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The role of regeneration in plant niche differentiation and habitat specialization

For Synara

Though I do not believe
that a plant will spring up
where no seed has been,
I have great faith in a seed.
Convince me that you have a seed there,
and I am prepared to expect wonders.

Henry David Thoreau

The role of regeneration in plant niche differentiation and habitat specialization

Dirk-Jan ten Brink

ACADEMIC DISSERTATION

For the degree of Doctor of Philosophy in Plant Ecology and Systematics,
to be publicly defended on January 24th 2008 at 10.00 a.m. in Blå Hallen at the
Department of Ecology, Ecology Building, Sölvegatan 37, Lund,
by permission of the Faculty of Science of the University of Lund.

The thesis will be defended in English.

Faculty opponent: Professor Jonathan Silvertown, The Open University, UK

Lund 2007

A doctoral thesis at a university in Sweden is produced either as a monograph or as a collection of papers. In the latter case, the introductory part constitutes the formal thesis, which summarizes the accompanying papers. These have either already been published or are manuscripts at various stages (in press, submitted or in ms).

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The role of regeneration in plant niche differentiation and habitat specialization	9
Acknowledgements	25
This thesis is based on the following papers:	
I ten Brink, D. and Bruun, H.H. Habitat specialization through germination cueing: a comparative study of herbs from open and shaded habitats – submitted	27
II ten Brink, D. and Bruun, H.H. Seedling phase strategies as a means of habitat specialization in herbaceous plants - submitted	47
III Bruun, H.H. and ten Brink, D. The recruitment advantage of big seeds over small seeds is larger in shaded than in open vegetation - submitted	63
IV ten Brink, D., Olsson, P. A., Tyler, G. and Bruun, H.H. Plant trait dispersion along a soil phosphorus gradient in rocky habitats. - manuscript	81
V ten Brink, D. and Bruun, H.H. Abiotic control of plant community assembly: a microcosm experiment - manuscript	97

The role of regeneration in plant niche differentiation and habitat specialization

Introduction

The niche and the habitat of a plant

Plant species exhibit various degrees of specialization to the environment they are found in. Specialization of plants to their environment is regulated by trade-offs. Some generalist species are able to occupy a wide range of HABITATS (see Table 1), but they are usually not able to dominate in all (or any) of the occupied habitats, since the jack-of-all-trades is a master of none (MacArthur 1972). Other species are only found under a more restricted set of conditions and thus more limited in their distribution along environmental

gradients. The extent of the distribution depends on the environmental tolerances and resource requirements of the species. These tolerances and requirements together form the NICHE of the species (Silvertown 2004). For a population of plants to successfully persist in a habitat, the individuals should be able to tolerate the prevailing environmental conditions and withstand competition from coexisting species; the a-biotic and biotic conditions should meet the minimum niche requirements of the species (Hutchinson 1957).

Plant ecologists are interested in studying plant distribution along environmental gradients in order to reveal niche dimensions of species. These

Table 1. Definitions of terms

Term	Definition	Source
Alpha niche	The features of the species' niche which differentiates it from co-occurring species at the local scale	2, 4, 5
Beta niche	The region of a species' niche that corresponds to the habitat(s) where it is found	2, 4
Fundamental niche	The region of its niche that a species is able to occupy in the absence of interspecific competition and natural enemies	1
Habitat	The kind of environment where a species occurs, defined by its physical conditions	2
Niche	An n-dimensional hypervolume defined by axes of resource use and/or environmental conditions and within which populations can sustain a viable population	1
Realized niche	The region of its niche that a species is able to occupy in the presence of interspecific competition and natural enemies	1
Regeneration niche	The dimensions of the niche which are relevant for the regeneration of plants such as seed production, germination and seedling establishment and required to replace one mature individual by another	3

The definitions are based on the following sources: 1. (Hutchinson 1957); 2. (Whittaker 1975); 3. (Grubb 1977); 4. (Pickett & Bazzaz 1978); 5. (Silvertown 2004)

findings can be important in predicting the effect of environmental change on plant communities and can be applied in the development of management strategies for conservation purposes. However, experimental studies reporting the position of certain plants species along one or several niche dimensions, as well as observational studies of plant distribution across environmental gradients elucidating the realized niches of plants are usually limited to the mature phase of the plant life cycle. These studies have led to important and frequently applied plant classification and strategy schemes like Raunkjær's (1904; 1934) life forms and Grime's (1974; 2001) CSR strategy scheme. These studies are based on the assumption that niches are uniform and constant throughout all life stages (e.g. MacArthur & Levins 1967). As a result, the importance to habitat specialization of plant traits and requirements of the regeneration phase, the REGENERATION NICHE (GRUBB 1977), is much less studied. The assumption that plant requirements and tolerances are constant ignores the fact that niche requirements may change during the life of individuals (Eriksson 2002). This phenomenon is called 'ontogenetic niche shifts' and is common to plants that occupy environments that change during their life span. For example in grassland plants that germinate in a vegetation gap which exists just temporarily, or the succession of an open plant community into a forest, whereby trees alter their own environment and that of the coexisting ground vegetation when they mature and develop into a forest.

Plants can only successfully establish and persist in a community if the long-term establishment rates outweigh the losses of individuals. The success of their establishment depends on the local environmental conditions being suitable for the regeneration requirements of the species. As plants advance through the life cycle from seed germination to reproduction and seed set, every stage of the life cycle passes through an environmental sieve allowing some individuals to advance whilst filtering out others (Harper 1977).

The most critical stages are germination, early seedling growth and seedling survival (Harper 1977; Schupp 1995).

Aim of this thesis

The aim of this work is to investigate the role of regeneration plant niche differentiation and habitat specialization. I investigated the role of germination strategies (paper I) and seedling traits (paper II) in habitat specialization. The recruitment advantage of large seeds in relation to the environment is discussed in paper III. The question whether co-occurring species are generally similar or different in regenerative and established traits is addressed in paper IV, while in paper V the importance of deterministic processes in the assembly of plant communities is investigated.

Before presenting the individual studies of this thesis, I will introduce some important aspects of the biology and ecology of plants and plant communities addressed in the papers of this thesis.

The ecology of seed dormancy and germination

Seed germination is a crucial stage in the life history of plants, since this life phase is generally accompanied by high mortality. The prerequisites of seeds to germinate are the availability of water and oxygen, which are consumed during the process of germination and a suitable temperature. Some species are able to germinate immediately after seed shedding, once the basic requirements have been fulfilled. However, it is common for many species to be dormant upon shedding. There are several types of seed dormancy (Baskin & Baskin 1998), and they are considered an adaptation to the uncertainty and temporal variation of the environment the seed faces after dispersal. The most common type of seed

dormancy, physiological dormancy, is regulated by a physiologically inhibiting mechanism that prevents growth of the embryo. It is assumed that a relieve in dormancy widens the temperature range over which seeds can germinate, and vice versa (Vegis 1964; Vleeshouwers *et al.* 1995). In general, there is a linear trend between the rate of seed germination and temperature (Probert 2000). In contrast to the relatively high minimum requirements for germination, found in many wetland plants (Grime *et al.* 1981; Baskin & Baskin 1998) inhibition of germination by high temperatures has been found in several sedges (Schütz & Rave 1999). Many species respond better to alternating temperatures (Probert 2000). Seeds located below the soil surface can sense depth by detecting fluctuating temperatures, since diurnal temperature fluctuations are higher in shallow than deeper soil layers (Fenner & Thompson 2005). Detection of fluctuating temperatures can also serve to sense gaps in the vegetation for the same reason of differences in diurnally fluctuations, which are reduced under a leaf canopy.

Another important environmental factor serving as cue for germination in many species is light (Grime & Jarvis 1975; Thompson 1989; Hilhorst 1993; Vleeshouwers *et al.* 1995; Pons 2000). Species with small seeds more often have light requirement than do larger-seeded species. The reason for this is probably that the small seeds with their small amount of reserves can't support the seedlings to reach the soil surface in case a seed germinates too deep in the soil (Pons 2000). A light requirement is also common for species in disturbed habitats and wetlands (Grime *et al.* 1981). The light environment of a seed or plant can vary both quantitatively and qualitatively. A leaf canopy above a seed strongly reduces PFD of all wavelengths, but much more in the photosynthetically active part of the spectrum (400-700 nm) than in the near infra-red (700-1000) due to the strong absorption of chlorophyll. Therefore, canopy shaded light is rich in far-red light (FR) and poor in red light (R). The light environment

of the seed is therefore often characterized by the R:FR ratio. Seed maturation under canopy-filtered light with a low R:FR ratio may induce a light requirement for germination (Smith 1982). The ability of a seed to detect the R:FR ratio of the light provides it with information about a possible presence of a leaf canopy in its neighbourhood. The presence of vegetation would indicate potential competition, which may lead to a strategy to remain ungerminated and await a disturbance that alters the conditions. In many light requiring species, light with a low R:FR ratio inhibits germination (e.g. Gorski *et al.* 1977; Silvertown 1980).

Seedling establishment, environmental stress and the importance of seed size

The seed sizes of species that co-occur in a community usually vary several orders of magnitude. This is the result of two opposing selection pressures acting on seed size. A larger number of seeds can potentially result in a larger number of offspring, meaning higher fitness. This selects for species that allocate their reproductive effort into many small seeds. However, since seedlings of larger seeded species are more competitive and better able survive adverse environmental conditions (Westoby *et al.* 2002), there is opposite selection pressure for increased seed size. The seed size/number trade-off (SSNT) model (Geritz 1995; Rees & Westoby 1997) predicts stable coexistence of species with a range of seed masses, whereby seed mass both influences the seed number as well as the per-seed recruitment success. The importance of seedling-seedling competition as cause of seedling mortality is believed to be low. The density of seedling in experiment where density-dependent seedling mortality has been observed is usually much larger than the density of seedlings in natural populations (Moles & Westoby 2004).

However, Silvertown & Bullock (2003) observed density-dependent seedling competition grassland gaps. Herbivory, drought and fungal attack were found to be among the most common causes of seedling mortality in a review of the literature (Moles & Westoby 2004). Besides the effects on germination as described above, the light environment also plays an important role in the performance of seedlings and established plants. Morphological shade avoidance responses include elongation of leaves, petioles and/or internodes (Smith & Whitelam 1997) resulting in more slender plants. Shade tolerance in plants is, among other things, characterized by an increased leaf area per unit leaf mass (specific leaf area, SLA) (Lambers *et al.* 1998). Leaf area is also influenced by water availability, and drought stressed plant typically develop leaves with a reduced SLA (Field 1991). A lower SLA reduces the potential growth rate, thereby reducing biomass accumulation in plants (Lambers *et al.* 1998).

The assembly of plant communities

A plant community is a group of populations of plant species that coexist in place and time and interact. The ability of species to disperse their seeds to a local community is a very important factor in plant community assembly (Chambers & MacMahon 1994), and an important attribute of the regeneration niche (Grubb 1977). However it is beyond the scope of this thesis. Some authors assert that species are functionally equivalent and that species composition will change randomly with time (e.g. Hubbell 2001). However, most observational and experimental evidence points to deterministic processes of plant community assembly. The abiotic and biotic environment act as filters, selecting on morphological, physiological and life-history traits of species from the regional species pool that are dispersed into the community (Weiher & Keddy 1999; McGill *et al.* 2006). The abiotic filter consists of environmental conditions

such as soil fertility, water availability and the light regime sort species into different habitats, based on their BETA NICHES (Pickett & Bazzaz 1978; Silvertown *et al.* 2006). Within habitats, plant interactions, e.g. resource competition, and others processes, are thought to determine local species coexistence based on ALPHA NICHES (Pickett & Bazzaz 1978; Silvertown *et al.* 2006). Traditionally, coexistence of plant species is believed to have resulted from previous interspecific competition for limiting resources, a process which has led to trait divergence between coexisting species (Gause 1934; MacArthur & Levins 1967). In contrast, empirical observations of vegetation often reveal similarities in life history, physiology and morphology of coexisting species, which indicates trait convergence (Keddy 1992; Grime 2006). This has led to a controversy about the role of competition within plant communities which is thought to play a minor role, especially in environments where abiotic stress is higher and resources more scarce (Brooker *et al.* 2005; Pierce *et al.* 2007). Trait divergence within plots should rather be explained as a result from disturbance rather than competition, according to Grime (2006).

Specific objectives and methodology

The specific objectives of the studies presented in this thesis are to investigate:

1. How germination requirements contribute to the habitat specialization of herbaceous plants from open and shaded habitats, and whether species are thereby able to utilize different opportunities in space (habitat) and time (season).
2. The association between seedling traits and specialization to contrasting habitats.
3. The importance of seed size for recruitment success along a gradient from open to shaded herbaceous plant

- communities.
4. Whether traits of coexisting plant species converge or diverge and whether traits related to regeneration are more variable at a local scale than are established traits.
 5. The extent to which deterministic processes in plant community assembly can result in similar species assemblages at a particular set of environmental conditions when the 'chance' factor dispersal is controlled for.

Paper I. In the first paper we examined the adaptive association between germination requirements and specialization to either forests or open habitats across a wide range of evolutionary lineages. We adopted a comparative approach using a large number of closely related plant species (congeners) with contrasting habitat preferences, to control for phylogenetic dependence. We studied seed germination in controlled climate rooms where we varied temperature and light in a full factorial design. Seeds were exposed to the various treatment combinations during a first incubation period of two weeks, after which ungerminated seeds received a chilling treatment for three months to allow the seeds to break possible physiological dormancy. A second incubation period followed the chilling, with identical conditions as during the first incubation.

Paper II. In the second study we continued with the comparative approach to study the adaptation seedlings of herbaceous plants to forest vs. open habitats and to dry vs. wet habitats. Here we also tested whether the effects of shade and drought vary independently or if shade has an amplified effect on drought-stressed seedlings. In a factorial design we varied the light and the watering regime and checked for differences in growth rate, height, slenderness, specific leaf area (SLA) and degree of elongation (longest internode, longest petiole or longest leaf sheath, depending on the species).

Paper III. The coexistence between species with

a range of seed sizes is explained by the seed size /seed number trade-off model, whereby small seeded species have a numeric advantage and larger seeded species are assumed to be more competitive and better withstand adverse conditions. This suggests that the value to plant species of possessing large seeds may differ between plant communities along environmental gradients. In this paper, the recruitment advantage of large seeds over small seeds in relation to the environmental conditions along a gradient from open to shaded habitats was studied. Data on seedling number and the reproductive output of fertile ramets per area (Perttula 1941) were used to analyze the relation between seed mass and per-seed recruitment success across 12 different plant community types.

Paper IV. Here the question was addressed whether plant traits converge or diverge within local assemblages relative to between-community trait variations in relation to soil parameters in rocky habitats. Using a trait-based method, we quantified trait dispersion in vegetation of southern Swedish rocky habitats in on shallow soils, along gradient of soil exchangeable phosphate and pH, and decomposed the variation in a within-plot (alpha) and a between-plot (beta) component. Additionally, we investigated whether traits related to regeneration vary more among co-occurring species than traits of established plants.

Paper V. The structure of plant communities is determined by the arrival history of species and local deterministic mechanisms, such as environmental filtering and competitive exclusion. Contrasting ecological theories predict co-occurring species to either exhibit trait convergence as a result of environmental filtering or trait divergence as a result of competition relative to a random assembled community. We studied deterministic processes in the assembly of species assemblages from a constrained species pool in a microcosm experiment across soil fertility and disturbance

regimes. We addressed the question whether community assembly patterns are repeatable within treatments.

Main results in a nutshell

Paper I: Germination strategies

Forest species tended to germinate only after the chilling period, which suggests they need to overcome physiological dormancy. Most open habitat species, in contrast, were shown to germinate instantly during the first incubation period without a need for chilling (Fig. 1). Species from open habitats germinated better under

high and fluctuating temperatures than under low and constant temperatures, whereas forest species performed equally well at low and high temperatures, preferring fluctuating over constant temperatures.

Paper II: Seedling adaptations

Seedling growth generally decreased with increasing shade and reduced watering frequency. Seedling height was generally largest at intermediate light. Specialization to shaded habitats was associated with a more conservative growth strategy, i.e. showing a more modest growth response to increasing light. Species from all habitats showed the highest relative elongation at intermediate

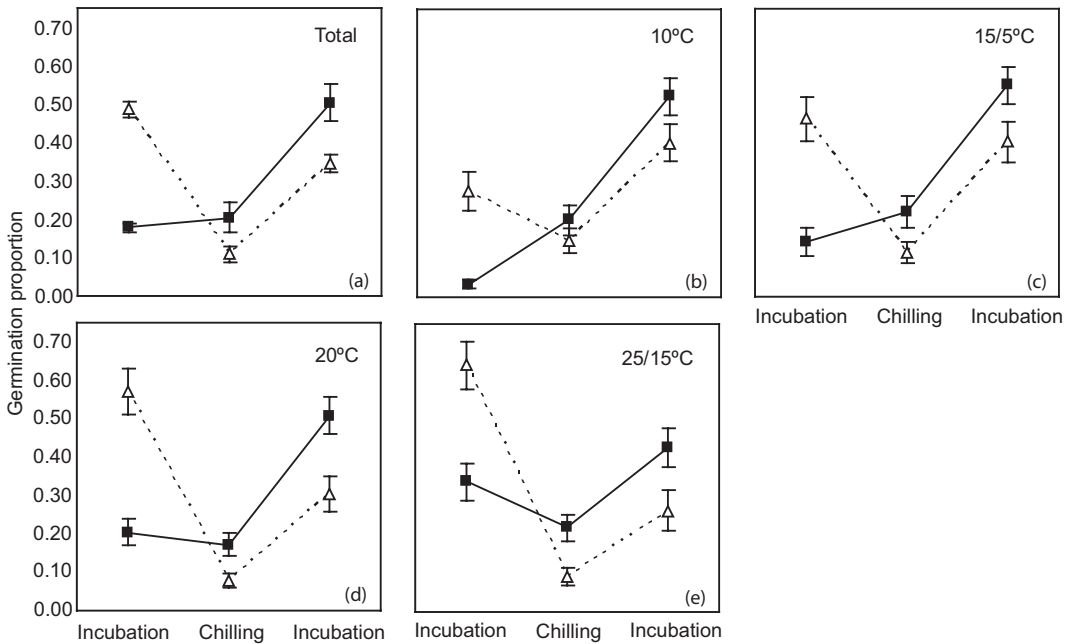


Figure 1 a-e. Germination of species from shaded habitats (filled squares) and open habitats (open triangles) in response to temperature (averaged over the light treatments) throughout the three successive treatment periods in the different treatment conditions. Error bars show 95% confidence intervals.

light, except for the moist-habitat species which increased elongation with shade. Species from dry habitats outperformed species from moist habitats in all treatments. SLA responded to light treatment, but not to watering regime.

Paper III: Seed size dependent recruitment across habitats

Larger seeds had a greater recruitment success relative to smaller seeds in all plant community types. However, the recruitment advantage of large seeds relative to small seeds strongly increased from grassland and open forest to closed-canopy forest. Canopy closure was the environmental variable with the highest explanatory power of this increase in recruitment success. Soil moisture was an additional explanatory factor. Litter cover, moss cover, and soil pH did not contribute to explaining the variation in relative recruitment success of larger seeds.

Paper IV: Trait dispersion in rocky habitat vegetation

Within-plot trait variation appeared to be larger than between-plot variation for seed mass, plant height, lateral spread and species indices of competition, stress and ruderality. The data did not support the hypothesis that traits related to regeneration vary more among co-occurring species than do traits of established individuals. The results indicate that traits diverge at local scale relative to the between-community level, indicating that limiting similarity plays a role in the investigated plant communities.

Paper IV: Abiotic control of community assembly

Patterns in species composition were highly repeatable at each level of soil fertility, while the assemblages were highly dissimilar between fertility treatments. The disturbance treatments did not affect species composition. A severe

drought resulted in high mortality in the high fertility treatment which was characterized by a dense sward of fast-grown and hence drought-susceptible plant individuals, while the intermediate and low fertility microcosms were less affected by the drought. At high fertility, the dominant *Holcus lanatus* and most of the seedlings died back as a result of the drought, after which the formerly subordinate *Festuca rubra* quickly colonized and dominated the microcosms.

General discussion

Regeneration strategies as habitat specialization

The findings in this thesis strongly suggest that habitat or beta niche specialization of plant species is linked to specialization in their regeneration niche. Based on the different germination responses of species from open and shaded habitats appeared to have (paper I), a model was developed showing how specific germination strategies and seasonal environmental conditions interact, enabling some species to successfully establish in shaded habitats, whereas other species fail to do so. The first of four strategies describes non-dormant species with a high temperature requirement, which fail to establish in forests due to the presence of the forest canopy, which suppresses germination. The second strategy is found among species with a requirement for chilling and high temperatures for germination. They also fail to establish in forest since the favourable temperature conditions in spring coincide with the presence of the forest canopy. The third category of species has non-dormant seeds which are able to germinate at low temperatures. They can potentially germinate during late autumn after the opening of the canopy, but their seedlings are exposed to the harsh winter conditions resulting in high mortality. The fourth and only successful strategy is a combination of a chilling requirement and an ability to germinate at low temperatures, enabling species to utilize the

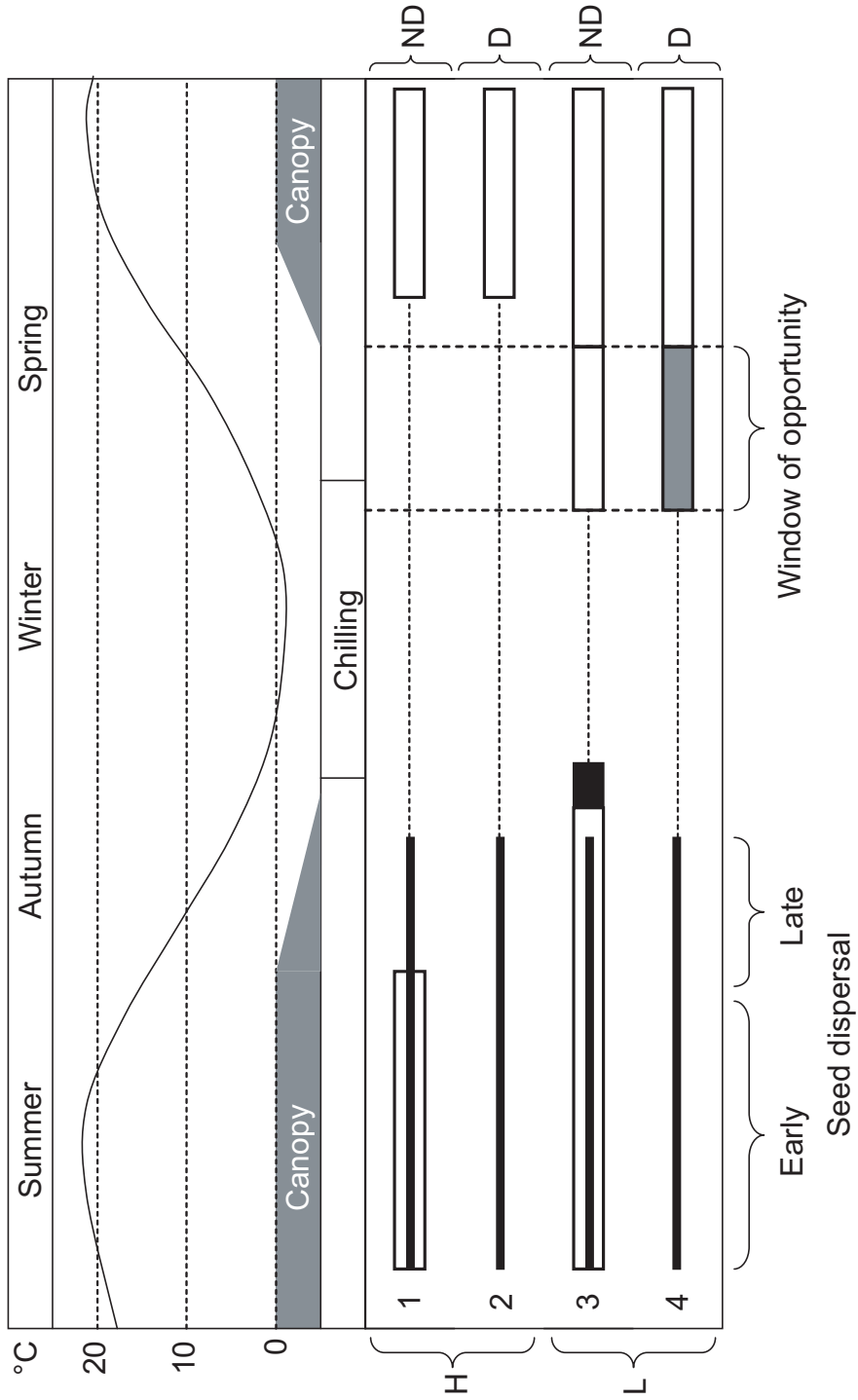


Figure 2. See opposite page for figure caption and figure legend

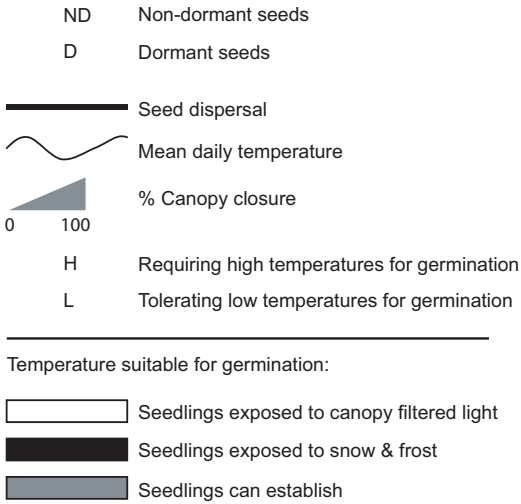


Figure 2. (Legend above; figure on opposite page) A conceptual model of how seasonal temperature and tree canopy presence interact with different germination strategies resulting in four potential scenarios explaining success and failure of species to establish in shaded habitats. Depending on the period of seed dispersal, species with a high temperature requirement for germination can potentially germinate directly upon seed dispersal or have to postpone germination until the next summer (1). Species tolerating low temperatures can germinate during late autumn or early spring (3). When seeds are dormant, a period of chilling is often required, delaying germination until after the winter (2, 4). Beside restrictions for germination due to temperature requirements, opportunities for germination and establishment are further constrained by snow and frost hazards for seedlings emerging just before winter as well as the low R:FR ratio of the light passing through the tree canopy which hinders germination in many species and also dramatically reduces seedling survival. The temperature curve is based on monthly mean temperatures from the period 1961-2004 from a weather station in Southern Sweden

short window of opportunity between snowmelt and canopy closure to germinate and establish in forests. The results in paper I suggest that most of the forest species tend to conform to the latter strategy.

This conceptual model is of course a simplification of reality, but nevertheless stresses how critical the early life stages of plants are, and how they can contribute to habitat specialization. The model is partly based on the assumption that seedling performance is reduced in harsh winter conditions and due to the adverse conditions of canopy shaded light. Tolerance of seedlings to low temperatures and frost is not investigated in this thesis. However, it is known that plants, especially in arctic and alpine climates, have developed a range of mechanisms to tolerate and/or avoid freezing (Larcher 2003). However tolerance against low temperatures or stress-tolerance in general, is accompanied by slow growth due to a reduced metabolism. The resource acquisition of plants at low temperatures is reduced due to a lower photosynthetic capacity and nutrient uptake from the soil (Woodward & Kelly 1997). Low temperature reduces enzyme function and water availability and uptake is greatly reduced at low temperatures, which negatively affects photosynthesis and nutrient uptake. Seedling establishment at low temperatures is therefore unlikely.

The slow growth of plants at low temperatures is in contrast with what is observed in seedlings; a short phase of accelerating growth which reaches its maximum approximately at the moment when the seedling shifts from relying on the reserves in the cotyledons for mineral nutrition to being completely autotrophic (Hanley *et al.* 2004). This period of fast growth is necessary for seedlings to overcome the critical initial establishing period, in which a shift away from the favourable conditions that have triggered germination are likely to be detrimental. The limited root system makes the young seedling extremely sensitive to a drought spell, while the seedling quickly needs to shift the disproportionate large advantage of the established vegetation in the competition for light and nutrients (Schwinning & Weiner 1998). Paper II showed that seedling growth decreased with increasing shade and decreasing water availability,

thereby validating the second assumption in the conceptual model. Although many abiotic stress factors such as shade and drought stress might not lead to direct mortality, it may make them more susceptible to herbivory and pathogens (Augsburger 1984). Mortality in the study in paper II was higher among the shade adapted species. Overall mortality, however, was low, probably because the lack of resources was not too severe.

Species from open and shaded habitats are differentially adapted to the light environment. Species from shaded habitats were characterized by a more conservative growth strategy, i.e. showing a more modest growth response to increasing light (paper II). The results in the seedling study suggest that open habitat species are better able to respond to a decrease in light availability by growing tall through stem elongating. This type of shade avoidance response is generally more adaptive among species from open habitats than forest species, since the latter benefit less from an increased vertical growth, which possibly allows them to overtop other plants in the forest floor, but does not release them from adverse effects of the tree canopy shade (Donohue *et al.* 2000). The SLA of the seedlings decreased increased with increasing shade for both open habitat species and shaded habitat plants. Contrary to our expectations, SLA did not differ between these habitat groups. The size of the leaves and the SLA is also influenced by water availability, and drought stressed plant typically develop leaves with a reduced SLA which helps them to prevent excess water loss through evapotranspiration. Paper II shows that SLA was higher in species from moist habitats than dry-habitat species across all light levels, but SLA was not affected by the watering regime imposed on the seedlings in the experiment. Species from dry habitats were expected to exhibit slower growth as an adaptation to stress, analogous to the slower growth in shade and as indicated by the lower SLA in dry habitat species. Dry habitat species were less affected by reduced water availability than were species from

moist habitats, indicating a conservative strategy. But contrary to the expectations, the species from dry habitats outperformed moist-habitat species in biomass production at both watering levels. For species from shaded habitats, the results showed that drought has an amplified effect at low light levels for growth and plant height. However, for other plant traits and other habitats, amplified effects were not observed.

The results in paper II indicate that in addition to the adaptive specialization of plants in the germination phase, also the seedlings contribute to habitat specialization. Species from contrasting habitats are differently affected and constrained by shortages of light and water in the seedling phase. The stronger exhibition of shade-avoidance in open habitats, contributes to the conceptual model which suggests that seedlings which establish in forests when the tree canopy has already been formed are less likely to be successful. Increased vertical growth by stem and petiole elongation bears a cost of increased susceptibility to drought due to a reduced root to shoot ratio (Maliakal *et al.* 1999) and a higher slenderness of plants makes plants more vulnerable to mechanical damage. The conceptual model explains how open habitat species are not adapted to establish in forests based on the combination of their germination ecology and seedling performance.

The experimental results in paper I do not offer an explanation for the fact that forest species generally do not grow in open habitats. However, the results in paper II suggest a trade-off between adaptations to the shaded environment in forests and competitive ability in open habitats with abundant light and hence in competitive exclusion of forest species in open habitats. Open habitat species grew larger, and were better able elongate with decreasing light, which is adaptive in herbaceous vegetation. This makes them better competitors than the forest species. Such a pattern of habitat partitioning could be called shared preferences, whereby competitively subordinate forest species occupy the region on

the environmental gradient with suboptimal (light) resources (Wisheu 1998; Keddy 2001).

The results in paper III indicate that the success of seedlings across different environments is also related to their seed size. It is well established in the literature that larger seeded species have a recruitment advantage over smaller seeded species (reviewed in Westoby *et al.* 2002), and this is also confirmed in paper III. However, the results indicate that the recruitment advantage increases with increasing environmental adversity, that is, species have more benefit of having larger seeds in closed canopy forests with low light levels, than in open forests and meadows. The abiotic environment, and in particular the light regime, has a major, seed size-dependent impact on seedling survival.

Since seed mass is a compromise between quantity and quality as indicated before, and the relative advantage of having large seeds in recruitment decreases towards open habitats it can be expected that forest species generally have larger seeds than do open habitat species. This is indeed reported in several studies (Salisbury 1942; Foster & Janson 1985; Mazer 1989; Hammond & Brown 1995). The dataset in the germination study (paper I) however, did not show a significant difference in seed size between forest and open habitat species.

Community assembly and niche differentiation

The first three papers focus on the performance of individual species; relating their beta niche to habitat specialization. However, the distribution of species does not only depend on the individual, physiological response of the species to the abiotic environment, or *FUNDAMENTAL NICHE*, but also on the interactions with all other species present in the habitat. These interactions include interspecific competition and facilitation. The abiotic and biotic environment of the species together form their *REALIZED NICHE*. In the last two papers I have looked into the community assembly processes

that shape patterns of species distribution along environmental gradients, based on features of both the species alpha niche and beta niche.

In paper IV, following the trait based method (Ackerly & Cornwell 2007), an attempt was made to quantify the alpha niche and beta niche components of species in terms of their trait values. Here, the beta trait value of a species is a measure of the beta niche position along a specific trait gradient. The alpha trait value represents the species' trait position relative to that of the co-occurring species and is a measure of the alpha niche position of the species. The trait-based gradient analysis in paper IV showed that within-community variation (the range of alpha values) was larger than between-community variation (the range of beta values) for seed mass, plant height, lateral spread and species indices of competition, stress and ruderality. Grime (2006) hypothesized that species tend to diverge in traits related to resource economy at the between-plot level as a result of the strong filtering effects of site productivity, which can be said to be beta niche filtering. This, however, would require the range of beta values to be larger than the range of alpha values which was not observed in the rocky habitat vegetation. According to Grime (2006), trait variation within plots should mainly be attributed to the diversifying effect of disturbance on recruitment opportunities of species and the altering of competitive hierarchies between dominants and subordinates. The advocates of the importance of competition based assembly rules (e.g. Wilson 2007) would ascribe local trait variation to the role of competition and the requirement of limiting similarity for species to coexist (Gause 1934; MacArthur & Levins 1967).

The lack of support for the idea that productivity acts as a strong beta niche filter, resulting in strong trait similarity within plots, and differences between plots along a fertility gradient might be explained by the abiotic stress factors operating in the rocky habitats. The vegetation sampling took place along a gradient of increasing

in soil exchangeable PO_4 with and simultaneous decrease along a wide ranging pH gradient. The pH gradient brings about adverse conditions at both of its extremes; low phosphorus availability at high pH and an increased solubility of phytotoxic aluminium species at low pH. Moreover, the shallow soils on the bedrock along the whole gradient are extremely drought-prone. These combined stress conditions might override the significance of the soil fertility gradient for the productivity of the vegetation. The length of the sampled productivity gradient strongly affects the range of beta values, which would likely increase with a longer gradient, while the range of alpha values would probably stay constant.

The effect of a strong gradient is illustrated by the patterns in community assembly driven by the abiotic conditions in the microcosms in paper V. The fertility gradient, and associated productivity, imposed on the developing plant communities was so strong that it resulted in very similar species assemblages between high fertility microcosms on the one hand and intermediate and low fertility microcosms on the other hand. This suggests an important role for deterministic processes in the assembly of plant communities.

The microcosm experiment (paper V) did not disentangle the effects of environmental filtering on the one hand and competition and other species interactions on the other hand. However, it can still be concluded that the abiotic regime effectively acts as beta-niche filter through direct selection on plant traits and indirectly via the effect on species composition and competitive hierarchies. The plant communities in paper V were assembled from a restricted species pool, incorporating a wide range of seed sizes. The disturbance treatments, which included vegetation gaps varying in size and seasonal timing, may be expected to have diversifying effects on regeneration strategies, thereby resulting in different species compositions across disturbance treatments. However, a severe drought early during summer had an overriding effect on the assembly process, killing off many

vulnerable seedlings and suppressing drought sensitive species, which might otherwise have benefited from the competitive relaxation caused by the disturbance treatments. The agents and extent of disturbance are not determined for the rocky habitat vegetation (paper IV). Here, no evidence was found for the notion of Grime (2006) that there is more variation among regeneration traits than established traits at a local scale due to the converging effects of productivity and diversifying effects of disturbance. Incorporation of more traits related to the juvenile life stage of plants in a trait-based gradient analysis than only seed mass would be valuable to assess of the importance of regeneration in these abiotically stressed communities.

Concluding remarks

The findings in this thesis reveal an important role of regeneration in niche differentiation between species and the resulting habitat specialization. Plants strategies in the regenerative phase contribute strongly to the beta niche of species and their adaptation to specific habitats. The abiotic environment acts a strong filter during the regenerative life phase of plants, sorting species into habitats based on differentiation in beta niches. The use of multiple species pairs in paper I & II is one of the strengths of the studies, as it allows generalizing beyond particular species. Caution, however, needs to be exercised when attempting to make sweeping statements about the importance of regeneration in habitat specialization in general. The ecological contrast in the germination study was between open and shaded habitats, and also included dry vs. moist habitats in the seedling experiment. Shade and drought, however, are just two of several environmental stress factors that can influence species distribution over habitats. Adaptations to low temperatures and a short growing season in alpine and arctic habitats, nutrient stress in low

fertility soils and other stresses like soil salinity and acidity (Crawford 1989) all require plants to trade-off stress-adaptations against competitive ability. When simultaneous operating stress factors act on a vegetation, the effects of a particular stress factor like shade on vegetation patterns and plant distribution might be obscured by the amplification, co-variation or constraint of other factors. This is reflected in the complex of abiotic stress factors operating on the rocky habitat vegetation. At the plant community level, deterministic processes play an important role in the assembly of plant communities. The abiotic environment causes species to separate along productivity gradients, and simultaneously affect local biotic interactions by governing species' relative abundances. The findings in this thesis on beta niche differentiation in the regenerative phase of the life cycle urge for more studies of the importance of regeneration at the alpha level. Including the whole suite of important life cycle stages that are here in this thesis shown to be important in beta niche differentiation such as germination ecology, seed size and seedling establishment in community level studies would be valuable.

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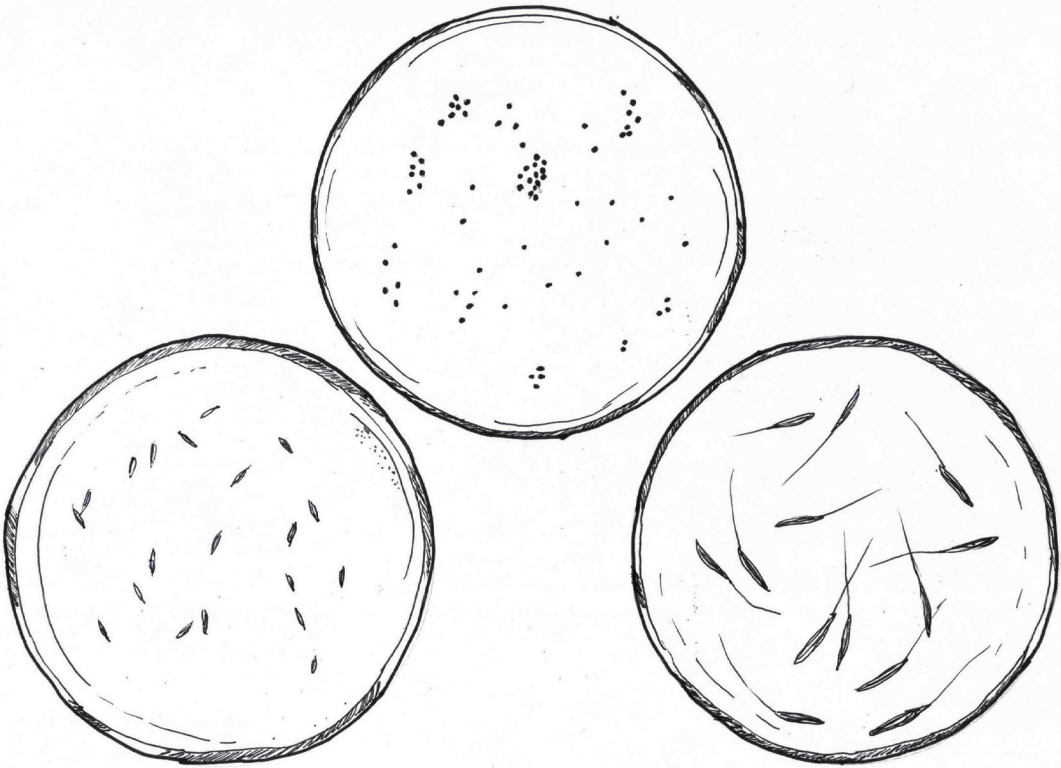
PS:

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Habitat specialization through germination cueing: a comparative study of herbs from open and shaded habitats

Dirk-Jan ten Brink and Hans Henrik Bruun

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Habitat specialization through germination cueing: a comparative study of herbs from open and shaded habitats

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We examined the adaptive association between germination ecology and specialization to either forest or open habitats across a range of evolutionary lineages of seed plants. Seed germination response to temperature, light and stratification was tested for 18 congeneric pairs, each consisting of one forest species and one open-habitat species. We used a factorial design involving four temperature treatments (constant-low, fluctuating-low, constant-high, fluctuating-high), two light levels and a cold stratification treatment. The congeneric species pair design took phylogenetic dependence into account. Species from open habitats germinated better under high and fluctuating temperatures than under low and constant; whereas forest species performed equally well at low and high temperatures. Forest species tended to germinate only after a period of cold stratification that could break/relax dormancy, whereas species from open habitats generally germinated without cold stratification. We connected the empirically-derived germination strategies to establishment opportunities in nature. Annual changes in temperature and light regime in temperate forest delimit windows of opportunity for germination and establishment. Germination strategies of forest plants are adaptations to utilize such narrow windows in time. Conversely, lack of fit between germination ecology and environment may explain why species of open habitats fail to establish in forests. Germination strategy is a likely mechanism of specialization of temperate herbs to shaded habitats. This finding strongly suggests that other phases in the plant life cycle than the established phase should be considered important in adaptive specialization.

Introduction

During the assembly of plant communities, environmental sieves act upon morphological, physiological and life-history traits, selectively filtering out certain species while allowing others to pass through (Harper 1977; Grime 2006; Keddy 1992; Bazzaz 1991). Trait-filtering may be driven by resource competition and plant interactions within habitats (corresponding to niche diversification

(Whittaker 1975) or differentiation in alpha niches (Pickett and Bazzaz 1978; Silvertown et al. 2006) and by environmental conditions at the between-habitat level (habitat diversification (Whittaker 1975) or differentiation in beta niches (Pickett and Bazzaz 1978; Silvertown et al. 2006)). The latter mechanism is the focus of this study.

Traditionally, plant ecologists have aimed at identifying links between habitat specialization and traits of established individuals, e.g. Raunkiaer's

(1904; 1934) life forms and Grime's (1974; 2001) CSR strategy scheme, assuming that niches are uniform and constant throughout all life stages (e.g. MacArthur and Levins 1967). However, niche requirements may change during the life of individuals, for example among plants establishing in environments that characteristically change during a plants' life span (Eriksson 2002). This phenomenon, called 'ontogenetic niche shifts', is rarely addressed in plant ecology. The importance to habitat specialization of plant traits and requirements of the regenerative phase, i.e. their regeneration niche (Grubb 1977), is therefore much less studied.

Plants appear to provide their seed with mechanisms to assure that germination takes place in a certain habitat and/or at a certain time of the year. Seed dormancy is one mechanism by which seed germination can be effectively delayed until favourable conditions for seedling establishment are more likely. In temperate regions, physiological dormant seeds often require a period of chilling to break dormancy, which in practice means that dormancy is broken during winter. Germination can subsequently take place, usually later on during spring since temperatures required to break dormancy are often too low to also trigger germination. Light and temperature are the major environmental factors influencing germination. Species differ in temperature ranges over which germination can take place (Probert 2000) and fluctuating temperatures have shown to stimulate germination in many species (Knapp 1956; Schütz 1999; Thompson and Grime 1983). Light serves as cue for germination in many species (Vleeshouwers et al. 1995; Pons 2000; Grime and Jarvis 1975; Thompson 1989), and seed maturation under canopy-filtered light with a low red to far red ratio (R:FR ratio) may induce a light requirement for germination (Smith 1982).

Habitat specialization results from the requirements of all life stages of the plant, both regenerative phase (germination, seedling establishment) and vegetative (adult). One may ask

whether a certain life stage is choosier than others, and thus, for any sessile organism, effectively governs habitat specialization. However, the question is hard to answer since for all life stages passed through by established plants found in nature, the needs have apparently been satisfied. Nevertheless, germination cueing was found to determine habitat specialization of six cold-temperate cespitose *Carex* species (Schütz 1997). Species from wooded wetlands germinated at low and constant temperatures, whereas species from open wetlands germinated at higher temperatures with large diurnal fluctuation. A similar role of germination cueing in microhabitat preference was found for two *Erodium* species inhabiting grasslands gaps (Rice 1985). Diurnal soil temperature fluctuation was found to promote germination rate and to be much larger in gaps than in the surrounding vegetation.

In this study we wanted to investigate the general applicability of the adaptive association between specific germination traits and habitat preference by testing across a wide range of evolutionary lineages. We adopted a comparative approach, using a large number of congeneric species pairs with contrasting habitat preference to investigate whether germination traits of seeds co-vary predictably with habitat preference. Each pair provides us with an independent replicate of evolutionary divergence in habitat preference. Additionally, the shared evolutionary history until divergence within pairs would allow unequivocal exclusion of confounding effects of unmeasured traits that species might share through common descent rather than through independent evolution (Rees 1995; Ackerly 1999) or niche divergences deeper in the phylogeny.

The ecological contrast investigated is between species from open versus shaded habitats, and the germination responses to environmental conditions typical for these two contrasting habitats. The microclimate of temperate forests is characterised by a closure of the tree canopy in late spring with an accompanying reduction of

Table 1. Species used in the study. Seed origin refers to species from open habitats. C: commercial supplier, RV: road verge, G: grassland, CD: coastal dune, U: urban area. All forest species origin from deciduous forests, except *Urtica dioica* which is purchased from a commercial supplier.

Genus	Forest	Open habitat	Seed origin
<i>Brachypodium</i>	<i>syvaticum</i>	<i>pinnatum</i>	C
<i>Bromus</i>	<i>benekenii</i>	<i>erectus</i>	RV
<i>Bromus</i>	<i>ramosus</i>	<i>inermis</i>	G
<i>Campanula</i>	<i>trachelium</i>	<i>rotundifolia</i>	G
<i>Carex</i>	<i>syvatica</i>	<i>lepidocarpa</i>	G
<i>Carex</i>	<i>remota</i>	<i>ovalis</i>	G
<i>Festuca</i>	<i>altissima</i>	<i>arundinacea</i>	CD
<i>Festuca</i>	<i>gigantea</i>	<i>pratensis</i>	C
<i>Geum</i>	<i>urbanum</i>	<i>rivale</i>	G
<i>Hordeylmus/Hordeum</i>	<i>europaeus</i>	<i>murinum</i>	U
<i>Hypericum</i>	<i>hirsutum</i>	<i>perforatum</i>	G
<i>Poa</i>	<i>nemoralis</i>	<i>pratensis</i>	G
<i>Primula</i>	<i>elatior</i>	<i>farinosa</i>	G
<i>Rumex</i>	<i>sanguineus</i>	<i>obtusifolius</i>	RV
<i>Silene</i>	<i>dioica</i>	<i>latifolia</i>	CD
<i>Stellaria</i>	<i>holostea</i>	<i>graminea</i>	G
<i>Stellaria</i>	<i>nemorum</i>	<i>media</i>	C
<i>Urtica</i>	<i>dioica</i>	<i>urens</i>	C

the temperature level and daily amplitude relative to open habitats (Fig. 4; upper panel), as well as a reduction of light quantity (photosynthetic photon flux density; PPFD) and R:FR ratio. In open habitats, the absence of a covering tree canopy results in larger diurnal temperature fluctuations. The light environment in open habitats depends on the height and density of the herbaceous vegetation.

The aim of this study is to test the hypotheses that 1) species' specialization to open and shaded habitats, respectively, is consistently accompanied by specialization in the regeneration niche and 2) that species are thereby adapted to utilise different windows of opportunity in time (season) and space (habitat). If species from contrasting habitats are

able to germinate in each others' habitat and the seedlings are able to emerge and establish, it can be concluded that the environmental requirements and competitive ability of the mature individuals govern the habitat specialization of the species. If, on the other hand, species with contrasted habitat preference respond differently to environmental conditions already in the early life stages, the conclusion can be drawn that the regeneration niche is indeed significant for the habitat specialization.

Materials and methods

Species selection

When selecting species, we considered as candidates all congeneric pairs of herbaceous species occurring in southernmost Sweden and adjacent eastern Denmark and forming an open-shaded habitat match. Low availability of sufficient amounts of ripe seeds during the period of collection excluded some species, e.g. having too sparse populations and common species with continuous seed release, heavy seed predation etc. This constrained the number of species to a workable number, so no random selection of study species pairs from a gross list of candidates was done, as otherwise recommended by Ackerly (1999) and Westoby (2002). Species were considered to prefer shaded habitats if they predominantly occur in habitats with a tree canopy, and the opposite for open-habitat species. Judgement of habitat preference was based on field experience of the authors and their colleagues. Species tending to occur in both open and shaded habitats were excluded. A total of 36 species in 18 congeneric pairs were used in this study. Each species pair consisted of two congeners, except *Hordehymus europaeus* and *Hordeum murinum*, which belong to closely related genera (Table 1). The species included were seven pairs of grasses, two pairs of sedges (*Carex*) and nine pairs of forbs. Four genera (*Bromus*, *Carex*, *Festuca* and *Stellaria*) were represented with two species pairs each. The grouping into pairs within these genera was based on taxonomic relatedness (Table 1).

Seed collection

Freshly matured seeds were collected from various locations in the southernmost part of Sweden, and adjacent eastern Denmark between July and October 2004. Seed collections came from one population per species, but from several individuals per population. Special care was taken to only

collect seeds of open-habitat species from open sites without any tree canopy, e.g. grasslands, road verges, ruderal sites and urban areas and, likewise, seeds of shaded-habitat species were collected from forests only. This was done to avoid confounding effects of induced light requirement (Cresswell and Grime 1981) and local ecological differentiation within species. Collected seeds were air-dried at room temperature and subsequently stored in paper bags at room temperature until further use. Field-collected species were complemented with seeds from commercial suppliers in case of five species, all but one open-habitat species (Table 1). These species were cultivated for one generation from seed collected in the wild and thus were not selected for specific seed characteristics.

After collection, seeds were visually checked and only firm, filled seeds were used. Seeds of aberrant colour or shape or with any sign of predation or underdevelopment were discarded. Two additional pairs selected for the experiment (*Campanula latifolia* / *C. persicaria* and *Ranunculus ficaria* / *R. acris*) were excluded from the analysis since the seeds of *C. latifolia* and *R. ficaria* did not germinate in any of the treatment combinations, probably due to immaturity or unusual germination behaviour.

Germination tests and experimental design

In February 2005, germination tests were initiated in temperature controlled climate rooms equipped with 400 W metal halide lamps (~200 $\mu\text{mol}/\text{m}^2/\text{s}$). The germination tests were performed as a full factorial design with two treatment factors, temperature with four levels: constant-low (10°C), constant-high (20°C), fluctuating-low (15/5°C) and fluctuating-high (25/15°) temperatures and light/darkness. The temperature conditions used correspond to daily means and diurnal fluctuations in spring (April) and summer (June) in southern Sweden. Diurnal temperature fluctuations corresponded to a light regime of 12 hours (higher temperature) and 12 hours of darkness (lower

temperature).

For each species, 5 replicates of 50 seeds were placed in 5 cm or 9 cm Petri dishes on filter paper (Munktell 00K, Grycksbo, Sweden) and wetted with distilled water. A lower number of seeds per Petri dish were used due to limited availability of seeds for *Hordelymus europaeus* (45), *Hordeum murinum* (40) and *Stellaria holostea* (30). In the dark treatment, the Petri dishes were wrapped in a double layer of aluminium foil immediately after wetting. The Petri dishes were randomly distributed on benches in the climate rooms.

Initially, seeds were exposed to the various treatment combinations for a period of 14 days (hereafter called 'first incubation period'), during and after which germinated seeds were counted and removed from the Petri dishes. The germination criterion used was the visible protrusion of the radicle. Subsequently, the Petri dishes were put in plastic bags (to avoid desiccation) and stored at 2°C for stratification during 3.5 months to allow the ungerminated seeds to break possible (physiological) dormancy. At the end of the stratification period, germinated seeds were counted and removed from the Petri dishes again. The stratification was followed by a second incubation period of 14 days with identical conditions as in the first incubation period. A 14 days period is recommended by Baskin & Baskin (1998, pp. 19). No new seeds germinated at the end of each incubation period and species which did not germinate at all during the first incubation did so after stratification. At the end of the second incubation period, the germination experiment was terminated and a final count of germinated seeds was made. In the light treatments, germinated seeds were counted regularly and removed from the Petri dish. In the dark treatment, germinated seeds were counted once after each of the three treatment periods. The counts in the dark treatment were made under dim green light. Petri dishes were kept moist continuously during the experiment. Petri dishes in light treatment periods received distilled water when needed during the incubation. The

wrapping of the Petri dishes in aluminium foil (dark treatments) and additional plastic during the stratification period prevented desiccation of the seeds and filter paper. Some replicate Petri dishes were discarded due to mould contamination and hence were not included in statistical analyses (see appendix 1 for sample sizes).

Data analysis

Germination proportions were arcsine square-root transformed to improve normality (Sokal and Rohlf 1995) and analyzed with factorial Analysis of Variance (ANOVA) (SPSS 12.0 for Windows, SPSS Inc, 2003). Germination proportion (final or divided into periods; after first incubation, after stratification, after second incubation) was the response variable. All analysis included species pair identity as a block factor (random). Post-hoc Tukey tests were performed to analyse the differences in means of the various treatment combinations in the ANOVA analyses. An alpha value of 0.05 was used.

Results

Two sets of analysis are presented in this section: (1) effects of the treatment factors temperature and light and their interaction with the habitat type and (2) the importance of stratification for germination. Mean germination proportions and standard deviations for each treatment combination are listed in appendix 1. A t-test revealed that species from open and shaded habitats did not differ significantly in seed size (results not shown).

Treatment effects (analysis I)

An ANOVA was performed with final germination proportion as response variable and habitat type, temperature and light as fixed factors (Table 2). Significant main effects were found for habitat,

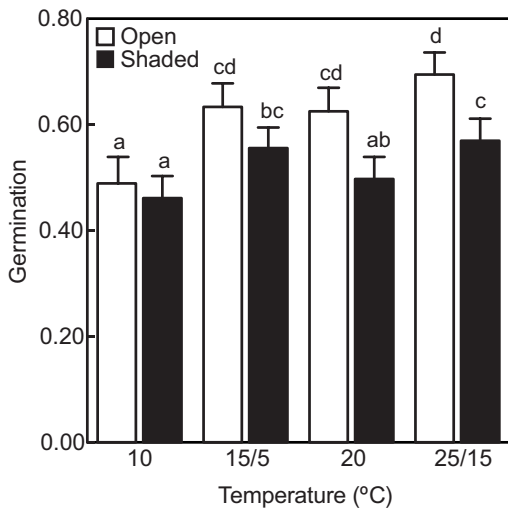


Figure 1. Germination response of species from open and shaded habitats in different temperature treatments. Different letters indicate statistically significant differences between treatments and habitat groups (germination values have been back-transformed following arcsine-square-root transformation used in ANOVA). Error bars show 95% confidence intervals.

temperature and light. Also the species pair identity, used as a random block factor in the analysis, was significant. The two-way interactions between habitat and temperature as well as between temperature and light were significant. The three-way interaction between habitat, temperature and light was not significant (Table 2).

Temperature

The Tukey post-hoc test revealed that germination proportions were significantly lower at 10°C constant temperature than in any of the other temperature treatments. Germination proportions at 20°C constant temperature also differed significantly from those at 25/15°C fluctuating temperature. Germination proportions at 15/5°C fluctuating temperature did not differ significantly from those at 25/15°C (Fig. 1).

A significant interaction between habitat

type and temperature indicated that species from open and closed habitats responded differently to the temperature treatments. The germination proportions of the species from closed and open habitats were not significantly different at 10°C and 15/5°C, but were significantly higher for species from open habitats than for forest species at 20°C and 25/15°C (Fig. 1).

Light

The species from both habitat types germinated to a higher degree in light than in darkness. The interaction between habitat type and light was not significant (Table 2).

Visual representation of treatment and interaction effects

Figure 2 gives a visual representation of the effects of treatments on germination proportion and their respective interactions. Effect sizes for the dependent factors were obtained from the ANOVA. Effect sizes, measured as partial eta squared values (Table 2), describe the proportion of the total variability that is attributable to a factor. Ignoring species pair identity, germination proportions were most strongly affected by temperature followed by light and habitat (Table 2, Fig. 2). Habitat, however, was used as first discriminating factor, as the study aims to compare species from open and shaded habitats. The partial eta squared values were used to determine the order of the other two variables along the x-axis. The different temperature treatments explained more of the variability in the whole data set than did the light treatments (Fig. 2). The figure reveals that the open-habitat species showed a higher overall germination response than the forest species.

Effect of stratification (analysis 2)

An ANOVA was performed to test the importance of stratification for germination (Table 3). The

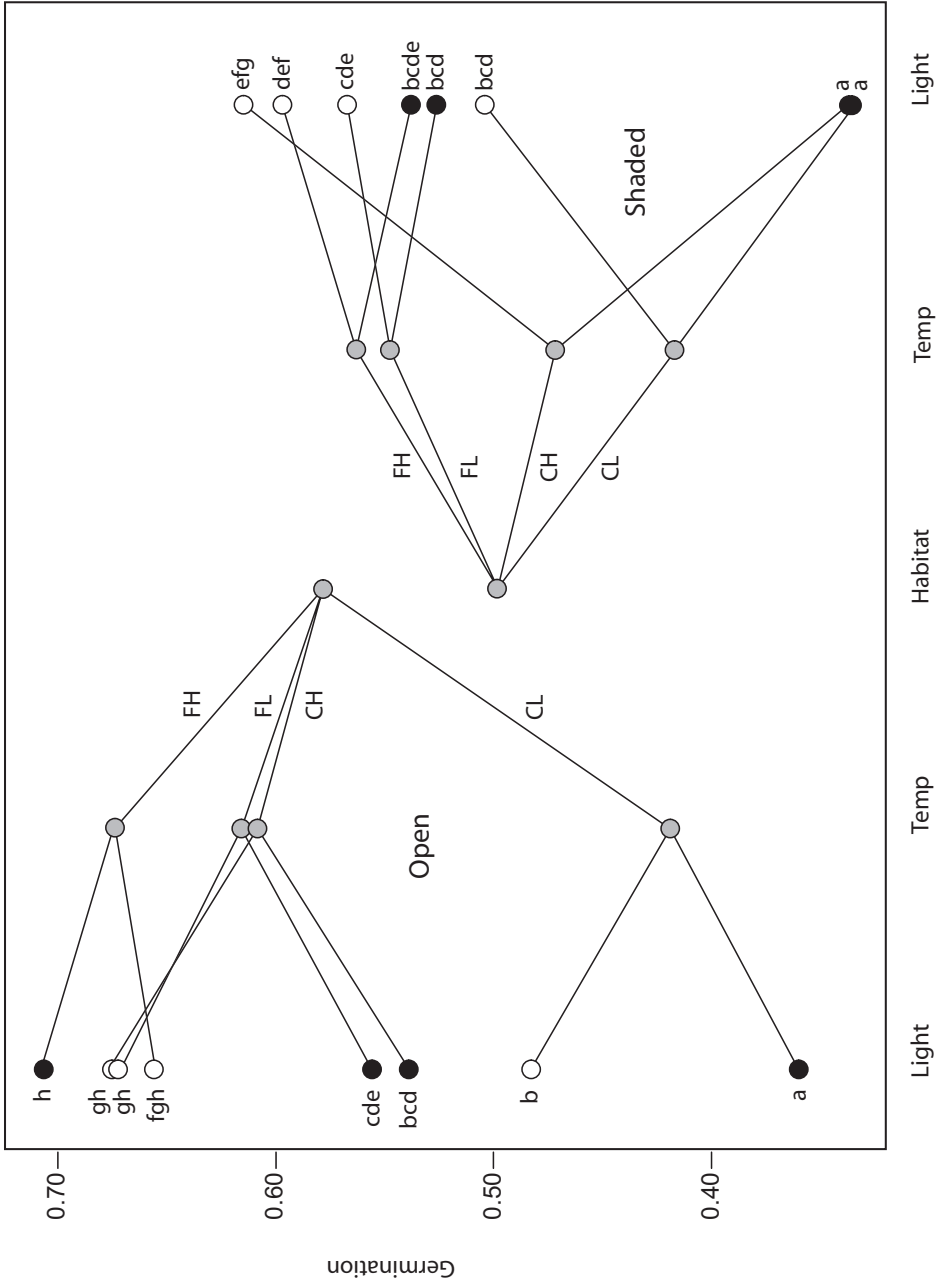


Figure 2. Interaction plot showing germination responses for the levels of and the interactions between habitat (species from open habitats left; from shaded habitats right), temperature (FH: fluctuating high, FL: fluctuating low, CH: constant high, CL: constant low) and light (open circles: light, filled black circles: darkness) in decreasing importance in ANOVA. Different small letters indicate statistically significant differences between the levels of the interactions.

Table 3. ANOVA of the effect of ‘time’ on germination proportions (germination response partitioned after census period). See main text for a detailed explanation.

Factor	df	F	p
Habitat	1, 4219	3.696	0.055
Time	2, 4219	297.910	<0.001
Spec. pair	17, 4219	16.445	<0.001
Habitat x Time	2, 4219	256.790	<0.001

germination data were grouped according to census point (after first incubation, after stratification period and after second incubation), which resulted in three germination values for each Petri dish. In the ANOVA, the three census periods were treated as three levels of the fixed factor ‘time’. Habitat

was used as a second fixed factor and the species pair identity was again used as a random block factor. The data were pooled over temperature and light treatments. The interaction between time and habitat was significant (Table 3). The species from closed habitats germinated largely after the stratification period, during the second incubation, whereas the species from open habitats exhibited considerable germination already during the first incubation period (Fig. 3a). Germination during the stratification period itself was low for species from both habitat types, since the stratification temperature of 2°C is too low for most species to germinate. Considerable proportions of the seeds of certain grass species, however, germinated during the stratification period.

Two similar tests of the importance of stratification for germination were performed on subsets of the data set corresponding to

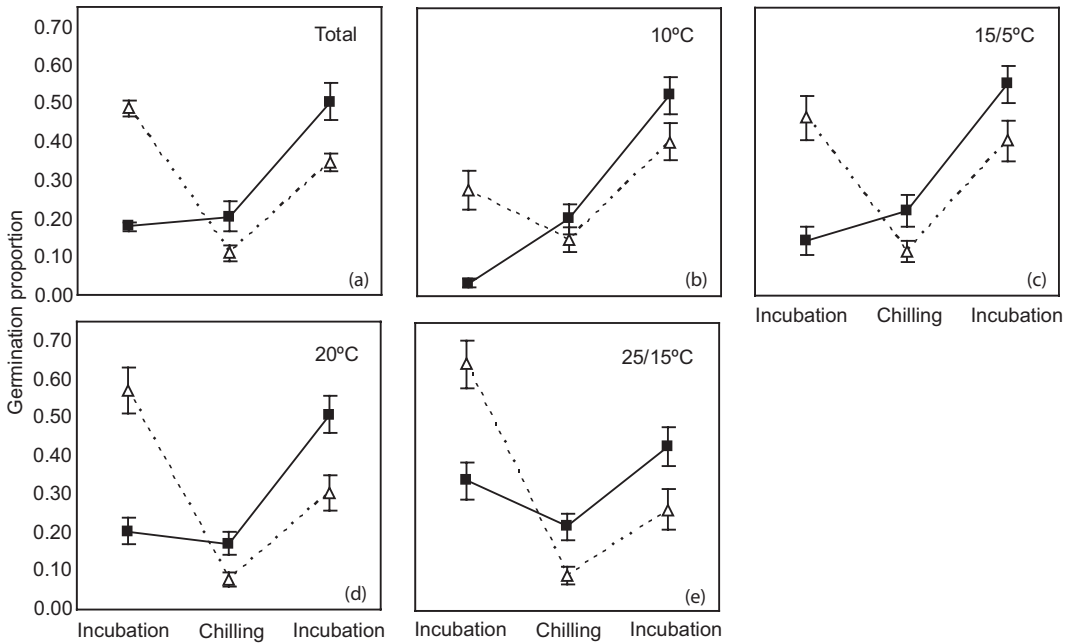


Figure 3 a-e. Germination of species from shaded habitats (filled squares) and open habitats (open triangles) in response to temperature (averaged over the light treatments) throughout the three successive treatment periods in the different treatment conditions. Error bars show 95% confidence intervals.

the different levels of the treatments factors temperature and light respectively. In all subsets, the interaction between time and habitat was significant (results not shown in detail). The interaction plots however, reveal that fluctuating temperatures and/or high temperature substitute the need for stratification to some extent (Fig. 3b-e). Open-habitat species germinated more during the first incubation period when the treatment temperatures were fluctuating and/or high. The forest species also germinated slightly more during the first incubation period when the treatment temperature was high, fluctuating or a combination of both, but in all cases the majority of seeds germinated after the stratification period (Fig. 3b-e).

Discussion

This study showed that specialization of plant species to either open or shaded habitats is linked to specialization in the regeneration niche. We found a strong tendency for species from shaded habitats to need a period of chilling to break seed dormancy. Such a requirement effectively delays germination until spring. In contrast, most species from open habitats were shown to germinate when incubated without chilling (Fig. 3a), enabling germination at any favorable point in time after dissemination. Species from open habitats were shown to germinate to higher degrees at fluctuating and/or high temperatures than at constant-low temperatures, whereas forest species performed equally well at low and high temperatures (within the constant and fluctuating levels respectively), but apparently preferring fluctuating over constant temperatures (Fig. 1). Tolerances and requirements for germination, as described above, combined with the occurrence in time and space of these environmental conditions in nature, circumscribe temporal windows of establishment opportunity specific to habitat-defined species group as summarized in the conceptual model in Fig. 4.

The need of a cold period to break seed dormancy prevents most forest species from germinating during the autumn following seed dispersal. In temperate forest, rising temperature in spring is followed by canopy closure. However, the period between snowmelt and canopy closure constitutes a short window of opportunity for species able to germinate at low temperature (Fig. 4, scenario 4). The absence of a chilling requirement common among species from open habitats contributes to explaining why such species do not establish in forest habitats (Fig. 4, scenario 1 & 3). A similar tendency for forest species to respond to low temperatures was found in a screening of 32 *Carex* species (Schütz and Rave 1999; Schütz 2000) Our experimental results suggest that species from open habitats in general come under scenario 1 or 3, whereas forest species tend to conform to scenario 2 or 4. However, under scenario 2 they cannot utilize the window of opportunity to establish in forests.

While the general trend was that open-habitat species germinate instantaneously and forest species delay germination until after a chilling period (Fig. 3a), the various temperature treatments showed quite some variation. Species from open habitats showed high germination responses before chilling in the treatments with high and/or fluctuating temperatures (Fig. 3c-e), whereas they showed consistently low germination rates before chilling at 10°C (Fig. 3b). Also among the forests species a higher fraction of the seeds germinated before chilling at high and fluctuating temperatures (Fig. 3b-e). These trends can be explained with Vleeshouwers' (1995) concept of dormancy. He states that dormancy varies on a continuous scale, and the degree of dormancy is influenced by external temperature, whereby chilling is a common way seeds lose dormancy. As dormancy decreases, the temperature range over which seeds can germinate widens (Vleeshouwers et al. 1995). Seeds can be dormant to such an extent that they only respond to high and/or fluctuating temperatures as shown in Fig 3, leading

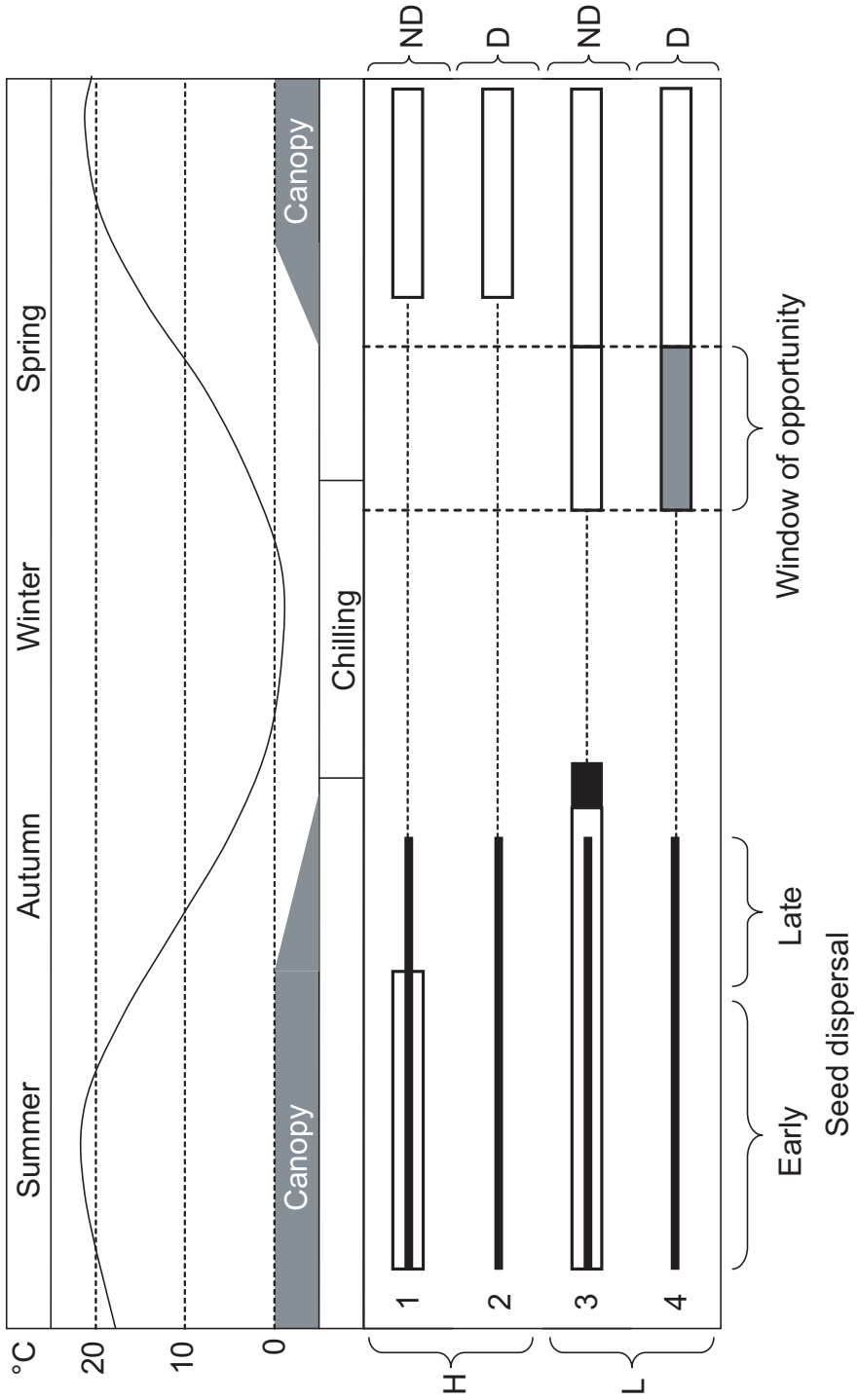


Figure 4. See opposite page for figure caption and figure legend

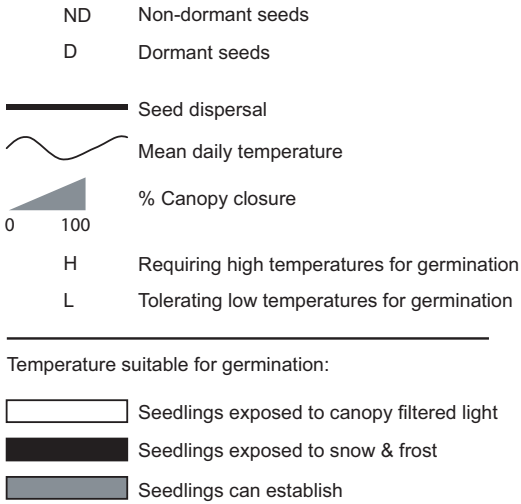


Figure 4. (Legend above; figure on opposite page) A conceptual model of how seasonal temperature and tree canopy presence interact with different germination strategies resulting in four potential scenarios explaining success and failure of species to establish in shaded habitats. Depending on the period of seed dispersal, species with a high temperature requirement for germination can potentially germinate directly upon seed dispersal or have to postpone germination until the next summer (1). Species tolerating low temperatures can germinate during late autumn or early spring (3). When seeds are dormant, a period a chilling is often required, delaying germination until after the winter (2, 4). Beside restrictions for germination due to temperature requirements, opportunities for germination and establishment are further constrained by snow and frost hazards for seedlings emerging just before winter as well as the low R:FR ratio of the light passing through the tree canopy which hinders germination in many species and also dramatically reduces seedling survival. The temperature curve is based on monthly mean temperatures from the period 1961-2004 from a weather station in Southern Sweden

to a dramatically reduced germination response when exposed to constant low temperatures.

Seeds of open-habitat and forest species appeared to differ consistently in the degree of dormancy when shed from the mother plant.

However, two different groups of species could be observed among the species with an apparently high degree of dormancy: 1) a group of species, all of them monocots, which showed no or minimal germination before chilling over all treatments (*Brachypodium pinnatum*, *B. sylvatica*, *Carex lepidocarpa*, *C. remota*, *C. sylvatica*, *Festuca altissima*) and 2) a group of species that responded to fluctuating-high temperatures only before chilling, sometimes along with a light requirement (*Bromus benekenii*, *Campanula trachelium*, *Carex ovalis*, *Festuca gigantea*, *Geum rivale*, *G. urbanum*, *Hordehymus europaeus*, *Hypericum hirsutum*, *Primula elatior*, *P. farinosa*, *Urtica urens*, *Stellaria holostea*, *S. nemorum*). The presence of a few open-habitat species in both groups suggests that either a chilling requirement is shared in some congeneric species despite different habitat preferences - pointing to phylogenetic constraints - or, more likely, that some other mechanism of habitat specialization than germination cueing underlies the habitat differentiation within some congeneric species pairs.

We did not find any causal relation between germination response to light and habitat differentiation. Both open-habitat and forest species germinated better in light than in darkness. For the forest species, light appeared to serve as a substitute for temperature fluctuations, because at constant temperature, light improved the germination response dramatically compared to the dark treatments (Fig. 2). The same trend, albeit much weaker, was visible for the open habitat species, and applied even to the fluctuating-low temperature treatment. Both light and temperature fluctuations are known to serve as indicator of soil burial depth for seeds (Pons 2000; Fenner and Thompson 2005).

The experimental results offer an explanation why species adapted to conditions in open habitats, fail to establish in forests (Fig. 4). Our findings, however, are not incompatible with previous studies showing forest species to have adaptive traits in the established phase to survive in shaded habitats. But, as said before,

germination and establishment are the first critical life stages of plants, and can therefore play an important role. Open-habitat species appear not to be adapted to establish in forests. The opposite explanation, why forest species do not grow in open habitats, cannot be derived from our results. An explanation could perhaps be sought in a trade-off between adaptations to the shaded environment in forests and competitive ability in open habitats with abundant light and hence in competitive exclusion of forest species in open habitats. Such a pattern of habitat partitioning could be called shared preferences, whereby competitively subordinate forest species occupy the region on the environmental gradient with suboptimal (light) resources (Wisheu 1998; Keddy 2001). The ecological contrast we have investigated was between open and shaded habitats. Shade, however, is just one of several environmental stress factors that can influence species distribution over habitats. Adaptations to low temperatures and a short growing season in alpine and arctic habitats, low water availability in dry habitats, nutrient stress in low fertility soils and other stresses like soil salinity and acidity (Crawford 1989) all require plants to trade-off stress-adaptations against competitive ability. When simultaneously operating stress factors act on a vegetation, the effects of a particular stress factor like shade on vegetation patterns and plant distribution might be obscured by the amplification, co-variation or constraint of other factors (Keddy 1992).

Our selection of 36 different herbaceous species representing different functional groups from different plant communities and the conceptual model (Fig. 4) enables us to generalize beyond particular species. When extrapolating from laboratory germination test to field situation, however, it should be noted that seasonal temperature development varies between years and, connected with that, the development and senescence of the tree canopy. Also, the timing of the canopy closure and light environment on the forest floor depend on the tree species.

Nevertheless, we are able to identify important causal effects of certain environmental cues on germination response of forest and open habitat species.

We conclude that there is a strong linkage between germination traits and habitat preference of herbs, and that germination strategies are a likely mechanism behind the coarse partitioning in open vs. shaded habitats of herbs in temperate regions. Our results may be valuable to mechanistic approaches to community assembly (Shipley et al. 2006) as well as in assessing possible effects of global warming on the distribution of plant species, when this global warming leads to a reduced chilling period and introduces unpredictable warm spells during winter.

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Appendix 1. Mean germination proportions (μ), and standard deviation (sd) and number of replicate Petri dishes (n) of all species under the different treatment combinations used in the study. O: open habitat. F: forest. Time: germination response partitioned after census period (1: first incubation, 2: cold stratification, 3: second incubation)

		Time						Darkness						Light														
		10°C		15/5°C		20°C		25/15°C		10°C		15/5°C		20°C		25/15°C												
		μ	sd	n	μ	sd	n	μ	sd	n	μ	sd	n	μ	sd	n	μ	sd	n									
<i>Brachypodium</i>	pinnatum	O	1	0.00	0.00	5	0.00	0.01	5	0.00	0.00	5	0.00	0.00	5	0.00	0.00	5	0.00	0.00	5							
		2	0.00	0.00	5	0.02	0.02	5	0.00	0.00	5	0.01	0.01	5	0.00	0.00	5	0.00	0.00	5	0.00	0.00	5					
		3	0.79	0.14	5	0.86	0.07	5	0.77	0.07	5	0.93	0.03	5	0.82	0.05	5	0.73	0.05	5	0.81	0.08	5	0.81	0.04	5		
	sylvaticum	F	1	0.00	0.00	5	0.00	0.00	5	0.00	0.00	5	0.00	0.00	5	0.00	0.00	5	0.00	0.00	5	0.00	0.00	5	0.00	0.00	5	
		2	0.00	0.00	5	0.00	0.00	5	0.00	0.00	5	0.00	0.01	5	0.00	0.00	5	0.00	0.00	5	0.00	0.00	5	0.00	0.00	5		
		3	0.60	0.11	5	0.56	0.14	5	0.50	0.04	5	0.27	0.24	5	0.66	0.06	5	0.60	0.09	5	0.65	0.13	5	0.46	0.06	5		
	<i>Bromus</i>	erectus	O	1	0.76	0.08	5	0.80	0.05	5	0.77	0.05	5	0.81	0.06	5	0.61	0.08	5	0.63	0.03	5	0.70	0.06	5	0.66	0.04	5
			2	0.00	0.00	5	0.00	0.00	5	0.00	0.00	5	0.00	0.00	5	0.10	0.06	5	0.06	0.04	5	0.02	0.03	5	0.01	0.03	5	
			3	0.00	0.00	5	0.00	0.00	5	0.00	0.00	5	0.00	0.00	5	0.01	0.02	5	0.01	0.03	5	0.01	0.01	5	0.00	0.00	5	
benekei		F	1	0.00	0.00	5	0.08	0.04	5	0.02	0.04	5	0.26	0.03	2	0.00	0.00	5	0.00	0.00	5	0.04	0.04	5	0.04	0.05	5	
		2	0.44	0.12	5	0.42	0.18	5	0.20	0.10	5	0.05	0.01	2	0.49	0.09	5	0.56	0.05	5	0.34	0.05	5	0.15	0.05	5		
		3	0.07	0.07	5	0.07	0.07	5	0.06	0.03	5	0.00	0.00	2	0.09	0.06	5	0.08	0.02	5	0.04	0.04	5	0.00	0.00	5		
<i>Bromus</i>		inermis	O	1	0.38	0.07	5	0.82	0.06	5	0.88	0.07	5	0.89	0.05	5	0.14	0.03	5	0.65	0.11	5	0.75	0.08	5	0.82	0.06	5
			2	0.13	0.05	5	0.03	0.03	5	0.00	0.00	5	0.00	0.00	5	0.16	0.06	5	0.07	0.06	5	0.02	0.03	5	0.00	0.00	5	
			3	0.32	0.10	5	0.02	0.02	5	0.00	0.00	5	0.00	0.00	5	0.38	0.06	5	0.16	0.05	5	0.06	0.04	5	0.01	0.02	5	
	ramosus	F	1	0.03	0.02	5	0.05	0.05	5	0.20	0.09	5	0.66	0.09	5	0.00	0.00	5	0.00	0.00	5	0.25	0.17	5	0.25	0.13	5	
		2	0.61	0.11	5	0.60	0.12	5	0.26	0.04	5	0.02	0.03	5	0.61	0.11	5	0.64	0.07	5	0.33	0.16	5	0.21	0.14	5		
		3	0.02	0.02	5	0.03	0.02	5	0.07	0.05	5	0.00	0.00	5	0.08	0.09	5	0.06	0.04	5	0.03	0.03	5	0.00	0.01	5		
	<i>Campanula</i>	rotundifolia	O	1	0.00	0.01	5	0.16	0.08	5	0.56	0.14	5	0.52	0.12	3	0.25	0.37	5	0.52	0.18	5	0.74	0.12	5	0.63	0.07	4
			2	0.00	0.00	5	0.00	0.00	5	0.00	0.00	5	0.01	0.02	3	0.03	0.04	5	0.04	0.08	5	0.07	0.02	5	0.08	0.08	4	
			3	0.06	0.04	5	0.01	0.02	5	0.03	0.03	5	0.00	0.00	3	0.20	0.18	5	0.02	0.03	5	0.01	0.01	5	0.00	0.00	4	
trachelium		F	1	0.00	0.00	5	0.00	0.00	5	0.04	0.02	5	0.16	0.04	4	0.00	0.00	5	0.00	0.00	5	0.22	0.11	5	0.21	0.09	5	
		2	0.00	0.00	5	0.08	0.05	5	0.00	0.00	5	0.04	0.01	4	0.00	0.00	5	0.00	0.00	5	0.03	0.03	5	0.02	0.04	5		
		3	0.32	0.08	5	0.25	0.08	5	0.23	0.06	5	0.26	0.03	4	0.58	0.13	5	0.40	0.12	5	0.29	0.17	5	0.19	0.16	5		
<i>Carex</i>		lepidocarpa	O	1	0.00	0.00	5	0.00	0.00	5	0.00	0.00	5	0.00	0.00	5	0.00	0.00	5	0.00	0.00	4	0.00	0.00	5	0.02	0.03	5
			2	0.00	0.00	5	0.00	0.00	5	0.00	0.00	5	0.00	0.00	5	0.00	0.00	5	0.00	0.00	4	0.00	0.00	5	0.01	0.03	5	
			3	0.02	0.03	5	0.38	0.14	5	0.23	0.15	5	0.40	0.05	5	0.00	0.00	5	0.18	0.08	4	0.18	0.13	5	0.23	0.12	5	
	sylvatica	F	1	0.00	0.00	5	0.00	0.00	4	0.00	0.00	5	0.00	0.00	5	0.00	0.00	5	0.00	0.00	5	0.00	0.00	5	0.00	0.00	5	
		2	0.00	0.00	5	0.00	0.00	4	0.00	0.00	5	0.00	0.00	5	0.03	0.07	5	0.00	0.00	5	0.00	0.00	5	0.00	0.00	5		
		3	0.71	0.06	5	0.94	0.03	4	0.57	0.03	5	0.84	0.03	5	0.54	0.09	5	0.91	0.03	5	0.87	0.21	5	0.82	0.06	5		

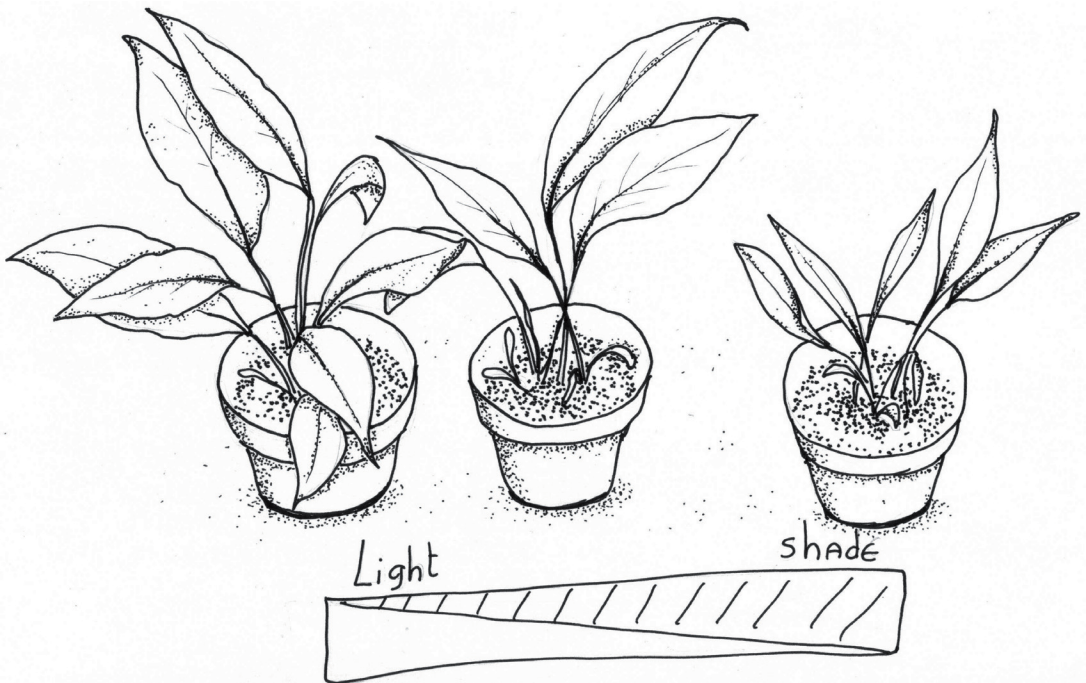
<i>Carex</i>	<i>onans</i>	O	1	0.00	0.00	5	0.00	0.00	5	0.00	0.00	5	0.00	0.00	5	0.11	0.05	5	0.55	0.08	5						
			2	0.00	0.00	5	0.00	0.00	5	0.00	0.00	5	0.00	0.00	5	0.00	0.00	5	0.00	0.00	5	0.06	0.06	5			
			3	0.01	0.02	5	0.87	0.04	5	0.20	0.09	5	0.82	0.03	5	0.30	0.14	5	0.88	0.09	5	0.74	0.07	5	0.28	0.05	5
	<i>remota</i>	F	1	0.00	0.00	5	0.00	0.00	5	0.00	0.00	5	0.00	0.00	5	0.00	0.00	5	0.00	0.00	5	0.02	0.04	4			
			2	0.00	0.00	5	0.00	0.00	5	0.00	0.00	5	0.00	0.00	5	0.00	0.00	5	0.00	0.00	5	0.00	0.00	5	0.05	0.07	4
			3	0.00	0.00	5	0.06	0.03	5	0.03	0.03	5	0.46	0.23	5	0.28	0.24	5	0.89	0.04	5	0.72	0.30	5	0.80	0.09	4
<i>Festuca</i>	<i>arundinacea</i>	O	1	0.01	0.01	5	0.41	0.05	5	0.81	0.07	5	0.81	0.06	5	0.08	0.09	5	0.32	0.18	5	0.86	0.04	5	0.80	0.09	5
			2	0.00	0.00	5	0.01	0.02	5	0.00	0.01	5	0.00	0.01	5	0.06	0.03	5	0.25	0.13	5	0.02	0.02	5	0.01	0.02	5
			3	0.79	0.05	5	0.37	0.07	5	0.01	0.02	5	0.02	0.03	5	0.70	0.13	5	0.13	0.09	5	0.01	0.02	5	0.01	0.03	5
	<i>altissima</i>	F	1	0.00	0.00	5	0.00	0.01	5	0.00	0.00	5	0.00	0.01	5	0.00	0.00	5	0.01	0.01	4	0.00	0.00	5	0.00	0.00	5
			2	0.00	0.01	5	0.00	0.01	5	0.00	0.00	5	0.02	0.04	5	0.00	0.00	5	0.01	0.01	4	0.00	0.00	5	0.00	0.01	5
			3	0.54	0.11	5	0.54	0.10	5	0.30	0.12	5	0.39	0.14	5	0.67	0.10	5	0.56	0.04	4	0.36	0.14	5	0.33	0.12	5
<i>Festuca</i>	<i>pratensis</i>	O	1	0.76	0.03	5	0.82	0.06	5	0.93	0.04	5	0.97	0.01	5	0.22	0.24	5	0.47	0.16	5	0.84	0.06	5	0.73	0.11	5
			2	0.10	0.03	5	0.01	0.02	5	0.01	0.01	5	0.00	0.00	5	0.23	0.13	5	0.09	0.10	5	0.02	0.02	5	0.08	0.06	5
			3	0.09	0.01	5	0.08	0.02	5	0.02	0.00	5	0.00	0.01	5	0.50	0.13	5	0.30	0.16	5	0.00	0.01	5	0.01	0.02	5
	<i>gigantea</i>	F	1	0.00	0.00	5	0.00	0.00	5	0.00	0.00	5	0.00	0.00	5	0.00	0.00	5	0.00	0.00	5	0.02	0.05	5	0.52	0.08	5
			2	0.00	0.01	5	0.02	0.02	5	0.00	0.00	5	0.16	0.09	5	0.01	0.02	5	0.00	0.00	5	0.05	0.04	5	0.19	0.06	5
			3	0.94	0.03	5	0.94	0.02	5	0.96	0.01	5	0.82	0.10	5	0.92	0.03	5	0.95	0.04	5	0.89	0.11	5	0.23	0.11	5
<i>Cicuta</i>	<i>rivale</i>	O	1	0.00	0.00	5	0.00	0.00	5	0.01	0.01	5	0.04	0.03	5	0.00	0.01	5	0.00	0.00	5	0.36	0.11	5	0.80	0.06	5
			2	0.00	0.00	5	0.00	0.00	5	0.00	0.00	5	0.00	0.00	5	0.00	0.00	5	0.03	0.07	5	0.00	0.00	5	0.00	0.00	5
			3	0.66	0.10	5	0.90	0.06	5	0.56	0.10	5	0.80	0.03	5	0.74	0.04	5	0.89	0.06	5	0.47	0.09	5	0.10	0.06	5
	<i>urbanum</i>	F	1	0.00	0.00	5	0.00	0.00	5	0.14	0.04	5	0.08	0.06	5	0.00	0.00	5	0.02	0.02	5	0.20	0.09	5	0.49	0.11	5
			2	0.00	0.01	5	0.02	0.02	5	0.00	0.00	5	0.16	0.09	5	0.01	0.02	5	0.00	0.00	5	0.05	0.04	5	0.19	0.06	5
			3	0.08	0.04	5	0.41	0.06	5	0.14	0.07	5	0.23	0.08	5	0.61	0.10	5	0.66	0.08	5	0.52	0.09	5	0.16	0.03	5
<i>Hordeum</i>	<i>murinum</i>	O	1	0.93	0.04	5	0.91	0.02	5	0.96	0.05	5	0.89	0.07	5	0.47	0.06	5	0.39	0.06	5	0.69	0.04	5	0.57	0.12	5
			2	0.01	0.02	5	0.01	0.01	5	0.01	0.02	5	0.00	0.00	5	0.44	0.08	5	0.29	0.08	5	0.19	0.06	5	0.28	0.10	5
			3	0.00	0.00	5	0.00	0.00	5	0.00	0.00	5	0.00	0.00	5	0.00	0.00	5	0.00	0.00	5	0.01	0.01	5	0.01	0.01	5
<i>Hordeolymus</i>	<i>europaeus</i>	F	1	0.00	0.00	5	0.08	0.04	5	0.14	0.06	5	0.17	0.13	4	0.00	0.00	5	0.00	0.00	5	0.00	0.00	5	0.03	0.05	5
			2	0.17	0.07	5	0.00	0.01	5	0.18	0.06	5	0.05	0.04	4	0.32	0.12	5	0.26	0.10	5	0.33	0.12	5	0.27	0.13	5
			3	0.02	0.02	5	0.02	0.02	5	0.01	0.03	5	0.03	0.04	4	0.05	0.04	5	0.04	0.09	5	0.02	0.03	5	0.02	0.02	5
<i>Hypericum</i>	<i>perforatum</i>	O	1	0.00	0.00	5	0.00	0.00	5	0.05	0.02	5	0.20	0.06	5	0.02	0.04	5	0.40	0.29	5	0.35	0.42	5	0.32	0.39	4
			2	0.00	0.00	5	0.00	0.00	5	0.00	0.00	5	0.00	0.00	5	0.01	0.02	5	0.14	0.23	5	0.00	0.01	5	0.00	0.00	4
			3	0.07	0.09	5	0.24	0.06	5	0.04	0.08	5	0.14	0.09	5	0.56	0.20	5	0.26	0.19	5	0.51	0.35	5	0.47	0.32	4
	<i>hirsutum</i>	F	1	0.00	0.00	5	0.00	0.00	5	0.00	0.00	5	0.00	0.00	5	0.00	0.00	5	0.00	0.00	5	0.07	0.13	5	0.23	0.31	5
			2	0.00	0.00	5	0.00	0.00	5	0.00	0.00	5	0.00	0.00	5	0.00	0.00	5	0.00	0.00	5	0.00	0.01	5	0.08	0.12	5
			3	0.00	0.00	5	0.68	0.13	5	0.48	0.30	5	0.83	0.07	5	0.26	0.20	5	0.34	0.24	5	0.77	0.09	5	0.38	0.41	5
<i>Poa</i>	<i>pratensis</i>	O	1	0.54	0.08	5	0.89	0.04	5	0.66	0.03	5	0.72	0.07	3	0.32	0.07	5	0.32	0.11	5	0.63	0.10	5	0.76	0.08	5
			2	0.07	0.04	5	0.02	0.03	5	0.00	0.01	5	0.00	0.00	3	0.33	0.09	5	0.18	0.09	5	0.06	0.07	5	0.02	0.03	5
			3	0.07	0.04	5	0.00	0.01	5	0.01	0.01	5	0.01	0.02	3	0.15	0.09	5	0.14	0.05	5	0.13	0.08	5	0.02	0.02	5

<i>nemoralis</i>	F	1	0.10	0.03	5	0.72	0.08	5	0.24	0.09	5	0.37	0.07	4	0.04	0.06	5	0.15	0.11	5	0.49	0.21	5	0.59	0.32	5
		2	0.01	0.01	5	0.01	0.01	5	0.00	0.00	5	0.00	0.00	4	0.07	0.04	5	0.06	0.02	5	0.05	0.02	5	0.01	0.02	5
		3	0.27	0.07	5	0.06	0.02	5	0.03	0.03	5	0.18	0.04	4	0.59	0.09	5	0.44	0.16	5	0.10	0.08	5	0.04	0.03	5
<i>jamosa</i>	O	1	0.00	0.00	5	0.00	0.00	5	0.00	0.00	5	0.00	0.00	5	0.00	0.00	5	0.00	0.00	5	0.00	0.01	5	0.10	0.10	5
		2	0.00	0.00	5	0.00	0.00	5	0.00	0.00	5	0.00	0.00	5	0.00	0.00	5	0.00	0.00	5	0.01	0.01	5	0.01	0.01	5
		3	0.00	0.00	5	0.01	0.02	5	0.01	0.01	5	0.00	0.01	5	0.09	0.09	5	0.45	0.29	5	0.72	0.14	5	0.29	0.40	5
<i>elatior</i>	F	1	0.00	0.01	5	0.10	0.02	5	0.00	0.01	5	0.00	0.00	5	0.00	0.00	5	0.00	0.00	5	0.02	0.02	5	0.02	0.02	5
		2	0.06	0.03	5	0.11	0.04	5	0.19	0.06	5	0.11	0.08	5	0.09	0.13	5	0.22	0.10	5	0.02	0.01	5	0.08	0.11	5
		3	0.08	0.02	5	0.12	0.06	5	0.10	0.03	5	0.10	0.05	5	0.10	0.06	5	0.11	0.05	5	0.58	0.15	5	0.34	0.14	5
<i>obtusifolius</i>	O	1	0.02	0.02	5	0.10	0.10	5	0.83	0.06	5	0.81	0.04	5	0.01	0.03	5	0.78	0.07	5	0.72	0.12	5	0.89	0.08	5
		2	0.01	0.02	5	0.02	0.04	5	0.00	0.01	5	0.08	0.02	5	0.41	0.17	5	0.05	0.03	5	0.08	0.07	5	0.02	0.04	5
		3	0.45	0.06	5	0.37	0.11	5	0.12	0.06	5	0.11	0.02	5	0.42	0.16	5	0.10	0.11	5	0.10	0.12	5	0.00	0.00	5
<i>sanguineus</i>	F	1	0.01	0.01	5	0.12	0.07	5	0.09	0.02	5	0.26	0.06	5	0.02	0.02	5	0.80	0.15	5	0.28	0.19	5	0.93	0.03	5
		2	0.11	0.04	5	0.00	0.00	5	0.01	0.01	5	0.00	0.00	5	0.35	0.09	5	0.08	0.10	5	0.04	0.05	5	0.00	0.01	5
		3	0.30	0.07	5	0.54	0.12	5	0.32	0.04	5	0.39	0.04	5	0.58	0.11	5	0.10	0.10	5	0.52	0.17	5	0.02	0.04	5
<i>latifolia</i>	O	1	0.17	0.07	5	0.44	0.07	5	0.44	0.08	5	0.82	0.06	5	0.12	0.05	5	0.69	0.09	5	0.61	0.07	5	0.77	0.13	5
		2	0.06	0.03	5	0.05	0.03	5	0.08	0.04	5	0.02	0.01	5	0.00	0.00	5	0.02	0.04	5	0.02	0.01	5	0.00	0.01	5
		3	0.22	0.12	5	0.07	0.06	5	0.10	0.07	5	0.03	0.03	5	0.17	0.03	5	0.07	0.06	5	0.05	0.04	5	0.03	0.04	5
<i>dioica</i>	F	1	0.00	0.00	5	0.06	0.04	5	0.06	0.03	5	0.24	0.02	5	0.01	0.01	5	0.26	0.12	5	0.50	0.10	5	0.61	0.21	5
		2	0.11	0.06	5	0.65	0.07	5	0.30	0.10	5	0.65	0.07	5	0.02	0.02	5	0.06	0.03	5	0.08	0.03	5	0.03	0.02	5
		3	0.80	0.07	5	0.21	0.03	5	0.49	0.08	5	0.09	0.05	5	0.71	0.09	5	0.29	0.06	5	0.24	0.07	5	0.20	0.12	5
<i>graminea</i>	O	1	0.09	0.04	5	0.28	0.06	5	0.46	0.08	5	0.75	0.07	2	0.16	0.25	5	0.46	0.28	5	0.26	0.30	5	0.42	0.25	5
		2	0.03	0.03	5	0.00	0.00	5	0.02	0.04	5	0.09	0.04	2	0.07	0.05	5	0.09	0.07	5	0.06	0.01	5	0.10	0.07	5
		3	0.02	0.03	5	0.06	0.06	5	0.02	0.02	5	0.00	0.00	2	0.16	0.11	5	0.05	0.10	5	0.06	0.06	5	0.04	0.05	5
<i>holostea</i>	F	1	0.07	0.04	5	0.10	0.06	5	0.00	0.00	5	0.13	0.03	5	0.00	0.00	5	0.00	0.00	5	0.00	0.00	5	0.01	0.02	5
		2	0.15	0.06	5	0.14	0.10	5	0.01	0.03	5	0.26	0.14	5	0.05	0.06	5	0.11	0.08	5	0.11	0.18	5	0.37	0.15	5
		3	0.12	0.06	5	0.14	0.14	5	0.06	0.05	5	0.07	0.05	5	0.25	0.11	5	0.17	0.04	5	0.10	0.08	5	0.01	0.03	5
<i>media</i>	O	1	0.13	0.09	5	0.13	0.03	4	0.21	0.06	5	0.42	0.11	2	0.06	0.08	5	0.16	0.07	5	0.13	0.04	5	0.21	0.04	5
		2	0.01	0.01	5	0.00	0.00	4	0.00	0.01	5	0.04	0.06	2	0.02	0.03	5	0.06	0.05	5	0.04	0.03	5	0.10	0.08	5
		3	0.02	0.02	5	0.01	0.01	4	0.04	0.02	5	0.01	0.01	2	0.04	0.04	5	0.05	0.03	5	0.02	0.02	5	0.00	0.00	5
<i>nemorum</i>	F	1	0.01	0.01	5	0.01	0.01	5	0.01	0.01	5	0.04	0.02	5	0.00	0.00	5	0.00	0.00	5	0.05	0.07	5	0.37	0.11	5
		2	0.09	0.06	5	0.27	0.04	5	0.01	0.01	5	0.27	0.09	5	0.01	0.02	5	0.00	0.01	5	0.06	0.06	5	0.07	0.07	5
		3	0.02	0.03	5	0.04	0.03	5	0.01	0.02	5	0.03	0.02	5	0.12	0.10	5	0.07	0.03	5	0.19	0.10	5	0.01	0.02	5
<i>urens</i>	O	1	0.00	0.00	5	0.00	0.00	5	0.09	0.05	5	0.26	0.09	4	0.00	0.00	5	0.00	0.00	5	0.00	0.00	5	0.00	0.00	5
		2	0.00	0.00	5	0.01	0.01	5	0.00	0.00	5	0.05	0.05	4	0.01	0.02	5	0.00	0.00	5	0.01	0.02	5	0.00	0.01	5
		3	0.12	0.08	5	0.23	0.09	5	0.13	0.03	5	0.20	0.03	4	0.04	0.04	5	0.13	0.12	5	0.05	0.03	5	0.00	0.01	5
<i>dioica</i>	F	1	0.00	0.01	5	0.06	0.06	5	0.22	0.12	5	0.52	0.09	3	0.00	0.00	5	0.00	0.01	5	0.20	0.06	5	0.24	0.18	5
		2	0.00	0.01	5	0.01	0.01	5	0.00	0.00	5	0.07	0.06	3	0.00	0.00	5	0.00	0.00	5	0.02	0.02	5	0.08	0.12	5
		3	0.24	0.19	5	0.33	0.11	5	0.11	0.06	5	0.12	0.11	3	0.19	0.10	5	0.41	0.28	5	0.30	0.13	5	0.26	0.19	5

Seedling phase strategies as a means of habitat specialization in herbaceous plants

Dirk-Jan ten Brink and Hans Henrik Bruun

II



Seedling phase strategies as a means of habitat specialization in herbaceous plants

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Aspects of the regeneration niche have been underexposed in studies of community assembly and plant distribution. We examined the adaptive association between seedling traits and habitat specialization. Two habitat contrasts were investigated, i.e. species specialized to forest vs. open habitats and to dry vs. wet habitats, across several evolutionary lineages of seed plants. We also tested whether the effects of shade and drought vary independently or if shade has an amplified effect on drought-stressed plants. Seedling response in terms of growth rate, height, slenderness, specific leaf area (SLA) and degree of elongation (longest internode, longest petiole or longest leaf sheath depending on species) to experimental light and watering treatments was assessed. We used a factorial design involving three light regimes and two watering frequencies. The open-shaded habitat contrast and the dry-wet habitat contrast were investigated using six and five pairs of congeneric species, respectively. The congeneric species pair design controlled for confounding effects of evolutionary history prior to habitat divergence. Seedling growth rate generally decreased with shade and reduced watering frequency. Plant height was generally largest at intermediate light. Specialization to shaded habitats was associated with more conservative growth strategy, i.e. showing a more modest growth response to increasing light. Species from all habitats showed the highest relative elongation at intermediate light, except for the moist-habitat species which increased elongation with shade. Contrary to our expectations, species from dry habitats grew bigger than species from moist habitats in all treatments. SLA responded to the light treatment, but not to watering regime. The contrasting light and moisture conditions across habitats appear to not have selected for differences in SLA. We conclude that seedling phase strategies of resource allocation in temperate herbs contribute to their habitat specialization. Habitat-specific seedling strategies and trade-offs in response to resource availability and environmental conditions may be important to adaptive specialization.

Introduction

The assembly of plant communities are assembled by filtering at two levels, abiotic and biotic (Keddy 1992). Filtering acts through plant traits and allows species into habitats, or prevents their establishment. This leads to trait convergence at the between-habitat scale as a result of the general abiotic regime, whereas diversifying trait filters operate at the within-community scale (Grime

2006). At the between-habitat level, functional plants traits can be said to constitute the beta-niche (Pickett & Bazzaz 1978; Silvertown et al. 2006). Within habitats, plant interactions, e.g. resource competition, and others processes, are thought to determine local species coexistence based on alpha-niches (Pickett & Bazzaz 1978; Silvertown et al. 2006; Pierce et al. 2007). Filtering takes places at all plant life cycle stages, but the

importance to habitat specialization of traits and requirements at the regenerative stage has been underexposed in studies of community assembly and plant distribution. Aspects of the regeneration niche (Grubb 1977) like seed germination, seedling establishment and early seedling survival must be of primary significance to long-term survival. Germination cueing has been shown to be important in habitat specialization of temperate forest herbs (Schütz 1997, ten Brink and Bruun, Paper I).

In this paper we test the generality of the association between seedling traits and habitat specialization across several evolutionary lineages by using congeneric species pairs from contrasting habitats. We investigate whether divergence in habitat preference is accompanied by a simultaneous divergence in traits. The focus is on contrasting species pairs from shaded vs. open habitats, as well as dry vs. moist habitats. The congeneric species pair selection ensures phylogenetic independence because the pairs are independent replicates of evolutionary divergence in habitat specialization. Furthermore, potential confounding effects of unmeasured traits due to shared evolutionary history can be excluded (Rees 1995; Ackerly 1999).

Another objective of the study was to test for the combined effects of water and light availability, the two major axes of variation among the habitats in our study, in relation to the adaptation of species to contrasting habitats, which we tested within each of the habitats separately. Smith & Huston (1989) found an amplified effect of drought on shaded plants, driven by a trade-off in shade and drought tolerance. Greater allocation to shoots (reducing root to shoot ratio) and specifically to leaves and leaf area in response to shade or as adaptation to shaded habitats would compromise resistance to drought (Smith & Huston 1989). Others have found shade to alleviate drought effects and have a weaker impact with increasing shade because of a predominating limitation by light (Canham et al. 1996) or facilitation through decreasing air

temperature and decreasing transpiration in shade (Holmgren 2000). Holmgren et al. (1997) found the effects of drought to impact most at high and low light levels and to be weaker in intermediate shade. Finally, Sack and Grubb (2002) and Sack (2004) found the effects of shade and drought to vary independently of each other, and thus be additive.

Morphological shade avoidance responses include elongation of leaves, petioles and/or internodes (Smith & Whitelam 1997) resulting in more slender plants, i.e. having an increased height to biomass ratio. Shade tolerance in plants is, among other things, characterized by an increased leaf area per unit leaf mass (specific leaf area, SLA) (Lambers et al. 1998). Leaf area is also influenced by water availability, and drought stressed plant typically develop leaves with a reduced SLA (Field 1991). A lower SLA reduces the potential growth rate, thereby reducing biomass accumulation in plants (Lambers et al. 1998). Henry & Aarssen (1997) predicted shade avoidance and shade tolerance, although not always mutually exclusive, to be negatively correlated along light gradients. Shade avoidance strategies should be more common among species from early and mid-successional stages whereas shade tolerance should be encountered among late successional species.

To test the adaptive habitat specialization of seedlings and their response to combined shade and drought stress, we performed a greenhouse experiment using several congeneric pairs of herbaceous vascular plants from contrasting habitats (open and shaded as well as dry and moist). We varied light availability by manipulating photosynthetically active radiation (PAR) and red to far-red ratio (R:FR) as well as watering frequency. Besides testing the before-mentioned hypotheses on the combined effects of drought and shade, we address the following hypotheses: Shade-adapted plants, as compared to open-habitat plants, 1) are less affected in growth rate with decreasing light, 2) exhibit a weaker shade avoidance response and 3) have a greater SLA across all light levels. We

Table 1. Congeneric species pairs used in the study and their respective plant traits analysed for their elongation response.

Shaded-habitat species	Elongation measure	Open-habitat species	Elongation measure
<i>Bromus hordeaceus</i>	internode	<i>Bromus benekenii</i>	internode
<i>Carex ovalis</i>	leaf-sheath	<i>Carex sylvatica</i>	leaf-sheath
<i>Festuca arundinacea</i>	leaf-sheath	<i>Festuca gigantea</i>	leaf-sheath
<i>Geum rivale</i>	petiole	<i>Geum urbanum</i>	petiole
<i>Rumex crispus</i>	petiole	<i>Rumex sanguineus</i>	petiole
<i>Silene latifolia</i>	petiole	<i>Silene dioica</i>	petiole
Dry-habitat species	Elongation measure	Moist-habitat species	Elongation measure
<i>Achillea millefolium</i>	petiole	<i>Achillea ptarmica</i>	internode
<i>Agrostis capillaris</i>	internode	<i>Agrostis stolonifera</i>	internode
<i>Carex ovalis</i>	leaf-sheath	<i>Carex lepidocarpa</i>	leaf-sheath
<i>Geum urbanum</i>	petiole	<i>Geum rivale</i>	petiole
<i>Rumex crispus</i>	petiole	<i>Rumex hydrolapathum</i>	petiole

also predict seedling mortality to increase with decreasing light and decreasing watering frequency and to be lower in shaded-habitat species than for open-habitat species and lower for among dry-habitat species than for moist-habitat species. Finally, we hypothesize specialization to dry habitats to be associated with a smaller growth reduction in response to drought than species from moist habitats due to higher water use efficiency and lower SLA; and we hypothesize growth rate to be lower for plant species from dry habitats than for species from moist habitats.

Methods

Species and seed selection

We selected 18 herbaceous species to form 11 congeneric species pairs with contrasting habitat preference (Table 1). The shaded-open habitat

contrast was represented by six species pairs and the dry-moist habitat contrast by five species pairs. Two single species and one species pair were used in both contrasts.

In the shaded-open habitat contrast, species were carefully selected as shaded or open-habitat species if they predominantly occur in habitat with or without a tree canopy, respectively. A similar selection criterion for was used for the dry-moist habitat contrast, where by the distinction was made between well-drained vs. continuously moist habitats.

The experiment was performed with seeds from previous collections (2004-2005) and additionally some species were purchased from commercial seed providers. During seed collection, freshly matured seeds were collected from various locations in southernmost Sweden. Seeds were obtained from several plants of a single population per species. Collected seeds were air-dried at room temperature and stored in paper bags at room

temperature until further use.

Experimental design and conditions

The experiment was performed during May and June 2006 in a greenhouse, where temperatures gradually increased from 25 to 35°C (daytime) and 13 to 16°C (night) during the experiment. The greenhouse was equipped with photosynthetically active radiation (PAR) at midday outside the greenhouse varied from about 170 $\mu\text{mol m}^{-2} \text{s}^{-1}$ on an overcast day, to about 1450 $\mu\text{mol m}^{-2} \text{s}^{-1}$ on a cloudless, sunny day. The ambient light climate in the greenhouse was less variable due to the automatic blinds which avoided excess radiation and was about 150-250 $\mu\text{mol m}^{-2} \text{s}^{-1}$ at midday, depending on the weather conditions, and the red to far red ratio (R:FR) was 1.15.

The seedling experiment was performed in a full factorial design with a watering treatment (low and high frequency of watering) and a light treatment (low, intermediate and high light). Seedlings were placed on two adjacent elongated benches with one watering treatment each. The levels of the light treatment were replicated on each bench. To make the light treatments, seedlings were placed under frames covered with different plastic films, approximately 40 cm above the benches. The high light treatment had a transparent plastic film, which reduced ambient greenhouse PAR by 25% and did not affect the R:FR ratio. In the intermediate light treatment, frames were covered with green plastic film (#138 Lee filters, Andover, UK) which reduced ambient greenhouse PAR to 56% and the R:FR ratio to 0.65. The low light treatment was applied using another green plastic film (#122 Lee filters, Andover, UK), reducing ambient greenhouse PAR to 30% and the R:FR ratio to 0.21.

Seeds of species known to need a period of chilling to relax seed dormancy (ten Brink and Bruun, Paper I) were subjected to a cold stratification treatment for 11 weeks. In May 2006, seeds were germinated in Petri dishes on moist

filter paper. Five seedlings in the cotyledon stage were transplanted within 2 days after germination into 9 cm pots filled with a nutrient enriched peat soil, equally spaced from each other and the sides of the pot. Seedlings of all species were transplanted into the pots within three days of each other (*Geum rivale* one week later because of slightly later seed germination). Five replicate pots per species per treatment combination were used. The pots were randomly placed beneath their respective light treatments with sufficient distance among pots to prevent interaction between individuals from different pots. The pots were regularly relocated. The pots were watered every second day (high frequency) or every 6-9 days (low frequency). At each watering event, the bench was filled with one cm water and pots were allowed to absorb water through their drainage holes for 30 minutes after which excess water was drained off from the bench.

After 34 days, the seedlings were harvested. Seedling mortality was recorded. On all individuals, total height was measured as well as longest internode, longest petiole or longest leaf sheath depending on the species (Table 1). A representative sample of 2-5 fully expanded leaves per pot from different individuals was collected and scanned on a flatbed scanner. Plant material was dried at 40°C until constant weight. Seedling dry weight was determined and the SLA was calculated from the dry weight and the surface area of the sampled leaves. SLA was averaged for each pot. Due to technical difficulties, height measurements are missing for *Rumex* species and SLA values are lacking for *Achillea* and *Rumex* species. Biomass at harvest is used as an estimate of growth rate, since all plants as newly germinated seedlings in the cotyledon stage.

We placed control pots, filled with soil but without plants, to check for water loss as result of evaporation. The infrequent watering regime reduced water content in the control pots to ~40 % before next watering as compared to the water content immediately after watering. In the frequent

Table 2. Mean seedling mortality values in percentages per habitat and treatment.

Light	Watering	Habitat			
		Shaded	Open	Dry	Moist
High	infrequent	6.0	4.0	6.4	2.4
	frequent	6.0	1.3	4.0	5.6
Intermediate	infrequent	10.0	4.0	4.8	8.8
	frequent	6.0	5.3	8.8	4.8
Low	infrequent	10.0	6.0	12.0	5.6
	frequent	6.0	6.7	7.2	3.2

watering regime the water content was reduced to only ~85%. The rate of desiccation in pots with seedlings depended on plant species and biomass.

Thermometers were placed at several positions underneath the plastic shading films. There was minimal variation in temperature between the different light treatments.

Data analysis

The nature of the watering treatment (each level was bound to a greenhouse bench) together with space and resource limitation due to the large number of species and replicate seedlings were constraining the statistical analysis. The levels of the watering treatment were not replicated in space. However, the spatial configuration of two adjacent elongated benches, combined with the uniform light and temperature conditions in the greenhouse did not allow for variation between the benches other than the large difference between the watering regimes which we imposed on the seedlings.

Factorial analysis of variance (ANOVA) was used to test for the effects of watering, light and habitat type on height, biomass, SLA and relative elongation measure (internode, petiole, or sheath length). The main effects of habitat, light and watering regime on mortality was analysed using non-parametric tests, as the data were not normally distributed. Relative elongation for each treatment

combination for each species was calculated as the respective internode, petiole or sheath length (see Table 1) standardized to (divided by) the mean value in the high light/frequent watering treatment. Analyses were performed on the shaded-open habitat contrast and the dry-moist habitat contrast separately, as well as for each habitat-group separately. Genus was treated as a random factor in all analyses. In the analysis of mortality and SLA, each pot was considered a replicate, whereas in the other analyses the five seedlings were treated as replicates within pot, which was then used as a random factor nested within each combination of the other factors. SLA, biomass, and height values were log-transformed in order to get normal-distributed data. In addition, the log-transformation assured phylogenetic independence, because it removed the correlations between the pair mean and the pair difference which otherwise would have resulted in non-independence between the congeneric species pairs (Freckleton 2000).

Results

Seedling mortality

Seedling mortality varied between treatments and habitats. (Table 2). In both habitat contrasts, mortality tended to be higher with decreasing

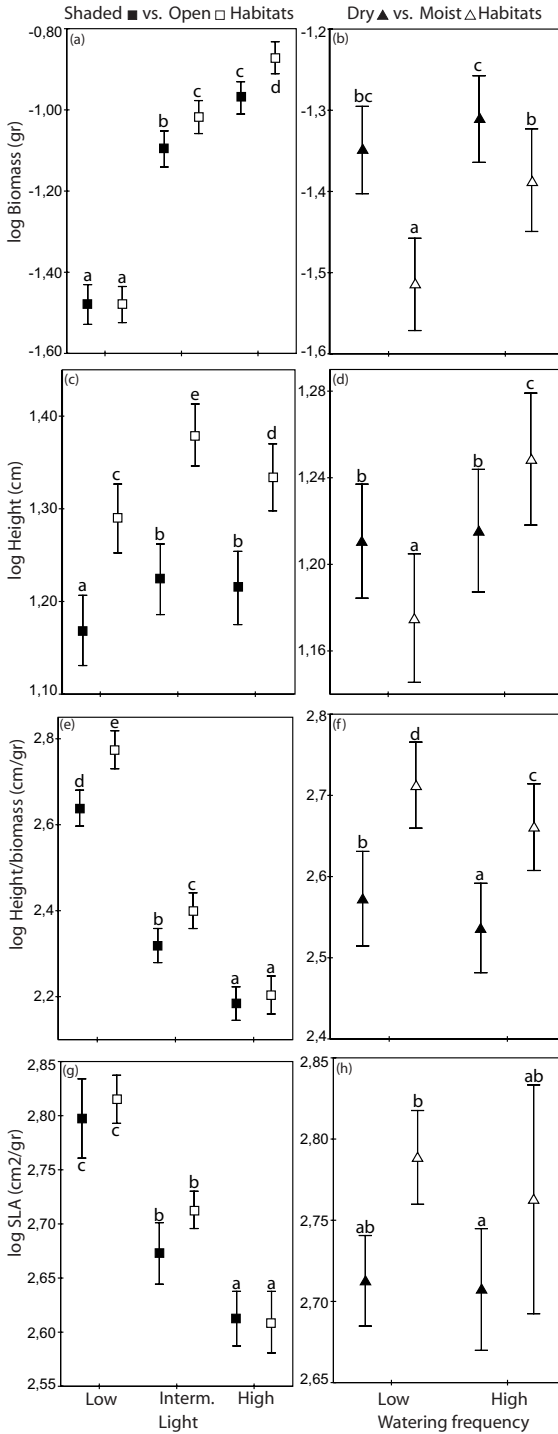


Figure 1. Log-transformed values of growth (biomass), height, slenderness (height/biomass) and SLA of species from shaded vs. open habitats (left) and dry vs. moist habitats. Error bars show 95% confidence intervals. Response variables in the open-shaded habitat contrast are plotted against the light treatment and for the dry-moist habitat contrast against the watering frequency. Different letters indicate significant differences between treatment-habitat combinations ($p < 0.05$).

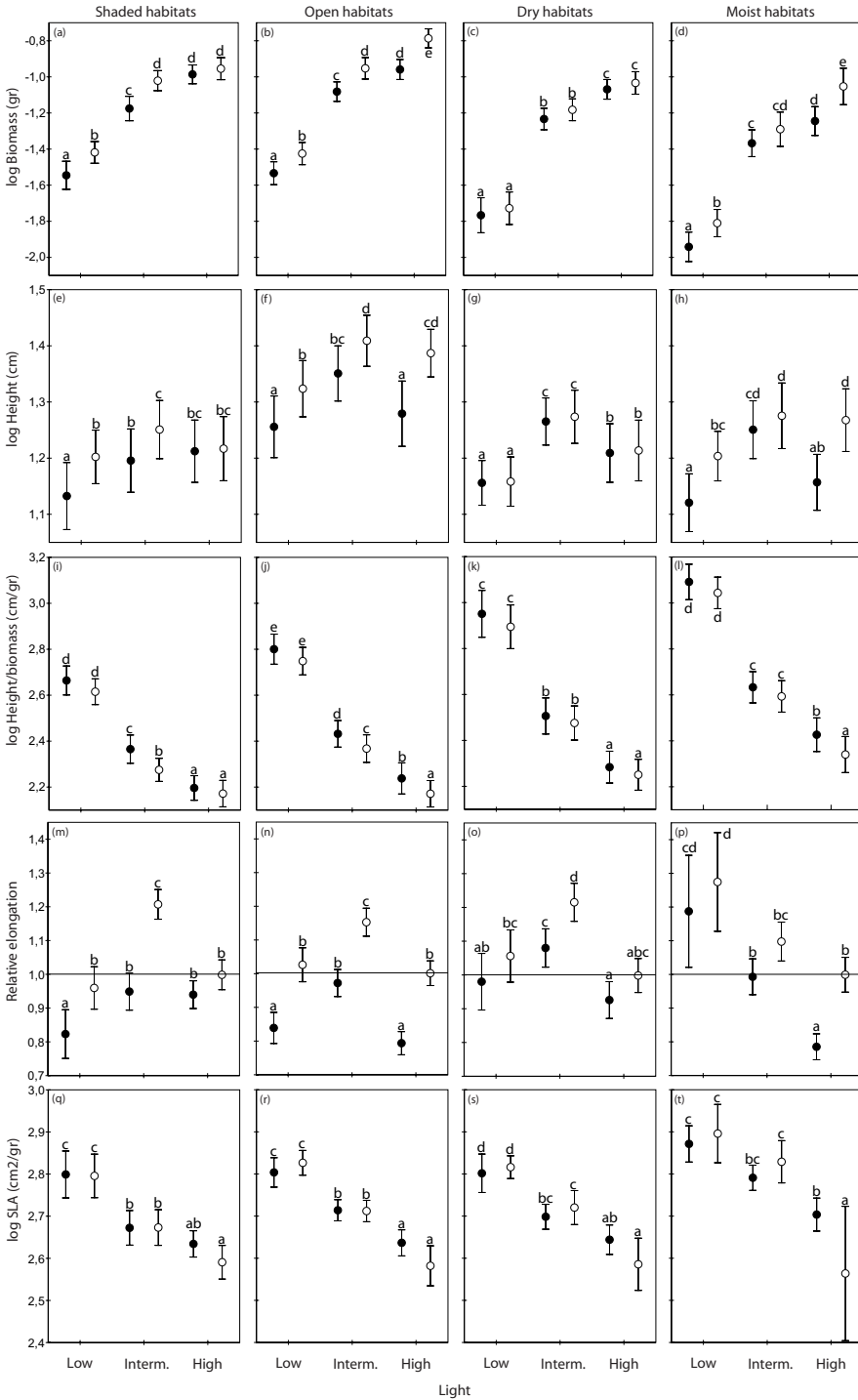


Figure 2. The combined effects of light and watering frequency (filled circles: low frequency; open circles: high frequency) on growth (biomass), height, slenderness (height/biomass) and SLA response of species from shaded, open, dry and moist habitats. Error bars show 95% confidence intervals. All variables except relative elongation are log-transformed. Different letters indicate significant differences between treatment combinations ($p < 0.05$). Note that relative elongation is a ratio (see main text more an explanation), so the x-axis of each graph for relative elongation is independent of the x-axis of the other graphs.

light and decreasing watering frequency, but these differences were not significant (statistics not shown). Species from shaded habitats showed significantly higher mortality than species from open habitats, but no difference was found between species from dry and moist habitats.

Growth rate (biomass)

Growth was strongly reduced with decreasing light (Table 3, upper panel). Species from open habitats performed better than shaded-habitat species at intermediate and high light (Fig 1a). Dry-habitat species performed better than moist-adapted species across all light levels. Growth was generally reduced with reduced watering frequency (Table 3, upper panel) but not much for the dry-habitat species (Fig 1b). Species from dry habitats had a higher growth rate than those from moist habitats. Open-habitat species performed better than shade-adapted species at both watering frequencies. There was no significant interaction between watering frequency and light on growth in any of the habitats. Reduced watering frequency however, significantly reduced growth in all but the dry-habitat species (Table 3, lower panel, Fig 2a-d).

Height

Plant height varied with light (Table 3, upper panel) and mean height was largest at intermediate light. Open-habitat species grew taller than shade-habitat species (Fig 1c), but no difference in height between species from moist and dry habitats was found. Height was not affected by watering frequency in dry-habitat species, but did increase with increasing watering frequency for moist-habitat species (Fig 1d) as well as the open- and shaded-habitat species. The interaction between habitat (dry-moist) and watering frequency was significant (Table 3, upper panel). Low frequency watering generally reduced plant height, except for the dry-habitat species. The interaction between

watering frequency and light was not significant (Table 3, lower panel, Fig 2e-h).

Slenderness

Slenderness (height corrected for biomass) strongly increased with decreasing light (Table 3, upper panel). The interaction between slenderness and habitat (open-shaded) was significant; at high light, slenderness did not differ between seedlings from the open and shaded habitats, but at intermediate and low light, the shaded-habitat seedlings were less slender than the open-habitat seedlings (Fig. 1e). Moist-habitat seedlings were more slender than dry-habitat seedlings across all light levels. Reduced watering frequency increased slenderness in both habitat comparisons (Table 3, upper panel, Fig 1f). Infrequent watering led to an increase in slenderness but not at all light levels and not for the dry-habitat species (Table 3; lower panel, Fig 2i-l). The interaction between watering frequency and light was not significant.

Elongation

Because of the way relative elongation was calculated (see methods), only analyses for the species within each habitat separately could be performed. Relative elongation was significantly affected by both light and watering frequency (Table 3, lower panel). Reduced watering frequency generally reduced the ability of seedlings to respond to shade (Fig 2 m-p). The intermediate light level elicited the largest response in elongation, analogue to the plant height response, except for the moist-habitat seedlings (Fig 2m-p).

SLA

SLA increased with decreasing light (Table 3, upper panel). Species from open and shaded habitats did not differ in SLA across the light levels (Fig 1g). Species from moist habitats had a higher SLA than dry-habitat species over all light levels. Watering

Table 3. Results of ANOVAs for growth (biomass), height, slenderness (height corrected for biomass), SLA and relative elongation. All variables except relative elongation are log transformed prior to analysis. The upper panel shows the analysis for the habitat-treatment analysis for both habitat contrasts. The lower panel shows the analysis where the treatment interaction is investigated per habitat separately. Mean squares (MS) and degrees of freedom (d.f.) are reported. Because of the unbalanced design due to seedling mortality, F-ratios for each independent variable were obtained using computed error terms (MS and d.f.) using Satterthwaite's method. Variables with values in bold are significant ($p < 0.05$).

Habitat contrast		Biomass		Height		Slenderness		SLA	
		MS	d.f.	MS	d.f.	MS	d.f.	MS	d.f.
Shade/ Open	Habitat	1.119	1	5.472	1	2.699	1	0.024	1
	Water	3.808	1	1.299	1	0.952	1	0.013	1
	Light	30.762	2	0.685	2	32.609	2	0.948	2
	Pot	0.126	343	0.035	284	0.080	284	-	-
	Genus	11.540	5	22.186	4	21.735	4	0.093	4
	H x W	0.010	1	0.047	1	0.010	1	<0.001	1
	H x L	0.117	2	0.030	2	0.514	2	0.012	2
	W x L	0.145	2	0.010	2	0.019	2	0.024	2
	H x W x L	0.179	2	0.081	2	0.024	2	0.002	2
	Error MS	0.056	1095	0.012	1095	0.030	1095	0.008	282
Dry/ Moist	Habitat	1.078	1	0.014	1	3.993	1	0.190	1
	Water	1.507	1	0.444	1	0.389	1	0.012	1
	Light	34.274	2	1.032	2	42.887	2	0.742	2
	Pot	0.178	284	0.048	225	0.091	225	-	-
	Genus	21.126	4	14.065	3	36.936	3	0.118	2
	H x W	0.461	1	0.215	1	0.013	1	0.004	1
	H x L	0.012	2	0.015	2	0.007	2	0.026	2
	W x L	0.021	2	0.015	2	0.055	2	0.077	2
	H x W x L	0.046	2	0.063	2	0.026	2	0.011	2
	Error MS	0.050	875	0.012	875	0.024	875	0.012	165

Table 3. Continued

Habitat		Biomass		Height		Slenderness		SLA		Elongation	
		MS	d.f.	MS	d.f.	MS	d.f.	MS	d.f.	MS	d.f.
Shaded	Water	2.072	1	0.044	2	0.487	1	0.009	1	4.803	1
	Light	13.416	2	0.257	2	12.214	2	0.445	2	2.494	2
	Pot	0.106	169	0.031	140	0.048	140	-	-	0.186	169
	Genus	5.871	5	11.850	4	9.442	4	0.306	4	5.702	5
	W x L	0.189	2	0.044	2	0.035	2	0.007	2	0.572	2
	Error MS	0.070	534	0.012	534	0.034	534	0.030	140	0.039	653
Open	Water	1.740	1	0.942	1	0.466	1	0.006	1	7.455	1
	Light	17.508	2	0.463	2	21.097	2	0.520	2	2.230	2
	Pot	0.065	169	0.022	140	0.043	140	-	-	0.122	169
	Genus	8.519	5	10.854	4	14.880	4	0.069	4	1.451	5
	W x L	0.134	2	0.047	2	0.007	2	0.018	2	0.024	2
	Error MS	0.044	561	0.013	561	0.026	561	0.050	138	0.040	679
Dry	Water	0.149	1	0.020	1	0.073	1	0.002	1	1.174	1
	Light	16.576	2	0.569	2	20.134	2	0.282	2	2.282	2
	Pot	0.172	140	0.042	111	0.078	111	-	-	0.228	140
	Genus	7.013	4	6.309	3	21.249	3	0.051	2	6.133	4
	W x L	0.043	2	0.002	2	0.024	2	0.014	2	0.068	2
	Error MS	0.047	428	0.008	428	0.025	428	0.040	81	0.051	546
Moist	Water	1.840	1	0.656	1	0.379	1	0.015	1	3.067	1
	Light	17.730	2	0.473	2	22.865	2	0.495	2	6.524	2
	Pot	0.074	140	0.033	111	0.044	111	-	-	0.708	140
	Genus	18.203	4	8.468	3	18.108	3	0.384	2	16.837	4
	W x L	0.023	2	0.100	2	0.015	2	0.074	2	0.267	2
	Error MS	0.052	447	0.015	447	0.024	447	0.011	82	0.080	563

regime did not affect SLA in any of the habitat comparisons (Table 3, upper panel, Fig 1h). In all habitats, SLA was lowest at combined high light and frequent watering. The interaction between light and watering regime was significant in all habitats except for the shaded habitats (Table 3,

lower panel, Fig q-t).

Discussion

Seedling mortality was low in our experiment; the

only significant difference found was between open and shaded habitats, although mortality also tended to be higher with increasing drought and decreasing light. Moles & Westoby (2004) screened the literature and found herbivory, drought and fungal attack to be the major causes of seedling mortality in nature, whereas physical damages and competition with established vegetation and other seedlings were of minor importance. However, in our experiment, it seems likely that a shortage of resources led to competition and subsequent mortality of some seedlings, as the low mortality rates indicate that resource shortage, e.g. drought, was apparently not so severe to kill off large number of seedlings.

Are different plant strategies in the seedling phase underlying habitat specialization?

Species from open habitats grew faster than species from shaded habitats. The increase in growth rate with increasing light indicates that biomass production was limited by shade for all species. Our experiment also showed that open-habitat species increased growth with increased light relatively more than did shade-adapted species. These findings confirm the idea that shade-tolerant seedlings are adapted to conserve energy by growing slowly in order to secure long-term survival, rather than to maximize growth, which is the more successful strategy for species from less shade-stressed environments (Grime 2001). Shade-tolerant plants are adapted to efficiently harvest light under constant low irradiance by increasing net carbon fixation per unit leaf protein (Givnish 1988). This ability is, among other things, made up of thin leaves, which have a low internal self-shading, and a low light compensation point (Lieth & Ashton 1961; Boardman 1977; Givnish et al. 2004). Open-habitat species responded to decreasing light by increasing their height at the intermediate light level, despite having lower biomass. The resulting higher height-to-biomass ratio with increasing shade was relatively more increased in open-habitat species than among

shade-adapted species. This strategy of elongation increases plant performance only when the investment in vertical growth leads to increased light interception. Plants from open habitats perceive shade from neighbouring herbaceous vegetation, and may improve their light climate greatly by growing taller and overtopping neighbours. In contrast, for species from shaded habitats like forests, height increase does not improve light interception substantially.

Our hypothesis that shade-adapted species would have a higher SLA than open-habitat species could not be confirmed by the results. SLA did not differ between shaded- and open-habitat species, although shade-adapted species are often reported to have thinner leaves and thus a higher SLA (Wilson et al. 1999). High plasticity in SLA in response to varying light levels of species from both shaded and open habitats was reported already more than a century ago (Haberlandt 1884) and is also manifest among our study species, but the contrasting environments have apparently not selected for differences in mean SLA between these two groups.

The results confirm our hypothesis that dry-habitat species are less affected by drought than are moist-habitat species. In contrast, it was contrary to our expectations that dry-habitat species outperformed moist-habitat species in biomass production. We expected that species confined drought-prone habitats would adopt a more conservative growth strategy analogous to that of shade-adapted species (Grime 2001). The lower SLA of the dry-habitat species is an indicator of this conservative growth strategy, as SLA in general is correlated with growth rate (Westoby 1998; Lambers et al. 1998). Growth rate, however, is also a function of assimilation rate and dry matter content (Lambers et al. 1998). The lower SLA of dry-habitat species could thus also be attributed to higher water-use efficiency due to a smaller leaf surface reducing evapotranspiration.

Effect of light and watering on shade avoidance and shade tolerance

Shade avoidance was primarily influenced by light in all habitats, and was also manifest in the species from shaded habitats, despite the fact that elongation is generally not adaptive for forest species since they often do not compete for light with other herbaceous species on the forest floor, but are shaded by the tree canopy. Shaded-habitat species however, were shorter and more compact than the open-habitat species.

The costs of expressing shade avoidance are reduced water use efficiency due to a lower root to shoot ratio (Maliakal et al. 1999). Expressing shade avoidance traits could lead to an increased vulnerability to drought stress and the adaptive value of petiole and stem elongation is generally reduced when plants experience drought stress (Schmitt et al. 2003). Reduced watering limited shade-avoidance expression in species from both open and shaded habitats; relative elongation and plant height were lower. Dry-habitat species showed no difference in shade avoidance between low and high watering, except for relative elongation at intermediate light. This is probably due to the minimal fitness advantage of elongation in dry habitats, where increased elongation leads to increased water-loss.

In our study, SLA was greatly influenced by the light environment. SLA increased with decreasing light availability. An increased leaf area increases evapotranspiration, and a smaller increase in SLA with reducing light would be expected under the low watering regime, but this was not observed in our study.

Interactive effects of drought and shade on plant fitness

Reducing watering frequency had negative effects on growth and height, and increased slenderness, in all but the dry-habitat species (Table 3; lower panel, Fig 2). The absence of a significant interaction between the watering treatment and the light treatment on growth, plant height and slenderness

in all habitats (Table 3, lower panel), suggests that the effects of drought do not amplify the negative impact of shade on the species. However drought reduced growth and height significantly for the shaded-habitats plants at the low and intermediate light levels, but not at high light. This suggests a trade-off between drought and shade tolerance in species from shaded habitats resulting in amplified effects of drought at low light levels. Among open-habitat species and moist-habitat species, drought and shade appeared to impact growth and orthogonally, corresponding to the findings of Sack and Grubb (2002) and Sack (2004) that drought has a proportional effect on growth and height independent of the light level. A third variant was observed among the dry-habitat species, where watering frequency had no impact on growth and height across the light levels. This could be attributed to the stress tolerant strategy of dry-habitat species, which are adapted to grow slowly rather than utilizing higher water availability for increasing biomass accumulation. Facilitation by shade, which would hypothetically relieve evapotranspiration by reducing temperatures, did not vary with the shading treatments as the temperatures beneath the plastic shading films only differed marginally.

Although the watering regime strongly influenced the water availability in the soil, we had no direct control of the actual water availability in the pots as this was also influenced by the plant species and plant biomass in the pot. Pots with larger plants dried out faster than pots with smaller plants. When interpreting the results, it should further be noted that the 'high' light level in our treatment is high relative to the other levels, and similar to full day light as perceived by plants in nature on an overcast day. The ambient light level of 150-250 $\mu\text{mol m}^{-2} \text{s}^{-1}$ at midday, however, corresponds to normal greenhouse light conditions. A higher light intensity at the high light level could possibly have revealed patterns and differences between species from different habitats that now are not shown, like bigger differences in SLA with increasing light and possible more

pronounced trade-offs between shade and drought tolerance. Photoinhibition, which can occur when light exceeds the saturation point, did not occur in our study. Under field conditions, especially plants from shaded habitats would be affected by high light as their saturation point is low and the leaves are adapted to function under low light levels.

Species traits and habitat specialization

We conclude that the two focal habitat contrasts in our study have imposed divergence between species in growth related traits. Plants from contrasting habitats are differently affected and constrained by shortages of light and water, which, among other factors, contribute to their habitat specialization. Segregation of plants along gradients of light or water availability, however, is also influenced by other factors such as nutrient availability, competition, disturbance, pathogens and herbivory. Regeneration of plants, like seed germination has been shown to be very important for habitat specialization in forest herbs (Schütz 1997, ten Brink and Bruun (Paper I)). This study shows that seedling phase of species contribute to habitat specialization, and suggest that also other phases in the plant life cycle than the established phase are important in adaptive specialization.

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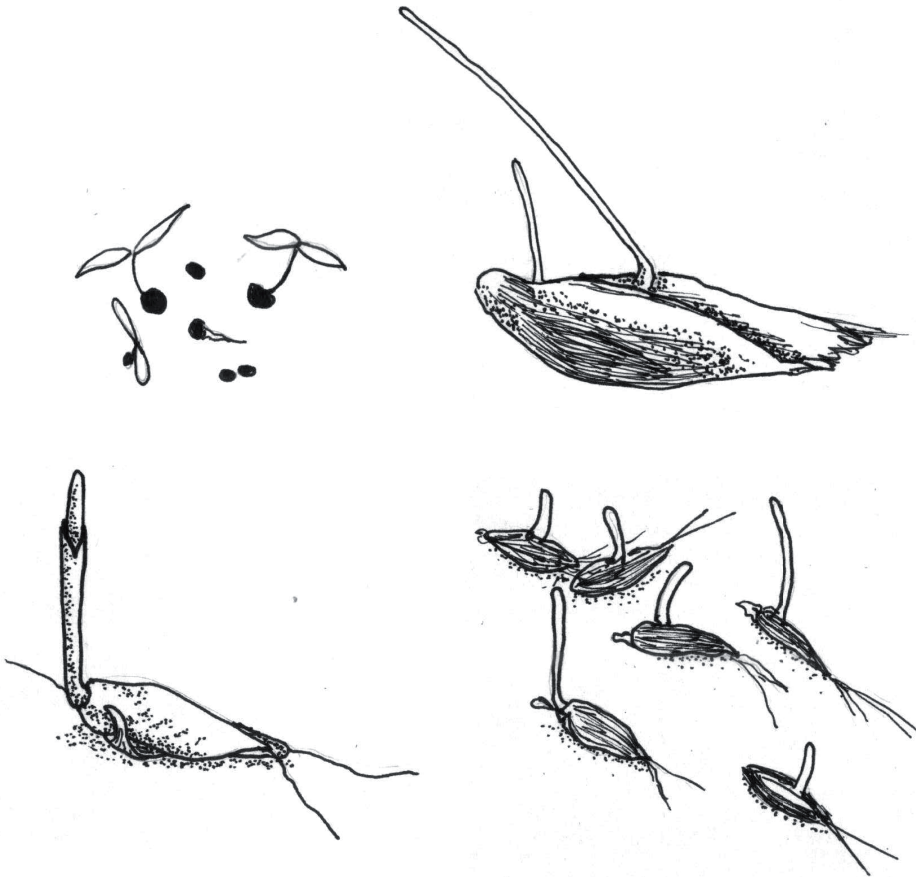
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The recruitment advantage of big seeds over small seeds is larger in shaded than in open vegetation

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III



The recruitment advantage of big seeds over small seeds is larger in shaded than in open vegetation

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Large seeds are assumed to have higher probability of successful recruitment than small seeds. This is because larger seeds give rise to larger seedlings and larger seedlings better withstand environmental hazards like deep shade and drought. Biotic and abiotic limitations to seedling growth and survival, and conversely availability of safe sites for recruitment, vary along environmental gradients and between habitat types. Thus, the value to plant species of possessing large seeds may differ between plant communities. We analyzed the relationship between seed mass and per-seed recruitment success (seedlings established per quantity seed produced) along an environmental gradient from open grassland to closed-canopy forest. We found that larger seeds have greater recruitment success relative to smaller seeds in all investigated communities. However, the recruitment success of large seeds relative to small seeds strongly increased from grassland and open forest to closed-canopy forest. Of the measured environmental variables, canopy closure most strongly explained this increase. This indicates a major direct effect of deep shade on seedling survival in natural plant communities. Additional explanatory power was associated with soil moisture. Litter cover, moss cover, and soil pH did not contribute to explaining the variation in relative recruitment success of larger seeds. Thus, the advantage of large seeds in terms of a recruitment success is pronounced in deeply shaded forest, but may be insignificant in open vegetation.

Nomenclature: Tutin et al. (1964-1980)

Introduction

In most plant communities, all suitable space is usually filled, and the reproductive capacity of the sum of all species in the community greatly exceeds what is necessary to successively populate empty space as it appears. Thus, only a small fraction of the seeds produced result in eventual recruitment of new individuals. At the same time, seed size of species inhabiting a plant community usually varies one or more orders of magnitude. These observations together open the question whether seed size matters to recruitment success

of species.

Over the last decade, a body of theory has built up attempting to explain the within-community variation in seed size as the result of an evolutionary game, known as the seed size/seed number trade-off (SSNT) model (Geritz, 1995; Rees & Westoby, 1997; Geritz, van der Meijden & Metz, 1999). The idea is that large-seeded and small-seeded species may coexist due to a trade-off between their abilities to reach and compete for vacant microsites. The theory has gained much attention and has generated numerous studies of the relationship between seed size and abundance

in plant communities (reviewed by Murray et al., 2002). The theory is based on two assumptions: (1) With reference to the negative relationship between seed mass and number of seeds per plant per year per unit reproductive effort (Harper, Lovell & Moore, 1970; Shipley & Dion, 1992; Moles et al., 2004), it is assumed that greater seed output enables mother plants to distribute their off-spring to more vacant microsites (Eriksson & Jakobsson, 1999; Bullock et al., 2002). This assumption has received scanty interest and empirical evidence is equivocal (Leishman, 2001; Jakobsson, Eriksson & Bruun, 2006). (2) There is a positive relationship between seed mass and seedling competitive ability (Geritz, 1995; Rees & Westoby, 1997). While there is little empirical evidence for the importance of seedling-seedling competition (Moles & Westoby, 2004b), many experimental studies have shown that larger-seeded species better survive environmental stress, e.g. drought, deep shade, deep litter or defoliation, including competitive suppression by established plant individuals (reviewed by Westoby et al., 2002). Thus, the positive linear relationship between seed mass and per-seed recruitment success predicted by SSNT model appears to be well-supported empirically (Gross, 1984; Burke & Grime, 1996; Jakobsson & Eriksson, 2000; Kidson & Westoby, 2000; Turnbull, Manley & Rees, 2005; but see counterexamples in Moles & Westoby, 2004a). It remains, however, largely untested to what degree the magnitude and importance of the positive relationship between seed mass and recruitment success is dependent on plant community context and local environment. It has sometimes been assumed that seed mass is of less importance to recruitment in open and disturbed vegetation (Salisbury, 1942; Gross, 1984; Westoby, Leishman & Lord, 1996; Jakobsson & Eriksson, 2000), but the question has never been investigated thoroughly.

If one assumes that, across species differing in seed mass, the same proportion of dispersed seeds will produce seedlings, and that all seedlings face the same hazards and are affected by these

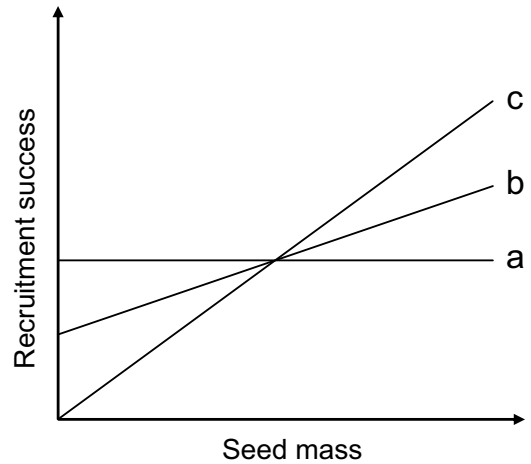


Figure 1. Relationship between recruitment success and seed mass in three hypothetical plant communities (a-c), differing in one or more environmental factors. In community a, the slope equals 0, i.e. recruitment success is independent of seed mass. In community b and c, slopes are greater than 0, i.e. larger seeds have higher per-capita recruitment success than smaller seeds. A greater slope is expected in environments in which larger-seeded species have a large recruitment success relative to smaller-seeded species. Hence, the seed-mass dependent recruitment percent is interacting with the environmental feature that differs between communities b and c.

in similar ways across community types, we should expect identical slopes of the seed-mass : recruitment-success relationship for all communities (Fig. 1). If, on the other hand, the relative recruitment success of large seeds changes along a gradient in environmental stress, such as shade, we should expect different slopes of the seed-mass : recruitment-success relationship. In the specific case of shade, we expect the steepest slope in the most deeply shaded environment (Fig. 1).

We aim at investigating if the importance of seed size for recruitment success changes along a gradient from open to shaded herbaceous plant communities (dry grassland to closed forest). We hypothesize that the relative recruitment success

of larger seeds relative to smaller increases towards forest communities. This could be expected to result from deeper shade and a thicker layer of leaf litter on the ground (Suding & Goldberg, 1999; Fröborg, 2001; Dzwonko & Gawroński, 2002). Conversely, small-scale disturbances in grassland may provide competition-free space for recruitment (Bullock et al., 1995; Eriksson et al., 2006), rendering seed size less important to recruitment success. We define the recruitment success as the number of seedlings divided by the number of seeds of the same species in the seed rain within the same delimited area.

We tested the hypothesis using data collected by Uno Perttula in southern Finland in 1934 (Perttula, 1941). An important reason why quantitative assessments of the relationship between seed size and recruitment success under field conditions are lacking, is that it requires knowledge of the seed rain and tedious counting of seedlings. Perttula's data are unique in offering both these variables over a range of plant communities.

Material and methods

Study area

The study of Perttula (1941) is a representative of a Finnish-Russian tradition for empirical studies of regeneration of herbaceous plant communities by surveying seedling emergence and survival (Bogdanovskaja-Guihéneuf, 1926; Linkola, 1932; Söyrinki, 1938; Rabotnov, 1950) long before the regeneration niche entered mainstream ecology (Grubb, 1977). The study was undertaken in southern Finland, some 110 km to the West of Helsinki and a few kilometres from the Baltic Sea, at the forest reserve Solböle (60° N, 23° E). The landscape is undulating, with granite outcrops and depressions with lakes and deposits from ice or sea. At the time of investigation, the vegetation cover consisted of a mosaic of forest, open meadows and rock outcrops with

shallow soils. Forests consisted mostly of mixed stands dominated to variable degree by *Picea abies*, *Betula* spp., *Populus tremula*, *Quercus robur*, and *Acer platanoides*. Less abundant was heath-forest, which was characterized by a field layer dominated by ericaceous dwarf-shrubs and with overstories dominated by *Picea abies*, *Pinus sylvestris*, and *Betula* spp. in mesic sites and by *Pinus sylvestris* in dry sites. Embedded in the forest matrix were mesic and dry meadows and rock outcrops. Mesic meadows were wooded meadows with a sparse canopy layer formed by *Pinus sylvestris* and deciduous trees like *Populus tremula* and an understory of *Juniperus communis*, *Corylus avellana* and other shrubs. Dry meadows had scattered *Juniperus communis*, while rock outcrops had no woody plant cover at all. Rock outcrop vegetation was characterized by almost no soil, but also dry meadow and *Calluna*-type dry heath-forest occurred on shallow soil over bedrock, whereas other communities were found on deeper soils. Perttula (1941) delimited 12 types of field-layer communities from different forest types and from adjacent meadows and rock outcrops (Table 1).

A number of environmental variables were measured or estimated in each habitat type. Top soil pH was measured on dried soil samples and soil moisture estimated subjectively on a 10-grade ordinal scale. Only minimum and maximum values were given in the original published source, so median values were calculated in addition. The following descriptors of vegetation structure were assessed: percentage cover of litter/thatch and moss layers and, where present, their depth; percentage cover of the field layer (ranges given in Table 1); light penetration (in percent of light above the tree canopy, Eder-Hecht-Graukeil photometer); and the canopy closure as an estimated percentage.

Data collection

In each plant community type, approximately ten 50 m² plots were placed, which totals 100 over all

Table 1. The 12 plant community types investigated and features of their environment.

No	Type	Pod- zol	Soil pH	Soil mois- ture	Litter cover (%)	Litter depth (cm)	Moss cover (%)	Moss depth (cm)	Field layer cover (%)	Light (%)	Canopy cover (%)
1	Dry heath-forest of Calluna type	1	3.9	2	100	1	10	4.5	15-50	45	20
2	Dry heath-forest of Vaccinium vitis-idaea type	1	3.9	2	0	2	65	4	20-60	28	43
3	Rock outcrop	0	4.8	2.5	0	0	15	2	5-80	100	0
4	Mesic meadow	0	5.1	4	100	2	25	2.5	65-100	75	10
5	Dry meadow	0	4.9	3	100	4	100	4	45-100	100	0
6	Mixed forest of Hepatica-Oxalis-type	0	5.6	4.5	82.5	2.5	20	2.5	45-100	16	50
7	Mesic heath-forest of Oxalis-Vaccinium myrtillus-type	1	4.4	4	100	2	80	4	10-70	15	65
8	Alder forest of fern-type	0	5.3	7	17.5	0	20	0	26-75	9	100
9	Mesic heath-forest of Vaccinium myrtillus-type	1	4.2	3	0	0	100	7	10-75	20	53
10	Mixed forest of Melica- Lathyrus type	0	6.0	3	100	4.5	0	0	30-98	30	100
11	Alder forest of Silene dioica type	0	5.3	4.5	0	0	20	0	45-100	37	58
12	Mixed forest of Oxalis- Maianthemum-type	0	5.2	4.5	100	1.5	45	5	30-80	3	90

12 communities. In each plot, four 1 m² subplots were placed for estimates of seed production and seedling recruitment. In each subplot, the number of sterile and fertile ramets was counted for each species on at least 5 occasions during the summer and autumn. The average number of fully developed undamaged seeds per fertile ramet was estimated from ramets collected in the plots, but not in close vicinity to the subplots, in order not to change the seed rain in these. The number of fertile ramets multiplied by the average number of seeds per ramet gave the seed productivity per unit area (seed yield in the terminology of Rabotnov 1950). In each subplot, seedlings were counted twice, in May/June and in August. For species with larger and more conspicuous seedlings, all seedlings were counted. For smaller species, seedlings were counted in two destructive 0.125 m² microplots per subplot on each occasion, and the average density for the whole subplot calculated. By assuming that all seeds produced by one species within a subplot were dispersed inside that subplot only, one may calculate the average recruitment success for each species in each community (Perttula, 1941, Tab. 10). Unfortunately, only the range, not the mean and spread, in seed production and seedlings per m² are given, and the original plot-wise data have been lost. Thus, the otherwise appropriate use of binomial regression, requiring both successes and failures, was not possible.

Data on seed size (seed mass [mg], throughout) were retrieved from various sources, chosen by geographical proximity, which in practice means that most records (75%) were from Scandinavia, and the rest mainly from Central Europe and the British Isles. Seed size data and their sources are listed in Appendix 1. For one species, *Calamagrostis purpurea*, which rarely sets seed, no data were available. Consequently, it was omitted from the analysis.

Data analysis

Differences between community types in average

seed mass and average seed number per ramet per year between plant communities (both log transformed) were investigated by one-way analysis of variance. The overall relationship between seed mass and seed number per ramet per year (both log transformed) was analysed using linear regression, and its interaction with plant community was investigated using analysis of covariance (with the factor community added first) as implemented in R version 2.5.1. The assumptions of normal distribution and homoscedasticity were justified for the log transformed data.

Recruitment success was calculated, for each species in each community separately, as the proportion of seedlings to total yield of fully developed, undamaged seeds (average over ten plots). Standardised Major Axis (SMA) regression was used to estimate the slope for each community type of the seed-mass : recruitment-success relationship. This model II regression is more appropriate than the usual model I regression for estimating the slope of a relationship when the independent variate is not controlled by an experimenter and has an associated measurement error and when the purpose is to identify the best line of fit, not to make predictions (Sokal & Rohlf, 1995; Warton et al., 2006). We used the R package SMATR ver. 2.1 (by Daniel Falster, David Warton and Ian Wright) to estimate slopes and test for between-community differences. Recruitment success was arcsine transformed (Sokal & Rohlf, 1995) and seed mass was log₁₀ transformed prior to analysis. The SMA procedure was as follows: an SMA slope common to both groups in a pair of community types was estimated using a likelihood ratio method following Warton & Weber (2002). The significance of this estimate was determined by testing for significant heterogeneity among slope estimates by permutation (Manly, 1997). After fixing the position of individual points along the estimated common slope, residuals were permuted among groups 999 times, with the common slope and test statistic recalculated after each iteration.

In order to investigate if differences in relative recruitment success of large seeds between community were attributable to differences in environment (Table 1), the SMA slopes (of the recruitment success to seed mass relationship) for communities were regressed on environmental variables using linear regression and statistical significance was tested by a permutation test using 999 permutations of residuals of the full regression model (Legendre & Legendre, 1998, pp. 606-612; Anderson & Legendre, 1999) as implemented in the programme Regressn (Legendre, 2002). Reported p-values are two-tailed.

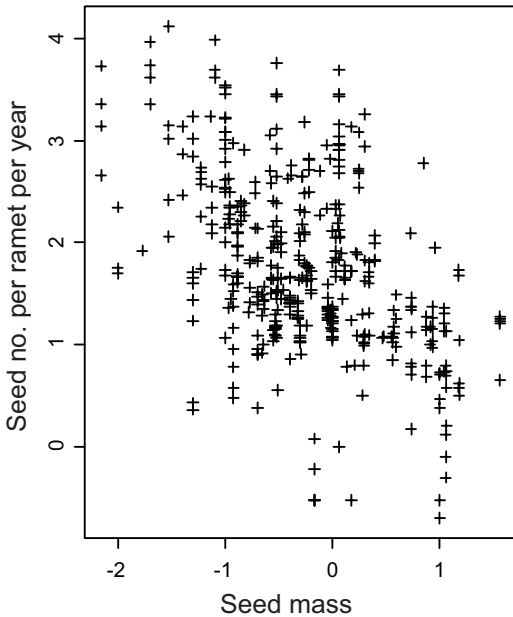


Figure 2. Relationship between log seed size and log seed number per ramet ($n = 414$). A linear regression model was highly significant ($p \ll 0.001$), but had low predictive power ($r^2 = 0.225$). Species may be represented by more than one point if they occurred in more than one plant community, but all points for a particular species have the same x coordinate.

Results

Average seed mass (log10 transformed) varied among communities (Table 2), but differences were not statistically significant (one-way ANOVA; $N = 422$, d.f. = 11, $F = 1.02$, $p = 0.43$). The variation between communities in average seed number per ramet was statistically significant (one-way ANOVA; $N = 414$, d.f. = 11, $F = 1.95$, $p = 0.03$), but had no systematic relationship with differences in light, moisture or other environmental factors. The relationship between log seed mass and log seed number was significantly negative (Fig. 2) and did not differ between communities (Table 3). However, most of the variation in seed number per ramet per year was not explained by log seed mass ($r^2 = 0.23$).

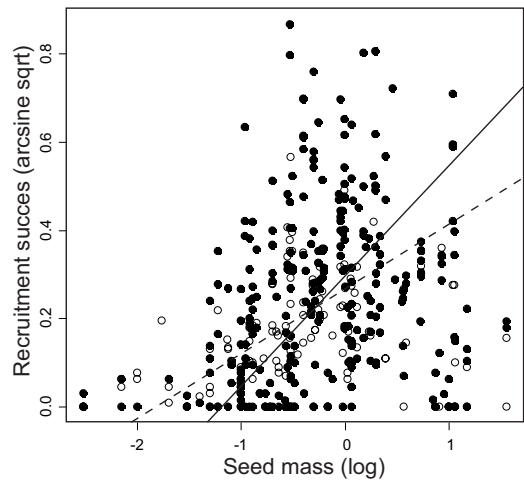


Figure 3. Recruitment success in relation to seed mass in 12 plant communities ranging from closed-canopy forest to grassland and rock outcrop, with Standardised Major Axis (SMA) regression lines. Two groups of community types are shown separately, deeply shaded forest field-layer communities (type 6-12, filled circles, full line) and open forest field layer, grassland and rock outcrop communities (type 1-5, empty circles, broken line). The slope of a regression line indicates the strength of the relative recruitment success of heavy seeds.

Table 2. The 12 plant community types investigated and the number of species encountered as seedlings, the median no. seedlings per m², mean seed mass (un-weighted average) and standard deviation (SD) around the mean, Standardised Major Axis (SMA) slope with lower and upper 95% confidence interval (CI).

No.	Community type	No. species	Median no. seedlings m ⁻²	Mean seed mass (mg)	Seed mass SD	SMA slope CI	SMA lower CI	SMA upper CI
1	Dry heath-forest of Cal-luna type	4	6	2.14	3.55	0.13	0.11	0.16
2	Dry heath-forest of Vaccinium vitis-idaea type	6	29	1.50	2.92	0.17	0.08	0.36
3	Rock outcrop	20	3823	2.67	8.23	0.13	0.08	0.20
4	Mesic meadow	55	3798	4.04	6.75	0.14	0.11	0.19
5	Dry meadow	45	1996	2.54	6.41	0.16	0.11	0.21
6	Mixed forest of Hepatica-Oxalis-type	75	11135	3.39	5.95	0.22	0.18	0.28
7	Mesic heath-forest of Oxalis-Vaccinium myrtillus-type	34	1140	2.32	3.95	0.26	0.19	0.36
8	Alder forest of fern-type	48	4252	3.62	14.15	0.28	0.21	0.37
9	Mesic heath-forest of Vaccinium myrtillus-type	17	696	3.00	5.00	0.27	0.17	0.44
10	Mixed forest of Melica-Lathyrus type	72	4248	4.25	7.36	0.23	0.18	0.29
11	Alder forest of Silene dioica type	39	3774	1.61	1.54	0.33	0.25	0.45
12	Mixed forest of Oxalis-Maianthemum-type	13	678	3.79	4.65	0.37	0.20	0.67

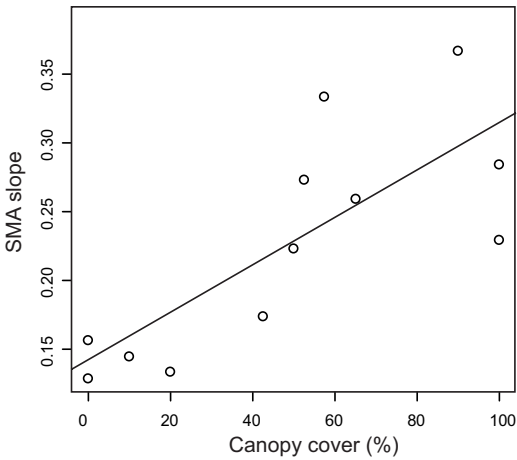


Figure 4. Relative recruitment success of heavy seeds (SMA slope of recruitment success vs. seed mass) in relation to canopy cover (%) in 12 plant community types ranging from grassland to heavily shaded forest field-layer communities.

All communities had positive SMA slopes of the seed-mass : recruitment-success relationship (Table 2). The Standardised Major Axis regression procedure showed significantly different slopes of the recruitment success to seed mass relationship between communities. Two rather distinct groups of communities appeared (Fig. 3). Communities 1 to 5 had relatively shallow slopes, meaning relatively small differences in recruitment success related to seed size. In contrast, communities 6 to 12 had relatively steep slopes, suggesting a relatively large gain in recruitment success with

increased seed size (Table 2). SMA slopes for the two groups, open and shaded, were significantly different (0.14 and 0.25, respectively; $p < 0.01$).

Four environmental variates had significant relationships with the variation in seed-size related relative recruitment success among communities, namely two related to shade (canopy cover and light penetration percentage) and two related to water regime (minimum and average moisture). Canopy cover had the strongest explanatory power ($r^2 = 0.610$, $p = 0.004$; Fig. 4). Minimum moisture ($r^2 = 0.544$, $p = 0.010$) and light penetration ($r^2 = 0.537$, $p = 0.005$) were somewhat weaker, and average moisture the weakest ($r^2 = 0.369$, $p = 0.037$). Multiple regression entering canopy cover first gave no significant two-factor models, meaning that shade and moisture related variates explained the same part of the variation in SMA slope between plant communities. Thus, the communities in which heavier-seeded species had the largest relative recruitment success (steep SMA slope) were field layer vegetation under deep shade with a relatively moist microclimate. Conversely, the communities in which heavier-seeded species had a comparatively small relative recruitment success (shallow SMA slope) were open forests, grasslands (meadow) and rock outcrop.

Discussion

Our analysis of Perttula's data provided evidence that larger seeds ensure plant species more

Table 3. Analysis of covariance of the seed size : seed number relationship over 12 plant community types ranging from closed-canopy forest to grassland and rock outcrop. For each model term, the degrees of freedom (d.f.), sum-of-squares (Sum Sq), F-value and p-value are given.

	d.f.	Sum Sq	F	<i>p</i>
Plant community	1	9.5	2.96	0.09
Log seed mass	1	323.1	100.15	< 0.001
Community x log seed mass	1	0.1	0.04	0.84
Residuals	430	1387.0		

successful per-seed recruitment, i.e. more seedlings established for a given number of seed produced. In addition, a markedly higher relative recruitment success of large seeds was found in field-layer communities under closed forest canopies. Thus, larger-seeded species appeared to have a much greater recruitment advantage relative to smaller-seeded species in closed forest than in open forest and grassland. This overall pattern was clear despite much variation in recruitment success unrelated to seed size variation (scatter in Fig. 3). This variation is attributable to idiosyncratic behaviour of species. A number of species appeared to deviate more systematically from the predicted relationship. These were all relatively large-seeded plant species with very low recruitment success, which was explicable by their association with specialized seed predators dwelling inside the seed (and thus seeds being scored as fully developed and undamaged by the investigator; *Lathyrus*, 4 spp.; *Vicia*, 3 spp.; *Convallariaceae*, 4 spp.; *Actaea spicata*) and species with idiosyncratic germination requirements (*Geranium bohemicum* and *Rubus saxatilis*). For these species, seed productivity and hence the seed rain density may have been overestimated.

Contrary to our expectation, no differences in relative recruitment success of large seeds were seen between open forest and meadow, or between meadow and rock outcrop vegetation. This may suggest that seed-size related recruitment success changes abruptly at a certain – high – threshold in light extinction, rather than gradually along the clinal variation in shade at the forest-floor level. This is in accordance with the finding of Leishman & Westoby (1994) that there was no seed-size related difference in seedling survival when experimentally varying shading from 50 – 95%, only at 99% shade.

The difference between plant communities in seed-size related recruitment success was not confounded by systematic variation in seed size, since differences in average seed size were insignificant. This observation is slightly at odds with the frequently found pattern that shade

tolerant species tend to have large seeds (Salisbury, 1942; Grubb, 1998; Thompson & Hodkinson, 1998), but perhaps reflects the rather forested character of all communities, even the meadow and rock outcrop communities investigated, which were situated as isolates in a forest matrix.

The cause for the observed difference among communities in relative recruitment success of large seeds appeared to be variation in shade. Light penetration percentage and canopy cover correlated strongly, and soil moisture weakly, with this difference, whereas litter cover and depth appeared to have no effect. This indicates a major direct effect of deep shade, probably acting on seedling survival. This is in accordance with evidence from greenhouse experiments on herbaceous (Leishman & Westoby, 1994) and woody species (Walters & Reich, 2000). However, both the mentioned studies found the seed-size related shade : seedling-survival relationship to be mainly driven by seed sizes above 10-100 mg. In the present – field-based – study, the positive relationship (Fig. 3) was driven by all seed size classes, except the largest, probably due to a disproportionately strong seed-predation pressure on these. In addition, shade might work already on the level of seed germination since smaller-seeded species have a light requirement for germination more often than larger -seeded species (Grime et al., 1981; Milberg, Andersson & Thompson, 2000) and because canopy-filtered light may induce a light requirement for germination (Pons, 2000).

Deep litter has previously been shown to hamper recruitment of field-layer species, particularly species with seed mass below 10 mg (Fröberg, 2001; Dzwonko & Gawroński, 2002). In the present study, accumulation of a deep litter layer was found in several of the studied communities (up to 100% cover, 4.5 cm deep on average in Mixed forest of *Melica-Lathyrus* type). However, litter appeared to be of less importance than deep shade in influencing recruitment differences among species with differently sized seeds.

The direct effect of shade suggests that environmental adversity, not competition among seedlings, is a selective force acting on seedling size (and hence seed mass). Had recruitment taken place in gaps below the canopy level, and the relative recruitment success of large seeds been expressed through a competitive superiority of larger seedlings, one would have expected stronger correlation with features of the forest floor environment, such as field layer cover, moss cover, or litter cover. This result is in line with the literature survey of Moles and Westoby (2004b) finding that seedlings more often die from environmental hazards (herbivory, pathogens, drought) than from seedling-seedling competition. In slight contrast to this survey, Silvertown & Bullock (2003) found that seedling mortality in grassland gaps was somewhat density dependent. Only when seedlings stand closely next to each other, seedling-seedling competition may matter, and probably those arisen from larger seeds will have an advantage.

Recruitment success of ericaceous dwarf-shrubs was very low. It is well-known that, despite insurmountable annual fruit production, seedlings are rarely observed in the field (Vander Kloet & Hill, 1994; Eriksson & Fröborg, 1996). However, although dominant in some of the investigated communities, only three species ericaceous dwarf-shrubs were found. Omission of four dwarf-shrub dominated communities on podzolic soils (see Table 1) did not change results.

A number of assumptions were made in the course of this study. The first assumption was that all seeds produced within a 1 m² subplot were dispersed inside that subplot only, or at least that seeds outflux was balanced by influx from the surroundings. Of course, seeds may be dispersed further away from the mother plant than 1 m, but for all practical purposes it was a reasonable assumption, as most seeds fall in the close vicinity of their mother plant (Harper, 1977; Verkaar, Schenkeveld & van de Klashorst, 1983; Jongejans & Telenius, 2001). Pre-dispersal seed

predation, but not post-dispersal, was taken into account. Larger seed are thought to be more prone to seed predation by both rodents and insects (Thompson, 1987; Reader, 1997; but see Moles, Warton & Westoby, 2003), and empirical studies have found support for this idea in both grassland (Hulme, 1994) and forest (Fröborg, 2001). Thus, if larger seeds were disproportionately consumed by seed predators, one would expect them to have a lower recruitment advantage, making the present estimate of a relative recruitment advantage of larger seeds a conservative one.

Perttula (1941) counted seedlings, but did not follow their survival to reproductive maturity, let alone to the next year. This means that there is a big leap from recruitment success to plant fitness. However, the seed dispersal and seedling germination and establishment phases are usually considered most critical in the plant life cycle. In most cases, only a negligible fraction of the seeds produced get as far as to become established seedlings. Moreover, the reserves provided by the mother plant are spent during this short but critical phase. For the purpose of a study of the seed-mass effect on recruitment, the seedling establishment phase is appropriate to consider, while survival during the juvenile phase is much less dependent on seed size (Moles & Westoby, 2004a).

In conclusion, the importance of seed size for recruitment success changed along the gradient from dry grassland to closed forest. This change, however, was not gradual, but abruptly shifting between forest with dense canopy cover and open forest, with little difference between open forest, grassland and rock outcrop communities. This knowledge is important in attempts of turning SSNT theory into mechanistic models of colonization and competition (e.g. Eriksson & Jakobsson, 1998), comparison of model results from different formations or geographic areas (e.g. Bruun, 2001) and in reviews and meta-analyses (e.g. Murray et al., 2002; Murray et al., 2005).

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Appendix 1. List of species with seed mass data and their sources.

<i>Species</i>	Seed mass (mg)	source
<i>Achillea millefolium</i>	0.13	5
<i>Actaea spicata</i>	6.91	7
<i>Aegopodium podagraria</i>	2.59	12
<i>Agrostis capillaris</i>	0.06	19
<i>Alchemilla sp.</i>	0.49	5
<i>Anemone nemorosa</i>	3.82	7
<i>Anemone ranunculoides</i>	1.10	3
<i>Angelica sylvestris</i>	3.60	10
<i>Anthoxanthum odoratum</i>	0.61	10
<i>Anthriscus sylvestris</i>	2.83	5
<i>Arabidopsis thaliana</i>	0.03	5
<i>Avenula pubescens</i>	1.31	4
<i>Briza media</i>	0.23	19
<i>Bromus hordeaceus</i>	2.90	22
<i>Calamagrostis arundinacea</i>	0.55	12
<i>Calamagrostis epigejos</i>	0.10	25
<i>Calamagrostis purpurea</i>	NA	
<i>Calluna vulgaris</i>	0.03	5
<i>Caltha palustris</i>	0.99	19
<i>Campanula persicifolia</i>	0.07	10
<i>Campanula rotundifolia</i>	0.08	18
<i>Cardamine bulbifera</i>	98.60	12
<i>Carex digitata</i>	0.64	12
<i>Carex pallescens</i>	1.36	10
<i>Carum carvi</i>	2.46	10

The recruitment advantage of large seeds - III

<i>Centaurea jacea</i>	1.06	5	<i>Hypochaeris maculata</i>	1.11	5
<i>Cerastium fontanum ssp. triviale</i>	0.12	5	<i>Impatiens noli-tangere</i>	4.50	9
<i>Chelidonium majus</i>	0.76	7	<i>Knautia arvensis</i>	2.47	5
<i>Circaea alpina</i>	0.51	14	<i>Lathyrus montanus</i>	15.00	5
<i>Cirsium helenoides</i>	3.56	23	<i>Lathyrus pratensis</i>	10.12	5
<i>Cirsium palustre</i>	2.00	19	<i>Lathyrus sylvestris</i>	37.44	20
<i>Cirsium vulgare</i>	2.64	19	<i>Lathyrus vernus</i>	15.30	7
<i>Convallaria majalis</i>	18.38	7	<i>Leucanthemum vulgare</i>	0.44	5
<i>Corydalis solida</i>	1.70	16	<i>Linnaea borealis</i>	2.40	10
<i>Crepis tectorum</i>	0.32	2	<i>Listera ovata</i>	0.00	8
<i>Dactylorhiza maculata</i>	0.00	8	<i>Luzula multiflora</i>	0.40	17
<i>Deschampsia cespitosa</i>	0.20	10	<i>Luzula pilosa</i>	0.59	12
<i>Deschampsia flexuosa</i>	0.63	18	<i>Lychnis viscaria</i>	0.06	5
<i>Dianthus deltoides</i>	0.14	5	<i>Lysimachia vulgaris</i>	0.29	20
<i>Elymus caninus</i>	3.44	9	<i>Maianthemum bifolium</i>	9.97	7
<i>Epilobium angustifolium</i>	0.05	19	<i>Melampyrum pratense</i>	7.45	10
<i>Epilobium montanum</i>	0.13	19	<i>Melampyrum sylvaticum</i>	13.00	12
<i>Euphrasia sp.</i>	0.23	10	<i>Melica nutans</i>	1.75	10
<i>Fallopia dumetorum</i>	4.30	16	<i>Milium effusum</i>	1.25	12
<i>Festuca ovina</i>	0.47	10	<i>Moebria trinervia</i>	0.22	19
<i>Festuca rubra</i>	1.04	18	<i>Mycelis muralis</i>	0.31	11
<i>Filipendula ulmaria</i>	0.30	12	<i>Myosotis stricta</i>	0.10	3
<i>Filipendula vulgaris</i>	0.52	5	<i>Oxalis acetosella</i>	0.99	4
<i>Fragaria vesca</i>	0.30	13	<i>Paris quadrifolia</i>	5.84	7
<i>Galium boreale</i>	0.75	10	<i>Pimpinella saxifraga</i>	1.07	5
<i>Galium palustre</i>	0.91	19	<i>Plantago lanceolata</i>	1.92	5
<i>Galium uliginosum</i>	0.29	10	<i>Poa angustifolia</i>	0.19	19
<i>Galium verum</i>	0.66	5	<i>Poa nemoralis</i>	0.17	12
<i>Geranium bohemicum</i>	7.00	1	<i>Polygonatum odoratum</i>	37.30	21
<i>Geranium robertianum</i>	2.02	12	<i>Potentilla argentea</i>	0.07	5
<i>Geranium sylvaticum</i>	5.33	10	<i>Potentilla crantzii</i>	0.44	10
<i>Geum rivale</i>	1.34	5	<i>Potentilla erecta</i>	0.77	10
<i>Glyceria fluitans</i>	1.20	19	<i>Prunella vulgaris</i>	0.61	5
<i>Hepatica nobilis</i>	2.18	12	<i>Pulmonaria officinalis</i>	6.11	12
<i>Hieracium sect. Vulgata</i>	0.44	11	<i>Ranunculus acris</i>	1.10	18
<i>Hieracium umbellatum</i>	0.44	11	<i>Ranunculus auricomus</i>	0.28	12
<i>Hierochloë australis</i>	1.20	3	<i>Ranunculus ficaria</i>	1.35	21
<i>Hypericum maculatum</i>	0.04	12	<i>Ranunculus polyanthemos</i>	2.50	3

<i>Ranunculus repens</i>	2.96	12
<i>Rhinanthus angustifolius</i>	2.30	6
<i>Rhinanthus minor</i>	1.87	5
<i>Rubus idaeus</i>	1.80	13
<i>Rubus saxatilis</i>	10.93	10
<i>Rumex acetosa</i>	1.29	10
<i>Rumex acetosella</i>	0.40	19
<i>Rumex crispus</i>	1.33	19
<i>Rumex longifolius</i>	1.88	15
<i>Scrophularia nodosa</i>	0.08	19
<i>Sedum acre</i>	0.03	19
<i>Sedum telephium</i>	0.05	12
<i>Silene dioica</i>	0.67	12
<i>Silene nutans</i>	0.35	4
<i>Solidago virgaurea</i>	0.74	10
<i>Stachys sylvatica</i>	1.67	7
<i>Stellaria graminea</i>	0.27	5
<i>Stellaria holostea</i>	3.70	21
<i>Stellaria longifolia</i>	0.10	3
<i>Stellaria nemorum</i>	0.34	7
<i>Trientalis europaea</i>	0.56	10
<i>Trifolium medium</i>	2.77	12
<i>Trifolium pratense</i>	2.33	10
<i>Trifolium repens</i>	0.23	5
<i>Urtica dioica</i>	0.16	12
<i>Vaccinium myrtillus</i>	0.20	10
<i>Vaccinium vitis-idaea</i>	0.24	10
<i>Valeriana officinalis ssp. officinalis</i>	0.67	24
<i>Valeriana sambucifolia</i>	1.08	10
<i>Veronica chamaedrys</i>	0.26	7
<i>Veronica officinalis</i>	0.14	5
<i>Vicia cracca</i>	14.29	19
<i>Vicia hirsuta</i>	2.19	19
<i>Vicia sepium</i>	16.70	7
<i>Vicia sylvatica</i>	16.52	7
<i>Vicia tetrasperma</i>	3.60	16
<i>Viola canina</i>	0.91	5
<i>Viola canina ssp. montana</i>	0.91	5

<i>Viola mirabilis</i>	1.65	12
<i>Viola palustris</i>	0.63	19
<i>Viola riviniana</i>	1.35	10
<i>Viola tricolor</i>	0.50	5

Sources

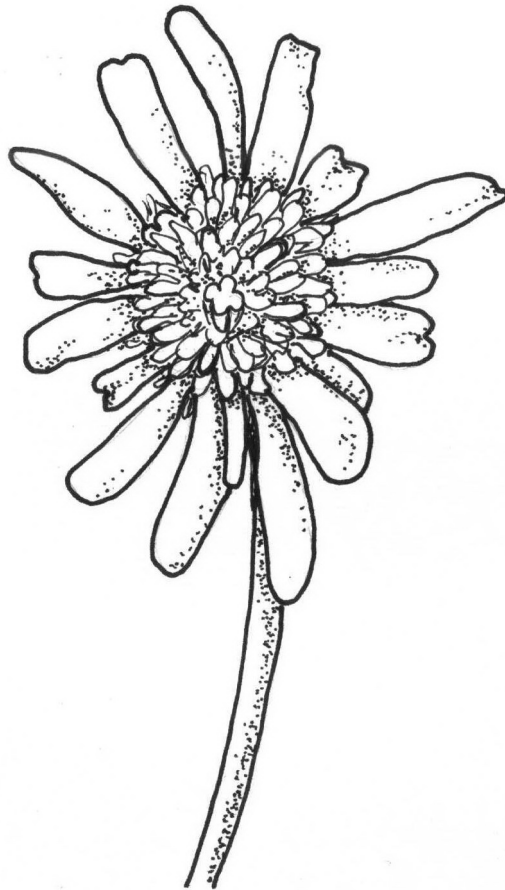
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Plant trait dispersion along a soil phosphorus gradient in rocky habitats

Dirk-Jan ten Brink, Pål Axel Olsson, Germund Tyler and Hans Henrik Bruun

IV



Plant trait dispersion along a soil phosphorus gradient in rocky habitats

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Abiotic and biotic filters act upon plant traits sorting species into local assemblages. We addressed the questions whether plant traits converge or diverge within local assemblages relative to between-community trait variations in rocky habitats and how trait dispersion relates to soil parameters. We also investigated whether traits related to regeneration varied more within plots than traits of established individuals, which is hypothesized to result from the diversifying effect of disturbance on recruitment opportunities. We quantified trait variation in southern Swedish rocky habitats, i.e. plant communities on shallow soils spanning a gradient in pH from very acid to calcareous with parallel variation in exchangeable phosphate. Within-community variation appeared to be larger than between-community variation for seed mass, plant height, lateral spread and species indices of competition, stress and ruderality. Our data do not support the hypothesis that traits related to regeneration vary more among co-occurring species than do traits of established individuals. We also found higher degree of trait divergence within communities relative to the between-community level. This would indicate that limiting similarity plays a role in structuring plant communities in rocky habitats if the underlying environmental gradient was mainly varying in productivity. However, rocky habitats are drought-prone by nature and chemical stress from aluminium increases towards low soil pH parallel to phosphorus.

Introduction

Plant communities are assembled from a pool of potential colonists, scaled by the vagility of species, through abiotic and biotic filtering processes selecting on plant traits (Weiher & Keddy 1999; McGill *et al.* 2006). These processes result in assemblages of species coexisting in sites. In classical competition theory, stable coexistence of plant species is believed to have resulted from previous interspecific competition for limiting resources, a process which has led to trait divergence between coexisting species (Gause 1936; MacArthur & Levins 1967). In contrast, empirical observations of vegetation often reveal similarities in life history, physiology and morphology of coexisting species (Keddy

1992; Grime 2006). In particular soil fertility and the associated site productivity appear to impose a strong selection on a set of interlinked physiological and morphological attributes, such as growth rate, leaf toughness, nutrient content and litter decomposition rate (Grime *et al.* 1997; Wright *et al.* 2004; Diaz *et al.* 2004). Accordingly, the dominant role of interspecific competition in trait divergence and species diversity in plant communities has been questioned; abiotic factors like drought, low nutrient availability, extreme temperatures and salinity may be more important in less benign environments (Brooker *et al.* 2005; Pierce *et al.* 2007). The net interactions between plants in alpine and arid environments, for example, may be facilitative rather than competitive (Bertness & Callaway 1994; Holmgren *et al.* 1997; Callaway

et al. 2002). Grime (2006) argued that disturbance rather than resource competition is the overriding mechanism creating and maintaining plant trait variation within plant communities. Disturbances in plant communities may vary in place, nature, intensity and timing. Besides creating opportunities for coexistence of potential dominant and subordinate species by altering competitive hierarchies (Richard 1979), disturbances can bring about a variety of regenerative mechanisms by which species can utilize temporally and spatially varying recruitment opportunities (Grubb 1977). The general spatial and temporal uniformity of productivity within the habitat, on the other hand, is hypothesized to impose convergence on physiological and morphological traits related to the resource economy of plants (Grime 2006).

We used vegetation data from a large number of Southern Swedish rocky habitats (Tyler 1996), comprising plant communities along a wide-ranging complex environmental gradient including parallel variation in soil pH and exchangeable phosphate (PO_4) gradient. The overall objective of this study was to quantify trait variation in the rocky habitats in relation to the soil parameters and to address the question whether traits converge or diverge at the local scale (within plots) relative to between-plot trait variation in the investigated communities. Furthermore we tested whether regenerative traits exhibit a higher degree of within-plot variation than established traits, as follows from the hypothesis of Grime (2006). To this end, we compared the dispersion of a number of traits of established plants related to the resource economy of plants with the variation in seed mass, being an important trait related to recruitment success across environments (Westoby *et al.* 1996; Bruun & ten Brink Paper III).

We employed a trait-based approach (Ackerly & Cornwell 2007) which provides a decomposition of species' trait values into alpha (α) and beta (β) components. The terms alpha and beta follow from definitions of the hierarchical division of the spatial components of plant diversity (Whittaker

1975). In this context, the α niche refers to characteristics of the species' realized niches that differentiate co-occurring species and thereby maintain diversity at a local scale where interactions among species occur. The β niche refers to the region of the species niche that corresponds to the habitat they occur in, and this feature must be at least partially shared among co-occurring species (Pickett & Bazzaz 1978; Silvertown *et al.* 2006).

The aims of the study were 1) to investigate for the rocky habitats in our study whether traits converge or diverge at the within-plot scale relative to the between-plot scale by comparing species mean alpha and beta values. 2) to test whether traits related to regeneration are more variable among co-occurring species than traits of established.

Methods

Study area

The research was carried out in southern Sweden including the Baltic islands Öland and Gotland. Sampling was limited to localities with exposed bedrock or shallow soil over bedrock. Bedrock characteristics varied greatly, although it was usually dominated by siliceous igneous rocks, such as granite. Various types of dark igneous rocks were widespread as well as several types of limestone. Quartzitic sandstone occurred locally. For more details on the study area and the floristic composition of the rocky habitats, see Tyler (1996).

The current vegetation in southern Sweden developed after the retreat of the Weichselian icecap some 8 500 y BCE; in coastal areas, however, only after the sea regression some 4 000 y BCE.

Natural weathering and ecosystem processes have led to a slow acidification of soils North Western Europe - a process that has been accelerated by anthropogenic acid deposition during the latest decades (Rozema *et al.* 1985; Falkengren-Grerup 1989) during the last decades. The gradient in

pH_{KCl} ranged from around 2.5 in the most acid soils to close to 8 in the limestone soils.

Vegetation sampling

A total of 439 plots of rocky habitats were sampled during July and August of 1993 and 1994. The floristic composition was recorded within 4 m² plots and the abundance of each species was estimated by the percent cover of the aboveground biomass (vertical projection on the ground). A total of 290 species were found in the plots (Table A1; see appendix). Nomenclature follows Flora Europaea (Tutin *et al.* 1964-1980). All plots were open, i.e. with negligible tree cover, but in some cases with scattered shrubs.

Soil analysis

Maximum soil depth was averaged from nine individual, regularly spaced, measurements in each plot. Soil was sampled at five points down to the bedrock (or to a maximum depth of 20 cm) and pooled to form one composite sample. Soil samples were stored at 5°C until analysis. In the laboratory, the soil was sieved, and only the fraction <6 mm was used. pH_{KCl} was determined electrometrically on all samples (10 g soil and 50 mL 0.2 m KCl) in supernatants obtained by 2 h extraction in a rotator. Exchangeable phosphate was determined using a flow injection analysis application of the stannous chloride-molybdate method following 30 min. extraction of 10 g soil at field moisture with unbuffered 100 mL 0.05 m Na_2SO_4 + 0.02 m NaF solution.

Trait selection

The selection of traits was restricted by the serial information available in the literature. Seed mass was chosen to represent the regenerative life stage of plants. Data on seed mass was obtained from the literature and own measurements (in general, seed without appendages, air-dried at

room temperature). The other traits were related to the established stage of the plants life cycle and reflect important aspects of life form, growth strategy and resource economy. Plant height and vegetative lateral spread are classifications based on field observations and range from 1 to 8 and 1 to 5 on a categorical scale, respectively (Hodgson *et al.* 1995). The three components of the CSR strategy scheme (Grime 1974; 2001; Fig A1 in the appendix) were each used separately as measures of competitiveness, stress-tolerance and ruderality. The coordinate values in CSR space (hereafter referred to as competition, stress and ruderality index respectively), for each CSR combination were adopted from Hunt *et al.* (2004) and are listed in Table A2 (see appendix). The trait analysis as described below is carried out for each trait separately. Seed mass data was available for 92 % of the 290 species. Species that we had to omit from the analysis were all are rare species with low abundance in a few plots only. For plant height and lateral spread we had data on 56 % of the species and the analysis on the CSR was based on 72 % of the species. Details on the mean abundance and frequency of the omitted species in Table A3

The trait-gradient method and data analysis

The idea of the trait-gradient analysis, which was developed by Ackerly and Cornwell (2007), is to partition species traits into α and β components, and to quantify the niche breadth of a species in trait units. The trait-gradient analysis uses species abundance data for the sampled plots and species-specific trait values. In the equations, t_{ij} = the trait value and a_{ij} = the abundance of species i in plot j . S and P are the total number of species and plots in the dataset, respectively. Ackerly & Cornwell (2007) defined the abundance-weighted plot mean trait values and species mean trait values as

$$\bar{t}_j = \frac{\sum_{i=1}^S a_{ij} t_{ij}}{\sum_{i=1}^S a_{ij}} \quad \text{and}$$

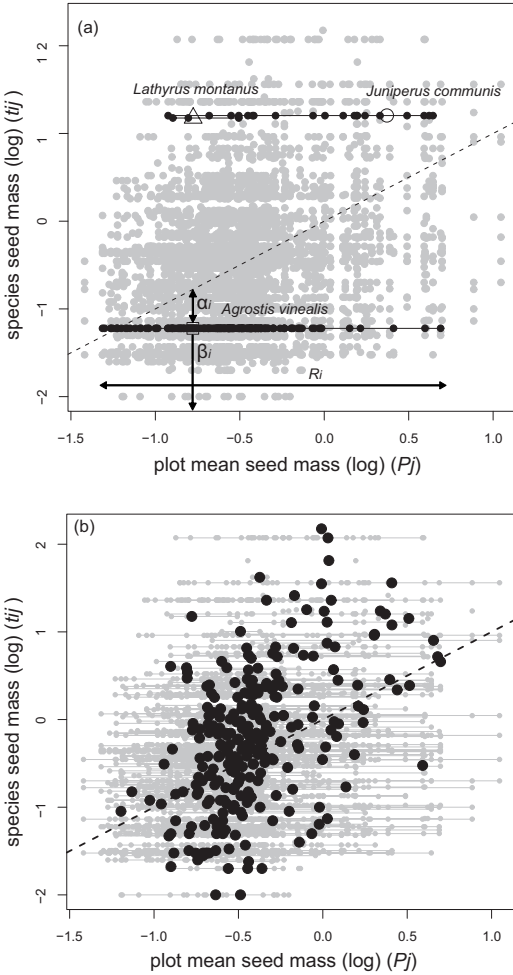


Figure 1 (a) Scatter plot of species trait values (t_{ij}) vs. abundance weighted plot mean trait values for log seed mass (mg). The dashed line is $X=Y$. The values for *Agrostis vinealis*, *Lathyrus montana* and *Juniperus communis* are highlighted for illustration. The large open points (square for *A.v.*, triangle for *L.m.* and circle for *J.c.*) show the mean position of occupied plots (β_i on the x-axis) and mean species trait values (\bar{t}_i) on the y-axis. The distance between these points and the $X=Y$ -line is α_i . The range of occupied plots on the x-axis is the niche breadth of the species (R_i). (b) Distribution of trait means for all species.

$$\bar{t}_j = \frac{\sum_{i=1}^P a_{ij} t_{ij}}{\sum_{i=1}^P a_{ij}}, \text{ respectively.}$$

Note that in our study, we lack plot-specific in-situ measured species trait data. So the trait value for each species is constant throughout all the plots. The relationship between traits (here: log seed mass) of species and plot level means is shown in Fig. 1a. Every point in the figure represents one species in one plot. Species that are vertically aligned co-occur in one plot and have a specific value for P_j . The $X=Y$ -line the ordinary least squares regression line of t_{ij} vs. P_j (weighted each point by abundance) with intercept zero and slope 1. We have highlighted three species in Fig. 1a to illustrate the method. *Agrostis vinealis* occurred in 179 plots, with $t_i = -1.22$. *A. vinealis* occurs in 179 out of 439 plots which span a range in P_j from -1.31 to 0.69. The mean location of the species along the trait-gradient is defined as the abundance-weighted mean of P_j for all the plots occupied by the species:

$$\beta_i = \frac{\sum_{j=1}^P P_j \bar{t}_{ij}}{\sum_{j=1}^P a_{ij}}$$

This value is called the β value of the species, and is a measure of the β niche position along the specific trait gradient. The open square indicates the mean position of *A. vinealis* along the gradient (β_i), and its trait value (t_i) (Fig. 1a). The novel concept introduced by Ackerly & Cornwell (2007) is the α trait value (α) which is defined as the difference between the species (mean) trait value and its β value according to the equation

$$\bar{t}_i = \beta_i + \alpha_i$$

where α_i is the deviation of (β_i and t_i) from the $X=Y$ -line and represents the species' trait position relative to that of the co-occurring species and is a measure of the α niche position of the species.

Table 1. Pearson correlation of soil pH and soil PO₄ and maximum soil depth with each other and with plot mean trait values. * Correlation significant at the 0.05 level. **Correlation significant at the 0.01 level.

	pH	PO ₄ (log)	Max. soil depth
Exch. PO ₄ (log)	-0.78**	-	
Max. soil depth	0.11*	-0.16**	-
Seed mass	0.16**	-0.21**	0.40**
Plant height	0.10*	-0.15**	0.34**
Lateral spread	-0.46**	0.35**	-0.03
Competition (c)	-0.35**	0.22**	0.29**
Stress (s)	0.22**	-0.27**	-0.09
Ruderality (r)	-0.09	0.21**	-0.15**

$\beta_i = -0.78$ for *A. vinealis*, indicating that it tends to occupy the low end of the seed mass gradient. The negative α value of *A. vinealis* ($\alpha_i = -0.44$) mean that within the plots it occupies, its seed mass value is on average lower than the mean of the co-occurring species. *Lathyrus montanus*, which occurred in three plots, has a similar β value as *A. vinealis* ($\beta_i = -0.78$) meaning that it also occurs at the low end of the plot mean seed mass gradient, but the high α value ($\alpha_i = 1.95$) indicates that within the plots it occurs, *L. montanus* has a far higher seed mass value than the species it co-occurs with. For *L. montanus* $t_i = 1.17$. A third species, *Juniperus communis* (20 occurrences), has a similar trait value as *L. montanus* ($t_i = 1.20$), but the species has a high β value ($\beta_i = 0.37$). Also, *J. communis* has on average a higher seed mass value than its co-occurring species ($\alpha_i = 0.83$).

Finally the niche breadth (R_j) of the species along the gradient is defined as the range of P_j values of occupied plots, which is $0.69 - (-1.31) = 2.00$ for *A. vinealis*.

The trait-gradient analyses were performed using the R software (R Development Core Team, 2007) with R scripts developed by Ackerly & Cornwell (2007).

We correlated soil pH, exchangeable PO₄ and maximum soil depth with plot mean trait levels values. Regression analysis was performed

to investigate the relation between exchangeable PO₄ and the standard deviation of α values of the traits in the plots, as a measure of trait variation within the plots (weighted by species abundance). The correlations and regressions analyses were performed (SPSS 15 for Windows, SPSS Inc, 2006). To improve normality, seed mass was log transformed and the competition and ruderality indices were square root transformed before analysis.

Results

The plot mean trait values give a biotic measure of the vegetation in a plot, defined by the species traits. The plot mean trait values of several of the traits correlate significantly with measurements of soil pH and exchangeable PO₄ and maximum soil depth (Table 1).

Exchangeable PO₄ decreased with increasing pH. Over this pH gradient we found seed mass and plant height to increase with pH. Seed mass and plant height also increased with maximum soil depth. We found that the species at high pH were more stress selected and species at low pH were more competition selected. Competition selection increased with soil depth. Ruderality showed strongest positive correlation to exchangeable

Table 2. Summary statistics for six traits across 439 plots of rocky habitat vegetation. Sqrt = square root transformed.

	Seed mass (mg. log)	Plant height	Lateral spread	Competition (sqrt)	Stress	Ruderality (sqrt)
Species characteristics						
t_j mean	-2.22	2.56	2.44	0.43	0.39	0.51
t_j min-max	- 2, 2.18	1, 8	1, 5	0, 1	0, 1	0, 1
β_j mean	-0.40	2.10	2.78	0.38	0.51	0.46
β_j min-max	-1.20, 0.70	1.30, 4.28	1.69, 4.10	0.15, 0.66	0.36, 0.73	0.17, 0.70
α_j mean	0.18	0.47	-0.34	0.06	-0.12	0.05
α_j min-max	-1.51, 2.18	-2.28, 5.43	-2.52, 2.55	-0.45, 0.57	-0.65, 0.42	-0.67, 0.64
R_j mean	0.85	1.21	1.18	0.26	0.23	0.28
R_j min-max	0, 2.46	0, 3.35	0, 2.99	0, 0.65	0, 0.74	0, 0.72
Plot characteristics						
P_j mean	-0.53	1.95	3.03	0.38	0.52	0.46
P_j min-max	-1.42, 1.05	1.03, 4.75	1.12, 4.13	0.04, 0.71	0.22, 0.96	0, 0.78

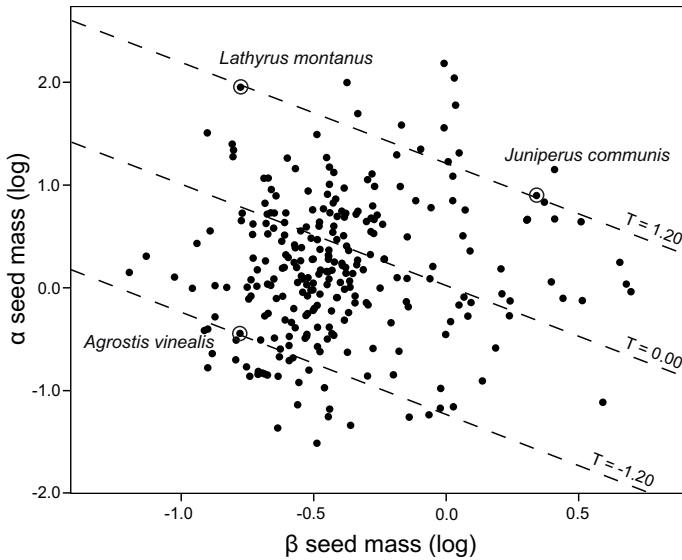


Figure 2. Scatter plot of β_i vs. α_i for seed mass. Species trait values (t_i) are equal to the sum of these two components. Highlighted points are the species illustrated in Fig. 1a

PO₄.

Details of the trait-gradient analysis of the rocky habitat vegetation are illustrated in figures for the seed mass data for interpretation of the used method and the evaluation of the results. The main results for the other traits are summarized in tables. The scatter plot of species values vs. abundance-weighted plot mean seed mass values illustrates the position of all species in the seed mass trait space (Fig 1b). Species that occurred in at least three plots are connected with a line. The niche breadth, representing the range of occupied plots for a specific species, varied from zero for species that occurred only in one plot, to 2.46 for *Festuca ovina*, spanning almost the entire gradient (Fig. 1b, Table 2). Every species is represented by a filled black circle indicating their mean position of occupied plots (β_i) on the x-axis and the species trait value (t_i) on the y-axis (Fig. 1b). All calculated parameters are provided in Table A1 (see appendix).

The range of α_i values for seed mass was 3.69 log seed mass units, while β_i spanned a range of 1.99 (Fig 2, Table 2). Also for all other traits the range in α_i was larger than the range of β_i . This means that, on average, species vary more in their

trait values relative to co-occurring species, than they do in the mean trait values of the plots in which they occur (Table 2).

We found a significant negative relation between exchangeable PO₄ and the within-plot variation in trait values for all traits (Fig 3). The competition index showed the strongest negative relation with exchangeable PO₄.

Discussion

Grime's (2006) hypothesis that species tend to diverge in traits related to resource economy at the between-plot level as a result of the strong filtering effects of site productivity, would require the range of β values to be larger than the range of α values. We did not find support for this hypothesis in our data with respect to the dispersion of the resource related traits like the competition index and the measures of height and lateral spread. The length and direction of the environmental gradient, however, is critical in this aspect. Apart from the wide range in soil pH and associated PO₄ availability, the harsh abiotic conditions as a result of the limited soil depth act as a strong selection

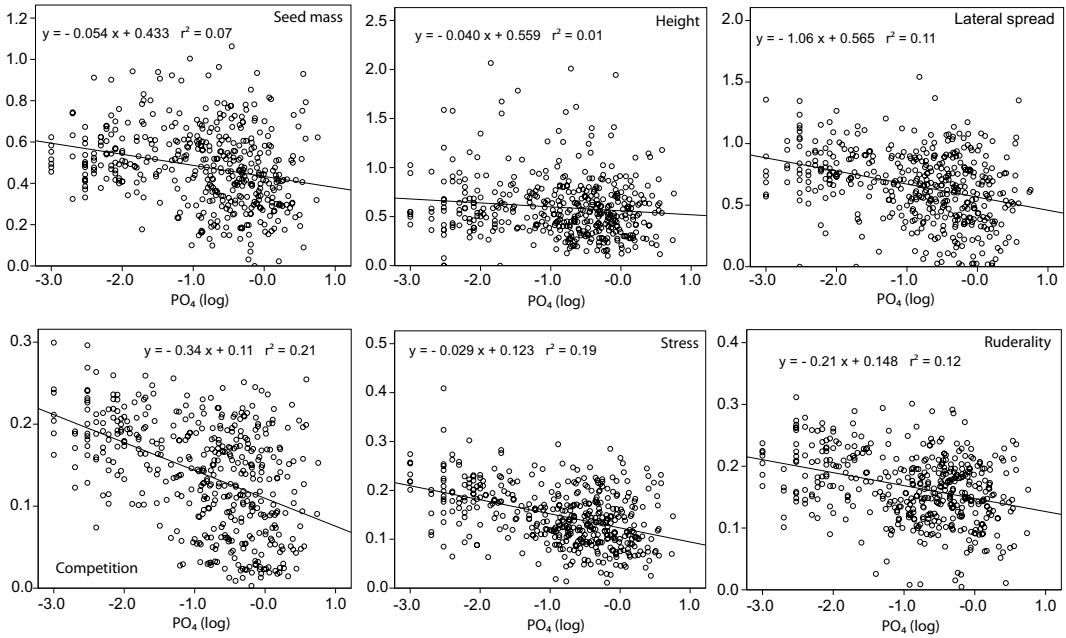


Figure 3. The relation between exchangeable phosphate (PO_4 log $\mu\text{mol g}^{-1}$) and the plot-wise standard deviations of the alpha trait values of the component species (weighted by abundance). Since second order regression equations gave virtually the same results as first-order regression equations in terms of explained variation, we used the latter.

filter on the species composition. Species that are sensitive to drought or need deeper soil for purposes of growth morphology are effectively excluded. Stressful conditions in the rocky habitat select against very competitive species.

The potential effect on productivity of the considerable increase in soil exchangeable PO_4 with decreasing pH, is probably counterbalanced by a parallel increase of solubility of phytotoxic aluminium species (Kinraide 1991). In the rocky habitats, soil aluminium concentration (Al^{3+} ; the most toxic form) showed a marked increase below a threshold of pH 5 (Tyler, 1996). Thus, there are adverse conditions at both ends of the wide pH gradient and this, in combination with the extremely drought-prone shallow soils, may be by far the strongest constraint. This reduces the relative importance of the soil exchangeable phosphate gradient and effectively means that

the gradient runs between two different kinds of stress regimes rather than from low to high stress. If we had sampled vegetation along a longer soil nutrient gradient, the range of β values would probably increase, while the range of α values would probably stay constant. In general, species are limited to a restricted range along environmental gradients, i.e. they have certain maximum niche breadths, and this will lead to a turnover in species associated with different environmental conditions. The relatively narrow mean niche breadths, compared to the range of beta values, were also seen in our study (Table 2). The wide range of α values relative to the range of β values for all investigated traits implies that traits diverge at the local scale, relative to the between-plot trait dispersion. The question whether this dissimilarity at local scale results from competition-driven limiting similarity, hypothesized to be

required for coexistence (MacArthur & Levins 1967), calls for an experimental test of the importance of competitive interactions relative to facilitative interactions in structuring rocky habitat plant communities. This could be done by removal experiments in the field (e.g. Herben *et al.* 1997; Kikvidze *et al.* 2005). By selecting target and neighbour with varying degree of similarity in terms of traits, one could also approach the question of limiting similarity.

The regression analysis revealed that for all traits, the similarity between species was higher with increasing exchangeable PO_4 and decreasing soil pH. Although this is partly attributable to decreasing species density at low soil pH – a pattern common to European vegetation (Pärtel 2002; Ewald 2003), the variation around the mean might logically be expected to increase with the mean value. For competition, lateral spread and ruderality (all positively correlated with exchangeable PO_4), however, the standard deviation of alpha trait values decreased with increasing plot mean trait values. This may mean that with increasing exchangeable PO_4 and increasing competition within the plots, the variation in competition strategies decreased among coexisting species, i.e. more similarity in competition indices with increasing exchangeable PO_4 . The same goes for the ruderality index. Ruderal plants, just like competitive plants, are placed at the same (high resource demanding) end of the slow-and-tight to fast-and-leaky spectrum (Grime 2006).

Our results did not confirm the idea that there is less trait divergence in the regenerative traits than in established ones. Ackerly & Cornwell (2007) applied their trait-gradient method to species in woody plant communities of coastal California, and found that the range of α values for specific leaf area, leaf size, wood density and maximum height were all larger than the range of their respective β values. The difference, however, between the ranges of α and β values were much larger for seed mass than for SLA, suggesting less trait convergence in the latter. In contrast, Franzén

(2004) found species with a similar seed mass in a semi-natural grassland to co-occur more frequently than expected from a random model.

Trait convergence and trait divergence may occur simultaneously during community assembly, and result in contrasting effects on trait similarity of coexisting species (Grime 2006). There seems to be no real conflict between the views of Grime and the advocates of the importance of assembly rules, amongst others represented by Wilson (2007), other than the dispute whether competition is the main mechanism leading to trait divergence within plant communities. This might depend on the type of habitat under investigation and the length and direction of the environmental gradients.

In the habitats we investigated, the vegetation experiences multiple stress factors as a result of the shallow soils; extreme variation in soil moisture, temperature, pH and nutrient availability (Tyler 1996). Maximum soil depth was significantly negatively and positively correlated with the ruderality index and the competition index, respectively (Table 1). The vegetation is sparse and inter-specific competition does not seem to be an important factor. The adverse conditions favours many species with a typical R or S or intermediate strategy, from Grime's (1974) CSR spectrum. There are relatively many annuals among the observed species. The short lifespan of annuals – they establish from seed during autumn or spring and complete their life cycle during spring or early summer - make them less susceptible to the severe summer drought (Tyler 1996). Olsson and Tyler (2004) found a significant positive relation between exchangeable soil phosphorus and the relative occurrence of non-mycorrhizal species in these communities. They concluded that having arbuscular mycorrhizal associations for plants is more a competitive strategy than a stress-tolerance strategy. A non-mycorrhizal strategy was most common at very low or very high pH and this was assumed to be due to higher environmental stress in these soils. A non-mycorrhizal strategy was most successful at low pH values probably due to

less phosphorus limitation in such soil, reflecting the importance of arbuscular mycorrhiza in phosphorus nutrition (Smith & Read 1997).

Because of the high number of plants throughout the plots, including many rare species specialized to the extreme conditions in the rocky habitats, we did not have trait data for all species. However, since most of the species that were excluded from the analysis occurred only in few plots and/or low abundance, we think this has not compromised the validity of our findings. Also the nature of the traits we have chosen was limited by available information in the literature and the applicability in the trait-gradient method we used. Seed mass is not the only regenerative trait of interest; reproductive output, seasonal germination timing and germination requirements and seed dormancy characteristics would all give valuable information on the importance of species coexistence in these habitats. Also information on traits related to tolerance of adverse conditions (drought, high acidity, high alkalinity etc) would have been of great value. Data on these traits is unfortunately not well documented in the literature for the majority of the species and some can only be expressed qualitatively.

We conclude that traits diverge at local scale relative to the between plot level, indicating that limiting similarity plays a role in our investigated plant communities. The hypothesis of Grime (2006) that the abiotic environment imposes a strong selection resulting in convergence of traits related to the resource economy of plants at local scale relative to the between plot trait variation could not be confirmed by our study. In this context it would be interesting to study a longer soil fertility gradient which also includes the study of other soil nutrients like nitrogen which is usually the limiting factor at higher productivity. We also did not find evidence for the notion of Grime (2006) that there is more variation among regeneration traits than established traits at a local scale. Incorporation of more traits related to the juvenile life stage of plants in a trait-based gradient

analysis than only seed mass would be valuable.

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Appendices

Table A1 can be found online at: <http://www.planteco.lu.se/people/hhb/rocky-habitats>

Table A2. Standard coordinate values within C-S-R space. Corners have value 1, opposite sides have value 0, intermediates are equidistant from each parent, and $C + S + R = 1$

	C	S	R
C	1.0000	0.0000	0.0000
C/CR	0.7500	0.0000	0.2500
C/CSR	0.6667	0.1667	0.1667
C/SC	0.7500	0.2500	0.0000
CR	0.5000	0.0000	0.5000
CR/CSR	0.4167	0.1667	0.4167
CSR	0.3333	0.3333	0.3333
R	0.0000	0.0000	1.0000
R/CR	0.2500	0.0000	0.7500
R/CSR	0.1667	0.1667	0.6667
R/SR	0.0000	0.2500	0.7500
S	0.0000	1.0000	0.0000
S/CSR	0.1667	0.6667	0.1667
S/SC	0.2500	0.7500	0.0000
S/SR	0.0000	0.7500	0.2500
SC	0.5000	0.5000	0.0000
SC/CSR	0.4167	0.4167	0.1667
SR	0.0000	0.5000	0.5000
SR/CSR	0.1667	0.4167	0.4167

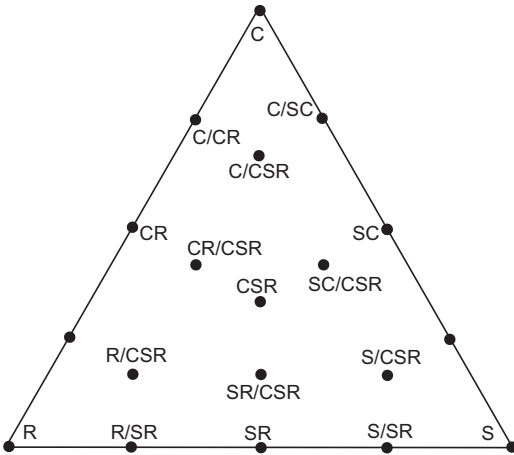


Figure A1 The positioning of the three primary plant functional types (C, S, R) and their intermediates in CSR space See Hunt *et al* (2004) for more details. This space is bounded by the units C = 0 to 1, S = 0 to 1 and R = 0 to 1.

Table A3. Mean frequency of the species omitted from the trait and analysis and species with the highest frequency and highest abundance among those. For all traits, the data are presented for three subsets of the total species lists used for the respective trait analyses. The trait analyses for the three components of the CSR classification as well as plant height and lateral spread used the same subset of species data.

Traits	Mean frequency (% of plots)	Max. frequency (% of plots)	Highest abundance (%)
Seed mass	2.95	18.0 (<i>Silene rupestris</i>) ¹	25 (<i>Sedum rupestre</i>) ³
CSR	4.57	18.0 (<i>Silene rupestris</i>) ¹	50 (<i>Spergula morisonii</i>) ⁴
Plant height & Lateral spread	5.60	18.6 (<i>Sedum album</i>) ²	50 (<i>Spergula morisonii</i>) ⁴

¹ Mean abundance: 2.28 % ³ Mean abundance: 3.06 %

² Mean abundance: 4.58 % ⁴ Mean abundance: 3.32 %

Abiotic control of plant community assembly: a microcosm experiment

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The structure of plant communities is determined by the arrival history of species and local deterministic mechanisms, such as environmental filtering and competitive exclusion. Contrasting ecological theories predict co-occurring species to either exhibit trait convergence as a result of environmental filtering or trait divergence as a result of competition relative to a random assembled community. We studied the effect of deterministic processes on the reproducibility of species assemblages, when the stochastic factor of seed dispersal is controlled for. Synthetic plant communities (microcosms) were established, initially by sowing seven common grassland species, with later addition of 25 target species. We varied fertility, target species sowing density and applied several disturbance treatments in a full factorial design. Patterns in species composition were highly repeatable at each level of soil fertility, while the assemblages were highly dissimilar between treatments, indicating strong deterministic processes in community assembly. Species richness was highest at intermediate fertility. The disturbance treatments did not affect species composition. A severe drought resulted in disproportionately high mortality in the high fertility treatment which was characterized by a dense sward of fast-grown and hence drought-susceptible plant individuals. This led to deflected community development in this treatment. The intermediate and low fertility microcosms were less affected by the drought. At high fertility, the dominant *Holcus lanatus* and most of the seedlings died back as a result of the drought, after which the formerly subordinate *Festuca rubra* quickly colonized and dominated the microcosms. We conclude that deterministic processes play an important role in the assembly of plant communities

Introduction

Plant community assembly may be thought of as the cross-product of arrival history and deterministic local mechanisms, or between chance and necessity in the words of Monod (1971). Environmental filtering and competitive exclusion are deterministic by nature. In contrast, dispersal of seeds and their arrival at a habitat is to high degree a matter of chance (Chambers & MacMahon 1994). Seed addition experiments have shown that many types of community are

dispersal limited (Turnbull *et al.* 2000; Clark *et al.* 2007). Once in a habitat, species will be put to the test for their adaptedness to the environment, from their arrival as propagules and throughout their life cycle. This filtering process acts on plant traits that have been collectively defined as the species' beta niche (Silvertown *et al.* 2006). Environmental selection alone must be expected to result in communities in which all constituent species share important functional traits, i.e. show trait convergence. However, in addition to environmental filters, competitive hierarchies

may define assembly rules (*sensu* Wilson 1999). Combined with the contention that “it is the most closely allied forms...which come into the severest competition with each other” (Darwin 1859: p. 110), competitive assembly rules must be expected to result in communities in which constituent species are dissimilar, i.e. show trait divergence. Contrasting ecological theories predict co-occurring species to either exhibit trait convergence as a result of environmental filtering or trait divergence as a result of competition relative to a random assembled community. Grime (2006), in a paper emphasizing the former view, hypothesized that species tend to converge in resource-economy related traits along productivity gradients. Fukami *et al.* (2005) showed that assembly of plant communities on former arable land was both deterministic and historically contingent. The assemblages converged in within-plot trait composition, but the initial manipulated compositional variation caused the communities to remain divergent in species identities.

Here we study to what extent community assembly patterns are repeatable under a certain set of environmental conditions, when constituent species are drawn from the same species pool and how the patterns vary along an environmental gradient. If deterministic processes dominate community assembly, resulting assemblages should be highly repeatable for each particular set of environmental conditions. Eliminating the chance effects of dispersal and species availability, enables evaluation of the combined effects of abiotic and biotic filtering. We performed a microcosm experiment (Fraser & Keddy 1997) to study community assembly from a restricted species pool under treatments varying fertility and disturbance.

The question whether community patterns are repeatable within treatments can be answered by comparing the within-treatment similarity with the between-treatment similarity. Another objective was to test whether the degree of intra-community similarity changes along a fertility gradient.

Dominant species in communities that developed under high fertility might be expected to faster fill up empty space leaving fewer opportunities for other species to establish. In contrast, the higher mortality which is expected at lower fertility, gives room for a higher species turnover and thus a relatively larger role of random processes, which could lead to a lower similarity among assemblages with decreasing fertility.

We also evaluate the results in the in context of drought resistance and community resilience in relation to soil fertility as the microcosms experienced a severe natural drought, which differentially affected the microcosms depending on the soil fertility levels.

Methods

Species and seed selection

We selected 25 herbaceous species, representing a range of seed sizes and plant life-history strategies (Table 1). We aimed to cover a range of plant CSR strategies (Grime 1974; 2001) related to resource dynamics varying from the acquisitive (‘fast and leaky’; CR) to retentive (‘slow and tight’; S) (Grime *et al.* 1997). The values on the resource dynamics continuum were related to the Grime’s CSR classification (Hodgson *et al.* 1999). CSR strategy values of species as published in the electronic appendix of Hunt *et al.* (2004) were adopted.

Freshly matured seeds were collected from various locations in the southernmost part of Sweden, and adjacent eastern Denmark between July and September 2006. Seeds were collected in open habitats, which included grasslands, road verges and disturbed sites with ruderal vegetation. Seeds were air-dried at room temperature and stored in paper bags at room temperature until further use.

Experimental design and treatments

To establish the microcosms, we used 15 plastic containers with an internal dimension of 55.5 cm × 35.5 cm × 27.5 cm (l × w × h). Five holes (2 cm diameter) were drilled in the bottom of each container to ensure free drainage of excess rainwater. The microcosm containers were placed in a common garden on a sheet of plastic foil to avoid roots from penetrating the soil below the microcosms. Each microcosm was divided in twelve plots (3 by 4; 11 cm × 13 cm) with a border zone of 2 cm between the plots and the edge of the container. The borderlines between the 12 plots in each microcosm were marked with metal pins on the edge of the containers; no physical demarcation was put. The containers were filled with a 3:1 volume ratio mixture of sand and unimproved peat. Early September 2006, five common grassland grass species (*Agrostis capillaris*, *Festuca rubra*, *Holcus lanatus*, *Poa pratensis* and *Poa trivialis*) were sown into the microcosms to establish an initial vegetation, as well as two herbaceous species common in grasslands (*Trifolium pratensis* and *Trifolium repens*). All of these species, except *Holcus lanatus*, were purchased from a commercial supplier.

The microcosms were treated with three levels of a full liquid NPK fertilizer (including micronutrients and trace elements) corresponding to 0, 10 and 40 g N/m²/year (referred to as low, intermediate and high fertility). Each microcosm received only one level of fertilizer, resulting in 5 replicate microcosms per fertilizer level. A single dose of fertilizer, corresponding to 1/4 of the yearly dose, was added in September 2006, whereby the low fertility treatment received the same level as the intermediate treatment in order to promote initial establishment of a plant cover. During 2007, the fertilizer application was equally spread over four monthly doses (April – July).

Mid-November, we added seeds of the 25 target species to the microcosms. Target species were applied at two densities, 15 and 45 seeds per

Table 1. List of species used in the microcosm experiment with their seed mass and CSR strategy. Species marked with (*) were sown to form the initial vegetation.

Species	seed mass	Strategy
<i>Achillea millefolium</i>	0.14	CSR
<i>Agrostis capillaris</i> *	0.07	CSR
<i>Alopecurus pratensis</i>	0.84	C/CSR
<i>Antbriscus sylvestris</i>	3.21	C/CR
<i>Briza media</i>	0.35	S/CSR
<i>Centaurea jacea</i>	2.15	S/CSR
<i>Cynosurus cristatus</i>	0.59	R/CSR
<i>Dactylis glomerata</i>	0.42	C/CSR
<i>Deschampsia cespitosa</i>	0.27	SC/CSR
<i>Festuca rubra</i> *	0.79	CSR
<i>Filipendula ulmaria</i>	0.41	C/SC
<i>Filipendula vulgaris</i>	0.75	S/CSR
<i>Galium boreale</i>	0.51	S/CSR
<i>Geum rivale</i>	1.24	S/CSR
<i>Holcus lanatus</i> *	0.42	CSR
<i>Hypericum maculatum</i>	0.04	CR/CSR
<i>Hypericum perforatum</i>	0.09	CR/CSR
<i>Medicago lupulina</i>	1.69	R/CSR
<i>Pastinaca sativa</i>	2.79	CR
<i>Plantago lanceolata</i>	1.56	CSR
<i>Primula veris</i>	0.86	S/CSR
<i>Ranunculus acris</i>	1.58	CSR
<i>Poa pratensis</i> *	0.18	CSR
<i>Poa trivialis</i> *	0.14	R/CSR
<i>Rumex crispus</i>	1.29	CR/CSR
<i>Rumex obtusifolius</i>	1.14	C/CSR
<i>Serratula tinctoria</i>	2.20	SC/CSR
<i>Silene vulgaris</i>	0.52	CSR
<i>Tblaspi arvense</i>	1.39	R
<i>Trifolium pratense</i> *	2.14	CSR
<i>Trifolium repens</i> *	0.69	CR/CSR
<i>Trollius europaeus</i>	0.66	SC/CSR

species per plot at low and high sowing density, respectively. The different densities were randomly assigned to either half in each microcosm (6 plots for each density). The microcosms were watered frequently until the 25 species were sown in November. Hereafter, they received water four times only, that is when the liquid fertilizer was applied.

The disturbance treatments included: (1 & 2) clipping one gap with a diameter of 6 cm either in autumn (1) or spring (2); (3 & 4) clipping 4 gaps with a diameter of 3 cm, adding up to the same combined area as a single 6 cm gap, either in autumn (3) or spring (3); (5) clipping the whole vegetation in the plot in both autumn and spring; and (6) untreated control. The autumn clipping took place immediately after the seed addition and the spring clipping in mid-May. The vegetation was clipped down to 1.5 cm above the soil surface on each occasion. The disturbance treatments were randomly distributed within each half of the microcosms, such that every microcosm included one plot of every combination of disturbance treatment and seed density.

Late august 2007, the microcosms were harvested in random order. The vegetation was clipped at the soil surface, sorted to species and dried at 70 °C to constant weight.

During the harvest, it appeared that the two *Hypericum* species could not be distinguished easily, and they have therefore been lumped into one group. *Alopecurus pratensis*, *Cynosurus cristatus*, *Dactylis glomerata*, *Filipendula ulmaria* and *Poa trivialis* were not observed in any of the plots. Either they have not emerged or survived until the harvest period or they have been overlooked because of very low abundance.

Data analysis

The effects of the fertility and disturbance treatments and the sowing density on species diversity, biomass production and litter production were analyzed with a factorial Analyses of Variance

(ANOVA).

We ran a PCA ordination on the relative species abundance (biomass) in plots on a variance-covariance matrix to reduce the variation in the original floristic data. The data were transformed such that the ordination was based on the Hellinger distance instead of Euclidian distance, which is more suitable for data that is a proportional frequency value (Legendre & Legendre 1998; Legendre & Gallagher 2001). Loadings on the first Principal Component (PC-1) were subsequently analyzed with an ANOVA to test for the effects of fertility, disturbance treatment and sowing density.

A measure of dissimilarity in species composition between all subplots was obtained by calculating all between-subplot Czekanowski dissimilarity coefficients. These inter-plot dissimilarities were analyzed with a one-way ANOVA.

The ANOVAs were carried out using SPSS (SPSS 15.0 for Windows, SPSS Inc, 2006). For factors with more than two levels, ANOVAs were followed by a post-hoc Tukey test to compare the effects the different levels. An alpha value of 0.05 was used in all analysis. The PCA ordination and the calculation of Czekanowski dissimilarity coefficients was done using the program PC-ORD (McCune & Mefford 1997)

Results

Vegetation development in the microcosms

A couple of weeks after sowing of the five grasses and the *Trifolium* species, the microcosms were dominated by *Holcus lanatus*, *Festuca rubra* and *Trifolium repens* (in decreasing order of abundance). The vegetation in the HF microcosms was dense and relatively tall, while the other microcosms, which received the same fertilizer dose in autumn, were characterized by a more open and shorter sward. During winter, the above-ground

Table 2. Means \pm standard deviation for species richness, biomass and litter in the different treatment combinations.

Treatment		Species richness	Biomass	Litter
Fertility	High	7.78 \pm 1.92	1.47 \pm 0.78	1.22 \pm 0.57
	Intermediate	15.55 \pm 2.43	1.69 \pm 0.44	1.11 \pm 0.45
	Low	12.1 \pm 2.54	0.56 \pm 0.22	0.51 \pm 0.34
Disturbance	Spring small	12.03 \pm 3.95	1.24 \pm 0.61	0.98 \pm 0.58
	Spring large	11.83 \pm 3.77	1.34 \pm 0.68	1.03 \pm 0.58
	Autumn small	11.6 \pm 4.29	1.28 \pm 0.75	0.98 \pm 0.54
	Autumn large	11.8 \pm 3.93	1.29 \pm 0.82	0.89 \pm 0.48
	No gaps	11.87 \pm 4.40	1.47 \pm 0.83	1.16 \pm 0.62
	Clipped	11.73 \pm 3.48	0.83 \pm 0.47	0.63 \pm 0.40
Sowing Density	High	12.96 \pm 3.69	1.29 \pm 0.73	1.13 \pm 0.55
	Low	10.67 \pm 3.84	1.20 \pm 0.72	0.75 \pm 0.49

Table 3. ANOVA of sowing density, fertility and disturbance treatments on litter, species richness and biomass.

Treatment		d.f.	F	p
Sowing density	Litter	1	40.845	0.000
	Species richness	1	55.837	0.000
	Biomass	1	1.608	0.207
Fertility	Litter	2	55.662	0.000
	Species richness	2	215.188	0.000
	Biomass	2	94.896	0.000
Disturbance	Litter	5	6.059	0.000
	Species richness	5	0.147	0.981
	Biomass	5	6.201	0.000
S x F	Litter	2	0.065	0.937
	Species richness	2	3.388	0.036
	Biomass	2	3.228	0.043
S x D	Litter	5	0.583	0.713
	Species richness	5	0.135	0.984
	Biomass	5	1.184	0.320
F x D	Litter	10	1.241	0.270
	Species richness	10	1.249	0.265
	Biomass	10	1.964	0.041
S x F x D	Litter	10	0.784	0.644
	Species richness	10	0.364	0.960
	Biomass	10	1.467	0.157

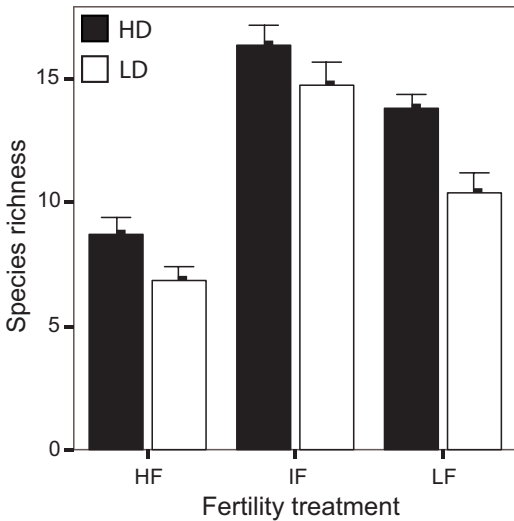


Figure 1. Effects of fertility and sowing density on species richness in the microcosms. HF, IF and LF stand for high, intermediate and low fertility, respectively. HD and LD are high and low density seed sowing. Main effects of fertility and density were significantly different at all levels. See table 2 for details on the ANOVA.

shoots died back to a large extent. Low winter temperatures were interrupted by a relatively warm spell during January 2007, after which the microcosms were covered with snow until early March 2007. A very dry period during the first half of June caused the aboveground vegetation to die-back considerable. The drought had the largest impact on the vegetation in the HF microcosms, leaving a lot of litter from dead *Holcus lanatus* shoots which had been dominating the sward until the drought. After the drought, *Festuca rubra*, which was only present at very low abundance before the die-back of *Holcus lanatus*, quickly colonized the HF microcosms.

Species diversity was higher in IM and LF microcosms than in the HF plots. Despite the massive dieback of *Holcus lanatus* during the drought, the standing biomass was highest in the HF plots. Also the amount of litter differed significantly across fertility treatments (Table 2 and

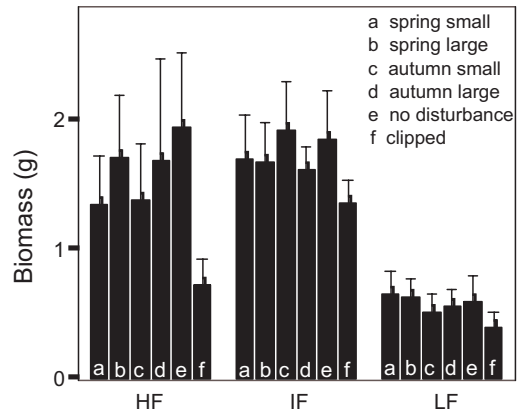


Figure 2. Effects of fertility and disturbance treatment on species richness in the microcosms. HF, IF and LF stand for high, intermediate and low fertility, respectively. Main effects of fertility levels, the clipped treatments differed significantly from the other disturbance treatments. See table 2 for details on the ANOVA.

3). The amount of litter and biomass differed with disturbance treatment, but species diversity did not vary with disturbance level. Sowing density, on the other hand, caused significant differences in litter production and species diversity, but did not affect the total biomass (Table 2 & 3; Fig. 1 & 2).

Species composition and similarity

The fertilizer application treatment had resulted in a striking, highly reproducible species composition pattern across the microcosms. The HF microcosms were relatively species poor; *Festuca rubra* dominated all plots, while *Rumex crispus* and *Rumex obtusifolius* were present in the majority of the plots and *Cardamine hirsuta* had colonised the plots spontaneously and was present in all HF microcosms, but absent in the others. Other species found frequently in HF plots included *Silene vulgaris*, *Agrostis capillaris*, *Hypericum* spp, *Plantago lanceolata*, *Geum rivale* and *Galium boreale*. Despite the large difference in fertilizer application, the

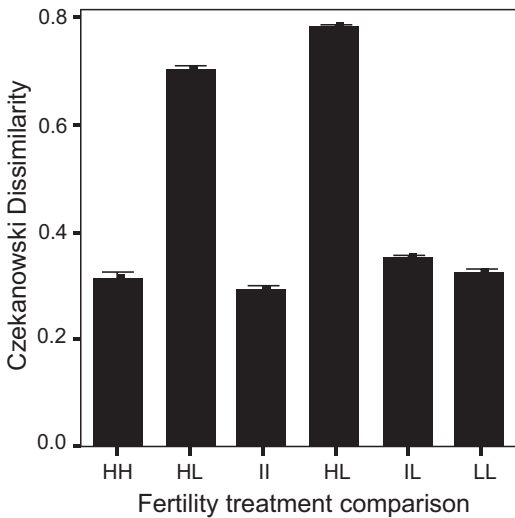


Figure 3. Pairwise comparison of similarity in species composition of all high (H), intermediate (I) and low (L) fertility subplots.

IF and LF microcosms showed a relatively high similarity in species composition. Both were characterized by a relatively high abundance of *Holcus lanatus* and were generally more species rich than were the HF microcosms. However, due to the apparent shortage of nutrients, the vegetation in the LF microcosms was scarcer and *Holcus* was less dominant and the shoots were shorter. *Festuca rubra* was present as subordinate species throughout all LF and IF plots. The remainder of the target species (except both *Rumex* species and *Silene vulgaris*) were found regularly in the IF and LF plots, although more species were absent from the LF plots and low sowing density plots.

The PCA ordination revealed one major floristic gradient, separating the HF microcosms from the IF and LF microcosms. The first PCA axis explained 65% of the variation in the original distance matrix. Since the second axis added only 11 % to the total variation explained, we did not consider more than one axis. The loadings of the first PCA-axis differed significantly across fertility treatments ($F_{2,14} = 1064$, $p < 0.001$) but not across

disturbance treatments ($F_{5,14} = 0.597$, $p = 0.702$) or seed density ($F_{1,14} = 0.008$, $p = 0.930$).

One-way ANOVA on pair-wise Czekanowski distance between pairs of subplots within and between fertility treatments showed that the dissimilarity in species composition was smaller within fertility treatments than between any combination of different fertility levels ($F_{5,16} = 6223.2$, $p < 0.01$, Fig 3). The highest dissimilarities were found between HF-IF and HF-LF. The degree of similarity was highest within IF subplots. Within HF and within LF were equally similar.

Discussion

Before one can answer the question whether species assemblages are repeatable, the question 'how similar is similar?' (Wilson *et al.* 1996) needs to be answered. Wilson *et al.* (1996) addressed the question 'do species assemblages ever recur?' and defined a baseline of similarity. If the similarity of two sites transgresses this baseline, they can be regarded as similar. In their study of road verge vegetation in Spain and New Zealand, the baseline was set as the average similarity of a large number of pairs of adjacent or nearby vegetation quadrates.

In our experiment, we compare the within-treatment similarity of plots to the between-treatment similarity. Our results clearly show that patterns in species composition were repeatable at similar levels of soil fertility, indicating strong deterministic processes in community assembly. High average dissimilarity between the HF microcosms on the one hand and the IF and LF microcosms on the other hand was found, while average dissimilarity within treatments was much lower. However, average within-treatment dissimilarity did not increase with decreasing fertility as expected from the assumption that competitive asymmetry would be more pronounced in the HF microcosms, resulting in lower invisibility (*sensu* Bruun & Ejrnæs 2006).

Due to the severe drought during early summer, mortality was highest in the HF microcosms, apparently leaving much open ground for colonization. The high availability of nutrients resulted in a sward of fast-grown plants with larger and thinner leaves, features which are common among competitive plants in highly productive environments (Gaudet & Keddy 1988; Hodgson *et al.* 1999) but which has the disadvantage of increased drought susceptibility (Wang *et al.* 2007).

After the drought and dieback of the dominant *Holcus lanatus*, the vegetation recovered quickly through re-growth of the formerly subordinate *Festuca rubra*. Many seedlings or small plants of target species that were present in the HF plots before the drought had died. The drought had also opened up the possibility for *Agrostis capillaris* to co-dominate some of the subplots at HF, whereas they were scarce or absent from some others. The apparently stochastic occurrence of *Agrostis capillaris*, *Silene latifolia*, *Deschampsia cespitosa* and *Briza media* in some HF plots has probably led to the slightly higher Czekanowski dissimilarity within the HF treatment than within the IF microcosms. Dissimilarity within the LF treatment was higher than within the IF treatment, probably due to a more open/stochastic community assembly, but also to higher mortality in the LF treatment allowing for faster turnover of individuals and species. The amount of litter in the LF microcosms was on the same order of magnitude as the remaining living biomass, whereas it was relatively smaller at IF (Table 2).

The natural drought acted as a strong disturbance and impacted the HF microcosms most. In the LF treatment, the plants were severely stressed by the low availability of mineral nutrients. A conservative growth strategy, i.e. slow growth, high longevity of tissues, and resistance to damage, are common features of stress adapted plants. Stress adapted plants can therefore be expected to be tolerant of several stress factors simultaneously (Levitt 1975; Grime 2001). However, stress factors

are also reported to have additive effects, e.g. shade and flooding (Lenssen *et al.* 2003) or shade and water availability (ten Brink & Bruun 2007). The combination of the severe drought and chronic low nutrient availability has led to high mortality in the microcosms. The similar species composition between IF and LF microcosms - all species occurring at IF have also been observed at LF - suggest that our species pool did not include any species that were really adapted to the combined effects of drought and low fertility. The range of CSR values in Table 1 shows that stress-adapted plants were underrepresented in our study. This may have arisen from our practical need for easily collectable seeds in the field in order to obtain sufficient quantities for a replicated experiment.

The compositional differences along the fertility gradient were accompanied by a clear trend in species richness, biomass and litter production. The overall productivity in the microcosms was relatively low, likely due to soil moisture limitation. Visual inspection before the drought revealed that the sward was higher and denser in the HF microcosms, with a number of seedlings of several species also found in the IF and LF plots. We can only speculate whether the seedlings would have been outcompeted by the dominating *Holcus* if the drought spell had not come. If the period of drought had been shorter, *Holcus* might have survived owing to their better developed root system, whereas even a short drought would likely be fatal for most seedlings.

The species composition did not vary with disturbance. The clipping treatment resulted in a lower biomass than in all other treatments. This difference increased with fertility explaining the significant interaction between disturbance treatments and fertility (cf. Foster *et al.* 2004). The clipping apparently affected the dominant grass species before they could initiate their lateral expansion underground, which in turn apparently prevented the re-growth and re-colonisation to cancel out the initial differences. Despite the low productivity, the disturbance treatments did not

create strong significant differences in biomass production apart of the complete clipping; neither did it affect the species richness. This may be explained in several ways. One explanation is that the drought had such a overwhelming effect on the assembly process, suppressing many drought sensitive species, which might otherwise have benefited from the competitive relaxation caused by the disturbance treatments. Alternatively, the disturbance treatments may not have created sufficient differences in the structure of the sward to benefit species that otherwise would have been suppressed. A higher intensity and more frequent disturbance would likely have yielded different results.

We conclude that deterministic processes play an important role in the assembly of plant communities. Although our microcosm experiment did not disentangle the effects of environmental filtering on the one hand and competition and other species interactions on the other hand, we conclude that the abiotic environment strongly influences community assembly. The abiotic regime effectively acts as beta-niche filter through direct selection on plant traits and indirectly via the effect on species composition and competitive hierarchies. Furthermore, we conclude that microcosm experiments are useful tools to address questions on the abiotic and biotic controls of plant community processes. However, our experience with the severe drought emphasizes the importance of recognizing the effects of processes other than those experimentally manipulated in the interpretation of experimental results (Huston 1997; Huston & McBride 2002).

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