

Positive diversity–invasibility relationship in species-rich semi-natural grassland at the neighbourhood scale

Michaela Zeiter^{1,2,*} and Andreas Stampfli^{1,2}

¹Institute of Plant Sciences, University of Bern, Altenbergrain 21, CH 3013 Bern, Switzerland and ²School of Agricultural, Forest and Food Sciences HAFL, Bern University of Applied Sciences, Länggasse 85, CH 3052 Zollikofen, Switzerland

*For correspondence. E-mail michaela.zeiter@ips.unibe.ch

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- **Background and Aims** Attempts to answer the old question of whether high diversity causes high invasion resistance have resulted in an invasion paradox: while large-scale studies often find a positive relationship between diversity and invasibility, small-scale experimental studies often find a negative relationship. Many of the small-scale studies are conducted in artificial communities of even-aged plants. Species in natural communities, however, do not represent one simultaneous cohort and occur at various levels of spatial aggregation at different scales. This study used natural patterns of diversity to assess the relationship between diversity and invasibility within a uniformly managed, semi-natural community.
- **Methods** In species-rich grassland, one seed of each of ten species was added to each of 50 contiguous 16 cm² quadrats within seven plots (8 × 100 cm). The emergence of these species was recorded in seven control plots, and establishment success was measured in relation to the species diversity of the resident vegetation at two spatial scales, quadrat (64 cm²) within plots (800 cm²) and between plots within the site (approx. 400 m²) over 46 months.
- **Key Results** Invader success was positively related to resident species diversity and richness over a range of 28–37 species per plot. This relationship emerged 7 months after seed addition and remained over time despite continuous mortality of invaders.
- **Conclusions** Biotic resistance to plant invasion may play only a sub-ordinate role in species-rich, semi-natural grassland. As possible alternative explanations for the positive diversity–invasibility relationship are not clear, it is recommended that future studies elaborate fine-scale environmental heterogeneity in resource supplies or potential resource flows from resident species to seedlings by means of soil biological networks established by arbuscular mycorrhizal fungi.

Key words: Biotic resistance, environmental heterogeneity, forbs, graminoids, grassland, invasibility, neighbourhood scale, recruitment, seed addition, seedling establishment, species diversity, species richness.

INTRODUCTION

Understanding why some plant species establish in a new site while others do not is central to ecology, in particular to community assembly, exotic invasions and the restoration of native communities. The successful establishment of a species in a site depends on the capacity of the species to invade (invasiveness), the propagule pressure of the arriving species and the invasibility of the ‘target’ community, i.e. the susceptibility of the community to be invaded (Lonsdale, 1999). This community property emerges from the local climate, the level of disturbance and the resident species’ competitive ability and resistance to disturbance (Lonsdale, 1999). Although disturbance has been widely recognized as a mechanism favouring plant invasions in the context of intermittent resource pulses (Davis *et al.*, 2000), disturbance has a surprisingly low power to explain global patterns of invasion (Moles *et al.*, 2012). Disturbance may reduce the ability of the resident vegetation to compete and increases the chances of survival of invaders in competition with resident species, e.g. by reducing the effect of asymmetric competition for light (Tilman, 1993; Hautier *et al.*, 2009; Souza *et al.*, 2011).

A further factor often held to influence community invasibility is species diversity. Elton (1958) considered whether diversity ‘begets’ resistance to invasion and argued that species-rich communities should be more resistant to invasion by new species than species-poor communities. Large-scale observational studies and small-scale experiments have found both positive and negative diversity–invasibility relationships, which suggests that the answer to Elton’s question is not straightforward (Levine and D’Antonio, 1999). This ‘invasion paradox’ might arise from the combining of different processes operating at different scales (Shea and Chesson, 2002; Fridley *et al.*, 2007). At large spatial scales, the relationship between diversity and invasibility is mainly positive due to processes related to a positive association between area and spatial environmental heterogeneity (see reviews by Herben *et al.*, 2004; Fridley *et al.*, 2007). At small spatial scales, where heterogeneity in environmental conditions is minimized, biotic resistance (Elton, 1958) is often thought to be more influential (Fridley *et al.*, 2007). So far, observational multiscale studies have typically looked for changes in the diversity–invasibility relationship at the square-metre-to-landscape scale, neglecting smaller scale variability in environmental conditions (but see Sandel and Corbin, 2010).

Biotic resistance results from competitive exclusion and explains a negative diversity–invasibility relationship by reduced resource availability for potential invaders due to increased resource utilization in species-rich communities. A further assertion of the biotic resistance hypothesis is that resident species should most strongly inhibit the establishment of species of the same functional group because of their similar resource requirements. Biotic resistance is supported by many studies conducted in plant communities which were artificially constructed with the aim of assessing the causal relationship between biodiversity and ecosystem functioning (Levine *et al.*, 2004; Mwangi *et al.*, 2007; Lanta and Lepš, 2008). Such experiments have indeed shown that the inhibition of introduced species is strongest when they are functionally similar to resident species (Fargione *et al.*, 2003; Mwangi *et al.*, 2007).

However, it remains unclear how important these findings are for predicting invasion success in natural communities. Constructed communities are generally quite species poor, as species richness gradients in these experiments mimic depauperate versions of the natural communities. These reductions in species richness *per se* therefore result in the creation of empty niches (Moore *et al.*, 2001). Moreover, young artificial communities are originally composed of even-aged plants, whereas mature natural communities mostly have more complex structures, with plants at various levels of spatial aggregation at different scales.

For an understanding of the invasion resistance along species richness gradients under natural site conditions, the processes which regulate species richness in the resident community are crucial (Moore *et al.*, 2001). Competitive dominance indicated by a negative relationship between resident species richness and community productivity might cause high invasion resistance, while empty niches due to lack of species (dispersal limitation) may lead to low invasion resistance. Seed-addition experiments have shown that many natural plant communities are open to immigration (e.g. Burke and Grime, 1996; Tilman, 1997; Foster *et al.*, 2004; Zeiter *et al.*, 2006; Stein *et al.*, 2008). Interestingly, even studies performed in communities of high species richness at small scales found dispersal limitation (Zobel *et al.*, 2000; Eriksson *et al.*, 2006), indicating that there are situations in which high diversity does not necessarily prevent invasions. Seed-addition studies have not found a consistent relationship between resident diversity and invasibility. This might be due to productivity or other environmental conditions which vary in their effects, from promoting species coexistence to competitive exclusion (Davies *et al.*, 2007).

One of the problems in invasion ecology is that time since invader introduction is often unknown (Strayer *et al.*, 2006). In correlative studies of the diversity–invasibility relationship, diversity at the time of invader introduction is often missed. This problem is eliminated in invader-addition studies, but the time point of the invasibility assessment may influence the outcome of such studies (Levine and D'Antonio, 1999). Unequivocal evidence of establishment success requires at least showing that the introduced populations can reach the reproductive stage, which is rarely done (Turnbull *et al.*, 2000). Investigating the temporal development of the diversity–invasibility relationship might also help to identify the timing of a driving process behind the emerging pattern. Factors

controlling invasibility may also change throughout the invasion process and cause a temporal shift in the diversity–invasibility relationship, as has recently been found in a study using fast-growing sessile marine communities as a model system (Clark and Johnston, 2011). The diversity–invasibility relationship in a system with perennial terrestrial plants might also be expected to shift with time because conditions favouring successful germination are not necessarily optimal for seedling survival and growth. We are not aware of any study which has examined the development of a diversity–invasibility relationship in terrestrial plant communities.

Here we assess the importance of biotic resistance in an old species-rich temperate grassland using two grain sizes at the neighbourhood scale. Biological invasion was mimicked by introducing seeds of ten species, and habitat match of the potential invaders was ensured by selecting these species from the species pool of the target community. The fate of the sown individuals was followed in order to answer the following questions. (1) Is establishment success of the introduced species related to the species richness of the resident plant community? The manner in which this relationship develops across 4 years after species introduction was particularly investigated. (2) Is establishment success of the introduced species negatively related to the abundance of resident species of the same functional group? (3) Is the diversity–invasibility relationship dependent on the scale considered?

If biotic resistance is an important phenomenon, a negative relationship between resident diversity and invasibility or a suppressed establishment success of invader species by residents of the same functional group would be expected. If biotic resistance arises from competition for light, a negative relationship between resident species phytomass and invasibility would also be expected.

MATERIALS AND METHODS

Study site

This study was performed in the control plots of a drought experiment established in March 2004 in regularly mown old grassland. The site was located adjacent to the long-term study site of San Carlo di Negrentino (820 m a.s.l., 46°27'51"N, 8°55'29"E) in southern Switzerland (Stampfli, 1992; Stampfli and Zeiter, 2004, 2008), had been used for hay-making twice a year for at least one century and no fertilizer had been added for over two decades. The slope is mainly inclined 11° towards south–south-east. The soil is a moderately acid sandy loam (*sensu* FAL, 1997; pH = 5.2, top soil sample measured in 2010 in water) with a low nutrient and a high silt content, and a water storage capacity of approx. 600 L m⁻². The climate is temperate humid (*sensu* Walter and Lieth, 1964) and shows high year-to-year variability in precipitation and duration of periods of low soil moisture between March and October (Stampfli and Zeiter, 2008). High species richness qualified this meadow as a site of national importance (Anonymous, 2010).

The existing uniform management regime was maintained during our study, and rainfall was regulated in seven areas of 1.5 × 1.5 m located within a range of 15 × 25 m from 16 July to 1 October 2004 and from 24 March to 11 May 2005.

Rainout shelters conducted water from unpredictable precipitation away, and an irrigation device gently added rainwater to the quadrats. Average seasonal amounts and frequencies of rainfall were simulated by adding 6–30 mm rainwater weekly as 1–3 nocturnal precipitation events per week. During natural precipitation events, waterproof screens fixed at a height of >0.5 m above the ground on four wooden poles automatically covered areas of 25 m² and rolled up soon after. The total reduction in radiation due to intermittent coverage by the screen during the period of controlled precipitation was 1.3 % based on high-frequency measurements of photosynthetically active radiation (PAR) below and above the screen, i.e. at 25 cm and 200 cm above-ground (A. Stampfli, HAFL, Zollikofen, Switzerland, unpubl. res.).

Invader addition experiment

In March 2004, two plots of 8 × 100 cm were set up 56 cm apart within each of the seven 1.5 × 1.5 m areas, and 4 cm quadratic grids made of 1 mm thick stainless steel were fixed to the ground. Each grid delineated 2 × 25 contiguous cells (16 cm²), each consisting of four sub-cells (4 cm²). Between 18 and 20 September 2004, one seed of each of ten species was dropped into each cell, of one randomly selected plot, using a funnel with a square opening matching the cell area. The other plot served as a control. This treatment simulated a propagule pressure of 6250 seeds m⁻². We sowed seeds of three grasses: *Bromus erectus*, *Danthonia decumbens* and *Helictotrichon pubescens*, and seven forbs: *Hypochaeris radicata*, *Primula veris*, *Plantago lanceolata*, *Ranunculus bulbosus*, *Salvia pratensis*, *Sanguisorba minor* and *Scabiosa columbaria* (species names follow Lauber and Wagner, 2001). The seeds of all species had been randomly collected in June and July 2004 as mixed samples from hundreds of mature inflorescences from at least ten local populations in the area surrounding the study site, cleaned and stored at room temperature. Tests of dry stored seeds in a growth chamber revealed high germination (>50 %) for all species except *P. lanceolata* and *P. veris* (<10 %).

Measurements of species diversity and establishment success

In July 2004, 2–5 weeks after the first harvest, rooted frequency of resident species in the 200 sub-cells (4 cm²) of each plot was recorded. We recorded 56 species in total, 18 of which were graminoids (Poaceae, *Carex* and *Luzula*) and 38 of which were forbs (non-gramineous herbs including a few species of legumes, woody dwarf shrubs and ferns). Species richness varied from 28 to 37 between plots (800 cm², $n = 7$). Mean richness was 32.9. At the quadrat scale (64 cm², $n = 84$), richness varied from 4 to 19 around a mean of 10.2. Species richness of plots and of within-plot means of quadrats showed a correlation of $r = 0.654$ ($n = 7$).

Graminoids contributed to 76 % of total rooted frequency and to 53 % of the hay yield harvested in July 2005. The graminoid–forb species richness ratio increased with decreasing spatial scale, 0.65 (800 cm²), 1.32 (64 cm²), 2.14 (16 cm²) and 3.01 (4 cm²). The rank abundance distribution in sub-cells ($n = 1400$) showed *Carex caryophylla* (19.0 %), *Festuca rubra* (15.9 %) and *Agrostis capillaris* (10.3 %) as the

highest ranking species among eight graminoids, with frequencies >3.6 %. All forbs had frequencies <2.3 %.

Recruitment success was measured as the number of living recruits of the introduced species. Seedling censuses were performed in late October 2004, April 2005, July 2005, April 2006, July 2006, April 2007, July 2007 and July 2008. Seedlings were marked with coloured ringlets, their position in cells recorded, and their survival observed. Establishment success in this grassland community can be reliably estimated 3–4 years after seed addition (Zeiter *et al.*, 2006; Zeiter and Stampfli, 2008). During the colonization phase of the invasion process investigated in this study, invader addition did not affect resident species diversity (data not shown). Two species were excluded from further analysis as no seedlings had emerged until 1 year after seed introduction (*D. decumbens*), or emerging seedlings did not differ in number between plots in which propagules had been introduced and control plots (*P. lanceolata*). The remaining eight species showed only 9 % emergence in control plots (Supplementary Data Table S1). Propagule pressure from natural populations does not seem to have an important effect (see ‘Data analysis’ in Supplementary Data).

In June 2008, recruits were harvested by cutting them at 5 cm above the ground with scissors. The phytomass of all eight invader species was pooled per plot, and weighed after drying at 80 °C for 24 h. Mean phytomass of invader individuals is total phytomass per plot divided by number of living individuals.

Measurements of environmental covariables

Seasonal productivity was calculated based on phytomass harvests of the plot areas in June and September. A 9-cm wide electric lawn mower was used to cut phytomass 5 cm above the ground, and separated fractions of graminoids and forbs were weighed after drying at 80 °C for 24 h. The sum of the harvests in September 2004 and June 2005 was used to test whether seedling establishment was related to productivity across plots. It was found that resident species richness was not related to productivity across plots ($r = 0.035$).

The proportion of space taken by gaps in the vegetation was recorded using a sample of 78 grid points per plot in October 2004. This variable was used to test whether open space caused a gradient of seedling emergence and establishment between plots.

Data analysis

Our analysis of the diversity–invasibility relationship uses resident species diversity in July 2004, before invader introduction, as the explanatory variable and establishment success of invaders (proportion of living invader individuals out of the number of introduced seeds) as the response variable measured during eight censuses.

Two different diversity measures were used as explanatory variables: species richness and the Shannon Index [$H' = -\sum (p_i \ln p_i)$], a diversity measure combining information of species richness and frequency. Both measures of diversity were calculated at two spatial scales: plot (8 × 100 cm, $n = 7$) and quadrat (8 × 8 cm, $n = 12$) nested within plot. Separate models were fitted for each of these diversity variables, as the

two are correlated with each other ($r = 0.837$ at the plot scale, $r = 0.941$ at the quadrat scale), and for each spatial scale.

All statistical analyses were performed with R (version 2.12.1; R Development Core Team, 2008). For more details on these analyses, see ‘Data analysis’ in Supplementary Data. We fitted and evaluated generalized linear mixed models (GLMMs) with binomial error distributions, using a logit-link function, and with Laplace approximation (lme4 package; Bates *et al.*, 2010) for maximum likelihood estimation of the parameters (Bolker *et al.*, 2009). Diversity and time (months since seed were added) were coded as continuous fixed factors. The models fitted at the plot scale had random effects for: invader species, plot, seedling census, the interaction between invader species and census, and subject, which was each invader species in each plot. We also fitted random slopes for time, for each subject. At the scale of the quadrat, within plot, the models included a further random factor, quadrat nested within plot, and the factor subject was then invader species within quadrat. Akaike’s Information Criterion (AIC) was used to compare the goodness of fit of models. Significance of fixed effects was also assessed by removing them from the model and comparing models with likelihood ratio tests. The significance of fixed effects was further confirmed by using Wald z statistics. When we found significant time \times diversity interactions, we subsequently carried out separate analyses for each seedling census. These post-hoc analyses used a simplified random effects structure.

We further tested whether invaders are inhibited by functionally similar residents during their first 10 months. Again, we applied generalized linear mixed modelling. We used the phytomass of resident graminoids or forbs as the explanatory variable at the scale of plot, and the number of living graminoid or forb invaders as the response variable. The two models fitted at the plot scale had random effects for invader species and plot.

TABLE 1. Generalized linear mixed model (GLMM) statistical table of establishment success of eight invader species in eight censuses over 46 months after seed addition in quadrats (64 cm^2 , $n = 12$) within plots (800 cm^2 , $n = 7$) and Shannon diversity H' as explanatory variable

Estimated variance component	Variance	s.d.	
Plot	0.09054	0.30091	
Quadrat	0.00923	0.09607	
Census	0.00000	0.00000	
Invader species	1.32268	1.15008	
Census \times Invader	0.15771	0.39712	
Subject: intercept	0.67457	0.82133	
Subject: time	0.01112	0.10544	
Fixed factors	Estimate	s.e.	z
Intercept	-1.50727	0.63151	-2.39*
Diversity (Shannon H')	0.00038	0.22599	0.00
Time	-0.37953	0.04876	-7.78***
Diversity \times Time	0.10083	0.02334	4.32***

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Final establishment success was based on phytomass of the invaders measured in 2008. Due to the low number and/or size of surviving individuals, the inclusion of invader species as a random factor was not feasible. Therefore, we analysed accumulated data, i.e. means or sums over eight invader species per plot. We fitted separate models with species diversity, resident phytomass or gap proportion as explanatory variables, and mean phytomass of invader individuals, total phytomass of invaders per plot or proportion of established invaders as response variables. We applied linear regressions for phytomass data, and generalized linear modelling (GLM) for proportions. We used quasi-binomial errors and performed F -tests to account for overdispersion.

RESULTS

Most of the seedlings of the invader species emerged in autumn, within 1 month of seed addition. A minority of individuals (16 %) emerged the following spring (Supplementary Data Table S1). From October 2004 onwards, the number of invader individuals continuously declined as mortality exceeded new emergences (Supplementary Data Fig. S1).

Fine-scale diversity–invasibility relationships within plots

At the spatial scale of the quadrat (64 cm^2 , $n = 12$) within plot, the relationship between diversity (Shannon H') and invasibility changed significantly with time after invader introduction (diversity \times time interaction, $\chi^2 = 20.84$, d.f. = 1, $P < 0.001$; Table 1). Results with species richness were similar (Supplementary Data Table S2), although the model fit was slightly less good (Shannon diversity AIC 609.4 vs. species richness AIC 609.8).

One month after seed addition, invasibility was not yet related to diversity (Shannon H') in the resident community (Fig. 1A, Table 2), but from April 2005 onwards, a significantly positive relationship between resident species diversity and invasibility was found at three censuses (7, 10 and 19 months after seeds had been added; Fig. 1A, Table 2). The relationship was no longer significant in the later censuses. The same pattern resulted when resident species richness was used as the measure of diversity (Supplementary Data Table S3).

Diversity–invasibility relationship between plots

At the scale of plot (800 cm^2) within site, approx. 400 m^2 , the relationship between diversity and invasibility significantly changed with time after invader introduction (diversity \times time interaction, $\chi^2 = 9.98$, d.f. = 1, $P = 0.002$, Table 3). The same pattern resulted when resident species richness was used as the measure of diversity (Supplementary Data Table S4).

One month after seed addition, invasibility was not yet related to diversity in the resident community (Fig. 1B, Table 2). From April 2005 onwards, a significantly positive relationship between resident species diversity and invasibility was found at every census across the 4 years (Fig. 1B, Table 2), despite the continuous mortality of invaders after seeds had been added. The same pattern resulted when species richness was used as the measure of diversity (Supplementary Data Table S3).

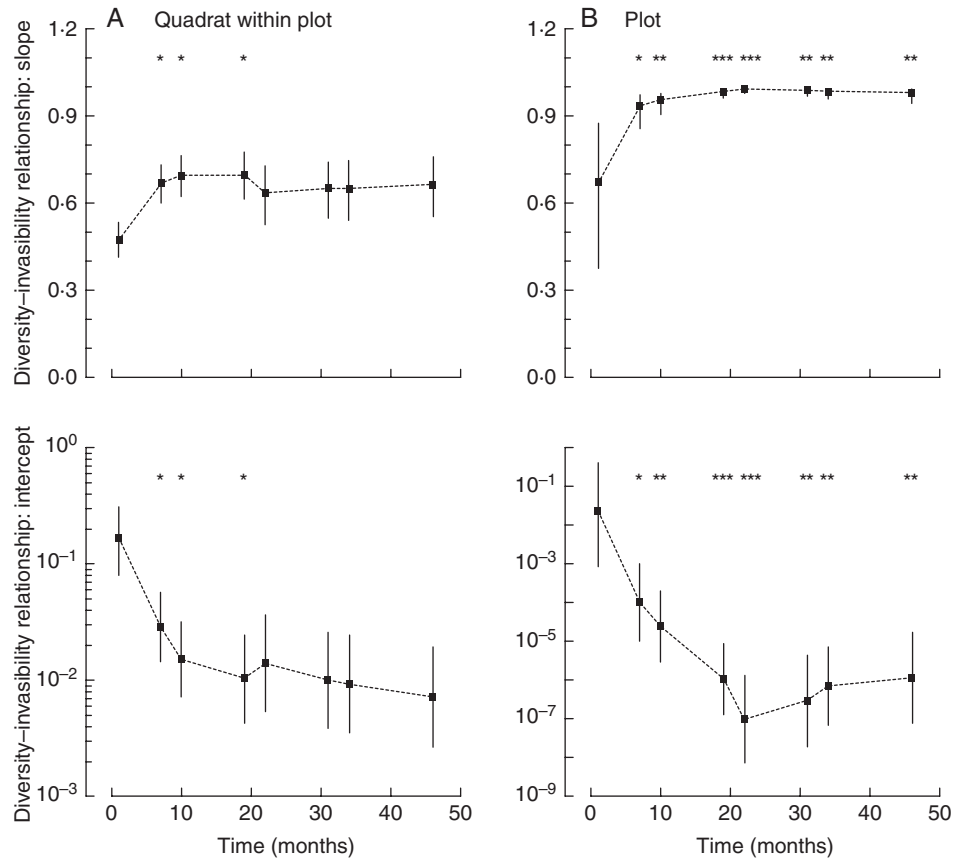


FIG. 1. Estimated regression parameters of diversity–invasibility relationships at eight censuses after seed addition of eight species. Slopes and intercepts at the scales of (A) quadrats (64 cm², 12 quadrats within seven plots) and (B) plots (800 cm², seven plots). All values (estimates ± s.e.) back-transformed after generalized linear mixed modelling with binomial error distribution and logit-link function, fitted separately at each census. χ^2 values after removing diversity from the models and comparing models with likelihood ratio tests (d.f. = 1), * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

TABLE 2. Invasion success in relation to resident diversity (Shannon H'), measured at scales of plot (800 cm², n = 7) and quadrat (64 cm², n = 12) within plot

Census (months)	Plot			Quadrat within plot		
	$z_{\text{intercept}}$	z_{slope}	χ^2	$z_{\text{intercept}}$	z_{slope}	χ^2
1	-1.11	0.58	0.33	-1.91	-0.45	0.20
7	-3.83***	3.03**	5.58*	-4.88***	2.35*	5.10*
10	-4.95***	3.89***	7.73**	-5.37***	2.48*	5.56*
19	-6.44***	5.27***	10.95***	-5.22***	2.20*	4.34*
22	-6.17***	5.11***	10.91***	-4.33***	1.23	1.34
31	-5.47***	4.37***	9.35**	-4.79***	1.43	1.80
34	-6.05***	4.75***	10.32**	-4.74***	1.36	1.44
46	-4.96***	3.77***	8.02**	-4.87***	1.44	1.54

Results of GLMMs fitted separately with data at eight censuses across 46 months after invader introduction. χ^2 values after removing diversity from the model and comparing models with likelihood ratio tests (d.f. = 1)
 * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Inhibition of invaders by functionally similar residents

Establishment success of the forb invaders was not related to the phytomass of resident forbs ($\chi^2 = 0.0001$, d.f. = 1, $P = 0.994$) and establishment success of graminoid invaders was not related to the phytomass of resident graminoids ($\chi^2 = 0.002$, d.f. = 1, $P = 0.969$).

Final establishment success and effect of environmental covariables

In July 2008, some individuals of the introduced species had reached the reproductive stage (Supplementary Data Table S1). The positive diversity–invasibility relationship was confirmed when invader phytomass, summed over all invader individuals,

TABLE 3. Generalized linear mixed model (GLMM) statistical table of establishment success of eight invader species in eight censuses over 46 months after seed addition in plots (800 cm², n = 7) and Shannon diversity H' as explanatory variable

Estimated variance component	Variance	s.d.	
Plot	0.02249	0.14998	
Census	<0.00001	<0.00001	
Invader species	0.98206	0.99099	
Census × Invader	0.15144	0.38916	
Subject: intercept	0.12704	0.35642	
Subject: time	0.00010	0.00314	
Fixed factors	Estimate	s.e.	z
Intercept	-5.6147	1.9797	-2.84**
Diversity (Shannon H')	1.4544	0.7243	2.01*
Time	-0.4304	0.1158	-3.71***
Diversity × Time	0.1384	0.0429	3.23**

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

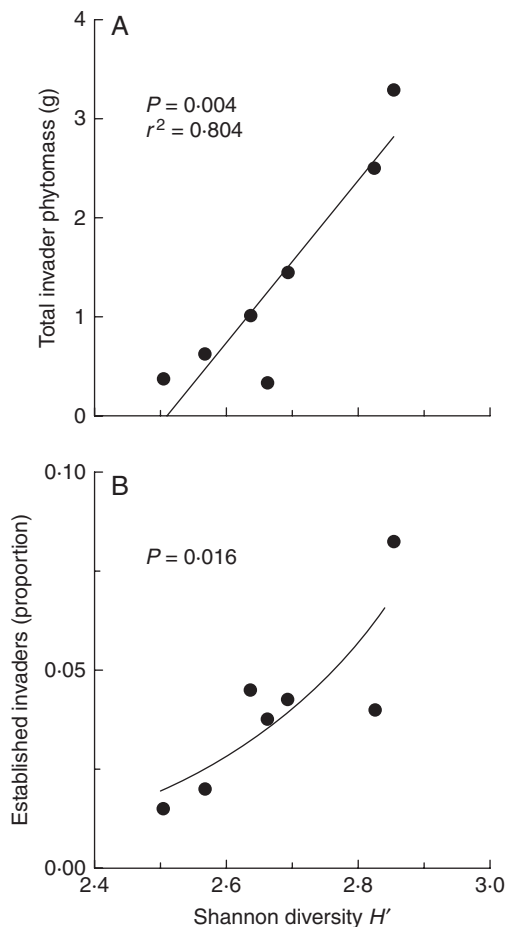


FIG. 2. Relationship between resident species diversity (Shannon H') and (A) total invader phytomass (sum over eight species) and (B) proportion of established out of added invader individuals of eight species in plots ($n = 7$), 4 years after seed addition.

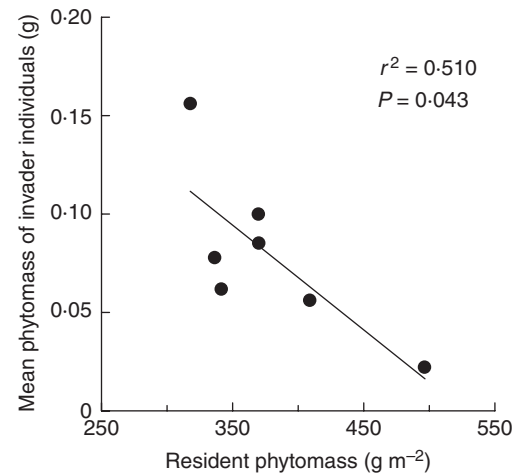


FIG. 3. Relationship between resident phytomass and mean phytomass of invader individuals, 4 years after seed addition in plots ($n = 7$).

was used as a measure of establishment success ($F_{1,5} = 25.6$, $P = 0.004$, Fig. 2A). This pattern was more strongly shaped by the number of invader individuals than by their size, as resident diversity was positively related to invader abundance ($F_{1,5} = 13.1$, $P = 0.015$, Fig. 2B) but not to mean phytomass of invader individuals ($F_{1,5} = 3.17$, $P = 0.130$, $r^2 = 0.274$).

Mean phytomass of invader individuals was negatively related to resident phytomass ($F_{1,5} = 7.25$, $P = 0.043$, $r^2 = 0.510$, Fig. 3), but neither invader abundance ($F_{1,5} = 0.12$, $P = 0.745$) nor total invader phytomass per plot ($F_{1,5} = 0.78$, $P = 0.417$) was affected by resident phytomass. Thus, although competition for light seems to reduce the growth of seedlings, biotic resistance, operating through competition for light, does not constrain overall invasion success.

Gap proportion did not influence final invader abundance ($F_{1,5} = 2.22$, $P = 0.197$), mean phytomass of invader individuals ($F_{1,5} = 0.28$, $P = 0.617$) or total invader phytomass ($F_{1,5} = 1.46$, $P = 0.281$). Thus, there is no evidence that a gap gradient between plots influenced the establishment of invaders.

DISCUSSION

Importance of biotic resistance for invasibility

With native species as test invaders, a positive diversity–invasibility relationship in an old grassland was found. No relationship between invader success and phytomass of residents of the same functional group was found. Both results contradict the biotic resistance hypothesis that species-rich communities contain fewer empty niches than species-poor communities. As a predictor of invasibility in this grassland, biotic resistance is therefore of lower importance than other factors which are not yet clear.

Our results seem to be inconsistent with those of studies performed in artificial grassland (Levine *et al.*, 2004) for two reasons. First, studies using depauperate communities find increased biotic resistance with diversity because artificially created species-poor plots include empty niches (Moore *et al.*, 2001), whereas the species-poor plots in our study are

comparatively species rich and have a complex spatial structure of plant aggregation. Secondly, studies differ in site productivity. Artificial experimental communities have often been created on fertile former arable land. They typically show an increase in phytomass with increasing diversity and therefore possibly also reduced invasibility due to competition for light (Hautier *et al.*, 2009). Studies conducted in harsh serpentine sites (Davies *et al.*, 2007) or coastal prairie grasslands (Sandel and Corbin, 2010) revealed that the slope of the diversity–productivity relationship can change from positive to negative with increasing site productivity.

The negative relationship between invader size, measured as mean phytomass of invader individuals, and resident phytomass in our study suggests that competition for light might have reduced establishment success of invaders (Hautier *et al.*, 2009). However, competition for light can be excluded as a major predictor of invasion resistance in our study, because invader success, quantified by total phytomass or number of invaders per plot, was not related to the phytomass of the resident vegetation. This is confirmed by the observation that the diversity–invasibility relationship developed during 7 months in winter following invader introduction when standing phytomass was very low.

With native species as test invaders, we ensured habitat match of the potential invaders. The diversity–invasibility relationship may differ with non-native invaders which have evolved in different biotic interaction networks. These networks may play an important role in affecting invasion success, as has been shown for soil fungal communities (Klironomos, 2002; Callaway *et al.*, 2004).

The positive diversity–invasibility relationship found in our study site at the neighbourhood scale challenges the importance of biotic resistance to plant invasion in semi-natural grassland. More studies performed in more sites, and also in more habitats, are needed to assess the generality of this pattern.

Processes contributing to positive diversity–invasibility relationships at a small spatial scale

At the neighbourhood scale, a positive diversity–invasibility relationship can result from three processes (Fridley *et al.*, 2007): generalist facilitation, biotic acceptance and environmental heterogeneity. Facilitative interactions are often assumed to increase in importance with the harshness of the abiotic environment (Bertness and Callaway, 1994). Holmgren and Scheffer (2010) recently argued that facilitation may even be stronger under moderate compared with extreme environmental conditions, e.g. because species of mild environments are less adapted to abiotic stress compared with species of extreme environments. During the 7 months following the addition of seeds in autumn, the complex architectural structure of a highly diverse community might have improved the microclimatic conditions for early seedling establishment at a small spatial scale. Facilitation in highly diverse communities may also operate by means of a higher probability that an invader will encounter compatible symbiotic organisms in the soil (van der Heijden, 2004; Spence *et al.*, 2011). Networks between plant roots and mycorrhizal fungal hyphae maintained by carbohydrate supplies from neighbouring adult plants may

link seedlings soon after germination with resident species and support them with otherwise growth-limiting soil mineral nutrients. Plant–mycorrhizal fungal networks may be spatially structured at a scale of <1 m in a seemingly homogenous grassland (Mummey and Rillig, 2008). Heterogeneity in specific functional support from soil biological networks and the pattern of positive relationship between invader success and resident species diversity in our study match in spatial scale.

The second process, biotic acceptance (Stohlgren *et al.*, 2006; Souza *et al.*, 2011), is based on the assumption that residents and invaders both generally prefer resource-rich conditions. Foster *et al.* (2002) explained a positive diversity–invasibility relationship in abandoned successional grassland by extrinsic factors, such as light availability and soil disturbance, which co-varied with species richness at the local scale. In our study, variation between plots in environmental conditions such as climate, soil type or management regime can safely be excluded. Minor variability in gap proportion had no influence on invader establishment. Nevertheless, without more comprehensive fine-scale measurement of environmental factors including soil nutrients, we cannot exclude biotic acceptance as a driver of the observed diversity–invasibility relationship.

The third process, spatial heterogeneity in resource conditions (Davies *et al.*, 2005), relies on the assumption that different invader species have different environmental requirements and that these are met in different parts of a heterogeneous site. Again, fine-scale measurements of environmental conditions within plots would be needed to elucidate the role of spatial environmental heterogeneity as a driver of the observed diversity–invasibility relationship.

Small-scale spatial heterogeneity

The processes which explain a positive diversity–invasibility relationship share the issue of spatial heterogeneity (Melbourne *et al.*, 2007). Facilitation relates to spatial heterogeneity of biotic interactions, while spatial heterogeneity in abiotic conditions at the between-site (Shea and Chesson, 2002) or the within-site scale (Davies *et al.*, 2005) are involved with biotic acceptance or environmental heterogeneity. The conceptual models of Shea and Chesson (2002) and Davies *et al.* (2005) consider spatial heterogeneity to be important only at larger spatial scales. Both models assume homogeneity in environmental conditions at the plant–neighbourhood scale which would allow competitive exclusion and result in a negative diversity–invasibility relationship. However, the positive diversity–invasibility relationship across small spatial grains of 64 cm² within 800 cm² in our study indicates that spatial heterogeneity is also important at the neighbourhood scale.

Temporal development of the diversity–invasibility relationship

The positive diversity–invasibility relationship emerged early, i.e. during the first months after invader introduction. As diversity was not related to invader abundance shortly after seed addition when invader abundance was influenced by seedling emergence, there is no evidence of any influence of resident diversity on seedling emergence. The positive

relationship emerged at the moment when seedling mortality exceeded new emergences. This implies that the early survival of seedlings is positively related to resident diversity. From 7 months after invader introduction onwards, the positive diversity–invasibility relationship remained stable at the scale of plot, indicating that the later survival of invaders and invader growth were not related to resident diversity. The temporal stability of this pattern over several years implies that resident species diversity has an important influence on early establishment of seedlings and indicates that processes affecting early seedling establishment are crucial for community assembly in this species-rich grassland, where individuals normally require several years to reach reproductive maturity (Zeiter *et al.*, 2006; Stampfli and Zeiter, 2008; Zeiter and Stampfli, 2008). The early influence of residents on invaders combined with the temporal stability of the diversity–invasibility pattern is rather surprising, as Levine *et al.* (2004) argued that biotic interaction mainly constrains the spread and impact of invaders, rather than their initial establishment. The disappearance of the positive diversity–invasibility relationship at the scale of quadrat within plot after 2 years might be due to insufficient statistical power with a declining number of individuals.

Conclusions

In our study, high species diversity co-occurred with high invasibility at the neighbourhood scale of a uniformly managed species-rich grassland. This challenges the importance of biotic resistance to plant invasion and calls for an alternative explanation based on fine-scale environmental heterogeneity. As potentially influential processes are unclear, future studies should elaborate resource supplies across fine-scale environmental gradients and potential resource flows from resident species to seedlings by means of networks with arbuscular mycorrhizal fungi.

SUPPLEMENTARY DATA

Supplementary data are available online at www.aob.oxfordjournals.org and consist of the following. Data analysis: detailed description of the methods of data analysis. Table S1: invasion success of single invader species. Table S2: generalized linear mixed model (GLMM) statistical table of establishment success over 46 months after seed addition in quadrats (64 cm²) and species richness as explanatory variable. Table S3: results of GLMMs fitted separately with data at eight censuses across 46 months after introduction of invaders and species richness as explanatory variables. Table S4. GLMM statistical table of establishment success over 46 months after seed addition in plots (800 cm²) and species richness as explanatory variable. Fig. S1: establishment success of invaders over time.

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