The effect of roots and ectomycorrhizal fungi on carbon cycling in forest soils

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Abstract

Though the input of labile substrates into the rhizosphere by roots is known to promote decomposition of both soil organic matter (SOM) and surface litter, the presence of ectomycorrhizal (ECM) fungi living in symbiosis with plant roots has been shown to coincide with decreased litter decomposition rates in some systems. In a series of field experiments, techniques including forest girdling and soil trenching were used to exclude roots and ECM fungi in order to investigate the mechanisms controlling litter decomposition in forest soils.

Soil trenching was carried out in combination with litter bag incubations, and measurements of soil CO_2 flux in a 20 year-old *Pinus contorta* stand. The use of mesh in-growth collars allowed the influence of ECM fungal hyphae on litter mass loss, and their contribution to soil CO_2 flux, to be established separately to that of roots. A specialised irrigation system allowed moisture effects caused by root/ECM hyphal water uptake to be investigated. Neither the presence of roots, nor ECM fungi had any influence on litter decomposition, and soil temperature was the only factor found to correlate with litter mass loss.

The exclusion of roots and ECM hyphae led to increased utilisation of a simple substrate, ^{13}C-labelled glucose. Results of incubations of four substrates, varying in structural complexity and nitrogen (N) content, suggested that the rapid utilisation of simple substrates by r-strategist microorganisms might be suppressed in the presence of ECM fungi. Though N content appeared to have a positive influence on substrate decomposition, the results were not significant.

In contrast, when forest girdling was used in a nearby *Tsuga heterophylla* stand to exclude plant-assimilate C supply to the soil, a significant reduction in the rate of litter mass loss was observed.

The results presented in this thesis indicate a potentially large role of ECM fungi in controlling decomposition in forest soils, and the mechanisms underlying their influence require further investigation.

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Author's declaration

I, Naomi Rachel Voke, hereby certify that I am the sole author of this thesis and that it has not been submitted previously for a higher degree. This work reported in this thesis was carried out by me, with the following exceptions:

I was responsible for the experimental design for Chapters 2 and 3, but I received assistance in the field, establishing the soil collars and the automated irrigation system from Jens-Arne Subke and Phil Ineson. One of the data loggers involved in soil temperature measurements was established in the *Pinus contorta* stand by Luke Smallman, as part of his Master's project. I also received assistance taking soil CO_2 flux measurements from Jens-Arne Subke on several occasions.

I designed and managed the litter decomposition component of the published paper, Subke *et al*. (2011) presented as Chapter 4 of this thesis. On one occasion, litter bags were collected from the field by Jens-Arne Subke. The overall design and management of the girdling treatment, and the soil CO_2 measurements was the responsibility of Jens-Arne Subke. However, I helped to facilitate the whole experiment, taking occasional measurements of soil CO_2 efflux and transporting collaborators to the field. The bulk of the writing of the paper presented was done by Jens-Arne Subke, however, I contributed the information contained in the methods section for the litter bag experiments, the litter mass loss data, and was involved in the writing of the results and discussion sections.

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Chapter 1 General introduction

It has been estimated that up to 40% of the world's terrestrial below-ground carbon (C) is stored in forests, as part of soil organic matter (SOM) (Dixon *et al*., 1994; Hyvonen *et al*., 2007). There is uncertainty regarding whether forest soils will remain a sink for carbon dioxide (CO_2) under future climate scenarios, mitigating the effects of rising atmospheric CO_2 (Fitter, 2005). Consequently, factors influencing the stability and turnover of large carbon stores such as forest SOM should be further investigated (Schulze and Freibauer, 2005) and, models updated to incorporate them as they are elucidated (Medlyn *et al*., 2005).

1.1 Carbon input to the soil

Surface litter decomposition is a fundamental process in forest soils, and is one of the major routes for carbon to be incorporated into SOM (Swift *et al*. 1979; Chadwick *et al*., 1998; Aerts, 2006). In a ^{13}C-labelled litter decomposition experiment in a *Populus nigra* L. plantation in Italy, Rubino *et al*. (2010) estimated that after 11 months, the litter had lost 80% of its mass, with c. 67% of the litter C incorporated into SOM. Around 30% of litter-derived C had been released into the atmosphere as carbon dioxide (CO_2) respired by microorganisms, including bacteria and fungi, which are the most important litter decomposers in forest soils (Berg & McClaugherty, 2003). Soil CO_2 efflux (R_s) is the main route by which C taken up by forest trees through photosynthesis is returned to the atmosphere (Janssens *et al*., 2001), and contributes around 60-80% of total ecosystem respiration (Kuzyakov & Larionova, 2005). R_s can be further subdivided, generally into heterotrophic respiration (R_h), and autotrophic respiration ($R_{a)}$ which in the current work is defined as the CO_2 flux from roots, and closely associated microorganisms in the rhizosphere (Subke *et al*., 2006).

Litter decomposition involves physical and chemical processes, including leaching, fragmentation and catabolism, breaking down the substrate into its chemical constituents, thus ensuring nutrient supply for microbial and plant growth (Swift *et al*., 1976; Aerts, 2006; Cotrufo *et al*., 2010). Litter is made up of a variety of substrates which vary in complexity, and is broken down by a succession of decomposer microorganisms, each adapted to a specific 'niche' (Frankland, 1969; 1998). The

composition of the microbial community changes alongside that of the litter, as the substrate becomes progressively more recalcitrant (McGuire & Treseder, 2010).

During the first stage of litter decomposition, soluble, low molecular weight substrates, including simple sugars are lost from the litter, mostly through leaching (McClaugherty, 1983). Following this, hemicelluloses, then cellulose, and finally lignin decomposition are the dominant processes (Berg & McClaugherty, 2003). The loss of the different organic-chemical components of litter over time from Scots pine needle litter is displayed in Fig. 1.1 (Berg *et al*., 1982). Though litter decomposition follows this general pattern, it is not strictly sequential, with decomposition of all the components occuring at the same time, to varying degrees (Berg & McClaugherty, 2003). Mindermann (1968) thought that it should be possible to calculate individual exponential decay rates for each of the litter components, the sum of which would represent the overall decomposition rate of the litter. Though mass loss from litter does follow an exponential pattern, there are interactions between the decomposition of the different components of litter which means that overall decomposition rates of complex substrates cannot merely be predicted by knowledge of the decomposition rates of the individual components included (Wider *et al*. 1982; Berg & McClaugherty, 2003).

The rate at which litter decomposes is controlled by three main variables; the activity of the decomposer organisms present, the physico-chemical environment, and the quality of the litter, all of which are linked (Swift *et al*., 1979). In hierarchical order, climate is generally held to be the most important controller of litter decomposition, as it affects the quality of the litter, and also directly influences the activity of soil microorganisms. This is then followed in order of importance by litter quality, and then the activity of the soil microorganisms themselves (Lavelle *et al*., 1993; Aerts, 2006).

Both moisture and temperature can be limiting to decomposition. Soil moisture dictates the availability of oxygen for aerobic decomposition, and the movement of enzymes, dissolved organic carbon (DOC) and nutrients through the soil solution (Goebel *et al*., 2007). Temperature affects the production and catalytic ability of

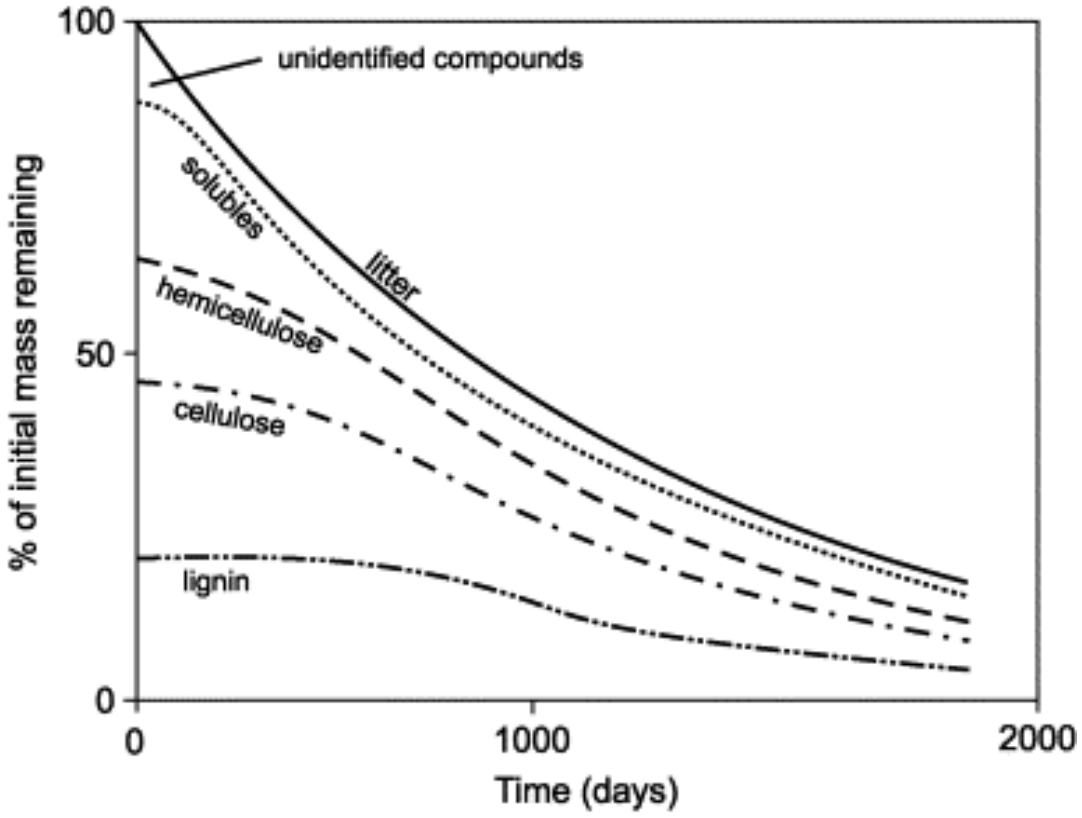

Fig. 1.1 The mass loss of organic-chemical components of Scots pine litter over time in a boreal Scots pine forest. From Berg *et al*. (1982).

enzymes involved in the decomposition process (Atkin & Tjoelker, 2003). When moisture falls below a certain threshold, an increase in temperature will not result in increased decomposition, just as in cold conditions, increased moisture will not necessarily result in increased decomposition (Berg & McClaugherty, 2003). Therefore the effects of moisture and temperature on decomposition can be strongly linked.

Aerts (1997) analysed first-year decomposition data from 44 locations spanning a range of climates, including temperate and tropical sites and found that though litter quality was associated with litter mass loss, AET (actual evapotranspiration; an index of temperature and moisture, used by large-scale studies to represent climate) had the highest association of all the variables tested. This was supported in another study of litter decomposition across climatic zones, by Meentemeyer (1978), who found that AET was considerably more important than litter quality (measured by lignin content). Berg (1993) also observed a high association between AET and litter mass loss over a range of 39 sites with differing climates, when standard litter was incubated at each of the sites. Berg (1993) found that when AET was combined with the average July temperature and average annual temperature, this could explain 70% of the mass loss observed, and that litter quality was more important at a local scale. However, this study, and others using standard litters, were criticised by Chadwick *et al*. (1998), who found that the chemistry of the underlying native litter layer affected the mass loss of standard *Pinus sylvestris* L. litter. Therefore this may have influenced the results of studies incubating standard litters over wide geographic scales, such as Berg *et al*. (1993) and may have given a false impression of the relative importance of climate and litter quality in controlling litter decomposition.

In addition to litter decomposition, another major route for C to be introduced into the soil and incorporated into SOM is via plant roots. In recent years, there has been increased interest in the interactions between soil components on decomposition in forest soils with, in particular, how plants exert control of decomposition through the input of C and organic acids to the soil via their roots, influencing the activity and interactions of soil microbial communities (Kuzyakov, 2002; Subke *et al*., 2004; Kuzyakov, 2010).

Plants allocate up to 50% of the C fixed from the atmosphere via photosynthesis below-ground to roots, c. 20% of which is cycled into the soil as lysates, exudates and root litter (reviewed in Kuzyakov & Domanski, 2000; Chaudhry *et al*. 2005). The area surrounding, and directly influenced, by the plant root is referred to as the rhizosphere. and is regarded as being a 'hotspot' of microbial activity with high microbial turnover rates, due to the input of labile photoassimilate C making the area rich in terms of energy for soil microorganisms (Kuzyakov & Larionova, 2005; Buee *et al*., 2009). In comparison, microorganisms have been shown to be C-limited in the bulk soil, which consists of partially degraded, more recalcitrant SOM (Killham, 1994; Ekblad & Nordgren, 2002) requiring more complex enzymes for decomposition and, therefore, turnover rates are comparatively low (Nehls *et al.* 2007).

1.1.1 Priming of SOM decomposition

Pulses of labile C into the soil, whether as a result of the presence of plant roots (Subke *et al*. 2004; Cheng, 2009), through the leaching of simple soluble sugars from litter (Rasmussen *et al*., 2007), or through decaying microbial or animal cells have been observed on numerous occasions to lead to priming effects (reviewed by Kuzyakov, 2002). Priming is defined as: an increase in the decomposition of recalcitrant SOM brought about by an increase in activity of soil microorganisms following the introduction of the labile substrates which are found in fresh organic matter (FOM) (Fontaine *et al*., 2004; Kuzyakov *et al.*, 2007). A schematic of the priming process, including both positive and negative priming effects is displayed in Fig. 1.2.

There has been knowledge of priming effects since the 1920's, but it has only been possible to quantify this process following the development of sophisticated isotopic labelling techniques since the 1940's, allowing more accurate separation of component fluxes of soil CO_2 than was previously possible (Kuzyakov & Larionova, 2005, Kuzyakov, 2010) The term 'priming' was coined by Bingeman *et al*. (1953) but the scale and mechanisms behind priming are still poorly understood (Fontaine *et al*., 2004). Despite this, the existing evidence of priming indicates the direct control of SOM turnover through plant activity, and this link between soil compartments could

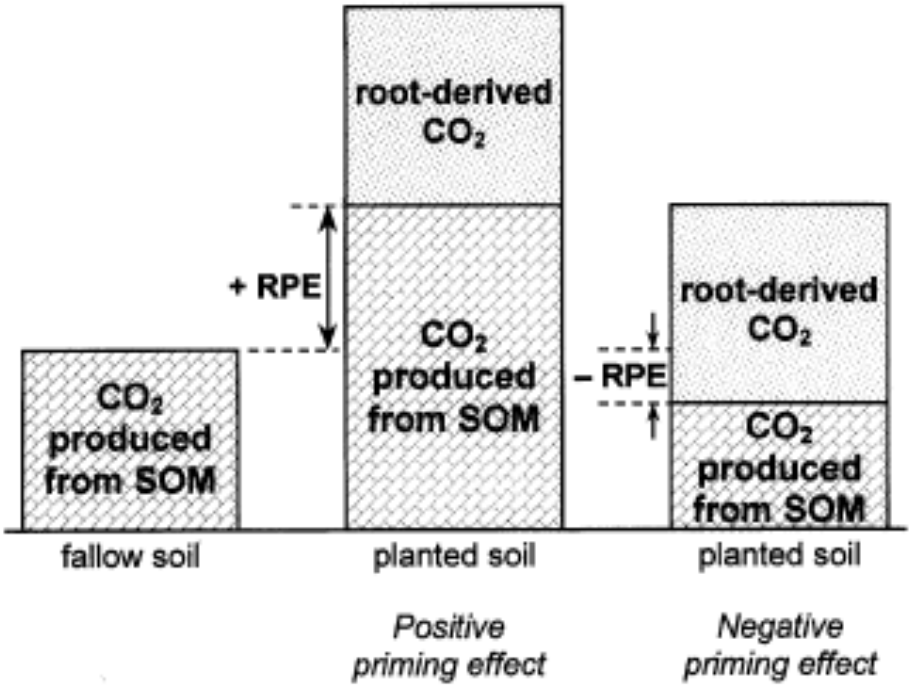

Fig 1.2 Schematic of carbon priming effects. Where roots are present in a planted soil, inputs of labile carbon results in either additional CO_2 being released from the soil from increased decomposition of SOM (a positive priming effect), or a reduction in SOM decomposition (a negative priming effect), in comparison to the CO_2 efflux measured in the absence of roots. Taken from Kuzyakov, (2002).

be a factor which could reduce the ability of forests to act as carbon sinks in the future (Subke *et al*., 2004).

Priming effects associated with the input of labile C to the soil were observed by Nottingham *et al*. (2009) following the addition of sucrose, which over their experiment released an additional 3.3% of the SOM C pool. The initial pulse of primed CO_2 they observed was also described in previous substrate addition studies, and could be an 'apparent priming effect' attributed to the replacement of C in microbial biomass, and not due to SOM decomposition (Dalenberg & Jager, 1981). Following the initial pulse of primed C, Nottingham *et al*. (2009) observed a pulse of substrate-derived CO_2 followed by a smaller, more sustained flux of primed CO_2, a 'real priming effect'. This same pattern was observed by Hamer & Marschner (2005), following the addition of ^{14}C-labelled fructose, alanine, oxalic acid and catechol to different horizons of two forest soils, and one arable soil. They observed particularly strong priming of SOM following the addition of ^{14}C-labelled glucose and alanine, with almost double the mineralisation of SOM compared to control soils. Catechol additions caused negative priming effects, reducing SOM decomposition by 43% in one of the soils, and the addition of oxalic acid caused both positive and negative priming effects. The results of Hamer & Marschner (2005) suggest that whether a positive or negative priming effect is induced depends on the substrate added and the soil type. Fontaine *et al*. (2007) introduced plant derived-C into sub-layers of soil, stimulating the decomposition of c. 2000 year old SOM. This demonstrated how the addition of labile fresh organic matter (FOM) has the potential to release C from previously stable SOM. However, the approach of Fontaine *et al*. (2007) could be criticised on the basis that they must also have added oxygen as well, which could have had the same promoting influence on SOM decomposition in deep anaerobic peat layers.

Priming effects have also been observed following litter addition. When Crow *et al*. (2009) conducted litter decomposition studies where the amount of litter input was doubled, they observed a greater release of C from the soil than would be predicted from the amount of additional litter C added. Rasmussen *et al*. (2007) studied the decomposition of ^{13}C-labelled ponderosa pine litter, and observed priming of SOM within the first 20 days of a 90 day study, the timing of which suggested that the

priming of SOM decomposition coincided with the release of the simple soluble components of litter.

1.1.2 Suggested mechanisms for the priming of SOM

Kuzyakov (2002) proposed a series of mechanisms by which the priming of SOM could be explained, the most important of which were deemed to be microbial activation following the introduction of labile substrates to the soil, and preferential substrate utilisation. Microbial activation is where the input of labile substrates causes increased microbial growth/turnover, leading to the decomposition of the added substrate alongside co-metabolism of SOM by the soil microorganisms. Cheng (2009) observed that SOM decomposition was increased by up to 380% in the presence of roots, in comparison to SOM decomposition in a control soil, though this was dependent on the plant species studied. They measured total microbial biomass, and found it was the same between treatments, leading them to the conclusion that the priming effects they observed were caused by increased activity and microbial turnover where roots were present, rather than an increase in the number of decomposer microorganisms in the system. De Nobilli *et al*. (2001) found that the addition of trace amounts of glucose, amino acids and root exudates to soil caused 2-5 times more C to be released as CO_2 than was added as part of the 'trigger' solution, and that the microbial community could be re-activated with repeat additions.

The alternative mechanism to explain priming offered by Kuzyakov (2002), preferential substrate utilisation, is where the addition of a labile substrate causes the microbial community to switch to the utilisation of that substrate, rather than existing more recalcitrant substrates, leading to a negative priming effect. This could explain the results of Chigineva *et al*. (2009) who found decreased litter decomposition following the addition of sucrose. This was coupled with a shift in the microbial community to microorganisms more suited to decomposing simple substrates (r-strategists), at the expense of K-strategist microorganisms, characterised by slow growth rates, and the ability to produce enzymes capable of degrading more complex substrates such as plant litter (Berg & McClaugherty, 2003, Fierer *et al*., 2007).

It was suggested in a conceptual model by Fontaine *et al*. (2003; 2004) that priming effects are dependent on competition between r- and K-strategist microorganisms. If r-strategist microorganisms are able to respond more rapidly to the input of labile substrates, and prevent access to the K-strategists, with their greater decomposer abilities, then there would not be expected to be a priming effect of labile C on SOM decomposition (Fontaine *et al*., 2003; 2004). However, Fontaine *et al*., (2004) suggest that if just a small proportion of the labile substrate was intercepted and utilised by K-strategists, they could become dominant upon the exhaustion of the labile substrate, and potentially decompose r-strategist biomass. The theory proposed by Fontaine *et al*. (2003; 2004) was supported by the findings of Wu *et al*. (2003) who observed greater priming of SOM following the application of ryegrass, a complex substrate, than following the addition of a simple substrate, ^{13}C-labelled glucose. The application of the ryegrass would be expected to increase the activity of K-strategist microorganisms to a greater extent than that of r-strategists, and therefore a greater amount of additional complex SOM decomposition was observed.

1.1.3 The priming of litter decomposition and the 'Gadgil effect'

In a girdling experiment, Subke *et al*. (2004) observed the priming of both SOM and litter decomposition following the input of labile C, cycled through roots and associated rhizosphere microorganisms in a Norway spruce plantation in Germany. There was also evidence of positive feedback, with the addition of the litter to the soil increasing rhizosphere activity, which in turn stimulated litter decomposition. This positive relationship between an intact rhizosphere and litter decomposition is the opposite to that found by Gadgil & Gadgil (1971; 1975). Using trenching, they found that litter decomposition was reduced in the presence of roots and associated ectomycorrhizal (ECM) fungi. Studies since have also observed a negative influence of roots on litter decomposition (Faber & Verhoef, 1991; Chuyong *et al*., 2002; Koide & Wu, 2003, Henkel, 2003). Gadgil & Gadgil (1971; 1975) suggested that the decrease in litter decomposition they observed was a result of the suppression of saprotrophic decomposers by ECM fungi.

1.2 Ectomycorrhizal (ECM) fungi

In most temperate and boreal pine forest systems the majority of the input of plant assimilates to soil is utilised by ectomycorrhizal (ECM) fungi living in symbiosis with roots (Högberg *et al*., 2001; Koide *et al*., 2011), and mycorrhizal networks can effectively replace fine roots in these systems (Read *et al*., 2004). The term 'mycorrhiza' has been in use since 1885 to describe modifications of the root structures of trees (Zak, 1964; Finlay, 2008), and describes a mutualistic symbiosis between a fungus and a plant root (Smith & Read, 1998). Ectomycorrhizal fungi are generally basiodiomycetes or ascomycetes, and globally as many as 10,000 fungal, and 8000 plant species could be involved in ectomycorrhizal associations, which is characterised by a mantle or sheath developing round fine roots, which are suppressed (Finlay, 2008; Nehls, 2008).

ECM fungi provide their host plant with nutrients such as N and P as well as potentially improving water absorption, and protecting the roots against pathogens (Chakravarty & Hwang, 1991; Conn & Dighton, 2000; Lindahl *et al*., 2002; Kipfer *et al*., 2011). In exchange, they are able to benefit from their close association with plant roots, and intercept an un-rivalled supply of energy-rich substrates allocated to the roots by the plant, thus the nutrient limitations of both the plant and the fungus are overcome through the association (Nehls, 2007; 2008). There is also evidence of nutrient exchange between plants connected by the same mycelial network (Leake *et al*., 2004).

ECM fungi are capable of forming extensive mycelial networks in the soil, with the mycelia making up 80% of the fungal biomass of certain species (Wallander *et al*., 2001; Rillig & Mummey, 2006). The production of mycelia allows a maximal surface area for nutrient exchange, and the supply of labile C from their host enables ECM fungi to proliferate and decompose or scavenge nutrients from heterogenous locations in the soil (Cairney, 2005; Drigo *et al*., 2012). It also potentially gives them an advantage over other, more carbon limited decomposer microorganisms in the soil, including saprotrophic fungi, as was suggested by Gadgil & Gadgil (1971; 1975). The outcome of competitive interactions between the mycelia of different species of

fungus has been shown to be related to the size of the resource from which each of the combatants has grown (Lindahl *et al*., 1999; Cairney, 2005).

Free-living saprotrophic fungi are considered to be better decomposers of recalcitrant substrates in the soil than ECM fungi (Colpeart & van Tichelen, 1996), therefore, it has been largely assumed that ECM fungi are reliant on the decomposer activities of the saprotrophs (Lindall *et al*., 1999). However, the ability of ECM fungi to decompose complex N containing substrates is greater than was previously thought (Bending & Read, 1995; Wu, 2011), though different species have been shown to have different decomposer abilities (Entry *et al*., 1991; Zeller *et al*., 2007). It has been suggested that if ECM fungi are able to use the advantage of their labile C supply and selectively take up N from decomposing substrates, they could suppress the activity of the more effective saprotrophic decomposers, and reduce litter decomposition (Abuzinadah *et al*., 1986; Bending, 2003).

An estimated 10-50% of the carbon fixed by trees is received from the roots by ECM fungi where they are present, which they then distribute throughout their mycelial networks (Smith & Read, 1998). This large loss of carbon from the host plant could be considered as wasteful (Killham, 1994), or evidence of parasitism. However, there is evidence that plants have developed mechanisms to prevent the cost of the symbiosis from being too high (Nehls *et al*., 2008), and it is the plant that is in control of the mycorrhizal symbiosis through its allocation of plant C (Fitter *et al*., 1998).

When the supply of plant-assimilates is reduced, ECM fungi can become very carbon limited, which can lead to problems with toxic accumulation of ammonium that is no longer assimilated (Wallander *et al*., 2011). Vallack *et al*. (2012) observed that following fertilisation of the soil with N in a boreal forest, the contribution of CO_2 efflux from ECM fungi to total soil CO_2 efflux decreased, while the component flux from soil heterotrophs increased. Therefore the tree would appear to be allocating less C to its roots when inorganic N is not limiting (Vogt *et al*., 1993). Through the use of isotopic pulse labelling and hyphal-ingrowth collars, Vallack *et al*. (2012) were able, for the first time, to measure how this influences the activity of the fungal partner, directly in the field. However, a three-year study by Wallander *et al*. (2011) found that whether

N fertilisation impacts the fungus depends on the effect it has on the growth of the tree. They studied ECM hyphal growth using mesh in-growth bags at two Norway spruce stands in Sweden, alongside fertilisation regimes. They found that where tree growth was stimulated by N fertilisation, ECM growth was not affected, but where tree growth was less stimulated by fertilisation with N, there was a decrease in ECM growth. In a meta-analysis of numerous independent field studies, Treseder (2004) found that on average, there was a 15% decrease in mycorrhizal abundance alongside N deposition, but that large variation between studies in terms of N responses means that predictability of microbial biomass according to the level of N deposition is low.

Kuzyakov (2002; 2010) proposed that the ECM mycorrhizal symbiosis is an evolved strategy by plants and involved in SOM priming. He suggested that when the plant provides labile C in the rhizosphere, microbial activation takes place, which results in the breakdown of substrates, including SOM by the fungal partner in order to gain N. In the process, N becomes available to the plant host. Cheng *et al*. (2008) observed that the increased turnover of microbial populations following labile C input results in more extracellular enzymes being produced, making a greater amount of nutrients available in solution for plant uptake, and if plant roots are longer lived than the fungi, then the plant would benefit over the long-term.

One individual ECM fungus, of the species *Armillaria bulbosa,* is reportedly one of the largest and oldest organisms in the world, spanning 15 hectares and weighing over 10,000 Kg (Smith *et al*., 1992). In boreal forest systems, ECM mycelia has been estimated to make up 30% of the total microbial biomass (Wallander *et al*., 2001), and they have been shown to contribute up to 50% of soil respiration (Högberg *et al*., 2001). ECM fungi are potentially huge players in forest soil carbon cycling, and if they do suppress the activity of saprotrophic microorganisms, as was suggested by Gadgil & Gadgil (1971; 1975), as well as being an important sink for photoassimilates, they could be contributing to increased C storage in SOM. Despite this, it is only relatively recently that attempts have been made to assess the turnover of ECM fungal mycelium in forest soils (Wallander *et al*., 2012), and their input has largely been ignored in carbon modelling (Lindahl *et al*., 2002; Heinemeyer *et al*., 2007). Further work is required in

order to establish the relative influence of plant assimilate-derived C, and the influence of ECM fungi on litter decomposition in forest soils.

1.3 Techniques: trenching and girdling

In order to separate out the effects of the input of plant assimilates, or the presence of roots and ECM fungal mycelia in order to measure their influence on litter decomposition rates and their contribution to total soil CO_2 efflux, it is necessary to exclude them from areas of the soil. Two methods that have been used successfully to achieve this in previous research are "trenching" (Gadgil & Gadgil, 1971; 1975; Heinemeyer *et al*., 2007; 2012; Comstedt *et al*., 2011, Vallack *et al*., 2012) and girdling (Högberg *et al*., 2001; Subke *et al*., 2004; Wu *et al*., 2011).

In the current work, the term 'trenching' will be used to refer to all situations where roots and mycorrhizal hyphae have been severed by cutting the soil to a depth below where the majority of roots and mycorrhizal hyphae are known to be concentrated. Roots and mycorrhizal hyphae are then excluded from an area of soil, either by repeated cutting, the establishment of an air gap, or through a physical barrier such as plastic or metal. By severing roots and ECM hyphae, the input of plant-assimilate C through these routes is cut off (reviewed in Subke *et al*., 2006). Trenching has the disadvantage that it is associated with an increase in the amount of decaying root and fungal material in the soil. It can also result in soil moisture differences between treatments, as there is no longer root water uptake (Saiz *et al*., 2006; Comstedt *et al*., 2011). In order to separate out ECM hyphae from roots, Heinemeyer *et al*. (2007) trenched the soil using sections of PVC pipe. These were hammered into the soil, so that four mesh-filled windows were positioned just below the soil surface, where most of the ECM hyphae had been observed to proliferate. The use of 41 µm mesh in the windows of the collars allowed ECM hyphae to grow into the trenched soil, whilst excluding roots. The use of 1 µm mesh in collar windows excluded both roots and ECM hyphae. They were able to take measurements of soil CO_2 efflux using an infra-red gas analyser (IRGA) with a survey chamber, which could be positioned directly on top of the PVC soil collars. By comparing the CO_2 efflux between the exclusion collars with CO_2 efflux measured from the total soil, they were able to separate out, the relative

contributions of roots (R_r), ECM hyphae (R_m) and background soil heterotrophs (R_h), to total soil CO_2 efflux (R_s), for the first time in the field.

Forest girdling is a sophisticated technique which prevents the flow of plant assimilate-derived C reaching the roots of the tree by severing the phloem. A comparison between measurements of soil CO_2 efflux from girdled and non-girdled plots can be used to separate out autotrophic (R_a) and heterotrophic (R_h) respiration. Following girdling, root and rhizosphere respiration decrease, as the supply of labile C from photosynthesis has been removed and any stored carbohydrates in roots are quickly depleted, therefore, any CO_2 evolved from these plots can be used as an estimate of R_h (Högberg *et al*., 2001; Bhupinderpal-Singh *et al*., 2003). Girdling also removes the labile C supply to ECM fungi present in the system, effectively taking away any competitive advantage conferred on them by their association with plant roots (Nehls, 2008). As girdling leaves the xylem intact, root water uptake is maintained, which is an advantage of girdling as opposed to trenching, as it avoids the confounding influence of soil moisture differences (Subke *et al*., 2004).

1.4 Thesis aims and structure

The aims of the current thesis were to investigate the factors controlling litter decomposition and soil CO_2 efflux in a temperate coniferous forest soil. An attempt was made to investigate the seemingly contradictory results in the literature surrounding the influence of ECM roots on litter decomposition, alongside consideration of the controls on decomposition by environmental variables including soil moisture and temperature. The following questions were addressed:

1. Is there an effect of roots and ECM fungi on litter decomposition rates?
2. Can any differences in litter decomposition between trenched and control soils, as observed by Gadgil & Gadgil (1971; 1975), be actually explained by soil moisture differences or does competition between mycorrhizal fungi and other soil microorganisms retard litter decomposition rates?
3. What is the effect of roots and ECM fungi on soil CO_2 efflux (R_s)? How are root respiration (R_r), mycorrhizal hyphal respiration (R_m) and heterotrophic

respiration (R_h) influenced differently by controlling factors such as temperature and soil moisture?

4. How important are ECM fungi in terms of their contribution to soil CO_2 efflux in a temperate coniferous forest?
5. Does the presence of mycorrhizal systems influence the decomposition of simple and complex substrates to the same extent?

Questions 1 & 2 are addressed in Chapter 2 of this thesis, which contains the results of a litter bag experiment, in combination with soil trenching used to selectively exclude roots and ECM hyphae. A specially developed irrigation system was used to control soil moisture between trenched and control soils. These questions are also addressed in Chapter 4, (now published in Subke *et al*., 2011), and report the results of a litter bag experiment carried out in conjunction with forest girdling. Questions 3 & 4 are addressed in Chapter 3 of this thesis, which includes the results of a series of measurements of soil CO_2 efflux carried out in conjunction with the litter bag experiment described in Chapter 2. Question 5 is addressed in Chapter 5 of this thesis, where the results of two incubations of ^{13}C-labelled substrates of varying structural complexity and N content are presented.

Chapter 2 Litter decomposition and the 'Gadgil effect'

2.1 Introduction

Studies have demonstrated a positive priming of soil organic matter (SOM) decomposition, following the addition of labile substrates into the soil (Kuzyakov, 2002; Fontaine *et al*., 2004). Subke *et al.* (2004) showed that priming of SOM decomposition can be accompanied by accelerated litter decomposition. Both SOM and litter decomposition are promoted by the presence of an intact rhizosphere where easily utilizable plant carbon derived from photosynthesis enters the soil through roots, and any associated soil microorganisms, including ectomycorrhizal (ECM) fungi (Subke *et al*., 2004; 2006).

The results of Subke *et al.* (2004) support those of Entry *et al*. (1991) who found increased litter decomposition where mycelial mats of the ECM fungus *Hysterangium setchellii* (Fischer) was present in a Douglas Fir stand, compared to adjacent experimental plots where there were no mycelial mats. Zhu & Ehrenfeld (1996) found that the presence of *Pinus rigida* (Mill.) roots stimulated litter decomposition by 18.7% over the course of a 29 month study in the New Jersey Pinelands. Dighton *et al*. (1987) used a mesocosm system to study decomposition of hide powder, chitin and cotton and found that the decomposition of all three substrates was enhanced where roots and mycorrhizas were present.

However, this evidence conflicts strongly with the classic studies of Gadgil & Gadgil (1971; 1975) which demonstrated the exact opposite conclusion, or so called 'Gadgil effect' i.e. a negative influence of the presence of an intact micorrhizosphere on litter decomposition. Gadgil & Gadgil (1971; 1975) conducted two field and one laboratory experiment, where they demonstrated decreased litter decomposition in the presence of *Pinus radiata* (L.) roots and their associated microorganisms, including ECM fungi. In both field experiments, the soil was cut with a spade (to a depth of 30 cm) to exclude both roots and ECM hyphae from 1 x 1 m plots in a *Pinus radiata* stand in the Kaingaroa State Forest, New Zealand. The second field experiment differed from the first by including a greater number of litter harvest days, and a more detailed analysis

of soil and litter samples to see how the chemical, physical, and microbial characteristics of the soil and the litter affected litter accumulation. Methodology may play a key role in explaining the apparent contradiction between the results of Gadgil & Gadgil (1971; 1975) and those of more recent studies (i.e. Subke *et al*., 2004).

Both field experiments conducted by Gadgil & Gadgil (1971; 1975) demonstrated increased amounts of litter accumulated on the soil surface in the presence of ECM roots in comparison to where roots had been excluded. The lowest mass of litter recorded at 3 and 6 months were from plots that had been cut, dug over, and roots removed. The lowest mass of litter accumulated on the surface were recorded in the treatment where plots were cut and dug, but the roots were not removed. The results of their laboratory experiment (Gadgil & Gadgil, 1975) also demonstrated decreased litter decomposition in the presence of mycorrhizal roots when compared to non-mycorrhizal roots.

The 'Gadgil effect' has been observed in subsequent litter decomposition studies. Chuyong *et al.* (2002) found that litter decomposed faster in plots where there was a low abundance of ECM trees, than it did with a high abundance of ECM trees. Faber & Verhoef (1991) conducted a trenching experiment, where roots were allowed to grow back into some experimental plots, but were permanently excluded from others in a 33 year old *Pinus nigra* (Arnold) plantation in the Netherlands. Where ECM roots were present, they observed a 2% decrease in litter decomposition rate. Fisher & Gosz (1986) conducted a cellulose decomposition experiment and found decreased decomposition of this substrate in the presence of roots in a mixed conifer forest in New Mexico. Genney *et al.* (2004) also found that decomposition of fluorene (a polycyclic aromatic hydrocarbon), was slower in the presence of *Pinus sylvestris* (L.) roots and their associated ECM fungi in a microcosm experiment.

So the influence of the presence of roots and associated ECM fungi on litter decomposition is complicated, with evidence for both positive and negative interactions. In addition, several studies have demonstrated no effect at all. Staaf (1988) found that the presence of ECM roots had no effect on litter decomposition during a trenching experiment in two forest sites in southern Sweden, one with mor

soil and the other with mull. Cuenca *et al*. (1983) selectively excluded vesicular-arbuscular mycorrhizal roots from litter bags, by periodically lifting the bags from the soil surface in a coffee plantation. They found that the presence of roots tended to positively influence the rate of litter mass loss, but this was only significant on one occasion. Chigineva *et al*. (2009) studied litter decomposition in the presence and absence of ECM roots with a sucrose addition treatment. They found a slight increase in decomposition in the presence of ECM roots one year, and a slight decrease the next year. A recent litter decomposition experiment in combination with girdling was carried out in 2 year old and 24 year old Eucalyptus plantations in Guangdong Province, southern China and also showed no effect of the presence of an intact rhizosphere on litter mass loss (Wu *et al.,* 2011).

In the tropical forests of Guyana, Henkel (2003) reported that the depth of litter was higher in two plots where the ectomycorrhizal tree *Dicymbe corymbosa* (Spruce ex Benth) had achieved monodominance, than in adjacent mixed forest plots. Mayor & Henkel (2006) conducted an experiment where, in the same location, trenching took place in the monodominant stand alongside a litter bag experiment. They found no difference in the decomposition rate of mixed or *D.corymbosa* litter in the monodominant stand between trenched and un-trenched plots. Though the *D. corymbosa* litter decomposed more slowly than the mixed species litter when incubated in the mixed forest, there was no difference in the decomposition of the mixed litter between the adjacent monodominant, and mixed forest plots. It was suggested that there may have been more specialised saprotrophic decomposers present in the *D. corymbosa* stand which meant the native litter was decomposed more effectively.

Gadgil & Gadgil (1971; 1975) suggested that the decrease in litter decomposition they observed in the presence of ECM roots could be explained by ECM fungi suppressing the activity of saprotrophic microorganisms, which are known to have a greater ability to decompose complex substrates (Colpaert & Van Tichelen, 1996; Hobbie *et al.,* 1999); this suppression would then lead to a decrease in the rate of litter decomposition in the non-trenched plots. Saprotrophic fungi rely on decaying organic matter as their sole energy source, with ECM fungi having an advantage by receiving

an easily utilisable energy source through their association with the plant root. A variety of explanations for the suppression of saprotrophic decomposers by ECM fungi have been proposed. They include: the creation of a physical barrier by proliferating and occupying space (Marx, 1969); the ability of ECM fungi to make their surroundings more acidic and therefore create conditions unsuitable for other soil microorganisms (Rasanayagam & Jeffries, 1992; Schelkle & Peterson, 1996), the secretion of antimicrobial compounds (Garrido *et al*., 1982; Duchesne *et al.*, 1988), or through resource competition, taking up and immobilising key nutrients such as nitrogen and phosphorus so they are unavailable for use by other soil microorganisms (Stark, 1972; Abuzinadah *et al*., 1986; Bending & Read, 1995). So there is evidence to support the observation of the suppression of saprotrophic decomposers by ECM fungi, via a variety of potential mechanisms, causing a decrease in litter decomposition.

However, there is an alternative explanation which could explain the results of Gadgil & Gadgil (1971; 1975). Koide & Wu (2003) studied the decomposition of litter and F-layer material in a *Pinus resinosa* (Aiton) plantation, relating the number of ECM root tips present in "envelopes" containing litter to the mass loss of the litter, and also the gravimetric moisture content of the litter in each bag. They found that high densities of ECM roots were associated with lower litter decomposition rates, and also with decreased litter moisture content. This work of Koide & Wu (2003) suggests that the effect of the presence of ECM roots on water availability could offer an explanation for the 'Gadgil effect'.

This alternative explanation is consistent with criticisms of the field experiments of Gadgil & Gadgil (1971; 1975), that there was no control for water uptake by ECM roots. Gadgil & Gadgil (1971; 1975) noted higher soil moistures in the trenched plots when compared to control plots, and they also acknowledged that more extreme differences between treatments in terms of soil moisture could have occurred, undetected between the sampling dates. It is conceivable that differences in litter decomposition by Gadgil & Gadgil (1971; 1975) were caused simply by soil moisture differences. The experiment carried out here was designed specifically to investigate this potential moisture explanation for the 'Gadgil effect'.

Litter decomposition rates have been studied using a number of approaches such as creating fibre glass mesh layers, where litter 'sandwiches' of litter cohorts are separated (Anderson, 1973; Singh & Gupta, 1977; Binkley, 2002). Others have measured carbon loss from litter via respiration (Anderson & Ineson, 1982) or used isotopic tracers including ^{14}C, and more recently, ^{13}C (Hobbie *et al.,* 2004; Hanson *et al.,* 2005). Couteaux *et al.* (2001) used ^{13}C-labelled wheat straw to study decomposition in soils along a climatic transect, giving an estimate of the level of biological activity in the different soils. Isotopic measurements have the advantage that they avoid the disturbance involved with confining litter, though uniform labelling of isotopically labelled substrates can be difficult and expensive (Bromand *et al.,* 2001). Non-invasive methods have the advantage of not interfering with any of the processes leading to litter breakdown, including leaching, catabolism and fragmentation, and therefore are considered to give the best representation of litter mean residence time (MRT) in the field, when compared to other methods (Swift *et al*., 1979; Cotrufo *et al.,* 2010).

The majority of studies of litter decomposition in the field confine litter by tethering leaves or use litter decomposition bags. The litter bag technique, where litter is contained in small bags of inert material, is most commonly used as it is a cheap and relatively simple method to use, providing an estimate of leaching and microbial catabolism of litter components (see Cotrufo *et al*., 2010). Accurate measurements of mass loss can be obtained with the contents of the bag being weighed prior to, and after incubation, with no confounding of results created by litter blown in from outside experimental plots. It is acknowledged that mass loss is often slower from litter bags than from tethered or unconfined litter, as less fragmentation takes place (Bocock & Gilbert, 1957). Also a consideration of mesh size is important when using litter bags, as the exclusion of soil animals can result in an underestimate of decomposition (Anderson, 1973). All the methods available for measuring litter decomposition in the field have drawbacks, and care should be taken when comparing studies where different techniques have been used (Aerts, 1997).

For the current experiment, a mesh exclusion collar trenching technique outlined in section 1.3 (Heinemeyer *et al*., 2007) was used to selectively exclude roots and / or

ECM hyphae. It was decided that litter bags incubated inside the mesh collars would provide the most sensible solution for confining the decomposing litter, and enable an accurate comparison of litter mass loss between the various treatments. This experimental design also allowed the influence of the presence of an intact rhizosphere, and the input of simple carbon into the soil, cycled through roots and associated microorganisms to be investigated, and also any potential impacts of soil moisture on litter decomposition to be controlled. Control for water uptake by roots and mycorrhizal hyphae in the top 6 cm of the soil, was achieved using a specially developed automated feedback irrigation system. The following hypotheses were tested:

1 Under conditions of similar soil moisture, there is a slower rate of litter decomposition in treatments when roots and any associated microorganisms have been excluded.

2 In treatments where soil moisture is allowed to decrease due to root and mycorrhizal presence, there is a slower rate of litter decomposition, compared to treatments where water taken up by roots and mycorrhizal fungi has been replaced.

2.2 Materials and methods

2.2.1 Site description

The study site was a 20 year old stand of Lodgepole pine (*Pinus contorta,* Douglas) with occasional silver birch (*Betula pendula,* Roth.), situated approximately 8 km south-east of York, United Kingdom (53º54′34″N, 0º59′48″W; c. 20 m asl) with little understory vegetation. The soil was a well-draining sandy gley soil, mean annual air temperature was 9.0 ˚C and mean annual precipitation was *c*. 627 mm (Subke *et al*., 2011). For a fuller site description, see Heinemeyer *et al.* (2007).

2.2.2 Experimental treatment

PVC tubing (20 cm diameter, Plumb Centre, Wolseley, UK) was cut to 30 cm lengths to create 16 "deep" soil collars. Four windows were cut (width 6 cm, height 4 cm, situated 5 cm from the top of the collar and evenly spaced) and covered with one of two meshes (Normesh Ltd., Oldham, UK) of different gauge, to create two treatments (following the design of Heinemeyer *et al.,* 2007). The windows were positioned directly below the soil surface. Treatment S (soil only) was created using 1 µm mesh, and excluded roots and ECM hyphae. Treatment MS (mycorrhizal hyphae + soil) was created using 41 µm mesh, excluding roots but permitting ECM hyphal access (see Fig. 2.1).

Thirty shallow collars (20 cm diameter) were randomly assigned positions in the *Pinus contorta* stand on 15th April 2008**,** with potential collar locations being rejected if the distance from the nearest tree was less than 0.5 m or greater than 2 m, collars were spaced at least 1 m apart. Measurements of background CO_2 efflux were conducted using a Li-8100 infrared gas analyser (IRGA) with a 20 cm survey chamber (Li-Cor Bioscience, Lincoln, NE, USA).

Based on these pre-existing CO_2 fluxes the locations were ranked, and this ranking used to assign each collar to a specific block, in a randomised block design. Collars showing outlying CO_2 efflux were excluded from the experiment. Replacing the shallow collars used for the background survey of soil CO_2 efflux, 16 deep collars were

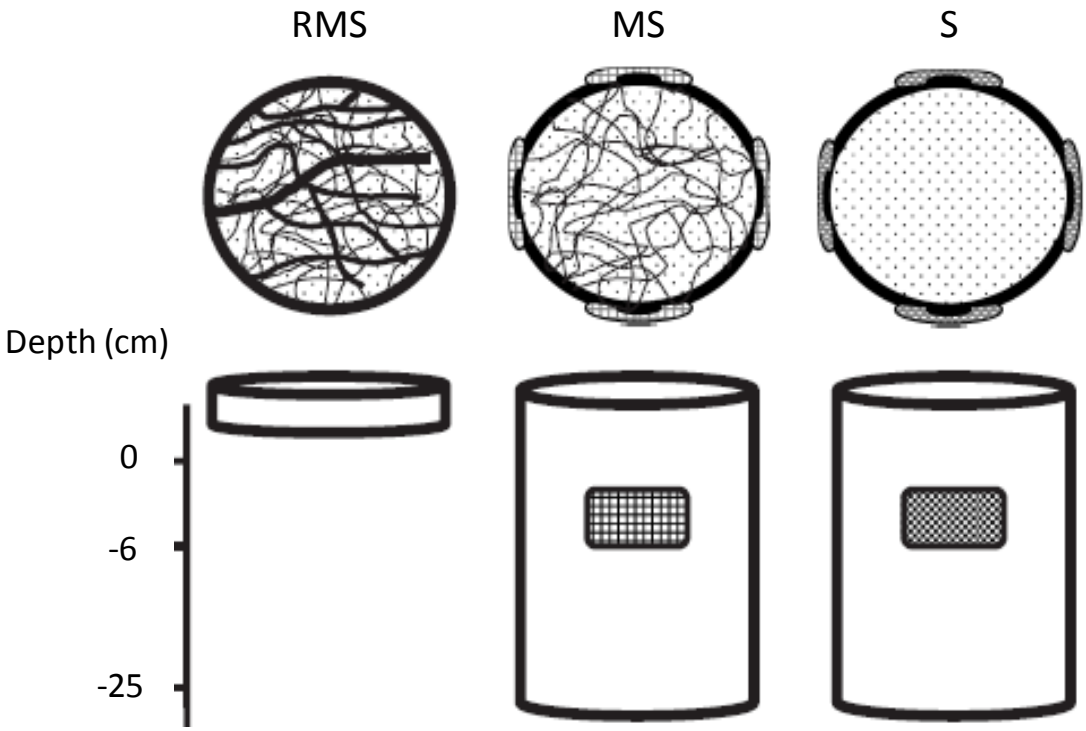

Fig. 2.1 Diagram adapted from Heinemeyer *et al*. (2007) showing the collar depth (cm) of the three PVC collar types used to create the three treatments: RMS, MS and S. Treatment RMS consisted of shallow surface collars where no soil components were excluded. Treatments MS and S involved trenching with 30 cm deep collars hammered into the soil to a depth of 25 cm ± 2 cm. Mesh, 41 µm, in the windows of treatment MS allowed in-growth of fungal hyphae, but excluded roots. Mesh, 1 µm, in the windows of treatment S excluded both roots and fungal hyphae.

hammered 25 cm ± 2 cm into the soil in the *Pinus contorta* stand in July 2008, cutting through the main concentration of roots. At the same time, 8 surface collars (20 cm diameter, Plumb Centre, Wolseley, UK), remained to create treatment RMS (roots + mycorrhizas + soil), where none of the soil components were excluded (see Fig. 2.1). Each treatment was represented in each block, with a random assignment of the treatment collars within the block.

In order to reduce variability in soil moisture over the course of the experiment, above-ground incident throughfall was excluded from all collars using PVC shields. These were constructed by cutting transparent corrugated PVC sheeting (Corulux, Ariel Plastics, Stavely, UK) into rectangles measuring 30 cm x 40 cm which were suspended at ~25 cm above the collars with four bamboo canes, and secured using cable ties (see Plate 2.1). Incident throughfall was replaced once a week with the area-weighted average volume of throughfall which had fallen during the previous week, measured using nine randomly placed throughfall collectors. The throughfall collectors were made from 6 L plastic bottles, each with a 20 cm funnel fitted with a removable nylon mesh filter to remove solid debris. The replacement volume of throughfall per collar was calculated and supplemented, when necessary, with additional throughfall collected in a large centralised water butt with incoming drainage from two large corrugated PVC sheets. No watering took place between 4th December 2009 and 19th January 2010 as there was heavy snowfall during this period and the contents of the centralised water butt were frozen.

The PVC shields also prevented the ingress of incident litter, the dry mass of which was determined and replaced by the mean dry mass of litter fall calculated for the area (*c.* 18.3 g m^{-2}), some of which was added as part of the litter bag experiments, and also on one other occasion (see Section 2.2.4).

2.2.3 Automated irrigation system

An additional two treatments were also established in order to determine any putative impacts of the various mesh treatments caused by changes in moisture status. This involved maintaining the same moisture content in the upper soil level in these additional treatments, targeted to the average measured upper soil level moisture

Plate 2.1 Photograph showing PVC shields suspended above soil collars in the *Pinus contorta* stand at Wheldrake Forest prior to the installation of the automated irrigation system and litter bag incubations.

content for treatment S. This was achieved through the addition of deionised water by a specially developed automated irrigation system (see Fig. 2.2), in addition to the weekly watering with throughfall described in Section 2.2.2. These additional treatment collars (four replicates of each treatment) are referred to as RMS(W), and MS(W). In addition, a further treatment was created to act as a control for adding deionised water. Four replicates of the S collars received the same deionised water input as treatment RMS(W), and are referred to as S(W) (see Table 2.1) For the purposes of distinguishing collars in terms of whether they received additional input from the automated irrigation system, the collars that did (RMS(W), MS(W) and S(W)) are referred to on occasion as the 'corrected collars' and those that did not (RMS, MS and S) are referred to on occasion as the 'non-corrected collars').

The irrigation system was controlled using a data logger (CR1000, Campbell Scientific Ltd., Loughborough, UK), which compared average surface soil moisture readings (top 6 cm of the soil) from three SM200 soil moisture sensors (Delta-T Devices Ltd., Cambridge, UK), in each of the treatments [RMS(W), MS(W), S); (nine in total); (see Fig. 2.2)] every five minutes. When the average soil moisture from the three soil moisture sensors in treatments RMS(W), or MS(W) fell below the average from treatment S, three submerged water garden pumps (Blagdon A4000 amphibious garden water pump) supplied fixed aliquots (3.2 ± 5% ml per minute) of deionised water to each of the RMS(W) and MS(W) collars over a three minute period.

Water was distributed to each of the collars via a 12 m length of tubing (1 x 3 mm, Silicone tubing, VWR, Lutterworth, UK), with four tubes from each of the three pumps attached to a four-way manifold. The opposite end of each 12 m length of tubing was knotted and regular perforations made along 40 cm from the knot. Previous testing had determined that arranging the tube in a spiral formation in the collar allowed an even spread of water across the soil surface. The data logger was programmed so that whenever the RMS(W) pump was activated, the S(W) pump was also switched on (see Fig. 2.2). A copy of the Campbell program used to control the automated watering feedback is given in Appendix 1. There were technical problems with the system during summer 2009, leading to fine-tuning and increased moisture control of treatment

Table 2.1 Summary of six treatments created using PVC soil collars, and water correction from the automated irrigation system. Treatments RMS and RMS(W) consisted of shallow surface collars where no soil components were excluded. Treatments MS, MS(W), S, S(W) consisted of 30 cm collars hammered to a depth of 25 cm ± 2 cm. Each treatment had 4 replicates.

Treatment Code	Brief treatment description	Collar Depth	Mesh Gauge
RMS	Roots+ ECM hyphae + background soil	Surface	-
RMS(W)	RMS + automated moisture input	Surface	-
MS	ECM hyphae + background soil with roots excluded	25 cm	41 µm
MS(W)	MS plus automated moisture input	25 cm	41 µm
S	Background soil with roots and ECM hyphae excluded	25 cm	1 µm
S(W)	Control for adding deionised water	25 cm	1 µm

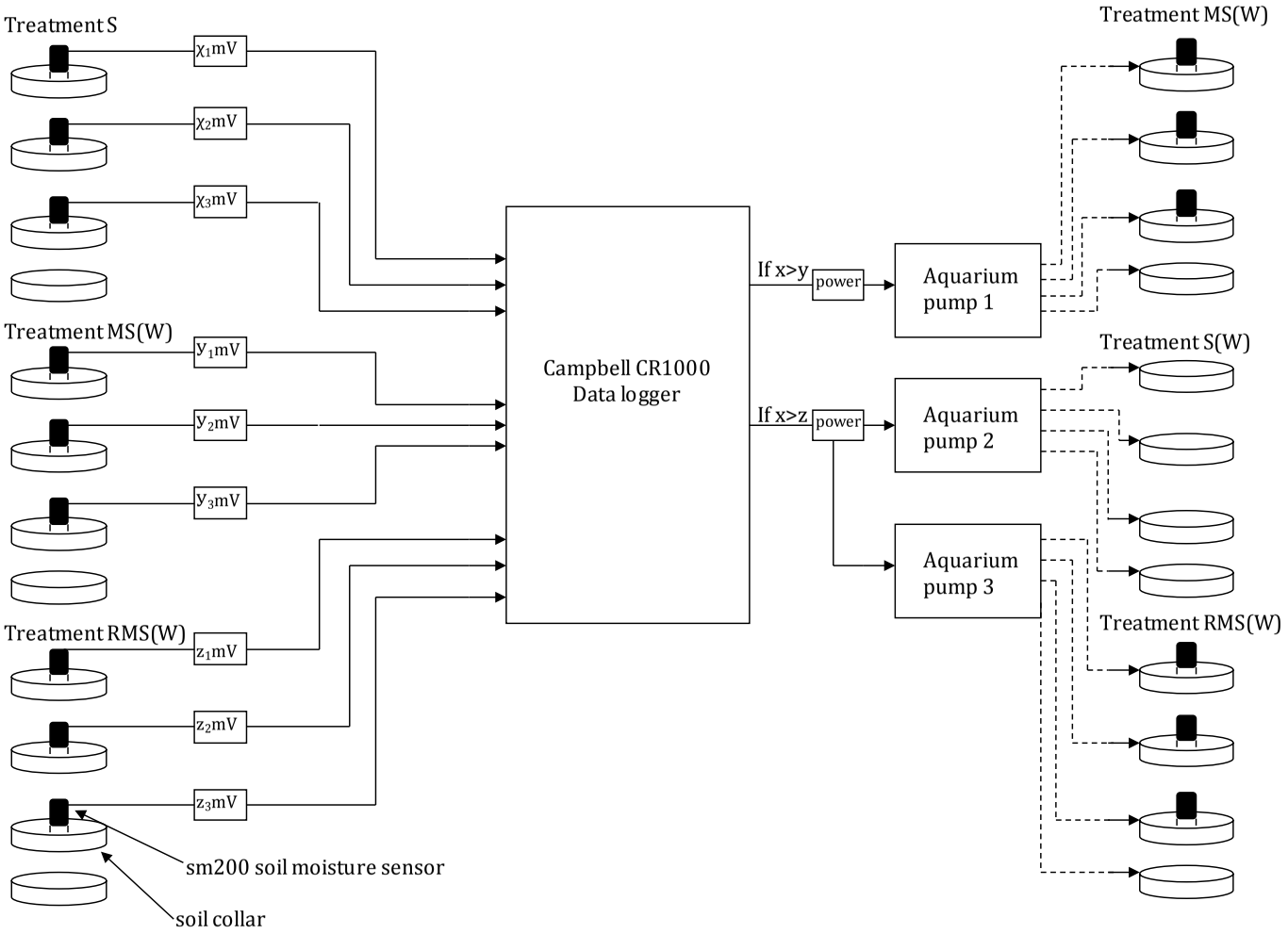

Fig. 2.2 The automated irrigation system used to create treatments RMS(W), MS(W) and S(W). The system was coordinated by a Campbell CR1000 data logger, linked to 9 SM200 soil moisture probes and three aquarium pumps. When the average soil moisture from treatments MS(W) and RMS(W) fell below that of treatment S, Aquarium pump 1 and/or pumps 2 and 3 were switched on to deliver fixed aliquots of water from a storage tank.

RMS(W) in particular during summer 2010.

2.2.4 Litter bag incubations

2.2.4.1 I2009 (Installed 2009) litter bags

Recently fallen Lodgepole pine (*Pinus contorta*) litter was taken from the soil surface at the experimental site on 10th April 2008 and returned to the laboratory, where it was air-dried over several weeks. Litter bags, *ca.* 5 x 9 cm, were constructed from polyester net curtain material with an aperture size of 0.5 mm^2. Initially two pieces of material were sewn together along three sides to form the bag using polyester thread. A noted mass, *ca.* 1 g, of dried litter was added to each litter bag alongside a piece of Dymo tape (9 mm embossing tape, Dymo Store, Cambridgeshire, UK) as recommended by Berg & Lazkowski (2006). Each piece of tape had a unique code punched into it which was then recorded alongside the initial litter dry mass in each bag for use in subsequent mass loss calculations.

Eleven litter bags were deployed in each of the 24 collars on 1st July 2009, with one additional bag being installed in four collars and then immediately harvested and brought back to the laboratory. These immediately returned four bags were used to calculate the small average physical mass loss associated with the construction, transit, moistening and installation of the litter bags.

To ensure the same total dry mass of litter was present in each of the collars, surface litter was removed before the litter bags were installed. A litter survey was carried out on 10th April 2008 with 40 locations being randomly sampled using a 0.5 x 0.5 m quadrat. The average fresh mass of litter was measured, with samples being dried in order to establish the fresh to dry mass ratio. Based on these measurements, average mass of dried native litter (5.8 g dry mass) were then moistened and placed in the collars surrounding the litter bags. Additional litter was added to the cores on 21st December 2009 (as part of I2010 litter bags, see below) and also a smaller amount on 18th April 2010 in order to simulate litterfall at the site, which although greatest in the Autumn, occurred to a certain extent all year round. Litterfall was quantified by

measuring the dry mass of the needles accumulated on the PVC shields suspended above the soil collars.

2.2.4.2 I2010 (Installed 2010) litter bags

Following a failure of the automated irrigation system in summer 2009 (insufficient moisture addition to treatment RMS(W) from 11th August 2009 to 14th October 2009), a supplementary set of litterbags (six in each of the 24 collars) were deployed on 21st December 2009 in order to extend the experiment. They were constructed using the same method as for the I2009 litter bags, but using bag material marked with a serrated edge to make them easily distinguishable from the original series of litter bags already in the collars. The litter added in order to simulate litterfall, and also that used in the I2010 litter bags was from the same collection of laboratory-dried native litter that had been used in the construction of the I2009 litter bag series and stored in dry conditions for later use.

2.2.5 Harvesting and processing of the litter bags

For the I2009 series of litter bags, one litter bag per collar was harvested on nine occasions; 10th July 2009, 11th August 2009, 30th September 2009, 26th November 2009, 28th January 2010, 07th April 2010, 4th June 2010, 18th August 2010 and 05th October 2010. For the second, I2010 series of litter bags, four harvests were carried out on 2nd March 2010, 14th May 2010, 17th August 2010 and 17th November 2010 with the same method of processing employed for both series of litter bags. Processing involved the careful removal of litter from the bags, with any fine roots and other additional material being gently removed. Fresh mass (g) of the litter was recorded and then the litter was dried at 75^{o}C for five days (to constant dry mass), before the dry mass (g) was recorded in order to establish mass loss.

2.2.6 Measurement of environmental parameters

Soil temperatures at depths of 5 cm and 10 cm were logged from replicate temperature probes (3 per depth, per site) linked to a DL2e Data Logger (Delta-T Devices Ltd., Cambridge, UK) every 10 minutes during the experiment. Average soil

temperatures at depths of 5 cm and 10 cm were calculated from these data, with periods when individual probes were faulty excluded from data analysis.

Soil moisture data were logged every 10 minutes from the nine SM200 soil moisture probes (Delta-T Devices Ltd., Cambridge, UK) permanently situated in soil collars from treatments RMS(W), MS(W) and S (three probes per treatment) as part of the automated irrigation system (see Fig. 2.2).

Additionally, average soil % moisture readings were taken from three locations in each of the 24 soil collars during hand-held surveys, generally prior to throughfall additions. A hand-held soil moisture probe was used, which consisted of a Meterman DM7B multimeter (Amprobe, Washington, USA) linked via a custom battery interface (Electronics Workshop, Department of Biology, University of York) to an SM200 soil moisture probe. These readings provided a comparison of the soil moisture between treatments with and without water addition by the automated irrigation system. The three readings per collar taken during hand-held moisture surveys also provided a check of whether water from the automated irrigation system was being evenly distributed across the soil surface, and were used in subsequent analyses to compare the function of the automated irrigation system between the summers of 2009 and 2010.

Measurements of the throughfall volumes collected from nine throughfall collectors during the experiment were also recorded, alongside the average amount of throughfall added to each collar under the PVC shields.

2.2.7 Data Analysis

The experiment followed a randomised block design, and a variety of statistical approaches were used during data analysis. Where possible, treatment differences were tested using two-way ANOVA with block and treatment (the six soil collar and watering treatments summarised in Table 2.1) as factors. Where sample distributions deviated from normal, data were transformed or the equivalent non-parametric tests were carried out. Further specific information regarding the use of different statistical

tests is detailed in the Results section. All statistical tests were carried out using SAS software package v. 9.2 (SAS Institute Inc., Cary, NC, USA).

2.2.7.1 Litter mass loss

The initial litter dry mass for each litter bag prior to incubation was adjusted with a correction term derived from the mass loss associated with litter bag construction, transit, moistening and installation, which was measured using the four bags that were harvested immediately following litter bag installation (see Section 2.2.4). Litter mass loss was expressed as the percentage mass remaining of the corrected initial litter dry mass (see Eq. 1).

$$\% \text{ mass remaining} = \frac{\text{litter dry mass after incubation (g)}}{\text{corrected litter dry mass before incubation (g)}} \times 100 \qquad \text{(Eq. 1)}$$

2.2.7.2 Litter moisture content

Following each harvest, the fresh and dry mass of each litter bag were used to calculate the litter % moisture content (see Eq. 2, after Allen *et al*., 1989).

$$\text{Litter \% moisture} = \frac{\text{loss in mass on drying (g)}}{\text{initial sample mass (g)}} \times 100 \qquad \text{(Eq. 2)}$$

2.2.7.3 Soil moisture content

Voltage output readings from the nine SM200 soil moisture probes permanently situated in collars as part of the automated irrigation system were converted to soil % moisture content (volumetric) by the Campbell CR1000 logger (Campbell Scientific Ltd., Loughborough, UK) using an equation for organic soils provided in supporting information for the SM200 soil moisture probes (Delta-T Devices Ltd., Cambridge, UK) (see Eq. 3).

$$\text{Soil \% moisture (volumetric)} = (-0.039 + 2.091V - 5.029V^2 + 7.907V^3 - 5.98V^4 + 1.758V^5) \times 100$$

(where V is the SM200 output in volts) (Eq. 3)

In order to convert voltages obtained during hand-held soil moisture surveys of the 24 soil collars into soil water content values, a calibration was undertaken, using soil from

the *Pinus contorta* stand at Wheldrake Forest. Soil samples from the top 10 cm of soil below the litter layer were brought back to the lab. Soil was sifted and then equal amounts were weighed into four plant pots with mesh at the bottom. Deionised water was added to the pots until the soil was fully saturated. Mass loss was measured at intervals over the next few days, alongside voltage outputs from the hand-held soil moisture probe (SM200), and the results were used to calculate gravimetric soil moisture content (see Eq. 4).

$$SWC_g = \frac{m_s - m_d}{m_d} \times 100 \qquad \text{(Eq. 4)}$$

(where SWC_g is the gravimetric soil water content (g water (g soil)$^{-1}$), m_s is the total soil mass, and m_d is the mass of dry soil)

A 4th degree polynomial regression (r^2 = 0.9966) was used to convert voltage readings obtained using the SM200 probe to SWC_g values (see Eq. 5)

$$SWC_g = -923.4\ V^4 + 2001\ V^3 - 1451\ V^2 + 512\ V - 10.69 \qquad \text{(Eq.5)}$$

(where V is the SM200 output in volts)

2.2.7.4 Regression analysis

Values of k, the decomposition constant (after Jenny *et al*. 1949; Olson, 1963) were calculated for each of the 24 collars for both the I2009 (16 months) and I2010 (11 months) litter bag incubations. This was achieved by plotting a linear regression of the natural log of the % mass remaining data against time, for each collar individually, the slope of each line being k.

Individual simple regressions and forward stepwise multiple regressions (SAS v 9.2) were used to assess whether environmental factors could explain any variation in litter mass loss. These environmental factors included soil temperature at depths of 5 cm and 10 cm, soil moisture content, and the throughfall volumes added to the collars at 7-10 day intervals during the experiment.

2.3 Results

2.3.1 Litter mass loss

The I2009 litter bag series showed a slow decline in % mass remaining during the 491 days of the experiment (see Fig. 2.3). The greatest rate of mass loss occurred over the first 121 days, and was followed by a steadier decline with an average total mass loss of 22.8% by the end of the experiment. Percentage mass remaining data were arcsine square root transformed to achieve normality, followed by repeated measures ANOVA with treatment as the main effect. There were no significant differences in the mass loss of litter between the different treatments, and no obvious trends due to the treatments ($F=0.712$, $p>0.622$, see Fig. 2.3).

A slow decline was also observed in the % mass remaining in the I2010 litter bag incubation (see Fig. 2.4). This set of supplementary litter bags showed an average total mass loss of 15.9% over the 331 day experimental period. Unlike the I2009 series, the I2010 series of litter bags had consistent moisture control from the automated irrigation system for the whole incubation period. This ensured that the moisture contents in the top 6 cm of soil in treatments RMS(W),and MS(W) were maintained at the same level as treatment S for the I2010 series.

For the I2010 series, the initial rapid rate of decomposition remained for 144 days, until the second harvest. At the third harvest, litter mass loss was not as great as for the second harvest, deviating somewhat from the expected exponential decay curve. Again, data were arcsine square root transformed to achieve normality, and there were no significant differences in mass loss between the treatments during the I2010 series, and no obvious trends due to the treatments ($F=0.667$, $p>0.645$, see Fig. 2.4). Despite being established in the soil collars in winter, at the end of December 2009, litter from the I2010 litter bag series had a greater initial rate of mass loss than litter from the litterbags in the I2009 series which had been left to decompose from the start of June 2009 (see Fig. 2.5). However, after the second harvest, the rate of decomposition of litter contained in the I2010 litter bags slowed, and for the following two harvests the total average mass remaining was greater than for the I2009 series.

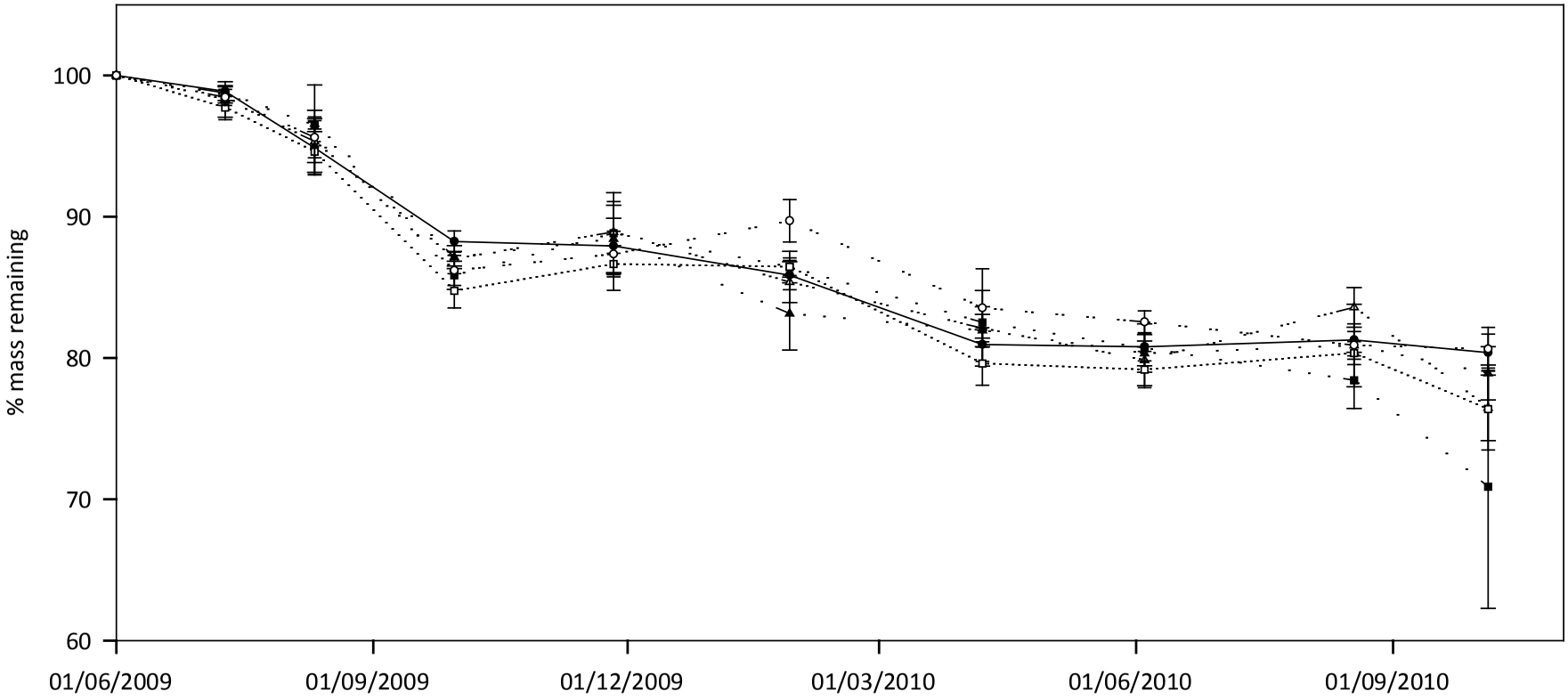

Fig. 2.3 Percentage mass remaining over time for the I2009 litter bag series. Treatments RMS(W), MS(W) and S(W) are represented by solid squares ■ , circles ● and triangles ▲, respectively. Treatments RMS, MS and S are represented by open squares □, circles ○ and triangles Δ, respectively. Error bars represent ± 1 SE (n = 4).

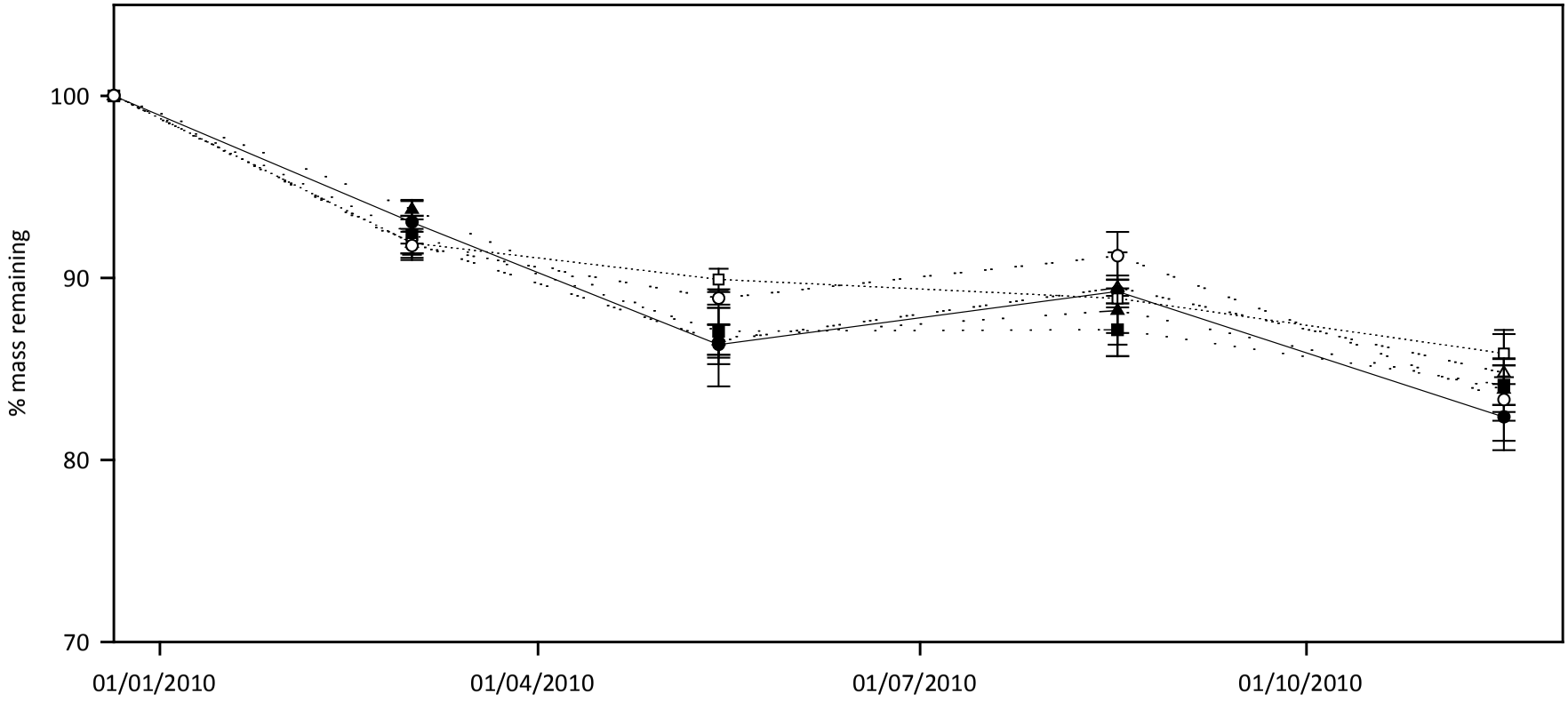

Fig. 2.4 Percentage mass remaining over time for the I2010 litter bag series. Treatments RMS(W), MS(W) and S(W) are represented by solid squares ■ , circles ● and triangles ▲, respectively. Treatments RMS, MS and S are represented by open squares □, circles ○ and triangles Δ, respectively. Error bars represent ± 1 SE (n = 4).

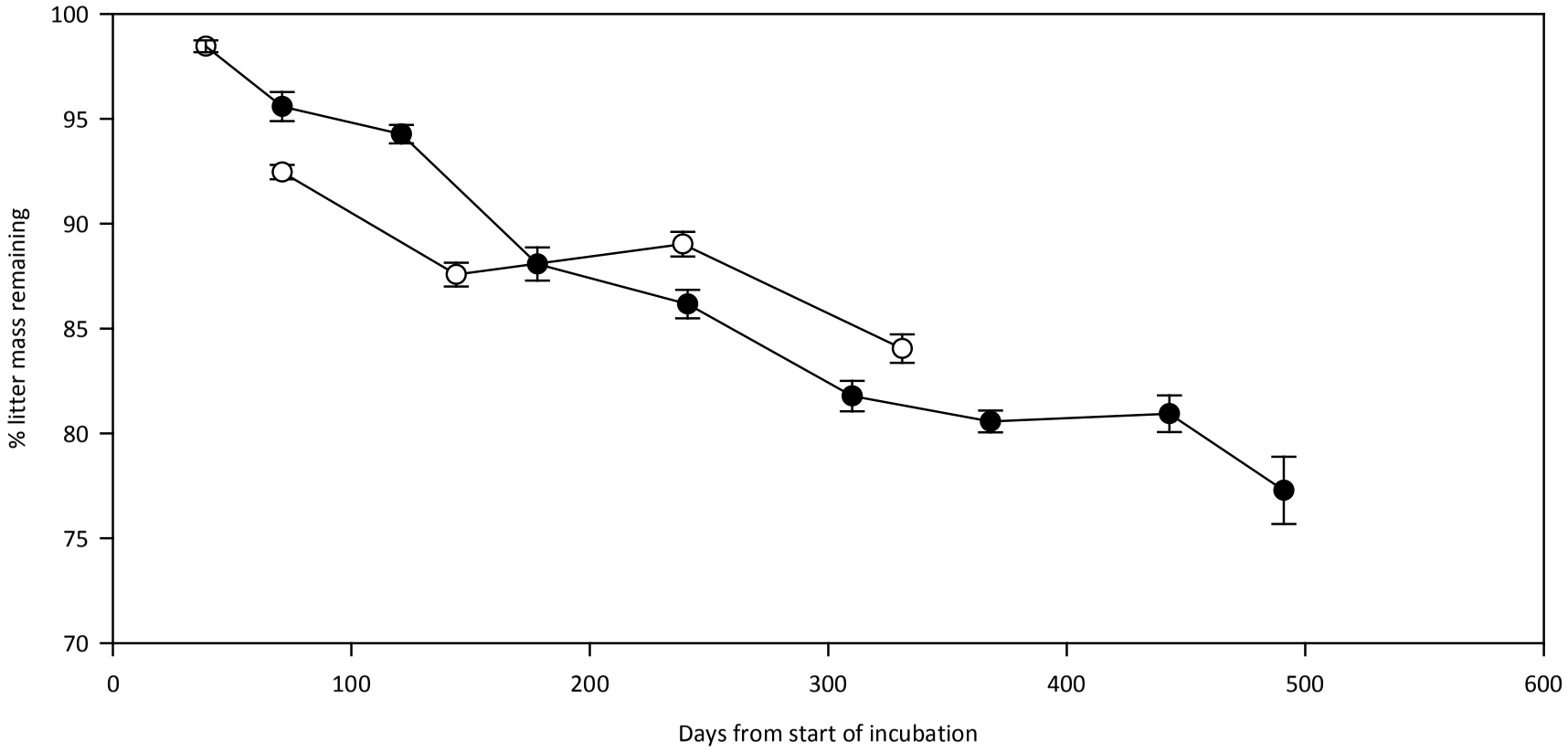

Fig. 2.5 Average mass remaining of litter, from all collars plotted against the number of days since the start of the two litter bag incubations. Series I2009 and I2010 are represented by solid circles ● and open circles ○, respectively. Error bars represent ± 1 SE (n = 24).

2.3.2 Litter moisture content

Analysis of the % moisture of litter for both the I2009 and I2010 series litterbags showed fluctuations in the moisture content of the litter across the experimental periods, with lower average litter moisture contents during the summer than the winter months (see Figs. 2.6 and 2.7). The average litter % moisture was calculated for each of the 24 collars for the I2009 and I2010 series separately. The resulting 24 values were compared using Friedman's non-parametric two-way analyses of variance with treatment and block as factors to identify any significant differences between the average litter % moisture of the different treatments at harvest. Results showed that there was a significant difference between treatments for litter % moisture for the I2009 litter bag series ($F= 3.56$, $p< 0.03$). *Post-hoc* analysis (Duncan's multiple range test on ranked data) showed that litter harvested from collars in treatments S(W) and RMS had a significantly higher average % moisture than litter harvested from collars in treatments S and MS (see Fig. 2.8). The same effect was not observed for the I2010 litter bag series, with no significant differences between treatments in terms of average litter moisture content ($F= 0.96$, $p>0.46$, see Fig. 2.8).

In order to establish whether fluctuations in the litter moisture between harvests could be explained by the underlying soil moisture, litter % moisture values from the nine collars permanently containing SM200 soil moisture probes (averages from treatments S, MS(W) and RMS(W), n=3) were natural log transformed to achieve normality. These data were then plotted against the average soil moisture content (top 6 cm of soil, % volumetric) on litter harvest days (between 9 am and 11 am prior to watering). The average soil moisture content values used in the correlation had been logged by the data logger running the automated irrigation system. There was a weak association ($r^2 = 0.1831$, $F= 5.6$, $p < 0.03$) between litter moisture and volumetric soil moisture on the litter harvest days for the I2009 litter bag series, and no pattern in terms of the treatments (see Fig. 2.9).

As there were only four harvest days for the I2010 litter bag series, these data were amalgamated with those from the I2009 series and the regression analysis was

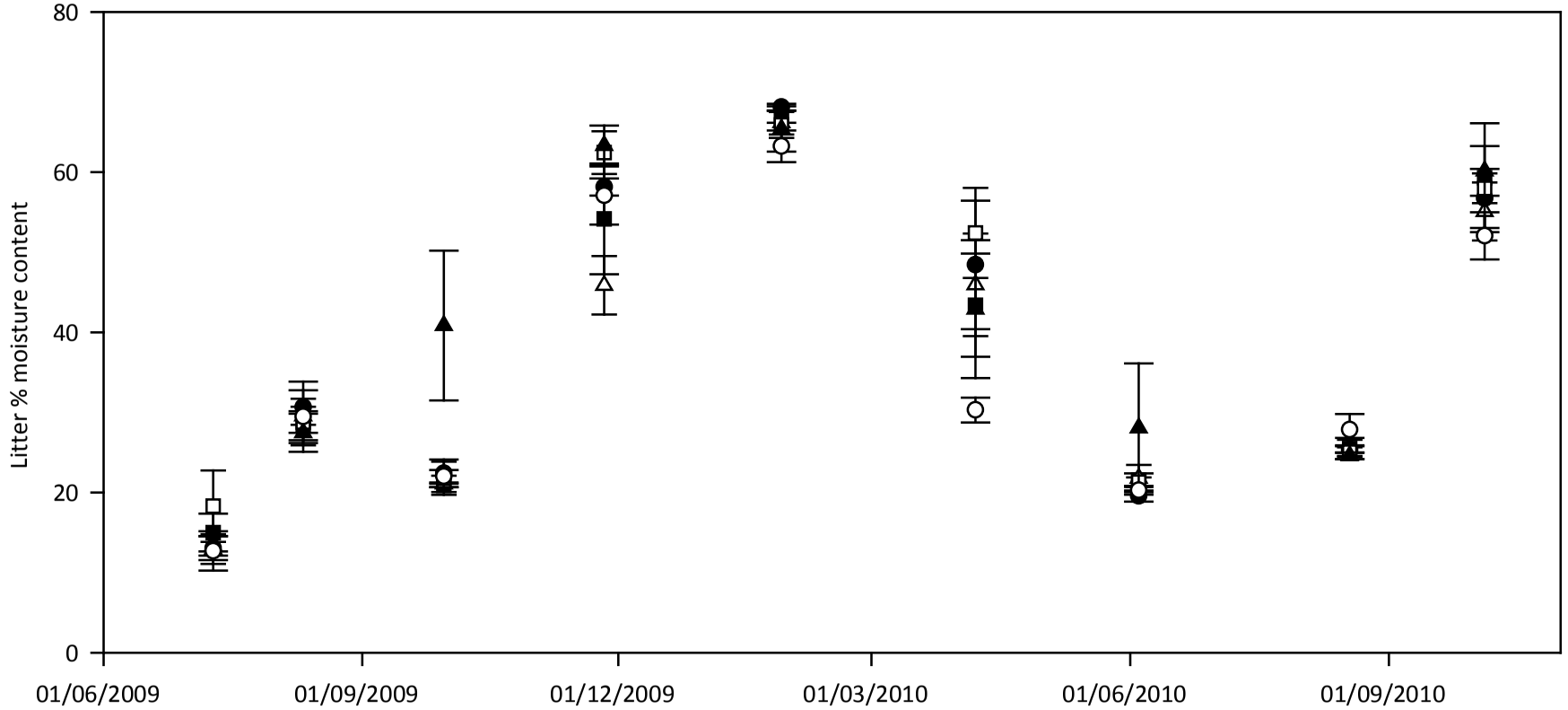

Fig. 2.6 Percentage moisture content of litter over time for the I2009 litter bag series. Treatments RMS(W), MS(W) and S(W) are represented by solid squares ■ , circles ● and triangles ▲, respectively. Treatments RMS, MS and S are represented by open squares □, circles ○ and triangles Δ, respectively. Error bars represent ± 1 SE (n = 4).

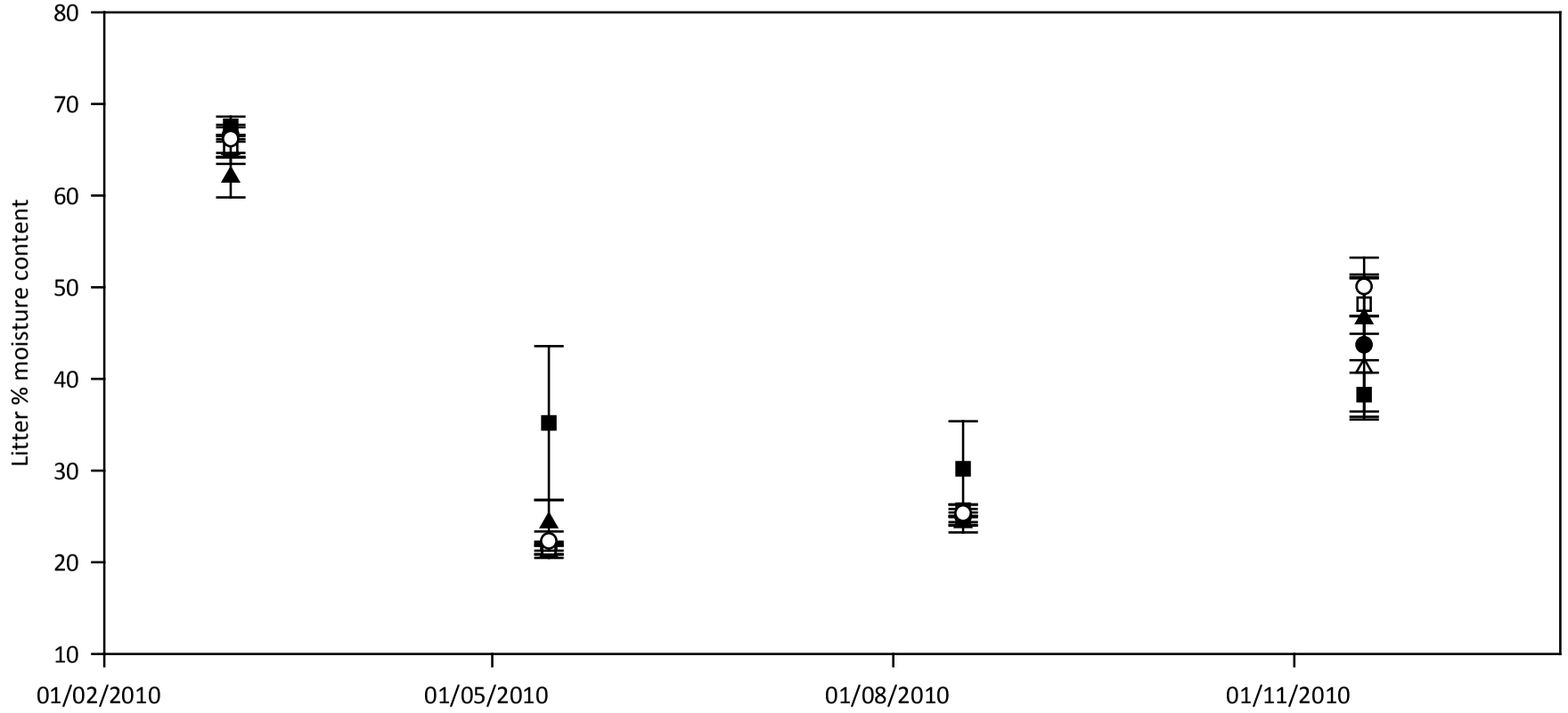

Fig. 2.7 Percentage moisture content of litter over time for the I2010 litter bag series. Treatments RMS(W), MS(W) and S(W) are represented by solid squares ■ , circles ● and triangles ▲, respectively. Treatments RMS, MS and S are represented by open squares □, circles ○ and triangles Δ, respectively. Error bars represent ± 1 SE (n = 4).

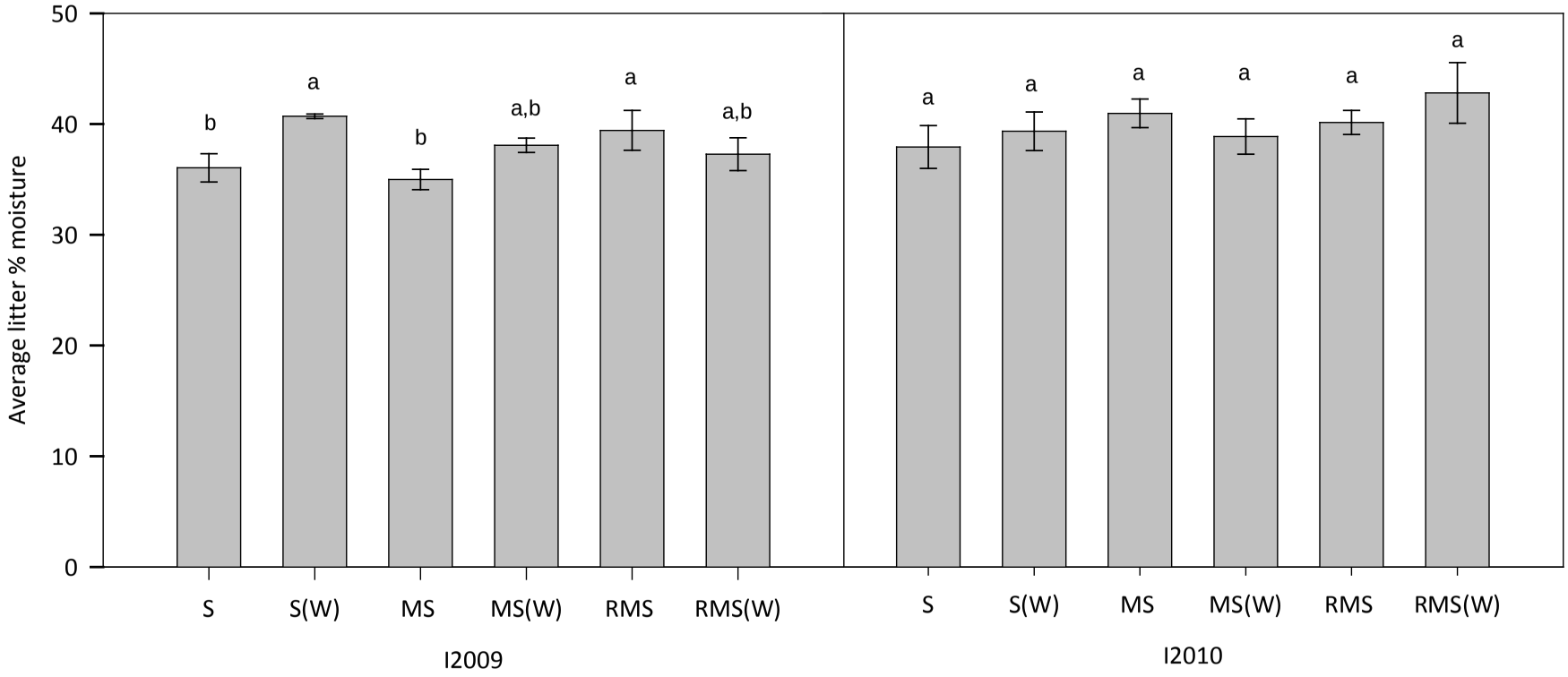

Fig. 2.8 Average moisture content of litter in the different treatments for the I2009 and I2010 litter bag series. Within each series, treatments which differ significantly in terms of litter moisture content have different letters. Error bars represent ± 1 SE (n = 4).

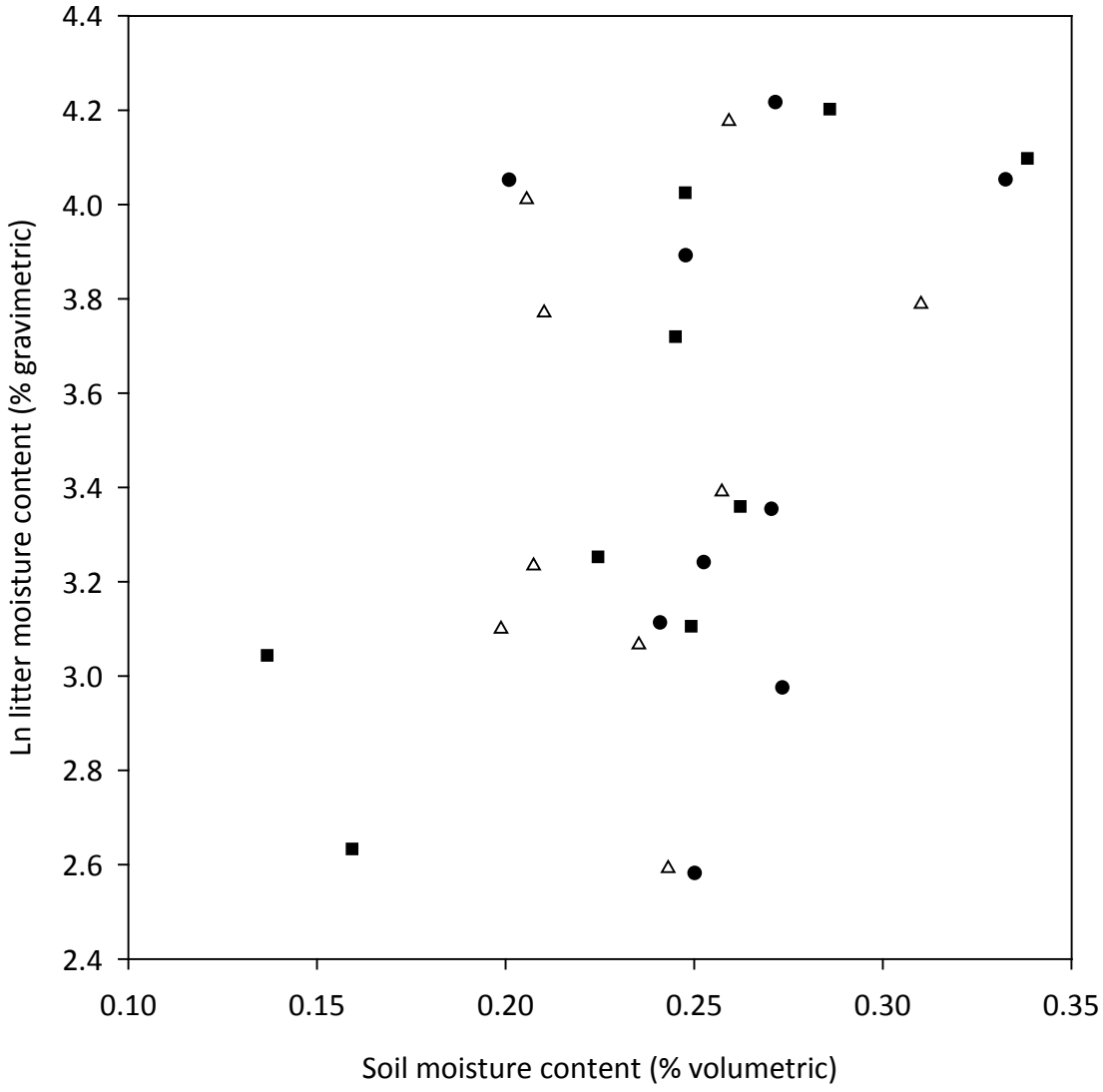

Fig. 2.9 Average volumetric soil moisture contents for collars containing permanent SM200 soil moisture probes as part of the automated irrigation system (averages from treatments S, MS(W) and RMS(W), three probes from each), plotted against the natural log of the average litter % moisture measured on litter harvest from the same collars during the I2009 litter bag incubation series. Treatments RMS(W) and MS(W) are represented by solid squares ■ and circles ●, respectively. Treatment S is represented by open triangles Δ. For each data point n=3.

repeated. There was no significant association between the variables (r^2 = 0.0682, F= 2.71, p > 0.1) and there was no pattern in terms of the treatments (see Fig. 2.10).

2.3.3 Soil moisture content

Fig. 2.11 shows the moisture content (% gravimetric) in the top 6 cm of the soil of treatments S, S(W), MS(W) and RMS(W) measured by hand held moisture survey. These surveys were conducted prior to watering of collars with throughfall, and the soil moistures were consequently likely to have been at times when the soil was at its driest. Initial technical problems with the automated irrigation system are evident during summer 2009 (see Fig. 2.11), when treatment RMS(W) had a consistently lower soil moisture than treatments MS(W) and S. Treatment S(W) had a higher soil moisture than the other corrected treatments during the summer, which is to be expected as it was receiving irrigation to the same level as treatment RMS(W), but with roots excluded.

For the treatments not receiving additional moisture through the automated irrigation system, the hand held surveys revealed clear differences in soil moisture in the presence of plant roots, with treatment RMS generally having lower soil % moisture than treatments MS and S (see Fig. 2.12), and shows that the presence of plant roots has the effect of drying the soil to a much greater extent than the presence of any in-growth of mycorrhizal hyphae.

Figs. 2.13 and 2.14 show more clearly the differences in soil moisture between treatments RMS and RMS(W) and MS and MS(W) during the experiment. Treatment RMS has a lower soil % moisture content than treatment RMS(W), as would be expected during the summer months. The difference in soil % moisture between treatments MS and MS(W) is less pronounced, as the presence of ECM hyphae did not affect soil moisture to the same extent as the presence of roots.

Friedman's non-parametric two-way analyses of variance (see Section 2.3.2 for a description of how the data were averaged to avoid pseudo replication) with treatment and block as factors were conducted for three distinct periods: (A) prior to

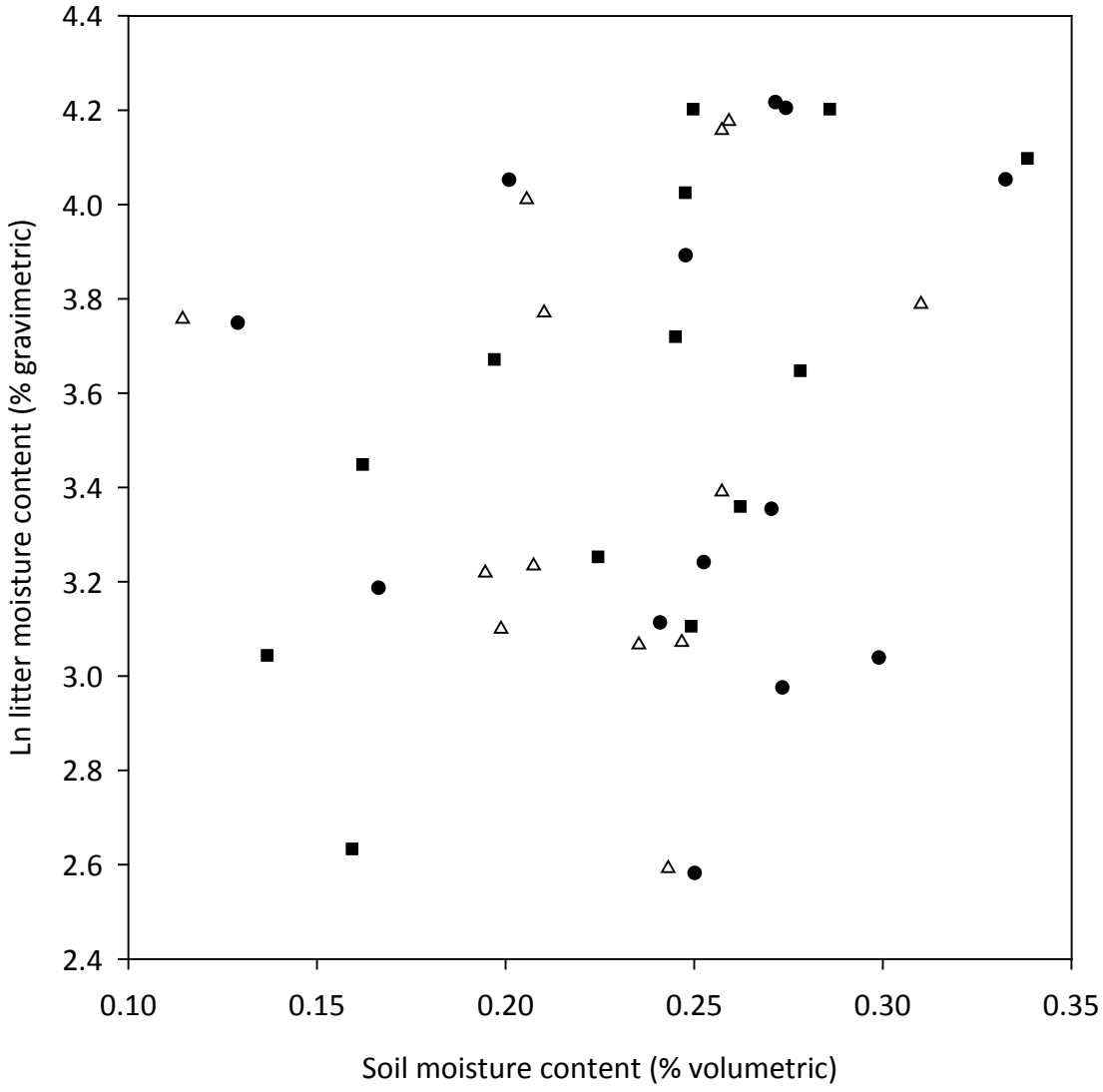

Fig. 2.10 Average volumetric soil moisture contents for collars containing permanent SM200 soil moisture probes as part of the automated irrigation system (averages from treatments S, MS(W) and RMS(W), three probes from each), plotted against the natural log of the average litter % moisture measured on litter harvest from the same collars during both the I2009 and I2010 litter bag incubation series. Treatments RMS(W) and MS(W) are represented by solid squares ■ and circles ●, respectively. Treatment S is represented by open triangles Δ. For each data point n=3.

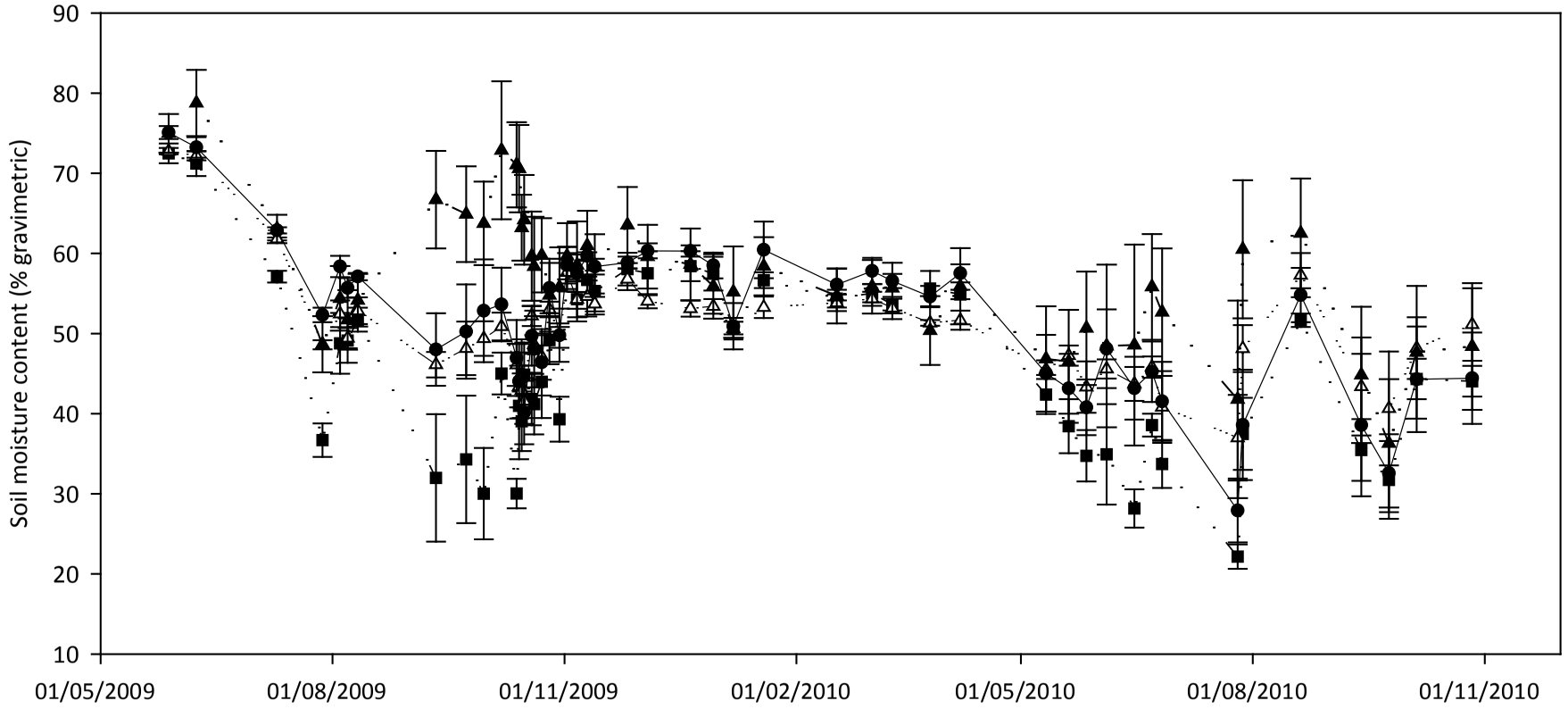

Fig. 2.11 Average gravimetric soil moisture contents from treatments RMS(W), MS(W), S(W) and S over time, measured during hand-held moisture surveys. Treatments RMS(W) ,MS(W) and S(W) are represented by solid squares ■ circles ● and triangles ▲, respectively. Treatment S is represented by open triangles Δ. Error bars represent ± 1 SE (n = 4).

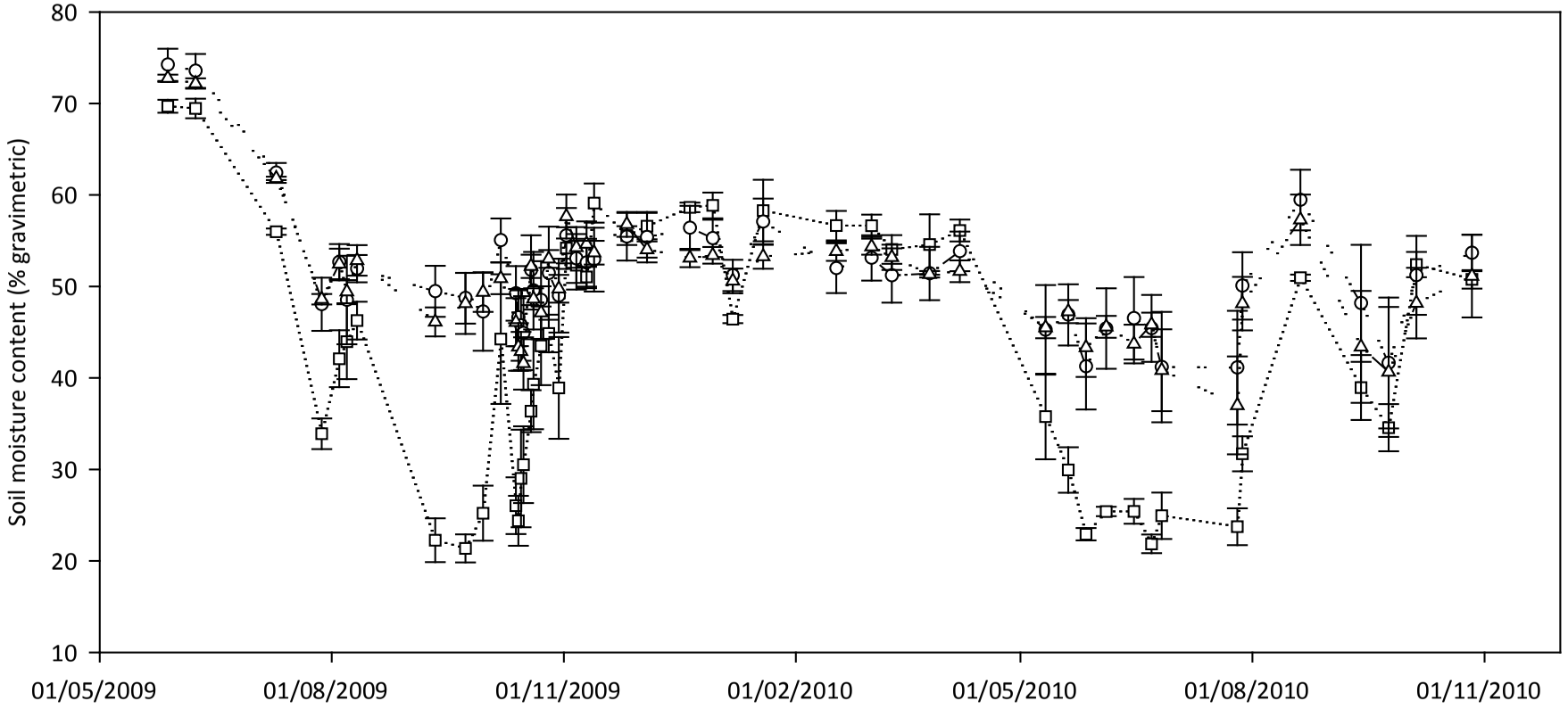

Fig. 2.12 Average gravimetric soil moisture contents from treatments RMS, MS and S over time, measured during hand-held moisture surveys. Treatments RMS, MS and S are represented by open squares □, circles ○ and triangles Δ, respectively. Error bars represent ± 1 SE (n = 4).

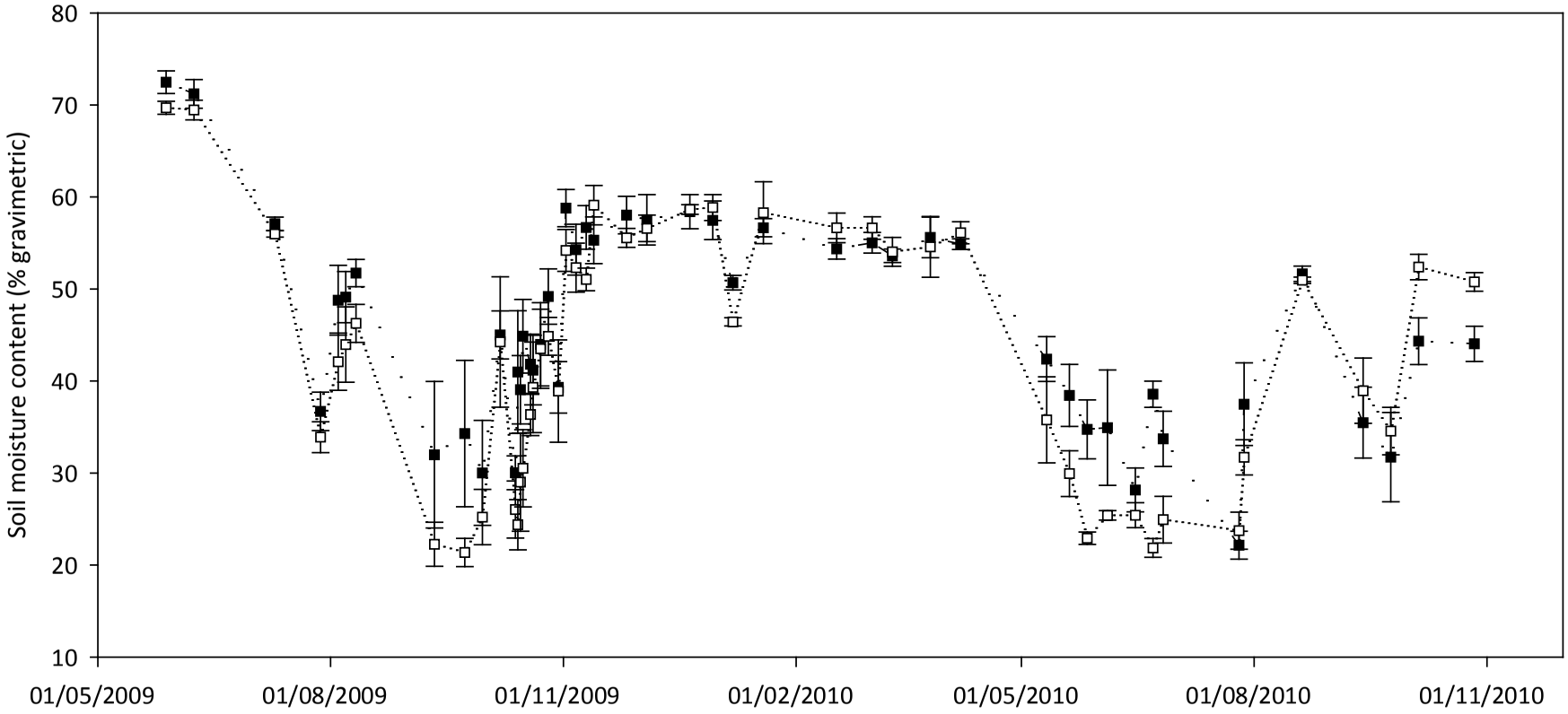

Fig. 2.13 Average gravimetric soil moisture contents from treatments RMS, and RMS(W) over time, measured during hand-held moisture surveys. Treatments RMS and RMS(W) are represented by open squares □ and solid squares ■ respectively. Error bars represent ± 1 SE (n = 4).

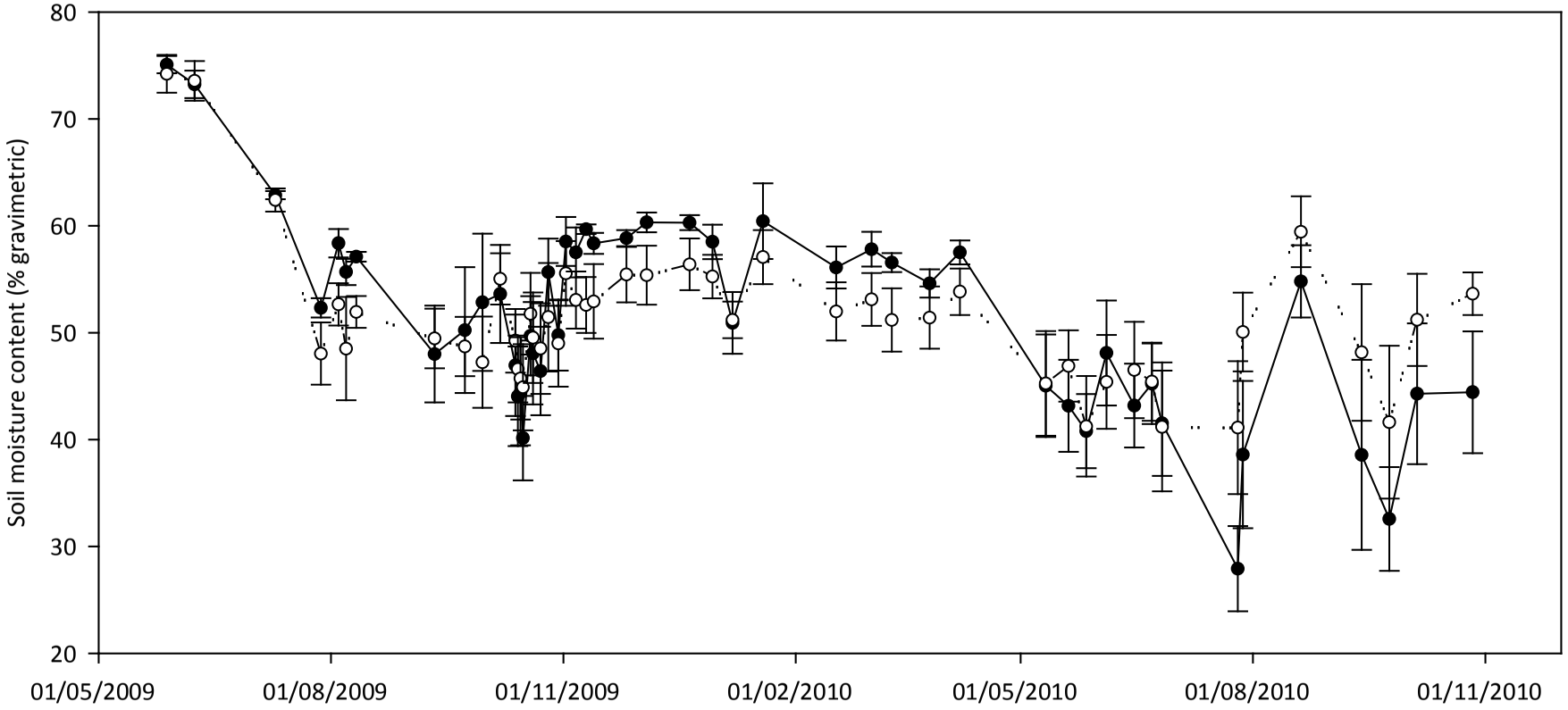

Fig. 2.14 Average gravimetric soil moisture contents from treatments MS, and MS(W) over time, measured during hand-held moisture surveys. Treatments MS and MS(W) are represented by open circles ○ and closed circles ●, respectively. Error bars represent ± 1 SE (n = 4).

development of the fault with the automated irrigation system, (B) during the fault with treatment RMS(W) excluded, and (C) after the fault, to see if treatments were significantly different in terms of the % moisture content in the top 6 cm of the soil.

Prior to the fault there was a significant difference between treatments ($F= 3.46$, $p<0.03$) in terms of soil % moisture (gravimetric) measured on hand-held moisture survey days. *Post- hoc* analysis (Duncan's multiple range test) revealed that treatment RMS had significantly lower soil moisture than all other treatments apart from RMS(W), and that S(W) had a significantly higher soil moisture than all other treatments apart from MS(W) (see Fig. 2.15).

During the fault, there was a significant difference between treatments ($F= 11.12$, $p<0.0006$). *Post-hoc* analysis revealed that the treatment RMS had a significantly lower soil moisture than the other treatments and treatment S(W) had a significantly higher soil moisture than all other treatments apart from MS(W) (see Fig. 2.15).

After the fault there was, again, a significance difference between treatments ($F= 3.18$ $p<0.04$), with treatment RMS having a significantly lower soil moisture than all other treatments apart from treatment RMS(W). RMS(W) was not significantly different from any of the treatments following the re-establishment of moisture control by the automated irrigation system (see Fig. 2.15).

Friedman's non parametric two- way ANOVA was repeated for just the months of June to September 2009 and 2010, in order to establish whether there were differences between the average soil % moisture contents of the collars during the summer months. For these analyses, treatment RMS(W) was not excluded. During summer 2009, there was a significant difference between treatments ($F = 5.57$, $p < 0.0045$) with treatment RMS having lower average soil moisture than all the other treatments apart from RMS(W) (see Fig. 2.16). Surprisingly, the average soil moisture content of treatment RMS(W) was not significantly different from that of treatment S, despite problems with the irrigation system. Also, treatments MS(W) and S(W) had significantly higher soil moisture than the other treatments.

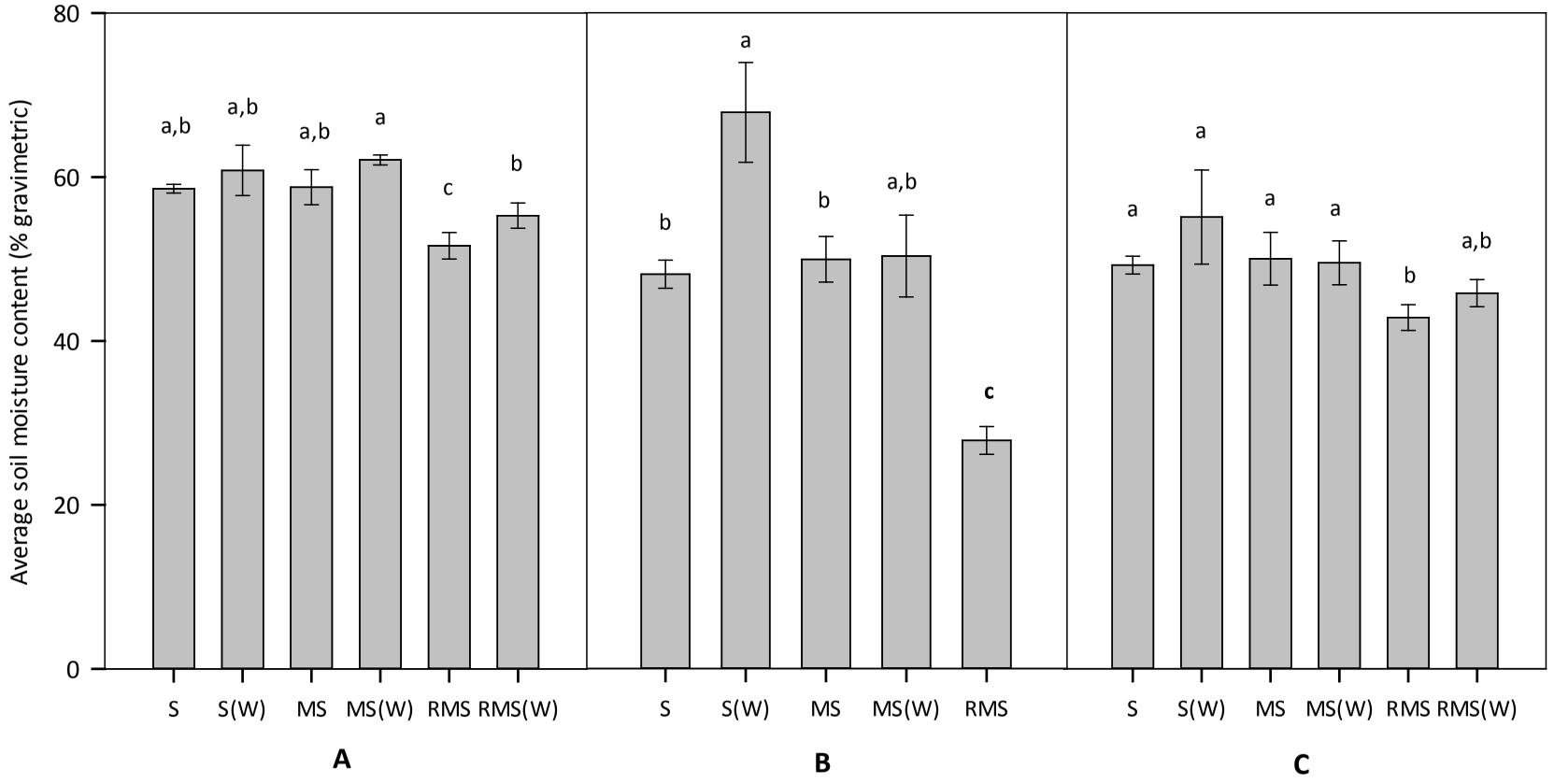

Fig. 2.15 Average gravimetric soil moisture contents recorded during hand-held soil moisture surveys for each of the six soil collar treatments: (**A**) prior to the fault with the automated irrigation system, (**B**) during the time of a fault with the automated irrigation system (where data from treatment RMS(W) are excluded), (**C**) after the fault. Within each period, treatments which differ significantly have different letters. Error bars represent ± 1 SE (n = 4).

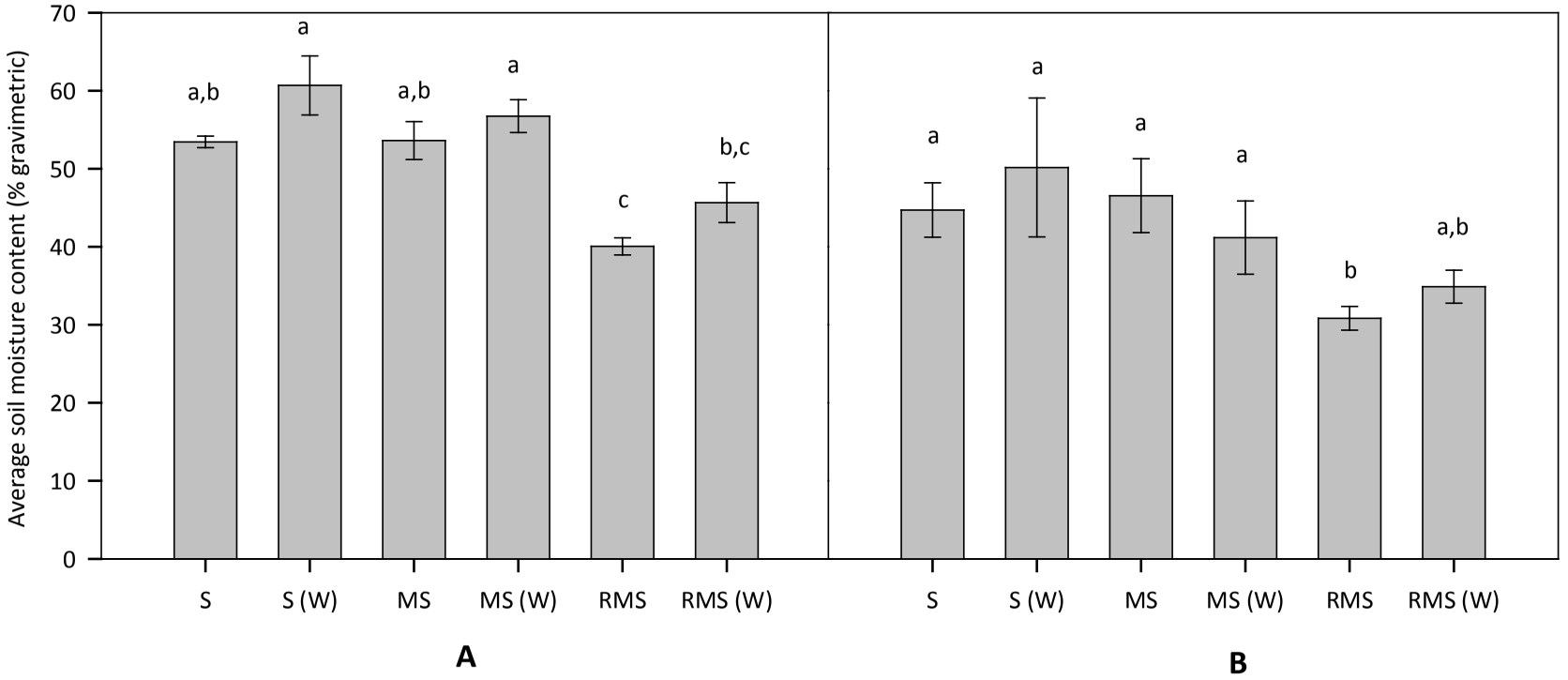

Fig. 2.16 Average gravimetric soil moisture contents recorded during hand-held soil moisture surveys for just the summer months: (**A**) June to September 2009, (**B**) June to September 2010. Within each period, treatments which differ significantly have different letters. Error bars represent ± 1 SE (n = 4).

During summer 2010 there were also overall differences between the treatments in terms of the average moisture measured in the collars ($F= 3.36$, $p<0.031$) with treatment RMS having significantly lower soil % moisture content than all other treatments apart from RMS(W) (see Fig. 2.16).

2.3.4 Regression Analysis

In order to see whether there was any significant difference in the decomposition rates between the different treatments, decomposition constants (k values) were calculated for each of the 24 collars for both the I2009 (16 months) and I2010 (11 months) litter bag incubations (see Table 2.2). The resulting k values were then tested for normality using Kolmogorov-Smirnov tests. A two-way ANOVA with block and treatment as factors showed that there was no significant difference in k values between the treatments for either the I2009 ($F=0.946$, $p>0.47$) or I2010 ($F=0.638$, $p>0.67$) series.

The I2009 and I2010 litter bag incubations began at different times of the year, and in order to assess whether variation in the residuals for the I2009 series could be explained by prevailing weather conditions, regression analyses were carried out. As there were no significant differences in litter mass loss between the mesh collar treatments, it was possible to split the I2009 litter bag incubation into nine periods based on the harvest dates and calculate the average mass (mg) remaining per period using the litter bags harvested from all 24 collars. Fig. 2.17 shows the natural log (ln) of the average mass (mg) remaining for each period plotted against time ($y=-0.0005x +25.687$, $R^2 = 0.952$). As expected, the transformed data fall on a straight line, following an exponential decay curve and a linear regression was fitted and used to calculate model values of average mass remaining (mg). Residuals were calculated and expressed as percentages of the model mass remaining (mg) in order to normalise the residuals, thus taking into account their position on the exponential decay curve. Average values per period (based on the litter harvest dates) were also calculated for environmental variables. Results from simple regression analyses showed that both the soil temperature at 5 cm and at 10 cm were significantly correlated with these residuals for the I2009 series ($p <0.007$ and $p<0.01$ respectively; see Fig. 2.18).

Table 2.2 Values of k, the decomposition constant calculated for the I2009 and I2010 litter bag incubations. Values for treatments are means, with standard errors in parentheses, n=4.

	Litter decomposition series			
Treatment	I2009		I2010	
S	-0.000475	(-0.000062)	-0.00022	(0.000066)
S(W)	-0.0005	(0.00007)	-0.000375	(0.000047)
MS	-0.00045	(0.000028)	-0.000325	(0.000075)
MS(W)	-0.0005	(0.00004)	-0.0003675	(0.0001)
RMS	-0.000525	(0.000047)	-0.00025	(0.000064)
RMS(W)	-0.000675	(0.00014)	-0.00032	(0.000089

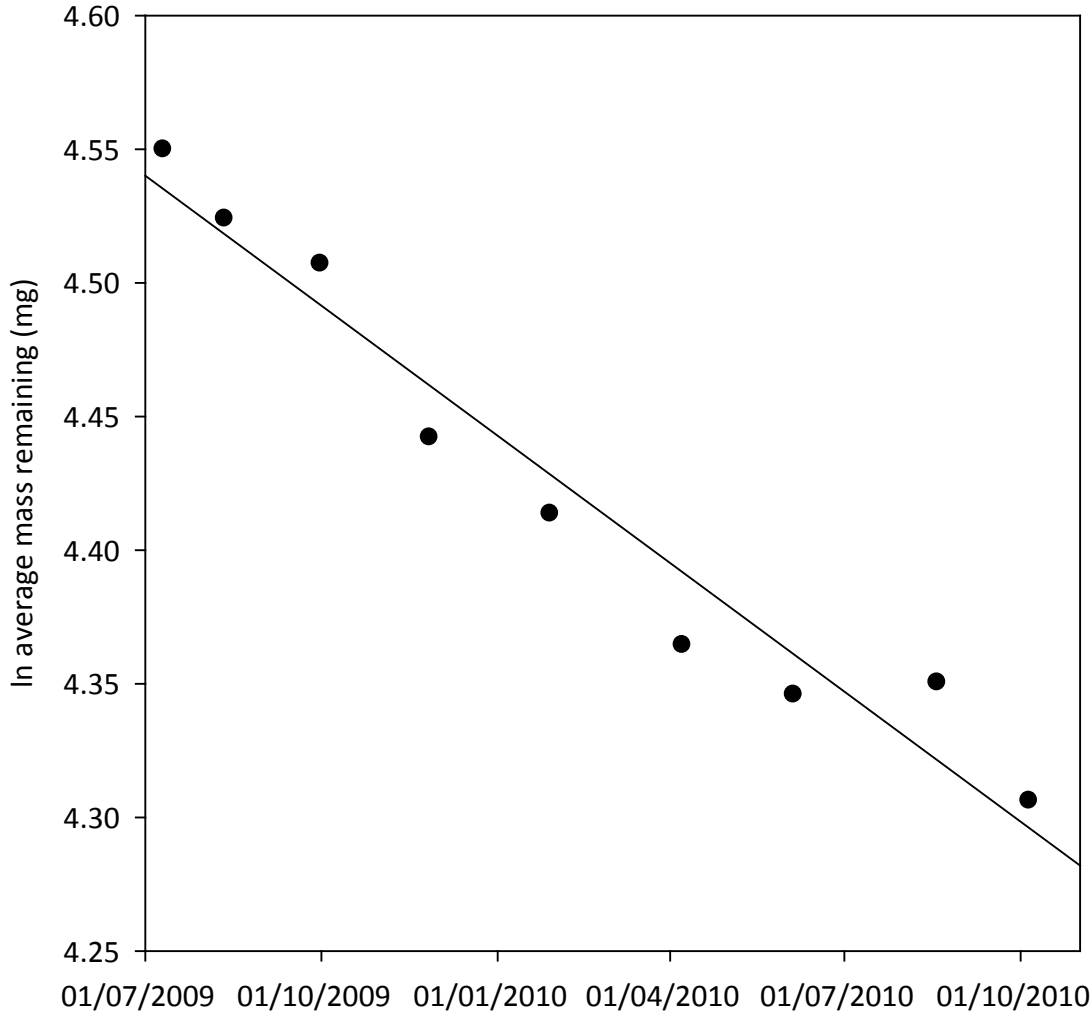

Fig. 2.17 Natural log (ln) of the average mass remaining for the I2009 litter bag incubation over the periods of decomposition defined by the litter bag harvests. Each point is the mean of 24 values.

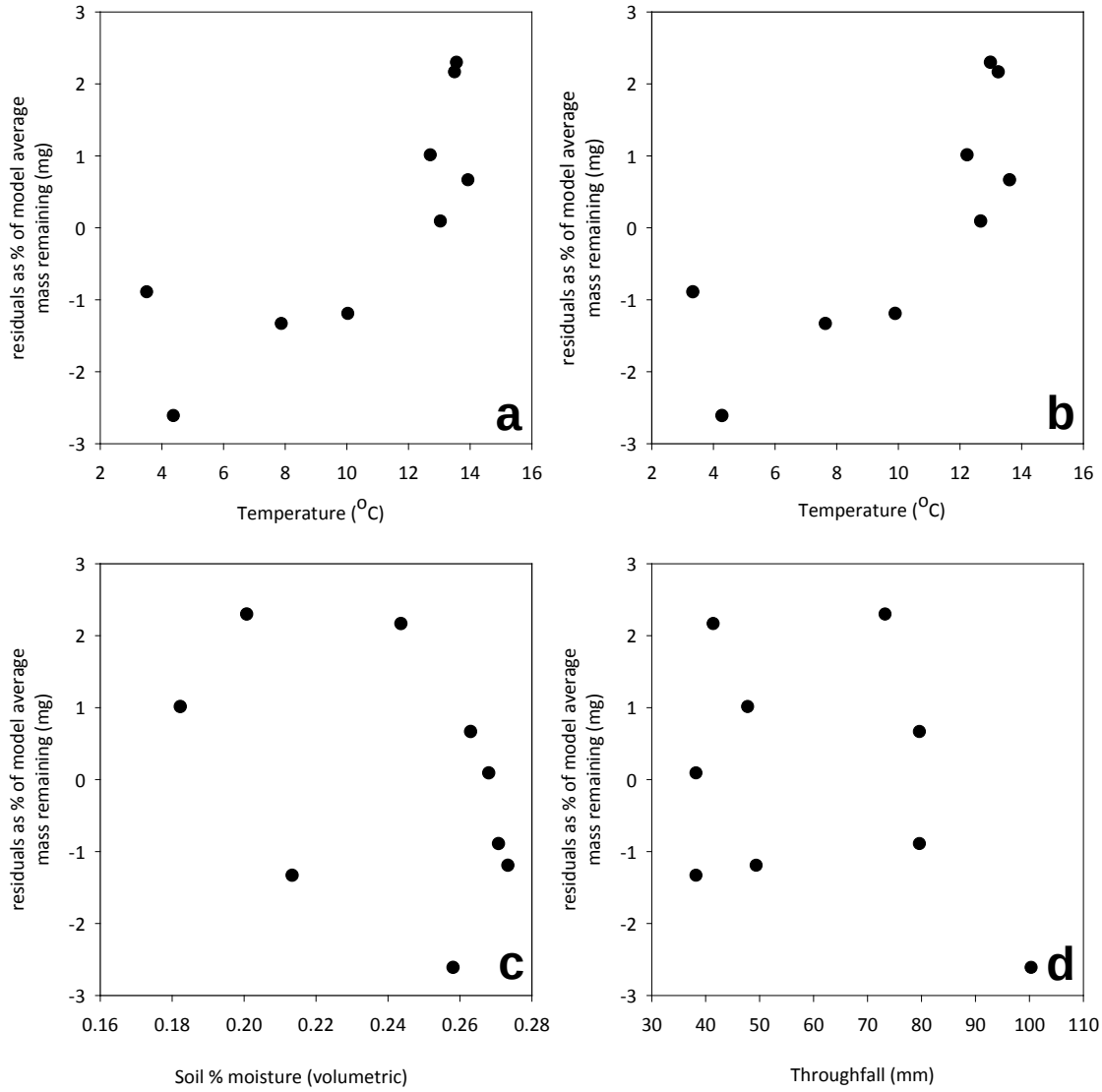

Fig. 2.18 Correlation between residuals as a percentage of model average mass remaining per litter decomposition period for the I2009 litter decomposition series plotted against: (**a**) soil temperature at 5 cm, (**b**) soil temperature at 10 cm, (**c**) volumetric soil moisture, (**d**) throughfall.

From Figs. 2.18a and 2.18b, when the soil temperatures were higher than 12˚C, the residuals were all positive, with litter mass loss being greater than predicted by the model. At lower temperatures, the residuals were negative, indicating that low temperatures were related to litter mass loss being less than predicted by the model.

The results of the forward stepwise multiple regression displayed in Table 2.3 show that soil temperature at a depth of 5 cm was significantly correlated with the residuals from the model ($p < 0.0074$, $r^2 = 0.6645$), and was the only variable that met the 0.05 significance level criteria for inclusion into the model. Table 2.3 also includes the results when the significance level criteria for entry into the model was set to 0.5, in order to demonstrate the poor association between the other environmental variables and the residuals from the model values.

The regression analyses were repeated for the I2010 series, but since there were only four periods, these data were amalgamated with those from the I2009 series in order to assess whether the observed effect of soil temperature was robust across the two series. Again, linear regression analysis showed that soil temperature at 5 cm significantly correlated with the residuals from the modelled values, as did the soil temperature at 10 cm, both at a significance level of $p < 0.02$. Figs. 2.19a and 2.19b show that although the correlations are not as strong as for the I2009 litter bag series, when temperatures were below 12˚C, the residuals tended to be negative, with less decomposition taking place at low temperatures than would be predicted by the overall regression model.

The results of the forward stepwise multiple regression for the amalgamated I2009 and I2010 data are also displayed in Table 2.3. The results show again that soil temperature at a depth of 5 cm was the only variable that met the 0.05 significance level criteria for entry into the model, and was highly significantly correlated with the residuals from the model values ($p < 0.018$, $r^2 = 0.4120$).

Table 2.3 Results from a forward stepwise multiple regression to explain variation in residuals for the I2009 litter decomposition series and for the I2009 and I2010 series combined.

I2009 litter decomposition series

Environmental factor	partial r^2	model r^2	F value	P value
soil temperature at 5 cm	0.6645	0.6645	13.86	0.0074
soil temperature at 10 cm	0.0827	0.7472	1.96	0.2108

Combined I2009 and I2010 litter decomposition series

Environmental factor	partial r^2	model r^2	F value	P value
soil temperature at 5 cm	0.412	0.412	7.71	0.018
soil temperature at 10 cm	0.0724	0.4845	1.41	0.2633
volumetric soil moisture	0.0569	0.5414	1.12	0.3182
throughfall volume (mls)	0.0335	0.5749	0.63	0.4498

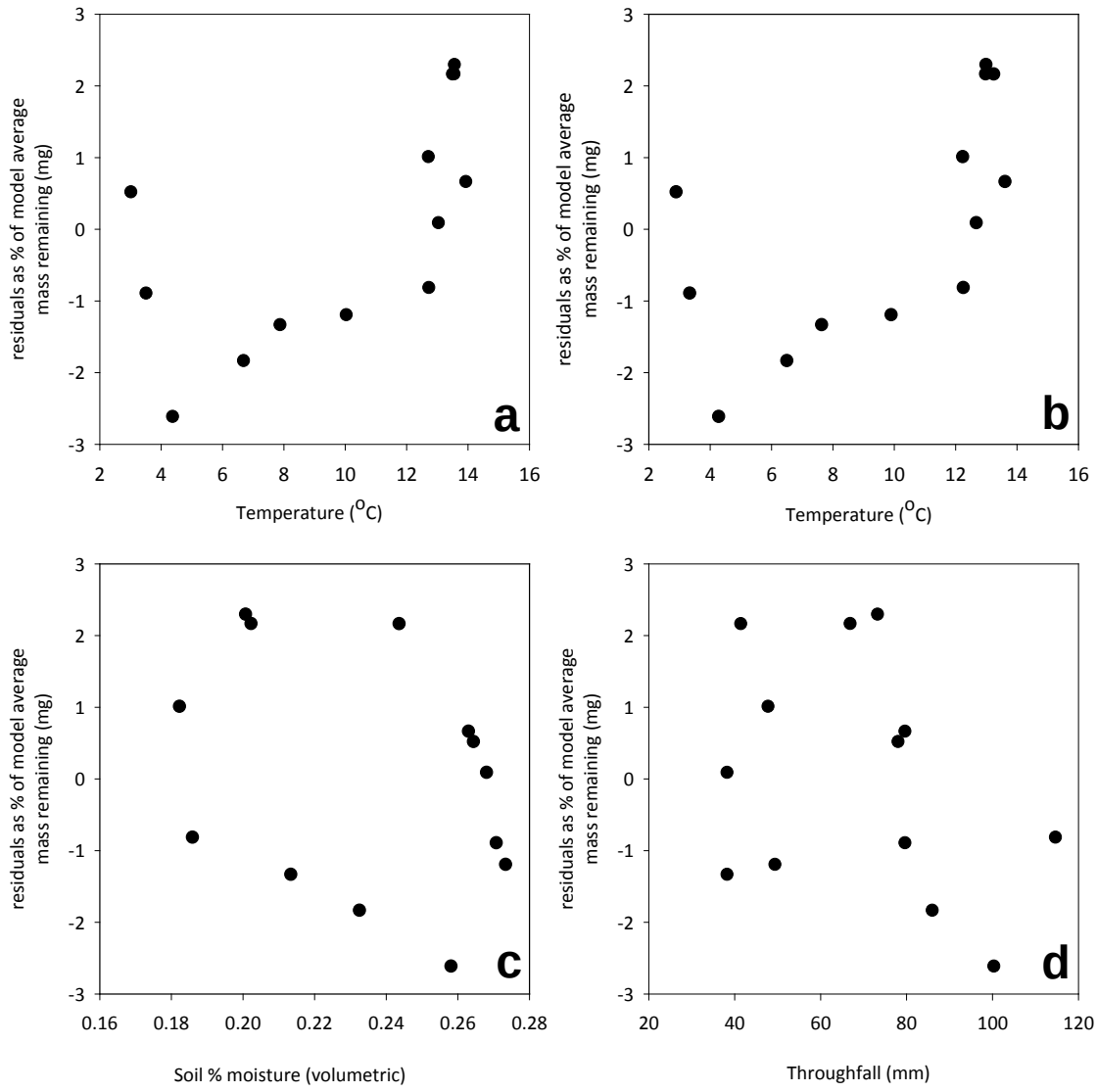

Fig. 2.19 Correlation between residuals as a percentage of model average mass remaining per litter decomposition period for the I2009 and 12010 litter decomposition series combined plotted against: (**a**) soil temperature at 5 cm, (**b**) soil temperature at 10 cm, (**c**) volumetric soil moisture, (**d**) throughfall.

2.4 Discussion

The aim of the current work was to investigate the 'Gadgil effect', by measuring litter decomposition in the presence and absence of roots and associated ECM hyphae, but with control for the influence of water uptake. The absence of any significant difference in the rate of litter mass loss between total-soil, root and ECM hyphal exclusion treatments does not support the hypothesis that the input of simple carbohydrates into the soil, derived from roots and associated microorganisms, including ectomycorrhizal (ECM) fungi, has a promoting effect on needle litter decomposition. Nor does the current work provide support for the findings of Gadgil & Gadgil (1971; 1975), who observed a decrease in litter decomposition in the presence of roots and associated ectomycorrhizal (ECM) hyphae.

Litter bags were successfully used to study mass loss of litter samples during this experiment based on the widely used technique, introduced by Bocock & Gilbert (1957), using confined litter to study field decomposition rates. In this experiment, the mesh size of the litter bags was 0.5 mm^2, preventing needle loss and avoiding excessive loss through fragmentation of the litter; larger soil animals, such as earthworms may have been excluded. However, despite frequent digging at the site whilst setting up experiments, earthworms were never observed.

A negative relationship between earthworm numbers and the abundance of other soil animals has been demonstrated (Räty & Huhta, 2003; Huhta & Räty, 2005). Schaefer & Schauermann (1990) found an inverse relationship in terms of the numbers of enchytraieds and earthworms present in forest soils. Enchytraeids were regularly found in the litter during litter-bag processing, and it may be that they were more able to tolerate the acidic soils present at the current site than earthworms (Räty & Huhta, 2003) and, consequently, the potential exclusion of earthworms from the litter bags was not of importance at this site. The mesh size used to construct the litter bags was large enough to allow the in-growth of fine roots and mycorrhizal hyphae.

The use of litter bags deployed in soil collars had the advantage that destructive harvesting of small defined samples of litter could take place at intervals from each collar. This allowed the accurate start and end mass of the litter to be measured, and

consequently an accurate measure of litter mass loss. A criticism of the field experiments of Gadgil & Gadgil (1971; 1975) is that they did not present a starting mass for their *Pinus radiata* litter prior to decomposition, or confine their experimental litter in any way. Instead they established the mass of litter accumulated at the soil surface of their 1 x 1 m plots at the different harvest dates, which means that, despite their block design, substantial error may have been introduced through litter blown into plots from surrounding areas.

Replicating the 1 x 1 m plots of Gadgil & Gadgil (1971; 1975) with accurate control of soil surface moisture over such a large area would be very challenging to achieve, and therefore smaller soil collars where the moisture could more easily be controlled were used. Despite the differences in experimental design, ECM roots were excluded in the current study, just as they were in the research by Gadgil & Gadgil (1971; 1975), and any treatment effects caused by trenching on litter decomposition should be evident, despite differences in the technique used to measure litter mass loss.

Though decomposition over long periods of time is best described by asymptotic decay curves, it is generally the case that simple exponential models are used as to describe litter mass loss during the initial stages of decomposition (Minderman, 1968; Latter *et al.,* 1998). In the current work the litter mass loss of both the I2009 and I2010 litter bag series followed expected exponential decay curves. Litter mass loss dynamics for both series shown in Figs. 2.3 and 2.4 could be divided into two phases, with a classic sharp initial phase of mass loss, consistent with the leaching of soluble organic components including simple sugars from the litter (Berg, 2000), followed by litter mass loss at a decreased rate, as more complex substrates including polysaccharides, cellulose and hemicelluloses and potentially lignin were decomposed by soil microorganisms (Swift *et al.,* 1979).

Decay constants (k values, after Olson, 1963) are widely used to incorporate the information contained in an exponential decay curve into a single figure which may then be used compare litter decomposition rates from different treatment regimes, or between species (Meentemeyer, 1978; Aerts, 1997, Guo & Sims, 2001). In the current study, the lack of difference between treatments in terms of litter mass loss revealed

by repeated measures ANOVA was also seen in no differences in the decomposition constant (k) values between treatments. The calculated values of mass loss of litter observed in the current experiment of 22.8% for the I2009 series (491 days), and 15.9% (121 days) for the I2010 series, were low. The average k value was -0.00052 for the I2009 series, with no significant difference in k between the treatments (see Table 2.2). For the I2010 series, there was an overall average k value of -0.00031, again with no significant difference in k between the six treatments. Meentemeyer (1978) observed an average value for k of -0.19 over the first year of a mixed litter decomposition experiment at Moorhouse in the UK. In a Scots pine plantation in Ehrhorn, Germany, which has a similar climate to the current field site, Berg *et al*. (1993) observed a first-year % mass loss of 38% from Scots pine litter, which is considerably greater than that observed during the current study. However, Berg & Staaf (1980) conducted two incubations in series of litters ranging in N content in central Sweden. They found that the first-year mass loss of litter with ambient N content was 20.7% for their first incubation, and for the second incubation, there was a greater first-year mass loss of 32.7%. Therefore, even for the same site there can be considerable variation between years in initial litter mass loss. It is possible that even with the weekly watering with average throughfall amounts, having PVC shield over the collars, may have made conditions in the collars drier than they would have been naturally, resulting in slower rates of decomposition than are generally seen in temperate coniferous forests.

Though the current work did not observe either the 'Gadgil effect' or a promoting influence of plant roots and associated microorganisms on litter mass loss, it was possible to relate decomposition rates to other variables. Aspects of climate are regulators of the metabolism of microorganisms responsible for decomposition and litter mass loss, with their activity frequently being related to climatic variables, including temperature and moisture (Lavelle *et al.,* 1993; Aerts, 1997; Chadwick *et al*., 1998; Davidson & Janssens, 2006). Variation in rates of litter decomposition over broad climatic scales have been explained using actual evapotranspiration (AET), an index of combined temperature and moisture, which includes a measure of the interaction between the two. Correlations between AET and annual decomposition rates have

produced r^2-values as high as 0.98 (Meentemeyer, 1978; Berg *et al*., 1993; Aerts, 1997).

Although AET is applicable over broad regional scales it cannot account for variation within microsites (Meentemeyer, 1978; Liski *et al.*, 2003, Aerts, 2006). Consequently, in the current work, the effects of temperature and moisture on litter decomposition were considered separately.

The initial litter mass loss shown in Fig. 2.5 occurred faster for the I2010 litter bag series than for the I2009 series, despite being installed in the winter, at the end of December, as opposed to June for the I2009 series. The litter used in the bags would be expected to be at the same initial stage of decomposition to begin with, as it was taken from the site on the same day, dried and stored before use. The difference in the initial decomposition rate could potentially be attributed to differences between the level of activity of soil microorganisms between the two seasons. However, measurements of soil CO_2 efflux have shown that though there is low level microbial activity through the winter months, microbial communities are much more active in the summer, associated with warmer temperatures (Heinemeyer *et al*., 2007). Though, it is also possible that the microbial community is more starved of easily attainable carbon in the winter months, so that rates of removal of these components of the litter may have proceeded more quickly during the winter.

The higher litter % moisture and soil % moisture during the winter months suggests that there may have been greater leaching of soluble components from the litter during the winter than during the summer months. Virzo de Santo *et al*. (1993), in a comparison of five coniferous sites under varying climates, found that litter moisture content was the rate-limiting factor for initial litter decomposition. Up to 50% of soluble components may be lost from decomposing litter via leaching, with the level of leaching being dependent on how much water comes into contact with the litter, and to a lesser extent, temperature (Witkamp, 1969; Swift *et* al., 1979; McClaugherty, 1983). The total volume of throughfall during the first few months of the I2010 incubation was slightly less than that for the I2009 litter bags during summer 2009.

However, the litter bags installed for the I2010 collars were quickly covered with snow during January 2010, which may have led to increased leaching as the snow thawed.

Seasonal fluctuations in throughfall and temperature resulted in higher litter moisture in the winter months than during the summer. There were only differences in litter % moisture between the treatments during the I2009 litter bag series with treatment S(W) (root and hyphal exclusion collars with the same water additions as treatment RMS(W)) having the highest average litter % moisture overall. This is to be expected as collars from treatment S(W) were receiving a considerable amount of water from the automated irrigation system, particularly during the summer months. There appeared to be no association between the litter % moisture and the underlying soil % moisture (see Fig. 2.9), an observation also made by others (eg. Virzo de Santo *et al*., 1991).

Treatment RMS, which received no water correction had relatively high average litter % moisture, despite the soil underneath having the lowest soil % moisture of all the treatments during the summer months. This could potentially be explained by soil microorganisms moving water to the litter layer where a large proportion of decomposition takes place. Hydraulic lift occurs when dry surface layers receive water from deeper layers as water travelling upwards in roots or hyphae passively enters dry soil along water potential gradients (Lehto & Zwiazek, 2011). The extent to which the large mycelial network in forest soils (Smith *et al*., 1992) can move water is poorly researched, although we know that it is important for nitrogen translocation to litter from deeper soil layers as suggested by Staaf & Berg (1977), and demonstrated using a ^{15}N tracer (Hart & Firestone, 1991). In a mesocosm experiment, Querejeta *et al.* (2003) used tracers to unambiguously demonstrate that during the night, hydraulic lift allowed water to be transferred from plants to associated fungal mycelium. It is possible via this mechanism that trees enhance nutrient uptake by supplying water to their mycorrhizal partners, increasing soil moisture in the litter layer so that the fungal mycelium is maintained, and litter decomposition can continue (Querejeta *et al*., 2003; 2007).

During the current study, significant drying of the soil only occurred in the total soil treatment (RMS) and there was no specific evidence of water uptake by ECM hyphae.

Figs. 2.11 and 2.12 show that the soil % moisture contents in the mycorrhizal treatments (MS and MS(W)) were similar to that of the soil only treatment (S) for the duration of the experiment. The contentious statement that enhanced water uptake is one of the benefits for the host plant in ectomycorrhizal symbiosis (see Lehto & Zwiazek, 2011) was not demonstrated here.

ECM hyphae have been shown to function as extensions of the root system, particularly during drought conditions (Duddridge *et al.,* 1980; Allen, 2007) and it has been suggested that water may be transported to the host plant via a mass-flow mechanism in large diameter 'vessel' hyphae, but direct evidence for this in the field is lacking (Cairney, 2005). As host trees tend to have well developed deep root systems, it is perhaps more beneficial for the plant to supply water to the fungus in order that decomposition can be continued in the drier surface layers, leading to enhanced nutrient acquisition (Lehto & Zwiazek, 2011).

In the current research, it seems likely that any soil moisture differences were not severe enough to limit litter decomposition, and the trees at the study site did not appear to facilitate additional water uptake through their fungal partners. Instead, the trees may have provided the ECM fungi in the litter layer with additional water during the summer months, taken up from deeper in the soil profile.

The results of Gadgil & Gadgil (1971; 1975) are difficult to interpret for several reasons. The main criticism of their work has been that in both of their field studies, they did not control for soil water uptake by roots and it is therefore not possible to discount the effect of soil moisture, or to attribute the decrease in litter decomposition they observed solely to a single factor. Soil moisture was controlled in the laboratory experiment of Gadgil & Gadgil (1975), where sterilised litter was left to decompose in microcosms containing mycorrhizal and non-mycorrhizal seedlings. In support of their field studies, they observed that the presence of ECM roots had a negative influence on litter decomposition. However, caution should be exercised when extrapolating results from experiments conducted under laboratory conditions to the field, where conditions are more variable, with extremes of temperature and moisture and other factors which would be expected to influence decomposition (Dighton *et al.* 1987).

Despite initial problems with the automated irrigation system during summer 2009, the control of soil moisture necessary to investigate the 'Gadgil Effect' was achieved. During summer 2009 and 2010, the effect root water uptake had, drying the soil, was clear as treatment RMS (total soil without correction) had significantly lower soil % moisture content than treatments MS and S, where roots had been excluded. However, these clear differences in soil moisture between treatments did not lead to any differences in litter decomposition rates, as might be expected considering the key role moisture can play in controlling litter decomposition (Robinson *et al.,* 1995; Murphy *et al.,* 1998). Results from the regression analysis showed no relationship between litter mass loss and soil moisture, or the amount of throughfall added to collars, supporting the conclusion that soil moisture is not a limiting factor for litter decomposition at this particular site.

Although there was no relationship between soil moisture and the rate of litter decomposition, temperature at a depth of 5 cm was found to be correlated. This apparent controlling effect of temperature on litter decomposition has been observed many times (Kirschbaum, 1995; Hobbie, 1996; Panikov, 1999; Guo & Sims, 2001). Domisch *et al*. (2006) investigated the effects of different soil temperatures on the decomposition of *Pinus sylvestris* needles in a growth chamber experiment, where moisture levels were maintained and found that the accumulating soil temperature sum dictated the rate of mass loss, with faster mass loss observed at 15˚C than at 10˚C or 5˚C.

Greatest litter decomposition rates generally occur under conditions of high temperature, where there is sufficient moisture (Bloomfield *et* al., 1993) but, in the natural environment, high temperatures tend to occur at times when soil moisture is at its lowest (Gonçalves & Carlyle, 1994). This was found for the current study, with the summer high temperatures coinciding with the lowest soil moistures, particularly in treatment RMS (total soil, no water correction).

Gadgil & Gadgil, and others since, have postulated that the mechanism behind the 'Gadgil effect' involves direct suppression of saprotrophic decomposers by ECM fungi, and a variety of explanations for this suppression have been proposed. ECM fungi

could suppress the activity of more effective litter decomposing saprotrophs by creating a physical barrier and occupying microsites in the soil, or via anti-microbial action, both of which have been demonstrated to provide protection for host-plant roots against pathogens (Zak *et al*., 1964; Garrido *et al*., 1982). Marx (1969) found that the fungal mantle and Hartig net of *Pinus echinata* (Mill.) ectomycorrhizas provided a physical barrier against infection of seedling roots by the pathogenic fungus *Phytophthora cinnamoni* (Rands), in comparison to non-mycorrhizal roots which were heavily infected.

The synthesis of antibiotics by the ECM fungus *Paxillus involutus* was shown to inhibit the growth of the root-rot fungus *Fusarium oxysporum*, with the production of these antibiotic compounds being stimulated by *Pinus resinosa* root exudates in vitro (Duchesne *et al*., 1988). There is also evidence that ECM fungi suppress the activity of pathogenic microorganisms *in vitro* by acidifying the surrounding medium, rather than secreting antibiotics (Schelkle & Peterson, 1996). Rasanayagam & Jeffries (1992) tested 19 isolates of ectomycorrhizal fungi to see if they inhibited the growth of the common plant pathogen *Pythium ultimum* and found that through a mechanism of acidification of the surrounding media, the presence of 16 of the isolates caused the hyphal tips of the pathogen to burst.

Another mechanism by which ECM fungi could suppress the activity of saprotrophic microorganisms is via the uptake and immobilisation of key nutrients. In a comparison of the concentration of ten biologically important elements including N and P between pine needles which had been decomposing for one year, and that contained in basidiomycete fungal fruiting bodies, Stark (1972) found that the fruiting bodies were enriched in N (94-340%), P (312-698%), K (60-2046%) and Cu (278-711%) relative to the pine needles, indicating that significant amounts of nutrients may be immobilised as part of the fungal mycelium. Co-evolution of ECM fungi, alongside their host plants may have created a system where ECM fungi are capable of outcompeting saprotrophic microorganisms for N and P in the nutrient poor litter produced by their host plant and from decomposing root material, whilst immobilising the labile carbon substrates from the plant roots (Henkel, 2003; Read *et al*., 2004). Bending & Read (1995) investigated the nutrient status of fermentation horizon organic matter (from a

pine forest soil) before and after colonisation by two species of ECM fungi in symbiosis with *Pinus sylvestris,* and found that the C:N ratio of the material was increased following colonisation by the two fungal species, when compared to un-colonised material.

Though it has been observed that different species of mycorrhizal fungi have differing abilities to extract N and P (Perez-Moreno & Read, 2000) N starvation of saprotrophic microorganisms following the uptake of N by mycorrhizal fungi could result in suppression of saprotrophic activity (Bending & Read, 1995). It has previously been assumed that ECM fungi depend on the decomposing activity of saprotrophs to extract organic N from recalcitrant compounds and laboratory studies have shown that ECM fungi are capable of extracting organic protein N, thus allowing them to compete even more effectively with saprotrophic decomposers (Abuzinadah *et al*., 1986). However, the ability of ECM fungi to extract N from recalcitrant polyphenolic compounds in natural conditions has been questioned (Koide & Wu, 2003).

As the 'Gadgil effect' was not observed during the current work, these results do not provide any insight into the observations of Gadgil & Gadgil (1971; 1975). Nor did the current work provide any support for the hypothesis that the input of simple carbohydrates into the soil, derived from roots and associated microorganisms, including ECM fungi, has a promoting effect on needle litter decomposition, as observed by Subke *et al.* (2004).

Interpretation of the literature surrounding the influence of labile carbon into the soil through roots and associated microorganisms in the rhizosphere is complicated, and whether the 'Gadgil effect', when observed, can best be explained by direct competition between ECM fungi and saptrotrophs, or by a moisture effect is uncertain. An attempt has been made here (see Table 2.4) to separate out the literature surrounding the Gadgil effect into studies which saw a positive, negative or no effect of the presence of ECM roots on decomposition, and also whether there was a difference between whatever treatments were implemented in the studies in terms of soil or litter moisture. This has only been possible where sufficient information has been provided in papers, with some papers (Cuenca *et al.,* 1983; Entry *et al*., 1991;

Table 2.4 Information from papers including, and following the work of Gadgil & Gadgil (1971; 1975), which have investigated the influence of ECM roots on the decomposition of litter and simple substrates.

Authors	Influence of ECM roots		Substrate(s)	Tree species present	Soil type	Annual air temperature	Annual precipitation
	on decomposition rate	on soil/litter moisture					
Chigineva *et al.* (2009)	(o)	Litter (-)	Litter (separate bags for each species): *Berlinia bracteosa* (Benth.), *Picea abies* (L.) H. Karst., *Populus tremula* (L.)	*Picea abies* (L.) H. Karst.	Albeluvisol	Mean: 2.7 – 3.8 ˚C	580 – 620 mm
Cuenca *et al.* (1983)	(o)	NA	Litter (separate bags for each species): *Coffee arabica* (L.), *Erithrena* sp., *Inga* spp.	*Coffee arabica* (L.), *Erithrena* sp., *Inga* spp.	Acid, derived from mica schists	Mean: 19 ˚C	1200 mm
Chuyong *et al.* (2002)	(-)	NA	Litter (separate bags for each species): *Berlinia bracteosa* (Benth.), *Didelotia africana* (Baill.), *Microberlinia bisulcata* (A.) Chev. *Tetraberlinia bifoliolata* (Harms) Hauman *Cola verticillata* (Thonn) Stapf. Ex A. Chev., *Oubanguia alata* (Bak.f.), *Strephonema pseudocola* (A.Chev)	Plots with either high or low abundance of ECM trees. High species diversity (for more information see Newbery *et al.* (1997)	Sandy (taken from Gartlan *et al.* 1986)	Mean: 23.7 – 30.2 ˚C (taken from Gartlan *et al.* 1986)	5460 mm (taken from Gartlan *et al.* 1986)

Table 2.4	Continued						
Authors	Influence of ECM roots		Substrate(s)	Tree species present	Soil type	Annual air temperature	Annual precipitation
	on decomposition rate	on soil/litter moisture					
Dighton *et al*. (1987)	(+)	NA	Hide Powder Chitin Cotton	*Pinus contorta*	Perlite/peat (95: 5)	Laboratory incubation	Laboratory incubation
Entry *et al.* (1991)	(+)	NA	Litter: *Pseudotsuga menziesii* (Mirb.) Franco	*Pseudotsuga menziesii* (Mirb.) Franco	Gravelly loam	Range: 2.7 - 18.5 ˚C	1905 mm
Faber & Verhoef (1991)	(-)	(o)	Litter: *Pinus nigra* (Arnold)	*Pinus nigra* (Arnold)	Sandy	Mean : 8.5 ˚C	NA
Fisher & Gosz (1986)	(-)	(-)	Cellulose	*Pseudotsuga menziesii, Abies concolor* (Lindl.), *Pinus ponderosa* (Laws), *Pinus flexilus* (James), *Populus tremuloides* (Michx.)	Sandy loam	Range: -5 - 15˚C	450-650 mm
Gadgil & Gadgil (1971; 1975)	(-)	(-)	Litter: *Pinus radiata*	*Pinus radiata*	Silty sand	Mean: 10 ˚C	1524 mm

Table 2.4	Continued						
Authors	Influence of ECM roots		Substrate(s)	Tree species present	Soil type	Annual air temperature	Annual precipitation
	on decomposition rate	on soil/litter moisture					
Mayor & Henkel (2006)	(o)	NA	Litter: Bags containing just *Dicymbe corymbosa* litter and bags containing mixed species litter (without *Dicymbe corumbosa*)	*Dicymbe corymbosa* in monodominant plots, also highly diverse mixed species plots (for further information, see Henkel *et al*., 2002)	Soils variable in texture, but uniformly acid	Range: 19 - 29˚C	3866 mm
McGuire *et al.*(2010)	(o)	NA	Litter: Bags containing just *Dicymbe corymbosa* litter and bags containing mixed species litter (without *Dicymbe corumbosa*)	*Dicymbe corymbosa* in monodominant plots, also highly diverse mixed species plots (for further information, see Henkel *et al*., 2002)	Soils variable in texture, but uniformly acid	Range: 19 – 29 ˚C	3866 mm
Staaf (1988)	(o)	(-) only on driest occasions	Litter: *Fagus sylvatica* (L.)	*Fagus sylvatica*, with occasional *Quercus* sp. and *Fraxinus* sp. (taken from Staaf, 1987)	Mor site: orthic podsol Mull site: gleyic cambisol, acid	Mean: 7 ˚C	650 – 800 mm
Subke *et al.* (2004)	(+)	NA	Litter: ^{13}C-labelled, *Picea abies* (L.) H. Karst	*Picea abies*	Sandy loam	Mean: 6 ˚C	1000 mm
Wu *et al*. (2011)	(o)	(-)	Litter: Eucalyptus	Eucalyptus	Acrisol	Mean: 22.5 ˚C	1534 mm

Table 2.4	Continued						
Authors	Influence of ECM roots		Substrate(s)	Tree species present	Soil type	Annual air temperature	Annual precipitation
	on decomposition rate	on soil/litter moisture					
Zhu & Ehrenfeld (1996)	(+)	(o)	Litter: *Pinus rigida* (Mill.)	*Pinus rigida*	Acid podzol	Range: 0 – 24˚C	1067 – 1168 mm

Chuyong *et al.,* 2002; Mayor & Henkel, 2006; McGuire *et al*. 2010) not stating whether there was a difference in soil or litter moisture between their treatments.

Studies which show a decrease in the decomposition of cellulose (Fischer & Gosz, 1986), fluorene (Genney *et al*., 2004) and litter (Koide & Wu, 2003) alongside decreases in soil or litter moisture in the presence of ECM roots provide support for the findings of Gadgil & Gadgil (1971; 1975). They also provide evidence for the theory that the 'Gadgil effect' is caused by indirect suppression of saprotrophic decomposers following the uptake of soil water by ECM roots. However, as with the findings of Gadgil & Gadgil, direct suppression of saprotrophs by ECM fungi cannot be ruled out as a possible explanation for the differences in decomposition observed in these studies.

Koide & Wu (2003) found that 36% of the variation in litter decomposition could be explained by the density of ECM root tips, and that variation in litter water content could explain 23% of the variation in litter decomposition. Their results show the importance of moisture for litter decomposition, but they also highlighted the complexity of the decomposition process, as although moisture effects could potentially explain the decrease in litter decomposition observed by Gadgil & Gadgil (1971; 1975), they cannot explain all the results from subsequent studies (see below). A large proportion of the variation in litter decomposition in the experiment of Koide & Wu (2003) remains unexplained.

Studies demonstrating a decrease in litter decomposition where ECM roots are present with no change in soil moisture (eg. Faber & Verhoef, 1991) may provide evidence for direct suppression of saprotrophs by ECM fungi as a cause of the 'Gadgil effect'. As there was no significant decrease in moisture associated with ECM roots, moisture differences caused by trenching could not explain these results.

Further evidence for the promoting effect of the presence of ECM roots on decomposition observed by Subke *et al*. (2004) comes from studies that have shown an increase in decomposition in the presence of ECM roots, alongside no change in soil moisture, as with the work of Zhu & Ehrenfeld (1996), and also Dighton *et al*. (1987). These studies do not argue against moisture differences causing the 'Gadgil effect', but

there is no evidence that ECM fungi were directly suppressing more effective saprotrophic decomposers in these studies.

There have been studies that have shown that the presence of ECM roots has little effect on soil or litter moisture, with no change in litter decomposition rates, (eg. Staaf 1988), who only observed differences in moisture between trenched and control plots on the driest of occasions. The study by Staaf (1988) did not provide evidence to repudiate that moisture differences may have caused the 'Gadgil effect' but it did provide evidence against a promoting effect of ECM roots, and also direct suppression of soil saprotrophs by ECM roots.

There have also been studies which support the current work, where a significant decrease in moisture has led to no change in litter decomposition (eg. Chigineva *et al*., 2009; Wu *et al*., 2011). The importance of soil moisture on decomposition is highlighted by the fact that, to our knowledge, no study has demonstrated an increase in litter decomposition alongside a decrease in soil moisture in the presence of ECM roots. This lends support to the hypothesis of Subke *et al*. (2004) that moisture effects may have been strong enough to mask any promoting effect of an intact rhizosphere in the studies of Gadgil & Gadgil (1971; 1975).

In the current work, the supply of labile carbon from plant roots to ECM fungi was excluded for some time from treatment collars, and this would be expected to take away any competitive advantage of the ECM fungi and alleviate any suppression of free-living soil saprotrophs taking place. Koide *et al*. (2011) found that the species of ECM fungus present in the system was critical for controlling root interactions with decomposition of dead root material and it may be also true for litter decomposition. The composition of the microbial community associated with the rhizosphere could dictate whether or not litter is decomposed at a faster rate, depending on the saprotrophic capabilities of the fungi in question (Koide & Wu, 2003).

Dighton *et al*. (1987) found that the presence of roots and mycorrhizas increased the decomposition of hide powder, chitin and cotton in mesocosms, and that different species of fungus differed in their decomposer capabilities, with the mycorrhizal fungus *Suillus luteus* (L.) being the most effective decomposer. They also found that

competition between fungi had an effect on substrate decomposition, with the observed enhancement of degradation of hide powder and cotton in the presence of roots and mycorrhizal fungi disappearing in the presence of the saprotrophic fungus *Mycena galopus* (Pers.). However the presence of the saprotroph had a 'synergistic effect' on chitin decomposition. Dighton *et al.* (1987) showed that in the absence of a plant host, there was very little difference in the level of decomposition taking place in the presence of the mycorrhizal or non-mycorrhizal fungi studied, though the presence of the plant host enhanced the decomposition of all three substrates significantly. It is possible therefore, that the differences between studies in terms of whether the presence of ECM roots had a positive, negative, or no effect at all on litter decomposition could be related to the microbial community present at a given experimental site. The work of Dighton *et al.* (1987) and Genney *et al*. (2004) has demonstrated that different species of ECM fungi have different decomposer abilities, and this, along with how capable these fungi are of competing with free-living saprotrophs, may dictate how decomposition is then affected by their exclusion (Koide & Wu, 2003).

Koide & Wu (2003) have also offered one further explanation which could potentially explain the inconsistencies in the literature in terms of why the moisture effect appears to be affecting litter decomposition in some studies (eg. Gadgil & Gadgil, 1971; 1975; Fischer & Gosz, 1986; Koide & Wu, 2003; Genney *et al*., 2004) but not for those of Chigineva *et al*. (2009), Wu *et al*. (2001) or at the current site. Koide & Wu (2003) have suggested that changes in soil moisture, following the exclusion of roots and associated microorganisms, may have an effect on litter decomposition depending on prevailing weather conditions. Koide and Wu (2003) suggested that the reason why their study produced an apparent soil moisture effect, and a decrease in litter decomposition in the presence of ECM roots (when other studies had not) was that their study was conducted during a particularly dry year, with levels of soil moisture falling below a 'threshold' level which would inhibit the decomposing activity of saprotrophic microorganisms. It is probable that during wet years, the same effect would not be observed (Bending, 2003; Virzo De Santo, 1993; Mayor & Henkel, 2006). It may be that although there was a significant difference between treatments in terms

of soil moisture content, overall soil moisture was high enough that ECM roots and hyphae did not take up sufficient water to affect the activity of litter decomposers during the current work.

As the experimental design of Koide & Wu (2003) did not have an ECM root exclusion treatment, it is not possible to directly compare the level of drying caused by ECM roots between their study and the current work. However, it is possible to make a crude comparison of average soil moistures measured at the current site, and those measured by Gadgil & Gadgil during their second field study (Gadgil & Gadgil, 1975) to see if there was greater inherent moisture limitation during their field studies than was apparent at Wheldrake Forest during the current work.

The field studies of Gadgil & Gadgil (1971; 1975) were carried out in a 22 year old un-thinned *Pinus radiata* stand in Kaingaroa State Forest, New Zealand. The region has an average rainfall of 1524 mm and an average temperature of 10˚C (Will, 1959; Seaton *et al*., 2008). The average temperature is similar to the average temperature of 9˚C experienced at Wheldrake Forest, however rainfall at Wheldrake Forest is less than half that experienced at the New Zealand site, with a yearly average of *c.* 630 mm (Heinemeyer *et al.*, 2007; Subke *et al.,* 2011). Consequently, it might be expected that moisture conditions were less limiting at the Gadgil site than at Wheldrake Forest. However, when the average soil % moisture contents from treatments RMS (total soil, no exclusion) and S (where roots and ECM hyphae excluded) from the current work, and the average soil % moisture (calculated from the four harvest days) from the cut and control treatments from the Gadgil & Gadgil (1975) paper are compared, the opposite appears to be true. The average soil moisture contents of treatment RMS and S were 42% and 50%, respectively (with a difference between the treatments of *c.* 8%). The average soil moisture contents of the Gadgil (1975) cut and control treatments were 36% and 43%, respectively (so a difference between treatments of *c.* 7%). The soil at Wheldrake is a well-draining sandy gley soil, and the soil at the Gadgil field site was classified as silty sand, so both are comparable in terms of drainage.

The conditions at the field site of Gadgil & Gadgil (1975) were likely to be more moisture limited than at the current site, and potentially the explanation of Koide and

Wu (2003), that the soil moisture would need to cross a 'threshold' value beyond which additional water uptake by ECM roots could affect litter decomposition might explain why Gadgil & Gadgil (1971; 1975) observed a decrease in litter decomposition in the presence of ECM roots, which was not observed in the current study.

There is no mention in either the Gadgil & Gadgil 1971 or 1975 paper of whether their field studies took place during particularly dry years. This is also true of most of the papers following on from the work of Gadgil & Gadgil (1971; 1975) with studies conducted over relatively short time periods of up to two years, where variations between years in the levels of litter and soil moisture, could be critically important for decomposition rates.

An attempt was made to conduct a meta-analysis from all the relevant published studies by plotting the average annual rainfall reported in each of the relevant studies against the observed % change in litter weight loss between treatments. Unfortunately, differences in methodologies, methods of reporting decomposition rates and lack of local specific weather data make such an analysis impossible.

The results of the current study, and those from the literature show that there are complex interactions between abiotic soil environment effects (soil moisture) and biotic effects (microbial suppression / competition), which prevent a direct evaluation of studies in contrasting systems. It is also likely that the nutrient content of the decomposing substrate and the nutrient status of the soil are important (Dighton *et al*., 1987; Dormaar 1990), factors not measured during the current work, but which warrant further consideration. Pickles *et al*. (2010) used spatial analysis to investigate the distribution of individual species of mycorrhizal fungi in the field and found that it is extremely patchy, and that the distribution changes considerably over time. This highly dynamic nature of microbial community distribution is likely to create real difficulty when trying to draw out mechanisms of control of decomposition within and between different systems.

In terms of understanding what caused the 'Gadgil effect', further work needs to be done and comparisons need to be made between average, wet and dry years. In an ideal world, an experiment such as the current work, with control for water uptake by

roots and ECM fungi should be conducted at the original field site in New Zealand. It would also be useful if papers reporting results from litter decomposition studies are uniform in terms of including the mass of litter prior to the start of the experiment and at the end, allowing easy comparison between studies, and also provide more comprehensive meteorological data.

Measurements of litter mass loss only constitute one method for estimating the level of decomposition taking place in forest soils. Though the results of the current experiment demonstrated that there was no difference in the rate of litter mass loss between the different soil collar treatments, the same may not be true for soil organic matter (SOM) decomposition as a whole. In Chapter 3, results from regular surveys of soil CO_2 efflux taken from the 24 soil collars will be presented alongside some results from periods when high resolution measurements of soil CO_2 were taken using an automated system. These results allow a comparison of the activity of the soil microbial community between the different mesh collar treatments, and an investigation of the influence of correction for water uptake by roots, and environmental factors on soil respiration.

Chapter 3 Forest soil CO_2 efflux measurements with controlled soil moisture

3.1 Introduction

Measurements of soil respiration are a proxy for measurements of decomposition and biological activity, giving an indication of the number of viable organisms active in a system (Song *et al.*, 1986; Panikov, 1999; Margesin *et al.*, 2000). There has been considerable recent interest in the links and feedbacks between above ground and below ground carbon cycling (Högberg *et al*., 2001; Subke *et al*., 2004; Vallack *et al*., 2012). Above ground activity, determining plant-derived C inputs to the soil, whether through leaf/needle litter or through root exudates/turnover has strong links with below ground processes, governing microbial activity in the soil, and this biological activity is reflected in the amount of C released through respiration (Kuzyakov & Domanski, 2000).

Subke *et al.* (2006) identified the following sources of CO_2 from the soil: (A) root respiration (from growth and maintenance of roots), (B) rhizomicrobial respiration (heterotrophic decomposition of live root-derived carbohydrates), (C) decomposition of fresh organic matter (FOM) such as surface litter, (D) decomposition of old soil organic matter (SOM), including priming of SOM decomposition following input of labile substrates, (F) weathering of soil carbonates. Consequently, soil CO_2 efflux (R_s) is a complex flux with contributions from a variety of sources (Sulzman *et al.,* 2005). However, it is difficult to separate and study all the contributing fluxes to soil CO_2 efflux simultaneously, and generally, when studies separate soil CO_2 efflux into component fluxes, they measure autotrophic respiration (R_a), and heterotrophic respiration (R_h). R_a includes CO_2 derived from the respiration of plant assimilate-derived C by roots, and the respiration of microorganisms directly associated with roots in the rhizosphere, including mycorrhizal fungi. R_h includes CO_2 derived from the decomposition of FOM such as leaf/needle litter and SOM carried out by microorganisms, and soil animals (Kirschbaum, 2005). When CO_2 data are used in a modelling context, feedbacks between the soil components are usually ignored, for example with the potential priming of the decomposition of SOM by labile substrates

in litter and root exudates (Kuzyakov, 2002; Subke *et al.,* 2004). Another problem is that by only separating out R_s into R_a and R_h, and not their component parts, there is the potential that full understanding of the control of the contributors to R_a and R_h by abiotic factors is not properly established. However, some individual studies have attempted to subdivide R_a and R_h (eg. Taneva & Gonzalez-Meler, 2012). Albanito *et al*. (2012) studied the contribution of R_a and R_h to R_s using inherent differences in $\delta^{13}C$ of the fluxes from the various sources, and a three-way mixing model. These authors were able to further separate out R_h into the contributions from litter decomposition, and the component flux from the decomposition of older SOM. Through the use of labelled litter, Rubino *et al*. (2010) found that over a period of 11 months, 30% of the mass loss of C from litter was released as CO_2, and the rest of the C was incorporated into SOM. Fahey *et al*. (2005) have also attempted to separate root and rhizosphere-derived components of R_a using a mass balance approach, incorporating data on root turnover rate and root respiration from excised roots in a hardwood system. Other attempts have been made to separate the components of R_a, and gain an estimate of the contribution of rhizomicrobial respiration (from microorganisms such as ECM fungi in the rhizosphere) to R_s using pulse-labelling of ECM seedlings in pots, data from the respiration of excised roots, and mass balance calculation (Fahey *et al.* 2005; Phillips & Fahey, 2005) but these methods are subject to a high degree of estimation and error.

ECM fungi are important contributors to the global carbon cycle as along with other tree species, they form symbioses with the *Pinaceae,* species of which make up boreal and temperate coniferous forests in the northern hemisphere (Smith & Read, 1997; Hilszczańska *et al.*, 2011). Heinemeyer *et al*. (2007) were the first to measure the contribution of ECM fungi to R_s in the field, in a 6 month experiment, divided into three measurement campaigns. This research was conducted in the same *Pinus contorta* stand in Wheldrake Forest used in the current work, with the same collar exclusion collar design. During their third measurement campaign, Heinemeyer *et al*. (2007) showed that R_h contributed 65% to R_s, and that R_a contributed 35%. They were able to show that the % contribution of ECM hyphae to R_s at Wheldrake forest in November to December 2005 was considerable (25%), and higher than that of roots, which contributed only 10%. Importantly, Heinemeyer *et al.* (2007) also demonstrated

that R_h and the components of R_a (roots and ECM hyphae) responded differently to environmental controlling factors. For example, they found that R_h was more affected by temperature than the mycorrhizal component of R_a. They also found that during a drought period, there was a decrease in respiration from both soil heterotrophs and mycorrhizal hyphae, but that the mycorrhizal flux contribution was particularly reduced, recovering quickly with the onset of rainfall.

Soil moisture is an important factor in forest soils, and along with temperature, is a key determinant of the rate of decomposition and release of C (Moyano *et al*., 2012). Soil water films critically allow the diffusion of extracellular enzymes involved in substrate breakdown through the soil medium (Steinweg *et al*., 2012). Therefore, during drought or freezing conditions, a decrease in decomposition and, hence a reduction in CO_2 release would be expected. On the other hand, the enzymes associated with anaerobic respiration generally catalyse reactions at a slower rate than those used in aerobic respiration, therefore, when soils become water-logged there is also a decrease in CO_2 efflux (Davidson & Janssens, 2006). Davidson *et al.* (1998) studied the effect of different levels of soil drainage on soil CO_2 efflux at six different sites and found that the better drained soils had higher rates of soil CO_2 efflux. However, a drought event caused a big decline in soil respiration rates during the summer months in five sites, but had no influence on soil CO_2 efflux from a swamp site.

Linn & Doran (1984) observed that non-tilled soils which had a higher percentage of water filled pores (% WFP) had higher soil CO_2 efflux when compared to ploughed soils which had a lower % WFP, though water-soluble C was also found to be important in determining CO_2 efflux. They found that below 60% WFP, soil moisture limited decomposition, but above 60%, restrictions in the diffusion, and availability of oxygen decreased the rate of decomposition. In two laboratory experiments using trenched and control soils, Fischer and Gosz (1986) found that there were significant moisture differences between trenched and control soils, and that once these moisture differences were eliminated, there was no difference in the soil CO_2 efflux from trenched versus control soils.

As was outlined in Chapter 2, soil moisture differences caused by water uptake by roots was offered as an explanation for the decrease in litter decomposition observed in control soils when compared to soils where roots had been cut, during the work of Gadgil & Gadgil (1971; 1975) by Koide & Wu (2003) and Subke *et al*. (2004). One of the main criticisms of excluding roots from soil to separate out the various components of R_s is that plots without active roots generally have higher soil moisture contents than control soils. This difference in soil moisture would be expected to affect soil respiration, leading to an over-estimation of R_h, and an underestimation of R_a. Water uptake by roots in non-trenched soils could potentially be decreasing soil moisture to a degree that would decrease R_s through decreasing R_h and, possibly the mycorrhizal component. This may have occured during the work of Heinemeyer *et al*. (2007) who did not measure or consider soil moisture differences between their treatments. However, their third measurement campaign was carried out during November and December, so any moisture differences caused by trenching would not be expected to be as severe as they would be during the summer months.

The current experimental set-up was designed primarily to test the hypotheses outlined in Chapter 2 (see Section 2.1). However, the control of soil moisture involved in the litter decomposition experiment also allowed a unique opportunity to study the effect of surface soil moisture differences on the various components of R_s. Few data are available concerning the contribution of ECM hyphae to forest soil carbon cycling, and to our knowledge, a direct correction of water uptake by roots in the field and a study of how the localised decrease of soil moisture in the presence of roots affects decomposition has not been attempted.

The aim of the current work was primarily to investigate the influence that soil moisture has on soil CO_2 efflux, and its component fluxes from roots, mycorrhizal hyphae and free-living soil heterotrophs. By running over two summers, the current study also gave greater information about the seasonality of the contributions from the various flux components. A major difference between the current work, and that of Heinemeyer *et al*. (2007) is that they used high resolution hourly data throughout, compared in the current work, where conclusions were drawn from fortnightly measurements of soil CO_2 efflux obtained using a hand-held CO_2 survey system. For

this reason it was decided to make a short-term comparison of data from an automated system (after Heinemeyer *et al*., 2007) and the hand-held soil CO_2 survey system. During the current work, the following hypothesis was tested:

The correction of soil moisture differences between soil where roots and / or ECM fungi are present, and where they have been excluded will result in an increase in R_s.

3.2 Materials and methods

3.2.1 Site description

The study site was a 20 year old stand of Lodgepole pine (*Pinus contorta,* Douglas) as described for the litter decomposition experiment covered in Chapter 2, with details provided in section 2.2.1.

3.2.2 Experimental treatment

Twenty four soil collars, comprising 16 'deep' soil collars (30 cm in height, hammered 25 cm into the soil) and 8 'shallow surface' collars were installed on 10th June 2008, in a randomised block design based on a survey of background CO_2 efflux (for further details of this survey, and of soil collar construction, see section 2.2.1).

A fortnight following collar installation, PVC shields were erected above the 24 soil collars, and weekly additions of average amounts of collected throughfall commenced (see section 2.2.2).

Between 10th June 2008 and 9th April 2009, there were effectively three treatments (RMS, MS and S, n=8). Following a survey of soil CO_2 efflux on 25th November 2008, replicates of treatments RMS, MS and S were ranked, and this ranking used to assign the 8 replicates in each collar type to the 'corrected' (receiving additional water from the automated irrigation system) and 'non-corrected' (control) treatments. This subdivision created an additional three treatments, and following the installation of the automated irrigation system on 9th April 2009, consequently four replicates each of RMS, MS and S. The new treatments are referred to as RMS(W), MS(W) and S(W), respectively, the (W) designated to describe 'corrected' water. For a summary of the six treatments, see Table 2.1.

3.2.3 Hand-held surveys of soil CO_2 efflux

Following collar installation, regular hand-held surveys of soil CO_2 efflux were carried out prior to watering, on a roughly fortnightly basis between 17th June 2008 and 27th October 2010, using a Li-8100 infra-red gas analyser (IRGA) with an 8100-103 20 cm survey chamber (Li-Cor Bioscience, Lincoln, NE, USA) (see Fig. 3.1). Measurements

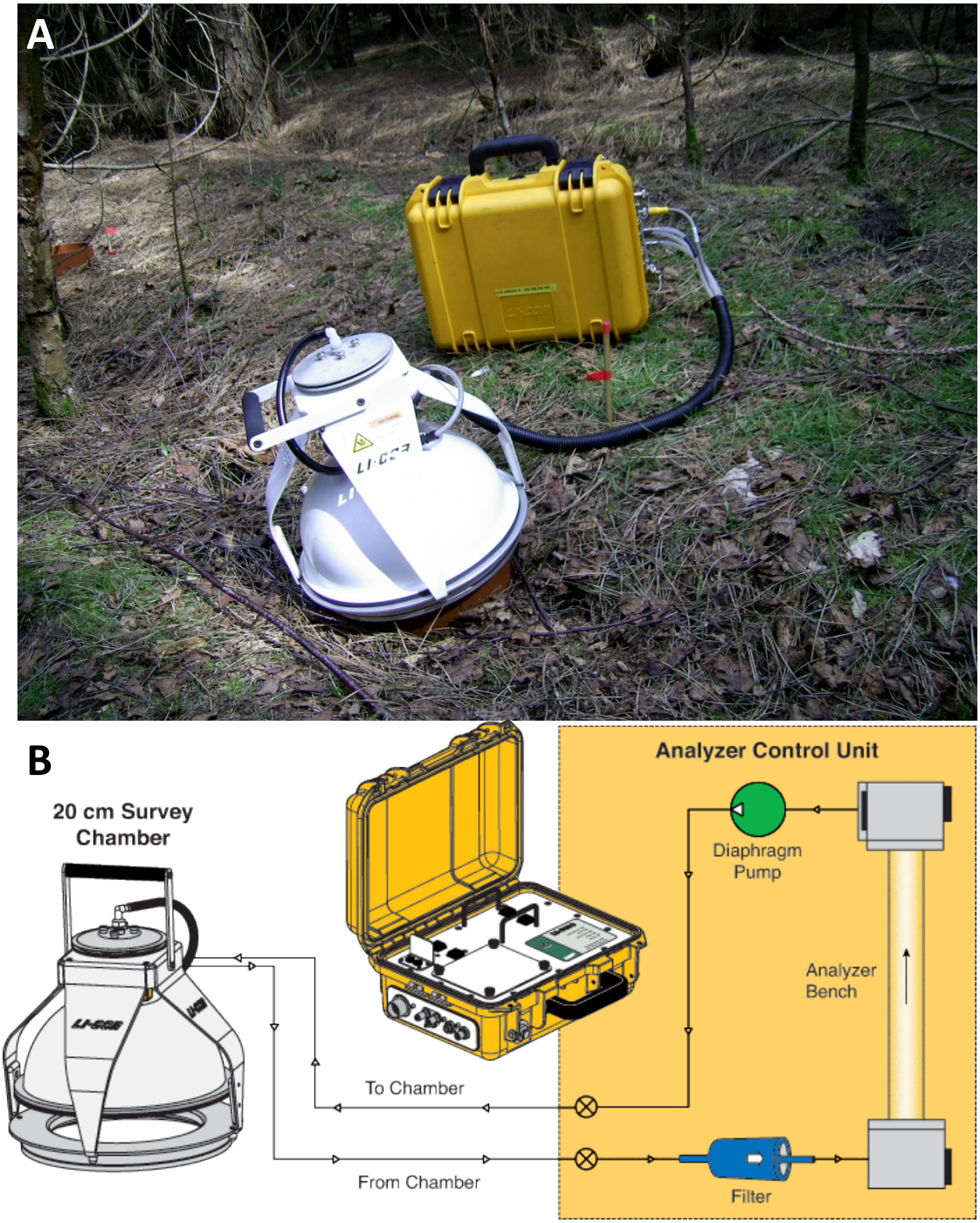

Fig. 3.1 (A) A photograph taken during a hand-held survey of soil CO_2 efflux showing the Li-Cor Li-8100 infrared gas analyser with an 8100-103 20 cm survey chamber in position on top of one of the PVC soil collars in the *Pinus contorta* stand at Wheldrake Forest (B) Schematic taken from www.licor.com showing the Li-8100 system configuration.

were always taken shortly after midday, (between 12:00 hrs and 15:00 hrs), and always in the same order, which included a random ordering of the six treatments. During each measurement, the survey chamber was closed for 2 minutes (a short enough time to limit any effects of the closed chamber on CO_2 diffusion gradients), during which time the rate of increase of CO_2 was measured by the Li-Cor software using both CO_2 and H_2O concentrations. The Li-Cor software offers two options, either a linear or polynomial function can be fitted to CO_2 vs. time data. As the polynomial is sensitive to the length of time of the measurement, for the current work, the linear function was selected (see Li-Cor, 2005). A dead-band of 10 seconds was set, so that the period immediately following the positioning of the survey chamber on each collar, and the chamber closing, was excluded from the flux calculation, thereby allowing time for stable mixing to be established prior to the start of the measurement. Care was taken not to breathe in close proximity to the chamber when it was closing, or to remain standing on soil within one metre of the chamber, as this could cause increased release of CO_2 from the soil, and an overestimation of the flux.

3.2.4 Automated hourly measurements of soil CO_2 efflux

Continuous hourly measurements of soil CO_2 efflux were taken using an automated soil CO_2 efflux measurement system, comprising a Li-cor 8100 IRGA, linked via a custom made multiplexer unit (Electronics Workshop, Department of Biology, University of York) to 12 automatic 8100-101 long-term chambers. This system was deployed for two measurement 'Runs'. Run 1 took place between 14th October 2009 and 26th October 2009, where the 12 automated chambers were positioned on top of four replicates each of treatments RMS, MS and S (the 'non-corrected' collars, see Section 2.2.3). Run 2 took place between 28th October 2009 and 16th November 2009, where the 12 automated chambers were re-positioned on top of four replicates of treatments RMS(W), MS(W) and S (the 'corrected collars', see Section 2.2.3). During each measurement, the chamber was closed for 3 minutes during which time the rate of increase of CO_2 was measured by the Li-Cor software using both CO_2 and H_2O concentrations. Again, a dead-band of 10 seconds was set, so that the period immediately following the positioning of the survey chamber on each collar, and the chamber closing, was excluded from the flux calculation, thereby allowing time for

stable mixing to be established prior to the start of the measurement. Once the measurement had finished, the chambers opened automatically, and remained open, so that the presence of the chamber did not affect soil processes.

3.2.5 Measurement of environmental parameters

For details of the measurements of soil temperature, and of hand-held soil moisture surveys refer to Section 2.2.6.

Measurements of regional light intensity (lux) and air temperature (^{o}C) were logged from four replicate sensors (HOBO Pendant temperature/light data logger Model: UA-002-64, Tempcon Instrumentation Ltd, Sussex, UK), suspended using cable ties from the top of 6 ft seedlings in the middle of a nearby stand (to avoid shading of the sensors by mature trees).

3.2.6 Data analysis

Linear regressions calculated by the in-built Li-Cor version 2 software were visually inspected, and where the date were 'noisy' and the coefficient of variation was large, occasionally measurements were excluded from later analysis. Each flux was corrected according to the individual collar it was measured from, as collars differed slightly in terms of their height above the soil surface (5 ± 1 cm), which affected the total volume over which the flux values were calculated (see Li-Cor, 2005) .

The experiment followed a randomised block design, and a variety of statistical approaches were used during data analysis. Where possible, parametric analyses were carried out but generally sample distributions deviated from normal so data were transformed where possible or the equivalent non-parametric tests were carried out, usually Friedman's non-parametric two-way ANOVA with *Post-hoc* analyses using Duncan's multiple range test (see Section 2.3.2 for a description of how the data were averaged to avoid pseudo replication). Further specific information regarding the use of different statistical tests is detailed in the Results section. All statistical tests were carried out using SAS software package v. 9.3 (SAS Institute Inc., Cary, NC, USA).

Overall percentage flux contributions from roots (see Eq. 3.1), ECM hyphae (see Eq. 2), and soil heterotrophs (R_h) (see Eq. 3) to total soil respiration (R_s) were calculated using the method of Heinemeyer *et al*. (2007). This process was repeated for the treatment RMS(W) and MS(W) collars, and for all treatments during summers 2009 and 2010 (1st June-30th Sept), and also for winter 2009/2010 (1st Nov-28 Feb).

$$\text{Root}\% = \left(\frac{(\text{RMS flux} - \text{MS flux})}{\text{RMS flux}}\right) \text{X } 100 \qquad (\text{Eq. } 3.1)$$

$$\text{ECM}\% = \left(\frac{(\text{MS flux} - \text{S flux})}{\text{RMS flux}}\right) \text{X } 100 \qquad (\text{Eq. } 3.2)$$

$$\text{Soil heterotroph } \% = \left(\frac{\text{S flux}}{\text{RMS flux}}\right) \text{x } 100 \qquad (\text{Eq. } 3.3)$$

where RMS flux is the average soil CO_2 efflux measured during hand-held surveys from treatment RMS, MS flux is the average soil CO_2 efflux from the treatment MS collars, and S flux is the average soil CO_2 efflux measured during hand-held surveys from treatment S collars.

3.2.7 Correlation between CO_2 fluxes and environmental variables

Individual simple correlations and a forward stepwise multiple regressions (SAS v 9.3) were used to assess whether environmental factors could explain any variation in soil CO_2 efflux. These environmental factors included soil temperature at depths of 5 cm, soil moisture content, and light intensity. For the forward stepwise multiple regression, the significance level for entry into the model was set at 0.05.

3.3 Results

3.3.1 Hand-held surveys of soil CO_2 efflux prior to installation of the automated irrigation system

At the first hand-held survey of soil CO_2 efflux, conducted seven weeks following collar installation, there was already a clear difference in the soil CO_2 efflux between treatments RMS, MS and S. The greatest CO_2 efflux was measured from total soil collars where neither roots nor mycorrhizal fungi had been excluded (treatment RMS) followed by where roots had been excluded (treatment MS), with the lowest flux values measured from the collars where roots and mycorrhizal hyphae had been excluded (treatment S). These treatment effects had a seasonal trend, being most pronounced during the summer months (see Fig. 3.2).

The annual cumulative soil CO_2 efflux was calculated for each of the 24 soil collars prior to the installation of the automated irrigation system (see Fig. 3.3). Results of Friedman's two-way ANOVA with treatment and block as factors showed that there was a highly significant difference between the soil collar treatments for average cumulative soil CO_2 efflux ($F= 27.52$, $p < 0.0001$). *Post-hoc* analysis (Duncan's multiple range test) showed that treatments RMS, MS and S were all significantly different from each other (see Fig. 3.3). There were no inherent significant differences between the collars of treatment RMS, and those which were later to become treatment RMS(W), once the automated irrigation system was installed. The same was true for the treatment MS collars, and those collars soon to be allocated to treatment MS(W), and also for the collars of treatments S and S(W).

3.3.2 Hand-held surveys of soil CO_2 efflux following the installation of the automated irrigation system

Fig. 3.4 shows the soil CO_2 efflux over time from all six treatments following installation of the automated irrigation system, and as with the data prior to automated irrigation, there were clear treatment differences, which were more pronounced during the summer months. In order that the data be viewed more clearly

and to aid comparison, the data from the six treatments displayed in Fig. 3.4 have been separated out and re

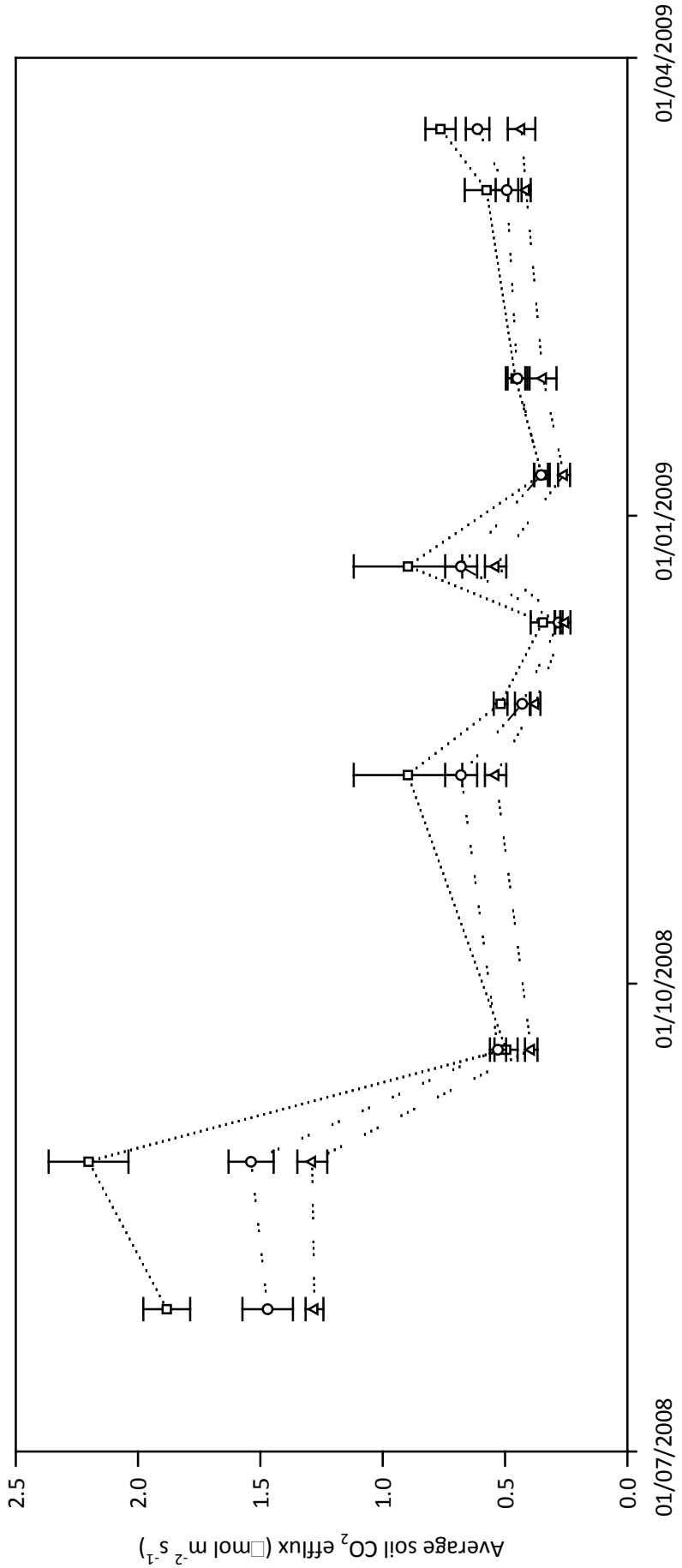

Fig. 3.2 Average soil CO_2 efflux (μmol m^{-2} s^{-1}) measured from the 24 soil collars prior to installation of the automated irrigation system. Treatments RMS, MS and S are represented by open squares □, circles ○ and triangles Δ, respectively. Error bars represent ± 1 SE (n = 8, apart from 18/09/2008, 11/12/2008 and 6/3/2009, where n = 4).

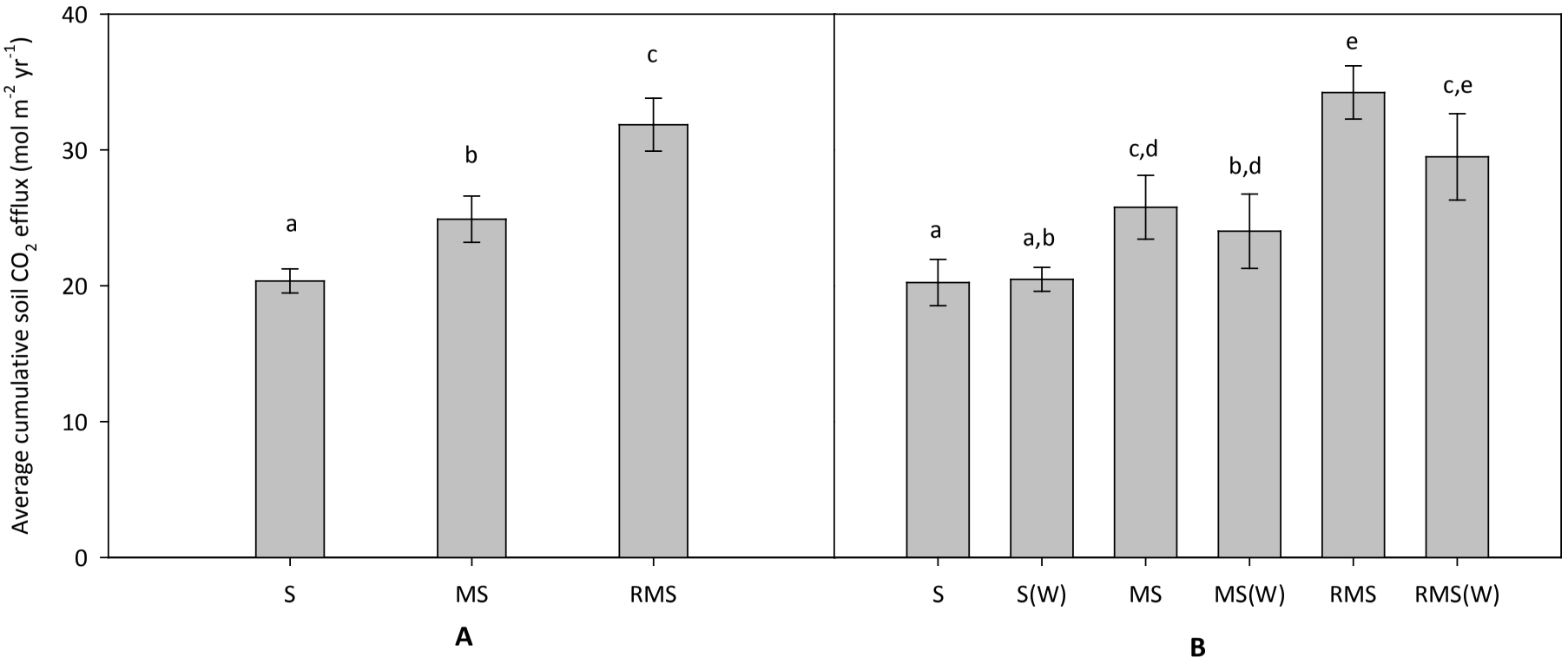

Fig. 3.3 Average cumulative soil CO_2 efflux (mol m^{-2} yr^{-1}) calculated prior to the installation of the automated irrigation system (**A**) for the three soil collar treatments, S, MS and RMS. Error bars represent ± 1 SE (n = 8), (**B**) for the purposes of establishing any inherent significant differences between the collars that were to remain as treatments S, MS and RMS, and those which were destined to receive additional correction for root and hyphal water uptake and become treatments S(W), MS(W) and RMS(W). Error bars represent ± 1 SE (n = 4). Within each treatment classification, treatments which differ significantly have different letters.

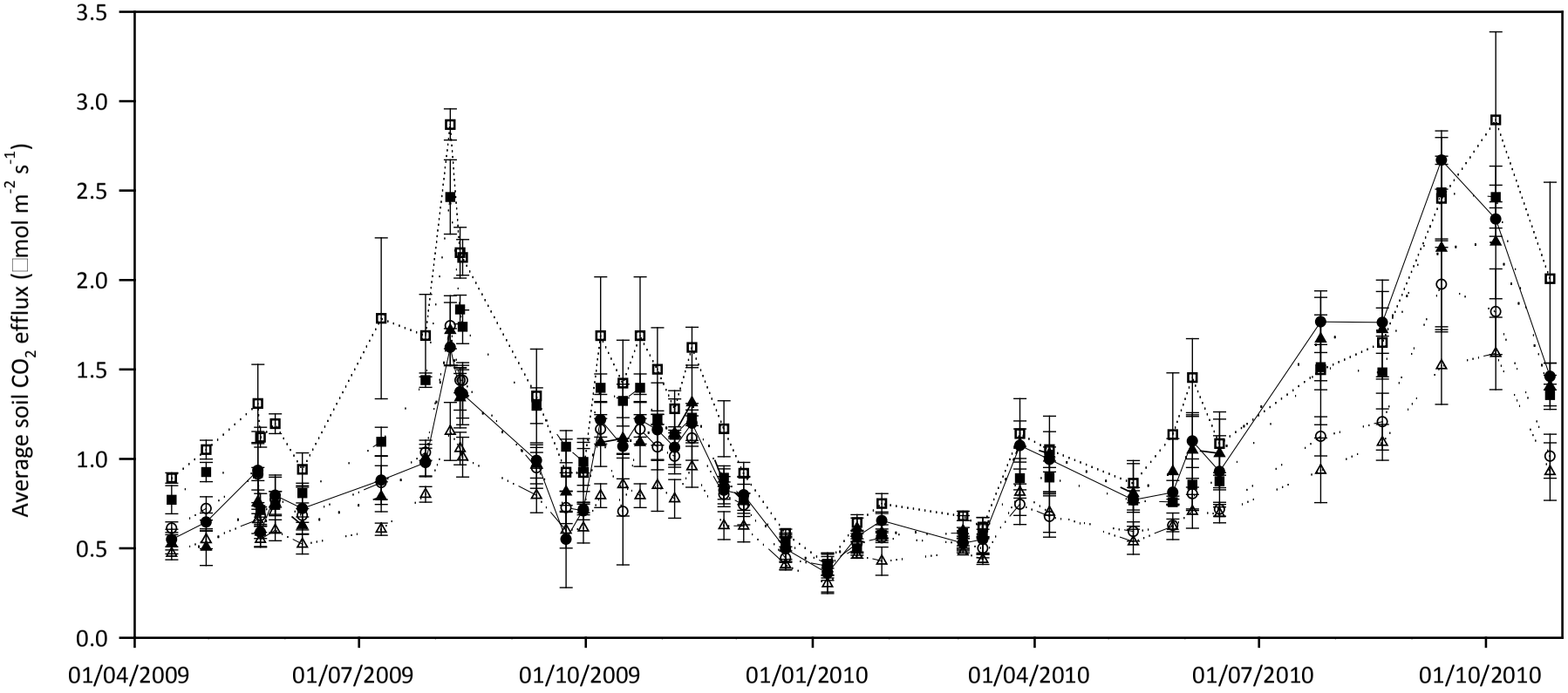

Fig. 3.4 Average soil CO_2 efflux (µmol m^{-2} s^{-1}) measured from the six soil collar treatments following installation of the automated irrigation system. Treatments RMS, MS and S are represented by open squares □, circles ○ and triangles Δ, respectively. Treatments RMS(W), MS(W) and S(W) are represented by solid squares ■, circles ● and triangles ▲, respectively. Error bars represent ± 1 SE (n = 4).

drawn over the next group of figures, beginning with the soil CO_2 efflux from collars that did not receive additional water from the automated irrigation system, the 'non-corrected' collars (treatments RMS, MS and S, see Fig. 3.5). There was a continuation of the treatment differences that were observed prior to the installation of the automated irrigation system, with treatment RMS collars consistently displaying the greatest soil CO_2 efflux, followed by treatments MS and S.

The same pattern was observed for the 'corrected collars', treatments RMS(W), MS(W) and S(W) during summer 2009, with treatment RMS(W) generally having a higher soil CO_2 efflux than treatments MS(W) S and S(W) (see Fig. 3.6). However, in contrast to the non-corrected treatments, during summer 2010, it would appear that the highest soil fluxes recorded during several of the hand-held CO_2 surveys were from treatment MS(W). Treatment S(W), which had received the same amount of water from the automated irrigation system as treatment RMS(W), had a higher flux than the treatment S control collars, and on several occasions during summer 2010 had a higher average soil CO_2 efflux than that of the treatment RMS(W) collars.

A direct comparison of treatments RMS and RMS(W) (see Fig. 3.7) shows that generally, greater soil CO_2 efflux was observed from treatment RMS than treatment RMS(W).The opposite was true for the mycorrhizal treatments, with a higher average soil CO_2 efflux measured on most occasions from treatment MS(W) (see Fig. 3.8). As previously mentioned for Fig. 3.4, this treatment effect was especially pronounced during summer 2010, with treatment MS(W) having the highest flux of all the six collar treatments (see Fig. 3.8).

Treatment S(W) collars had a consistently higher average soil CO_2 efflux than treatment S collars, again, with the greatest differences observed during the summer months. As with treatment MS(W), the difference between S and S(W) appears to be greater for summer 2010, than it was for 2009 (see Fig. 3.9).

The cumulative soil CO_2 efflux was calculated using the results of the hand-held surveys of soil CO_2 efflux for each of the 24 soil collars following the installation of the automated irrigation system (see Fig. 3.10). Results of a Friedman's two-way ANOVA with block and treatment as factors showed that overall there was a significant

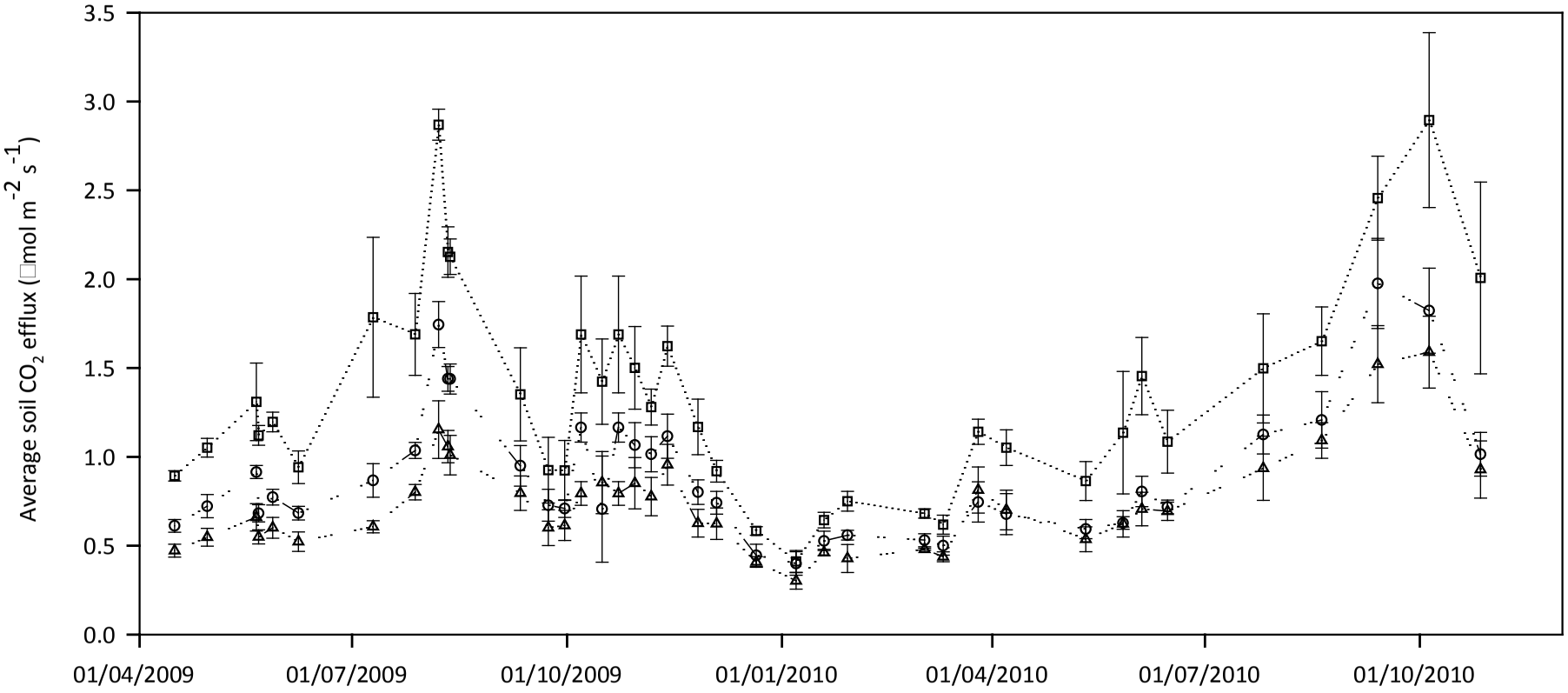

Fig. 3.5 Average soil CO_2 efflux (µmol m^{-2} s^{-1}) measured from collars not receiving additional water correction via the automated irrigation system. Treatments RMS, MS and S are represented by open squares □, circles ○ and triangles Δ, respectively. Error bars represent ± 1 SE (n = 4).

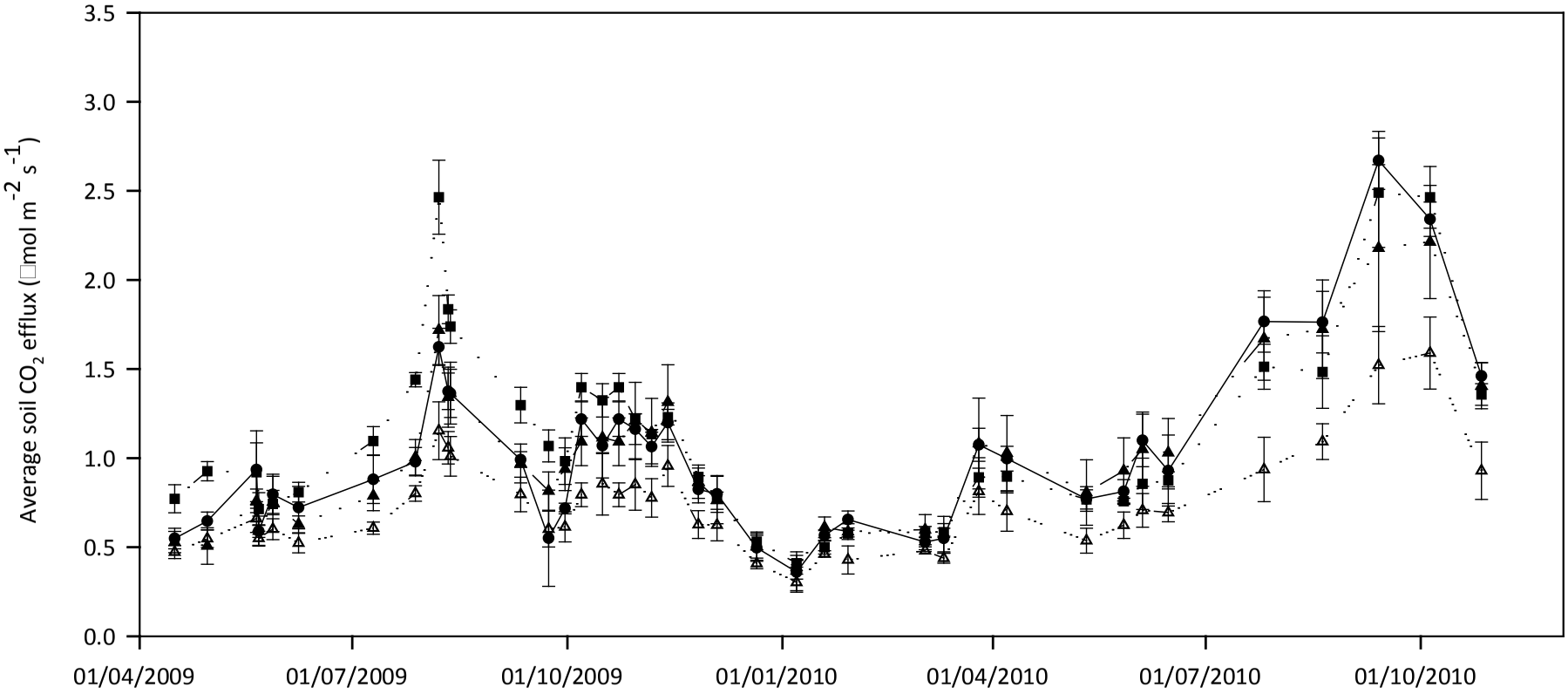

Fig. 3.6 Average soil CO_2 efflux (µmol m^{-2} s^{-1}) measured from collars receiving additional water correction via the automated irrigation system, and also the control treatment (treatment S). Treatments RMS(W), MS(W) and S(W) are represented by solid squares ■, circles ● and triangles ▲, respectively. Treatment S is represented by open triangles Δ. Error bars represent ± 1 SE (n = 4).

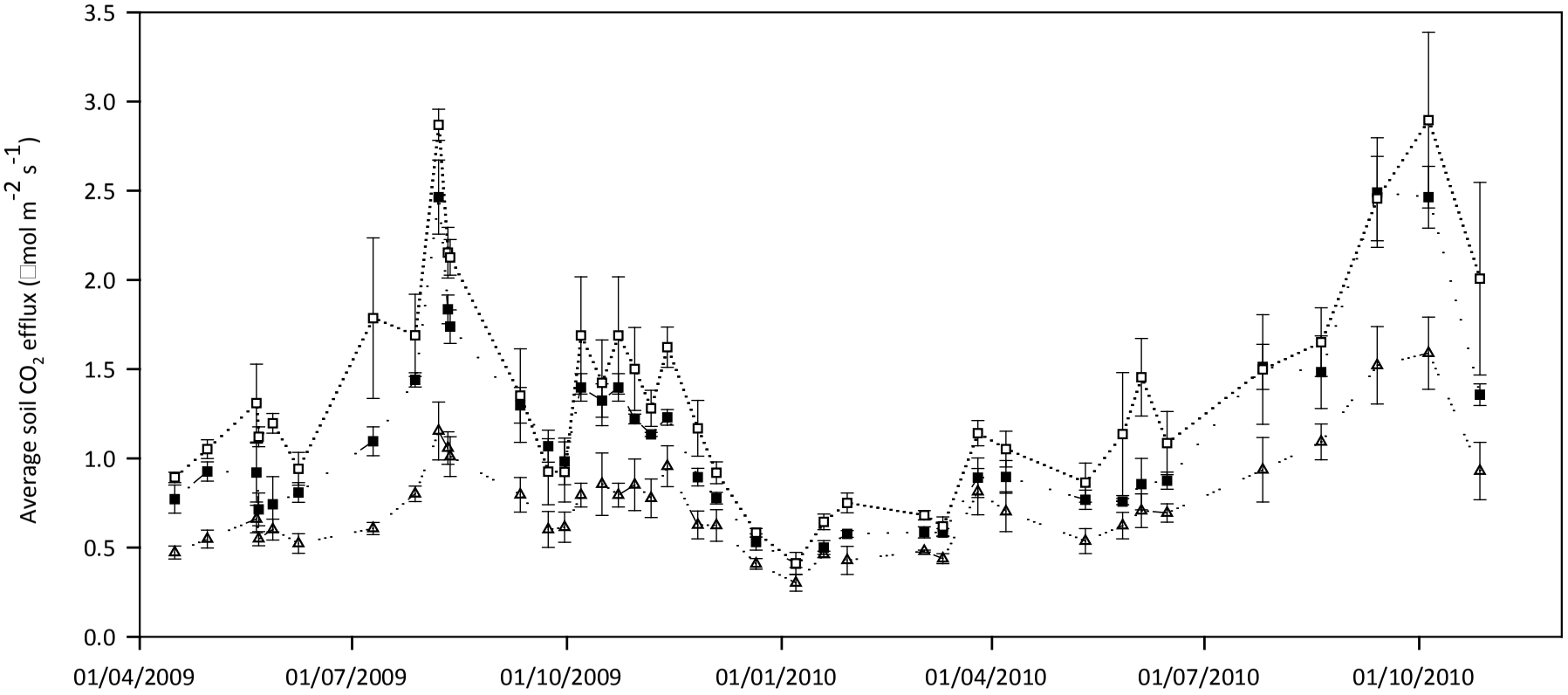

Fig. 3.7 Average soil CO_2 efflux (µmol m^{-2} s^{-1}) measured from the total soil, with and without correction from the automated irrigation system (treatments RMS(W) and RMS), and also the control treatment (treatment S). Treatments RMS(W) and RMS are represented by solid squares ■ and open squares □, respectively. Treatment S is represented by open triangles Δ. Error bars represent ± 1 SE (n = 4).

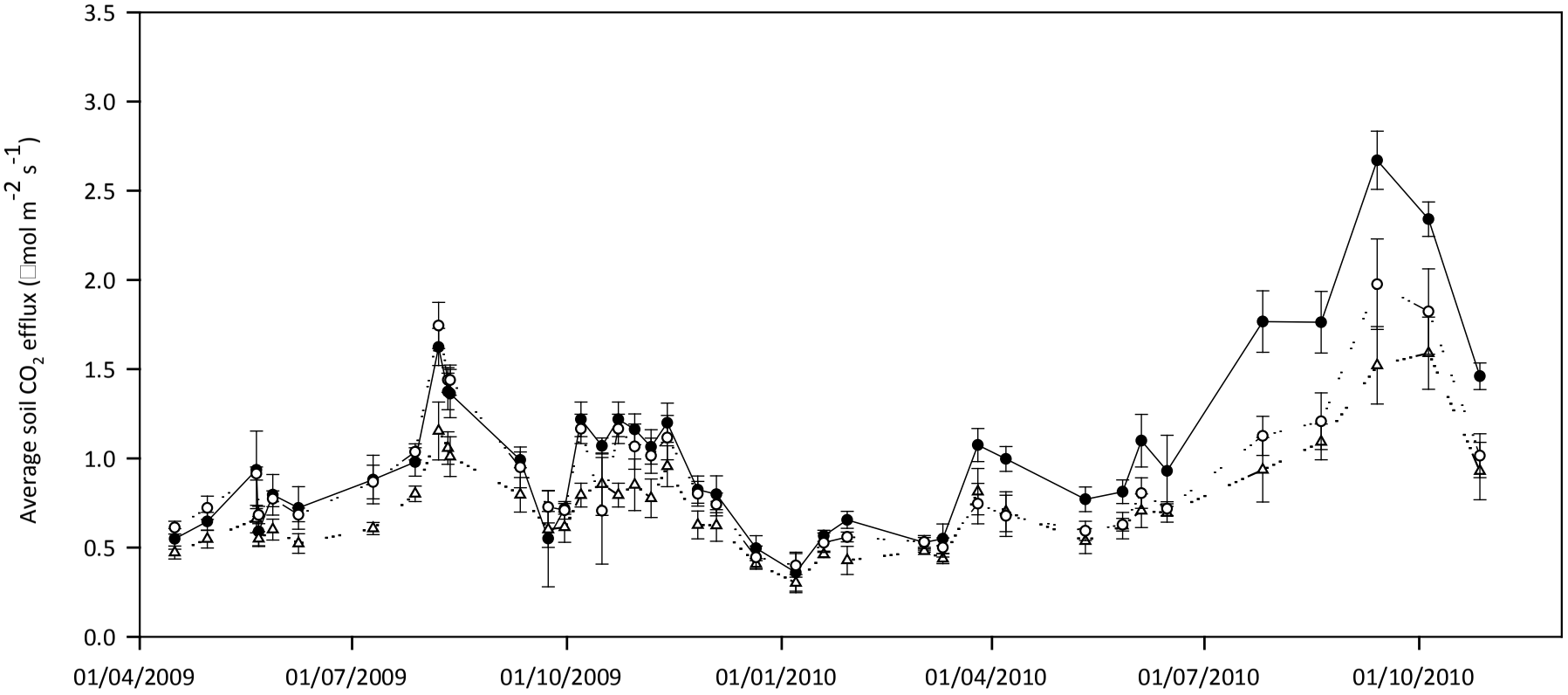

Fig. 3.8 Average soil CO_2 efflux (µmol m^{-2} s^{-1}) measured from the mycorrhizal treatments, with and without correction from the automated irrigation system (treatments MS(W) and MS), and also the control treatment (treatment S). Treatments MS(W) and MS are represented by solid circles ● and open circles ○, respectively. Treatment S is represented by open triangles Δ. Error bars represent ± 1 SE (n = 4).

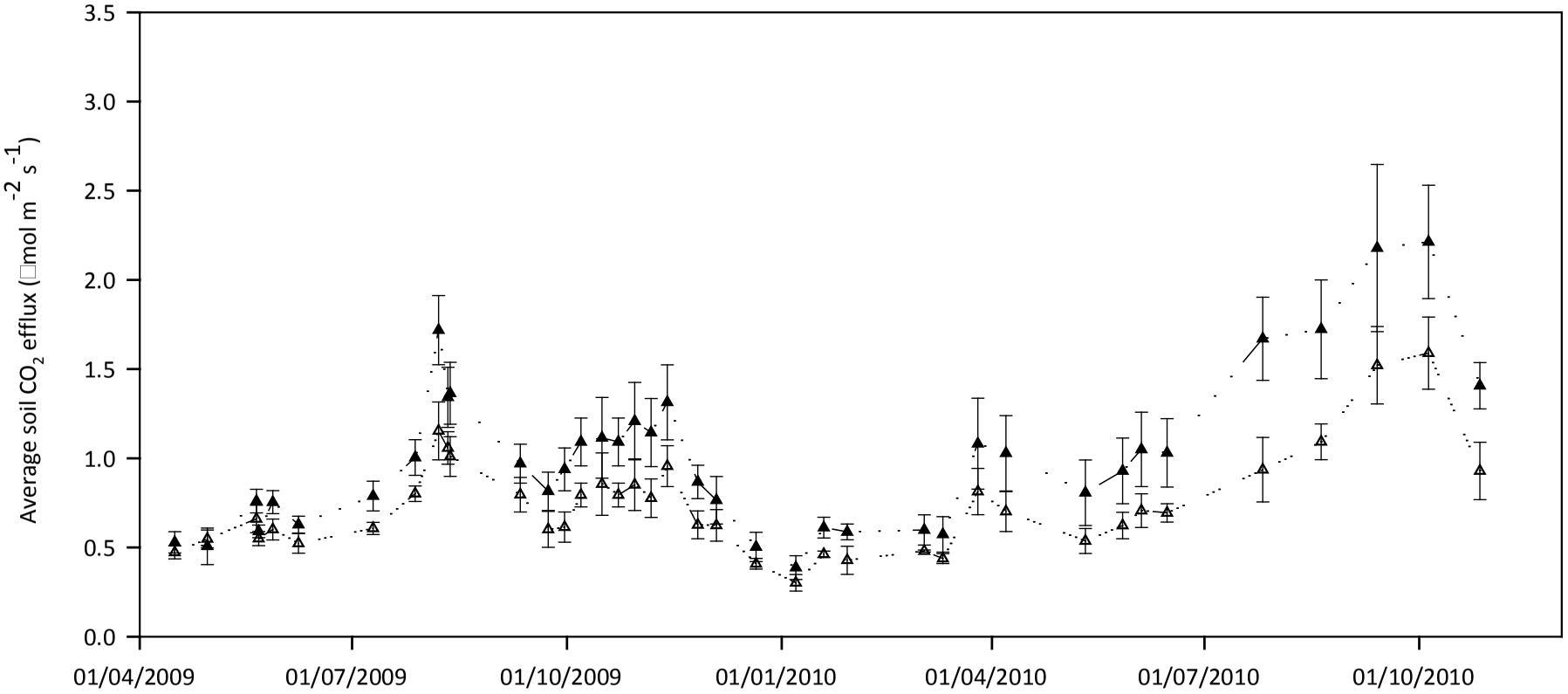

Fig. 3.9 Average soil CO_2 efflux (µmol m^{-2} s^{-1}) measured from soil-only treatments, with and without correction from the automated irrigation system (treatments S(W) and S). Treatments S(W) and S are represented by solid triangles ▲, and open triangles Δ , respectively. Error bars represent ± 1 SE (n = 4).

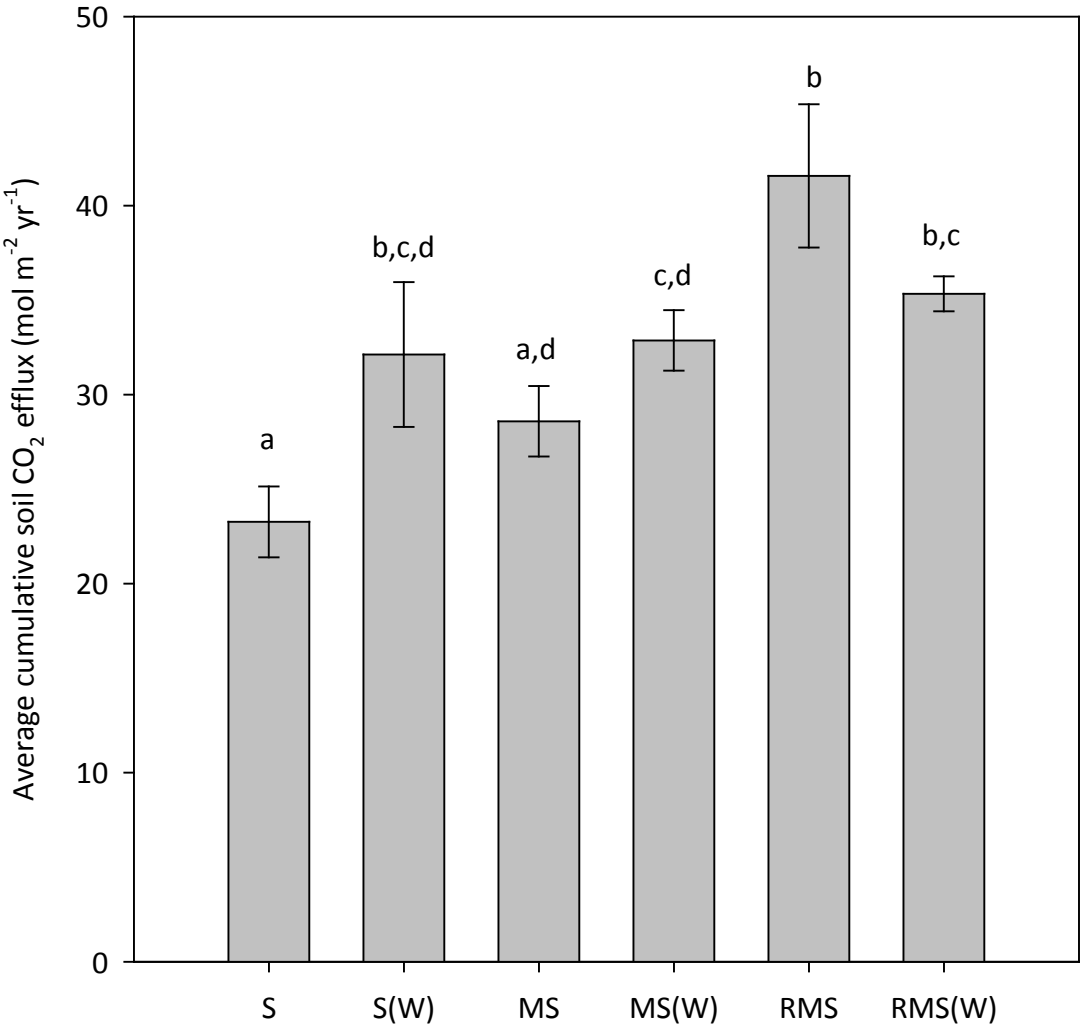

Fig. 3.10 Average cumulative soil CO_2 efflux (mol m^{-2} yr^{-1}) calculated following the installation of the automated irrigation system. Error bars represent ± 1 SE (n = 4), treatments which differ significantly have different letters.

difference between the treatments in terms of their average cumulative soil CO_2 efflux ($F= 6.33$, $p < 0.003$).

Fig. 3.10 clearly shows that overall, the greatest emissions of CO_2 were from collars where roots were not excluded (treatments RMS(W) and RMS) and *post-hoc* analysis showed that there was no overall significant difference between these two treatments. The opposing trend in terms of the effect of irrigation on the CO_2 efflux from the root and mycorrhizal collars mentioned previously (Figs. 3.7 and 3.8) can be seen clearly here. Treatment RMS appeared to have a higher cumulative soil CO_2 efflux than treatment RMS(W), though this was not significant, and as there was a higher flux from the RMS collars prior to the establishment of the automated irrigation system, it is not possible to attribute this apparent difference in the root contribution to soil CO_2 efflux to correction for root water uptake via automated irrigation.

For the mycorrhizal collars, the treatment effect observed prior to automated irrigation was reversed following the installation of the automated irrigation system, with the higher flux coming from MS(W), rather than MS (see Figs. 3.3 and 3.10). The difference between the cumulative soil CO_2 efflux from treatments MS and MS(W) compared to treatment S suggests that with automated irrigation, the overall contribution of mycorrhizal hyphae to soil CO_2 efflux is enhanced, compared to the mycorrhizal contribution from the non-corrected MS collars. However, the differences between treatments MS(W) and MS in terms of the cumulative soil CO_2 efflux were not significant either prior to, or following the start of automated irrigation.

Interestingly, despite roots and mycorrhizal hyphae being excluded, the average cumulative soil CO_2 efflux from treatment S(W) was not significantly different from that of the root or mycorrhizal treatments. Treatment S(W) had a significantly higher CO_2 efflux than that of treatment S (see Fig. 3.10).

As the treatment differences in terms of soil CO_2 efflux were most pronounced during the summer, the average cumulative soil CO_2 efflux for each of the treatments was recalculated for just the summer months (1st June to 30th Sept, 2009 and 1st June to 30th Sept 2010, see Fig. 3.11). Results of Friedman's two-way ANOVA with block and treatment as factors showed that there was a significant difference between the

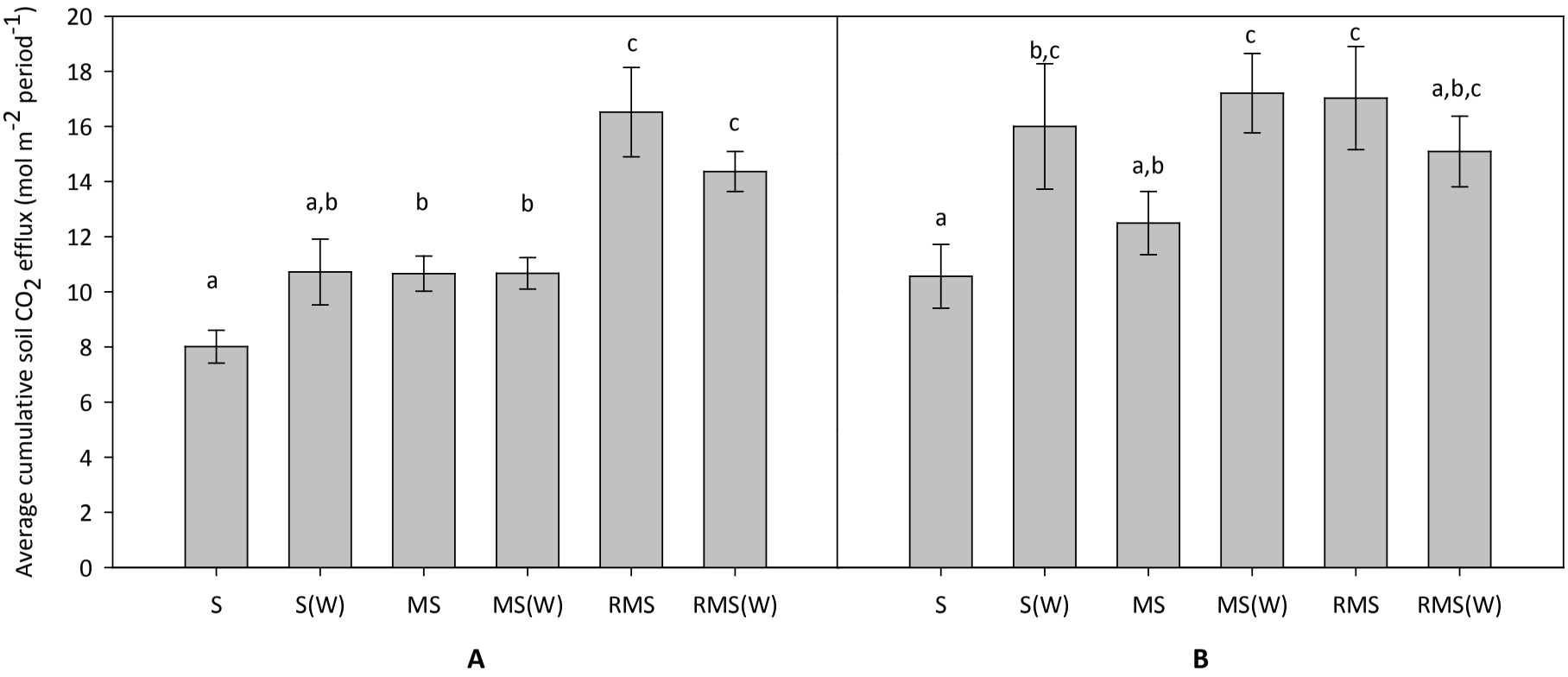

Fig. 3.11 Average cumulative soil CO_2 efflux (mol m^{-2} period-^{1}) (**A**) calculated for summer 2009 (1st June – 30th Sept 2009), and (**B**) calculated for summer 2010 (1st June – 30th Sept 2010). Error bars represent ± 1 SE (n = 4). Within each period, treatments which differ significantly have different letters.

treatments for both summer 2009 ($F=10.13$, $p<0.0003$) and summer 2010 ($F=3.36$ $p<0.04$) for average cumulative soil CO_2 efflux (see Fig. 3.11).

The magnitude of the fluxes from all treatments was greater for summer 2010 than summer 2009, apparently driven by an increase in the underlying soil heterotrophic flux (treatment S) (see Fig. 3.11). The relationship between treatments RMS and RMS(W), with RMS having the higher soil CO2 efflux (though not significant), was maintained over the two summers. However, there was a clear difference between summer 2009 and 2010 in terms of the cumulative soil CO_2 efflux measured from treatments MS and MS(W). The cumulative fluxes from treatments MS and MS(W) during summer 2009 were not significantly different. However for summer 2010, the average cumulative flux from the MS(W) collars was significantly greater than that from the MS collars, and unlike 2009, it was not significantly different from the cumulative fluxes from treatments RMS and RMS(W). There was also a significant difference in the cumulative soil CO_2 efflux between the treatment S(W) and S collars in summer 2010 that was not present during summer 2009. In 2010, as was seen for the MS(W) treatment collars, the soil CO_2 efflux from the S(W) collars did not differ from that of the RMS and RMS(W) collars.

Figs. 3.5 and 3.6 show that during the winter months, fluxes from both the corrected and non-corrected treatments had decreased relative to the fluxes measured during the summer months, and that any differences between the treatments were smaller (see Figs. 3.5 and 3.6). The average cumulative soil CO_2 efflux was calculated for each of the six treatments for just the winter months (1^{st} Nov to 28^{th} Feb) in 2009 and 2010 (see Fig. 3.12). There was a similar treatment effect to that observed during summer 2010, but the magnitudes of all the fluxes was smaller, and the difference between the treatments was not significant ($F = 2.38$, $p>0.08$). Treatments S and MS had higher cumulative fluxes relative to the other treatments than they did during summer 2009 and summer 2010. Treatments MS(W) and S(W) had also increased relative to the other treatments, compared to during summer 2009, however, unlike treatments S and MS the higher relative cumulative fluxes remained during summer 2010 (see Figs 3.11 and 3.12).

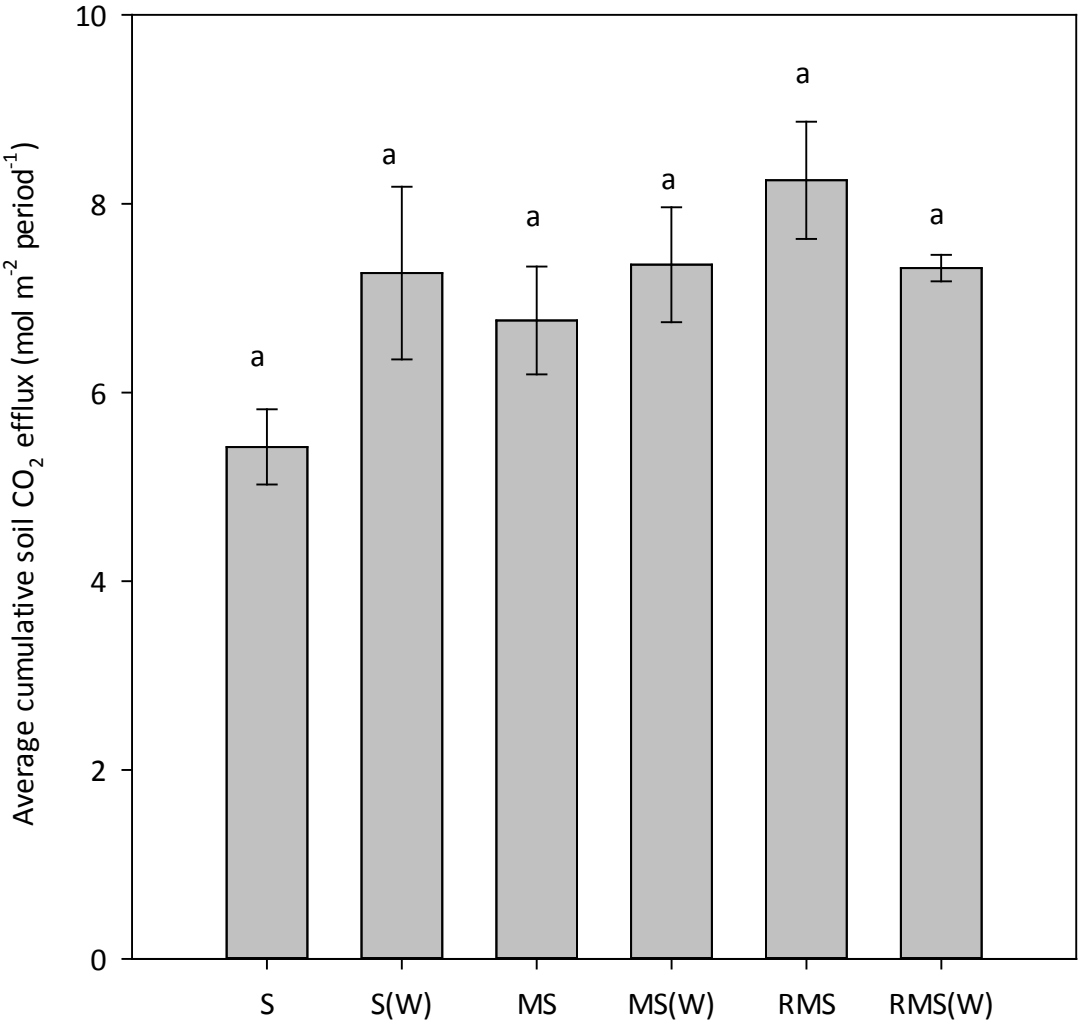

Fig. 3.12 Average cumulative soil CO_2 efflux (mol m^{-2} $period^{-1}$) calculated for the six soil collar treatments for winter 2009/10 (1st Nov 2009 – 28th Feb 2010). Error bars represent ± 1 SE (n = 4), treatments which differ significantly have different letters.

In order to assess whether there was an interaction between the soil collar treatments and the watering treatments (i.e. whether the collars received water via the automated irrigation system), the Friedman's two-way ANOVA were repeated, only this time with soil collar treatment (RMS, MS or S) and water correction treatment ('corrected' or 'non-corrected') as factors. The data were not normal, and transformation to achieve normality was not possible. As there was no appropriate non-parametric alternative to a three-way ANOVA available, it was decided to perform a Friedman's non-parametric two-way ANOVA, ignoring the block.

Overall, there was no significant interaction between the collar treatments and whether they received additional water correction via the automated irrigation system ($F=2.05$, $p>0.15$). When the analysis was repeated for just summer 2009, again the interaction was not significant ($F=0.96$, $p>0.4$). However for summer 2010, though the difference between the soil collar treatments RMS, MS and S was not significant ($F=1.18$, $p>0.32$), there was a significant interaction between the treatment collar type and whether it received additional water correction via the automated irrigation system or not ($F=3.77$, $p<0.05$).

Table 3.1 shows the contributions (%) of roots, mycorrhizal hyphae (Ra flux components) and background soil heterotrophs (R_h) to the total cumulative soil CO_2 efflux (R_s), and also their contributions for just summer 2009, summer 2010 and winter 2009/2010. For the non-corrected treatments (RMS, MS and S), the overall contribution of roots, mycorrhizal hyphae and background soil heterotrophs to R_s were, 31.2%, 12.8% and 56.0% respectively. The % contributions from roots and mycorrhizal hyphae were less for summer 2010 than for summer 2009, with an increase in the % flux derived from background soil heterotrophs (R_h), reflecting the greater increase in the soil CO_2 efflux from treatment S between summer 2009 and summer 2010, than for treatments MS and RMS (see Fig. 3.11). The % contribution of roots to total soil CO_2 efflux in the non-corrected treatments fell from 35.5% in summer 2009, to 17% in winter 2009/2010. Though the fluxes during winter were evidently smaller (see Fig. 3.5), the relative contribution of mycorrhizal hyphae remained fairly constant between summer 2009 and winter 2009/2010, though the mycorrhizal hyphal component appeared to contribute less to R_s in summer 2010.

Table 3.1 Average contribution of roots, mycorrhizal hyphae and background soil heterotrophs to the cumulative soil CO_2 efflux (mol m^{-2} yr^{-1}) following the installation of the automated irrigation system, and also for summer 2009 (June- Sept), summer 2010 (June-Sept) and winter 2009/10 (Nov-Feb) for both the non-corrected (RMS, MS and S) and corrected (RMS(W), MS(W)) treatments. Values are percentages, calculated using the method of Heinemeyer *et al*. (2007).

	% Contribution to total soil CO_2 efflux			
	Overall	Summer 2009	Summer 2010	Winter 2009/10
Non corrected treatments (RMS, MS, S)				
roots	31.2	35.5	26.6	17.0
mycorrhizal hyphae	12.8	16.0	11.3	16.3
background soil heterotrophs (R_h)	56.0	48.5	62.0	65.7
autotrophic respiration (R_a)	44	51.5	37.9	33.3
Corrected treatments (RMS(W), MS(W), S)				
roots *	7.0	25.7	-14.0	-0.5
roots**	19.1	25.8	17.2	7.6
mycorrhizal hyphae	27.2	18.5	44.0	26.4
background soil heterotrophs (R_h)	65.9	55.8	70.0	74.1

*calculated using MS(W) as the underlying mycorrhizal flux component

**calculated using MS as the underlying flux component, for comparison

The high cumulative MS(W) flux, relative to the RMS(W) in 2010 previously discussed (see Figs. 3.6 and 3.11) resulted in a high % contribution of mycorrhizal hyphae to the R_S for the corrected collars, and a low value for the % contribution of roots to R_S (7 % compared to treatment RMS, which contributed 31.2%). Because of the high MS(W) collar flux, the contributions from roots during winter 2009/2010 and summer 2010 were calculated as being negative (-0.5 % and -14.0 %, respectively). For comparison, the % contribution of roots from treatment RMS(W) was recalculated using the mycorrhizal contributions from the MS, rather than MS(W) treatment, this resulted in a % contribution of 19.1 % overall, and more reasonable flux values for summer 2009, summer 2010 and winter 2009/2010 (25.8 %, 17.2 % and 7.6 %, respectively). Because the RMS(W) cumulative flux was lower than the RMS cumulative flux (see Fig. 3.11) the % flux component from background soil heterotrophs was increased for the irrigated treatments compared to the non-irrigated treatments.

3.3.3 Soil moisture content

Analysis of the soil moisture contents of the six collar treatments are covered in detail in Section 2.3.3, and are summarised in the current section. Figs. 2.12, 2.13 and 2.14 show that over the year, the soil moisture measured during the hand-held soil moisture surveys varied, with the greatest differences between the treatments being observed during the summer months. Generally, there was little difference between treatments S, MS and MS(W) in terms of soil moisture content. However, during the summer months, there was a dramatic decrease in the soil moisture content measured from treatments RMS, and to a lesser extent RMS(W) (see Fig. 2.13).

Fig. 3.13 (a copy of Fig. 2.16 added to this Section for ease of reference) shows that overall, there was a decrease in the average soil moisture contents of all six treatments between summer 2009 and summer 2010, which is the opposite of what occurred in terms of the soil CO_2 efflux (see Fig. 3.11). Despite problems with the automated irrigation system in summer 2009, there was no significant difference between treatments RMS(W) and S for both summer 2009 and 2010 in terms of the average soil moisture contents, though there was a significant difference between treatments RMS and S.

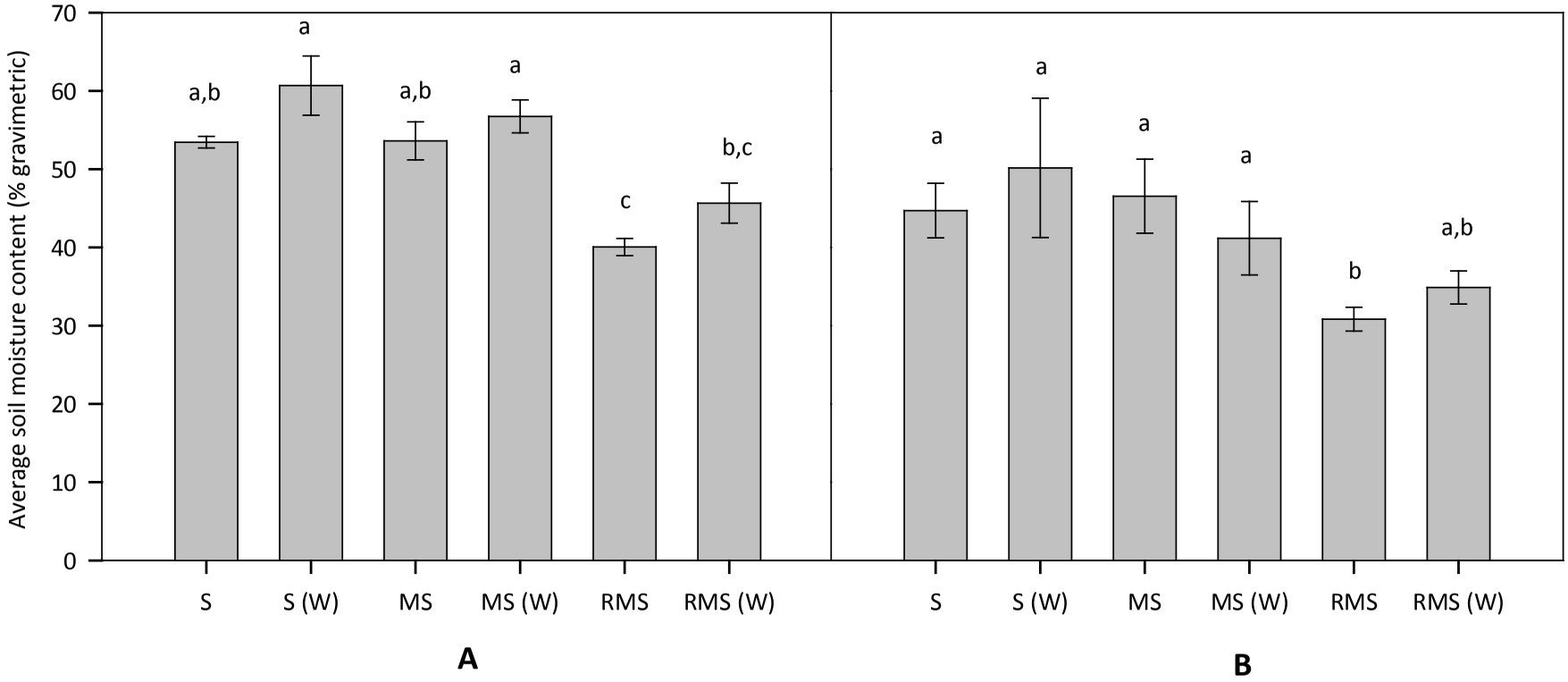

Fig. 3.13 Replica of Fig. 2.16, showing the average gravimetric soil moisture contents recorded during hand-held soil moisture surveys for just the summer months: (**A**) 1st June – 30th Sept 2009, (**B**) 1st June – 30th Sept 2010. Within each period, treatments which differ significantly have different letters. Error bars represent ± 1 SE (n = 4).

Treatment RMS, which had the lowest soil moisture content of all the treatments during summers 2009 and 2010 (see Fig. 3.13), had the highest cumulative soil CO_2 efflux in 2009, and the second highest in summer 2010 (see Fig. 3.11). This seemingly negative effect of soil moisture on soil CO_2 efflux was also demonstrated by treatments MS(W) and MS. In summer 2010, the average soil moisture content was lower (though not significantly) for treatment MS(W) than treatment MS (see Fig. 3.13), but the average cumulative soil CO_2 efflux from treatment MS(W) was higher than that of all the other treatments, and significantly higher than the cumulative soil CO_2 efflux from treatment MS (see Fig. 3.11).

Treatment S(W) had a higher soil moisture content than all the other treatments during both summers, as it was receiving the same level of water correction from the automated irrigation system as treatment RMS(W), whilst not having any soil water uptake by roots (see Fig 3.13). There was no significant difference in terms of soil moisture between treatments S(W) and S for summer 2009 or summer 2010. However, this was not mirrored by the cumulative soil CO_2 efflux, where there was a much higher average flux measured from treatment S(W) than S during summer 2010, compared to summer 2009 (see Fig. 3.11).

During winter 2009/2010, the average soil moisture contents from the six treatments were very similar, and the difference between the treatments was not significant ($F = 1.24$, $p>0.3$, see Fig. 3.14). For treatments MS, MS(W) S and S(W), the average soil moisture contents were slightly higher during winter 2009/2010 than during the two summers. However treatments RMS and RMS(W) had a noticeably higher average soil moisture contents during the winter months than during summers 2009 and 2010. The differences between the treatments in terms of soil CO_2 efflux for winter 2009/10 were not significant ($F= 2.38$, $p>0.08$, see Fig. 3.12), but, treatment RMS had a higher cumulative soil CO_2 efflux than the other treatments, and treatment S had the lowest cumulative soil CO_2 efflux of all the treatments despite all treatments having similar average soil moisture contents. So, as for the data from summers 2009 and 2010, the variation in the soil CO_2 efflux did not appear to reflect what was happening in terms of the soil moisture contents (see Figs. 3.12 and 3.14).

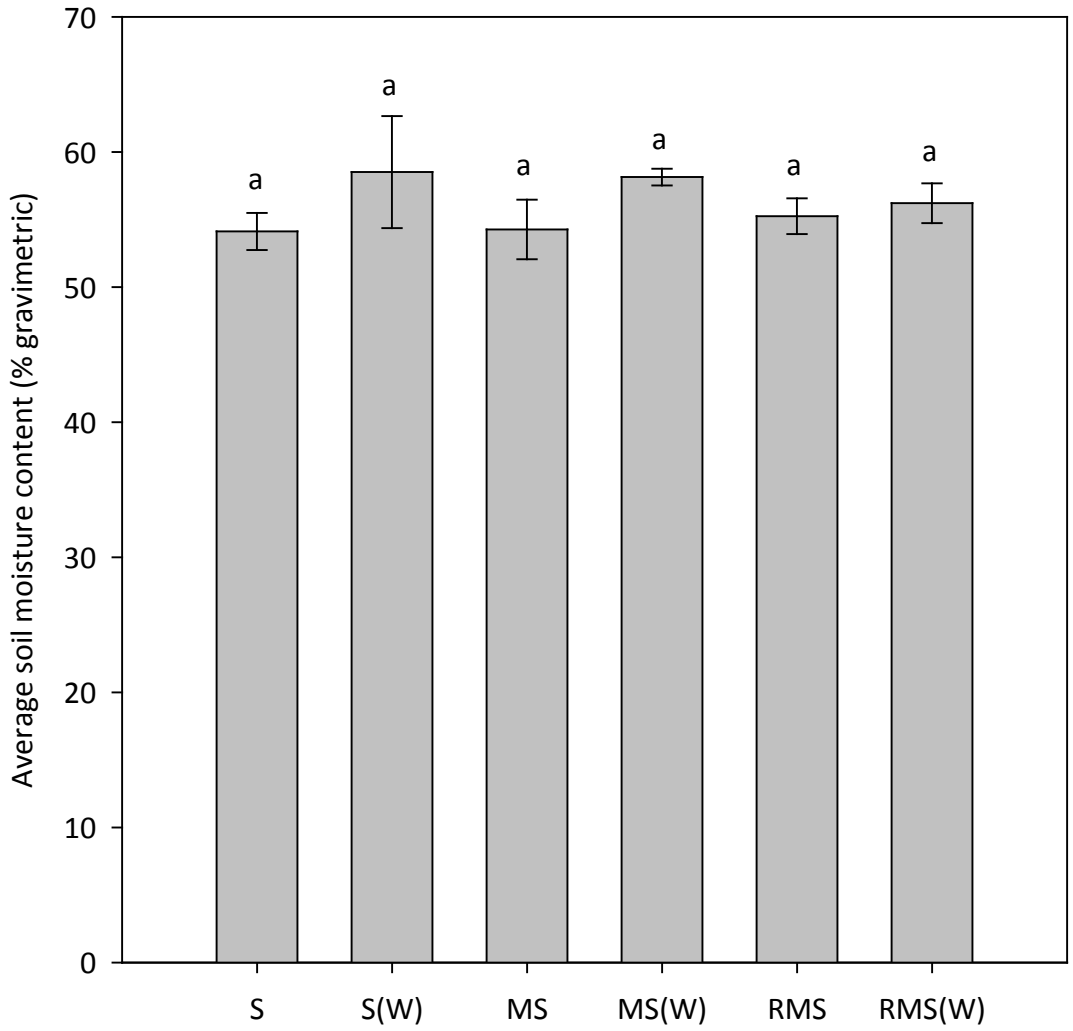

Fig. 3.14 Average soil moisture contents (% gravimetric) for the six soil collar treatments calculated from hand-held soil moisture surveys during winter 2009/10 (1st Nov – 28th Feb). Error bars represent ± 1 SE (n = 4). Treatments which differ significantly have different letters.

In order to ascertain whether there was any association between the soil CO_2 efflux and the soil moisture measured during hand-held surveys for the whole period following the installation of the automated irrigation system, these data were natural log- transformed to achieve normality, then the average soil CO_2 efflux per measurement day was plotted against the average soil moisture (% gravimetric) measured on the hand-held CO_2 efflux survey days for each of the six treatments individually (see Fig. 3.15), then a Pearsons product-moment correlation was carried out (see Table 3.2). The associations between soil moisture content (% gravimetric) and the soil CO_2 efflux measured from treatments RMS and MS were non-significant. There were weak, but significant negative associations between the average soil CO_2 efflux and the average soil surface moisture contents for treatments MS(W), S(W), S and RMS (see Fig. 3.15 and Table 3.2).

3.3.4 Temperature and Light intensity

Environmental data measured during the period following installation of the automated irrigation system is displayed in Fig. 3.16, and show clear seasonal trends. This seasonal change in average soil surface temperature is reflected by the soil CO_2 fluxes displayed in Figs. 3.4 to 3.9, with the higher average temperatures measured during the summer months, coinciding with the highest soil CO_2 fluxes. The maximum daily soil temperature was also recorded, and used in subsequent correlations (see Table 3.2), but as it showed a similar pattern to the average daily temperature it is not displayed in Fig. 3.16.

Data were transformed where necessary to achieve normality and scatter plots were produced to show any associations between the soil CO_2 efflux measured on hand-held survey days for the different treatments, and environmental variables logged on the same days including: the average soil surface temperature at a depth of 5 cm (see Fig. 3.17), the maximum soil surface temperature at a depth of 5 cm (see Fig. 3.18), the average air temperature measured above the canopy (see Fig. 3.19) and the average light intensity measured above the canopy (see Fig. 3.20). These scatter plots show that there appears to be a positive association between soil CO_2 efflux and soil temperature, and that there are variations between the different treatments. As with

Table 3.2 Results of Pearson product-moment correlations to investigate any association between the average soil CO_2 efflux measured from the six soil collar treatments and environmental variables.

Average soil moisture (% gravimetric)						
	S	S(W)	MS	MS(W)	RMS	RMS(W)
R^2	0.2	0.27	0.05	0.32	0.02	0.13
Sig.	p<0.008	p<0.001	p>0.18	p<0.0005	p>0.36	p<0.04
Daily average soil temperature at a depth of 5 cm (^oC)						
	S	S(W)	MS	MS(W)	RMS	RMS(W)
R^2	0.48	0.48	0.57	0.48	0.65	0.64
Sig.	p<0.0001	p<0.0001	p<0.0001	p<0.0001	p<0.0001	p<0.0001
Daily maximum soil temperature at a depth of 5 cm (^oC)						
	S	S(W)	MS	MS(W)	RMS	RMS(W)
R^2	0.44	0.46	0.53	0.48	0.61	0.59
Sig.	p<0.0001	p<0.0001	p<0.0001	p<0.0001	p<0.0001	p<0.0001
Daily mean air temperature above the canopy (^oC)						
	S	S(W)	MS	MS(W)	RMS	RMS(W)
R^2	0.58	0.59	0.50	0.53	0.64	0.58
Sig.	p<0.0001	p<0.0001	p<0.0001	p<0.0001	p<0.0001	p<0.0001
Daily mean light intensity above the canopy (Lux)						
	S	S(W)	MS	MS(W)	RMS	RMS(W)
R^2	0.08	0.08	0.06	0.07	0.17	0.10
Sig.	p>0.1	p>0.1	p>0.15	p>0.15	p<0.02	p>0.08

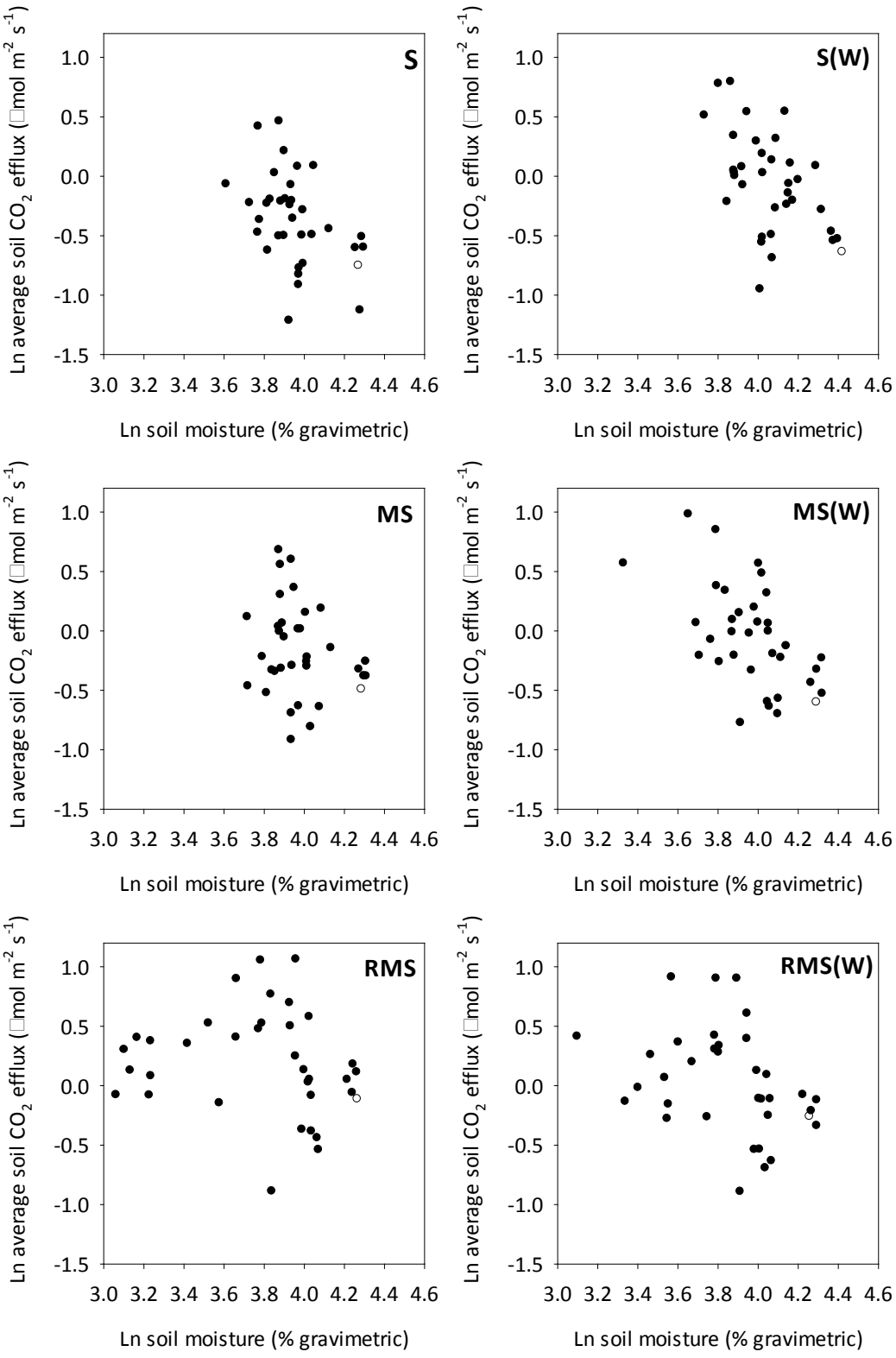

Fig. 3.15 Correlations between the natural log of the average soil CO_2 efflux (µmol m^{-2} s^{-1}) and the natural log of the average soil moisture contents (% gravimetric) for treatments S, S(W), MS, MS(W), RMS and RMS(W). Each point is the mean of 4 values.

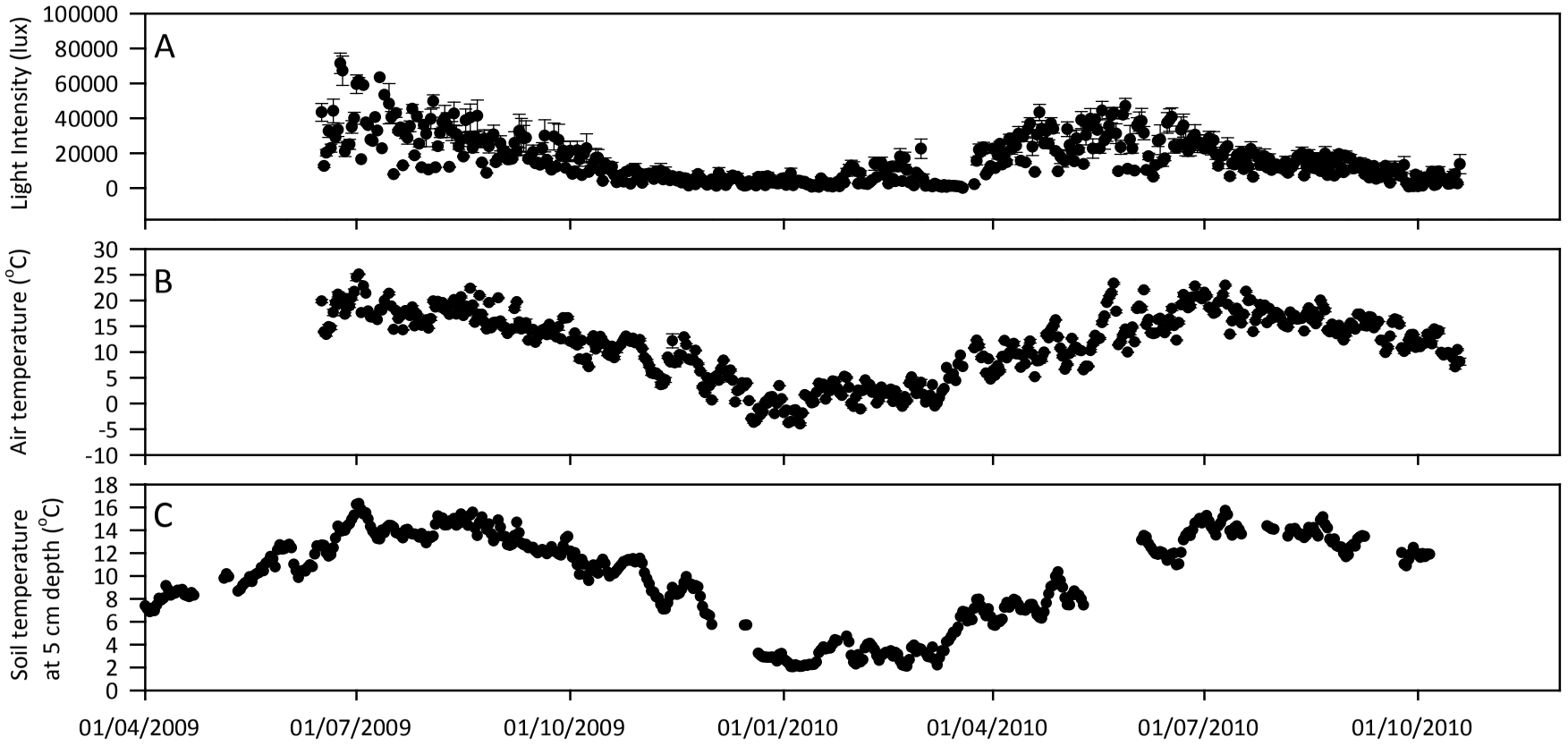

Fig. 3.16 (**A**) Daily average light intensity (lux) above the canopy, (**B**) daily average air temperature (^{o}C) above the canopy and (**C**) daily average soil temperature (^{o}C) at a depth of 5 cm measured during the period following installation of the automated irrigation system. For (**A**) and (**B**), error bars represent ± 1 SE (n = 4).

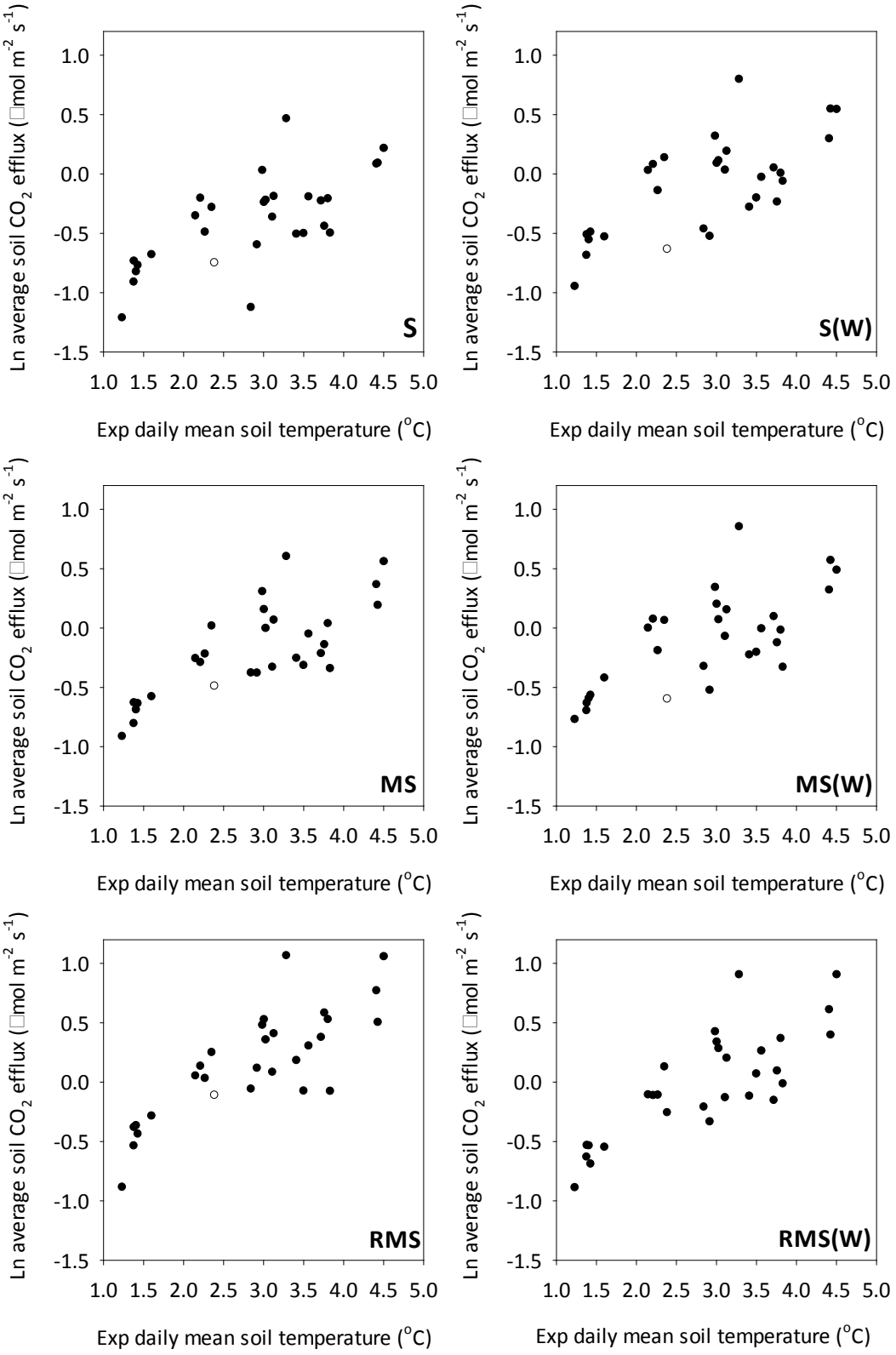

Fig. 3.17 Correlations between the natural log of the average soil CO_2 efflux (µmol m^{-2} s^{-1}) for treatments S, S(W), MS, MS(W), RMS and RMS(W) and the exponential of the mean soil temperature at a depth of 5 cm (°C) measured on the hand-held CO_2 efflux survey days. Each point is the mean of 4 values.

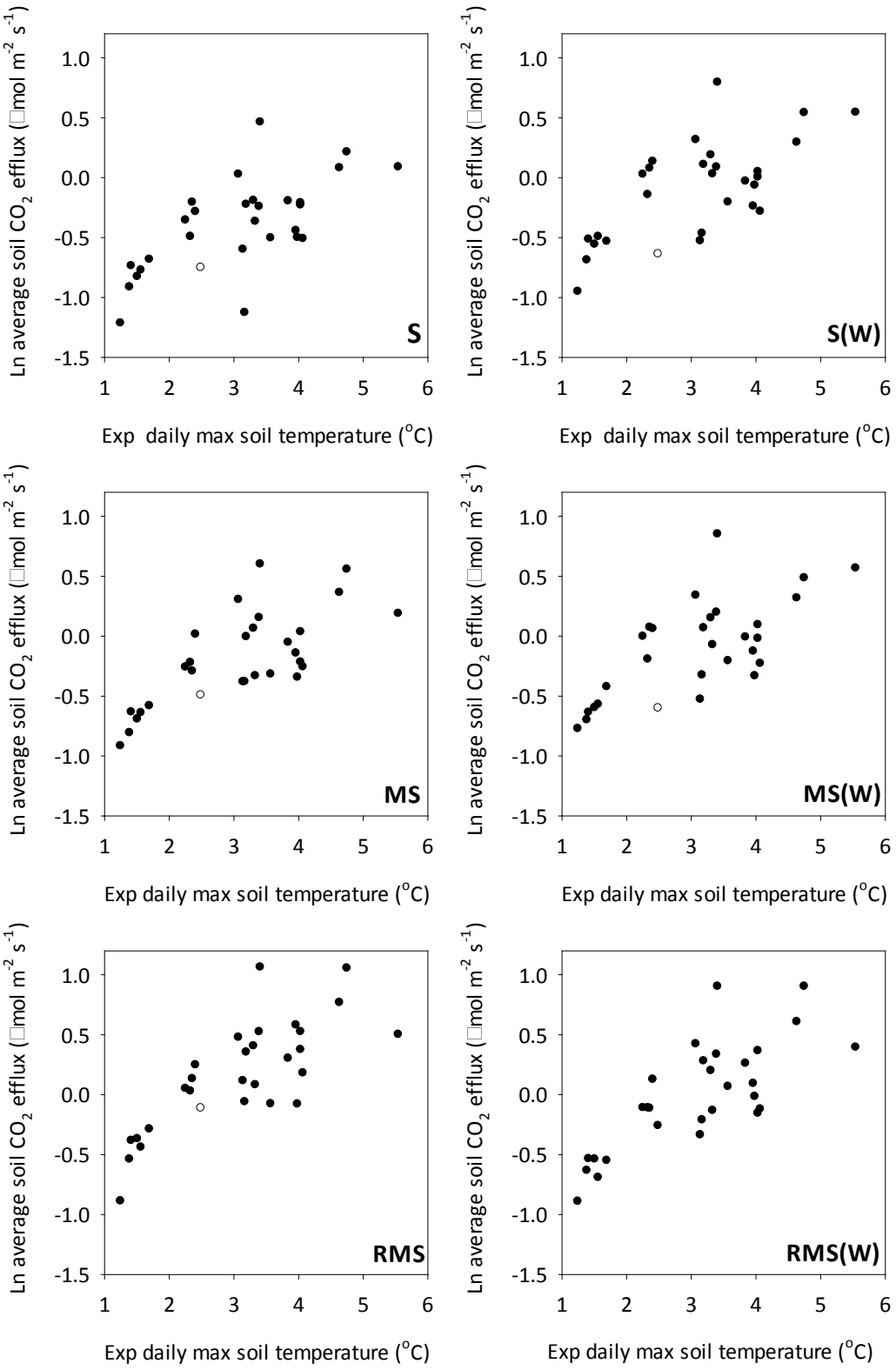

Fig. 3.18 Correlations between the natural log of the average soil CO_2 efflux (µmol m^{-2} s^{-1}) for treatments S, S(W), MS, MS(W), RMS and RMS(W) and the exponential of the maximum soil temperature at a depth of 5 cm (°C) measured on the hand-held CO_2 efflux survey days. Each point is the mean of 4 values.

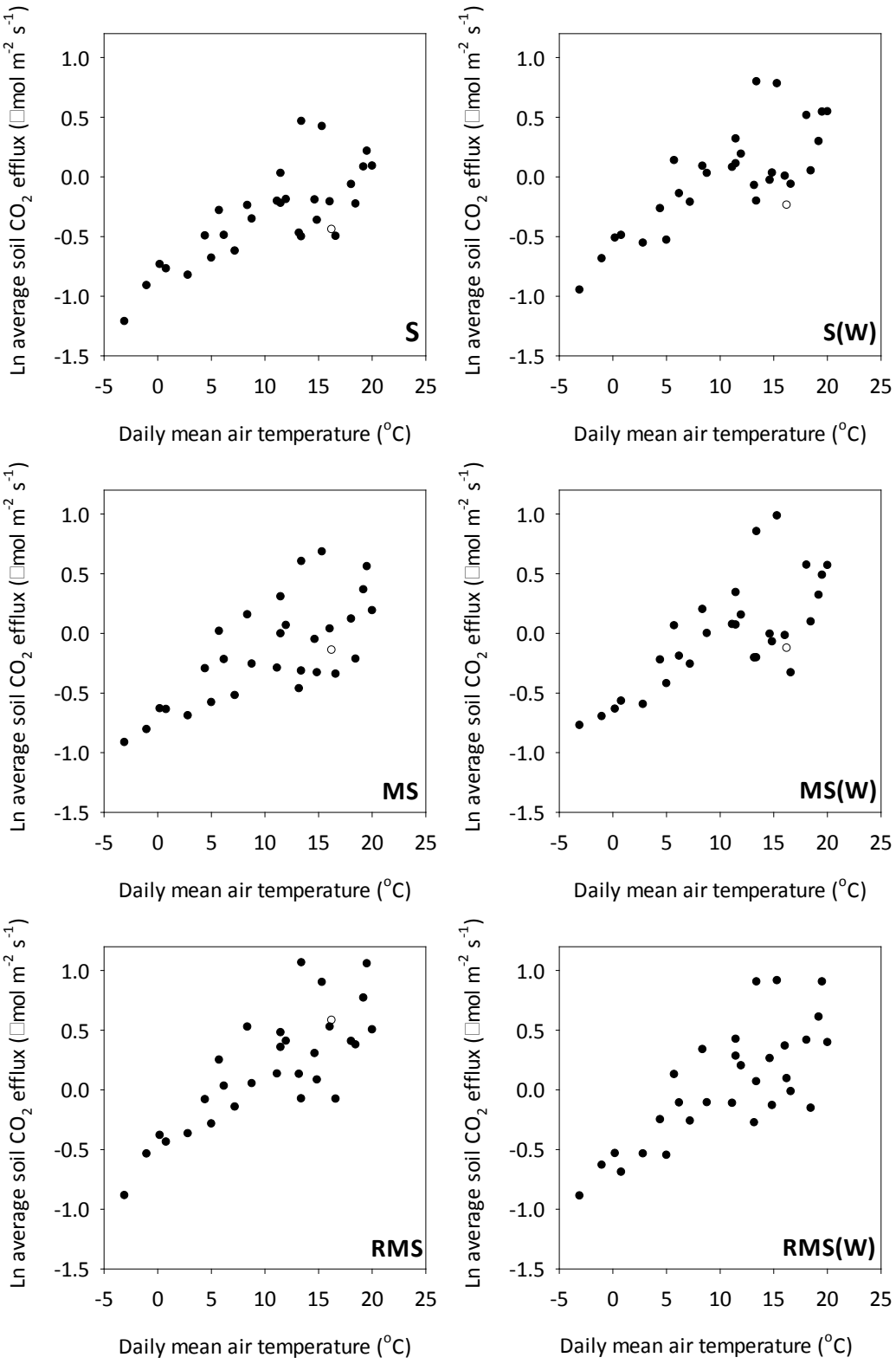

Fig. 3.19 Correlations between the natural log of the average soil CO_2 efflux (µmol m^{-2} s^{-1}) for treatments S, S(W), MS, MS(W), RMS and RMS(W) and the mean air temperature (°C) above the canopy measured on hand-held CO_2 survey days. Each point is the mean of 4 values.

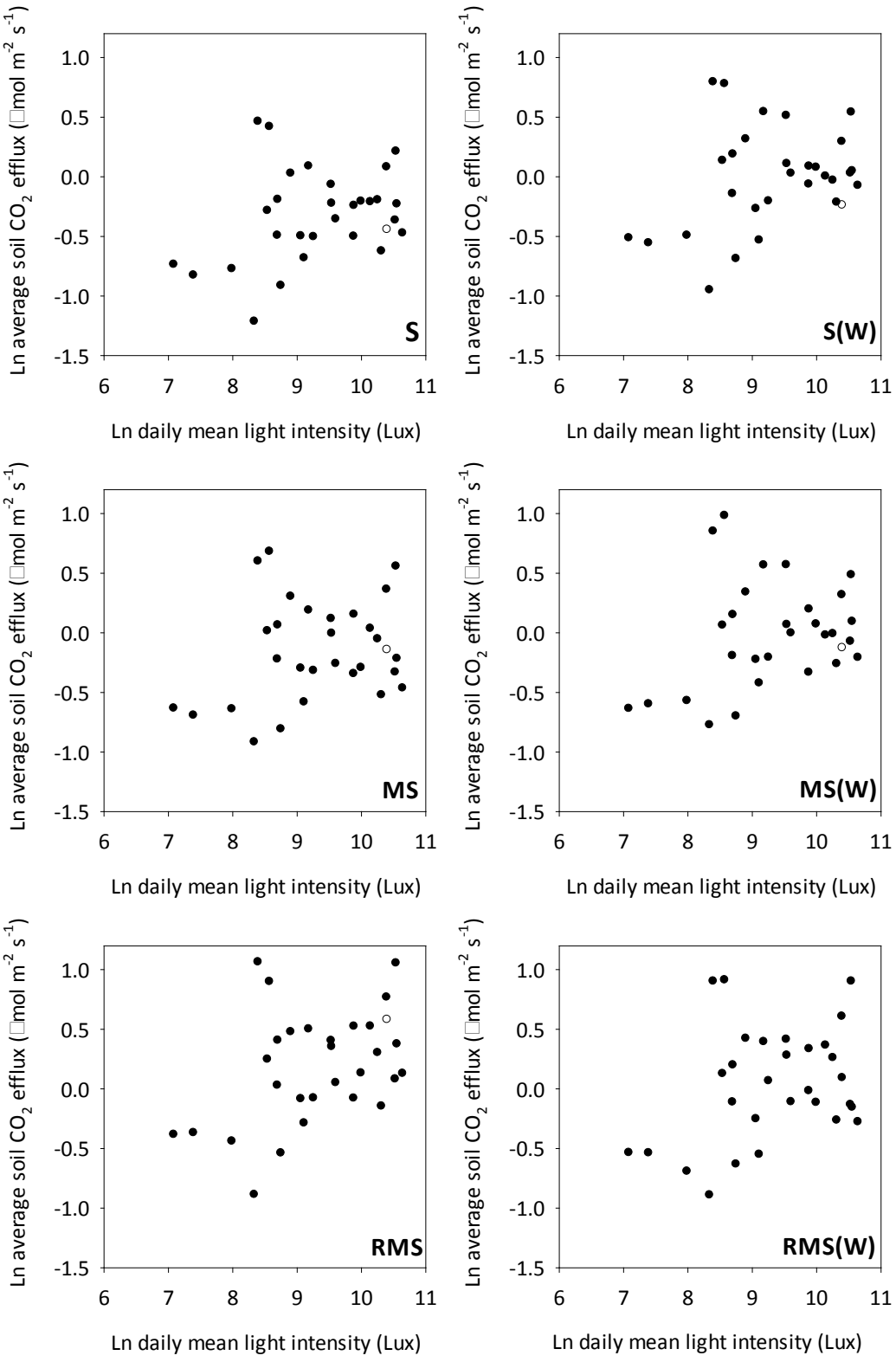

Fig. 3.20 Correlations between the natural log of the average soil CO_2 efflux (µmol m^{-2} s^{-1}) for treatments S, S(W), MS, MS(W), RMS and RMS(W) and the natural log of the mean light intensity (lux) above the canopy on hand-held CO_2 survey days. Each point is the mean of 4 values.

the soil moisture contents, Pearson product-moment correlations were carried out to assess the degree of association. The co-efficient of determination (R^2) values obtained, and the significance of the relationship between the variables for each treatment are also displayed in Table 3.2.

The results displayed in Table 3.2 show that temperature appears to be more important in terms of driving soil CO_2 than soil moisture, for all six treatments. Water correction from the automated irrigation system did not appear to influence the strength of the various correlations, with the corrected and non-corrected collars from each soil collar type being relatively similar in terms of the strength of correlation between the temperature measurements and soil CO_2 efflux, apart from in the case of treatments MS and MS(W). Soil CO_2 efflux from treatment MS(W) tended to have a lower correlation than the soil CO_2 efflux from treatment MS to temperature, with correlation similar to that of treatments S and S(W). However, soil CO_2 efflux measured from treatment MS(W) did have a higher correlation with air temperature than the soil CO_2 efflux measured from treatment MS. The soil CO_2 efflux from treatments S and S(W) tended to have the lowest correlations with mean and maximum soil temperature at a depth of 5 cm, but had higher correlations with mean air temperature than treatments MS and MS(W). The correlations between the soil CO_2 efflux measured from treatments RMS and RMS(W), and the various temperature measurements, were the highest of all the soil collar types, and were similar to each other. Therefore, from the individual correlations (see Table 3.2) the difference in the strength of the associations between total soil CO_2 efflux (R_s) (R^2 = 0.65), and soil temperature, and that of the correlations for treatments MS and S (R^2= 0.57, R^2 = 0.48, respectively) indicates that both R_h (treatment S) and R_a were positively correlated with soil temperature, and that the correlations were stronger for R_a than R_h. From the separation out of the flux components of R_a, root respiration would appear to have a higher correlation with soil temperature than mycorrhizal respiration.

There were weak correlations between the soil CO_2 efflux measured from treatments MS, MS(W), S and S(W) and light intensity measured above the canopy. There was a slightly stronger association between the soil CO_2 efflux from treatments RMS and RMS(W) with light intensity, which was significant in the case of treatment RMS (R^2 =

0.17, $p<0.02$), indicating that root respiration was more influenced by light intensity than mycorrizal or soil heterotrophic respiration (R_h).

In order to formally assess the relative importance of the relationship between soil CO_2 efflux from the six treatments, and the environmental variables shown in Table 3.2, where necessary, data were transformed to achieve normality and a forward stepwise multiple regression was carried for each treatment individually. The results of the forward stepwise multiple regression displayed in Table 3.3 show that the treatments differ in the environmental variables deemed most influential in driving the soil CO_2 efflux by the model, with the mean soil temperature (5 cm depth) being the variable most highly related to the soil CO_2 efflux for the corrected treatments RMS, RMS(W) and MS and the mean daily air temperature above the canopy explaining the most variation in soil CO_2 efflux from treatments S, S(W) and MS(W). These results, along with the results from the individual correlations suggest that temperature is generally positively associated with soil CO_2 efflux for all six of the soil collar treatments, with the association being slightly stronger for treatments RMS and RMS(S) than for the other treatments, suggesting that the root component of autotrophic respiration (R_a) is more influenced by temperature than the other components of Rs.

Lagged Pearson product-moment correlations were carried out to see if there was a stronger association between soil CO_2 efflux measured from the six treatments, and light intensity measurements taken up to four days prior to the soil CO_2 efflux measurements. The results displayed in Table 3.4 confirm this, with the associations being noticeably stronger, and significant for all treatments when the soil CO_2 efflux is correlated with the mean light intensity measured the day prior to the hand-held surveys. The association is strongest for treatments RMS (R^2 = 0.29, $p<0.003$) and RMS(W) (R^2= 0.21, $p<0.01$), followed by treatments S(W) and S, then treatments MS and MS(W). Following this, for the other lagged correlations, the associations are lower again, returning to similar levels as the original low association displayed in Table 3.2.

Table 3.3 Results from a forward stepwise multiple regression to explain variation in soil CO_2 efflux from six soil collar treatments using environmental variables.

Treatment S				
Environmental variable	partial r^2	model r^2	F value	Sig.
mean daily air temperature	0.5962	0.5962	32.49	p<0.0001
Treatment S(W)				
Environmental variable	partial r^2	model r^2	F value	Sig.
mean daily air temperature	0.5748	0.5748	29.74	p<0.0001
Treatment MS				
Environmental variable	partial r^2	model r^2	F value	Sig.
mean daily soil temperature (5 cm depth)	0.5779	0.5779	30.12	p<0.0001
Treatment MS(W)				
Environmental variable	partial r^2	model r^2	F value	Sig.
mean daily air temperature	0.5412	0.5412	25.95	p<0.0001
Treatment RMS				
Environmental variable	partial r^2	model r^2	F value	Sig.
mean daily soil temperature (5 cm depth)	0.6479	0.6479	40.49	p<0.0001
soil moisture content (% gravimetric)	0.1037	0.7516	8.77	p<0.008
Treatment RMS(W)				
Environmental variable	partial r^2	model r^2	F value	Sig.
mean daily soil temperature (5 cm depth)	0.6627	0.6627	43.23	p<0.0001

Table 3.4 Results of Pearson product-moment correlations to investigate associations between the average soil CO_2 efflux measured from the six soil collar treatments and the lagged daily mean light intensity logged for the same measurement day, and up to four days prior to the hand-held surveys of soil CO_2 efflux.

Daily mean light intensity above the canopy (Lux)						
	S	S(W)	MS	MS(W)	RMS	RMS(W)
R^2	0.08	0.08	0.06	0.07	0.17	0.10
Sig.	p>0.1	p>0.1	p>0.15	p>0.15	p<0.02	p>0.08
Daily mean light intensity above the canopy (Lux) one day prior to CO_2 efflux measurements						
	S	S(W)	MS	MS(W)	RMS	RMS(W)
R^2	0.20	0.21	0.16	0.18	0.29	0.21
Sig.	p<0.01	p<0.02	p<0.03	p<0.02	p<0.003	p<0.01
Daily mean light intensity above the canopy (Lux) two days prior to CO_2 efflux measurements						
	S	S(W)	MS	MS(W)	RMS	RMS(W)
R^2	0.05	0.06	0.05	0.04	0.11	0.09
Sig.	p>0.19	p>0.17	p>0.2	p>0.25	p>0.06	p>0.1
Daily mean light intensity above the canopy (Lux) three days prior to CO_2 efflux measurements						
	S	S(W)	MS	MS(W)	RMS	RMS(W)
R^2	0.08	0.10	0.08	0.08	0.15	0.16
Sig.	p>0.12	p>0.09	p>0.12	p>0.13	p<0.04	p<0.03
Daily mean light intensity above the canopy (Lux) four days prior to CO_2 efflux measurements						
	S	S(W)	MS	MS(W)	RMS	RMS(W)
R^2	0.06	0.06	0.06	0.06	0.14	0.11
Sig.	p>0.17	p>0.18	p>0.19	p>0.3	p<0.05	p>0.07

3.3.5 Automated hourly measurements of soil CO_2 efflux

From the hourly measurements of soil CO_2 efflux it is possible to see that there were clear temporal variation in the fluxes from treatments RMS, MS and S measured during Run 1 of the automated soil CO_2 efflux measurement system (see Fig. 3.21), and that those fluctuations were similar to the fluctuations in surface soil temperature recorded during that period of time (see Fig. 3.22). The steep decrease, followed by a increase in soil temperature (see Fig. 3.22) was mirrored by a fall, then rise in soil CO_2 efflux from treatments RMS, MS and S (see Fig. 3.21). Error bars have been removed from Fig. 3.21 for ease of distinguishing between treatments. During the two week period the automated system was running, generally the highest soil CO_2 efflux was measured from treatment RMS, followed by the CO_2 efflux from treatment MS, with treatment S consistently having the lowest soil CO_2 efflux.

In order to ascertain how much information was lost due to the intermittent nature of hand-held surveys of soil CO_2 efflux, when compared to the high resolution hourly data recorded using the automated soil CO_2 efflux measurement system, the cumulative soil CO_2 efflux was calculated for the period of Run 1, using the data from two hand-held surveys conducted during Run 1, and also the automated system data (see Fig. 3.23). For the non-corrected collars measured during Run 1, there was no significant difference between treatments RMS, MS and S in terms of the cumulative soil CO_2 efflux calculated from the high resolution hourly data. The two methods used to calculate the cumulatives produced extremely similar results, with the same treatment pattern, and no significant difference in the cumulative soil CO_2 efflux between the treatments, regardless of whether the high resolution ($F=0.69$, $p>0.5$), or more sporadic hand-held survey data ($F=1$, $p>0.4$) were used to calculate the cumulatives.

In order to assess the diurnal pattern in terms of soil CO_2 efflux during Run 1, a period of two days is shown in Fig. 3.24 A, alongside air temperature measurements (see Fig. 3.24 B) and soil surface temperature measurements (see Fig. 3.24 C). The surface soil temperature peaked in the afternoon on 21.10.2009, following a slight peak in air

temperature. The same occurred in the afternoon of the 22.10.2009. It is difficult to detect a diurnal pattern in the soil CO_2 efflux, though treatment S appears to have

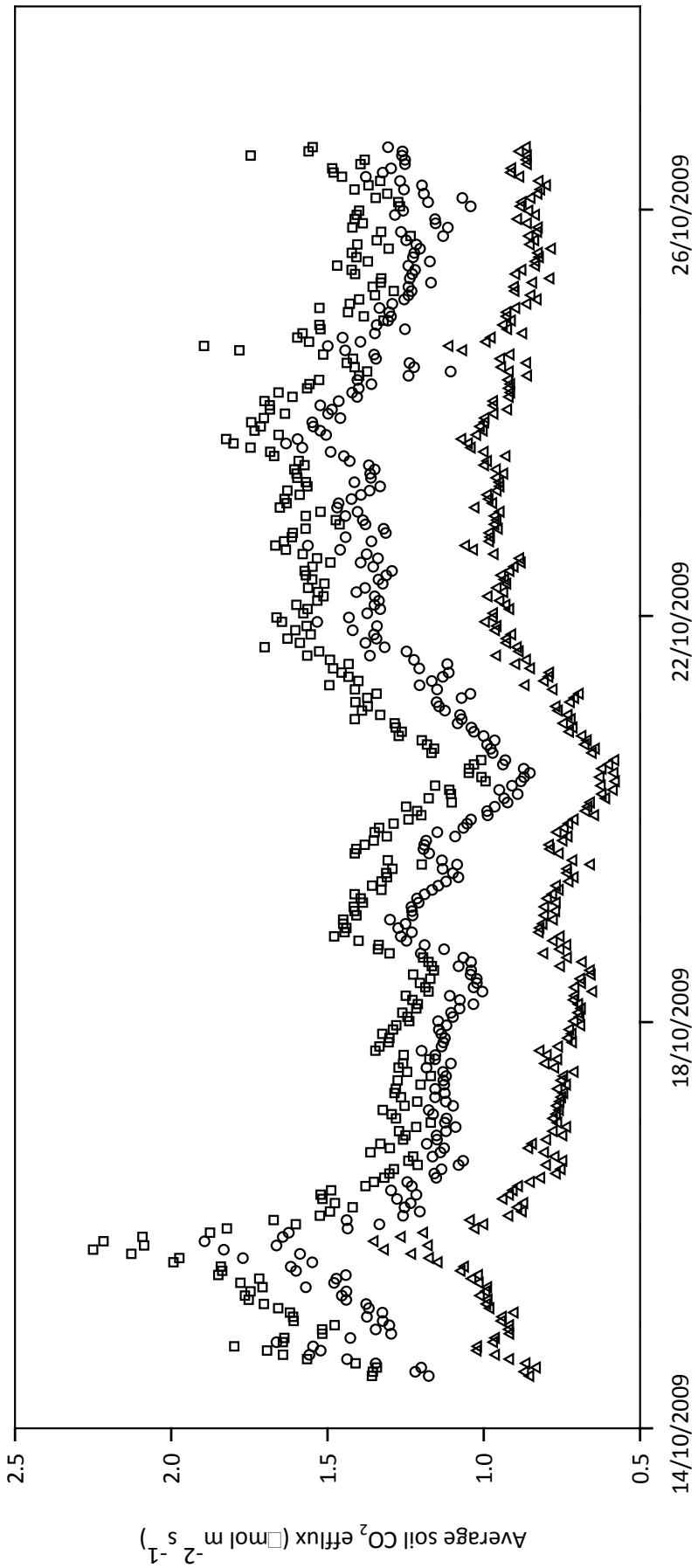

Fig. 3.21 Average hourly soil CO_2 efflux measurements from collars not receiving additional water correction via the automated irrigation system, taken during Run 1 of the automated soil CO_2 efflux measurement system. Treatments RMS, MS and S are represented by open squares □, circles ○ and triangles Δ, respectively.

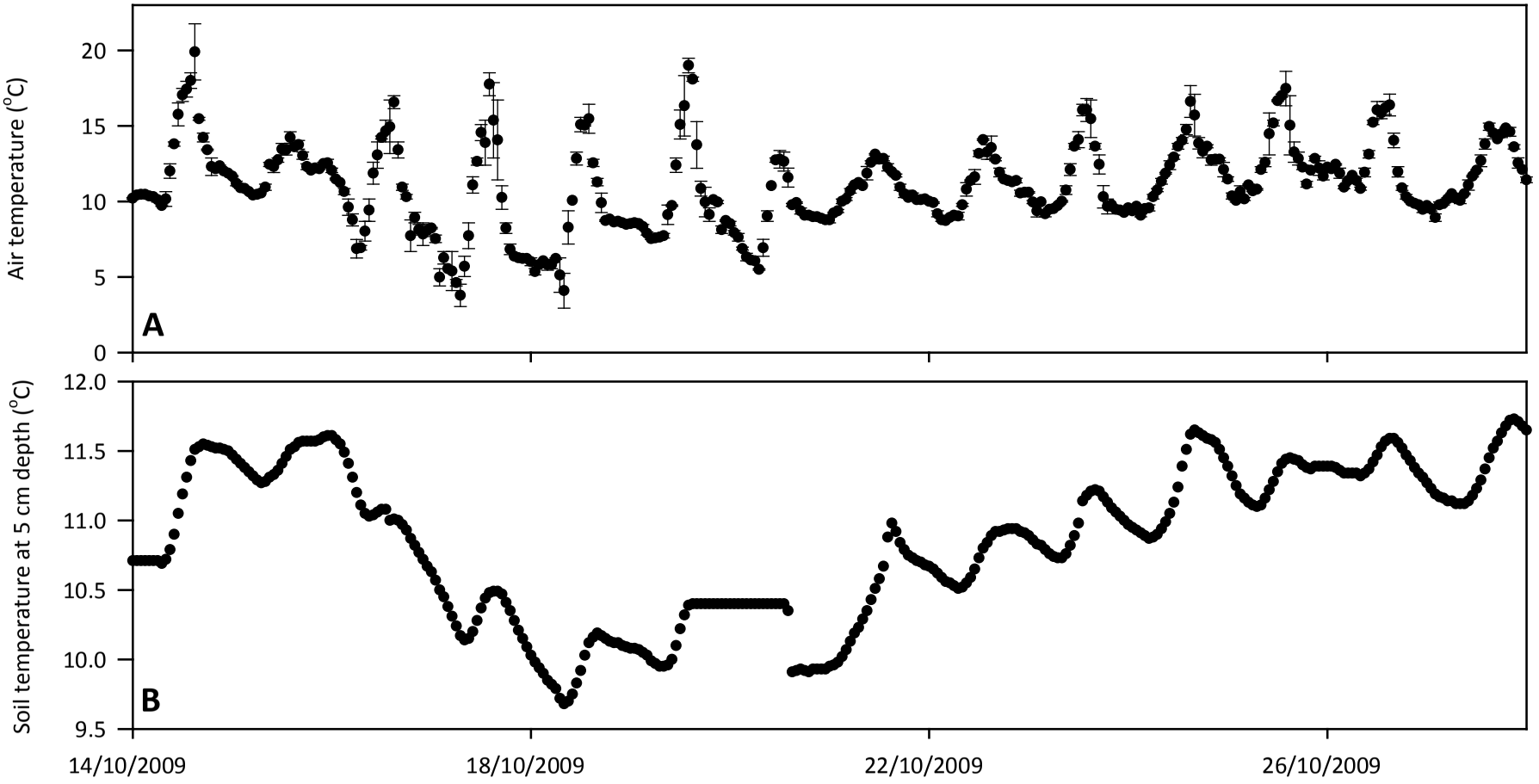

Fig. 3.22 Hourly average (**A**) air temperature above the canopy (°C) and (**B**) soil temperature at a depth of 5 cm (°C) measured during Run 1 of the automated CO_2 efflux measurement system. Where displayed, error bars represent ± 1 SE (n = 4).

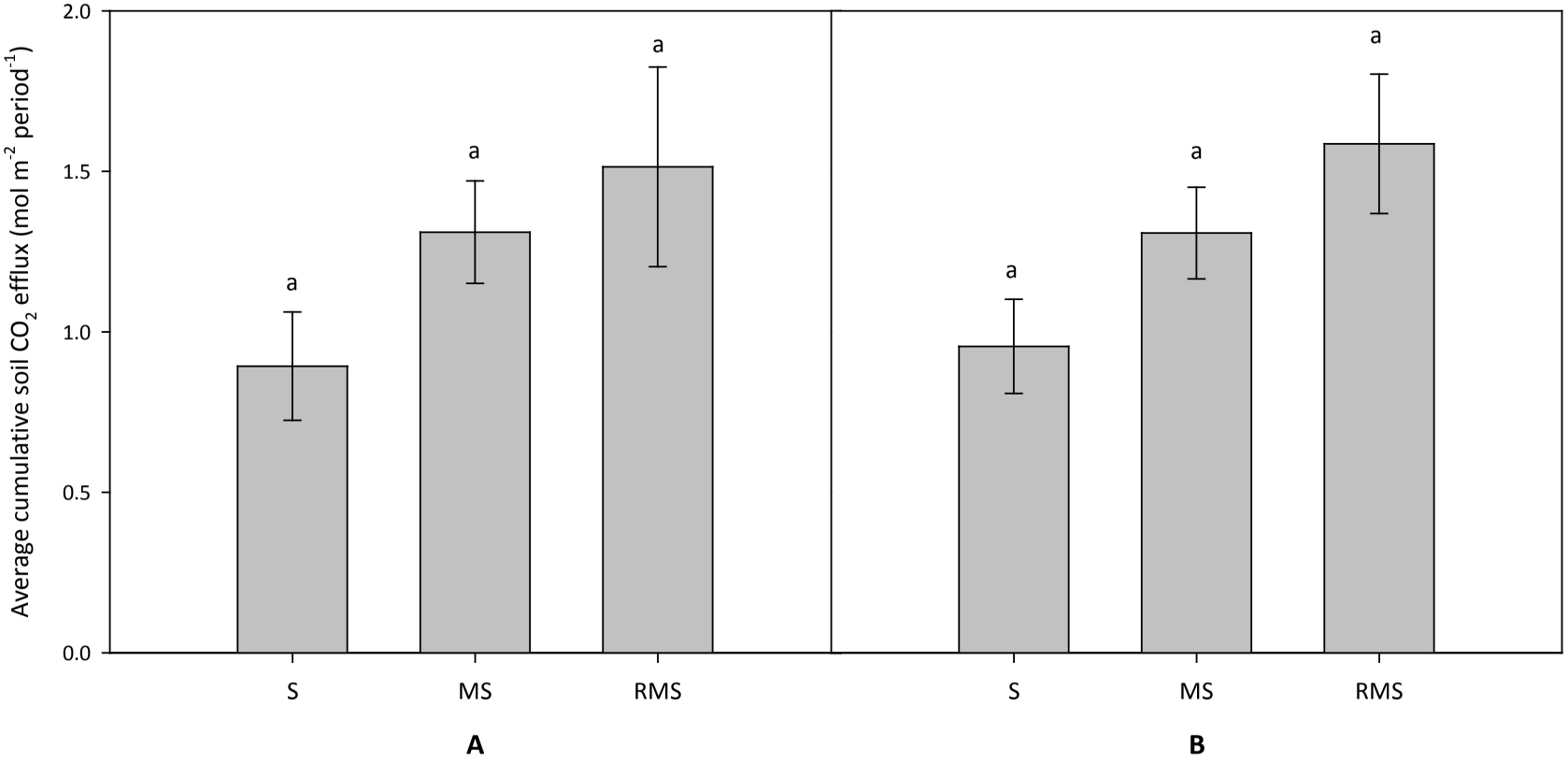

Fig. 3.23 A comparison of the average cumulative CO_2 efflux calculated for soil collars over the time period 14th Oct 2009 to 28th Oct 2009 (Run 1) using (**A**) hourly data from the automated CO_2 efflux measurement system, and (**B**) data from two hand-held surveys of soil CO_2 efflux carried out during this time. Error bars represent ± 1 SE (n = 4). Within each method of calculation, treatments which differ significantly have different letters.

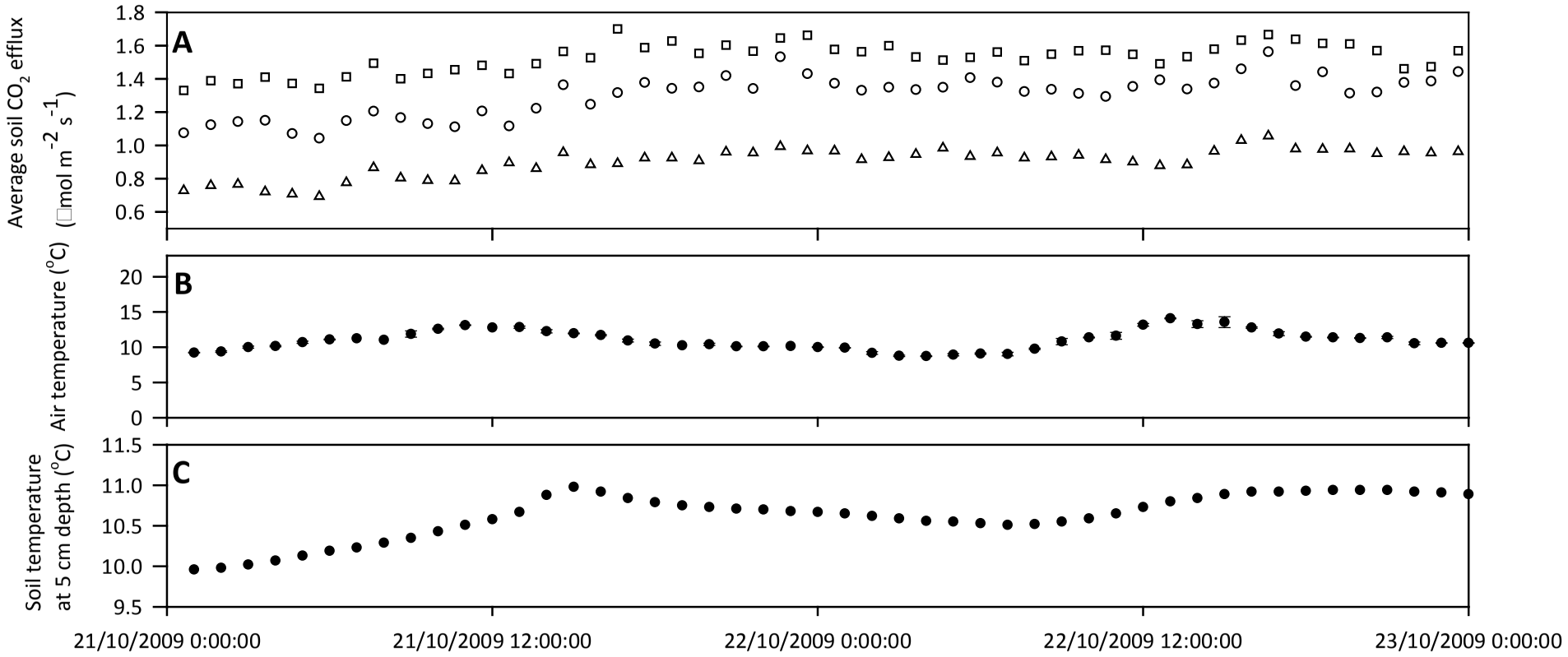

Fig. 3.24 Hourly average (**A**) soil CO_2 efflux, treatments RMS, MS and S are represented by open squares □, circles ○ and triangles Δ, respectively, (**B**) air temperature above the canopy (°C) and (**C**) soil temperature at a depth of 5 cm (°C), measured over two days during Run 1 of the automated CO_2 efflux measurement system. Where displayed, error bars represent ± 1 SE (n = 4).

roughly followed soil temperature, rising towards the end of the two days. The CO_2 efflux from treatments RMS and MS rose and fell, frequently in opposite directions, overall though, as with treatment S, there was a general rise in both fluxes, over the two day period, following the rise in surface soil temperature.

Fig. 3.25 displays the data from Run 2 of the automated CO_2 efflux measurement system on the corrected collars (treatments RMS(W), MS(W) and the control collars, treatment S). The distinction between treatments RMS(W) and MS(W) is not as clear as the distinction between treatments RMS and MS measured during Run 1 (see Fig. 3.21). Because of this, in a change of formatting, treatment MS(W) is represented here by open circles in order that it can be distinguished from treatment RMS(W). There were more occasions where treatment MS(W) had an equal or higher flux than treatment RMS(W). As with Run 1, treatment S consistently had the lowest soil CO_2 efflux. Despite Run 2 starting only two weeks after the start of Run 1, Fig. 3.26 shows that, in comparison to the soil surface temperature during Run 1, which ranged between 9.5^{o}C and 11.5^{o}C (see Fig. 3.22), during Run 2, the soil surface temperature was more variable. The temperature fell below 7^{o}C on occasion, and the shape of the soil temperature data was mirrored by the soil CO_2 efflux data, falling gradually during the three week measurement period (see Figs. 3.25 and 3.26).

A comparison between the cumulative soil CO_2 efflux calculated using the high resolution hourly measurements, and the sporadic handheld surveys of soil CO_2 efflux was also done for Run 2 (see Fig. 3.27). Unlike the comparison of the two methods conducted on the data from Run 1, here there were differences between results produced. The cumulatives calculated using the high resolution automated data reflect what was shown in Fig. 3.25, that though the difference between treatments RMS(W) and MS(W) was not great, the greater soil CO_2 efflux was mostly from treatment MS (see Fig. 3.27 A). However, the cumulatives calculated using the two hand-held soil CO_2 efflux surveys during Run 2 show a different treatment pattern, suggesting that the higher flux was from treatment RMS (see Fig. 3.27 B). Both methods reflect that the lowest flux was consistently from treatment S, but this difference was significant according to the hand held survey data ($F=13$, $p<0.007$, see Fig 3.27 B), whereas there was no significant difference between the cumulatives calculated using the high

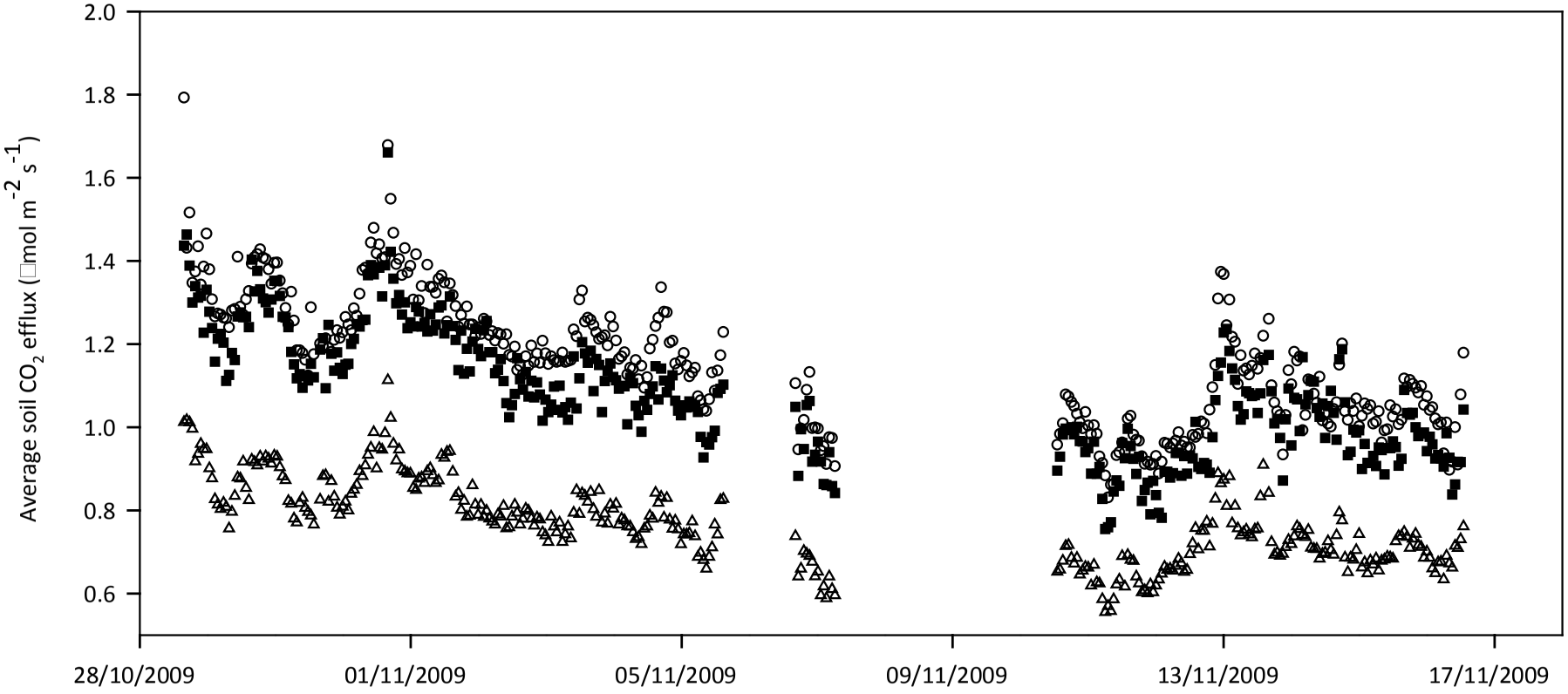

Fig. 3.25 Average hourly soil CO_2 efflux measurements from collars receiving additional water correction via the automated irrigation system and the control treatment (treatment S), taken during Run 2 of the automated soil CO_2 efflux measurement system. Treatments RMS(W) and MS(W) are represented by solid squares ■ and open circles ○, respectively. Treatment S is represented by open triangles Δ.

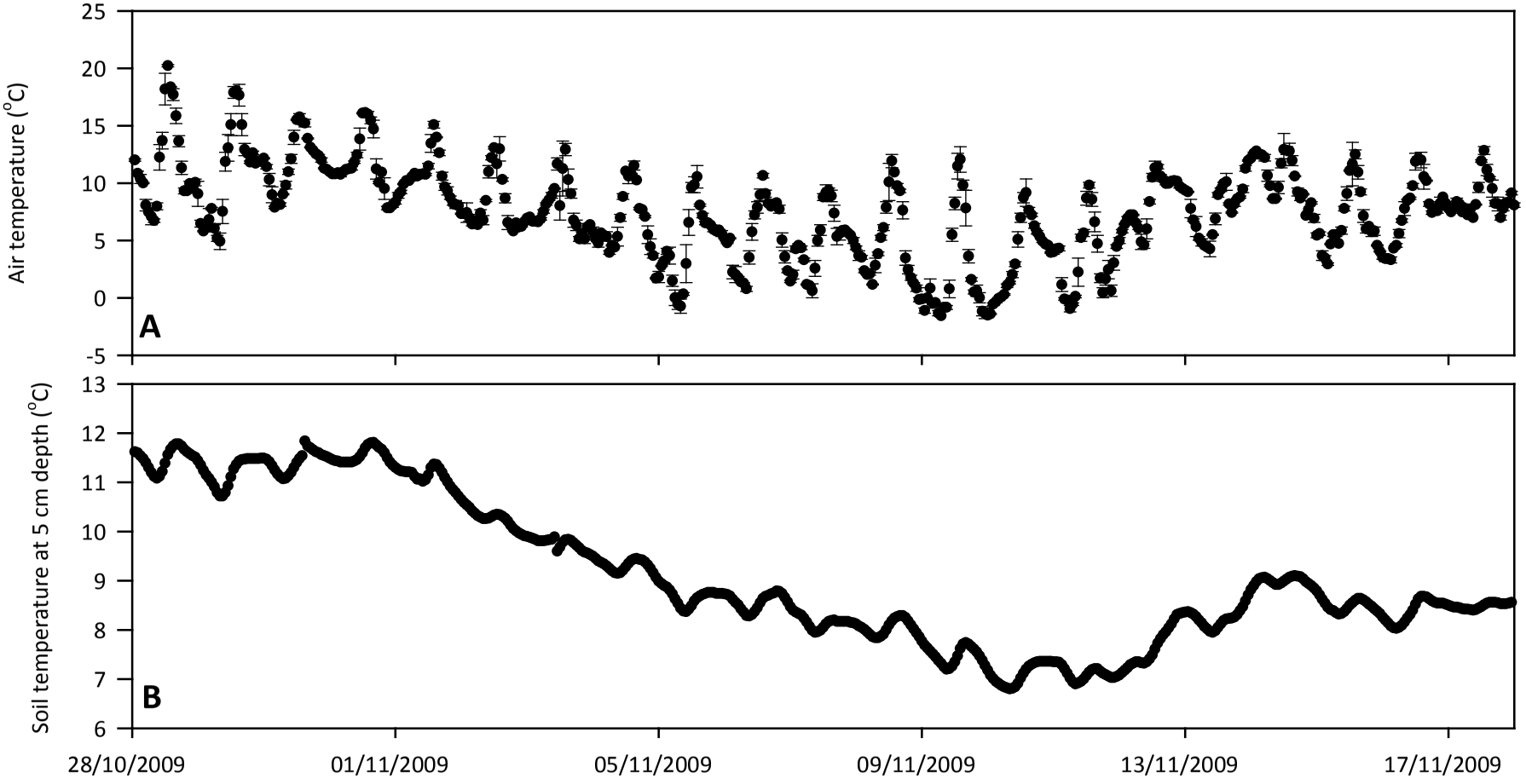

Fig. 3.26 Hourly average (**A**) air temperature above the canopy (°C) and (**B**) soil temperature at a depth of 5 cm (°C) measured during Run 2 of the automated CO_2 efflux measurement system. Where displayed, error bars represent ± 1 SE (n = 4).

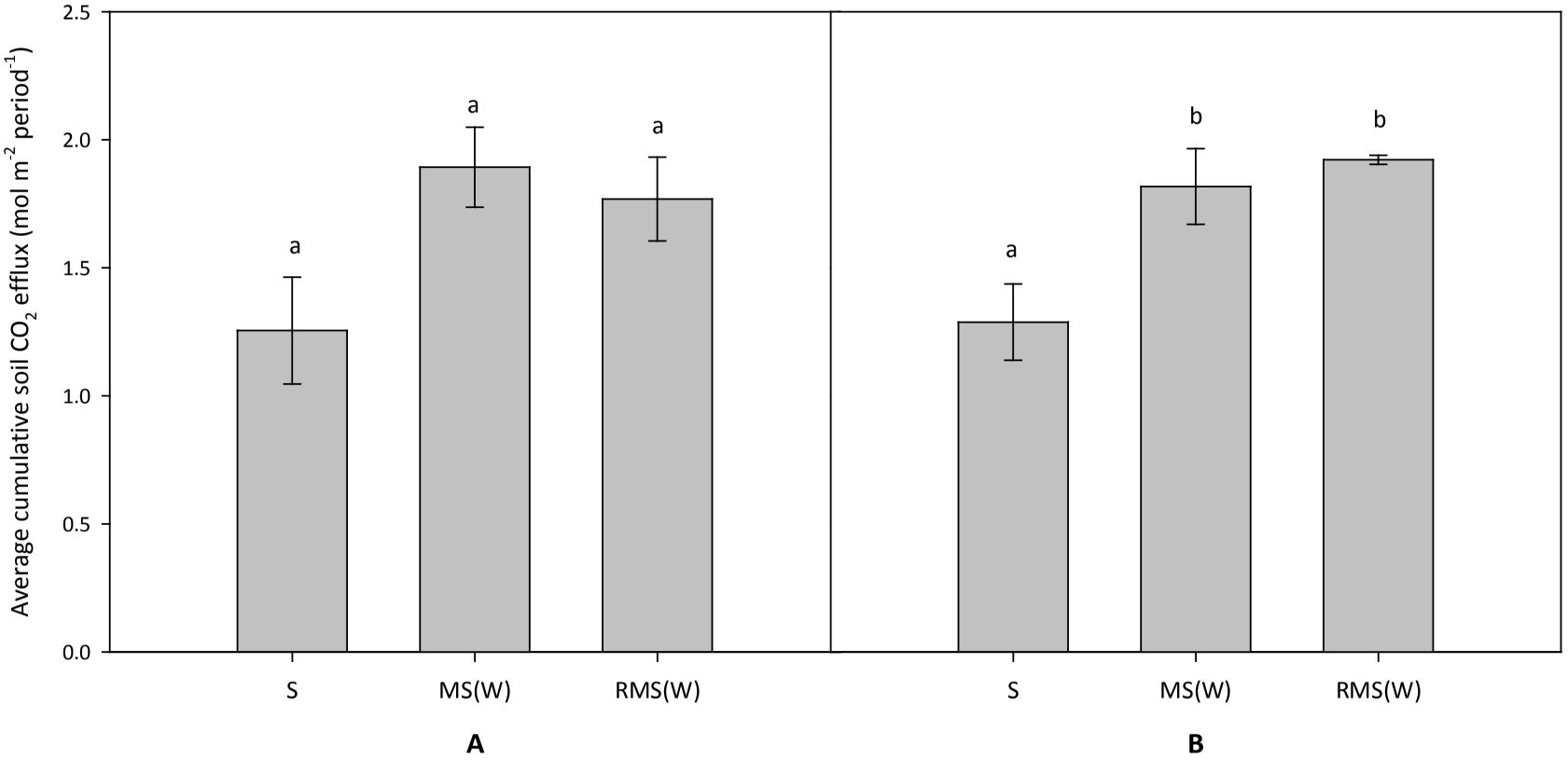

Fig. 3.27 A comparison of the average cumulative CO_2 efflux calculated for soil collars over the time period 28th Oct 2009 to 16th Nov 2009 (Run 2) using (**A**) hourly data from the automated CO_2 efflux measurement system, and (**B**) data from two hand-held surveys of soil CO_2 efflux carried out during this time. Error bars represent ± 1 SE (n = 4). Within each method of calculation, treatments which differ significantly have different letters.

resolution hourly data (F=2.33, p>0.17, see Fig. 3.27 A). Runs 1 and 2 of the automated CO_2 measurement system were conducted over different periods of time, and therefore cannot be directly compared.

In order to assess the diurnal pattern in terms of soil CO_2 efflux during Run 2, again a period of two days is shown in Fig. 3.28 A, alongside air temperature measurements (see Fig. 3.28 B) and soil surface temperature measurements (see Fig. 3.28 C). Soil surface temperature peaked in the afternoon on 31.10.2009, falling to its lowest point before midday on 1.11.2009, before peaking again in the afternoon on 1.11.2009. The peaks in soil surface temperature appear to be at the same time, or just following peaks in air temperature measured above the canopy (see Fig. 3.28 B). There was a peak in the soil CO_2 efflux measured from treatment RMS(W), MS(W) and S collars in the mid afternoon of 1.11.2009, at the same time as the peak in soil temperature, and immediately following the peak in air temperature. During this short period of time, consistent with the complete time series (see Fig. 3.25), the greatest soil CO_2 efflux was measured from treatment MS(W), this was followed by treatment RMS, then the lowest fluxes were measured from treatment S collars.

The high resolution hourly data for soil CO_2 efflux (Runs 1 and 2) were correlated with the surface soil temperature, using Spearman's Rank correlations for treatments RMS, MS and S during Run 1 (see Table 3.5), and for treatments RMS(W), MS(W) and S during Run 2 (see Table 3.6). The associations between soil CO_2 efflux and soil temperature were all highly significant for all treatments over Runs 1 and 2. However, the correlations were stronger for treatments RMS(W), MS(W) and S(W) during Run 2, than the correlations for Run 1. The correlations were noticeably stronger between the hourly average soil CO_2 efflux and the hourly mean soil surface temperature, than between daily average soil temperature and sporadic hand-held soil CO_2 efflux survey data shown previously in Table 3.2. Unlike the hand-held correlations, with the higher resolution correlations, treatment S had a higher correlation than treatments RMS and MS during Run 1 (see Table 3.5), whereas for the hand held surveys, treatment RMS had the highest correlation. During Run 2, CO_2 efflux from treatment RMS(W) had the highest correlation with soil temperature, followed by treatment MS(W) and S, which is consistent with the hand-held data, though here MS(W) had a higher correlation

Table 3.5 Results of Spearman's Rank correlations between the average soil CO_2 efflux measured during Run 1 of the automated CO_2 efflux measurement system from treatments RMS, MS and S, and the lagged hourly mean surface temperature (depth 5 cm) logged for the same measurement hour, and the four hours prior to each measurement of soil CO_2 efflux.

Hourly average soil CO_2 efflux (µmol m^{-2} s^{-1}) and the hourly average soil surface temperature (^oC)			
	S	MS	RMS
ρ	0.70	0.62	0.58
Sig.	p<0.0001	p<0.0001	p<0.0001
Hourly average soil CO_2 efflux (µmol m^{-2} s^{-1}) and the hourly average soil surface temperature (^oC) from one hour prior			
	S	MS	RMS
ρ	0.70	0.62	0.58
Sig.	p<0.0001	p<0.0001	p<0.0001
Hourly average soil CO_2 efflux (µmol m^{-2} s^{-1}) and the hourly average soil surface temperature (^oC) from two hours prior			
	S	MS	RMS
ρ	0.70	0.61	0.57
Sig.	p<0.0001	p<0.0001	p<0.0001
Hourly average soil CO_2 efflux (µmol m^{-2} s^{-1}) and the hourly average soil surface temperature (^oC) from three hours prior			
	S	MS	RMS
ρ	0.68	0.60	0.56
Sig.	p<0.0001	p<0.0001	p<0.0001
Hourly average soil CO_2 efflux (µmol m^{-2} s^{-1}) and the hourly average soil surface temperature (^oC) from four hours prior			
	S	MS	RMS
ρ	0.66	0.58	0.54
Sig.	p<0.0001	p<0.0001	p<0.0001

Table 3.6 Results of Spearman's Rank correlations between average soil CO_2 efflux measured during Run 2 of the automated CO_2 efflux measurement system from treatments RMS(W), MS(W) and S, and the lagged hourly mean surface temperature (depth 5 cm) logged for the same measurement hour, and the four hours prior to each measurement of soil CO_2 efflux.

Hourly average soil CO_2 efflux (µmol m^{-2} s^{-1}) and the hourly average soil surface temperature (^oC)			
	S	MS(W)	RMS(W)
ρ	0.84	0.85	0.86
Sig.	p<0.0001	p<0.0001	p<0.0001

Hourly average soil CO_2 efflux (µmol m^{-2} s^{-1}) and the hourly average soil surface temperature (^oC) from one hour prior			
	S	MS(W)	RMS(W)
ρ	0.84	0.84	0.86
Sig.	p<0.0001	p<0.0001	p<0.0001

Hourly average soil CO_2 efflux (µmol m^{-2} s^{-1}) and the hourly average soil surface temperature (^oC) from two hours prior			
	S	MS(W)	RMS(W)
ρ	0.83	0.84	0.86
Sig.	p<0.0001	p<0.0001	p<0.0001

Hourly average soil CO_2 efflux (µmol m^{-2} s^{-1}) and the hourly average soil surface temperature (^oC) from three hours prior			
	S	MS(W)	RMS(W)
ρ	0.82	0.84	0.85
Sig.	p<0.0001	p<0.0001	p<0.0001

Hourly average soil CO_2 efflux (µmol m^{-2} s^{-1}) and the hourly average soil surface temperature (^oC) from four hours prior			
	S	MS(W)	RMS(W)
ρ	0.80	0.83	0.84
Sig.	p<0.0001	p<0.0001	p<0.0001

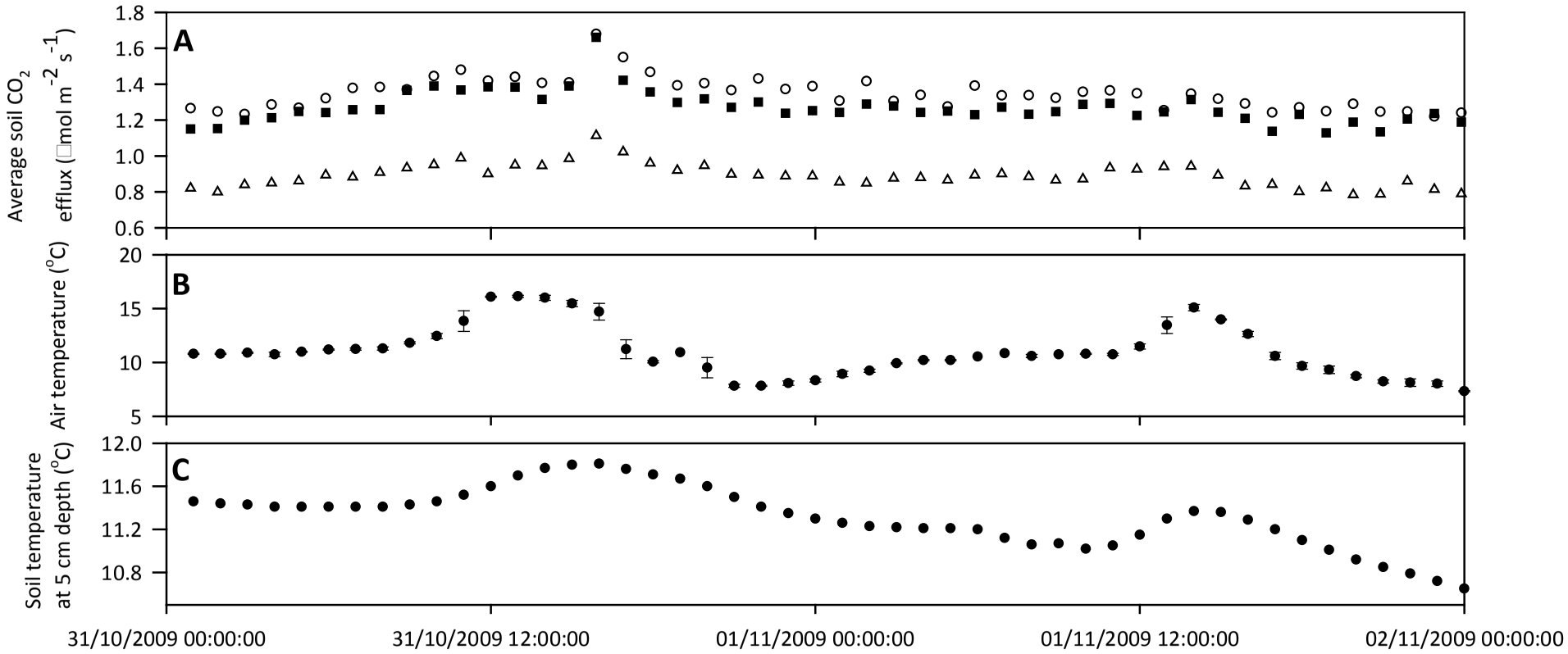

Fig. 3.28 Hourly average (**A**) soil CO_2 efflux, treatments RMS(W), MS(W) and S are represented by solid squares ■, open circles ○ and open triangles Δ, respectively, (**B**) air temperature above the canopy (°C) and (**C**) soil temperature at a depth of 5 cm (°C), measured over two days during Run 2 of the automated soil CO_2 efflux measurement system. Where displayed, error bars represent ± 1 SE (n = 4).

than treatment S (see Table 3.6), which previously had the same level of correlation in the hand-held data (see Table 3.2). Runs 1 and 2 were carried out over a much shorter period of time than the hand-held surveys, which were conducted throughout the entire year, and therefore the soil surface temperature will have varied to a greater extent in the hand-held data, than it did during Runs 1 and 2.

In order to assess whether there was a delay in the response in terms of soil respiration to soil temperature, a series of lagged correlations were carried out, comparing the soil CO_2 efflux to the average soil temperature logged for the four previous hours (see Tables 3.5 and 3.6). However, it would appear that soil respiration responded rapidly to soil temperature changes, with the strongest associations between soil CO2 efflux and soil temperature being for the same hour, as lagging the temperature data only decreasing the level of association for all treatments.

The average soil moisture measured from the soil collars during Runs 1 and 2 is shown in Fig. 3.29. During Run 1, overall, there was a highly significant difference between the treatments in terms of the average soil moisture ($F=13$, $p<0.007$). Consistent with the hand-held surveys of soil CO_2 efflux, treatment RMS had significantly lower soil moisture than treatments MS and S, whilst having generally the highest (though not significantly higher) soil CO_2 efflux of the three treatments (see Fig. 3.23). Treatments MS and S had very similar soil moisture contents (see Fig. 3.29), but the soil CO_2 efflux from treatment MS was higher than that of treatment S. During Run 2, there was no significant difference in the average soil moisture content of treatments RMS(W), MS(W) or S ($F=0.69$, $p>0.5$).

The sporadic hand-held surveys of soil CO_2 efflux were only ever measured in the early afternoon, and never at night. In order to see whether the pattern in terms of the treatments differed between night and day, the high resolution hourly data was split into two time periods, so that the average cumulative soil CO_2 efflux could be determined, per treatment for ‘day time’ (defined here as 08:00-20:00hrs), and ‘night time’ (20:00-08:00hrs, see Fig. 3.30).

The same magnitude of fluxes appeared to be observed from both the corrected and non-corrected fluxes during the day and the night, again with no significant differences

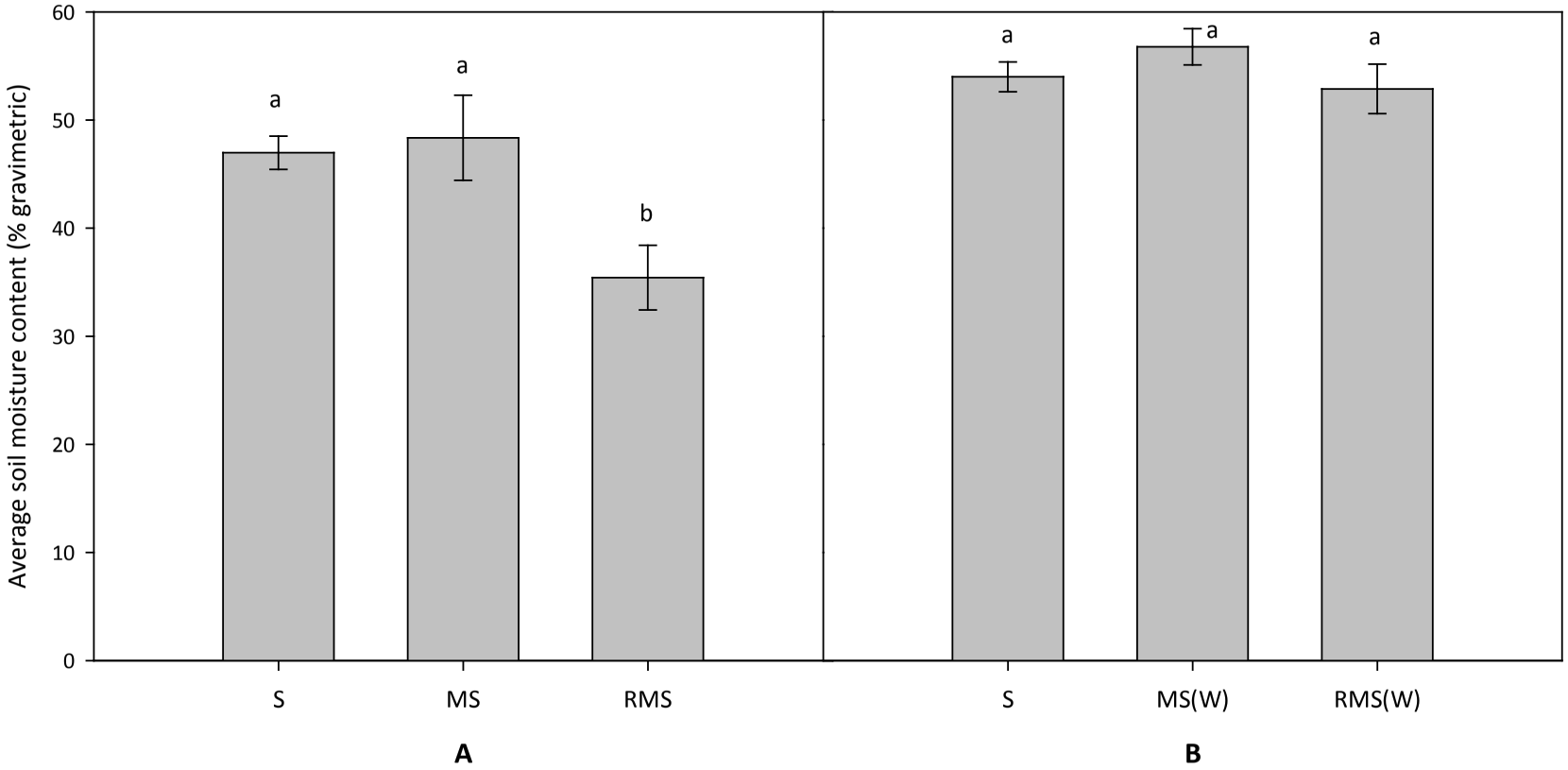

Fig. 3.29 Average soil moisture content (% gravimetric) CO_2 efflux calculated from hand-held soil moisture surveys during (**A**) Run 1, and (**B**) Run 2 of the automated CO_2 efflux measurement system. Error bars represent ± 1 SE (n = 4). Within each run, treatments which differ significantly have different letters.

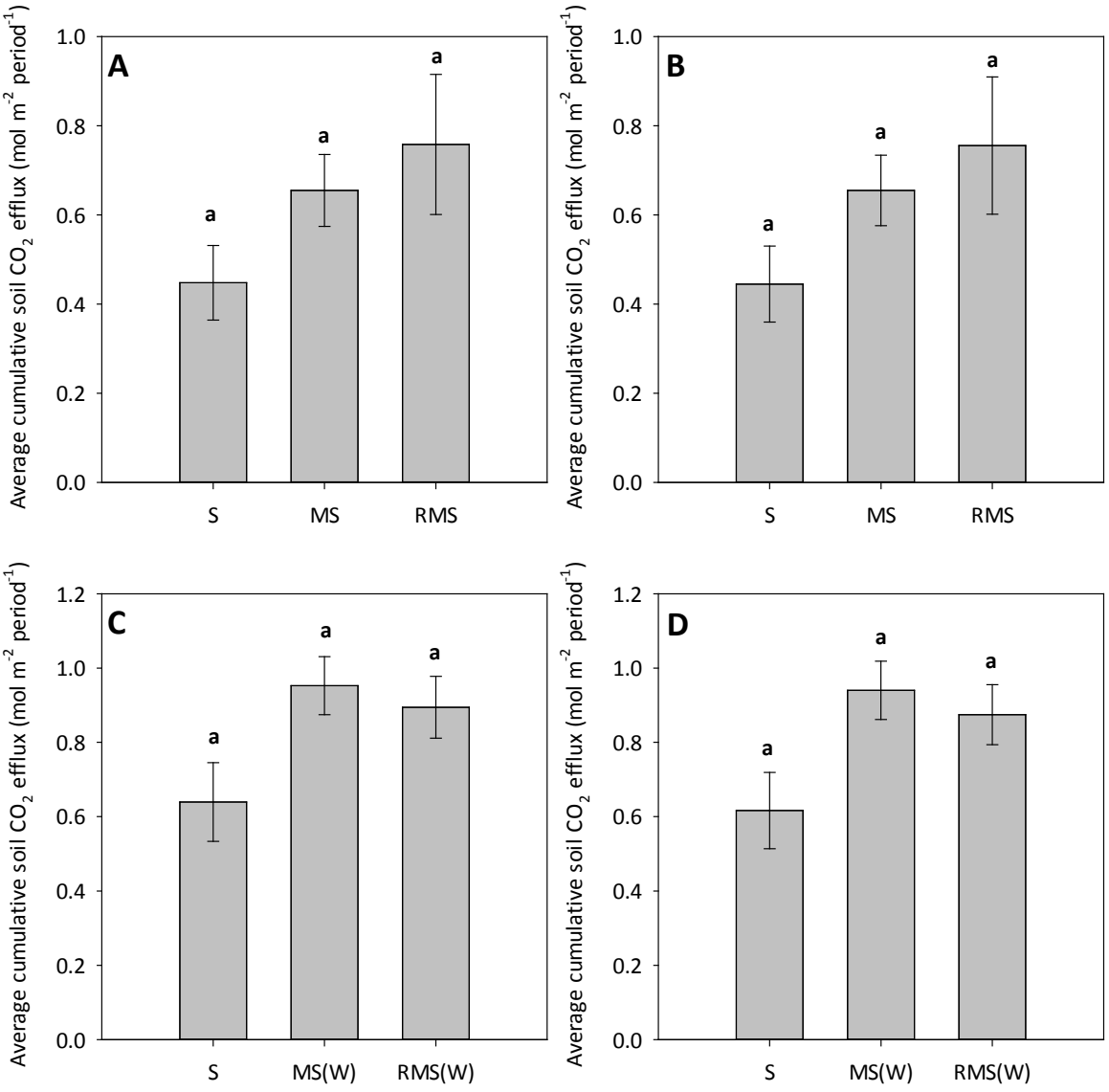

Fig. 3.30 Average cumulative soil CO_2 efflux measured using the automated soil CO_2 efflux measurement system from (**A**) treatments RMS, MS and S (Run 1) during the day (08:00 to 20:00 hrs), (**B**) treatments RMS, MS and S (Run 1) during the night (20:00 to 08:00 hrs), (**C**) treatments RMS(W), MS(W) and S (Run 2) during the day (08:00 to 20:00 hrs), (**D**) treatments RMS(W), MS(W) and S (Run 2) during the night (20:00 to 08:00 hrs). Within each sample period, treatments which differ significantly have different letters. Error bars represent ± 1 SE (n = 4).

between the cumulative fluxes observed at any time. The pattern in terms of the treatments was also the same between the day and the night time.

3.4 Discussion

The aim of the current work was to investigate differences in total soil respiration (R_s) caused by soil trenching, and the role of abiotic factors including soil moisture, temperature and light intensity on R_s and its component heterotrophic (R_h) and autotrophic (R_a) fluxes, and also on the components fluxes from roots and ECM hyphae that make up R_a. The addition of water to the various soil collar treatments via the automated irrigation system allowed an investigation of how correcting any soil moisture differences caused by trenching would affect the CO_2 efflux from the different soil collar treatments. The results suggest that water correction had no effect on root respiration, but there is evidence of increased CO_2 efflux from mycorrhizal hyphae and background soil heterotrophs, and this apparent difference between the soil components in their response to water correction is discussed here.

Trenching was used successfully to separate out total soil CO_2 efflux (R_s) into its component autotrophic (R_a) and heterotrophic (R_h) fluxes. The use of different sized mesh in the collar windows was successful in allowing an estimate of the contributions of roots (R_r) and ectomycorrhizal hyphae (R_m) to R_a. The average contribution of R_h to R_s was 56%, compared to 44% from R_a, the contributions of the autotrophic flux components from root respiration and mycorrhizal respiration were 31.2% and 12.8%, respectively.

Previous studies of the relative contributions of the different flux components to R_s have shown a range of results, varying between sites and plant species, the methodology used to separate out the flux components, and also the length of time used to calculate the average contributions (Subke *et al.*, 2006). Taneva & Gonzalez-Meler (2012) reported average flux components of 30% from R_a, and 70% from R_h during the daytime, and 34% from R_a and 66% from R_h during the night time. In contrast to the current work, they subdivided R_h into the components from litter decomposition (26% during the day) and background SOM decomposition (44% of during the day). The root contributions to R_s reported by Heinemeyer *et al*. (2012) from a four-year experiment in a temperate deciduous forest in south-east England were 38%, with contributions from mycorrhizal fungi of 18%, and the contribution

from R_h was 44%. Comstedt *et al*. (2011) showed in a trenching study combined with modelling to account for moisture differences, that the autotrophic and heterotrophic components contributed c. 50% each to R_s.

The results reported by Heinemeyer *et al.* (2007) from the trenching study conducted previously in the *Pinus contorta* stand at Wheldrake Forest, were also different to those of the current work. In contrast to the 56% contribution of R_h to R_s in the current work, their estimate of the R_h contribution was 65%, they also reported a higher contribution of 25% from mycorrhizal hyphae, compared to 12.8% in the current work, and 10% from roots, which was lower than the 31.2% in the current study. The third measurement campaign in the study by Heinemeyer *et* al. (2007), which included treatment RMS took place between 25th October and 29th December 2005, compared to the 16 months of measurements in the current work (including two summers), so it is likely that the difference in the average contributions from the various components can be explained by seasonality (see discussion below).

A common problem with trenching as a technique is resulting increases in R_s caused by decay following the severing of existing roots and mycorrhizal hyphae (Comstedt *et al*., 2011). In their review of 30 years of literature surrounding separating out soil components, Subke *et al*. (2006) noted that authors commonly acknowledge this as a problem and some try to deal with the additional CO_2 efflux from decaying roots by accounting for the amount of dead roots likely to be present in collars following trenching and applying decay constants from meta-analyses of regional trends in root decay dynamics (Silver & Miya, 2001; Saiz *et al.*, 2006). There are problems with this approach as the correction factor is constant and does not take into account seasonal variations in the autotrophic and heterotrophic fluxes (Saiz *et al.,* 2006). In contrast, using a PLS-model, Comstedt *et al.* (2011) compared what they assumed to be 'root free' trenched plot data from trenched plots in 2004 (the field season following when trenching took place) with the data from the same plots in 2003 in order to gain an estimate of the proportion of the flux which could be attributed to trenching-induced decay. Another approach used to avoid confounding effects of decaying roots and hyphae following trenching is to wait until the contribution from this source is likely to be minimal (Boone *et al.,* 1998; Sulzman *et al*., 2005). In the current work, it is

acknowledged that the treatment effect observed following trenching for treatments MS and S shown in Figs. 3.2 and 3.3 will probably have been overestimated, though collars had been established for nine months prior to the start of automated irrigation, so this confounding effect should have been reduced. Suseela *et al.* (2012) waited 15 months after trenching before taking CO_2 efflux measurements in order to estimate the size of microbial populations, which they felt was adequate to remove any such problems. Sayer and Tanner (2010) trialled a new trenching technique where the R_a was estimated by measuring soil CO_2 efflux prior to, and quickly following trenching, before decay processes were significantly affecting the CO_2 efflux; this was compared to bi-weekly measurements from trenched plots, where they found that the data could not be used for seven months due to the additional CO_2 release from decomposing roots.

The current work shows clear seasonal variation in total soil CO_2 efflux (R_s), measured from the RMS collars on the time-series graphs, showing peaks during the summer months and lower fluxes during the winter months. This seasonality of soil respiration has also been demonstrated by numerous previous studies (eg. Tang *et al.,* 2005; Heinemeyer *et al.*, 2012), and it has been shown to be caused by variations in the different flux components. Taneva & Gonzalez-Meler (2012) found that there were clear fluctuations in both R_a and R_h, which caused changes in overall R_s over the seasons.

There were also fluctuations of R_a and R_h in the current work, with a greater contribution from treatment S (R_h) during the winter months, 2009/2010 than during both summer 2009 and 2010. In contrast to an increase in the cumulative R_h flux for the winter months, the R_a contribution decreased, driven by a decrease in the CO_2 efflux from roots, consistent with roots becoming dormant during the winter (Franck, 2011). The mycorrhizal component of R_a stayed relatively consistent between summer 2009 and winter 2009/2010, though it did decrease for summer 2010. These results are supported by Heinemeyer *et al.* (2012), albeit for a deciduous system. They found that generally, background soil heterotrophic respiration was higher than autotrophic respiration during the winter. They did find that occasionally, during the growing season, the autotrophic flux component was higher than the heterotrophic flux

component, which was also the case in the current work for summer 2009, but not for summer 2010.

The increase in Ra during the growing season that was observed in the current study, and by Heinemeyer *et al*. (2012) was also observed by Comstedt *et al*. (2011) in a Norway spruce forest in Sweden. They estimated that the maximum contribution of autotrophic respiration in their study ranged between 0% to 43% from April to June, but that it ranged from 35% to 72% from June onwards, peaking in the growing season (August to September), this was consistent with other field experiments in Sweden (eg. Högberg *et al*., 2001; Bhupinderpal-Singh *et al*., 2003). Though in the current work, the mycorrhizal flux component stayed relatively constant between summer 2009 and winter 2009/2010, it is possible that a period of rapid growth of mycorrhizal hyphae, as reported for the autumn by Wallander *et al*. (2001), was somehow missed. It is also possible that if a period of rapid mycorrhizal growth spanned September to October at Wheldrake forest in 2009, it may have been averaged across the two periods. Heinemeyer *et al*. (2007) reported a fall in the mycorrhizal flux component in the autumn, following fruiting body production, though they did still report a higher mycorrhizal flux component compared to the root flux component in late October to December and it may be that for 2009 when the current work took place, there was not the same level of mycorrhizal growth as in previous years.

The peaks for treatment RMS shown in Fig. 3.11 are nearly the same height, which suggests that between 2009 and 2010, R_s stayed relatively constant, and only through flux separation is it possible to see that there were hidden differences in the components of R_s between the two summers. The increase in the flux component from R_h (treatment S) between summers 2009 and 2010, also led to an increase in the cumulative flux measured from treatment MS. Both the components of R_a fluctuated between the years, with a decrease in the contribution from roots between summers 2009 and 2010, from both the corrected, and non-corrected collars (RMS and RMS(W)). There was also a decrease in the mycorrhizal component from treatment MS, leading to a decrease in R_a compared to R_h between 2009 and 2010.

The main aim of the current work was to investigate the effect of water uptake by roots on R_s, and its component fluxes. The successful flux separation in the current work was accompanied, as for previous trenching studies (Gadgil & Gadgil 1971, 1975; Fischer & Gosz, 1986), with significant differences between the treatments in terms of soil moisture content, particularly during the summer months for treatment RMS, as was shown in Fig. 3.13. Correcting moisture differences caused by the uptake of water by roots and mycorrhizal fungi had no effect on the total soil CO_2 efflux (R_s), as shown by a comparison of the CO_2 efflux from treatments RMS and RMS(W). Though Fig. 3.11 and Table 3.1 show that there was a lower contribution from irrigated roots (from RMS(W)) to total soil CO_2 efflux, there were no significant differences between treatments RMS and RMS(W) in terms of soil CO_2 efflux overall, for summer 2009 or for summer 2010, and there had been a lower (though not significant) cumulative flux from treatment RMS(W) collars compared to RMS collars prior to the start of automated irrigation. In the winter months, when the soil moistures were extremely similar, the same difference in terms of RMS(W) having a slightly lower, but not significant difference in terms of soil CO_2 efflux remained, and the relationship never changed despite changes in water stress over the year. If there had been an effect of water uptake by roots affecting Rs, potentially by decreasing the component underlying fluxes from mycorrhizal hyphae and background soil heterotrophs (R_h), there should have been an increase in the flux from treatment RMS(W), in comparison to treatment RMS.

Comstedt *et al*. (2011) used the strength of association between soil moisture and soil CO_2 efflux from trenched and control soils to model the underlying R_h flux component where roots were present in the control soils, so taking the flux contributions of Heinemeyer *et al*. (2007) further and introducing a modelled correction for moisture differences. They estimated that had this correction not been applied, there would have been a considerable overestimation of the R_h flux component (~18-24%), caused by trenched soils having a 3-7% higher moisture content than control soils. One problem with the current work is that rather than decreasing the moisture in treatment S, as was achieved by Comstedt *et al*. (2011) using modelling, the other drier treatments were watered, which is the opposite of what is natural. However the

current work allowed the mechanisms behind the control of litter decomposition and soil CO_2 efflux to be investigated in the field. It would seem from the current results that the work of Heinemeyer *et al*. (2007), may not have been confounded by the effects of soil moisture differences between the treatments previously at the current site, assuming there is no difference between years in terms of moisture stress caused by water uptake by roots. Though the results of the current work suggest that the soil moisture differences caused by root water uptake were not having an effect on R_s, soil moisture is still an acknowledged controlling factor on soil respiration in different circumstances (Fischer & Gosz, 1986), and should always be measured in combination with soil trenching. In a later study, in a deciduous forest in SE England, Heinemeyer *et al*. (2012) did take treatment-specific moisture measurements on a monthly basis, which they increased to high resolution measurements during the final year of their experiment. They found significant differences between their trenched and control soil in terms of soil moisture, and put shields above the collars to block incident throughfall in just the S and MS collars, in an attempt to dry the soil to the same level as that of RMS. Lavigne *et al.* (2006) found that modest water stress caused a substantial reduction in R_s of 25-50%, which recovered when soil moisture increased. However, Mosier *et al.* (2003) observed that increased soil moisture resulted in a decrease in soil CO_2 efflux when compared to controls in the Colorado shortgrass steppe, but when high water was combined with N addition, the highest CO_2 flux values were observed. Though there was a 30% drop in the surface moisture content of treatment RMS compared to treatment S during the summer months, the soil moisture content in the current work never fell below 20% for any of the treatments, which is potentially a reason why there was no positive influence of irrigation on R_s. The drought-induced drop in mycorrhizal CO_2 efflux observed by Heinemeyer *et al.* (2007) involved soil moisture content (measured outside the collars) falling to less than 15%.

During summer 2009, irrigation appears to have had no influence on the CO_2 efflux from treatment MS, with practically identical cumulative fluxes calculated for treatments MS and MS(W). It was noted during this time that water was very rarely supplied to these treatment collars by the automated irrigation system, so although the cumulative CO_2 efflux for treatments MS and MS(W) were significantly higher than

for treatment S (see Fig. 3.11) indicating successful colonisation of the collars, ECM hyphae appeared to take up very little soil moisture. However, during summer 2010, the cumulative soil CO_2 efflux from treatment MS(W) had increased significantly compared to treatment MS, suggesting that irrigation was positively influencing the mycorrhizal flux component. The same apparent effect of irrigation was also observed for the R_h flux component, with a higher cumulative soil CO_2 efflux from treatment S(W) compared to treatment S, in contrast to summer 2009, where the difference was not significant.

The sudden significant interaction for summer 2010 between soil collar treatment, and whether the collar was receiving irrigation, when there had been no significant interaction for summer 2009, was driven mostly by sudden increases in the soil CO_2 efflux from the MS(W) and S(W) collars relative to the treatment MS and S collars. It was noted that during summer 2010, the reservoir housing the three aquarium pumps that irrigated treatments RMS(W), MS(W) and S(W) required filling more frequently, which would partly be explained by better water correction of treatment RMS(W), but it could also indicate that the MS(W) pump was being activated to a greater extent than it had been in summer 2009. Also, the lack of difference between a new set of MS and S collars of the same depth, in terms of soil CO_2 efflux led to these new collars being dug up after 18 months. The collars, which had been intended for use in the substrate decomposition experiment presented in Chapter 5 were found to contain a large quantity of roots which had grown up from underneath, although the mesh windows were still intact. Therefore an alternative hypothesis to irrigation causing the significant increase in soil CO_2 efflux from the treatment MS(W) and S(W) collars is that roots had grown up into the collars, possibly as early as winter 2009/10. This is supported by the observation that the CO_2 efflux measured from treatments MS(W) and S(W) was the same or slightly greater than for treatments RMS and RMS(W), which is consistent with them also becoming total soil (R_s) treatments. Alternatively, that CO_2 efflux from root proliferation near to/underneath the collars may have been diffusing through the soil collars and increasing the soil CO_2 efflux from these treatments.

Therefore, there is problem with the technique employed in the current work, and in other studies (eg. Suseela *et al*., 2012) of waiting for a period of time following trenching for the confounding addition of CO_2 from decaying roots and hyphae to decrease, in that one runs the risk of roots growing back in again, effectively removing treatment effects. The collar depth at this site was chosen based on the observation of Heinemeyer *et al.* (2007) that the main mass of roots was concentrated in the top 30 cm of the soil, with a low density of roots below this point. It was difficult to hammer the collars to this depth, as below was a hard sandy layer. In retrospect, it may have been better to use deeper collars, and to dig in and backfill the collars. However, this would have caused additional disturbance which should be avoided. Also, it would be extremely difficult to guarantee that all roots were excluded, and impossible to see whether they were present in the treatment collars during the experiment. Other studies running for two years have encountered the similar problems, with Comstedt *et al*. (2011) observing colonisation of their collars by mycorrhizal mycelium during the second year of their study. A further problem with the current experimental design was that the 'shallow' surface collars used to create treatment RMS and RMS(W), and the 'deep' collars used to create treatments MS, MS(W), S and S(W) will have impacted the soil in slightly different ways. It is acknowledged that the 'deep' collars will have compacted the soil to a greater degree than the surface collars. It would have been better to have used deep collars for all treatments and allowed roots to re-grow in through windows, following cutting the soil, as was done for mycorrhizal hyphae. However, this strategy would likely take longer, and potentially also allow problems with roots growing under the collars, further compromising the treatments.

It is possible that the irrigation may have been the cause of the proliferation of roots and /or mycorrhizal hyphae, and potentially bacteria into the MS(W) and S(W) collars. Plants have been known to adapt their root morphology in response to soil moisture, with such plasticity allowing them to compete with other species/ individuals for limiting resources (Fitter & Hay, 1987). In the fast-draining sandy soils at Wheldrake Forest, the majority of roots are concentrated at the soil surface (Heinemeyer *et al*., 2007) allowing them to intercept moisture and to take up nutrients from the decomposing litter. So for treatments MS(W) and S(W), it is likely there will have been

increased leaching of substrates from the litter layer alongside increased moisture from irrigation, so roots or ECM hyphae which had grown into the collars may have proliferated to a greater degree in these collars than they had in the non-corrected MS and S collars. This is supported by Ludovici & Morris (1996) who observed Loblolly pine root proliferation in response to a high water treatment. Also, Huang *et al.* (1997) observed that of four warm-season turfgrass species, the two which exhibited greatest root plasticity had the highest drought resistance, as they were quickly able to avoid surface drought by proliferating in lower areas of the soil profile.

So, if the greater CO_2 efflux from treatments MS(W) and S(W) can be explained by roots growing into the collars, it would appear that there was no significant effect of irrigation on the respiration from the different collar treatments, nor did the surface soil moisture content of the collars appear to have a controlling influence on R_s at this site. It is possible that moisture differences lower down in the soil profile had an effect on the soil CO_2 efflux from the different treatments, but only surface soil moisture contents (top 6 cm of the soil) were measured during the current work.

The correlations between soil CO_2 efflux and the soil moisture contents for the six soil collar treatments showed that there were significant but weak negative associations between soil moisture content and soil CO_2 efflux for the corrected treatments (RMS(W), MS(W), S(W)) and the control, treatment S. None of the associations were particularly strong, and the association between soil CO_2 efflux and soil moisture for treatments RMS, MS were weaker still, and non significant. Soil moisture content was only included in the forward stepwise multiple regression model on one occasion, for treatment RMS, which is odd as this contradicts what the individual correlations showed. However, this treatment consistently had a high soil CO_2 efflux, whilst having the lowest soil moisture content during the summer months for 2009 and 2010. For the individual correlations, the strongest negative relationship was for treatment MS(W), explaining 32% of the variation, followed by treatment S(W), potentially caused by those treatments having high CO_2 efflux during summer 2010.

This negative association between soil moisture and CO_2 efflux has been observed previously, by Davidson *et al.* (1998) in a hardwood forest, which they attributed to

other factors important in terms of governing soil respiration, such as temperature and substrate availability being high at times of the year when soil moisture is at its lowest. As with the current study, Sulzman *et al*. (2005) logged the lowest temperatures and the highest soil moistures in the winter months, whilst the peak in soil CO_2 efflux over their three year study (2001-2004) occurred in August, July and May respectively, when moisture conditions were relatively low. They also reported a low association between soil moisture content and CO_2 efflux.

It would seem that R_h was not limited by soil moisture content in this study, as despite the soil moisture content of treatment S decreasing between summers 2009 and 2010 (see Fig. 3.13), there was an increase in the cumulative soil CO_2 efflux (see Fig 3.11) and the contribution from R_h had increased (see Table 3.1). The decrease in contribution from mycorrhizal hyphae between summers 2009 and 2010 could potentially indicate a moisture limitation, and because of the potential confounding effects of roots growing into treatment MS(W) it is not possible to see if irrigation had any effect, the same applies to treatment S(W).

It has been shown that soil moisture, when at extremes, can cause decreases in CO_2 efflux from all flux components, and affect the influence of other abiotic factors, including temperature sensitivity on respiration (Davidson & Janssens, 2006; Suseela *et al.*, 2012). Heinemeyer *et al.* (2007) noticed that the mycorrhizal flux fell rapidly following a period of drought, but with rainfall, the flux component recovered quickly. Moisture surveys were taken sporadically in the current study, therefore short-term changes in soil moisture will have been missed. Also regular weekly watering, though it followed average rainfall meant that inputs were regular and drought conditions may have been avoided.

The same lack of influence of soil moisture on soil CO_2 was shown for the high-resolution data taken during Runs 1 and 2 of the automated soil CO_2 efflux measurement system. Treatment RMS had the highest soil CO_2 efflux during Run 1, but the average soil moisture content was significantly lower than the soil moistures of treatments MS and S (see Fig. 3.29). During Run 2, there was no significant difference between the treatments in terms of the average soil moisture contents, but there was

a significantly higher soil CO_2 efflux from treatment RMS and MS collars, than there was from treatment S. There are limitations here as the soil moisture content was taken on two occasions, and averaged, and high resolution moisture data would have made a better comparison to the high resolution soil CO_2 efflux data.

The average soil moisture content during the winter months was very similar for all six treatments, and not much higher than the average during the summer months, but the lower contents from treatment RMS(W), RMS, MS(W) and MS had disappeared (Fig. 3.14). There were still variations between the treatments in terms of soil CO_2 efflux however, further reflecting that soil moisture was not the main controller of soil CO_2 efflux at this site. The cumulative soil CO_2 efflux from all treatments was reduced during the winter, alongside a general increase in soil moisture, thus reinforcing any negative associations.

In contrast with soil moisture content, temperature is known to have a positive association with biological processes, such as soil respiration, peaking at the same time as R_s (Sulzman *et al.,* 2005). The results of the surveys of soil CO_2 efflux conducted in the *Pinus contorta* stand at Wheldrake Forest clearly suggest that temperature was the main controlling factor for respiration, having a positive association with soil CO_2 efflux from the six treatments. The clear seasonal pattern in terms of soil CO_2 efflux shown over time in Figs. 3.4 -3.9 was reflective of the seasonal variations in soil temperature shown in Fig. 3.16. This association was supported by the results of the forward stepwise multiple regression, which showed that, for all treatments, between 53% and 67% of the variation in soil CO_2 efflux could be explained by temperature, whether air temperature, or soil surface temperature (measured at a depth of 5 cm).

The results from simple correlations showed that R_s (treatment RMS) correlated most strongly with soil surface temperature (R^2 = 0.65), correlations between soil temperature and soil CO_2 efflux from treatments MS and S, (R2 = 0.57, and R2 =0.48), respectively, suggest that R_a was more influenced by soil temperature than R_h, and that of the components of R_a, root respiration was more influenced by soil temperature than respiration from mycorrhizal hyphae. Had the associations been of the same strength for treatments RMS, MS and S, this would have indicated that there

was no difference in the association strength between all the components of R_s, but this was not the case.

Differing effects of temperature on autotrophic (R_a) and heterotrophic (R_h) respiration have been observed previously. Boone *et al*. (1998) demonstrated in a trenching and litter input experiment in a temperate hardwood forest, that the association between soil respiration and soil temperature was stronger for control plots (R^2=0.91) compared to trenched plots (R^2=0.73), where roots had been excluded using fibre glass screens. In a girdling experiment in a *Vitis vinifera* vineyard, Franck *et al.* (2011) demonstrated that root respiration was more sensitive than background soil heterotrophic respiration to soil temperature.

However, other studies have shown no difference between the temperature sensitivity of R_a and R_h (Irvine *et al*., 2005). During a girdling experiment in northern Sweden, Bhupinderpal-Singh *et al.* (2003) reported a 20-day period when temperature dropped by 6˚C, accompanied by a rapid decline in R_h, but not R_a, suggesting that temperature was in fact more important in the control of R_h, than R_a. This was supported by Hartley *et al.* (2007), who demonstrated that R_h was more sensitive than R_a to changes in temperature. Their experiment included soils at ambient temperature, and soils that were warmed to 3˚C above ambient, and a comparison of planted (maize or wheat) and unplanted soils. They found that during the growing season, there were increases in respiration in all the soils, but that the greater the contribution of R_a to R_s, the less temperature sensitivity was observed.

Previously, at the current site, Heinemeyer *et al.* (2007) demonstrated that there were strong associations between soil temperature and soil CO_2 efflux from treatments S (R^2=0.88) and MS (R^2=0.68). However, when the difference in flux between these two treatments was subtracted to gain an estimate of just the mycorrhizal flux component, and this was correlated against soil temperature, there was no association, meaning that R_h was more affected by temperature than the mycorrhizal component of R_a. Also, Heinemeyer *et al*. (2012) demonstrated that a greater temperature sensitivity of R_s during the winter months could be explained by the increase in the R_h flux component relative to R_a.

There is contention regarding the relative temperature sensitivity of autotrophic and heterotrophic respiration (Högberg, 2010; Luo & Zhou, 2010; Zhou *et al.,* 2010). Subke & Bahn (2010) have offered a suggestion which explains why studies such as Boone *et al.* (1998) and Franck *et al.* (2011) have apparently shown the opposite to others in terms of the relative sensitivity of R_a and R_h to temperature. They suggest that studies which show increased temperature sensitivity of R_a relative to R_h overlook the effects of substrate supply and plant phenology on R_a, mistaking their effect to be that of temperature. Though temperature certainly has an effect on kinetic properties of enzymes and is therefore likely to influence respiration, low substrate supply during the winter months could also be a factor explaining the decrease in root respiration observed in the current work (Subke & Bahn, 2010). Atkin & Tjoelker (2003) point out that even if temperatures are optimal for respiration, a limit in substrate supply would restrict the level of respiration taking place. Also, during cold periods, the maximum catalytic activity (V_{max}) of respiratory enzymes is reduced, so even if there are substrates available, respiration is reduced. Clearly, the interpretation of what factors are driving respiration is complicated, Franck *et al.* (2011) acknowledge that the difference in sensitivity to temperature they observed could also be explained by the C availability to the roots in the non-trenched treatments. Bhupinderpal-Singh *et al.* (2003) suggest that though soil temperature may be used to model R_h, that plant photosynthetic activity, and the allocation of C to roots would give a more accurate prediction of R_a, and though temperature may still have an effect on root respiration, roots have been shown to acclimate quickly, reducing the temperature response (Atkin *et al*. 2000, Atkin & Tjoelker, 2003). Heinemeyer *et al.* (2007; 2012) were able to demonstrate that it is not only the root component of R_a that changes in response to substrate supply and plant phenology. Mycorrhizal respiration was shown to alter around the time of fruiting body appearance in the autumn, which increased the apparent temperature sensitivity, though they commented that it was more likely due to changes in the substrate supply to the fungi from their mycorrhizal partners.

The high resolution hourly CO_2 efflux data and the hourly average temperatures measured during Runs 1 and 2 of the automated CO_2 efflux measurement system show that the same association with temperature appeared to remain for the high

resolution measurements as it had for the more sporadic hand-held measurements. During Run 1, in contrast to the previous temperature regressions, treatment S had the highest positive association with temperature (ρ=0.70), followed by treatments MS and RMS (ρ= 0.62 and ρ= 0.58, respectively), so it would appear that diurnally, R_h was more affected by fluctuations in temperature, than R_a. As these high-resolution measurements were taken over a short two-week period, there was much less temperature variation than was seen seasonally, but also potentially less variation in the substrate supply available for roots and mycorrhizal hyphae, with starch reserves in roots potentially having a buffering effect on R_a, which may explain why they were less affected by short-term temperature change than soil heterotrophs, which in the trenched plots may have been more substrate limited.

However, there was no evidence from the lagged temperature correlations that R_h responded more quickly to changes in soil temperature than the autotrophic flux components, with soil CO_2 efflux from all three treatments having the stongest association with soil temperature for the hour when the CO_2 measurements were taken (see Table 3.5). During Run 2 with the corrected collars, the associations were equal across treatments S, MS(W) and RMS(W), suggesting that with irrigation, soil heterotrophs were almost as affected by temperature as roots and mycorrhizal hyphae. The comparison of the treatment effects between day and night showed that there was no difference in the relative contributions of the different flux components to the cumulative soil CO_2 efflux during runs 1 or 2, if the measurements were taken during the day compared to during the night. Overall, the cumulative fluxes measured during the day and the night were of the same magnitude. Taneva & Gonzalez-Meler (2011) found that overall, both autotrophic and heterotrophic respiration were slightly higher during the night time, and that there were significant differences between day and night time during the summer months. The current measurements were taken during October and November, and therefore considering the variations that Taneva & Gonzalez-Meler (2011) observed during the summer months, it is perhaps surprising that no differences were observed here. If CO_2 release from a system were to be modelled accurately though, it would be a good idea to include night time measurements, particularly, it would seem, during the summer.

So, it has been demonstrated that assimilate supply to the soil is likely to be an important driver of R_a as well as temperature, and that care must be taken not to mistake an increase in CO_2 efflux due to substrate supply as an increase in soil CO_2 efflux driven solely by an increase in soil temperature (Olsson *et al.,* 2005; Subke and Bahn, 2010). The importance of assimilate supply as a controller of R_a was demonstrated by Hogberg et al (2001), who found a clear coupling between aboveground plant activity and R_a in a large-scale girdling study experiment in a Boreal pine forest in Sweden, where the effect of cutting off photoassimilates to roots on R_s was studied. They noticed a reduction in respiration in the girdled plots of 56%, and the loss of ECM fruiting bodies, demonstrating the dependence of not only roots, but also mycorrhizal fungi on photoassimilates in these systems.

In the current work, though light intensity did not meet the 0.05 significance level for inclusion into the forward stepwise multiple regression model, there was a significant but weak association between soil CO_2 efflux and light intensity for treatment RMS (R^2 = 0.17, $p<0.02$). An attempt to investigate the timeframe over which R_a is influenced by photosynthesis was unsuccessful, with higher associations for all treatments, including R_h being observed between soil CO_2 efflux and the mean light intensity measured one day prior to the hand-held surveys of soil CO_2 efflux. It is surprising that this lagged association was higher for treatment S than for treatment MS (R^2= 0.21 and R^2=0.16, respectively), when it would be expected to be higher for treatment MS, which included the CO_2 from the mycorrhizal flux component. Treatment RMS did still have the highest association of CO_2 with light intensity (R2= 0.29), as would be expected as root respiration relies on photoassimilates. A previous study by Ekblad & Högberg (2001) in a mixed conifer forest in northern Sweden found a strong relationship between the isotope ratio ($\delta^{13}C$) of soil CO_2 efflux with the relative humidity in the air, 3-4 days prior to the measurements of soil CO_2 efflux. As they had deduced that RH affects the $\delta^{13}C$ of photosynthates (low RH leads to a high $\delta^{13}C$), they were able to come up with an estimate that photoassimilates are available for use in root respiration 1-4 days after photosynthesis has taken place. Heinemeyer *et al.* (2012) were able to show that the time lag for associations between GPP and mycorrhizal respiration was 2-8 days, and this was a longer time lag than for root

respiration, and also that factors such as herbivory from caterpillars, and mildew increased the time lag for GPP correlation with both root and mycorrhizal respiration. Bowling *et al.* (2002) found peaks in correlation coefficients between $\delta^{13}C$ of soil CO_2 efflux and the vapour saturation deficit (vpd) of air (which alters the $\delta^{13}C$ of photosynthates) for 5, 9, 5 and 10 days for four different conifer forests along a precipitation gradient transect in Oregon, USA. They explained the lag time as being a result of a variety of processes, including phloem loading, transport time, unloading, the allocation of C by the plant, the phenology of leaves and roots, root exudation, mycorrhizal and microbial activity and fine root turnover. All of these factors, and whether the plant stores the photoassimilates, for example as root reserves in the winter months, would be expected to affect the lag time for different sites (Bowling *et al.*, 2002; Irvine *et al*., 2005; Brüggemann *et al.,* 2011). Therefore, the reason why there was such a low association between light intensity and soil CO_2 efflux, and why a stronger association with the respiration from R_a components was not seen, could be due to any or all of these factors, and there is potential that lagging over a longer period of time would result in identifying stronger associations.

The aim of using the automated CO_2 efflux measurement system on the soil collars at Wheldrake forest was to ascertain whether obvious bias was introduced by only carrying out sporadic surveys of soil CO_2 efflux. It would appear that when there is a clear difference between treatments over time, with little overlap, as was the case for treatments RMS, MS and S as shown in Fig. 3.21, then there is less chance of a sporadic measurement taken during that time leading to a false conclusion of what was happening generally. This was the case for the two sporadic surveys of soil CO_2 efflux taken during Run 1 of the automated system, where there was no difference in the cumulative soil CO_2 efflux for that period calculated using the high resolution data, compared to when it was calculated using the low resolution data.

However, when treatments RMS(W) and MS(W) were similar in terms of the soil CO_2 efflux, with considerable overlap, as was the case for Run 2 of the automated system, the cumulatives calculated using the high automated system data and the low resolution data from the two hand-held surveys of soil CO_2 efflux showed a different treatment effect. Interestingly, the automated system was switched off for the hour

when the hand-held surveys were taken, but when the automated data was assessed from the hour prior to the hand-held surveys, and the first survey following, to see if the same result of treatment RMS(W) having the higher flux (as shown by the hand-held surveys) was shown in the nearest automated data, treatment MS(W) was still shown to have the higher soil CO_2 efflux.

Each automated CO_2 efflux measurement took 3 minutes in every hour, which although is at a much greater frequency than that of the two one-off hand-held measurements of soil CO_2 efflux, still leaves quite a large period of time unmeasured. Therefore, it is not surprising that when there is overlap between two treatments in terms of soil CO_2 efflux, different methods can produce different cumulative fluxes.

Another reason why there may have been differences in the estimates of the cumulative fluxes from the hand-held surveys of soil CO_2 efflux using the Li-Cor LI-8100 IRGA and 20 cm survey chamber, compared to the high-resolution automated surveys using the multiplexed system is down to an artefact recently discovered by Nottingham *et al*. (2012, In press). Whilst conducting soil CO_2 efflux measurements in a tropical montane cloud forest in Peru, they noticed that soil CO_2 efflux was significantly higher when measured using a multiplexed system as in the current work, than it was using a hand-held IRGA and survey chamber. They conducted swap tests with the two systems on the cloud forest soil and two other soil types in order to isolate the problem. They found that the difference was only encountered during measurements of CO_2 efflux from the cloud forest soil. This soil had a low bulk-density and the mass flow of CO_2 was promoted by the automated system. Their swap test technique could be criticised on the basis that the multiplexed system was always tested first, prior to the hand-held system. However they showed a clear artefact of using two different designs of system, which they were able to solve by simply changing the angle of the air inflow tube and reducing the flow rates in the automated chambers. The difference between the high and low resolution measurements in terms of the cumulative soil CO_2 efflux during Run 2 highlights the need for caution when interpreting data measured using two different systems, even when the majority of the components are the same.

In summary, the current work has demonstrated that despite there being no significant difference between the treatments in terms of litter decomposition (Chapter 2), there were clear significant differences between the six soil collar treatments in terms of soil CO_2 efflux. There were potential problems with roots and /or mycorrhizal hyphae growing into deep soil collars towards summer 2010, which highlights the need for caution when using soil trenching as a technique to separate out the various flux components of R_s. Though there was no evidence from this research to support that moisture differences caused by water uptake by roots influences soil CO_2 efflux in trenched compared to control soils, or that moisture in general was limiting soil CO_2 efflux at this site, the need to measure soil moisture and its effect on the components of soil CO_2 efflux at alternative sites, under more severe moisture conditions is apparent.

Temperature was clearly the most important driving factor of soil CO_2 efflux at Wheldrake Forest, though whether the seeming greater response of root respiration to soil temperature was in fact at least in part explained by variations in assimilate supply is a possibility, and warrants further investigation.

The use of the high resolution data from the automated soil CO_2 efflux measurement system allowed an investigation of the degree of bias introduced by basing assumptions in terms of the treatment effects on sporadic hand-held surveys of soil CO_2 efflux. It would seem that unless two treatments are extremely close in terms of their soil CO_2 efflux, the high and low resolution measurements generally give consistent results.

Certainly the differences demonstrated in terms of the responses of roots and mycorrhizal hyphae to seasonal changes, whether driven by temperature, moisture or assimilate/substrate supply support the view of Heinemeyer *et al*. (2007, 2012) that it is important to consider the different flux components of R_s and the factors important for driving them separately, for modelling of carbon dynamics in forest systems to be accurate.

In the next Chapter, results of a litter decomposition experiment in combination with forest girdling and measurements of soil CO_2 efflux are presented, these data have been published (Subke *et al*., 2011).

Chapter 4 Dynamics and pathways of autotrophic and heterotrophic soil CO_2 efflux revealed by forest girdling (Subke *et al*., 2011).

Chapter 5 ECM fungi and the decomposition of ^{13}C-labelled simple and complex substrates

5.1 Introduction

As previously described and demonstrated in Chapter 4, the introduction of labile substrates, including plant assimilate C to the soil have been shown to cause increased microbial activity and turnover of complex substrates including litter (Subke *et al*., 2004; 2006), and SOM (Kuzyakov, 2002; Fontaine *et al*., 2004). In temperate and boreal forest systems, much of the labile C entering the soil is via ECM fungi in symbiosis with plant roots. Godbold *et al*. (2006) estimated that in these systems, *ca.* 60% of the C input to the soil is cycled through ectomycorrhizal (ECM) fungi, a greater input than from leaf litter decomposition and the turnover of fine roots.

Forest soils are complex systems and competition between microorganisms of differing decomposer abilities can influence the rate of carbon cycling taking place. There is evidence that the C supply to ECM fungi gives them a competitive advantage in forest soils, and their dominance, combined with their limited decomposer abilities has the effect of slowing down litter decomposition (Gadgil & Gadgil, 1971; 1975). In a study comparing litter decomposition and microbial diversity, between a mono-dominant *Dicymbe corumbosa* stand, with associated ECM fungi, and a mixed-species stand where AM associations were dominant, McGuire *et al*. (2010) reported increased decomposition of litter collected from both the mono-dominant and the mixed litter stands when they were incubated in the soil of the mixed stand. The decreased rate of decomposition of litter native to both stands in the presence of ECM fungi in the mono-dominant stand was accompanied by decreased microbial diversity in the soil. Gadgil & Gadgil (1971; 1975) and Abuzinadah *et al*. (1986) suggested that a potential mechanism through which ECM fungi could inhibit decomposition is selective uptake of nutrients such as N from the litter, making them unavailable for soil saprotrophs. This is one key mechanism that could explain the decrease in diversity in the ECM-dominated stand observed by McGuire *et al*. (2010). It has been largely assumed that ECM fungi intercept N released from complex substrates decomposed by saptrotrophs, since ECM fungi reportedly cannot access organic N on their own (Bending & Read,

1995). Though the decomposer abilities of free-living saprotrophic fungi are considered to be much greater than for ECM fungi, there is evidence of certain species of ECM fungi directly accessing organic N substrates (Abuzinadah *et al*., 1986; Lindahl *et al*., 1999a). In contrast to the findings of Gadgil & Gadgil (1971; 1975) and McGuire *et al*. (2010), the results of girdling studies (Subke *et al*., 2004) and the litter decomposition experiment presented in Chapter 4 suggest that the presence of ECM fungi actively promoted the decomposition of complex substrates. In the current Chapter, the results from two experiments are presented, where the influence of roots and associated ECMs on the decomposition of substrates differing in terms of structural complexity and nitrogen (N) content was investigated.

In an initial experiment, conducted prior to the litter decomposition experiments presented in Chapters 2, 3 and 4 of this thesis, it was decided to assess the influence of the presence of ECM roots, ECM hyphae excluding roots, and free living soil saprotrophs on the decomposition of a simple substrate, ^{13}C-labelled glucose. High resolution measurements of soil CO_2 efflux and its isotopic composition were measured continuously from four replicate collars each of treatments RMS, MS and S, with a field-deployed mass spectrometer. This allowed a unique opportunity to study the addition of a labile C source to the soil, in the presence or absence of ECM fungi in a field situation.

Further, in order to investigate the apparent emerging discrepancy between the results of this initial ^{13}C-labelled glucose decomposition experiment, and the results of the girdling experiment presented in Chapter 4 (Subke *et al*., 2011), a further field experiment was conducted. During the current experiment, the decomposition rates of the following four substrates: ^{13}C-labelled glucose, ^{13}C-labelled cellulose, ^{13}C-labelled lysine and ^{13}C-labelled fungal cell wall material were studied in the presence or absence of ECM hyphae (treatments MS and S). These four substrates were selected in terms of their differing structural complexities and N contents; Table 5.1 provides the coding structure and basic rationale behind these treatments. Glucose is a simple sugar and a common component of rhizodeposits (Kuzyakov, 2010) and contains no nitrogen (N). Lysine is an N-containing amino acid, but is also relatively simple in terms of structure. In contrast, fungal cell wall material is estimated to contain up to 60%

chitin (Treseder & Allen, 2000), a complex N containing substrate which constitutes an important structural component of microbial cells.

A considerable amount of work has been conducted into studying the influence of the turnover of roots on forest C cycling (Silver & Maya, 2001), but surprisingly little attention has been given to the turnover of ECM fungal biomass (Wallander, 2012). We now realise that ECM fungal biomass constitutes one of the major sinks for plant photoassimilates in temperate and boreal forest systems (Godbold *et al*., 2006) and their turnover is likely to provide a substantial resource in terms of both C and N for other soil microorganisms (Wilkinson *et al*., 2011, Fernandez & Koide, 2012). A handful of recent studies have assessed the mass loss of laboratory cultured ECM mycelia in mesh bags (Koide & Malcolm, 2009; Koide *et al*., 2011; Fernandez & Koide, 2012). Drigo *et al*. (2012) studied the turnover of ^{13}C-labelled fungal mycelia in a controlled laboratory study. However in the current field-based study, the use of the ^{13}C isotopic label, in combination with soil trenching allowed an estimate of the turnover of the complex structural components of ECM fungal mycelium in the soil, in the presence or absence of labile C inputs through roots and associated ECM fungi, which to our knowledge has not previously been attempted. The hypotheses under test in the current work were:

^{13}C-labelled glucose decomposition experiment:

There will be a faster decomposition rate of simple substrates in the presence of an intact rhizosphere, compared to where roots and ECM fungi are excluded.

^{13}C-labelled simple and complex substrate decomposition experiment:

1. There will be a faster decomposition of structurally complex substrates in the presence of ECM fungi, compared to where they are excluded.

2. There will be faster decomposition of N-containing substrates in the presence of ECM fungi, compared to where they are excluded.

5.2 Materials and methods

5.2.1 Site description

The study site was the same 20 year old stand of Lodgepole pine (*Pinus contorta,* Douglas) used for the litter decomposition experiment (see Chapter 2, and further study site information given in Section 2.2.1).

5.2.2 Experiment 1: ^{13}C-labelled glucose decomposition experiment

5.2.2.1 Experimental treatment

The aim of this experiment was to investigate the impacts of root/root + mycorrhizal exclusion on field decomposition rates of added ^{13}C labelled glucose. This involved the construction of appropriate exclusion collars and field use of an isotope ratio mass spectrometer to monitor *in situ* $^{13}CO_2$ production from these collars. As previously mentioned, 24 soil collars, 16 'deep' soil collars (30 cm in height, hammered 25 cm into the soil) and 8 shallow surface collars were installed on 10th June 2008, in a randomised block design based on a survey of background CO_2 efflux (for further details of this survey, and of soil collar construction, see section 2.2.1). There were three treatments (RMS, MS and S, n=8; see previous Chapters for treatment naming conventions).

There are experimental limitations imposed by the field use of the field IRMS (see below and Fig. 5.1) which includes the maximum distance between cores and the IRMS (50 metres) and a maximum of 16 analytical lines (and hence the number of cores) which can be monitored automatically; this line limitation means that the number of treatments or replicates has to be reduced (as in this case) or the lines have to be moved manually between cores at regular (e.g. daily) intervals (as for Experiment 2, below). In the current experiments, initial CO_2 flux measurements were made using a hand-held IRGA to optimally allocate treatments and blocks to minimise experimental heterogeneity and, hence, the number of replicates required.

Two weeks after collar installation, PVC shields were erected above the 24 soil collars, and weekly additions of average amounts of collected throughfall commenced; this

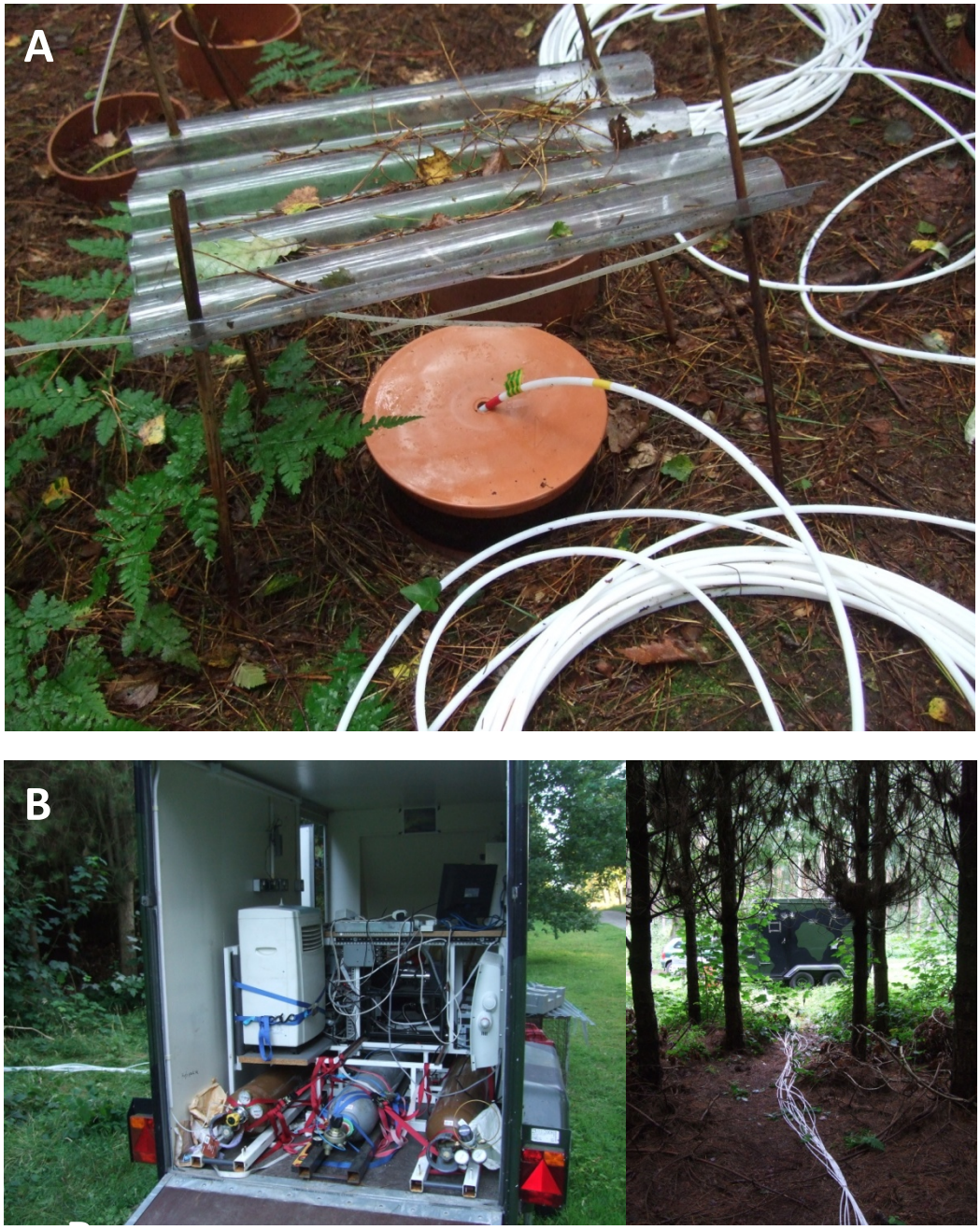

Fig. 5.1 Photographs (A) showing a flow-through chamber positioned on top of a soil collar, secured using a section of tyre inner-tubing (B) the mobile laboratory containing an IRMS in position at Wheldrake Forest, with treatment lines running out to the treatment collars.

also greatly reduced the heterogeneity in CO_2 fluxes whilst fully maintaining the chemistry and quantity of throughfall inputs.

Following a hand-held survey of soil CO_2 efflux using an Li-8100 infrared gas analyser (IRGA) with a 20 cm survey chamber (Li-Cor Bioscience, Lincoln, NE, USA) on 25th November 2008, replicates of treatments RMS, MS and S were ranked, and the ranked collars were split into four sets of pairs. Randomly within each pair, one of the collars was assigned for use in the current study. This left four replicates of each of the three treatments: RMS (surface collars, nothing excluded) MS (deep collars, 41 µm mesh windows, roots excluded) and S (deep collars, 1 µm mesh windows, roots and mycorrhizal hyphae excluded).

There was an expected significant initial difference between the three soil treatments in terms of soil CO_2 efflux, measured during the hand-held survey on 25th November 2008 (Friedman's non parametric two-way ANOVA, $F= 12.84$, $p<0.007$), with the greatest soil CO_2 efflux measured from treatment RMS, followed by treatments MS and S. This indicated successful exclusion of the various soil components, and confirmed the in-growth of ECM hyphae into the treatment MS collars.

Flow-through chambers (20 cm diameter, 10 cm height) were placed on top of the 12 soil collars, and a tight seal achieved using a section of tyre inner tubing (see Fig. 5.1 A). Following the method of Subke *et al*. (2009), soil CO_2 efflux (R_s) from the treatment collars and its isotopic composition ($\delta^{13}C$) were measured directly using a field-deployed mobile laboratory containing an isotope ratio mass spectrometer (IRMS) (see Fig. 5.1 B), linked to a specially developed continuous flow gas sampling interface, drawing air through up to 15 measurement lines, and from a reference cylinder at a constant rate of 50 ml min^{-1} (see Fig. 5.1; for further information, see Subke *et al.,* 2009). In addition to the 12 sample lines running to treatment collars, two sample lines were allocated to two new surface collars (of the same type as the treatment RMS collars, see section 2.2.3), deployed at the start of the experiment in order to gain a measurement of the natural abundance of ^{13}C in soil CO_2 efflux (referred to here as NS control collars). A further line was positioned near to the gas intake point on the flow-through chambers in order to provide measurements of the concentrations and $\delta^{13}C$

values of CO_2 in ambient air (referred to here as the Air Line). Soil CO_2 efflux and its isotopic composition ($\delta^{13}C$) was measured from each soil collar on an hourly basis, from 11th until 21st December 2008. The flux of $^{13}CO_2$ was used to estimate the microbial utilization of the ^{13}C labelled glucose in the different treatments.

^{13}C-labelled glucose solution (300 cm^3, 5 g l^{-1} glucose, 60 mg l^{-1} ^{13}C-labelled glucose (99 atom %), made using throughfall as the solvent, was added to the four replicate treatment collars of treatments RMS, MS and S between 15:00 and 16:00 hrs on 12th December 2008. The amount of throughfall used to make the solution was based on the average amount collected over the plot for the week prior, corrected for the collar surface area. To avoid any osmotic effects, the concentration of glucose added was based on the amount normally included in growth media (Marx, 1969), considered optimal for microbial growth. The NS control collars received 300 cm^3 of throughfall without glucose, whilst the 12 remaining collars which were not included in the current experiment received 300 $cm^3$of glucose solution without the isotopic label.

5.2.3 Simple and complex substrate decomposition experiment

For a full list of substrates used in the experiment, and the treatment coding system, see Table 5.1. The total amount and 'dilution' ratio with unlabelled substrate for each of the substrates added was carefully planned so that both identical amounts of total C and ^{13}C were added to each of the experimental cores.

5.2.3.1 Preparation of ^{13}C-labelled fungal cell wall material

Mycelium of the generalist ECM basidiomycete *Paxillus involutus* (Batsch) Fr. (Isolate MAI) was grown on agar in Petri dishes containing ¼ strength modified MMN medium (Marx, 1969; for full list of ingredients, see Appendix 2), incubated at 25˚C until the mycelia covered the surface of the agar. Small 1 x 1 cm plugs of agar were then aseptically transferred to 500 cm^3conical flasks, each containing 250 cm^3 of ¼ strength modified MMN liquid media. For one third of the flasks, 10% of the ^{12}C glucose was replaced with 99 atom % ^{13}C-labelled glucose (Cambridge Isotope Laboratories, Inc., Andover, USA), without the malt extract so that the glucose was the only C source in

Table 5.1 Summary of ten treatments created using PVC soil collars, (30 cm hammered to a depth of 25 ± 2 cm) and the addition of ^{13}C-labelled substrates. Each treatment had 3 replicates.

Treatment code	Substrate	Mesh gauge	Soil collar treatment
GLS	^{13}C-labelled glucose	1 µm	Roots and ECM hyphae excluded
LYS	^{13}C-labelled lysine	1 µm	Roots and ECM hyphae excluded
CES	^{13}C-labelled cellulose	1 µm	Roots and ECM hyphae excluded
FUS	^{13}C-labelled fungal cell wall material	1 µm	Roots and ECM hyphae excluded
NSS	Control (no substrate)	1 µm	Roots and ECM hyphae excluded
GLM	^{13}C-labelled glucose	41 µm	Roots excluded
LYM	^{13}C-labelled lysine	41 µm	Roots excluded
CEM	^{13}C-labelled cellulose	41 µm	Roots excluded
FUM	^{13}C-labelled fungal cell wall material	41 µm	Roots excluded
NSM	Control (no substrate)	41 µm	Roots excluded

the media. The flasks were incubated at ambient room temperature and left to rock gently until the mycelia had grown to fill the flasks.

Following this, the mycelia were removed from the flasks, and cut away from any of the original plug that was remaining before being frozen at -20 ˚C for several days. The fungal cell wall material was then extracted following the method of Moreno *et al*. (1969), where the frozen fungus was crushed 25 times with a manual French press. Samples of the crushed fungus were then suspended individually in approximately ten times their volume of deionised water before treatment in a sonicator for 20 minutes. The crude cell walls were isolated by centrifugation for 10 minutes at 3000 rcm (Metafuge 1.0, Heraeus Sepatech), and the supernatant solution was removed and replaced with deionised water; this process was repeated until the supernatant solution became clear. The pellet containing the cell wall material was then freeze-dried and stored in a dessicator until prior to use when it was hand-ground using a pestle and mortar to a fine powder.

5.2.3.2 Preparation of ^{13}C-labelled cellulose

^{13}C cellulose was extracted from wheat straw using the method of Sun *et al*. (2004) which had been grown under a $^{13}CO_2$ enriched atmosphere (^{13}C atom enrichment of 10%, δ^{13}C of 8890‰). To remove waxes, a soxhlet extraction was carried out using 200 cm^3toluene and 100 cm^3 of ethanol (for c. 7g of cellulose). The residue was then thoroughly rinsed with deionised water in cellulose-free thimbles. Lignin was removed using acid digestion, with a mixing ratio of 30:60:10, formic acid: acetic acid: water, including the addition of 0.1% HCl, carried out for 4 hours at 85 ˚C. The residue was then washed with deionised water and ethanol, and dried in an oven at 60˚C for 24 hours before being stored in a dessicator prior to use.

5.2.3.3 Preparing substrates for addition to the soil collars

The C content of the fungal cell wall material was estimated at 40.1 % (based on the % C content of glucosamine), consistent with assumptions made in previous studies (Ek, 1997). Based on this assumption, the amount of fungal cell wall material C available for

addition to each of the three FUS (see Table 5.1), and each of the three FUM collars, was 0.234 g, with a ^{13}C atom enrichment of 3.35%.

Accordingly, the ^{13}C-labelled glucose was diluted with ^{12}C glucose (D-glucose anhydrous, Fisher Scientific Ltd, Loughborough, UK) whilst the ^{13}C-labelled lysine (99 atom %, Cambridge Isotope Laboratories, Inc., Andover, USA) was appropriately diluted with ^{12}C-lysine (L-Lysine dihydrochloride, Acros Organics, Geel, Belgium). The ^{13}C-labelled cellulose was diluted with ^{12}C-cellulose made under identical conditions to the labelled material but never exposed to $^{13}CO_2$ (see section 5.2.3.2). The most limited material was the extracted fungal cell walls and all substrates were diluted to match the amount of cell wall material added to the collars in terms of C content and atom enrichment.

5.2.3.4 Experimental treatment

Using the same technique as previously described in Section 2.2.2, PVC tubing (20 cm diameter, Plumb Centre, Wolseley, UK) was cut to 30 cm lengths to create 40 soil collars. Four windows were cut (width 6 cm, height 4 cm, situated 5 cm from the top of the collar and evenly spaced) and covered with one of two meshes (Normesh Ltd., Oldham, UK) of different gauge, to create two treatments (following the design of Heinemeyer *et al.,* 2007). The cores were inserted to a depth which positioned the mesh windows to just below the soil surface. Treatment S (soil only) was created using 1 µm mesh, and excluded roots and ECM hyphae; treatment MS (mycorrhizas + soil) used 41 µm mesh, excluding roots but permitting ECM hyphal access (see Fig. 2.1).

Forty-four shallow collars (20 cm diameter) were randomly assigned positions in the *Pinus contorta* stand on 8th September 2011**,** with potential collar locations being rejected if the distance from the nearest tree was less than 0.5 m or greater than 2 m, collars were spaced at least 1 m apart . Measurements of background CO_2 efflux were conducted using a Li-8100 infrared gas analyser (IRGA) with a 20 cm survey chamber (Li-Cor Bioscience, Lincoln, NE, USA).

Based on these initial CO_2 flux measurements, the locations were ranked, and this ranking used to assign each collar to a specific pair, in a randomised design. Any

collars with outlying CO_2 flux rates were excluded from the experiment. The 40 collars were hammered 25 cm ± 2 cm into the soil in the *Pinus contorta* stand on 19th September 2011, cutting through the main concentration of roots. Two weeks after collar installation, small PVC roofs were erected above the 24 soil collars, and weekly additions of average amounts of collected throughfall commenced.

Following a hand-held survey of soil CO_2 efflux on 3rd August 2012, when there was a significant difference between the treatment MS and S soil collars ($P<0.0258$, Wilcoxon signed-rank test), the pairs of MS and S collars were ranked, according to the magnitude of the MS flux. The two pairs with the highest MS fluxes, and the three pairs with the lowest MS fluxes were excluded, to leave 30 collars (15 pairs). Within each of the three new blocks, each containing five pairs of MS and S collars, the pairs were randomly assigned to one of five substrate treatments (see Table 5.1)

On 22nd August 2012, the mobile IRMS laboratory was established at the experimental site, and a series of measurements began. The three blocks, each of which contained one replicate from each of the 10 substrate treatments described in Table 5.1, were measured in series with one 'run' of the mobile laboratory in the morning, one in the early afternoon and one in the evening. Prior to each run of the mobile laboratory, flow-through chambers were deployed as described for the ^{13}C-labelled glucose decomposition experiment (see Section 5.2.2.1) with one sample line dedicated to calibration by drawing air from the reference gas cylinder of known CO_2 concentration and $\delta^{13}CO_2$ value. For this experiment, a further two analytical lines were positioned near to the gas intake point on the flow-through chambers to provide measurements of the concentration and $\delta^{13}C$ of CO_2 in ambient air (referred to here as the Air Lines). For each run of the mobile lab, a minimum of four hourly cycles were carried out with the data from the first measurement cycle being discarded. As previously, the flow rate of gas through all the sample lines was controlled at 50 cm^3 min^{-1}.

Between 10:00 hrs and 11:00 hrs on 23rd August 2012, the four isotopically labelled substrates were applied as dry powder to the three replicates of each of the treatments shown in Table 5.1, and 200 cm^3 of deionised water was applied to wash

the substrates gently into the litter later. The NS control collars received 200 cm^3 of deionised water, but without any added substrate.

On 29th August 2012, a slightly different measurement strategy was followed with all the replicates from treatments GLS, GLM, LYS and LYM measured during one run of the mobile laboratory, all the replicates from treatments CES, CEM, FUS and FUM measured in a second run, and all the replicates of treatments NSS, NSM, FUS and FUM measured in a final run. This was done in order to enable direct comparisons between the MS and S collar treatments for each of the substrates, whilst reducing the error associated with spreading the three replicates for each of the treatments out over each measurement day. The mobile laboratory ran from 22nd August 2012 to 2nd September 2012, when power supply difficulties stopped the measurements. The 'return' flux of ^{13}C as $^{13}CO_2$ was used to estimate the microbial utilization of the substrates in the different treatments.

5.2.4 Data analysis

5.2.4.1 Calculating soil CO_2 efflux, and its isotopic composition ($\delta^{13}C$)

Following the method of Subke *et al*. (2009), the concentration of CO_2 measured in the flow-through chamber headspace air was used to calculate the soil CO_2 efflux (F) for each soil collar, for each measurement cycle of the mobile laboratory (see Eq. 5.1). This calculation was identical for the data obtained from both the initial ^{13}C-labelled glucose decomposition experiment, and the later ^{13}C-labelled simple and complex substrate decomposition experiment.

$$\text{Soil } CO_2 \text{ flux (F)} = \left(\frac{C_{sample} - C_{air} \text{ x flow}}{A}\right) \qquad \text{(Eq. 5.1)}$$

Where C_{sample} is the concentration of CO_2 (($\mu l\ l^{-1}$) in the chamber headspace air, C_{air} is the concentration of CO_2 ($\mu l\ l^{-1}$) entering the flow-through chambers in the ambient forest air (an average of the concentrations measured from the two Air Lines), flow is

the flow rate of air through the chamber (cm^3 min), and A is the area of soil covered by the soil collar. The isotope ratio ($\delta^{13}C$) of soil CO_2 efflux was calculated for each soil collar, for each measurement cycle using a two-source mixing model (see Eq. 5.2).

$$\delta_{Soil} = \left(\frac{\left(\delta_{Sample} C_{Sample} - \delta_{Air} C_{Air} \right)}{C_{Sample} - C_{Air}} \right) \quad \text{(Eq. 5.2)}$$

:

Where C is the CO_2 concentration, and δ is the $^{13}C/^{12}C$ isotope ratio of the gas. The suffix 'Soil' refers to CO_2 derived from the soil, the suffix 'Sample' refers to the CO_2 measured from the chamber headspace air, and the suffix 'Air' refers to the CO_2 entering the flow through chamber (an average of the two Air Lines).

5.2.4.2 Statistical analysis

Both experiments used a randomised block design, with a variety of statistical approaches used during data analysis. Where possible, parametric analyses were carried out but generally sample distributions deviated from normal so data were transformed where possible or the equivalent non-parametric tests were carried out, usually Friedman's non-parametric two-way ANOVA with *post-hoc* analyses using Duncan's multiple range test (see Section 2.3.2 for a description of how the data were averaged to avoid pseudo replication). Further specific information regarding the use of different statistical tests is detailed in the Results section. All statistical tests were carried out using SAS software package v. 9.3 (SAS Institute Inc., Cary, NC, USA).

5.3 Results

5.3.1 ^{13}C-labelled glucose decomposition experiment

5.3.1.1 Soil CO_2 flux

Prior to the addition of ^{13}C-labelled glucose, as expected, there was a consistently higher concentration of CO_2 in the headspace air leaving the chambers than in the incoming air (see Fig. 5.2). There was also a larger soil-derived CO_2 flux (see Fig. 5.3) from treatment S collars, than from treatment RMS and MS collars. In order to view these data more clearly, Fig. 5.4 displays the same data as Fig. 5.3, but with the error bars removed. A Friedman's non parametric one-way ANOVA was performed on the of the cumulative soil CO_2 flux data, and demonstrated a highly significant difference between the treatments in terms of the cumulative soil CO_2 flux calculated for each collar during the period prior to substrate addition ($F = 29.64$, $p<0.0001$). A *post-hoc* analysis showed that all treatments were significantly different (see Fig. 5.5).

As described in Section 5.2.2.1, two NS (no substrate) control collars were placed on the soil surface when the flow-through chambers were established. Therefore, the soil inside the NS control collars had not received the same treatment in terms of the exclusion of incident throughfall, and the weekly watering regime which the treatment RMS, MS and S collars had been subject to for a period of six months prior to the start of the current experiment. This treatment difference is reflected in the particularly low cumulative soil CO_2 flux from the NS control collars when compared to the other treatments (see Fig. 5.5).

Following the addition of ^{13}C-labelled glucose, between 15:00 and 16:00 hrs on 12th December 2008, an increase in CO_2 flux was quickly evident from all collars (see Figs. 5.3 and 5.4). Once started, this rise in respiration occurred at a similar rate for all three treatments, but began particularly quickly in treatment S, two hours after substrate addition. Though there was a transient rise in soil CO_2 flux measured from treatment RMS collars three hours after substrate addition, the response from both the RMS and MS collars started to occur approximately 11 hours after the addition of the ^{13}C-labelled glucose. CO_2 flux peaked at a much greater level in treatment S collars, and

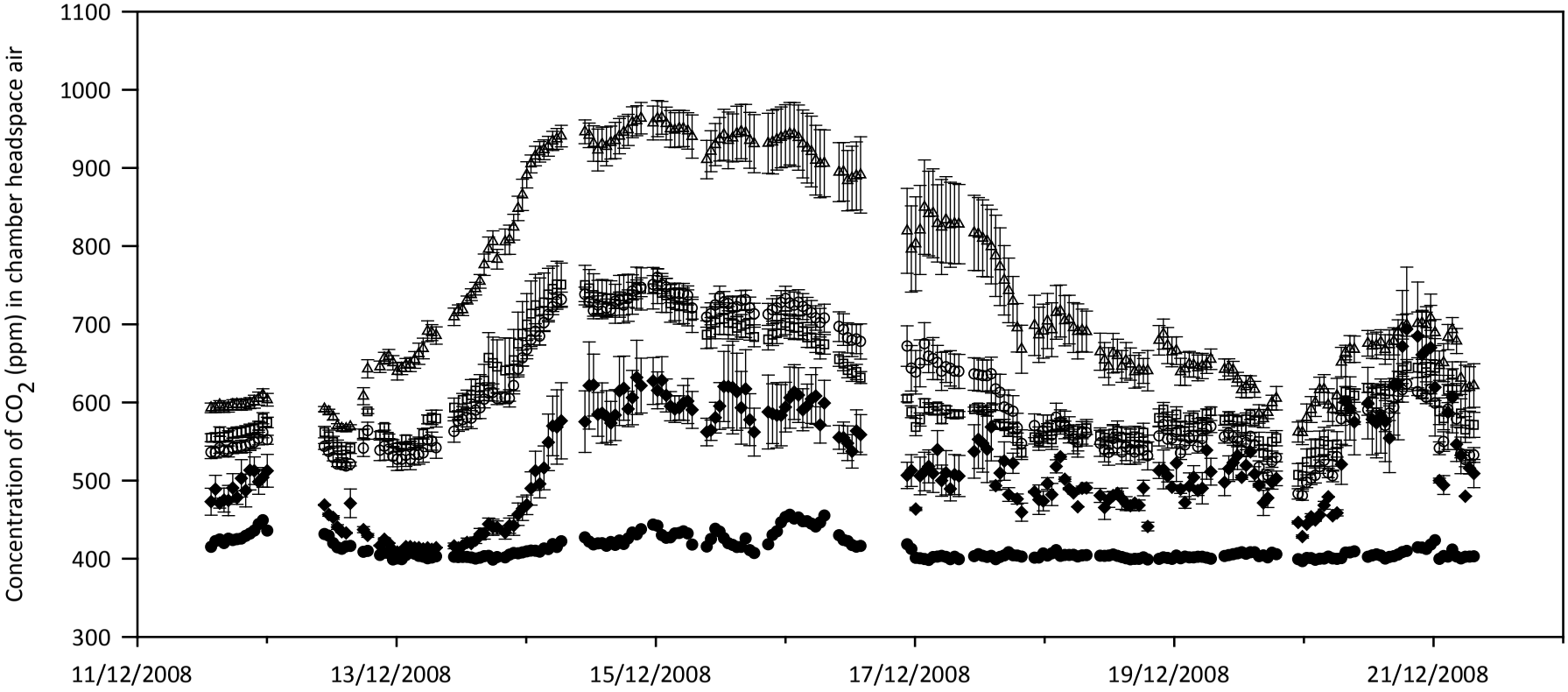

Fig. 5.2 CO_2 concentration (ppm) of the chamber headspace air measured over time, with the addition of ^{13}C-labelled glucose between 15:00 and 16:00 hrs on 12/12/2008. Treatments are RMS (□), MS (○) and S (Δ), error bars represent ± 1 SE (n = 4) NS collars are represented by solid diamonds, and error terms are the range, with n = 2. Incoming ambient air, measured from the Air Line is represented by solid circles, n = 1.

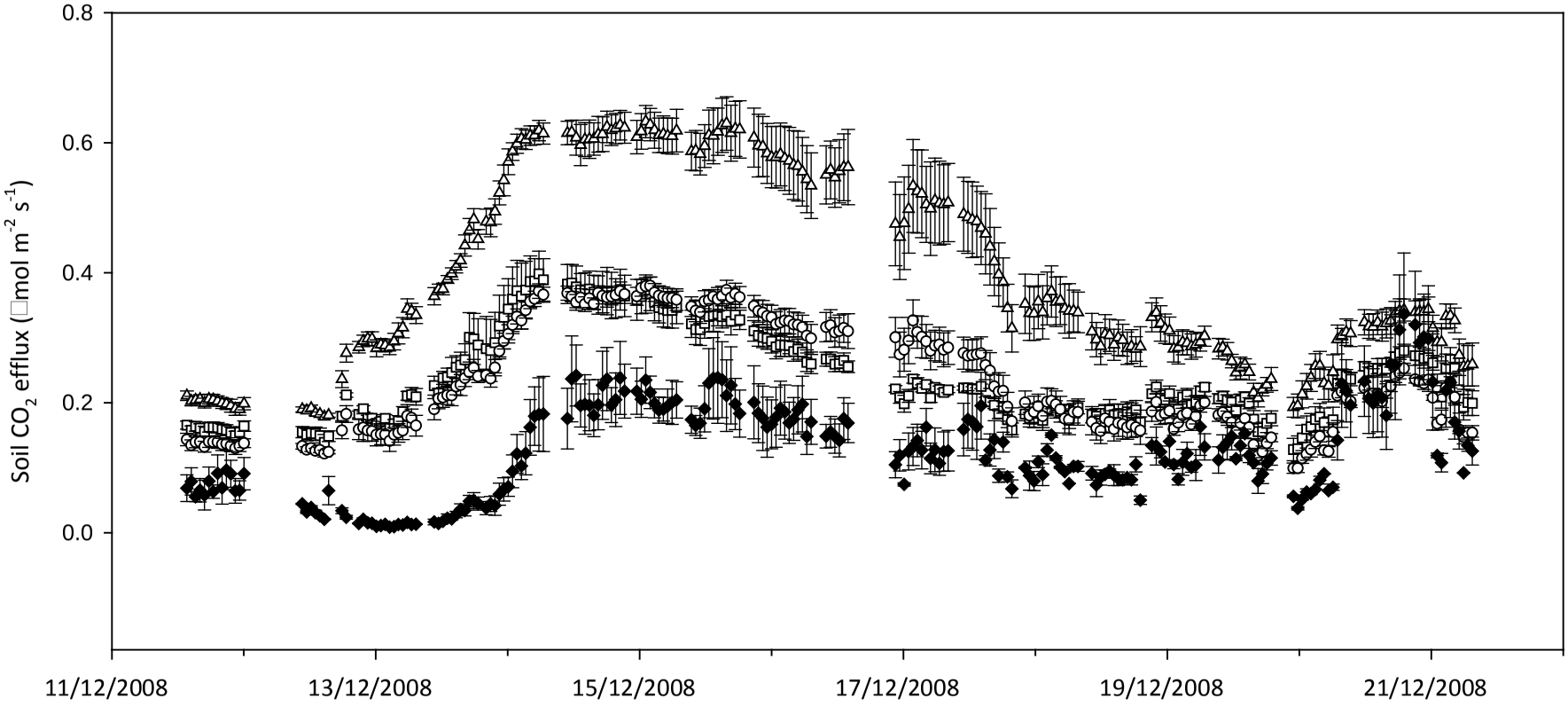

Fig. 5.3 Soil CO_2 efflux (µmol m^{-2} s^{-1}) measured over time, with the addition of ^{13}C-labelled glucose between 15:00 and 16:00 hrs on 12/12/2008. Treatments are RMS (□), MS (○) and S (Δ), error bars represent ± 1 SE (n = 4) NS collars are represented by solid diamonds, and error terms are the range with n = 2.

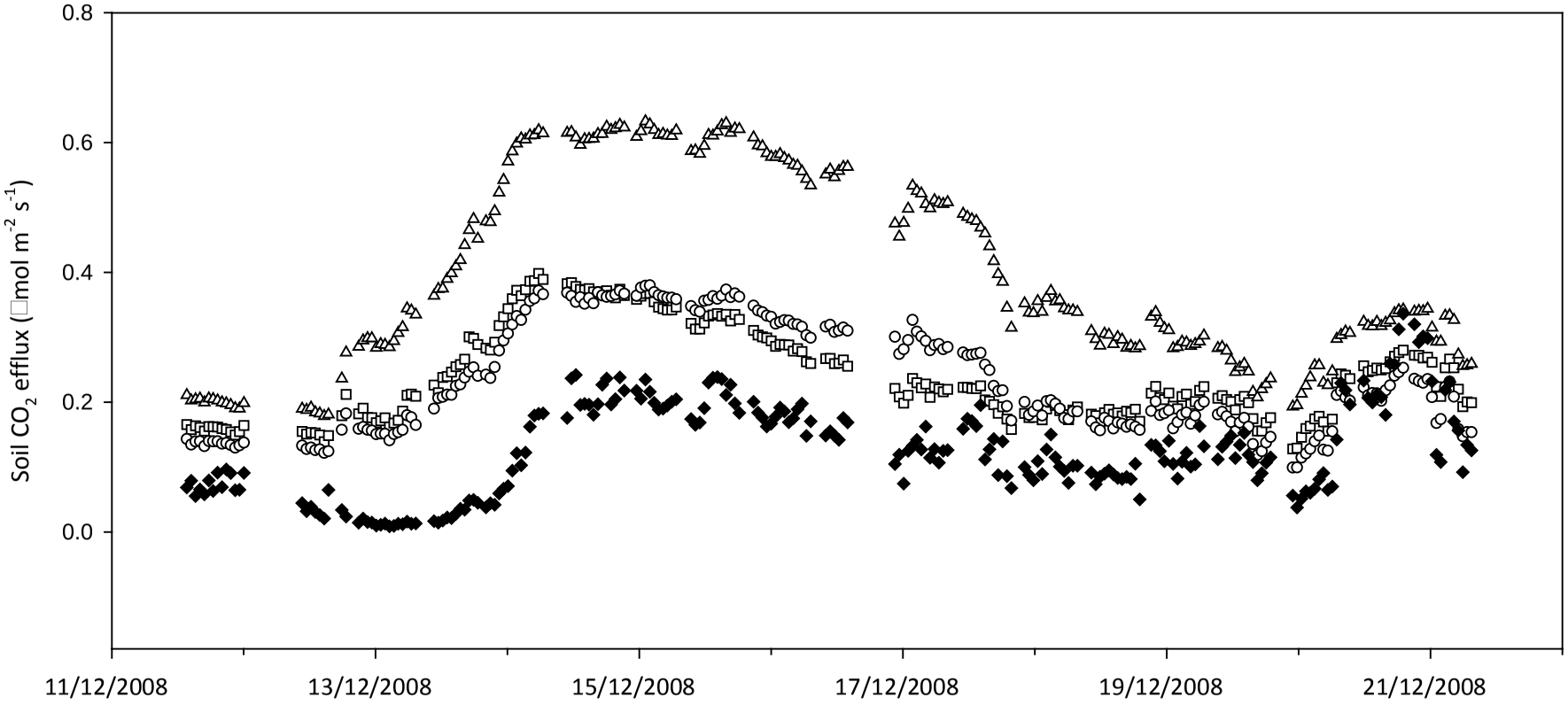

Fig. 5.4 Soil CO_2 efflux (µmol m^{-2} s^{-1}) measured over time, with the addition of ^{13}C-labelled glucose between 15:00 and 16:00 hrs on 12/12/2008. Treatments are RMS (□), MS (○) and S (Δ), (n = 4). NS control collars are represented by solid diamonds, (n = 2). Error bars have been removed to allow the data points to be viewed more clearly.

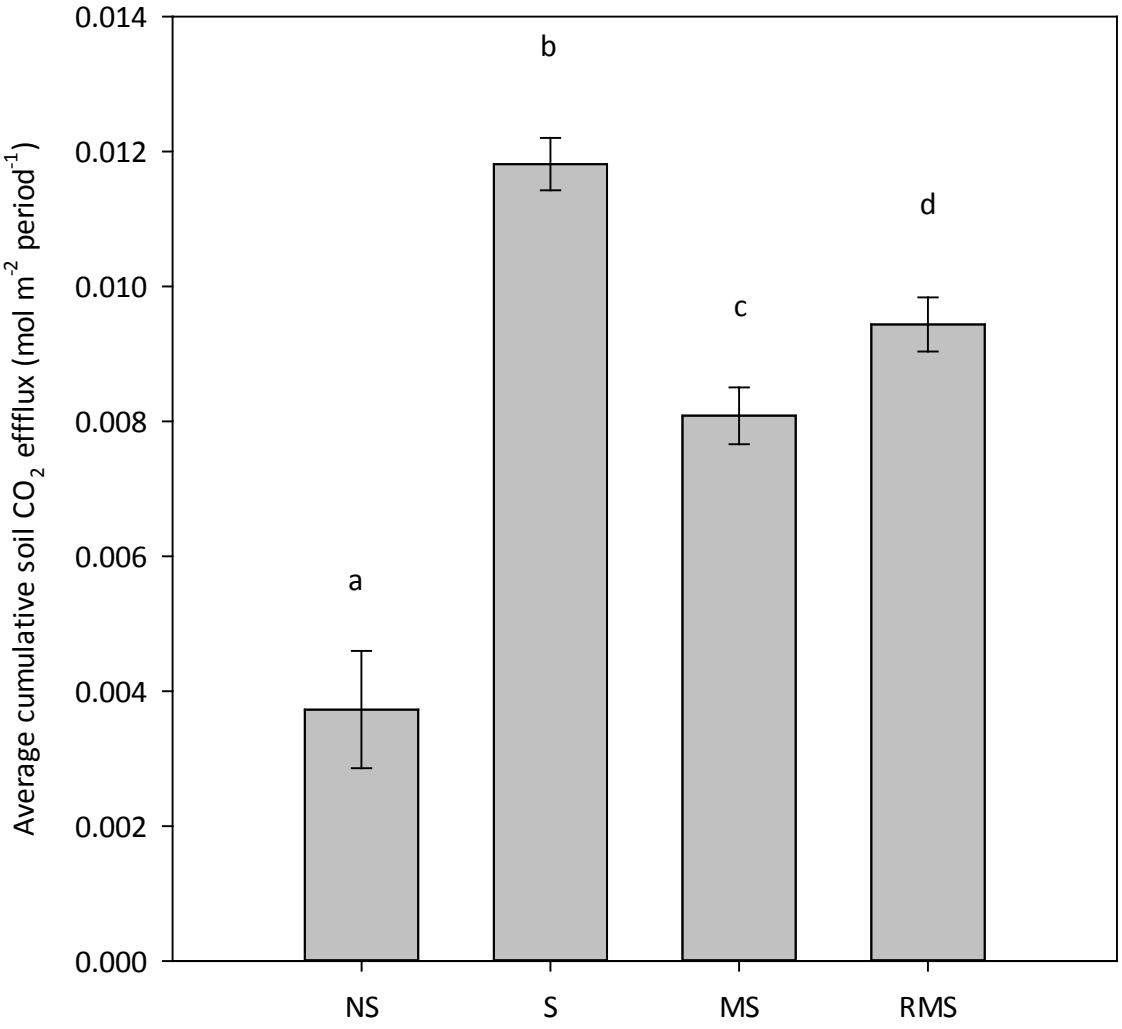

Fig. 5.5 Average cumulative soil CO_2 efflux (mol m^{-2} $period^{-1}$) during the period following the installation of flow-through chambers, prior to the addition of ^{13}C-labelled glucose (a period of 16 hours). Treatments which differ significantly have different letters. For treatments S, MS and RMS, error bars represent ± 1 SE (n = 4). For the control (NS) collars and error terms are the range, with n = 2.

was sustained for longer than in treatment MS and RMS collars, though this was also contributed to by treatment S collars having an initial higher background soil CO_2 flux prior to substrate addition (see Figs 5.3 and 5.4). Friedman's two-way ANOVA, with treatment and block as factors, revealed a highly significant difference between the treatments (NS control collars were excluded from this analysis as they were not part of the original block structure) in terms of their cumulative soil CO_2 flux following substrate addition ($F= 13$, $p<0.007$). *Post-hoc* analysis showed that treatment S collars had a significantly higher average cumulative CO_2 flux than treatments MS and RMS, which did not differ significantly (see Fig. 5.6).

As there was a significant difference between the treatments in terms of the cumulative Soil CO_2 flux, it justified to carry out repeated Friedman's two-way ANOVA at each time point to see whether there were occasions when treatments MS and RMS differed significantly. These analyses revealed that for a short period of time following 16th December 2008, the soil CO_2 flux was greater from treatment MS than treatment RMS (see Figs. 5.3 and 5.4).

There was a second peak in soil CO_2 flux, which began on 20th December 2008 and occurred in all treatments, including the NS control collars. It is probable therefore, that this response in the CO_2 flux was not caused by substrate addition, but by background changes in soil temperature (see Figs. 5.3 and 5.4). Watering took place on 16.12.2008 between 13:45 and 15:15 hours, so it is unlikely to have contributed to this second peak in flux rate.

5.3.1.2 $\delta^{13}C$ soil CO_2 flux

The $\delta^{13}C$ of soil CO_2 flux over time is displayed in Fig. 5.7. Again, in order to make viewing the data easier, Fig. 5.8 shows the same data as Fig. 5.7, but with the error bars removed. As there was no significant difference in the average $\delta^{13}C$ of soil CO_2 flux between treatments RMS, MS, S and the NS control collars prior to substrate addition ($F=2.29$, $p>0.14$), any rise in the $\delta^{13}C$ from the treatments above that of the NS control $\delta^{13}C$ must be attributed to utilisation of the substrate. The $\delta^{13}C$ of soil CO_2 flux displayed in Figs. 5.7 and 5.8 matches the data shown in Figs. 5.3 and 5.4, with treatment S having a greater and more sustained peak in flux, though there was not as

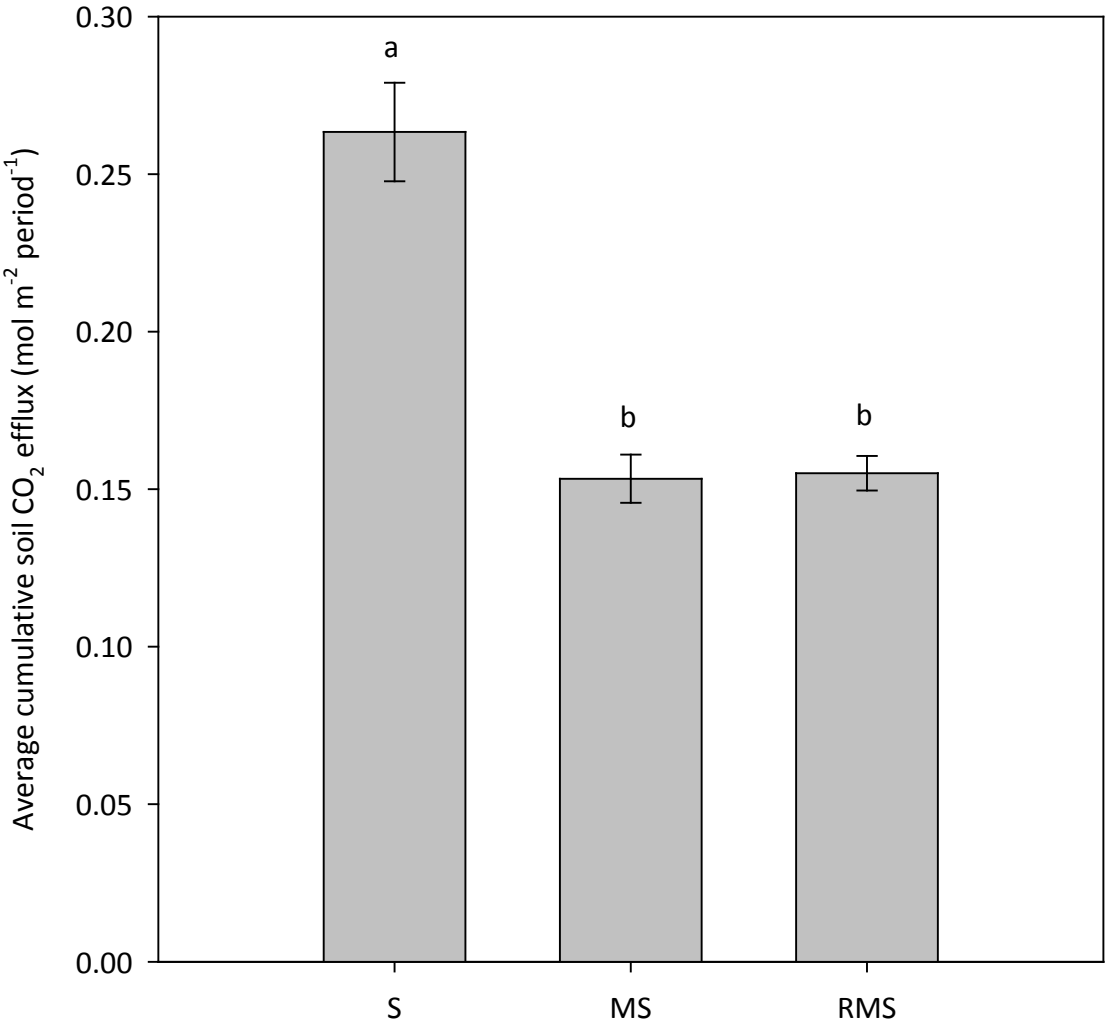

Fig. 5.6 Average cumulative soil CO_2 efflux (mol m^{-2} $period^{-1}$) following the addition of ^{13}C-labelled glucose (a period of 175 hours). Treatments which differ significantly have different letters, error bars represent ± 1 SE (n = 4).

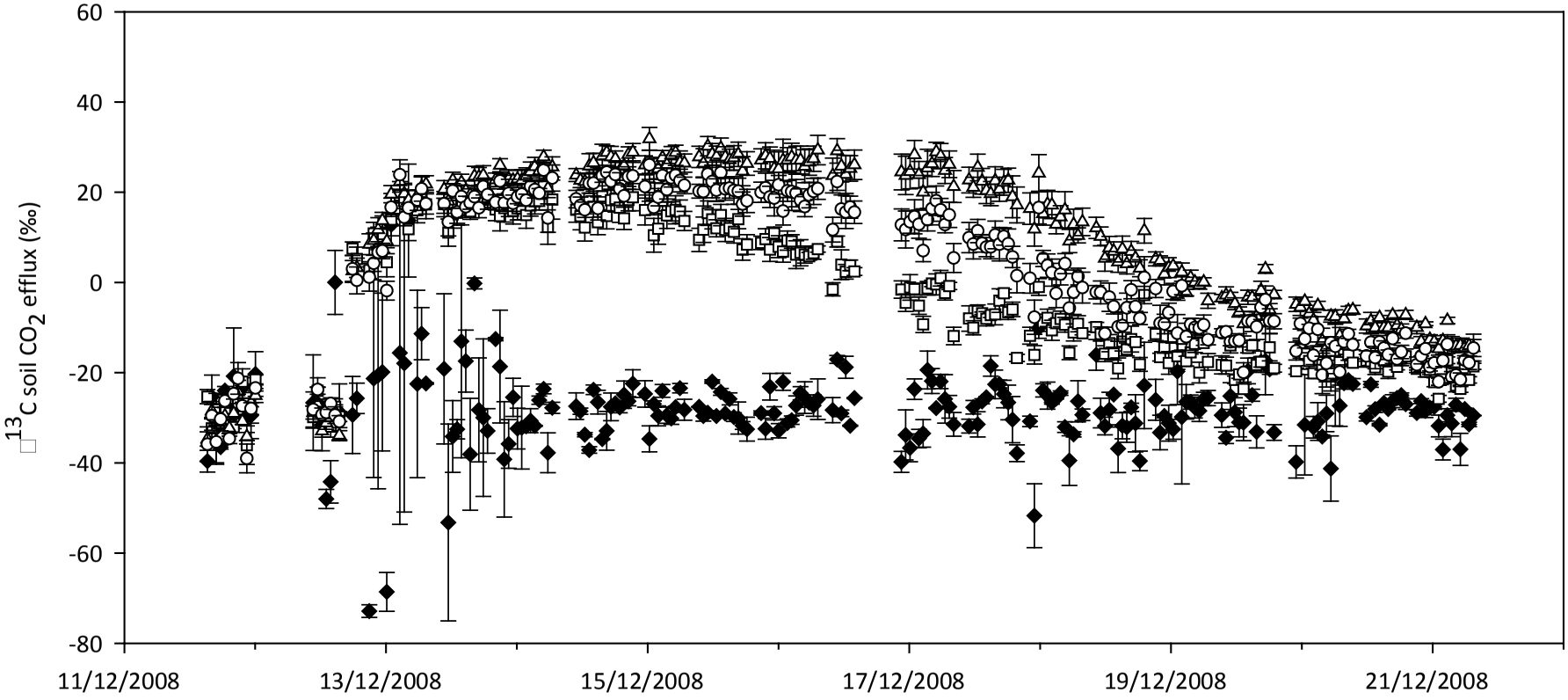

Fig. 5.7 δ ^{13}C soil CO_2 efflux (‰) measured over time, with the addition of ^{13}C-labelled glucose between 15:00 and 16:00 hrs on 12/12/2008. Treatments are RMS (□), MS (○) and S (Δ), error bars represent ± 1 SE (n = 4). NS collars are represented by solid diamonds, and error terms are the range with, n = 2.

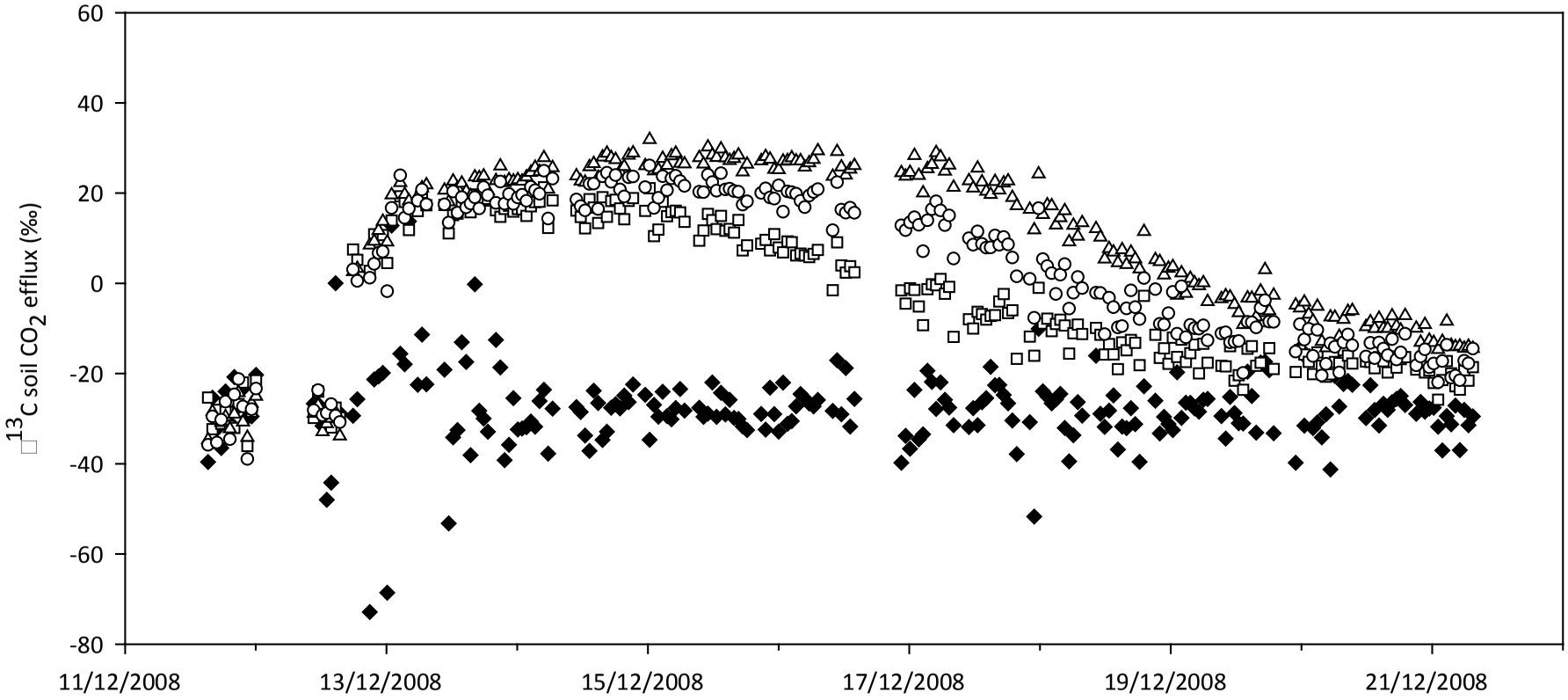

Fig. 5.8 δ ^{13}C soil CO_2 efflux (‰) measured over time, with the addition of ^{13}C-labelled glucose between 15:00 and 16:00 hrs on 12/12/2008. Treatments are RMS (□), MS (○) and S (Δ), error bars represent ± 1 SE (n = 4). NS collars are represented by solid diamonds, error terms are the range, n = 2.

dramatic a difference between the $\delta^{13}C$ of the fluxes from each treatment as there was in the soil CO_2 flux (see Figs. 5.3 and 5.4). This is because the soil CO_2 flux data, also incorporates any pre-existing differences in background soil CO_2 fluxes from the different treatments. As previously mentioned, it was clear that treatment S had a higher soil CO_2 flux than treatments MS and RMS, so this accentuated the treatment S peak in Figs. 5.3 and 5.4, relative to the peak in the $\delta^{13}C$ displayed in Figs. 5.7 and 5.8. The differences between the treatments in terms of the average $\delta^{13}C$ of soil CO_2 flux following substrate addition was highly significant ($F=45.59$, $p<0.0001$) with all treatments significantly different from each other (see Fig. 5.9). The second peak in the soil CO_2 flux shown in Figs. 5.3 and 5.4 is not reflected in Figs. 5.7 and 5.8, again suggesting that this increase was not due to substrate addition.

Following the addition of ^{13}C-labelled glucose, there is a period when the $\delta^{13}C$ of soil CO_2 flux from the NA control collars was very noisy, after which the value settled down and remained in a fairly constant range for the remainder of the experiment (see Figs. 5.7 and 5.8); initial contamination with CO_2 from human breath may have contributed to these unusual values. In order to ensure that subsequent calculations involving the $\delta^{13}C$ of soil CO_2 flux from the NS control collars were not compromised, it was decided that an average $\delta^{13}C$, be calculated over the time period after these initial noisy data, until the end of the experiment. This value was substituted for the raw NS control collar values at each time point following substrate addition and was used to recalculate ^{12}C and ^{13}C concentrations, used in all subsequent analysis.

5.3.1.3 Percentage return of glucose-derived C

In order to estimate the mass of total C, ^{13}C, and ^{12}C released from each collar during the experiment, a simple calculation mass balance was carried out using the total CO_2 concentration measured from the chamber headspace air, and the re-calculated $^{13}CO_2$ and $^{12}CO_2$ concentrations. By working out the cumulative mg C, ^{12}C, and ^{13}C measured, then subtracting the cumulative mg of C from the Air Line, and the average cumulative mg C, ^{12}C and ^{13}C from the NA control collars, an estimate of the mass of glucose-derived C emitted (including any additional C released from priming of SOM) from the collars during the experiment was obtained (see Fig. 5.10). Friedman's two-way

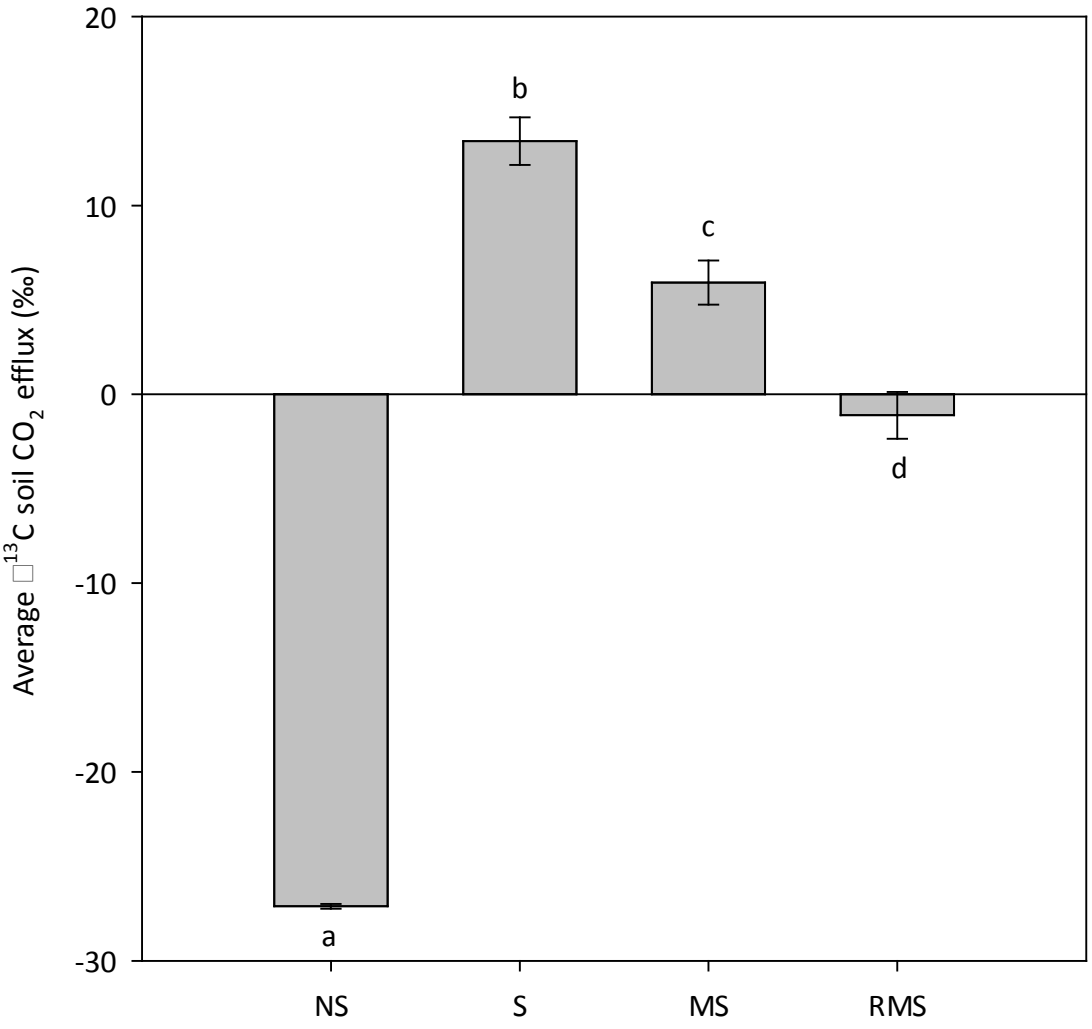

Fig. 5.9 Average $\delta^{13}C$ soil CO_2 efflux (‰) following the addition of ^{13}C-labelled glucose. Treatments which differ significantly have different letters, error bars for treatments S, MS, RMS represent ± 1 SE (n = 4), error terms for NS control collars represent the range, with n = 2.

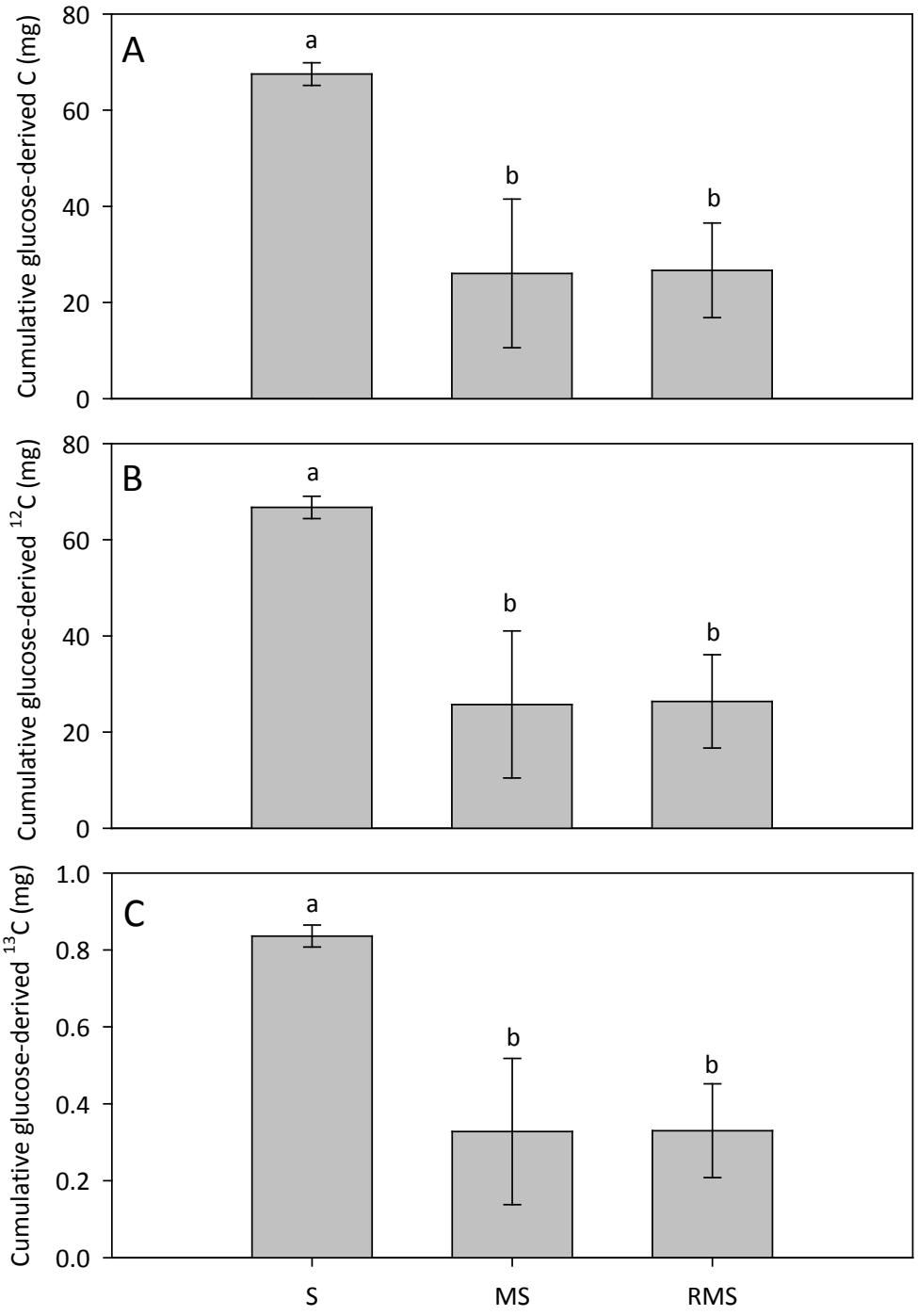

Fig. 5.10 Average cumulative additional return as CO_2 of: (A) C, (B) ^{12}C, (C) ^{13}C (mg) from treatment collars over the experimental period (see text for calculation method). Within each chart, treatments which differ significantly have different letters. Error bars represent ± 1 SE (n = 4).

ANOVA, with block and treatment as factors, showed there was a highly significant difference between the treatments in terms of the mass of total C, ^{13}C and ^{12}C released during the experiment ($F=13$, $p<0.007$), with treatment S having a significantly higher release of C than treatments MS and RMS which did not differ significantly in terms of the mass of C released (see Fig. 5.10). The higher % return of glucose-derived C from treatments RMS and MS, estimated using this method, does not support what is shown by the $\delta^{13}C$ of soil CO_2 flux (see Figs. 5.7 and 5.8). One reason for this discrepancy is that the use of the mass of C emitted from the NS control collars as a proxy for the background release of C from the treatment collars does not take into account the inherent differences in soil CO_2 flux between the treatments, and the NS control collars, prior to substrate addition (see Fig. 5.5).

In order to resolve this problem, and to gain a more treatment-specific estimate of the background soil CO_2 being emitted from the treatment collars, two alternative calculation methods were attempted. The first, using the proportional difference between each individual treatment collar, and the NS control collars prior to substrate addition to alter the NS control collar concentration (ppm) at each time point, creating a control for each individual collar, proved unsuccessful. Initially, this seemed the best available option, as it allowed for variation in CO_2 flux driven by environmental factors, such as temperature, to be taken into account (see Figs. 5.3 and 5.4). However, the values obtained using this method for the mass of C released from the 'controls' were higher than the mass from each collar when the substrates had been added.. Clearly, the proportional differences between the NS control collar CO_2 flux and that of the treatment collars prior to substrate addition must have decreased during the experiment. In support of this, Fig. 5.2 shows that initially the difference was large, with the NS collar CO_2 concentration being close to that of the Air Line, but then the concentration appears to increase relative to that of the Air Line, suggesting that it was becoming more similar to the underlying background CO_2 concentration of treatments RMS, MS and S.

A second, alternative calculation method was applied, where the average mass of soil-derived C from each collar prior to substrate addition was subtracted from the mass of soil-derived C after substrate addition to give an estimate of the mass of C returned

following utilisation of the glucose. This method is not ideal, as it does not take into account fluctuations in the background soil C flux due to changing factors such as soil temperature and moisture. However, the results obtained do show that there was a greater mass of C released from treatment MS than treatment RMS, as was shown by the $\delta^{13}C$ of soil CO_2 flux in Figs. 5.7 and 5.8 (see Fig. 5.11). Furthermore, the error bars displayed for each treatment in Fig. 5.11 are much smaller than in Fig. 5.10, suggesting that this method is at least more precise than the use of the NS control collars. The average mass of C (mg) returned per treatment was used, alongside the original mass of total C, ^{12}C and ^{13}C added to estimate the % return of glucose-derived per treatment during this experiment (see Table 5.2). Results of Friedman's two-way ANOVA, with block and treatment as factors, showed there was a highly significant difference between the treatments in terms of the mass of total C, ^{13}C and ^{12}C released during the experiment ($F=13$, $p<0.007$), with treatment S having a significantly higher release of C, and a higher % return of glucose-derived C than treatments MS and RMS. Though the mass of C from treatments MS and RMS calculated using this method seems more reflective of what is displayed in Figs. 5.7 and 5.8, these two treatments did not differ significantly in terms of the mass of C released (see Fig. 5.11 and Table 5.2).

5.3.1.4 Soil moisture surveys

Results of Friedman's non-parametric two way ANOVA comparing the average soil moisture per treatment and block from the three hand-held soil moisture surveys conducted on 5th, 16th and 22nd December 2008 showed that there was a significant difference between the treatments in terms of soil moisture ($F=13$, $p<0.007$), with treatment RMS having a significantly higher average soil % moisture (gravimetric) than treatments MS and S, which did not differ significantly.

5.3.2 Simple and complex ^{13}C-labelled substrate decomposition experiment

5.3.2.1 Soil CO_2 flux

Prior to the establishment of flow-through chambers, a hand-held survey of soil CO_2 flux was carried out (see Section 5.2.3.4) in order to ascertain whether there was a significant difference between the treatment MS and S collars, which would indicate

Table 5.2 The percentage return as CO_2 of glucose-derived C, ^{12}C and ^{13}C from added ^{13}C-labelled glucose for treatments RMS, MS and S, calculated using the soil-derived C from each of the collars prior to the addition of ^{13}C-labelled glucose. Treatments which differ significantly have different letters. Values for treatments are means, with standard errors in parentheses, n=4.

Treatment	% return of glucose-derived C
S	8.7 (0.9)[a]
MS	4.3 (0.3)[b]
RMS	3.5 (0.4)[b]
	% return of glucose-derived ^{12}C
S	8.7 (0.9)[a]
MS	4.3 (0.3)[b]
RMS	3.5 (0.4)[b]
	% return of glucose-derived ^{13}C
S	8.9 (0.9)[a]
MS	4.4 (0.3)[b]
RMS	3.6 (0.4)[b]

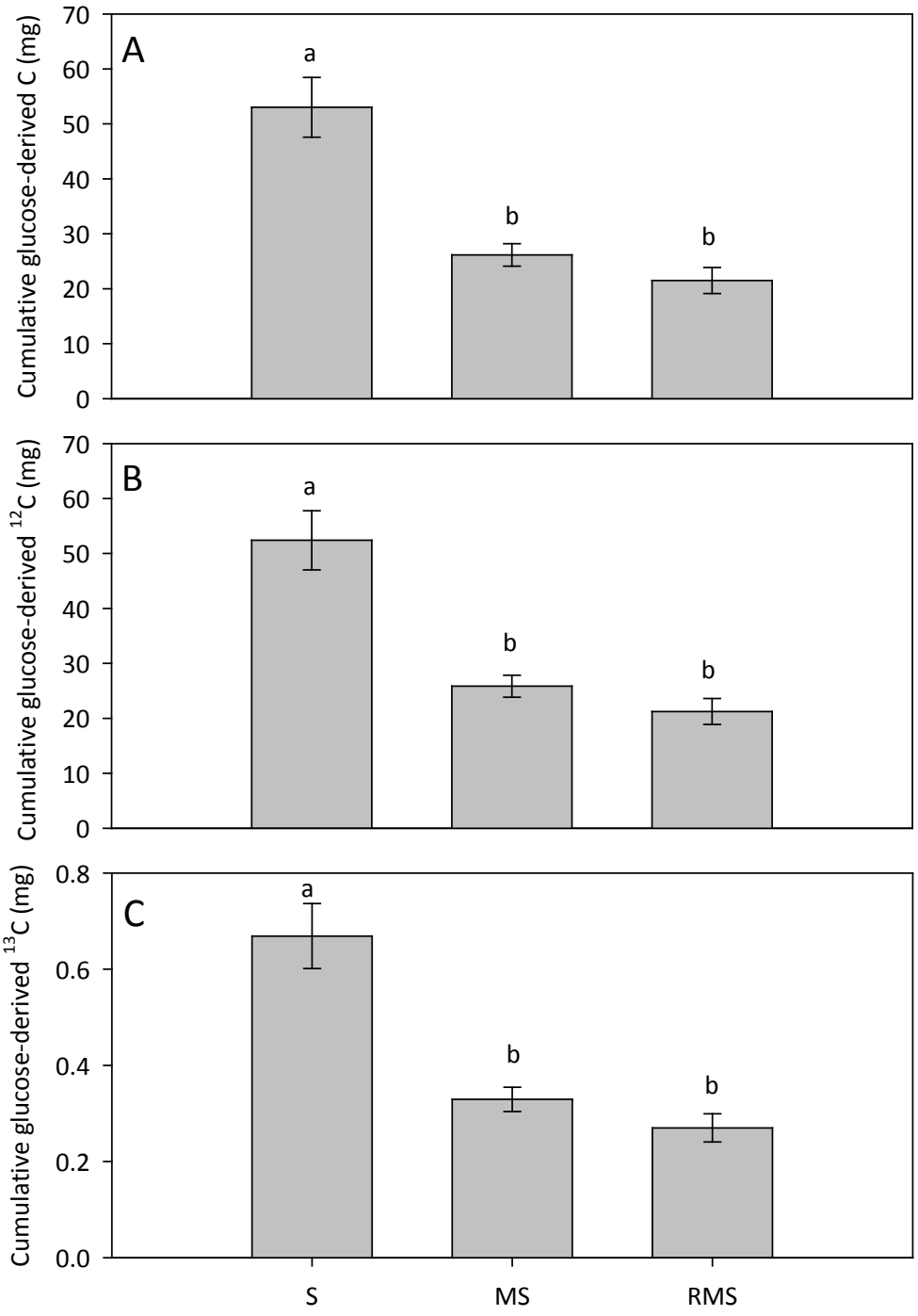

Fig. 5.11 Average cumulative additional return of: (A) C, (B) ^{12}C, (C) ^{13}C (mg) from treatment collars over the experimental period (see text for calculation). Within each chart, treatments which differ significantly have different letters. Error bars represent ± 1 SE (n = 4).

successful ingrowth of ECM hyphae into the MS collars. Analysis using a Wilcoxon signed-rank test showed that there was greater soil CO_2 flux measured from treatment MS collars than from treatment S collars ($p<0.03$). However, during the period following flow-through chamber establishment, prior to the addition of the ^{13}C-labelled substrates, the data recorded using the mobile laboratory indicated that significantly higher values of soil CO_2 flux were obtained from treatment S collars, rather than the treatment MS collars (Friedman's non parametric two-way ANOVA, $F=3.5$, $p<0.02$).

Fig. 5.12 displays the cumulative soil CO_2 fluxes for the MS and S collars prior to substrate addition, calculated according to the substrates they were due to receive on 24th August 2012. This was done to allow later comparison with the cumulative soil CO_2 flux following substrate addition. The highest average soil CO_2 flux prior to substrate addition was from treatment GLS, though there were large differences in the cumulative soil CO_2 flux between the three replicate GLS collars, which meant that the error term was large (see Fig. 5.12). *Post-hoc* analysis showed that the collars which were to make up treatment LYS had a significantly higher soil CO_2 flux than all the other treatments apart from FUS, GLS and NSS, prior to substrate addition. This difference was not reflected by the results of the Li-Cor hand-held survey of soil CO_2 flux which had been conducted previously. When the data were re-analysed from the original Li-Cor survey using a Friedman's two-way ANOVA, separating the collars out according to the substrates they had been assigned to receive, there was a significant difference between the treatments in terms of the average soil CO_2 flux ($p<0.05$). However, *post-hoc* analysis revealed that just three weeks prior to flow through collar establishment, the MS collars consistently had a higher soil CO_2 flux than the S collars, so there was a discrepancy here between the two sets of soil CO_2 flux measurements.

Following the addition of the ^{13}C-labelled substrates on 24th August 2012, the average soil CO_2 flux measured from all treatments that had received substrates increased, whereas the soil CO_2 flux from treatment NSS declined slightly (see Fig. 5.13). In order to view these data more clearly, the various treatments have been separated over the next group of figures. The rise in soil CO_2 flux was particularly pronounced for treatment GLS, with an equally rapid, though less sustained, rise in GLM (see Fig. 5.14),

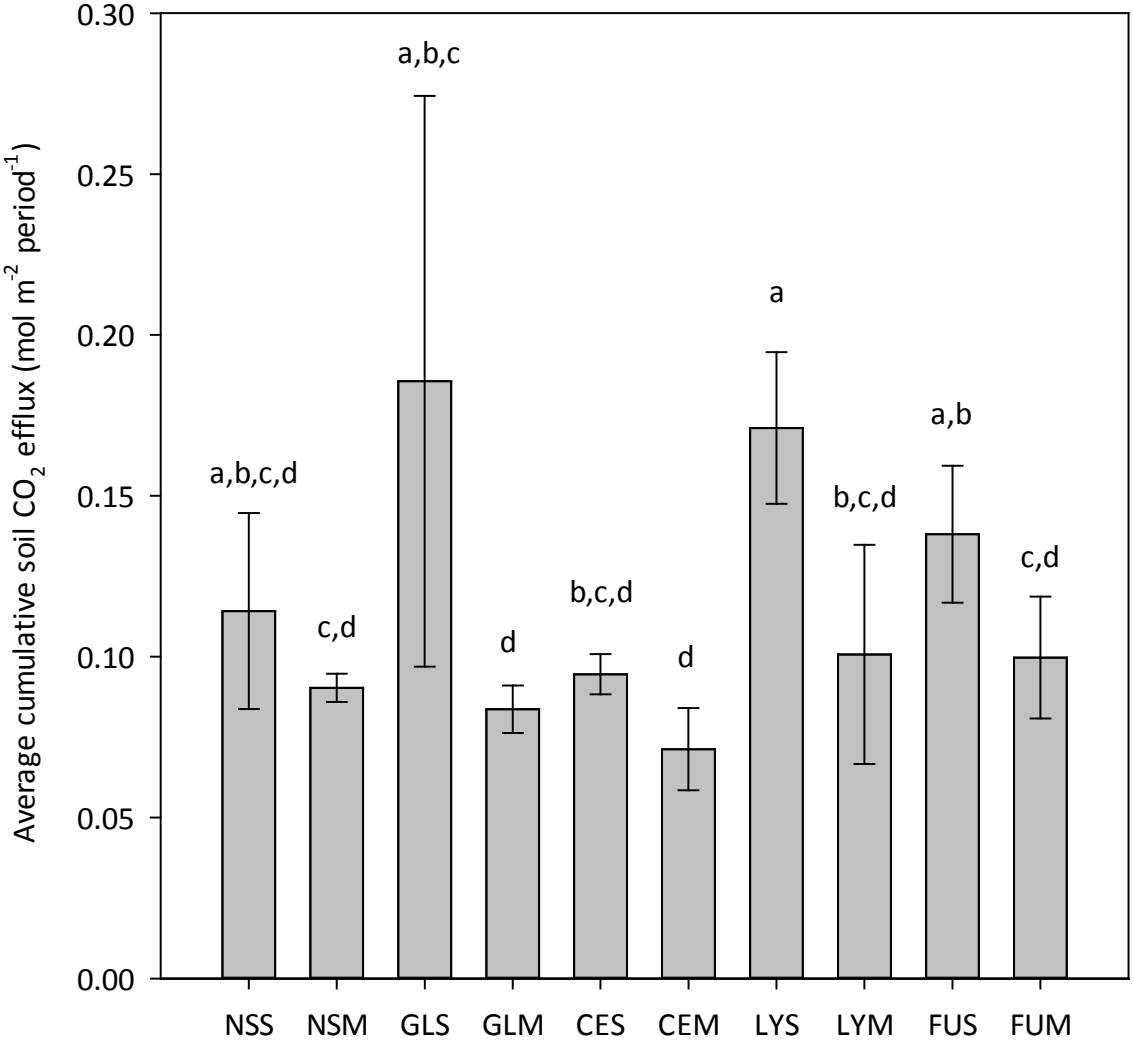

Fig. 5.12 Average cumulative soil CO_2 efflux (mol m^{-2} $period^{-1}$) during the period following the installation of flow-through chambers, prior to the addition of ^{13}C-labelled substrates (a period of 29 hours). Treatments which differ significantly have different letters. Error bars represent ± 1 SE (n = 3).

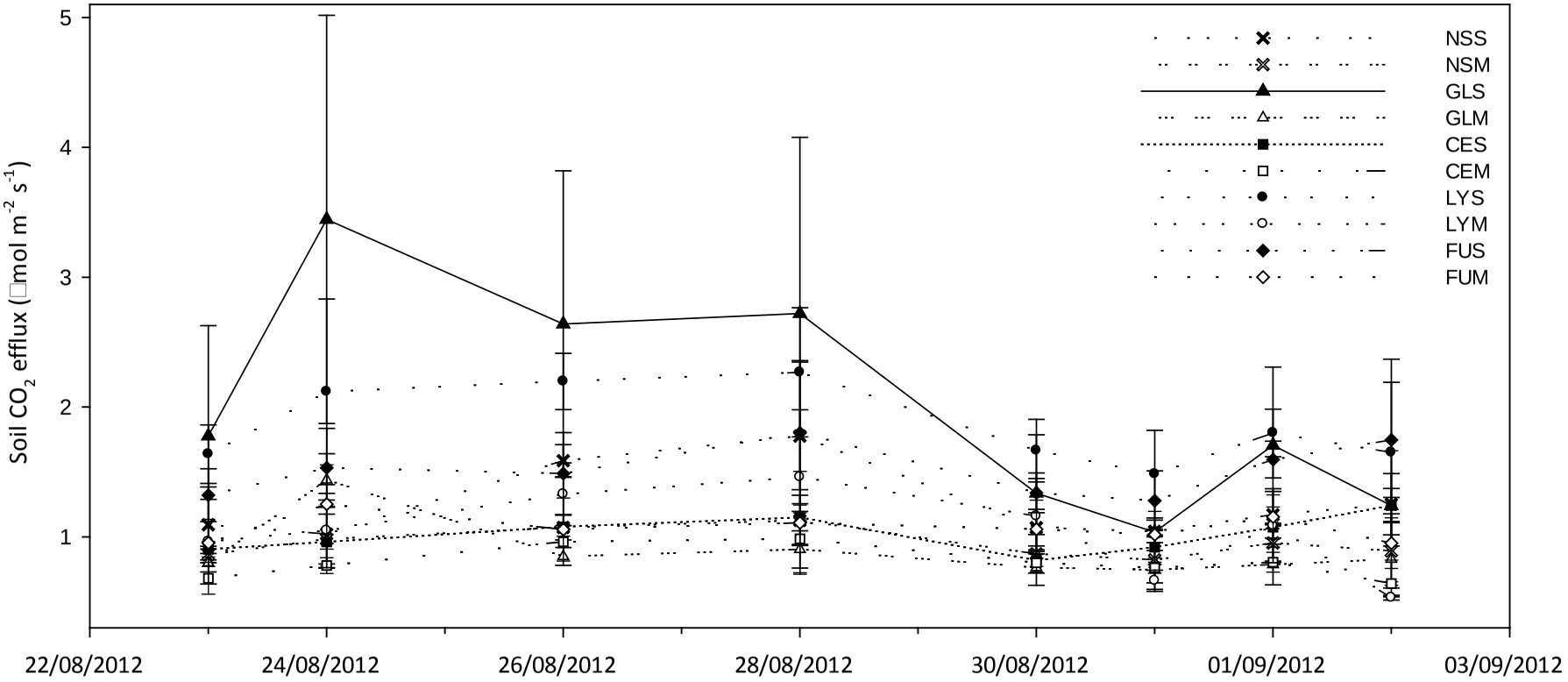

Fig. 5.13 Average soil CO_2 efflux (µmol m^{-2} s^{-1}) measured over time, with the addition of ^{13}C-labelled substrates immediately prior to measurements on 24/08/2012. Error bars represent ± 1 SE (n = 3). Treatment codes are described fully in Table 5.1.

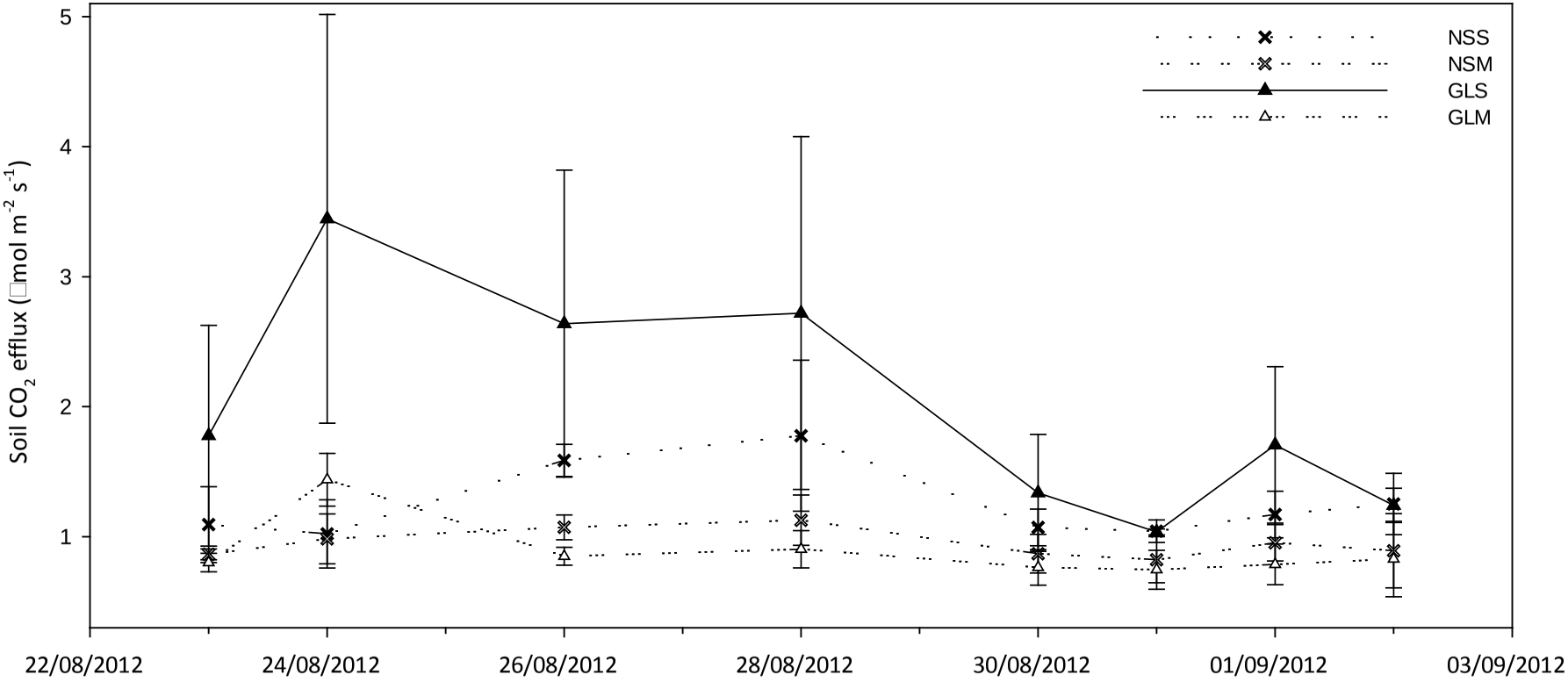

Fig. 5.14 Average soil CO_2 efflux (µmol m^{-2} s^{-1}) measured over time, from treatments GLS, GLM, NSS and NSM. The addition of ^{13}C-labelled substrates took place immediately prior to measurements on 24/08/2012. Error bars represent ± 1 SE (n = 3). Treatment codes are described fully in Table 5.1.

reflecting what was shown previously in the ^{13}C-glucose decomposition experiment (see Fig. 5.3). This rise in soil CO_2 flux following substrate addition was short-lived for treatment GLS, and it tended to have a lower soil CO_2 flux than both the control treatments (NSS and NSM), which was also the case prior to substrate addition (see Figs. 5.12 and 5.14).

Though there was a slight increase in the soil CO_2 flux measured from treatments CEM and CES following ^{13}C-labelled cellulose addition, this was much less pronounced than had been observed in treatments GLS and GLM. The soil CO_2 flux was higher from treatment CES than treatment CEM (see Fig 5.15), though both treatments had a consistently lower average soil CO_2 flux than treatment NSS, where no ^{13}C-labelled substrates had been added, which was also the case prior to substrate addition (see Fig. 5.12). The same lack of difference between the relative treatment fluxes prior to and following substrate addition was also observed in treatments LYS and LYM (see Fig. 5.16). Treatment LYS had a higher flux than treatment NSS, both prior to and following treatment addition, and treatment LYM had a lower average soil CO_2 flux than treatment LYS prior to and following substrate addition. Fig. 5.17 shows that there no was no clear peak in soil CO_2 flux in treatments FUS and FUM following the addition of ^{13}C-labelled fungal material, during the time of the current work. Again, treatment FUS consistently had a higher average soil CO_2 flux than treatment FUM, which was also the case prior to substrate addition (see Fig 5.12).

The cumulative soil CO_2 flux was calculated for each soil collar, in order to determine whether the addition of the ^{13}C-labelled substrates had caused any change in soil CO_2 flux from the different treatments (see Fig 5.18). The treatment pattern in terms of the cumulative soil CO_2 flux was identical prior to (see Fig. 5.12), and following the addition of the substrates (see Fig 5.18). Results of a Friedman's two-way ANOVA, with block and treatment as factors, showed that there was a highly significant difference between the treatments ($F= 4.88$, $p<0.003$). Within each substrate treatment, significant differences between MS and S collars occurred for treatments GLS and GLM, LYS and LYM and also FUS and FUM. However, there were significant differences between these treatments prior to the addition of the substrates (see Fig. 5.12). There was no difference between the MS and S collars in terms of cumulative soil CO_2 flux

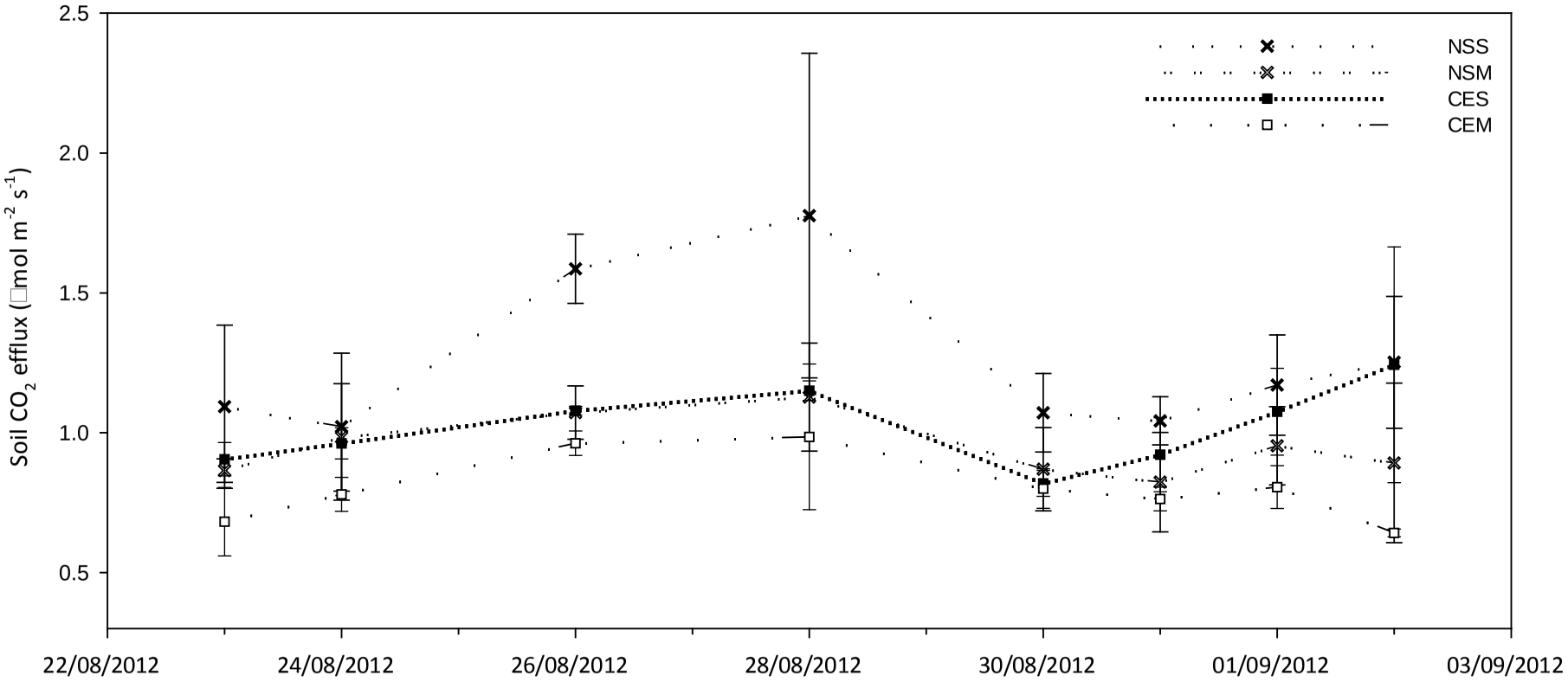

Fig. 5.15 Average soil CO_2 efflux (µmol m^{-2} s^{-1}) measured over time, from treatments CES, CEM, NSS and NSM. The addition of ^{13}C-labelled substrates took place immediately prior to measurements on 24/08/2012. Error bars represent ± 1 SE (n = 3). Treatment codes are described fully in Table 5.1.

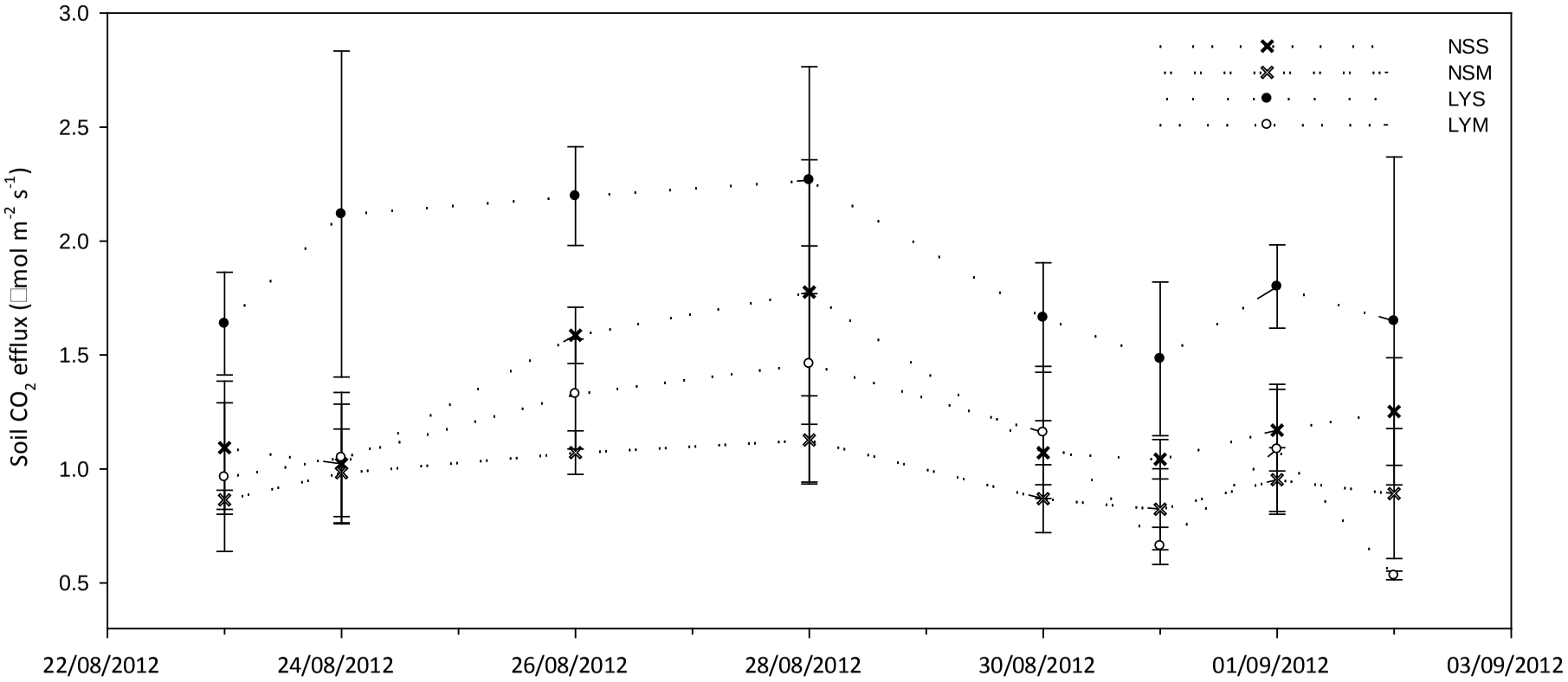

Fig. 5.16 Average soil CO_2 efflux (µmol m^{-2} s^{-1}) measured over time, from treatments LYS, LYM, NSS and NSM. The addition of ^{13}C-labelled substrates took place immediately prior to measurements on 24/08/2012. Error bars represent ± 1 SE (n = 3). Treatment codes are described fully in Table 5.1.

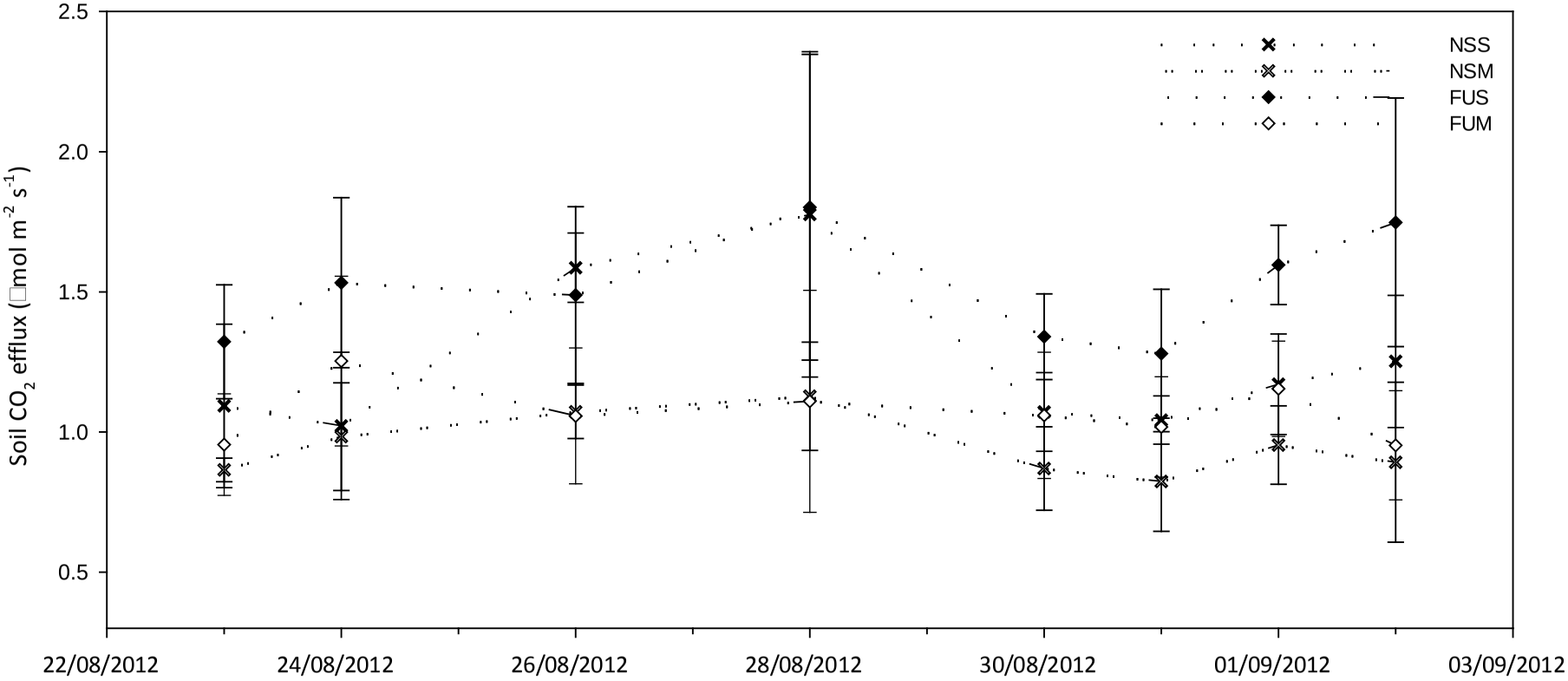

Fig. 5.17 Average soil CO_2 efflux (µmol m^{-2} s^{-1}) measured over time, from treatments FUS, FUM, NSS and NSM. The addition of ^{13}C-labelled substrates took place immediately prior to measurements on 24/08/2012. Error bars represent ± 1 SE (n = 3). Treatment codes are described fully in Table 5.1.

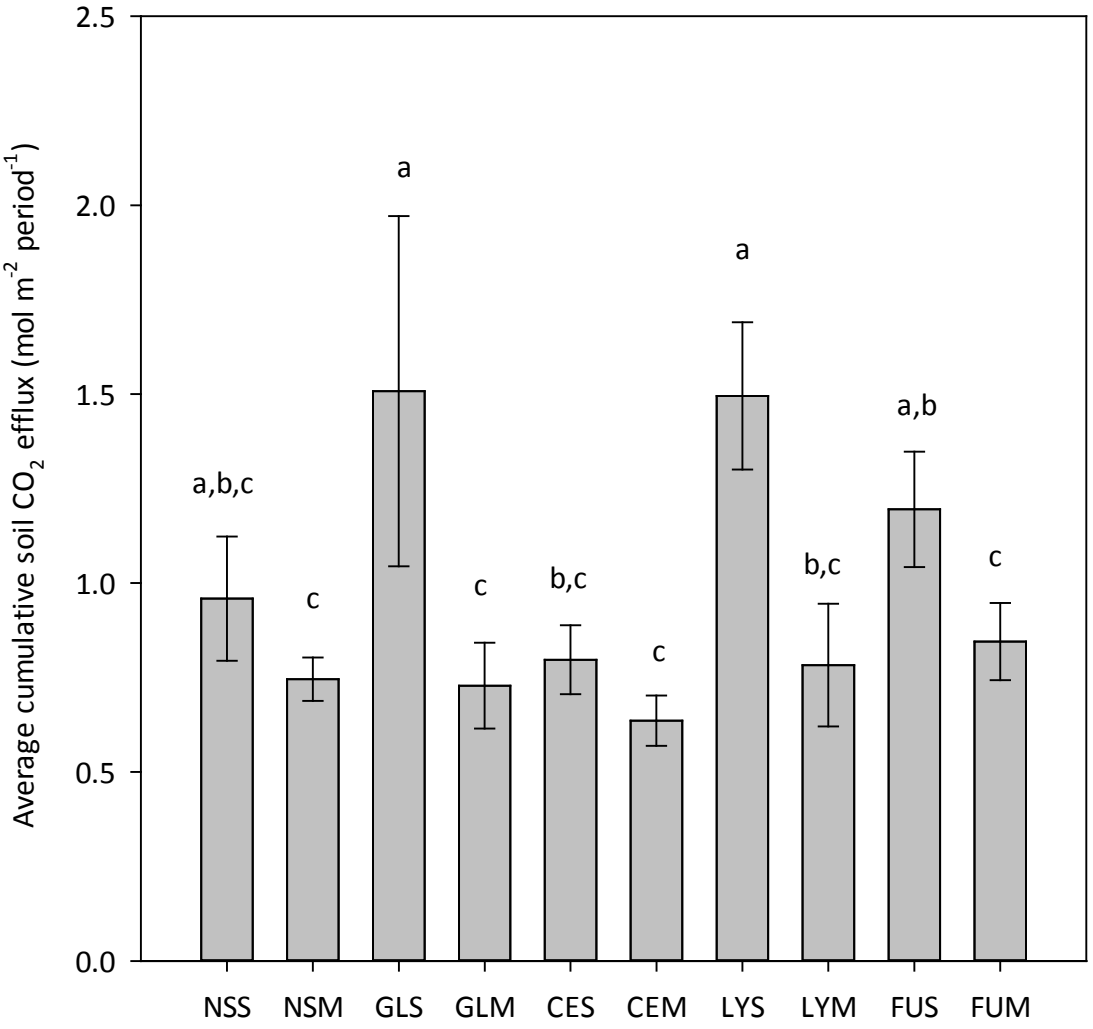

Fig. 5.18 Average cumulative soil CO_2 efflux (mol m^{-2} $period^{-1}$) during the period following the addition of ^{13}C-labelled substrates (24th August 2012 to 2nd September 2012, a period of 220 hours). Treatments which differ significantly have different letters. Error bars represent ± 1 SE (n = 3).

either prior to, or following the addition of ^{13}C-labelled cellulose to treatments CES and CEM (see Figs. 5.12 and 5.18).

When the return of soil CO_2 flux from all the replicates of substrate treatment were measured simultaneously on 29th August 2012, there was a switch in the treatment effects, with higher average soil CO_2 flux recorded from treatment GLM, when compared to treatment GLS (see Fig. 5.19). The same 'switch' was observed for treatments LYS and LYM, though results of a paired t-test showed that the differences between the MS and S collars for both the ^{13}C-labelled glucose and lysine treatments were not significant. There was a significant difference between the average soil CO_2 flux measured from treatments GLS and GLM, though again, this was shown to be the case prior to substrate addition, so could not necessarily be attributed to utilisation of the substrate (see Fig. 5.19).

5.3.2.2 δ^{13}C Soil CO_2 flux

The use of the ^{13}C-label allowed a comparison of the decomposition of the different substrates between the S and MS collars, whilst removing any variation caused by the magnitude of the fluxes from the collars prior to, and following substrate addition. Fig. 5.20 shows the δ^{13}C of soil CO_2 flux plotted against time for all of the ^{13}C-labelled substrates, and it was possible to attribute any increase in the δ^{13}C, above that of the NSS and NSM collars, to use of the ^{13}C-labelled substrates. The δ^{13}C results show that there was an immediate peak in utilisation of the glucose, with a slightly higher utilisation from treatment S collars than treatment MS collars, in support of what was shown in the previous ^{13}C-labelled glucose experiment. The peak in utilisation of ^{13}C-labelled lysine took place later than that of ^{13}C-labelled glucose, occurring at around four days following substrate addition, but the utilisation was more sustained than that for glucose, with no apparent difference between the MS and S collars at any time point (see Fig. 5.20).

In order to see what was happening overall, the average δ^{13}C of soil CO_2 flux was calculated for each treatment (see Fig. 5.21) and showed that there was a highly significant difference between the utilisation of the different substrates during this

experiment (F = 21.20, p<0.0001). Though the CO_2 output from the ^{13}C-labelled glucose

Fig. 5.19 Average soil CO_2 efflux (μmol m^{-2} s^{-1}) measured from treatment collars on 29th August 2012, when each replicate from the different ^{13}C-labelled substrate treatments was measured in the same cycle, rather than being split over the day. Results of paired t-tests comparing the average soil CO_2 efflux between MS and S collars for each ^{13}C-labelled substrate treatment are displayed. Error bars represent ± 1 SE (n = 3). Treatment codes are described fully in Table 5.1.

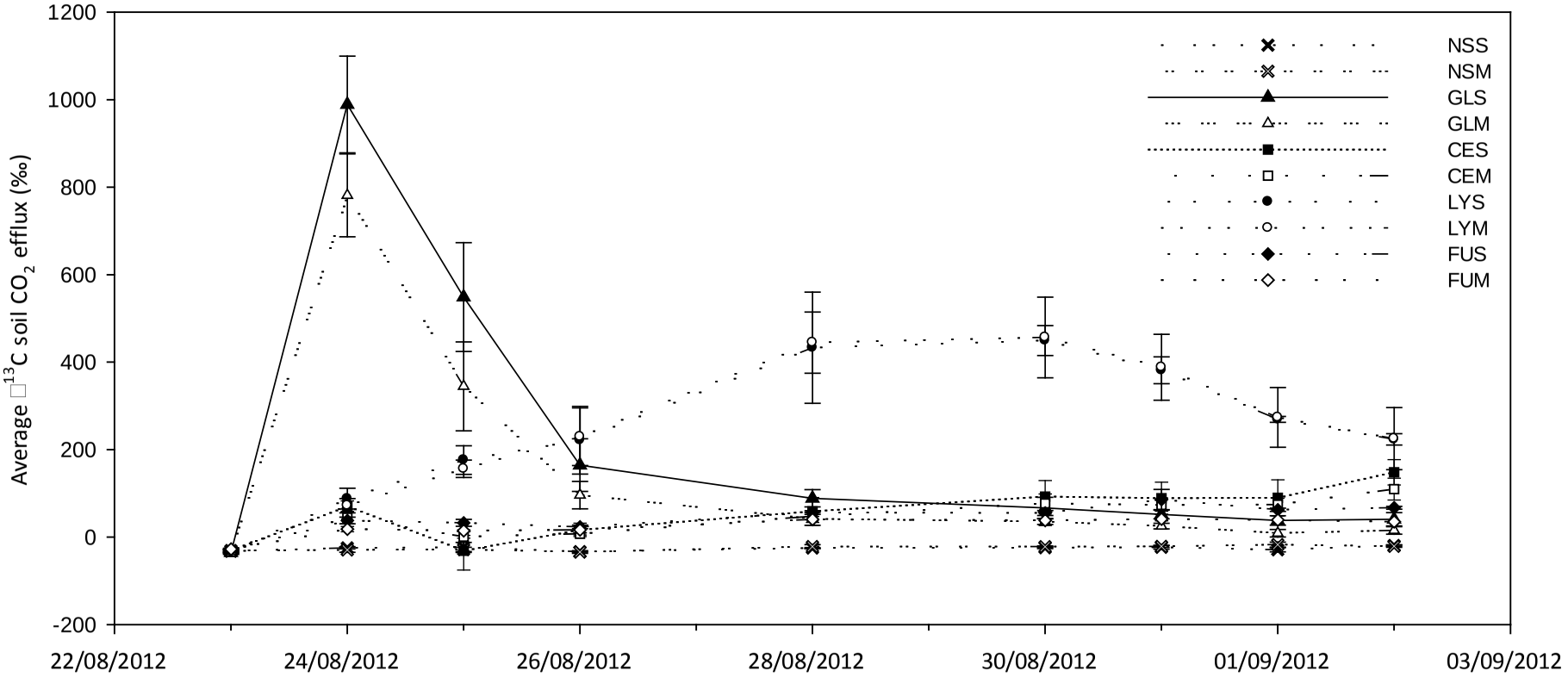

Fig. 5.20 Average $\delta^{13}C$ of soil CO_2 efflux (‰) measured from all treatment collars over time. Error bars represent ± 1 SE (n = 3). Treatment codes are described fully in Table 5.1.

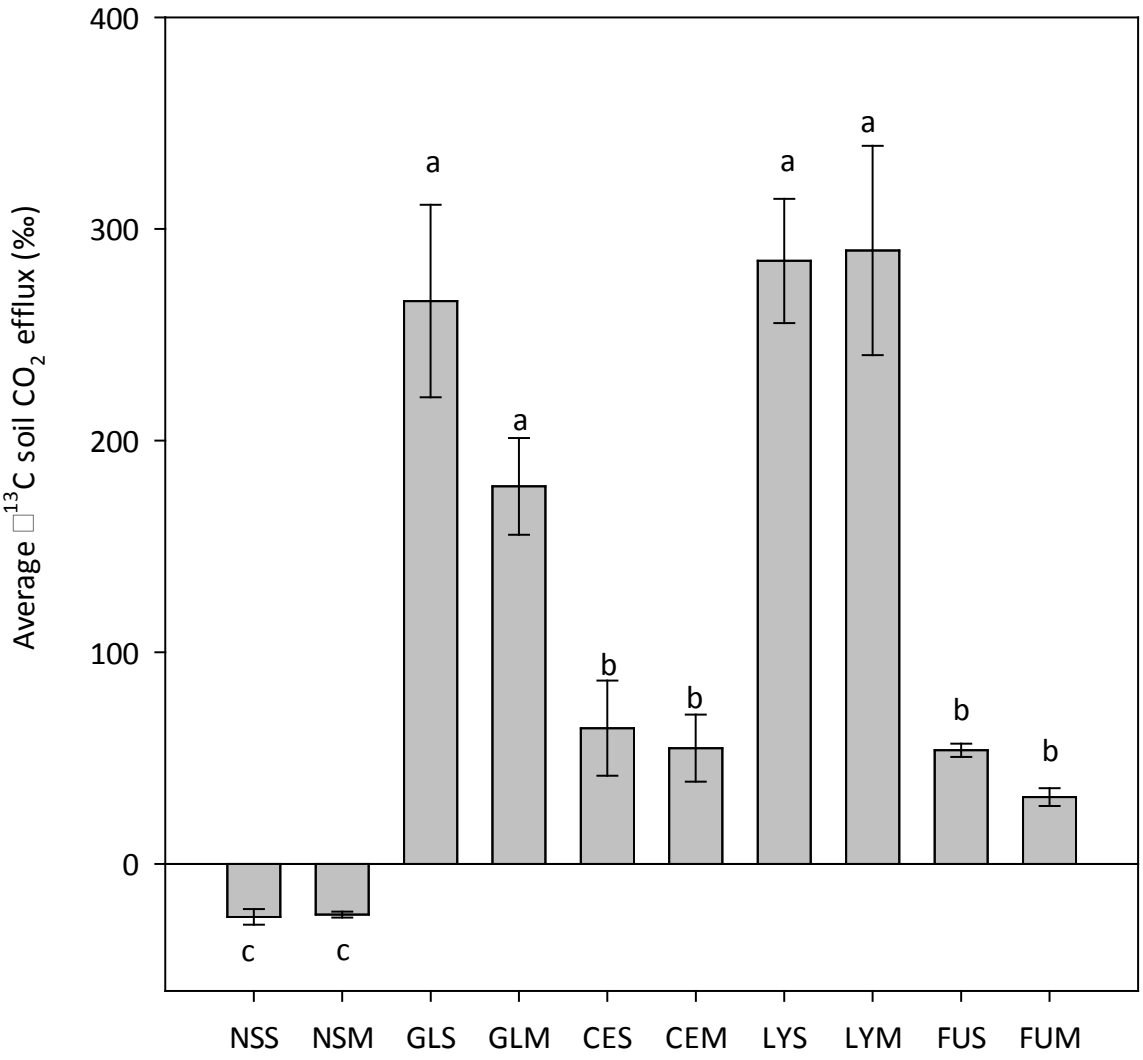

Fig. 5.21 Average $\delta^{13}C$ of soil CO_2 efflux (‰) measured from treatment collars following the addition of ^{13}C-labelled substrates. Error bars represent ± 1 SE (n = 3).

in treatments GLS and GLM peaked faster, there was more sustained use of the ^{13}C-labelled lysine in treatments LYS and LYM, resulting in the highest average $\delta^{13}C$ values of all the treatments. Though the $\delta^{13}C$ of soil CO_2 flux measured from the glucose treatments appeared higher on average than from treatment S collars than from treatment MS collars, the difference was not significant. There were no significant differences between the MS and S collars for any of the substrates in terms of the average $\delta^{13}C$ of soil CO_2 flux. The significant difference between the $\delta^{13}C$ of soil CO_2 flux from treatments GLS, GLM, LYS and LYM, compared to CES, CEM, FUS and FUM would suggest that there was a significantly higher utilisation of the simple substrates than of the more complex substrates during this experiment.

A comparison of the average $\delta^{13}C$ of soil CO_2 flux was also made for 29th August 2012 (see Fig. 5.22), when all the replicates from each substrate treatment were measured at the same time; the line number limitations referred to above meant that single substrate comparisons necessitated a temporary re-arrangement of sampling order. Paired t-tests were used to compare MS and S collars in terms of the average $\delta^{13}C$ of soil CO_2 flux, and as with the comparison using the overall average (see Fig. 5.21), there were no significant differences between the MS and S collars for any of the substrates. Though there appeared to be a higher average $\delta^{13}C$ of soil CO_2 flux in treatment FUS, when compared to FUM (see Fig. 5.21) the difference was not significant.

5.3.2.3 Percentage return of substrate-derived C

As with the previous ^{13}C-glucose decomposition experiment, the mass of substrate-derived C (mg), ^{12}C and ^{13}C from each of the collars during the current experiment was calculated by subtracting the estimated cumulative soil-derived C prior to substrate addition from the cumulative soil-derived C following substrate addition. It was decided not to use the cumulative soil derived C from treatments NSS and NSM as there were occasions, particularly for treatment NSS, when these collars had a higher soil CO_2 flux than the substrate collars (see Figs. 5.13 to 5.17), and therefore were not reflective of the underlying soil-derived CO_2 flux of the other treatments. The estimated substrate- derived C, ^{12}C and ^{13}C for each of the treatments are displayed in

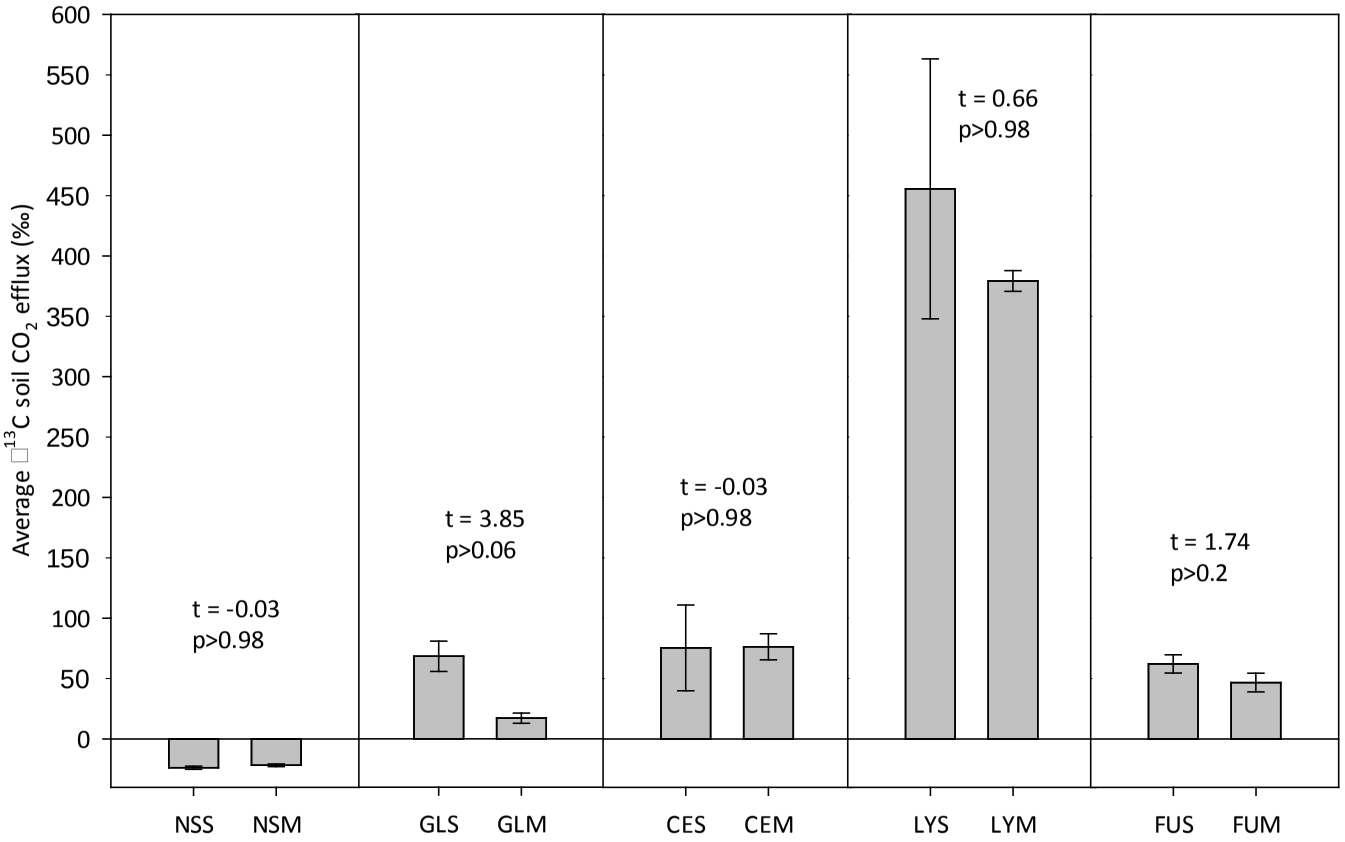

Fig. 5.22 Average $\delta^{13}C$ of soil CO_2 efflux (‰) measured from treatment collars on 29th August 2012, when each replicate from the different ^{13}C-labelled substrate treatments measured over one cycle. Results of paired t-tests comparing the average $\delta^{13}C$ of soil CO_2 efflux between MS and S collars for each ^{13}C-labelled substrate treatment are displayed. Error bars represent ± 1 SE (n = 3).

Fig. 5.23, and the % return values for the added C, ^{12C} and ^{13}C are displayed in Table 5.3.

The NS collars are also included, as the additional C calculated from these collars indicated that there was an increase in the underlying soil respiration from the other treatments during the time of the experiment that was not caused by ^{13}C-substrate addition. However, there were differences between the collars prior to substrate addition and the increase observed in the NS collars following substrate addition is unlikely to be an estimate of an underlying increase in C release from the other treatments.

There was no significant difference between the treatments in terms of the estimated additional substrate-derived C ($F= 0.96$, $p>0.5$), or ^{12}C ($F= 0.96$, $p>0.5$) emitted from the collars. However, there was a highly significant difference between the additional ^{13}C derived from the treatments ($F=11.26$, $p<0.0001$), with a significantly higher substrate-derived ^{13}C flux observed from treatment LYS than LYM. There was no significant difference between the MS and S collars in terms of the substrate-derived ^{13}C emitted from the remaining substrates (see Fig. 5.23, Table 5.3). The simple substrates, ^{13}C-labelled lysine and glucose, were clearly utilised to a greater extent than the more complex ^{13}C-labelled fungal cell wall material, and these results suggest that ^{13}C-labelled cellulose was the least utilised substrate during this experiment (see Fig. 5.23, Table 5.3).

5.3.2.4 Soil moisture surveys

Prior to flow through chamber addition, results of a hand-held survey of soil moisture conducted on 3rd August 2012, analysed using a paired t-test showed that there were no significant differences between treatment MS and S collars in terms of soil moisture ($t=1.17$, $p>0.25$). Another soil moisture survey was conducted following substrate addition on 30th August 2012, and again there were no significant differences between the treatments in terms of soil moisture ($t=0.31$, $p>0.7$).

Table 5.3 The percentage return as CO_2 of substrate-derived C, ^{12}C and ^{13}C from added ^{13}C-labelled substrates for treatments MS and S, calculated using the soil-derived C from each of the collars prior to the addition of ^{13}C-labelled substrates. For each substrate, collar treatments which differ significantly have different letters. Values for treatments are means, with standard errors in parentheses, n=4.

	^{13}C-labelled glucose	^{13}C-labelled cellulose	^{13}C-labelled lysine	^{13}C-labelled fungal cell wall material
Collar treatment	% return of substrate-derived C			
S	20.3 (32.4)[a]	15.0 (7.2)[a]	35.7 (15.4)[a]	26.9 (19.5)[a]
MS	17.0 (10.3)[a]	17.0 (4.7)[a]	5.3 (16.0)[b]	16.6 (11.9)[a]
	% return of substrate-derived ^{12}C			
S	19.9 (33.4)[a]	15.2 (7.4)[a]	35.7 (15.7)[a]	27.4 (20.0)[a]
MS	17.0 (10.6)[a]	17.3 (4.8)[a]	5.0 (16.5)[b]	16.9 (12.1)[a]
	% return of substrate-derived ^{13}C			
S	37.9 (6.3)[a]	9.5 (3.2)[a]	37.3 (6.6)[a]	14.8 (7.1)[a]
MS	16.6 (3.7)[a]	8.8 (1.8)[a]	15.4 (5.4)[b]	8.4 (4.1)[a]

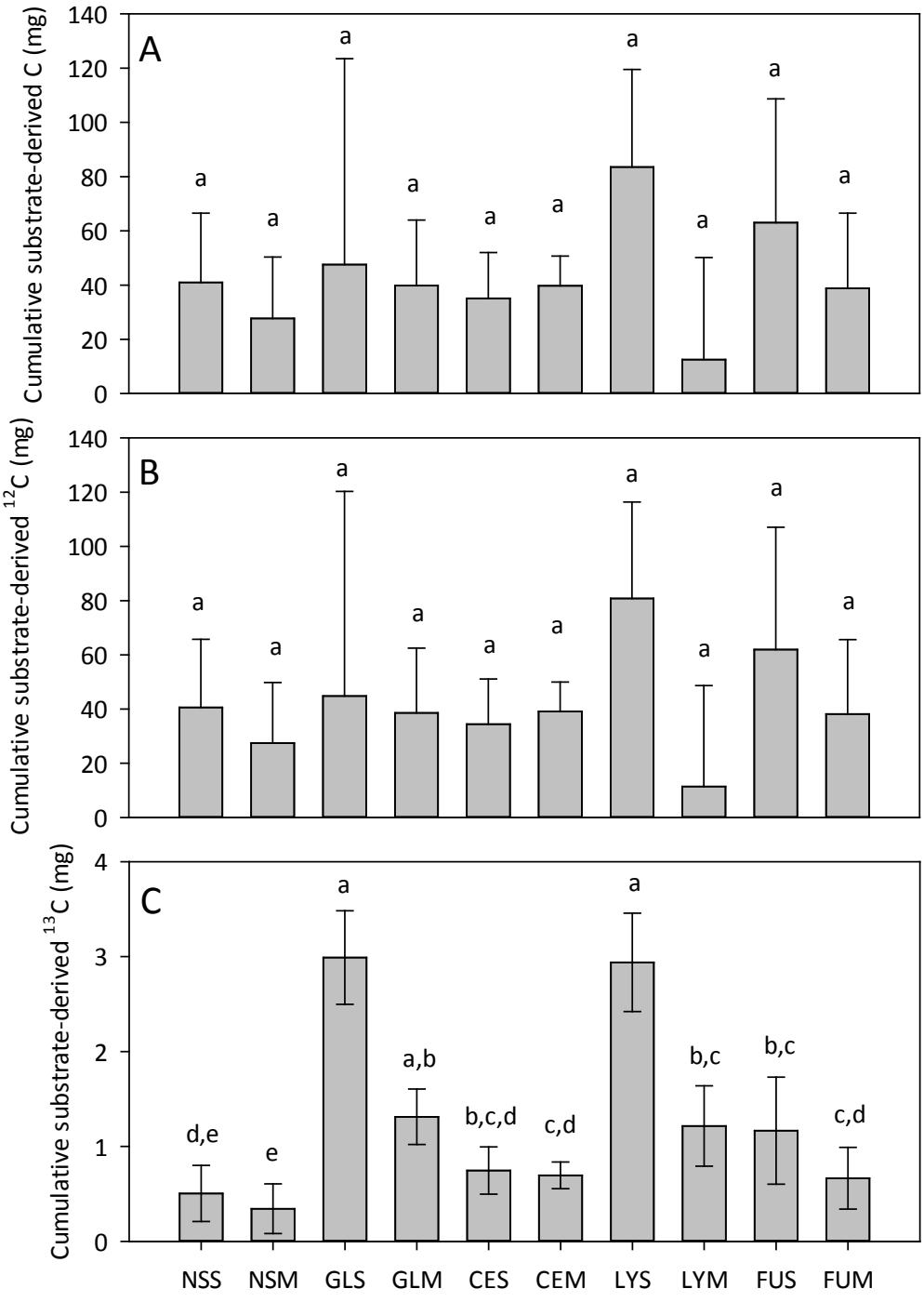

Fig. 5.23 Average cumulative additional return as CO_2 of: (A) C, (B) ^{12}C, (C) ^{13}C (mg) from treatment collars over the experimental period. Within each chart, treatments which differ significantly have different letters. Error bars represent ± 1 SE (n = 3).

5.4 Discussion

The aim of the initial ^{13}C-glucose decomposition experiment was to investigate the decomposition of a simple substrate in the presence of an intact rhizosphere, or where plant photoassimilate-C input to the soil, cycled through roots and ECM fungi was excluded by trenching. Previous studies have demonstrated a promoting effect of labile C input to the soil on the decomposition of needle litter (Zhu & Ehrenfeld, 1996; Subke *et al.,* 2004), and this finding was supported by the results of a more recent girdling study (Subke *et al*., 2011, constituting Chapter 4 of this thesis).

The results of the current ^{13}C-labelled glucose decomposition experiment, showed a clear enhanced utilisation of the ^{13}C-labelled glucose with root and ECM hyphal exclusion (see Table 5.2). This suggests some form of difference in the microbial community between the soil collar treatments affecting the utilisation of simple and more complex substrates. It is possible that this resulted from the complexity of the substrates in terms of their structure, and the enzyme complement required by microorganisms for their decomposition, or more simply due to the nitrogen (N) content of the substrates.

The ^{13}C-labelled simple and complex substrate decomposition experiment was designed to investigate this further using four substrates varying in terms of their structural complexity and N content. In contrast to the work of Subke *et al*. (2004), and Subke *et al*. (2011) (see Chapter 4), the results presented in the current Chapter suggest that the decomposition of all the substrates investigated, whether simple or complex, was promoted when roots and ECM fungi were excluded. However, the two experiments showed slightly different results for glucose, with no significant difference in the utilisation of this substrate in the second experiment, in contrast to the initial experiment (see Tables 5.2 and 5.3). CO_2 data from both of the experiments presented in the current Chapter suggest that this greater substrate utilisation was caused by a higher level of microbial activity in the treatment S collars, observed prior to the application of the ^{13}C-labelled substrates.

5.4.1 The influence of structural complexity on ^{13}C-labelled substrate decomposition

Labile C substrates, such as glucose, are required by soil microorganisms for growth, and to provide energy for the production of extracellular enzymes that break down the more complex nutrient-containing SOM (Mary *et al*., 1992; Schnekenberger *et al*., 2008). By excluding plant assimilates from the soil of the S collars in the current experiment, the soil was essentially starved of labile C and the microbial community appears to have altered in response to increased labile C limitation (Ekblad & Nordgren, 2002), though further work is required to confirm this. In the S collars it is likely that the rapid response observed following the addition of the labile substrates (^{13}C-labelled glucose and lysine; see Figs. 5.4, 5.14 and 5.16) was from microorganisms with an 'r' life strategy. This functional group of microorganisms includes species characterised by having the ability to respond rapidly following the sudden input of labile substrates (Panikov, 1999).

In the current work, the addition of ^{13}C-labelled glucose in both experiments resulted in a rapid increase in the activity of the microbial community. This was reflected in the increase in soil CO_2 flux following substrate addition on 12th December 2008 for the initial ^{13}C-labelled glucose decomposition experiment (see Figs. 5.3 and 5.4), and on 24th August 2012 for the later ^{13}C-labelled simple and complex substrate decomposition experiment (see Fig. 5.14). In both cases, the initial rate of increase appeared the same for the S and MS collars (and also the RMS collars in the initial experiment). However, the magnitude of the flux was greater where roots and ECM fungi had been excluded, as shown by the higher average cumulative soil CO_2 flux from treatment S collars than the other treatments following substrate addition (see Figs. 5.6 and 5.18). In both experiments, there was a higher average cumulative soil CO_2 flux from the treatment S collars prior to the addition of the substrates (see Figs. 5.5 and 5.12), therefore substrate addition did not cause the treatment difference observed.

When the initial difference in CO_2 flux from each treatment was taken into account by studying the $\delta^{13}C$ of soil CO_2 flux over time for the addition of ^{13}C-labelled glucose in the initial experiment (see Fig. 5.7), the rate of response to substrate addition was the same for all collar treatments, though it was sustained to a greater extent in the S collars compared to the RMS and MS collars. Following ^{13}C-labelled glucose addition in the second experiment, the response of the treatment S and MS collars was also

similarly quick as reflected by the average $\delta^{13}C$ of soil CO_2 flux over time (see Fig. 5.20), with the peak immediately following substrate addition. Fig 5.20 also shows that there was no difference at any time point in the average $\delta^{13}C$ of soil CO_2 flux measured from treatment MS or S collars following ^{13}C-labelled lysine addition. Previous studies (eg. Anderson & Domsch, 1978; Dalenberg & Jager, 1981; 1989) report the same rapid response of the soil microbial community to glucose as was observed in the current work. Following ^{13}C-glucose addition, Dilly & Zyakun (2008) observed a peak in soil CO_2 flux three days following addition of C4 plant-derived glucose to soil in a Beech forest in Germany.

Only a small percentage of the C added as part of the ^{13}C-labelled glucose was returned as CO_2 during the time of both the current experiments. The amount differed between the MS and S collars, but ranged from 3.5% to 8.7% for the initial ^{13}C-labelled glucose decomposition experiment (see Table 5.2) and from 17% to 20.3% for the later ^{13}C-labelled simple and complex decomposition experiment (see Table 5.3). However, this low return of substrate % return is not unusual, Brant *et al.,* (2006) reported a peak in respiration within two days of the addition of ^{13}C-labelled glucose to soil in a Douglas Fir and Western Hemlock forest in Oregon. They calculated that 70 - 80% of the added C was incorporated into microbial cells, so the percentage of the added substrate returned as respired CO_2 was of the same order as was reported in the current work. Dalenberg & Jager (1981) reported that during the 2 week period following labelled glucose addition, 75% of the added C returned as CO_2, with 25% remaining in microbial biomass under laboratory conditions. Therefore, had the current work continued for longer it might be expected that a greater amount of ^{13}C added as part of the simple ^{13}C-labelled substrates would have been released as $^{13}CO_2$.

The lower percentage of added glucose-C returned during the ^{13}C-labelled glucose decomposition experiment, when compared to the later ^{13}C-labelled simple and complex substrate decomposition experiment (see Tables 5.2 and 5.3) is potentially a seasonal effect. The initial ^{13}C-labelled glucose decomposition experiment was conducted in December 2008, whereas the later ^{13}C-labelled simple and complex substrate decomposition experiment was conducted in August and September 2012. Temperature is likely to have had an effect on the level of activity of the microbial

community decomposing the substrates, alongside the availability of substrates already in the soil (Subke & Bahn, 2010), which is reflected in the lower soil CO_2 fluxes displayed in Figs. 5.3 and 5.4 for the initial ^{13}C-labelled glucose decomposition experiment, when compared to those displayed in Fig. 5.13 for the ^{13}C-labelled simple and complex substrate decomposition experiment.

There is also the potential that the difference observed in terms of the level of soil CO_2 flux and % return of the substrates (see Tables 5.2 and 5.3) could be explained by the amount of ^{13}C-labelled substrates added. The amount of C added to the soil in glucose in the initial experiment was 0.6 g C 314 cm^{-2} (collar surface area), in the second simple and complex substrate decomposition experiment, the amount added was considerably lower, 0.23 g C 314 cm^{-2}, with the amount of C added constrained by the estimated amount available for use as part of the cultured ^{13}C-labelled fungal biomass. Dilly & Zyakun (2008) found that when they altered the amount of ^{13}C-glucose added to soil, the amount dictated the level of stimulation of the microbial community reflected by soil CO_2 flux. It is possible that this could explain why the return of substrate-derived ^{13}C from glucose was significantly different between the MS and S collars in the initial experiment (see Fig 5.11 and Table 5.2), but not significant for the later ^{13}C-labelled simple and complex substrate decomposition experiment (see Fig. 5.23 and Table 5.3). Potentially, the amount of glucose added in the second experiment was not sufficient enough to prime the activity of the soil microbial community to the same extent, so the same effect was not observed. The results displayed in Table 5.3 show that the percentage return of added ^{13}C from the treatment S collars for the simple substrates lysine and glucose were similar (37.3% and 37.9%, respectively). However the difference between the MS and S collars in terms of the substrate-derived ^{13}C returned was significant for lysine, but not for glucose (see Fig. 5.23 and Table 5.3).

In the current work, it is likely that the decomposition of the more complex, polymeric substrates would require K-strategist microorganisms, with the ability to produce a greater complement of specialist enzymes (Panikov, 1999). This is supported by Dighton *et al*. (1987) who demonstrated greater decomposition of complex substrates including hide powder, cotton and chitin by the K-strategist mycorrhizal fungus *Suillus*

luteus (L.) Gray, a later coloniser of substrates than the r-strategist fungus *Hebeloma crustuliniforme* (Bull. Ex St. Amans.) Quél. K-strategist microorganisms can be at a competitive disadvantage in comparison to r-strategists (Fontaine 2003; 2004) because they develop more slowly and tend to dominate in the later stages of litter decomposition, following the utilisation of more labile substrates by the r-strategists (Chigineva *et al*., 2009). Therefore it is not surprising that the same rapid response seen following the introduction of the labile glucose and lysine was not observed for the structurally complex substrates, cellulose and fungal cell wall material. Unlike simple substrates, there did not appear to be a convincing peak in the $\delta^{13}C$ of soil CO_2 flux from treatments CES, CEM, FUS or FUM (see Fig. 5.20). The average $\delta^{13}C$ of soil CO_2 flux was significantly greater from treatments CES, CEM, FUS and FUM than from the NS treatments, showing that the substrates were being decomposed, but at a much reduced level when compared to the decomposition rates of the simple substrates (see Fig. 5.21). There was a hint that there was greater utilisation of the complex substrates, ^{13}C-labelled fungal cell wall material, and ^{13}C-labelled cellulose from the S collars as was seen with the simple substrates (see Table 5.3). However, the difference in the amount of substrate-derived ^{13}C returned between treatments MS and S was not significant for either the ^{13}C- labelled cellulose or the ^{13}C-labelled fungal cell wall material (see Fig. 5.23). The second experiment was cut short due to technical difficulties, and it is possible that the experiment did not run long enough to draw appropriate conclusions regarding the influence of the presence of an intact rhizosphere on the decomposition of the more complex substrates.

Of all the substrates studied in the current work, the least return of substrate-derived C (see Fig. 5.23), was observed following the addition of ^{13}C-labelled cellulose. There was a return of 9.5% of the ^{13}C added to treatment CES, and an 8.8% return of the ^{13}C added to treatment CEM (see Table 5.3), The returns of ^{13}C from treatments CES and CEM were greater than the additional ^{13}C released from the control treatments NSS and NSM, indicating that though the utilisation of this structurally complex component was relatively low, it was decomposed to some extent during the time of the experiment. The results of the current experiment differed from those of Fontaine *et al*. (2004) who observed that the addition of cellulose to the soil stimulated the

microbial community, with almost double the production of control soils at day 70. They observed a short lag phase of three days before exponential growth until substrate exhaustion at day 17. In contrast, the response in terms of soil CO_2 flux for the current study was comparatively low (see Fig. 5.15) with the average soil CO_2 flux over time from treatments CES and CEM being lower than that of treatment NSS. The treatment NSS collars had a higher average cumulative soil CO_2 flux than the CES and CEM collars prior to substrate addition (see Fig. 5.12), and the addition of cellulose to treatments CES and CEM did not appear to stimulate CO_2 production relative to that of the control collar NSS following substrate addition either (see Fig. 5.18).

The amount of each substrate added in this experiment was dictated by the available amount of cultured ^{13}C-labelled fungal cell wall material, where there was difficulty in culturing a large amount of biomass. Therefore it is possible that the small amount of the complex substrates added in the second experiment may have affected the results observed. This is highlighted by the fact that Fontaine *et al*. (2004) were able to recover 85% of the labelled cellulose C they added by day 13 of their experiment, which is a large amount compared to the % returns observed for cellulose-derived C in the current study (see Table 5.3). The savannah-derived soils studied by Fontaine *et al*. (2004) were incubated at 28 ˚C, which might also explain the difference in the level of stimulation of soil CO_2 flux in the current work, compared to that of Fontaine *et al*. (2004); temperatures in the *Pinus contorta* stand at Wheldrake forest during September were considerably lower, with an average air temperature for September 2010 of 14˚C.

Mycorrhizal mycelia is a relatively recalcitrant substrate because of its chitin content (Wallander *et al*., 2012), but a surprisingly large amount of fungal cell wall material was decomposed during the current work. The percentage return of ^{13}C added as fungal cell wall material during the nine days of incubation was 14.8% from treatment S and 8.4 % from the treatment MS collars (see Table 5.3). The % return of substrate-derived ^{13}C from the structurally simple glucose and lysine was greater, but not hugely different (see Table 5.3). Following the addition of ^{13}C-labelled fungal cell wall material on 24th December, there was an immediate noticeable rise in the soil CO_2 flux (see Fig. 5.17), which was steeper for treatment FUM than treatment FUS. However, as for the

addition of ^{13}C-labelled cellulose, there was obvious peak in soil CO_2 flux, suggesting that perhaps too small an amount was added to stimulate the microbial community, or that the experiment did not run long enough to observe a peak, with insufficient time for K-strategists to respond to the addition of this complex substrate (Janssens *et al*., 2010).

This apparent lack of recalcitrance of ECM fungal biomass has also been observed in other recent studies. Koide *et al*. (2011) simultaneously compared the decomposition of laboratory cultured fungal mycelia in litter bags with fine roots finding that during their 37 day experiment the mass loss of fungal mycelia was 56.8%, compared with 19.4% for fine roots. Drigo *et al*., (2012) studied the decomposition of ^{13}C-labelled fungal biomass, and found that, within seven days of substrate addition, ^{13}C was incorporated into fungal DNA in the soil, with the labile fractions of the substrate being rapidly utilised by saprotrophic fungi. Fernandez & Koide (2012) studied the decomposition of laboratory cultured ECM fungal tissue from five isolates, collected from root tips in a *Pinus resinosa* plantation with the decomposition of the cultured mycelia being studied in a mesh bag decomposition experiment spanning 28 days. The % mass loss they observed varied significantly between fungal isolates, ranging between 15-50% at 14 days and 20-60% at 28 days, which is not inconsistent with the results of the current work. They observed that the fungal chitin was not recalcitrant in comparison to other tissues, indeed higher concentrations of chitin in fungal tissues was associated with higher levels of decomposition.

The current work differed slightly from the handful of studies that have investigated the decomposition of laboratory cultured fungal biomass, as generally others have used intact mycelia (eg. Koide & Malcolm, 2009). The decay of fungal mycelia has been described as similar to the decay of plant litter, as they both contain fractions of varying complexity, which are broken down by a series of different microorganisms with varying decomposer abilities (Wallander *et al*., 2012; Drigo *et al*., 2012). In the current work, in order to avoid a confounding effect of the decomposition of the more labile cell contents, these were extracted leaving the more recalcitrant cell wall material. However, this did not appear to have reduced mass loss when compared to these other studies.

5.4.2 Evidence of competition between ECM fungi and free living saprotrophic microorganisms

Clearly there was a difference between the decomposition of simple and complex substrates in the current experiment. In the current work, there was significantly higher use of simple substrates, ^{13}C-labelled glucose in the initial experiment, and ^{13}C-labelled lysine in the second experiment by the treatment S collars, where ECM roots had been excluded. In the girdling experiment presented in Chapter 4, there was significantly greater decomposition of a complex substrate where ECM roots had not been excluded. This difference is likely explained by the ability of the microorganisms in the different treatments to respond to the added substrates. In the current work, though there was evidence that there might have been a promotion of decomposition of complex substrates in the absence of roots and ECM fungi, the difference between the MS and S collars was not significant, and therefore does not contradict the findings in Chapter 4. It is likely then that the differences observed were to do with structural complexity and specifically the ability of r-strategist decomposers to respond to added simple substrates between the MS and S collar treatments.

The suppression hypothesis proposed by Gadgil and Gadgil (1971; 1975) can potentially explain why they, and others since (Faber & Verhoef, 1991; Chuyong *et al.,* 2002) have observed decreased litter decomposition in the presence of roots and ECM fungi. The hypothesis could potentially be applied to explain the seeming lack of ability of r-strategist microorganisms in the MS collars to respond to the addition of the simple substrates, when compared to the r-strategists in the treatment S collars. It involves the ability of ECM fungi to utilise the competitive advantage they have through receiving labile plant photoassimilate C, in order to outcompete saptrotrophic fungi, both r- and K-strategists, potentially through the uptake of essential nutrients such as N, making them unavailable to saprotrophs (Abuzinadah *et al*., 1986; Bending, 2003).

Lindahl *et al*. (1999b) demonstrated that the outcome of competitive interaction experiments between a wood-decomposing fungus and an ECM fungus in microcosms was dictated by the size of resource available to the wood-decomposing fungus. When

the size of the experimental wood blocks was large, the wood decomposer was able to outcompete and capture resources from the ECM fungus, but the outcome was reversed when the wood blocks, and consequently, the available resource was low. This is potentially what occurs in the *Pinus contorta* stand at Wheldrake Forest. The resources available to ECM fungi in terms of labile C were high, therefore they are able to utilise this advantage to selectively uptake N and other essential nutrients, thereby suppressing other soil decomposers. Hence, when ECM fungi were excluded there was a much larger response of r-strategists to the addition of both the simple substrates in the S collars compared to the MS collars. Conditions were not limited for ECM fungi in terms of labile C, therefore they did not respond in the same way to the addition of the labile substrate.

One of the most interesting and unexpected findings of the current work was that in both experiments there was a reversal in the impacts of the exclusion treatments for soil CO_2 flux prior to substrate addition (see Figs. 5.5 and 5.12). Prior to the deployment of the mobile laboratory, when the soil CO_2 flux was measured with the Li-Cor IRGA (see sections 5.2.2.1 and 5.2.3.4) the higher average soil CO_2 fluxes were measured from treatment RMS, followed by the flux from treatments MS then S. For the later substrate decomposition experiment, the higher average fluxes were measured from treatment MS when monitored using the Li-Cor hand-held system, prior to substrate addition. However, when measured with the field-deployed IRMS, the day prior to substrate addition, in both cases this treatment effect had switched, with the higher flux consistently occurring from the treatment S collars.

This difference, with the higher level of soil CO_2 flux measured by the mobile laboratory from the treatment S collars, (roots and ECM fungi excluded), is not consistent with the general view that the rhizosphere is a hotspot of microbial activity in comparison to the bulk soil (Kuzyakov, 2010). Several days prior to substrate addition in both experiments, the collars were watered with a considerable amount of throughfall to ensure no differences in soil moisture content between treatments. With hindsight, it is possible that this altered the respiration from the collars. The 1 µm mesh in the S collar windows would be expected to retain moisture to a greater extent than the 41 µm mesh in the MS collar windows, and it is possible that the retention of

a higher soil moisture in the S collars resulted in a higher respiration rate when measured by the IRMS. The results presented in Chapter 3 showed that for summer 2009, the irrigation of treatment S(W) with relatively large amounts of water did lead to increased respiration rate. Measurements with the Li-Cor IRGA were never made immediately following the addition of the large amounts of throughfall to the collars in either the ^{13}C-labelled glucose decomposition experiment, or the ^{13}C-labelled simple and complex substrate decomposition experiment; this apparent effect of the addition of large volumes of water requires further consideration. Though soil moisture surveys conducted during both experiments showed that there was no significant difference between the collars in terms of soil surface moisture, the moisture lower down in the soil profile was never measured, and this could have had an impact.

There is also another potential explanation for the apparent increase in the activity of soil microorganisms in the S collars relative to the MS collars. Throughfall is known to contain soluble carbohydrates, but with varying concentration during the year (McClaugherty, 1983). It is possible that these labile compounds, leached from the canopy could have increased the activity of r-strategist microorganisms prior to the addition of the ^{13}C-labelled substrates. De Nobilli *et al*. (2001) observed that repeated pulses of labile C inputs can cause a state of metabolic alertness (Kuzyakov, 2002; Cheng, 2009), resulting in a greater response from the soil microbial community with the next pulse of labile C input (Kuzyakov, 2010). The observation that even this pre-priming effect appears to have been suppressed in the MS collars, in the presence of ECM fungi is further evidence for the suppression hypothesis (Gadgil & Gadgil, 1971; 1975) prior to the beginning of the current experiments.

Though the analysis systems were analytically checked, and both measurement systems were found to be functioning properly, previous studies (Janssens *et al*., 2010; Nottingham *et al*., 2012) have observed artefacts involving chamber design and soil type on estimates of soil CO_2 flux. It would be useful to conduct a series of comparison tests, in conjunction with contrasting addition of large volumes of throughfall or deionised water to elucidate these processes more fully.

5.4.3 The influence of substrate nitrogen (N) content on ^{13}C-labelled substrate decomposition rates

In addition to the input of labile C substrates, which dictates the growth of saprotrophic microbial biomass, nitrogen (N) is also a limiting factor for soil microorganisms (Mary *et al*., 1992), and has been shown to control the decomposition rate of complex substrates such as plant litter (Cotrufo *et al*. 2004). There is the possibility that the switch observed in the decomposition rates of simple (the current Chapter, enhanced when ECM fungi excluded) and complex substrates (Chapter 4, decreased when plant C through ECM fungi excluded by girdling) could be explained by the N contents of the substrates used rather than their polymeric complexity. The litter used as a substrate in the girdling experiment contained N but, in addition to being structurally more simple than plant litter, glucose does not contain N. Though ECM fungi have a competitive advantage in terms of their labile C supply provided by their plant host, they are also known to be limited in N or P, and have been shown to proliferate where there is a good supply of N (Bending and Read 1995). Indeed, Vallack *et al*. (2012) demonstrated decreased CO_2 flux from ECM fungi following N fertilisation in a boreal forest. When N is plentiful, this suggests that the plant allocates less assimilate C below-ground to the fungus. ECM fungal scavenging for N has been given as an explanation for priming of SOM decomposition following labile C input in the rhizosphere (Kuzyakov, 2010). Therefore, when N was introduced in substrates in the current experiment, if it was the limiting growth of ECM fungi at Wheldrake forest, there would have been a similar utilisation of the N containing substrates between treatments MS and S. The lack of a difference between the δ^{13}C of soil CO_2 flux over time shows this to be the case for the ^{13}C-labelled lysine (see Fig. 5.20), suggesting that both ECM fungi and free-living soil saprotrophs were utilising the substrate.

There seemed to be a trend towards increased lysine decomposition over glucose, with the average δ^{13}C of soil CO_2 flux from treatment LYS appearing larger, but this was not significant to that measured from treatment GLS (see Fig. 5.21). Dalenberg & Jager (1981) observed rapid release of CO_2 following glucose addition, which they attributed to a turnover of C in microbial biomass. Due to its N content, there is the potential that a greater amount of lysine C could have been immobilised into the

microbial biomass, rather than being released as CO_2, though it was beyond the scope of the current work to test this. Certainly the utilisation of the ^{13}C-labelled lysine was more gradual and sustained than that of the glucose, as is reflected by the $\delta^{13}C$ of soil CO_2 flux over time (see Fig. 5.20), suggesting that N content was of importance in maintaining enhanced microbial activity in this experiment.

There was also greater return of ^{13}C from the complex N containing substrate, ^{13}C-labelled fungal cell wall material, than for cellulose in both the MS and S collars (see Fig. 5.23). Vance & Chapin (2001) noted that there was a greater immobilisation of N following the addition of cellulose to laboratory incubated soils, indicating the reliance of cellulose decomposers on assimilation of additional N, which may have restricted the decomposition of the cellulose, compared to the fungal cell wall material in the current work (Janssens *et al*., 2010).

The fungal cell wall material which was used as the high N, high structural complexity substrate in the current work was from one species of ECM fungus, *Paxillus involutus*. With the rising interest in the decomposition of ECM biomass, studies have investigated how the N content of the microbial biomass affects its decomposition. Koide & Malcolm (2009) observed that the rate of decomposition of ECM fungal mycelia differed between species, with a strong association between mass loss from and tissue N content, in a mesh bag experiment. Koide *et al*. (2011) tested whether the N content of mycorrhizal mycelia, could lead to increased decomposition of fine roots in symbiosis with ECM fungi. They found that colonisation of roots either had no effect on their decomposition rate, or was slightly increased depending on the isolate of fungus, which they attributed to different isolates having different C:N ratios. Wilkinson *et al*. (2011) measured the decomposition of fungal necromass in mesocosms, and observed that the greater the species richness of the fungal isolates represented in a sample, the greater the decomposition rate. Nearly all of these recent studies acknowledge that fungal mycelia grown in optimum conditions in the laboratory may have very different N content to naturally-produced decaying mycelia in the field. It is possible that the relatively fast decomposition of the ^{13}C-labelled fungal cell wall material observed in the current experiment may have been partly attributed to a relatively high N content, and may not be representative of natural

ECM mycelial turnover rates. Recently, Wallander *et al*. (2012) highlighted the need for considerably more research in this area given the clear importance of ECMs in forest C cycling.

Though it was beyond the scope of this experiment to directly measure the loss of N from the substrates, and subsequent uptake by the different components of the soil community, the increased use of both simple and complex substrates (see Fig 5.23) when they contained N (as opposed to containing no N) would suggest that this was of importance; had the experiment run over a longer period, effects of N limitation on use of the substrates may have become more evident.

Exclusion techniques were successfully used in the current experiments to separate out the components of soil CO_2 flux. The measurements of the decay of isotopically labelled substrates *in situ* using the field-deployed mass spectrometer allowed an accurate estimate of substrate decomposition over time, with the in-growth collar technique allowing a unique investigation of the influence of ECM fungi on decomposition processes. The initial ^{13}C-labelled glucose experiment informed the later experiment in terms of the resolution of measurements required to be able to assess the decomposition of the substrates between the different collar types. Unfortunately, constraints were put on the experimental design in terms of the number of lines available for measurement at any one time which meant a large error term resulting from spreading three replicate measurements for each of the substrates over the course os a day. When all the replicate collars for each substrate were measured at once on 29th August, the same patterns were observed In that there was no difference between the S and MS collars in terms of the $\delta^{13}C$ of soil CO_2 flux for any of the substrates (see Fig. 5.22). However, the pattern in terms of the average soil CO_2 flux was slightly different from over the whole time series (see Figs. 5.19), suggesting that the greater magnitude in terms of soil CO_2 flux was from treatment GLM, compared to treatment GLS, when the cumulative soil CO_2 flux measured for the nine days following substrate addition suggested the opposite treatment effect (see Fig. 5.18). A possible explanation for this discrepancy is that weekly watering took place on the morning of the 30th August, and prior to this on the 29th, the soil will probably have been at its driest. As was mentioned previously, the impact of soil moisture

extremes are something that needs to be investigated more fully, to find out if they can influence soil CO_2 flux to the extent that treatment effects may have been altered in the current experiments.

Though an attempt was made to improve on the design of the first experiment, by including soil collar-specific NS control collars (treatments NSS and NSM), it was found that even when blocked, the variation between individual collars was so large that it was not really possible to use these collars as a proxy for the underlying flux for the other treatments, when calculating the estimated substrate-derived return of C.

Whatever the explanation for the higher activity prior to substrate addition in both experiments, there was consistently higher activity where ECM fungi had been excluded (treatment S) in comparison to where they were not excluded (treatments MS and RMS) observed in both experiments. This higher level of activity resulted in a higher turnover of all the substrates from treatment S collars, reflected by the substrate-derived return of ^{13}C data shown in Figs. 5.11 and 5.23, and the greater % return of added substrate ^{13}C from treatment S shown in Tables 5.2 and 5.3. The faster turnover of both simple and complex substrates in the absence of ECM fungi in the current experiment contrasted the results of the previous girdling experiment (Chapter 4), where the input of labile substrates into the soil, cycled through roots and ECM fungi had a positive input on litter decomposition. It is possible that there is a difference in the level of C limitation caused by root/mycorrhizal exclusion and girdling on saprotrophic microorganisms, which would explain why in forest systems where the better decomposers are saprotrophs, excluding ECM fungi promoted decomposition of complex substrates alongside trenching, but reduced decomposition alongside girdling.

In summary, the differences observed between MS and S collars in terms of the relative utilisation of simple and complex substrates observed in the initial ^{13}C-labelled glucose decomposition experiment, and in the girdling experiment (see Chapter 4), are probably explained by the structural complexity of the substrates. Evidence was provided of suppression of r-strategist microorganisms in the presence of ECM fungi. There was evidence of N limitation of r-strategists following the potential input of labile substrates in throughfall, though further investigation is required. There was also

evidence that N content could influence the decomposition of more complex substrates, yet a longer-term experiment would be required to investigate this more fully. The current work has also added to the existing small body of literature concerning the turnover time of ^{13}C-labelled fungal cell wall material in forests, suggesting that even the more recalcitrant components of this little-studied substrate decompose over a relatively short period of time, when placed in the field.

Chapter 6 General Discussion

The aim of this thesis was to investigate the role of roots and associated ECM fungi on carbon (C) cycling in forest soils, including their contribution to soil CO_2 flux and their influence on other soil organisms. Soil organic matter (SOM) is a globally important sink for C, and it is becoming increasingly important that the fluxes of C in and out of the SOM are investigated (Fontaine *et al*. 2003; Schulze & Freibauer, 2005). The experiments presented in this thesis have focussed on the three main factors which control decomposition, as outlined by Swift *et al*. (1979): namely the quality of the substrate; the physico-chemical environment; the activity of the decomposer microorganisms. Throughout the litter decomposition and soil CO_2 flux studies presented in Chapters 2 and 3, the influences of temperature and moisture on litter decomposition were investigated. The influence of the quality of the substrate in terms of its N content and structural complexity was investigated in the simple and complex substrate decomposition experiment presented in Chapter 5.

6.1 The influence of ectomycorrhizal (ECM) fungi on decomposition in forest soils

The potential influence of ECM fungi on the C cycle was highlighted by Gadgil & Gadgil (1971; 1975), who observed decreased litter decomposition in the presence of ECM roots. This was attributed to suppression of saprotrophic microorganisms, which are believed to be the better decomposers (Lindahl *et al*., 1999). The observations of Gadgil & Gadgil (1971; 1975) were supported by later studies which also observed the 'Gadgil effect' (Fischer & Gosz, 1986; Genney *et al*., 2004; Chuyong *et al*., 2002). In contrast, results from other studies found an increase in decomposition rates in the presence of ECM roots (Dighton *et al*., 1987; Entry *et al*., 1991; Subke *et al*., 2004).

Through the use of exclusion collars, the experiments conducted as part of this thesis considered the influence of the ECM fungi, separately from that of roots. This allowed an investigation of how litter decomposition and also the decomposition of ecologically relevant substrates are influenced by these microorganisms. The current work has provided strong evidence to support the theory of Gadgil & Gadgil (1971; 1975), that competition between ECM fungi and saprotrophic decomposers in forest soils causes decreased decomposition. The results from the incubations of ^{13}C-labelled

simple and complex substrates (Chapter 5) showed that there was a significantly higher % of ^{13}C-labelled glucose mineralised to CO_2 under treatment S, than from treatments MS and RMS (see Table 5.2) and a significantly higher % mineralisation of ^{13}C-labelled lysine from treatment S than treatment MS (see Table 5.3). The higher glucose mineralisation rates in the absence of roots and ECM hyphae in the initial ^{13}C-labelled glucose decomposition experiment, and in the absence of ECM fungi in the later ^{13}C-labelled simple and complex substrate decomposition experiment, was attributed to changes in the r-strategy microorganisms. There appeared to be a trend towards greater decomposition of the structurally complex substrates, ^{13}C-labelled fungal cell wall material, and ^{13}C-labelled cellulose in the absence of ECM hyphae. However, this particular experiment did not run for sufficiently long to establish whether there would have been significantly higher turnover of substrates by K-strategist microorganisms when ECM fungi had been excluded.

In contrast to the results obtained for the individual substrates in the current work (Chapter 5) there was no evidence for a suppression of litter decomposition in the presence of roots and ECM fungi (Chapter 2). This inconsistency could potentially be explained by the species of ECM fungi dominant in the *Pinus contorta* stand at Wheldrake forest, and its ability to compete with other K-strategist saprotrophic decomposers (Dighton *et al*., 1987; Lindahl *et al*. 1999). Had the ^{13}C-labelled simple and complex substrate experiment run for longer, it may have been possible to see if the observed trend of higher decomposition of the structurally complex substrates in the absence of ECM fungi continued, and whether ECM fungi in that system also suppress the decomposition of complex, as well as simple substrates, and then potentially try to establish why this was not observed for the litter bag experiment described in Chapter 2. One possible explanation is that the nature of the positioning of the bags in a lattice design in the soil collars resulted in ECM fungi and other decomposer microorganisms having less access to the litter. In the girdling experiment presented in Chapter 4, and the ^{13}C-labelled simple and complex substrate experiments presented in Chapter 5, the added litter and substrates had a greater level of contact with the soil, and less interference from litter bag material, which has

been shown to alter the decomposition dynamics of litter, when compared to other methods (Bocock & Gilbert, 1957; Cotrufo *et al*., 2010).

Despite this inconsistency with the complex substrates, in the current work, there was certainly a much more rapid response to the addition of the structurally simple substrates, suggesting that in the presence of ECM fungi, r-strategist microorganisms are suppressed, and that 'microbial activation' (Kuzyakov, 2002) was much more pronounced where mycorrhizal hyphae had been excluded.

During the ^{13}C-labelled simple and complex substrate decomposition experiment (Chapter 5), the difference between treatments MS and S in terms of the return of ^{13}C added as part of the ^{13}C-labelled glucose approached significance ($p<0.051$) (see Table 5.3). This lack of significance, in comparison to the result of the previous ^{13}C-labelled glucose incubation (Table 5.2) was attributed to the smaller amounts of substrates added during the second experiment, leading to reduced CO_2 production. Indeed, these results highlight the power of isotopic labelling as a technique to differentiate between the various sources of soil CO_2 efflux (Subke *et al*., 2006; Kuzyakov, 2006). Without the isotope data ($\delta^{13}C$), it would not have been possible to detect differences in the decomposition rates of the substrates between the treatments. In contrast to the results obtained for the individual substrates in the current work (Chapter 5) there was no evidence for a suppression of litter decomposition in the presence of root and ECM fungi in the current work.

Suggested mechanisms by which ECM roots could potentially suppress saprotrophic decomposers include: the creation of a physical barrier and occupying microsites in the soil (Marx, 1969), anti-microbial action (Zak *et al*., 1964; Duchesne *et al*., 1988), the uptake of water by ECM roots drying the soil (Koide & Wu, 2003), and the selective uptake and immobilisation of key nutrients such as N (Stark, 1972; Bending & Read, 1995; Abuzinadah *et al*., 1986). As has already been discussed, the ability to extract N and P differs between ectomycorrhizal species but they are thought to be capable of bypassing parts of the N cycle, and making N unavailable for use by other soil microorganisms (Perez-Moreno & Read, 2000). The results of the experiment described in Chapter 2, which was designed specifically to test whether the influence

of ECM roots observed by Gadgil & Gadgil (1971; 1975) could be attributed to the uptake of water by roots also showed no evidence to support the hypothesis of Koide & Wu (2003). Also the lack of a significant moisture difference between the MS and S collars in the ^{13}C-labelled simple and complex substrate decomposition experiment means that the suppression effect observed could also not be explained by this mechanism. These results, for the first time, refute the frequent criticism of the original Gadgil & Gadgil (1971) studies in that they failed to control for the potential impacts of trenching on soil moisture. It would therefore appear that the impacts of the presence of ECMs on litter decomposition rates, as described by Gadgil & Gadgil (1971), can potentially largely be attributed to changes in microbial populations rather than physico-chemical or substrate quality changes.

Previous results have demonstrated that the N content of substrates affects their decomposition (Vance & Chapin, 2001; Cotrufo *et al*. 2004), and this was reflected in the current work with increased decomposition of ^{13}C-labelled lysine in comparison with ^{13}C-labelled glucose (Chapter 5). There is evidence that the influence of ECM fungi may change as a result of N deposition (Vallack *et al*. 2012), though this has also been shown to be dependent on the system, and the effect that N addition has on the growth of trees (Wallander *et al*., 2011). With future increased anthropogenic deposition of N over large areas of the globe (see Phoenix *et al*., 2006), it may be that plant C allocation patterns will change, and the current dominance of ECM fungi in temperate and boreal pine forests reduced. The evidence from the current work, and from studies such as McGuire *et al*. (2010), would suggest that ECM fungi may be retarding decomposition on a large scale, and reducing the amount of C released as a result of decomposition in forest soils. Though the addition of N has been shown to have varying effects on the decomposition of litter, there is evidence that when it is added to low quality litters with high lignin content, there is a stimulation of litter decomposition (Knorr *et al*. 2005). Therefore, it is important to assess alongside predicted changes in litter quality, whether the amount of C released from litter decomposition is altered by deposition of N, and the impact of a potential loss of dominance of ECM fungi in forest systems.

The current work included two studies where the contribution of ECM hyphae to total soil CO_2 efflux (R_s) was measured in the field. The contributions of ECM mycelium to soil CO_2 flux estimated by Heinemeyer *et al*. (2007; 2012) and Moyano *et al*. (2008) ranged between 5% to 30%, which is consistent with the results obtained in the current work. During the 18 months of CO_2 measurements presented in Chapter 3, ECM fungi contributed, on average, 12.8% of total soil CO_2 flux (Table 3.1). The contribution of mycorrhizal hyphae to R_s remained fairly constant throughout the year, suggesting that despite their potential conserving effect of SOM through retarding decomposition processes, ECM fungi are responsible for the return of an appreciable amount of plant-assimilate derived C to the atmosphere as CO_2. The CO_2 flux results from the trenching experiment presented in Chapter 3, and those from the girdling experiment presented in Chapter 4, were consistent in that they both showed that the underlying root and mycorrhizal components of R_a can react independently. Both experiments indicated that the decrease in R_a during the winter months was due to a decrease in the root component. This finding supports the arguments of Heinemeyer *et al*. (2007; 2012) that the mycorrhizal component, and sensitivity to environmental controls, should be considered separately from that of roots, and the other soil component fluxes, if modelling of forest soil C dynamics is to be accurate.

Not only do ECM fungi have a role in controlling the rate of decomposition through suppressing other microorganisms, they also provide a substrate for decomposition which in the past has been overlooked (Wallander, 2012). It has been estimated that 10-50% of photoassimilate-derived C allocated below ground is received by ECM fungi, which effectively replace fine roots (Smith & Read, 2008), and form extensive mycelial networks in the soil (Cairney, 2005). Högberg and Högberg (2002) stated that their estimate of 32% contribution of ECM mycelia to microbial biomass was likely to be an underestimate, because it didn't take into account the utilisation of ECM mycelium as a substrate by other soil microorganisms. The current work has also added to a small, but developing body of knowledge surrounding the nature of fungal mycelial turnover in forest soils (Koide & Malcolm, 2009, Wilkinson *et al*., 2011; Drigo *et al*., 2012), showing that in a period of nine days, *ca.* 14.8% of ^{13}C added as part of the fungal cell

wall material was returned from treatment S, and *ca.* 8.4% was returned from treatment MS (Chapter 5).

6.2 A promoting influence of the presence of roots on litter decomposition

Though there has been increased interest in C priming since the 1980's, work still needs to be done to investigate the mechanisms controlling the priming of SOM decomposition through labile C input, with studies showing contrasting effects of the addition of labile C on SOM decomposition (Dormaar, 1990; Kuzyakov, 2010). In the Norway spruce plantation where Subke *et al*. (2004) carried out their ^{13}C-labelled litter decomposition experiment in conjunction with forest girdling, it would appear that the input of labile substrates into the soil through both the presence of roots, and leached from added litter, had a positive priming effect on both SOM and litter decomposition.

In the current work, the litter decomposition experiment conducted alongside forest girdling (Chapter 4, Subke *et al*. 2011) supported the work of Subke *et al*. (2004), demonstrating a clear link between the activity of plants, and their allocation of C to the soil and surface litter mass loss. This finding was in contrast with the results of the litter decomposition experiment conducted alongside soil trenching (Chapter 2) where there was no difference in mass loss, expressed as k-values observed between any of the six treatments (see Table 2.1), and the results of the ^{13}C-labelled simple and complex substrate decomposition experiment (Chapter 5).

It is possible that these apparent contradictions can be explained by differences in the severity of the method used to exclude ECM roots between the different studies. There is no disputing that ECM fungi appear to be causing reduced decomposition where they are present (Chapter 5). However, the complete disruption of labile plant-assimilate C input, alongside other substrates in plant root exudates during girdling is likely to cause much more severe C-limitation in girdled soil than is observed in trenched soil, which could decrease the decomposition of more complex substrates.

The word 'trenching' has been used in this thesis to describe methods where plant roots and mycorrhizal hyphae have been severed and then excluded from areas of soil. In the experiments presented in Chapters 2, 3 and 5, this was done using exclusion

collars made of PVC. Other studies have used metal barriers (Faber & Verhoef, 1991), or plastic linings (Zhu & Ehrenfeld, 1996). Gadgil & Gadgil (1971; 1975) refer to 'cutting' the soil with a spade, and then re-cutting every two weeks. Staaf *et al*. (1988) also cut with a spade, but then only re-cut twice a year. Clearly, there is a large amount of variation in the methodologies used to exclude roots and mycorrhizal hyphae and these variations may go some way towards explaining the differing results.

The literature assessed in Chapter 2 (see Table 2.4) with regards to the Gadgil effect needs to be re-evaluated to see if there is a pattern with regards to the effect of ECM roots and the exclusion method used. For example, studies where there was a promotion of litter decomposition in the presence of ECM roots tended to be where girdling had been used (Subke *et al*., 2004; Subke *et al*., 2011), or where there had been a physical barrier installed following cutting, as in the case of Zhu & Ehrenfeld (1996), who lined their trenches with plastic. Faber & Verhoef claim that there was a reduction of decomposition rates of 2% in the presence of roots, compared to where they had been excluded using metal screens. However, upon closer inspection of their results, in the control treatment (their study was designed to look at the impact of collembolan species), after 7 months, there was greater weight loss where roots were not excluded (see Fig. 1, Faber & Verhoef, 1991). In contrast, in studies where girdling or no physical barriers were used to exclude the influence of roots (e.g. Gadgil & Gadgil, 1971; 1975; Cuenca *et al*., 1983; Staaf, 1988; Chuyong *et al*., 2002; Koide & Wu, 2003) there tended to be no effect, or a negative effect, of roots on decomposition. An exception to this pattern is a girdling study conducted by Wu *et al*. (2011), where no effect of the exclusion of plant assimilate-derived C on litter decomposition was observed.

In the litter decomposition experiment presented in Chapter 2, the exclusion collars used did not block the input of soil water into the collars, which could flow in through the mesh windows. Therefore any dissolved organic carbon (DOC), alongside other dissolved materials would be able to enter the collars. This could also have occurred in studies where no physical barrier was used, with studies tending not to indicate the size of the air gap created by their cutting techniques. It is possible that the conditions of C-limitation caused in girdled plots, or the more elaborate trenched plots have a

greater retarding effect on litter decomposition than in environments where there is still plant-assimilate input through root or ECM exudates available to saprotrophic organisms as DOC. Högberg & Högberg (2002) demonstrated that following girdling, there was a 45% decrease in DOC in girdled plots, when compared to controls.

It is possible in the currrent *Pinus contorta* stand that plant assimilate-derived C was still priming the decomposition of litter in the exclusion collars to a certain extent. This could explain why there was not the same indication of the reliance of soil decomposers on plant-assimilate C in the litter decomposition experiment presented in Chapter 2, as there was in the girdling study presented in Chapter 4 of the current work. Schaefer *et al*. (2009) used both trenching and girdling in the same plot to estimate the R_h flux and found that it appeared lower from the girdled plots. However, the same comparison was done in Subke *et al*., (2011, Chapter 4) and there was no significant difference between the R_h flux measured using the PVC exclusion collars in the control plots, or between the girdled and control plots (see Fig. 3, Subke *et al*., 2011). Overall, plant-assimilate C input to the soil is clearly important, priming the activity of soil microorganisms, which has been reported on numerous occasions (reviewed in Dormaar, 1990; Kuzyakov, 2002), and when this supply is comprehensively removed from large areas of soil using techniques such as girdling (Subke *et al*., 2006), this may override any suppressing effect that ECM fungi have on litter decomposition in forest soils.

6.3 The influence of moisture and temperature on decomposition

Climate has been acknowledged to be one of the most important drivers of decomposition processes in forest soils, affecting the rate at which the different stages of decomposition can proceed (Swift *et al*., 1979).

In contrast to previous studies (Virzo de Santo *et al*., 1993; Koide & Wu, 2003), soil moisture was shown not to be limiting to either litter decomposition (Chapter 2), or soil CO_2 efflux (Chapter 3) during the current work. Though soil moisture was significantly correlated with the 'watered' treatments, RMS(W), MS(W), S(W) and also treatment S, the associations were weak. This lack of correlation between soil

moisture and soil CO_2 flux is a common finding as, with seasonal changes, levels of moisture are generally low when other influencing variables such as temperature and assimilate supply are peaking (Davidson *et al*., 1998; Sulzman *et al*., 2005).

Soil moisture contents in the *Pinus contorta* stand at Wheldrake forest, even in treatment RMS which had the lowest soil moisture contents of all the treatments during the summer months, never fell below 20%. Though previous work by Heinemeyer *et al*. (2007) at the same site demonstrated that soil moisture limited the ECM flux component during a period of drought, no such effects were observed in the current study.

Temperature on the other hand explained *ca*. 66% of the variation in litter mass loss (Table 2.3), and 53-67 % of the variation in soil CO_2 efflux in the *Pinus contorta* stand at Wheldrake Forest (Table 3.3). In the current work, there was evidence of the flux component from roots having a higher temperature sensitivity than the components from mycorrhizal hyphae or soil heterotrophs (Table 3.2). This is in contrast with previous results at the same site, for a shorter period of time by Heinemeyer *et al*. (2007), who observed that R_h was more influenced by temperature than R_a. Moyano *et al*. (2008) reported that the flux components from all sources were correlated with temperature, apart from the mycorrhizal flux component at one of the two sites they studied. The results from the current work, and those of Heinemeyer *et al*. (2007; 2012) and Moyano *et al*. (2008) have provided evidence that the mycorrhizal flux component responds differently to environmental drivers than the root component, and that therefore these two components of R_a should be modelled separately in the future.

6.4 Suggestions for further work

This thesis has highlighted the link between above-ground and below-ground activity, in terms of the promoting influence of plant-assimilate C, cycled into the soil via roots on litter decomposition. It has also clearly demonstrated that the presence of ECM fungi has a negative influence on the turnover of substrates in forest soils. In light of the results presented in the current work, there is a need for future studies excluding ECM fungi from areas of soil, and measuring their influence on decomposition, and to

use molecular techniques in order to measure the associated changes in microbial communities. This could be achieved using a combination of pyrosequencing of bacterial and fungal communities and analysis of functional genes involved in the decomposition of cellulose such as the fungal cellobiohydrolase (exocellulase) gene *cbh-I,* as used by Baldrian *et al.* (2012). This would hopefully lead to a better understanding of the mechanisms driving suppression.

Though the experiment presented in Chapter 5 incorporated substrates which included or did not include N, the influence of N on the role of ECM fungi has not been properly considered as part of the current work. Different ECM fungi have been demonstrated to have different capabilities with regards to decomposing complex N-containing substrates. The suggested suppression mechanism regarding the uptake of N by ECM fungi (Abuzinadah *et al*., 1986) should be investigated to a greater extent in the field, in order to fully appreciate the role that ECM fungi play in carbon storage, and how this may change with N deposition, as highlighted by the work of Vallack *et al*. (2012).

Further work is required where the ECM flux component is studied in the field, between a range of environments, alongside the measurement of environmental parameters, and measurements of photo-assimilate supply, in order to separate out effects of temperature, and substrate supply. It would be interesting to study whether overall, there is a balance between the conservation of C as part of SOM by ECM fungi, and their release of photo-assimilate derived C as CO_2. For example, does the presence of ECM fungi in a system result in net increased or decreased C storage, compared to systems where they are not present or have been excluded?

Such work should be combined with further attempts to study the decomposition dynamics of fungal biomass in forest soils, where if possible, fungal mycelia has been cultured under more 'natural' conditions, and not in media optimal for microbial growth.

Considering the importance of the global C cycle in the context of climate change (IPCC, 2007), particular attention should be paid in future work to fully understand the mechanisms controlling the potentially huge role ECM fungi could be playing in the global C cycle.

Appendix 1 Example Campbell CR1000 program

```
'CR1000

'Created by Short Cut (2.5)

'Declare Variables and Units

Dim Voke

Dim TCount

Public Badger1

Public Badger2

Public Batt_Volt

Public SM200(9)

Public SWC(9)

Public SoilOnly_SWC

Public SoilMyc_SWC

Public SoilMycRoot_SWC

'Public T_mV(4)

'Public T_kOhm(4)

'Public T_C(4)

Public PanelT


Units Batt_Volt=Volts

Units SM200=V

Units SWC=% volumetric

'Units T_mV=mV

'Units T_kOhm = kOhm

'Units T_C = deg C

Units PanelT = deg C
```

```
'Define Data Tables
DataTable(Table1,True,-1)
DataInterval(0,10,Min,10)
'Sample (4,T_mV(1),IEEE4)
'Sample (4,T_kOhm(1),IEEE4)
Sample (1,PanelT,IEEE4)
'Sample (9,SM200(1),FP2)
Sample (9,SWC(1),FP2)
Sample (1,SoilOnly_SWC,IEEE4)
Sample (1,SoilMycRoot_SWC,IEEE4)
Sample (1,SoilMyc_SWC,IEEE4)
Sample (1,Badger1,IEEE4)
 Sample (1,Badger2,IEEE4)
'Sample(1,SM200(2),FP2)
'Sample(1,SWC(2),UINT2)
'Sample(1,SM200(3),FP2)
EndTable

DataTable(Table2,True,-1)
DataInterval(0,10,min,10)
Minimum(1,Batt_Volt,FP2,False,False)
EndTable

'Main Program
BeginProg
Scan(10,Min,1,0)
Badger1=0Badger2=0
```

```
'Default Datalogger

Battery Voltage measurement Batt_Volt:

Battery(Batt_Volt)

'Turn AM16/32 Multiplexer On

'PortSet(1,1)

'Turn SM200s On

PortSet(9,1)

Voke=1

 'Delay  scan for 2 second warm-up period

Delay(0,2,Sec)

'SubScan(0,0,2)

'Switch to next AM16/32 Multiplexer channel

'PulsePort(2,10000)

'SM200 soil moisture content measurements mV on the AM16/32 Multiplexer:

VoltSe(SM200(Voke),9,mV2500,1,0,0,250,.001,0)

'Voke=Voke+1

'NextSubScan

'Conversion of mV signal to SWC

For Voke=1 To 9

SWC(Voke)=-0.039+2.091*(SM200(Voke))-
(5.029*(SM200(Voke))^2)+(7.907*(SM200(Voke))^3)-
(5.978*(SM200(Voke))^4)+(1.758*(SM200(Voke))^5)

Next

'Turn SM200s Off

PortSet(9,0)

'Turn AM16/32 Multiplexer Off

'PortSet(1,0)
```

```
'Average Treatments

SoilOnly_SWC=(SWC(1)+SWC(2)+SWC(3))/3

SoilMycRoot_SWC=(SWC(4)+SWC(5)+SWC(6))/3

SoilMyc_SWC=(SWC(7)+SWC(8)+SWC(9))/3

'Irrigation instruction; switches 12 V output if SWC in root collars is lower than average of "soil only" and "Mycorrhiza" collars

'Badger is in there just to log when the pumps were switched on and off

If SoilOnly_SWC-SoilMycRoot_SWC>0.03 Then

PortSet(3,1)

Badger1=1

EndIf

Delay(0,15,Sec)

PortSet(3,0)

If SoilOnly_SWC-SoilMyc_SWC>0.03 Then

PortSet(5,1)

Badger2=1

EndIf

Delay(0,15,Sec)

PortSet(5,0)

'Measure Air Temperature

'TCount = 1

BrHalf(T_mV(TCount),4,mV2500,10,VX1,4,1000,1,0,_50Hz,1,0)

'Convert voltage to resistance

'T_kOhm(TCount) = 2*(1-T_mV(TCount))/(T_mV(TCount))

'T_C(TCount) = -21.042*LN(T_kOhm(TCount)) + 39.406

'Generate ratio of resistance, where calculated resistance is devided by resistace at 0 deg C (needed as input for following instruction)

'RR_T(TCount) = T_kOhm(TCount)/6.53
```

```
'Convert resistance to temperature in deg C
'PRT(T_C(TCount),1,RR_T(TCount),1,0)
PanelTemp(PanelT,_50Hz)

'Call Data Tables and Store Data
CallTable(Table1)
CallTable(Table2)
NextScan
EndProg
```

Appendix 2

Components of Modified Melin Norkrans (MMN) growth medium (Marx, 1969) used to culture ^{13}C-labelled fungal cell wall material for use in the simple and complex substrate decomposition experiment (Chapter 5). ¼ strength solution was used.

	Full strength: In 1 litre distilled H_2O	¼ strength: In 1 litre distilled H_2O
Diammonium hydrogen orthophosphate $(NH_4)_2HPO_4$	0.25 g	62.5 mg
Potassium dihydrogen orthophosphate KH_2PO_4	0.50 g	0.125 g
Magnesium sulphate $MgSO_4.7H_2O$	0.15 g	37.5 mg
Calcium chloride $CaCl_2.2H_2O$	0.05 g	12.5 mg
Sodium chloride NaCl	0.025 g	6.25 mg
FeEDTA (2%)	24 mg	6.0 mg
Thiamine (not autoclaved, filtered separately	0.1 mg	0.025 mg
D-glucose (not autoclaved, filtered separately)*	1.0 g	1.0 g
Malt extract**	3.0 g	0.75 g
Agar (oxoid No. 3)***	15.0 g	15.0 g
Adjusted to pH 4.7 with HCl and autoclaved		

*^{13}C-labelled medium contained 10% ^{13}C-labelled glucose (99 atm%), 90% non-labelled glucose

**excluded from the media when culturing the non-labelled fungal biomass (malt extract)

***agar excluded from liquid media

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