Recruitment in species-rich grasslands: the effects of functional traits and propagule pressure

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Abstract

Aims

The assembly of plant communities is a complex process which combines impacts from the species pool, dispersal and propagule pressure, niche requirements of colonizing species and the niche structure of the community. Recent theory development has incorporated all these aspects, e.g. in 'stochastic niche theory'. We investigated recruitment into a species-rich grassland community, using an experimental approach where we manipulated the trait composition of the community and examined the success of colonizing species entering with various propagule pressure. Specifically, we examined two predictions: (i) colonization success increases with increasing difference between traits of the colonizing species and the trait profile of the community and (ii) colonization success increases with increasing propagule pressure.

Methods

The examined communities were species-rich semi-natural grasslands located in southern Sweden. After a careful documentation of the composition of the plant communities at the experimental sites, we manipulated the trait profile of species-rich grassland plots based on the plant functional trait specific leaf area (SLA), which is correlated with several key life history functions. In addition to SLA, seed mass was also used to describe the trait profile of grassland plots. Seeds of 12 plant species from the regional species pool, varying in SLA and seed mass, were sown into plots using four different levels of propagule pressure. Recruitment was examined after 1 year. We also planted juvenile 'plug plants' of the same species which allowed us to examine survivorship and growth beyond the seedling stage.

Important Findings

Overall we found very limited evidence for relationships between the traits of the colonizing species and the trait profile of the community and for recruitment after sowing these relationships were contrary to the prediction. Survival of plug plants after two seasons of growth was high irrespective of the trait profile of the community, but growth of plug plants was affected by the trait profile of the surrounding community. For four of the species there was a positive effect of increased propagule pressure on colonization. The results suggest that species assembly in species-rich grasslands is not strongly dependent on the niche structure of the community. However, the finding that colonization of only a third of the species responded positively to increased propagule pressure indicates that there might be niche-related effects that were not captured by our treatments. Overall, our results indicate that the factors determining colonization in this community are species specific. Some species are able to colonize irrespective of niche relationships, provided that the propagule pressure is sufficiently high to overcome stochastic mortality after seed arrival. For other species, however, we cannot exclude that niche assembly occurred, but we failed to identify the relevant niche factor.

Keywords: assembly rules • recruitment limitation • seed size • semi-natural grassland • SLA

INTRODUCTION

A central question in both basic and applied ecology is how communities are assembled from available species pools. Community assembly is a complex process that incorporates both the mechanisms affecting the entry of species into a community, and the mechanisms that allow species to persist after colonization. The present understanding of assembly processes can be described as recognition of a continuum of mechanisms where the end points represent 'niche assembly' and 'neutral (or

stochastic) assembly' (Gravel *et al.* 2006). Niche assembly is based on inter-specific competition as a dominating interaction structuring communities. Species utilize and divide available niche space and are constrained by limiting similarity, i.e. species with similar niche requirements cannot coexist (Chase and Leibold 2003; Hutchinson 1957; MacArthur and Levins 1967). Also facilitative mechanisms may influence assembly processes, thus broadening the impact of niche relationships to not just competition (Gilbert *et al.* 2009). Neutral assembly implies that species are considered neutral with respect to each other and that they have an equal ability to colonize a community (Hubbell 2001). Local species composition would thus reflect the inflow of species from the regional species pool, i.e. the propagule pressure, which implies that communities are basically open for colonization irrespective of the traits of colonizers. Most likely, the assembly of natural communities involves both these kinds of mechanisms (e.g. Ernest *et al.* 2008; Myers and Harms 2009a, 2009b), calling for a conceptual unification of assembly theories (Gravel *et al.* 2006; Tilman 2004; Vellend 2010). Tilman (2004) suggested a 'stochastic niche theory' based on the premises that successful recruitment into a community depends both on passing a sieve of stochastic mortality immediately following the colonization, and a sieve reflecting available resources which depend on the niche utilization of the resident species. Instead of focusing on niche vs. neutrality as alternative models of assembly, the real issue is instead the quantitative impact of these various mechanisms, incorporating those suggested by niche, neutral and stochastic models.

A corollary of the insight that assembly is dependent on a mixture of mechanisms involving community niche structure, species niche requirements, the inflow of species into communities as well as stochastic processes relating to the success of incoming species, is that species may vary among themselves in how they are influenced by these mechanisms. The prime objective of this study was to examine some mechanisms affecting community assembly, focusing on a selection of species examined individually. We investigated recruitment processes in species-rich grassland communities in Sweden, using 12 species that were experimentally introduced into communities subjected to various treatments. These kinds of grasslands, often called 'semi-natural grasslands', previously covered extensive areas in Northern Europe, as a result of a long tradition of grazing and mowing, but they are now much reduced and fragmented due to land use change (Eriksson *et al.* 2002). The investigated grasslands have very high small-scale species richness with up to 41 species per 0.09 m^2 (results, this paper) and would thus a priori be considered as having an almost saturated niche space. However, in contrast to that expectation, previous studies have indicated that these grassland communities, despite being extremely species rich, may be open for colonization for species occurring in the regional species pool (Eriksson *et al.* 2006). This is congruent with studies from other similar systems, which have documented a high species turnover (Stampfli and Zeiter 2004; Sykes *et al.* 1994) as well as continual regeneration of annual, biennial and short-lived perennial species (Grubb 1976).

Most previous studies on how the functional trait composition of the resident plant community affects recruitment and establishment have used discrete functional groups as the niche variable, often based on alleged differences in resource use among groups. Some commonly studied groups are grasses (C3 and C4), legumes and forbs (Emery 2007; Fargione *et al.* 2003; Symstad 2000; Turnbull *et al.* 2005a). For example, Fargione *et al.* (2003) found that introduced species in experimental plots established in old fields performed worst in plots where the same functional guild (expressed as C3 grasses, C4 grasses, legumes and forbs) was abundant. In our study, we used a different approach. Instead of manipulating the abundance of discrete functional groups, the distribution of a continuous functional trait was manipulated. We used the trait specific leaf area (SLA) (ratio of leaf mass to surface area), which has been shown to be correlated with several important life history characters such as resource acquisition and use, plasticity and relative growth rate (Diaz *et al.* 2004; Weiher *et al.* 1999; Westoby *et al.* 2002). Thus, our assumption was that SLA is useful as a proxy for describing and manipulating the available niche space, where species positions along the SLA axis represent different life history strategies and niche requirements. In addition, we also described the manipulated communities in terms of seed mass. Seed mass has been linked to trade-offs involved in niche differentiation, such as the competition–colonization trade-off and the establishment–colonization trade-off (e.g. Coomes and Grubb 2003). Generally, these trade-offs predict that a larger seed mass gives an advantage during establishment, either by improving competitive ability or by increasing tolerance to harsh environmental conditions. We were not able to manipulate both SLA and seed mass because the design would then become too large if kept orthogonal. Instead we used the natural variation in seed mass among plots and any variation that was caused indirectly by the SLA manipulation.

For each of the 12 species, we included in the experiment, we examined the prediction that recruitment is lower for a colonizing species if the community has a functional trait profile similar to the colonizing species (i.e. limiting similarity operates) compared to communities that are functionally dissimilar to the colonizing species. We added seeds of species with different functional trait values to grassland communities where the trait profile was either manipulated (SLA) or described (seed mass). Juvenile plants were transplanted into grassland plots in order to examine if any effects also appear beyond the seedling stage. We also examined the prediction that an increased propagule pressure increases recruitment of a colonizing species, irrespective of the trait profile of the resident community. We did this by sowing species at four different propagule pressures, both higher and lower than the observed natural seed rain.

MATERIALS AND METHODS

Field site

The study was carried out in Nynäs nature reserve, c. 100 km south of Stockholm, Sweden (50°50′N, 17°24′E). Nynäs

nature reserve contains a mosaic of species-rich semi-natural grasslands, arable fields, ex-arable fields and various types of forests, mainly coniferous or mixed deciduous–coniferous forests. The experiment was established in semi-natural grasslands in the reserve, within a 2.0 × 2.5 km area. The semi-natural grasslands are herb-rich, have high species evenness and are characterized by very high small-scale species richness, containing up to 41 species per 0.09 m^2 (results, this study). The soil conditions of the grasslands are fairly homogeneous with pH in the range 5.6–6.1, available phosphorous content in the range 0.9–1.8 mg/100 g dry soil and available ammonium content is in the range 0.11–1.83 mg/100 g dry soil (Öster, unpublished work). Mean temperature in the summer averages 16°C (July) and in winter 3°C (February), and the annual mean precipitation is 550 mm.

Experimental set-up: seed sowing

Within the semi-natural grasslands a total of 96 rectangular areas of the size 2.0 × 1.0 m were established in May 2007. Each rectangle was subdivided into eight 0.3 × 0.3 m plots, with a 0.2 m buffer zone between them (Fig. 1). Plots were assigned community treatments and four propagule pressures using a design where all used levels of propagule pressure (see below) were used within the same large (2.0 × 1.0 m) rectangle. We did not have one single homogenous area of spe-

Buffer zone

0.5 m
0.3 m
0.2 m
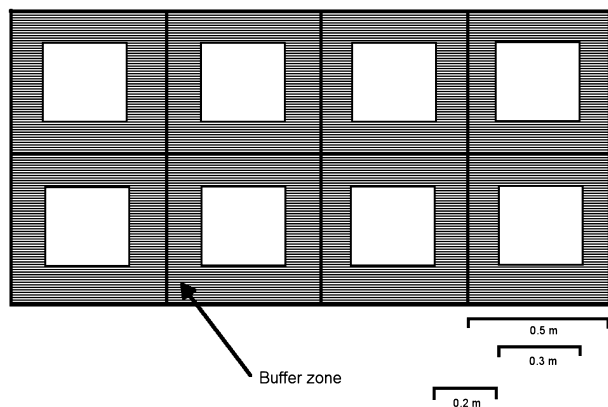
Treatment combinations for each row

C 0	R 0	N 0	Empty	× 4 rows
C 12	C 30	C 55	C 75	× 4 rows
R 12	R 30	R 55	R 75	× 4 rows
N 12	N 30	N 55	N 75	× 12 rows

Figure 1: a schematic illustration of the design of each 2 × 1 m rectangle containing eight 0.3 × 0.3 m plots separated by a 0.2 m buffer zone. One row contains one treatment [SLA treatment (N), random removal treatment (R) or control (C)] and all four levels of propagule pressure (12, 30, 55, 75 seeds per plot) or the propagule pressure controls (0 seeds). The inset figure shows the allocation of treatments among the 24 rows, using 12 rectangles per site. The eight different sites contained a similar and balanced set of treatments.

cies-rich grassland available that could accommodate the whole experimental design. Instead, eight different grassland sites were used, and 12 rectangles were placed within each site. These 12 rectangles were spatially close to each other (within a 10 × 20 m area) and together they contained all combinations of propagule pressure and treatment, in a constant proportion among sites. The focus of this study is the response of seed, seedlings and juveniles to their local surrounding community, and the distribution of all treatments (as described below) was balanced across the sites. Thus, we considered the response of each species to plots-wise conditions as the basic unit of study.

During the period 2 July–10 August 2007, each of the 768 plots was surveyed, and identity and percent cover of each plant species were recorded. The total cover of all species could sum to >100% since the canopy of species sometimes overlapped. Cardboard pieces of known sizes were used to aid the measurements in order to minimize bias. Bare ground was estimated in the same way as for species. Due to the omission of an initially planned treatment concerning the effects of bryophytes on recruitment, 32 plots were not included in the final analyses, so the total number of plots was 736.

We used SLA and seed size to describe the functional trait profile in the plots. Data on SLA were collected from a previous study in the nature reserve (Quested *et al.* 2007) and from a parallel study in the same study sites as ours (Marteinsdottir and Eriksson, unpublished work), where leaf trait measurements were collected for the most common species (up to 80% cumulative field layer), using standard protocols (Cornelissen *et al.* 2003). Data on seed mass was compiled from studies also conducted in the study area (Jakobsson and Eriksson 2000; Quested and Eriksson, unpublished work).

A manipulation of the functional trait profile (SLA) was done by removing (by hand pulling) species from the plots. Usually five or more species were removed, but in some cases, depending on the species composition in the plot, only two to three species were removed. In order to create a between-plot variation in the SLA profile, species with similar SLA values (comparatively 'low' or 'high' SLA, respectively) were removed within each of four propagule pressures (12, 30, 55 and 75 seeds × species^{-1} × plot^{-1}, see below), keeping the propagule pressure treatment independent from the SLA treatment. As most grassland species are perennial, and it was impossible to also remove their roots and rhizomes, we expected them to re-sprout with time. Thus, the created disturbance of the communities can be viewed as 'windows of opportunity for recruitment.

To describe the position of each species in trait space (SLA, seed mass) compared to all other species in the plot, we calculated a trait similarity value (called *S*) for each sown species in each plot. This was done by summing, for all species in the plot (*n*), the product between abundance (percent cover) of species *i* (p_i) and the difference (absolute value) in the selected trait between the sown species *j* (trait$_j$) and the species *i* (trait$_i$) in the community. A 0 value means that the community is totally similar to the sown target species.

$$S_j = \sum_{i=1}^{n} p_i \times |\text{trait}_j - \text{trait}_i|.$$

Using species abundance data for each species (p_i) in each plot and species trait values (trait_i), we also calculated aggregated plant trait values for each plot using the formula:

$$\text{trait}_{\text{plot}} = \sum_{i=1}^{n} p_i \times \text{trait}_i.$$

In addition to the trait manipulations, treatment controls were established. In one control, species were removed randomly irrespective of trait value (SLA), creating similar sized gaps (in terms of cover and number of removed species) in the sward. In a second control, the whole community was left intact.

Into the plots, we added seeds from 12 plant species, consisting of three grasses, seven forbs and two legumes, which differed in SLA and seed size (Table 1). All 12 species occur in the semi-natural grasslands within the reserve. However, for practical survey reasons, the selected species were not among the most abundant species in the grasslands. Wild collected seeds from southern Sweden were delivered from a commercial supplier (Pratensis AB, www.pratensis.se), except for *Anthriscus sylvestris* which we collected within the study area. Seeds were sown in September 2007. The viability of the seeds was confirmed in greenhouse germination trials. Seeds were hand broadcast into the plots, and the sward was gently shaken in order for the seeds to fall down and come in contact with the soil.

Four different propagule pressures were used based on previous research in the study area, which reported an average natural seed rain of 3 820 seeds $\times$ m^{-2} with a range up to 17 600 seeds $\times$ m^{-2} (Jakobsson *et al.* 2006). Using equal numbers of seeds for each of the 12 species, the total propagule pressures, we used were 1 600, 4 000, 7 333 and 10 000 seeds $\times$ m^{-2}, translating to 12, 30, 55 and 75 seeds $\times$ species^{-1} $\times$ plot^{-1}. This is also similar to the propagule pressure (12–100 seeds $\times$ dm^{-2}) used by Lindborg (2006), who reported negative density effects of propagule pressures at 100 seeds $\times$ dm^{-2}, similar to our highest propagule pressure. The four chosen levels of propagule pressure were thus in the range of the normal seed rain and encompassed a relevant range up to a threshold (2.5 times the average natural seed rain) where previous research have reported negative density effects on the seedlings. For each of the community treatment and treatment controls, plots were also left without seed sowing, in order to document natural recruitment. Recruitment in all plots was recorded in August 2008 reflecting germination and first summer survival.

We used 416 plots for the SLA treatment (96 for each of the four levels of propagule pressure and 32 left unsown), 160 plots for the random removal treatment (32 for each level of propagule pressure and unsown) and 160 plots for intact community (32 for each level of propagule pressure and unsown), making a total of 736 plots.

Experimental set-up: planted juvenile plants

Effects observed on germination and early survival may not translate to later life cycle stages, and therefore, we also planted established (near flowering size) juvenile plants ('plug plants') of the same species in 1 m^2 plots adjacent to the seed sowing plots. Plug plants were obtained from the same commercial supplier as the seeds (Pratensis AB, www.pratensis.se) and were grown using the same seed sources. These plants were of uniform size and had been growing for a full summer in ~93 cm^3 of soil. *Anthriscus sylvestris* could not be obtained so only plants from 11 of the species were planted. A total of 2 058 plug plants were planted in early October 2007, with 44 plants per 1 m^2 plot (four individuals of each species), in a total of 47 plots, using a fixed planting schedule. Survival and performance data of the plug plants were collected in 2008 and 2009 but only the last measurement (September 2009) was used in the analyses. Performance of plug plants was estimated by measuring leaf width and rosette diameter (when applicable). Leaf width and rosette diameter were assumed to reflect plant size, and they were also tightly correlated ($r = 0.92$, $P < 0.001$). We did not have any data on the species community in the plots where plug plants were planted. Instead, we used mean values of the four adjacent experimental plots.

Analyses

Two species (*Campanula persicifolia* and *Dianthus deltoides*) had too low seedling recruitment to be analyzed individually. Recruitment of the species was zero inflated and showed overdispersion in tests using Poisson errors. To handle this problem, we used zero-inflated negative binomial 'hurdle' models, with species richness and trait similarity values as predictors. Random factors cannot be included in these models. In hurdle models, zeroes are considered 'true zeroes', i.e. no occurrence

Table 1: plant species that were used in the experiment and their trait values

Species	Life form	SLA (mm^2/mg)	Seed mass (mg)
Anthriscus sylvestris (L.)	Forb	38.6	3.730
Briza media L.	Grass	22.7	0.230
Campanula persicifolia L.	Forb	16.1	0.053
Centaurea jacea L.	Forb	20.2	1.064
Dianthus deltoides L.	Forb	24.9	0.141
Festuca pratensis L.	Grass	22.8	1.250
Helictotrichon pubescens (Huds.) Pilg.	Grass	15.9	1.310
Hypericum maculatum Crantz	Forb	33.0	0.030
Knautia arvensis (L.) Coult.	Forb	19.5	2.470
Lotus corniculatus L.	Legume	21.6	1.320
Plantago media L.	Forb	14.9	0.283
Trifolium pratense L.	Legume	24.3	1.625

Measurements of SLA and seed mass are described in the text.

of a seedling has been missed (Zuur *et al.* 2009). Zeroes versus non-zeroes were modeled using a binomial distribution, and any relationship describes the requirement for successful recruitment in a plot (irrespective of number of seedlings). The non-zero presence values were modeled with a zero-truncated negative binomial model, and any relationship describes the requirement for establishing more than one seedling. The analyses were made for each species individually. For each species, we used a Bonferroni adjustment of the significance level to 0.00625 (0.05/8), since we made two types of analyses for each of the four different levels of propagule pressure.

Differences in survival of plug plants between species were examined with a Kruskal–Wallis analysis of variance (ANOVA) due to a non-normal distribution. Relationships between survival of plug plants (using plot means of species) and plot-wise abundance weighted trait values were examined with Spearman Rank Correlations. Leaf width was standardized for each species by dividing with the mean, allowing for comparisons across species. Relationships between leaf width and plot variables were examined with multiple regressions, including plot-wise species richness in and trait values. Analyses were performed using STATISTICA 8.0 except for the hurdle models that were performed using the pscl package in R version 2.9.2.

The overall result from the propagule pressure treatment was analyzed with ANOVA, with total recruitment as response variable. The effect of propagule pressure on the recruitment of the individual species was analyzed with Kruskal–Wallis ANOVA due to the data not being normally distributed.

RESULTS

In total, 113 species were found in the 736 plots, with a mean of 25.47 ($\pm$4.8 SD) species per subplot (range 15–41 species). A total of 16 663 seedlings were recorded, with a mean of 22.6 $\pm$ 12.9 SD (range 0–69) seedlings per plot. There was a significant positive relationship between species richness and natural recruitment in the unmanipulated and unsown control subplots ($n = 32$, $R^2 = 0.58$, $P < 0.001$). All further analyses were therefore computed by using species richness as a covariate or as an additional variable in multiple regressions or by using the residuals of the regression between species richness and recruitment as a response variable.

Experimental recruitment was related to species richness, at least for some of the different levels of propagule pressure, for 5 of the 10 species for which an analysis could be performed (Table 2). Of these five species, there was a positive effect of species richness for four species. Strong evidence for a positive

Table 2: recruitment of 10 grassland plant species in relation to two aspects of the trait profile of the community, SLA and seed mass, and four different propagule pressures (12, 30, 55 and 75 seeds $\times$ species^{-1} $\times$ plot^{-1})

Species	Model	Species richness				S (SLA)				S (seed size)			
		12	30	55	75	12	30	55	75	12	30	55	75
Anthriscus sylvestris	Hurdle (count)												
	Hurdle (zeroes)												
Briza media	Hurdle (count)				+								
	Hurdle (zeroes)												
Centaurea jacea	Hurdle (count)	+	+	+									
	Hurdle (zeroes)	+											
Festuca pratensis	Hurdle (count)												
	Hurdle (zeroes)												
Helictotrichon pubescens	Hurdle (count)												
	Hurdle (zeroes)												
Hypericum maculatum	Hurdle (count)												
	Hurdle (zeroes)				−	−		−	−				
Knautia arvensis	Hurdle (count)		+	+									
	Hurdle (zeroes)												
Lotus corniculatus	Hurdle (count)												−
	Hurdle (zeroes)												
Plantago media	Hurdle (count)												
	Hurdle (zeroes)												
Trifolium pratense	Hurdle (count)	+	+	+	+								
	Hurdle (zeroes)												−

Hurdle models were used, including species richness as a variable in order to correct for any relationship between recruitment and species richness in the target plots. A significant positive slope of the model is indicated with a plus (+) and a negative slope with a minus (−). $\alpha = 0.0065$ (Bonferroni adjusted significance level).

relationship, i.e. a significant relationship for more than one propagule pressure, was found for *Centaurea jacea*, *Knautia arvensis* and *Trifolium pratense* (Table 2). For one species, *Hypericum maculatum*, the relationship was negative, but only for the highest propagule pressure (Table 2). There was no effect of amount of bare ground on recruitment (corrected for effects of species richness), neither in the controls nor in the manipulated plots.

The SLA profile in the manipulated plots was related to the recruitment for only 1 of the 10 species, *H. maculatum* (Table 2). The relationship was negative for three levels of propagule pressure, meaning that recruitment decreased when the difference in SLA trait value between *H. maculatum* and the resident community increased. A significant recruitment response to the seed mass profile was found for two species, *Lotus corniculatus* and *T. pratense* (Table 2). For both species, the effect was found for only one level of propagule pressure. As for the single relationship with the SLA profile, the relationships to the seed mass profile were negative, meaning that recruitment decreased when the difference in seed mass between the colonizing species and the resident community increased.

Survival of plug plants was generally high with a mean survival rate of 81.4% after two seasons of growth (Table 3). There were significant differences between species in their survival rate, with *C. jacea* having the highest survival (93.1%) and *H. maculatum* having the lowest survival (54.2%) (Table 3). Survival of plug plants was not related to species richness, plot-wise aggregated SLA value or plot-wise aggregated seed mass value.

Average leaf size of all plug plants in the plots showed a negative relationship with species richness in the plots, indicating that plants were smaller if many species were present ($R^2 = 0.25$, $P = 0.0004$). When investigated individually, five species had a smaller leaf size as species richness increased

(Table 4). Two species had a larger leaf size as plot-wise SLA increased and two species has a larger leaf size as plot-wise seed mass decreased (Table 4). Overall, between 9 and 29% of the variation in leaf size was explained by three plot variables (SLA, seed size, species richness) (Table 4).

The propagule pressure treatment was independent from the species richness gradient (ANOVA, $F_{4,155} = 0.12$, $P = 0.97$), so the general effect of propagule pressure could be analyzed on uncorrected recruitment data. Total recruitment in control plots increased with increased propagule pressure (Fig. 2). Linear and a logarithmic relationships could be fitted equally well ($n = 5$, $P < 0.05$, $r = 0.94$–0.95) to the means in Fig. 1, so it was not possible to determine whether recruitment will increase linearly or asymptotically if propagule pressure is increased beyond 75 seeds × species^{-1} × plot^{-1}. Examined individually, recruitment of four of the species, *Anthriscus sylvestris*, *C. jacea*, *K. arvensis* and *T. pratense*, increased with increasing propagule pressure, indicating that these four species were mostly responsible for the general effect of increased propagule pressure on recruitment (Fig. 3).

DISCUSSION

Empirical research from various systems has so far shown little congruence regarding plant community assembly models. It has been shown, in both experimental and natural successional grasslands, that species abundance distributions are predicted by niche rather than neutral processes and that species competitive ability in terms of uptake of soil nitrogen, governs how communities are assembled (Harpole and Tilman 2006). Several studies have found empirical evidence for niche assembly (Fargione *et al.* 2003; Levine and HilleRisLambers 2009; Questad and Foster 2008; Turnbull *et al.* 2005a, 2005b), while

Table 3: fraction of survivors among transplanted plug plants of 11 grassland species after two seasons of growth in species-rich grasslands

Species	Survival	n
Briza media	0.920[a]	188
Campanula persicifolia	0.644[c,d]	180
Centaurea jacea	0.931[a]	188
Dianthus deltoides	0.904[a]	188
Festuca pratensis	0.872[a,b]	188
Helictotrichon pubescens	0.920[a]	188
Hypericum maculatum	0.542[d]	188
Knautia arvensis	0.856[a,b]	188
Lotus corniculatus	0.747[a,b,c]	186
Plantago media	0.904[a]	188
Trifolium pratense	0.702[b,c,d]	188
In total	0.814	2 058

Due to a shortage of plug plants, the sample was smaller for *C. persicifolia* and *L. corniculatus*. Letters after survival rate depict significant differences ($P < 0.05$) between species (Kruskal–Wallis ANOVA, $H = 218.1$, $P < 0.001$).

Table 4: results from multiple regressions between plant size, measured as leaf width, of juvenile plug plants of 10 grassland species after two seasons of growth and three properties of the target community, species richness (SR), SLA and seed mass trait profile

	SR	SLA	Seed mass	Model summary
Briza media	n.s.	n.s.	n.s.	$R^2 = 0.09$, $P = 0.07$
Campanula persicifolia	n.s.	0.04**	n.s.	$R^2 = 0.29$, $P < 0.001$
Centaurea jacea	n.s.	n.s.	n.s.	$R^2 = 0.13$, $P < 0.05$
Dianthus deltoides	n.s.	n.s.	n.s.	$R^2 = 0.14$, $P < 0.05$
Festuca pratensis	n.s.	n.s.	n.s.	n.s.
Helictotrichon pubescens	−0.03**	n.s.	n.s.	$R^2 = 0.29$, $P < 0.001$
Hypericum maculatum	−0.04**	n.s.	n.s.	$R^2 = 0.25$, $P < 0.01$
Knautia arvensis	n.s.	n.s.	n.s.	$R^2 = 0.16$, $P < 0.05$
Lotus corniculatus	−0.02**	n.s.	−0.16*	$R^2 = 0.26$, $P < 0.05$
Plantago media	−0.03*	0.06**	−0.20*	$R^2 = 0.28$, $P < 0.001$
Trifolium pratense	−0.03*	n.s.	n.s.	$R^2 = 0.15$, $P < 0.05$

n.s. = not significant.
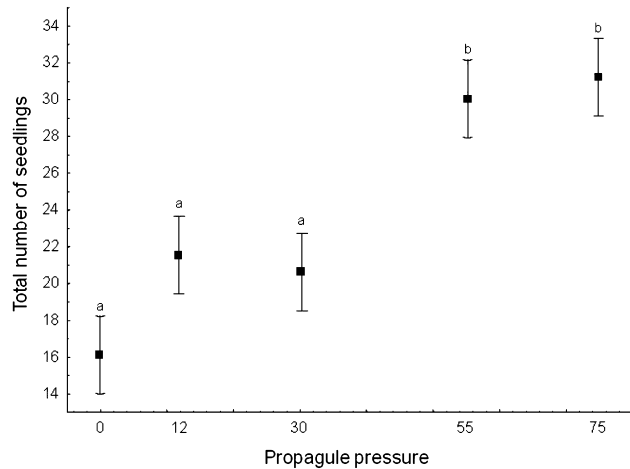*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

Figure 2: total number of seedlings in unmanipulated grassland plots in relation to the propagule pressure expressed as number of added seeds species^{-1} × plot^{-1}. Recruitment increases with higher propagule pressure (ANOVA, $F_{4,155} = 9.48$, $P < 0.001$). Letters indicate significant differences between groups ($P > 0.05$). Error bars show ±1 standard error.

others have found no or only weak evidence (Emery 2007; Symstad 2000; Von Holle and Simberloff 2004). Some studies have found evidence for a combination of niche and neutral (or stochastic) assembly (Ernest *et al.* 2008; Fukami *et al.* 2005; Myers and Harms 2009a), supporting recent theoretical developments that combine both models (Adler *et al.* 2007; Gravel *et al.* 2006; Tilman 2004; Vergnon *et al.* 2009).

This study aimed at examining recruitment with a focus on individual species, based on the notion that species may vary with regard to the extent their recruitment depends on the niche structure of the resident community. In contrast to most previous experimental studies on this topic (but see Harpole and Tilman 2006), we did not use classifications of functional types (i.e. functional guilds) but instead manipulated the distribution of a continuous trait, SLA, assumed to reflect species resource economy and thereby their niche requirements. The reason for the importance of SLA as a 'soft trait' is its correlation with important life history characters ('hard traits'), including potential relative growth rate, leaf lifespan, residence time of nutrients in plant tissues and mass-based net photosynthetic capacity (Poorter and Remkes 1990; Reich
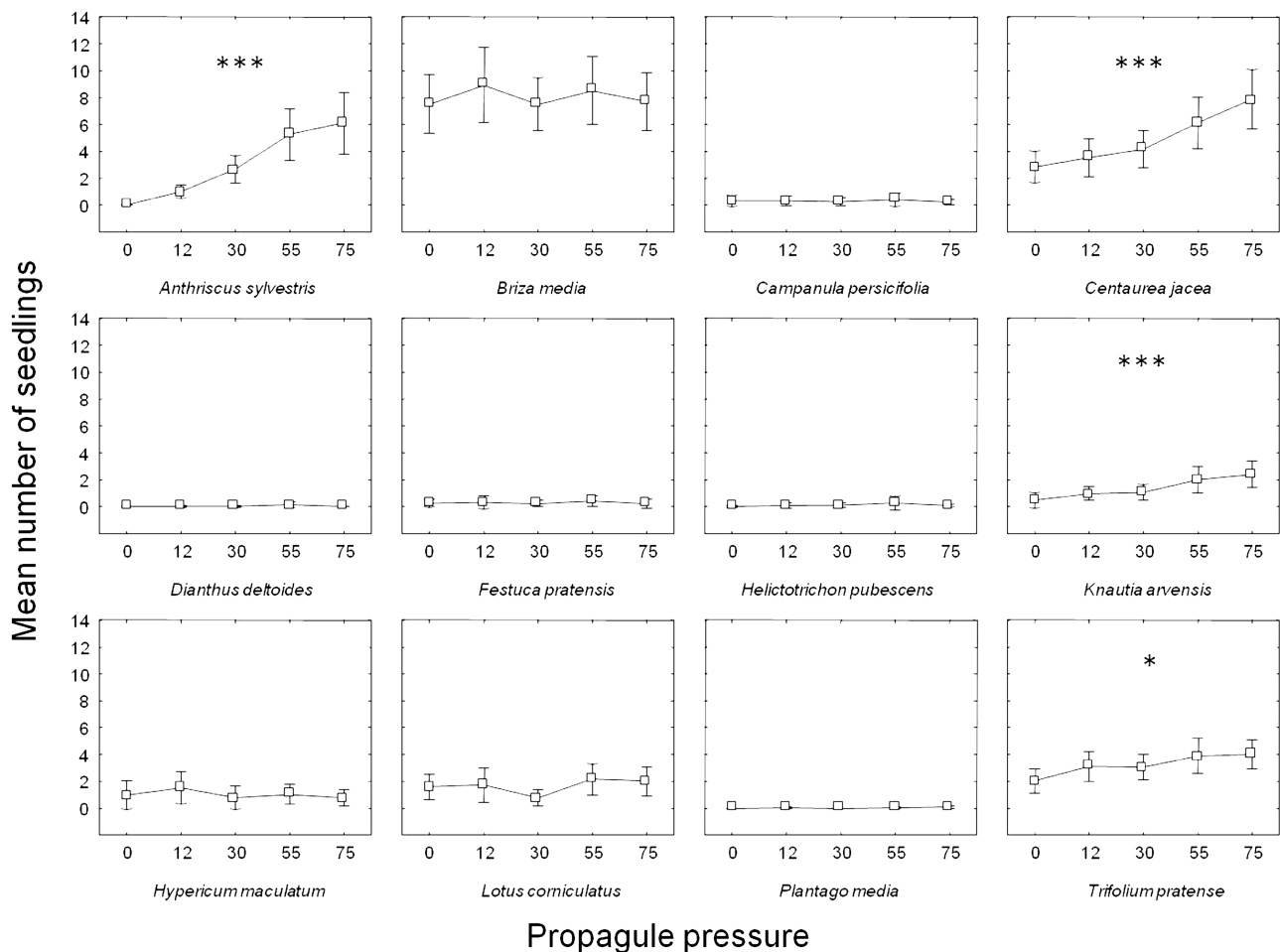

Figure 3: recruitment of 12 sown grassland species in unmanipulated grassland plots depending on the propagule pressure (12, 30, 55 and 75 seeds × species^{-1} × plot^{-1}). $n = 32$ for each point in each graph. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

et al. 1992; Weiher *et al.* 1999; Westoby 1998; Westoby *et al.* 2002). These correlations make SLA an important part of a fundamental trade-off between rapid acquisition of resources and conservation of resources (Diaz *et al.* 2004). In addition, we used seed mass, another trait shown to be of importance to plant function (Weiher *et al.* 1999; Westoby 1998; Westoby *et al.* 2002). Seed mass affects dispersal and recruitment ability (Leishman *et al.* 2000; Moles and Westoby 2004) and is associated with important trade-offs, including the competition–colonization trade-off and the establishment–colonization trade-off (Coomes and Grubb 2003).

We did not find any evidence supporting the prediction that recruitment would be higher if the recruiting species were functionally dissimilar to the surrounding community. There were only weak effects of trait dissimilarity (between the resident community and the colonizers) on recruitment, and these effects were contrary to the prediction. In four of five species where recruitment was related to species richness, the effect was positive. Furthermore, survival of the plug plants was generally high for most of the species and was not related to the trait profile of the community. In contrast, plant size showed some relationship to trait profile of the community. A relationship to the community aggregate SLA was found for two of the species, and there was a negative relationship between plant size and the community aggregate seed mass for two of the species. Also the negative relationship between plug plant growth and surrounding species richness may indicate interactions such as increased competition, and thus ongoing niche structuring processes in the community, which has been previously shown in another study of plug plants (Mwangi *et al.* 2007). Taken together, this indicates that the composition of the surrounding community has an effect on plant performance. Based on the regression models this effect may be fairly large in some species with a maximum of 29% of the variation in plants size explained by the investigated community factors.

One important difference between our study and most previous studies is that we manipulated natural grassland communities with a very high richness (up to 41 species per 0.09 m²). In these types of communities, most species have moderate to low abundance. In our study, only 15% of the experimental plots contained species with a percent cover above 30%. When most species are rare in a community, effects of demographic stochasticity and dispersal limitation may be more pronounced and niche effects of less importance (Scheffer and van Nes 2006). This would imply that species are able to colonize irrespective of the trait profile of the community, provided that they reach a propagule pressure sufficient to overcome the stochastic mortality after seed arrival. Some results from our study support this interpretation. The total recruitment of all species together increased with increased propagule pressure. The results did not clearly indicate whether there is an asymptotically declining recruitment at high propagule pressure, even though the propagule pressure was 2.5 times higher than the average natural seed rain. As survival of the plug plants was high, even after two seasons of growth, we expect that the effects of propagule

pressure treatment will remain even after the seedlings reach maturity, and they will thus influence the community structure (Zeiter *et al.* 2006). However, the effect of propagule pressure was mainly dependent on 4 of the 12 species sown, implying that the effect of propagule pressure is not universal among species in these grasslands. There are numerous examples of seed limitation of recruitment (Clark *et al.* 2007; Myers and Harms 2009b), but only a handful of studies have examined how recruitment changes with increased propagule pressure. In our study, recruitment increased approximately linearly even at our highest propagule pressure indicating that, at least for some of the species, these species-rich communities are basically open for colonization (Eriksson *et al.* 2006). Of the four species that responded to propagule pressure, we find the three species with the largest seeds in the study (*Anthriscus, Knautia, Trifolium*; Table 1). It may thus be that the effect of propagule pressure is most likely among species with a high seed competitive ability during recruitment (assuming that this ability is positively correlated to seed size). If this is so, it indicates a further level of complexity in the relative influence of stochastic vs. niche-related processes during community assembly.

In conclusion, using a different approach to manipulate niche structure than most previous studies, we found limited support for the prediction that trait dissimilarity between colonizers and resident species influences recruitment. For a subset of the species, increasing propagule pressure enhances recruitment, suggesting that these species may overcome the mortality sieve after seed arrival, provided that there is sufficient density of propagules. We cannot, however, exclude the impact of niche structuring on colonization and assembly in these species-rich grasslands. The majority of species did not increase recruitment despite increasing propagule pressure, and there were effects of species richness and to a lesser degree effects of the trait profile, on the growth of transplanted plug plants. On balance, this study therefore supports recent theory based on a reconciliation of niche and neutral (stochastic) theory for community assembly (e.g. Gravel *et al.* 2006; Tilman 2004). The species density in the studied grasslands may be the result of both a subtle and intricate division of niche space, and (for some species at least) a basically open community in which species can colonize provided that there is a sufficient number of incoming seeds. The long continuity of grassland management has created environmental conditions allowing for continuous addition of species from the regional species pool to local sites, over time leading to an exceptionally high small-scale species density. Species-rich grassland communities in Northern Europe are presently considered very important from a conservation viewpoint. The landscape surrounding remaining grassland fragments have been strongly transformed due to modern agriculture and forestry. Thus, the species-rich grasslands are probably subjected to a propagule pressure reflecting the transformed landscape. A possible implication of our results is that this changed propagule pressure may over time result in a shift in species composition, even if the present management regime is maintained.

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