage, and flowering performances of plant community. Low abundance, growth, the plant structures (height and coverage), and flowering performance of the plant community across all months were associated with the shallowest depth of 50 mm, while higher and the greatest performance occurred at 100 mm and 200 mm substrate depths respectively. Many previous vegetation-related studies for green roof were also observed with a pattern which deeper substrates promoted better plant performances (e.g. Dunnett et al., 2008b; Getter and Rowe, 2008; Durhman et al., 2007; Dunnett and Nolan, 2004). This could be due to the advantages that deeper substrates have. Deeper substrate depths would be likely to provide greater moisture retention, to protect root from temperature fluctuations, and to allow more space for plant roots to grow. VanWoert et al. (2005) investigated substrate moisture contents of three substrate types to evaluate relationship between watering regime factor and growth of *Sedum spp*.. The three substrate types that were used were 2 cm depth, 2 cm depth with an extra moisture retention fabric layer, and 6 cm, consisting of 40 % heat-expanded slate, 40 % United States Golf Association (USGA) grade sand, 10 % Michigan Peat, 5 %dolomite, 3.33 % composted yard waste, and 1.67 % composted poultry litter. The result showed that the deep substrate (6 cm) treatment retained higher moisture content and produced greater plant growth. Indeed, the abundance and growth pattern of the plant community at the shallow substrate depth exhibited dramatic decrease from the initial values, during August to October in 2007, which were some of the hotter and drier months (Figure 4.2). In contrast, deeper substrates maintained an increasing pattern of abundance and growth during the hot and dry period, although showing decline patterns from peak performance from September. The same is true for the second year, which was associated with significant higher abundance and growth in deeper substrates. This indicates that substrate depth might be related to a factor that could affect plant overwintering survival. According to a study by Herrick and Perry

(1995) evaluating the lowest survival temperature of 23 herbaceous perennials, many herbaceous perennials were subject to low temperatures and wide temperature fluctuations. Thus, more freezing injury of plants would be likely to occur at shallower substrate depths compared to deeper substrate depths. In a study by Boivin et al. (2001), who carried out an experiment to evaluate the effect of three substrate depths (5 cm, 10 cm, and 15 cm) on freezing injury of six herbaceous perennials: *Ajuga reptans*, *Arenaria verna* 'Aurea', *Armeria maritima*, *Draba aizoides*, *Gypsophila repens*, and *Sedum xhybridum*, it was shown that the shallow substrate depth experienced significantly lower temperature (5 cm; - 0.4 °C) and much larger temperature fluctuations (5 cm; 8.3 °C) than deeper substrates (10 cm for 0.9 °C, 5.9 °C; 15 cm for 1.6 °C, 4.7 °C) in Quebec city Canada in October and November 1995, and found that plant had more low temperature injury at 5 cm than at 10 cm or 15 cm depths. In addition, these temperature factors, which occurred at shallower depth, could reduce water availability to plants, resulting in reduction of mineral nutrients and phytohormones transport necessary for basic metabolism (Ali et al., 1998; Wraith and Ferguson, 1994).

It is interesting to note that in the flowering performance patterns of the plant community, the deeper substrate depth (200 mm depth) appears to have little significant benefit compared to 100 mm depth, although 200 mm depth had generally higher performance. However, more plants in the deeper substrate depths in both watering treatments produced longer length of flowering display and more numbers of flowers in the plant communities. This may be caused by low abundance rate and smaller size of plants resulting from higher recurrent moisture stress and nutrient limitation in shallower substrates than in the deeper substrates. In a study by Schmitt (1983) on the relationship between individual flowering phenology, plant size, and reproductive success in *Linanthus androsaceus*, a California grassland annual, it was found that flower number was positively correlated with plant size (height) and the duration of flowering. In the current study, similar observations were also revealed; there was a significant positive correlation between plant size (height and coverage) and flowering numbers, and between abundance of shoots and flowering numbers in both watering treatments (data not presented in the results).

4.5.1.2 Effect of watering

Application of additional watering had significant benefit in abundance, height, coverage, biomass, and flowering performances of the plant community. In this study, watering was an important factor relating to abundance, growth, and performance, especially at substrates with shallower depths (50 mm and 100 mm depth). As noted above, this experiment indicated that 50 mm substrate depth is not suitable for satisfactory abundance, growth, and performance of the plant community without additional watering. The result also showed that in comparison with the first year, the growth of the plant community at 50 mm depth decreased in both watering regimes, while the opposite responses were revealed at 100 mm and 200 mm [Figure 4.9 (c)]. According to other studies by Nonami and Boyer (1990) and Acevedo et al. (1971), who evaluated the relationship between plant growth (Soybean; *Glycine max* [L.] Merr. *cv* Williams and Maize plants; *Zea mays* L., var. WF9 X M14, Crow's hybrid corn, Milford, Ill. respectively) and soil water potential, the growth of leaves and stems is rapidly inhibited by inadequate water condition. Supplemental watering during the first growing season produced significantly higher growth at all substrate depth than non-supplementary watering, and produced statistically similar growth of plant community in the shallower substrate to that of the deeper substrate without it [Figure 4.9 (a)]. This pattern appeared to be weak in the second year [Figure 4.9 (b)]. This is not surprising because application of irrigation was carried out on one occasion over the growing season of 2008. However, one of most striking aspects of these results is that, even with one watering event, the plant community still had significant higher productivity in the second year than without additional watering between same substrate depths, although not statistically significant different in 100 mm and 200 mm depth. This result may support the finding that additional watering during the establishment period (first year) is an important factor for plants to perform better and to persist long-term, and indicate that frequent additional watering over the growing season is not as critical when the plant community is established. Supplementary watering significantly improved abundance of the plant community at all substrate depths compared to non-supplementary watering. However, in 200 mm depth, supplementary watering did not have significant benefit to structural characteristic of plant community such as height and coverage, and flowering performance. In case of flowering performance, the benefit

of supplementary watering was revealed at 50 mm depth only. Interestingly, under non-watering condition, at all depths flowering commenced a month earlier than under watering conditions. It is probable that plant community without additional watering may give less competition from plants growing more vigorously in watering. Drought stress may also encourage flowering in some species. In an experiment by Boot et al. (1986), who examined the effect of drought stress on timing of flowering in *Urtica dioica* and *U. urens*, it was found that although prolonged drought strongly inhibited vegetative flowering in both species, flowering in *U. urens* occurred earlier than in *U. dioica* and was less inhibited by drought stresses which strongly inhibited in *U. dioica*.

Again, under non-watering conditions, there were apparent differences in plant community development between substrate depths, while under watering conditions, this was not the case. Namely, additional watering diluted these differential responses to substrate depth, especially between 100mm and 200mm substrate depth. Other studies also suggested that the key factor that influences plant growth was water availability rather than depth of substrate on its own (Rowe et al., 2006; Dunnett and Nolan, 2004). The plant communities with additional watering, as indicated by abundance, growth and performance, developed constantly at all substrate depths over the first growing season. As expected, this may be because supplementary watering would assist to solve problems of growth environment caused by shallower substrate depths, and to support stable environment for plant community development, for example, by removing drought conditions and increasing essential mineral nutrients achieved through the uptake of water.

4.5.1.3 Effect of substrate type

The result showed that the Limestone-based substrate produced significantly greater abundance, growth, and flowering performance than the Zinco substrate. In flowering performance, many more plants in the Limestone-based substrate prolonged flowering time one month later compared to the Zinco substrate. This is perhaps not surprising, given that the species were specifically chosen as being native to calcareous grassland. Jefferies and Willis (1964) studied four species, *Juncus squarrosus* L., *Nardus stricta* L.,

Sieglingia decumbens (L) Bernh., and *Origanum vulgare* L. to evaluate the influence of calcium on the growth and establishment of the species in different types of soil, Fen peat, Carboniferous limestone, and Sand-dune soil. They found that the plants had successful growth in soils which had similar nutritional conditions their natural habitats and that the calcium concentration relative to the concentration of other nutrients in the soil was an important factor affecting the growth and establishment of the plants.

By early in the second year, abundance and growth of the plant community in the limestone-based substrate performed better than in the Zinco substrate. However, by the end of second growing season, in terms of dry weight, the growth of the plant community in the limestone-based substrate was significantly reduced compared to the first growing season, and recorded lower growth than the Zinco substrate, although showed difference was not significant, whilst the Zinco substrate resulted in the opposite. It would appear from the findings of Jefferies and Willis (1964) that this may be because the ratio of calcium concentration to other nutrients in the limestone-based substrate may become insufficient from organic matter decomposition, which is accompanied by nitrogen mineralization. According to Handreck and Black (2005, p.25), organic materials play an important role as a source of nutrients for plants and as water holding capacity, however, rates of decomposition of organic materials are various and they decompose rapidly. In an experiment by Emilsson and Rolf (2005), it was shown that 3 % and 10 % peat organic matter contained in two kinds of green roof substrate almost completely decomposed during the first year on the roof. Under the exposed roof environmental condition, the frequent rainfall in the UK could make much more repetitive drying and rewetting cycle of substrate. This repeated cycle may result in increased rate of decomposition of organic matter (Sørensen, 1974). The decomposition of the organic matter in the Limestone-based substrate may lead to decrease in nutrient availability and turnover of the physical characteristics of the substrate (water holding capacity and air filled porosity), and consequently may have negative impact on the growth of the plant community.

4.5.1.4 Effect of fertiliser application

Results from the fertiliser study also showed similar performance pattern of plant community as the one in substrate type study. As a whole, NPK slow release fertiliser used in this study produced greater abundance, structural characteristics, flowering performance, and growth of plant community. This trend was also observed in the second growing season, although no additional fertiliser was applied. As in the supplemental watering treatment, additional fertiliser application may not be necessary to maintain better development and performance of the plant community after the plant community was established. This is also probably related to the greater availability of nutrients. Similar results with the positive effect of fertilisation on plant establishment and growth were shown in other studies (Retzlaff et al., 2009; Rowe et al., 2006; Emilsson, 2004; Kircher, 2004). The exception was also observed in abundance of the plant community in the second growing season. Although the plant community in both treatments performed better over the 2-year growing season compared to the initial season, fertiliser application was not directly beneficial to the abundance of the plant community in the second year compared to the non-fertilised plant community. The abundance of the plant community in both fertiliser treatments showed no significant difference [Figure 4.8 (e)]. This may be a result of the dramatic increase in the abundance of the plant community in the non-fertiliser treatment in the second growing season. This is possibly caused by the influence of seedlings from overgrown plants in the fertiliser treatment. In the fertiliser treatment, less open space was available for seedling establishment and growth because the plants were closely packed.

Chapter 5. Plant Selections for calcareous grasslands on green roofs in the UK: Effect of substrate depth, irrigation, substrate type, and fertiliser on establishment and performance of seventeen native perennial species

5.1 Introduction

In the previous chapter, it has been demonstrated that the establishment and performance of plant communities were influenced by environmental tolerances: substrate depths and types, irrigation, and fertiliser. In a pilot study of this experiment (Choi and Dunnett, 2008), some of the individual species showed different responses. As it has been stated in the previous chapter, for a wider application to planting design for green roofs elsewhere, evaluation of establishments and performances of individual species over a period of years is needed. Thus, this chapter focused on the evaluation of individual species' establishment and performance characteristics pertaining to survival, growth, phenological growth pattern, flowering period, and effects of environmental factors on the characteristics. Therefore, in this chapter, the following five main questions are considered.

i) What native species are suitable on different depths of substrate?
ii) What native species grow successfully with and without supplementary watering?
iii) Is there any difference in quality and quantity of plant performance between commercial green roof substrate (Zinco semi-intensive) and Limestone-based substrate for calcareous grassland species?
iv) Does fertiliser influence plant establishment, growth, and flowering performance of each species in substrate with shallow depth?
v) How do plant structural characteristics and flowering of each individual species change over time?

5.2 Plant profiles

Brief descriptions are given below of the seventeen species included in the experiments.

5.2.1 *ACHILLEA MILLEFOLIUM*

Achillea millefolium is a strong aromatic, winter-green, rhizomatous perennial growing up to 300 mm and 450 mm in height of foliage and flowering shoot respectively (Grime, et al., 2007, p.84; Warwick and Black, 1982, p.163). The species produces creamy/white flowers from June to August and fruits from July onwards, and reproduces by seeds and from fragments of rhizomes. The seeds germinate in autumn or spring. The species is found on soils with pH ranging from 4.0 to 8.0, and in relatively fertile soil conditions (Grime, et al., 2007, p.84; Dixie and Swift, 1996, p.38). *A. millefolium* is found on a range of habitats including grazed or ungrazed grassland, waysides, spoil heaps and rock outcrops, but is absent from wetland and woodland. It is tolerant of drought due to its deep and extensive root systems that allow it to survive long dry spells, and a relatively high tolerance to shade (Warwick and Black, 1982, p.172/174) but susceptible to water-logging. It is relatively intolerant to competition from taller and more robust herbs (Grime, et al., 2007, p.84).

5.2.2 *AGRIMONIA EUPATORIA*

Agrimonia eupatoria is a slight aromatic, deciduous, medium to tall perennial with an erect stem reaching a height of up to 800 mm and producing yellow flowers from June to August. Its leaves remain during spring to autumn. *A. eupatoria* occurs widely in fertile soils to neutral soils but is found abundantly in well-drained soils with pH 6.5 approximately. It can also be found on dry, open, circum-neutral and calcareous grassland. The species regenerates from a subterranean rhizome and via seeds. The seeds germinate in late winter or spring (Grime et al., 2007, p.650/664/677; Blamey, et al., 2003, p.134; Dixie and Swift, 1996, p.5).

5.2.3 *CAMPANULA GLOMERATA*

Campanula glomerata is a perennial herbaceous plant with upright stems reaching about 200 to 400 mm. The species produces deep blue-purple flowers from June to October (Blamey, et al., 2003, p.250). It prefers well-drained calcareous soils with over pH 6.5 and low fertility, and is restricted to southern central Britain. It is typically found in chalk and limestone grasslands and in open woodlands (Dixie and Swift, 1996, p.10). It is vegetatively propagated by rhizomes (Bachmann, et al., 2005, p.258).

5.2.4 *CLINOPODIUM VULGARE*

Clinopodium vulgare is a scarcely aromatic, partially evergreen perennial herb that grows between 100 mm to 750 mm. Pink-purple flowers appear from July to September. It requires dry calcareous soils of around pH 7.0. The species regenerates vegetatively by means of lateral spread. *C. vulgare* can be found on dry grassy and bushy places, especially on lime (Grime et al., 2007, p.653/666/679; Blamey, et al., 2003, p.220).

5.2.5 *GALIUM VERUM*

Galium verum is a winter-green, long-lived, and short to medium height and often low-growing perennial which grows typically up to 300 mm in height of foliage and flowers. The species produces yellow flowers from July to August and seeds from September to November. It regenerates primarily vegetatively by means of stolons. It is found on soils in the pH range of 4.0 to 8.0, but is mainly found in soils with pH between 5.5 and 6.5. *G. verum* is eventually suppressed by taller and more robust species. It can be found on relatively infertile neutral to calcareous soils including grazed or ungrazed calcareous grassland, rocky outcrops, and waysides, and on limestone quarry tips, and is also common on sandy soils due to the deep root system (Grime et al., 2007, p.326; Dixie and Swift, 1996, p.21).

5.2.6 *HELIANTHEMUM NUMMULARIUM*

Helianthemum nummularium is a slow-growing, shrubby, winter-green, mat-forming perennial. The species grows to less than 200 mm in height of foliage, overtopped by the inflorescence. The flowering period appears from June to July and seeds shed in July and August. *H. nummularium* is commonly associated with species-rich, short turf on well-drained, infertile soils. *H. nummularium* is restricted to calcareous soils on chalk and limestone with pH ranging from 4.5 to 8.0, and is also found on acid soil with pH 3.8 or less in Scotland. The species is tolerant of drought conditions due to having deep tap-root and shedding leaves at the extreme and adult plant is also frost-resistant. *H. nummularium* is intolerant to competition in taller grasslands which develops in response to dereliction or fertiliser application. Seeds tend to germinate in spring and seedlings are intolerant of drought. For successful seedling establishment, it requires a short turf with local areas of bare soil (Grime et al., 2007, p.342).

5.2.7 *HYPOCHAERIS RADICATA*

Hypochaeris radicata is a partially winter-green (in very cold weather no leaves overwinter), and short to medium perennial that grows up to 600 mm in height of scape. The foliage is typically appressed to the ground. It produces yellow flowers appearing from June to September and seed from July onwards. *H. radicata* is found on a range of habitats on dry, sandy, and slightly acidic soils including short turf in pastures, open meadows, heaths, derelict pastures and waysides. The species is found commonly and abundantly in soils with pH range between 4.5 and 5.5. The species is absent from very fertile soils in places susceptible to water-logging, strong acidic soils, or calcareous soils in areas with a less oceanic climate. The species colonise new sites by means of its windblown seed but once established it regenerates both by seed and by vegetative means. Germination tends to peak in spring and autumn. *H. radicata* is very tolerant of drought due to the deep root system (Grime, et al., 2007, p.364; Dixie and Swift, 1996, p.9).

5.2.8 *KNAUTIA ARVENSIS*

Knautia arvensis is a medium to tall hairy, fairly stout perennial herb of dry, well-drained calcareous and neutral grassland that can be found on chalk and limestone meadow, rough pasture, hedgerows, verges and grassy waste places. The species grows up to 1000 mm and produces large bluish-lilac to purple flowers on the end of the tall stem from July to September (Blamey, et al., 2003, p.262; Dixie and Swift, 1996, p.15). It occurs in soils of around pH 7.0 and in sands, clays and limestone. *K. arvensis* regenerates seasonally by seed and germinates in autumn (Grime, et al., 2007, p.656/682).

5.2.9 *LEONTODON AUTUMNALIS*

Leontodon autumnalis is a winter-green and rosette-forming perennial that grows up to 300 mm in height of scape. The leaves are usually appressed to the ground. It produces flowers from June to October and seeds from July to October. The species can be found on sites such as road verges and pasture on moist fertile soil with pH 5.0 or over. *L. autumnalis* is absent from woodland and tall herbaceous vegetation due to its intolerance to shading. It regenerates by means of wind-dispersed seeds. Germination is affected by temperature (Grime et al., 2007, p.396).

5.2.10 *LEUCANTHEMUM VULGARE*

Leucanthemum vulgare is a winter-green, short-lived perennial found commonly on spoil heaps and limestone quarry waste, and in meadow, abandoned pasture, and railway banks, but absent from wet sites and woodland habitats. It prefers soils of low to moderate fertility and is found in soils of pH 5 to 8. It tends to be tolerant of drought. The plant grows to 400 mm and 700 mm in height of foliage and flowers respectively. The flower head is produced on the end of each stem in June and July. *L. vulgare* regenerates mainly by seed because of its limited capacity for vegetative spread.

Germination occurs in autumn or spring and exhibits rapidly at the surface of dry soils (Grime et al., 2007, p.400; Dixie and Swift, 1996, p.25).

5.2.11 *LINARIA VULGARIS*

Linaria vulgaris is a partially winter-green perennial of open and artificial habitats on dry soils including cinder tips, beside railways, hedgerows, and roadside. *L. vulgaris* is found in soils with pH of 5.5 to 8 and in dry, unshaded condition with low fertility, and is also found occasionally on acidic soil down to pH 3.5. It grows to about 500 mm in height of foliage, overtopped by the inflorescence with yellow flowers with an orange bulge and a long straight spur from July to October. Flowering shoots die back during autumn. Short leafy shoots produced in autumn overwinter and elongate in late spring. *L. vulgaris* is spread by wind-dispersed seed and once established it regenerates by means of adventitious buds produced on its roots. Seeds are produced from September onwards and germinate in spring and early summer. It is relatively tolerant of drought due to its deep and extensive root system (Grime et al., 2007, p.402; Dixie and Swift, 1996, p.40).

5.2.12 *LOTUS CORNICULATUS*

Lotus corniculatus is a long-lived, the commonest legume of unproductive grasslands and is probably the most ecologically wide-ranging legume in Britain Isles, which extend from maritime to montane environments, from moderately low to high soil pH, from infertile to moderately fertile soils, and spoil and open habitats to grassland. It is, however, absent from tall grasses, woodland and wetland, and very acidic soils, and very fertile sites. *L. corniculatus* is found abundantly in soils within two ranges of pH from 5.5 to 6.0 and from 7.5 to 8.0. It grows to less than 200 mm in height of foliage overtopped by flowers appearing in June and July. Most shoots die back in late autumn. It regenerates mainly by seed germinating in spring and has the limited capacity for clonal expansion. *L. corniculatus* is intolerant of high nitrogen levels and of drought species (Grime et al., 2007, p.410; Dixie and Swift, 1996, p.6).

5.2.13 *ORIGANUM VULGARE*

Origanum vulgare is a downy and faintly aromatic, partially winter-green, tall herb that reaches up to 800 mm in height of foliage overtopped by inflorescence with clusters of pink petaled flowers appearing from July to September. Seed sheds from August to November and germinates in spring. *O. vulgare* has limited means of lateral vegetative spread and regenerates mainly by seed. Shoots die back in autumn. The species cannot survive well in non-disturbed sites because it is suppressed by taller and fast-growing species. *O. vulgare* is relatively tolerant of drought by having deep root system with very long and numerous root hairs that can exploit subsoil water during periods of drought. *O. vulgare* is associated with dry and infertile, and calcareous soils. It occurs frequently and abundantly in soils with around pH 7.0 and is not recorded below pH 5.5. It is found on a range of habitats including rocky limestone habitats, hedge banks, road verges and scrub, but is absent from arable land, woodland, spoil heaps, enclosed pasture, meadows and wetlands (Grime et al., 2007, p.448; Dixie and Swift, 1996, p.34).

5.2.14 *PILOSELLA AURANTIACA*

Pilosella aurantiaca is a winter-green, short to medium perennial with erect scape having orange or brownish flowers from June to September that reaches up to 400 mm. The plant is spread through its wind-dispersed seed, and once established it regenerates by stolons and shallow rhizomes. It occurs abundantly in soils with around pH 6.5 and is found primarily on spoil and wasteland habitats (Grime et al., 2007, p.658/671/684; Blamey, et al., 2003, p.300).

5.2.15 *PILOSELLA OFFICINARUM*

Pilosella officinarum is a winter-green, low-growing stoloniferous herb associated with dry and calcareous soils. Habitats include calcareous pastures, scree, wasteland, rocky outcrops, and road verges. It is, however, absent from wetlands and woodland. Foliage

usually is appressed to the ground, but grows up to 100 mm in taller vegetation. The plant reaches up to 300 mm in height of scape producing lemon-yellow flowers from May to June or to August. *P. officinarum* tends to be susceptible to drought due to shallow root system and to shade of taller plants due to the low stature of the shoot. It is stimulated by addition of nitrogen in production and flowering. *P. officinarum* is spread by wind dispersed seed and it regenerates by means of long stolons and of seed germinating during autumn (Grime et al., 2007, p.466).

5.2.16 *PRIMULA VERIS*

Primula veris is a spring-flowering, long-lived, winter-green, rosette-forming perennial characteristic of short, species-rich grasslands. *P. veris* thrives in moist, well-drained calcareous soils and is found occasionally in dry, non-calcareous soils. It is intolerant of waterlogged soils and is rarely observed in shaded habitats. It is also necessary for it to have enough amount of light for flowering (Grime *et al*., 2007, p.492) and germination (Milberg, 1994, p.7). Thus, it is not successful in woodland or under tall plants. Its preferred habitats include open grasslands, stabilized scree slopes and limestone quarry heaps, railway banks, and road verges. The plant grows up to 150 mm in height of foliage in taller grassland and flower stalk reaches up to 300 mm. *P. veris* produces new leaves during winter and flowers in April and May but reaches maximum growth in summer and seeds shed from July to September onwards. It replicates rarely vegetatively by means of branching of the rhizome, although this may be the main means of regeneration in stable communities (Grime et al., 2007, p.492; Dixie and Swift, 1996, p.12).

5.2.17 *SCABIOSA COLUMBARIA*

Scabiosa columbaria is a winter-green, long-lived, rosette-forming, strict calcicolous herb associated with moist to dry in fertile calcareous soils with a pH of over 5.5 in semi-natural grassland. It is found in habitats including grazed and ungrazed grassland,

scree, quarry spoil, cliffs, rocky outcrops, waysides, and railway ballast, but is absent from arable, wetland and woodland habitats. The plant grows up to less than 100 mm and 700 mm in height of foliage and flower stalk respectively. Flowering appears from July to August and seed sets from August to October. *S. columbaria* is considered to be tolerant of drought. This is because it has long tap-root which is able to tap subsoil water during dry periods. It cannot thrive in tall or productive communities because its low stature and limited capacity for lateral vegetative spread. *S. columbaria* is strongly dependent upon seed for regeneration which germinates mainly in autumn (Grime et al., 2007, p.544; Dixie and Swift, 1996, p.30).

5.3 Measurements

Under the same experiment as discussed in Chapter 4, a number of different measurements were made to assess the effects of environmental factors on survival, performance, and growth of each individual species. As in Chapter 4, the height, number of leaves, shoots or stems, and inflorescences, and coverage of each individual plant were recorded monthly throughout the period of the experiment. The total number of survived shoots of individual species per tray each was counted. The mean total number of individual species per tray was converted to percentages as a percentage of original number of species in June 2007. The number of inflorescences produced was counted for individual species. The mean height of individuals of each species was recorded over the period. Nine individuals of each species were measured for this purpose. For species that had more than nine individuals due to propagation, nine highest heights were taken; consequently, means were calculated from the resulting nine measurements. Measurements of plant coverage of each species were recorded by taking monthly digital images. The digital images were analysed as percentages at a ratio to tray size by using the Photoshop programme (Figure 5.1). At the end of both growing seasons, dry-weights of all plants above ground were recorded, and were sorted by species and its tray number.

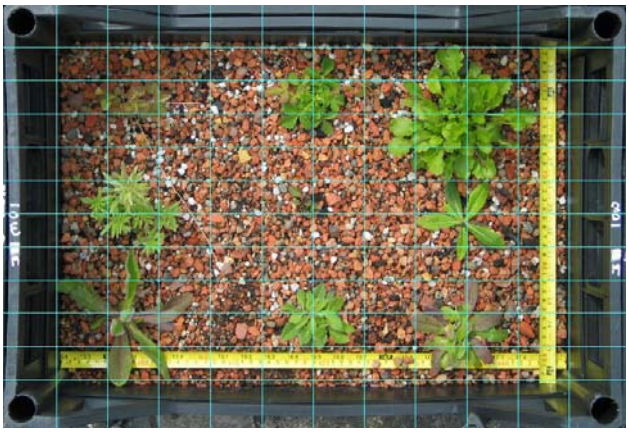


Figure 5.1. Coverage measurement.

Statistical analysis

For statistical analysis, all the mean values of each species was assessed to determine significant differences in response to environmental factors across the periods, between the initial value and values of each month for the same environmental factor (substrate depths and types, irrigation regimes, and fertiliser applications), and between same environmental factors for the same month using Kruskal-Wallis test and Mann-Whitney *U*-test to find where the differences existed (Dytham, 2003).

5.4 Results

5.4.1 Abundance of individuals

As in chapter 4, the total number of shoots of individual species was counted monthly over the 2 years of the experiments. The total number of survived shoots of individual species of each tray was converted to percentages as a percentage of original number of individual species in June 2007. For statistical analysis, the mean total number of survived shoots per tray was assessed to investigate differences between original numbers of shoot in June 2007 and every month for the same environmental factor and between same environmental factors (substrate depths, substrate types, and fertiliser applications) for the same month using Kruskal-Wallis test and after that Mann-Whitney *U*-test were carried out to find where differences were (Dytham, 2003).

5.4.1.1 Effect of substrate depth without supplementary watering

Table 5.1 shows the mean percentage of survived plants of each species at 50 mm, 100 mm, and 200 mm substrate depth across the 2-year period of the experiment under non-watering conditions. All species survived at 100 mm and 200 mm depth over the period and all species survived at 50 mm depth into August 2007. However by the second year, 6 out of 17 species, *A. millefolium, A. eupatoria, C. glomerata, H. nummularium, P. aurantiaca,* and *P. veris,* did not survive. The majority of the species, 10 out of 17 at 100 mm depth and 11 out of 17 at 200 mm depth, increased in numbers. At 50 mm depth, only two species increased in numbers. 13 at 100 mm and 14 species at 200 mm depth maintained 50 % or more of their original numbers, whilst only 3 species maintained 50 % or more of the original numbers at 50 mm depth, which were *H. radicata, L. vulgare, and S. columbaria*.

At the end of the experiment in April 2008, the abundance of individual species showed significant differences between 50 mm, 100 mm, and 200 mm substrate depth. However,

Table.5.1. Abundance of individual species at 50, 100, and 200 mm substrate depth in non-watering treatment over the period 2007-2008.

Non-watering treatment	Substrate depth																	
	500mm						100mm						200mm					
	2007					2008	2007					2008	2007					2008
	Jun.	Jul.	Aug.	Sep.	Oct.	Apr.	Jun.	Jul.	Aug.	Sep.	Oct.	Apr.	Jun.	Jul.	Aug.	Sep.	Oct.	Apr.
A.. millefolium	100 Aa	95 Aa	79 Aa	0	0	0	100 Aa	107 Aa	114 Ba	140 Ba	153 Bb	184 Bb	100 Aa	104 Aa	125 Ba	128 Ba	172 Bb	254Bb
A. eupatoria	100 Aa	100 Aa	100 Aa	0	0	0	100 Aa	100 Aa	100 Aa	111 Ba	100 Ba	67 Ba	100 Aa	133 Aa	111 Aa	156 Bb	122 Ba	78Ba
C. glomerata	100 A	100 A	100 A	33 A	22 A	0	100 A	100 Aa	100 A	100 B	100 B	56 B	100 Aa	100 Aa	100 Aa	100 Ba	100 Ba	78 Ba
C. vulgare	100 Aa	101 Aa	89 Aa	6 Ab	3 Ab	25 Ab	100 Aa	107 Aa	119 Ba	163 Ba	134 Ba	224 Ba	100 Aa	99 Aa	116 Ba	142 Ba	142 Ba	147 Ba
G. verum	100 Aa	193 Ab	241 Ab	98 Aa	35 Ab	23 Ab	100 Aa	167 Ab	259 Bb	217 Bb	211 Ba	136 Ba	100 Aa	165 Ab	248 Cb	245 Bb	213 Bb	229 Cb
H. nummularium	100 A	100 A	100 A	33	11	0 A	100 A	100 A	100 A	100	100	11 A	100 A	100 A	100 A	100	100	11A
H. radicata	100 Aa	106 Aa	100Aa	25Ab	16 Ab	197 ABb	100 Aa	85 Aa	85 Aa	76 Ba	85 Ba	288 Ab	100 Aa	97 Aa	97 Aa	91 Ba	118 Ba	132 Bb
K. arvensis	100 Aa	106 Aa	106 Aa	33 Ab	11 Ab	17 Ab	100 Aa	113 Aa	119 Aa	119 Aa	94 Ba	219 Bb	100 Aa	167 Ab	208 Ab	192 Bb	175 Ba	333 Bb
L. autumnalis	100 Aa	131 Aa	128 Aa	72 Aa	13 Ab	28 Ab	100 Aa	98 Aa	91 Aa	104 Ba	100 Ba	89 Ba	100 Aa	102 Aa	116 Aa	123 Ba	120 Ba	191 Ca
L. vulgare	100 A	100 A	100 A	22 A	11 A	389 A	100 A	100 A	100 A	100 B	100 B	511 A	100 Aa	100 Aa	100 Aa	100 Ba	100 Ba	256 Ab
L. vulgaris	100 Aa	143 Ab	176 Ab	106 Aa	43 Aa	33 Ab	100 Ba	136 Aa	193 Bb	252 Bb	196 Bb	254 Bb	100 Ba	154 Bb	200 Bb	208 Bb	169 Ba	168 Ba
L. corniculatus	100 Aa	143 Ab	196 Ab	6 Ab	0	16 Ab	100 Aa	168 Bb	392 Bb	459 Bb	134 Ba	45 Ab	100 Aa	171 Bb	607 Cb	717 Cb	491 Cb	98 Aa
O. vulgare	100 Aa	118 Aa	138 Aa	103 Aa	47 Ab	14 Ab	100 Aa	107 ABb	156 Bb	177 Bb	150 Ba	47 Ba	100 Aa	135 Bb	168 Bb	219 Bb	243 Bb	42 Cb
P. aurantiaca	100 A	100	100	33 A	11 A	0	100 Aa	100 Aa	400 Ab	364 Bb	327 Bb	327 Bb	100 Aa	500 Bb	660 Bb	570 Bb	510 Cb	690Cb
P. officinarum	100 Aa	100 Aa	118 Aa	127 Aa	27 Ab	18 Ab	100 Aa	182 Bb	555 Bb	364 Bb	264 Ba	118 Ba	100 Ba	294 Cb	350 Bb	328 Bb	311 Cb	133 Ba
P. veris	100 Aa	88 Aa	45 Ab	18 Ab	9 Ab	0	100 Ba	37 Ab	23 Ab	15 Ab	17 Bb	14 Bb	100 Aa	90 Aa	62 Aa	26 Ab	36 Bb	15 Bb
S. columbaria	100 A	100 A	100	56 A	33 A	56 A	100 A	100 A	100	100	100	311 B	100 A	100 A	133	144 B	167 B	389 B

Different capital letters indicate significant difference at p=0.05 (Mann-Whitney *U*-test after Kruskal-Wallis test) between substrate depths for the same month. Different lower-case letters indicate significant difference at p=0.05 (Mann-Whitney *U*-test after Kruskal-Wallis test) between original number and each month for the same substrate depth.

3 species, *H. nummularium, L. vulgare, L. corniculatus,* did not show any significant differences in abundance between the three depths. Of the 14 species showing significant differences between three depths, abundance was significantly the highest for *G. verum, L. autumnalis,* and *P. aurantiaca* at 200 mm and for *H. radicata* and *O. vulgare* at 100 mm depth. As mentioned above, however, *H. nummularium* and *P. veris* were significantly lower in abundance than 50 % of their original numbers at all substrate depths. Of the 14 species, 9 species that did not have significant differences in abundance between 100 mm and 200 mm depth were: *A. millefolium, A. eupatoria, C. glomerata, C. vulgare, K. arvensis, L. vulgaris, P. officinarum, P. veris* and *S. columbaria*. *H. radicata* showed significant higher abundance at 100 mm depth than at 200 mm depth, with intermediate at 50 mm depth and no significant difference to both 100 mm and 200 mm depth.

Overall, the most common pattern of abundance of the species over time was for an increase in numbers at 100 mm and 200 mm depth, and for a reduction in numbers at 50 mm depth. 6 and 7 out of increased species at the first two depths exhibited a statistically-significant response. 15 species at the last depth showed a decreasing pattern with having a significant response. 3 species, *H. nummularium, O. vulgare,* and *P. veris* declined in numbers with less than 50 % of original numbers across all substrate depths, whilst 2 species, *H. radicata* and *L. vulgare,* increased in abundance at all depths; however, these two species over the first year declined in numbers at 50 mm depth.

5.4.1.2 Effect of substrate depth with supplementary watering

In contrast to the abundance of species in the non-watering treatment, it is notable that under watering treatment conditions, all species survived at all substrate depths over the 2-year period of the experiment with the exception of *H. nummularium* at 100 mm and 200 mm depth (Table 5.2). The majority of the species maintained 50 % or more of their original numbers across all depths, which were 14 species out of the 17 at 50 mm and 15species out of 16 at 100 mm and 200 mm. At 100 mm and 200 mm, more species

Table.5.2. Abundance of individual species at 50, 100, and 200 mm substrate depth in watering treatment over the period 2007-2008.

Watering treatment	Substrate depth																	
	500mm						100mm						200mm					
	2007					2008	2007					2008	2007					2008
	Jun.	Jul.	Aug.	Sep.	Oct.	Apr.	Jun.	Jul.	Aug.	Sep.	Oct.	Apr.	Jun.	Jul.	Aug.	Sep.	Oct.	Apr.
A.. millefolium	100 Aa	129 Aa	137 Aa	147 Ab	165 Ab	165 Ab	100 Aa	106 Aa	117 Aa	162 Aa	197 Ab	295 Bb	100 Aa	144 Aa	172 Ab	198 Ab	262 Ab	538 Bb
A. eupatoria	100 A	100 A	100 A	144 A	144 A	89 A	100 Aa	111 Aa	122 Aa	156 Aa	144 Aa	122 Aa	100 A	100 A	133 A	156 A	156 A	133 A
C. glomerata	100 Aa	100 Aa	100 Aa	100 Aa	100 Aa	78 Aa	100 Aa	100 Aa	100 Aa	100 Aa	100 Aa	78 Aa	100 Aa	100 Aa	100 Aa	100 Aa	89 Aa	100 Aa
C. vulgare	100 Aa	100 Aa	106 Aa	200 Ab	225 Ab	110 Aa	100 Aa	113 Aa	144 Aa	240 Ab	284 Ab	300 Aa	100 Aa	110 Aa	118 Aa	197 Ab	226 Ab	215 Aa
G. verum	100 Aa	184 Ab	231 Ab	275 Ab	259 Ab	114 Aa	100 Aa	215 Ab	314 Bb	411 Bb	396 ABb	243 Bb	100 Aa	239 Bb	317 Bb	386 Bb	383 Bb	275 Bb
H. nummularium	100 A	100 A	100 A	100 A	100 A	22 A	100 A	100 A	100 A	100 A	100 A	0 A	100 A	100 A	100 A	100 A	100 A	0 A
H. radicata	100 Aa	107 Aa	93 Aa	100 Aa	95 Aa	71 Ab	100 Aa	82 Aa	84 Aa	95 Aa	105 Aa	124 Aa	100 Aa	81 Aa	91 Aa	102 Aa	91 Aa	74 Ab
K. arvensis	100 Aa	130 Aa	130 Aa	130 Aa	130 Aa	230 Ab	100 Aa	129 ABa	129 Aa	150 Aa	143 Aa	321 Ba	100 Aa	126 Ba	126 Aa	132 Aa	132 Aa	289 Bb
L. autumnalis	100 Aa	111Aa	98 Aa	109 Aa	104 Aa	87 Aa	100 Aa	98 Aa	98 Aa	114 Aa	116 Aa	166 Ba	100 Aa	92 Aa	98 Aa	125 Aa	134 Aa	234 Ba
L. vulgare	100 Aa	100 Aa	100 Aa	100 Aa	100Aa	111 Aa	100 Aa	100 Aa	100 Aa	100 Aa	100 Aa	133 Aa	100 Aa	100 Aa	100 Aa	100 Aa	100 Aa	122 Aa
L. vulgaris	100 Aa	186 Ab	208 Ab	233 Ab	183 Aa	117 Aa	100 Aa	235 Bb	231 Bb	225 Ab	144 Aa	113 Aa	100 Ba	185 Bb	188 Bb	229 Bb	140 Ba	155 Ba
L. corniculatus	100 Aa	164 Ab	475 Ab	781 Ab	839 Ab	110 Aa	100 Aa	176 Ab	709 Bb	988 Ab	939 ABb	193 Ba	100 Aa	186 Ab	1002 Cb	1278 Bb	1080 Bb	315 Ba
O. vulgare	100 Aa	116 Aa	145 Aa	180 Ab	170 Ab	15 Ab	100 Aa	153 Ab	219 Bb	292 Bb	339 Bb	116 Ba	100 Aa	203 Ab	290 Bb	410 Cb	490 Bb	236 Ba
P. aurantiaca	100 Aa	110 Aa	120 Aa	190 Ab	160 Aa	360 Ab	100 Aa	422 Bb	489 Bb	511 Bb	444Bb	478 Ab	100 Aa	490 Bb	530 Bb	580 Bb	530 Bb	750 Ab
P. officinarum	100 A	111 A	200 A	233 A	256 A	189 A	100 A	444 B	500 B	533 B	411 B	156 A	100 Aa	462 Bb	446 Bb	477 Bb	392 Bb	285 Bb
P. veris	100 Aa	89 Aa	86 Aa	89 Aa	91 Aa	23 Ab	100 Aa	97 Aa	51 Ab	66Aa	60 Aa	43 Ab	100 Aa	94 Aa	79 Aa	64 Aa	67 Aa	24 Ab
S. columbaria	100 A	100 A	100 A	100 A	100 A	289 A	100 A	144 A	144 A	167 A	178 B	511 B	100 A	111 A	111 A	122 A	189 B	567 B

Different capital letters indicate significant difference at p=0.05 (Kruskal-Wallis, and Mann-Whitney U-test) between substrate depths for the same month. Different lower-case letters indicate significant difference at p=0.05 (Kruskal-Wallis, and Mann-Whitney U-test) between original number and each month for the same substrate depth.

increased in number than at 50mm; 10 species at 50 mm, 14 species at 100 mm, and 13 of the 17 species at 200 mm depth. Each species also showed a statistically significant increased or decreased abundance pattern at three substrate depths over the two years period of the experiment. Kruskal-Wallis test revealed that 9 species at 50 mm, 10 at 100 mm, and 11 at 200 mm depth out of the increased species had statistically significant responses. Species that did not show statistically significant responses in abundance pattern at all depths throughout the period were *C. glomerata, L. autumnalis,* and *L. vulgare*. 3 species, *A. eupatoria*, *H. radicata*, and *K. arvensis* showed no significant response only at 100 mm substrate depth.

A. millefolium, C. vulgare, G. verum, L. vulgaris, L. corniculatus, P. aurantiaca, P. officinarum, and *S. columbaria* showed a significant increase in number across the period at all depths, whereas *H. nummularium* and *P. veris* significantly decreased and was recorded with fewer than 50 % of the original numbers across all substrate depths. *H. nummularium* maintained 100 % of their original shoot numbers into the first year of the experiment, however by the second year, it declined 22 % at 50 mm depth and did not survive at 100 mm and 200 mm depth. *H. radicata* exhibited a significant reduction in number at 50 mm and 200 mm in the second year, but an increase at 100 mm although it was not a statistically significant response compared to the original numbers. *O. vulgare* declined significantly into 15 % of the original numbers at 50 m depth (P = 0.0009), whilst it increased at 100 mm and 200 mm depth.

In the final abundance of the individual species between three substrate depths, 8 out of the 17 species showed no significant differences in abundance between all substrate depths. Of 9 species that showed significant difference in abundance across substrate depths, two species, *L. vulgaris* and *P. officinarum* were significantly the highest at 200 mm depth. The others, seven species at 100 mm and 200 mm, were significantly higher in abundance compared to 50 mm depth, however, there were no significant differences between 100 mm and 200 mm depth.

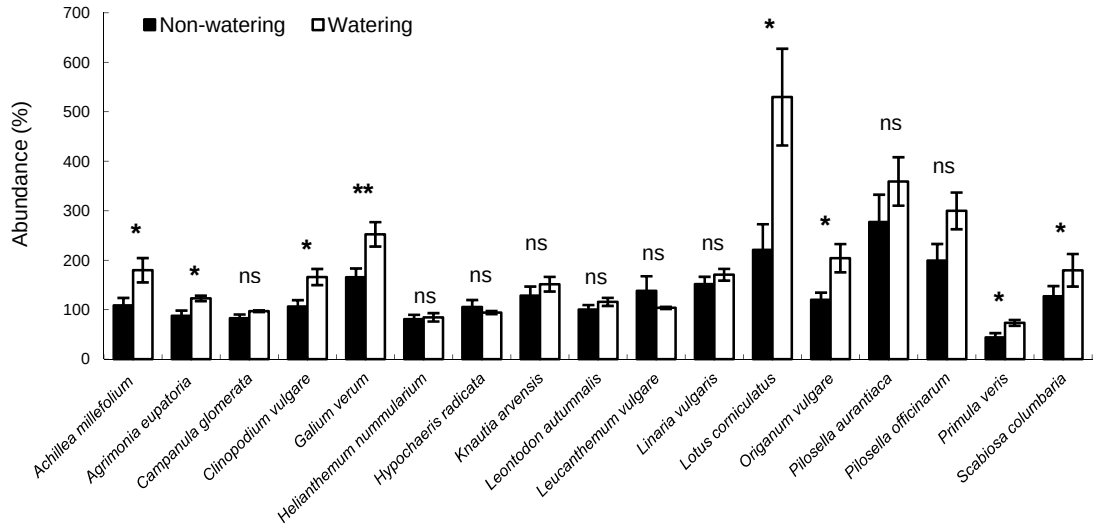


Figure 5.2. Abundance of individual species across all substrate depths and period in response to watering treatments. Error bars represent standard error of the mean value. Significant differences (Mann-Whitney *U*-test) between non-watering and watering treatments are indicated by: *P=0.05; **P=0.01; ***P=0.001; ns, not significant.

5.4.1.3 Effect of watering

The mean percentage abundance of individual species in response to watering treatments across all substrate depths over the 2-years period of the experiment is shown in Figure 5.1. The majority of the species exhibited higher abundance in watering treatment except in *H. radicata* and *L. vulgare*, whether the differences were statistically significant or not. 9 out of the 17 species that showed no significant difference between non-watering and watering treatment were: *C. glomerata*, *H. nummularium*, *H. radicata*, *K. arvensis*, *L. autumnalis*, *L. vulgare*, *L. vulgaris*, *P. aurantiaca*, and *P. officinarum*. Abundance of species showing significant differences between the two watering treatments was higher in watering treatment. Abundance was the lowest in *P. veris* with non-watering treatment and highest in *L. corniculatus* with watering treatment.

5.4.1.4 Effect of substrate type

Table 5.3 shows that all plants survived in the both substrate types over the 2 years of the experiment except for *H. nummularium* in the Zinco substrate. All species in the Limestone-based substrate and 15 out of 17 species in the Zinco substrate maintained 50 % or more of their original numbers over time. The majority of the species, 12 out of the 16 species in the Zinco substrate and 14 out of the 17 in the Limestone-based substrate, increased abundance in number into the second growing season. 7 species of the increasing species significantly improved their numbers in both substrate types into the second year. *C. glomerata*, *H. radicata*, and *O. vulgare* did not show any significant responses in both substrate types across the period. In the Limestone-based substrate, *C. vulgare* and *L. autumnalis* showed significant increase in numbers over the period, whilst they showed no statistically significant response in the Zinco substrate. *H. nummularium* and *P. veris* did not survive or drew significantly declining pattern at either substrate types throughout the period, although in the Limestone-based substrate

Table 5.3. Abundance of individual species in response to substrate types over the period 2007-2008.

	Zinco commercial substrate						Limestone-based substrate					
	2007					2008	2007					2008
	Jun.	Jul.	Aug.	Sep.	Oct.	May	Jun.	July	Aug.	Sep.	Oct.	May
A. millefolium	100 Aa	131 Aa	137 Aa	176 Ab	180 Ab	278 Ab	100 Aa	118 Aa	128 Aa	179 Ab	288 Bb	418 Bb
A. eupatoria	100 Aa	122 Aa	133 Aa	156 Aa	167 Ab	100 Aa	100 Aa	111 Aa	156 Aa	189 Ab	189 Ab	189 Aa
C. glomerata	100 Aa	100 Aa	100 Aa	100 Aa	100 Aa	89 Aa	100 Aa	100 Aa	100 Aa	100 Aa	100 Aa	89 Aa
C. vulgare	100 Aa	129 Aa	143 Aa	169 Aa	231 Aa	363 Aa	100 Aa	154 Aa	354 Bb	391 Bb	537 Bb	751 Aa
G. verum	100 Aa	171 Ab	269 Ab	359 Ab	378 Ab	310 Ab	100 Aa	201 Ab	420 Bb	459 Bb	563 Bb	499 Bb
H. nummularium	100	100	100	100	100	0	100	100	100	100	100	56
H. radicata	100 Aa	103 Aa	100 Aa	83 Aa	93 Aa	192 Aa	100 Aa	94 Aa	94 Aa	97 Aa	103 Aa	132 Aa
K. arvensis	100 Aa	105 Aa	110 Aa	105 Aa	105 Aa	290 Ab	100 Aa	120 Aa	127 Aa	180 Ab	187 Ab	528 Ab
L. autumnalis	100 Aa	109 Aa	112 Aa	118 Aa	124 Aa	189 Aa	100 Aa	121 Aa	124 Aa	133 Aa	158 Ab	278 Ab
L. vulgare	100	100	100	100	100	867 A	100	100	100	100	100	1021 A
L. vulgaris	100 Aa	128 Aa	146 Aa	224 Ab	215 Ab	250 Ab	100 Ba	212 Ab	356 Ab	521 Ab	506 Bb	924 Bb
L. corniculatus	100 Aa	150 Ab	360 Ab	832 Ab	920 Ab	452 Aa	100 Aa	165 Ab	460 Ab	963 Ab	991 Ab	513 Aa
O. vulgare	100 Aa	105 Aa	112 Aa	158 Aa	244 Aa	87 Aa	100 Aa	154 Aa	150 Aa	172 Aa	265 Aa	356 Aa
P. aurantiaca	100 Aa	278 Aa	400 Ab	522 Ab	500 Ab	646 Ab	100 Aa	182 Aa	618 Bb	691 Bb	736 Bb	1145 Bb
P. officinarum	100 Aa	154 Aa	285 Ab	300 Ab	300 Ab	162 Aa	100 Aa	89 Aa	356 Bb	472 Bb	450 Bb	156 Aa
P. veris	100 Aa	98 Aa	68 Aa	64 Aa	64 Aa	17 Ab	100 Aa	98 Aa	98 Aa	80 Aa	64 Ab	51 Ab
S. columbaria	100 Aa	100 Aa	110 Aa	110 Aa	110 Aa	522 Ab	100 Aa	100 Aa	111 Aa	278 Ab	300 Bb	700 Ab

Different capital letters indicate significant difference at p=0.05 (Mann-Whitney U-test) between substrate types for the same month. Different lower-case letters indicate significant difference at p=0.05 (Mann-Whitney U-test after Kruskal-Wallis test) between original number and each month for the same substrate type.

they maintained at least 50 % of the original numbers. *C. glomerata* exhibited same pattern at both substrates, which maintained 100 % of the initial number into the first year; however by the second year it declined into 89 %. As mentioned above, although *O. vulgare* showed no statistically significant response at either substrate over the period, the species in the Zinco substrate increased more than 200 % of the original numbers into the first year and declined in number by the second year, whilst it increased abundance throughout the 2-year period in the Limestone-based substrate.

For the final abundance in May 2008, 14 out of the 17 species had higher abundance in Limestone-based substrate than in Zinco commercial substrate. 4 of these species, *A. millefolium*, *G. verum*, *L. vulgaris*, and *P. aurantiaca*, had a significant difference between substrate types. However, in the case of *H. radicata* and *P. officinarum* in the Zinco substrate, abundance was higher than in the Limestone-based substrate, although it was not a statistically significant response.

5.4.1.5 Effect of fertiliser application

The percentage abundance of each species in response to fertiliser application across the 2 years of the experiment is shown in Table 5.4. There was a similarity in the response across the species to both fertiliser treatments over time. The majority of the species responded with the maintenance of the original numbers or increase in number across the 2 years, and the greatest abundance appeared in the second year at both fertiliser treatments. All species survived at both fertiliser treatments over the 2-year period of the experiment. 15 out of the 17 species at non-fertiliser treatment and 16 species at fertiliser treatment maintained 50 % or more of their original number. Of these species, 10 species without fertiliser application and 12 species with it increased significantly in abundance over the period. *A. eupatoria* at non-fertiliser treatment maintained their original number over the period, while at fertiliser treatment it exhibited a significant increase with ($P < 0.001$). *C. glomerata* did not show significant response at either fertiliser treatment throughout the 2-year period. 3 species, *H. nummularium*, *O. vulgare*, and *P. veris* declined significantly over the 2-year period at both fertiliser treatments,

Table 5.4. Abundance of individual species in response to fertiliser treatments over the period 2007-2008.

	Non-fertiliser treatment						Fertiliser treatment					
	2007					2008	2007					2008
	Jun.	Jul.	Aug.	Sep.	Oct.	May	Jun.	Jul.	Aug.	Sep.	Oct.	May
A. millefolium	100 Aa	110 Aa	117 Aa	134 Aa	200 Ab	153 Aa	100 Aa	100 Ab	247 Bb	427 Bb	510 Bb	932 Bb
A. eupatoria	100 a	100 a	122 a	133 Aa	122 Aa	100 a	100	111	200	211 B	211 B	144
C. glomerata	100 a	100 a	100 a	100 a	89 Aa	100 a	100 a	100 a	100 a	100 a	78 Aa	100 a
C. vulgare	100 Aa	126 Aa	126 Aa	196 Ab	299 Ab	491 Ab	100 Aa	154 Ab	402 Bb	498 Bb	1306 Bb	1013 Ab
G. verum	100 Aa	191 Ab	265 Ab	334 Ab	301 Ab	352 Ab	100 Aa	296 Bb	674 Bb	1245 Bb	1588 Bb	856 Bb
H. nummularium	100	100	100	100	100	67 A	100	100	100	100	100	67 A
H. radicata	100 Aa	94 Aa	89 Aa	92 Aa	92 Aa	874 Ab	100 Aa	100 Aa	96 Aa	89 Aa	107 Aa	180 Bb
K. arvensis	100 Aa	105 Aa	110 Aa	105 Aa	100 Aa	289 Ab	100 Aa	100 Aa	433 Bb	527 Bb	733 Bb	890 Ab
L. autumnalis	100 Aa	97 Aa	103 Aa	111 Aa	109 Aa	453 Ab	100 Aa	131 Aa	128 Aa	203 Bb	219 Bb	567 Ab
L. vulgare	100	100	100	100	100	2293 A	100	100	100	100	100	3375 A
L. vulgaris	100 Aa	138 Aa	163 Aa	171 Aa	87 Aa	237 Ab	100 Ba	546 Bb	750 Bb	794 Bb	922 Bb	1341 Bb
L. corniculatus	100 Aa	184 Ab	562 Ab	754 Ab	762 Ab	621 Ab	100 Aa	290 Ab	663 Ab	880 Ab	1110 Ab	802 Ab
O. vulgare	100 Aa	124 Aa	106 Aa	118 Aa	116 Aa	31 Ab	100 Aa	130 Aa	88 Aa	79 Aa	85 Aa	21 Ab
P. aurantiaca	100	289 A	344 A	433 A	433 A	964 A	100	867 A	1113 A	1211 A	1178 A	1760 A
P. officinarum	100 Aa	200 Aa	264 Ab	293 Ab	307 Ab	107 Aa	100 Aa	436 Bb	929 Bb	921 Bb	786 Bb	79 Aa
P. veris	100 Aa	61 Ab	43 Ab	58 Ab	35 Ab	31 Ab	100 Aa	90 Aa	69 Aa	50 Bb	45 Ab	60 Aa
S. columbaria	100	100	122 A	133 A	133 A	813 A	100	100	567 A	656 A	789 A	920 A

Different capital letters indicate significant difference at p=0.05 (Mann-Whitney U-test) between substrate types for the same month. Different lower-case letters indicate significant difference at p=0.05 (Mann-Whitney U-test after Kruskal-Wallis test) between original number and each month for the same substrate type.

except for *P. veris* with fertiliser application. *O. vulgare* at both fertiliser treatments and *P. veris* at non-fertiliser treatment were recorded with fewer than 50 % of their original numbers. For the second growing season, of 3 species that showed higher abundance at the non-fertiliser treatment than at the fertiliser treatment, *H. radicata* exhibited a significant difference (P = 0.0013).

5.4.2 Plant growth of individuals

As in Chapter 4, in order to cancel out variations in the initial size of planting stock, the mean total dry weight (g) of individual species was converted to a percentage of the mean total dry weight in 100 mm depth in 2007 and 2008 [Tables 5.5 and 5.6]. The dry weight (g) of species in 2008 was converted to a percentage of their mean total dry weight in 2007 [Table 5.7]. For dry weight in substrate type and fertiliser treatment was converted to a percentage of the mean total dry weight in Zinco substrate in 2007 and in non-fertiliser treatment in 2007 respectively [Tables 5.8 and 5.9]. For statistical analysis, Kruskal-Wallis test was used to investigate significant differences between data set and

after that Mann-Whitney *U*-test was carried out to find where the differences were (Dytham, 2003).

5.4.2.1 Effect of substrate depth and supplementary watering

5.4.2.1.1 Growth of individual species in 2007

The effects of substrate depth and supplementary watering on plant growth (assessed by dry weight) of individual species across the 2-year period of the experiment are shown in Tables 5.5 and 5.6 respectively. It was shown that although substrate depth and/or supplementary watering had a significant effect on growth of plant community, there were substantial variations in the response of individual species.

In the first growing season, *C. glomerata* and *P. veris* were not significantly affected by substrate depth in either watering treatment, while in *C. vulgare* no significant response to substrate depth was shown in watering treatment. Except for the above 3 species, substrate depth had a significant effect on dry weight of the species. In the non-watering treatment, of the species, 7 species, which were *H. nummularium*, *H. radicata*, *L. autumnalis*, *L. vulgare*, *L. corniculatus*, *P. aurantiaca*, and *P. officinarum*, had significantly the greatest dry weight at 200 mm depth and the lowest at 50 mm depth, with 100 mm depth intermediate and significantly different to both 50 mm and 200 mm depths. In the other 7 species, *A. millefolium*, *C. vulgare*, *G. verum*, *K. arvensis*, *L. vulgaris*, *O. vulgare*, and *S. columbaria* showed significantly higher dry weight at 100 mm and 200 mm depths than at 50 mm depth, but no significant differences were revealed between 100 mm and 200 mm depths.

In the watering treatment, 6 species, *A. millefolium*, *K. arvensis*, *L. autumnalis*, *L. vulgare*, *L. vulgaris*, and *L. corniculatus*, were significantly greater at 200 mm depth than at 50 mm and 100 mm depths. Dry weights of the species at 100 mm depth were intermediate, and significantly different to both 50 mm and 200 mm depths. 5 species, *H. nummularium*, *H. radicata*, *O. vulgare*, *P. aurantiaca*, and *S. columbaria*, had

Table 5.5. Dry weight of species in 2007 as a percentage of dry weight in 100 mm depth* in 2007.

	Non- watering treatment			Watering treatment			
	50 mm	100 mm*	200 mm	50 mm	100 mm	200 mm	*P* - value [1]
A. millefolium	15.6 a	100.0 c	138.5 c	44.1 b	142.3 c	222.6 d	0.001
	±1.81	±14.3	±29.11	±4.36	±21.11	±36.14	
A. eupatoria	44.7 a	100.0 bc	52.0 a	57.0 ab	108.7 c	72.3 ac	0.003
	±4.87	±19.71	±10.50	±9.87	±13.52	±12.36	
C. glomerata	58.5 a	100.0 ac	115.9 ab	161.0 b	140.2 bc	136.6 ab	0.011
	±13.06	±17.2	±23.43	±13.19	±15.71	±31.42	
C. vulgare	27.6 a	100.0 b	135.3 b	88.2 b	107.8 b	119.0 b	0.001
	±3.46	±25.73	±32.75	±10.35	±21.75	±20.17	
G. verum	42.8 a	100.0 bcd	99.2 cd	59.8 ab	85.1 bc	136.9 d	0.001
	±8.73	±26.78	±12.06	±7.98	±12.85	±16.47	
H. nummularium	29.4 a	100.0 b	228.7 c	100.2 b	195.2 c	316.8 c	0.001
	±6.02	±20.61	±40.50	±10.96	±28.82	±54.08	
H. radicata	34.9 a	100.0 bc	166.3 d	67.3 b	133.9 cd	221.4 d	0.001
	±6.54	±12.64	±16.69	±5.06	±23.52	±42.13	
K. arvensis	43.2 a	100.0 b	141.4 b	64.5 a	121.6 b	217.8 c	0.001
	±5.34	±11.47	±24.34	±7.82	±13.63	±20.88	
L. autumnalis	29.9 a	100.0 b	190.1 c	77.6 b	177.4 c	300.0 d	0.001
	±5.21	±15.92	±27.87	±5.89	±24.74	±44.61	
L. vulgare	23.1 a	100.0 c	164.4 d	49.8 b	143.0 cd	234.3 e	0.001
	±3.36	±15.39	±15.19	±6.29	±18.73	±28.47	
L. vulgaris	20.0 a	100.0 bc	160.9 bc	27.3 a	92.0 b	136.1 c	0.001
	±3.88	±15.82	±31.36	±4.43	±18.72	±11.45	
L. corniculatus	26.2 a	100.0 c	262.8 b	271.7 b	418.7 d	606.5 e	0.001
	±1.66	±9.42	±34.63	±13.26	±27.57	±44.98	
O. vulgare	31.8 a	100.0 bd	162.3 cd	56.8 b	199.1 ce	262.4 e	0.001
	±9.15	±20.40	±29.37	±8.29	±12.52	±34.97	
P. aurantiaca	15.4 a	100.0 c	143.6 d	53.5 b	133.9 cd	162.3 cd	0.001
	±2.24	±13.23	±9.67	±9.04	±24.03	±27.49	
P. officinarum	56.2 a	100.0 b	180.2 c	104.6 ab	192.0 bc	221.5 c	0.001
	±7.22	±10.69	±24.19	±18.29	±55.15	±27.47	
P. veris	58.9 a	100.0 a	174.4 a	108.9 a	100.0 a	78.9 a	0.096
	±9.20	±15.37	±66.90	±13.17	±24.83	±7.35	
S. columbaria	36.3 a	100.0 bd	183.3 cd	68.5 b	199.8 c	211.1 c	0.001
	±4.64	±15.41	±39.10	±7.61	±30.57	±28.02	

The same letters are not statistically different at $p = 0.05$ (Mann-Whitney *U*-test after Kruskal-Wallis test[1]).

statistically same dry weights between 100 mm and 200 mm depths, and the both depths had significantly higher dry weight than 50 mm depth.

In contrast to the above species, in *A. eupatoria*, *G. verum*, and *P. officinarum*, different patterns appeared. For *A. eupatoria*, dry weight was significantly greater at 100 mm depth than at 50 mm and 200 mm depths in the non-watering treatment, and there were no significant differences between the latter two depths. While in the watering treatment,

dry weight of *A. eupatoria* at 200 mm depth was statistically the same at the ones at both 50 mm and 100 mm depths. *G. verum* in the watering treatment showed that 200 mm depth had significantly higher dry weight than 50 mm and 100 mm depths, and there were no significant differences between the latter two depths. In the case of *P. officinarum,* dry weight at 200 mm depth was significantly greater than at 50 mm depth. 100 mm depth was not statistically different to either 50 mm and 200 mm depths (Table 5.5).

For the two watering regimes comparison at the same substrate depths, 5 species, *A. eupatoria, G. verum*, *L. vulgaris, P. officinarum,* and *P. veris,* were not significantly affected by supplementary watering at all substrate depths, while 3 species, *L. autumnalis*, *L. corniculatus,* and *O. vulgare*, showed significant response to watering treatment at all depths. In *C. glomerata, C. vulgare*, *H. radicata,* and *P. aurantiaca,* significant differences were revealed at 50 mm depth only. *K. arvensis* showed a significant difference at 200 mm depth, for *A. millefolium* and *L. vulgare* at 50 mm and 200 mm, and for *H. nummularium* and *S. columbaria* at 50 mm and 100 mm depths. All the species that had significant response to watering treatment at any depth showed significantly higher dry weight in the watering treatment.

5.4.2.1.2 Growth of individual species in 2008

In the second growing season, 7 species, *A. millefolium, A. eupatoria, C. glomerata, G. verum, O. vulgare, P. officinarum,* and *P. veris,* did not survive at 50 mm depth by the end of the season, whereas in the watering treatment, all species survived at all depths. In the case of *H. nummularium,* plants did not survive at 50 mm depth in the non-watering treatment, while in the watering treatment it did not survive at 100 mm and 200 mm depths. A Kruskal-Wallis test on data in Table 5.6 revealed that in 3 species, *C. vulgare, H. nummularium*, and *H. radicata,* substrate depth did not have a significant effect on dry weight in either watering treatment. *C. glomerata* did not show a significant response to substrate depths in the watering treatment. *L. corniculatus* and *S. columbaria* in the non- watering treatment and *A. millefolium* in the watering treatment

Table 5.6. Dry weight of species in 2008 as a percentage of dry weight in 100 mm depth* in 2008.

	Non- watering treatment			Watering treatment			
	50 mm	100 mm*	200 mm	50 mm	100 mm	200 mm	*P* - value [1]
A. millefolium	0.0 —	100.0 b	156.2 bc	22.3 a	115.2 b	233.2 c	0.001
	±0.00	±15.82	±27.09	±5.65	±15.22	±35.72	
A. eupatoria	0.0 —	100.0 b	67.4 ab	27.2 a	112.8 b	135.8 b	0.001
	±0.00	±18.37	±14.81	±8.86	±20.81	±39.43	
C. glomerata	0.0 —	100.0 a	923.1 b	253.8 ac	546.2 cd	1553.8 bc	0.001
	±0.00	±43.34	±308.75	±110.07	±177.05	±625.33	
C. vulgare	16.4 a	100.0 a	41.8 a	33.0 a	69.0 a	94.6 a	0.082
	±10.80	±47.69	±12.42	±6.20	±25.91	±56.54	
G. verum	0.0 —	100.0 ab	185.8 b	54.7 a	107.2 b	190.8 b	0.001
	±0.00	±34.21	±32.18	±10.60	±20.74	±40.82	
H. nummularium	0.0 —	100.0 a	80.2 a	35.2 a	0.0 —	0.0 —	0.290
	±0.00	±86.98	±80.25	±24.57	±0.00	±0.00	
H. radicata	13.7 a	100.0 a	29.8 a	19.7 a	22.9 a	16.8 a	0.172
	±6.07	±39.77	±7.18	±6.85	±8.92	±2.41	
K. arvensis	1.9 a	100.0 bc	144.8 c	41.7 b	118.3 bc	146.1 c	0.001
	±1.90	±32.68	±34.38	±5.58	±35.62	±41.31	
L. autumnalis	9.8 a	100.0 bc	216.1 cd	54.2 b	195.3 bcd	437.0 d	0.001
	±7.46	±35.66	±72.85	±17.37	±62.46	±142.20	
L. vulgare	8.8 a	100.0 bc	187.2 c	54.1 b	131.4 bc	360.6 d	0.001
	±3.43	±25.16	±40.91	±10.21	±31.81	±50.97	
L. vulgaris	10.0 a	100.0 bc	156.6 c	14.6 a	51.3 b	93.0 bc	0.001
	±6.35	±28.77	±36.65	±5.11	±16.81	±25.07	
L. corniculatus	2.7 a	100.0 c	300.0 e	18.1 b	188.5 d	279.4 de	0.001
	±0.80	±19.32	±16.75	±3.48	±23.45	±28.47	
O. vulgare	0.0 —	100.0 ab	138.4 ab	90.6 a	326.7 b	417.0 ab	0.005
	±0.00	±61.93	±70.56	±87.76	±111.50	±145.81	
P. aurantiaca	32.1 a	100.0 bc	276.4 c	55.3 ab	158.2 bc	174.5 c	0.010
	±15.16	±29.72	±81.22	±12.02	±44.22	±62.02	
P. officinarum	0.0 —	100.0 b	1743.5 c	417.4 a	234.8 ab	903.9 c	0.001
	±0.00	±71.48	±639.10	±172.30	±78.53	±155.26	
P. veris	0.0 —	100.0 ab	104.8 ab	9.5 a	42.9 ab	100.0 b	0.024
	±0.00	±50.51	±69.66	±9.52	±30.30	±34.99	
S. columbaria	41.6 a	100.0 b	350.0 c	134.9 b	268.4 c	620.0 c	0.001
	±29.23	±33.96	±95.19	±59.75	±55.21	±227.24	

The same letters are not statistically different at $p = 0.05$ (Mann-Whitney *U*-test after Kruskal-Wallis test[1]).

had significant differences between all substrate depths. 200 mm depth had significantly higher dry weight than 50 mm depth. Dry weights of the species at 100 mm depth were intermediate, and significantly different to both 50 mm and 200 mm depth. While, *A. millefolium*, *K. arvensis*, *L. autumnalis*, *L. vulgare*, *O. vulgare P. aurantiaca*, and *P. veris* in the non-watering treatment, and *L. corniculatus* and *S. columbaria* in the watering treatment, and *A. eupatoria*, *G. verum*, and *L. vulgaris* in both watering

treatments, had statistically the same dry weights between 100 mm and 200 mm depths with significantly higher values than at 50 mm depth. Of the above species, *K. arvensis*, *L. autumnalis*, *P. aurantiaca*, and *P. veris* in the watering treatment, were intermediate at 100 mm depth and not statistically different to either 50 mm and 200 mm depths. In the case of *O. vulgare* in the watering treatment, dry weight at 200 mm depth was intermediate and not statistically different to either 50 mm and 100 mm depths. *L. vulgare* and *P. officinarum* had significantly higher dry weight at 200 mm than 50 mm and 100 mm depth, and no significances were revealed between 50 mm and 100 mm depths.

For the two watering regimes comparison in the same substrate depths, 4 species, *C. vulgare, H. radicata, L. vulgaris*, and *P. aurantiaca*, showed no significant differences between the watering treatments at all substrate depths. *C. glomerata* at 100 mm depth, *K. arvensis* and *L. autumnalis* at 50 mm, and *L. vulgare* at 50 mm and 200 mm, and *L. corniculatus* and *S. columbaria* at 50 mm and 100 mm depths had significantly higher dry weight in the watering treatment.

5.4.2.1.3 Growth of individual species as percentage of dry weight in 2007

Compared to the dry weight in 2007, 5 species of the surviving 9 species at 50 mm depth in non-watering treatment showed significant decrease in dry weight, except for 4 species (*H. radicata, L. vulgaris, P. aurantiaca,* and *S. columbaria*) that did not have significant difference between the two growing seasons. While in the watering treatment, 9 species showed no significant difference between the two years. The species were; *A. millefolium, A. eupatoria, G. verum, K. arvensis, L. autumnalis, L. vulgare, L. vulgaris, P. aurantiaca,* and *S. columbaria* (Table 5.7). At 100 mm depth, in the non-watering treatment, 6 species, *C. glomerata, H. nummularium, O. vulgare, P. aurantiaca, P. officinarum,* and *P. veris* exhibited significant decrease, whereas in the watering treatment, 3 species, *H. radicata, P. officinarum, P, veris*, showed the same. In contrast, *A. millefolium* and *L. corniculatus* at 100 mm and 200 mm in both watering treatments, and *A. eupatoria* and *C. glomerata* at the watered 200 mm depth, and *G. verum* at the

Table 5.7. Dry weight of species in 2008 as a percentage of dry weight in 2007.

	Non- watering treatment			Watering treatment		
	50 mm	100 mm	200 mm	50 mm	100 mm	200 mm
A. millefolium	0.0 —	321.8 ***	387.1 **	158.8 ns	289.4 **	346.4 **
	±0.00	±21.63	±57.00	±35.96	±52.73	±44.09
A. eupatoria	0.0 —	142.8 ns	164.3 ns	55.7 ns	138.6 ns	236.2 *
	±0.00	±28.96	±28.51	±10.90	±19.42	±25.75
C. glomerata	0.0 —	14.2 **	161.7 ns	27.3 **	58.1 ns	167.3 *
	±0.00	±6.36	±41.6	±10.86	±16.36	±59.47
C. vulgare	50.0 *	152.8 ns	36.5 **	48.3 **	81.1 ns	74.3 ns
	±29.27	±54.05	±11.78	±10.61	±27.63	±35.82
G. verum	0.0 —	322.5 ns	441.2 **	209.8 ns	289.8 **	347.5 **
	±0.00	±143.82	±78.64	±32.65	±31.42	±92.66
H. nummularium	0.0 —	18.9 **	5.9 ***	7.2 ***	0.0 —	0.0 —
	±0.00	±15.99	±5.94	±4.83	±0.00	±0.00
H. radicata	63.0 ns	125.1 ns	20.8 ***	39.0 **	23.0 **	12.0 ***
	±27.98	±50.56	±4.03	±14.06	±8.25	±3.44
K. arvensis	14.1 ***	183.6 ns	214.3 ns	136.9 ns	194.2 ns	134.3 ns
	±14.14	±37.80	±52.58	±7.66	±51.76	±26.47
L. autumnalis	73.2 **	84.4 ns	155.4 ns	64.1 ns	98.4 ns	124.8 ns
	±64.54	±25.15	±73.80	±20.37	±32.59	±41.06
L. vulgare	39.3 **	96.2 ns	120.4 ns	149.2 ns	114.2 ns	168.8 ns
	±11.80	±16.21	±26.55	±45.94	±28.25	±20.66
L. vulgaris	279.4 ns	319.5 ns	373.8 **	232.8 ns	201.3 ns	260.9 ns
	±144.63	±72.96	±52.83	±86.11	±53.69	±61.75
L. corniculatus	46.9 **	426.9 *	593.3 ***	31.5 ***	210.0 **	210.2 ***
	±15.47	±73.84	±87.92	±6.82	±28.29	±21.06
O. vulgare	0.0 —	37.0 *	45.3 *	44.6 **	76.5 ns	67.4 ns
	±0.00	±24.25	±24.45	±43.05	±25.73	±19.53
P. aurantiaca	121.4 ns	51.3 *	112.3 ns	72.6 ns	61.0 ns	50.3 ns
	±47.72	±9.71	±32.90	±21.53	±15.25	±12.48
P. officinarum	0.0 —	3.5 ***	40.0 **	19.9 ***	6.1 ***	17.3 ***
	±0.00	±2.54	±12.84	±9.55	±2.92	±4.62
P. veris	0.0 —	24.9 **	16.0 **	2.0 ***	13.9 **	31.0 **
	±0.00	±11.05	±12.58	±2.02	±9.42	±9.93
S. columbaria	101.5 ns	122.6 ns	234.3 ns	230.1 ns	154.4 ns	288.3 ns
	±76.76	±36.19	±45.62	±109.42	±24.10	±77.99

Significant differences (Mann-Whitney *U*-test) between dry weight of 2007 and 2008 are indicated by: **P* = 0.005; ***P* = 0.01; ****P* = 0.001; ns, not significant.

non-watered 200 mm and the watered 100 mm and 200 mm depth, *L. vulgaris* at the non-watered 200 mm depth increased significantly in dry weight. At 200 mm depth, species that showed significant decrease were; *C. vulgare*, *H. nummularium*, and *O. vulgare* in the non-watering treatment, and *H. radicata*, *P. officinarum*, and *P. veris* in both watering treatments.

5.4.2.2 Effect of substrate type

In 2007, the majority of the species (12 out of 17) had significantly higher dry weight in the Limestone-based substrate than the Zinco substrate, whereas in 2008, only 2 species, *C. glomerata* and *K. arvensis*, exhibited this trend. *P. veris* in 2007 and *L. corniculatus* in 2008 showed the opposite tendency. In 2007, 4 species, *A. eupatoria*, *H. nummularium*, *L. corniculatus*, and *O. vulgare*, were not affected by substrate type, while in 2008, 13 species exhibited this tendency.

Throughout the two growing seasons, dry weights of 5 species, *H. radicata*, *L. autumnalis*, *P. aurantiaca*, *P. officinarum*, and *P. veris*, decreased significantly in both substrates, while *A. millefolium* showed a significant increase. 6 species, *A. eupatoria*, *C. vulgare*, *K. arvensis*, *L. vulgare*, *O. vulgare*, and *S. columbaria*, did not show significant difference in either substrate between the two growing seasons. *G. verum* and *L. vulgaris* in the Zinco substrate increased significantly, whereas they did not show

Table 5.8. Dry weight of species in 2007 and 2008 as a percentage of dry weight in Zinco substrate treatment* in 2007.

	2007				2008				
	Zinco*		Lime		Zinco		Lime		
	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	*P* - value [1]
A. millefolium	100.0 a	14.86	212.8 b	31.88	352.7 bc	57.56	444.8 c	62.57	< 0.001
A. eupatoria	100.0 a	21.92	169.0 a	36.80	137.2 a	37.99	275.4 a	83.27	0.087
C. glomerata	100.0 a	32.48	556.6 b	119.16	260.0 a	157.01	1681.0 c	732.75	< 0.001
C. vulgare	100.0 a	18.87	471.4 b	79.79	145.4 a	44.94	367.5 ab	141.41	0.007
G. verum	100.0 a	10.69	408.5 b	48.20	355.1 b	114.19	539.2 b	168.62	0.003
H. nummularium	100.0 a	19.46	75.4 a	5.47	0.0 —	0.00	21.3 b	9.97	< 0.001
H. radicata	100.0 a	9.65	164.0 b	27.51	56.4 c	26.75	44.0 c	12.33	< 0.001
K. arvensis	100.0 a	13.21	289.5 bc	52.31	186.1 ab	41.32	358.9 c	60.95	0.002
L. autumnalis	100.0 a	11.04	283.6 b	36.89	47.2 c	10.19	126.2 ac	38.58	< 0.001
L. vulgare	100.0 a	9.23	601.1 b	224.84	175.2 a	33.99	297.3 ab	79.42	0.002
L. vulgaris	100.0 a	17.42	308.1 b	61.34	224.8 b	47.90	335.6 ab	93.31	0.053
L. corniculatus	100.0 a	17.94	90.6 a	12.67	170.1 b	21.09	30.7 c	6.28	< 0.001
O. vulgare	100.0 a	23.46	236.8 a	62.53	62.0 a	34.84	195.5 a	91.14	0.106
P. aurantiaca	100.0 a	10.34	291.1 b	38.96	55.4 c	14.92	149.6 ac	45.14	< 0.001
P. officinarum	100.0 a	12.84	331.8 b	50.43	6.2 c	2.37	52.1 c	38.36	< 0.001
P. veris	100.0 a	9.69	59.4 b	9.98	1.9 c	1.26	11.4 c	6.85	< 0.001
S. columbaria	100.0 a	14.57	333.0 b	67.71	280.5 ab	96.06	455.4 b	102.32	0.007

Zinco: Zinco substrate treatment; Lime: Limestone-based substrate treatment.
The same letters are not statistically different at $p = 0.05$ (Mann-Whitney *U*-test after Kruskal-Wallis test[1]).

significant difference in the Limestone substrate between the two years. *C. glomerata* exhibited the opposite pattern, increasing in the Limestone substrate and having no significant response in the Zinco substrate. In the second season, *H. nummularium* did not survive in the Zinco substrate and decreased significantly in the Limestone substrate. *L. corniculatus* increased significantly in the Zinco substrate, while it decreased in the Limestone substrate.

5.4.2.3 Effect of fertiliser application

In 2007, 12 species except for 5 species, *C. glomerata*, *H. nummularium*, *L. corniculatus*, *O. vulgare*, and *P. veris*, had significantly higher dry weight in fertiliser treatment than in non-fertiliser treatment, while in 2008, 6 species exhibited this trend. In contrast, *L. corniculatus* showed the opposite tendency, having higher dry weight in the non-fertiliser treatment.

Table 5.9. Dry weight of species in 2007 and 2008 as a percentage of dry weight in non-fertiliser* in 2007.

	2007				2008				
	NF*		F		NF		F		
	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	*P* - value [1]
A. millefolium	100.0 a	14.46	2734.7 b	377.98	221.6 b	38.27	3720.1 c	376.36	< 0.001
A. eupatoria	100.0 a	20.10	364.3 b	56.76	198.4 ac	39.87	415.4 bc	107.15	0.003
C. glomerata	100.0 a	24.96	877.8 a	391.94	243.8 a	131.71	1381.3 a	895.49	0.259
C. vulgare	100.0 a	11.94	1768.4 b	425.27	138.5 a	39.64	1015.5 b	381.63	< 0.001
G. verum	100.0 a	14.17	1487.0 b	223.99	388.1 c	42.96	2524.2 b	750.11	< 0.001
H. nummularium	100.0 a	17.79	183.6 a	46.09	38.0 b	21.28	121.4 ab	66.01	0.014
H. radicata	100.0 a	15.76	609.4 b	188.48	92.5 a	29.46	62.4 a	23.33	0.001
K. arvensis	100.0 a	14.82	1912.1 b	400.20	158.5 c	21.72	1571.2 b	271.43	< 0.001
L. autumnalis	100.0 a	13.10	912.6 b	146.05	83.9 a	14.99	75.6 a	22.10	< 0.001
L. vulgare	100.0 a	15.58	2133.0 b	189.24	147.7 a	58.09	1342.6 c	168.86	< 0.001
L. vulgaris	100.0 a	12.06	4148.2 b	599.69	166.1 c	20.05	2378.9 b	611.41	< 0.001
L. corniculatus	100.0 a	9.58	119.4 a	26.04	266.4 b	24.50	49.3 c	18.84	< 0.001
O. vulgare	100.0 a	30.14	420.3 ab	243.94	26.8 b	17.89	22.7 b	22.68	0.012
P. aurantiaca	100.0 a	14.00	772.3 b	121.80	62.0 a	15.06	128.4 a	30.11	< 0.001
P. officinarum	100.0 a	20.71	547.5 b	147.73	20.5 c	11.15	30.4 c	30.41	< 0.001
P. veris	100.0 a	25.35	60.6 a	26.52	31.5 a	25.42	246.8 a	157.00	0.130
S. columbaria	100.0 a	9.79	1189.1 b	290.89	344.5 c	85.15	1126.4 abc	484.46	0.002

NF: non-fertiliser treatment; F: fertiliser treatment.
The same letters are not statistically different at $p = 0.05$ (Mann-Whitney U-test after Kruskal-Wallis test[1]).

Throughout the two growing seasons, *A. millefolium* in both fertiliser treatments, *G. verum, K. arvensis, L. vulgaris, L. corniculatus,* and *S. columbaria* in the non-fertiliser treatment increased significantly. In the fertiliser treatment, no species exhibited significantly increased pattern except for *A. millefolium*. In contrast, *P. officinarum* in both treatments, and *H. nummularium* and *O. vulgare* in non-fertiliser treatment, and *H. radicata, L. autumnalis, L. vulgare, L. corniculatus*, and *P. aurantiaca* in fertiliser treatment exhibited significant reductions over the 2-year period.

5.4.3 Plant structural characteristics of individuals

5.4.3.1 Effect of substrate depth and supplementary watering

The plant structural characteristics of individual species (height and coverage) in response to substrate depth and watering treatment are shown in Table 5. 10. Kruskal-Wallis test revealed that substrate depth had a significant effect on the majority of the species. However, there were some exceptions. In the non-watering treatment, *P. veris* showed no significant difference in coverage between substrate depths. In the watering treatment, more species exhibited this tendency: *A. eupatoria, C. vulgare, G. verum, L. corniculatus* and *P. officinarum* in plant height, and *C. glomerata, H. nummularium,* and *P. veris* in both height and coverage. In most of the species, no significant differences were found between 100 mm and 200 mm depths and those depths were significantly higher than 50 mm depth. Exceptionally, *A. eupatoria* showed significant higher plant coverage at 100 mm depth than 50 mm and 200 mm depths. Effects of substrate depth on plant structural characteristics tended to be more clearly revealed in the non-watering treatment compared to one in the watering treatment. 10 species in the non-watering treatment had significantly the greatest plant structures in height and/or in coverage at 200 mm depth and the lowest at 50 mm depth, with 100 mm depth intermediate and significantly different to both 50 mm and 200 mm depths. However, in the watering treatment, only 4 species, *A. millefolium, K. arvensis,* and *L. vulgaris* in coverage, and, *L. vulgare,* exhibited this tendency.

Table 5.10. Mean height (cm) and mean total coverage (%) of individual species in response to substrate depth in non-watering treatment and watering treatment across the 2-year period of the experiment.

		Non-watering treatment							Watering treatment						
		50 mm		100 mm		200 mm			50 mm		100 mm		200 mm		
		Mean	SE	Mean	SE	Mean	SE	*P*-value*	Mean	SE	Mean	SE	Mean	SE	*P*-value*
A. millefolium	Ht. (cm)	2.1 Aa	0.310	4.9 Ab	0.220	6.6 Ab	0.793	0.000	4.2 Ba	0.167	6.3 Bb	0.264	6.0 Ab	0.325	0.000
	Co. (%)	0.68 Aa	0.118	3.56 Ab	0.323	4.91 Ac	0.475	0.000	1.90 Ba	0.153	4.68 Bb	0.387	6.58 Ac	0.593	0.000
A. eupatoria	Ht. (cm)	2.9 Aa	0.449	6.3 Ab	0.929	4.7 Ab	0.384	0.000	5.0 Ba	0.375	7.7 Aa	0.996	5.2 Aa	0.469	0.272
	Co. (%)	0.92 Aa	0.142	1.97 Ab	0.259	1.31 Ab	0.156	0.001	1.42 Ba	0.122	2.08 Ab	0.164	1.27 Aa	0.099	0.000
C. glomerata	Ht. (cm)	0.6 Aa	0.077	1.4 Ab	0.330	1.9 Ac	0.374	0.000	1.3 Ba	0.188	1.2 Aa	0.089	1.5 Aa	0.134	0.093
	Co. (%)	0.24 Aa	0.037	0.31 Aa	0.031	0.53 Ab	0.037	0.000	0.35 Ba	0.028	0.43 Ba	0.035	0.45 Aa	0.045	0.423
C. vulgare	Ht. (cm)	2.9 Aa	0.413	7.7 Ab	0.635	7.2 Ab	0.599	0.000	7.2 Ba	0.622	6.6 Aa	0.521	7.0 Aa	0.563	0.875
	Co. (%)	0.48 Aa	0.069	1.39 Ab	0.138	1.76 Ab	0.165	0.000	1.07 Ba	0.104	1.27 Aa	0.120	1.71 Ab	0.140	0.003
G. verum	Ht. (cm)	2.9 Aa	0.347	5.2 Ab	0.401	5.2 Ab	0.257	0.000	4.7 Ba	0.199	5.0 Aa	0.252	4.9 Aa	0.258	0.945
	Co. (%)	0.74 Aa	0.102	1.89 Ab	0.192	2.30 Ab	0.178	0.000	1.29 Ba	0.097	1.62 Aab	0.133	2.14 Ab	0.177	0.002
H. nummularium	Ht. (cm)	0.9 Aa	0.122	1.6 Ab	0.138	1.9 Ab	0.196	0.000	1.8 Ba	0.155	1.9 Aa	0.150	1.8 Aa	0.180	0.390
	Co. (%)	0.47 Aa	0.073	1.21 Ab	0.132	2.52 Ac	0.334	0.000	1.33 Ba	0.177	2.08 Aa	0.311	2.85 Aa	0.457	0.116
H. radicata	Ht. (cm)	3.0 Aa	0.647	7.0 Ab	1.285	6.6 Ab	1.215	0.000	1.8 Aa	0.089	5.3 Ab	1.017	3.8 Ab	0.586	0.000
	Co. (%)	1.07 Aa	0.163	2.48 Ab	0.265	2.94 Ab	0.319	0.000	0.97 Aa	0.062	2.13 Ab	0.234	3.23 Ab	0.384	0.000
K. arvensis	Ht. (cm)	1.5 Aa	0.182	3.3 Ab	0.200	3.8 Ab	0.209	0.000	2.4 Ba	0.155	3.2 Ab	0.229	3.6 Ab	0.226	0.000
	Co. (%)	0.94 Aa	0.124	2.35 Ab	0.163	2.62 Ab	0.198	0.000	1.47 Ba	0.100	2.6 Ab	0.223	3.36 Bc	0.237	0.000
L. autumnalis	Ht. (cm)	2.0 Aa	0.345	4.3 Ab	0.758	6.0 Ac	0.788	0.000	2.5 Ba	0.126	4.7 Bb	0.659	7.1 Ab	0.824	0.000
	Co. (%)	0.85 Aa	0.105	2.05 Ab	0.189	3.11 Ac	0.310	0.000	1.58 Ba	0.142	3.01 Bb	0.280	3.98 Ab	0.358	0.000
L. vulgare	Ht. (cm)	3.0 Aa	0.311	6.5 Ab	0.610	10.4 Ac	1.337	0.000	3.9 Aa	0.149	6.6 Ab	0.474	7.8 Ac	0.444	0.000
	Co. (%)	2.54 Aa	0.715	5.56 Ab	0.542	6.98 Ac	0.545	0.000	2.71 Ba	0.215	5.77 Ab	0.409	7.9 Ac	0.491	0.000
L. vulgaris	Ht. (cm)	3.6 Aa	0.403	7.6 Ab	0.502	8.7 Ab	0.539	0.000	5.2 Ba	0.227	8.2 Ab	0.376	8.2 Ab	0.440	0.000
	Co. (%)	0.68 Aa	0.097	2.72 Ab	0.288	3.86 Ac	0.362	0.000	1.03 Ba	0.120	2.65 Ab	0.314	3.62 Ac	0.353	0.000
L. corniculatus	Ht. (cm)	3.6 Aa	0.539	6.7 Ab	0.623	7.3 Ab	0.655	0.000	7.6 Ba	0.517	8.9 Ba	0.714	9.3 Ba	0.682	0.108
	Co. (%)	4.26 Aa	0.866	12.3 Ab	1.962	20.2 Ac	2.781	0.000	15.3 Ba	1.860	22.2 Bab	2.783	25.0 Ab	2.778	0.025
O. vulgare	Ht. (cm)	4.2 Aa	0.545	6.4 Ab	0.574	7.6 Ab	0.738	0.001	4.4 Aa	0.388	9.3 Bb	0.762	7.9 Ab	0.511	0.000
	Co. (%)	0.59 Aa	0.072	1.44 Ab	0.176	1.96 Ab	0.200	0.000	0.95 Ba	0.084	2.37 Bb	0.210	2.41 Ab	0.217	0.000
P. aurantiaca	Ht. (cm)	1.72 Aa	0.202	9.2 Ab	1.446	13.8 Ab	1.925	0.000	3.6 Ba	0.441	8.6 Ab	1.279	8.4 Bb	1.304	0.000
	Co. (%)	0.72 Aa	0.105	2.57 Ab	0.271	4.30 Ac	0.419	0.000	1.68 Ba	0.169	3.71 Ab	0.411	3.88 Ab	0.417	0.000
P. officinarum	Ht. (cm)	1.06 Aa	0.125	2.0 Ab	0.378	2.8 Ab	0.442	0.001	1.6 Ba	0.173	2.5 Aa	0.489	2.8 Aa	0.475	0.171
	Co. (%)	0.73 Aa	0.100	1.43 Ab	0.150	2.56 Ac	0.282	0.000	1.44 Ba	0.156	2.68 Aab	0.492	3.08 Ab	0.374	0.012
P. veris	Ht. (cm)	0.9 Aa	0.122	1.4 Ab	0.116	1.4 Ab	0.133	0.016	1.3 Ba	0.083	1.6 Aa	0.149	1.4 Aa	0.093	0.485
	Co. (%)	0.34 Aa	0.051	0.42 Aa	0.055	0.45 Aa	0.065	0.188	0.42 Aa	0.042	0.64 Aa	0.080	0.44 Aa	0.048	0.250
S. columbaria	Ht. (cm)	1.0 Aa	0.084	2.4 Ab	0.646	5.9 Ac	1.491	0.000	1.5 Ba	0.111	6.48 Bb	1.514	3.8 Ab	0.804	0.000
	Co. (%)	0.79 Aa	0.113	1.29 Ab	0.136	1.95 Ac	0.248	0.000	0.99 Ba	0.084	2.32 Bb	0.262	1.96 Ab	0.232	0.000

Ht., Height; Co., Coverage.
*Significant differences at p =0.05 between substrate depths for the same watering treatment (Kruskal-Wallis test). Different capital letters indicate significant difference at p = 0.05 (Mann-Whitney *U*-test after Kruskal-Wallis test) between watering treatments for the same depth. Different lower-case letters indicate significant difference at p = 0.05 (Mann-Whitney *U*-test after Kruskal-Wallis test) between substrate depths for the same watering treatment.

Mann-Whitney *U*-test on data shown in Table 5.10 found that individual species showed significant responses to the watering treatment. In the majority of the species, the plants with watering treatment exhibited significantly higher plant growth in height and/or coverage. 16 species at 50 mm, 6 species (*A. millefolium*, *C. glomerata*, *L. autumnalis*,

L. corniculatus, *O. vulgare*, and *S. columbaria*) at 100 mm, and 3 species (*K. arvensis*, *L. corniculatus*, and *P. aurantiaca*) at 200 mm of the 17 species studied showed the tendency. Exceptionally, *P. aurantiaca* at 200 mm depth exhibited significantly lower height in the watering treatment. However, some species did not have significant response to watering treatment at all substrate depths. The species were *H. radicata* in both of height and coverage, *L. vulgare* in height, and *P. veris* in coverage. *L. corniculatus* showed significant differences in height at all the substrate depths.

5.4.3.2 Effect of substrate type

Mann-Whitney *U*-test reveled differences in the majority of the species between substrate types in terms of mean height and mean total coverage of individual species across the 2-year period of the experiment (Table 5.11). The limestone-based substrate had significantly higher plant height and coverage. Some species, *A. millefolium*, *P. aurantiaca*, *O. vulgare*, and *P. veris*, in height, and *A. eupatoria* in coverage, and *H. nummularium* and *L. corniculatus* in both of height and coverage, did not show significant differences between substrate types.

5.4.3.3 Effect of fertiliser application

The mean height and mean total coverage of individual species with and without fertiliser application are also shown in Table. 5.11. Similar to the effect of substrate type, the majority of the species (16 out of 17 species) exhibited significant differences in plant height and/or coverage. Plants with fertiliser application had significantly higher values than plants with non-fertiliser application. Only 3 species, *O. vulgare* in both plant structural characteristics, and *H. nummularium* and *L. corniculatus*, showed no significant responses to fertiliser treatment.

Table 5.11. Mean height (cm) and mean total coverage (%) of individual species in response to substrate type and fertiliser treatment across the 2-year period of the experiment.

		Substrate type					Fertiliser treatment				
		Zinco		Lime		*P*-value*	NF		F		*P*-value*
		Mean	SE	Mean	SE		Mean	SE	Mean	SE	
A. millefolium	Ht. (cm)	5.9 a	0.25	6.5 a	0.33	0.1557	4.6 a	0.19	18.5 b	1.64	< 0.001
	Co. (%)	4.14 a	0.38	7.89 b	0.70	< 0.001	4.14 a	0.42	25.04 b	2.22	< 0.001
A. eupatoria	Ht. (cm)	5.0 a	0.61	7.2 b	0.98	0.0291	3.7 a	0.44	15.0 b	1.86	< 0.001
	Co. (%)	1.69 a	0.14	2.19 a	0.20	0.1306	1.86 a	0.10	3.86 b	0.29	< 0.001
C. glomerata	Ht. (cm)	1.4 a	0.12	2.3 b	0.22	0.0014	1.2 a	0.08	3.7 b	0.40	< 0.001
	Co. (%)	0.49 a	0.04	1.43 b	0.13	< 0.001	0.65 a	0.04	2.88 b	0.45	< 0.001
C. vulgare	Ht. (cm)	6.4 a	0.50	9.9 b	0.90	0.0140	6.7 a	0.52	13.7 b	1.08	< 0.001
	Co. (%)	1.28 a	0.13	3.76 b	0.40	< 0.001	2.28 a	0.23	11.38 b	1.99	< 0.001
G. verum	Ht. (cm)	5.2 a	0.23	6.8 b	0.38	0.0041	5.2 a	0.26	11.4 b	0.82	< 0.001
	Co. (%)	1.72 a	0.12	5.24 b	0.47	< 0.001	2.39 a	0.16	11.83 b	1.16	< 0.001
H. nummularium	Ht. (cm)	1.8 a	0.17	2.0 a	0.22	0.7982	1.4 a	0.08	2.4 b	0.19	< 0.001
	Co. (%)	2.98 a	0.54	2.44 a	0.30	1.0000	2.97 a	0.53	4.04 a	0.54	0.1511
H. radicata	Ht. (cm)	4.1 a	0.72	12.3 b	1.71	0.0095	4.1 a	0.81	15.6 b	1.81	< 0.001
	Co. (%)	2.51 a	0.19	4.98 b	0.54	0.0260	4.75 a	0.46	9.36 b	1.12	0.0016
K. arvensis	Ht. (cm)	3.0 a	0.18	5.2 b	0.86	0.0084	3.1 a	0.20	18.6 b	2.36	< 0.001
	Co. (%)	2.41 a	0.18	5.96 b	0.52	< 0.001	3.74 a	0.26	16.05 b	1.50	< 0.001
L. autumnalis	Ht. (cm)	5.1 a	0.90	9.6 b	1.18	0.0001	5.8 a	0.79	14.7 b	1.40	< 0.001
	Co. (%)	3.07 a	0.54	6.07 b	0.59	< 0.001	4.01 a	0.36	11.81 b	1.02	< 0.001
L. vulgare	Ht. (cm)	5.2 a	0.23	10.7 b	1.29	< 0.001	5.1 a	0.19	20.0 b	1.55	< 0.001
	Co. (%)	4.06 a	0.25	9.65 b	0.83	< 0.001	5.75 a	0.73	21.62 b	1.71	< 0.001
L. vulgaris	Ht. (cm)	7.6 a	0.48	11.7 b	1.17	0.0320	7.6 a	0.44	20.5 b	1.43	< 0.001
	Co. (%)	2.52 a	0.24	5.87 b	0.68	< 0.001	3.16 a	0.35	22.72 b	2.38	< 0.001
L. corniculatus	Ht. (cm)	10.1 a	0.60	9.6 a	0.64	0.3828	9.3 a	0.64	11.3 b	0.74	0.0441
	Co. (%)	21.54 a	2.65	22.44 a	2.24	0.6630	24.85 a	2.41	22.32 a	2.28	0.4421
O. vulgare	Ht. (cm)	6.9 a	0.68	8.4 a	0.94	0.3963	4.7 a	0.56	5.4 a	0.87	0.5187
	Co. (%)	1.50 a	0.17	3.00 b	0.39	0.0297	1.10 a	0.12	2.39 a	0.49	0.5127
P. aurantiaca	Ht. (cm)	8.1 a	1.14	9.1 a	1.09	0.0613	7.2 a	1.19	12.2 b	1.46	< 0.001
	Co. (%)	3.01 a	0.31	7.88 b	0.76	< 0.001	4.12 a	0.36	14.96 b	1.34	< 0.001
P. officinarum	Ht. (cm)	1.5 a	0.17	3.6 b	0.61	< 0.001	2.6 a	0.47	5.9 b	1.14	0.0152
	Co. (%)	1.63 a	0.17	5.24 b	0.67	< 0.001	2.78 a	0.30	8.36 b	1.23	0.0019
P. veris	Ht. (cm)	1.4 a	0.10	1.8 a	0.23	0.1326	1.5 a	0.11	2.5 b	0.33	0.0023
	Co. (%)	0.40 a	0.05	0.62 b	0.06	0.0058	0.62 a	0.06	0.89 b	0.10	0.0471
S. columbaria	Ht. (cm)	3.3 a	0.97	5.6 b	1.08	0.0040	3.4 a	0.80	14.7 b	2.14	< 0.001
	Co. (%)	1.85 a	0.15	4.27 b	0.45	< 0.001	2.54 a	0.21	10.49 b	1.27	< 0.001

Zinco, Zinco commercial substrate; Lime, Limestone-based substrate; NF, non-fertiliser treatment; F, fertiliser treatment.
The same letters in substrate type treatment and fertiliser treatment are not significantly different at $p = 0.05$ (Mann-Whitney *U*-test).

5.4.4 The growth pattern of individual species over time

From the results of the above section, plant structural characteristics exhibited at 100 mm depth with watering treatment tended to be intermediate in range among the treatments. This section deals with plant growth patterns of individual species at the 100

mm substrate depth. Plant growth pattern of each individual species exhibited at all treatments are summarised in detail in Chapter 6 throughout the following analysis.

Changes of plant height and coverage of the individual species over time are shown in Tables 5.12 and 5.13, and Figures 5.3 and 5.4 for the representative species over the first growing season. Kruskal-Wallis test on data in Table 5.12 revealed that 2 species, *L. autumnalis* (P = 0.069) and *P. officinarum* (P = 0.062), had no significant changes in plant height over time. In the case of *H. nummularium* and *H. radicata*, they exhibited this tendency during the first season. Throughout the first season, 10 species showed a significant increase in height and they were stable after reaching maximum growth. These species were; *A. millefolium*, *A. eupatoria*, *C. glomerata*, *C. vulgare*, *G. verum*, *S. columbaria*, *L. vulgare*, *L. vulgaris*, *L. corniculatus*, and *O. vulgare*. The first 6 species reached a peak height in September. The last 4 species did so in August. *P. veris* exhibited a steady decline by the end of the first season. *K. arvensis* and *P. aurantiaca* exhibited a hump-backed pattern over the first season, increasing steadily by August, and levelling off. 8 species were recorded with maximum heights of more than 10 cm, 5 species between 5 cm and 10 cm, and 4 species with less than 5 cm. In maximum height, *P. aurantiaca* exhibited the highest height, followed by *L. corniculatus*, and *S. columbaria*. *C. glomerata* was the species with the lowest height.

In the second growing season, 4 species (*A. eupatoria*, *C. vulgare*, *H. radicata*, and *O. vulgare*) showed significantly lower heights compared to the initial height in the first season, whereas 8 species (*A. millefolium*, *C. glomerata*, *G. verum*, *K. arvensis*, *L. vulgare*, *L. vulgaris*, *P. aurantiaca*, and *S. columbaria*) exhibited the opposite tendency. 4 species (*L. autumnalis*, *L. corniculatus*, *P. officinarum*, and *P. veris*) statistically recovered the initial height. *H. nummularium* had completely disappeared in the second season.

For change of coverage of individual species, the majority of the species (15 out of 17 species) exhibited a significant increase pattern over the first growing season, whereas *A. eupatoria* did not change significantly. *P. veris* exhibited a hump-backed pattern over

Table 5.12. Mean height (cm) of individual species in response to 100 mm depth in watering treatment over time.

Watering treatment	2007										2008		
100 mm	Jun.		Jul.		Aug.		Sep.		Oct. 07		Apr. 08		*P*- value[1]
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	
A. millefolium	4.2 a	0.236	5.4 b	0.587	6.4 bc	0.355	7.5 c	0.446	6.6 bc	0.530	7.6 c	0.817	0.001
A. eupatoria	7.1 a	0.623	4.9 b	0.428	8.7 ab	1.920	11.5 ab	3.610	11.5 ab	3.690	2.2 c	0.160	0.001
C. glomerata	0.8 a	0.075	0.9 a	0.096	1.2 b	0.127	1.4 b	0.175	1.2 b	0.132	1.9 b	0.377	0.003
C. vulgare	3.2 a	0.320	5.7 b	0.491	9.5 c	1.170	10.1 c	1.070	8.8 c	0.979	2.4 d	0.354	0.001
G. verum	2.9 a	0.315	4.4 b	0.471	5.5 bc	0.559	5.6 bc	0.574	5.1 bc	0.560	6.4 c	0.568	0.001
H. nummularium	2.3 a	0.287	2.1 a	0.180	2.1 a	0.138	2.5 a	0.430	2.2 a	0.227	0.0 —	0.000	0.001
H. radicata	2.5 a	0.274	2.3 a	2.907	5.8 a	3.423	10.3 a	3.475	4.1 a	1.119	1.5 b	0.201	0.021
K. arvensis	2.1 a	0.249	2.9 ab	0.538	3.6 b	0.507	2.9 ab	0.529	2.6 ab	0.341	5.3 c	0.500	0.001
L. autumnalis	2.6 a	0.345	2.9 a	0.211	9.7 a	3.165	5.1 a	1.590	3.9 a	0.360	3.8 a	0.411	0.069
L. vulgare	4.5 a	0.361	6.8 bc	0.460	8.1 bc	2.448	6.3 b	0.551	6.3 bc	0.675	7.8 c	0.899	0.005
L. vulgaris	4.7 a	0.451	7.3 b	0.623	10.1 c	0.778	10.0 c	0.527	8.0 bc	1.130	9.4 c	0.360	0.001
L. corniculatus	3.3 ab	0.283	4.6 b	0.448	14.9 c	1.130	10.4 d	0.393	13.0 cd	1.510	7.4 abd	1.690	0.001
O. vulgare	4.7 a	0.548	8.4 b	0.932	13.7 c	1.520	13.0 c	1.450	13.6 c	1.440	2.5 d	0.624	0.001
P. aurantiaca	1.9 a	0.107	6.2 bc	0.444	20.0 bc	5.100	7.7 b	1.460	10.3 bc	3.490	5.2 c	0.385	0.001
P. officinarum	1.4 a	0.097	1.8 a	0.285	7.3 a	2.360	1.9 a	0.386	1.6 a	0.155	1.0 a	0.330	0.062
P. veris	1.8 a	0.125	2.2 a	0.205	1.4 ab	0.415	1.1 b	0.180	1.1 b	0.151	2.1 ab	0.675	0.019
S. columbaria	2.0 ab	0.331	1.4 a	0.165	3.2 bc	0.688	14.8 bc	5.960	13.7 bc	5.640	3.8 c	0.436	0.008

[1] Significant differences at $p = 0.05$ between the initial height and each month (Kruskal-Wallis test).
Different low-case letters indicate significant difference (Mann-Whitney *U*-test) between months for the same species.

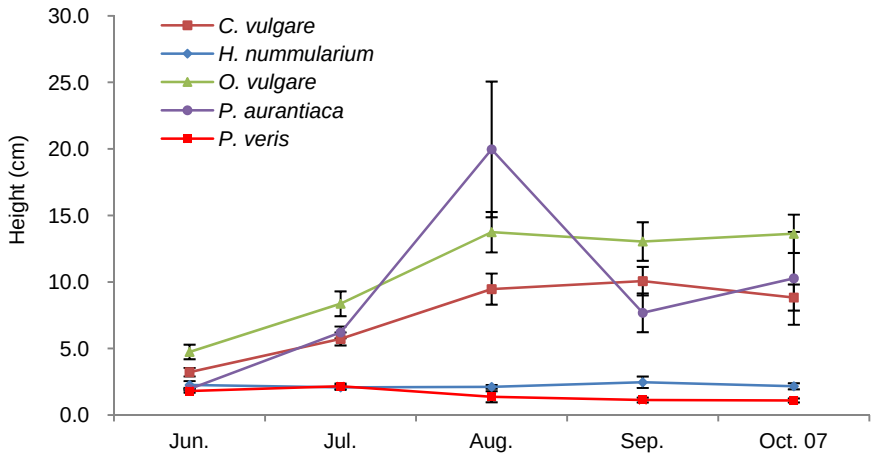


Figure 5.3. Change of height of the representative individual species over the first growing season (2007). Error bars represent standard error.

the season, increasing by July, and then declining back to the initial coverage. Of the species that had a significant increase in coverage, 12 species reached peak coverage in October, 2 species (*H. radicata* and *L. vulgare*) in August, and *P. officinarum* in September. *L. corniculatus* was the largest species (47.91%) in maximum coverage. 6 species had coverage ranging from 5.00 % to 10.00 %, and 10 species with less than

Table 5.13. Mean total coverage (%) of individual species in response to 100 mm depth in watering treatment over time.

Watering treatment	2007										2008		
	Jun.		Jul.		Aug.		Sep.		Oct. 07		Apr. 08		*P*- value[1]
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	
A. millefolium	0.57 a	0.046	4.19 b	0.505	4.76 b	0.480	5.10 bc	0.939	7.41 c	0.832	6.05 bc	0.742	0.001
A. eupatoria	2.12 a	0.237	2.78 a	0.403	2.73 a	0.314	2.53 a	0.251	2.14 a	0.287	0.15 b	0.040	0.001
C. glomerata	2.51 a	0.037	4.20 b	0.055	4.20 b	0.050	5.30 b	0.066	5.91 b	0.101	0.89 c	0.043	0.001
C. vulgare	0.58 a	0.115	1.27 b	0.182	1.98 c	0.175	1.90 c	0.275	1.99 c	0.285	0.30 d	0.094	0.001
G. verum	0.52 a	0.066	1.54 b	0.211	1.97 b	0.213	2.06 bc	0.202	2.86 c	0.304	0.79 a	0.070	0.001
H. nummularium	0.56 a	0.084	1.13 b	0.147	2.07 c	0.213	3.23 cd	0.497	5.50 bd	0.974	0.00 —	0.000	0.001
H. radicata	0.77 a	0.071	2.81 b	0.291	3.58 b	0.551	3.18 b	0.567	3.10 b	0.709	0.44 c	0.146	0.001
K. arvensis	1.32 a	0.161	2.52 b	0.204	3.34 bc	0.395	3.69 bc	0.483	4.19 c	0.484	0.55 d	0.138	0.001
L. autumnalis	1.18 a	0.170	3.07 b	0.267	3.79 bc	0.388	4.57 cd	0.463	5.64 d	0.413	0.40 e	0.087	0.001
L. vulgare	2.72 a	0.403	6.40 b	0.501	8.06 b	0.689	7.87 b	0.622	7.81 b	0.812	2.14 a	0.413	0.001
L. vulgaris	0.28 a	0.036	3.03 b	0.443	3.62 b	0.550	3.64 b	0.507	5.11 b	0.845	0.24 a	0.096	0.001
L. corniculatus	1.69 a	0.141	9.92 b	0.723	32.22 c	2.178	41.33 d	4.936	47.91 d	6.096	4.31 a	1.456	0.001
O. vulgare	0.95 a	0.163	2.63 b	0.270	3.39 bc	0.380	3.46 c	0.315	3.61 c	0.275	0.20 d	0.097	0.001
P. aurantiaca	0.88 a	0.087	3.38 b	0.464	5.28 c	0.651	6.04 c	0.795	6.84 c	0.878	0.22 d	0.042	0.001
P. officinarum	0.89 a	0.128	2.36 b	0.638	4.09 b	1.423	4.88 b	1.941	3.81 b	1.057	0.05 c	0.026	0.001
P. veris	0.45 a	0.038	1.13 b	0.093	1.04 ab	0.262	0.68 a	0.241	0.49 a	0.128	0.06 c	0.024	0.001
S. columbaria	1.21 a	0.119	2.03 b	0.205	2.44 bc	0.328	3.04 c	0.409	4.69 bc	1.024	0.52 d	0.119	0.001

[1] Significant differences at $p = 0.05$ between the initial height and each month (Kruskal-Wallis test).
Different low-case letters indicate significant difference (Mann-Whitney *U*-test) between months for the same species.

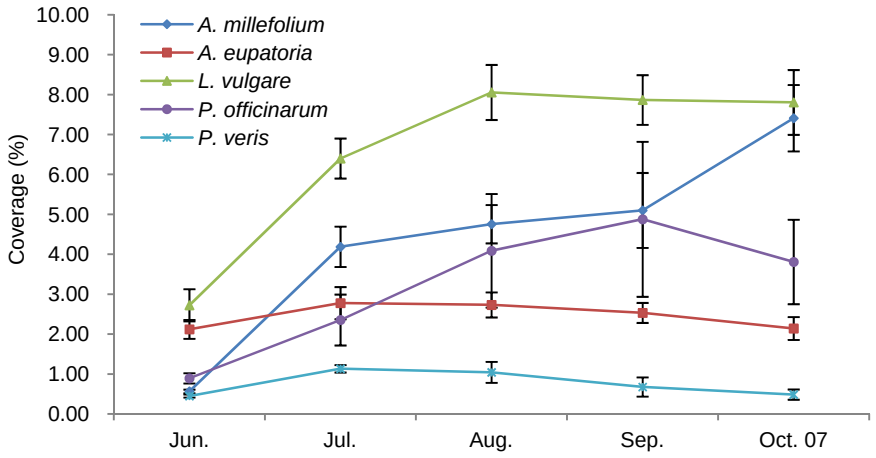


Figure 5.4. Change of coverage of the representative individual species over the first growing season (2007). Error bars represent standard error.

5.00 %. Species with the smallest coverage was *P. veris*. In the second growing season, the majority of the species (11 out of 17 species) had significantly lower coverage than the initial, whereas *A. millefolium* showed significantly increased coverage. 4 species, *G. verum*, *L. vulgare*, *L. vulgaris*, and *L. corniculatus*, had statistically the same coverage to the initial.

5.4.5 Flowering performance of individual species

5.4.5.1 Effect of substrate depth without supplementary watering

Under the non-watering condition, Kruskal-Wallis test on data shown in Table 5.14 found that about half of the species (8 out of 17) showed significant differences between substrate depths in terms of inflorescences number. The species were; *C. vulgare*, *H. radicata*, *L. autumnalis*, *L. vulgare*, *L. vulgaris*, *L. corniculatus*, *P. aurantiaca*, and *P. officinarum*. 7 species, *A. millefolium*, *A. eupatoria*, *C. glomerata*, *G. verum*, *H. nummularium*, *O. vulgare*, and *S. columbaria*, did not have significant response to substrate depths. Better performance in flowering was mostly shown in the deeper substrates. However, there were no significant differences between 100 mm and 200 mm depths in all the species that showed significant response to substrate depths.

Table 5.14. Mean total number of inflorescence of individual species in response to substrate depth in non-watering treatment and watering treatment across the 2-year period of the experiment.

	Non-watering treatment							Watering treatment						
	50 mm		100 mm		200 mm		*P*-value*	50 mm		100 mm		200 mm		*P*-value*
	Mean	SE	Mean	SE	Mean	SE		Mean	SE	Mean	SE	Mean	SE	
A. millefolium	0.0 a	0.00	0.0 a	0.00	0.6 a	0.56	0.368	0.0	0.00	0.0	0.00	0.0	0.00	—
A. eupatoria	0.0 a	0.00	0.1 a	0.11	0.0 a	0.00	0.368	0.0 a	0.00	0.1 a	0.00	0.0 a	0.11	0.368
C. glomerata	0.0 a	0.00	0.1 a	0.11	0.0 a	0.00	0.368	0.1 a	0.11	0.0 a	0.00	0.0 a	0.00	0.368
C. vulgare	0.2 Aa	0.15	1.8 Ab	0.43	1.9 Ab	0.42	0.005	0.9 Ba	0.11	1.0 Aa	0.33	1.3 Aa	0.41	0.766
G. verum	0.0 a	0.00	1.3 a	1.33	0.1 a	0.11	0.594	0.0	0.00	0.0	0.00	0.0	0.00	—
H. nummularium	0.1 Aa	0.11	0.0 a	0.00	0.1 Aa	0.11	0.595	0.1 Aa	0.11	0.0 a	0.00	0.1 Aa	0.11	0.595
H. radicata	0.0 a	0.00	1.2 Ab	0.40	1.7 Ab	0.75	0.026	0.0 a	0.00	0.4 Ab	0.29	1.4 Ab	0.53	0.025
K. arvensis	0.0	0.00	0.0	0.00	0.0	0.00	—	0.0	0.00	0.0	0.00	0.0	0.00	—
L. autumnalis	0.1 Aa	0.11	0.8 Ab	0.22	1.7 Ab	0.55	0.018	0.2 Aa	0.15	0.8 Aab	0.28	1.6 Ab	0.29	0.007
L. vulgare	0.0	0.00	0.1 Ab	0.22	1.4 Ab	0.69	0.041	0.0 a	0.00	0.0	0.00	0.2 Aa	0.22	0.368
L. vulgaris	0.1 Aa	0.11	1.3 Ab	0.37	1.9 Ab	0.56	0.003	0.6 Aa	0.18	1.4 Aa	0.63	1.3 Aa	0.37	0.272
L. corniculatus	2.3 Aa	0.90	10.2 Aab	3.51	26.1 Ab	10.05	0.042	8.0 Ba	1.11	16.7 Ab	2.97	31.0 Ab	4.89	0.001
O. vulgare	0.1 a	0.11	0.2 Aa	0.22	0.3 Aa	0.17	0.452	0.0	0.00	1.0 Bb	0.33	0.3 Ab	0.17	0.011
P. aurantiaca	0.0	0.00	0.3 Ab	0.17	0.6 Ab	0.18	0.039	0.1 a	0.11	0.7 Ab	0.17	1.1 Bb	0.11	0.001
P. officinarum	0.0	0.00	1.0 Ab	0.29	0.9 Ab	0.31	0.007	0.4 a	0.34	0.7 Aa	0.24	0.6 Aa	0.29	0.530
P. veris	0.0	0.00	0.0	0.00	0.0	0.00	—	0.0	0.00	0.0	0.00	0.0	0.00	—
S. columbaria	0.0 a	0.00	0.4 Aa	0.44	0.6 Aa	0.38	0.366	0.0 a	0.00	0.6 Aa	0.38	0.1 Aa	0.11	0.312

Significant differences at p =0.05 between substrate depths for the same watering treatment (Kruskal-Wallis test). Different capital letters indicate significant difference at p = 0.05 (Mann-Whitney *U*-test) between watering treatments for the same depth. Different lower-case letters indicate significant difference at p = 0.05 (Mann-Whitney *U*-test after Kruskal-Wallis test) between substrate depths for the same watering treatment.

Flowering pattern of the species at each substrate depth varied considerably. *Knautia arvensis* and *Primula veris* did not flower at all across all substrate depths, while *C. vulgare*, *L. autumnalis*, *L. vulgaris*, *L. corniculatus*, and *O. vulgare* did so. Of other species, *A. millefolium* flowered at 200 mm, for *A. eupatoria* and *C. glomerata* at 100 mm, and for *G. verum*, *H. radicata*, *L. vulgare*, *P. aurantiaca*, *P. officinarum*, and *Scabiosa columbaria* at 100 and 200 mm, and for *H. nummularium* at 50 mm and 200 mm.

5.4.5.2 Effect of substrate depth with supplemental watering

Under the watering condition, only 5 species, *H. radicata*, *L. autumnalis*, *L. corniculatus*, *O. vulgare*, and *P. aurantiaca*, were influenced by substrate depth in terms of flowering performance (Table 5.14). As in the non-watering treatment, the species had significantly higher inflorescences at 100 mm and 200 mm depths than 50 mm depth. In the above 5 species, no significant differences were found between 100 mm and 200 mm depths, except for *L. autumnalis* which was intermediate at 100 mm depth and showed no significant difference to either 50 mm or 200 mm depth. 4 species did not flower at all depths: *A. millefolium*, *G. verum*, *K. arvensis*, and *P. veris*. In *C. vulgare*, *L. autumnalis*, *L. vulgaris*, *L. corniculatus*, *P. aurantiaca*, and *P. officinarum*, inflorescences appeared across all substrate depths. Of the other species, *C. glomerata* at 50 mm, *H. nummularium* at 50 mm and 200 mm, and *H. radicata*, *O. vulgare*, and *S. columbaria* at 100 mm and 200 mm, *A. eupatoria* at 100 mm depth, and *L. vulgare* at 200 mm depth, flowered.

Mann-Whitney *U*-test on data shown in Table 5.14 found that only 4 species showed significant responses to watering treatment: *C. vulgare* and *L. corniculatus* at 50 mm, *O. vulgare* at 100 mm, and *P. aurantiaca* at 200 mm depth. Higher inflorescences number was observed in substrate depth with watering treatment.

Table 5.15. Mean total number of inflorescence of individual species in response to substrate type and fertiliser treatment across the 2-year period of the experiment.

	Substrate type					Fertiliser treatment				
	Zinco		Lime			NF		F		
	Mean	SE	Mean	SE	*P*-value*	Mean	SE	Mean	SE	*P*-value*
A. millefolium	0.0	0.000	0.0	0.000	—	0.0	0.000	2.8	0.925	—
A. eupatoria	0.1 a	0.111	0.1 a	0.111	1.000	0.1 a	0.111	0.4 a	0.176	0.1396
C. glomerata	0.0	0.000	0.0	0.000	—	0.0	0.000	0.0	0.000	—
C. vulgare	1.1 a	0.309	5.0 b	1.247	0.0031	1.0 a	0.373	15.3 b	2.819	0.0032
G. verum	0.0	0.000	1.1	0.754	—	0.0	0.000	4.3	2.380	—
H. nummularium	0.0	0.000	0.2	0.147	—	0.0	0.000	0.1	0.111	—
H. radicata	1.1 a	0.484	3.4 a	1.156	0.0837	0.9 a	0.455	5.4 b	1.842	0.013
K. arvensis	0.0	0.000	0.0	0.111	—	0.0	0.000	3.3	1.041	—
L. autumnalis	0.9 a	0.200	1.8 a	0.521	0.1754	0.8 a	0.222	5.6 b	0.818	0.0004
L. vulgare	0.0	0.000	1.2	0.795	—	0.0	0.000	13.6	5.247	—
L. vulgaris	1.0 a	0.441	4.4 b	1.345	0.0135	0.4 a	0.242	17.7 b	3.189	0.0004
L. corniculatus	19.8 a	7.380	17.0 a	4.072	1.000	7.9 a	2.983	5.1 a	1.504	0.5930
O. vulgare	0.3 a	0.236	1.3 a	0.500	0.1183	0.0	0.000	1.8	0.795	—
P. aurantiaca	0.6 a	0.176	1.9 a	0.696	0.0669	0.7 a	0.236	5.2 b	0.940	0.0010
P. officinarum	0.4 a	0.176	1.1 a	0.309	0.0970	0.4 a	0.242	0.7 a	0.236	0.4569
P. veris	0.0	0.000	0.0	0.000	—	0.0	0.000	0.0	0.000	—
S. columbaria	0.0	0.000	0.1	0.111	—	0.3 a	0.333	5.1 b	2.508	0.0457

Zinco, Zinco commercial substrate; Lime, Limestone-based substrate; NF, non-fertiliser treatment; F, fertiliser treatment. The same letters in substrate type treatment and fertiliser treatment are not significantly different at $p = 0.05$ (Mann-Whitney *U*-test*).

5.4.5.3 Effect of substrate type

Mann-Whitney *U*-test on data shown in Table 5.15 revealed that substrate type had a significant effect on inflorescence number in only 2 of the 17 species studied, *C. vulgare* and *L. vulgaris*. The species had significantly higher mean total number of inflorescence in the Limestone-based substrate than the Zinco substrate. 7 species did not have a significant response to substrate type: *A. eupatoria*, *H. radicata*, *L. autumnalis*, *L. corniculatus*, *O. vulgare*, *P. aurantiaca*, and *P. officinarum*. Of other species, 4 species (*G. verum*, *H. nummularium*, *L. vulgare*, and *S. columbaria*) flowered only in the Limestone-based substrate. In another 4 species, *A. millefolium*, *C. glomerata*, *K. arvensis*, and *P. veris*, no inflorescences were present in either substrate type.

5.4.5.4 Effect of fertiliser

Mann-Whitney *U*-test on data in table 5.15 revealed differences in 6 species between fertiliser treatments. The species were *C. vulgare*, *H. radicata*, *L. autumnalis*, *L. vulgaris*, *P. aurantiaca*, and *S. columbaria*. In other species, *A. eupatoria* (P = 0.139), *L. corniculatus* (P = 0.5930), and *P. officinarum* (P = 0.4569) fertiliser did not significantly affect inflorescence number of plants. *C. glomerata* and *P.* veris did not have flowering plants in either fertiliser treatment. 6 species, *A. millefolium*, *G. verum*, *H. nummularium*, *K. arvensis*, *L. vulgare*, and *O. vulgare*, had flowering plants only in the fertiliser treatment.

5.4.5.5 Summary of flowering performance of individual species over time

A summary of flowering plant percentage of individual species over time is shown in Figures 5.4 (sorted by low percentage) and 5.5 (sorted by high percentage). The percentage of flowering plant of individual species shown was converted from the mean total flowering plant of individual species per tray across all treatments (n=90). Flowering performance of each species in each treatment was summarised in detail in Chapter 6.

In particular, *C. vulgare* and *L. corniculatus* showed higher percentage (over 50.0 %) of flowering plants in maximum value and their peak was September and August respectively. *L. vulgaris* showed relatively constant percentage of flowering plants. *H. radicata*, *L. autumnalis*, and *P. officinarum* started flowering earlier (from June) compared to the others. *A. millefolium*, *C. glomerata*, *K. arvensis*, *L. vulgare*, and *S. columbaria* showed lower percentage of flowering plants but the species reached a peak flowering by the end of the first growing season. A mixture of earlier flowering and later flowering species could be useful to extend the term of flowering.

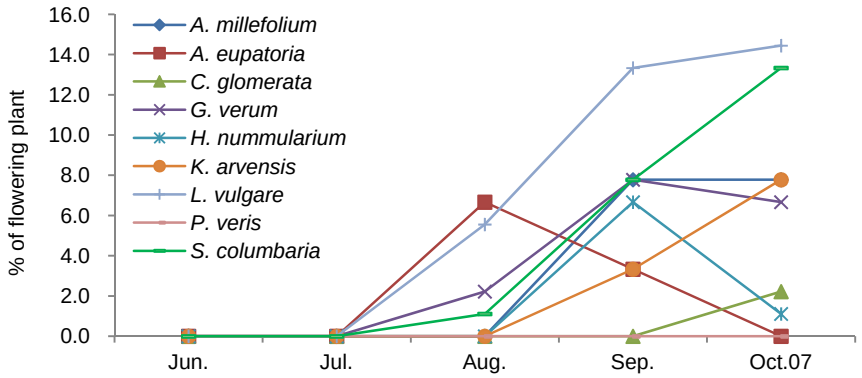


Figure 5.5. Summary of percentage of flowering plant of the individual species per tray (n=90) over the first growing season (sorted by low percentages).

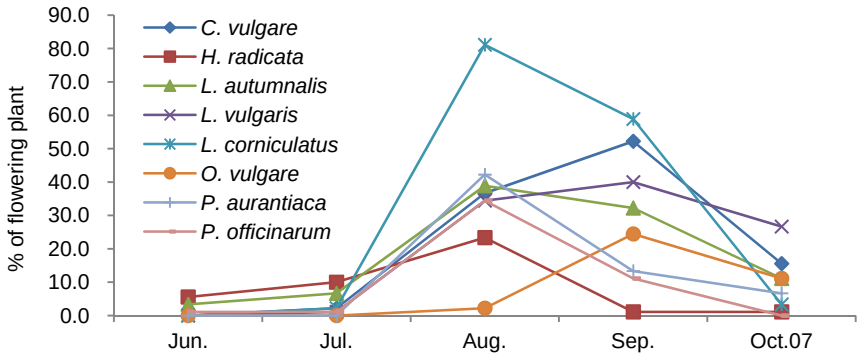


Figure 5.6. Summary of percentage of flowering plant of the individual species per tray (n=90) over the first growing season (sorted by high percentages).

Across all species, no flowering was observed in April 2008, while no month was found without flowering over the first growing season. At least 3 species had flowering plants in each month. From July, the number of flowering species increased and in September 2007, the greatest number of flowering species (15 species) was shown. August and September had the most flowering. *P. veris* did not flower at all. *H. radicata* and *L. autumnalis* showed the longest flowering period, which was over 5 months, while *C. glomerata* had the shortest period with 1 month. For *A. millefolium* and *A. eupatoria*, it was 2 months, and for *G. verum*, *L. vulgare*, *O. vulgare*, *P. aurantiaca*, and *S. columbaria*, 3 months and for *C. vulgare*, *L. vulgaris*, *L. corniculatus*, and *P. officinarum*, it was 4 months.

5.5 Discussion

5.5.1 Plant abundance

5.5.1.1 Effect of substrate depth without supplementary watering

At the end of the first growing season, plant abundance was influenced by substrate depth for all species. All species had higher abundance in deeper substrates than in shallower substrate. At the 50 mm substrate depth, *A. millefolium*, *A. eupatoria*, and *L. corniculatus* did not survive at all and other species had very low abundance. At the deeper substrates all species tended to have higher abundance, except for *P. veris* which showed very low abundance across all depths. This result suggests that although most of the species included in this experiment are tolerant of drought, the species might not be able to withstand an extreme drought condition that occurred frequently at 50 mm depth due to little precipitation during August in 2007 (*ref.* Appendix 8). Additionally, *L. corniculatus* is not a drought tolerant species (Grime, et al., 2007). In the current experiment, measurement of moisture content of substrate with supplementary watering during the period showed that 50 mm depth experienced more frequent soil moisture stress with below 12.0 % compared to 100 mm and 200 mm depths. It was also observed that 50 mm deep substrate surface dried out quickly between precipitation events compared to the deeper substrates. This finding could be supported by VanWoert et al. (2005) who found that shallower substrate depths had lower moisture content than deeper substrate depths. In the study by Nagase and Dunnett (2010) on drought tolerance of three types of plant groups (Sedums, grasses, and herbaceous plants including *Armeria maritima*, *Origanum vulgare*, *Prunella vulgaris*, and *Silene uniflora*), it was found that under dry conditions, with watering at 3 weeks interval, all the herbaceous species were not able to survive at all in mixture planting. Abundance of individual species may have been influenced by soil temperature as well as the moisture stress. In the study by Dunnett (1995), who carried out an experiment to evaluate 5 species (*Achillea millefolium*, *Cirsium arvense*, *Dactylis glomerata*, *Festuca rubra*, and *Poa pratensis*) in response to unusual high temperatures in late winter and spring, and to

severe late frosts and to severe drought in summer, it was found that shoot numbers of *A. millefolium* with other species declined by prolonged high soil temperature.

In the early second growing season, the overall trend still appeared to associate lower abundance with shallower substrate and higher abundance with deeper substrates. Except for 4 species, *H. nummularium, H. radicata, L. vulgare,* and *L. corniculatus,* all the species were influenced by substrate depth in terms of plant abundance. At 50 mm depth, more species did not survive at all: *A. millefolium, A. eupatoria, C. glomerata, H. nummularium, P. aurantiaca,* and *P. veris*. In addition, *A. eupatoria, C. glomerata, H. nummularium, L. autumnalis, L. corniculatus, O. vulgare,* and *P. veris* did not recover to the original numbers at deeper substrate depths. This might indicate that substrate depth influences the overwintering survival and/or re-establishment of the species for the new growing season. Boivin et al. (2001) found that shallower substrate depths experienced much more severe temperature fluctuations than deeper substrates, which in turn resulted in more low temperature injury to plant growth. According to Dunnett's experiment (1995), in mixture planting, *A. millefolium* appeared to be weakened by extreme low temperature as well as high soil temperature and drought condition. Of the species, *H. radicata* and *L. vulgare* were highlighted with dramatically increased abundance at 50 mm depth as well as the other deeper substrates due to abundant occurrences of seedling of the species in the early second year. The species also had significantly higher abundance in the non-watered treatment than in the watered treatment. According to Grime et al. (2007, p364/400), these 2 species regenerate by means of seedling which germinates over much of the year with peaks in autumn and spring, and between March and September respectively. Experiment by Oomes and Elberse (1976) on germination of six grassland herbs (*Achillea millefolium, Hypochaeris radicata* ssp. *radicata, Leucanthemum vulgare, Plantago lanceolata, Prunella vulgaris* and *Rumex acetosa*) in micro-sites with different water contents showed that seed of *L. vulgare* had rapid germination on the drier soil surface. This could support the finding of the current study which *L. vulgare* showed higher abundance in the non-watering treatment at all depths than in the watering treatment.

5.5.1.2 Effect of substrate depth with supplementary watering

Compared to the non-watering treatment, fewer species under the watering condition showed significant response to the substrate depths. At the end of the first season, 7 species were influenced by substrate depth, which were *G. verum*, *L. vulgaris*, *L. corniculatus*, *O. vulgare*, *P. aurantiaca*, *P. officinarum*, and *S. columbaria*. While in the early second growing season 9 species, *A. millefolium*, *G. verum*, *K. arvensis*, *L. autumnalis*, *L. vulgaris*, *L. corniculatus*, *O.* v*ulgare*, *P. officinarum*, and *S. columbaria*, showed a response to the depths. As a whole, supplementary watering tended to support that most of the species maintained high plant abundance at all substrate depths throughout the two growing seasons of the experiment, although only one watering event was applied in the second year. The most striking difference in comparison with the non-watering condition was that most of the species at 50 mm depth showed high or very high abundance. This may be due to additional watering application, which is the only different factor compared to the non-watering condition, assuming that other environmental and physiological conditions are same. The effect of substrate depth on plant abundance of the species tended to disappear with having additional watering. Dunnett and Nolan (2004) discussed that "application of additional watering during the establishment phase especially was important factor to provide significant benefit to plant performance" (p.308). However, the additional watering did not positively affect *L. autumnalis* and *L. vulgare* at all depths (*ref.* Appendix 9). *H. nummularium* did not survive at the two deeper substrates. This may be due to a more shaded and competitive condition from the higher growth of neighbours due to the additional watering. According to Grime et al. (2007), the three species tend to appear intolerant to competition and shade, and to have a limited capacity for lateral vegetative spread.

5.5.1.3 Effect of substrate type

During the two growing seasons, most of the species had high percentage of plant abundance in both substrate types. This indicates that supplementary watering may greatly work as a key factor to individual plant abundance in both substrates. There

were some species that showed plant abundance rate below the original numbers in the early second season. The species were: *C. glomerata*, *H. nummularium*, and *P. veris* in both substrates, and *O. vulgare* in the Zinco substrate. This result indicates that plant abundance of the species may be associated with habitat characteristics of the species itself. In the experiments by Jefferies and Willis (1964), it was shown that *O. vulgare* had higher seedling development on limestone soil with pH 7.4 and cannot survive in soil with low calcium concentrations. In the case of *Helianthemum nummularium*, it only survived with high abundance rate in the Limestone-based substrate in the second growing season. Tansley (1965[b], p.533) stated that *H. nummularium* is categorised as exclusive species that occurred only in chalk grassland.

Higher plant abundance for all species was nearly always associated with the Limestone-based substrate whether they showed a significant response or not. This may be because limestone consisted of the limestone-based substrate and there was less moisture stress during drought period in summer (*ref*. Appendix 8) compared to the Zinco substrate. According to the study by Kirkham et al. (2008), evaluating the species-richness and plant functional characteristics of hay meadow communities in response to application of inorganic and organic fertilisers and lime, liming treatment increased a capacity for nutrient uptake and positively influenced increasing number of species.

5.5.1.4 Effect of fertiliser

Most of species showed greater abundance rates at fertiliser treatment than at non-fertiliser treatment. In the end of the first season, species that showed a significant response to fertiliser treatment were: *A. eupatoria*, *C. vulgare*, *K. arvensis*, *L. autumnalis*, *P. officinarum*, *A. millefolium*, *G. verum*, and *L. vulgaris*. Of the species, the last 3 species and *H. radicata* exhibited a significant response in the early second season. This may be a result of greater availability of nutrients from NPK slow release fertiliser. In the study by Rowe et al. (2006), who carried out an experiment to evaluate the effect of fertiliser levels on Sedum species and Michigan native herbaceous

perennials and grasses, it was found that during the establishment period and overwintering, most of the species had high abundance rates across the various fertiliser levels, however, some of native species did so without fertiliser treatment. The similar tendency was observed in the current study. In the early second growing season, the species that exhibited the tendency were: *H. radicata*, *O. vulgare*, and *P. officinarum*. *H. radicata* showed a significantly greater abundance rate at non-fertiliser application than at fertiliser application. This may be result of less competition and relatively greater proportion of open space at non-fertiliser treatment resulting in more opportunities for seedling emergence from its own and/or an influence of seedling from fertiliser treatment. Relatively less favourable condition may give rise to less shading and competition from vigorously growing neighbours compared to more favourable condition. Grime et al. (2007) describe that "*O. vulgare* is unable to coexist with taller, fast-growing species" (p. 448) and "*P. officinarum* is vulnerable to the shade of taller plants" (p. 466).

Except for 3 species, *H. nummularium*, *O. vulgare*, and *P. veris*, other species maintained high or very high abundance at both fertiliser treatments. As discussed above, this result indicates that water availability may be a more important factor to abundance of native species than fertiliser (Rowe et al., 2006). Goldberg and Novoplansky (1997) also pointed out that nutrient availability primarily affects plant growth but not survival. In addition, under the condition of allocating trays with both fertiliser treatments adjacent, it might be additional result of nutrient leaching from fertiliser treatment that could affect plants in tray with non-fertiliser.

5.5.1.5 Summary of abundance of individual species

From the results in terms of abundance of individual species, at 50 mm substrate depth not all species applied in this study survived well when they were not watered, except for *H. radicata, L. vulgare*, and *S. columbaria*. At both of 100 mm and 200 mm depths, however, individual species were observed with successful abundance (more than 50.0 %). The species include *A. millefolium*, *A. eupatoria*, *C. glomerata*, *C. vulgare*, *G.*

verum, H. radicata, K. arvensis, L. autumnalis, L. vulgare, L. vulgaris, P. aurantiaca, P. officinarum, and *S. columbaria,* and in case of *L. corniculatus* for 200 mm depth only. The higher abundance rates occurred in treatments where watering was given, indicating that it might be necessary to apply supplementary watering especially during hot and dry weather. However, *Primula veris* was not successful even under more favourable conditions. The species exhibited constant decrease pattern in abundance across all treatments. This may be because that the species is vulnerable to the shade of taller plants and competitive conditions (Grime et al., 2007, p.492). In the study by Tamm (1972) on survival and flowering of *Primula veris* on 3 different sites (Site I, shaded meadow area by closing canopy of ash grove; Site II, situated near the edge of an open meadow exposed to full sunlight but partly shaded; Site III, dry meadow with drier and less competitive condition than the other sites, but with similar light conditions to site II) in wooded meadow in eastern Sweden, it was found that in sites I and II, the population of *P. veris* declined rapidly or constantly. However, in site III, which was relatively a stable and favourable environment, the species maintained a stable population by vegetative propagation. In the early second growing season, *O. vulgare* showed low abundance at all depths without supplementary watering and in 50 mm depth with supplementary watering and at both fertiliser treatment. This suggests that *O. vulgare* may not survive effectively under severe drought and/or shading condition that result from robust growth of other species. In the study by Van Tooren and Pons (1988) on effects of temperature and light on the germination in chalk grassland species, it was found that seed of *O. vulgare* germinating mainly in spring had poor germination at low temperature and light.

5.5.2 Plant growth

5.5.2.1 Effect of substrate depth without supplementary watering

Results of the experiment over the 2 growing seasons showed that most of the individual species had a significant response to substrate depth, except for *C. glomerata*

and *P. veris* in the first growing season, and *C. vulgare*, *H. nummularium*, and *H. radicata* in the second season. This indicates that substrate depth have profound effect on plant growth of the individual species as well as abundance. As discussed above, in many previous studies (i.e. Dunnett and Nolan, 2004), deeper substrates promote greater plant performance due to relatively higher moisture availability. In this study, the trend was observed in terms of plant growth of the individual species. Moreover, the trend appeared to be clearer over the second season because many species did not survive at 50 mm depth by the end of the season. The species were *A. millefolium*, *A. eupatoria*, *C. glomerata*, *G. verum*, *H. nummularium*, *O. vulgare*, *P. officinarum*, and *P. veris*. This suggests that they may be the most sensitive species of the individual species to moisture stress. Grime et al. (2007) reported that "*P. officinarum* appears to show some susceptibility to drought" (p. 466).

Although the overall plant growth was significantly greater at 200 mm substrate depth than 50 mm and 100 mm (Figure 4.6), there were individual species that showed no significant difference in plant growth between 100 mm and 200 mm depths. In the first growing season, the species were *A. millefolium*, *C. vulgare*, *G. verum*, *K. arvensis*, *O. vulgare*, and *S. columbaria*. *A. eupatoria* at 100 mm depth, and *H. nummularium*, *H. radicata*, *L. autumnalis*, *L. vulgare*, *L. corniculatus*, *P. aurantiaca*, and *P. officinarum* at 200 mm depth had the best plant growth. In the second season, 10 of the 14 species that showed significant response to substrate depth were observed with the trend, except for *C. glomerata*, *L. corniculatus*, *P. officinarum*, and *S. columbaria*, which showed significantly the greatest growth at 200 mm depth. The species include *A. millefolium*, *A. eupatoria*, *G. verum*, *K. arvensis*, *L. autumnalis*, *L. vulgare*, *L.* vulgaris, *O. vulgare*, *P. aurantiaca*, and *P. veris*. This indicates that the difference between moisture content in 100 mm and 200 mm depths may not be a key limitation to plant growth of those species.

In the research by Dunnett et al. (2008b), who investigated influence of substrate depth (100 mm and 200 mm) on plant performance of planted perennial herbaceous species originating from dry habitat and colonising species over 6 growing seasons, it was found that although some species benefited from low substrate depth (100 mm) in the

initial years after establishment, this advantage appeared to be lost in long-term. In the current study, many species were observed with a significant reduction in plant growth at 100 mm depth as well as the other two depths during the second season. The species were *C. vulgare* at both 50 mm and 200 mm depths, *K. arvensis*, *L. autumnalis, L. vulgare*, and *L. corniculatus* at 50 mm depth; *C. glomerata* and *P. aurantiaca* at 100 mm depth; *H. nummularium, O. vulgare, P. officinarum,* and *P. veris* at both 100 mm and 200 mm depths and *H. radicata* at 200 mm depth. Some of the species increased in plant growth. The species were; *A. millefolium* and *L. corniculatus* at both 100 mm and 200 mm depths, and *G. verum* and *L. vulgaris* at 200 mm depth. This indicates that it may need long-term observation to decide stable species for green roof environment.

5.5.2.2 Effect of substrate depth with supplementary watering

Compared to drought condition, the effect of substrate depth tended to be relatively weakened under watering treatment conditions. More species, 3 and 4 species in the first and the second growing season respectively, were observed with no significant response to substrate depth. The species were *C. glomerata* and *C. vulgare* in both growing seasons, and *P. veris* in the first season, and *H. nummularium* and *H. radicata* in the second season. The other species tended to produce higher plant growth in the deeper substrate depths. This indicates that under watering condition, shallower substrates might experience more and frequent moisture stress than deeper substrates, and thus the moisture stress differently affect each species in plant growth and development. Carter et al. (1997) investigated the combined effects of elevated CO_2 (350 ppmv and 700 ppmv), temperature (18/10 °C and 25/15 °C) and drought (100 % and 60 % water condition) on growth and plant development of *Lotus corniculatus*. They found that under the drought condition *L. corniculatus* was reduced in shoot biomass, and that growth temperature and water availability was the main environmental variables which affected shoot biomass of the species.

In comparison between watering regimes at the same substrate depth, in contrast to plant growth at 50 mm depth without watering application, supplementary watering

supported all species' survival by the end of the second growing season. Also, most of the species tended to produce higher plant growth at the depth over the 2-year period of the experiment than non-watering treatment, despite that one supplementary irrigation event was carried out over the second season. This indicates that supplementary watering may be an important factor for plant growth of the individual species at shallower substrate, especially under green roof conditions during the establishment phase (Dunnett and Nolan, 2004). However, watering application at deeper substrates (100 mm and 200 mm) appeared to have little direct benefit, although many species had higher plant growth compared to those with non-watering treatment. The species that exhibited a significant response to watering treatment were: in the first season, *H. nummularium* and *S. columbaria* at 50 mm and 100 mm depths, and *A. millefolium* and *L. vulgare* at 50 mm and 200 mm depths, and *C. glomerata*, *C. vulgare, H. radicata*, and *P. aurantiaca* at 50 mm, *K. arvensis* at 200 mm depth, and *L. autumnalis*, *L. corniculatus*, and *O. vulgare* at all depths. In the second growing season, less species showed clear advantages of additional watering. As expected, it is likely to be one event of additional watering over the second season. The species were; *K. arvensis* and *L. autumnalis* at 50 mm depth, *C. glomerata* at 100 mm depth, *L. corniculatus* and *S. columbaria* at 50 mm depth and 100 mm depth, and *L. vulgare* at 50 mm and 200 mm depths.

Throughout the 2-year period of the experiment, *A. eupatoria* and *C. glomerata* at 200 mm depth, and *A. millefolium*, *G. verum*, and *L. corniculatus* at both of 100 mm and 200 mm depths improved significantly in plant growth, whereas *C. glomerata*, *C. vulgare*, *H. nummularium*, *L. corniculatus*, and *O. vulgare* at 50 mm depth, and *H. radicata*, *P. officinarum*, and *P. veris* across all of the substrate depths were reduced in plant growth. This indicates that for successful establishment of the latter 3 species and *H. nummularium* (did not survive at 100 mm and 200 mm depths) it may be necessary to have more stable and favourable environment.

5.5.2.3 Effect of substrate type

The results show that plant growth of individual species had different response to substrate type for the 2 growing seasons. Throughout the first growing season, *A. millefolium, C. glomerata, C. vulgare, G. verum, H. radicata, K. arvensis, L. autumnalis, L. vulgare, L. vulgaris, P. aurantiaca, P. officinarum,* and *S. columbaria* produced significantly higher plant growth in the limestone-based substrate than the Zinco substrate. This may be because most of the species originate from calcareous grassland habitat. Another factor, as discussed above, may be due to increased nutrient availability by limestone (Kirkham et al., 2008) and less moisture stress in the Limestone-based substrate. Jefferies and Willis (1964) found that important factors for successful growth were soil conditions with similar nutritional conditions to the soils of natural habitats which the species occurred and calcium concentration relative to the concentration of other nutrients in the soil. The study has shown that *Origanum vulgare* had vigorous growth in soil with high amount of calcium. In the current study, similar result was observed in *O. vulgare*, although not a statistically significant response. The opposite tendency was also observed in *P. veris*, producing higher plant growth in the Zinco substrate. This may be supported by the study of Whale (1984), which investigated response of *Primula* species to competition in different soil water-logging and soil drought condition. The study showed that *P. veris* in both mixture and monoculture planting had a lower biomass on saturated soil than on freely drained soil. In the current study, it was observed that the Limestone-based substrate maintained higher moisture content for longer period than the Zinco substrate (*ref.* Appendix 8)

In the second growing season, however, 13 species did not have a significant response to the substrate types, while in the first season 4 species showed the same response. This result indicates that the advantage of the Limestone-based substrate appeared to be lost although many species indicated a trend towards higher plant growth without having a statistically significant response. As discussed in Chapter 4, it may be linked to a decrease in nutrient availability and a change of the physical characteristics of the substrate that result from an increase of decomposition of organic matter included in the Limestone-based substrate (Birch, 1958) and/or more severe moisture stress due to one

occasion of additional watering over the second growing season, compared to the first season. Only 3 species, *C. glomerata*, *K. arvensis*, and *L. corniculatus*, had significant plant growth in response to substrate types. *H. nummularium* did not survive in the Zinco substrate. Of these species, *L. corniculatus* exhibited greater plant growth in the Zinco substrate than in the Limestone-based substrate, resulting from a significant sharp decline in the Limestone-based substrate, whereas it showed a significant increase in the Zinco substrate compared to the first growing season. According to Grime et al (2007), it was reported that "*L. corniculatus* is restricted to unproductive plots and is strongly suppressed by nitrogenous manures" (p. 410).

5.5.2.4 Effect of fertiliser

Fertiliser application tended to improve plant growth of the individual species over the two growing seasons whether they had a statistically significant response or not. However, the result over the second growing season indicates that it appeared to weaken the benefit of fertiliser application to plant growth of the individual species. 12 species in the first growing season were significantly influenced by fertiliser application, while in the second season 7 were influenced; *A. millefolium*, *C. vulgare*, *G. verum*, *K. arvensis*, *L. vulgare*, *L. vulgaris*, and *L. corniculatus*. Interestingly, as in the Limestone-based substrate, *L. corniculatus* was observed with a negative response to fertiliser application, decreasing significantly at fertiliser treatment in the second growing season compared to the first season, while increasing at non-fertiliser treatment. The species thrived better at non-fertiliser treatment. It, however, did better at fertiliser treatment in the first season, although not with a statistically significant response. This indicates that in the first season, nutrient availability may mainly affect plant growth of the species, while in the second season moisture stress may strongly affect the growth. This result could be supported by the study of Carter et al. (1997) and Briggs (1990). Carter et al. (1997) found that drought stress inhibited plant growth of the species. In the study by Briggs (1990), who examined effects of nitrogen source on plant growth, reproduction and chemistry of *L. corniculatus*, it was found that under daily watered condition, *L. corniculatus* had higher shoot biomass in the nitrogen fertiliser treatment (received 0.13

g ammonium nitrate 5 days a week) than in the unfertilised treatment.

For the same fertiliser treatment, the individual species showed changes in plant growth throughout the growing season. *A. millefolium*, *A. eupatoria*, *C. glomerata*, and *G. verum* were species that improved in plant growth at both fertiliser treatments. This indicates that for the species, fertiliser application may ameliorate some of the effects of drought that occurred in the second season. *P. veris* decreased in plant growth at non-fertiliser treatment, while increasing at fertiliser treatment, although the difference was not statistically significant. A similar result with *P. veris* was observed in the experiment by Ash et al. (1994), which examined survival and establishment of a range of native herbaceous species on 4 waste sites with and without fertiliser. In this study, *Primula veris* on alkaline industrial wastes declined in plant growth when fertiliser was not applied, but also the species grew poorly in spite of having fertiliser applied. *H. nummularium*, *H. radicata*, *O. vulgare*, *L. autumnalis*, *P. aurantiaca*, and *P. officinarum* were reduced in plant growth at both fertiliser treatments. In the case of the species, *K. arvensis*, *L. vulgaris*, *L. corniculatus*, and *S. columbaria*, plant growth increased at non-fertiliser treatment, while it decreased at fertiliser treatment.

5.5.2.5 Summary of plant growth of individual species

In terms of mean total dry weight biomass across all treatments at the second growing season, 3 groups can be identified: species with low (< 2.000 g), medium (2.000 to 5.000 g) and high growth (> 5.000 g) (Figure 5.6). The species group can thus be separated by those with low (*A. eupatoria*, *C. glomerata*, *C. vulgare*, *H. nummularium*, *H. radicata*, *L. autumnalis*, *O. vulgare*, *P. aurantiaca*, *P. officinarum*, and *P. veris*), medium (*G. verum*, *K. arvensis*, *L. vulgaris*, and *S. columbaria*), and high plant growth (*A. millefolium*, *L. corniculatus*, and *L. vulgare*). The later 3 species tended to be dominant across all treatments over the 2-year period (Figure 5.6). This is probably related to the plant functional characteristics of the species and the differences in competitive ability between the species. According to Grime's CRS theory (2002), the three species are a relative large and fast-growing species, and are classified as

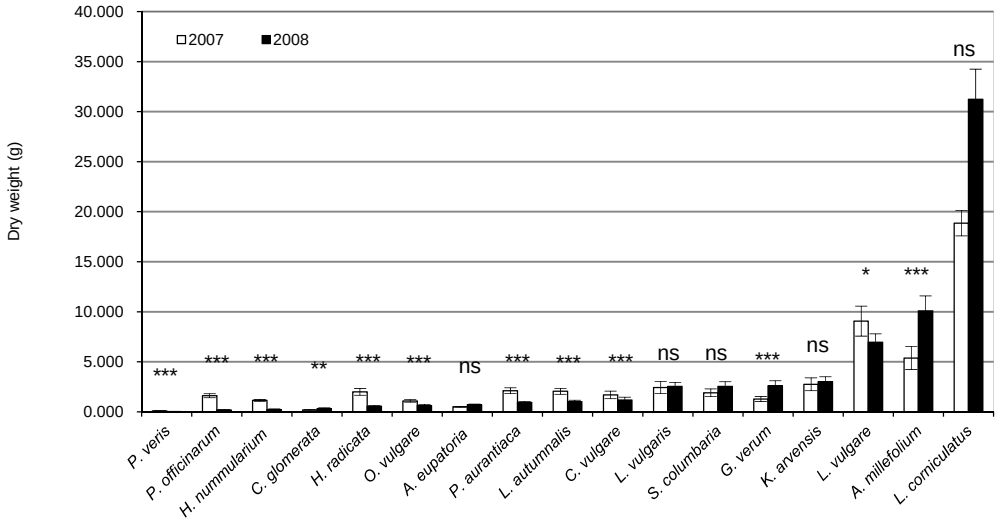


Figure 5.7. The mean total dry weight across all treatments in the two growing season for individual species used in the study. Error bars represent S.E. Significant differences (Mann-Whitney *U*-test) between 2007 and 2008 are indicated by: * $P < 0.05$; ** $P < 0.01$; $P < 0.001$; ns, not significant.

competitive-ruderals for *A. millefolium* and as 'CRS' strategists for *L. corniculatus* and *L. vulgare*. Grime (2002) described that perennial species included within competitive-ruderals are "most abundant in circumstances in which the impact of disturbance is less immediate or catastrophic" (p. 122) and C-S-R strategists are "adapted to habitats in which the level of competition is restricted by moderate intensities of both stress and disturbance" (p.116). The result of the dominant tendency of the three species could be supported by the findings of Buckland and Grime (2000). The result showed that CSR strategists group produced higher shoot biomass at low and medium soil fertility compared to the other three groups. They found that in the high fertility treatment all vegetations were not only dominated by potentially fast-growing perennial plant species, as observed in natural plant communities in unmanaged habitats, but the same tendencies were also occurred in the moderate and low fertility treatment (Buckland and Grime, 2000, p.344). They suggested that:

> "the potentially fast-growing plant species normally restricted to fertile soils, although much reduced in vigour by mineral nutrient stress, retained their competitive advantage relative to slower-growing species" (p.344).

The mean total dry weight of individual species across all treatments also showed the overall trends of plant growth pattern (stable, increase, or decrease) of the species throughout the 2-year period of the experiment. The increased species were; *A. millefolium*, *A. eupatoria*, *C. glomerata*, *G. verum*, *K. arvensis*, *L. vulgaris*, *L. corniculatus*, and *S. columbaria*. The decreased species include *C. vulgare*, *H. nummularium*, *H. radicata*, *L. autumnalis*, *L. vulgare*, *O. vulgare*, *P. aurantiaca*, *P. officinarum*, and *P. veris*. However, the increased species did not change significantly throughout the period except for *A. millefolium* and *G. verum*, while the decreased species did so significantly.

5.5.3 The plant structural characteristics

5.5.3.1 Effect of substrate depth and supplemental watering

The results that show mean height and mean total coverage of each species in response to substrate depth in non-watering and watering treatment across the period demonstrated that under non-watering treatment conditions, plant structural characteristics were clearly influenced by substrate depth for all species. An exception was *P. veris*, which exhibited no significant response in coverage. As stated above, plant growth of *P. veris* was associated with nutrient level (Ash et al., 1994). In contrast, under watering treatment conditions, more species were not influenced by substrate depths. The species were *A. eupatoria*, *C. vulgare*, *G. verum*, *L. corniculatus*, and *P. officinarum* in height, and *C. glomerata*, *H. nummularium*, and *P. veris* in both height and coverage. This may be due to significant increases in both plant structures for more species at 50 mm depth by additional watering. Deeper substrate tended to support higher plant size in both watering treatments. This result of positive effects of deeper substrates on plant size is observed in the findings of Dunnett et al. (2008b); 200 mm depth produced significantly higher plant height and diameter than 100 mm depth. However, in the current study, some species showed no significant differences in plant size between 100 mm and 200 mm depths. Among the species that exhibited a

significant response to substrate depth, in non-watering treatment 13 in height and 6 species in coverage tended not to have a significant difference between 100 mm and 200 mm, while in watering treatment 8 species in height or coverage did so.

The results also show that supplementary watering had a significant effect on plant structural characteristics. Higher plant structural characteristics were nearly always associated with the watering treatment, except for *P. aurantiaca* at 200 mm depth where it showed a significantly lower vertical structure. However, 7 and 13 species at 100 mm and 200 mm respectively tended to have lower structure in the watering treatment, although these differences were not statistically significant. This may be linked to higher competition resulting from the more productive condition of greater moisture supply. In the most of the species, the effect of watering treatment was more clearly revealed at 50 mm substrate depth. This may be because under non-watering conditions shallower substrate depth experienced more severe drought stress to the species.

5.5.3.2 Effect of substrate type

From the results that showed mean values across the period (Table 5.11), plant structural characteristics of individual species were also influenced by substrate type for most of the species (15 species out of 17 species) except for *H. nummularium* and *L. corniculatus*. Of the species, *C. glomerata, C. vulgare, G. verum, H. radicata, K. arvensis, L. autumnalis, L. vulgare, L. vulgaris, P. officinarum,* and *S. columbaria* had significant responses in both structural characteristics. Species that exhibited a significant response only in coverage structure were *A. millefolium, O. vulgare, P. aurantiaca,* and *P. veris*. *A. eupatoria* exhibited a significant difference in vertical structure only. Higher plant structural characteristics were nearly always associated with the Limestone-based substrate. This is presumably because higher nutrient availability from organic matter and limestone contained in the substrate stimulated plant size of the species.

5.5.3.3 Effect of fertiliser

Throughout the 2-year period of the experiment, overall plant structural characteristics were affected by fertiliser with higher height and coverage occurring at fertiliser treatment for most of the species (Table 5.11). Retzlaff et al. (2009) found Sedum plants with fertiliser exhibited the greatest diameter growth during establishment period. However, it was not always true for some species. The exceptions were *Origanum vulgare*, showing no significant differences in both height and coverage, and *H. nummularium* and *L. corniculatus* with no significant difference in coverage.

5.5.4 Flowering of individual species

5.5.4.1 Effect of substrate depth

In both watering treatments, it was shown that most of the species produced higher flowering performance at deeper substrates (100 mm and 200 mm) whether significant difference was revealed or not. In a study by Dunnett et al. (2008b), it was also found that flowering performance of herbaceous perennial grass and herb species tended to be higher in deeper substrate. This may be because that flowering performance of individual species could be directly influenced by water availability. In a study by Carter et al. (1997), investigating an effect of elevated CO_2, temperature and draught on plant growth and development of *Lotus corniculatus*, it was found that number of flowers per plant of *L. corniculatus* was significantly reduced by drought treatment. Many previous studies of plant selection for green roofs also showed that in general, water availability of substrate is an important factor for plant survival, establishment, and growth on green roof (Rowe, et al., 2006; Monterusso, et al., 2005; Dunnett and Nolan, 2004). In the current study, flowering performance of the individual species also indicated this trend.

5.5.4.2 Effect of watering

In the watering treatment comparisons at the same substrate depth, effect of watering was much more clearly revealed at shallow substrate (50 mm depth); only 4 species flowered at the depth in the non-watering treatment, while 8 species in the watering treatment did. However, additional watering at deeper substrates (100 mm and 200 mm depths) did not appear to have an effect on flowering performance. There existed some exceptional species that were stimulated in flowering performance by supplementary watering: *C. vulgare* and *L. corniculatus* at 50 mm, *O. vulgare* at 100 mm, and *P. aurantiaca* at 200 mm depth. With the exception of these species, some species at 100 mm and/or 200 mm that received no supplementary watering produced higher flowering performance although it was not statistically significant. The species were; *C. vulgare*, *H. radicata*, and *P. officinarum* at both of 100 mm and 200 mm depths, and *L. vulgare*, *L. vulgaris*, and *S. columbaria* at 200 mm depth. As discussed in Chapter 4, this indicates that some extent of drought stress condition may stimulate flowering performance of the species. In the study by Boot et al. (1986), it was shown that more plants of *Urtica urens* flowered in less severe drought treatment compared to non-drought treatment.

5.5.4.3 Effect of substrate type

The species that flowered in both of substrate types produced higher flower performance in number in the Limestone-based substrate than the Zinco substrate. Only 2 species, however, *C. vulgare* and *L. vulgaris*, were significantly influenced by substrate types. *L. corniculatus* did not have this tendency, producing more flowers in the Zinco substrate although not statistically significant. This may be associated with nutrient availability, especially the amount of nitrogen, from organic matter contained in the Limestone-based substrate. Same tendency was also found in the fertiliser treatment in this study. According to Briggs (1990), *L. corniculatus* in no additional nitrogen treatment produced more flowers than in the high nitrogen treatment.

5.5.4.4 Effect of fertiliser

In this study fertiliser had a significant effect on flowering performance of the species (6 of the 17 species studied). Higher flowering performance was nearly always associated with the fertiliser whether significant differences were revealed or not, which were likely to provide higher nutrient availability to plants for longer period. As in the effect of substrate type, however, *L. corniculatus* was suppressed in flowering performance. This result could be supported by Briggs (1990).

5.5.4.5 Flowering time and duration of individual species

From this study, it was shown that the flowering time and duration of individual species on the green roof differed from those at field, although there was no great discrepancy (*ref*. Appendix 14). Almost all species that flowered showed trends that started blooming later and/or had a shorter flowering duration than at field across all treatments, except for *C. vulgare* and *H. radicata* with fertiliser application, which coincided with flower starting time at field and had a longer duration. This is perhaps not surprising because the initial size of plants grown from small plugs used in these experiments was small (Table 4.5) and the experiments started in June. This result could be supported by Schmitt (1983) who found that flowering phenology had a significantly positive correlation with plat size (height) and flower number in *Linanthus androsaceus*, a California grassland annual. Another reason may be caused by more limitation of resource supply on green roof than field. Fox (1990) reported that moisture limitation delayed flowering in annual plants. However, in comparisons between watering regimes, the onset of flowering of some species occurred a month earlier in non-watering treatment than in watering treatment. The species were; *Hypochaeris radicata* at 100 mm, *Leontodon autumnalis* at all depths, *Origanum vulgare* and *Pilosella officinarum* at 200 mm, and *Scabiosa columbaria* at 100 mm and 200 mm. Of the species that flowering later, some species had longer flowering duration. The species were *G. verum* at 100 mm depth in the non-watering treatment, *L. vulgare* at 200 mm depth and in fertiliser treatment, and *L. corniculatus* at 100 mm with additional watering, in the

Limestone-based substrate, and in fertiliser treatment.

5.5.5 Summary of performance of individual species

For successful plant species for green roof systems, it is necessary to select the plants which have high emergence and abundance, good growth and stable growth pattern, and flowering for visual interest over long-term. In this study, *C. glomerata*, *H. nummularium*, *P. officinarum* and *P. veris* were not successful across all the substrate depths and both watering treatments, while *A. millefolium*, *G. verum*, *K. arvensis*, *L. vulgare*, and *S. columbaria* showed good performance, except at 50 mm depth without watering treatment. *C. glomerata* and *H. nummularium* are strongly recommended to have additional nutrient application for good growth. *O. vulgare* was only suitable at the deeper substrate depths (at 100 mm and 200 mm depths) with additional watering and in the Limestone-based substrate. Of the other species, under the condition of relying on natural rainfall only, *A. millefolium*, *A. eupatoria*, *G. verum*, *K. arvensis*, *L. autumnalis*, *L. vulgare*, *L. vulgaris*, *P. aurantiaca*, and *S. columbaria* performed well in both of the deeper substrates (at 100 mm and 200 mm depths). *C. vulgare* and *H. radicata* at 100 mm depth, and *L. corniculatus* at 200 mm depth fulfilled these requirements. Under watering condition, *H. radicata* was not successful at all substrate depths. *O. vulgare* required depths with more than 100 mm at least for good abundance rate and growth. *L. corniculatus* showed good plant performance across all substrate depths, whereas *C. vulgare* was successful at 200 mm depth only. The other species, *A. eupatoria*, *L. autumnalis*, *L. vulgaris*, *O. vulgare*, and *P. aurantiaca*, showed better performance at all the deeper substrate depths. Limestone-based substrate and fertiliser treatment promoted greater abundance, growth, and flowering for nearly all species tested. However, *L. corniculatus* were not suitable in conditions with high nutrient level. Across all treatments, *L. corniculatus* was the most dominant species that covered the most area, followed by *A. millefolium* and *L. vulgare*, whilst *H. nummularium* and *P. veris* were the most recessive species. However, the plant size of most species on the roof tended to be smaller compared to their normal growth on the ground, mainly because of stress

caused by shallow substrate, drought and exposure. According to Köhler (2004b), taller plants on the ground generally display smaller size on extensive green roofs.

Chapter 6 Conclusion

6.1 Introduction

This thesis is mainly concerned with the possibility of plant selection and communities from calcareous grassland for roof greening vegetation. Calcareous grassland plant communities have great potential for roof greening vegetations because calcareous grasslands, which are some of the most species-rich plant communities occurring over limestone or chalk substrates, can offer abundant sources of native plant species. They also have similar soil characteristics, typically thin, low nutrient, and free draining soils (Tansley, 1965, p.98), to that on green roof systems. The plant communities, including regional distinctive native species that have evolved and are adapted to the local climate and conditions over a long period of time, are naturally stress-tolerant, and adapted to limited water availability.

However, as mentioned in Chapter 1, there are few studies of recreating calcareous grassland plant communities on green roofs and few specific recommendations for native plant communities or assemblages on green roofs in the UK. This explores the possibility through a series of experiments which were designed to investigate the nature of the potential substrates that support the plant community from calcareous grasslands, and to examine a standardised plant screening methodology to investigate the environmental tolerances of a range of species and to explore patterns of plant growth and flowering performance at the community and individual species level.

In this chapter, the experiments described in this thesis are concluded and performances of each individual species are summarized, using the results from those experiments.

6.2 Substrates for calcareous grassland vegetations on green roofs

In terms of the physical and chemical characteristics of the experimental substrates used in this study, all of the substrates could be classified as lightweight substrates for green

roofs. Of the substrates, the LECA substrate type was recorded as the lightest weight, the Brick rubble substrate type was intermediate, and the heaviest was the Limestone substrate type. In terms of air filled porosity, pH value, and salt content, all of the substrates met the minimum requirements that conform to FLL standards. The pH range of the substrates (7.42 to 8.33) is suitable for species originating from calcareous grassland. Under dry environmental condition of the roof, however, some of the Limestone types, the LECA, and the Brick rubble are not effective substrates for use of green roof systems to support successful plant development and performance; this is due to insufficient air filled porosities and/or moisture content, and/or soil compaction leading to poor water use and restricted nutrient uptake for the brick rubble especially. In the case of the LECA substrate with 100:0:0 composition rate, the substrate completely dried out by the next day with 0.0 % water holding capacity at 22.3 °C, RH 57.0 % (*Ref*. Appendix 2). Therefore, it is suggested that LECA must be mixed with other materials and would have to be used as a soil amendment, and that for use of brick rubble substrate, another amendment such as loam with organic matter together would be necessary to advance soil permeability for effective root penetration and water use of plant for successful establishment and growth of the species.

In terms of application to practical use on green roofs, successful substrates are likely to combine the ideal balance of moisture content and air filled porosity, and to support high survival, rapid establishment, and high ground cover density (Getter and Rowe, 2006; Beattie and Berghage, 2004; White and Snodgrass, 2003). The result shows that the regular supplementary watering for this experiment might be an important factor in all of the substrates being able to support high seedling survival. For dry environmental conditions on the roof, it would be valuable to investigate drought tolerance of the species on the different substrate types under conditions with different irrigation regimes for further research. This would lead to minimum frequency of watering to maintain stable growth of the species. It was shown that at least 10 % organic matter and addition of loam is essential and 60:20:20 composition rate was the best to achieve a good balance between moisture content and air filled porosity and to sufficiently support the emergence and initial growth of seedling of the species. In this study, the substrate that most consistently met these criteria across all of the species was the Limestone 2 substrate type with 60:20:20 composition rate. The substrate could also

support wider range of calcareous grassland species on the roof compared to the two Zinco commercial substrates. In future studies, more detailed work on the physical and chemical characteristics of the substrate, especially for water transfer efficiency and root penetrability, and nutrient characteristics, would be needed to evaluate the relationship between substrate and plant performance. In addition, the different responses of each species to the substrates would be useful to investigate performance of various species originating from the habitat on the substrates and/or other substrate components.

6.3 Establishment and performance of the plant community

The abundance, structural characteristics, biomass, and flowering performance of the plant community were influenced by substrate depth, irrigation, substrate type, and fertiliser application. As expected, favourable conditions, deeper substrate depths, and using a calcareous substrate containing organic matter, supplementary watering, and fertiliser addition promote greater abundance, height, coverage, biomass, and flowering performance of the plant community. This is probably because they provide the advantage of moisture retention, less temperature fluctuation, and high availability of nutrients to the plant community. It was also shown that additional watering and fertiliser application was not as critical when the plant community was established. However, for successful establishment during the first growing season in order for the plant community to persist over long-term, it is desirable to have at least 100 mm substrate depth and supplementary watering during drought period in the UK climate. Of all the treatments, drought stress produced the most severe effects on the plant community development. Peak flowering time was revealed as the time from August to October at all environment conditions. It is important to understand that in some parts, these favourable conditions can cause problems for development of the plant community over time, for example, decreased biomass in the limestone-based substrate and in the fertiliser treatment in the second growing season compared to the first growing season. It is, therefore, important to maintain observations into the long-term.

6.4 The growth characteristics of individual plant species over time

In previous study by Nagase (2008) on the green roof in Rotherham, Northern England from February to November 2006, the growth characteristics of individual species over the time was analysed in three sections: coverage, vertical (height) and best growing season. The analysis method was adapted for this study. In this study, plant structural characteristics of individual species were evaluated over the first growing season. In vertical growth and coverage, two categories were used, growth form (maximum size in height and coverage) and growth pattern (phenology). Three growth forms were identified across all treatment, low (plant height < 50 mm), medium (50 mm ≤ plant height < 100 mm), and high (plant height ≥ 100 mm), and coverage (small: plant coverage < 5.00 %; medium: 5.00 % ≤ plant coverage < 10.00 %; large: plant coverage ≥ 10.00 %). The growth patterns were also divided into four types (stable: coverage or height does not change significantly over time; bell shaped: coverage or height increases and reaches a maximum in a certain time and then declines; increase: coverage or height increases and they are stable after they reach maximum growth, or increase steadily; decrease: coverage or height decrease steadily). The best growing season in this study indicates a month when the plant reached maximum size in terms of height and coverage of plant. Plant abundance, plant growth (dry-weight), and, flowering times are also added. Three groups can be classified for plant abundance and growth of individual species: for abundance, species unlikely to survive (< 50%), moderate (50 ≤ plant abundance < 100 %), and best abundance (≥ 100 %); for plant growth, species with low (< 2.000 g), and medium (2.000 ≤ plant growth < 5.000 g), high growth (5.000 g ≤ plant growth < 10.000 g), and greater growth (≥ 10.000 g). This section summarises plant growth characteristics and responses to environmental variables of each individual plant species.

6.4.1 *ACHILLEA MILLEFOLIUM*

Plant abundance, structural characteristics, and/or growth of *Achillea* were influenced by substrate depth, irrigation, substrate type, and fertiliser, except for plant abundance in 2007 in substrate depth treatment with watering, structure (height) in substrate type treatment (Table 6.1). *Achillea* showed successful plant establishment and performance on favourable conditions with deeper substrate depths and all treatments with watering, except for 50 mm depth. Supplementary watering was clearly advantageous to plant abundance at 50 mm and 200 mm depths, structures at 50 mm and 100 mm depth, and plant growth at 50 mm and 200 mm in 2007 and at 50 mm depth in 2008.

The favourable growing conditions tended to produce medium to large plant size, high abundance, and medium to greater growth, and the species exhibited the increase growth pattern over time. The plants tended to show greater overwintering survival and increase in plant growth with more than medium range in the second growing season across all treatments. In contrast, *Achillea* did not thrive on substrate with 50 mm substrate depth in both watering treatments during the 2-years period. The plants tended to be small in plant size and growth. The plant structures at the 50 mm deep substrate without watering showed a decreased growing pattern continuously or a bell-shaped pattern over time, while the plant with watering did not change much in plant height over time but coverage increased to October.

Achillea started flowering in later season (September) and continued until October, compared to the ground level from June to August. They were found only at 200 mm deep substrate without watering, and at substrate with fertiliser application.

Table 6.1. Summary of plant performance of *Achillea millefolium* in response to environmental variables.

			50 mm				100 mm				200mm			
		Response to environm-ental variables*	Max. size	Growth form	Growth pattern	Best growing season	Max. size	Growth form	Growth pattern	Best growing season	Max. size	Growth form	Growth pattern	Best growing season
Non-watering treatment	Vertical (cm)	D	4.7	Low	Decrease	Jul	5.5	Medium	Increase	Oct	8.4	Medium	Increase	Sep
	Coverage (%)	D	1.86	Small	Bell	Aug	4.47	Small	Increase	Jul	6.06	Medium	Increase	Sep
	Abundance 2007	D	n.s.											
	Abundance 2008	D	n.s.											
	Growth 2007	D	Low				Low				Medium			
	Growth 2008	D	n.s.				High				High			
	Flowering	I	–				–				Sep. to Oct.			
Watering treatment	Vertical (cm)	D	4.5	Low	Stable	Jul	7.5	Medium	Increase	Sep	7.2	Medium	Increase	Sep
	Coverage (%)	D	3.18	Small	Increase	Oct	7.41	Medium	Increase	Oct	10.30	High	Increase	Oct
	Abundance 2007	I												
	Abundance 2008	D												
	Growth 2007	D	Low				Medium				Medium			
	Growth 2008	D	Low				High				Greater			
	Flowering	D	–				–				–			

Substrate type	Zinco substrate	Vertical (cm)	I	7.9	Medium	Increase	Sep	Limestone-based substrate	Vertical (cm)	8.7	Medium	Increase	Sep
		Coverage (%)	D	5.74	Medium	Increase	Oct		Coverage (%)	10.5	High	Increase	Oct
		Abundance 2007	D						Survival 2007				
		Abundance 2008	D						Survival 2008				
		Growth 2007	D	Medium					Growth	Medium			
		Growth 2008	D	High					Growth 2008	High			
		Flowering		–					Flowering	–			
Fertiliser treatment	Non-fertiliser application	Vertical (cm)	D	5.7	Medium	Increase	Aug	Fertiliser application	Vertical (cm)	30.7	High	Increase	Sep
		Coverage (%)	D	7.97	Medium	Increase	Oct		Coverage (%)	49.2	High	Increase	Oct
		Abundance 2007	D						Survival 2007				
		Abundance 2008	D						Survival 2008				
		Growth 2007	D	Medium					Growth	Greater			
		Growth 2008	D	Medium					Growth 2008	Greater			
		Flowering		–					Flowering	Sep. to Oct.			

*Significant responses to environmental variables (substrate depth, substrate type, fertiliser treatment);
D: dependent response (statistical significance); I: Independent response (no statistical significance).

Vertical: Low: plant height < 50mm; Medium; 50 mm ≤ plant height < 100 mm; High: plant height ≥ 100 mm.

Coverage: Small: plant coverage < 5.00 %; Medium: 5.00 % ≤ plant coverage < 10.00 %; Large: plant coverage ≥ 10.00 %

Abundance: Unlikely to survive (< 50%) n.s.; not survived at all. Moderate abundance (50 ≤ plant abundance < 100 %) Best abundance (≥ 100 %)

Growth: Low (< 2.000 g); Medium (2.000 ≤ plant growth < 5.000 g); High (5.000 g ≤ plant growth < 10.000 g); Greater (≥ 10.000 g).

6.4.2 *AGRIMONIA EUPATORIA*

Plant abundance, structural characteristics, and/or growth of *Agrimonia* were influenced by the given environmental variables. However, in the substrate type treatment, the plant was not influenced, except for plant height (Table 6.2). *Agrimonia* plants appeared to be promoted in abundance, structural characteristics, and/or growth by 100 mm and 200 mm depth, watering, the Limestone-based substrate, and fertiliser application. In the substrate depth treatment with both watering regimes plant structures appeared to be advantageous at 100 mm depth although no significant differences were found between the two deeper substrate depths. Supplementary watering promoted plant structures and abundance at 50 mm and 100 mm depth, and plant growth at 50 mm in 2008.

Agrimonia tended to produce small plant growth in all treatments, except for the Limestone-based substrate that produced medium plant growth in the second growing season, and to show a decreased growth pattern at all depths without watering and at 50 mm depth with it, and in the non-fertiliser treatment. *Agrimonia* did not survive at all in the non-watered 50 mm depth during the 2-years period, while in more favourable conditions, the deeper substrate depths (100 mm and 200 mm) and all treatments with supplemental watering, it did successfully over time. However, in the following season plants in the watered 50 mm depth, and in the non-watered 100 mm and 200 mm depth showed reduced plant abundance.

Agrimonia flowered during the month of August in the non-watered 100 mm depth and in both fertiliser treatments, and in September in the watered 200 mm depth and in both substrate types. The flowering performance was independent of the environmental variables.

Table 6.2. Summary of plant performance of *Agrimonia eupatoria* in response to environmental variables.

			50 mm				100 mm				200mm			
		Response to environm-ental variables*	Max. size	Growth form	Growth pattern	Best growing season	Max. size	Growth form	Growth pattern	Best growing season	Max. size	Growth form	Growth pattern	Best growing season
Non-watering treatment	Vertical (cm)	D	7.1	Medium	Decrease	Jun	9.8	Medium	Decrease	Sep	7.9	Medium	Decrease	Jun
	Coverage (%)	D	1.90	Small	Decrease	Jul	3.12	Small	Decrease	Jun	1.60	Small	Stable	Aug
	Abundance 2007	D	n.s.											
	Abundance 2008	D	n.s.											
	Growth 2007	D	Low				Low				Low			
	Growth 2008	D	n.s.				Low				Low			
	Flowering	I	–				Aug				–			
Watering treatment	Vertical (cm)	I	7.8	Medium	Decrease	Jun	11.5	High	Stable	Sep	7.2	Medium	Stable	Jun
	Coverage (%)	D	1.79	Small	Stable	Jun	2.78	Small	Stable	Jul	1.70	Small	Stable	Sep
	Abundance 2007	I												
	Abundance 2008	I												
	Growth 2007	D	Low				Low				Low			
	Growth 2008	D	Low				Low				Low			
	Flowering	I	–				–				Sep			

Substrate type	Zinco substrate	Vertical (cm)	D	7.3	Medium	Stable	Sep	Limestone-based substrate	Vertical (cm)	12.2	High	Stable	Sep
		Coverage (%)	I	2.23	Small	Stable	Aug		Coverage (%)	2.99	Small	Increase	Oct
		Abundance 2007	I						Survival 2007				
		Abundance 2008	I						Survival 2008				
		Growth 2007	I	Low					Growth	Low			
		Growth 2008	I	Low					Growth 2008	Medium			
		Flowering	I	Sep					Flowering	Sep			
Fertiliser treatment	Non-fertiliser application	Vertical (cm)	D	5.4	Medium	Decrease	Aug	Fertiliser application	Vertical (cm)	27.4	High	Increase	Sep
		Coverage (%)	D	1.99	Small	Stable	Jun		Coverage (%)	5.42	Medium	Increase	Aug
		Abundance 2007	D						Survival 2007				
		Abundance 2008	–						Survival 2008				
		Growth 2007	D	Low					Growth	Low			
		Growth 2008	I	Low					Growth 2008	Low			
		Flowering	I	Aug					Flowering	Aug			

*Significant responses to environmental variables (substrate depth, substrate type, fertiliser treatment);
D: dependent response (statistical significance); I: Independent response (no statistical significance).

Vertical: Low: plant height < 50mm; Medium; 50 mm ≤ plant height < 100 mm; High: plant height ≥ 100 mm.

Coverage: Small: plant coverage < 5.00 %; Medium: 5.00 % ≤ plant coverage < 10.00 %; Large: plant coverage ≥ 10.00 %

Abundance: Unlikely to survive (< 50%); Moderate abundance (50 ≤ plant abundance < 100 %); Best abundance (≥ 100 %)

n.s.; not survived at all.

Growth: Low (< 2.000 g); Medium (2.000 ≤ plant growth < 5.000 g); High (5.000 g ≤ plant growth < 10.000 g); Greater (≥ 10.000 g).

6.4.3 *CAMPANUMLA GLOMERATA*

Campanula was influenced by substrate depth with the non-watering treatment, substrate type, and fertiliser, but not substrate depth with watering treatment (Table 6.3). Plant abundance, structures, and/or growth appeared to be promoted by 100 mm and 200 mm depth, the Limestone-based substrate, and fertiliser application. Supplementary watering promoted plant establishment and performance of *Campanula* at 50 mm and/or 100 mm depth.

In the first growing season, *Campanula* plants tended to maintain high abundance rates in 100 mm and 200 mm depth without supplemental watering, 50 mm depth and 100 mm depth with it, and the Zinco substrate and the Limestone-based substrate treatment, while in 200 mm depth with supplemental watering and both fertiliser applications plant exhibited medium survival rate. In the following season, *Campanula* appeared to reduce plant abundance in the former treatments and in 50 mm depth the plants did not survive at all, while in the latter treatments plants have recovered completely. *Campanula* plants tended to produce small plant structure and growth over time. After summer (August) in the first growing season *Campanula* showed a decreased growth pattern in the non-watered 50 mm depth (for both plant height and coverage) and in the non-fertiliser application (for plant height).

The flowers were found only during the month of October in the non-watered 100 mm depth and in the watered 50 mm.

Table 6.3. Summary of plant performance of *Campanula glomerata* in response to environmental variables.

			50 mm				100 mm				200mm			
		Response to environm-ental variables*	Max. size	Growth form	Growth pattern	Best growing season	Max. size	Growth form	Growth pattern	Best growing season	Max. size	Growth form	Growth pattern	Best growing season
Non-watering treatment	Vertical (cm)	D	1.0	Low	Decrease	Jul	2.5	Low	Stable	Sep	2.9	Low	Increase	Sep
	Coverage (%)	D	0.49	Small	Decrease	Aug	0.48	Small	Stable	Sep	0.71	Small	Stable	Aug
	Abundance 2007	D												
	Abundance 2008	D			n.s									
	Growth 2007	I			Low				Low				Low	
	Growth 2008	D			n.s.				Low				Low	
	Flowering	I			–				Oct				–	
Watering treatment	Vertical (cm)	I	2.0	Low	Increase	Oct	1.4	Low	Increase	Sep	1.9	Low	Bell	Sep
	Coverage (%)	I	4.69	Small	Stable	Oct	5.91	Medium	Increase	Oct	6.30	Medium	Increase	Jul
	Abundance 2007	I												
	Abundance 2008	I												
	Growth 2007	I			Low				Low				Low	
	Growth 2008	I			Low				Low				Low	
	Flowering	I			Oct				–				–	

Substrate type	Zinco substrate	Vertical (cm)	D	1.9	Low	Increase	Sep	Limestone-based substrate	Vertical (cm)	3.2	Low	Increase	Sep	
		Coverage (%)	D	0.67	Small	Stable	Oct		Coverage (%)	2.02	Small	Increase	Sep	
		Abundance 2007	I						Survival 2007					
		Abundance 2008	I						Survival 2008					
		Growth 2007	D			Low			Growth 2007			Low		
		Growth 2008	D			Low			Growth 2008			Low		
		Flowering	–			–			Flowering			–		
Fertiliser treatment	Non-fertiliser application	Vertical (cm)	D	1.5	Low	Decease	Aug	Fertiliser application	Vertical (cm)	4.6	Low	Bell	Aug	
		Coverage (%)	D	0.69	Small	Stable	Aug		Coverage (%)	4.71	Small	Stable	Oct	
		Abundance 2007	I						Survival 2007					
		Abundance 2008	–						Survival 2008					
		Growth 2007	I			Low			Growth 2007			Low		
		Growth 2008	I			Low			Growth 2008			Low		
		Flowering	–			–			Flowering			–		

*Significant responses to environmental variables (substrate depth, substrate type, fertiliser treatment);
D: dependent response (statistical significance); I: Independent response (no statistical significance).

Vertical: Low: plant height < 50mm; Medium; 50 mm ≤ plant height < 100 mm; High: plant height ≥ 100 mm.

Coverage: Small: plant coverage < 5.00 %; Medium: 5.00 % ≤ plant coverage < 10.00 %; Large: plant coverage ≥ 10.00 %

Abundance: Unlikely to survive (< 50%); Moderate abundance (50 ≤ plant abundance < 100 %); Best abundance (≥ 100 %)

n.s.; not survived at all.

Growth: Low (< 2.000 g); Medium (2.000 ≤ plant growth < 5.000 g); High (5.000 g ≤ plant growth < 10.000 g); Greater (≥ 10.000 g).

6.4.4 *CLINOPODIUM VULGARE*

Plant abundance, structures, and/or growth of *Clinopodium* appeared to be influenced by substrate depth, irrigation, substrate type, and fertiliser (Table 6.4). However, under the watering condition, plant structure characteristics only showed different response to substrate depths (for plant coverage). The more favourable conditions with deeper substrate depths, supplementary watering, the Limestone-based substrate, and fertiliser addition tended to support higher plant abundance, growth, and/or structural characteristics. Supplementary watering showed clear advantages to plant structures and growth in 2007 at 50 mm depth, and plant abundance at 50 mm depth and 200 mm depth.

In all treatments, *Clinopodium* plants tended to maintain high abundance and increased growth pattern to September or October, except for 50 mm depth without supplementary watering. In the non-watered 50 mm depth, plants did not survive successfully and were retarded by drought condition during August in the first growing season. The plants tended to produce low plant growth in all treatments, except for the Limestone-based substrate that did medium plant growth in the first growing season.

Flowering of *Clinopodium* was influenced by substrate depth, irrigation, substrate type, and fertiliser. *Clinopodium* produced more flowers in 100 mm and 200 mm depth, the Limestone-based substrate, and fertiliser treatment. Supplementary watering promoted flowering performance at 50 mm depth. Flowering started mostly in August and continued until September. However, plant with fertiliser application had an extended flowering season from July to October.

Table 6.4. Summary of plant performance of *Clinopodium vulgare* in response to environmental variables.

			50 mm				100 mm				200mm			
		Response to environm-ental variables*	Max. size	Growth form	Growth pattern	Best growing season	Max. size	Growth form	Growth pattern	Best growing season	Max. size	Growth form	Growth pattern	Best growing season
Non-watering treatment	Vertical (cm)	D	7.3	Medium	Bell	Aug	12.1	High	Bell	Sep	11.4	High	Increase	Aug
	Coverage (%)	D	1.10	Small	Decrease	Aug	2.37	Small	Bell	Aug	2.89	Small	Bell	Aug
	Abundance 2007	D												
	Abundance 2008	D												
	Growth 2007	D	Low				Low				Low			
	Growth 2008	D	Low				Low				Low			
	Flowering	D	Aug to Sep				Aug to Sep				Aug to Sep			
Watering treatment	Vertical (cm)	I	11.4	High	Increase	Sep	10.1	High	Increase	Sep	10.2	High	Increase	Sep
	Coverage (%)	D	1.75	Small	Increase	Oct	1.99	Small	Increase	Oct	2.41	Small	Increase	Sep
	Abundance 2007	I												
	Abundance 2008	I												
	Growth 2007	I	Low				Low				Low			
	Growth 2008	I	Low				Low				Low			
	Flowering	I	Aug to Sep				Aug to Sep				Aug to Sep			

Substrate type	Zinco substrate	Vertical (cm)	D	9.9	Medium	Increase	Sep	Limestone-based substrate	Vertical (cm)	17.5	High	Increase	Oct
		Coverage (%)	D	1.67	Small	Increase	Sep		Coverage (%)	6.63	Medium	Increase	Sep
		Abundance 2007	D						Survival 2007				
		Abundance 2008	I						Survival 2008				
		Growth 2007	D	Low					Growth 2007	Medium			
		Growth 2008	I	Low					Growth 2008	Low			
		Flowering	D	Aug to Sep					Flowering	Aug to Oct			
Fertiliser treatment	Non-fertiliser application	Vertical (cm)	D	9.6	Medium	Increase	Sep	Fertiliser application	Vertical (cm)	21.3	High	Increase	Sep
		Coverage (%)	D	2.64	Small	Stable	Oct		Coverage (%)	24.7	Large	Bell	Aug
		Abundance 2007	D						Survival 2007				
		Abundance 2008	I						Survival 2008				
		Growth 2007	I	Low					Growth 2007	Low			
		Growth 2008	I	Low					Growth 2008	Low			
		Flowering	D	Aug					Flowering	Jul to Oct			

*Significant responses to environmental variables (substrate depth, substrate type, fertiliser treatment);
D: dependent response (statistical significance); I: Independent response (no statistical significance).

Vertical: Low: plant height < 50mm; Medium; 50 mm ≤ plant height < 100 mm; High: plant height ≥ 100 mm.

Coverage: Small: plant coverage < 5.00 %; Medium: 5.00 % ≤ plant coverage < 10.00 %; Large: plant coverage ≥ 10.00 %

Abundance: Unlikely to survive (< 50%); Moderate abundance (50 ≤ plant abundance < 100 %); Best abundance (≥ 100 %)

n.s.; not survived at all.

Growth: Low (< 2.000 g); Medium (2.000 ≤ plant growth < 5.000 g); High (5.000 g ≤ plant growth < 10.000 g); Greater (≥ 10.000 g).

6.4.5 *GALIUM VERUM*

Plant abundance, structural characteristics, and growth of *Galium* were influenced by substrate depth, irrigation, substrate type, and fertiliser, except for plant height and abundance in 2007 in substrate depth treatment with supplementary watering, and plant abundance in 2007 and growth in 2008 in substrate type treatment (Table 6.5). Plant establishment and performance of the species was promoted by deeper substrates, watering, the Limestone-based substrate, and fertiliser. Plant with supplementary watering produced higher plant abundance at all depths, structures at 50 mm depth, and growth at 50 mm depth in the second growing season.

Galium plants survived successfully in all treatments over time, except for 50 mm depth without supplemental watering that exhibited low plant abundance with less than 50% of the original value. In the first growing season, *Galium* produced low plant growth, except for plants with fertiliser that showed high plant growth. In the following season, however, plant growth increased in 200 mm depth in the two watering treatments, the Limestone-based substrate, and fertiliser treatment. The plants tended to produce medium height and small coverage in all treatments, excluding plants in the Limestone-based substrate and with fertiliser application, and showed an increased growth pattern to September or October, except for the non-watered 50 mm and 100 mm depth.

Galium produced flowers in 100 mm from August to October, and in the non-watered 200 mm depth during the month of August, and in the Limestone-based substrate and fertiliser application from September to October.

Table 6.5. Summary of plant performance of *Galium verum* in response to environmental variables.

			50 mm				100 mm				200mm			
		Response to environm-ental variables*	Max. size	Growth form	Growth pattern	Best growing season	Max. size	Growth form	Growth pattern	Best growing season	Max. size	Growth form	Growth pattern	Best growing season
Non-watering treatment	Vertical (cm)	D	5.3	Medium	Bell	Aug	6.6	Medium	Stable	Aug	5.9	Medium	Increase	Aug
	Coverage (%)	D	1.43	Small	Bell	Aug	2.84	Small	Bell	Sep	3.32	Small	Increase	Sep
	Abundance 2007	D												
	Abundance 2008	D												
	Growth 2007	D	Low				Low				Low			
	Growth 2008	D	n.s.				Low				Medium			
	Flowering	I	–				Aug to Oct				Aug			
Watering treatment	Vertical (cm)	I	5.1	Medium	Increase	Sep	5.6	Medium	Increase	Sep	5.0	Medium	Increase	Sep
	Coverage (%)	D	2.04	Small	Increase	Oct	2.86	Small	Increase	Oct	3.92	Small	Increase	Oct
	Abundance 2007	I												
	Abundance 2008	D												
	Growth 2007	D	Low				Low				Low			
	Growth 2008	D	Low				Low				Medium			
	Flowering	–	–				–				–			

Substrate type: Zinco substrate	Vertical (cm)	D	5.9	Medium	Increase	Sep	Limestone-based substrate	Vertical (cm)	8.7	Medium	Increase	Sep		
	Coverage (%)	D	2.68	Small	Increase	Oct		Coverage (%)	8.95	Medium	Increase	Oct		
	Abundance 2007	I						Survival 2007						
	Abundance 2008	D						Survival 2008						
	Growth 2007	D	Low					Growth 2007	Low					
	Growth 2008	I	Low					Growth 2008	Medium					
	Flowering	–	–					Flowering	Sep to Oct					
Fertiliser treatment: Non-fertiliser application	Vertical (cm)	D	5.5	Medium	Increase	Aug	Fertiliser application	Vertical (cm)	15.1	High	Increase	Sep		
	Coverage (%)	D	3.54	Small	Increase	Oct		Coverage (%)	21.0	Large	Increase	Oct		
	Abundance 2007	D						Survival 2007						
	Abundance 2008	D						Survival 2008						
	Growth 2007	D	Low					Growth 2007	High					
	Growth 2008	D	Medium					Growth 2008	Greater					
	Flowering	–	–					Flowering	Sep to Oct					

*Significant responses to environmental variables (substrate depth, substrate type, fertiliser treatment);
D: dependent response (statistical significance); I: Independent response (no statistical significance).

Vertical: Low: plant height < 50mm; Medium; 50 mm ≤ plant height < 100 mm; High: plant height ≥ 100 mm.

Coverage: Small: plant coverage < 5.00 %; Medium: 5.00 % ≤ plant coverage < 10.00 %; Large: plant coverage ≥ 10.00 %

Abundance: Unlikely to survive (< 50%); Moderate abundance (50 ≤ plant abundance < 100 %); Best abundance (≥ 100 %)

n.s.; not survived at all.

Growth: Low (< 2.000 g); Medium (2.000 ≤ plant growth < 5.000 g); High (5.000 g ≤ plant growth < 10.000 g); Greater (≥ 10.000 g).

6.4.6 *HELIANTHEMUM NUMMULARIUM*

Plant abundance, structural characteristics, and/or growth of *Helianthemum* were influenced by the given environmental variables. The effects of the environmental variables were observed only in plant structures (for both height and coverage), abundance, and growth in the substrate depth treatment without supplementary watering, and plant growth in substrate depth treatment with it, and plant structure (height) in fertiliser treatment (Table 6.6). Supplementary watering showed clear advantages to plant structures and abundance at 50 mm depth, and plant growth at 50 mm and 100 mm depth in 2007 and only at 50 mm in 2008.

Helianthemum plants showed successful plant abundance in the first growing season, except for 50 mm depth without supplementary watering. However, in the following season, the plant abundance decreased across all treatments. Of the treatments, plants did not survive at all in the non-watered 50 mm depth, and in the watered 100 mm and 200 mm depth, and in the Zinco substrate treatment. *Helianthemum* produced low plant growth in all treatments, except for the watered 200 mm depth that showed medium plant growth in 2007. The plants tended to maintain low height and coverage, and showed an increased growth pattern for coverage in the favourable conditions, deeper substrate and all treatments with supplementary watering.

Helianthemum produced flowers in later season (September), compared to the ground level (June to July). The flowers were found in 50 mm and 200 mm depth with and without watering, the Limestone-based substrate, and fertiliser application. In the Limestone-based substrate the flowering period extended from September to October.

Table 6.6. Summary of plant performance of *Helianthemum nummularium* in response to environmental variables.

			50 mm				100 mm				200mm			
		Response to environm-ental variables*	Max. size	Growth form	Growth pattern	Best growing season	Max. size	Growth form	Growth pattern	Best growing season	Max. size	Growth form	Growth pattern	Best growing season
Non-watering treatment	Vertical (cm)	D	1.9	Low	Decrease	Jun	2.2	Low	Stable	Sep	3.1	Low	Decrease	Jun
	Coverage (%)	D	1.13	Small	Bell	Jul	2.11	Small	Increase	Sep	5.01	Medium	Increase	Oct
	Abundance 2007	D												
	Abundance 2008	I	n.s.											
	Growth 2007	D	Low				Low				Low			
	Growth 2008	I	n.s.				Low				Low			
	Flowering	I	Sep				–				Sep			
Watering treatment	Vertical (cm)	I	2.5	Low	Stable	Jun	2.5	Low	Stable	Sep	3.0	Low	Stable	Sep
	Coverage (%)	I	3.24	Small	Increase	Oct	5.50	Medium	Increase	Oct	7.89	Medium	Increase	Oct
	Abundance 2007	I												
	Abundance 2008	I					n.s.				n.s.			
	Growth 2007	D	Low				Low				Medium			
	Growth 2008	I	Low				n.s.				n.s.			
	Flowering	I	Sep				–				Sep			

Substrate type: Zinco substrate	Vertical (cm)	I	2.5	Low	Stable	Jun	Limestone-based substrate	Vertical (cm)	3.6	Low	Increase	Oct		
	Coverage (%)	I	6.48	Medium	Increase	Sep		Coverage (%)	5.82	Medium	Increase	Oct		
	Abundance 2007	–						Survival 2007						
	Abundance 2008	–	n.s.					Survival 2008						
	Growth 2007	I	Low					Growth 2007	Low					
	Growth 2008	–	n.s.					Growth 2008	Low					
	Flowering	–	–					Flowering	Sep to Oct					
Fertiliser treatment: Non-fertiliser application	Vertical (cm)	D	1.6	Low	Stable	Aug	Fertiliser application	Vertical (cm)	3.9	Low	Increase	Oct		
	Coverage (%)	I	8.43	Medium	Increase	Oct		Coverage (%)	8.47	Medium	Increase	Oct		
	Abundance 2007	–						Survival 2007						
	Abundance 2008	I						Survival 2008						
	Growth 2007	I	Low					Growth 2007	Low					
	Growth 2008	I	Low					Growth 2008	Low					
	Flowering	–	–					Flowering	Sep					

*Significant responses to environmental variables (substrate depth, substrate type, fertiliser treatment);
D: dependent response (statistical significance); I: Independent response (no statistical significance).

Vertical: Low: plant height < 50mm; Medium; 50 mm ≤ plant height < 100 mm; High: plant height ≥ 100 mm.

Coverage: Small: plant coverage < 5.00 %; Medium: 5.00 % ≤ plant coverage < 10.00 %; Large: plant coverage ≥ 10.00 %

Abundance: Unlikely to survive (< 50%); Moderate abundance (50 ≤ plant abundance < 100 %); Best abundance (≥ 100 %)

n.s.; not survived at all.

Growth: Low (< 2.000 g); Medium (2.000 ≤ plant growth < 5.000 g); High (5.000 g ≤ plant growth < 10.000 g); Greater (≥ 10.000 g).

6.4.7 *HYPOCHAERIS RADICATA*

Plant abundance, structural characteristics, and growth of *Hypochaeris* were influenced by substrate depth, irrigation, substrate type, and fertiliser. However, the influences were not revealed in plant abundance in 2007 in the substrate depth treatment with watering and in fertiliser treatment, and in 2008 in the substrate depth treatment without watering, and in both growing seasons in substrate type treatment, and in plant growth in 2008 in all treatments (Table 6.7). In the second growing season, the favourable growing conditions tended not to promote plant growth and abundance, except for plant abundance at fertiliser treatment. Supplementary watering did not improve plant structural characteristics and abundance, but produced higher plant growth at 50 mm depth in 2007.

Hypochaeris plants survived successfully in all treatments during the 2-year growing seasons, except for the non-watered 50 mm depth. *Hypochaeris* produced low plant growth in all treatments, except for the watered 200 mm depth, the Limestone-based substrate, and fertiliser application in the first season. In all treatment with supplementary watering, *Hypochaeris* plants tended to exhibit increasing plant structures to September or October, while in the non-watering treatment, plant structures decreased after draught period.

Hypochaeris produced flowers across all treatments, except for 50 mm depth in both watering treatments. The flowering was influenced by substrate depth in both watering treatments and fertiliser treatment. More flowers were produced in deeper substrates and fertiliser treatment. Flowers appeared between June and October. The plant with fertiliser treatment had an extended longer flowering period from June to October.

Table 6.7. Summary of plant performance of *Hypochaeris radicata* in response to environmental variables.

		Response to environm-ental variables*	50 mm				100 mm				200mm			
			Max. size	Growth form	Growth pattern	Best growing season	Max. size	Growth form	Growth pattern	Best growing season	Max. size	Growth form	Growth pattern	Best growing season
Non-watering treatment	Vertical (cm)	D	8.1	Medium	Decrease	Jul	17.6	High	Bell	Sep	14.3	High	Bell	Aug
	Coverage (%)	D	1.84	Small	Decrease	Aug	4.64	Small	Bell	Aug	5.63	Medium	Bell	Aug
	Abundance 2007	D												
	Abundance 2008	I												
	Growth 2007	D	Low				Low				Low			
	Growth 2008	I	Low				Low				Low			
	Flowering	D	–				Jul to Sep				Jul to Oct			
Watering treatment	Vertical (cm)	D	2.2	Low	Stable	Sep	10.3	High	Stable	Sep	7.4	Medium	Increase	Aug
	Coverage (%)	D	1.33	Small	Increase	Sep	3.58	Small	Increase	Aug	5.49	Medium	Increase	Sep
	Abundance 2007	I												
	Abundance 2008	D												
	Growth 2007	D	Low				Low				Medium			
	Growth 2008	I	Low				Low				Low			
	Flowering	D	–				Aug to Oct				Aug to Oct			

		Response to environm-ental variables*	50 mm				200mm			
			Max. size	Growth form	Growth pattern	Best growing season	Max. size	Growth form	Growth pattern	Best growing season
Substrate type: Zinco substrate	Vertical (cm)	D	8.7	Medium	Bell	Jul				
	Coverage (%)	D	3.28	Small	Increase	Sep				
	Abundance 2007	I								
	Abundance 2008	I								
	Growth 2007	D	Low							
	Growth 2008	I	Low							
	Flowering	I	Jul to Oct							
Substrate type: Limestone-based substrate	Vertical (cm)						23.1	High	Increase	Sep
	Coverage (%)						9.08	Medium	Increase	Sep
	Survival 2007									
	Survival 2008									
	Growth 2007						Medium			
	Growth 2008						Low			
	Flowering						Jul to Oct			
Fertiliser treatment: Non-fertiliser application	Vertical (cm)	D	6.9	Medium	Stable	Sep				
	Coverage (%)	D	5.64	Medium	Increase	Oct				
	Abundance 2007	I								
	Abundance 2008	D								
	Growth 2007	D	Low							
	Growth 2008	I	Low							
	Flowering	D	Jun, Aug, Oct							
Fertiliser treatment: Fertiliser application	Vertical (cm)						29.5	High	Bell	Aug
	Coverage (%)						17.4	Large	Increase	Oct
	Survival 2007									
	Survival 2008									
	Growth 2007						High			
	Growth 2008						Low			
	Flowering						Jun to Oct			

*Significant responses to environmental variables (substrate depth, substrate type, fertiliser treatment);
D: dependent response (statistical significance); I: Independent response (no statistical significance).

Vertical: Low: plant height < 50mm; Medium; 50 mm ≤ plant height < 100 mm; High: plant height ≥ 100 mm.

Coverage: Small: plant coverage < 5.00 %; Medium: 5.00 % ≤ plant coverage < 10.00 %; Large: plant coverage ≥ 10.00 %

Abundance: Unlikely to survive (< 50%); Moderate abundance (50 ≤ plant abundance < 100 %); Best abundance (≥ 100 %)

n.s.; not survived at all.

Growth: Low (< 2.000 g); Medium (2.000 ≤ plant growth < 5.000 g); High (5.000 g ≤ plant growth < 10.000 g); Greater (≥ 10.000 g).

6.4.8 *KNAUTIA ARVENSIS*

Plant abundance, structural characteristics, and/or growth of *Knautia* were influenced by substrate depth, irrigation, substrate type, and fertiliser, except for plant abundance in substrate depth treatment with supplementary watering (Table 6.8). The favourable growing conditions with deeper substrate depths, the Limestone-based substrate, and fertiliser addition tended to support greater abundance, plant structures, and growth. Supplementary watering promoted plant abundance and coverage at 50 mm and 100 mm, height at 50 mm depth, and growth at 200 mm in 2007 and at 50 mm depth in 2008.

Knautia plants survived successfully in all treatments over time, except for 50 mm depth without supplemental watering. In the first growing season, *Knautia* plants produced low plant growth in all treatments with the exception of plants in the Limestone-based substrate and with fertiliser addition. In the following season, plant increased plant growth at 200 mm depth in the two watering treatments and maintained medium and greater growth rate in the Limestone-based substrate and fertiliser addition treatment respectively. The plants tended to maintain low height and coverage, and to exhibit an increasing growth pattern for coverage in all treatments with supplementary watering. However, in the non-watering treatment, *Knautia* plant structures tended to exhibit a decreasing pattern after draught period, except for plant coverage at 200 mm depth.

Knautia flowered only in the Limestone-based substrate during the month of October and in fertiliser treatment from September to October.

Table 6.8. Summary of plant performance of *Knautia arvensis* in response to environmental variables.

			50 mm				100 mm				200mm			
		Response to environm-ental variables*	Max. size	Growth form	Growth pattern	Best growing season	Max. size	Growth form	Growth pattern	Best growing season	Max. size	Growth form	Growth pattern	Best growing season
Non-watering treatment	Vertical (cm)	D	2.6	Low	Decrease	Jun	3.8	Low	Decrease	Aug	4.1	Low	Bell	Aug
	Coverage (%)	D	1.84	Small	Decrease	Aug	3.22	Small	Bell	Sep	3.42	Small	Increase	Aug
	Abundance 2007	D												
	Abundance 2008	D												
	Growth 2007	D	Low				Low				Low			
	Growth 2008	D	Low				Low				Medium			
	Flowering	–	–				–				–			
Watering treatment	Vertical (cm)	D	2.6	Low	Decrease	Jul	3.6	Low	Bell	Aug	3.9	Low	Bell	Aug
	Coverage (%)	D	1.98	Small	Increase	Sep	4.19	Small	Increase	Oct	4.86	Small	Increase	Sep
	Abundance 2007	I												
	Abundance 2008	I												
	Growth 2007	D	Low				Low				Low			
	Growth 2008	D	Low				Low				Medium			
	Flowering	–	–				–				–			

Substrate type: Zinco substrate	Vertical (cm)	D	2.8	Low	Stable	Aug	Limestone-based substrate	Vertical (cm)	8.8	Medium	Increase	Oct		
	Coverage (%)	D	3.40	Small	Increase	Oct		Coverage (%)	9.29	Medium	Increase	Oct		
	Abundance 2007	D						Survival 2007						
	Abundance 2008	D						Survival 2008						
	Growth 2007	D	Low					Growth 2007	Medium					
	Growth 2008	D	Low					Growth 2008	Medium					
	Flowering	–	–					Flowering	Oct					
Fertiliser treatment: Non-fertiliser application	Vertical (cm)	D	3.3	Low	Decrease	Aug	Fertiliser application	Vertical (cm)	36.7	High	Increase	Sep		
	Coverage (%)	D	5.63	Medium	Increase	Oct		Coverage (%)	29.2	Large	Increase	Oct		
	Abundance 2007	D						Survival 2007						
	Abundance 2008	D						Survival 2008						
	Growth 2007	D	Low					Growth 2007	Greater					
	Growth 2008	D	Low					Growth 2008	Greater					
	Flowering	–	–					Flowering	Sep to Oct					

*Significant responses to environmental variables (substrate depth, substrate type, fertiliser treatment);
D: dependent response (statistical significance); I: Independent response (no statistical significance).

Vertical: Low: plant height < 50mm; Medium; 50 mm ≤ plant height < 100 mm; High: plant height ≥ 100 mm.

Coverage: Small: plant coverage < 5.00 %; Medium: 5.00 % ≤ plant coverage < 10.00 %; Large: plant coverage ≥ 10.00 %

Abundance: Unlikely to survive (< 50%); Moderate abundance (50 ≤ plant abundance < 100 %); Best abundance (≥ 100 %)

n.s.; not survived at all.

Growth: Low (< 2.000 g); Medium (2.000 ≤ plant growth < 5.000 g); High (5.000 g ≤ plant growth < 10.000 g); Greater (≥ 10.000 g).

6.4.9 *LEONTODON AUTUMNALIS*

Plant abundance, structures, and/or growth of *Leontodon* appeared to be influenced by substrate depth, irrigation, substrate type, and fertiliser. However, influences of environmental variables were not revealed in plant abundance in 2007 in substrate depth treatment with watering, plant abundance in both growing season and growth in 2008 in substrate type treatment, and plant abundance and growth in 2008 in fertiliser addition treatment (Table 6.9). The favourable growing conditions, deeper substrate depths, the Limestone-based substrate, supplementary watering, and fertiliser addition produced greater abundance, height, coverage, and growth of the species. Advantages of supplementary watering occurred in plant structures at 50 mm and 100 mm depth, and in plant growth at all substrate depths in 2007 but at 50 mm depth only in 2008.

In all treatments, *Leontodon* plants tended to successfully maintain plant abundance throughout the 2-year growing season, except for 50 mm depth without supplementary watering, and to produce low plant growth. Of the favourable growing conditions, the watered 200 mm depth in both growing seasons, the Limestone-based substrate in 2007, and fertiliser addition treatment in 2007 produced plant growth with more than medium range.

Leontodon produced flowers in all treatment between June and October. The flowers in the fertiliser treatment had the longest flowering display which started in June and continued until October. The flowering was influenced by substrate depth in both watering treatments and fertiliser treatment, and was prompted by deeper substrates (100 mm and 200 mm depth) and fertiliser treatment.

Table 6.9. Summary of plant performance of *Leontodon autumnalis* in response to environmental variables.

			50 mm				100 mm				200mm			
		Response to environm-ental variables*	Max. size	Growth form	Growth pattern	Best growing season	Max. size	Growth form	Growth pattern	Best growing season	Max. size	Growth form	Growth pattern	Best growing season
Non-watering treatment	Vertical (cm)	D	4.5	Low	Bell	Aug	13.2	High	Bell	Aug	13.0	High	Bell	Aug
	Coverage (%)	D	1.56	Small	Decrease	Aug	3.11	Small	Bell	Aug	5.06	Medium	Increase	Aug
	Abundance 2007	D												
	Abundance 2008	D												
	Growth 2007	D	Low				Low				Low			
	Growth 2008	D	Low				Low				Low			
	Flowering	D	Jul				Jul to Sep				Jul to Sep			
Watering treatment	Vertical (cm)	D	3.0	Low	Stable	Jun	9.7	Medium	Stable	Aug	18.2	High	Bell	Aug
	Coverage (%)	D	2.93	Small	Increase	Oct	5.64	Medium	Increase	Oct	6.86	Medium	Increase	Oct
	Abundance 2007	I												
	Abundance 2008	D												
	Growth 2007	D	Low				Low				Medium			
	Growth 2008	D	Low				Low				Medium			
	Flowering	D	Sep				Aug to Sep				Aug to Oct			

Substrate type	Zinco substrate	Vertical (cm)	D	10.7	High	Stable	Aug	Limestone-based substrate	Vertical (cm)	19.9	High	Increase	Oct
		Coverage (%)	D	7.22	Medium	Increase	Oct		Coverage (%)	10.7	Large	Increase	Sep
		Abundance 2007	I						Survival 2007				
		Abundance 2008	I						Survival 2008				
		Growth 2007	D	Low					Growth 2007	Medium			
		Growth 2008	I	Low					Growth 2008	Low			
		Flowering	I	Aug to Oct					Flowering	Aug to Oct			
Fertiliser treatment	Non-fertiliser application	Vertical (cm)	D	12.8	High	Stable	Aug	Fertiliser application	Vertical (cm)	26.1	High	Bell	Aug
		Coverage (%)	D	8.29	Medium	Increase	Oct		Coverage (%)	19.4	Large	Increase	Oct
		Abundance 2007	D						Survival 2007				
		Abundance 2008	I						Survival 2008				
		Growth 2007	D	Low					Growth 2007	High			
		Growth 2008	I	Low					Growth 2008	Low			
		Flowering	D	Jun, Aug, Oct					Flowering	Jun to Oct			

*Significant responses to environmental variables (substrate depth, substrate type, fertiliser treatment);
D: dependent response (statistical significance); I: Independent response (no statistical significance).

Vertical: Low: plant height < 50mm; Medium; 50 mm ≤ plant height < 100 mm; High: plant height ≥ 100 mm.

Coverage: Small: plant coverage < 5.00 %; Medium: 5.00 % ≤ plant coverage < 10.00 %; Large: plant coverage ≥ 10.00 %

Abundance: Unlikely to survive (< 50%); Moderate abundance (50 ≤ plant abundance < 100 %); Best abundance (≥ 100 %)

n.s.; not survived at all.

Growth: Low (< 2.000 g); Medium (2.000 ≤ plant growth < 5.000 g); High (5.000 g ≤ plant growth < 10.000 g); Greater (≥ 10.000 g).

6.4.10 *LEUCANTHEMUM VULGARE*

Plant structural characteristics and growth of *Leucanthemum* were influenced by substrate depth, substrate type, and fertiliser, while plant abundance was not influenced by the given environmental variables, except for plant abundance in 2007 in substrate depth treatment without watering (Table 6.10). Greater plant abundance, structures, and growth of the species occurred in 200 mm depth, in the Limestone-based substrate, and in fertiliser addition treatment. Supplementary watering improved plant structure (coverage only) at 50 mm depth, and growth at 50 mm and 200 mm depth in both growing seasons.

Leucanthemum plants successfully survived in all treatments over time, except for 50 mm depth without supplementary watering. In all treatments with the exception of 50 mm depth with both watering regimes, *Leucanthemum* produced plant growth with more than medium range during the 2-year growing seasons.

Leucanthemum produced flowers in the non-watered 100 mm depth from August to September and in the non-watered 200 mm depth from August to October, and in watered 200 mm depth from August to September, and in the Limestone-based substrate from September to October, and in the fertiliser treatment from August to October. Supplementary watering did not prove to be advantageous to plant flowering performance at all substrate depths. The flowering performance was only influenced by substrate depths without watering.

Table 6.10. Summary of plant performance of *Leucanthemum vulgare* in response to environmental variables.

			50 mm				100 mm				200mm			
		Response to environm-ental variables*	Max. size	Growth form	Growth pattern	Best growing season	Max. size	Growth form	Growth pattern	Best growing season	Max. size	Growth form	Growth pattern	Best growing season
Non-watering treatment	Vertical (cm)	D	5.4	Medium	Decrease	Jul	8.1	Medium	Bell	Aug	14.5	High	Bell	Sep
	Coverage (%)	D	7.24	Medium	Bell	Jul	9.17	Medium	Bell	Sep	10.5	Large	Bell	Sep
	Abundance 2007	D												
	Abundance 2008	I												
	Growth 2007	D	Low				Medium				High			
	Growth 2008	D	Low				Medium				High			
	Flowering	D	–				Aug to Sep				Aug to Oct			
Watering treatment	Vertical (cm)	D	4.5	Low	Stable	Jun	8.1	Medium	Increase	Aug	8.0	Medium	Increase	Oct
	Coverage (%)	D	3.70	Small	Increase	Sep	8.06	Medium	Increase	Aug	10.34	Large	Increase	Oct
	Abundance 2007	I												
	Abundance 2008	I												
	Growth 2007	D	Low				Medium				High			
	Growth 2008	D	Low				Medium				Greater			
	Flowering	I	–				–				Aug to Sep			

Substrate type	Zinco substrate	Vertical (cm)	D	5.7	Medium	Increase	Sep	Limestone-based substrate	Vertical (cm)	18.4	High	Increase	Sep
		Coverage (%)	D	5.61	Medium	Increase	Oct		Coverage (%)	15.8	Large	Increase	Sep
		Abundance 2007	I						Survival 2007				
		Abundance 2008	I						Survival 2008				
		Growth 2007	D	Medium					Growth 2007	Greater			
		Growth 2008	I	Medium					Growth 2008	High			
		Flowering	–	–					Flowering	Sep to Oct			
Fertiliser treatment	Non-fertiliser application	Vertical (cm)	D	6.4	Medium	Stable	Aug	Fertiliser application	Vertical (cm)	31.6	High	Increase	Sep
		Coverage (%)	D	6.85	Medium	Stable	Oct		Coverage (%)	31.1	Large	Bell	Aug
		Abundance 2007	I						Survival 2007				
		Abundance 2008	I						Survival 2008				
		Growth 2007	D	Medium					Growth 2007	Greater			
		Growth 2008	D	Medium					Growth 2008	Greater			
		Flowering	–	–					Flowering	Aug to Oct			

*Significant responses to environmental variables (substrate depth, substrate type, fertiliser treatment);
D: dependent response (statistical significance); I: Independent response (no statistical significance).

Vertical: Low: plant height < 50mm; Medium; 50 mm ≤ plant height < 100 mm; High: plant height ≥ 100 mm.

Coverage: Small: plant coverage < 5.00 %; Medium: 5.00 % ≤ plant coverage < 10.00 %; Large: plant coverage ≥ 10.00 %

Abundance: Unlikely to survive (< 50%); Moderate abundance (50 ≤ plant abundance < 100 %); Best abundance (≥ 100 %)

n.s.; not survived at all.

Growth: Low (< 2.000 g); Medium (2.000 ≤ plant growth < 5.000 g); High (5.000 g ≤ plant growth < 10.000 g); Greater (≥ 10.000 g).

6.4.11 *LINARIA VULGARIS*

Plant abundance, structures, and/or growth of *Linaria* appeared to be influenced by substrate depth, irrigation, substrate type, and fertiliser. However, it also appeared that influences of environmental variables on the species were not revealed in plant abundance in substrate depth treatment with watering in both growing seasons, plant growth in 2008 in substrate type treatment (Table 6.11). The favourable growing conditions, deeper substrate depths, the Limestone-based substrate, supplementary watering, and fertiliser addition supported greater abundance, plant structures, growth of the species. Advantages of supplementary watering occurred at 50 mm depth only for plant structures and abundance.

Linaria plants survived successfully in all treatments over time, except for 50 mm depth without supplemental watering. In the first growing season, *Linaria* plants produced low plant growth in all treatments with the exception of plants with fertiliser application that produced greater growth in both growing seasons. In the following season, however, plant growth increased at 200 mm depth in both watering regimes and in the Limestone-based substrate.

Flowers were produced in the non-watered 50 mm depth and non-fertiliser addition treatment during the month of August, and in the non-watered 100 mm and 200 mm depth from August to September, in all substrate depths with watering and two substrate types from August to October, and in fertiliser addition treatment from July to October. The flowering was influenced by substrate depth without watering, substrate type, and fertiliser, and was prompted by deeper substrates (100 mm and 200 mm depth), the Limestone-based substrate, and fertiliser treatment.

Table 6.11. Summary of plant performance of *Linaria vulgaris* in response to environmental variables.

			50 mm				100 mm				200mm			
		Response to environm-ental variables*	Max. size	Growth form	Growth pattern	Best growing season	Max. size	Growth form	Growth pattern	Best growing season	Max. size	Growth form	Growth pattern	Best growing season
Non-watering treatment	Vertical (cm)	D	6.5	Medium	Bell	Aug	9.5	Medium	Bell	Aug	11.1	High	Bell	Sep
	Coverage (%)	D	1.34	Small	Bell	Aug	4.80	Small	Increase	Oct	5.78	Medium	Increase	Sep
	Abundance 2007	D												
	Abundance 2008	D												
	Growth 2007	D	Low				Low				Low			
	Growth 2008	D	Low				Medium				Medium			
	Flowering	D	Aug				Aug to Sep				Aug to Sep			
Watering treatment	Vertical (cm)	D	6.5	Medium	Bell	Sep	10.1	High	Increase	Aug	9.7	Medium	Increase	Aug
	Coverage (%)	D	2.16	Small	Increase	Oct	5.11	Small	Increase	Oct	6.43	Medium	Increase	Oct
	Abundance 2007	I												
	Abundance 2008	I												
	Growth 2007	D	Low				Low				Low			
	Growth 2008	D	Low				Low				Medium			
	Flowering	I	Aug to Oct				Aug to Oct				Aug to Oct			

Substrate type	Zinco substrate	Vertical (cm)	D	9.5	Medium	Increase	Oct	Limestone-based substrate	Vertical (cm)	9.5	Medium	Increase	Sep
		Coverage (%)	D	3.84	Small	Increase	Oct		Coverage (%)	9.02	Medium	Increase	Oct
		Abundance 2007	D						Survival 2007				
		Abundance 2008	D						Survival 2008				
		Growth 2007	D	Low					Growth 2007	Low			
		Growth 2008	I	Low					Growth 2008	Medium			
		Flowering	D	Aug to Oct					Flowering	Aug to Oct			
Fertiliser treatment	Non-fertiliser application	Vertical (cm)	D	10.1	High	Bell	Aug	Fertiliser application	Vertical (cm)	30.3	High	Increase	Sep
		Coverage (%)	D	3.74	Small	Stable	Sep		Coverage (%)	34.1	Large	Stable	Oct
		Abundance 2007	D						Survival 2007				
		Abundance 2008	D						Survival 2008				
		Growth 2007	D	Low					Growth 2007	Greater			
		Growth 2008	D	Low					Growth 2008	Greater			
		Flowering	D	Aug					Flowering	Jul to Oct			

*Significant responses to environmental variables (substrate depth, substrate type, fertiliser treatment);
D: dependent response (statistical significance); I: Independent response (no statistical significance).

Vertical: Low: plant height < 50mm; Medium; 50 mm ≤ plant height < 100 mm; High: plant height ≥ 100 mm.

Coverage: Small: plant coverage < 5.00 %; Medium: 5.00 % ≤ plant coverage < 10.00 %; Large: plant coverage ≥ 10.00 %

Abundance: Unlikely to survive (< 50%); Moderate abundance (50 ≤ plant abundance < 100 %); Best abundance (≥ 100 %)

n.s.; not survived at all.

Growth: Low (< 2.000 g); Medium (2.000 ≤ plant growth < 5.000 g); High (5.000 g ≤ plant growth < 10.000 g); Greater (≥ 10.000 g).

6.4.12 *LOTUS CORNICULATUS*

Plant establishment and performance of *Lotus* was mainly influenced by substrate depth and supplementary watering. Substrate type and fertiliser application did not affect plant abundance, structures, and growth of the species, except for plant growth in 2008 (Table 6.12). Deeper substrate depths and supplementary watering tended to support greater plant abundance, structures, and growth of the species. Advantages of supplementary watering were shown at all substrate depths for plant abundance, height, and plant growth in 2007, and at 50 mm and 100 mm depth for coverage and plant growth in 2008.

Lotus plants survived successfully in all treatments over time, except for 50 mm depth and 100 mm depth without watering, and produced high plant growth, except for the non-watered 50 mm depth that showed low growth. *Lotus* produces large plant structures in all treatments and exhibited increased growth pattern until September or October in the favourable growing conditions.

Lotus produced flowers in all treatments and usually in the later season from August to October, compared to the ground level from June to July. Flowers were produced during the month of August in the non-watered 50 mm depth, from August to September in the non-watered 100 mm and 200 mm depth, in the watered 50 mm and 200 mm depth, in the Zinco substrate, and in the non-fertiliser treatment, and from August to October in the watered 100 mm depth and in the Limestone-based substrate, and from July to August and October again in fertiliser addition treatment. The flowering was influenced by substrate depth in both watering treatments. Deeper substrate depths and supplementary watering at 50 mm depth improved flowering performance.

Table 6.12. Summary of plant performance of *Lotus corniculatus* in response to environmental variables.

			50 mm				100 mm				200mm			
		Response to environm-ental variables*	Max. size	Growth form	Growth pattern	Best growing season	Max. size	Growth form	Growth pattern	Best growing season	Max. size	Growth form	Growth pattern	Best growing season
Non-watering treatment	Vertical (cm)	D	10.3	High	Bell	Aug	13.2	High	Bell	Aug	12.1	High	Increase	Aug
	Coverage (%)	D	17.10	Large	Bell	Aug	30.00	Large	Bell	Sep	39.4	Large	Increase	Sep
	Abundance 2007	D	n.s.											
	Abundance 2008	I												
	Growth 2007	D	Low				High				Greater			
	Growth 2008	D	Low				Greater				Greater			
	Flowering	D	Aug				Aug to Sep				Aug to Sep			
Watering treatment	Vertical (cm)	I	13.4	High	Bell	Sep	14.9	High	Increase	Aug	13.2	High	Increase	Sep
	Coverage (%)	D	31.12	Large	Increase	Oct	47.91	Large	Increase	Oct	50.82	Large	Increase	Oct
	Abundance 2007	I												
	Abundance 2008	D												
	Growth 2007	D	Greater				Greater				Greater			
	Growth 2008	D	High				Greater				Greater			
	Flowering	D	Aug to Oct				Aug to Oct				Aug to Oct			

Substrate type	Zinco substrate	Vertical (cm)	I	14.2	High	Increase	Aug	Limestone-based substrate	Vertical (cm)	13.5	High	Increase	Aug
		Coverage (%)	I	41.5	Large	Increase	Sep		Coverage (%)	40.9	Large	Increase	Sep
		Abundance 2007	I						Survival 2007				
		Abundance 2008	I						Survival 2008				
		Growth 2007	I	Greater					Growth 2007	Greater			
		Growth 2008	D	Greater					Growth 2008	High			
		Flowering	I	Aug to Oct					Flowering	Aug to Oct			
Fertiliser treatment	Non-fertiliser application	Vertical (cm)	I	11.8	High	Increase	Aug	Fertiliser application	Vertical (cm)	15.6	High	Increase	Oct
		Coverage (%)	I	46.9	Large	Increase	Oct		Coverage (%)	34.6	Large	Increase	Oct
		Abundance 2007	I						Survival 2007				
		Abundance 2008							Survival 2008				
		Growth 2007	I	Greater					Growth 2007	Greater			
		Growth 2008	D	Greater					Growth 2008	High			
		Flowering	I	Aug					Flowering	Jul to Oct			

*Significant responses to environmental variables (substrate depth, substrate type, fertiliser treatment);
D: dependent response (statistical significance); I: Independent response (no statistical significance).

Vertical: Low: plant height < 50mm; Medium; 50 mm ≤ plant height < 100 mm; High: plant height ≥ 100 mm.

Coverage: Small: plant coverage < 5.00 %; Medium: 5.00 % ≤ plant coverage < 10.00 %; Large: plant coverage ≥ 10.00 %

Abundance: Unlikely to survive (< 50%); Moderate abundance (50 ≤ plant abundance < 100 %); Best abundance (≥ 100 %)

n.s.; not survived at all.

Growth: Low (< 2.000 g); Medium (2.000 ≤ plant growth < 5.000 g); High (5.000 g ≤ plant growth < 10.000 g); Greater (≥ 10.000 g).

6.4.13 *ORIGANUM VULGARE*

Plant establishment and performance of *Origanum* were mainly influenced by substrate depth and supplementary watering, except for plant growth in 2008 in the substrate depth treatment with watering. Substrate type and fertiliser application affected only plant coverage and growth in both growing seasons respectively (Table 6.13). The favourable growing conditions produced greater abundance, structures and growth. Supplementary watering promoted plant abundance and growth in 2007 at all substrate depths, and plant growth in 2008 at 50 mm depth, and plant structures at 100 mm or both of 50 mm and 100 mm depth.

In the first growing season, *Origanum* survived successfully in all treatments with the exception of the non-watered 50 mm depth, whereas in the following season, the species did not do so in 50 mm depth without and with watering, 200 mm depth without it, and fertiliser application. *Origanum* tended to produce low plant growth in all treatments, except for the watered 200 mm depth in 2007, the Limestone-based substrate in 2007, and fertiliser addition treatment in both growing seasons, which produced plant growth with more than medium range.

Flowers were produced during the month of September in the non-watered 50 mm depth, in the watered 200 mm depth, and in the Zinco substrate, and from August to September in the non-watered 100 mm and 200 mm depth, and from August to October in the watered 100 mm depth, and from September to October in the Limestone-based substrate and fertiliser addition treatment. The flowering was only influenced by substrate depths with watering treatment and prompted by deeper substrate depths.

Table 6.13. Summary of plant performance of *Origanum vulgare* in response to environmental variables.

			50 mm				100 mm				200mm			
		Response to environm-ental variables*	Max. size	Growth form	Growth pattern	Best growing season	Max. size	Growth form	Growth pattern	Best growing season	Max. size	Growth form	Growth pattern	Best growing season
Non-watering treatment	Vertical (cm)	D	9.3	Medium	Bell	Aug	9.6	Medium	Bell	Sep	11.3	High	Increase	Sep
	Coverage (%)	D	1.12	Small	Bell	Aug	2.28	Small	Bell	Aug	2.89	Small	Increase	Sep
	Abundance 2007	D												
	Abundance 2008	D												
	Growth 2007	D	Low				Low				Low			
	Growth 2008	D	n.s.				Low				Low			
	Flowering	I	Sep				Aug to Sep				Aug to Sep			
Watering treatment	Vertical (cm)	D	6.2	Medium	Bell	Sep	13.7	High	Increase	Aug	10.4	High	Increase	Aug
	Coverage (%)	D	1.52	Small	Stable	Aug	3.61	Small	Increase	Oct	3.92	Small	Increase	Oct
	Abundance 2007	D												
	Abundance 2008	D												
	Growth 2007	D	Low				Low				Medium			
	Growth 2008	I	Low				Low				Low			
	Flowering	D					Aug to Oct				Sep			
Substrate type: Zinco substrate	Vertical (cm)	I	10.0	Medium	Increase	Sep								
	Coverage (%)	D	2.10	Small	Increase	Aug								
	Abundance 2007	I												
	Abundance 2008	I												
	Growth 2007	I	Low											
	Growth 2008	I	Low											
	Flowering	I	Sep											
Substrate type: Limestone-based substrate	Vertical (cm)	I									14.0	High	Increase	Oct
	Coverage (%)	D									4.56	Small	Increase	Sep
	Survival 2007	I												
	Survival 2008	I												
	Growth 2007	I									Medium			
	Growth 2008	I									Low			
	Flowering	I									Sep to Oct			
Fertiliser treatment: Non-fertiliser application	Vertical (cm)	I	6.6	Medium	Bell	Jul								
	Coverage (%)	I	1.53	Small	Stable	Jun								
	Abundance 2007	I												
	Abundance 2008	I												
	Growth 2007	D	Low											
	Growth 2008	D	Low											
	Flowering	–	–											
Fertiliser treatment: Fertiliser application	Vertical (cm)	I									8.0	Medium	Stable	Aug
	Coverage (%)	I									3.42	Small	Stable	Aug
	Survival 2007	I												
	Survival 2008	I												
	Growth 2007	D									Greater			
	Growth 2008	D									high			
	Flowering	–									Sep to Oct			

*Significant responses to environmental variables (substrate depth, substrate type, fertiliser treatment);
D: dependent response (statistical significance); I: Independent response (no statistical significance).

Vertical: Low: plant height < 50mm; Medium; 50 mm ≤ plant height < 100 mm; High: plant height ≥ 100 mm.

Coverage: Small: plant coverage < 5.00 %; Medium: 5.00 % ≤ plant coverage < 10.00 %; Large: plant coverage ≥ 10.00 %

Abundance: Unlikely to survive (< 50%); Moderate abundance (50 ≤ plant abundance < 100 %); Best abundance (≥ 100 %)

n.s.; not survived at all.

Growth: Low (< 2.000 g); Medium (2.000 ≤ plant growth < 5.000 g); High (5.000 g ≤ plant growth < 10.000 g); Greater (≥ 10.000 g).

6.4.14 *PILOSELLA AURANTIACA*

Plant abundance, structure characteristics, and growth of *P. aurantiaca* were influenced by the given environmental variables, except for plant abundance and growth in 2008 in substrate depth treatment with watering and in fertiliser treatment, and plant height and growth in 2008 in substrate type treatment (Table 6.14). The favourable growing conditions improved plant abundance, growth, and structural characteristics of the species. Supplementary watering showed clear advantages to plant abundance, structures, and plant growth in 2007 at 50 mm depth.

P. aurantiaca exhibited high abundance and low plant growth in all treatments during the 2-year growing seasons, except for the non-watered 50 mm depth with low plant abundance, and the Limestone-based substrate and fertiliser application with medium and high plant growth respectively during the first growing season. Plants without supplementary watering tended to exhibit bell-shaped growth pattern, whereas in all treatments with it, the plant exhibited an increased pattern in structure characteristics until the end of growing season.

P. aurantiaca flowered in all treatments, except for the non-watered 50 mm depth, and the flowering appeared in August in the non-watered 100 mm and 200 mm depth, in October in the watered 50 mm depth, in August and October again in the watered 100 mm and 200 mm depth, from August to September in both substrate types, and from August to October in fertiliser treatment. The flowering performance was influenced by substrate depth in both watering treatments and fertiliser treatment, and was greater in deeper substrate depths and fertiliser addition treatment.

Table 6.14. Summary of plant performance of *Pilosella aurantiaca* in response to environmental variables.

			50 mm				100 mm				200mm			
		Response to environm-ental variables*	Max. size	Growth form	Growth pattern	Best growing season	Max. size	Growth form	Growth pattern	Best growing season	Max. size	Growth form	Growth pattern	Best growing season
Non-watering treatment	Vertical (cm)	D	3.6	Low	Bell	Jul	20.3	High	Bell	Aug	32.4	High	Bell	Aug
	Coverage (%)	D	1.86	Small	Bell	Aug	4.70	Small	Bell	Sep	7.17	Medium	Bell	Sep
	Abundance 2007	D												
	Abundance 2008	D	n.s.											
	Growth 2007	D	Low				Low				Low			
	Growth 2008	D	Low				Low				Low			
	Flowering	D	–				Aug				Aug			
Watering treatment	Vertical (cm)	D	5.8	Medium	Increase	Oct	20.0	High	Bell	Aug	25.8	High	Bell	Aug
	Coverage (%)	D	3.32	Small	Increase	Oct	6.84	Medium	Increase	Oct	6.79	Medium	Increase	Oct
	Abundance 2007	D												
	Abundance 2008	I												
	Growth 2007	D	Low				Low				Low			
	Growth 2008	I	Low				Low				Low			
	Flowering	D	Oct				Aug, Oct				Aug, Oct			

Substrate type	Zinco substrate	Vertical (cm)	I	17.1	High	Bell	Aug	Limestone-based substrate	Vertical (cm)	21.4	High	Bell	Sep
		Coverage (%)	D	5.27	Medium	Increase	Oct		Coverage (%)	13.6	Large	Increase	Sep
		Abundance 2007	D						Survival 2007				
		Abundance 2008	D						Survival 2008				
		Growth 2007	D	Low					Growth 2007	Medium			
		Growth 2008	I	Low					Growth 2008	Low			
		Flowering	I	Aug to Sep					Flowering	Aug to Sep			
Fertiliser treatment	Non-fertiliser application	Vertical (cm)	D	20.7	High	Bell	Aug	Fertiliser application	Vertical (cm)	32.0	High	Bell	Aug
		Coverage (%)	D	8.11	Medium	Increase	Oct		Coverage (%)	25.89	Large	Increase	Oct
		Abundance 2007	D						Survival 2007				
		Abundance 2008	I						Survival 2008				
		Growth 2007	D	Low					Growth 2007	High			
		Growth 2008	I	Low					Growth 2008	Low			
		Flowering	D	Aug to Oct					Flowering	Aug to Oct			

*Significant responses to environmental variables (substrate depth, substrate type, fertiliser treatment);
D: dependent response (statistical significance); I: Independent response (no statistical significance).

Vertical: Low: plant height < 50mm; Medium; 50 mm ≤ plant height < 100 mm; High: plant height ≥ 100 mm.

Coverage: Small: plant coverage < 5.00 %; Medium: 5.00 % ≤ plant coverage < 10.00 %; Large: plant coverage ≥ 10.00 %

Abundance: Unlikely to survive (< 50%); Moderate abundance (50 ≤ plant abundance < 100 %); Best abundance (≥ 100 %)

n.s.; not survived at all.

Growth: Low (< 2.000 g); Medium (2.000 ≤ plant growth < 5.000 g); High (5.000 g ≤ plant growth < 10.000 g); Greater (≥ 10.000 g).

6.4.15 *PILOSELLA OFFICINARUM*

Plant abundance, structures, and/or growth of *P. officinarum* were influenced by substrate depth, watering, substrate type, and fertiliser (Table 6.15). The favourable growing conditions, deeper substrate depths, the Limestone-based substrate, supplementary watering, and fertiliser addition produced greater abundance, plant structures and growth of the species. Advantages of supplementary watering occurred at 50 mm depth for plant growth in 2008 and structures, and at 50 mm and 200 mm depth for plant abundance.

P. officinarum survived successfully and produced low plant growth in all treatments during the 2-year growing seasons, except for the non-watered 50 mm depth exhibited low plant abundance, and the Limestone-based substrate and fertiliser application produced medium plant growth. Plants without supplementary watering showed a decreased or bell-shaped growth pattern, while in all treatments with supplementary watering, the plant showed an increased pattern until the end of growing season.

P. officinarum produced flowers in all treatments, except for 50 mm depth without watering treatment. The flowers appeared during the month of August in the non-watered 100 mm depth and the watered 200 mm depth and in non-fertiliser treatment, from July to August in the non-watered 200 mm depth, from August to September in the watered 50 mm and 100 mm depth and in the substrate type treatment, and from June to August again in fertiliser addition treatment. The flowering was not influenced by the environmental variables, except for substrate depth without watering treatment. Deeper substrate depths (100 mm and 200 mm depth) produced higher flowering performance.

Table 6.15. Summary of plant performance of *Pilosella officinarum* in response to environmental variables.

			50 mm				100 mm				200mm			
		Response to environm-ental variables*	Max. size	Growth form	Growth pattern	Best growing season	Max. size	Growth form	Growth pattern	Best growing season	Max. size	Growth form	Growth pattern	Best growing season
Non-watering treatment	Vertical (cm)	D	1.8	Low	Decrease	Jul	5.1	Medium	Bell	Aug	7.8	Medium	Bell	Aug
	Coverage (%)	D	1.52	Small	Decrease	Aug	2.50	Small	Bell	Aug	4.52	Small	Bell	Sep
	Abundance 2007	D												
	Abundance 2008	D												
	Growth 2007	D	Low				Low				Low			
	Growth 2008	D	n.s.				Low				Low			
	Flowering	D	–				Aug				Jul to Aug			
Watering treatment	Vertical (cm)	I	2.5	Low	Increase	Aug	7.3	Medium	Stable	Aug	8.5	Medium	Bell	Aug
	Coverage (%)	D	2.63	Small	Increase	Oct	4.88	Small	Increase	Sep	5.43	Medium	Increase	Oct
	Abundance 2007	I												
	Abundance 2008	I												
	Growth 2007	I	Low				Low				Low			
	Growth 2008	D	Low				Low				Low			
	Flowering	I	Aug to Sep				Aug to Sep				Aug			

Substrate type	Zinco substrate	Vertical (cm)	I	2.1	Low	Stable	Aug	Limestone-based substrate	Vertical (cm)	11.0	High	Bell	Sep
		Coverage (%)	D	2.97	Small	Increase	Sep		Coverage (%)	10.2	Large	Increase	Oct
		Abundance 2007	I						Survival 2007				
		Abundance 2008	I						Survival 2008				
		Growth 2007	D	Low					Growth 2007	Medium			
		Growth 2008	I	Low					Growth 2008	Low			
		Flowering	I	Aug to Sep					Flowering	Aug to Sep			
Fertiliser treatment	Non-fertiliser application	Vertical (cm)	D	5.0	Medium	Bell	Aug	Fertiliser application	Vertical (cm)	21.7	High	Bell	Aug
		Coverage (%)	D	5.37	Medium	Increase	Oct		Coverage (%)	17.0	Large	Increase	Sep
		Abundance 2007	D						Survival 2007				
		Abundance 2008	I						Survival 2008				
		Growth 2007	D	Low					Growth 2007	Medium			
		Growth 2008	I	Low					Growth 2008	Low			
		Flowering	I	Aug					Flowering	Jun, Aug			

*Significant responses to environmental variables (substrate depth, substrate type, fertiliser treatment);
D: dependent response (statistical significance); I: Independent response (no statistical significance).

Vertical: Low: plant height < 50mm; Medium; 50 mm ≤ plant height < 100 mm; High: plant height ≥ 100 mm.

Coverage: Small: plant coverage < 5.00 %; Medium: 5.00 % ≤ plant coverage < 10.00 %; Large: plant coverage ≥ 10.00 %

Abundance: Unlikely to survive (< 50%); Moderate abundance (50 ≤ plant abundance < 100 %); Best abundance (≥ 100 %)

n.s.; not survived at all.

Growth: Low (< 2.000 g); Medium (2.000 ≤ plant growth < 5.000 g); High (5.000 g ≤ plant growth < 10.000 g); Greater (≥ 10.000 g).

6.4.16 *PRIMULA VERIS*

Plant abundance, structural characteristics, and/or growth of *Primula* were influenced by the given environmental variables. The influences of the environmental variables on the species were observed in plant height, abundance, and growth in substrate depth treatment without supplementary watering, and in plant abundance in 2007and growth in 2008 in substrate depth treatment with it, and plant coverage and growth in 2007 in substrate type treatment, and in plant structures in fertiliser treatment (Table 6.16). The favourable growing conditions tended to support higher plant establishment and growth of *Primula*. Supplementary watering showed clear advantages to plant abundance at all substrate depths, and to plant structure (height) and growth in 2008 at 50 mm depth.

In the first growing season, *Primula* did not survive successfully without additional watering and in fertiliser treatment. In the following season, low abundance was appeared in all treatments with supplementary watering as well as the treatment without it. *Primula* only produced successful plant abundance in the Limestone-based substrate and fertiliser addition treatment. *Primula* plants tended to produce small plant structure and low plant growth over time. In the second growing season, plant growth decreased at all substrate depths in both watering regimes and in both substrate types, while plant with fertiliser treatment did not do so.

P. veris did not produce flowers at all in all treatments.

Table 6.16. Summary of plant performance of *Primula veris* in response to environmental variables.

			50 mm				100 mm				200mm			
		Response to environm-ental variables*	Max. size	Growth form	Growth pattern	Best growing season	Max. size	Growth form	Growth pattern	Best growing season	Max. size	Growth form	Growth pattern	Best growing season
Non-watering treatment	Vertical (cm)	D	2.1	Low	Decrease	Jul	1.7	Low	Bell	Jul	2.2	Low	Decrease	Jul
	Coverage (%)	I	0.71	Small	Decrease	Aug	0.93	Small	Bell	Jul	1.02	Small	Bell	Jul
	Abundance 2007	D												
	Abundance 2008	D			n.s.									
	Growth 2007	D			Low				Low				Low	
	Growth 2008	D			n.s.				Low				Low	
	Flowering	–			–				–				–	
Watering treatment	Vertical (cm)	I	1.8	Low	Decrease	Jun	2.2	Low	Decrease	Jul	2.1	Low	Bell	Jul
	Coverage (%)	I	0.63	Small	Decrease	Jul	1.13	Small	Bell	Jul	0.90	Small	Bell	Jul
	Abundance 2007	I												
	Abundance 2008	I												
	Growth 2007	I			Low				Low				Low	
	Growth 2008	D			Low				Low				Low	
	Flowering	–			–				–				–	

Substrate type	Zinco substrate	Vertical (cm)	I	2.2	Low	Stable	Jul	Limestone-based substrate	Vertical (cm)	2.0	Low	Stable	Aug
		Coverage (%)	D	0.94	Small	Bell	Jul		Coverage (%)	0.88	Small	Bell	Sep
		Abundance 2007	I						Survival 2007				
		Abundance 2008	I						Survival 2008				
		Growth 2007	D			Low			Growth 2007			Low	
		Growth 2008	I			Low			Growth 2008			Low	
		Flowering	–			–			Flowering			–	
Fertiliser treatment	Non-fertiliser application	Vertical (cm)	D	2.0	Low	Stable	Jul	Fertiliser application	Vertical (cm)	2.7	Low	Bell	Aug
		Coverage (%)	D	1.09	Small	Decrease	Jun		Coverage (%)	1.13	Small	Decrease	Jun
		Abundance 2007	I						Survival 2007				
		Abundance 2008	I						Survival 2008				
		Growth 2007	I			Low			Growth 2007			Low	
		Growth 2008	I			Low			Growth 2008			Low	
		Flowering	–			–			Flowering			–	

*Significant responses to environmental variables (substrate depth, substrate type, fertiliser treatment);
D: dependent response (statistical significance); I: Independent response (no statistical significance).

Vertical: Low: plant height < 50mm; Medium; 50 mm ≤ plant height < 100 mm; High: plant height ≥ 100 mm.

Coverage: Small: plant coverage < 5.00 %; Medium: 5.00 % ≤ plant coverage < 10.00 %; Large: plant coverage ≥ 10.00 %

Abundance: Unlikely to survive (< 50%); Moderate abundance (50 ≤ plant abundance < 100 %); Best abundance (≥ 100 %)

n.s.; not survived at all.

Growth: Low (< 2.000 g); Medium (2.000 ≤ plant growth < 5.000 g); High (5.000 g ≤ plant growth < 10.000 g); Greater (≥ 10.000 g).

6.4.17 *SCABIOSA COLUMBARIA*

Plant abundance, structures, and/or growth of *Scabiosa* appeared to be influenced by substrate depth, irrigation, substrate type, and fertiliser. However, in the second growing season, the influences of the environmental variables were not revealed in plant abundance and growth in substrate type and fertiliser treatment (Table 6.17). The favourable growing conditions, deeper substrate depths, the Limestone-based substrate, supplementary watering, and fertiliser addition produced greater plant abundance, structures, and growth of the species. Supplementary watering promoted plant structures, survival, and growth at 50 mm and 100 mm depth.

In all treatments, *Scabiosa* plants tended to successfully maintain plant abundance throughout the 2-year growing seasons, except for 50 mm depth without supplementary watering that exhibited low abundance with less than 50 %. *Scabiosa* showed an increasing plant growth pattern in the favourable growing conditions. In the first growing season, *Scabiosa* produced low plant growth in all treatments, except for the Limestone-based substrate and fertiliser application. In the following growing season, however, 200 mm depth with both watering regimes, and both substrate types and fertiliser applications produced plant growth with more than medium range.

Scabiosa flowered in all treatments, except for 50 mm depth in both watering treatment and the Zinco substrate. Flowers were produced from August to September in the non-watered 100 mm depth and from September to October in the non-watered 200 mm depth and in fertiliser treatment, and during the month of October in the watered 100 mm and 200 mm depth, in the Limestone-based substrate, and in non-fertiliser treatment. The flowering performance was only influenced by fertiliser treatment.

Table 6.17. Summary of plant performance of *Scabiosa columbaria* in response to environmental variables.

			50 mm				100 mm				200mm			
		Response to environm-ental variables*	Max. size	Growth form	Growth pattern	Best growing season	Max. size	Growth form	Growth pattern	Best growing season	Max. size	Growth form	Growth pattern	Best growing season
Non-watering treatment	Vertical (cm)	D	1.5	Low	Decrease	Jun	5.5	Medium	Stable	Aug	13.5	High	Increase	Oct
	Coverage (%)	D	1.28	Small	Decrease	Aug	2.00	Small	Bell	Sep	3.06	Small	Stable	Oct
	Abundance 2007	D												
	Abundance 2008	D												
	Growth 2007	D	Low				Low				Low			
	Growth 2008	D	Low				Low				Medium			
	Flowering	I	–				Aug to Sep				Sep to Oct			
Watering treatment	Vertical (cm)	D	1.8	Low	Increase	Sep	14.8	High	Increase	Sep	6.7	Medium	Increase	Oct
	Coverage (%)	D	1.54	Small	Increase	Oct	4.69	Small	Increase	Oct	3.33	Small	Increase	Oct
	Abundance 2007	D												
	Abundance 2008	D												
	Growth 2007	D	Low				Low				Low			
	Growth 2008	D	Low				Low				Medium			
	Flowering	I	–				Oct				Oct			

Substrate type	Zinco substrate	Vertical (cm)	D	6.1	Medium	Increase	Sep	Limestone-based substrate	Vertical (cm)		16.9	High	Increase	Oct
		Coverage (%)	D	3.17	Small	Increase	Oct		Coverage (%)		8.43	Medium	Increase	Oct
		Abundance 2007	D						Survival 2007					
		Abundance 2008	I						Survival 2008					
		Growth 2007	D	Low					Growth 2007		Medium			
		Growth 2008	I	Medium					Growth 2008		Medium			
		Flowering	–	–					Flowering		Oct			
Fertiliser treatment	Non-fertiliser application	Vertical (cm)	D	6.2	Medium	Increase	Sep	Fertiliser application	Vertical (cm)		32.2	High	Increase	Oct
		Coverage (%)	D	4.52	Small	Increase	Oct		Coverage (%)		20.9	Large	Increase	Oct
		Abundance 2007	D						Survival 2007					
		Abundance 2008	I						Survival 2008					
		Growth 2007	D	Low					Growth 2007		Greater			
		Growth 2008	I	Medium					Growth 2008		High			
		Flowering	D	Oct					Flowering		Sep to Oct			

*Significant responses to environmental variables (substrate depth, substrate type, fertiliser treatment);
D: dependent response (statistical significance); I: Independent response (no statistical significance).

Vertical: Low: plant height < 50mm; Medium; 50 mm ≤ plant height < 100 mm; High: plant height ≥ 100 mm.

Coverage: Small: plant coverage < 5.00 %; Medium: 5.00 % ≤ plant coverage < 10.00 %; Large: plant coverage ≥ 10.00 %

Abundance: Unlikely to survive (< 50%); Moderate abundance (50 ≤ plant abundance < 100 %); Best abundance (≥ 100 %)

n.s.; not survived at all.

Growth: Low (< 2.000 g); Medium (2.000 ≤ plant growth < 5.000 g); High (5.000 g ≤ plant growth < 10.000 g); Greater (≥ 10.000 g).

6.5 The growth pattern and flowering performance of individual plant species over time

Plant growth type and pattern of each individual species varies according to the environmental variables. Overall growth form and pattern of the individual plant species across all treatments can be divided into 6 patterns using the categories in the above section. The classification of growth patterns of individual plant species and the species allocating to the classification is shown in Table 5.18. Most of the species showed maximum vertical and lateral growth and best flowering season from the late summer to the autumn. The species included in Pattern 1 and Pattern 2 tended to grow slowly and vertical and/or lateral growth did not change much over time. The difference between Pattern 1 and Pattern 2 was vertical growth form and pattern. Pattern 1 tended to be low growing and decrease in height over time, whereas Pattern 2 tended to have medium height and keep the height over time. They were relatively prominent in the summer throughout the year. In Pattern 1, *P. veris*, and in Pattern 2, *A. eupatoria* were included. Similar to Pattern 1 and Pattern 2, Pattern 3 and Pattern 4 tended to be low growing form. The difference between these two Patterns was that the growth of Pattern 3 was

Table 6.18. The classification of growth forms and patterns of individual plant species.

		Coverage		Vertical			
	Species	Growth type	Growth pattern	Growth type	Growth pattern	Best growing season	Flowering season
Pattern 1	*P. veris*	Small	bell	Low	Decrease	July	
Pattern 2	*A. eupatoria*	Small	stable	Medium	stable	August	Aug to Sept
Pattern 3	*C. glomerata*	Small	stable	Low	Increase	October	Oct
Pattern 4	*H. nummularium*	Medium	Increase	Low	Stable	September	Sept to Oct
Pattern 5	*H. radicata*	Small	Increase	High	Bell	September	Jun to Oct
	P. officinarum	Medium	Increase	Medium	Bell	August	July to Sept
	L. autumnalis	Medium	Increase	High	Bell	August	Jun to Oct
Pattern 6	*O. vulgare*	Small	Increase	Medium	Increase	September	Aug to Oct
	C. vulgare	Small	Increase	High	Increase	September	July to Oct
	G. verum	Medium	Increase	Medium	Increase	September	Aug to Oct
	S. columbaria	Medium	Increase	Medium	Increase	October	Aug to Oct
	K. arvensis	Medium	Increase	Medium	increase	October	Sept to Oct
	L. vulgare	Medium	Increase	High	Increase	September	Aug to Oct
	L. vulgaris	Medium	Increase	High	Increase	September	July to Oct
	P. aurantiaca	Medium	Increase	High	Increase	August	Aug to Oct
	A. millefolium	Large	Increase	Medium	Increase	October	Sept to Oct
	L. corniculatus	Large	Increase	High	Increase	September	July to Oct

prominent in vertical structure, whereas the growth of Pattern 4 was prominent in lateral growth. This is because the species in Pattern 3, *C. glomerata* has erect flowering stem, whereas the species in Pattern 4, *H. nummularium* have mat-forming and its flowers are just overtopped on prostrate stem. Pattern 5 and Pattern 6 tended to grow vigorously throughout the year and increase in coverage and/or height. The difference between the two Patterns was structure of flower stems. The species belonging to Pattern 5 have erect scapes and they tended to show maximum growth from the late summer to early autumn and then decrease in the late autumn. The species classified as Pattern 5 were; *H. radicata, L. autumnalis, and P. officinarum*. In this study, a large number of species was classified in Pattern 6. The species included; *A. millefolium, C. vulgare, G. verum, K. arvensis, L. vulgare, L. vulgaris, L. corniculatus, O. vulgare, P. aurantiaca*, and *S. columbaria*.

It would be possible to create aesthetic green roofs which have a long flowering and seasonal interest if the appropriate environments for the species were given. Throughout the first growing season, flowering performance of the species was present from June to October. The best flowering performance season was in September when the highest number of flowering species (15 species) was observed. Of the species, *H. radicata* and *L. autumnalis* showed particularly the longest flowering performance (5 months), followed by *C. vulgare*, *L. vulgaris*, and *L. corniculatus* (4 months), and *C. glomerata* for the shortest term (1 month). *P. veris* did not flower at all over time.

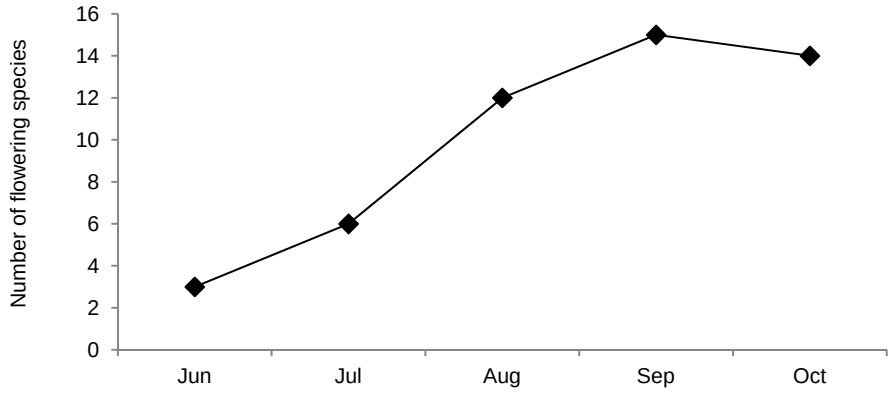


Figure 6.1. Change of number of flowering species over time across all treatments.

6.6 The influence of the environmental variables on plant performance.

In terms of application to practical use for green roofs, successful species are likely to demonstrate high emergence and survivorship, good growth and stable growth pattern, and flowering for visual interest over long-term. Suitability of the individual plant species at each treatment is shown in Table 6.19. Under the condition with limited water availability (relying on natural rainfall only), at shallow substrate depth (50 mm depth) plant performances of all individual species were very restricted due to low survival, low growth pattern, and/or poor flowering performance. Most of the species did not survive well over time. Only 2 species, *H. radicata* and *L. vulgare*, were able to survive well, and this was followed by *S. columbaria*. Not all species showed good plant growth. In contrast with the shallow substrate, deeper substrates (100 mm and 200 mm depth) support more than half of the species exhibiting successful plant performance. *A. millefolium*, *A. eupatoria*, *G. verum*, *K. arvensis*, *L. autumnalis*, *L. vulgare*, *L. vulgaris*, *P. aurantiaca*, and *S. columbaria* were suitable for use in both of 100 mm and 200 mm depth. *C. vulgare* and *H. radicata* showed successful performance in the depth of 100 mm only, while *L. corniculatus* showed this pattern in the depth of 200 mm only. The unsuccessful species across all substrate depths, which showed low survival, insufficient growth and/or no flowering, were *C. glomerata*, *H. nummularium*, *O. vulgare*, *P. officinarum*, and *P. veris*.

Under watering condition, at shallow substrate depth (50 mm depth), all species survived well, except for *H. nummularium*, *O. vulgare*, and *P. veris*. However, only six species showed good plant performance. The species were *A. millefolium*, *G. verum*, *K. arvensis*, *L. vulgare*, *L. corniculatus*, and *S. columbaria*. Deeper substrates (100 mm and 200 mm depth) were more suitable for supporting successful performance of most of the species. The successful species at both substrate depths were *A. millefolium*, *A. eupatoria*, *G. verum*, *K. arvensis*, *L. autumnalis*, *L. vulgare*, *L. vulgaris*, *L. corniculatus*, *O. vulgare*, *P. aurantiaca*, and *S. columbaria*. *C. vulgare* was suitable at 200 mm depth only. *C. glomerata*, *H. nummularium*, *H. radicata*, *P. officinarum*, and *P. veris* did not succeed in their plant performance across all substrate depths.

Table 6.19. Suitability of individual species for use on green roofs

Species	Non-watering condition			Watering condition				
	50 mm	100 mm	200 mm	50 mm	100 mm	200 mm	Limestone-based substrate	Fertiliser application
A. millefolium								
A. eupatoria								
C. glomerata								
C. vulgare								
G. verum								
H. nummularium								
H. radicata								
K. arvensis								
L. autumnalis								
L. vulgare								
L. vulgaris								
L. corniculatus							—	—
O. vulgare								
P. aurantiaca								
P. officinarum								
P. veris								
S. columbaria								

Best for plant performance

Suitable for plant performance

Unlikely succeed

—, Negative response

Compared to the Zinco commercial substrate, the Limestone-based substrate and additional fertiliser treatment that had higher nutrient availability tended to promote greater survival, growth, and flowering for nearly all species tested. However, in the case of *L. corniculatus*, the species exhibited clearly negative response to conditions with high nutrient level. In the fertiliser treatment there was a tendency for plant growth

to be very vigorous, which may not be able to withstand sudden environmental changes. At both of the treatments, *P. officinarum* and *P. veris* were not successful in their plant performance. *H. nummularium* in the fertiliser treatment and *O. vulgare* in the Limestone-based substrate were able to have successful performance. At the two treatments, the successful species were *A. millefolium*, *A. eupatoria*, *C. glomerata*, *C. vulgare*, *G. verum*, *H. radicata*, *K. arvensis*, *L. autumnalis*, *L. vulgare*, *L. vulgaris*, *L. corniculatus*, *P. aurantiaca*, and *S. columbaria*.

Overall, *C. glomerata* and *H. nummularium* across all treatments in a standard commercial green roof substrate without fertiliser addition, and *P. officinarum* and *P. veris* across all treatments including additional fertiliser treatment were not effective green roof plants under the given conditions of the experiment. *O. vulgare* was only suitable at the deeper substrate depths (at 100 mm and 200 mm depths) with additional watering and in the Limestone-based substrate.

6.7 Conclusion

Most of the calcareous grassland species studied here are suitable for the use of green roofs in the UK when sufficient substrate are designed. The species with high survival, good plant growth and flowering performance such as *A. millefolium*, *A. eupatoria*, *G. verum*, *K. arvensis*, *L. autumnalis*, *L. vulgare*, *L. vulgaris*, *L. corniculatus*, *O. vulgare*, *P. aurantiaca*, and *S. columbaria* could be recommended for the application. On the contrary, *C. glomerata*, *H. nummularium*, *P. officinarum*, and *P. veris* are best excluded using a standard commercial green roof substrate. The key factors in succeeding plant establishment and development, and flowering performance of the species would be mostly related to sufficient availability of moisture and nutrition of substrate and substrate depth. The survivorship and initial growth of the species would be more closely related to moisture availability rather than nutrient availability, and plant development and flowering performance with nutrition availability. Supplementary watering was an important factor regarding the initial plant establishment and growth, and treatments with high nutrient availability for better plant development and

flowering performance, especially with substrates of shallower depth in the first growing season. In order for successful plant assemblages to persist over long-term, it is desirable to have at least 100 mm substrate depth, and periods of limited irrigation during the first growing season and drought period in the UK. 50 mm substrate depth is not effective substrate supporting successful performance of the species, which are adapted to infertile and dry habitats. A treatment of a minimum additional nutrient enables rich plant assemblages to produce better plant development and flowering performance, consequently to be colourful and visually attractive for the longest possible throughout the year. However, it is important to be aware of the use of the treatment because some species did not follow this rule, and to consider planting density which results in increase of competition and interruption between plants.

For aesthetical planting design on green roofs, a wider ranges of the native plant species with different growing form and flowering season, especially early season, should be investigated for further research. Using the various growth forms and patterns of species and flowering season, more dynamic change of green roofs and colourful, visual attraction could be produced for the longest possible throughout the year. In this study, more detailed environmental measurements such as moisture and nutrient availability to plant and temperature fluctuation of the substrates would have been helpful to analyse relationships between the plant growth of the species and the environmental factors. For dry and insufficient nutrient environmental conditions on the roof, it would be valuable to investigate drought tolerance and nutrient stress of the species under conditions with different irrigation and nutrient regimes in a climate-controlled greenhouse for further research. This would lead to minimum frequency of watering and minimum amount or necessity of additional nutrient to maintain stable growth and visual attractions of the species. It is necessary to maintain observation in the long-term in order to understand how plant performance of the species would change over time, and to identify relationship between the plant performance of the species and the changes of substrate characteristics. In addition, it is recommended to investigate the establishment of such vegetations from seed as well as from planting for further research.

6.8 Summary

Key points from this study relevant to the possibility of plant selection and communities from calcareous grassland for green roof application were:

- Calcareous grassland plant communities are ideal candidates for roof greening vegetations because calcareous grasslands can offer abundant sources of native plant species. They also have similar soil characteristics, typically thin, low in nutrients and free draining soils to that of green roof systems.

- Successful substrates tend to be closely related to soil conditions with similar characteristics to the soils of natural habitats which the species occurred.

- In terms of application to practical use on green roofs, substrates require combining the well-balance of moisture content and air filled porosity in order to support high survival, rapid establishment, and high ground cover density.

- Organic matter and loam play an important role as a source of nutrients for plants and as an amendment for water holding capacity and air filled porosity of substrate

- LECA (Light Expanded Clay Aggregate) must be mixed with other materials and would have to be used as a soil amendment.

- For use of brick rubble substrate, another amendment such as loam with organic matter together would be necessary to advance soil permeability for effective root penetration and water use of plant for successful establishment and growth of the species.

- Limestone 2 substrate type with loam and organic matter in 60:20:20 composition rate tend to be the most successful substrate type among the experimental substrate. The substrate could also support a wider range of calcareous grassland species on the roof compared to the two Zinco commercial substrates.

- When the plant community is established, additional watering regularly and fertiliser application is not as critical. However, in order for successful plant assemblages to persist over long-term, it is desirable to have at least 100 mm substrate depth, and periods of limited irrigation during the first growing season and drought period in the UK.

- In terms of application to practical use for green roofs in the UK, most of the calcareous grassland species studied here are suitable when a sufficient depth of substrate and appropriate type are provided.

- The species with high survival, good plant growth and flowering performance could be recommended for the application. These species are; *A. millefolium*, *A. eupatoria*, *G. verum*, *K. arvensis*, *L. autumnalis*, *L. vulgare*, *L. vulgaris*, *L. corniculatus*, *O. vulgare*, *P. aurantiaca*, and *S. columbaria*.

- *C. glomerata*, *H. nummularium*, *P. officinarum*, and *P. veris* are best excluded using a standard commercial green roof substrate.

- A minimum additional nutrient enables rich plant assemblages to produce better plant development and flowering performance. As a consequence, plants are colourful and visually attractive for the longest possible duration throughout the year. However, it is important to be aware of the use of the treatment because some species did not follow this rule. It is also imperative to consider planting density which results in an increase in competition and interruption between plants.

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APPENDIX 1. The contemporary green roofs and policies around the world.

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Country/city	Descriptions
Australia **Brisbane**	• Introduction of incentives to developers to incorporate green roofs into buildings. • use of shallow, dry and low-nutrient soils, making Australian flora on extensive green roofs
Austria **Liz**	• Obligatory green roofs installation on all developments (in particular, greening on the tops of underground and multi-storey car parks)
Britain **London**	• Large areas of green roofs are planned, or have been constructed for biodiversity providing habitat for a protected bird species (the black redstart) by the use of demolition materials and rubbles • London Biodiversity Action Plans encourage green roof installation.
Sheffield	• The first city to develop an official green roof policy. - a programme of information and technical advice to stakeholders. - demonstration roofs, development of cost-benefit analysis. - a range of direct promotion of green roofs through policy development and regulation. • 'Green Roof Forum'; established by Sheffield City Council, the University of Sheffield, and Groundwork Sheffield, a city environmental regeneration charity, representatives from architects, environmental and wildlife agencies, and developers. - for formulating and implementing an ongoing strategy to increase green roof infrastructure in the region.
Canada **Toronto**	• GRHC (the Green Roof for Healthy Cities coalition - networking of researchers, policy-makers and industry representative - training and educational activities • Research activity centres for developing cost-benefit to the city; - The benefits of green roofs and other techniques for reducing the urban heat island effect of the city. • Demonstration green roof on Toronto City Hall. • The City of Toronto Act, 2006; - Section 108 of the Act provides the authority for the City to pass a by-law requiring and governing the construction of green roofs. • Toronto's Green Roof Strategy in the Environmental Benefits and Costs of Green Roof Technology for the City of Toronto. - reduced urban heat island - stormwater management implication (quality and quantity) - energy budgets of individual buildings - improved air quality
China	• Plan to construct green roofs of 40 million m^2 in total by the Beijing Olympic Games, with financial support for 50% of costs of the construction.

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Denmark **Copenhagen** (adapted from: Livingroofs.org[b])	• The first city in Scandinavia to have a mandatory green roof policy • Five requirements for green roof have been set out: - absorbing 50 to 80 % of the precipitation that falls on the roof. - providing a cooling and insulating effect of the building and reducing reflection - helping to make the city greener, reducing the urban heat island effect, counteracting the increased temperatures in the city. - contributing to a visual and aesthetic architectural variation that has a positive effect on the quality of life. - protecting the roof life of the roofing membrane by protecting it against UV rays, etc.
Germany	• The Federal Nature Protection Act, the Federal Building Code, state-level nature protection; - Mitigation for lost habitat or landscape due to built development • 14% of new flat roof constructions (13.5 million m^2) in the country in 2001 were greened. • 636 out of 1488 German municipalities (43%) had some kind of incentive or policy supporting green roofs. • Green roof design and construction guidelines published in 1998 by the Landscape Research, Development & Construction Society (FLL). • Three main tools for promotion of green roofs at the local level. - direct financial support; subsidies for construction. - stormwater fee discounts of between 50 and 100 %. - requirements for green roofs in local development plans.
Stuttgart	One of first cities in the country giving official support to roof greening Green Programme for Urban Renewal institute; subsidies for the costs of green roof construction and free technical advice
Berlin	• The Courtyard Greening Program by the west Berlin government; - reimbursed residents for half total cost of green roof installation ($37 to $75/$m^2$$ or 3.40 to $7.00/$ft^2$) • Installed 63,500 m^2 (684,000 ft^2) of extensive green roofs on renovated buildings in the central city of West Berlin. • Green roof installation for acquiring permission for large building construction • The Biotope Area Factor (BAF); - Property must meet target greening level by the plan to be issued a new building permit. - Green roofs are a favoured method of mitigation.
Japan **Tokyo**	• Mitigation of urban heat island effect through green roofs • Introduction of regulation in 2001 that all new private buildings with more than 1000 m^2 (10,760 ft^2) and public buildings with over 250 m^2 (2,690 ft^2) of floor space cover 20 % of the rooftop with vegetation for establishing 1200 hectares of green roofs by 2011, reducing the temperature of the city by 1°C (1.7 °F). • Financial support in forms of subsidies for green roof installation over 40 cities. • 23 cities in Tokyo have demonstration roofs.
Korea (South) **Seoul**	• Program promoting green roofs for increasing urban environment and creating

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Korea (South) **Seoul**	green spaces in the city. • Financial support for the cost of green roof constructions in range from 99m^2 to 992m^2. - 70 to 100% of the cost for public buildings - 50% to 70% for private buildings; 70 % (150,000 KRW or 85.00 GBP approximately) for inner Nam mountain visual regions. - 90,000 to 108,000 KRW or approximately 51.00 to 61.00 GBP per square meter • Design of green roof system and management manual made by the council in 2007 (in Korean) • 249 green roofs (69,184m^2) established by 2005; 201 green roofs (56,636m^2) constructed from 2002. • Installing new 122 green roofs (52,263m^2) on private buildings for 2010 • Completing 98 green roof constructions (50 for public and 48 private buildings) by Oct, 2010.
Norway	• Turf-roofed buildings are relatively commonplace; regarded as part of the national heritage and are linked to romantic notion of closeness to nature.
Russia **St Petersburg**	• Dealt with green roofs for food production by urban apartment dwellers.
Singapore	• To encourage roof gardens and vertical greening for ameliorating the urban heat island effect. • Roof gardens in the country are important as providing green space for the public.
Sweden **Malmö**	• The Botanical Roof Garden in Augustenborg; a model demonstration and research facility - showing the range of green roof types and commercial systems. - conducting research into green roof performance. • In the refurbished Augustenborg housing estate - An above-ground rainwater drainage system - Green roofs on public buildings • BO21 housing exhibition area; - green roofs were one of main measures to gain a required amount of green credits that all buildings in the development had to achieve.
Switzerland	• Replacement habitat and green space for what was lost through development. • Swiss Landscape Concept; compatibility with natural settings and landscape (with minimal interference with the natural environment) and 25% of all new commercial development for green roof in an attempt to maintain favourable microclimates
Basel	• Nature and Landscape Conservation Act 9; Building and Planning Act 72 - Compulsory implementation of green roof installation on new flat roofs. • Biodiversity is main drive for green roofs; the beginning of new concept of green roof to biodiversity • As supporting 20 % of the cost of green roof construction, 3 % of the existing flat roofs in the canton were converted into green roofs within 18 months. • 1929 flat roofs in total (1711 for extensive green roofs and 218 intensive green roofs), approximately 23% of flat roof area in the canton, were greened by 2006 • Design green roofs for creating ecological diversity (using local soils, seed mixes, and different depth of substrate) on roofs over 500 m^2 (5,380 ft^2)

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Basel	• No subsidy to support green roof installation as part of the normal cost of building; - For developer, installation of green roofs; routine and no objections.
United States	• The Green Infrastructure Action Plan (2007); - to promote the benefits of using green infrastructure in mitigating overflows from combined and separate sewers and reducing runoff. - addressing 7 broad areas: research; outreach and communication; Clean Water Act regulatory support; tools; demonstrations and recognition; partnerships and promotion. • To meet the National Pollutant Discharge Elimination System (NPDES) using green infrastructure, such as green roofs;
California	• To promote green roofs to support and conserve native habitat (California grassland) - e.g. the Headquarters of the Mormon Church in Salt Lake City supporting a large meadow of native grassland species
Chicago	• Energy saving is the main drive for green roof implementation. - Requirement of minimum standards of solar reflectivity and emissivity achieved by green roofs. • Green roofs are a major component to make the city the greenest in the United States.
Minneapolis	• Program reducing stormwater charges (up to 100%) through rain garden and green roofs.
New York	• PLANYC (2007) - recommendation of extensive green roofs program as a means to reduce storm water overflows, reduce CO_2 emissions, and modification of the urban heat island effect.
Portland	• To reduce or prevent polluted urban runoff reaching rivers for protecting important local salmon industry. • Green roof policy in 2005; green roof installation on all re-roofing buildings - 'Floor or to area' credits for green roof installations; increasing the permitted floor space of building that installed green roof. • The Ecoroof program; by the Bureau of Environmental Services and Office of Sustainable Development in 1999. - to investigate and explore green roofs as a stormwater management tool. - first project for the program; the Hamilton Building. - technical assistance to building owners. - monitoring stormwater on the Hamilton Building. - grants for demonstration green roof projects. - guided tours of green roofs for visitors; 'Ecoroof Tours': including details of construction, planting and costs. - vegetation and design monitoring. - promotional presentations to developers and consultants. - the investigation of design, performance, policy and economic issues.
Seattle	• Several cities of Seattle have policies and planning documents supporting sustainable building, including green roofs • Seattle Public Utilities; - considering green roofs for stormwater management and Low Impact Development

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Seattle	(LID) • Seattle Stormwater, Grading and Drainage Control code; - acceptance of green roofs as means of stormwater overflow management and impervious surface reduction credits.
Washington, DC	• For management of stormwater runoff, pursuing three approaches for green roof implementation in new development or redevelopment projects; - Regulations and legislation; the Green Building Act of 2006 (D.C. Act16-15) - Incentives; • Financial support; $500,000 available in subsidies for green roof installations. • Substantial reducing permit review period for projects incorporating practices such as green roofs. • stormwater fee reductions - Education and outreach; • 'stormwater audit' program about practices such as green roof for reducing stormwater runoff from the properties. • updating and revising the District's Storm Water Management Guidebook. • the recent completion of extensive green roofs - One judiciary Square (8,000 ft^2) - the Franklin d. Reeves Center (4,000 ft^2)

APPENDIX 2. Water holding capacity (%) of substrates

	1 day (29/08/06) (29.3℃,RH:43%)[1]	3 days (01/09/06) (22.3℃, RH:57%)		5 days (03/09/06) (23.4℃, RH:67%)		7 days (06/09/06) (24.8℃, RH:59%		9 days (08/09/06) (19.2℃, RH:58%)		11 days (10/09/06) (33.6℃, RH:30%)		13 days (12/09/06) (29.2℃, RH:51%)	
	W.H.C (%)[2]	W.H.C	as % day1	W.H.C	as % day1	W.H.C	as % day1	W.H.C	as % day1	W.H.C	as % day1	W.H.C	as % day1
Limestone 1 (100:0:0)	6.09	0.79	13.02	0.41	6.78	0.28	4.60	0.23	3.72	0.15	2.41	0.12	1.97
Limestone 1 (90:0:10)	6.97	3.81	54.64	2.15	30.91	1.39	20.00	0.55	7.94	0.14	2.01	0.10	1.44
Limestone 1 (80:0:20)	7.71	4.63	60.03	3.21	41.70	2.58	33.48	1.34	17.39	0.86	11.16	0.66	8.56
Limestone 1 (70:20:10)	11.36	5.31	46.71	4.26	37.50	3.93	34.62	2.09	18.43	1.32	11.62	1.07	9.45
Limestone 1 (60:20:20)	8.68	4.91	56.53	3.99	45.93	3.55	40.86	2.07	23.81	1.15	13.21	0.84	9.68
Limestone 2 (100:0:0)	24.77	12.24	49.42	9.07	36.64	6.70	27.05	2.68	10.82	1.79	7.21	2.00	8.08
Limestone 2 (90:0:10)	28.98	15.74	54.31	12.65	43.66	10.07	34.76	5.29	18.24	3.21	11.07	2.81	9.68
Limestone 2 (80:0:20)	28.07	16.27	57.98	12.53	44.63	9.65	34.39	5.03	17.93	3.25	11.59	2.75	9.79
Limestone 2 (70:20:10)	29.40	15.50	52.72	10.46	35.58	6.57	22.36	4.08	13.88	3.18	10.82	2.45	8.32
Limestone 2 (60:20:20)	22.97	11.99	52.19	8.39	36.52	5.83	25.37	4.39	19.13	3.38	14.72	2.89	12.57
Limestone 3 (100:0:0)	16.74	9.75	58.22	9.72	58.06	3.95	23.62	2.61	15.57	2.05	12.23	2.15	12.82
Limestone 3 (90:0:10)	19.45	10.91	56.10	8.15	41.91	6.17	31.70	3.21	16.52	2.48	12.75	2.24	11.51
Limestone 3 (80:0:20)	17.00	8.49	49.96	6.95	40.90	5.61	33.02	3.80	22.35	2.80	16.47	2.53	14.86
Limestone 3 (70:20:10)	22.91	12.42	54.22	8.69	37.95	6.09	26.57	4.29	18.71	3.71	16.18	3.55	15.48
Limestone 3 (60:20:20)	15.37	7.21	46.92	5.55	36.12	4.82	31.35	3.41	22.16	2.85	18.56	2.34	15.22
LECA (100:0:0)	2.06	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
LECA (90:0:10)	2.11	0.35	16.46	0.06	2.85	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
LECA (80:0:20)	3.62	3.57	98.53	2.39	66.11	1.96	54.14	0.23	6.26	0.20	5.52	0.00	0.00
LECA (70:20:10)	7.19	4.73	65.71	3.82	53.10	2.75	38.18	1.07	14.83	0.81	11.21	0.38	5.28
LECA (60:20:20)	10.24	6.44	62.89	4.98	48.63	3.52	34.38	1.22	11.91	0.65	6.32	0.75	7.36
John Innes	28.02	11.76	41.97	9.15	32.67	7.49	26.74	5.31	18.94	4.37	15.61	3.72	13.28
Z. Semi	24.63	11.61	47.13	8.83	35.87	7.07	28.70	5.53	22.44	4.59	18.65	3.97	16.11
Z. Sedum	22.23	10.79	48.56	7.95	35.78	6.73	30.29	4.69	21.09	3.46	15.57	2.99	13.47

[1] ℃ and RH inner greenhouse. [2] W.H.C.: Water holding capacity (%)

APPENDIX 2. Moisture contents (%) of substrates

	15 days (14/09/06) (20.8℃, RH:89%)		17 days (16/09/06) (22.8℃, RH:60%)		19 days (18/09/06) (22.5℃, RH:52%)	
	W.H.C	as % day1	W.H.C	as % day1	W.H.C	as % day1
Limestone 1 (100:0:0)	0.11	1.75	0.17	2.74	0.27	4.38
Limestone 1 (90:0:10)	0.34	4.88	0.17	2.39	0.13	1.82
Limestone 1 (80:0:20)	0.83	10.81	0.42	5.45	0.23	2.94
Limestone 1 (70:20:10)	0.95	8.33	0.63	5.58	0.35	3.05
Limestone 1 (60:20:20)	1.03	11.83	0.61	7.07	0.38	4.38
Limestone 2 (100:0:0)	2.04	8.24	1.99	8.05	1.95	7.86
Limestone 2 (90:0:10)	2.34	8.07	1.99	6.86	1.89	6.51
Limestone 2 (80:0:20)	2.63	9.36	2.05	7.29	1.65	5.89
Limestone 2 (70:20:10)	2.31	7.85	1.68	5.71	1.59	5.40
Limestone 2 (60:20:20)	2.42	10.54	1.95	8.48	1.73	7.52
Limestone 3 (100:0:0)	2.32	13.86	2.41	14.38	2.22	13.26
Limestone 3 (90:0:10)	2.03	10.45	1.67	8.57	1.59	8.19
Limestone 3 (80:0:20)	2.45	14.43	1.79	10.51	1.77	10.43
Limestone 3 (70:20:10)	3.01	13.13	2.45	10.68	1.94	8.47
Limestone 3 (60:20:20)	2.22	14.44	1.79	11.67	1.31	8.50
LECA (100:0:0)	0.00	0.00	0.00	0.00	0.00	0.00
LECA (90:0:10)	0.00	0.00	0.00	0.00	0.00	0.00
LECA (80:0:20)	0.00	0.00	0.00	0.00	0.00	0.00
LECA (70:20:10)	0.29	4.08	0.06	0.83	0.00	0.00
LECA (60:20:20)	0.43	4.17	0.34	3.32	0.07	0.65
John Innes	2.95	10.54	2.00	7.14	1.35	4.83
Z. Semi	3.61	14.67	2.53	10.26	1.91	7.77
Z. Sedum	2.71	12.21	2.35	10.59	1.46	6.57

[1] ℃ and RH inner greenhouse. [2] W.H.C.: Water holding capacity (%)

APPENDIX 2. Water holding capacity (%) of substrates

	1 day (29/08/06) (29.3℃,RH:43%)	3 days (01/09/06) (22.3℃, RH:57%)		5 days (03/09/06) (23.4℃, RH:67%)		7 days (06/09/06) (24.8℃, RH:59%		9 days (08/09/06) (19.2℃, RH:58%)		11 days (10/09/06) (33.6℃, RH:30%)		13 days (12/09/06) (29.2℃, RH:51%)	
	W.H.C	W.H.C	as % day1	W.H.C	as % day1	W.H.C	as % day1	W.H.C	as % day1	W.H.C	as % day1	W.H.C	as % day1
Brick A (60:20:20)	31.74	21.49	67.70	15.45	48.67	10.17	32.03	7.88	24.83	5.67	17.85	5.27	16.61
Brick A (80:0:20)	20.04	10.71	53.46	7.01	35.00	4.99	24.88	4.47	22.29	3.41	17.00	2.09	10.41
Brick B (60:20:20)	30.90	21.38	69.19	16.58	53.65	12.18	39.42	9.94	32.17	7.48	24.22	6.62	21.44
Brick B (80:0:20)	20.40	14.59	71.54	10.02	49.12	6.37	31.24	5.29	25.92	4.00	19.61	3.31	16.24
Brick A+B (60:20:20)	30.43	19.79	65.02	15.04	49.42	10.77	16.56	8.41	27.62	6.02	19.78	5.21	17.11
Brick A+B (80:0:20)	20.75	11.30	54.45	7.48	36.04	5.54	26.69	4.59	22.10	3.49	16.83	2.63	12.69

	15 days (14/09/06) (20.8℃, RH:89%)		17 days (16/09/06) (22.8℃, RH:60%)		19 days (18/09/06) (22.5℃, RH:52%)	
	W.H.C	as % day1	W.H.C	as % day1	W.H.C	as % day1
Brick A (60:20:20)	4.76	15.00	4.55	14.34	3.85	12.14
Brick A (80:0:20)	1.69	8.42	2.07	10.35	2.13	10.65
Brick B (60:20:20)	5.97	19.32	5.98	19.36	5.98	19.37
Brick B (80:0:20)	2.35	11.54	3.18	15.59	2.25	11.01
Brick A+B (60:20:20)	4.37	14.37	5.03	16.54	4.03	13.23
Brick A+B (80:0:20)	2.09	10.09	2.16	10.41	1.73	8.35

[1] ℃ and RH: inner greenhouse. [2] W.H.C.: Water holding capacity (%)

APPENDIX 3. Mean bulk density at dry and saturated condition in response to substrate type across all composition rates.

	Bulk density (Dry) (Mg/m^3)			Bulk density after saturation (Mg/m^3)			*P*-value[1]
	Mean	S.E		Mean	S.E.		
Limestone 1	1.47	0.013	Aa	1.57	0.008	Ba	0.001
Limestone 2	1.56	0.017	Ab	1.87	0.011	Bb	0.001
Limestone 3	1.71	0.018	Ac	1.94	0.014	Bc	0.001
LECA	0.41	0.008	Ad	0.54	0.024	Bd	0.001
Brick A	1.32	0.022	Ae	1.58	0.036	Baeg	0.002
Brick B	1.29	0.024	Ae	1.60	0.037	Bae	0.002
Brick A+B	1.32	0.016	Ae	1.61	0.032	Bae	0.002
John Innes	1.14	0.013	Af	1.63	0.016	Be	0.0112
Zinco semi	1.00	0.014	Ag	1.39	0.030	Bf	0.0117
Zinco sedum	1.16	0.022	Af	1.46	0.025	Bfg	0.0119
P-value[2]	0.001			0.001			

[1] Different capital letters indicate significant differences at $p = 0.05$ between dry and saturated particle density for the same substrate type (Mann-Whitney *U*-test).
[2] Different lower-case indicate significant differences at $p = 0.05$ between substrate types for the same particle density (Mann-Whitney *U*-test after Kruskal-Wallis test).

APPENDIX 4. Increase of bulk density in response to saturation[1], air filled porosity[2], water holding capacity[3], pH, and EC in response to composition rate and substrate type.

	% increase[1]			AFP[2]			WHC[3]			pH			EC (dS/m)		
Substrate type	Mean	S.E		Mean	S.E		Mean	S.E		Mean	S.E		Mean	S.E	
Limestone1	7.28	1.053	a	37.93	1.559	a	8.16	0.365	a	8.06	0.035	a	0.402	0.0228	ac
Limestone2	19.72	0.916	b	14.11	0.597	b	26.84	0.857	b	8.00	0.028	ab	0.383	0.0232	ab
Limestone3	13.48	0.641	c	15.24	0.644	b	18.29	0.681	c	7.95	0.033	bd	0.353	0.0296	b
LECA	31.84	4.265	d	46.01	1.375	c	5.04	0.511	d	7.65	0.037	c	0.505	0.0392	c
Brick A	19.79	0.828	b	30.05	3.142	d	25.89	1.331	be	7.90	0.019	d	1.076	0.0768	d
Brick B	23.79	0.587	df	25.69	2.471	d	26.32	1.219	be	7.88	0.016	d	1.340	0.0769	e
Brick A+B	22.00	1.044	bf	29.32	2.438	d	25.59	1.112	be	7.80	0.014	e	1.307	0.0565	e
John Innes	43.67	0.748	e	10.30	0.843	e	28.02	1.262	b	6.61	0.172	f	0.909	0.0582	d
Zinco semi	39.82	2.216	e	15.20	3.407	bef	24.63	1.466	be	7.34	0.044	g	1.904	0.0597	f
Zinco sedum	26.19	1.744	df	27.44	4.643	df	22.23	1.479	e	7.50	0.070	cg	1.936	0.0374	f
P - value [4]	< 0.001			< 0.001			< 0.001			< 0.001			< 0.001		

	% increase[1]			AFP[2]			WHC[3]			pH			EC (dS/m)		
Composition rate	Mean	S.E		Mean	S.E		Mean	S.E		Mean	S.E		Mean	S.E	
100:0:0	13.57	1.818	a	33.52	3.569	a	12.42	1.208	a	8.01	0.049	a	0.278	0.0113	a
90:0:10	13.80	1.407	a	32.09	3.510	a	14.38	1.458	ab	8.05	0.051	a	0.298	0.0074	a
80:0:20	17.10	1.210	a	32.02	2.162	a	17.12	0.839	b	7.90	0.013	b	0.741	0.0658	b
70:20:10	25.37	5.829	ab	23.91	2.934	b	17.72	1.397	be	7.77	0.053	b	0.460	0.0311	c
60:20:20	23.16	1.246	b	22.04	1.629	b	21.67	1.079	cd	7.83	0.029	b	0.904	0.0727	b
John Innes	43.67	0.748	c	10.30	0.843	c	28.02	1.262	c	6.61	0.172	c	0.909	0.0582	b
Zinco semi	39.82	2.216	c	15.20	3.407	bc	24.63	1.466	cd	7.34	0.044	d	1.904	0.0597	d
Zinco sedum	26.19	1.744	b	27.44	4.643	ab	22.23	1.479	de	7.50	0.070	d	1.936	0.0374	d
P - value [4]	< 0.001			< 0.001			< 0.001			< 0.001			< 0.001		

[4] significant differences at $P = 0.05$ (Kruskal-Wallis test) between values within the same column
Different lower-case letters indicate significant difference at $P = 0.05$ (Mann-Whitney *U*-test after Kruskal-Wallis test [4]) between values within the same column.

APPENDIX 5. Seedling emergence, survival, and growth in response to substrate type and composition rate.

Leucanthemum vulgare	Maximum % of emergence			Survival (%)			Dry weight (g)		
	Mean	S.E		Mean	S.E		Mean	S.E	
Limestone1	3.080	0.432	a	66.9	6.41	a	0.057	0.0145	ac
Limestone2	5.920	0.557	b	82.6	4.19	a	0.050	0.0094	ac
Limestone3	4.640	0.534	b	77.4	6.27	a	0.033	0.0084	ac
LECA	2.480	0.377	ac	56.5	8.80	a	0.069	0.0142	ac
John Innes	3.600	0.718	ab	76.7	18.63	a	0.018	0.0101	abc
Zinco semi	0.800	0.327	c	50.0	22.36	a	0.002	0.0010	b
Zinco sedum	2.000	0.667	ac	66.7	16.67	a	0.013	0.0070	bc
P - value [1]	0.001***			0.063 ns			0.011*		

Leucanthemum vulgare	Maximum % of emergence			Survival (%)			Dry weight (g)		
	Mean	S.E		Mean	S.E		Mean	S.E	
100:0:0	2.350	0.404	ad	53.1	7.61	a	0.004	0.0009	ab
90:0:10	3.600	0.527	ab	73.7	8.71	a	0.010	0.0023	a
80:0:20	4.200	0.619	b	73.9	6.70	a	0.020	0.0035	c
70:20:10	4.150	0.597	b	67.8	7.42	a	0.081	0.0129	d
60:20:20	5.850	0.583	c	85.7	6.35	a	0.131	0.0179	e
John Innes	3.600	0.718	abc	76.7	18.63	a	0.018	0.0101	abc
Zinco semi	0.800	0.327	d	50.0	22.36	a	0.002	0.0010	b
Zinco sedum	2.000	0.667	abd	66.7	16.67	a	0.013	0.0070	abc
P - value [1]	0.001***			0.166 ns			0.001***		

[1]Significant differences at $P = 0.05$ (Kruskal-Wallis test) between values within the same column are indicated by: *$P = 0.05$; **$P = 0.01$: ***$P = 0.001$; ns, not significant.
Different lower-case letters indicate significant difference at $P = 0.05$ (Mann-Whitney *U*-test after Kruskal-Wallis test[1]) between values within the same column.

(*continued on next page*)

APPENDIX 5. Seedling emergence, survival, and growth in response to substrate type and composition rate.

Briza media	Maximum % emergence			Survival (%)			Dry weight (g)		
	Mean	S.E		Mean	S.E		Mean	S.E	
Limestone 1	34.67	14.270	af	63.33	20.276	a	0.314	0.1404	aef
Limestone 2	70.67	10.439	bce	100.000	0.000	a	0.366	0.0765	acf
Limestone 3	84.00	3.386	c	100.000	0.000	a	0.391	0.0454	a
Brick A	46.67	9.888	abe	89.38	6.777	a	0.096	0.0249	be
Brick B	47.00	3.215	abe	94.75	2.507	a	0.142	0.0285	be
Brick A+B	61.00	7.793	abd	100.00	0.000	a	0.188	0.0523	ce
John Innes	84.67	9.262	cd	99.02	0.693	a	0.661	0.0668	d
Zinco semi	42.00	4.619	abe	100.00	0.000	a	0.160	0.0274	ef
Zinco sedum	33.33	6.360	ef	99.82	3.029	a	0.092	0.0209	e
P-value [1]	0.003**			0.384 ns			0.010**		

Prunella vulgaris	Maximum % emergence			Survival (%)			Dry weight (g)		
	Mean	S.E		Mean	S.E		Mean	S.E	
Limestone 1	63.67	8.090	abc	98.37	3.543	a	0.784	0.0868	af
Limestone 2	72.00	6.197	a	100.00	0.000	a	0.686	0.1085	acf
Limestone 3	88.67	3.955	bd	100.00	0.000	a	0.879	0.1368	af
Brick A	31.33	11.963	ce	100.00	0.000	a	0.219	0.0573	b
Brick B	47.00	9.983	ace	86.78	6.886	a	0.491	0.0662	cd
Brick A+B	45.63	11.167	ace	91.81	8.461	a	0.384	0.1072	bd
John Innes	94.00	3.559	d	100.00	0.000	a	2.825	0.1501	e
Zinco semi	58.67	0.943	ac	100.00	0.000	a	0.967	0.2078	a
Zinco sedum	47.33	2.055	e	70.10	5.002	a	0.625	0.0039	df
P-value [1]	0.002**			0.055 ns			0.001***		

[1] Significant differences at $P = 0.05$ (Kruskal-Wallis test) between values within the same column are indicated by: *$P = 0.05$; **$P = 0.01$: ***$P = 0.001$; ns, not significant.
Different lower-case letters indicate significant difference at $P = 0.05$ (Mann-Whitney *U*-test after Kruskal-Wallis test[1]) between values within the same column.

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APPENDIX 5. Seedling emergence, survival, and growth in response to substrate type and composition rate.

Briza media	Maximum % emergence		% survival		Dry weight (g)	
	Mean	S.E	Mean	S.E	Mean	S.E
80:0:20	56.56	7.386	84.81	7.517	0.167	0.0302
60:20:20	58.11	5.078	97.68	2.037	0.332	0.0540
P-value [2]	0.8472 ns		0.1074 ns		0.0354*	

Prunella vulgaris	Maximum % emergence		% survival		Dry weight (g)	
	Mean	S.E	Mean	S.E	Mean	S.E
80:0:20	54.78	5.810	92.54	3.687	0.463	0.0525
60:20:20	59.89	7.562	99.77	0.907	0.685	0.0875
P-value [2]	0.4285 ns		0.1255 ns		0.1249 ns	

[2] Significant differences at $P = 0.05$ (Mann-Whitney *U*-test) between values within the same column are indicated by: *$P = 0.05$; **$P = 0.01$: ***$P = 0.001$; ns, not significant.

Comparison between maximum percentage emergence and survival of *Briza media* and *Prunella vulgaris* across all substrates.

	Maximum % emergence		Survival (%)	
	Mean	S.E	Mean	S.E
Briza media	56.53	3.902	92.92	3.231
Prunella vulgaris	59.02	4.028	94.93	1.892
P-value [3]	0.6195 ns		0.7906 ns	

[3] Significant differences at $P = 0.05$ (Mann-Whitney *U*-test) between values within the same column are indicated by: *$P = 0.05$; **$P = 0.01$: ***$P = 0.001$; ns, not significant.

APPENDIX 6. Seedling emergence, survival, and growth of *Leucanthemum vulgare* in response to individual substrate.

	Maximum % of emergence		% survival		Dry weight (g)	
	Mean	S.E.	Mean	S.E	Mean	S.E.
L1 (100:0:0)	1.80	0.629	60.00	16.330	0.002	0.0007
L1 (90:0:10)	1.40	0.600	50.00	16.667	0.008	0.0046
L1 (80:0:20)	3.80	1.209	78.33	13.159	0.027	0.0103
L1 (70:20:10)	3.20	0.952	57.50	15.833	0.056	0.0191
L1 (60:20:20)	5.20	0.952	88.50	6.149	0.193	0.0497
L2 (100:0:0)	4.00	0.943	82.50	10.574	0.006	0.0022
L2 (90:0:10)	5.20	0.904	86.33	6.013	0.008	0.0021
L2 (80:0:20)	7.60	1.392	87.08	7.769	0.023	0.0054
L2 (70:20:10)	5.60	1.360	67.21	10.704	0.082	0.0175
L2 (60:20:20)	7.20	1.405	89.90	10.902	0.131	0.0272
L3 (100:0:0)	3.40	0.733	70.00	13.333	0.004	0.0012
L3 (90:0:10)	5.40	1.267	78.57	11.139	0.012	0.0037
L3 (80:0:20)	3.20	0.998	70.00	15.275	0.013	0.0045
L3 (70:20:10)	5.60	1.454	76.33	12.943	0.058	0.0211
L3 (60:20:20)	5.60	1.360	92.00	18.184	0.078	0.0310
LE (100:0:0)	0.20	0.200	0.00	0.000	0.000	0.0000
LE (90:0:10)	2.40	0.833	80.00	28.087	0.011	0.0074
LE (80:0:20)	2.20	0.629	60.00	16.330	0.017	0.0066
LE (70:20:10)	2.20	0.554	70.00	20.000	0.127	0.0373
LE (60:20:20)	5.40	0.945	72.33	13.597	0.121	0.0254
John Innes	3.60	0.718	76.67	18.626	0.018	0.0101
Zinco semi	0.80	0.327	50.00	22.361	0.002	0.0010
Zinco sedum	2.00	0.667	66.67	16.667	0.013	0.0070
P - value*	< 0.001		0.067		< 0.001	

* Significant differences at $P = 0.05$ (Kruskal-Wallis test) between values within the same column.
L1: Limestone 1, L2: Limestone 2, L3: Limestone 3, LE: LECA, Zinco semi: Zinco semi-extensive.

APPENDIX 7. The relationship (Spearman rank correlation test) between seedling emergence, survival, growth, and substrate characteristics (bulk density air filled porosity, water holding capacity, pH, and EC).

		Maximum % emergence		% survival		Dry weight (g)	
		r_s	*P*-value	r_s	*P*-value	r_s	*P*-value
Leucanthemum vulgare	Bulk density (Mg/m^3)	0.457	**0.0282**	0.290	01796	- 0.098	0.6553
	Air filled porosity (%)	- 0.666	**0.000518**	- 0.441	**0.035**	- 0.345	0.1064
	Water holding capacity (%)	0.572	**0.004365**	0.354	0.0972	0.138	0.5289
	pH	0.189	0.3887	0.193	0.377	- 0.185	0.3972
	EC	0.023	0.9179	0.000	0.9982	0.543	**0.007403**
Briza media	Bulk density (Mg/m^3)	0.311	0.2597	0.262	0.3457	0.182	0.5159
	Air filled porosity (%)	- 0.561	**0.0297**	- 0.471	0.0761	- 0.643	**0.00974**
	Water holding capacity (%)	- 0.193	0.491	0.141	0.6162	- 0.107	0.7039
	pH	0.138	0.6248	- 0.046	0.8696	0.082	0.7708
	EC	- 0.511	0.0517	0.024	0.9319	- 0.350	0.2009
Prunella vulgaris	Bulk density (Mg/m^3)	0.313	0.2563	0.253	0.3629	- 0.050	0.8595
	Air filled porosity (%)	- 0.643	**0.009654**	- 0.666	**0.006772**	- 0.496	0.0598
	Water holding capacity (%)	- 0.377	0.1658	0.438	0.1025	- 0.279	0.3147
	pH	0.107	0.7034	0.002	0.994	- 0.238	0.3936
	EC	- 0.568	**0.0271**	- 0.238	0.3927	- 0.168	0.5499

APPENDIX 8. Moisture contents of substrates, daily precipitation, and maximum air temperature.

2007 year		Moisture content (%)							P.* (mm)[3]	M.T.* (°C)[3]
Month	Day	50 mm[1]	100 mm[1]	200 mm[1]	Zinco[2]	Lime[2]	NF[2]	F[2]		
July	10	W	W	W	W		W	W	0.0	19.9
	11					17.2			0.0	17.9
	12								0.8	21.2
	13								19.0	16.8
	14								0.2	20.3
	15								17.4	14.0
	16								0.6	21.5
	17								4.6	19.3
	18								0.8	21.1
	19								4.4	22.1
	20								13.4	15.4
	21								5.8	13.3
	22								0.6	19.2
	23								0.8	18.4
	24	17.2	20.1	12.8	18.8	23.1	18.8	22.9	0.0	20.4
	25								7.4	18.1
	26								10.4	16.3
	27								0.6	20.0
	28								0.0	18.7
	29								0.6	18.4
	30	9.9[W]	13.0	13.2	12.6	19.6	14.4	15.9	0.0	19.0
	31	24.9	8.5[W]	9.5[W]	9.3[W]	15.4	8.9[W]	10.4[W]	0.0	21.9
August	1								0.0	22.2
	2								0.0	20.4
	3	5.5[W]	17.5	13.1	15.8	9.6[W]	16.5	17.8	0.0	21.1
	4								0.0	22.9
	5								0.0	26.9
	6	8.8[W]	9.4[W]	10.2[W]	6.4[W]	17.5	6.8[W]	7.7[W]	0.0	20.2
	7								0.0	19.2
	8	21.1	21.3	15.2	19.5	12.9	19.8	21.7	0.0	20.4
	9					10.0[W]			0.0	21.7
	10								0.0	23.4
	11								0.0	23.8
	12								0.4	19.6
	13								0.8	19.2
	14								11.2	18.1
	15								4.0	17.7
	16								0.2	16.8
	17								0.0	18.3
	18								5.6	16.2
	19								4.8	16.9
	20								0.0	15.6
	21								0.0	16.6
	22								0.0	19.3
	23						18.3	15.1	0.0	22.6
	24	9.4[W]	15.1	14.3	13.9	20.1	15.5	11.6[W]	0.0	24.4
	25								0.0	21.7
	26								0.0	19.9
	27								0.0	18.9
	28	17.8	10.4[W]	11.5[W]	8.3[W]	10.5[W]	9.4[W]	17.5	0.2	16.2
	29								0.0	19.3
	30	11.3[W]	28.8	20.0	26.6	29.8	29.2	13.7	0.0	19.6
	31								0.0	17.4
Sept.	1	36.5	24.9	18.8	22.4	22.4	22.6	11.7[W]	0.0	20.2
	2								2.2	18.2
	3								0.0	19.4
	4								0.0	19.3
	5								0.0	21.7
	6								0.0	24.8

[W] Application of watering after measurement when the moisture content of the substrate was less than 12 %
[1] mean value (n=36); [2] mean value (n=24)
[3] source from: Sheffield weather page.
Zinco: Zinco substrate treatment; Lime: Limestone-based substrate treatment; NF: Non-fertiliser treatment; F: Fertiliser treatment
*P: Precipitation; *M.T.; Maximum temperature

APPENDIX 8. Moisture contents of substrates, daily precipitation, and maximum air temperature.

2007 year		Moisture content (%)							P.* (mm)[3]	M.T.* (°C)[3]
Month	Day	50 mm[1]	100 mm[1]	200 mm[1]	Zinco[2]	Lime[2]	NF[2]	F[2]		
Sept.	7	8.4 [W]	11.9 [W]	11.9 [W]	10.4 [W]	10.0 [W]	12.0 [W]	13.5	0.0	23.6
	8								0.0	19.9
	9								0.0	18.9
	10	24.7	26.5	17.7	23.2	22.0	24.2	8.4 [W]	0.0	19.6
	11								0.0	20.1
	12	15.0	21.2	16.3	18.4	20.6	19.7	28.8	0.0	21.8
	13								0.0	19.8
	14	10.7 [W]	17.4	15.1	15.8	15.7	17.5	20.6	0.2	17.7
	15								0.0	17.8
	16								0.0	19.4
	17	28.9	13.2	13.6	11.1 [W]	9.9 [W]	12.5	13.5	0.2	14.9
	18								0.0	13.6
	19	19.3	12.0 [W]	11.3 [W]	30.6	28.6	10.9 [W]	9.8 [W]	0.2	18.1

[W] Application of watering after measurement when the moisture content of the substrate was less than 12 %
[1] mean value (n=36); [2] mean value (n=24)
[3] source from: Sheffield weather page.
Zinco: Zinco substrate treatment; Lime: Limestone-based substrate treatment; NF: Non-fertiliser treatment; F: Fertiliser treatment
*P: Precipitation; *M.T.; Maximum temperature

APPENDIX 9. Abundance of individual species in response to substrate depths, substrate types, and fertiliser treatments.

				Mean	SE		*P*- value[2]
Achillea millefolium	Non-watering	50 mm		46.9	7.35	Aa	
		100 mm		152.3	15.32	Ab	
		200 mm		158.6	12.25	Ab	
			P-value[1]	< 0.001			
	Watering	50 mm		148.8	7.30	Ba	< 0.001
		100 mm		186.1	16.99	Aa	0.1605
		200 mm		250.8	24.58	Bb	0.0039
			P-value[1]	0.019			
		Zinco		187.8	17.56	a	
		Lime		230.6	25.04	a	
			P-value[1]	0.4664			
		NF		158.7	13.25	a	
		F		430.7	53.03	b	
			P-value[1]	< 0.001			
Campanula glomerata	Non-watering	50 mm		59.3	6.75	Aa	
		100 mm		92.6	3.60	Ab	
		200 mm		96.3	2.59	Ab	
		P-value[1]	*P*-value[1]	< 0.001			
	Watering	50 mm		96.3	2.59	Ba	< 0.001
		100 mm		96.3	2.59	Aa	0.4074
		200 mm		98.1	1.85	Aa	0.5673
		P-value[1]	*P*-value[1]	0.815			
		Zinco		98.1	1.85	a	
		Lime		98.1	1.85	a	
		P-value[1]	*P*-value[1]	1.0000			
		NF		98.1	1.85	a	
		F		96.3	2.59	a	
		P-value[1]	*P*-value[1]	0.5673			
Galium verum	Non-watering	50 mm		127.2	16.899	Aa	
		100 mm		192.2	15.505	Ab	
		200 mm		255.1	28.775	Ab	
			P-value[1]	< 0.001			
	Watering	50 mm		201.8	14.353	Ba	0.0011
		100 mm		281.2	18.429	Bb	0.0007
		200 mm		296.4	19.254	Bb	0.0064
			P-value[1]	< 0.001			
		Zinco		278.2	20.23	a	
		Lime		388.1	30.15	b	
			P-value[1]	0.0092			
		NF		262.6	17.00	a	
		F		916.5	129.78	b	
			P-value[1]	< 0.001			
Agrimonia eupatoria	Non-watering	50 mm		50.0	6.87	Aa	
		100 mm		96.3	5.89	Ab	
		200 mm		116.7	6.87	Ab	
			P-value[1]	< 0.001			
	Watering	50 mm		113.0	5.32	Ba	< 0.001
		100 mm		125.9	6.57	Ba	0.0016
		200 mm		129.6	7.30	Aa	0.2493
			P-value[1]	0.178			
		Zinco		129.6	7.30	a	
		Lime		155.6	9.41	b	
			P-value[1]	0.0485			
		NF		113.0	5.94	a	
		F		163.0	8.48	b	
			P-value[1]	< 0.001			
Clinopodium vulgare	Non-watering	50 mm		57.3	8.01	Aa	
		100 mm		149.8	14.40	Ab	
		200 mm		131.5	12.36	Ab	
			P-value[1]	< 0.001			
	Watering	50 mm		148.1	11.06	Ba	< 0.001
		100 mm		211.3	23.97	Aa	0.0516
		200 mm		230.6	50.16	Ba	0.0202
			P-value[1]	0.165			
		Zinco		243.7	37.32	a	
		Lime		645.4	158.52	b	
			P-value[1]	0.0005			
		NF		299.0	47.08	a	
		F		788.7	138.23	b	
			P-value[1]	0.0005			
Helianthemum nummularium	Non-watering	50 mm		57.4	6.79	Aa	
		100 mm		85.2	4.88	Ab	
		200 mm		85.2	4.88	Ab	
			P-value[1]	0.001			
	Watering	50 mm		87.0	4.61	Ba	0.0006
		100 mm		83.3	5.12	Aa	0.7963
		200 mm		83.3	5.12	Aa	0.7963
			P-value[1]	0.829			
		Zinco		83.3	5.12	a	
		Lime		92.6	3.60	a	
			P-value[1]	0.1426			
		NF		94.4	3.15	a	
		F		94.4	3.15	a	
			P-value[1]	1.000			

[1]Significant differences in mean value across the period at $p = 0.05$ between environmental variables (Kruskal-Wallis test for substrate depth; Mann-Whitney U-test for substrate type and fertiliser application) are indicated by different lower-case. [2]Significant differences in mean value across the period at $p = 0.05$ between watering treatments at the same substrate depth (Mann-Whitney *U*-test) are indicated by different capital letters. Zinco: Zinco substrate treatment; Lime: Limestone-based substrate treatment; NF: Non-fertiliser application; F: Fertiliser application.

APPENDIX 9. Abundance of individual species in response to substrate depths, substrate types, and fertiliser treatments.

				Mean	SE		*P*- value[2]
Hypochaeris radicata	Non-watering	50 mm		102.7	18.67	Aa	
		100 mm		138.5	24.68	Aa	
		200 mm		115.8	10.25	Aa	
			P-value[1]	0.0530			
	Watering	50 mm		97.0	3.62	Aa	0.1352
		100 mm		102.2	5.76	Aa	0.7047
		200 mm		92.9	5.00	Ba	0.0148
			P-value[1]	0.3570			
		Zinco		166.8	33.83	a	
		Lime		190.8	35.64	a	
			P-value[1]	0.9017			
		NF		623.4	296.10	a	
		F		154.2	23.30	a	
			P-value[1]	0.7480			
Knautia arvensis	Non-watering	50 mm		68.8	8.69	Aa	
		100 mm		135.5	12.56	Ab	
		200 mm		210.2	19.45	Ac	
			P-value[1]	< 0.001			
	Watering	50 mm		147.2	11.66	Ba	< 0.001
		100 mm		183.3	21.62	Ba	0.0292
		200 mm		179.0	22.40	Ba	0.0496
			P-value[1]	0.307			
		Zinco		142.1	13.38	a	
		Lime		229.3	26.63	b	
			P-value[1]	0.0011			
		NF		164.7	19.39	a	
		F		620.4	93.96	b	
			P-value[1]	< 0.001			
Linaria vulgaris	Non-watering	50 mm		107.0	12.14	Aa	
		100 mm		203.6	17.93	Ab	
		200 mm		196.3	17.71	Ab	
			P-value[1]	< 0.001			
	Watering	50 mm		185.1	14.20	Ba	0.0002
		100 mm		252.5	25.07	Aa	0.2392
		200 mm		179.8	11.78	Aa	0.9534
			P-value[1]	0.154			
		Zinco		191.4	15.73	a	
		Lime		437.3	44.48	b	
			P-value[1]	< 0.001			
		NF		200.6	21.86	a	
		F		911.4	113.83	b	
			P-value[1]	< 0.001			

				Mean	SE		*P*- value[2]
Leontodon autumnalis	Non-watering	50 mm		82.6	9.97	Aa	
		100 mm		98.2	5.85	Aa	
		200 mm		132.4	7.85	Ab	
			P-value[1]	0.001			
	Watering	50 mm		105.8	4.28	Aa	0.0588
		100 mm		136.2	14.18	Aa	0.0961
		200 mm		137.8	11.48	Aa	0.7981
			P-value[1]	0.170			
		Zinco		156.1	16.76	a	
		Lime		175.5	16.50	a	
			P-value[1]	0.2513			
		NF		271.5	64.84	a	
		F		349.2	68.40	b	
			P-value[1]	0.0066			
Leucanthemum vulgare	Non-watering	50 mm		120.4	23.10	Aa	
		100 mm		168.5	43.98	Ab	
		200 mm		125.9	11.27	Ab	
			P-value[1]	0.003			
	Watering	50 mm		101.9	4.94	Aa	0.0935
		100 mm		105.6	3.15	Aa	0.1034
		200 mm		103.7	4.55	Aa	0.0527
			P-value[1]	0.516			
		Zinco		324.1	99.40	a	
		Lime		348.1	193.71	a	
			P-value[1]	0.7018			
		NF		677.8	293.95	a	
		F		1083.3	504.63	a	
			P-value[1]	0.928			
Lotus corniculatus	Non-watering	50 mm		77.4	11.04	Aa	
		100 mm		243.3	33.19	Ab	
		200 mm		399.5	45.48	Ac	
			P-value[1]	< 0.001			
	Watering	50 mm		440.4	50.69	Ba	< 0.001
		100 mm		543.9	64.99	Ba	0.0003
		200 mm		692.5	74.35	Ba	0.0065
			P-value[1]	0.054			
		Zinco		532.9	51.10	a	
		Lime		616.9	66.50	a	
			P-value[1]	0.7032			
		NF		628.6	60.11	a	
		F		670.8	94.07	a	
			P-value[1]	0.8273			

[1]Significant differences in mean value across the period at $p = 0.05$ between environmental variables (Kruskal-Wallis test for substrate depth; Mann-Whitney U-test for substrate type and fertiliser application) are indicated by different lower-case. [2]Significant differences in mean value across the period at $p = 0.05$ between watering treatments at the same substrate depth (Mann-Whitney *U*-test) are indicated by different capital letters. Zinco: Zinco substrate treatment; Lime: Limestone-based substrate treatment; NF: Non-fertiliser application; F: Fertiliser application.

APPENDIX 9. Abundance of individual species in response to substrate depths, substrate types, and fertiliser treatments.

				Mean	SE		*P*- value[2]
Origanum vulgare	Non-watering	50 mm		89.5	11.59	Aa	
		100 mm		174.9	28.29	Ab	
		200 mm		171.4	19.18	Ab	
			P-value[1]	0.001			
	Watering	50 mm		126.9	10.84	Ba	0.0139
		100 mm		218.9	17.81	Bb	0.0097
		200 mm		310.6	25.26	Bc	< 0.001
			P-value[1]	< 0.001			
		Zinco		140.0	14.42	a	
		Lime		214.3	29.48	a	
			P-value[1]	0.2154			
		NF		111.2	15.17	a	
		F		86.9	14.73	a	
			P-value[1]	0.0932			
Pilosella aurantiaca	Non-watering	50 mm		57.4	7.29	Aa	
		100 mm		303.7	30.47	Ab	
		200 mm		529.6	37.87	Ac	
			P-value[1]	< 0.001			
	Watering	50 mm		177.8	18.79	Ba	< 0.001
		100 mm		390.7	39.04	Ab	0.1068
		200 mm		514.8	43.78	Ab	0.5992
			P-value[1]	< 0.001			
		Zinco		455.6	46.97	a	
		Lime		658.0	67.24	b	
			P-value[1]	0.0418			
		NF		516.7	72.77	a	
		F		1074.1	93.95	b	
			P-value[1]	< 0.001			
Pilosella officinarum	Non-watering	50 mm		85.2	12.45	Aa	
		100 mm		297.2	39.38	Ab	
		200 mm		307.7	39.07	Ab	
			P-value[1]	< 0.001			
	Watering	50 mm		181.5	16.94	Ba	< 0.001
		100 mm		357.4	47.41	Ab	0.2709
		200 mm		467.1	51.27	Bb	0.0265
			P-value[1]	< 0.001			
		Zinco		246.3	25.22	a	
		Lime		321.8	39.01	a	
			P-value[1]	0.3843			
		NF		252.5	25.28	a	
		F		592.6	67.99	b	
			P-value[1]	0.0037			
Primula veris	Non-watering	50 mm		45.1	6.67	Aa	
		100 mm		35.0	4.70	Aa	
		200 mm		55.5	5.99	Aa	
			P-value[1]	0.070			
	Watering	50 mm		79.4	5.74	Ba	0.0001
		100 mm		70.0	5.71	Ba	< 0.001
		200 mm		72.7	5.70	Ba	0.0339
			P-value[1]	0.423			
		Zinco		69.8	5.71	a	
		Lime		82.6	4.73	a	
			P-value[1]	0.0746			
		NF		58.7	5.31	a	
		F		72.3	5.42	b	
			P-value[1]	0.0240			
Scabiosa columbaria	Non-watering	50 mm		74.1	8.84	Aa	
		100 mm		135.2	14.27	Ab	
		200 mm		172.2	17.22	Ac	
			P-value[1]	< 0.001			
	Watering	50 mm		131.5	12.90	Ba	< 0.001
		100 mm		207.4	25.47	Bb	0.0057
		200 mm		200.0	26.17	Ab	0.8895
			P-value[1]	0.014			
		Zinco		191.7	30.35	a	
		Lime		264.8	38.23	b	
			P-value[1]	0.0109			
		NF		338.9	82.98	a	
		F		538.9	55.35	b	
			P-value[1]	< 0.001			

[1]Significant differences in mean value across the period at $p = 0.05$ between environmental variables (Kruskal-Wallis test for substrate depth; Mann-Whitney U-test for substrate type and fertiliser application) are indicated by different lower-case. [2]Significant differences in mean value across the period at $p = 0.05$ between watering treatments at the same substrate depth (Mann-Whitney *U*-test) are indicated by different capital letters. Zinco: Zinco substrate treatment; Lime: Limestone-based substrate treatment; NF: Non-fertiliser application; F: Fertiliser application.

APPENDIX 10.1. Mean total dry weight of individual species in response to substrate depths in non-watering over the 2-year period of the experiment

Non-watering regime	Substrate Depth																		
	2007									*P*-value*	2008									*P*-value*
	50 mm			100 mm			200 mm				50 mm			100 mm			200 mm			
	Mean	SE		Mean	SE		Mean	SE			Mean	SE		Mean	SE		Mean	SE		
A. millefolium	0.280	0.032	— a	1.792	0.256	Ab	2.482	0.522	Ab	0.000	0.000	0.000	—	5.736	0.908	Ba	8.958	1.550	Ba	0.000
A.. eupatoria	0.239	0.026	— a	0.534	0.105	Ab	0.278	0.056	Aa	0.014	0.000	0.000	—	0.727	0.134	Aa	0.490	0.108	Aa	0.001
C. glomerata	0.053	0.012	— a	0.091	0.016	Aa	0.106	0.021	Aa	0.096	0.000	0.000	—	0.014	0.006	Ba	0.133	0.045	Ab	0.001
C. vulgare	0.150	0.019	Aa	0.544	0.140	Ab	0.737	0.178	Ab	0.001	0.098	0.064	Ba	0.596	0.284	Ab	0.249	0.074	Bb	0.013
G. verum	0.239	0.049	— a	0.558	0.149	Ab	0.553	0.067	Ab	0.004	0.000	0.000	—	1.246	0.426	Aa	2.314	0.401	Ba	0.001
H. nummularium	0.192	0.039	— a	0.654	0.135	Ab	1.497	0.265	Ac	0.001	0.000	0.000	—	0.180	0.157	Ba	0.144	0.144	Ba	0.340
H. radicata	0.349	0.066	Aa	1.001	0.127	Ab	1.664	0.167	Ac	0.001	0.162	0.072	Aa	1.181	0.470	Aa	0.352	0.085	Ba	0.056
K. arvensis	0.331	0.041	Aa	0.767	0.088	Ab	1.084	0.187	Ab	0.001	0.031	0.031	Ba	1.636	0.535	Ab	2.368	0.562	Ab	0.001
L. autumnalis	0.222	0.039	Aa	0.743	0.118	Ab	1.413	0.207	Ac	0.001	0.068	0.051	Ba	0.690	0.246	Ab	1.491	0.503	Ab	0.001
L. vulgare	0.799	0.116	Aa	3.461	0.533	Ab	5.689	0.526	Ac	0.001	0.322	0.126	Ba	3.661	0.921	Ab	6.850	1.500	Ab	0.001
L. vulgaris	0.141	0.027	Aa	0.704	0.111	Ab	1.133	0.221	Ac	0.001	0.270	0.172	Aa	2.700	0.777	Ab	4.229	0.989	Bb	0.000
L. corniculatus	1.649	0.105	Aa	6.302	0.594	Ab	16.56	2.180	Ac	0.001	0.754	0.226	Ba	28.41	5.490	Bb	85.40	4.760	Bc	0.001
O. vulgare	0.244	0.070	— a	0.769	0.157	Ab	1.248	0.226	Ab	0.007	0.000	0.000	—	0.353	0.219	Ba	0.489	0.249	Ba	0.006
P. aurantiaca	0.158	0.023	Aa	1.028	0.136	Ab	1.476	0.099	Ac	0.001	0.188	0.089	Aa	0.584	0.174	Bb	1.616	0.475	Ab	0.005
P. officinarum	0.404	0.052	— a	0.720	0.077	Ab	1.298	0.174	Ac	0.001	0.000	0.000	—	0.026	0.018	Ba	0.446	0.163	Bb	0.001
P. veris	0.059	0.009	— a	0.100	0.015	Aab	0.174	0.067	Ab	0.025	0.000	0.000	—	0.023	0.012	Ba	0.024	0.016	Ba	0.042
S. columbaria	0.186	0.024	Aa	0.511	0.079	Ab	0.937	0.200	Ab	0.001	0.238	0.167	Ba	0.572	0.184	Ab	2.003	0.517	Ab	0.001

*Significant differences at p =0.05 between substrate depths for the same year (Kruskal-Wallis test)

Different capital letters indicate significant difference at p = 0.05 (Mann-Whitney *U*-test) between years for the same depth

Different lower-case letters indicate significant difference at p = 0.05 (Mann-Whitney *U*-test after Kruskal-Wallis test) between substrate depths for the same year

APPENDIX 10.2. Mean total dry weight of individual species in response to substrate depths in watering treatment over the 2-year period of the experiment

Watering regime	Substrate Depth																		
	2007									2008									
	50 mm			100 mm			200 mm			*P*-value*	50 mm			100 mm			200 mm			*P*-value*
	Mean	SE		Mean	SE		Mean	SE			Mean	SE		Mean	SE		Mean	SE		
A. millefolium	0.790	0.078	Aa	2.550	0.378	Ab	3.989	0.648	Ac	0.000	1.280	0.324	Aa	6.609	0.873	Bb	13.38	2.050	Bc	0.000
A.. eupatoria	0.304	0.053	Aa	0.581	0.072	Ab	0.387	0.066	Aab	0.025	0.198	0.064	Aa	0.820	0.151	Ab	0.987	0.287	Bb	0.001
C. glomerata	0.147	0.012	Aa	0.128	0.014	Aa	0.124	0.029	Aa	0.591	0.037	0.016	Ba	0.079	0.026	Aa	0.224	0.090	Aa	0.115
C. vulgare	0.480	0.056	Aa	0.587	0.118	Aa	0.648	0.110	Aa	0.588	0.197	0.037	Ba	0.411	0.154	Aa	0.574	0.337	Aa	0.991
G. verum	0.333	0.045	Aa	0.474	0.072	Aa	0.763	0.092	Ab	0.004	0.681	0.132	Aa	1.336	0.258	Bb	2.377	0.508	Bb	0.005
H. nummularium	0.656	0.072	Aa	1.278	0.189	— b	2.073	0.354	— b	0.005	0.063	0.044	B —	0.000	0.000	—	0.000	0.000	—	0.125
H. radicata	0.673	0.051	Aa	1.340	0.235	Ab	2.217	0.422	Ab	0.002	0.232	0.081	Ba	0.270	0.105	Ba	0.199	0.029	Ba	0.892
K. arvensis	0.494	0.060	Aa	0.932	0.105	Ab	1.670	0.160	Ac	0.001	0.682	0.091	Aa	1.934	0.583	Aab	2.390	0.676	Ab	0.026
L. autumnalis	0.577	0.044	Aa	1.319	0.184	Ab	2.230	0.332	Ac	0.001	0.374	0.120	Aa	1.348	0.431	Aab	3.016	0.981	Ab	0.026
L. vulgare	1.724	0.218	Aa	4.949	0.648	Ab	8.108	0.985	Ac	0.001	1.980	0.374	Aa	4.809	1.160	Aa	13.20	1.870	Ab	0.001
L. vulgaris	0.192	0.031	Aa	0.648	0.132	Ab	0.959	0.081	Ac	0.000	0.393	0.131	Aa	1.386	0.431	Ab	2.512	0.677	Ab	0.004
L. corniculatus	17.12	0.835	Aa	26.38	1.740	Ab	38.23	2.830	Ac	0.001	5.134	0.988	Ba	53.55	6.660	Bb	79.38	8.090	Bc	0.001
O. vulgare	0.437	0.064	Aa	1.531	0.096	Ab	2.018	0.269	Ab	0.000	0.320	0.310	Ba	1.154	0.394	Aa	1.473	0.515	Aa	0.103
P. aurantiaca	0.550	0.093	Aa	1.377	0.247	Ab	1.668	0.283	Ab	0.002	0.323	0.070	Aa	0.924	0.258	Aa	1.020	0.362	Aa	0.135
P. officinarum	0.753	0.132	Aa	1.382	0.397	Aa	1.594	0.198	Aa	0.064	0.107	0.044	Ba	0.060	0.020	Ba	0.231	0.040	Bb	0.005
P. veris	0.109	0.013	Aa	0.100	0.025	Aa	0.079	0.007	Aa	0.394	0.002	0.007	Ba	0.010	0.007	Bab	0.023	0.008	Bb	0.043
S. columbaria	0.350	0.039	Aa	1.021	0.156	Ab	1.079	0.143	Ab	0.001	0.772	0.324	Aa	1.536	0.300	Aab	3.548	1.300	Ab	0.011

*Significant differences at p =0.05 between substrate depths for the same year (Kruskal-Wallis test)

Different capital letters indicate significant difference at p = 0.05 (Mann-Whitney *U*-test) between years for the same depth

Different lower-case letters indicate significant difference at p = 0.05 (Mann-Whitney *U*-test after Kruskal-Wallis test) between substrate depths for the same year

APPENDIX 10.3. Mean total dry weight of individual species in response to substrate depth in non-watering and watering treatment over the 2-year period of the experiment

2007	50 mm						100 mm						200 mm						
	NW			W			NW			W			NW			W			
	Mean	SE		Mean	SE		Mean	SE		Mean	SE		Mean	SE		Mean	SE		*P*-value*
A. millefolium	0.280	0.032	a	0.790	0.078	b	1.792	0.256	c	2.550	0.378	c	2.482	0.522	c	3.989	0.648	d	0.0010
A. eupatoria	0.239	0.026	a	0.304	0.053	ab	0.534	0.105	bc	0.581	0.072	c	0.278	0.056	a	0.387	0.066	ac	0.0030
C. glomerata	0.053	0.012	a	0.147	0.012	b	0.091	0.016	ac	0.128	0.014	bc	0.106	0.021	ab	0.124	0.029	ab	0.0110
C. vulgare	0.150	0.019	a	0.480	0.056	b	0.544	0.140	bc	0.587	0.118	bc	0.737	0.178	b	0.648	0.110	b	0.0010
G. verum	0.239	0.049	a	0.333	0.045	ab	0.558	0.149	bcd	0.474	0.072	bc	0.553	0.067	cd	0.763	0.092	d	0.0010
H. nummularium	0.192	0.039	a	0.656	0.072	b	0.654	0.135	b	1.278	0.189	c	1.497	0.265	c	2.073	0.354	c	0.0010
H. radicata	0.349	0.066	a	0.673	0.051	b	1.001	0.127	bc	1.340	0.235	cd	1.664	0.167	d	2.217	0.422	d	0.0010
K. arvensis	0.331	0.041	a	0.494	0.060	a	0.767	0.088	b	0.932	0.105	b	1.084	0.187	b	1.670	0.160	c	0.0010
L. autumnalis	0.222	0.039	a	0.577	0.044	b	0.743	0.118	b	1.319	0.184	c	1.413	0.207	c	2.230	0.332	d	0.0010
L. vulgare	0.799	0.116	a	1.724	0.218	b	3.461	0.533	c	4.949	0.648	cd	5.689	0.526	d	8.108	0.985	e	0.0010
L. vulgaris	0.141	0.027	a	0.192	0.031	a	0.704	0.111	bc	0.648	0.132	b	1.133	0.221	bc	0.959	0.081	c	0.0010
L. corniculatus	1.649	0.105	a	17.12	0.835	b	6.302	0.594	c	26.38	1.740	d	16.56	2.180	b	38.23	2.830	e	0.0010
O. vulgare	0.244	0.070	a	0.437	0.064	b	0.769	0.157	bd	1.531	0.096	ce	1.248	0.226	cd	2.018	0.269	e	0.0010
P. aurantiaca	0.158	0.023	a	0.550	0.093	b	1.028	0.136	c	1.377	0.247	cd	1.476	0.099	d	1.668	0.283	cd	0.0010
P. officinarum	0.404	0.052	a	0.753	0.132	ab	0.720	0.077	b	1.382	0.397	bc	1.298	0.174	c	1.594	0.198	c	0.0010
P. veris	0.059	0.009	a	0.109	0.013	a	0.100	0.015	a	0.100	0.025	a	0.174	0.067	a	0.079	0.007	a	0.0960
S. columbaria	0.186	0.024	a	0.350	0.039	b	0.511	0.079	bd	1.021	0.156	c	0.937	0.200	cd	1.079	0.143	c	0.0010

NW: non-watering treatment; W: watering treatment

*Significant differences at p =0.05 within same row (Kruskal-Wallis test)

Different lower-case letters indicate significant difference at p = 0.05 (Mann-Whitney *U*-test after Kruskal-Wallis test) within same row

APPENDIX 10.4. Mean total dry weight of individual species in response to substrate depth in non-watering and watering treatment over the 2-year period of the experiment

2008	50 mm						100 mm						200 mm						
	NW			W			NW			W			NW			W			
	Mean	SE		Mean	SE		Mean	SE		Mean	SE		Mean	SE		Mean	SE		*P*-value*
A. millefolium	0.000	0.000	–	1.280	0.324	a	5.736	0.908	b	6.609	0.873	b	8.958	1.550	bc	13.38	2.050	b	0.0010
A. eupatoria	0.000	0.000	–	0.198	0.064	a	0.727	0.134	b	0.820	0.151	b	0.490	0.108	ab	0.987	0.287	b	0.0010
C. glomerata	0.000	0.000	–	0.037	0.016	ac	0.014	0.006	a	0.079	0.026	bc	0.133	0.045	bc	0.224	0.090	bc	0.0010
C. vulgare	0.098	0.064	a	0.197	0.037	a	0.596	0.284	a	0.411	0.154	a	0.249	0.074	ab	0.574	0.337	a	0.0820
G. verum	0.000	0.000	–	0.681	0.132	a	1.246	0.426	ab	1.336	0.258	b	2.314	0.401	b	2.377	0.508	b	0.0010
H. nummularium	0.000	0.000	–	0.063	0.044	a	0.180	0.157	a	0.000	0.000	–	0.144	0.144	a	0.000	0.000	–	0.2900
H. radicata	0.162	0.072	a	0.232	0.081	a	1.181	0.470	a	0.270	0.105	a	0.352	0.085	a	0.199	0.029	a	0.1720
K. arvensis	0.031	0.031	a	0.682	0.091	b	1.636	0.535	bc	1.934	0.583	bc	2.368	0.562	c	2.390	0.676	c	0.0010
L. autumnalis	0.068	0.051	a	0.374	0.120	b	0.690	0.246	bc	1.348	0.431	bcd	1.491	0.503	cd	3.016	0.981	d	0.0010
L. vulgare	0.322	0.126	a	1.980	0.374	b	3.661	0.921	bc	4.809	1.160	bc	6.850	1.500	c	13.20	1.870	d	0.0010
L. vulgaris	0.270	0.172	a	0.393	0.131	a	2.700	0.777	bc	1.386	0.431	b	4.229	0.989	c	2.512	0.677	bc	0.0010
L. corniculatus	0.754	0.226	a	5.134	0.988	b	28.41	5.490	c	53.55	6.660	d	85.40	4.760	e	79.38	8.090	e	0.0010
O. vulgare	0.000	0.000	–	0.320	0.310	a	0.353	0.219	ab	1.154	0.394	b	0.489	0.249	ab	1.473	0.515	ab	0.0050
P. aurantiaca	0.188	0.089	a	0.323	0.070	ab	0.584	0.174	bc	0.924	0.258	bc	1.616	0.475	c	1.020	0.362	bc	0.0010
P. officinarum	0.000	0.000	–	0.107	0.044	a	0.026	0.018	b	0.060	0.020	ab	0.446	0.163	c	0.231	0.040	c	0.0010
P. veris	0.000	0.000	–	0.002	0.007	a	0.023	0.012	ab	0.010	0.007	ab	0.024	0.016	ab	0.023	0.008	b	0.0240
S. columbaria	0.238	0.167	a	0.772	0.324	b	0.572	0.184	b	1.536	0.300	c	2.003	0.517	c	3.548	1.300	c	0.0100

NW: non-watering treatment; W: watering treatment

*Significant differences at p =0.05 within same row (Kruskal-Wallis test)

Different lower-case letters indicate significant difference at p = 0.05 (Mann-Whitney *U*-test after Kruskcal-Wallis test) within same row

APPENDIX 10.5. Mean total dry weight (g) of individual species in response to substrate types over the 2-year period of the experiment

	Substrate type treatment											
	2007						2008					
	Zinco			Lime			Zinco			Lime		
	Mean	SE		Mean	SE		Mean	SE		Mean	SE		*P*-value*
Achillea millefolium	1.852	0.275	a	3.942	0.590	b	8.238	1.159	bc	6.533	1.066	c	< 0.001
Agrimonia eupatoria	0.416	0.091	a	0.702	0.158	a	0.570	0.153	a	1.144	0.346	a	0.087
Campanula glomerata	0.070	0.023	a	0.388	0.083	b	0.181	0.109	a	1.506	0.602	c	<0.001
Clinopodium vulgare	0.543	0.103	a	2.561	0.434	b	0.790	0.074	a	1.815	0.718	ab	0.007
Galium verum	0.433	0.046	a	1.770	0.209	b	1.539	0.495	b	2.337	0.731	b	0.003
Helianthemum nummularium	1.434	0.279	a	1.081	0.078	a	0.000	0.000	—	0.306	0.143	b	< 0.001
Hypochaeris radicata	1.286	0.124	a	2.109	0.354	b	0.558	0.249	c	0.566	0.159	c	< 0.001
Knautia arvensis	0.910	0.120	a	2.634	0.476	bc	1.693	0.376	ab	3.266	0.555	c	0.002
Leontodon autumnalis	1.113	0.123	a	3.158	0.411	b	0.478	0.112	c	1.406	0.430	ac	< 0.001
Leucanthemum vulgare	2.959	0.273	a	17.79	6.653	b	3.702	0.870	a	7.332	2.071	ab	0.002
Linaria vulgaris	0.628	0.109	a	1.934	0.385	b	1.411	0.301	b	2.107	0.586	ab	0.053
Lotus corniculatus	23.04	4.135	a	20.89	2.918	a	23.06	3.635	a	6.441	1.446	b	0.004
Origanum vulgare	0.891	0.209	a	2.110	0.557	a	0.552	0.310	a	1.742	0.812	a	0.106
Pilosella aurantiaca	1.288	0.133	a	3.749	0.502	b	0.714	0.192	c	1.927	0.581	ac	< 0.001
Pilosella officinarum	0.951	0.122	a	3.156	0.480	b	0.059	0.023	c	0.496	0.365	c	< 0.001
Primula veris	0.117	0.011	a	0.069	0.012	b	0.002	0.001	c	0.013	0.008	c	< 0.001
Scabiosa columbaria	0.929	0.135	a	3.093	0.629	b	2.403	0.823	ab	4.230	0.950	b	0.007

Zinco: Zinco substrate treatment; Lime: Limestone substrate treatment

*Significant differences at p =0.05 within same row (Kruskal-Wallis test)

Different lower-case letters indicate significant difference at p = 0.05 (Mann-Whitney *U*-test after Kruskal-Wallis test) within same row

APPENDIX 10.6. Mean total dry weight (g) of individual species in response to fertiliser treatment over the 2-year period of the experiment

	Fertiliser treatment												
	2007						2008						
	NF			F			NF			F			
	Mean	SE		Mean	SE		Mean	SE		Mean	SE		*P*-value*
Achillea millefolium	1.273	0.184	a	34.82	4.813	b	2.821	0.487	c	47.37	4.792	b	< 0.001
Agrimonia eupatoria	0.346	0.069	a	1.259	0.196	b	0.686	0.138	ac	1.436	0.370	bc	0.003
Campanula glomerata	0.091	0.015	a	0.702	0.303	a	0.195	0.103	a	1.263	0.798	a	0.259
Clinopodium vulgare	0.323	0.097	a	1.359	0.789	ab	0.087	0.058	b	0.073	0.073	b	0.012
Galium verum	0.487	0.069	a	7.237	1.090	b	1.889	0.209	c	12.28	3.651	b	< 0.001
Helianthemum nummularium	0.923	0.164	a	1.696	0.426	a	0.351	0.196	b	1.121	0.610	ab	0.014
Hypochaeris radicata	1.313	0.207	a	8.003	2.475	b	1.215	0.387	a	0.820	0.306	a	0.001
Knautia arvensis	0.927	0.137	a	17.72	3.708	b	1.469	0.201	c	14.56	2.515	b	< 0.001
Leontodon autumnalis	0.964	0.126	a	8.801	1.409	b	0.809	0.145	a	0.729	0.213	a	< 0.001
Leucanthemum vulgare	2.026	0.316	a	43.20	3.833	b	2.992	1.116	a	24.72	3.523	c	< 0.001
Linaria vulgaris	0.422	0.051	a	17.51	2.532	b	0.701	0.085	c	10.04	2.582	b	< 0.001
Lotus corniculatus	17.49	1.675	a	20.88	4.555	a	46.60	4.286	b	8.619	3.295	c	< 0.001
Origanum vulgare	0.573	0.068	a	10.14	2.438	b	0.794	0.227	a	5.822	2.188	b	< 0.001
Pilosella aurantiaca	1.122	0.157	a	8.667	1.367	b	0.696	0.169	a	1.441	0.338	a	< 0.001
Pilosella officinarum	0.899	0.186	a	4.921	1.328	b	0.184	0.100	c	0.273	0.273	c	< 0.001
Primula veris	0.060	0.015	a	0.036	0.016	a	0.019	0.015	a	0.148	0.094	a	0.130
Scabiosa columbaria	0.842	0.082	a	10.01	2.450	b	2.616	0.702	c	9.487	4.080	abc	0.002

NF: non-fertiliser treatment; F: fertiliser treatment

*Significant differences at p =0.05 within same row (Kruskal-Wallis test)

Different lower-case letters indicate significant difference at p = 0.05 (Mann-Whitney *U*-test after Kruskal-Wallis test) within same row

APPENDIX 11.1. Mean height (cm) and coverage (%) of individual species in response to substrate depths in non-watering treatment over the 2-year period of the experiment.

Non-watering regime			2007														2008			
			Jun.			Jul.			Aug.			Sep.			Oct.			Apr.			
			Mean	SE		Mean	SE		Mean	SE		Mean	SE		Mean	SE		Mean	SE		*P*- value[2]
Achillea millefolium	Ht. (cm)	50 mm	3.2	0.323	Aa	4.7	0.660	Aa	4.2	0.412	Aa	0.2	0.222	Ab	0.0	0.000	—	0.2	0.167	Ab	0.001
		100 mm	2.8	0.169	Aa	5.2	0.325	Ab	5.4	0.296	Bb	4.1	0.332	Bc	5.5	0.607	Abc	6.3	0.546	Bb	0.001
		200 mm	2.6	0.253	Aa	5.3	0.336	Ab	7.7	1.950	Bbc	8.4	3.350	Bbc	7.6	2.500	Ab	7.9	0.661	Bc	0.001
		P-value[1]			0.125			0.635			0.018			0.001			0.9295			0.001	
	Co. (%)	50 mm	0.59	0.063	Aa	1.41	0.242	Ab	1.86	0.244	Ab	0.22	0.222	Ac	0.00	0.000	—	0.00	0.000	—	0.001
		100 mm	0.59	0.085	Aa	4.47	0.600	Bb	4.27	0.588	Bb	4.40	0.415	Bb	3.13	0.595	Bb	4.49	1.189	Bb	0.001
		200 mm	0.54	0.067	Aa	5.54	0.666	Bb	5.08	0.709	Bb	6.06	1.085	Bb	5.53	1.488	Bb	6.71	1.250	Bb	0.001
		P-value[1]			0.897			0.001			0.001			0.001			0.001			0.001	
Agrimonia eupatoria	Ht. (cm)	50 mm	7.1	0.821	Aa	5.9	0.629	Aab	4.2	0.539	Ab	0.0	0.000	—	0.0	0.000	—	0.0	0.000	—	0.001
		100 mm	7.0	0.578	Aa	5.6	0.632	Aab	8.9	3.650	Aab	9.8	3.690	Aab	4.1	1.230	Abc	2.4	0.437	Ac	0.002
		200 mm	7.9	1.090	Aa	5.4	0.838	Aabc	4.9	0.573	Abc	4.8	0.612	Ac	3.1	0.859	Abd	2.2	0.208	Ad	0.001
		P-value[1]			0.834			0.771			0.321			0.1445			0.6578			0.4739	
	Co. (%)	50 mm	1.80	0.264	Aa	1.90	0.210	Aa	1.82	0.215	Aa	0.00	0.000	—	0.00	0.000	—	0.00	0.000	—	0.001
		100 mm	3.12	0.939	Aa	3.10	0.660	Aa	2.62	0.504	Aa	2.03	0.248	Ba	0.80	0.249	Bb	0.15	0.042	Bb	0.001
		200 mm	1.85	0.419	Aa	1.87	0.449	Aa	1.60	0.354	Aa	1.47	0.363	Ba	1.00	0.213	Ba	0.08	0.022	Bb	0.001
		P-value[1]			0.742			0.623			0.135			0.001			0.001			0.004	
Campanula glomerata	Ht. (cm)	50 mm	0.9	0.140	Aab	1.0	0.104	Aa	1.0	0.171	Aa	0.4	0.216	Ab	0.1	0.078	Ab	0.1	0.078	Ab	0.001
		100 mm	0.8	0.078	Aa	0.9	0.162	Aa	0.8	0.112	Aa	2.5	1.400	Aa	2.3	1.320	Ba	1.1	0.460	ABa	0.668
		200 mm	0.9	0.087	Aa	1.2	0.163	Ab	1.7	0.445	Bbc	2.9	1.580	Bbc	2.7	1.540	Bb	1.9	0.299	Bc	0.009
		P-value[1]			0.648			0.192			0.019			0.005			0.001			0.007	
	Co. (%)	50 mm	0.40	0.046	Aa	0.41	0.069	Aa	0.49	0.087	Aa	0.16	0.103	Ab	0.01	0.008	Ab	0.00	0.000	—	0.001
		100 mm	0.27	0.031	Aa	0.38	0.066	Aa	0.38	0.078	Aa	0.48	0.080	Ba	0.24	0.082	Bab	0.09	0.045	Bb	0.004
		200 mm	0.41	0.058	Aa	0.71	0.108	Bbc	0.64	0.063	Bc	0.70	0.047	Cc	0.50	0.062	Cab	0.21	0.065	Bad	0.001
		P-value[1]			0.072			0.024			0.021			0.003			0.001			0.004	

[1]Significant differences at p =0.05 between substrate depths for the same watering treatment (Kruskal-Wallis test). [2] Significant differences at p = 0.05 between months for the same depth (Kruskal-Wallis test). Different capital letters indicate significant difference between substrate depths for the same month. Different lower-case letters indicate significant difference between months for the same depth.
Ht.: Height, Co.: Coverage

APPENDIX 11.1. Mean height (cm) and coverage (%) of individual species in response to substrate depths in non-watering treatment over the 2-year period of the experiment.

Non-watering regime			2007 Jun.			Jul.			Aug.			Sep.			Oct.			2008 Apr.			
			Mean	SE		Mean	SE		Mean	SE		Mean	SE		Mean	SE		Mean	SE		*P*- value[2]
Clinopodium vulgare	Ht. (cm)	50 mm	3.2	0.336	Aa	4.7	0.518	Aab	7.3	0.991	Ab	1.2	0.841	Acd	0.1	0.133	Ac	1.1	0.251	Ad	0.001
		100 mm	3.6	0.320	Aa	7.1	0.725	Ab	11.5	1.030	Bc	12.1	1.030	Bc	9.0	1.990	Babc	3.1	0.143	Ba	0.001
		200 mm	3.6	0.379	Aa	6.8	0.807	Ab	11.4	1.340	Bc	11.1	1.420	Bc	7.8	0.956	Bbc	2.4	0.352	Ba	0.001
		P-value[1]			0.598			0.061			0.026			0.001			0.001			0.001	
	Co. (%)	50 mm	0.69	0.110	Aa	0.80	0.093	Aa	1.10	0.163	Aa	0.20	0.132	Abc	0.01	0.014	Ab	0.10	0.053	Ac	0.001
		100 mm	0.63	0.089	Aa	1.80	0.288	Bbc	2.37	0.203	Bc	2.37	0.125	Bc	1.40	0.329	Bab	0.18	0.087	Ad	0.001
		200 mm	0.63	0.075	Aa	2.01	0.252	Bbd	2.89	0.274	Bc	2.81	0.316	Bbc	1.91	0.388	Bd	0.45	0.150	Aa	0.001
		P-value[1]			0.984			0.001			0.001			0.001			0.001			0.073	
Galium verum	Ht. (cm)	50 mm	3.6	0.435	Aac	4.4	0.357	Aab	5.3	0.511	Ab	2.4	0.717	Ac	0.7	0.667	Ac	1.1	1.060	Ac	0.001
		100 mm	3.8	0.354	Aa	5.6	0.643	Aa	6.6	0.837	Aa	6.4	1.130	Ba	4.2	1.250	Ba	4.5	1.200	Ba	0.123
		200 mm	3.6	0.311	Aa	5.3	0.274	Ab	5.9	0.516	Ab	5.6	0.749	Bab	5.2	0.756	Bab	6.0	0.756	Bb	0.029
		P-value[1]			0.743			0.149			0.625			0.018			0.005			0.008	
	Co. (%)	50 mm	0.58	0.074	Aa	1.20	0.099	Ab	1.43	0.198	Ab	0.72	0.270	Aabc	0.32	0.311	Acd	0.20	0.197	Ad	0.001
		100 mm	0.80	0.189	Aa	2.13	0.325	Bbc	2.58	0.409	Bc	2.84	0.493	Bcd	1.88	0.594	Babcd	1.08	0.387	Bab	0.004
		200 mm	0.62	0.070	Aa	2.40	0.166	Bb	2.94	0.282	Bb	3.32	0.451	Bb	3.13	0.423	Bb	1.35	0.151	Bc	0.001
		P-value[1]			0.757			0.001			0.006			0.001			0.002			0.004	
Helianthemum nummularium	Ht. (cm)	50 mm	1.9	0.254	Aa	1.2	0.059	Ab	1.3	0.149	Aab	0.6	0.377	Ac	0.1	0.133	Ac	0.0	0.000	—	0.001
		100 mm	2.1	0.221	Aa	1.5	0.121	Bb	1.8	0.234	Bab	2.2	0.505	Bab	1.5	0.198	Bab	0.4	0.273	Ac	0.002
		200 mm	3.1	0.633	Aab	2.0	0.161	Cab	2.8	0.598	Ba	1.9	0.088	Bbc	1.5	0.140	Bc	0.2	0.167	Ad	0.001
		P-value[1]			0.148			0.001			0.002			0.006			0.001			0.5687	
	Co. (%)	50 mm	0.46	0.720	Aa	0.68	0.138	Aa	1.13	0.141	Ab	0.43	0.232	Aac	0.09	0.093	Ac	0.00	0.000	—	0.001
		100 mm	0.56	0.090	Aa	0.93	0.122	ABb	1.72	0.211	Bc	2.11	0.310	Bc	1.84	0.356	Bbc	0.10	0.070	Ad	0.001
		200 mm	0.96	0.242	Aa	1.59	0.218	Ba	2.81	0.419	Cb	4.71	0.812	Cb	5.01	0.951	Cb	0.02	0.016	Ac	0.001
		P-value[1]			0.144			0.004			0.002			0.001			0.001			0.312	

[1]Significant differences at p =0.05 between substrate depths for the same watering treatment (Kruskal-Wallis test). [2] Significant differences at p = 0.05 between months for the same depth (Kruskal-Wallis test). Different capital letters indicate significant difference between substrate depths for the same month. Different lower-case letters indicate significant difference between months for the same depth.
Ht.: Height, Co.: Coverage

APPENDIX 11.1. Mean height (cm) and coverage (%) of individual species in response to substrate depths in non-watering treatment over the 2-year period of the experiment.

Non-watering regime			2007 Jun.			Jul.			Aug.			Sep.			Oct.			2008 Apr.			
			Mean	SE		Mean	SE		Mean	SE		Mean	SE		Mean	SE		Mean	SE		*P*- value[2]
Hypochaeris radicata	Ht. (cm)	50 mm	2.8	0.358	Aa	8.1	2.670	Aa	4.6	1.940	Aab	0.5	0.241	Ac	0.4	0.306	Ac	1.4	0.124	Ab	0.001
		100 mm	2.2	0.240	Aac	5.2	2.760	Aa	13.8	3.890	Aab	17.6	3.560	Bb	1.7	0.262	Bc	1.7	0.156	Ac	0.001
		200 mm	2.1	0.140	Aa	5.9	2.650	Ab	14.3	3.790	Bb	14.0	3.810	Bb	1.8	0.232	Ba	1.6	0.217	Aa	0.001
		P-value[1]			0.236			0.396			0.034			0.001			0.014			0.548	
	Co. (%)	50 mm	1.11	0.256	Aac	1.66	0.191	Aa	1.84	0.185	Aa	0.24	0.108	Ab	0.04	0.044	Ab	1.39	0.575	Ac	0.001
		100 mm	1.11	0.191	Aa	3.86	0.516	Bbc	4.64	0.664	Bb	2.87	0.382	Bc	0.92	0.375	Ba	1.83	0.582	Aa	0.001
		200 mm	1.17	0.189	Aa	4.61	0.409	Bb	5.63	0.621	Bb	5.14	0.658	Bb	2.70	0.604	Ca	0.36	0.132	Bc	0.001
		P-value[1]			0.693			0.001			0.001			0.001			0.001			0.001	
Knautia arvensis	Ht. (cm)	50 mm	2.6	0.460	Aa	2.2	0.360	Aa	2.5	0.320	Aa	0.6	0.342	Abc	0.1	0.133	Ab	0.9	0.203	Ac	0.001
		100 mm	2.7	0.313	Aab	2.9	0.226	Aab	3.8	0.346	Bac	3.3	0.175	Ba	2.0	0.485	Bb	5.1	0.576	Bc	0.001
		200 mm	2.8	0.234	Aa	2.9	0.295	Aa	4.1	0.410	Bb	4.1	0.270	Cb	3.4	0.456	Bab	5.4	0.734	Bb	0.005
		P-value[1]			0.619			0.101			0.02			0.001			0.001			0.001	
	Co. (%)	50 mm	1.59	0.240	Aa	1.77	0.095	Aa	1.84	0.087	Aa	0.31	0.206	Ab	0.04	0.039	Ab	0.06	0.043	Ab	0.001
		100 mm	1.48	0.214	Aa	2.78	0.145	Bb	3.12	0.228	Bb	3.22	0.317	Bb	2.27	0.526	Bab	1.22	0.343	Ba	0.001
		200 mm	1.27	0.141	Aa	3.23	0.348	Bb	3.42	0.474	Bb	3.36	0.454	Bb	3.04	0.453	Bb	1.39	0.383	Ba	0.001
		P-value[1]			0.777			0.001			0.003			0.001			0.001			0.001	
Leontodon autumnalis	Ht. (cm)	50 mm	2.9	0.230	Aa	2.2	0.150	Ab	4.5	1.703	Aab	1.0	0.402	Acd	0.2	0.200	Ac	1.1	0.178	Ad	0.001
		100 mm	2.9	0.307	Aa	2.7	0.516	Aa	13.2	3.176	Bb	2.5	0.421	Ba	2.0	0.421	Ba	2.7	0.459	Ba	0.006
		200 mm	2.8	0.309	Aa	5.2	1.704	Aac	13.0	3.197	Bb	6.3	1.534	Ccb	3.5	0.871	Cab	3.8	0.360	Bab	0.040
		P-value[1]			0.968			0.143			0.041			0.001			0.001			0.001	
	Co. (%)	50 mm	1.27	0.185	Aab	1.47	0.147	Aa	1.56	0.171	Aa	0.66	0.246	Abc	0.07	0.058	Ac	0.10	0.065	Ac	0.001
		100 mm	1.18	0.186	Aa	2.76	0.255	Bbc	3.11	0.331	Bc	3.09	0.445	Bbc	1.73	0.450	Babd	0.45	0.192	ABd	0.001
		200 mm	1.17	0.140	Aa	4.23	0.588	Bb	5.06	0.852	Bb	4.59	0.623	Bb	3.34	0.449	Cb	0.58	0.094	Bc	0.001
		P-value[1]			0.837			0.001			0.001			0.001			0.001			0/006	

[1]Significant differences at p =0.05 between substrate depths for the same watering treatment (Kruskal-Wallis test). [2] Significant differences at p = 0.05 between months for the same depth (Kruskal-Wallis test). Different capital letters indicate significant difference between substrate depths for the same month. Different lower-case letters indicate significant difference between months for the same depth.
Ht.: Height, Co.: Coverage

APPENDIX 11.1. Mean height (cm) and coverage (%) of individual species in response to substrate depths in non-watering treatment over the 2-year period of the experiment.

Non-watering regime			2007 Jun.			Jul.			Aug.			Sep.			Oct.			2008 Apr.			
			Mean	SE		Mean	SE		Mean	SE		Mean	SE		Mean	SE		Mean	SE		*P*- value[2]
Leucanthemum vulgare	Ht. (cm)	50 mm	4.8	0.391	Aab	5.4	0.424	Aa	3.9	0.294	Ab	0.6	0.455	Ac	0.3	0.278	Ac	2.9	0.478	Ab	0.001
		100 mm	4.0	0.270	Aa	7.5	0.520	Bb	8.1	2.217	Bbc	7.5	2.435	Bc	4.6	0.432	Bac	7.5	1.209	Bbc	0.002
		200 mm	3.9	0.310	Aac	8.1	0.509	Bb	13.8	4.415	Bb	14.5	4.797	Bbc	5.0	4.076	Bc	10.2	1.247	Bb	0.001
		P-value[1]			0.154			0.006			0.001			0.001			0.001			0.001	
	Co. (%)	50 mm	2.61	0.346	Aa	7.24	3.960	Aab	4.21	0.420	Ab	0.58	0.424	Ac	0.00	0.000	—	0.78	0.196	Ad	0.001
		100 mm	2.40	0.392	Aa	8.19	0.982	Bb	8.98	1.140	Bb	9.17	1.050	Bb	3.69	0.915	Aa	1.79	0.521	Aa	0.001
		200 mm	2.28	0.354	Aa	9.80	0.733	Bb	10.4	0.644	Bb	10.5	0.589	Bb	7.46	0.596	Bc	1.99	0.385	Aa	0.001
		P-value[1]			0.556			0.007			0.001			0.001			0.001			0.120	
Linaria vulgaris	Ht. (cm)	50 mm	3.6	0.154	Aa	4.9	0.402	Abc	6.5	1.100	Ac	3.3	1.120	Aad	1.1	0.458	Ad	2.3	1.130	Aabd	0.001
		100 mm	3.8	0.252	Aa	6.6	0.826	Bb	9.5	1.290	ABb	9.2	1.230	Bb	7.3	0.987	Bb	8.9	1.388	Bb	0.003
		200 mm	4.4	0.481	Aa	7.5	0.590	Bbc	10.7	1.270	Bbd	11.1	1.480	Bbd	6.9	1.080	Bc	11.7	0.886	Bd	0.001
		P-value[1]			0.636			0.013			0.037			0.003			0.001			0.001	
	Co. (%)	50 mm	0.31	0.042	Aa	1.01	0.131	Ab	1.34	0.230	Ab	1.02	0.323	Aab	0.30	0.172	Aac	0.07	0.047	Ac	0.001
		100 mm	0.34	0.020	Aa	2.79	0.427	Bbc	3.10	0.426	Bbcd	3.89	0.574	Bcd	4.80	0.783	Bd	1.37	0.567	ABab	0.001
		200 mm	0.41	0.065	Aa	4.49	0.474	Cb	4.91	0.561	Cb	5.78	0.755	Bb	5.59	0.751	Bb	2.00	0.778	Bc	0.001
		P-value[1]			0.593			0.001			0.001			0.001			0.001			0.003	
Lotus corniculatus	Ht. (cm)	50 mm	3.7	0.346	Aa	5.8	0.636	Ab	10.3	1.070	Ac	0.3	0.333	Ad	0.0	0.000	—	1.6	0.258	Ae	0.001
		100 mm	3.5	0.251	Aac	4.5	0.361	Aa	13.2	1.250	Ab	10.4	0.644	Bb	5.4	1.480	Aac	3.0	0.374	Bc	0.001
		200 mm	2.4	0.242	Aa	3.5	0.463	Aa	12.1	1.010	Ab	10.9	0.957	Bb	11.1	0.985	Bb	3.7	1.100	Ba	0.001
		P-value[1]			0.023			0.053			0.117			0.001			0.001			0.011	
	Co. (%)	50 mm	2.06	0.264	Aa	6.41	0.469	Ab	17.1	1.500	Ac	0.41	0.387	Ad	0.00	0.000	—	0.06	0.019	Ae	0.001
		100 mm	2.34	0.487	Aa	9.21	0.706	Bb	29.8	5.180	Bc	30.0	4.670	Bc	7.85	2.790	Aab	0.28	0.167	ABd	0.001
		200 mm	2.14	0.411	Aa	9.56	1.150	Bb	36.8	6.300	Bc	39.4	6.220	Bc	34.5	5.180	Bc	1.04	0.505	Bd	0.001
		P-value[1]			0.906			0.007			0.011			0.001			0.001			0.017	

[1]Significant differences at p =0.05 between substrate depths for the same watering treatment (Kruskal-Wallis test). [2] Significant differences at p = 0.05 between months for the same depth (Kruskal-Wallis test). Different capital letters indicate significant difference between substrate depths for the same month. Different lower-case letters indicate significant difference between months for the same depth.
Ht.: Height, Co.: Coverage

APPENDIX 11.1. Mean height (cm) and coverage (%) of individual species in response to substrate depths in non-watering treatment over the 2-year period of the experiment.

Non-watering regime			2007 Jun.			Jul.			Aug.			Sep.			Oct.			2008 Apr.			
			Mean	SE		Mean	SE		Mean	SE		Mean	SE		Mean	SE		Mean	SE		*P*- value[2]
Origanum vulgare	Ht. (cm)	50 mm	3.4	0.266	Aa	6.0	0.784	Ab	9.3	1.310	Ac	4.5	1.470	Aabcd	1.5	0.823	Ade	0.3	0.206	Ae	0.001
		100 mm	4.4	0.362	Ba	7.3	0.600	Ab	9.1	1.380	Ab	9.6	1.320	Bb	6.1	1.800	Bab	1.8	0.553	Bc	0.001
		200 mm	3.7	0.352	Aa	7.1	0.722	Ab	10.7	1.930	Ab	11.3	1.930	Bb	10.9	1.800	Bb	2.2	0.573	Bc	0.001
		P-value[1]			0.107			0.447			0..576			0.021			0.004			0.025	
	Co. (%)	50 mm	0.65	0.068	Aa	0.97	0.114	Ab	1.12	0.122	Ab	0.59	0.198	Aab	0.21	0.141	Ac	0.02	0.020	Ac	0.001
		100 mm	0.80	0.149	Aa	2.04	0.358	Bb	2.28	0.477	Bb	2.09	0.454	Bb	1.31	0.433	Babc	0.11	0.048	Bc	0.001
		200 mm	0.80	0.082	Aa	2.58	0.272	Bb	2.66	0.476	Bb	2.89	0.454	Bb	2.72	0.435	Cb	0.10	0.057	Bc	0.001
		P-value[1]			0.569			0.001			0.008			0.004			0.002			0.032	
Pilosella aurantiaca	Ht. (cm)	50 mm	1.6	0.128	Aa	3.6	0.291	Ab	3.3	0.276	Ab	0.4	0.250	Ac	0.2	0.167	Ac	1.2	0.153	Aa	0.001
		100 mm	1.7	0.126	Aa	6.4	0.378	Bb	20.3	4.570	Bc	18.5	4.480	Bc	3.4	0.906	Bad	4.8	0.567	Bd	0.001
		200 mm	1.7	0.072	Aa	6.8	0.238	Bb	32.4	3.180	Bc	31.4	3.190	Bc	3.7	0.717	Bd	6.4	0.474	Bb	0.001
		P-value[1]			0.947			0.001			0.001			0.001			0.001			0.001	
	Co. (%)	50 mm	0.87	0.061	Aa	1.22	0.116	Ab	1.86	0.147	Ac	0.36	0.244	Ad	0.00	0.000	—	0.00	0.000	—	0.001
		100 mm	0.94	0.109	Aa	3.42	0.338	Bbc	4.40	0.384	Bcd	4.70	0.467	Bd	2.25	0.617	Aab	0.23	0.094	Ae	0.001
		200 mm	0.92	0.123	Aa	5.21	0.372	Cb	6.90	0.662	Cc	7.17	0.601	Cc	5.12	0.845	Bbc	0.48	0.080	Bd	0.001
		P-value[1]			0.918			0.001			0.001			0.001			0.001			0.001	
Pilosella officinarum	Ht. (cm)	50 mm	1.6	0.080	Aa	1.8	0.228	Aa	1.7	0.197	Aa	1.0	0.331	Aab	0.3	0.199	Aa	0.0	0.000	—	0.001
		100 mm	1.3	0.156	Aa	2.0	0.303	Ab	5.1	1.960	Bb	1.8	0.154	Ab	1.1	0.189	Aba	0.8	0.343	Aa	0.001
		200 mm	1.6	0.112	Aa	3.1	1.110	Aab	7.8	1.560	Bb	1.5	0.128	Aa	1.3	0.082	Ba	2.0	0.418	Aa	0.013
		P-value[1]			0.057			0.823			0.028			0.157			0.011			0.0502	
	Co. (%)	50 mm	1.09	0.201	Aa	1.06	0.085	Aa	1.52	0.179	Aa	0.71	0.279	Aa	0.00	0.000	—	0.00	0.000	—	0.001
		100 mm	0.60	0.078	Aa	1.91	0.113	Bb	2.50	0.232	Bb	2.40	0.303	Bb	1.11	0.330	Aa	0.05	0.026	Ac	0.001
		200 mm	0.76	0.065	Aa	2.69	0.363	Bb	4.06	0.653	Cc	4.52	0.726	Cc	3.12	0.397	Bbc	0.17	0.055	Ad	0.001
		P-value[1]			0.091			0.001			0.001			0.001			0.001			0.004	

[1]Significant differences at p =0.05 between substrate depths for the same watering treatment (Kruskal-Wallis test). [2] Significant differences at p = 0.05 between months for the same depth (Kruskal-Wallis test). Different capital letters indicate significant difference between substrate depths for the same month. Different lower-case letters indicate significant difference between months for the same depth.
Ht.: Height, Co.: Coverage

APPENDIX 11.1. Mean height (cm) and coverage (%) of individual species in response to substrate depths in non-watering treatment over the 2-year period of the experiment.

Non-watering regime			2007														2008			
			Jun.			Jul.			Aug.			Sep.			Oct.			Apr.			
			Mean	SE		Mean	SE		Mean	SE		Mean	SE		Mean	SE		Mean	SE		*P*- value[2]
Primula veris	Ht. (cm)	50 mm	1.8	0.150	Aab	1.9	0.104	Aa	1.4	0.193	Ab	0.3	0.170	Ac	0.1	0.133	Ac	0.0	0.000	—	0.001
		100 mm	1.7	0.121	Aa	2.1	0.109	Ab	1.5	0.226	Aac	0.8	0.195	Ad	0.8	0.235	Acd	1.8	0.416	Aabd	0.001
		200 mm	1.8	0.160	Aac	2.2	0.166	Aa	1.9	0.122	Aa	0.6	0.246	Ab	0.6	0.151	Ab	1.1	0.503	Abc	0.001
		P-value[1]			0.955			0.635			0.129			0.354			0.060			0.2413	
	Co. (%)	50 mm	0.56	0.063	Aa	0.69	0.070	Aa	0.71	0.125	Aa	0.08	0.078	Ab	0.03	0.026	Ab	0.00	0.000	—	0.001
		100 mm	0.36	0.048	Ba	0.93	0.046	Bb	0.80	0.133	Ab	0.30	0.101	Bad	0.02	0.011	Ac	0.11	0.043	Ad	0.001
		200 mm	0.39	0.053	ABa	1.02	0.093	Bb	0.86	0.170	Ab	0.37	0.142	ABac	0.01	0.010	Ac	0.06	0.042	Ac	0.001
		P-value[1]			0.047			0.018			0.821			0.040			0.429			0.003	
Scabiosa columbaria	Ht. (cm)	50 mm	1.5	0.156	Aa	1.1	0.058	Ab	1.0	0.078	Abc	0.8	0.259	Abc	0.6	0.290	Aabc	0.8	0.160	Ac	0.041
		100 mm	1.8	0.267	Aba	1.7	0.317	Aa	5.5	3.820	Ba	1.8	0.288	Ba	1.5	0.369	Aa	2.4	0.404	Ba	0.534
		200 mm	2.2	0.237	Bab	1.6	0.223	Aa	2.1	0.283	Ba	12.9	5.980	Babc	13.5	5.680	Bbc	3.2	0.328	Bc	0.039
		P-value[1]			0.049			0.134			0.002			0.008			0.003			0.001	
	Co. (%)	50 mm	1.27	0.460	Aab	1.08	0.130	Aab	1.28	0.108	Aa	0.60	0.226	Abc	0.45	0.247	Ac	0.08	0.063	Ac	0.001
		100 mm	0.93	0.070	Aa	1.63	0.168	Bb	1.84	0.417	Ab	2.00	0.426	Bb	1.17	0.238	Bab	0.17	0.039	Bc	0.001
		200 mm	1.68	0.460	Aa	2.0	0.165	Ba	2.00	0.295	Aa	2.70	0.641	Ba	3.06	1.050	Ba	0.27	0.054	Bb	0.001
		P-value[1]			0.417			0.004			0.113			0.002			0.007			0.013	

[1]Significant differences at p =0.05 between substrate depths for the same watering treatment (Kruskal-Wallis test). [2] Significant differences at p = 0.05 between months for the same depth (Kruskal-Wallis test). Different capital letters indicate significant difference between substrate depths for the same month. Different lower-case letters indicate significant difference between months for the same depth.
Ht.: Height, Co.: Coverage

APPENDIX 11.2. Mean height (cm) and coverage (%) of individual species in response to substrate depths in watering over the 2-year period of the experiment.

Watering regime			2007 Jun.			Jul.			Aug.			Sep.			Oct.			2008 Apr.			
			Mean	SE		Mean	SE		Mean	SE		Mean	SE		Mean	SE		Mean	SE		*P*- value[2]
Achillea millefolium	Ht. (cm)	50 mm	3.6	0.399	Aa	4.5	0.511	Aa	4.1	0.310	Aa	4.5	0.391	Aa	4.2	0.324	Aa	4.3	0.515	Aa	0.503
		100 mm	4.2	0.236	Aa	5.4	0.587	Ab	6.4	0.355	Bbc	7.5	0.446	Bc	6.6	0.530	Bbc	7.6	0.817	Bc	0.001
		200 mm	4.1	0.379	Aa	4.4	0.320	Aa	4.9	0.283	Aa	7.2	0.496	Bbc	6.6	0.656	Bb	8.9	0.770	Bc	0.001
		P-value[1]			0.264			0.294			0.001			0.003			0.002			0.001	
	Co. (%)	50 mm	0.69	0.186	Aa	1.52	0.213	Ab	2.03	0.315	Ab	2.41	0.380	Abc	3.18	0.333	Ac	1.59	0.197	Ab	0.001
		100 mm	0.57	0.046	Aa	4.19	0.505	Bb	4.76	0.480	Bb	5.10	0.939	Bbc	7.41	0.832	Bc	6.05	0.742	Bbc	0.001
		200 mm	0.62	0.084	Aa	4.97	0.561	Bb	5.61	0.551	Bbc	7.94	0.944	Bcd	10.30	1.090	Bd	10.04	1.710	Bcd	0.001
		P-value[1]			0.972			0.001			0.001			0.001			0.001			0.001	
Agrimonia eupatoria	Ht. (cm)	50 mm	7.8	0.871	Aa	6.0	0.559	Aab	5.3	0.717	Aab	4.9	0.810	Ab	4.3	0.830	Ab	1.6	0.185	Ac	0.001
		100 mm	7.1	0.623	Aa	4.9	0.428	Ab	8.7	1.920	Aab	11.5	3.610	Aab	11.5	3.690	Aab	2.2	0.160	Bc	0.001
		200 mm	7.2	0.721	Aa	5.1	0.583	Ab	4.7	0.926	Ab	6.2	1.520	Aab	5.8	1.700	Aab	2.2	0.105	Bc	0.001
		P-value[1]			0.846			0.301			0.108			0.505			0.298			0.012	
	Co. (%)	50 mm	2.01	0.370	Aa	1.79	0.260	Aa	1.70	0.186	Aa	1.58	0.161	Aa	1.36	0.152	Aa	0.08	0.021	Ab	0.001
		100 mm	2.12	0.237	Aa	2.78	0.403	Aa	2.73	0.314	Ba	2.53	0.251	Ba	2.14	0.287	Aa	0.15	0.040	Ab	0.001
		200 mm	1.33	0.157	Aa	1.51	0.226	Aa	1.39	0.184	Aa	1.70	0.185	Aa	1.43	0.267	Aa	0.25	0.078	Ab	0.001
		P-value[1]			0.051			0.069			0.008			0.012			0.066			0.179	
Campanula glomerata	Ht. (cm)	50 mm	0.8	0.056	Aa	0.9	0.062	Aab	1.3	0.196	Abc	1.9	0.595	Ac	2.0	0.878	Ac	1.0	0.250	Aabc	0.002
		100 mm	0.8	0.075	Aa	0.9	0.096	Aa	1.2	0.127	Ab	1.4	0.175	Ab	1.2	0.132	Ab	1.9	0.377	ABb	0.003
		200 mm	0.8	0.084	Aa	1.0	0.133	Aab	1.3	0.120	Abc	1.9	0.488	Ac	1.3	0.244	Aabc	2.6	0.316	Bd	0.001
		P-value[1]			0.856			0.394			0.688			0.669			0.972			0.007	
	Co. (%)	50 mm	3.20	0.027	Aa	3.50	0.031	Aa	3.20	0.041	Aa	4.20	0.050	Aa	4.69	0.078	Aa	0.21	0.009	Ab	0.001
		100 mm	2.51	0.037	Aa	4.20	0.055	Ab	4.20	0.050	Ab	5.30	0.066	Ab	5.91	0.101	Ab	0.89	0.043	ABc	0.001
		200 mm	2.30	0.038	Aac	6.30	0.104	Ab	3.70	0.061	Aab	5.40	0.124	Ab	5.26	0.144	Aabc	1.61	0.047	Bc	0.004
		P-value[1]			0.076			0.076			0.337			0.427			0.768			0.023	

[1]Significant differences at p =0.05 between substrate depths for the same watering treatment (Kruskal-Wallis test). [2] Significant differences at p = 0.05 between months for the same depth (Kruskal-Wallis test). Different capital letters indicate significant difference between substrate depths for the same month. Different lower-case letters indicate significant difference between months for the same depth.
Ht.: Height, Co.: Coverage

APPENDIX 11.2. Mean height (cm) and coverage (%) of individual species in response to substrate depths in watering treatment over the 2-year period of the experiment.

Watering regime			2007														2008			
			Jun.			Jul.			Aug.			Sep.			Oct.			Apr.			
			Mean	SE		Mean	SE		Mean	SE		Mean	SE		Mean	SE		Mean	SE		*P*- value[2]
Clinopodium vulgare	Ht. (cm)	50 mm	3.4	0.317	Aa	5.4	0.388	Ab	10.4	1.190	Ac	11.4	1.250	Ac	10.7	1.130	Ac	1.8	0.220	Ad	0.001
		100 mm	3.2	0.320	Aa	5.7	0.491	Ab	9.5	1.170	Ac	10.1	1.070	Ac	8.8	0.979	Ac	2.4	0.354	Ad	0.001
		200 mm	3.3	0.351	Aa	6.0	0.625	Ab	9.6	1.270	Ac	10.2	1.550	Ac	9.3	1.380	Ac	3.7	0.438	Ba	0.001
		P-value[1]			0.753			0.879			0.675			0.520			0.198			0.004	
	Co. (%)	50 mm	0.59	0.103	Aa	1.06	0.133	Ab	1.33	0.159	Ab	1.66	0.230	Ab	1.75	0.271	Ab	0.12	0.028	Ac	0.001
		100 mm	0.58	0.115	Aa	1.27	0.182	Ab	1.98	0.175	Bc	1.90	0.275	Ac	1.99	0.285	Ac	0.30	0.094	Ad	0.001
		200 mm	0.72	0.109	Aa	2.01	0.384	Ab	2.08	0.249	Bb	2.41	0.196	Ab	2.40	0.238	Ab	0.63	0.208	Aa	0.001
		P-value[1]			0.382			0.149			0.027			0.063			0.296			0.097	
Galium verum	Ht. (cm)	50 mm	3.1	0.377	Aa	4.5	0.488	Ab	4.9	0.485	Ab	5.1	0.312	Ab	4.6	0.402	Ab	5.8	0.411	Ab	0.005
		100 mm	2.9	0.315	Aa	4.4	0.471	Ab	5.5	0.559	Abc	5.6	0.574	Abc	5.1	0.560	Abc	6.4	0.568	Ac	0.001
		200 mm	2.9	0.276	Aa	4.1	0.312	Ab	4.7	0.368	Ab	5.0	0.360	Ab	4.6	0.174	Ab	8.2	0.434	Bc	0.001
		P-value[1]			0.937			0.775			0.514			0.904			0.878			0.007	
	Co. (%)	50 mm	0.53	0.060	Aa	1.18	0.146	Ab	1.62	0.166	Ac	1.82	0.120	Ac	2.04	0.189	Ac	0.57	0.097	Aa	0.001
		100 mm	0.52	0.066	Aa	1.54	0.211	Ab	1.97	0.213	ABb	2.06	0.202	Abc	2.86	0.304	Ac	0.79	0.070	Aa	0.001
		200 mm	0.52	0.056	Aa	1.78	0.209	Abe	2.46	0.247	Bbc	2.92	0.258	Bc	3.92	0.349	Bd	1.24	0.154	Be	0.001
		P-value[1]			0.961			0.185			0.047			0.0075			0.002			0.004	
Helianthemum nummularium	Ht. (cm)	50 mm	2.5	0.304	Aa	2.0	0.289	Aab	1.8	0.232	Ab	2.3	0.449	Aab	1.9	0.306	Aab	0.3	0.171	Ac	0.001
		100 mm	2.3	0.287	Aa	2.1	0.180	Aa	2.1	0.138	Aa	2.5	0.430	Aa	2.2	0.227	Aa	0.0	0.000	A —	0.001
		200 mm	2.2	0.273	Aa	1.8	0.135	Aa	1.8	0.114	Aa	3.0	0.758	Aa	1.7	0.110	Aa	0.0	0.000	A —	0.001
		P-value[1]			0.702			0.819			0.129			0.551			0.282			0.125	
	Co. (%)	50 mm	0.37	0.042	Aa	0.60	0.071	Ab	1.64	0.125	Ac	2.09	0.237	Acd	3.24	0.483	Ad	0.02	0.018	— e	0.001
		100 mm	0.56	0.084	Aa	1.13	0.147	Bb	2.07	0.213	Ac	3.23	0.497	ABcd	5.50	0.974	Abd	0.00	0.000	—	0.001
		200 mm	0.62	0.102	Aa	1.32	0.138	Bb	2.14	0.266	Ac	5.13	0.804	Bd	7.89	1.323	Bd	0.00	0.000	—	0.001
		P-value[1]			0.127			0.001			0.186			0.010			0.014			0.125	

[1]Significant differences at p =0.05 between substrate depths for the same watering treatment (Kruskal-Wallis test). [2] Significant differences at p = 0.05 between months for the same depth (Kruskal-Wallis test). Different capital letters indicate significant difference between substrate depths for the same month. Different lower-case letters indicate significant difference between months for the same depth.
Ht.: Height, Co.: Coverage

APPENDIX 11.2. Mean height (cm) and coverage (%) of individual species in response to substrate depths in watering treatment over the 2-year period of the experiment.

Watering regime			2007 Jun.			Jul.			Aug.			Sep.			Oct.			2008 Apr.			
			Mean	SE		Mean	SE		Mean	SE		Mean	SE		Mean	SE		Mean	SE		*P*- value[2]
Hypochaeris radicata	Ht. (cm)	50 mm	2.1	0.212	Aac	1.7	0.165	Aab	1.6	0.083	Ab	2.2	0.134	Ac	2.1	0.121	Ac	1.1	0.292	Ad	0.001
		100 mm	2.5	0.274	Aa	2.3	2.907	Aa	5.8	3.423	ABa	10.3	3.475	Aa	4.1	1.119	Ba	1.5	0.201	Ab	0.021
		200 mm	1.9	0.131	Aa	1.9	0.185	Aa	7.4	2.479	Bb	6.4	1.863	Ab	3.3	0.235	Bb	1.8	0.266	Aa	0.001
		P-value[1]			0.253			0.277			0.005			0.056			0.005			0.079	
	Co. (%)	50 mm	0.63	0.054	Aa	1.11	0.105	Ab	1.32	0.089	Ab	1.33	0.100	Ab	1.26	0.068	Ab	0.36	0.074	Ac	0.001
		100 mm	0.77	0.071	Aa	2.81	0.291	Bb	3.58	0.551	Bb	3.18	0.567	Bb	3.10	0.709	Bb	0.44	0.146	Ac	0.001
		200 mm	0.79	0.090	Aa	3.82	0.382	Bb	5.20	0.941	Bb	5.49	0.796	Cb	4.48	1.094	Bb	0.28	0.077	Ac	0.001
		P-value[1]			0.436			0.001			0.001			0.001			0.001			0.760	
Knautia arvensis	Ht. (cm)	50 mm	2.5	0.278	Aa	2.6	0.463	Aabc	2.4	0.469	Aabc	1.9	0.344	Ab	1.7	0.198	Ab	3.3	0.286	Ac	0.012
		100 mm	2.1	0.249	Aa	2.9	0.538	Aab	3.6	0.507	ABb	2.9	0.529	ABab	2.6	0.341	Bab	5.3	0.500	Bc	0.001
		200 mm	2.1	0.264	Aa	3.3	0.312	Abc	3.9	0.355	Bb	3.1	0.352	Bbc	2.8	0.332	Bac	6.2	0.495	Bd	0.001
		P-value[1]			0.399			0.312			0.048			0.037			0.018			0.001	
	Co. (%)	50 mm	1.31	0.135	Aa	1.42	0.185	Aab	1.79	0.161	Ab	1.98	0.206	Ab	1.93	0.238	Ab	0.42	0.104	Ac	0.001
		100 mm	1.32	0.161	Aa	2.52	0.204	Bb	3.34	0.395	Bbc	3.69	0.483	Bbc	4.19	0.484	Bc	0.55	0.138	Ad	0.001
		200 mm	1.41	0.154	Aa	3.49	0.305	Cb	4.28	0.360	Bbc	4.86	0.396	Bc	4.82	0.319	Bc	1.30	0.270	Ba	0.001
		P-value[1]			0.971			0.001			0.001			0.001			0.001			0.011	
Leontodon autumnalis	Ht. (cm)	50 mm	3.0	0.241	Aa	2.3	0.227	Aa	2.5	0.310	Aa	2.7	0.245	Aa	2.3	0.212	Aa	2.4	0.529	Aa	0.170
		100 mm	2.6	0.345	Aa	2.9	0.211	Aa	9.7	3.165	Ba	5.1	1.590	ABa	3.9	0.360	Ba	3.8	0.411	Ba	0.069
		200 mm	2.6	0.327	Aa	2.8	0.566	Aa	18.2	0.761	Bb	6.0	1.303	Bc	6.6	1.628	Bc	6.0	1.176	Bac	0.001
		P-value[1]			0.493			0.126			0.001			0.014			0.002			0.031	
	Co. (%)	50 mm	0.92	0.048	Aa	1.26	0.060	Ab	1.69	0.096	Ac	2.33	0.188	Ad	2.93	0.409	Ad	0.32	0.134	Ae	0.001
		100 mm	1.18	0.170	Aa	3.07	0.267	Bb	3.79	0.388	Bbc	4.57	0.463	Bcd	5.64	0.413	Bd	0.40	0.087	Ae	0.001
		200 mm	1.13	0.111	Aa	4.59	0.311	Cb	4.83	0.373	Bbc	5.94	0.517	Bc	6.86	0.849	Bc	0.88	0.214	Ad	0.001
		P-value[1]			0.518			0.001			0.001			0.001			0.002			0.154	

[1]Significant differences at p =0.05 between substrate depths for the same watering treatment (Kruskal-Wallis test). [2] Significant differences at p = 0.05 between months for the same depth (Kruskal-Wallis test). Different capital letters indicate significant difference between substrate depths for the same month. Different lower-case letters indicate significant difference between months for the same depth.
Ht.: Height, Co.: Coverage

APPENDIX 11.2. Mean height (cm) and coverage (%) of individual species in response to substrate depths in watering treatment over the 2-year period of the experiment.

Watering regime			2007 Jun.			Jul.			Aug.			Sep.			Oct.			2008 Apr.			
			Mean	SE		Mean	SE		Mean	SE		Mean	SE		Mean	SE		Mean	SE		*P*- value[2]
Leucanthemum vulgare	Ht. (cm)	50 mm	4.5	0.407	Aa	4.5	0.292	Aa	3.5	0.233	Aa	3.9	0.277	Aa	3.5	0.183	Aa	3.2	0.522	Aa	0.053
		100 mm	4.5	0.361	Aa	6.8	0.460	Bbc	8.1	2.448	Bbc	6.3	0.551	Bb	6.3	0.675	Bbc	7.8	0.899	Bc	0.005
		200 mm	4.9	0.305	Aa	6.8	0.408	Bb	7.2	1.016	Bb	7.7	0.360	Cb	8.0	0.615	Cb	12.5	1.446	Cc	0.001
		P-value[1]			0.805			0.001			0.001			0.001			0.001			0.001	
	Co. (%)	50 mm	2.21	0.455	Aa	2.90	0.431	Aab	3.27	0.489	Aab	3.70	0.437	Ab	3.59	0.335	Ab	0.57	0.187	Ac	0.001
		100 mm	2.72	0.403	Aa	6.40	0.501	Bb	8.06	0.689	Bb	7.87	0.622	Bb	7.81	0.812	Bb	2.14	0.413	Ba	0.001
		200 mm	2.99	0.300	Aa	9.10	0.958	Bb	9.99	0.871	Bb	10.11	0.841	Bb	10.34	0.753	Cb	4.88	0.544	Cc	0.001
		P-value[1]			0.298			0.001			0.001			0.001			0.001			0.001	
Linaria vulgaris	Ht. (cm)	50 mm	3.5	0.318	Aa	5.4	0.529	Abc	5.8	0.487	Abc	6.5	0.510	Ab	4.5	0.518	Aac	5.7	0.402	Ab	0.002
		100 mm	4.7	0.451	Aa	7.3	0.623	Ab	10.1	0.778	Bc	10.0	0.527	Bc	8.0	1.130	Bbc	9.4	0.360	Bc	0.001
		200 mm	4.1	0.328	Aa	6.6	0.508	Ab	9.7	0.949	Bc	9.6	0.806	Bc	9.2	0.973	Bbc	9.7	1.210	Bbc	0.001
		P-value[1]			0.129			0.096			0.001			0.004			0.005			0.001	
	Co. (%)	50 mm	0.25	0.031	Aa	0.93	0.141	Ab	1.06	0.154	Ab	1.68	0.201	Ac	2.16	0.309	Ac	0.12	0.030	Ad	0.001
		100 mm	0.28	0.036	Aa	3.03	0.443	Bb	3.62	0.550	Bb	3.64	0.507	Bb	5.11	0.845	Bb	0.24	0.096	Aa	0.001
		200 mm	0.37	0.050	Aa	3.66	0.311	Bb	4.87	0.926	Bbc	5.30	0.287	Ccd	6.43	0.407	Bd	1.08	0.284	Aa	0.001
		P-value[1]			0.172			0.001			0.001			0.001			0.001			0.076	
Lotus corniculatus	Ht. (cm)	50 mm	4.0	0.346	Aa	6.1	0.330	Ab	13.4	1.470	Ac	8.5	0.445	Ad	8.7	0.351	Ad	5.2	0.950	Aab	0.001
		100 mm	3.3	0.283	Aba	4.6	0.448	Bb	14.9	1.130	Ac	10.4	0.393	Bd	13.0	1.510	Bcd	7.4	1.690	ABabd	0.001
		200 mm	2.7	0.230	Ba	3.5	0.448	Bac	13.0	0.787	Ab	13.2	1.080	Cb	11.0	0.492	Bbc	12.6	1.120	Bbc	0.001
		P-value[1]			0.029			0.002			0.270			0.001			0.007			0.005	
	Co. (%)	50 mm	1.58	0.173	Aa	7.56	0.599	Ab	24.67	1.665	Ac	30.00	2.838	Ac	31.12	3.196	Ac	1.71	0.722	Aa	0.001
		100 mm	1.69	0.141	Aa	9.92	0.723	Ab	32.22	2.178	Bc	41.33	4.936	Ad	47.91	6.096	Bd	4.31	1.456	Aa	0.001
		200 mm	1.47	0.170	Aa	9.11	0.824	Ab	33.56	2.724	Bc	48.33	2.682	Bd	50.82	3.508	Bd	10.20	2.240	Bb	0.001
		P-value[1]			0.727			0.086			0.031			0.002			0.003			0.014	

[1]Significant differences at p =0.05 between substrate depths for the same watering treatment (Kruskal-Wallis test). [2] Significant differences at p = 0.05 between months for the same depth (Kruskal-Wallis test). Different capital letters indicate significant difference between substrate depths for the same month. Different lower-case letters indicate significant difference between months for the same depth.
Ht.: Height, Co.: Coverage

APPENDIX 11.2. Mean height (cm) and coverage (%) of individual species in response to substrate depths in watering treatment over the 2-year period of the experiment.

Watering regime			2007 Jun.			Jul.			Aug.			Sep.			Oct.			2008 Apr.			
			Mean	SE		Mean	SE		Mean	SE		Mean	SE		Mean	SE		Mean	SE		*P*- value[2]
Origanum vulgare	Ht. (cm)	50 mm	3.5	0.395	Aa	5.0	0.603	Ab	6.0	0.745	Ab	6.2	0.875	Ab	5.5	0.992	Aab	0.3	0.199	Ac	0.001
		100 mm	4.7	0.548	Aa	8.4	0.932	Bb	13.7	1.520	Bc	13.0	1.450	Bc	13.6	1.440	Bc	2.5	0.624	Bd	0.001
		200 mm	4.0	0.201	Aa	7.5	0.322	Bb	10.4	1.160	Bc	10.2	1.020	Bc	10.3	1.120	Bc	4.8	1.110	Ba	0.001
		P-value[1]			0.123			0.006			0.001			0.003			0.001			0.003	
	Co. (%)	50 mm	0.77	0.086	Aa	1.07	0.149	Aab	1.52	0.183	Ab	1.14	0.143	Aab	1.16	0.182	Aab	0.02	0.013	Ac	0.001
		100 mm	0.95	0.163	Aa	2.63	0.270	Bb	3.39	0.380	Bbc	3.46	0.315	Bc	3.61	0.275	Bc	0.20	0.097	Bd	0.001
		200 mm	0.73	0.041	Aa	2.41	0.239	Bb	3.26	0.302	Bbc	3.60	0.423	Bc	3.92	0.383	Bc	0.51	0.221	Ba	0.001
		P-value[1]			0.556			0.001			0.001			0.001			0.001			0.010	
Pilosella aurantiaca	Ht. (cm)	50 mm	1.6	0.093	Aa	3.7	0.147	Ab	4.0	0.337	Ab	3.0	0.536	Ab	5.8	2.440	Aab	3.6	0.462	Ab	0.002
		100 mm	1.9	0.107	Aa	6.2	0.444	Bbc	20.0	5.100	Bbc	7.7	1.460	Bb	10.3	3.490	Abc	5.2	0.385	Ac	0.001
		200 mm	1.5	0.155	Aa	6.0	0.251	Bb	25.8	3.960	Bc	5.5	0.200	Cb	6.8	2.030	Ab	4.8	0.624	Ab	0.001
		P-value[1]			0.051			0.001			0.001			0.001			0.110			0.054	
	Co. (%)	50 mm	0.72	0.064	Aa	1.26	0.094	Ab	2.12	0.209	Ac	2.73	0.228	Ad	3.32	0.277	Ad	0.09	0.014	Ae	0.001
		100 mm	0.88	0.087	Aa	3.38	0.464	Bb	5.28	0.651	Bc	6.04	0.795	Bc	6.84	0.878	Bc	0.22	0.042	Bd	0.001
		200 mm	0.87	0.055	Aa	3.72	0.316	Bb	4.96	0.485	Bbc	6.61	0.920	Bc	6.79	0.931	Bc	0.32	0.085	Bd	0.001
		P-value[1]			0.190			0.001			0.001			0.001			0.005			0.020	
Pilosella officinarum	Ht. (cm)	50 mm	1.2	0.120	Aa	1.4	0.148	Aac	2.5	0.671	Ab	1.8	0.189	Abc	2.1	0.510	Abc	0.7	0.343	Aa	0.007
		100 mm	1.4	0.097	Aa	1.8	0.285	Aa	7.3	2.360	Aa	1.9	0.386	Aa	1.6	0.155	Aa	1.0	0.330	Aa	0.062
		200 mm	1.1	0.066	Aa	2.1	0.327	Abd	8.5	1.970	Ac	1.5	0.155	Ab	1.6	0.126	Abd	2.1	0.231	Bd	0.001
		P-value[1]			0.149			0.130			0.186			0.593			0.952			0.015	
	Co. (%)	50 mm	0.73	0.063	Aa	1.22	0.062	Ab	1.51	0.126	Abd	2.47	0.301	Ac	2.63	0.489	Acd	0.05	0.019	Ae	0.001
		100 mm	0.89	0.128	Aa	2.36	0.638	ABb	4.09	1.423	Bb	4.88	1.941	ABb	3.81	1.057	Ab	0.05	0.026	Ac	0.001
		200 mm	0.90	0.110	Aa	2.54	0.331	Bb	4.09	0.625	Bbc	5.34	0.934	Bc	5.43	1.019	Ac	0.20	0.041	Bd	0.001
		P-value[1]			0.365			0.012			0.007			0.030			0.081			0.012	

[1]Significant differences at p =0.05 between substrate depths for the same watering treatment (Kruskal-Wallis test). [2] Significant differences at p = 0.05 between months for the same depth (Kruskal-Wallis test). Different capital letters indicate significant difference between substrate depths for the same month. Different lower-case letters indicate significant difference between months for the same depth.
Ht.: Height, Co.: Coverage

APPENDIX 11.2. Mean height (cm) and coverage (%) of individual species in response to substrate depths in watering treatment over the 2-year period of the experiment.

Watering regime			2007 Jun.			Jul.			Aug.			Sep.			Oct.			2008 Apr.			
			Mean	SE		Mean	SE		Mean	SE		Mean	SE		Mean	SE		Mean	SE		*P*- value[2]
Primula veris	Ht. (cm)	50 mm	1.8	0.073	Aa	1.7	0.133	Aab	1.6	0.108	Aabc	1.4	0.108	Abc	1.2	0.156	Ac	0.4	0.183	Ad	0.001
		100 mm	1.8	0.125	Aa	2.2	0.205	Aa	1.4	0.415	Aab	1.1	0.180	Ab	1.1	0.151	Ab	2.1	0.675	Bab	0.019
		200 mm	1.4	0.091	Ba	2.1	0.158	Ab	1.3	0.086	Aa	0.9	0.133	Ac	1.0	0.176	Aac	1.4	0.376	Babc	0.001
		P-value[1]			0.012			0.068			0.319			0.068			0.689			0.023	
	Co. (%)	50 mm	0.50	0.027	Aa	0.63	0.078	Aa	0.63	0.140	Aab	0.42	0.068	Aab	0.30	0.063	Ab	0.02	0.010	Ac	0.001
		100 mm	0.45	0.038	Aa	1.13	0.093	Bb	1.04	0.262	Aab	0.68	0.241	Aa	0.49	0.128	Aa	0.06	0.024	Ac	0.001
		200 mm	0.34	0.050	Aa	0.90	0.071	Bb	0.71	0.124	Ab	0.37	0.069	Aa	0.30	0.064	Aa	0.04	0.012	Ac	0.001
		P-value[1]			0.074			0.002			0.409			0.835			0.537			0.161	
Scabiosa columbaria	Ht. (cm)	50 mm	1.2	0.079	Aab	1.0	0.048	Aa	1.2	0.204	Aab	1.8	0.531	Abc	1.7	0.192	Acd	1.9	0.145	Ad	0.001
		100 mm	2.0	0.331	Aab	1.4	0.165	Ba	3.2	0.688	Bbc	14.8	5.960	Bbc	13.7	5.640	Abc	3.8	0.436	Bc	0.008
		200 mm	1.3	0.132	Aa	1.6	0.223	Bab	3.3	1.450	Bbc	5.3	3.020	Bbc	6.7	3.300	Abc	4.3	0.813	Bc	0.003
		P-value[1]			0.071			0.023			0.014			0.042			0.129			0.007	
	Co. (%)	50 mm	0.84	0.048	Aa	1.03	0.071	Aab	1.13	0.088	Ab	1.26	0.145	Ab	1.54	0.316	Ab	0.12	0.032	Ac	0.001
		100 mm	1.21	0.119	Ba	2.03	0.205	Bb	2.44	0.328	Bbc	3.04	0.409	Bc	4.69	1.024	Bbc	0.52	0.119	Bd	0.001
		200 mm	0.95	0.089	ABa	1.78	0.113	Bb	1.82	0.267	Bb	3.24	0.879	Bb	3.33	0.674	Bb	0.63	0.169	Ba	0.001
		P-value[1]			0.043			0.001			0.002			0.003			0.009			0.016	

[1]Significant differences at p =0.05 between substrate depths for the same watering treatment (Kruskal-Wallis test). [2] Significant differences at p = 0.05 between months for the same depth (Kruskal-Wallis test). Different capital letters indicate significant difference between substrate depths for the same month. Different lower-case letters indicate significant difference between months for the same depth.
Ht.: Height, Co.: Coverage

APPENDIX 12. Mean height (cm) and coverage (%) of individual species in response to substrate types over the 2-year period of the experiment.

			2007														2008							
			Jun.			Jul.			Aug.			Sep.			Oct.			Apr.					Mean value across the period	SE	
			Mean	SE		Mean	SE		Mean	SE		Mean	SE		Mean	SE		Mean	SE		*P*-value[2]				
Achillea	Ht. (cm)	Zinco	3.9	0.29	Aa	4.6	0.34	Aa	5.8	0.41	Ab	7.9	0.61	Ac	6.3	0.46	Abc	6.8	0.53	Abc	<0.001		5.9	0.25	A
millefolium		Lime	3.4	0.32	Aa	4.5	0.28	Ab	7.1	0.43	Ac	8.7	0.58	Ad	7.5	0.50	Acd	7.8	0.76	Acd	<0.001		6.5	0.33	A
		P-value[1]			0.4788			0.5357			0.0694			0.3314			0.1015			0.3760		*P*-value[3]			0.1557
	Co. (%)	Zinco	0.65	0.11	Aa	2.98	0.58	Ab	3.97	0.41	Abc	4.50	0.63	Abcd	5.74	0.87	Acd	7.04	1.02	Ad	<0.001		4.14	0.38	A
		Lime	0.50	0.08	Aa	6.80	0.70	Bb	6.76	0.74	Bb	9.12	0.65	Bc	10.5	1.13	Bc	13.7	2.05	Bc	<0.001		7.89	0.70	B
		P-value[1]			0.3314			0.0062			0.003			<0.001			<0.001			0.0273		*P*-value[3]			< 0.001
Agrimonia	Ht. (cm)	Zinco	5.6	0.56	Aa	4.2	0.31	Ab	3.7	0.27	Ab	7.3	2.39	Aab	7.3	2.42	Aab	2.1	0.25	Ac	<0.001		5.0	0.61	A
eupatoria		Lime	5.9	0.77	Aa	5.0	0.64	Aa	5.8	1.00	Aa	12.2	3.81	Aa	11.7	3.55	Aa	2.6	0.15	Ab	0.001		7.2	0.98	B
		P-value[1]			0.9647			0.479			0.1575			0.1116			0.2002			0.2147		*P*-value[3]			0.0291
	Co. (%)	Zinco	1.54	0.28	Aa	2.22	0.31	Aa	2.23	0.24	Aa	1.90	0.28	Aa	1.85	0.33	Aa	0.38	0.14	Ab	<0.001		1.69	0.14	A
		Lime	1.42	0.22	Aa	2.65	0.44	Ab	2.69	0.47	Ab	2.88	0.43	Ab	2.99	0.61	Ab	0.52	0.11	Ac	<0.001		2.19	0.20	A
		P-value[1]			0.9296			0.5962			0.6577			0.1022			0.1221			0.2692		*P*-value[3]			0.1306
Campanula	Ht. (cm)	Zinco	0.9	0.09	Aa	1.0	0.07	Aac	1.1	0.13	Aac	1.9	0.47	Ab	1.4	0.19	Abc	2.1	0.37	Ab	0.002		1.4	0.12	A
glomerata		Lime	0.8	0.08	Aa	1.2	0.19	Aa	2.0	0.35	Bb	3.2	0.55	Bc	2.7	0.51	Bbc	3.7	0.67	Bc	<0.001		2.3	0.22	B
		P-value[1]			0.7533			0.3981			0.0094			0.0117			0.0297			0.0267		*P*-value[3]			0.0014
	Co. (%)	Zinco	0.31	0.05	Aa	0.50	0.09	Aa	0.50	0.09	Aa	0.61	0.10	Aa	0.67	0.13	Aa	0.36	0.14	Aa	0.088		0.49	0.04	A
		Lime	0.37	0.05	Aa	1.50	0.30	Bb	1.46	0.29	Bb	2.02	0.34	Bb	1.92	0.24	Bb	1.31	0.34	Bb	<0.001		1.43	0.13	B
		P-value[1]			0.5069			0.0041			0.0041			0.0008			0.0011			0.0215		*P*-value[3]			< 0.001
Clinopodium	Ht. (cm)	Zinco	3.5	0.40	Aa	5.5	0.75	Ab	7.3	0.97	Abc	9.9	1.18	Ac	9.1	1.39	Ac	3.2	0.34	Aa	<0.001		6.4	0.50	A
vulgare		Lime	2.5	0.33	Aa	5.9	0.34	Ab	12.8	0.96	Bc	17.0	1.07	Bd	17.5	1.27	Bd	3.8	0.74	Aa	<0.001		9.9	0.90	B
		P-value[1]			0.1219			0.5361			0.0041			0.0015			0.0017			0.7904		*P*-value[3]			0.0140
	Co. (%)	Zinco	0.54	0.07	Aa	1.05	0.17	Ab	1.45	0.25	Ab	1.67	0.26	Ab	1.67	0.27	Ab	1.30	0.49	Aab	0.034		1.28	0.13	A
		Lime	0.32	0.03	Ba	4.22	0.57	Bb	4.24	0.56	Bb	6.63	1.00	Bc	6.12	0.79	Bc	1.85	0.22	Aa	<0.001		3.76	0.40	B
		P-value[1]			0.0189			<0.001			<0.001			<0.001			<0.001			0.9349		*P*-value[3]			< 0.001

[1]Significant differences at p =0.05 between substrate types for the same month (Mann-Whitney *U*-test). [2]Significant differences at p =0.05 between months for the same type (Kruskal-Wallis test). [3]Significant differences in mean value across the period at p = 0.05 between substrate types (Mann-Whitney U-test). Different capital letters indicate significant difference between substrate types for the same month and in mean value across the period. Different lower-case indicate significant difference between months for the same type. Ht.: Height, Co.: Coverage, Zinco: Zinco substrate treatment; Lime: Limestone-based substrate treatment

(*Continued on next page.*)

APPENDIX 12. Mean height (cm) and coverage (%) of individual species in response to substrate types over the 2-year period of the experiment.

			2007														2008							
			Jun.			Jul.			Aug.			Sep.			Oct.			Apr.					Mean value across the period	SE	
			Mean	SE		Mean	SE		Mean	SE		Mean	SE		Mean	SE		Mean	SE		*P*-value[2]				
Galium	Ht. (cm)	Zinco	3.5	0.18	Aa	4.3	0.32	Ab	5.5	0.40	Abc	5.9	0.48	Acd	5.0	0.42	Abc	7.2	0.51	Ad	<0.001		5.2	0.23	A
verum		Lime	3.2	0.22	Aa	4.8	0.41	Ab	7.8	0.56	Bc	8.7	0.65	Bc	7.7	0.88	Bc	8.5	0.94	Ac	<0.001		6.8	0.38	B
		P-value[1]			0.3979			0.5953			0.0047			0.0068			0.0192			0.232		*P*-value[3]			0.0041
	Co. (%)	Zinco	0.57	0.06	Aa	1.38	0.13	Ab	2.06	0.22	Ac	2.20	0.27	Ac	2.68	0.24	Ac	1.44	0.13	Ab	<0.001		1.72	0.12	A
		Lime	0.57	0.07	Aa	5.80	0.64	Bb	5.70	0.67	Bb	7.75	1.00	Bbc	8.95	0.89	Bc	2.66	0.20	Bd	<0.001		5.24	0.47	B
		P-value[1]			0.79			<0.001			<0.001			<0.001			<0.001			0.0011		*P*-value[3]			< 0.001
Helianthemum	Ht. (cm)	Zinco	2.5	0.28	Aa	1.8	0.40	Ab	2.2	0.38	Aab	2.2	0.41	Aab	2.3	0.32	Aa	0.0	0.00	—	<0.001		1.8	0.17	A
nummularium		Lime	1.8	0.36	Aa	1.6	0.26	Aa	1.9	0.25	Aab	2.3	0.39	Aab	3.6	0.90	Ab	0.5	0.26	— c	0.001		2.0	0.22	A
		P-value[1]			0.063			0.4515			0.722			0.5949			0.3306					*P*-value[3]			0.7982
	Co. (%)	Zinco	0.65	0.08	Aa	1.69	0.32	Ab	2.94	0.47	Ac	6.48	2.34	Acd	6.12	1.01	Ad	0.00	0.00	—	<0.001		2.98	0.54	A
		Lime	0.42	0.09	Aa	2.08	0.31	Ab	2.14	0.37	Ab	3.90	0.42	Ac	5.82	0.65	Ad	0.28	0.13	— a	<0.001		2.44	0.30	A
		P-value[1]			0.0572			0.2881			0.2508			0.6588			0.9648			—		*P*-value[3]			1.0000
Hypochaeris	Ht. (cm)	Zinco	3.5	0.89	Aa	8.7	3.20	Aa	4.7	2.36	Aa	3.0	0.36	Aa	2.9	0.27	Aa	1.6	0.28	Ab	0.019		4.1	0.72	A
radicata		Lime	1.9	0.23	Ba	9.9	2.94	Ab	14.5	4.54	Bb	23.1	4.53	Bbc	22.7	3.12	Bc	1.8	0.22	Aa	<0.001		12.3	1.71	B
		P-value[1]			0.0187			0.3536			0.0416			0.0088			0.0027			0.3066		*P*-value[3]			0.0095
	Co. (%)	Zinco	1.39	0.19	Aa	3.01	0.53	Abc	2.98	0.58	Abc	3.28	0.36	Ac	3.06	0.23	Ac	1.82	0.43	Aab	0.003		2.51	0.19	A
		Lime	1.20	0.15	Aa	6.83	0.92	Bb	7.15	1.11	Bb	9.08	1.15	Bb	6.93	1.42	Ab	1.21	0.41	Aa	<0.001		4.98	0.54	B
		P-value[1]			0.5962			0.010			0.0115			0.0012			0.0766			0.3836		*P*-value[3]			0.0260
Knautia	Ht. (cm)	Zinco	2.2	0.25	Aa	2.7	0.29	Aa	2.8	0.14	Aa	2.8	0.37	Aa	2.5	0.33	Aa	4.7	0.60	Ab	0.019		3.0	0.18	A
arvensis		Lime	2.3	0.36	Aa	2.5	0.36	Aa	3.9	0.93	Aab	5.0	1.25	Ab	8.8	4.53	Bb	8.6	0.92	Bc	<0.001		5.2	0.86	B
		P-value[1]			0.9295			0.535			0.3998			0.0929			0.0027			0.0047		*P*-value[3]			0.0084
	Co. (%)	Zinco	0.99	0.18	Aa	2.18	0.31	Ab	2.77	0.32	Ab	2.91	0.34	Ab	3.40	0.45	Ab	2.22	0.55	Aab	0.002		2.41	0.18	A
		Lime	1.07	0.14	Aa	6.12	0.81	Bbd	6.47	0.83	Bbcd	8.70	1.23	Bbc	9.29	1.12	Bc	4.29	0.97	Ad	0.001		5.96	0.52	B
		P-value[1]			0.9648			<0.001			0.0015			<0.001			<0.001			0.2057		*P*-value[3]			< 0.001

[1]Significant differences at p =0.05 between substrate types for the same month (Mann-Whitney *U*-test). [2]Significant differences at p =0.05 between months for the same type (Kruskal-Wallis test). [3]Significant differences in mean value across the period at p = 0.05 between substrate types (Mann-Whitney U-test). Different capital letters indicate significant difference between substrate types for the same month and in mean value across the period. Different lower-case indicate significant difference between months for the same type. Ht.: Height, Co.: Coverage, Zinco: Zinco substrate treatment; Lime: Limestone-based substrate treatment

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APPENDIX 12. Mean height (cm) and coverage (%) of individual species in response to substrate types over the 2-year period of the experiment.

			2007														2008								
			Jun.			Jul.			Aug.			Sep.			Oct.			Apr.			*P*-value[2]		Mean value across the period	SE	
			Mean	SE		Mean	SE		Mean	SE		Mean	SE		Mean	SE		Mean	SE						
Leontodon	Ht. (cm)	Zinco	2.9	0.41	Aa	3.0	0.46	Aa	**10.7**	4.15	Aa	8.6	2.51	Aa	2.6	0.27	Aa	3.1	0.82	Aa	0.089		5.1	0.90	A
autumnalis		Lime	3.4	0.28	Aac	2.8	0.39	Aa	6.4	2.08	Bac	19.0	3.10	Bb	**19.9**	1.31	Bb	5.8	0.98	Bc	<0.001		9.6	1.18	B
		P-value[1]			0.1566			0.9294			0.0216			0.0216			<0.001			0.0339	*P*-value[3]			0.0001
	Co. (%)	Zinco	1.23	0.16	Aa	2.50	0.41	Ab	2.92	0.40	Abc	3.44	0.33	Abc	7.22	2.82	Ac	1.10	0.18	Aa	<0.001		3.07	0.54	A
		Lime	1.01	0.12	Aa	6.43	0.98	Bb	6.88	0.74	Bb	10.7	1.20	Bc	10.1	1.01	Bc	2.19	0.52	Aa	<0.001		6.07	0.59	B
		P-value[1]			0.3536			0.0023			0.0017			0.004			0.0134			0.2384	*P*-value[3]			< 0.001
Leucanthemum	Ht. (cm)	Zinco	3.7	0.28	Aa	5.1	0.51	Aab	4.8	0.35	Ab	5.7	0.32	Ab	5.5	0.43	Ab	6.3	0.90	Ab	0.021		5.2	0.23	A
vulgare		Lime	3.9	0.28	Aa	5.8	0.31	Ab	9.8	0.89	Bc	18.4	4.36	Bd	17.6	4.52	Bcd	8.7	1.63	Abcd	<0.001		10.7	1.29	B
		P-value[1]			0.5955			0.4011			<0.001			<0.001			<0.001			0.2161	*P*-value[3]			< 0.0001
	Co. (%)	Zinco	2.30	0.18	Aa	4.26	0.53	Abc	4.77	0.55	Ac	5.41	0.59	Abc	5.61	0.47	Abc	2.82	0.42	Aab	<0.001		4.06	0.25	A
		Lime	1.87	0.19	Aa	12.2	1.14	Bbc	11.5	0.81	Bb	15.8	1.86	Bc	13.7	1.46	Bbc	4.58	1.25	Aa	<0.001		9.65	0.83	B
		P-value[1]			0.1221			<0.001			<0.001			<0.001			0.0011			0.5418	*P*-value[3]			< 0.001
Linaria	Ht. (cm)	Zinco	3.9	0.32	Aa	6.3	0.91	Ab	8.1	1.33	Ab	9.1	1.10	Ab	9.5	1.22	Ab	8.8	0.90	Ab	0.002		7.6	0.48	A
vulgaris		Lime	3.6	0.40	Aa	6.0	0.65	Ab	12.5	1.78	Acd	19.8	3.62	Bc	18.8	3.19	Bc	9.7	0.47	Ad	<0.001		11.7	1.17	B
		P-value[1]			0.3757			0.6582			0.0849			0.0151			0.0468			0.4788	*P*-value[3]			0.0320
	Co. (%)	Zinco	0.38	0.05	Aa	2.52	0.42	Abc	3.02	0.54	Abc	3.26	0.61	Abc	3.84	0.62	Ab	2.07	0.35	Ac	<0.001		2.52	0.24	A
		Lime	0.18	0.02	Ba	5.56	0.87	Bb	5.40	0.77	Bb	7.66	1.22	Bbc	9.02	1.32	Bc	7.38	2.78	Abc	<0.001		5.87	0.68	B
		P-value[1]			0.0012			0.0104			0.0468			0.0171			0.0081			0.0774	*P*-value[3]			< 0.001
Lotus	Ht. (cm)	Zinco	3.6	0.26	Aa	7.3	0.80	Ab	14.2	1.43	Ac	11.9	1.06	Ac	11.1	0.77	Ac	12.6	0.39	Ac	<0.001		10.1	0.60	A
corniculatus		Lime	3.4	0.33	Aa	6.1	0.67	Ab	13.5	2.00	Ac	11.0	0.66	Ac	12.6	0.87	Ac	10.9	0.77	Ac	<0.001		9.6	0.64	A
		P-value[1]			0.7234			0.3526			0.8598			0.7232			0.4011			0.1439	*P*-value[3]			0.3828
	Co. (%)	Zinco	1.92	0.32	Aa	7.37	1.71	Ab	33.9	5.78	Ac	41.5	6.42	Ac	39.4	6.51	Ac	11.0	2.12	Ab	<0.001		21.54	2.65	A
		Lime	1.55	0.19	Aa	27.2	3.06	Bb	30.1	3.27	Abc	40.9	4.69	Abc	37.5	2.93	Ac	7.35	2.10	Aa	<0.001		22.44	2.24	A
		P-value[1]			0.3314			0.0062			0.003			<0.001			<0.001			0.0273	*P*-value[3]			0.6630

[1]Significant differences at p =0.05 between substrate types for the same month (Mann-Whitney *U*-test). [2]Significant differences at p =0.05 between months for the same type (Kruskal-Wallis test). [3]Significant differences in mean value across the period at p = 0.05 between substrate types (Mann-Whitney U-test). Different capital letters indicate significant difference between substrate types for the same month and in mean value across the period. Different lower-case indicate significant difference between months for the same type. Ht.: Height, Co.: Coverage, Zinco: Zinco substrate treatment; Lime: Limestone-based substrate treatment

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APPENDIX 12. Mean height (cm) and coverage (%) of individual species in response to substrate types over the 2-year period of the experiment.

			2007															2008							
			Jun.			Jul.			Aug.			Sep.			Oct.			Apr.			*P*-value[2]		Mean value across the period	SE	
			Mean	SE		Mean	SE		Mean	SE		Mean	SE		Mean	SE		Mean	SE						
Origanum vulgare	Ht. (cm)	Zinco	3.8	0.25	Aa	7.6	0.78	Ab	8.6	1.56	Ab	10.0	2.03	Aab	9.5	1.91	Aab	1.7	0.54	Ac	<0.001		6.9	0.68	A
		Lime	3.3	0.37	Aa	5.6	0.61	Ab	9.1	1.16	Acd	13.8	2.93	Ad	14.0	2.96	Ad	4.4	1.36	Aabc	0.001		8.4	0.94	A
		P-value[1]			0.2157			0.0243			0.9648			0.3096			0.233			0.2247		*P*-value[3]			0.3963
	Co. (%)	Zinco	0.80	0.05	Aa	1.90	0.30	Ab	2.10	0.41	Aab	1.98	0.47	Aab	1.97	0.47	Aab	0.21	0.09	Ac	0.001		1.50	0.17	A
		Lime	0.59	0.04	Ba	3.60	0.80	Abc	3.49	0.90	Abc	4.56	1.20	Ac	4.32	1.17	Abc	1.44	0.54	Aab	0.022		3.00	0.39	B
		P-value[1]			0.0061			0.1575			0.3314			0.1447			0.1449			0.1274		*P*-value[3]			0.0297
Pilosella aurantiaca	Ht. (cm)	Zinco	2.0	0.12	Aa	6.8	0.41	Ab	17.1	4.47	Ab	11.9	3.61	Ab	6.0	0.33	Abc	5.0	0.44	Ac	<0.001		8.1	1.14	A
		Lime	1.5	0.08	Ba	5.1	0.61	Bb	10.2	0.86	Ac	21.4	3.85	Bd	9.8	1.38	Bc	6.6	0.41	Be	<0.001		9.1	1.09	A
		P-value[1]			0.0051			0.0338			0.7238			0.034			0.0011			0.0377		*P*-value[3]			0.0613
	Co. (%)	Zinco	0.90	0.06	Aa	2.82	0.27	Ab	4.31	0.44	Ac	5.10	0.63	Ac	5.27	0.86	Ac	0.69	0.23	Ad	<0.001		3.01	0.31	A
		Lime	0.56	0.05	Ba	8.67	0.90	Bb	9.07	0.89	Bb	13.6	1.15	Bc	13.4	0.96	Bc	1.94	0.31	Bd	<0.001		7.88	0.76	B
		P-value[1]			0.0017			<0.001			0.0011			<0.001			<0.001			0.0041		*P*-value[3]			< 0.001
Pilosella officinarum	Ht. (cm)	Zinco	1.2	0.07	Aa	1.4	0.15	Aa	2.1	0.68	Aa	2.1	0.71	Aa	1.3	0.13	Aa	1.1	0.23	Aa	0.522		1.5	0.17	A
		Lime	1.2	0.09	Aa	1.8	0.07	Bb	3.3	0.21	Bc	11.0	2.38	Bd	2.6	0.32	Bbc	1.6	0.44	Aab	<0.001		3.6	0.61	A
		P-value[1]			0.9283			0.0454			<0.001			0.0068			0.004			0.5615		*P*-value[3]			< 0.001
	Co. (%)	Zinco	0.60	0.07	Aa	1.44	0.13	Ab	2.17	0.22	Ac	2.97	0.38	Ac	2.48	0.44	Abc	0.16	0.05	Ad	<0.001		1.63	0.17	A
		Lime	0.75	0.13	Aa	5.34	0.76	Bb	5.78	1.03	Bb	8.94	1.50	Bc	10.2	1.97	Bc	0.43	0.17	Aa	<0.001		5.24	0.67	B
		P-value[1]			0.4797			<0.001			<0.001			<0.001			<0.001			0.1922		*P*-value[3]			< 0.001

[1]Significant differences at p =0.05 between substrate types for the same month (Mann-Whitney *U*-test). [2]Significant differences at p =0.05 between months for the same type (Kruskal-Wallis test). [3]Significant differences in mean value across the period at p = 0.05 between substrate types (Mann-Whitney U-test). Different capital letters indicate significant difference between substrate types for the same month and in mean value across the period. Different lower-case indicate significant difference between months for the same type. Ht.: Height, Co.: Coverage, Zinco: Zinco substrate treatment; Lime: Limestone-based substrate treatment

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APPENDIX 12. Mean height (cm) and coverage (%) of individual species in response to substrate types over the 2-year period of the experiment.

			2007															2008							
			Jun.			Jul.			Aug.			Sep.			Oct.			Apr.					Mean value across the period		
			Mean	SE		Mean	SE		Mean	SE		Mean	SE		Mean	SE		Mean	SE		*P*- value[2]			SE	
Primula	Ht. (cm)	Zinco	1.8	0.10	Aa	2.2	0.24	Aa	1.6	0.16	Aac	1.0	0.15	Ab	1.3	0.12	Abc	0.3	0.16	Ad	0.001		1.4	0.10	A
veris		Lime	1.5	0.13	Aa	1.6	0.16	Aa	2.0	0.36	Aac	1.7	0.16	Ba	1.3	0.11	Aa	2.5	1.36	Aa	<0.001		1.8	0.23	A
		P-value[1]			0.130			0.1011			0.3074			0.0083			0.9644			0.053		*P*-value[3]			0.1326
	Co. (%)	Zinco	0.45	0.04	Aa	0.94	0.10	Ab	0.54	0.12	Aad	0.16	0.07	Ace	0.28	0.05	Acd	0.03	0.01	Ae	0.001		0.40	0.05	A
		Lime	0.43	0.05	Aac	0.80	0.15	Aab	0.76	0.13	Ab	0.88	0.16	Bb	0.64	0.16	Aab	0.22	0.09	Ac	<0.001		0.62	0.06	B
		P-value[1]			0.790			0.4783			0.2689			0.0013			0.0772			0.0887		*P*-value[3]			0.0058
Scabiosa	Ht. (cm)	Zinco	1.4	0.13	Aa	1.3	0.16	Aa	1.8	0.65	Aa	6.1	4.25	Aab	5.9	3.96	Aab	3.3	0.33	Ab	0.006		3.3	0.97	A
columbaria		Lime	2.5	1.31	Aab	1.3	0.10	Aa	2.5	0.52	Ab	6.6	1.94	Bc	16.9	4.38	Bd	4.0	0.51	Ac	<0.001		5.6	1.08	B
		P-value[1]			0.6212			0.9645			0.0562			0.0419			0.008			0.2323		*P*-value[3]			0.0040
	Co. (%)	Zinco	0.79	0.09	Aa	1.51	0.16	Ab	1.92	0.19	Ab	2.80	0.29	Ac	3.17	0.41	Ac	0.92	0.13	Aa	<0.001		1.85	0.15	A
		Lime	0.90	0.12	Aa	4.05	0.53	Bb	4.17	0.55	Bb	6.61	1.04	Bc	8.43	1.03	Bc	1.43	0.26	Aa	<0.001		4.27	0.45	B
		P-value[1]			0.5065			<0.001			0.0014			0.0035			0.0014			0.1221		*P*-value[3]			< 0.001

[1]Significant differences at p =0.05 between substrate types for the same month (Mann-Whitney *U*-test). [2]Significant differences at p =0.05 between months for the same type (Kruskal-Wallis test). [3]Significant differences in mean value across the period at p = 0.05 between substrate types (Mann-Whitney U-test). Different capital letters indicate significant difference between substrate types for the same month and in mean value across the period. Different lower-case indicate significant difference between months for the same type. Ht.: Height, Co.: Coverage, Zinco: Zinco substrate treatment; Lime: Limestone-based substrate treatment

APPENDIX 13. Mean height (cm) and coverage (%) of individual species in response to fertiliser treatments over the 2-year period of the experiment.

			2007														2008							
			Jun.			Jul.			Aug.			Sep.			Oct.			Apr					Mean value across the period	SE	
			Mean	SE		Mean	SE		Mean	SE		Mean	SE		Mean	SE		Mean	SE		*P*- value[2]				
Achillea	Ht (cm)	NF	3.0	0.18	Aa	4.6	0.43	Abc	5.7	0.38	Ab	5.4	0.24	Ab	5.1	0.31	Ab	3.8	0.51	Ac	<0.001		4.6	0.19	A
millefolium		F	2.8	0.14	Aa	7.8	0.58	Bb	21.8	2.04	Bcd	30.7	3.21	Bc	29.3	3.28	Bc	18.6	1.48	Bd	<0.001		18.5	1.64	B
		P-value[1]			0.451			0.0012			0.004			0.004			0.004			0.004		*P*-value[3]			< 0.001
	Co (%)	NF	1.82	0.41	Aab	1.82	0.29	Aa	3.06	0.38	Ab	4.42	0.40	Ac	7.97	1.24	Ad	5.75	1.17	Acd	<0.001		4.14	0.42	A
		F	2.07	0.16	Aa	13.1	1.86	Bb	22.9	3.14	Bc	33.0	2.86	Bd	49.2	2.45	Be	30.0	1.11	Bd	<0.001		25.04	2.22	B
		P-value[1]			0.450			<0.001			<0.001			<0.001			<0.001			<0.001		*P*-value[3]			< 0.001
Agrimonia	Ht (cm)	NF	4.6	0.33	Aa	2.8	0.27	Ab	5.4	1.73	Aac	5.1	1.69	Aabc	2.5	0.60	Ab	1.8	0.18	Ac	0.002		3.7	0.44	A
eupatoria		F	5.2	0.79	Aa	4.1	0.45	Ba	22.8	3.77	Bb	27.4	4.73	Bb	25.3	4.54	Bb	5.2	0.94	Ba	<0.001		15.0	1.86	B
		P-value[1]			0.596			0.027			0.002			0.005			0.002			0.001		*P*-value[3]			< 0.001
	Co (%)	NF	1.99	0.22	Aa	1.76	0.16	Aa	1.76	0.27	Aa	1.72	0.25	Aa	1.84	0.31	Aa	2.07	0.35	Aa	0.919		1.86	0.10	A
		F	1.86	0.22	Aa	2.94	0.34	Bb	5.42	0.66	Bc	5.33	0.71	Bc	4.94	0.72	Bc	2.66	0.45	Aab	<0.001		3.86	0.29	B
		P-value[1]			0.720			0.010			<0.001			0.003			0.002			0.269		*P*-value[3]			< 0.001
Campanula	Ht (cm)	NF	0.9	0.08	Aa	1.1	0.09	Aac	1.5	0.23	Abc	1.3	0.16	Ab	0.8	0.18	Aab	1.8	0.22	Ac	<0.001		1.2	0.08	A
glomerata		F	1.0	0.07	Aa	1.7	0.20	Bb	4.6	0.74	Bc	4.5	0.89	Bc	3.6	0.87	Babc	6.8	1.24	Bc	<0.001		3.7	0.40	B
		P-value[1]			0.504			0.029			<0.001			0.001			0.033			0.008		*P*-value[3]			< 0.001
	Co (%)	NF	0.58	0.08	Aa	0.51	0.05	Aa	0.69	0.11	Aa	0.63	0.07	Aa	0.69	0.13	Aa	0.82	0.12	Aa	0.279		0.65	0.04	A
		F	0.91	0.09	Ba	0.88	0.18	Aa	2.70	0.63	Ba	3.88	1.17	Aa	4.71	1.60	Aa	4.20	1.34	Ba	0.172		2.88	0.45	B
		P-value[1]			0.006			0.071			0.008			0.051			0.145			0.042		*P*-value[3]			< 0.001
Clinopodium	Ht (cm)	NF	3.0	0.24	Aa	6.1	0.52	Ab	9.3	1.26	Ac	9.6	1.24	Ac	9.3	1.15	Ac	3.2	0.42	Ad	<0.001		6.7	0.52	A
vulgare		F	3.8	0.24	Aa	10.3	1.43	Bb	18.3	1.66	Bc	21.3	1.39	Bc	20.8	1.61	Bc	7.8	1.50	Bb	<0.001		13.7	1.08	B
		P-value[1]			0.076			0.030			0.003			<0.001			0.001			0.009		*P*-value[3]			< 0.001
	Co (%)	NF	1.45	0.15	Aa	1.47	0.19	Aa	2.22	0.34	Aa	2.07	0.22	Aa	2.64	0.43	Aa	3.14	0.78	Aa	0.353		2.28	0.23	A
		F	1.21	0.14	Aa	3.38	0.54	Bb	24.7	8.93	Bc	11.9	1.47	Bcd	15.8	2.38	Bcd	11.4	4.58	Aabd	<0.001		11.38	1.99	B
		P-value[1]			0.279			0.005			<0.001			<0.001			<0.001			0.430		*P*-value[3]			< 0.001

[1]Significant differences at p = 0.05 between substrate types for the same month (Mann-Whitney *U*-test). [2]Significant differences at p = 0.05 between months for the same fertiliser treatment (Kruskal-Wallis test). [3]Significant differences in mean value across the period at p = 0.05 between fertiliser treatments (Mann-Whitney U-test). Different capital letters indicate significant difference between fertiliser treatments for the same month and in mean value across the period. Different lower-case indicate significant difference between months for the same treatment.
Ht.: Height, Co.: Coverage, NF: Non-fertiliser treatment; F: Fertiliser treatment

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APPENDIX 13. Mean height (cm) and coverage (%) of individual species in response to fertiliser treatments over the 2-year period of the experiment.

			2007														2008						
			Jun.			Jul.			Aug.			Sep.			Oct.			Apr				Mean value across the period		
			Mean	SE		Mean	SE		Mean	SE		Mean	SE		Mean	SE		Mean	SE		*P*-value[2]				
Galium	Ht (cm)	NF	3.0	0.30	Aa	4.4	0.31	Ab	5.5	0.42	Ab	5.3	0.36	Ab	5.1	0.54	Ab	7.8	0.56	Ac	<0.001		5.2	0.26	A
verum		F	2.8	0.20	Aa	6.5	0.32	Bb	12.2	1.34	Bc	15.1	1.44	Bcd	15.0	1.32	Bcd	16.9	1.28	Bd	<0.001		11.4	0.82	B
		P-value[1]			0.596			0.003			<0.001			<0.001			<0.001			<0.001	*P*-value[3]			< 0.001
	Co (%)	NF	1.35	0.19	Aa	1.49	0.20	Aa	1.88	0.16	Aa	2.44	0.24	Ab	3.54	0.31	Ac	3.67	0.27	Ac	<0.001		2.39	0.16	A
		F	1.57	0.15	Aa	4.41	0.71	Ba	10.6	1.04	Bc	18.4	2.10	Bd	21.0	2.60	Bd	15.0	1.69	Bcd	<0.001		11.83	1.16	B
		P-value[1]			0.445			0.001			0.004			0.004			0.004			0.004	*P*-value[3]			< 0.001
Helianthemum	Ht (cm)	NF	1.8	0.13	Aa	1.4	0.10	Ab	1.6	0.14	Aab	1.6	0.13	Aab	1.5	0.21	Aab	0.6	0.21	Ac	0.003		1.4	0.08	A
nummularium		F	1.9	0.16	Aa	1.5	0.14	Aa	2.6	0.20	Bb	3.1	0.43	Bb	3.9	0.61	Bb	1.4	0.37	Aa	0.004		2.4	0.19	B
		P-value[1]			0.657			0.893			0.003			0.005			0.003			0.091	*P*-value[3]			< 0.001
	Co (%)	NF	1.11	0.27	Aad	1.56	0.22	Aa	2.53	0.41	Ab	3.64	0.61	Abc	8.43	2.27	Ac	0.57	0.24	Ad	<0.001		2.97	0.53	A
		F	0.89	0.13	Aa	1.60	0.18	Ab	4.06	0.55	Bc	7.46	1.21	Bd	8.47	1.62	Ad	1.75	0.72	Aab	<0.001		4.04	0.54	A
		P-value[1]			0.964			0.859			0.041			0.010			0.724			0.301	*P*-value[3]			0.1511
Hypochaeris	Ht (cm)	NF	2.6	0.55	Aab	1.7	0.15	Ab	4.7	1.92	Ac	6.9	2.71	Aac	6.6	3.39	Aac	2.3	0.17	Aab	0.011		4.1	0.81	A
radicata		F	4.3	1.87	Aab	7.6	1.64	Bbe	29.5	3.18	Bbd	28.4	2.96	Bc	18.8	4.41	Bcd	4.8	0.97	Aae	p<0.001		15.6	1.81	B
		P-value[1]			0.653			<0.001			<0.001			0.001			0.013			0.157	*P*-value[3]			< 0.001
	Co (%)	NF	3.06	0.32	Aa	2.99	0.38	Aa	3.32	0.27	Aa	4.42	0.56	Aab	5.64	0.95	Ab	7.05	1.40	Ab	0.004		4.75	0.46	A
		F	3.43	0.35	Aa	8.92	1.82	Bb	14.1	2.39	Bb	13.6	1.56	Bb	17.4	4.74	Bb	2.96	0.80	Ba	<0.001		9.36	1.12	B
		P-value[1]			0.503			0.003			<0.001			<0.001			0.010			0.010	*P*-value[3]			0.0016
Knautia	Ht (cm)	NF	2.7	0.45	Aab	3.2	0.45	Aac	3.3	0.46	Aac	2.6	0.28	Aab	2.0	0.24	Ab	4.7	0.51	Ac	0.006		3.1	0.20	A
arvensis		F	2.2	0.33	Aa	4.8	0.72	Ab	18.9	4.56	Bc	36.7	6.77	Bc	33.3	5.18	Bc	15.8	1.35	Bc	<0.001		18.6	2.36	B
		P-value[1]			0.377			0.215			<0.001			<0.001			<0.001			<0.001	*P*-value[3]			< 0.001
	Co (%)	NF	2.78	0.43	Aa	2.81	0.31	Aa	3.48	0.41	Aab	4.08	0.45	Aab	5.63	0.86	Ab	3.67	0.70	Aab	0.028		3.74	0.26	A
		F	2.74	0.35	Aa	7.71	0.92	Bb	16.3	2.40	Bc	23.3	1.96	Bde	29.2	3.19	Be	16.8	3.29	Babcd	<0.001		16.05	1.50	B
		P-value[1]			0.891			<0.001			<0.001			<0.001			<0.001			0.017	*P*-value[3]			< 0.001

[1]Significant differences at p = 0.05 between substrate types for the same month (Mann-Whitney *U*-test). [2]Significant differences at p = 0.05 between months for the same fertiliser treatment (Kruskal-Wallis test). [3]Significant differences in mean value across the period at p = 0.05 between fertiliser treatments (Mann-Whitney U-test). Different capital letters indicate significant difference between fertiliser treatments for the same month and in mean value across the period. Different lower-case indicate significant difference between months for the same treatment.
Ht.: Height, Co.: Coverage, NF: Non-fertiliser treatment; F: Fertiliser treatment

(*Continued on next page.*)

APPENDIX 13. Mean height (cm) and coverage (%) of individual species in response to fertiliser treatments over the 2-year period of the experiment.

			2007															2008								
			Jun.			Jul.			Aug.			Sep.			Oct.			Apr					Mean value across the period		
			Mean	SE		Mean	SE		Mean	SE		Mean	SE		Mean	SE		Mean	SE		*P*-value[2]				
Leontodon	Ht (cm)	NF	3.2	0.31	Aa	7.6	2.72	Aa	12.8	2.64	Aa	3.7	0.31	Aa	3.7	0.93	Aa	4.0	0.55	Aa	0.152		5.8	0.79	A
autumnalis		F	4.6	1.26	Aa	6.3	2.17	Aa	26.1	1.96	Bb	20.9	1.31	Bc	20.9	3.80	Bbc	9.2	0.97	Bd	<0.001		14.7	1.40	B
		P-value[1]			0.860			0.250			0.004			<0.001			0.001			0.002		*P*-value[3]			< 0.001
	Co (%)	NF	2.76	0.35	Aa	2.71	0.33	Aa	3.54	0.37	Aa	4.83	0.38	Ab	8.29	1.21	Ac	2.74	0.64	Aab	<0.001		4.01	0.36	A
		F	3.67	0.35	Aa	10.1	1.05	Ba	16.2	1.44	Ba	19.2	2.08	Bb	19.4	2.06	Bc	5.69	1.47	Aab	<0.001		11.81	1.02	B
		P-value[1]			0.056			<0.001			<0.001			<0.001			0.001			0.556		*P*-value[3]			< 0.001
Leucanthemum	Ht (cm)	NF	4.4	0.31	Aac	5.1	0.35	Aabc	6.4	0.48	Ab	5.8	0.45	Aab	5.0	0.44	Aac	4.1	0.36	Ac	0.008		5.1	0.19	A
vulgare		F	3.8	0.22	Aa	11.9	0.53	Bb	24.5	1.89	Bc	31.6	2.81	Bc	30.6	2.35	Bc	17.6	1.92	Bd	<0.001		20.0	1.55	B
		P-value[1]			0.171			<0.001			<0.001			<0.001			<0.001			<0.001		*P*-value[3]			< 0.001
	Co (%)	NF	4.78	0.44	Aa	5.02	0.46	Aa	4.94	0.52	Aa	5.11	0.60	Aa	6.85	0.82	Aa	7.42	3.50	Aa	0.189		5.75	0.73	A
		F	4.80	0.35	Aa	20.3	2.52	Bb	31.1	3.36	Bc	29.2	2.52	Bc	25.8	1.22	Bbc	19.4	4.86	Aabc	<0.001		21.62	1.71	B
		P-value[1]			0.894			<0.001			<0.001			<0.001			<0.001			0.125		*P*-value[3]			< 0.001
Linaria	Ht (cm)	NF	4.0	0.24	Aa	8.4	1.14	Abc	10.1	0.77	Ab	9.6	0.87	Abd	5.7	1.07	Aac	7.9	0.73	Acd	<0.001		7.6	0.44	A
vulgaris		F	4.3	0.55	Aa	13.6	1.43	Bb	23.7	2.00	Bce	30.3	2.03	Bd	29.5	2.24	Bcd	21.7	1.68	Be	<0.001		20.5	1.43	B
		P-value[1]			0.452			0.042			0.006			<0.001			<0.001			<0.001		*P*-value[3]			< 0.001
	Co (%)	NF	2.16	0.44	Aa	2.41	0.51	Aa	3.08	0.55	Aa	3.74	0.51	Aa	3.21	0.64	Aa	4.34	1.77	Aa	0.118		3.16	0.35	A
		F	2.24	0.38	Aa	9.33	1.13	Bb	18.9	2.65	Bc	31.6	3.81	Bd	34.1	3.83	Bd	40.2	6.79	Bd	<0.001		22.72	2.38	B
		P-value[1]			0.756			<0.001			<0.001			<0.001			<0.001			0.006		*P*-value[3]			< 0.001
Lotus	Ht (cm)	NF	3.3	0.31	Aa	7.1	0.73	Ab	11.8	1.47	Acd	8.9	0.70	Abc	11.4	0.79	Ad	13.5	1.93	Acd	<0.001		9.3	0.64	A
corniculatus		F	3.1	0.16	Aa	9.4	0.87	Ab	12.8	1.07	Acd	12.2	0.97	Bc	15.6	1.26	Bd	15.0	1.98	Acd	<0.001		11.3	0.74	B
		P-value[1]			0.565			0.093			0.565			0.017			0.013			0.537		*P*-value[3]			0.0441
	Co (%)	NF	3.85	0.76	Aa	13.9	2.10	Ab	30.0	4.35	Ac	34.7	3.32	Acd	46.9	5.05	Ad	21.3	5.15	Aabc	<0.001		24.85	2.41	A
		F	4.33	0.67	Aa	16.1	2.80	Ab	32.3	6.17	Abcd	30.0	5.78	Acd	34.6	5.00	Ad	16.6	3.82	Aabc	<0.001		22.32	2.28	A
		P-value[1]			0.533			0.757			0.965			0.199			0.052			0.689		*P*-value[3]			0.4421

[1]Significant differences at p = 0.05 between substrate types for the same month (Mann-Whitney *U*-test). [2]Significant differences at p = 0.05 between months for the same fertiliser treatment (Kruskal-Wallis test). [3]Significant differences in mean value across the period at p = 0.05 between fertiliser treatments (Mann-Whitney U-test). Different capital letters indicate significant difference between fertiliser treatments for the same month and in mean value across the period. Different lower-case indicate significant difference between months for the same treatment.
Ht.: Height, Co.: Coverage, NF: Non-fertiliser treatment; F: Fertiliser treatment

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APPENDIX 13. Mean height (cm) and coverage (%) of individual species in response to fertiliser treatments over the 2-year period of the experiment.

			2007														2008							
			Jun.			Jul.			Aug.			Sep.			Oct.			Apr					Mean value across the period		
			Mean	SE		Mean	SE		Mean	SE		Mean	SE		Mean	SE		Mean	SE		*P*-value[2]			SE	
Origanum vulgare	Ht (cm)	NF	3.4	0.36	Aa	6.6	0.85	Ab	6.4	1.61	Aab	5.6	1.66	Aab	5.0	1.79	Aab	0.9	0.49	Ac	0.003		4.7	0.56	A
		F	3.7	0.26	Aa	7.1	1.28	Aa	8.0	2.85	Aa	6.3	2.89	Aa	5.4	2.74	Aa	1.7	1.14	Aa	0.101		5.4	0.87	A
		P-value[1]			0.377			1.000			1.000			0.475			0.275			0.911		*P*-value[3]			0.5187
	Co (%)	NF	1.53	0.14	Aa	1.36	0.19	Aa	1.17	0.33	Aa	1.02	0.35	Aa	1.31	0.37	Aa	0.23	0.134	Ab	0.009		1.10	0.12	A
		F	1.57	0.17	Aa	3.08	1.06	Aa	3.42	1.51	Aa	3.27	1.64	Aab	2.82	1.51	Aab	0.21	0.179	Ab	0.037		2.39	0.49	A
		P-value[1]			1.000			0.929			1.000			0.531			0.242			0.655		*P*-value[3]			0.5127
Pilosella aurantiaca	Ht (cm)	NF	1.8	0.09	Aa	6.4	0.40	Abc	20.7	5.13	Ab	5.3	0.40	Acd	4.0	0.56	Ad	5.2	0.53	Acd	<0.001		7.2	1.19	A
		F	2.0	0.10	Aa	10.6	0.78	Bb	32.0	2.69	Ac	12.5	2.32	Bbd	9.2	1.81	Bde	6.8	0.99	Ae	<0.001		12.2	1.46	B
		P-value[1]			0.154			<0.001			0.184			0.012			0.001			0.144		*P*-value[3]			< 0.001
	Co (%)	NF	2.73	0.26	Aae	3.20	0.27	Aab	4.61	0.54	Abc	5.51	0.78	Acd	8.11	1.05	Ad	1.82	0.48	Ae	<0.001		4.12	0.36	A
		F	3.50	0.29	Aa	12.5	0.76	Bb	17.7	1.30	Bc	24.2	2.45	Bd	25.9	2.52	Bd	5.98	1.58	Ba	<0.001		14.96	1.34	B
		P-value[1]			0.109			<0.001			<0.001			<0.001			<0.001			0.009		*P*-value[3]			< 0.001
Pilosella officinarum	Ht (cm)	NF	1.3	0.11	Aad	4.5	2.14	Abc	5.0	1.41	Ab	1.9	0.47	Aac	1.2	0.10	Ad	1.6	0.44	Aabcd	0.004		2.6	0.47	A
		F	0.9	0.06	Ba	3.3	0.36	Ab	21.7	3.44	Bc	4.3	0.40	Bb	4.1	0.68	Bb	0.9	0.45	Aa	<0.001		5.9	1.14	B
		P-value[1]			0.014			0.121			0.003			0.005			0.006			0.331		*P*-value[3]			0.0152
	Co (%)	NF	1.97	0.35	Aa	2.50	0.47	Aa	3.02	0.51	Aab	3.03	0.59	Aab	5.37	0.98	Ab	0.80	0.39	Ac	0.001		2.78	0.30	A
		F	1.98	0.13	Aa	4.91	0.77	Bb	10.3	1.25	Bc	17.0	4.14	Bc	15.4	3.20	Bc	0.51	0.42	Ad	<0.001		8.36	1.23	B
		P-value[1]			0.581			0.017			0.008			0.012			0.017			0.288		*P*-value[3]			0.0019

[1]Significant differences at p = 0.05 between substrate types for the same month (Mann-Whitney *U*-test). [2]Significant differences at p = 0.05 between months for the same fertiliser treatment (Kruskal-Wallis test). [3]Significant differences in mean value across the period at p = 0.05 between fertiliser treatments (Mann-Whitney U-test). Different capital letters indicate significant difference between fertiliser treatments for the same month and in mean value across the period. Different lower-case indicate significant difference between months for the same treatment.
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(*Continued on next page.*)

APPENDIX 13. Mean height (cm) and coverage (%) of individual species in response to fertiliser treatments over the 2-year period of the experiment.

			2007														2008							
			Jun.			Jul.			Aug.			Sep.			Oct.			Apr					Mean value across the period		
			Mean	SE		Mean	SE		Mean	SE		Mean	SE		Mean	SE		Mean	SE		*P*- value[2]			SE	
Primula	Ht (cm)	NF	1.8	0.19	Aa	2.0	0.22	Aa	1.1	0.25	Aa	1.5	0.14	Aa	1.2	0.21	Aa	1.3	0.45	Aa	0.089		1.5	0.11	A
veris		F	1.5	0.14	Aa	2.5	0.23	Ab	2.7	0.39	Bb	1.8	0.46	Aab	1.4	0.26	Aa	5.2	1.58	Aab	0.019		2.5	0.33	B
		P-value[1]			0.182			0.077			0.007			0.626			0.374			0.059		*P*-value[3]			0.0023
	Co (%)	NF	1.09	0.07	Aa	0.92	0.15	Aac	0.42	0.13	Abd	0.48	0.09	Ab	0.61	0.09	Abc	0.20	0.09	Ad	<0.001		0.62	0.06	A
		F	1.13	0.11	Aa	1.12	0.09	Aa	0.91	0.21	Aab	0.63	0.19	Aab	0.43	0.15	Ab	1.13	0.43	Aab	0.042		0.89	0.10	B
		P-value[1]			0.853			0.254			0.067			0.756			0.112			0.088		*P*-value[3]			0.0471
Scabiosa	Ht (cm)	NF	1.5	0.20	Aab	1.3	0.12	Aa	2.2	0.51	Aabc	6.2	3.99	Abcd	4.9	2.54	Acd	4.2	0.44	Ad	0.001		3.4	0.80	A
columbaria		F	1.2	0.14	Aa	3.4	0.69	Bb	13.9	3.95	Bc	29.5	5.29	Bd	32.2	5.06	Bd	8.0	1.37	Bc	<0.001		14.7	2.14	B
		P-value[1]			0.225			0.002			<0.001			0.003			<0.001			0.030		*P*-value[3]			< 0.001
	Co (%)	NF	1.88	0.24	Aa	1.88	0.12	Aa	2.41	0.30	Aab	3.03	0.33	Abc	4.52	0.66	Ac	1.98	0.47	Aab	0.003		2.54	0.21	A
		F	2.40	0.19	Aa	5.51	0.55	Bb	10.9	0.72	Bc	17.9	3.88	Bd	20.9	3.66	Bd	5.86	1.50	Bab	<0.001		10.49	1.27	B
		P-value[1]			0.070			<0.001			<0.001			<0.001			<0.001			0.027		*P*-value[3]			< 0.001

[1]Significant differences at p = 0.05 between substrate types for the same month (Mann-Whitney *U*-test). [2]Significant differences at p = 0.05 between months for the same fertiliser treatment (Kruskal-Wallis test). [3]Significant differences in mean value across the period at p = 0.05 between fertiliser treatments (Mann-Whitney U-test). Different capital letters indicate significant difference between fertiliser treatments for the same month and in mean value across the period. Different lower-case indicate significant difference between months for the same treatment.
Ht.: Height, Co.: Coverage, NF: Non-fertiliser treatment; F: Fertiliser treatment

APPENDIX 14. Summary of flowering characteristics of individual species over the 2-year period of the experiment.

Achillea		Ref.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	P	S	
millefolium	NW	50 mm								0.0	0.0	– a
		100 mm								0.0	0.0	– a
		200 mm						0.2	0.3	11.1	0.6	– a
										P-value*	0.368	
	W	50 mm								0.0	0.0	
		100 mm								0.0	0.0	
		200 mm								0.0	0.0	
		Zinco								0.0	0.0	
		Lime								0.0	0.0	
		NF								0.0	0.0	
		F						1.3	1.4	66.7	2.8	
Agrimonia		Ref.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	P	S	
eupatoria	NW	50 mm								0.0	0.0	– a
		100 mm					0.1			11.1	0.1	– a
		200 mm								0.0	0.0	– a
										P-value*	0.368	
	W	50 mm								0.0	0.0	– a
		100 mm								0.0	0.0	– a
		200 mm						0.1		11.1	0.1	– a
										P-value*	0.368	
		Zinco						0.1		11.1	0.1	
		Lime						0.1		11.1	0.1	
										P-value*	1.000	
		NF					0.1			11.1	0.1	
		F					0.4			44.4	0.4	
										P-value*	0.14	
Campanula		Ref.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	P	S	
glomerata	NW	50 mm								0.0	0.0	– a
		100 mm							0.1	11.1	0.1	– a
		200 mm								0.0	0.0	– a
										P-value*	0.368	
	W	50 mm							0.1	11.1	0.1	– a
		100 mm								0.0	0.0	– a
		200 mm								0.0	0.0	– a
										P-value*	0.368	
		Zinco								0.0	0.0	
		Lime								0.0	0.0	
		NF								0.0	0.0	
		F								0.0	0.0	

Ref: flowering time and duration at ground level (source from Grime *et al*., 2007), P: percentage of flowering plants, S: the mean total number of inflorescences per replicate across time

NW: Non-watering treatment; W: Watering treatment, Zinco: Zinco substrate treatment; Lime: Limestone-based substrate treatment, NF: Non-fertiliser treatment; F: Fertiliser treatment

*Significant differences at $p < 0.05$ between substrate depths (Kruskal-Wallis test), substrate types (Mann-Whitney *U*-test), or fertiliser treatments (Mann-Whitney *U*-test)

Flowering time and the mean total number of inflorescences per replicate

Different capital letters indicate significant difference at $p < 0.05$ (Mann-Whitney *U*-test) between both watering treatments at the same depth

Different lower-case letters indicate significant difference at $p < 0.05$ (Mann-Whitney *U*-test after Kruskal-Wallis test) between substrate depths

(Continued on nest page)

APPENDIX 14. Summary of flowering characteristics of individual species over the 2-year period of the experiment.

Clinopodium		Ref.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.		P	S	
vulgare	NW	50 mm					0.1	0.1			22.2	0.2	Aa
		100 mm					0.3	1.4			88.9	1.8	Ab
		200 mm					1.2	0.7			77.8	1.9	Ab
										P-value*		0.005	
	W	50 mm					0.4	0.4			88.9	0.9	Ba
		100 mm					0.2	0.8			66.7	1.0	Aa
		200 mm					0.6	0.8			66.7	1.3	Aa
										P-value*		0.766	
	Zinco						0.1	1.0			66.7	1.1	
	Lime						0.2	2.7	2.1		100.0	5.0	
										P-value*		0.003	
	NF						1.0				55.6	1.0	
	F					0.2	2.0	8.0	5.1		88.9	15.3	
										P-value*		0.003	
Galium		Ref.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.		P	S	
verum	NW	50 mm									0.0	0.0	– a
		100 mm					0.4	0.4	0.4		11.1	1.3	– a
		200 mm					0.1				11.1	0.1	– a
										P-value*		0.594	
	W	50 mm									0.0	0.0	
		100 mm									0.0	0.0	
		200 mm									0.0	0.0	
	Zinco										0.0	0.0	
	Lime							0.6	0.6		22.2	1.1	
	NF										0.0	0.0	
	F							2.6	1.8		44.4	4.3	
Helianthemum		Ref.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.		P	S	
nummularium	NW	50 mm						0.1			11.1	0.1	Aa
		100 mm									0.0	0.0	– a
		200 mm						0.1			11.1	0.1	Aa
										P-value*		0.595	
	W	50 mm						0.1			11.1	0.1	Aa
		100 mm									0.0	0.0	– a
		200 mm						0.1			11.1	0.1	Aa
										P-value*		0.595	
	Zinco										0.0	0.0	
	Lime							0.1	0.1		22.2	0.2	
	NF										0.0	0.0	
	F							0.1			11.1	0.1	

Ref: flowering time and duration at ground level (source from Grime *et al*., 2007), P: percentage of flowering plants, S: the mean total number of inflorescences per replicate across time

NW: Non-watering treatment; W: Watering treatment, Zinco: Zinco substrate treatment; Lime: Limestone-based substrate treatment, NF: Non-fertiliser treatment; F: Fertiliser treatment

*Significant differences at $p < 0.05$ between substrate depths (Kruskal-Wallis test), substrate types (Mann-Whitney *U*-test), or fertiliser treatments (Mann-Whitney *U*-test)

Flowering time and the mean total number of inflorescences per replicate

Different capital letters indicate significant difference at $p < 0.05$ (Mann-Whitney *U*-test) between both watering treatments at the same depth

Different lower-case letters indicate significant difference at $p < 0.05$ (Mann-Whitney *U*-test after Kruskal-Wallis test) between substrate depths

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APPENDIX 14. Summary of flowering characteristics of individual species over the 2-year period of the experiment.

Hypochaeris radicata		Ref.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.		P	S	
	NW	50 mm									0.0	0.0	– a
		100 mm				0.2	0.7	0.3			66.7	1.2	Ab
		200 mm				0.3	0.6	0.7	0.1		44.4	1.7	Ab
										P-value*		0.026	
	W	50 mm									0.0	0.0	– a
		100 mm					0.2	0.1	0.1		22.2	0.4	Ab
		200 mm					0.8	0.4	0.2		55.6	1.4	Ab
										P-value*		0.025	
	Zinco					0.6	0.1	0.3	0.1		55.6	1.1	
	Lime					0.3	0.7	1.4	1.0		88.9	3.4	
										P-value*		0.084	
	NF				0.3		0.2		0.3		44.4	0.9	
	F				0.2	0.1	1.1	2.3	1.7		88.9	5.4	
										P-value*		0.013	
Knautia arvensis		Ref.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.		P	S	
	NW	50 mm									0.0	0.0	
		100 mm									0.0	0.0	
		200 mm									0.0	0.0	
	W	50 mm									0.0	0.0	
		100 mm									0.0	0.0	
		200 mm									0.0	0.0	
	Zinco										0.0	0.0	
	Lime								0.1		11.1	0.1	
	NF										0.0	0.0	
	F							0.6	2.8		77.8	3.3	
Leontodon autumnalis		Ref.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.		P	S	
	NW	50 mm				0.1					11.1	0.1	Aa
		100 mm				0.1	0.3	0.3			66.7	0.8	Ab
		200 mm				0.2	1.1	0.3			66.7	1.7	Ab
										P-value*		0.018	
	W	50 mm						0.2			22.2	0.2	Aa
		100 mm					0.7	0.1			55.6	0.8	Aab
		200 mm					1.1	0.3	0.1		88.9	1.6	Ab
										P-value*		0.007	
	Zinco						0.4	0.2	0.2		77.8	0.9	
	Lime						0.2	1.2	0.3		77.8	1.8	
										P-value*		0.175	
	NF				0.1		0.6		0.1		66.7	0.8	
	F				0.2	0.2	1.2	3.1	0.8		100.0	5.6	
										P-value*		0.0004	

Ref: flowering time and duration at ground level (source from Grime *et al*., 2007), P: percentage of flowering plants, S: the mean total number of inflorescences per replicate across time

NW: Non-watering treatment; W: Watering treatment, Zinco: Zinco substrate treatment; Lime: Limestone-based substrate treatment, NF: Non-fertiliser treatment; F: Fertiliser treatment

*Significant differences at $p < 0.05$ between substrate depths (Kruskal-Wallis test), substrate types (Mann-Whitney *U*-test), or fertiliser treatments (Mann-Whitney *U*-test)

Flowering time and the mean total number of inflorescences per replicate

Different capital letters indicate significant difference at $p < 0.05$ (Mann-Whitney *U*-test) between both watering treatments at the same depth

Different lower-case letters indicate significant difference at $p < 0.05$ (Mann-Whitney *U*-test after Kruskal-Wallis test) between substrate depths

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APPENDIX 14. Summary of flowering characteristics of individual species over the 2-year period of the experiment.

Leucanthemum		Ref.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.		P	S	
vulgare	NW	50 mm									0.0	0.0	
		100 mm					0.1	0.1			11.1	0.2	Ab
		200 mm					0.2	0.7	0.6		44.4	1.4	Ab
										P-value*		0.041	
	W	50 mm									0.0	0.0	– a
		100 mm									0.0	0.0	– a
		200 mm					0.1	0.1			11.1	0.2	Aa
										P-value*		0.368	
	Zinco										0.0	0.0	
	Lime							0.4	0.8		33.3	1.2	
	NF										0.0	0.0	
	F						0.1	7.7	5.8		77.8	13.6	
Linaria		Ref.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.		P	S	
vulgaris	NW	50 mm					0.1				11.1	0.1	Aa
		100 mm					0.7	0.7			88.9	1.3	Ab
		200 mm					1.0	0.9			77.8	1.9	Ab
										P-value*		0.033	
	W	50 mm					0.1	0.1	0.3		55.6	0.6	Aa
		100 mm					0.5	0.3	0.6		55.6	1.4	Aa
		200 mm					0.2	0.8	0.3		88.9	1.3	Aa
										P-value*		0.272	
	Zinco						0.3	0.2	0.4		55.6	1.0	
	Lime						0.2	2.7	1.6		88.9	4.4	
										P-value*		0.014	
	NF						0.4				33.3	0.4	
	F					0.1	6.7	8.6	2.3		100.0	17.7	
										P-value*		0.004	
Lotus		Ref.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.		P	S	
corniculatus	NW	50 mm					2.3				66.7	2.3	Aa
		100 mm					8.9	1.3			66.7	10.2	Aab
		200 mm					11.6	14.6			88.9	26.1	Ab
										P-value*		0.042	
	W	50 mm					6.0	2.0			100.0	8.0	Ba
		100 mm					8.0	8.6	0.1		100.0	16.7	Ab
		200 mm					10.2	20.8			100.0	31.0	Ab
										P-value*		0.001	
	Zinco						9.7	10.1			100.0	19.8	
	Lime						6.7	10.2	0.1		100.0	17.0	
										P-value*		1.000	
	NF						7.8	0.1			77.8	7.9	
	F					0.4	4.6		0.1		88.9	5.1	
										P-value*		0.593	

Ref: flowering time and duration at ground level (source from Grime *et al*., 2007), P: percentage of flowering plants, S: the mean total number of inflorescences per replicate across time

NW: Non-watering treatment; W: Watering treatment, Zinco: Zinco substrate treatment; Lime: Limestone-based substrate treatment, NF: Non-fertiliser treatment; F: Fertiliser treatment

*Significant differences at $p < 0.05$ between substrate depths (Kruskal-Wallis test), substrate types (Mann-Whitney *U*-test), or fertiliser treatments (Mann-Whitney *U*-test)

Flowering time and the mean total number of inflorescences per replicate

Different capital letters indicate significant difference at $p < 0.05$ (Mann-Whitney *U*-test) between both watering treatments at the same depth

Different lower-case letters indicate significant difference at $p < 0.05$ (Mann-Whitney *U*-test after Kruskal-Wallis test) between substrate depths

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APPENDIX 14. Summary of flowering characteristics of individual species over the 2-year period of the experiment.

Origanum vulgare		Ref.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.		P	S	
	NW	50 mm						0.1			11.1	0.1	– a
		100 mm					0.1	0.1			11.0	0.2	Aa
		200 mm					0.2	0.1			33.3	0.3	Aa
										P-value*		0.452	
	W	50 mm									0.0	0.0	–
		100 mm					0.2	0.7	0.1		66.7	1.0	Bb
		200 mm						0.3			33.3	0.3	Ab
										P-value*		0.011	
	Zinco							0.3			22.2	0.3	
	Lime							0.3	1.0		55.6	1.3	
										P-value*		0.118	
	NF										0.0	0.0	
	F							1.4	0.3		44.4	1.8	
Pilosella aurantiaca		Ref.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.		P	S	
	NW	50 mm									0.0	0.0	–
		100 mm					0.3				33.3	0.3	Ab
		200 mm					0.6				55.6	0.6	Ab
										P-value*		0.039	
	W	50 mm							0.1		11.1	0.1	– a
		100 mm					0.4		0.2		66.7	0.7	Ab
		200 mm					1.0		0.1		100.0	1.1	Bb
										P-value*		0.001	
	Zinco						0.3	0.2			55.6	0.6	
	Lime						0.1	1.8			88.9	1.9	
										P-value*		0.067	
	NF						0.4	0.1	0.1		55.6	0.7	
	F						4.9	0.2	0.1		100.0	5.2	
										P-value*		0.001	
Pilosella officinarum		Ref.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.		P	S	
	NW	50 mm									0.0	0.0	–
		100 mm					1.0				77.8	1.0	Ab
		200 mm				0.1	0.8				55.6	0.9	Ab
										P-value*		0.007	
	W	50 mm					0.1	0.3			22.2	0.4	– a
		100 mm					0.4	0.2			55.6	0.7	Aa
		200 mm					0.6				33.3	0.6	Aa
										P-value*		0.530	
	Zinco						0.3	0.1			44.4	0.4	
	Lime						0.2	0.9			77.8	1.1	
										P-value*		0.097	
	NF						0.4				33.3	0.4	
	F				0.1		0.6				55.6	0.7	
										P-value*		0.457	

Ref: flowering time and duration at ground level (source from Grime *et al*., 2007), P: percentage of flowering plants, S: the mean total number of inflorescences per replicate across time

NW: Non-watering treatment; W: Watering treatment, Zinco: Zinco substrate treatment; Lime: Limestone-based substrate treatment, NF: Non-fertiliser treatment; F: Fertiliser treatment

*Significant differences at $p < 0.05$ between substrate depths (Kruskal-Wallis test), substrate types (Mann-Whitney *U*-test), or fertiliser treatments (Mann-Whitney *U*-test)

Flowering time and the mean total number of inflorescences per replicate

Different capital letters indicate significant difference at $p < 0.05$ (Mann-Whitney *U*-test) between both watering treatments at the same depth

Different lower-case letters indicate significant difference at $p < 0.05$ (Mann-Whitney *U*-test after Kruskal-Wallis test) between substrate depths

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APPENDIX 14. Summary of flowering characteristics of individual species over the 2-year period of the experiment.

Primula veris		Ref.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	P	S	
	NW	50 mm									0.0	
		100 mm									0.0	
		200 mm									0.0	
	W	50 mm									0.0	
		100 mm									0.0	
		200 mm									0.0	
	Zinco										0.0	
	Lime										0.0	
	NF										0.0	
	F										0.0	

Scabiosa columbaria		Ref.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	P	S	
	NW	50 mm								0.0	0.0	– a
		100 mm					0.1	0.3		11.1	0.4	Aa
		200 mm						0.3	0.2	22.2	0.6	Aa
										P-value*	0.366	
	W	50 mm								0.0	0.0	– a
		100 mm							0.6	22.2	0.6	Aa
		200 mm							0.1	11.1	0.1	Aa
										P-value*	0.312	
	Zinco										0.0	
	Lime								0.1	11.1	0.1	
	NF								0.3	11.1	0.3	
	F							1.9	3.2	55.6	5.1	
										P-value*	0.046	

Ref: flowering time and duration at ground level (source from Grime *et al*., 2007), P: percentage of flowering plants, S: the mean total number of inflorescences per replicate across time

NW: Non-watering treatment; W: Watering treatment, Zinco: Zinco substrate treatment; Lime: Limestone-based substrate treatment, NF: Non-fertiliser treatment; F: Fertiliser treatment

*Significant differences at $p < 0.05$ between substrate depths (Kruskal-Wallis test), substrate types (Mann-Whitney *U*-test), or fertiliser treatments (Mann-Whitney *U*-test)

Flowering time and the mean total number of inflorescences per replicate

Different capital letters indicate significant difference at $p < 0.05$ (Mann-Whitney *U*-test) between both watering treatments at the same depth

Different lower-case letters indicate significant difference at $p < 0.05$ (Mann-Whitney *U*-test after Kruskal-Wallis test) between substrate depths