Plant functional traits and vegetation strategies

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Summary

In this thesis we tested the predictions from the CSR theory for the community membership. Predictions are that, in the absence of competition, species from all strategies (*Competitors*, *Ruderals* and *Stress-tolerants*) will persist in low-stress habitats, whereas in high-stress habitats, only species with *Stress-tolerant* traits will survive. CSR recognizes that species evolved similar traits to one universal stress. For this reason, we were interested in testing which strategies will survive in different sources of stress. Our results from field and greenhouse experiments suggest that CSR theory does not predict community membership from the initial stages of a plant life-cycle. Instead, we found that the habitat stress plays a major role in determining the species that are incorporated into a community.

In this thesis we also used a trait-based approach to evaluate: 1) the relationship between key traits using annuals species, and 2) the links between genome size and phenotypic variation within species. Firstly, we studied the triangular relationship reported for seed mass and leaf area in woody species. These traits are involved in the plant reproduction strategy and plant water and energy-use. We found a triangular relationship in annuals species, suggesting that is conserved across groups (woody and annuals). We also found that the driver of this relationship is related to soil fertility. Finally, for the relationship between genome size and phenotypic variation within species, we found that larger genome species display higher variation in traits than small genome species. This can be a potential advantage in heterogeneous environments where the amount of phenotypic variation would allow the species to adapt to them.

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Chapter 1

General Introduction

1.1 Plant functional traits

Plant functional traits (PFTs) are "the morphological, physiological and phenological characteristics that represent ecological strategies and determine how plants respond to environmental factors, affect other trophic levels and influence ecosystem properties" (Perez-Harguindeguy et al. 2013). PFTs reflect the organisms' role in the ecosystem (Diaz and Cabido 2001), affect the organismal performance (McGill et al. 2006), and are an indicator of the constraints and opportunities that an organism faces in its habitat (Perez-Harguindeguy et al. 2013). For this reason, PFTs represent trade-offs between growth and reproduction, resource capture and conservation, or light and water acquisition/nutrient acquisition (Lavorel and Garnier 2002).

In general, plant functional traits can be distinguished into 'soft traits', which are easy to measure or assess, and are considered surrogates of other 'hard traits'. The latter give more accurate information of the species effects and responses in the ecosystem, but they are not easy to quantify for many species, and hence are less explored (Hodgson et al. 1999). For example, seed mass and shape are considered soft traits and are indicators of seed persistence in the soil, which is a hard trait ((Thompson et al. 1993, Funes et al. 1999), but see (Leishman et al. 2000) for studies in Australia and New Zealand in which none relationship or the opposite trend was found). Some of the more studied plant functional traits are specific leaf area (or SLA), plant height, and seed mass (described in more detail below, Section 1.2). These traits are relatively easy to measure, and reflect the axes of resource conservation and leaf-turnover (specific leaf area or SLA), competitive ability (plant height), fecundity and success of establishment (seed mass) (Lavorel et al. 2007).

Functional traits display a great amount of variation within and across species. Despite this, global studies have identified trait-relationships that reveal the plants functioning across biomes. For example, the negative relationship between leaf nitrogen and leaf life-span shows resource-allocation in plants, and is related to herbivore interactions. Higher leaf nitrogen makes species more palatable, which would reduce the leaf life-span (Reich et al. 1997). This makes a set of plant characteristics useful predictors for communities and ecosystem functioning (Diaz et al. 2004), as they provide links of species distribution patterns to environmental gradients (Poorter 2007). For example, plant communities with large leaves favor moisture in soils, whereas communities of short leaved plants and high root length are associated to drought conditions (Gross et al. 2008).

1.2 Plant functional traits: environmental relationships and allometries

Seed mass (the weight of an average oven-dry seed [mg])

Seed mass is related to the dispersal, fecundity and recruitment of a plant (Lavorel et al. 2007). Small seeds are easier to bury in the soil and hence they form seed banks more readily than big seeds (Thompson 1987). Small seeded species also produce more seeds per unit of energy than larger seeded species (seed size/number trade-off, (Jakobsson and Eriksson 2000)), and have a higher relative growth rate (RGR (Westoby et al. 1992)). However, some short-lived species with bigger seeds can grow faster at a given size (Turnbull et al. 2012)). Also, large-seeded species have been shown to have a competitive advantage over small seeded species (Turnbull et al. 1999), through the colonization/competition trade-off. This means that large-seeded species have higher recruitment at higher densities than small-seeded species.

In relation to nutrient availability in the soil, conflicting evidence is found for seed size; seed size is positively (Marañón and Grubb 1993, Grubb and Coomes 1997) or negatively (Lee and Fenner 1989, Dainese and Sitzia 2013) or unrelated (Hammond and Brown 1995, Wright and Westoby 1999) to soil nutrient content. Seed mass tends to increase with rainfall (Moles et al. 2014) and temperature (Moles et al. 2005a, Pakeman et al. 2008).

Plant height (shortest distance between the upper boundary of the main photosynthetic tissues on a plant and the ground level [m]).

Plant height is positively related to its life span, and the ability of a species to compete for light (Moles et al. 2009). Plant height also shows allometric relationships with plant traits like seed mass (Leishman et al. 1995) and leaf area (Cornelissen et al. 2003). Short stature is considered a response to disturbance (Lavorel et al. 2007), and plants are much taller in the tropics than in temperate zones, related to the warmer, wetter and more productive conditions found in lower latitudes (Moles et al. 2009).

Leaf size; area (the projected surface area of one side of a leaf [mm^2]), and width (the largest imaginary circle that can be fitted on the leaf surface).

Leaf area is involved in the control of leaf energy and water balance (Givnish 1987, Cornelissen et al. 2003). Leaf area has allometric relationships with seed mass (Cornelissen 1999) and plant height (Cornelissen et al. 2003), and it varies significantly between non-woody (ferns, graminoids and forbs) and woody species (shrubs, trees and vines) (Niklas et al. 2007). Small leaves are generally interpreted as an adaptation to drought and high-radiation (Ackerly 1999, Ackerly and Reich 1999, Cornelissen et al. 2003).

Leaf width is related to the control of heat loss, with narrower leaves (associated to sunny environments) having more effective heat loss. This is because they have a smaller boundary layer (the static thin layer of still air holding the leaf surface) than broad leaves, thus avoiding overheating (Niinemets and Kull 1994).

Leaf thickness; leaf tissue density [mm]

The thickness of a leaf affects the amount of light that it can absorbed and the CO2 diffusion through the leaf tissues. Thicker leaves are long-lived, their construction is expensive to the plant and are associated with low growth and photosynthetic rates (Vile et al. 2005).

Specific leaf area or SLA (leaf area per unit dry mass [mm^2 mg^{-1}])

SLA reflects nutrient residence time, so species that live in nutrient-poor habitats will maximize the residence by having a low SLA (Lavorel et al. 2007). Species with high SLA are related to fertile habitats, have short-life leaves, and are more vulnerable to herbivores than species with low SLA values (Cornelissen et al. 2003). However, SLA is difficult to measure in plants with vertical leaves, or in plants like cacti (Wilson et al. 1999).

Leaf dry matter content or LDMC (dry mass of a leaf divided by its fresh mass [mg g^{-1}])

LDMC is related to the tissue density (Cornelissen et al. 2003), and it indicates the resource strategy of a species. A high LDMC is associated with low-fertility habitats, and resistance to hazards (Perez-Harguindeguy et al. 2013).

Guard cell length (in µm)

Guard cells surround the stomata and control their opening (Chater et al. 2014). Hence, guard cell length is used as a proxy for the size of the stomatal pore. Leaves with small guard cells are more efficient in water –use than leaves with larger guard cell length (Charles et al. 1997). Small cell size is associated with resistance to dry conditions, because cells can maintain turgor even in water deficit conditions, which controls stomatal opening (Cutler et al. 1977).

Stomatal density (number of stomata per mm2).

Stomatal density is related to the water economy of the plant, many small stomata are more efficient in terms of water loss than a few large stomata (Charles et al. 1997). In general, stomatal density decreases with the increase in CO_2 concentrations (Chater et al. 2014). An increase in stomatal density is usually accompanied by a decrease in stomatal size (Franks and Beerling 2009), and epidermal cell area. In dry habitats, many small stomata respond faster to water deficit and more stomata allow diffusion of CO_2 (Beaulieu et al. 2008).

Genome size: 2C DNA and 1Cx values [picograms (pg) or megabase pairs (Mbp)]

Genome size refers to the total amount of DNA content in a nucleus. It is a strong predictor of cell size and stomata density, with large-genome size species having larger cells and low stomatal density, and it is positively related to other plant traits like seed mass. This relationship is likely to be a consequence of the nucleotype effects, *i.e.* organs like cotyledons become larger because the cells forming them become larger (Beaulieu et al. 2008).

Given that the amount of DNA in a nucleus varies throughout the cell cycle, two measures are commonly used (Greilhuber et al. 2005, Pellicer and Leitch 2014): 1) Holoploid (2C-value) refers to the amount of DNA in a somatic cell at the first stage of the cell cycle, regardless of the ploidy level, and 2) Monoploid (1Cx value); is the amount of DNA taking into account the polyploidy level. Genome size (2C-value) in angiosperms varies nearly 2000-fold across species (Leitch and Leitch 2008). Much of this variation has been quantified between species and this is attributed to the accumulation of repeated sequences (Biemont 2008). Variation in genome size within species (at the individual or population level), remains controversial for plants (Bennett and Leitch 2005). This is because this variation is considered to be an artifact resulted from not using the appropriate technique or making taxonomic mistakes (Greilhuber 1998, 2005). In the particular case for *Zea mays*, intraspecific variation has been confirmed. Genome size has been reduced from the lower to the higher latitudes, which has been related to the reduction in the growing season, *i.e.* higher latitude varieties reduced their cell cycle, producing more cells (Bennett and Leitch 2005). One of the aims of this thesis is to explain the allometric relationships between plant functional traits in terms of habitat variables. Also, to evaluate if genome size variation across species is linked to functional trait variation within species.

1.3 Grouping plant functional traits into strategies

PFTs are used in broad functional classifications of species (Westoby 1998, Hodgson et al. 1999), which aim is to predict the community structure and the ecosystem processes. These classifications are varied, and are mainly based on the response of plant species to nutrient availability (or stress) and disturbance. Some of the most cited classifications are: 1) The CSR theory which groups species into vegetation strategies. These strategies, different to schemes like LHS (explained below), are sets of traits that respond similar to different levels of disturbance and stress. *Ruderal* species have short-life cycles, short time to flower production and duration of reproduction, and so they grow faster in respond to the high disturbance in their habitat. *Competitor* species have high canopy height, lateral spread and dense biomass above and below ground and grow in undisturbed and productive habitats, and *Stress-tolerant* species which grow slow and have long-lived tissues to cope with unproductive, non-disturbed habitats (Grime 1979), 2) The LHS scheme, which is based on single functional traits, and positions a species depending on three axes of variation: SLA which responds to favorable growth conditions, and canopy height and seed mass which respond to disturbance. This scheme recognizes that there can be viable strategies under high-stress and disturbance, contrary to CSR theory (Westoby 1999), and 3) The *r-K* model, which positions species in the slow or fast continuum depending on the species traits. Species in unpredictable, density-independent conditions, will be under *r*- selection, and so they will present traits like early reproduction, rapid development, and short-life cycles (which is similar to the *Ruderal* species' traits that evolved under high-disturbance, from CSR model). If growing under predictable, density-dependent environments, species will be *K*-selected and they will grow slow, longer life-cycles (similar to the *Stress-tolerant* traits in CSR theory) than *r*-selected species, but greater competitive ability (Pianka 1970).

One of the most cited and debated models is CSR theory. The initial classification was initially developed for herbaceous species in the UK (Grime et al. 2007), and it has been extended to woody and all vascular species (Pierce et al. 2014), and into organisms other than plants (Grime and Pierce 2012a). However, predictions from this theory have been rarely tested (Wilson and Lee 2000). Also, CSR theory has some other limitations; 1) the traits used to classify species into one particular strategy are also found in species with a completely different strategy. For example the lateral spread, which constitutes a *Competitor* trait is also found in *Stress-tolerant* species that have stoloniferous growth (Cerabolini et al. 2010). This could have important implications for the community membership predicted from CSR theory, 2) CSR theory considers one universal type of stress, and so species respond in the same way regardless of the source of stress. However, different types of stress can lead to different plant communities (Tilman 1988), suggesting that the type of stress could influence the strategies that are most beneficial towards being incorporated into a community, and 3) CSR theory is based on the species traits at the established phase (the regenerative and the established phase are considered to be uncoupled), and states that depending on the disturbance and stress levels it is possible to predict the adult (established) traits for a community. However, there is evidence showing that species are adapted to their regenerative niche and not to the adult niche (Poorter 2007). Suggesting that predictions from CSR theory in terms of the community membership might no be predictable from the initial phases of establishment.

For these reasons, the other aim of this thesis is to test whether CSR predictions on community membership, predict which species could be part of the community membership from the initial phases (regenerative) of the plant-life cycle.

Thesis outline

Chapter 2

In this chapter we explore the predictions of community structure from CSR theory. Can we predict which species become part of a community once competition is removed? We investigate this under field conditions in low and high-stress habitats.

Chapter 3

In the field there are many factors that are not possible to control for and that could have an effect on the species membership in low and high-stress habitats. For example, during the field experiment (chapter 2), many of the plots were affected by flooding. Whilst this is not expected to kill the seeds (Baskin and Baskin 2001), the water might have taken the seeds away of the plot, which would have caused a reduction in the observation of seedling emergence. In this chapter we explore the effects of low and high-stress habitats on species membership once competition is removed under controlled conditions.

Chapter 4

Plant allometries give us insight into the trade-offs underlying them. Seed mass and leaf area have been found to be related in a triangular way in woody species, *i.e.* plant species with big leaves can produce either big or small seeds, but species with small leaves can only produce small seeds. In this chapter we ask if this relationship can be generalized and extended to annual species that could potentially have different allometries, given that this species have a higher investment in reproduction. Also, how is this allometric relationship dependent upon environmental conditions of soil fertility and light.

Chapter 5

Genome size is related to functional plant traits across species, *e.g.* cell size, seed mass and leaf area. It has been hypothesized that large genome species pay costs for the accumulation of 'junk DNA', *i.e.* display less phenotypic variation. In this chapter we argue that if this is true, then this constraint should be detected within species. We explore this question using phylogenetic methods that allow us to control for species relatedness and hence evaluate for ecological significance of the possible relationships between genome size and other plant traits.

Chapter 2

Stress is the main filter for plant-community membership

2.1 Abstract

The CSR theory proposes that species have evolved sets of traits mainly in response to disturbance and stress (Grime 1979). These traits allow species to be grouped into different vegetation strategies: *Competitors*, *Stress-tolerators* and *Ruderals* or a combination of these. In fertile, low-disturbance habitats, *Competitor* species will dominate, but if stress is increased *Stress-tolerators* become dominant. Here we ask if the effects of the environment on seedling recruitment, in the absence of competition with established vegetation, can be predicted using CSR theory. We also explore the effects of seed mass asking whether large seeded species have better survival and if this varies with stress. We found that seedling survival was not in agreement with the predictions of CSR theory, and seed mass did not influence seedling survival. From the results of this experiment, we conclude that once competition is removed, stress is the main filter determining which species can be incorporated into a community, but this occurs regardless of the CSR strategy.

2.2 Introduction

Two major determinants of community membership are disturbance and stress (Grime 1979). Disturbance causes partial or total destruction of the plant biomass, whilst stress reflects the external constraints on photosynthetic production. These factors act like filters allowing species with specific sets of traits to persist and dominate in different habitats. Combinations of the levels of disturbance and stress, *e.g.* low disturbance and high stress, promote the evolution of three main plant strategies in the established phase of the plant life-cycle ((Grime 1979) , Fig. 1)). This means that in a particular type of habitat, species membership should be determined by the levels of disturbance and stress, and be reflected by the presence of particular plant vegetation strategies.

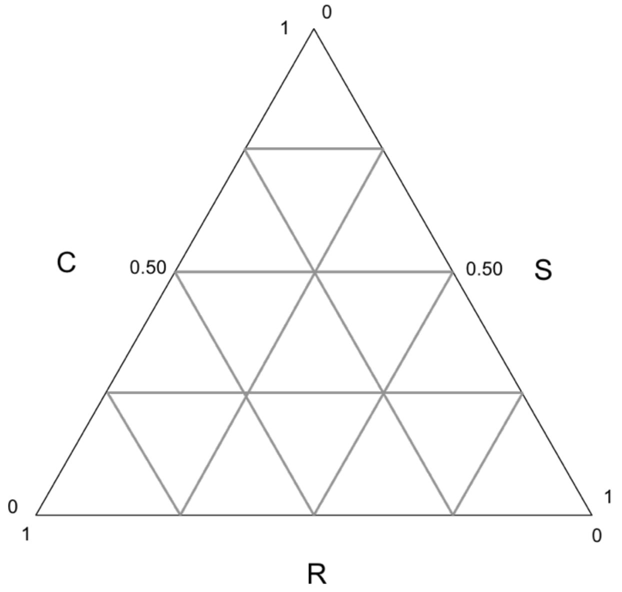

Figure 1. Triangular ordination of the vegetation strategies (modified from Grime et al. (2007)). Plant species have evolved a different strategy depending on the levels of stress and disturbance; *C*: *Competitors, R*: *Ruderals*, *S*: *Stress-tolerators*. Intermediate levels of disturbance and stress would cause combinations of the main vegetation strategies. For example: a *RC* (*Ruderal-Competitor*) species would have 0.5 of each vegetation strategies traits.

Competitors are species that dominate in productive, infrequently disturbed habitats. They compete for resources by growing fast, which can be achieved by a rapid leaf expansion and the development of plants with tall shoots and high root density. In these highly fertile habitats, competition is the main process that determines which species can persist. However, when stress is increased by low water availability, low temperature, low nutrient or plants shading other strategies, *Stress-tolerators* are expected to occur. *Competitors* do not occur in stressful habitats not because of competition exclusion but because the environment is too harsh for their populations to persist. *Stress-tolerators* are characterized by having slow growth and long-lived tissues, which allows them to cope with low resource availability. Other traits that are used to define the *Stress-tolerators* strategy are leaf weight, SLA and dry matter content, as they are thought to reflect species capacity to withstand herbivory, and slow-growth conservative resources strategy (Hodgson et al. 1999). The third plant strategy, *Ruderals*, consist of species specialized for fast growth, in habitats with frequent disturbances, but low stress. *Ruderal* species generally complete their life cycle quickly, *e.g.* annuals or short-lived perennials, during the periods between disturbances. In summary, there are three fundamental vegetation strategies. Nevertheless, along stress and disturbance gradients, there are species with a intermediate strategies, *e.g. Ruderal-Competitor* (Grime 1979).

This suggests that if competition is removed in a productive habitat then species of all strategies will successfully colonize, as competition is the main determinant of community membership. However, in stressful habitats, when competition is removed it is the environment that determines community membership and so only *Stress-tolerant* species can successfully colonize. Testing this predictions is important as it is a key assumption of CSR theory (Grime and Pierce 2012b), and has previously only been explored indirectly using data from simple competition experiments (*e.g.* (Kadmon 1993, 1995) data on *Stipa capensis* analysed by (Brooker and Kikvidze 2008)). Also, because predictions from CSR theory are rarely tested, but CSR strategies are widely used for characterizing species from a community (Austin and Gaywood 1994, Wilson and Lee 2000).

Studies of seed size have shown that higher recruitment success is associated with larger-seed mass (Jakobsson and Eriksson 2000). Also, species with bigger seeds have a competitive advantage over small-seeded ones (Gross 1984, Rees 1995, Turnbull et al. 1999), but see Fenner (1978) in which no relationship was found).They also experience less risk of mortality caused by herbivory (Moles and Westoby 2004), germinate faster and so are more likely to initially occupy space (Eriksson and Jakobsson 1998). These attributes become important for species with different vegetation strategies, as some might colonize faster than others and this, in part, will determine the community membership (Gough et al. 2000a).

2.3 Objectives

In this chapter we ask whether vegetation strategies (CSR) influence which species can establish in sites varying in their stress levels. This was done after removing the established vegetation so competition could not prevent recruitment. We tested the following predictions derived from the *CSR* theory:

1) In low-stress sites, seedlings from all the vegetation strategies can survive, because the environment is not stressful and the effects of competition have been removed. However, in high-stress sites only *Stress-tolerators* can survive, because even after removing the effects of competition, the environment is too stressful to allow establishment.

2) In low-stress sites, *Competitors* will have the highest seedling survival, as they are better adapted to low-stress conditions (*i.e.* they expand leaves faster, leading to a fast growth).

3) Seedling emergence would be greater in low-stress sites in comparison with high-stress sites ones, where low pH might prevent seeds from germinating.

4) Large seeded species would have higher seedling survival.

2.4 Methods

2.4.1 Study sites

A large field experiment examining seedling emergence and survival was conducted for five months at ten sites differing in stress. The sites were located in Sheffield and at the Longshaw Estate in England (Fig. 2). The sites were all between 84 and 131 m a.s.l., mean annual precipitation is 796 mm (average of 131 years; Met office data from the Sheffield station), and during the year of study, the total precipitation was 1146 mm.

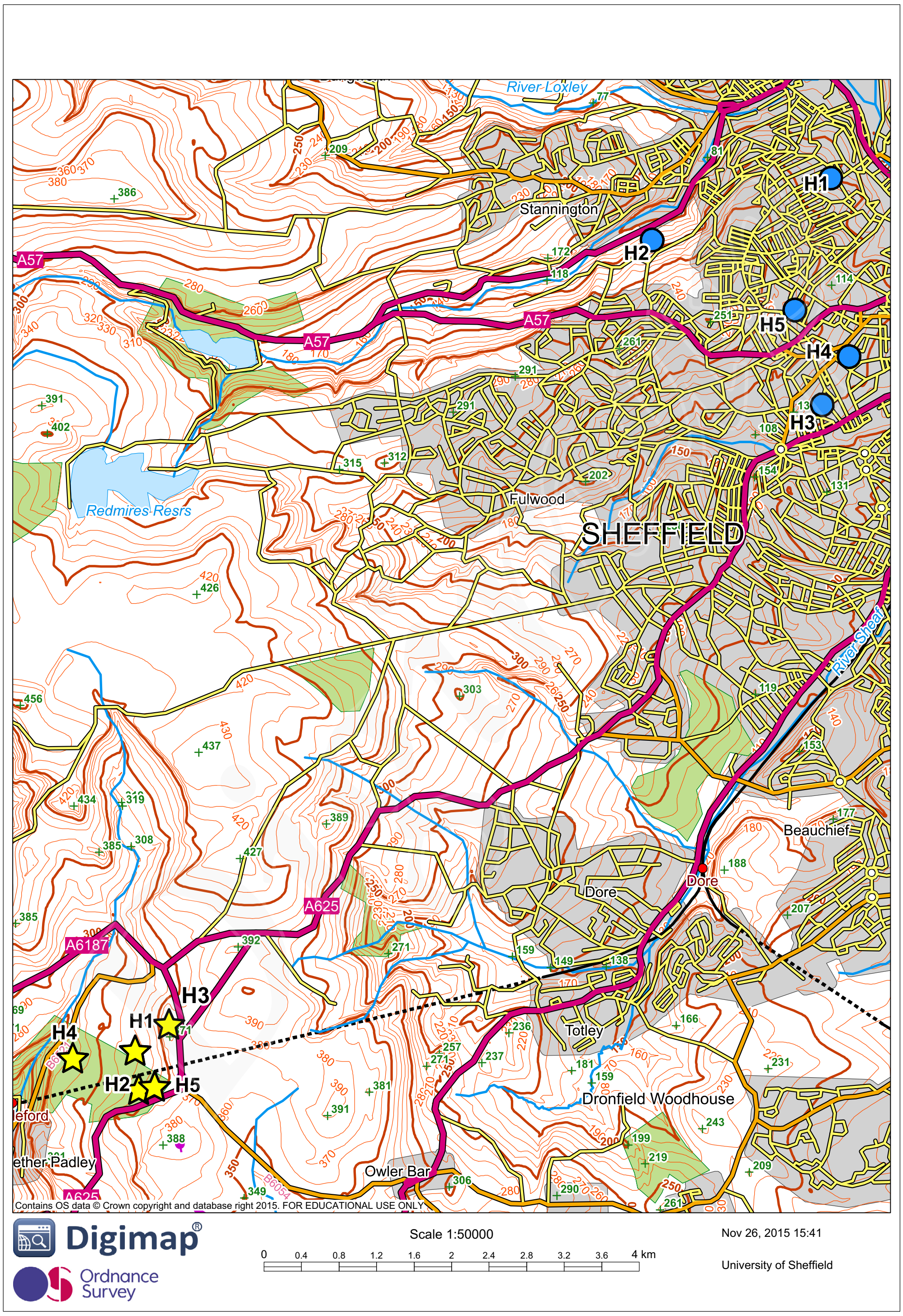

Figure 2. Map for the locations of the low-stress (blue circles), and high-stress (yellow stars) sites, used for the field experiment. Grid references for each site can be find in Table A1.

2.4.2 Generalities of the sites

Five acidic, high-stress sites were established at Longshaw Estate, in the Peak District. The dominant vegetation is moorland and acidic grassland (Table A1). The five low-stress sites had all been fertilized in the past, and were in public or private gardens, and allotments (Table A1).

2.4.3 Soil analysis: pH, nitrogen and phosphorus

We conducted a soil analysis to check that the sites we chose fall into the high-stress and low-stress categorization used for this study. To do this, a total of five soil samples (~200 g per sample) were collected from each of the sites using a 2.5 cm diameter corer. The upper 10 cm of soil was sampled. The plant-available nitrogen, phosphorus and pH were then measured as described below, and the raw data are shown in Table A1.

pH

The soil samples were stored in a fridge overnight at 5°C, and pH was measured within 24-hours of collection. To do this, the soil was sieved to exclude stones and roots. Then, a total of 20 g of soil for each sample was placed in 50 mL beakers, and 20 mL of distilled water were added and the mixtures were stirred. Before starting the pH measurements, mixtures were left to stand for 30 minutes, then stirred again and then left to stand for a further 30 minutes. The pH meter was calibrated with pH 4 and pH 7 buffers prior to use.

Plant-available nitrogen: Extraction of NO_3–N and NH_4–N with 2.0 M KCl

Soil samples were weighed (2.5 g) in an aluminum dish, and dried at 80°C for four days. After this, samples were placed into dispensing plastic bottles, and 25 mL 2.0 *M* of KCl added to each bottle. Bottles were then shaken for 30 min and then filtered with No. 42 filter paper into scintillation vials. Nitrogen content was analyzed using the flow injection analyser.

Plant-available phosphorus: Olsen's P

Soil samples were weighed (2.5 g) in an aluminum dish, and dried at 80°C for four days. After this, samples were placed into dispensing plastic bottles with 50 mL of standard P solution (0.5 *M* of sodium hydrogen orthophosphate in 1 L of dH_2O). Sample bottles were shaken for 30 minutes and then filtered with Whatman No. 42 filter paper into scintillation vials. Each sample was analyzed by pouring 0.5 mL of the extract into plastic cuvettes, followed by 0.2 mL of 0.1M ascorbic acid, and 0.5 mL of an ammonium molybdate and antimony potassium tartrate colour developing solution. Finally, the cuvettes were made up to 3.8 ml with 2.6 ml of distilled water and the optical density was measured at 882 nm on a spectrophotometer (Cecil CE1020) after 45 minutes to evaluate the phosphorus content.

2.4.4 Species selection and classification

We used 35 species from the Sheffield flora, belonging to eight families (Table 2). These species are classified by vegetation strategy using Grime et al. (2007), (Fig. A1). We used two approaches: 1) using the scores for the components (*C*, *S* or *R*) of the vegetation strategies as a *continuous* variable, and 2) using the vegetation strategy as a *categorical* variable. First, the three components *C*, *S*, *R* in the triangle sum to unity (Hunt et al. 2004), so for a species that has intermediate vegetation strategy between *C* and *R*, like *Agrostis stolonifera* (Grime et al. 2007), the scores for each component would be 0.5 at *C*, 0.5 at *R*, and 0 at *S*. Each score was fitted as a *continuous* variable. As the components sum to 1 they highly correlated, and so we carried out a separate analysis for each component. Throughout this chapter we use 'component' to refer to the score that each species has for each of the three components of the vegetation strategy, this would be competitiveness (*C*), ruderalilty

(*R*) and stress-tolerator (*S*). For the categorical classification of the vegetation strategy we use *Competitor*, *Stress-tolerant* and *Ruderal*, and we refer to this as ‘strategy’.

2.4.5 Seeds for the experiment

Seeds were obtained from different seed companies. Prior to sowing in the field, 40 seeds from each of the species were placed in Petri dishes on moist filter paper to test their viability. More than half of the species showed more than 80% germination (Table 1). To facilitate their germination *Medicago lupulina* and *Trifolium repens* were scarified with sand paper before sowing, and *Galeopsis tetrahit* and *Cirsium vulgare* were stratified at 3 °C for two months. For all species, five groups of 30 seeds were weighed, and then the average weight of a single seed was calculated (Table 2). Seed mass varied from 6.9×10^{-5} g in *Agrostis stolonifera* to 7.2×10^{-3} g in *Centaurea scabiosa* (~100-fold variation, Fig. A2).

Table 1. Species and families used for the field experiment classified with respect to CSR strategy. Families: AST: Asteraceae, POA: Poaceae, JUN: Juncaceae, LAM: Lamiaceae, CAR: Caryophyllaceae, FAB: Fabaceae, ROS: Rosaceae, RUB: Rubiaceae. Vegetation strategies; *C*: *Competitor*, *S*: *Stress-tolerant*, *R*: *Ruderal*, *CR*: *Competitor-Ruderal*, *CS*: *Competitor,Stress-tolerant*, *RS*: *Ruderal*, *Stress-tolerant*, and *CSR*: *Competitor, Stress-Tolerant, Ruderal*. Life-span: P: Perennial, A: Annual, (Grime et al. 2007).

Species name	**Family**	**Strategy**	**Seed weight (gr)**	**Germination test (%)**	**Life-span**
Agrostis stolonifera	POA	CR	6.92222×10^{-5}	93	P
Anthoxanthum odoratum	POA	CSR/SR	0.000708	90	P
Arctium minus	AST	CR	0.006137	93	P
Avenula pratensis	POA	SC/S	0.003254	40	P
Avenula pubescens	POA	CSR/SC	0.002236	40	P
Brachypodium sylvaticum	POA	SC/S	0.00068	93	P
Bromus hordeaceus	POA	R	0.002529	93	A
Centaurea scabiosa	AST	CSR/S	0.007189	90	P
Cerastium fontanum	CAR	CSR/R	0.000136	66	P
Cirsium arvense	AST	C	0.000802	40	P
Cirsium vulgare	AST	CR	0.002362	40	P
Dactylis glomerata	POA	CSR/C	0.000521	50	P
Deschampsia cespitosa	POA	CSR/SC	0.000205	90	P
Deschampsia flexuosa	POA	SC/S	0.000434	90	P
Festuca gigantea	POA	CSR	0.002249	90	P
Festuca ovina	POA	S	0.000327	90	P
Festuca rubra	POA	CSR	0.000868	90	P
Galeopsis tetrahit	LAM	CR/R	0.003514	40	A
Galium saxatile	RUB	S	0.003758	40	P
Geum urbanum	ROS	CSR/S	0.00249	66	P
Holcus lanatus	POA	CSR	0.000314	50	P
Juncus effusus	JUN	C/SC	0.00028	66	P
Juncus squarrosus	JUN	S	9.56667×10^{-5}	40	P
Koeleria macrantha	POA	S	0.000263	90	P
Lamium purpureum	LAM	R	0.00257	93	A
Luzula campestris	JUN	CSR/S	0.000737	40	P
Luzula multiflora	JUN	CSR/S	0.000521	60	P
Medicago lupulina	FAB	SR/R	0.002051	60	A-P
Poa annua	POA	R	0.000189	60	Short-live P
Poa trivialis	POA	CSR/CR	0.000196	90	P
Silene dioica	CAR	CSR	0.000802	60	P
Silene vulgaris	CAR	CSR	0.005244	80	P
Stachys sylvatica	LAM	CR/C	0.001431	40	P
Stellaria media	CAR	R	0.000504	86	A
Thymus polytrichus	LAM	S	0.000188	86	P
Trifolium repens	FAB	CSR/CR	0.000614	80	P

2.4.6 Field experiment

At each of the ten sites, five patches of 50 × 70 cm were selected, in a randomized block design, and each divided into 10 × 10 cm squares. Prior to sowing, all vegetation was removed from each patch, and 30 seeds of a single species were sown into each 10 × 10 cm square. Seedling emergence was recorded every week; each seedling was marked on a map so individuals could be identified. Height, leaf number, and survival were measured each week. The recordings lasted approximately for five months, ending in July 2012.

2.4.7 Statistics

Data were analyzed using generalized linear models, specifically a logistic regression analysis, which assumes a binomial distribution and a logit link function. Seedling emergence was defined as the number of seedlings observed per viable seeds sown, and seedling survival was fraction of seedlings that survived to the end of the experiment. We used a quasibinomial distribution to correct for overdispersion (residual deviance > residual degrees of freedom) when needed. The effects of the strategy (categorical variable), component (continuous variable), stress (categorical variable), were tested on seedling emergence and survival, and the effect of seed mass (continuous log–transformed variable) on seedling survival.

We fitted pH, phosphorus and nitrogen as continuous variables to test for the effect on seedling emergence and survival. When in a significant interaction, pH was categorized, into low or high-stress for plotting purposes. For the analysis of the soil characteristics between sites (low and high-stress), we first averaged the results for pH, phosphorus and nitrogen availability for each of the site categories (low or high-stress). A *t*-test was used to test for differences in nutrients available for the plant and pH between sites (low and high-stress). All the statistical analyses were conducted in R, (R Development Core Team 2010).

2.5 Results

2.5.1 Soil characteristics

In the stressful sites, pH was more acidic (mean = 4.40) than in the low-stress sites (mean = 6.99; *t* = 12, *d.f.* = 47.48, *P* < 0.0001, Fig. 3, see Table A1 for raw data). Also, the plant available phosphorus was lower in the high-stress sites (mean = 0.528), than in the low-stress sites (mean = 3.70; *t* = 6.25, *d.f.* = 24.26, *P* < 0.0001, Fig. 3, Table A1). However, the opposite was found for plant available nitrogen, *i.e.* the high-stress sites had a higher plant available nitrogen (mean = 0.105) in comparison to the low-stress sites (mean = 0.089; *t* = -2.05, *d.f.* = 47.18, *P* = 0.03; Fig. 3, Table A1), although the result was only marginally significant.

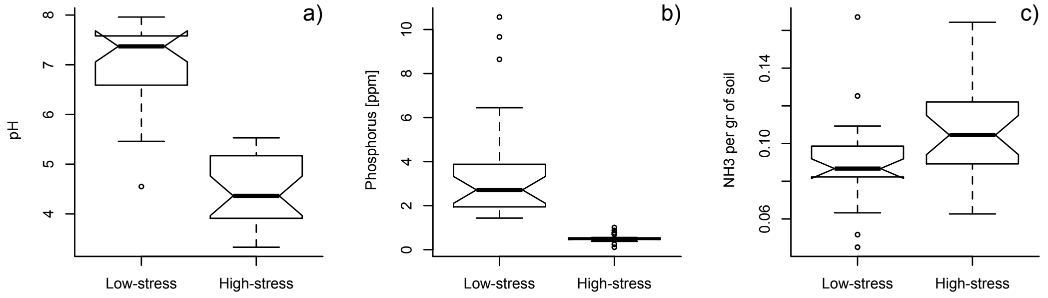

Figure 3. Soil nutrient availability and pH for: low-stress and high-stress sites. a) pH values; *t* = 12, *d.f.* = 47.48, *P* < 0.0001, b) Plant-available phosphorus (ppm); *t* = 6.25, *d.f.* = 24.26, *P* < 0.0001, and c) Plant-available nitrogen per gram of soil; *t* = -2.05, *d.f.* = 47.18, *P* = 0.03). The notch, where the box narrows around the median, shows the 95% confidence interval. The upper and low whiskers indicate the maximum and the minimum values (excluding outliers), respectively.

2.5.2 Seedling emergence

As we expected, total seedling emergence was higher in low-stress (1528 seedlings, or 8.31% of viable seeds), than in high-stress sites (368 seedlings, or 2% of viable seeds). Seedlings from 29 species were recorded at the low-stress sites, whereas only 20 species occurred at the stressful sites.

Strategies as a categorical variable

Contrary to our expectations, *Stress-tolerant* species did not have the highest emergence at the high-stress sites (Fig. 4). In addition, we also found that the level of stress (low or high) had a similar effect on all strategies (r^2 = 0.167, Table 2, Fig. 4). As expected seedling emergence was much higher at the low-stress than in the high-stress sites (Table 2, Fig. 4).

Components of the strategy as a continuous variable

The ruderality, *R* (χ^2 = 169.44, *d.f* = 1, $P < 0.0001$, r^2 = 0.113), and the tolerance to stress, *S* (χ^2 = 209.83, *d.f* = 1, $P < 0.0001$, r^2 = 0.113) of the species had an effect on seedling emergence, but the competitiveness *C,* did not (r^2 = 0.09, Fig. 5). However, this effect was the same at low and high-stress sites (Table 2), showing a negative relationship between seedling emergence and tolerance to stress, and a positive relationship between seedling emergence and ruderality (Fig. 5).

Table 2. Effect of stress and strategy, on seedling emergence and survival.

Seedling emergence analysis	χ^2	*d.f.*	*P*
Strategy as categorical variable			
Strategy	634.56	6	<0.0001
Stress	815.65	1	<0.0001
Strategy × Stress	60.93	6	0.33
Component as continuous variable			
C component	7.30	1	0.37
Stress	801.27	1	<0.0001
C × Stress	4.44	1	0.48
S component	209.83	1	<0.0001
Stress	805.33	1	<0.0001
S × Stress	15.25	1	0.19
R component	169.44	1	<0.0001
Stress	804.91	1	<0.0001
R × Stress	4.02	1	0.50

Seedling survival analysis	χ^2	*d.f.*	*P*
Strategy as categorical variable			
Strategy	8.0928	6	0.78
Stress	10.60	1	<0.05
Strategy × Stress	5.11	6	0.91
Component as continuous variable			
C component	2.96	1	0.25
Stress	10.25	1	<0.05
C × Stress	0.052	1	0.87
S component	0.025	1	0.91
Stress	9.81	1	<0.05
S × Stress	2.40	1	0.30
R component	1.05	1	0.49
Stress	9.4	1	<0.05
R × Stress	1.83	1	0.36
Seed mass effect			
Seed mass	0.0631	1	0.86
Stress	9.67	1	<0.05
Seed mass × Stress	1.11	1	0.48

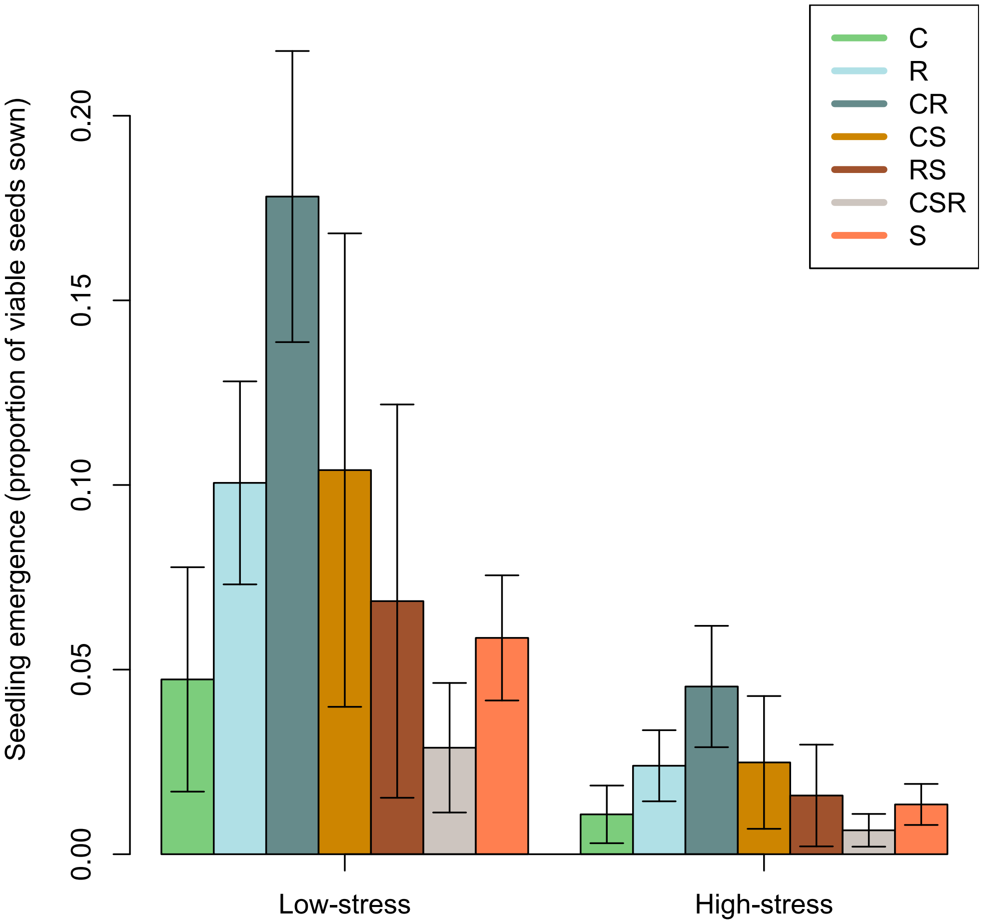

Figure 4. Seedling emergence (proportion of viable seeds sown) for different strategies at low and high-stress sites. *C*: *Competitor*, *R*: *Ruderal, CR*: *Competitor-Ruderal*, *CS*: *Competitor* and *Stress-tolerant, RS*: *Ruderal and Stress-tolerant*, *CSR*: *Competitor, Stress-tolerant* and *Ruderal*, *S*: *Stress-tolerant*. 95% confidence intervals are indicated on each bar.

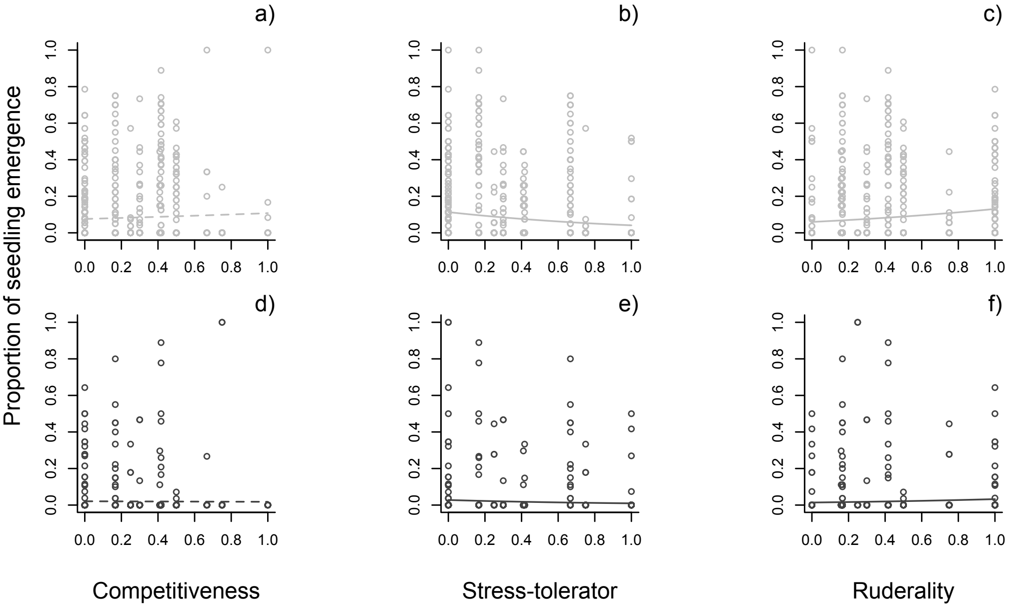

Figure 5. Seedling emergence (proportion of viable seeds sown) at each site for the different components: a) and d) *Competitiveness*, b) and e) *Stress-tolerator,* and c) and f) *Ruderality*. Gray symbols indicate data points for the low-stress sites and black symbols for the high-stress sites. Dashed lines indicate not significant effect on seedling emergence.

Site-soil characteristics and emergence

Seedling emergence was sensitive to differences in pH ($\chi^2 = 821.49$, *d.f.* = 1, $P < 0.0001$), but not to nitrogen ($\chi^2 = 0.73$, *d.f.* = 1, $P = 0.773$), or phosphorus availability ($\chi^2 = 3.04$, *d.f.* = 1, $P = 0.55$). However, we found an effect of the interaction between pH and phosphorus on seedling emergence ($\chi^2 = 107.65$, *d.f.* = 1, $P = 0.00047$), at low (more acidic) and high pH values the emergence increased with phosphorous (Fig. 6). However, the emergence of the seedlings was lower when the sites where more acidic (Fig.6). We also analyzed the possible interaction between the soil characteristics and the strategy on seedling emergence, but none was significant (for pH: $\chi^2 = 17.11$, *d.f.* = 6, $P = 0.902$, for phosphorus x pH: $\chi^2 = 29.88$, *d.f.* = 6, $P = 0.698$).

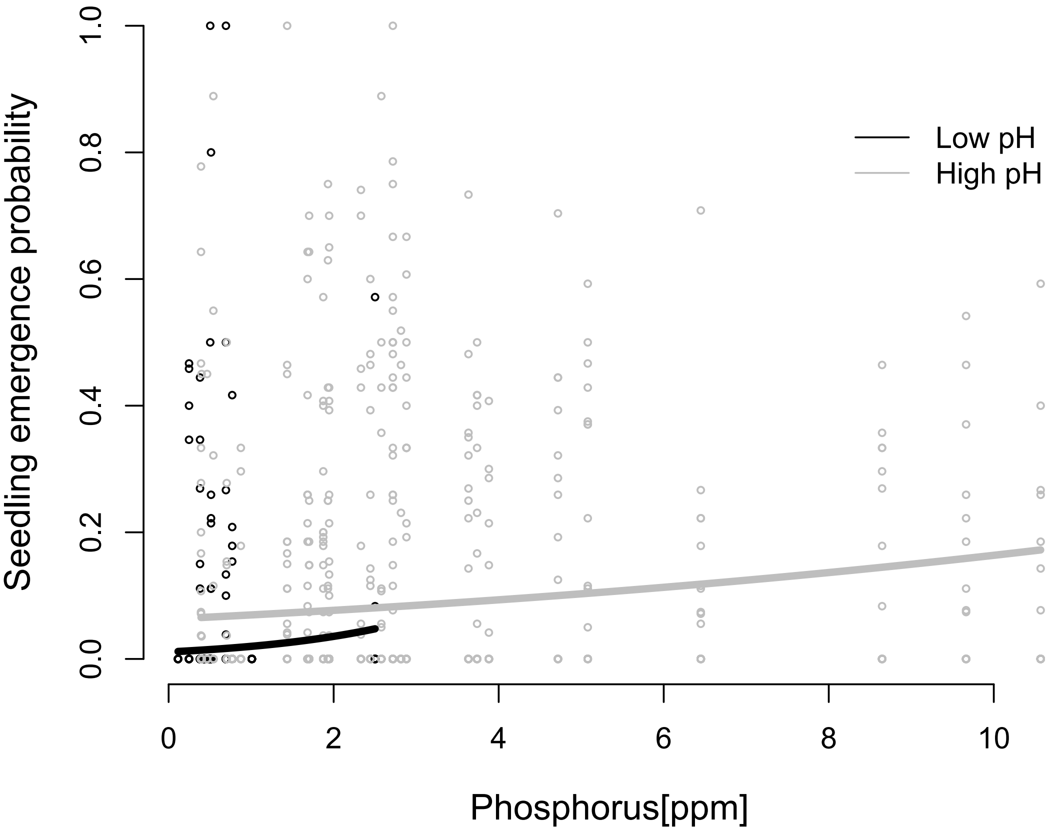

Figure 6. Effect of phosphorus and pH on seedling emergence probability. The points indicate the observed proportion of seedling emergence. The predicted values from the model are shown in categories, *i.e.* pH values were categorized into low pH (≤5) for the high-stress sites, and high pH (> 5) for the low-stress sites.

2.5.3 Seedling survival

Strategies as a categorical variable

As we expected, once competition is removed, seedling survival was negatively affected by the stressful conditions in the site (Table 2). Total seedling survival was higher in low-stress sites (82 survivors or 5.3% of seedlings) compared to high-stress ones (7 survivors, 1.9% of seedlings). In addition, we found that 16 species survived in the low-stress sites, whereas only 3 surviving at the high-stress sites (these were from the S, CR and R strategies). However, the effect of the strategy was not significant and neither was the interaction between the strategy and the stress-level (r^2 = 0.031, Table 2). So in the low-stress sites species with a *Competitor* strategy did not have a higher survival, and the *Stress-tolerators* were not the only survivors in the high-stress sites (Fig. 7).

Components of the strategy as a continuous variable

None of the components had a significant effect on seeding survival, nor was the interaction between component and stress-level (Table 2). Only the level of stress had a significant effect on seedling survival (r^2 =0.03, Table 2).

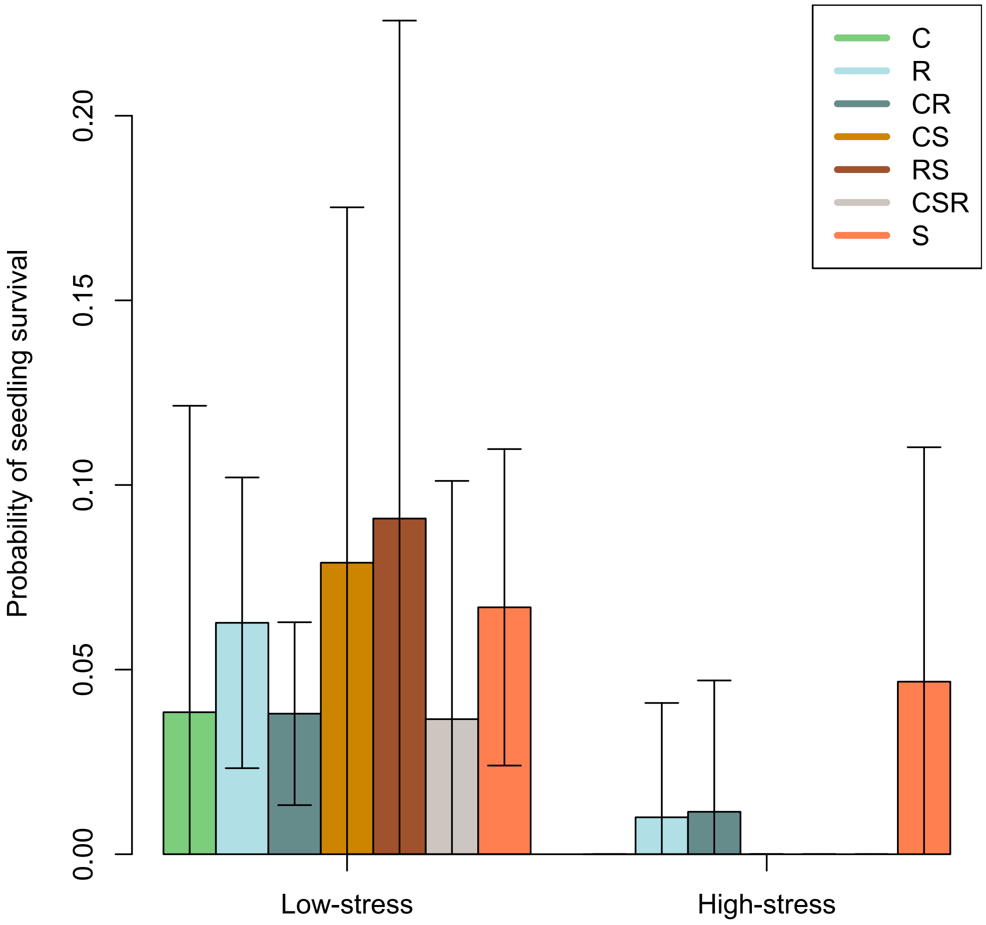

Figure 7. Probability of seedling survival for the vegetation strategies in low and high stress sites. *C*: *Competitor*, *R*: *Ruderal, CR*: *Competitor-Ruderal*, *CS*: *Competitor* and *Stress-tolerant, RS*: *Ruderal and Stress-tolerant*, *CSR*: *Competitor, Stress-tolerant* and *Ruderal*, *S*: *Stress-tolerant*. 95% confidence intervals are indicated on each bar. The effect of the main strategy on seedling survival was not significant.

Sites-soil characteristics and survival

Seedling survival was not affected by pH ($\chi^2 = 3.57$, *d.f.*= 1, $P = 0.23$), plant-available nitrogen ($\chi^2 = 0.06$, *d.f.* = 1, $P = 0.06$), or plant-available phosphorus ($\chi^2 = 7.78$, *d.f.* = 1, $P = 0.05002$). However, the interaction between pH × phosphorus was significant ($\chi^2 = 8.86$, *d.f.* = 1, $P = 0.036$), showing that at lower pH values and lower phosphorus, the survival probability of a seedling is increased, but as pH and phosphorus are increased the seedling survival slightly decreased (Fig.8).

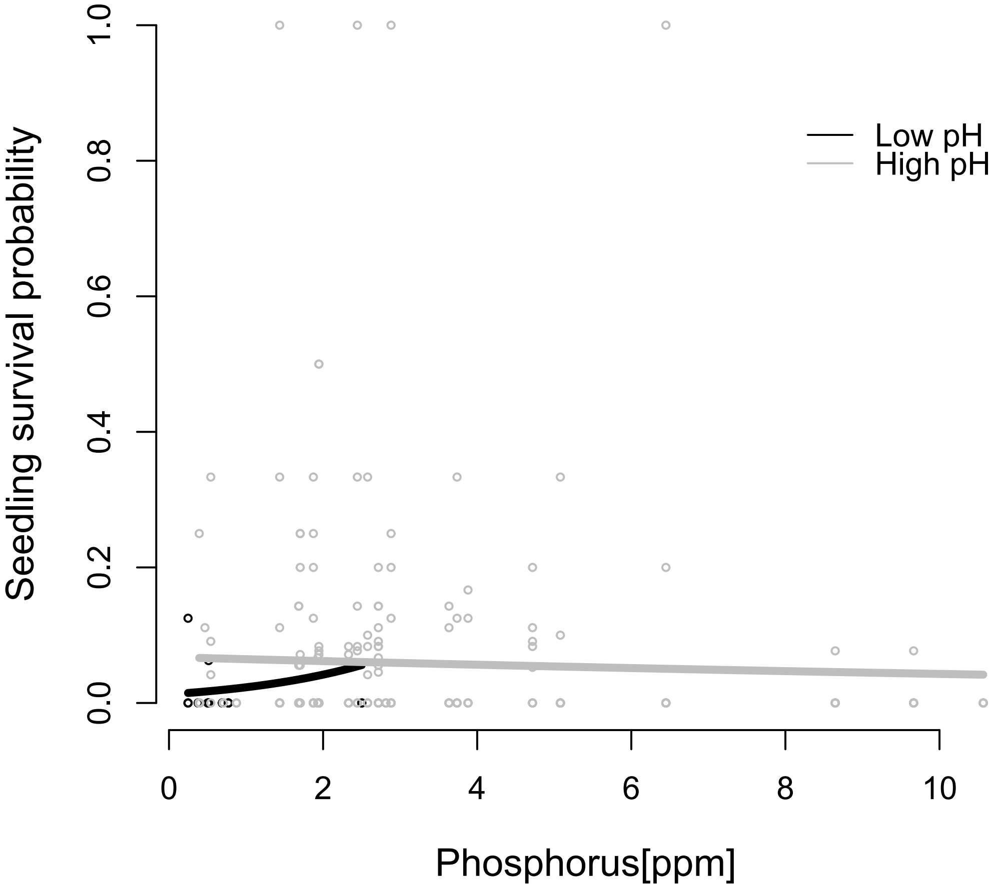

Figure 8. Effect of phosphorus and pH on seedling survival probability. The points indicate the observed proportion of seedling surviving at the end of the experiment. The lines were calculated by sub-setting the fitted values at different pH categories: low pH (≤5), and high pH (> 5). Lines present the expected seedling survival probability along the observed phosphorus range and mean observed pH within each category.

Seed mass and stress effect on seedling survival

We found an effect of the stress on seedling survival, but not for seed mass and for the interaction between seed mass and stress ($r^2 = 0.031$, Table 2).

2.6 Discussion

In this study we analyzed data on seedling emergence and survival from a large field experiment conducted in low and high-stress sites. This experiment included 35 species that are classified by CSR vegetation strategy (Grime et al. 2007). Our findings indicate that stress reduced the recruitment of species with different strategies in a similar way. This suggests that the vegetation strategies do not predict which species will be part of the community membership in low and high stress. Soil stress was an important filter in determining which species could colonize a particular habitat, however this was not predicted by the CSR theory.

2.6.1 Seedling emergence

We found that after removing competition, seedlings from all seven strategies emerged at both low and high-stress sites, but fewer seedlings from fewer species were recorded in the high-stress sites. The differences in seedling emergence between sites were related to the differences in pH values. In high-stress sites the mean pH was lower (mean = 4.3) than in the low-stress sites (mean = 7), and seeds generally germinate at pH values between 6.5 and 7 (Baskin and Baskin 2001). Similarly, in an Artic study by Gough et al. (2000b) found that soil was an important filter that determines local species richness, with fewer species occurring in more acidic soils (pH < 5.5).

Plant strategy affected seedling emergence (Fig. 4). However, no clear patterns in terms of the effect of the strategies were found. For example: seedlings from *R* and *CR* had the highest emergence in the low-stress site, but in the high-stress site seeds from *S* had one of the lowest seedling emergence (Fig. 4). Also, the stress affected in the same way to species with different CSR strategies. The CSR strategies operate at the established phase of a species (Hodgson et al. 1999). Nevertheless, the main bottlenecks in many populations occur at the seedling stage, and seedlings are typically the most environmentally sensitive stage in the lifecycle. In addition, the majority of the species used in this study regenerate from seeds (Grime et al. 2007), and so reduced emergence and seedling survival will influence adult community composition (Leishman and Westoby 1992).

2.6.2 Seedling survival

<u>Low and high- stress sites</u>

As expected, seedling survival was higher at the low-stress than in the high-stress sites (Fig.7). However, differences in seedling survival were not explained by the pH values in the soil. Although not measure in this study, high levels of Aluminium are known to inhibit plant growth by limiting nutrient acquisition, through its toxic effects on plant roots. It becomes increasingly available at low pH values (<5) (Ryan et al. 1992, Ryan and Kochian 1993, Kidd and Proctor 2000). Such conditions were present in the high-stress sites in this study (mean=4.3), and could have increased Aluminium availability and reduced seedling survival. Another possible factor that could have affected seedling survival was the temperature. We do not have values of the temperature for each of the sites; however, the general temperature for the low-stress sites was slightly warmer that the high-stress sites (average= 8.63 vs 8.04 °C), and this could have had a negative effect on survival.

Vegetation strategies, components and stress

We found that stress affected the different strategies in the same way, not what would be predicted by CSR theory. First, in the low-stress sites, seedling survival of the species with *Competitor* traits was not different from the other species. A controlled experiment conducted by Mahmoud and Grime (1976) showed that a *Competitor* species (*Arrhenatherum elatius*) had a greater performance when grown in monoculture, and under high-nitrogen levels, than a stress-tolerant species (*Festuca ovina*). However, we did not find this in field conditions. Second, we were expecting *Stress-tolerators* species to be better able to survive and established under high-stress because they share common traits to tolerate the stress imposed by the environment (Grime 1979). However, in this study we found no evidence that survival of *Stress-tolerators* differed between the sites of high and low-stress. Instead, it seems that in such high-stress sites, environmental filtering (*sensu* Kraft et al. (2015) appears to be acting strongly on individuals' survival and hence influencing which species are incorporated into the community. From our results, we can say that just a few species (*Poa trivialis* (CSR/CR), *Geum urbanum* (CSR/S), and *Stellaria media* (R), data not shown), rather than a type of species were filtered *i.e.* survived, by the conditions present in the high-stress site.

2.6.3 Seed mass effect on survival

Larger seeded species did not show an establishment advantage as we expected. In several studies (Turnbull et al. 1999, Jakobsson and Eriksson 2000), a recruitment advantage has been recorded in large-seeded species. In these studies, the seed mass effect was tested in the presence of other species and at different seed densities, and so comparison with the present study is difficult. However, we do not discard the possibility that we could have miss out the positive effects that larger seeds have on recruitment in the presence of interspecific competition and high densities. It is possible that the positive effects of larger seed mass are only observed in interaction with interspecific competition. As shown in Gross (1984), the effect of seed mass was positively related to seedling weight in vegetated cover, but there was no effect of seed mass in bare soil (or absence of competition).

In summary, CSR theory has been extended to be used for the management and restoration of vegetation. This is done by predicting the plant strategies that will be present in a certain vegetation type from initial conditions, *i.e.* the initial vegetation and the environmental or management scenario (Hunt et al. 1991). Recently, CSR theory has been extended to organisms other than plants (Grime and Pierce 2012b). However, it is only rarely that predictions from CSR theory have been tested (Wilson and Lee 2000). In this study we have shown that CSR theory does not predict community membership from the initial phases of the life-cycle of a plant. Our results are supported by the lack of interactions between strategies (or components) with stress, and also by the low r^2-values for each model.

Chapter 3

The type of stress plays a major role in determining community membership

3.1 Abstract

CSR theory (Grime 1979) predicts that in fertile, (low–stress) and undisturbed habitats *Competitor* species will succeed, whereas in habitats subject to stressful conditions only *Stress-tolerators* can persist. From theory we would expect that if competition, disturbance and stress sources were removed, all species would have a greater chance of survival, whereas in stressful conditions only *Stress-tolerators* can survive. In terms of seed size we expect larger seeded species to survive better particularly under stressful conditions. To test these ideas, we conducted a large greenhouse experiment using seeds from 35 species classified in terms of their CSR strategy. As we expected, seedling survival was higher in the low-stress conditions, but not when pH is controlled, suggesting that this factor can be an important filter in community membership. Our results were in partial agreement with predictions from CSR theory, suggesting that it is not a good predictor for community membership. We also found that species with a large seed mass did not show any advantage over species with small seeds.

3.2 Introduction

The CSR theory, proposed by Grime (1979), states that disturbance and stress are the main determinants of community structure, and that different levels of these two factors result in the evolution of three main vegetation strategies. In productive habitats, where disturbance is low, species are effective above– and below–ground competitors. These *Competitor* species have a constellation of traits for rapid growth and resource capture: rapid leaf and shoot expansion, which allows them to compete for foliage space. Their adaptations, evolved in fertile habitats, also confer competitive advantage under low–fertile conditions (Mahmoud and Grime 1976). However, as disturbance increases in productive habitats, *Ruderals* prevail, as these species can grow rapidly after disturbance. The high relative growth rate (RGR) at the seedling stage is important as it allows them to complete their life-cycle quickly (Grime et al. 2007). *Ruderals* allocate most of their resources to reproduction, and hence fail to produce higher shoot and root density, which are traits associated with success in non-disturbed habitats. Finally, when disturbance is low, but stress is high (stress being the external constraints on biomass production), the importance of competition is reduced, and only species able to persist in the stressful conditions can survive. These *Stress-tolerators* have low–growth rates, and long-lived tissues, which allows them to persist under stressful conditions. Although competition for resources occurs in these habitats, competition is not the main process determining its persistence. Because of the adaptations evolved to endure stress, *Stress-tolerators* are not expected to have a better performance when grown under high-nutrient conditions (Mahmoud and Grime 1976). The implications are that in productive, undisturbed environments competition will be the main process determining community membership, whereas in unproductive habitats, stress will become the main filter that determines which species can be members of the community.

Also, in relation to community membership, single traits rather than strategies (a set of traits), have been recognized to be important in determining plant performance. For example seed mass has been studied in many ecological studies (Moles et al. 2005b, Moles et al. 2007), and is positively related to competitive ability (Gross and Werner 1982) and survival under stressful conditions, like drought and shade (Westoby 1998), and in general large seeded species have higher survival rates (Moles and Leishman 2008). Under low–nutrient conditions, large seeded species can produce seedlings from their large seed reserves with extensive root systems (Lee and Fenner 1989), which improves survival (Dainese and Sitzia 2013). Therefore species with larger seeds would be expected to have a greater chance of becoming members of a community than small seeded species especially under stressful conditions.

In nature, many sources of disturbance and stress can interact to determine the recruitment of new species into a community. For instance, germination and seedling survival are affected by pH, temperature, herbivory and climatic conditions, such as drought and winter cold (Baskin and Baskin 2001, Eriksson 2008). We therefore carried out a greenhouse experiment to test, which species succeed after removing competition in both low and high-stress soils, differing in nutrient availability and pH. In the absence of competition, predictions from CSR theory are:

1) In the low–stress conditions, seedlings from all the strategies can survive, because the conditions are not stressful, and competition no longer excludes any species. However, under high–stress conditions, only *Stress-tolerators* can survive, because the stressful conditions prevent establishment.

2) The effect of the CSR strategy, would be different depending on the degree of stress:

a) For the *Competitors* and *Ruderals* we expect to see an increase in their performance in low-stress conditions. Under high stress conditions we expect a large reduction in their performance.

b) In the case of *Stress-tolerators* we expect to see a small improvement in performance under low stress, as they are slow growing.

Additionally, we tested the following predictions based on previous work:

3) Seedling emergence would be higher in low-stress where the low pH values can prevent seeds from germinating. However, in the absence of a low pH, nutrient availability should not affect seedling emergence.

4) The positive effect of large seed size on emergence and survival will be greater in high–stress conditions.

3.3 Methods

3.3.1 Experimental design

Thirty-five species categorized into the CSR strategies using Grime et al. (2007) were used in the experiment (Table 1 in Chapter 2, Fig. A1). The classification of the vegetation strategies used was the same as that described in Chapter 2 (see p. 18). A total of 10 seeds per species were sown in each cells (75 × 70 × 65 mm) of seed tray inserts, and these were placed into seed trays (38 × 24 cm, Fig. A4). We used 3 trays per replicate and 25 replicates were used per soil type, with five additional replicates for each control treatment. Each replicate was randomly arranged on a bench in the AWEC greenhouse facilities at the University of Sheffield, and randomly moved once a week. The trays were watered with distilled water every two days, and greenhouse temperature was set at 22 °C during daytime, and 15 °C at night–time with a 12/12 hr photoperiod.

Seedling emergence (the number of seedlings observed) was recorded weekly and seedlings were marked on a map to identify each individual. Seedling height, leaf number, and seedling survival (the fraction of seedlings that survived to the end of the experiment) were measured once a week for 9 weeks. At the end of the experiment, the aboveground part of each surviving plant was removed and dried in an oven at 80 °C for 3 days to calculate the dry weight of the above–ground mass biomass (g).

3.3.2 Soil treatments

Soil was collected from five high-stress and five low-stress sites located in and around Sheffield, UK (Table A1, Appendix, Fig. 2 in Chapter 2). In this experiment we chose low and high-stress sites based on their pH (acidic or non-acidic) and fertility (low or high nutrient availability) conditions (see the Results section for Soil characteristics in Chapter 2, p. 22). A total of 54 kg of soil was collected; this was approximately 5 kg per site divided into 5 × 1 kg replicates. The soil was dried and sieved to remove the roots and 30 g were placed in a 10 × 10 cm pot, and sprinkled with distilled water. In addition to these soil treatments, two more treatments were included in the experiment: a control with nutrients (nutrient–added) and a control without nutrients (nutrient–stress). These were added to eliminate the detrimental effects that low pH values might have on species that are calcicole, *i.e.* do not like acid (Baskin and Baskin 2001). The controls consisted of a mix of vermiculite and washed sand (1:1 ratio), the mix was added to the pots and watered with distilled water. For the Rorison's solution a 100 mL stock solutions of each element were made up (by Dr. David Johnson), and stored in a refrigerator. From these stock solutions, 1 mL from each was added together and made up to 1 liter with dH_2O, representing full strength Rorison's solution. For the control with nutrients, 20 ml of Rorison's full strength nutrient solution was added to each of the pots every week (Table 1).

Table 1. Full strength Rorison's nutrient solution, preparation of 1L, (Booth et al. 1993).

Element	mg / l^{-1} in Rorison's solution	Stock solutions	Gram of source compound in 100 ml stock solution	Ml of stock solution required to make 1 l of Rorison solution
Ca / N	80/56	$Ca(NO_3)_2 \cdot 4H_2O$	47.61	1
Mg	24	$MgSO_4 \cdot 7H_2O$	24.80	1
K/P	78/31	$K_2HPO_4 \cdot 3H_2O$	23.07	1
Fe	3	Fe EDTA	2.50	1
Trace elements:				
Mn	0.5	$MnSO4 \cdot 4H_2O$	0.20	1
B	0.5	H_2BO_3	0.29	1
Mo	0.1	$(NH_4)_6MoO_{24} \cdot 4H_2O$	0.18	1
Zn	0.1	$ZnSO_4 \cdot 7H_2O$	0.04	1
Cu	0.1	$CuSO_4 \cdot 5H_2O$	0.03	1

pH adjusted to 7.0 with HCl or NaOH as required

3.3.3 Statistics

We used generalized linear models, specifically a logistic regression analysis which assumes a binomial distribution and a logit link function. We tested the effects of the vegetation strategy and the stress treatment (analyzed as a categorical variable), upon both seedling emergence and survival. To do this, we used two different approaches. These are described in detail in Chapter 2 (p. 18), but in summary we analyzed the effects of the vegetation strategy in two separate ways, using the vegetation strategy as a categorical variable, and also as a continuous variable. In this chapter, we refer to them as the "strategy" when used as a categorical variable, and as "component" when used as continuous variable. The effect of seed mass (as a continuous variable) and treatment were tested on seedling survival. All the statistical analyses were conducted in R Development Core Team (2010).

3.4 Results

3.4.1 Seedling emergence in low and high–stress soils

Strategies as categorical variable

Contrary to our expectations, we found that the seedling emergence was not influenced by the stress treatment. Also, the effect of stress on seedling emergence did not depend on the plant strategy, although there was a highly significant difference in emergence between the strategy groups (r^2 = 0.178, Table 2, Fig. 1).

Components as a continuous variable

The stress did not affect seedling emergence (Table 2). Similarly, we did not find an effect of the *competitiveness* on emergence (r^2 = 0.0028, Table 2, Fig. 2 a & d). However, there was an interaction between the *stress-tolerator* component and the stress (r^2 = 0.04, Table 2, Fig. 2 b & e), but the effect was small.

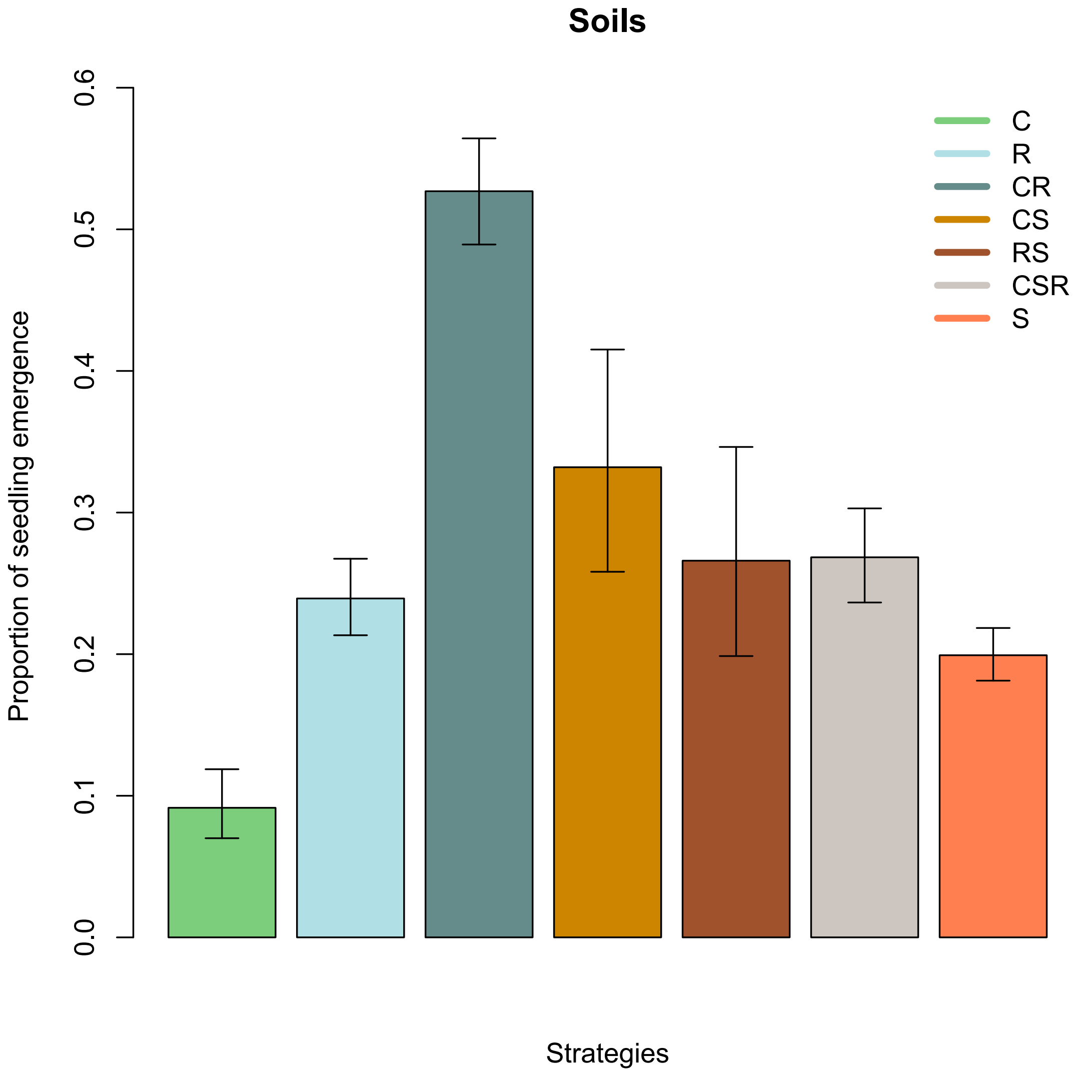

Figure 1. Proportion of seedlings emerging for the different CSR strategies for the soil treatment. *C*: competitor, *R*: ruderal, *CR*: competitor-ruderal, *CS*: competitor and stress-tolerant, *RS*: ruderal and stress- tolerant, *CSR*: competitor, stress-tolerant and ruderal, *S*: stress-tolerant. 95% confidence intervals are shown by each error bars.

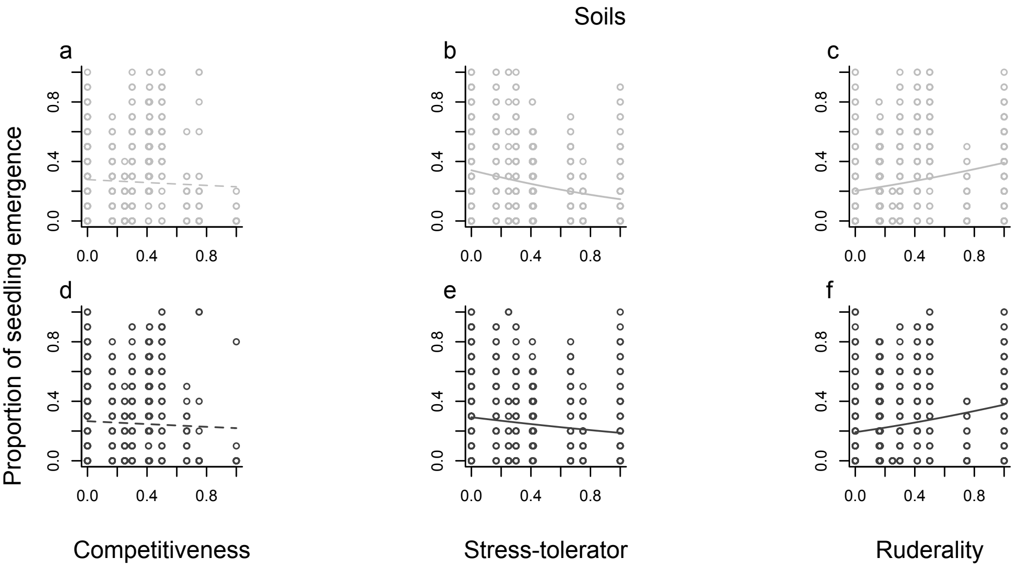

Figure 2. Seedling emergence for each of the components of the vegetation strategy at the soil treatments: (a) and (d) *Competitiveness,* (b) and (e) *Stress-tolerator*, (c) and (f) *Ruderality*. Dashed line indicates non-significant effects on seedling emergence. Gray symbols and lines; data point in the low-stress soils, black symbols and lines: data point in the high-stress soils. Note, the two lines for the *Ruderality* component are the same, there is no interaction with stress.

3.4.2 Seedling emergence under nutrient–added and nutrient–stress

Strategies as a categorical variable

As we expected, seedling emergence was not affected by the nutrient stress in the control experiments ($r^2 = 0.2013$, Table 3, Fig. 3), and the effect of stress on emergence did not significantly differ between strategies. However, we found a highly significant effect of the strategy (Table 3).

Components as a continuous variable

Our findings from the analysis of the strategies were reinforced by the analysis of the components; we found that emergence was not affected by the nutrient stress, or by the interaction between stress and the components (Table 3, Fig. 4). The main effect of the components was only significant for the *stress-tolerator* (r^2 = 0.037) and the *ruderality* (r^2 = 0.067), but not for the *competiveness* (r^2 = 0.012).

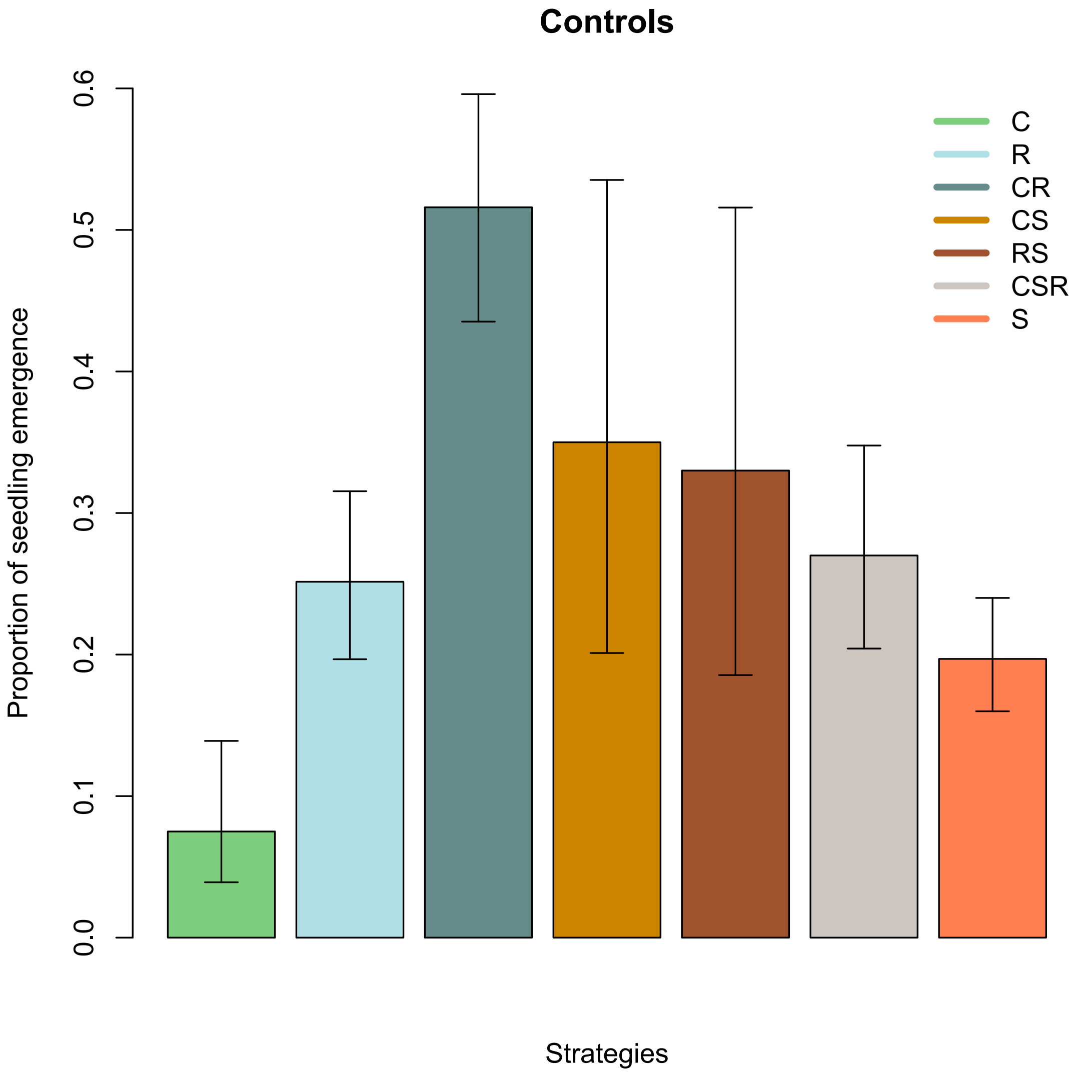

Figure 3. Proportion of seedlings emerging for the different CSR strategies for the control treatment. *C*: competitor, *R*: ruderal, *CR*: competitor-ruderal, *CS*: competitor and stress-tolerant, *RS*: ruderal and stress-tolerant, *CSR*: competitor, stress-tolerant and ruderal, *S*: stress-tolerant. 95% confidence intervals are shown by each error bar.

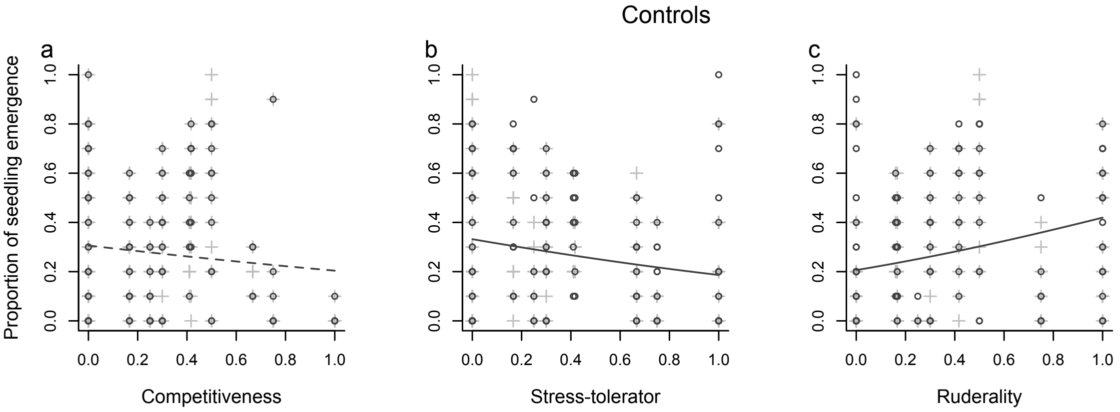

Figure 4. Proportion of seedlings emerging in the control treatments for each component of the vegetation strategy (a): *Competitiveness*, (b): *Stress-tolerator* and (c): *Ruderality*. In each plot, only one line is shown as there were no significant interactions (Table 3). Dashed line: not-significant, and solid line: significant effect of the component on the proportion of seed germination.

3.4.3 Seedling survival in low and high–stress soils

Strategies as a categorical variable

As expected, there were more seedlings surviving in the low-stress (total seedlings = 2102), than in the high-stress soils (total seedlings = 1903), (Table 2). Although the effect of stress on survival did depend on the vegetation strategy (Table 2), we found that *Competitors* did not perform better than the rest of the strategies in the low-stress soils, and neither the *Stress-tolerants* did not have higher performance in the high-stress soils (r^2 = 0.061, Fig. 5).

Components as a continuous variable

The analysis of the components showed a different outcome from the strategies analysis. First, we found that as we expected, the competitiveness of the species reduced its survival in the high–stress soils, but did not increase the survival in the low-stress conditions (r^2 = 0.024, Table 2, Fig. 6 a & d). Second, and also in agreement with predictions from CSR theory, the stress-tolerance of the species increased its probability of survival under high–stress soils, and decreased the chances of surviving in the low–stress soils (r^2 = 0.025, Table 2, Fig. 6 b & e). Finally, the ruderality of the species did increase the survival probability in the low–stress soils, and decreased it in the high–stress conditions (r^2 = 0.015, Table 2, Fig. 6 c & f).

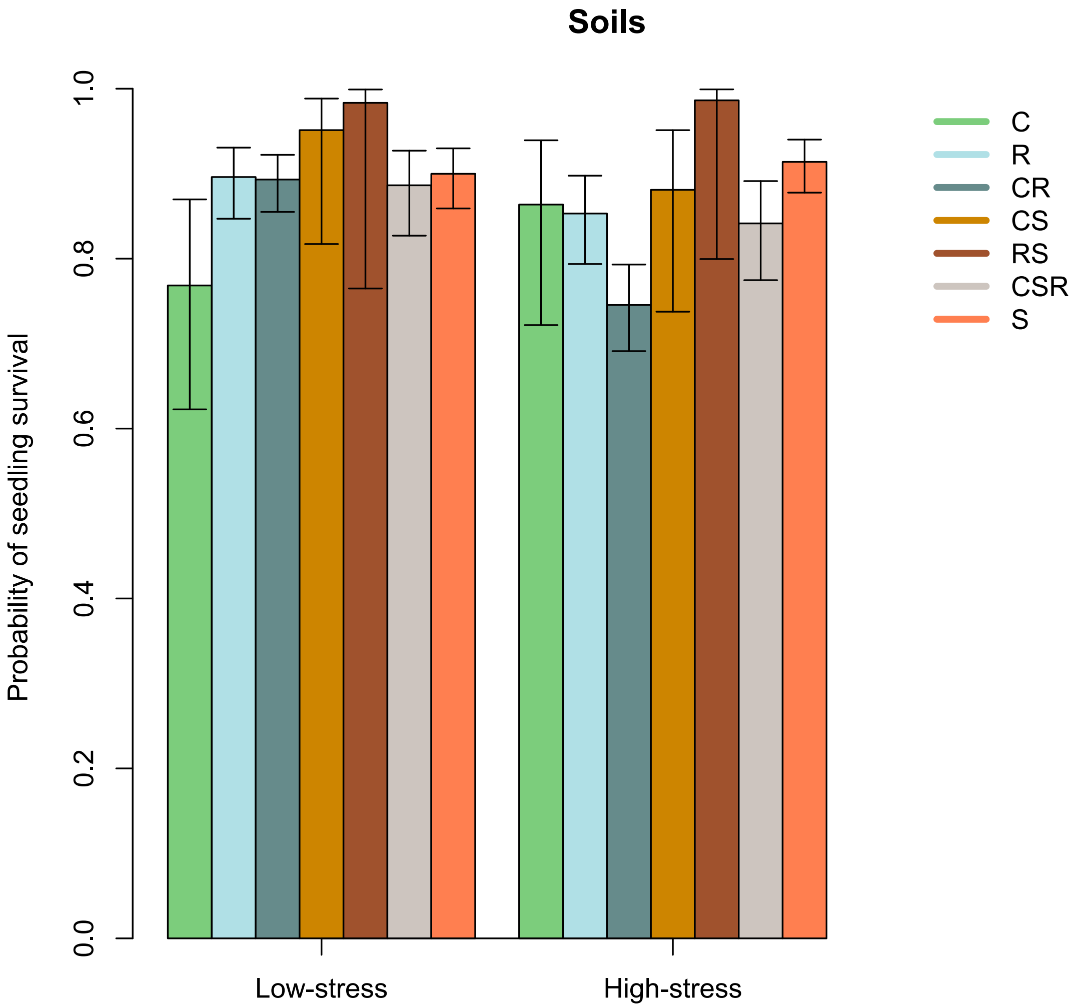

Figure 5. Probability of seedling survival for each vegetation strategy in both treatments (low–stress and high–stress). *C*: competitor, *R*: ruderal, *CR*: competitor-ruderal, *CS*: competitor and stress-tolerant, *RS*: ruderal and stress-tolerant, *CSR*: competitor, stress-tolerant and ruderal, *S*: stress-tolerant. 95% confidence intervals are shown by the error bars.

Table 2. Results from the analysis of the seedling emergence and survival for the low and high–stress soils.

Seedling emergence analysis	χ^2	*d.f.*	*P*
Strategy as a categorical variable			
Strategy	1314	6	< 0.00001
Stress	3.75	1	0.28
Strategy × Stress	31.58	6	0.14
Component as a continuous variable			
C component	13.14	1	0.056
Stress	2.94	1	0.36
C × Stress	3.64	1	0.31
S component	254.91	1	<0.00001
Stress	2.98	1	0.35
S × Stress	23.36	1	<0.01
R component	337.9	1	<0.0001
Stress	3.01	1	0.35
R × Stress	10.27	1	0.085

Seedling survival analysis	χ^2	*d.f.*	*P*
Strategy as a categorical variable			
Strategy	70.89	6	<0.0001
Stress	27.07	1	<0.001
Strategy × Stress	35.23	6	<0.01
Component as a continuous variable			
C component	17.69	1	<0.01
Stress	25.14	1	<0.001
C × Stress	8.46	1	0.043
S component	3.22	1	0.21
Stress	24.98	1	<0.001
S × Stress	24.39	1	<0.001
R component	0.9982	1	0.48
Stress	23.43	1	<0.001
R × Stress	9.01	1	0.037
Seed mass effect			
Seed mass	0.19	1	0.76
Stress	22.43	1	<0.01
Seed mass × Stress	1.5	1	0.39

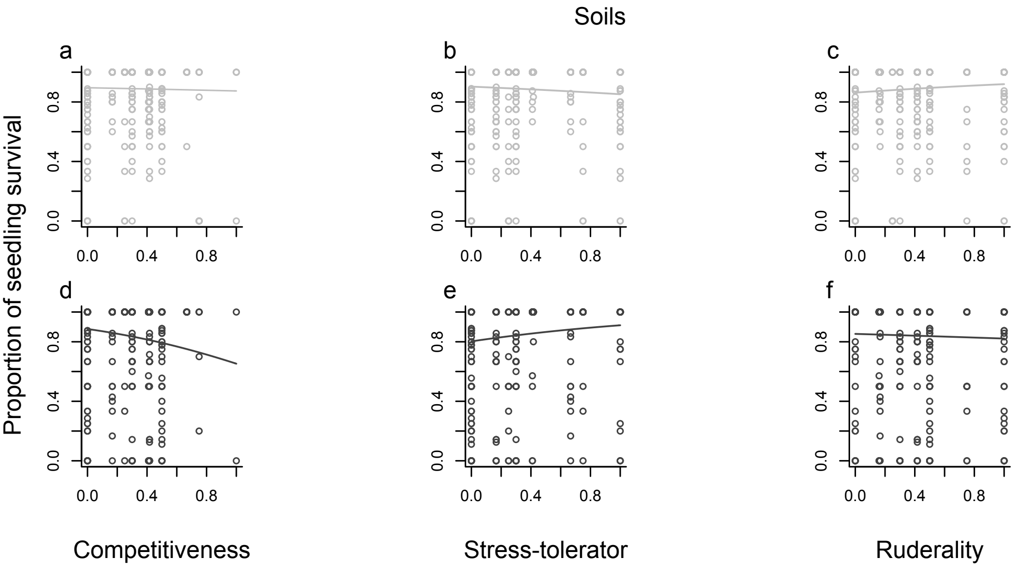

Figure 6. Probability of seedling survival for each of the components: (a) and (d) *Competitiveness*, (b) and (e) *Stress-tolerator*, and (c) and (f) *Ruderality*. Gray symbols indicate data points for the low–stress soils, and black symbols for the high–stress soils.

3.4.4 Seedling survival under nutrient–added and nutrient–stress controls

Strategies as a categorical variable

Seedling survival was not reduced by the stress in low nutrients conditions, once the effect of acidic pH is removed (Table 3). We did not find an effect of the strategy on seedling survival or of the interaction between strategy and stress (r^2 = 0.11, Table 3).

Components as a continuous variable

The results for the components were consistent with results from the analysis of the strategies. We did not find an effect of stress of the site, or of the *competitiveness* (r^2 =0.021) or the *ruderality* of the species (r^2 =0.033). However, we found that the more *stress-tolerator* a species is, the less probable is to survive in nutrient-stressed conditions, and that under nutrient-added conditions, the chances of surviving would increase (r^2 = 0.50, Fig.7 b & e). (Table 3, Fig. 7 b & e).

Table 3. Seedling emergence and survival analysis for nutrient–added, and nutrient–stress controls.

Seedling emergence analysis	χ^2	*d.f.*	*P*
Strategy as a categorical variable			
Strategy	273.90	6	<0.0001
Stress	2.92	1	0.32
Strategy × Stress	7.175	6	0.87
Component as a continuous variable			
C component	11.77	1	0.06
Stress	3.08	1	0.34
C × Stress	1.50	1	0.50
S component	43.97	1	<0.001
Stress	3.11	1	0.33
S × Stress	2.19	1	0.41
R component	81.89	1	<0.0001
Stress	3.15	1	0.32
R × Stress	4.7	1	0.22

Survival analysis	χ^2	*d.f.*	*P*
Strategy as a categorical variable			
Strategy	12.34	6	0.085
Stress	1.54	1	0.23
Strategy × Stress	6.64	6	0.42
Component as a continuous variable			
C component	2.14	1	0.18
Stress	1.61	1	0.25
C × Stress	0.79	1	0.42
S component	3.2	1	0.09
Stress	1.61	1	0.24
S × Stress	5.7	1	0.02
R component	0.70	1	0.44
Stress	1.61	1	0.24
R × Stress	4.27	1	0.06
Seed mass effect			
Seed mass	0.25	1	0.66
Stress	1.54	1	0.27
Seed mass × Stress	2.09	1	0.20

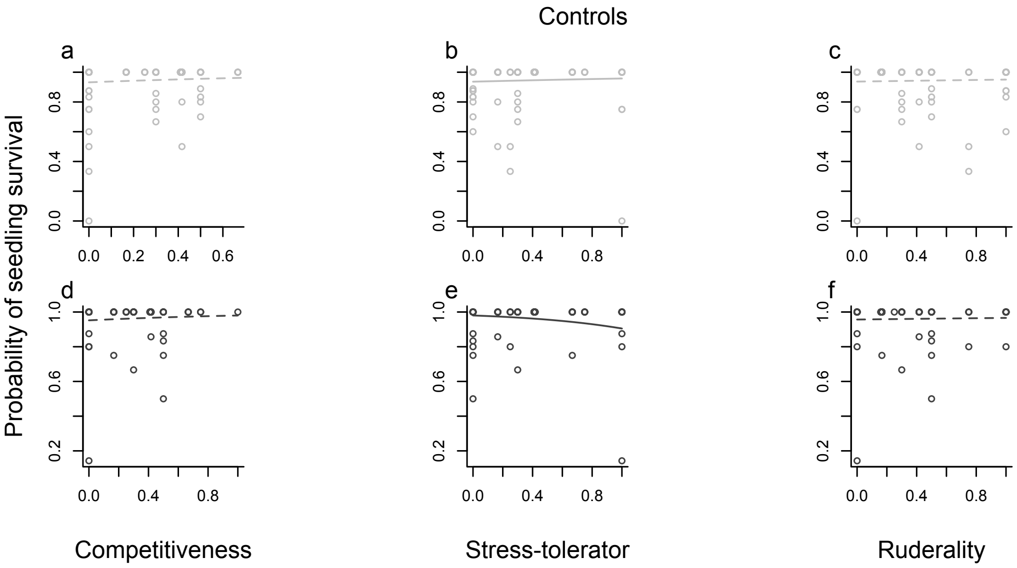

Figure 7. Probability of seedling survival in the control experiment for each component of the vegetation strategy; (a) and (d) *Competitiveness*, b) and (e) *Stress-tolerator*, (c) and (f) *Ruderality*. Dashed lines indicate a non- significant effect on seedling survival. Gray symbols indicate data points for the nutrient treatment; black symbols indicate data points for the no-nutrient treatment. For the *Stress-tolerant* component, differences in treatment are shown (gray line: nutrient–added, black line: nutrient–stress).

3.4.5 Seed mass effect on seedling survival under low and high–stress, and under nutrient – added and nutrient –stress controls.

Seed size did not affect the survival, and the stress did not interact with the seed size (Table 2 and 3). This was the same for all the treatments: low and high-stress soils (r^2 = 0.011), and nutrient-added and nutrient-stress controls (r^2 = 0.021).

3.4.6 Above–ground mass

The above–ground mass was significantly higher in the low-stress soils (mean = 0.077g) than in the high–stress soils (mean = 0.014g, *t*-test = 29.76, *d.f.* = 2058.17, $P < 0.00001$, Fig. A3a). Similar results were found for the nutrient–added control (mean = 0.073g) and the nutrient–stress control (mean = 0.008 g, *t*-test = 16.48, *d.f.* =452.26, $P < 0.00001$, Fig. A3b).

3.5 Discussion

In this chapter, we have used data collected from a large greenhouse experiment to test the predictions of CSR theory. We used 35 species with different vegetation strategies, sown into different stress treatments; 1) low and high-stress soils (differing in their pH, phosphorus and nitrogen availability), and 2) nutrient-added and nutrient-stress controls. Our findings show that species with a given strategy do not always respond to the level of stress, and when they do, the species with the same strategy can respond differently to the type of stress. The lack of consistency between our results and the predictions of CSR theory, suggests that simple categorization of species into functional categories does not allow prediction of community membership. Interestingly, we found that the type of stress could be an important filter for the species that are recruited into a community.

3.5.1 Seedling emergence

Stress treatments: 1) low and high-stress soils, and 2) nutrient added and nutrient-stress controls

Contrary to the expectations of CSR theory, seedling emergence was not different between individuals grown in low and high-stress soils. A cause of this unvarying emergence could be the favorable temperature of the greenhouse. Temperature, along with moisture, is a crucial factor regulating seed germination (Washitani 1985, Baskin and Baskin 1988). In our experiment, temperatures were between 15 and 22 °C. These are in the range of the optimal temperature for germination of grasses in temperate zones (16 and 25 °C (Baskin and Baskin 2001), and for species of temperate zones in general (Baskin and Baskin 1988, Kew 2016).

Vegetation strategies, components and stress

In this experiment, the seedling emergence of species with different vegetation strategies was not different at different stresses (the results were the same for both, the soil and the control treatments). However, the emergence of species with a *Stress-tolerator* component was slightly less negative in the high-stress treatments, which is an indicator of the advantages of *Stress-tolerators* under stress. This result did not hold for the emergence of the *Stress-tolerators* in the controls, suggesting that species with a *Stress-tolerator* components respond differently to different stresses, and not in the same way as suggested by (Grime 1979).

3.5.2 Seedling survival

Seed mass effect

Larger seeds have been shown to have an advantage on low soil moisture conditions (Leishman and Westoby 1994b), shade (Leishman and Westoby 1994a) and other hazards (Leishman et al. 2000). We did not find an effect of seed-size on seedling survival under stress conditions. This result could be the consequence of: a) the duration of the experiment (9-weeks), or b) the favorable growth conditions in the greenhouse. During the experimental period, the seedlings could still have been partially dependent on seed mass reserves, but also the potential advantages of large seed mass could have been hidden by the non-hazardous conditions presented in the greenhouse.

Stress treatments effect: 1) low and high-stress soils, and 2) nutrient added and nutrient-stress controls

In this study, the nutrient-stress did not influence survival; instead, variation in survival might have been driven by the presence of Aluminium in the soil treatments. As discussed in Chapter 2, low pH in the soil triggers the availability of Aluminium for plants, and this is highly toxic for the roots. Despite there being no difference in seedling survival for strategies at different nutrient levels, the mean dry weight was higher in the nutrient-added, than in the nutrient-stress controls, and this result was consistent for the low and high -stress soil (Fig. A3 a,b). Shoot biomass is generally higher under high-nutrient availability (McGraw and Chapin 1989). In addition, our result is similar to Hanley and Fenner (1997), who found that seedling mortality was not affected by nutrient deprivation after 12 weeks (as observed for the survival at the nutrient-stress treatment), but found that nutrient deprivation negatively affected species biomass. These findings suggest that there is a higher chance of becoming part of a vegetal community in low-stress habitats.

Effect of vegetation strategies, components and stress

Species with a stress-tolerant strategy did not have a higher survival at the high-stress treatment (Fig.5). However, survival increased for species with a *Stress-tolerator* component at the high-stress soils (Fig. 6e). Nevertheless, the predictive power from the model was low, and the results from the control treatment were opposite, *i.e.* survival decreased for species with a *Stress-tolerator* component at nutrient-stress (Fig.7 b, e). This indicates that different stresses would lead to different plant strategies. Grime’s definition of stress is universal (Grime, 1974), suggesting that species with *Stress-tolerant* characteristics will be present in sites with high intensities of stress no matter the source of it. However, different types of stress (low phosphorus or low calcium) can lead to different plant communities (Tilman 1988). Additionally, the type of stress will favor the presence of species with different traits. For example, low-nutrient habitats favor species with high allocation to roots, whereas low-light habitats will favor species with high allocation to shoots (Chapin 1980). Our results support the view of different stresses acting upon community membership, rather than one type of stress selecting for the same traits.

This atypical response of *Stress-tolerant* species has been reported elsewhere; Moog et al. (2005) found an increase in *Stress-tolerant* species under nutrient-stress conditions, but also under no-nutrient stress conditions. Gaxiola et al. (2010) found that *Stress-tolerant* species were tolerant to waterlogging; but also showed high-growth rates under fertile conditions, which is opposite to CSR predictions. This shows that the response to stressful conditions associated to *Stress tolerant* species might not always be predictable from the vegetation strategy. In addition McGraw and Chapin (1989) reported that species with *Stress-tolerant* attributes, have also *Ruderal* traits that allows them to outcompete their neighbors in the field.

The *Ruderality* and *Competitiveness* did increase the survival of the species in low-stress soils, as predicted from CSR theory (Fig.6 a,d). This is in agreement to the results reported in Mahmoud and Grime (1976), who found that *Arrhenatherum elatius* a *Competitor* species, showed a positive response to high-nitrogen supply. However, in our study the change in the type of stress resulted in a different response (Fig.7 a, d), implying that the nutrient availability is not the main cause of increased survival for the *Competitor* and *Ruderal* species.

In conclusion, the results from this study suggest that we cannot predict community membership from CSR theory. Instead, seedling survival seems to depend on the type of stress. Additionally, we found that the different types of stress cause the presence of species with different strategies, rather than only the Stress-tolerant strategy as predicted by CSR theory.

Chapter 4

The triangular seed mass-leaf size relationship holds for annual plants and is determined by habitat fertility

This work has been prepared for publication with the following authorship:

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4.1 Abstract

Plant allometries help us to understand resource allocation in plants and give us insight into how communities are structured. A triangular allometric relationship was reported for woody species in which seed mass and leaf size combinations are all possible, except for species with big seeds and small leaves, and it is explained in terms of variation between habitats. In this study we tested if the triangular relationship between seed mass and leaf size exist in annual plants, and if habitat indicators of soil fertility and light (Ellenberg numbers) are driving this relationship. We show that the triangular relationship also exists for annuals. This suggests that the allometric combinations between leaf size and seed mass are conserved across life-forms. We also found that the triangular relationship is driven by between-habitat variation in fertility (as measured by Ellenberg nitrogen numbers).

4.2 Introduction

In nature, leaves show large differences between species within climates (Givnish 1987). Leaves also display great variation in their morphology, anatomy, and physiology in response to growing conditions (Witkowski and Lamont 1991). Within species, leaf area varies in relation to both water and light availability (Xu et al. 2009). Despite this variation, comparative studies have shown that there are leaf strategies that can be recognized globally. For example, using a global database Wright et al. (2004) found that there is a positive relationship between leaf life-span and leaf dry-mass (LMA; dry mass of a leaf per unit of light-intercepting leaf area deployed). This means that longer-lived leaves are thicker and/or denser (Wright et al. 2004).

One of the most studied leaf traits is size or area, as it is easy to measure, and is involved in the control of leaf energy and water balance (Givnish 1987, Cornelissen et al. 2003). Leaf area is positively correlated with precipitation (Hamann 1979 in (Dolph and Dilcher 1980), and soil nutrient availability (Ashton and Hall 1992, McDonald et al. 2003), but negatively correlated to light availability (Niinemets and Kull 1994) and altitude (Milla and Reich 2011). Small leaves are therefore generally interpreted as an adaptation to drought and high-radiation (Ackerly 1999, Ackerly and Reich 1999, Cornelissen et al. 2003). Allometric studies show that leaf area is positively correlated with leaf dry mass (Niklas et al. 2009), twig-thickness (Yang et al. 2010), leaf width (Wilson et al. 1999). This means that larger leaves with broader, thicker laminas have less effective heat loss and lower photosynthetic rates (Givnish 1987). Allometric studies also give us insight into how communities are shaped, for example the relationship between wood density, and traits like stem water storage, leaf phenology, and resistance to hazards determines ecological distributions in trees (Enquist 2002).

In general, leaf area is negatively correlated with specific leaf area (SLA – leaf area/leaf mass; (Ackerly 1999, Ackerly and Reich 1999, Milla and Reich 2007), which is a good predictor of the relative growth rate (Cornelissen et al. 2003). The ecological significance of leaf area may relate to resource capture in productive habitats where big leaves are advantageous, and resistance to grazing, where small leaves are better (Diaz et al. 2001).

Midgley & Bond (Midgley and Bond 1989) found that leaf size and cone size are positively correlated in species from the *Leucadendron* genus. Further research was conducted by Cornelissen (1999) who hypothesized that if the infrutescence size and seed mass are positively correlated, then leaf area and seed mass should be positively correlated too. However, given the existence of pioneer species with large infrutescences and small seeds, there should be a deviation from this allometry, in which small seeded species can have either small or large leaves. In agreement with his hypothesis, Cornelissen (1999) found a triangular relationship between leaf area and seed mass in mature, woody species, suggesting that small-seeded species can have large or small leaves, whereas large-seeded species with small leaves do not occur. The triangular relationship seemed to be underpinned by variation between habitats in plant ecological strategies with: A) fast growing (ruderal) species with small seeds and large leaves found in early successional habitats, B) slow-growing (stress-tolerant) species with small seeds and leaves, found in stress-prone habitats, and C) slow-growing, tall plants (competitive) species with large leaves and seeds, found in mid-late successional habitats. So in summary the triangular relationship appears to be driven by variation between rather than within habitats. However, it should be noted that Cornelissen (1999) did not have any measures of habitat quality.

Given the clear links between variation in the environment and leaf size (Hamann 1979, Dolph and Dilcher 1980, Givnish 1987, Witkowski and Lamont 1991, Ashton and Hall

1992, Niinemets and Kull 1994, Ackerly and Reich 1999, Wright et al. 2004), it is therefore important to control for environmental variation when conducting comparative analyses. We therefore explored the relationship between seed mass and leaf size using a large database (provided by JG Hodgson, the Unit of Comparative Plant Ecology and Department of Archaeology, University of Sheffield), containing Ellenberg nitrogen and light numbers (which are proxies for assessing habitat characteristics; (Ertsen et al. 1998), or indicators for preference of soil fertility and light (Hill et al. 2004). The database contained trait information for 401 species of annuals from the UK. We examined two main questions: 1) Does the triangular relationship of seed mass-leaf size also occur in annual plants? 2) If so, does variation between environments drive this relationship?

To understand these two questions, we also explored the relationship between the components of the triangular relationship (leaf area and seed mass) separately in relation to Ellenberg numbers. Additionally, we looked at the relationship between leaf area and seed mass with plant height, as this trait plays an important role in determining the competitive capacity of the species for light and is positively correlated to leaf area (Cornelissen et al. 2003). In each case we ask how the relationship depends on habitat light and nitrogen availability.

4.3 Methods

The dataset contained information on 401 annual species from the UK, belonging to 37 families (Table 1). Two types of data were incorporated into the database:

1) **1)** Measurements from mature field specimens, with the range of per species sample sizes in brackets;
    a. Leaf area [mm^2] measured as the one sided surface area of a lamina (n = 1 - 17).

   b. Seed weight [mg], (*n* = 1-21), with some values extracted from Kew (2016).

The individual measurements for leaf area and seed weight were averaged at the species level and then log transformed.

2) Literature-based measurements; Ellenberg numbers for light and nitrogen for each of the species were extracted from the literature (Ellenberg et al. 1992), and for plant height class species were assigned into a class following (Stace 1987, Grime et al. 2007). This was done depending on the height of the species. In total there were nine plant height classes. In the database the species Ellenberg light numbers ranged from 4 to 9 (shade-semi shade plants, to plants in full light) and for the Ellenberg nitrogen numbers from 1 to 9 (low to high fertile habitats).

Table 1. Families and number of species per family used in the analysis. Mean leaf area and seed mass per family are shown.

Family	Number of species	Mean of seed mass (gr)	Mean of leaf area (mm^2)
Amaranthaceae	26	1.13	1219.15
Apiaceae	15	3.48	2456.59
Asteraceae	45	1.44	1595.68
Balsaminaceae	3	8.59	6524.83
Boraginaceae	10	3.21	938.02
Brassicaceae	40	2.15	1559.69
Campanulaceae	2	0.22	134.14
Caryophyllaceae	31	0.91	275.32
Cistaceae	1	0.06	436.51
Cyperaceae	1	0.05	398.10
Euphorbiaceae	6	0.98	263.67
Fabaceae	34	52.05	813.87
Gentianaceae	2	0.01	217.57
Geraniaceae	11	1.58	928.24
Juncaceae	1	0.02	117.48
Lamiaceae	13	1.71	803.69
Linaceae	1	1.41	48.97
Lythraceae	2	0.07	35.82
Malvaceae	7	4.81	2804.60
Montiaceae	3	0.59	522.23
Orobanchaceae	8	3.12	244.12
Papaveraceae	14	1.63	2073.24
Plantaginaceae	13	0.61	170.80
Poaceae	64	5.69	1393.27
Polygonaceae	12	4.12	1457.37
Portulacaceae	1	0.07	269.15
Primulaceae	2	0.45	120.18
Ranunculaceae	9	3.83	766.36
Resedaceae	1	1.14	275.42
Rosaceae	2	0.21	49.09
Rubiaceae	6	5.31	64.35
Saxifragaceae	2	0.02	71.31
Scrophulariaceae	1	0.01	25.118
Solanaceae	4	2.87	426.45
Urticaceae	1	0.51	512.86
Valerianaceae	5	0.82	426.45
Violaceae	2	0.56	244.48

We analyzed the allometric relationship between plant height class and leaf area, we calculated the mid-point values between each plant height class (mm) and then log transformed these. Ellenberg numbers were used as categorical variables in all the analyses.

By using gls models, we were able to incorporate the phylogenetic relatedness in our analysis. This is done using a variance-covariance matrix, which incorporates the distance between each species and its ancestor (variance) and the distance between each species (covariance) (Paradis 2012). These values were extracted from Daphne phylogeny, which is an ultrametric tree for 4685 species of vascular plants of the British Isles, Germany, The Netherlands, and Switzerland (Durka and Michalski 2012). Phylogenetic regression analyses were performed using the R packages ape (Paradis et al. 2004) and nlme (R Development Core Team 2010, Pinheiro et al. 2014). By using gls models we were able to incorporate the phylogenetic relatedness into our analysis. Variation in sample size of the explanatory variable, when available, was incorporated into the model.

To explore the relationship between leaf area and seed mass we constructed a range of different models. To test for a triangular relationship between seed mass and leaf area we allowed the variance about the fitted line to be a function of the fitted values. This assumes the variance of the residuals is given by,

$$\sigma^2 \exp\,(2 * t * \hat{y})$$

where $\hat{y}$ is the fitted values and t an estimated parameter. When t is negative the variance decreases as the fitted values become larger creating a triangular relationship.

To test whether the triangular relationship was a result of variation between habitats we fitted models including variation in Ellenberg numbers (as factors) for nitrogen or light, and tested if the variance function was still significant.

4.4 Results

4.4.1 Triangular relationship in leaf area and seed mass

The positive relationship between leaf area and seed mass described for woody species by Cornelissen (1999) was also found in annual plants (Fig. 1, $F_{1,\ 373}$ = 106.47 $P < 0.0001$, slope = 0.43 ± 0.042, r^2= 0.318). As in Cornelissen (1999), plants with small seeds had either small or large leaves, but the combination of large seeds with small leaves did not occur. As expected the variance about the fitted line decreased as the sample size became larger (Likelihood radio test = 7.81, P = 0.0052), but even after taking this effect into account, there was a highly significant decrease in the variance about the fitted line (Likelihood radio test = 10.66, P = 0.0011) resulting in a triangular relationship (Fig. 1A).

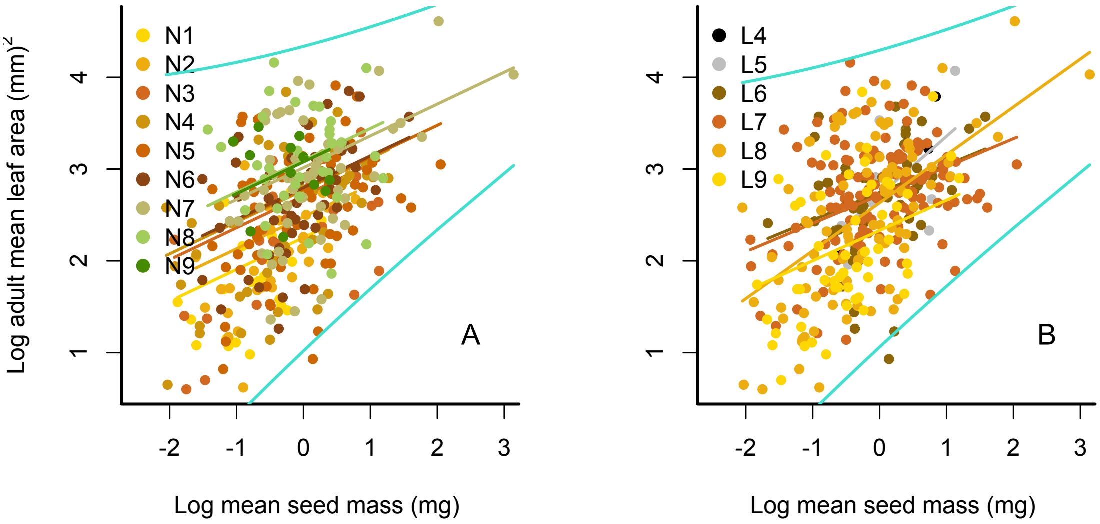

Figure 1. Leaf area (log) in relationship to seed mass (log scale) at different Ellenberg A) nitrogen and B) light numbers. The blue lines are the fitted variance function for each model. Ellenberg numbers for Nitrogen and Light are indicated by different colors, and represent a different line.

The relationship between leaf area and seed mass showed a non-significant interaction with Ellenberg nitrogen numbers (Ellenberg nitrogen × Seed mass, $F_{8,\ 321}$ = 1.83, P = 0.069; Fig.

1A), the main effect of Ellenberg nitrogen was however significant. In the model including Ellenberg nitrogen (r^2 = 0.43), there was no longer a significant decrease in the variance about the fitted line (Likelihood radio test= 0.043, P = 0.83). This suggests that the triangular relationship between seed mass and leaf area is a consequence of the variation in this relationship between habitats. The parameters for this model suggest that the intercepts increase with fertility, *i.e.* at a given seed mass, plants on fertile habitats typically have larger leaves than plants from less-fertile habitats (Fig. 1A, Table 2).

For Ellenberg light numbers, we found a significant interaction with the relationship between leaf area and seed mass (Ellenberg light × Seed mass, $F_{5,\ 332} = 2.56$ $P < 0.027$, r^2 = 0.31; Fig. 1B). In the model with the Ellenberg light × Seed mass interaction the variance about the fitted line still declined (Likelihood radio test = 8.41, P = 0.003). This result suggests that the triangular relationship between seed mass and leaf area is not driven by between-habitat variation in Ellenberg light numbers. The intercept values for the relationship between seed and leaf area tend to decrease as Ellenberg light numbers increased, *i.e.* at lighter conditions (Fig. 1B, Table 2),

Table 2. Intercept and slope values, for each relationship at different Ellenberg nitrogen and light numbers. The non-significant values are indicated in bold.

Plant trait	Ellenberg numbers	*Intercept* ± S.E.	*P*	*Slope* ± S.E.	*P*
Leaf area vs seed mass	Nitrogen				
	1	2.25±0.21	<0.0001	0.34±0.04	0.008
	2	2.47±0.21	<0.0001	0.34±0.04	0.003
	3	2.70±0.21	<0.0001	0.34±0.04	<0.0001
	4	2.76±0.20	<0.0001	0.34±0.04	<0.0001
	5	2.78±0.19	<0.0001	0.34±0.04	0.0002
	6	2.79±0.19	<0.0001	0.34±0.04	0.0003
	7	3.01±0.20	<0.0001	0.34±0.04	<0.0001
	8	3.09±0.20	<0.0001	0.34±0.04	0.27
	9	3.07±0.24	<0.0001	0.34±0.04	0.08
	Light				
	4	2.76±0.40	<0.0001	0.13±0.47	0.71
	5	2.71±0.26	<0.0001	0.64±0.27	0.02
	6	2.74±0.22	<0.0001	0.29±0.09	0.001
	7	2.71±0.22	<0.0001	0.30±0.06	<0.001
	8	2.63±0.24	<0.0001	0.52±0.06	<0.001
	9	2.33±0.27	<0.0001	0.33±0.10	0.002
Leaf area vs plant height	Nitrogen				
	1	1.59±0.43	0.0003	0.24±0.28	0.38
	2	1.50±0.33	<0.0001	0.52±0.21	0.01
	3	0.65±0.44	0.14	1.24±0.25	<0.001
	4	0.76±0.29	0.01	1.18±0.14	<0.001
	5	0.97±0.30	0.0018	0.99±0.14	<0.001
	6	1.19±0.32	0.0002	0.87±0.14	<0.001
	7	0.93±0.34	0.007	1.14±0.16	<0.001
	8	1.54±0.41	0.0002	0.86±0.20	<0.001
	9	2.39±0.55	<0.0001	0.34±0.29	0.24
	Light				
	4	1.35±0.35	<0.001	0.40±0.03	<0.0001
	5	1.13±0.27	<0.0001	0.40±0.03	<0.0001
	6	1.14±0.22	<0.0001	0.40±0.03	<0.0001
	7	1.16±0.22	<0.0001	0.40±0.03	<0.0001
	8	1.07±0.22	<0.0001	0.40±0.03	<0.0001
	9	0.89±0.03	0.225	0.40±0.03	<0.0001
Seed mass vs plant height	Nitrogen				
	1	-1.17±0.30	0.0002	0.36±0.09	0.0001
	2	-0.99±0.30	0.0013	0.36±0.09	0.0001
	3	-0.85±0.31	0.0079	0.36±0.09	0.0001

4	-0.81±0.30	0.0085	0.36±0.09	0.0001
5	-0.84±0.31	0.0076	0.36±0.09	0.0001
6	-0.72±0.31	0.02	0.36±0.09	0.0001
7	-0.62±0.32	0.054	0.36±0.09	0.0001
8	-0.67±0.32	0.036	0.36±0.09	0.0001
9	-1.02±0.34	0.0035	0.36±0.09	0.0001
Light				
4	-1.04±0.42	0.013	0.41±0.08	<0.0001
5	-0.74±0.29	0.01	0.41±0.08	<0.0001
6	-0.88±0.24	0.0005	0.41±0.08	<0.0001
7	-0.98±0.24	0.0001	0.41±0.08	<0.0001
8	-1.18±0.24	<0.0001	0.41±0.08	<0.0001
9	-1.25±0.24	<0.0001	0.41±0.08	<0.0001

4.4.2 Leaf area and habitat variables

Leaf area in our dataset varied by 4 orders of magnitude (from ~ 4 mm^2 to 41,000 mm^2), which represents a large proportion of the global variation among species (5–6 orders of magnitude (Wright et al. 2007). In our dataset leaf area was related to habitat quality, as in previous studies (Ashton and Hall 1992, Niinemets and Kull 1994, McDonald et al. 2003), (Ellenberg nitrogen - $F_{8, 330} = 8.79$, $P < 0.0001$, $r^2 = 0.30$; Fig. 2A and Ellenberg light $F_{5, 338} = 6.84$, $P < 0.0001$, $r^2 = 0.193$; Fig. 2B). To explore how leaf area varied with habitat quality we then refitted the models with Ellenberg numbers as an ordered factor. This allows us to partition the variation in leaf area between Ellenberg numbers into linear and quadratic components. We found there were significant linear ($t_{339} = 6.79$, $P < 0.0001$) and quadratic ($t_{339} = -2.19$, $P = 0.02$) terms for leaf area and Ellenberg nitrogen. However, for Ellenberg light numbers, only the linear term was marginally significant ($t_{344} = -1.97$, $P = 0.049$). So, leaf area increased with soil fertility (or higher Ellenberg nitrogen numbers), and possibly with shadiness (low Ellenberg light numbers).

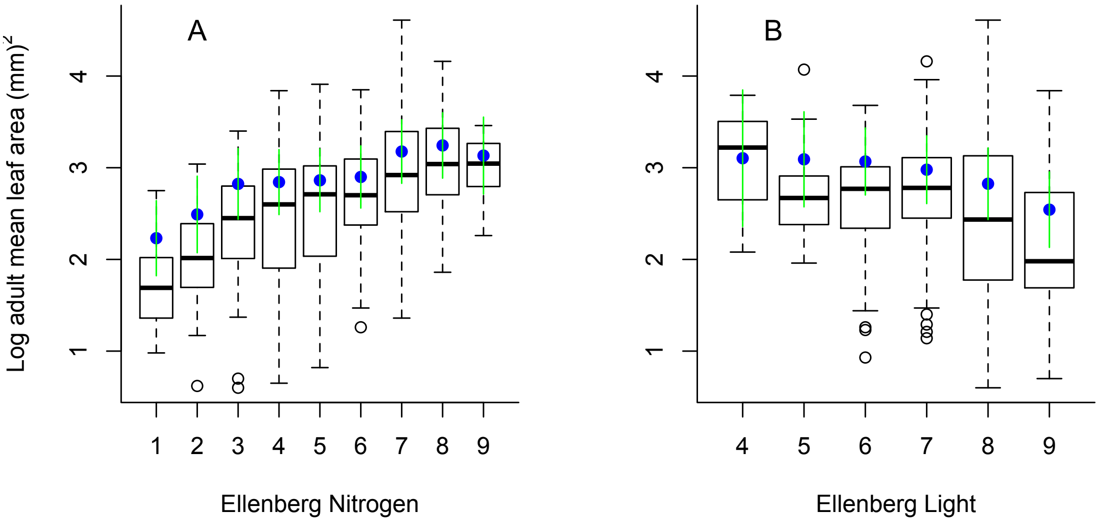

Figure 2. Relationship between leaf area (log) and Ellenberg values A) Nitrogen, and B) Light. Blue points are the fitted values and 95% confidence intervals are shown in green.

4.4.3 Seed mass and habitat variables

In our dataset, seed mass varied 5 orders of magnitude, from 10^{-3} to 10^{2} grams, which represents half of the global variation among species: 10 orders of magnitude (Harper et al. 1970). Seed mass varied with Ellenberg Nitrogen numbers ($F_{8,\ 330} = 4.821$, $P < 0.0001$, $r^2 = 0.108$; Fig. 3A) and Light numbers ($F_{5,\ 338} = 7.127$, $P < 0.0001$, $r^2 = 0.093$, Fig. 3B). In the model with Ellenberg nitrogen as an ordered factor, there were significant linear ($t_{339} = 3.16$, $P = 0.0017$) and quadratic ($t_{339} = -3.52$, $P < 0.001$) terms. However, neither of these terms was significant for Ellenberg light numbers: linear ($t_{344} = -1.27$, $P = 0.20$) or quadratic ($t_{344} = -1.91$, $P = 0.055$).

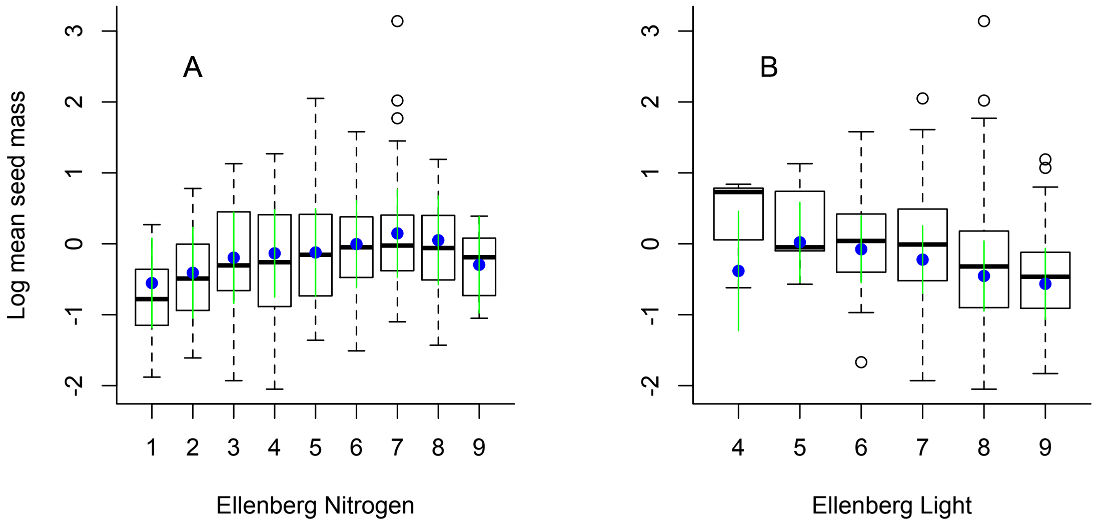

Figure 3. Relationship between seed mass (log) and Ellenberg values A) Nitrogen, and B) Light. Blue points are the fitted values and 95% confidence intervals are shown in green.

We also explore the relationship between seed mass and plant height (r^2 = 0.171), and its interaction to Ellenberg numbers. Our results showed that there was an effect of Ellenberg nitrogen numbers ($F_{8, 329}$ = 4.82, $P < 0.0001$) and plant height ($F_{1, 329}$ = 14.87, $P = 0.0001$) on seed mass, but the interaction was not significant (Ellenberg nitrogen × Plant height, $F_{8, 321}$ = 1.47, $P = 0.16$, $r^2 = 0.15$, Fig. 4A). Similar results were found for Ellenberg light numbers ($F_{5, 337}$ = 7.86, $P < 0.0001$), plant height ($F_{1, 337}$ = 22.54, $P < 0.0001$), and the interaction (Ellenberg light × Plant height, $F_{5, 332}$ = 0.64, $P = 0.66$, $r^2 = 0.07$, Fig. 4B). So larger seeds occurred on taller plants and the intercept of this relationship tended to increase with soil fertility and decreased with light.

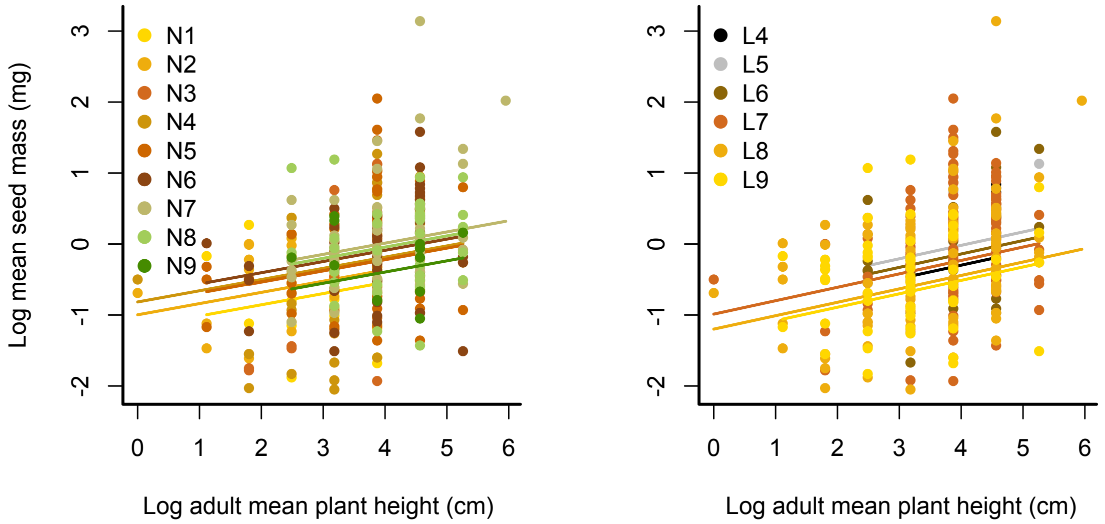

Figure 4. Relationship between seed mass and plant height (log scale) at different Ellenberg values for A) Nitrogen, and B) Light. Only lines with significant slopes are shown.

4.4.4 Leaf area in relation to habitat and other plant traits

Leaf area was positively related to plant height ($F_{1,\,373} = 214.62$, $P < 0.0001$, $r^2 = 0.55$; Fig. 5). The slope was 0.43 ±0.028 indicating that leaf area tends to increase more slowly than plant height. For this relationship there was a significant interaction with Ellenberg nitrogen ($F_{8,\,321} = 2.26$, $P = 0.022$, $r^2 = 0.73$), but not for Ellenberg light ($F_{5,\,332} = 1.67$, $P = 0.13$, $r^2 = 0.60$) numbers. As the relationship between plant height and Ellenberg Light number was similar in light levels 4 to 8 (Table 2), we collapsed the light levels into two groups (4-8 and 9) and compared this model to the full model (that included Ellenberg light numbers from 4-9). The resulting model was not significantly different from the full model (Likelihood ratio test = 3.80, $P = 0.43$). The reduced model showed, that there was an effect of plant height ($F_{1,\,340} = 177.76$, $P < 0.0001$) and Ellenberg light numbers ($F_{1,\,340} = 40.22$, $P < 0.0001$) on leaf area, and also there was evidence of an interaction between Ellenberg light level and plant height ($F_{1,\,340} = 6.34$, $P < 0.05$, $r^2 = 0.61$), suggesting that the slope of the relationship between leaf area and plant height was shallower in full light (0.28±0.06 *vs* 0.45±0.03). Therefore, at a given height, plants tended to have larger leaf areas in more fertile habitats (larger Ellenberg nitrogen numbers, Fig. 5A), and shady habitats (lower Ellenberg light numbers, Fig. 5B), although in the latter case this was largely driven by a single habitat.

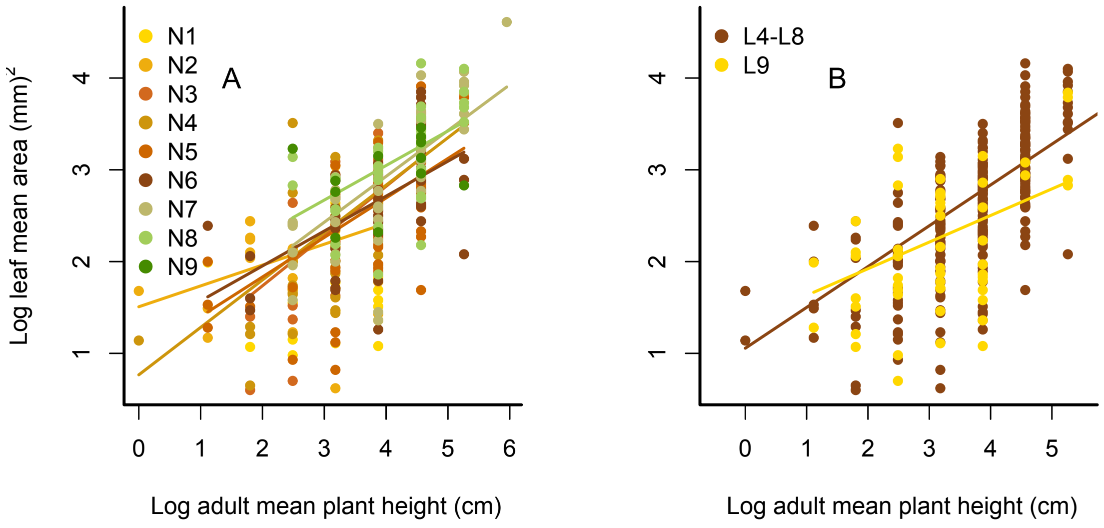

Figure 5. Leaf area (log) in relationship plant height (log) depending on Ellenberg: A) Nitrogen and B) light numbers (from the reduced model). Only lines with significant slopes are shown.

We also analyzed the relationship between plant height and habitat variables. We found that it was positively related to soil fertility, fitting Ellenberg nitrogen as an ordered factor there were significant linear ($t_{339} = 5.45$, $P < 0.0001$) and quadratic ($t_{339} = -2.15$, $P = 0.03$, Fig.6A) terms. However, for Ellenberg light numbers, neither the linear ($t_{344} = -1.57$, $P = 0.11$) nor the quadratic ($t_{339} = -1.74$, $P = 0.08$, Fig.6B) terms were significant.

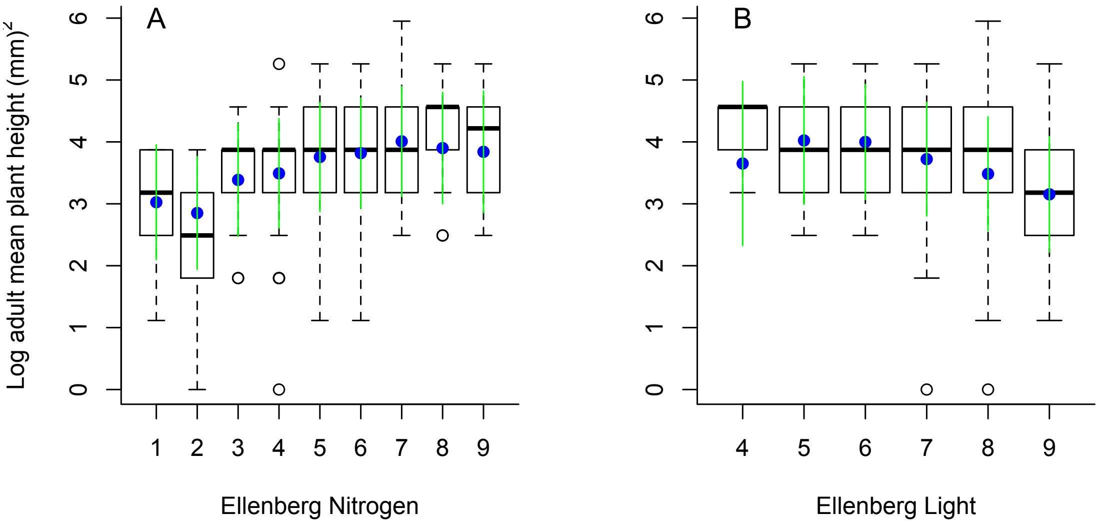

Figure 6. Relationship between plant height (log) and Ellenberg numbers for A) nitrogen, and B) light. Blue points are the fitted values and 95% confidence intervals are shown in green.

4.5 Discussion

4.5.1 Triangular relationship between leaf area and seed mass

We found that the triangular relationship described by Cornelissen (1999) for woody species, also occurred in our dataset of annual plants (Fig. 1). This triangular relationship was a consequence of no large seeded species having small leaves. Cornelissen (1999) suggested several possible explanations for this pattern, these included: 1) phylogeny, 2) allometry, and 3) ecology, specifically variation in life-history, successional stage, and between-habitat variation in nutrients and shade. Our analysis was restricted to annuals, and so the variation in life-history is likely to be small. Likewise, all our statistical analyses included phylogeny and so this is unlikely to explain the relationship. Our results suggest that allometry in combination with between-habitat variation in fertility (as measured by Ellenberg nitrogen numbers) could be driving the triangular relationship in annual species (Fig. 1A), since there is a significant decrease in the variance of the fitted leaf area – seed mass relationship when between habitat variation in fertility is ignored, but not when it is included in the model.

4.5.2 Leaf size, habitat variables and plant height

Several studies have demonstrated that leaf size is positively related to soil fertility (Ashton and Hall 1992, Fonseca et al. 2000, McDonald et al. 2003). Our results were in agreement with this (linear: $t_{339} = 6.79$, $P < 0.0001$, and quadratic term: $t_{339} = -2.19$, $P = 0.02$; Fig. 2A). Explanations for this pattern are usually framed in terms of high leaf construction costs, which limit leaf size in nutrient poor habitats (Givnish 1987, Xu et al. 2009). However, several studies suggest that leaf size forms part of a trade-off with leaf number (Falster and Westoby 2003, Westoby and Wright 2003, Kleiman and Aarssen 2007), and so plants could in principle produce either many small leaves or a few large ones. Alternatively, plant size might limit leaf area in infertile habitats, and in agreement with this hypothesis we found that plant height increased with soil fertility (Fig. 6A). It has also been suggested that higher transpiration in small leaves could be an adaptation for acquiring nutrients in low fertility habitats (Yates et al. 2010).

In the case of light, previous studies have reported a negative relationship with leaf area (Niinemets and Kull 1994, Markesteijn et al. 2007). However, for annuals, the relationship was only marginally significant ($P < 0.049$), with larger leaves occurring in shaded habitats, *i.e.* low Ellenberg light numbers (Fig. 2B). The negative relationship between leaf area and light availability is explained in terms of larger leaves allowing greater light interception in less lit habitats (Markesteijn et al. 2007), whereas in open habitats, plants construct smaller leaves, with low SLA, which would increased their photosynthetic capacity (Björkman 1981, Markesteijn et al. 2007).

Leaf area was positively related to plant height class (Fig. 5). A similar relationship was reported for woody species (Senn et al. 1992, Niinemets and Kull 1994, Cornelissen 1999). The increase in leaf area with plant height is not surprising as the size of an axis or stem is linked to the size of its appendages, in this case leaves (Cornelissen 1999). In our study, the intercept for the relationship between leaf size and plant height increased with Ellenberg nitrogen numbers (Fig. 5A). These results are expected from theory: bigger leaves and taller plants are adaptive in highly nutrient-competitive habitats (Falster and Westoby 2003, Wright et al. 2007), where competition for light is strong. However, in our study we found that only the slope, and not the intercept, declines with light (Ellenberg light = 9), suggesting that in full light environments, there is a restriction for leaf area values to increase even in taller plants (Fig. 5B).

4.5.3 Seed mass, habitat variables and plant height

Evidence for the relationship between seed mass and fertility is equivocal (Leishman et al. 2000, Pakeman et al. 2008) with positive (Grubb and Coomes 1997), negative (Lee and Fenner 1989, Parolin 2000, Dainese and Sitzia 2013) and no relationship (Hammond and Brown 1995, Wright and Westoby 1999, Pakeman et al. 2008), all being found. However, these studies often used relatively few species, for example: Lee and Fenner (1989) used 12 species in the *Chionochloa* genus. Repeating this analysis using the data from the paper, we found that the significant negative relationship ($P < 0.04$) was no longer significant if a single species was removed ($P = 0.42$).

Several explanations have been presented in the literature to account for the range of relationships found. Positive relationships might arise if small seeds were advantageous in low fertility habitats because they have higher RGR, and so potentially outcompeting their neighbours (Marañón and Grubb 1993). In contrast, negative relationships would occur if large seeds allowed greater seedling establishment in low fertile soils, say as a result of greater seed reserves (Lee and Fenner 1989, Dainese and Sitzia 2013). In our dataset we found a positive relationship between seed mass and soil fertility (for the linear term: $t_{339} = 3.16$, $P = 0.0017$; quadratic term: $t_{339} = -3.52$, $P < 0.001$, Fig. 3A). Another possible explanation for this positive relationship relates to plant height, since small plants can only support small seeds and taller plants can produce structures to bear bigger seeds, although this relationship is allometric (Aarssen 2005, Pierce et al. 2014). Also, larger plants typically occur in more productive habitats (Moles et al. 2009). So bigger plants in higher Ellenberg Nitrogen habitats would be able to produce bigger seeds. Our results for annuals showed plants were taller as soil fertility increased (Fig. 6A). Also, seed mass was positively related to plant height and this relationship tended to increase with soil fertility (Fig. 4A).

In the case of light, several studies have reported that bigger seeds are associated with closed habitats (Salisbury 1974, Metcalfe and Grubb 1995, Hodkinson et al. 1998, Thompson and Hodkinson 1998). Although, in some cases the relationship seems to depend on the taxonomic level at which comparisons are made. For example, Grubb and Metcalfe (1996) found larger seed species in shaded habitats when the comparison was made between genera within families, but no difference when comparisons are made within genera (see also Mazer (1990)).

In contrast to these studies, within annuals we found no evidence for a positive relationship between seed mass and shade, as neither the linear ($t_{344} = -1.27$, $P = 0.20$) nor the quadratic terms ($t_{344} = -1.91$, $P = 0.055$, Fig. 3B) were significant. We believe this is largely a consequence of annuals being restricted to open habitats (there were no species with Ellenberg light numbers less than 4). Nevertheless, when relating seed mass to plant height at different Ellenberg numbers for light, we found that taller plants produce bigger seeds, and this relationship tended to increased with shadiness (Fig. 4B). So, again this could be a consequence of plant height, as small plants are usually related to lit habitats, and not to shady ones, mainly because of the reduction in light availability created by taller plants (Moles et al. 2009).

As shown above, the allometries between leaf area and seed mass can be extended into plants with no secondary growth, such as annual species. This is relevant as it suggests that the resource allocation evolved in a similar way across plant species, even though annuals have a higher reproductive effort (seed number per fruit) than perennials (Primack 1979). As Cornelissen (1999) suggests, the lack of a forth corner could be the result of 1) biomechanical limitations between the mass of the seed and the length of the leaf, or 2) the balance between the leaf (source) and the seed (sink) in a plant. These ideas, however, remain to be tested.

Chapter 5

Plant functional trait variation and genome size

5.1 Abstract

The relationships between genome size and plant traits across species have shown that genome size is strongly correlated with other traits like cell size, seed mass and leaf area. Comparisons of phenotypic variation between small *vs*. large genome species lead to the hypothesis that species with large genomes pay costs for the accumulation of 'junk DNA', *i.e.* these species display less phenotypic variation in some functional traits, for example; large genome species can only display large seeds, while small genome species can do either big or small seeds. In this study we argue that if large genome species are restricted in the amount of phenotypic variation they can display, then this constraint should be detected within species. In general, our findings did not support the large genome constraint hypothesis. Instead, we found that large genome species have more phenotypic variation for seed mass, guard cell length and leaf area at the intraspecific level. If large genome species are frequent in variable environments, then having more phenotypic variability could be an advantageous strategy in those conditions.

5.2 Introduction

Plant functional traits are defined as species' characteristics that are linked to their role (effect or response) in the ecosystem (Diaz and Cabido 2001). They affect the organismal performance (McGill et al. 2006), and reflect adaptations of the species to their habitat conditions. For example, the specific leaf area (SLA) of a species is linked to leaf life span (long-lived leaves have lower SLA; (Lavorel et al. 2007)), and reflects the resource-content of a particular environment (high SLA indicates resource-rich habitats; (Cornelissen et al. 2003)).

Plant functional traits are usually compared across species mean values (Cornelissen et al. 2003, McGill et al. 2006, Violle et al. 2012). Nevertheless, variation in plant functional traits also occurs within species. For some traits, such leaf mass per area and leaf dry matter content, intraspecific variation is even higher than that at the interspecific (Messier et al. 2010). This indicates that intraspecific trait variation is important in promoting species coexistence and structuring communities (Jung et al. 2010). The variation in plant traits across species has been found to correlate with habitat and geographic variables (*e.g.* precipitation, nutrient availability, temperature and latitude (Leishman et al. 2000, Susko and Lovett-Doust 2000, Moles et al. 2014), plant allometries (*e.g.* plant height and leaf size (Cornelissen 1999, Moles et al. 2009)) and to genome size (Knight and Ackerly 2002, Beaulieu et al. 2008).

In relation to genome size, Knight et al. (2005), hypothesize that species with large genomes pay costs related to the accumulation and replication of ‘junk DNA’. The authors gathered information on the evolution, ecology and phenotype of species and related them to genome size (1Cx and 2C DNA). They show that species with increasingly large genome sizes are increasingly rare. They also show evidence for a constraint on the range of traits observed in large genome species. For example, large-genome species have only big seeds, while small genome species can have either small or big seeds, (but see ((Hodgson et al. 2010) where they reported that tiny seeded Orchids have big genome sizes). They also suggest that large genome species are also more environmentally constrained. Large genome species do not occur in extreme (cold or hot) temperatures, and this constrains the species’ ecological distributions. Knight et al. (2005), conclude that large genome species pay costs associated to having a large genome and that this constrains the possible phenotypic trait values of a species. However, the idea of 'junk DNA', has been debated in studies which have shown that transposable elements (TEs), which make up for 'junk DNA', have a function in the regulation and evolution of the genomes ((Biemont and Vieira 2006) and references therein). For example, TE's create diversity among maize individuals by copying gene segments into this species genome and also they are expressed into RNA (Biemont and Vieira 2006). This suggests that 'junk DNA' might have an actual function at the phenotypic level.

Previous studies have investigated the large genome constraint hypothesis by comparing between species trait-values. However, if there are phenotypic constraints for large genome species, we should be able to detect them within species. This can be tested by measuring the phenotypic variation that a specific species can display for a particular trait and relating this to its genome size. We predict that if large-genome species are constraint then: a) small genome species should display more variation *within* species in comparison to species with larger genomes. Also, b) we expect to see the large-genome constraints for other phenotypic traits *between* species. Alternatively, if 'junk DNA' has a function in the organism, then the large genome constraint hypothesis may not hold. Species may not pay a cost associated with having a larger genome and may not be phenotypically constrained. If this is true, then we would not expect to observe a reduction in the phenotypic variation large genome species.

In this study, we first evaluated trait variation at different taxonomic levels: family, genus, species and within species. This was described by the coefficient of variation of each functional trait at each taxon. Second, we analyzed the relationship between the trait-variance within species and genome size. We conducted this analysis using a phylogenetically corrected regression framework to account for non-independence of species traits resulting from their shared evolutionary history. Finally, we analyzed the relationship between genome size and mean traits between species; this analysis was done with both a generalized least square model (incorporating a phylogeny) and a quantile regression.

5.3 Methods

5.3.1 Data

We examined an unpublished dataset (provided by JG Hodgson) comprising 253 plant species; of these 59% are annuals, 34% are perennials and 7% biennials. Traits measurements come from a variety of habitats, from across Eastern and Western Europe and Middle East Asia. Several plant traits of mature individuals were recorded following the procedures described in Charles et al. (1997). In summary we used measurements of: a) leaf area (mm^2); measured on fresh leaves as the one sided surface area of a lamina, b) leaf dry matter content or LDMC (g), after leaves were dried for 2 days at 80° C, c) SLA (leaf area /leaf dry weight), d) stomatal density, the average for both the upper and lower surfaces (number of stomata per mm^2), recorded from acetates sheets taken for cell impressions, e) guard cell length (Links 1993-1996), leaf thickness [mm], measured with a dial gauge, g) seed mass (mg), h) leaf width was measured as the largest imaginary circle that can be fitted on the leaf surface (mm).

Individuals were sampled within populations and then average, so each data point is the mean for a particular population. The sample size (number of populations) per species varied depending on the plant trait (Table 1).

Genome size data was taken from (Bennett and Leitch 2012), together with additional data from references not yet incorporated into the Plant DNA C-values database (IJ Leitch, personal communication). The DNA amounts in the database have been estimated in different ways depending on the source of the data, this is because some of the data have been taken from the literature. However, the main approach was through the use of flow cytometry. Using this technique, a flurochrome stain was used to bind to DNA. The total amount was then estimated based upon the relative fluorescence intensities. For more information on flow cytometry protocol's see (Dolozel et al. 2007).

Genome size refers to the total amount of DNA content in a nucleus. Given that the amount of DNA in a nucleus varies throughout the cell cycle, two measures are commonly used: 1) Holoploid (2C DNA), which is the total amount of DNA in a somatic cell at the first stage of the cell cycle, regardless of the ploidy level, and 2) Monoploid (1Cx) which is the total amount of DNA taking into account the polyploidy level of the species. The holoploid (2C DNA) value is positively correlated to the cell volume, while the monoploid (1Cx) value is positively correlated to the cell cycle time (Bennett 1972, Beaulieu et al. 2007a, Beaulieu et al. 2007b, Münzbergová 2009). In this study species' 2C DNA–values ranged from 0.3 to 33.70 pg (112-fold). For the species of known ploidy we calculated monoploid genome size (1Cx) by dividing the 2C value by the ploidy level.

Table 1. Sample size (or number of populations) and range of values per species per plant trait.

Plant trait	Sample size (n)	Range of values
Specific leaf area (SLA)	1-26	8.10-61.6
Leaf dry matter content (LDMC)	1-16	8.11-35.9
Leaf area	1-17	16.51-16183.3
Leaf width	1-8	0.85-386
Leaf thickness	1-15	0.07-0.5
Stomatal density	1-12	26-399.9
Guard cell length	1-11	14.10-61.7
Seed mass	1-37	0.021-62.5

5.3.2 Analysis

To address the first question regarding the trait variation at different levels, we analyzed the coefficient of variation at different taxonomic levels (family, genus, species, within species). The coefficient of variation is given by

$$CV = \frac{\sigma}{\mu}$$

where σ is the standard deviation, and μ the trait mean.

To test the second idea, that larger genome species display less trait variation within species, we calculated the variance for each trait within species and related these values to the genome size of each species. We used a phylogenetic regression (Orme et al. 2012), expecting to see a negative relationship, indicating the decreased in variance with larger genome sizes. The phylogeny used for this analysis was trimmed from a published phylogeny that used molecular data for 32,223 species (Zanne et al. 2014). For species not in the phylogeny we used the pez package (Pearse et al. 2015) to add species within genera (Fig. 1).

As Genome size (2C DNA), and monoploid genome size (1Cx) have been showed to have different explanatory power (Beaulieu et al. 2007b) both were used in the analysis.

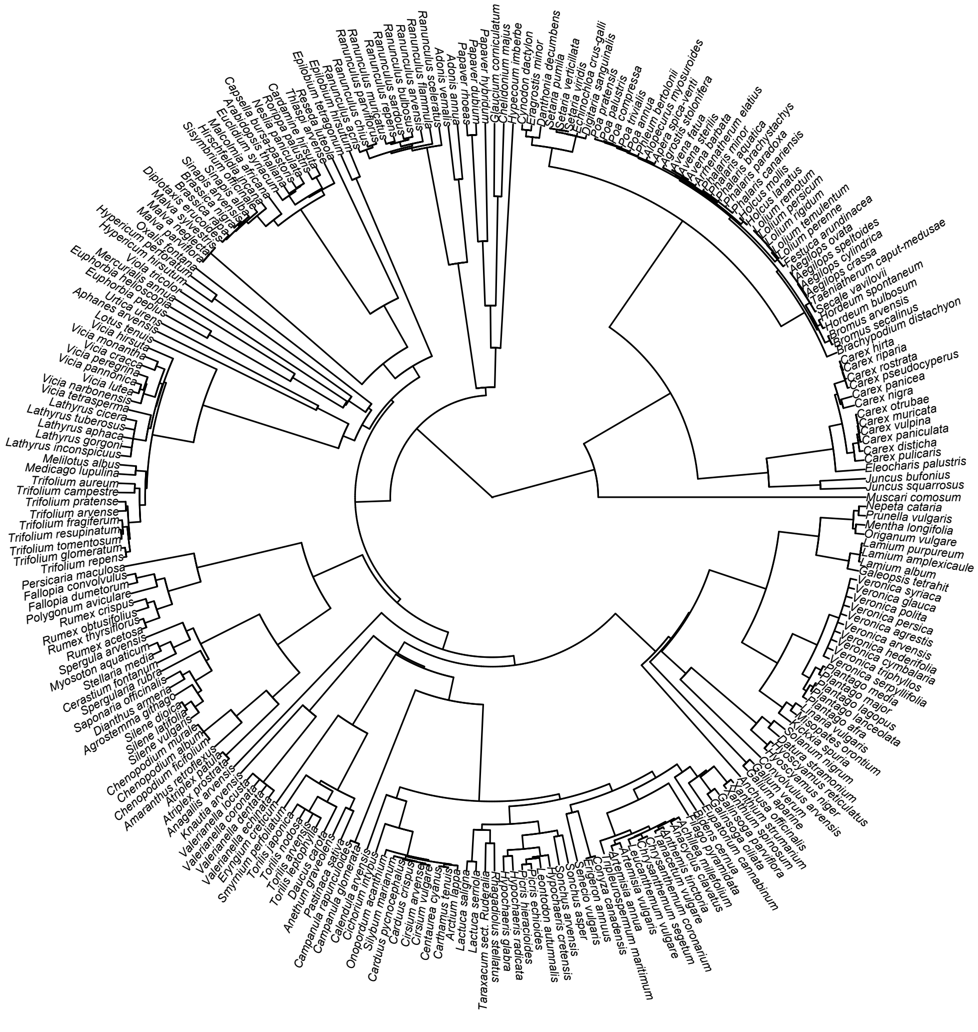

Figure 1. Phylogeny for the 253 species used in the analysis.

Finally, to address the relationship between genome size and mean traits between species, we used two approaches. First we used a phylogenetically corrected regression to test the relationship between genome size and trait values. We then assessed whether the variance in genome size increased with these trait values. To do this, a generalized least square regression was fitted using gls function from the nlme package (Pinheiro et al. 2014). In this analysis, the phylogenetic relatedness of different species is accounted for by measuring the phylogenetic distance between each species pair and incorporating this value into the models variance-covariance matrix (Paradis 2012). For the gls models the distributional assumptions were more appropriate when the response variable was log transformed. The log transformation is however a variance stabilizing transformation making the analysis difficult to interpret. Using the standard 1st order approximation to the variance we find,

$$\sigma_Y^2 \approx \mu_Y^2 \sigma_e^2 \exp{(2\beta F)}$$

where σ_Y^2 is the variance of the response variable on the untransformed scale, μ_Y the mean, σ_e^2 the fitted error variance, β a fitted parameter and F the fitted values from the regression model. This means we can test $\beta = 0$ to explore if the variance of the response variable is a function of genome size.

The second approach used was a quantile regression (Koenker 2011) , which is a non-parametric method. A quantile regression fits a regression line to different quantiles of the observations, not to the average of the response variable (as most of the regression models do). Quantile regression analysis is useful when describing complex patterns of variation, for example changes in the slope for different quantiles can be used to understand systematic changes in the variance (Cade and Noon 2003). We fitted regressions for the quantiles 0.05, 0.1, 0.25, 0.50, 0.75, 0.90, 0.95. To examine changes in the slopes of the relationship between genome size and plant traits we fitted quantile regressions for the range of 0.5 (5%) to 0.95 (95%) quantiles in steps of 0.01. If large genome species are constrained in the range of phenotypic variation they can display, then we expect to see changes in the slope of the relationships at different quantiles. However, quantile regression does not incorporate the phylogenetic relationship between species, and so for that reason the outcome must be interpreted carefully. In both methods (phylogenetic gls and quantile regression) we used species mean trait values, and so ask whether the interspecific trait variance depends on genome size. All the analyses were conducted in R (R Development Core Team 2010).

5.4 Results

For most of the traits studied (seed mass, SLA, leaf thickness, leaf size and leaf width), we found that the coefficient of variation (CV) was higher between species than within species (Fig. 2). However, the CV was higher within species for LDMC, stomata density and guard cell length (Fig. 2).

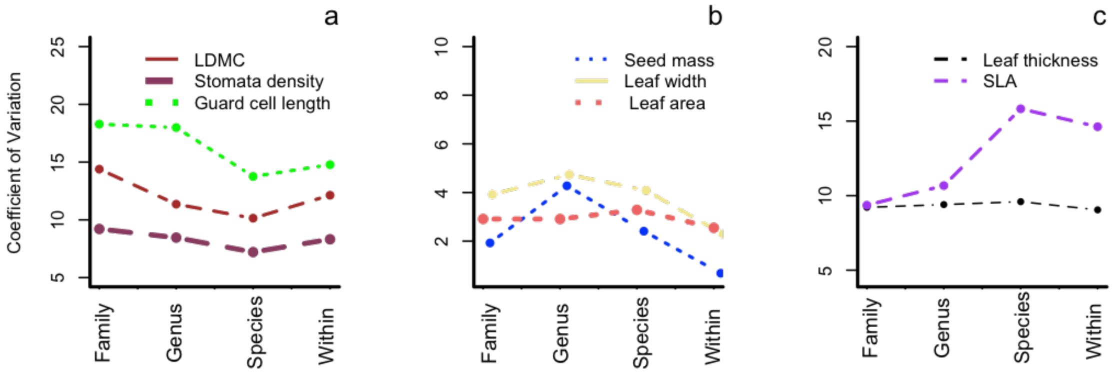

Figure 2. Coefficient of variation for plant functional traits at different levels: family, genus, species and within species, a) leaf dry matter content (LDMC), stomatal density, and guard cell length, b) seed mass, leaf width and leaf area, c) leaf thickness, and specific leaf area (SLA).

5.4.1 Variation in plant traits *within* species, and its relationship to genome size

5.4.1.1 Phylogenetic analysis

The variance within species for stomatal density was reduced as genome size increased (Table 2, Fig. 3d). However, this was the only negative slope between the variance within species of a trait and both 2C DNA and 1Cx (Table 2). We found a significant positive relationship for the variance within species of guard cell length, seed mass, and leaf area as both, 2C DNA and 1Cx, increased (Table 2, Fig. 3). However, for guard cell length and 1Cx values this relationship was not significant (Table 2). There was not a significant relationship between genome size and the variance within species of leaf thickness, LDMC and SLA, and leaf width (Table 2).

Table 2. Phylogenetic regressions for the variance within species for each trait and 2C and 1Cx.

Trait variance [log-scale]	2C DNA				1Cx			
	Slope	*r^2	*F*	*P*	Slope	*r^2	*F*	*P*
Guard cell length	0.19	(1)0.05 (2)0.03	5.46	**0.02**	0.10	(1)0.01 (2)0.006	1.03	0.31
Stomata density	-0.40	(1)0.09 (2)0.07	14.9	**<0.001**	-0.41	(1)0.09 (2)0.07	12.3	**<0.001**
Seed mass	1.62	(1)0.33 (2)0.17	43.74	**<0.0001**	1.71	(1)0.35 (2)0.22	47.73	**<0.0001**
Leaf area	0.81	(1)-0.04 ≈0 (2)0.061	13.46	**<0.001**	0.90	(1)0.11 (2)0.08	14.05	**<0.001**
Leaf width	0.16	(1)0.004 (2)0.007	0.76	0.38	0.18	(1)-0.03 ≈0 (2)0.007	0.73	0.39
Leaf thickness	0.13	(1)0.006 (2)0.01	2.05	0.15	0.11	(1)-0.003 ≈0 (2)0.006	1.08	0.29
LDMC	0.10	(1)0.0002 (2)-0.006 ≈0	3.03	0.08	0.07	(1)0.006 (2)0.003	1.04	0.30
SLA	0.15	(1)0.02 (2)0.01	2.86	0.092	0.017	(1)0.001 (2)-0.006 ≈0	0.028	0.86

* There are two ways to calculate r^2 for phylogenetic regressions(Paradis 2012), so here I present the results for both (1) and (2).

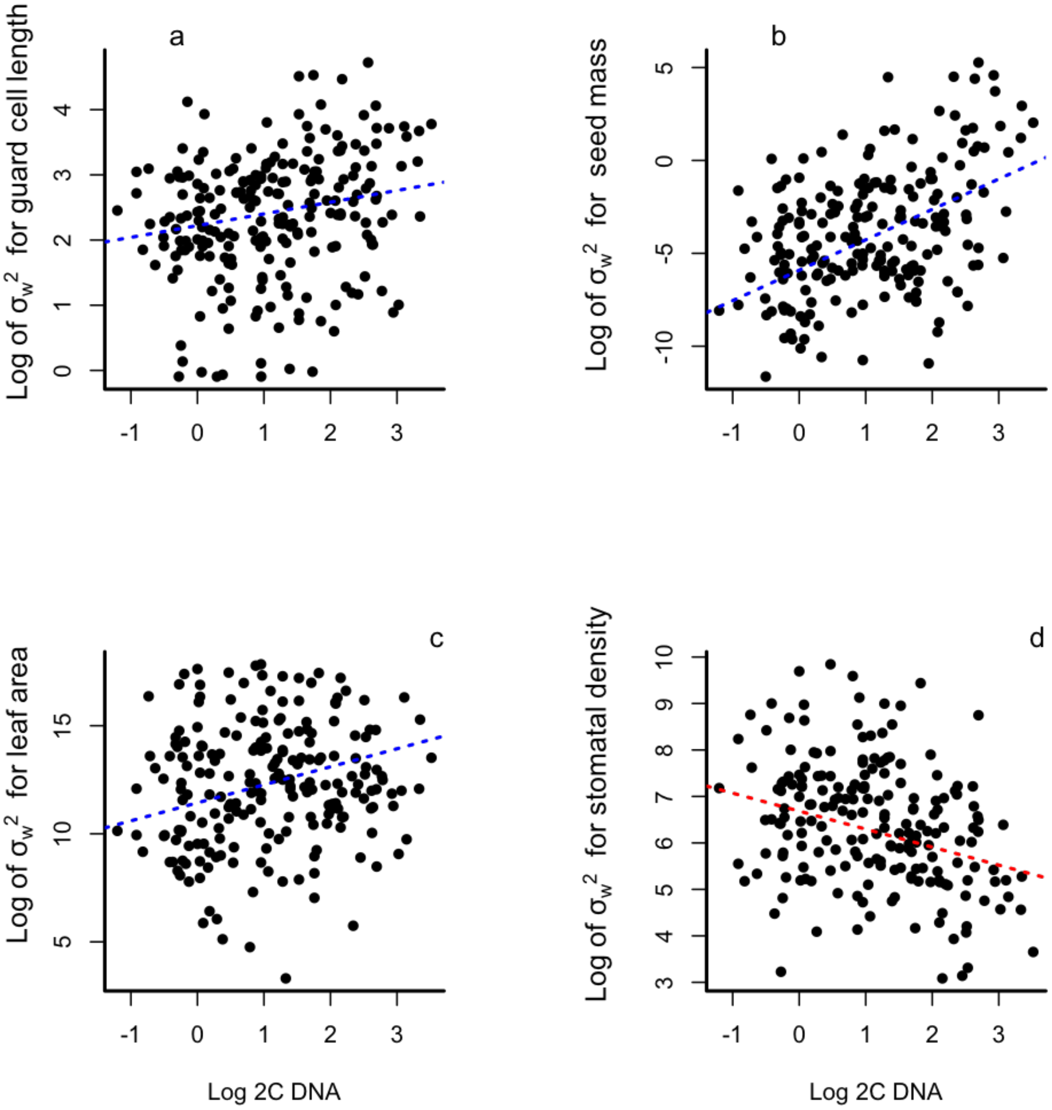

Figure 3. Phylogenetic analysis for the relationship between genome size (2C DNA) and the variance within species (σ_w^2 log scale) for functional traits: a) guard cell length, b) seed mass, c) leaf area, d) stomatal density. Blue lines indicate positive slopes, red lines negative slopes for 2C DNA values.

5.4.2 Variation in plant traits *between* species and its relationship to genome size

5.4.2.1 Phylogenetic analysis

The analysis for the mean values showed a reduction in the variance of stomatal density as genome size increased (only for 2C DNA, Fig. 4a) and an increase for guard cell length (only for 1Cx, Fig. 4b). For the other traits the variance about the fitted line did not vary significantly with the fitted values (Table 3).

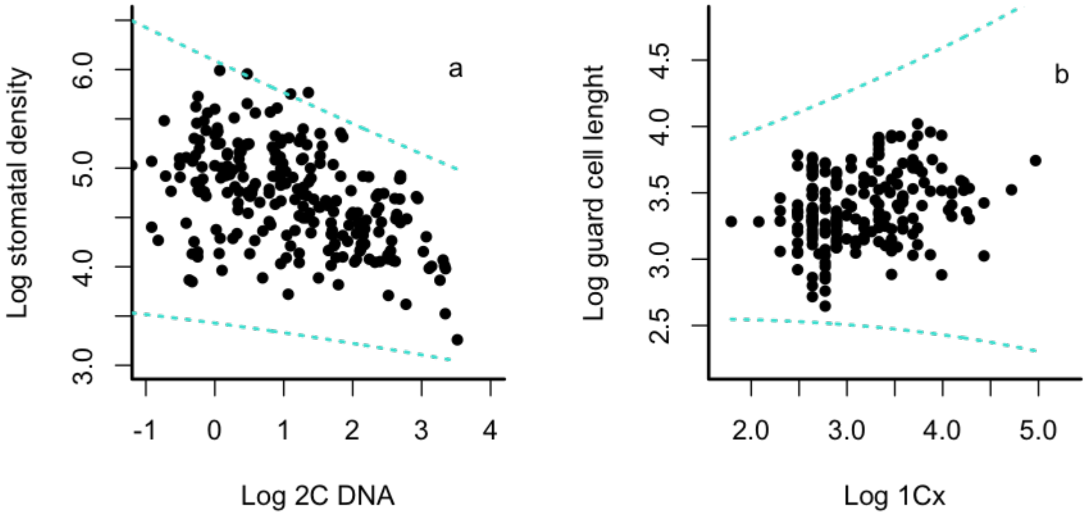

Figure 4. Phylogenetic regression between; a) stomatal density (log) and 2C DNA (log), and b) guard cell length (log) and 1Cx (log). The blue line represents the variance at each model.

Table 3. Phylogenetic analysis to test for a reduction on the variance of the mean traits between species for 2C DNA and 1Cx. Significant values are indicated in bold. *L.Ratio* = Likelihood Radio

Plant trait [log scale]	2C DNA			1Cx		
	Exponent	*L.Ratio*	*P*	Exponent	*L.Ratio*	*P*
Guard cell length	-0.236	0.97	0.32	1.63	7.28	**0.006**
Stomata density	0.415	4.35	**0.037**	-0.52	0.94	0.33
Seed mass	0.096	3.67	0.055	0.02	0.34	0.55
Leaf area	-0.18	2.74	0.09	0.98	3.22	0.07
Leaf width	0.30	1.83	0.17	1.04	0.17	0.67
Leaf thickness	-0.29	0.96	0.32	0.008	0.00005	0.99
LDMC	-0.51	0.42	0.51	0.86	0.74	0.38
SLA	0.043	0.0090	0.92	0.95	0.024	0.87

5.4.2.2 Quantile regression

The analysis at different quantiles for each plant trait (species means) and genome size suggests there was a reduction in the slope at the higher quantiles for stomatal density, leaf area, leaf thickness and leaf dry matter content (Fig. 5). For genome size and seed mass, the relationship was significantly positive at all quantiles and the slope tended to increase at higher quantiles, however this change was only significantly different (from the slope at the 0.5 quantile) at the 0.85 and 0.95 quantiles (Fig. 5b). The relationship between 2C DNA and guard cell length was significantly positive at all quantiles, but changes in the slope were not significantly different from the slope at the 0.5 quantile (Fig. 5a).

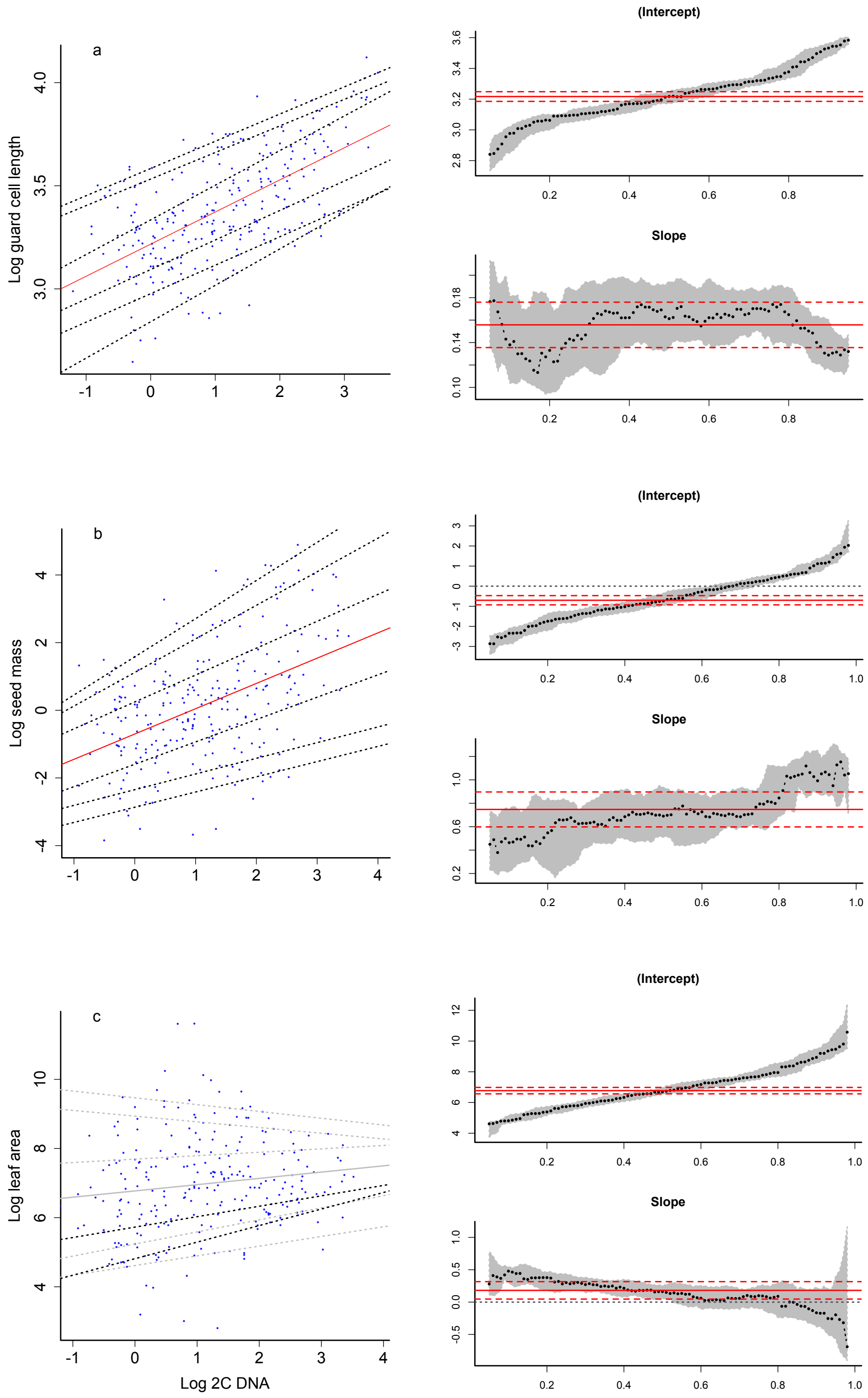

Figure 5. Relationship between mean values of 2C DNA (log) and plant traits (log): a) guard cell length, b) seed mass and c) leaf area. The regressions at different quantiles are represented in dashed-lines, and to the 0.5 quantile of the data in a continuous line. Gray color: non-significant relationships. Black and red colors: significant relationships for the quantiles, and linear model, respectively. The slope and the intercept for the entire range of quantiles are shown on the right side. In red are the slope and confidence interval for the linear model (0.5 quantile). The shaded area represents 95% confidence intervals of the linear predictor for each trait.

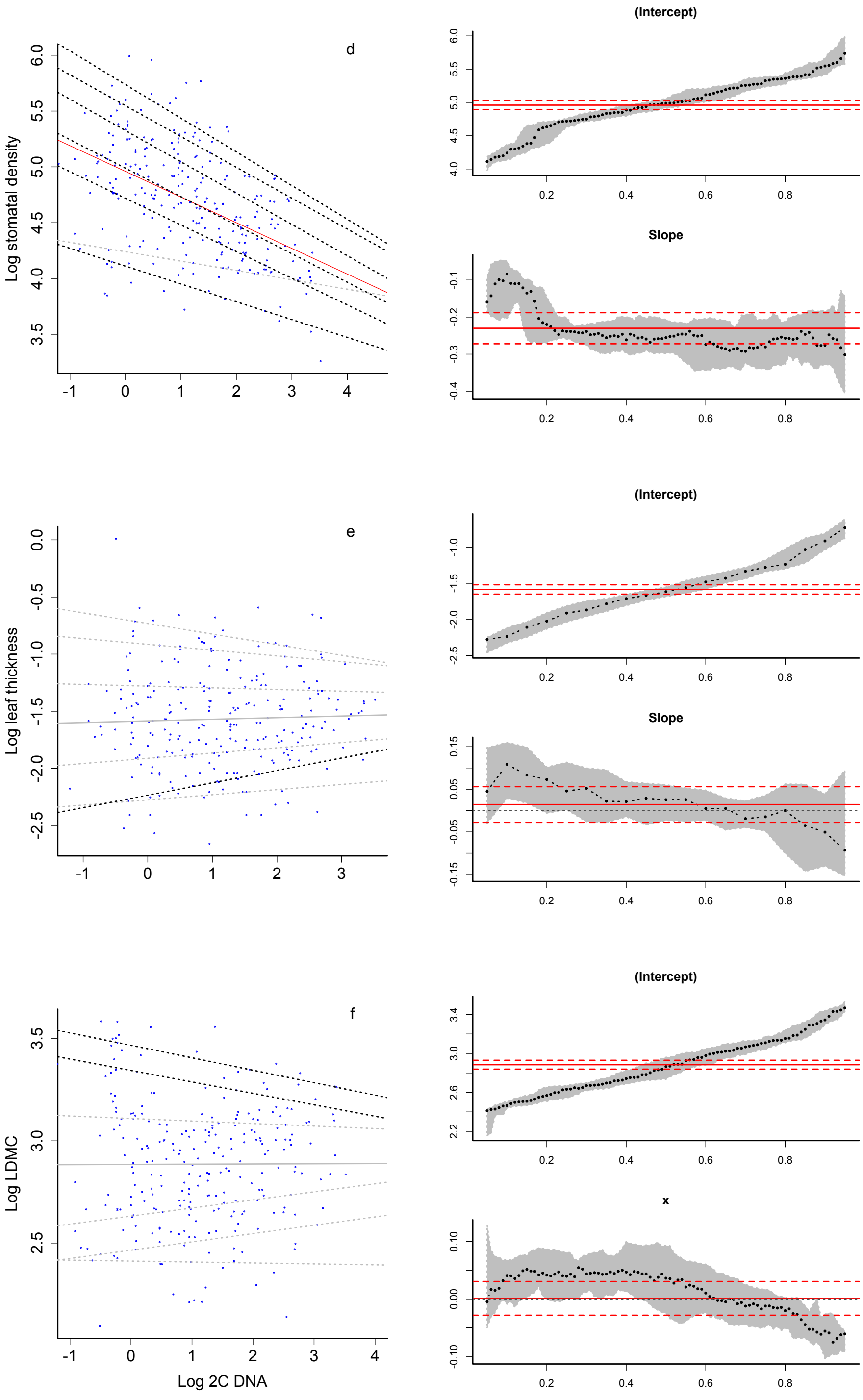

Figure 5. Relationship between mean values of 2C DNA (log) and plant traits (log): d) stomatal density, e) leaf thickness and f) LDMC. The regressions at different quantiles are represented in dashed-lines, and to the 0.5 quantile of the data in a continuous line. Gray color: non-significant relationships. Black and red colors: significant relationships for the quantiles, and linear model, respectively. The slope and the intercept for the entire range of quantiles are shown on the right side. In red are the slope and confidence interval for the linear model (0.5 quantile). The shaded area represents 95% confidence intervals of the linear predictor for each trait.

The quantile regressions for 1Cx and plant traits showed some differences from the analysis with 2C DNA values. We found a significant negative relationship with seed mass at the 0.05 quantile, but no significant changes in the slope were detected (Fig. 6b). Leaf area showed a significant positive relationship to 1Cx at the 0.95 quantile, and also showed an increase in the slope for the 0.95 quantile (Fig. 6c). For LDMC there was an increase in the slope for the positive relationship, and this change was significantly different from 0.5 towards the higher quantiles (Fig. 6f). The rest of the relationships showed a constant change in the slope (not significantly different from the slope at the 0.5 quantiles) across all quantiles (Fig. 6).

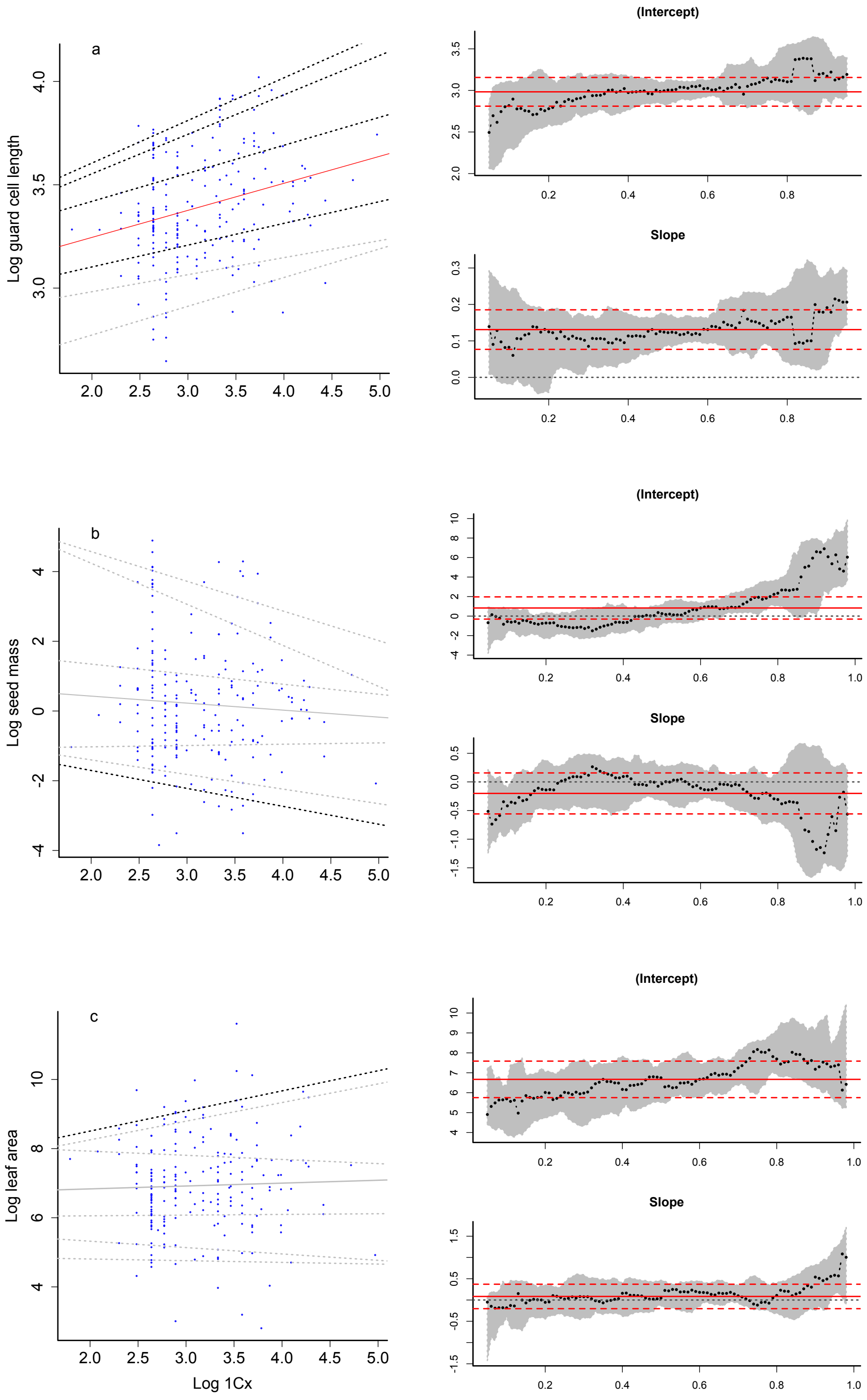

Figure 6. Relationship between mean values of 1Cx log) and plant traits (log): a) guard cell length, b) seed mass and c) leaf area. The regressions at different quantiles are represented in dashed-lines, and to the 0.5 quantile of the data in a continuous line. Gray color: non-significant relationships. Black and red colors: significant relationships for the quantiles, and linear model, respectively. The slope and the intercept for the entire range of quantiles are shown on the right side. In red are the slope and confidence interval for the linear model (0.5 quantile). The shaded area represents 95% confidence intervals of the linear predictor for each trait.

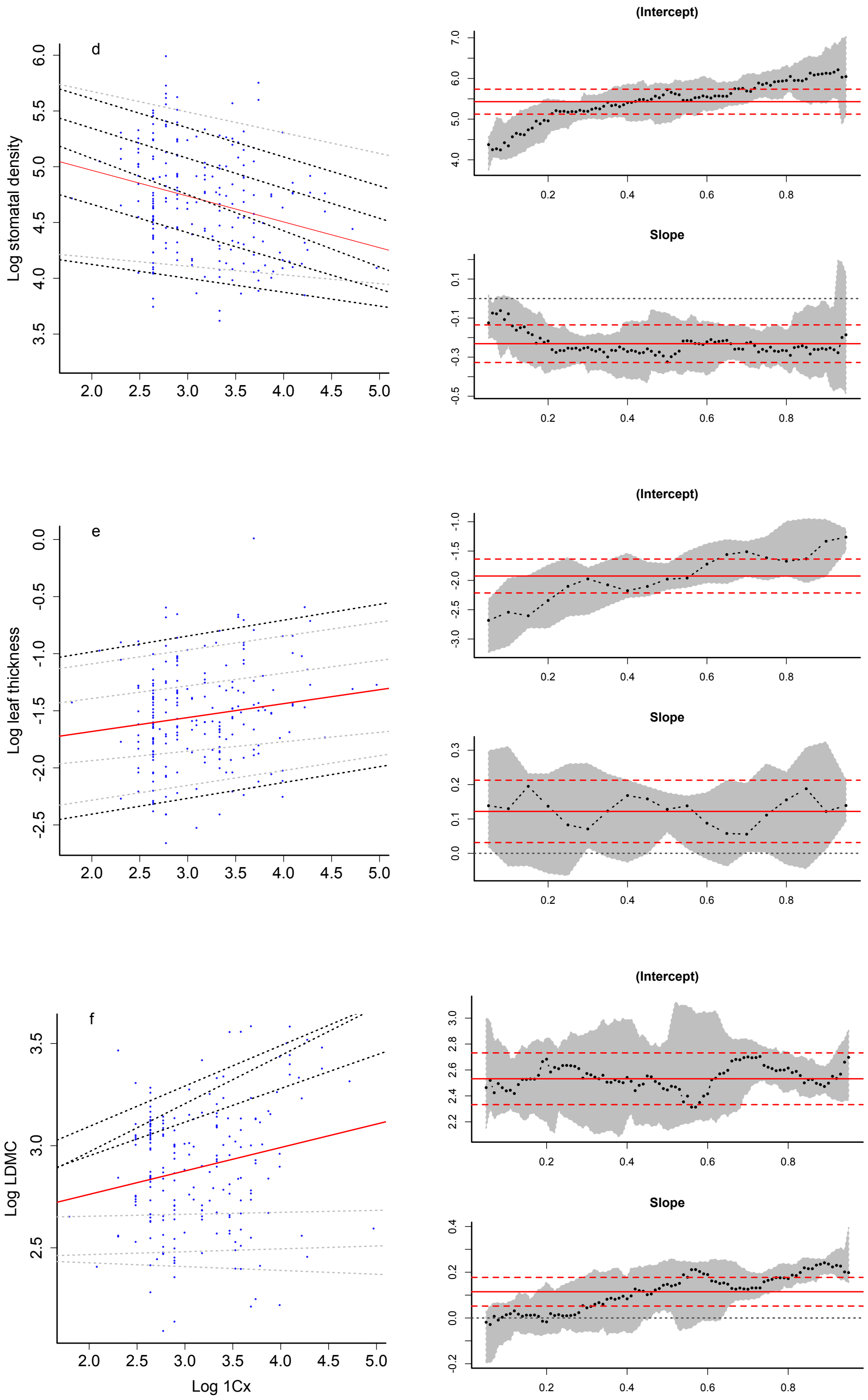

Figure 6. Relationship between mean values of 1Cx log) and plant traits (log): d) stomatal density, e) leaf thickness and f) LDMC. The regressions at different quantiles are represented in dashed-lines, and to the 0.5 quantile of the data in a continuous line. Gray color: non-significant relationships. Black and red colors: significant relationships for the quantiles, and linear model, respectively. The slope and the intercept for the entire range of quantiles are shown on the right side. In red are the slope and confidence interval for the linear model (0.5 quantile). The shaded area represents 95% confidence intervals of the linear predictor for each trait.

5.5 Discussion

We have presented an analysis of the genome size and its relationship to the variation in the phenotype. We do this within and between species. Particularly, we have shown that our data do not support the large-genome size constraint hypothesis for most of the traits studied.

First, we found that variation in traits was higher between species than within species for most of the traits, as previously reported (Cornelissen et al. 2003). However, we also found that LDMC, stomatal density and guard cell length had higher coefficients of variation within species (Fig. 2a). Similar results were reported by Jung et al. (2010) and Messier et al. (2010), with SLA and LDMC having greater variation within species. This suggests this variation cannot be ignored in ecological studies.

Also, an interesting finding was that genome size was significantly related to the variation of more traits within species than it was to variation between species. This suggests that functional traits predictors' work differently at different scales, and also that genome size could perhaps give us more information if we looked within species.

5.5.1 *Within* species variation

Within species, stomatal density was the only trait for which the variance was reduced as genome size increased (Fig. 3d). For most of the traits, the relationship between the intraspecific variance and genome size was either positive (seed mass, guard cell length and leaf area; Fig. 3), or not significantly different from zero (for SLA, LDMC, leaf thickness and leaf width; Table 2). Our results therefore do not support the large-genome size constraint hypothesis within species. Instead, our findings suggest that, as genome size increases, species display greater variation for some traits, suggesting that the amount of DNA is somehow linked to the variation at the phenotypic level. These traits, which presented greater variation as genome size increases, are involved in the overall reproductive strategy (seed mass) and in the energy and water balance of the plant (guard cell length and leaf area).

The greater variation found in traits within species can be understood by two possible explanations. Firs, large genome species are usually found in temperate zones, whereas small genome species are more frequent in the tropics (Levin and Funderburg 1979). This has been attributed to the idea that the latter are more specialized (Stebbins 1966), and cope with fewer environmental pressures than species in temperate zones (which are more variable in temperature and rainfall). Under this scenario (temperate zones), large genome species could potentially respond to the changing conditions by adjusting a particular trait to a broader range of conditions (Schellberg and Pontes 2012).

Second, large genome species are related to high-fertile habitats (Smarda et al. 2013) except for geophytes plants which are found in low-fertile habitats (Vesely et al. 2013). This is because Phosphorus and Nitrogen, required to build DNA, are limited in the natural ecosystems (Kang et al. 2015). The high phenotypic variation found in our study for large genome species could be a mechanism to reduce intraspecific competition under high-fertile conditions. Evidence for this idea is reported in (Kunstler et al. 2015), who found that maximum height dissimilarity lead to a reduction in competition in tree species. In addition, variation in seed mass and leaf area has been found to promote species' coexistence by reducing effects of neighbor competition in growth and survival (Uriarte et al. 2010).

5.5.2 *Between* species variation

For the quantile regressions we found a tendency of a reduction in the variation for stomatal density, leaf area, leaf thickness and LDMC, indicated by the negative slope towards the higher quantiles. This suggests a reduction in the phenotype as genome size is increased. However, these changes in the reduction of the slope were not significant at higher quantiles. We only detected a significant increment at higher quantiles for seed mass, indicating that the variance is higher at larger values for a particular trait (the was a significant difference between the slopes at 0.85 and 0.95 quantiles (Fig. 5b). This result does not support the large-genome constraint hypothesis.

In the case of 1Cx values the slope increased towards higher quantiles for LDMC, and the slope was significantly different from the rest at the 0.90 quantile (Fig.6f). For the other traits, we did not find a significant change in the slope that would indicate a reduction or an increase in the trait variance.

On the other hand, the phylogenetic analysis for the trait variation between species indicated that stomatal density variance decreases as 2C DNA values increase; this was also observed within species (Fig. 4a). These results are in agreement with the large-genome constraint hypothesis, *i.e.* large-genome species display less phenotypic variation. For guard cell length, however, we found that the variance was increased as 1Cx values did, which was also consistent within species (Fig. 4b).

For these two traits (stomatal density and guard cell length), the genome size effects are found between and also within species. In addition, the variation in the phenotype is retained at both levels of variation: for stomatal density, larger genome species showed constrained variation, whereas in guard cell length the opposite was true. Guard cell length (a proxy for pore size; (Charles et al. 1997)) and stomatal density are crucial for water and carbon control of the leaf. Our results suggest a strategy in which large-genome species would present a combination of either large (adaptive in wet conditions) or small guard cells (adaptive in drier conditions, (Hetherington and Woodward 2003)), and low stomatal density.

An important point to consider from our findings is that there were different outcomes when the phylogenetic relationships between species are accounted for, and when they were not (quantile analysis). Relationships between traits can be highly significant before incorporating the relatedness among different taxa. For example, (Kelly and Beerling 1995). re-analyzed data from Salisbury (1928). The results from the re-analysis, showed that stomatal density was not related to the degree of light to which the plant is exposed (trees having greater stomatal density than marginal herbs), after phylogenetic relatedness was taken into account. A similar example was reported in Beaulieu et al. (2007a) where they found a significant negative relationship between leaf mass per area (LMA; an indicator of leaf density), and 2C DNA across angiosperms, which was positive when analyzed in a phylogenetic framework. The authors also found a non-significant phylogenetic relationship for photosynthetic rate, which lead them to conclude that there is not enough evidence to support the large genome-constraint idea for this trait, as previously suggested (Knight et al. 2005).

Our results do not support the large genome constraint hypothesis at the phenotypic level, but actually the opposite, *i.e.* that species with larger genome size (2C DNA) can display more phenotypic variation. However, it is crucial to incorporate data from broader environmental conditions to detect if this holds for species in the tropics. Another important point to consider here is that we do not present data for the variation within species in genome size. This type of variation in genome size remains controversial (Bennett and Leitch 2005), as it has been shown to be result of either a technical artifact, or mistakes in the taxonomic classification (Greilhuber 1998, 2005). Despite this, it would be interesting to analyze the relationship for genome size and the phenotype within species for species for which the intraspecific variation in genome size has been proved, like *Zea mays* (Bennett and Leitch 2005).

Chapter 6

General Discussion

Classification of plants in terms of their function has been widely used in ecology (Raunkiaer 1937, Grime 1979, Suding and Goldstein 2008, Violle et al. 2012). This has been done by grouping species on their response to the environment to understand the community structure and the ecosystem processes (Cornelissen et al. 2003, Grime et al. 2007). In this thesis we tested and used this approach to firstly investigate predictions from CSR theory in terms of the community membership with empirical work. Finally, in the last two chapters we used individual plant functional traits (rather than a plant strategies) to test allometric constraints and linked them to habitat indicators. We also used variation in plant functional traits (PFT) in relation to genome size, and propose the possible ecological mechanism that might be acting upon this relationship.

In short, our findings suggest that CSR theory will not predict community membership, whereas the study of plant allometries might give us more insights into the drivers of community structure. Also, we suggest that genome size can give us understanding into the ecological processes, *i.e.* competition and coexistence, through trait-variation within species and their distribution.

Does CSR theory predict community membership?

The results from two large experiments conducted in the field and in the greenhouse, suggest that CSR theory predictions are not sufficient to predict which species will be part of a community from the early stages in a plant life cycle. Species from different vegetation strategies in the field were filtered into a community mainly by the level of stress, and not by the vegetation strategy. The fact that survival was higher at the low-stress than in the high-stress habitats shows that community membership is determined by strong environmental filters present in those habitats. That is, species that do not tolerate the environmental conditions, in the absence of neighbors (Kraft et al. 2015).

In terms of the vegetation strategies, we show that once competition is removed in low-stress habitats, *Competitors* were not predominant members of the community as predicted from CSR theory, and under stressful conditions, *Stress-tolerant* strategies responded different to the type of stress. CSR theory considers that species with certain characteristics will respond in the same way to a universal stress (Grime 1979). We show that the type of stress has important consequences in the community membership, as the type of stress would filtered not only species with *Stress-tolerant* traits, but rather other traits included in the classification of *Competitors* or *Ruderals*.

CSR theory classifies species in their *established* phase. We suggest this is a limitation for the predictive power of CSR. The *regenerative* phase or seedlings are the initial phase of the community assemblage (Keeley and van Mantgern 2008) and therefore play a crucial role in understanding how is a community structured (Leck and Outred 2008). Seedlings can be subjected to higher selective pressures than the adult phase (Leck et al. 2008). As shown by Poorter (2007) plant traits are closely related to the conditions experienced at the *regenerative* phase.

What information can we extract from relationships between plant functional traits (PFT)?

Understanding the general principles driving the organization of plant communities in different environments is a major challenge in ecology (Hooper et al. 2005). PFT are consistently related to environmental gradients (Knight and Ackerly 2002, Reich et al. 2003, Moles et al. 2014), indicating the adaptations that species evolve to distribute in a particular habitat, and also showing global trade-offs, for example that increasing maximum photosynthetic rate (A_m) will decrease leaf longevity (Reich et al. 1997, Wright et al. 2004, Shipley et al. 2006). We show that relationships between seed mass and leaf area (*e.g.* allometries) are consistent across life-forms and that they are driven by variation in habitat fertility. This indicates that plant species have evolved similar patterns in resource-use, regardless the life-cycle. We also show that the large amount of DNA contain in a cell, is linked somehow to the variation in PFT within species. This brings some interesting future research lines, first despite PFT are generally measured between species (Perez-Harguindeguy et al. 2013), we show that similar to other studies (Jung et al. 2010, Messier et al. 2010), variation in traits within species can not be disregarded. Also, that genome size variation is linked to this functional variation, and less to variation between species. However, we found that genome size is a good predictor for variation within species for seed mass, but not for guard cell length or stomatal density.

As reported by Knight and Beaulieu (2008), we also found that the relationship with PFT is likely to be less strong as the level of organization increases (from properties of cells to whole plant attributes, Appendix, Table A2). This suggests that genome size gives different inferences depending on the level of analysis. Finally, evidence suggests that species with larger genomes are distributed in more variable environmental conditions. This, together with more trait variability found in larger genome species, suggests that genome size could be use to predict species distributions, and could give insight into other ecological processes, such as the possible mechanisms by which species coexist (Violle et al. 2012).

Future research needs

The use of resources and plant traits changes with developmental stages (Fenner et al. 1999, Mediavilla and Escudero 2003, Goodger et al. 2006). Future research should incorporate in the trait relationships across life-stages, and on comparisons between plant functional groups. Studies suggest that the key set of traits influencing performance at one life stage might be very different to those at other stages (Niinemets 2006). Furthermore, ontogeny might have important effects on some groups of plants (deciduous species), but not for others (evergreen species, (Lusk and Warton 2007)). This can also have an impact in the predictions that we can make from the allometric relationships, or between traits and habitat variables.

The study of PFTs is a powerful tool to predict biota and its relation to the environment (Hooper et al. 2005). This research is often carried out on above-ground traits, mainly because they are easy to access. Genome size is negatively related to root meristem growth rate. Suggesting that large genome species, like parasitic plants, do not rely as much on root growth, as non-parasitic species (Gruner et al. 2010). However, we need to assess: how belowground PFTs are related to environmental conditions and genome size? It is unknown relatively how much the underground component contributes to our insight into ecological processes. These are intriguing questions and possibly leading to interesting findings, as roots are good indicators of habitat variables. For example, low specific root length (ratio between root length and root biomass) is linked to slow-growing species (Comas et al. 2002), and also mycorrhizae associations in roots could influence competition in low-productive habitats (Keddy et al. 2000).

Appendix

Table A1. Measurements for the pH, nitrogen and phosphorous available for the plant (values for each soil sample are shown). The data were sorted from the most acidic site to the less acidic site within each type of habitat: S*tressful*: Site 1; Longshaw down, Site2; Car park 2, Site 3; Longshaw top, Site 4; Poem, Site 5; Car park 1. *Low stress:* Site 1; Bex garden, Site 2; Allotment, Site 3; Botanical gardens, Site 4; Lynwood gardens, Site 5; Greenhouse.

Habitat type	Grid reference	pH	NH3 [mg/gr]	P [ppm]
High-stress				
Site 1	SK 2649879224	3.48	0.113	0.475
		3.48	0.113	0.475
		3.53	0.128	0.428
		3.33	0.164	0.475
		3.57	0.123	0.475
Site 2	SK 2675479026	3.911	0.062	0.494
		3.911	0.062	0.494
		4.71	0.089	0.114
		3.96	0.062	0.494
		3.99	0.062	0.494
Site 3	SK2668279540	4.363	0.104	0.513
		4.95	0.089	0.247
		5.18	0.139	0.703
		3.45	0.079	0.504
		4.363	0.104	0.513
Site 4	SK2612679271	4.23	0.101	0.770
		4.68	0.141	1.008
		5.53	0.102	0.875
		4.67	0.122	0.380
		4.46	0.116	0.694
Site 5	SK2670178996	5.29	0.103	0.392
		5.43	0.137	0.770
		5.17	0.102	0.542
		5.27	0.114	0.466
		5.31	0.091	0.399
Low-stress				
Site 1	SK3359188552	6.59	0.098	2.57
		6.55	0.051	3.63
		5.46	0.125	2.88
		4.55	0.167	2.50
		6.32	0.078	2.81
Site 2	SK3193788042	6.44	0.106	1.43
		6.62	0.085	1.94
		6.83	0.085	1.70
		6.62	0.085	1.94
		6.16	0.074	1.87
Site 3	SK 33665 86216	7.34	0.104	10.567
		7.38	0.063	4.717
		7.52	0.099	6.449
		7.68	0.086	8.646
		7.42	0.109	9.664

Site 4	SK 3395186777	7.96	0.04501	1.683
		7.58	0.07928	2.444
		7.59	0.08979	2.330
		7.42	0.09271	5.079
		6.95	0.09555	3.738
Site 5	SK 3336587236	7.37	0.082285	2.717
		7.60	0.082285	2.717
		7.55	0.08796	3.880
		7.601	0.082285	2.717
		7.88	0.08683	1.930

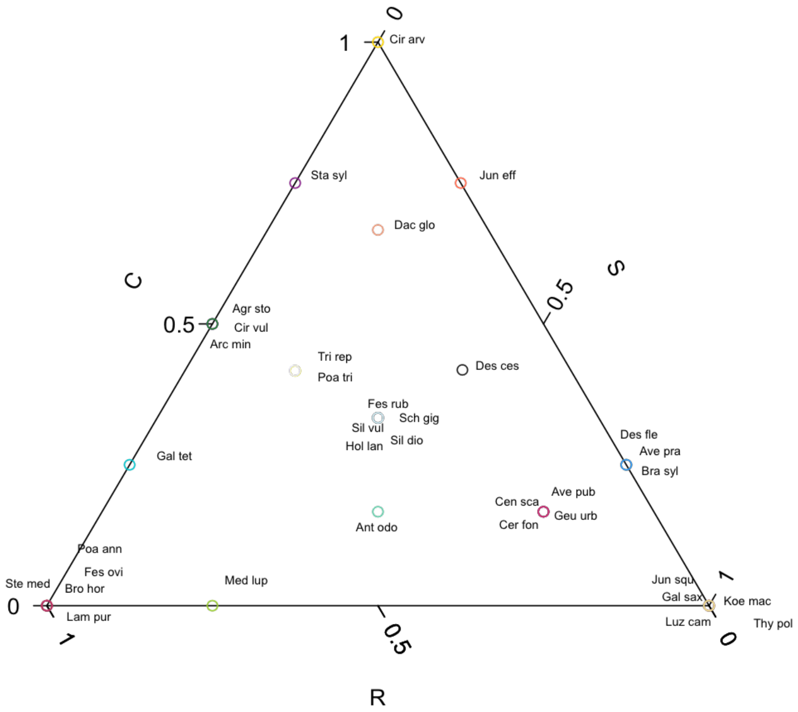

Figure A1. Triangular ordination for the species used in chapters 2 and 3.

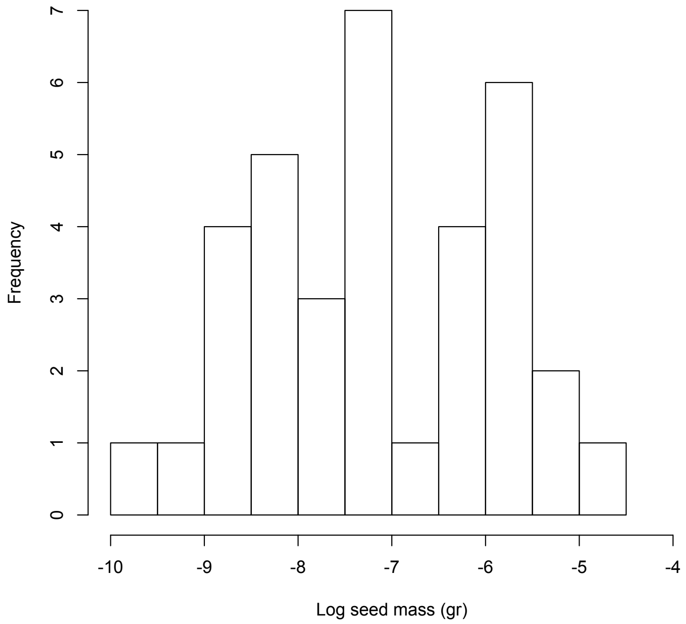

Figure A2. Log seed mass (gr) distribution for the species used in chapter 2 and 3.

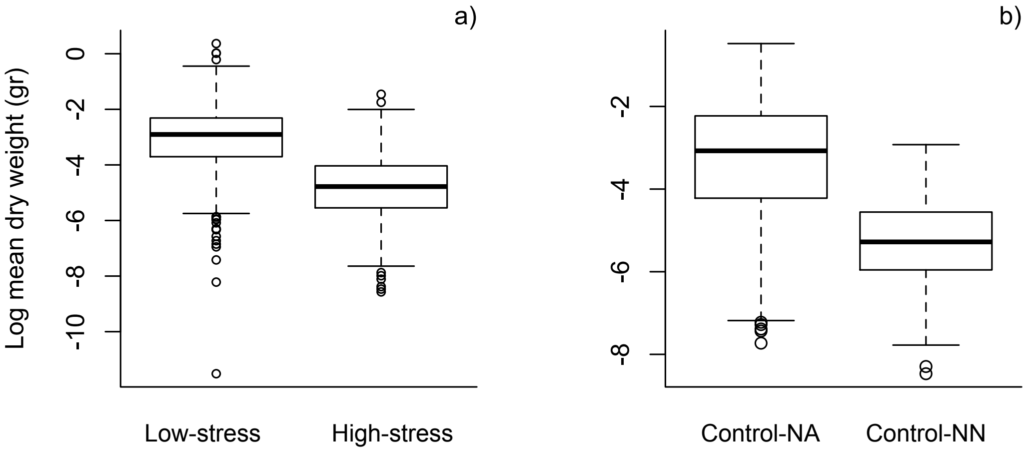

Figure A3. Mean dry weight (log) for all the species at a) Low and High-stress treatments, and b) the control with nutrients added (Control-NA), and the control with no-nutrients (Control-NN).

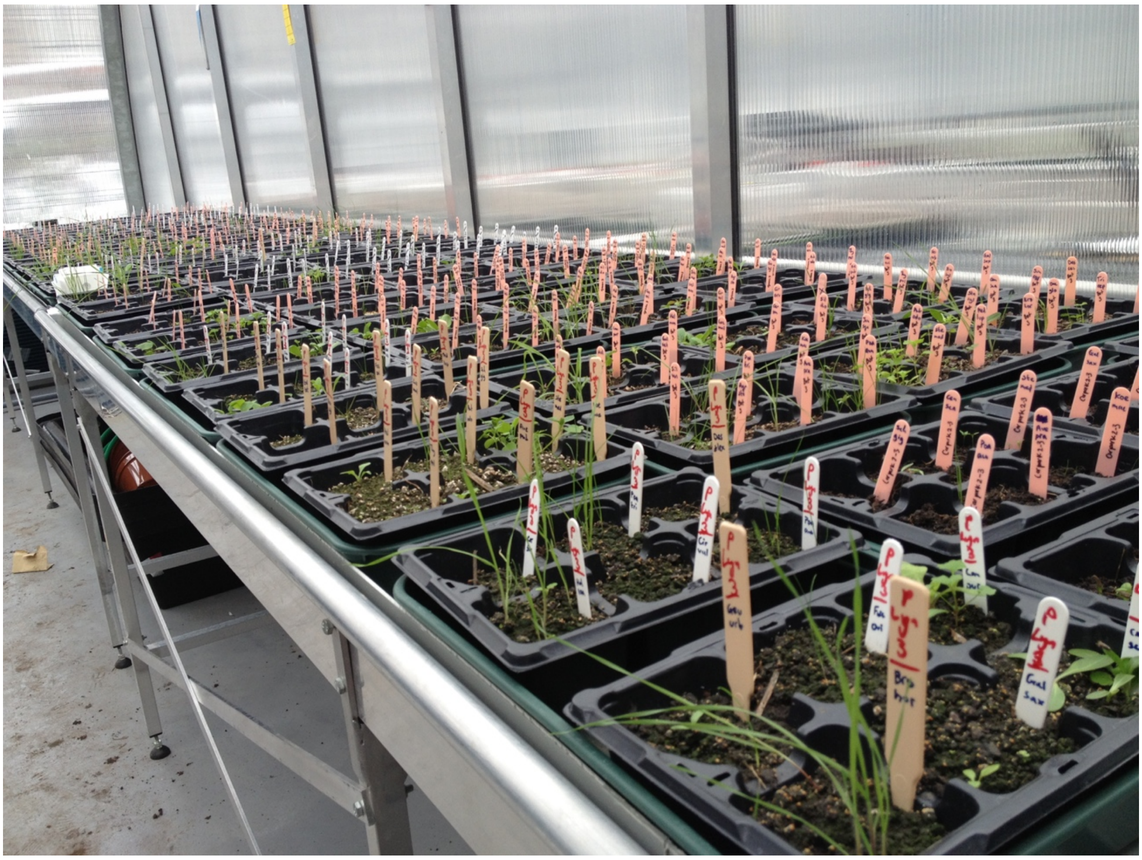

Figure A4. Greenhouse experiment

Table A2. Phylogenetic regression for the mean value of each trait and its relationship to genome size (2C DNA and 1Cx).

	2C DNA				1Cx			
Trait mean [log scale]	Slope	r^2*	*F*	*P*	Slope	r^2*	*F*	*P*
Guard cell length	0.17	(1) 0.51 (2) 0.36	141.95	<0.0001	0.14	(1) 0.50 (2) 0.25	66.22	<0.0001
Stomata density	-0.21	(1) 0.28 (2) 0.15	31.48	<0.0001	-0.17	(1) 0.34 (2) 0.11	16.21	<0.0001
Seed mass	0.84	(1) 0.55 (2) 0.18	34.34	<0.0001	1.03	(1) 0.61 (2) 0.27	72.60	<0.0001
Leaf area	0.41	(1) 0.03 (2) 0.07	12.49	<0.001	0.55	(1) 0.24 (2) 0.11	24.5	<0.0001
Leaf width	0.23	(1) -0.2≈0 (2) 0.02	4.34	0.03	0.20	(1)-0.13 ≈0 (2) 0.02	3.65	0.058
Leaf thickness	0.14	(1) 0.08 (2) 0.09	23.64	<0.0001	0.14	(1) 0.20 (2) 0.08	18.02	<0.0001
DMC	-0.05	(1) -0.0008≈0 (2) 0.02	6.85	0.009	-0.035	(1) -0.02≈0 (2) 0.017	2.58	0.10
SLA	-0.07	(1) -0.07 ≈0 (2) 0.04	10.01	0.0018	-0.035	(1) 0.02 (2) 0.02	?	<0.0001
Plant height	0.14	(1) 0.12 (2) 0.04	10.87	<0.001	0.17	(1) 0.20 (2) 0.08	12.24	<0.0001

* There are two ways to calculate r^2 for phylogenetic regressions(Paradis 2012), so here I present the results for both (1) and (2).

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