

Invasion in grassland gaps: the role of neighbourhood richness, light availability and species complementarity during two successive years

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Summary

1. We examined how the performance of three grass species, inserted as invaders in the gaps of synthesized communities, was affected by percentage light transmittance in these gaps and by the species richness and productivity of the surrounding neighbour plants. We also investigated whether these influences changed through time.

2. In both the first and the second season following invader establishment, realized growth (invader leaf length) was positively related to percentage light transmittance (which was the best predictor for invasibility), and negatively related to neighbour biomass, in all species examined. The richness–invasibility relationship became significant only in year 2, when increasing neighbourhood richness enhanced complementarity (demonstrated by means of I_{\max} , an index for assessing the degree of complementary resource use), which in turn negatively affected invader leaf length. In the first year such a relationship had not yet developed, probably because the communities were still young and plant interactions were still limited. However, even in year 2 the richness effect saturated at low richness, with no mixture over-yielding the best two-species mixture.

3. The data suggest that moderately productive, species-rich communities, preferably with some broad-leaved species, and with a nutrient input as low as possible, will offer the best defence against invasions.

Key-words: complementary resource use, diversity, invader, invasibility, species richness

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Introduction

The success of an invasive species in a certain habitat depends on its own characteristics, on the traits of the invaded ecosystem, and on the interaction between them. Traits that appear to be linked with invasiveness are a broad native range and rapid dispersal, which in turn, are associated with traits such as short generation time, long fruiting period, large seed crops, small seed size, prolonged seed viability and seed transport by wind or animals (Rejmánek & Richardson 1996; Williamson & Fitter 1996; Goodwin, McAllister & Fahrig 1999; Alpert, Bone & Holzapfel 2000; Kolar & Lodge 2001). It has also been suggested that greater phenotypic plasticity confers greater invasiveness (Williams, Mack & Black 1995; Schweitzer & Larson 1999), and life form or functional group might also correlate with invasiveness (Smith & Knapp 1999).

Important determinants of invasibility on a broad scale are disturbance regime; climate; level of environmental stress; abundance of natural enemies, competitors and mutualists; propagule pressure; resource availability; community structure; and ecosystem type (Crawley 1987; Smith & Knapp 1999; Alpert *et al.* 2000; Levine 2000; Kolar & Lodge 2001).

However, these factors do not explain variation in invader performance observed at the local scale, which probably arises from small-scale differences in invasibility interacting with invader traits. Elucidating these small-scale processes may have important implications for management, particularly if feasible techniques can be devised (e.g. altering nutrient input or mowing regime) locally to reduce community invasibility. A possible driver for microsite differences in invasibility might be local variation in species richness. The hypothesis that more diverse communities are less susceptible to invasion (Elton 1958; review by Levine & D'Antonio 1999) has attracted much attention and has recently been tested both with experimental studies on synthesized

communities (Knops *et al.* 1999; Levine 2000; Naeem *et al.* 2000; Prieur-Richard *et al.* 2000; Symstad 2000; Dukes 2001a, 2002; Hector *et al.* 2001); and with studies on large-scale natural invasion patterns (Planty-Tabacchi *et al.* 1996; Smith *et al.* 1999; Stohlgren *et al.* 1999; Foster *et al.* 2002). In studies of the second type, the most diverse areas are usually the most invaded, contrary to experimental studies that tend to support the hypothesis that diversity enhances invasion resistance. This controversy between the different types of studies can be resolved by distinguishing the causal effects of diversity on invasion resistance from the effects of factors associated with diversity across communities, such as disturbance, water and nutrient levels, percentage open canopy or bare ground, and climate (Levine, Kennedy & Naeem 2002). Despite support from theoretical models (Case 1991; Law & Morton 1996; Kokkoris, Troumbis & Lawton 1999), the results of the experimental approach have been debated by several authors (Aarssen 1997; Hodgson *et al.* 1998; Lawton *et al.* 1998; Wardle 2001). This is because the observed negative relationship between richness and invasibility can be explained by two different mechanisms: one based on complementary resource use, which is a true biological mechanism; the other based on the so-called sampling effect. In the mechanism of resource-use complementarity, increasing species richness results in more complete utilization of resources and consequently fewer available resources for possible invaders, which reduces invasibility. The sampling-effect model involves a greater probability of including the most competitive species in the assembled community as species richness is increased, which also reduces invasibility. Detecting the presence of effects of resource-use complementarity in reducing invasibility over and above the sampling effect might be very useful for developing management strategies, but has hardly been done to date (Dukes 2002). To this end, an experimental design is needed with monocultures of all the species present in the total species pool (Wardle 2001), and over-yielding (positive complementarity effect) of the mixtures has to be tested.

In addition, little is known about the mechanistic pathway by which species richness, through complementary resource use, affects invasion resistance. Some studies indicate that richness effects on invasibility are mediated by reduced resource availability (e.g. nutrients, light, water; Knops *et al.* 1999; Hector *et al.* 2001), which suggests that they are indirect. As a consequence, they might be weak compared to the effects on invasibility of variation in resource availability in the environment, such that manipulation of richness would have only little potential. On the other hand, because richness might influence several above- and below-ground resources at the same time, it could be a strong predictor of invasibility, and consequently a good management tool. In the current study we test how species richness influences invasibility, and whether richness, or rather the parameters modified by species richness such as percentage light transmittance and productivity (Naeem

et al. 2000; Foster *et al.* 2002), best predict invasion success.

In diversity experiments, two levels of species richness might be considered: overall community richness (number of species composing the community); and neighbourhood richness (number of species directly influencing the invader). In a community, the number of different neighbourhoods (comprising both the number of individuals composing the neighbourhood and their taxonomic identity) is determined not only by the species richness of the community, but also by the clumping and abundance distribution of the resident species (Troumbis, Galanidis & Kokkoris 2002). Because we are interested in the direct influence of the number of species surrounding an invader, we focus on neighbourhood richness instead of community richness. Moreover, the potential for invasions on a local scale is mainly determined by the nature of biotic interactions (competition for space, light and nutrients; presence of facilitators, etc.) and the identity of both resident and invading species at the neighbourhood scale (Naeem *et al.* 2000; Troumbis *et al.* 2002).

We set up an experiment to find out whether and how species richness, percentage light transmittance and plant productivity affect invader performance, and whether these effects depend on invader identity, or change through time. To this end, mixtures of different species richness (1, 2, 4, 8), composed of a series of native grass species varying in productivity, were created experimentally according to stringent rules. Each community contained gaps in which invader seeds (three species) were inserted. Eight neighbour plants, which together had the same species richness as the mixture as a whole, surrounded each gap (i.e. neighbourhood and community richness were the same). Germination, growth and survival during two growing seasons were used as measures of success of the three invader species. The specific research questions were: (1) Do species richness, percentage light transmittance and neighbour plant productivity influence invader performance, and how are they related? (2) Does richness affect the invaders through complementary resource use? (3) Which of the above parameters is the best predictor of invader growth? (4) Are the observed patterns the same in different invader species? (5) Concerning management, how can invasibility be reduced locally?

Materials and methods

Eight perennial grass species were selected, common to western European grasslands and exhibiting a range of productivities: *Agrostis tenuis* Sibth. (At); *Arrhenatherum elatius* L. (Ae); *Cynosurus cristatus* L. (Cc); *Dactylis glomerata* L. (Dg); *Festuca pratensis* Huds. (Fp); *Festuca rubra* L. (Fr); *Holcus lanatus* L. (Hl); and *Phleum pratense* L. (Pp). Grass species were chosen because grassland cultivation is a key activity in western European agriculture (e.g. 50% of the agricultural

Fr	At	Fr	Pp	At
Cc	Pp	Cc	At	Fr
At	Fr	Invader	Pp	Cc
Pp	Cc	At	Fr	Pp
At	Fr	Pp	Cc	At
Cc	At	Invader	Fr	Pp
Fr	Pp	Cc	At	Cc
Cc	At	Fr	Pp	Fr

Fig. 1. Example of positions of plants in a four-species mixture. The two central gaps in which invader seeds are inserted are surrounded with grey squares marking the neighbour plants (2×8) of those invaders. Plants in the outer border buffer for edge effects and are not used in the analyses. Pp, *Phleum pratense*; Cc, *Cynosurus cristatus*; At, *Agrostis tenuis*; Fr, *Festuca rubra*.

land in Belgium is grassland), and because invasions are often a cause for concern in grassland management (Watkinson & Ormerod 2001). The species were sown in trays between 11 and 14 March 2002, and seedlings were transplanted between 6 and 15 May 2002 into plastic containers placed outdoors ($30 \times 20 \times 14.5$ cm deep). The latter were filled with steam-sterilized sandy loam on a bottom layer (2 cm) of coarse-grained sand, and embedded in the soil to be level with the surrounding grassland vegetation. Following a substitutive design (40 plants per container), 24 different species mixtures were created, including monocultures of all species, eight bicultures, four mixtures of four species and four mixtures of eight species. In each container, two predefined positions were kept bare to form gaps in which invaders were inserted later on (Fig. 1). The set of 24 mixtures was replicated three times, and in each replicate another species was inserted as invader: *Festuca arundinacea* Schreb., *Lolium perenne* L. and *Poa trivialis* L., which were the three most invasive grass species from a previous experiment (Milbau *et al.* 2003). Weekly rotation of the containers diminished the influence of the position and height of adjacent communities on plant growth.

The communities were assembled according to the following rules: (1) each species composition is unique (to include as many combinations as possible); (2) all species occur in equal proportions, both within each level of species richness and within each mixture (maximum evenness); (3) species assemblages at a given diversity level differ maximally in composition (minimal number of species in common). In each mixture the plants were positioned as follows: (1) the eight neighbour plants surrounding each gap contain equal

proportions of every species present in the mixture; (2) the spatial arrangement of these neighbours is different for the two gaps within each container, with a maximum number of different interactions; (3) clumping is avoided in the mixtures to maximize interspecific interaction; (4) each of the four replicas of the eight-species mixture has a different internal arrangement (denoted as $N = 8$ (1); $N = 8$ (2); $N = 8$ (3); $N = 8$ (4), $N =$ species number).

Before the invader seeds were inserted (10 in each gap), the vegetation was cut twice (4 July and 2 August 2002). In keeping with extensive management, low amounts of fertilizer were supplied at planting and after the July and August harvests (total: $50 \text{ kg N ha}^{-1} \text{ year}^{-1}$; $50 \text{ kg K ha}^{-1} \text{ year}^{-1}$; $80 \text{ kg P ha}^{-1} \text{ year}^{-1}$, supplied in three equal fractions). In addition to natural rainfall, water was added by capillary rise during drier periods. Regular weeding removed all natural colonization. After inserting the invader seeds on 12 August 2002, we determined germination time and cumulative germination percentage until germination was complete. Only the first (out of 10) germinating seed in each gap was kept. Realized invader growth was measured as total leaf length per plant (laminae only, all leaves combined) and above-ground biomass (cut to 1 cm above ground level and oven-dried for 48 h at 75°C) at the end of the first growing season on 9 October 2002, and as total leaf length in the middle of the second season on 7 July 2003. Survival was monitored weekly during September and October 2002, 2-weekly in November and December, and monthly afterwards until July 2003. On 9 October 2002 and 8 July 2003, the invaded communities were cut to 3.5 cm above ground level, and the eight neighbour plants bordering each gap were dried and weighed together ('biomass of neighbour plants'). Shading of the invaders was estimated by measuring photosynthetic photon flux density above the canopy and in each gap at 2 cm above the soil surface, on 20 August 2002 and 10 September 2002, 2 and 5 weeks after the August harvest, respectively, in order to obtain data on a more open vegetation (percentage PAR transmittance O) and a more closed vegetation (percentage PAR transmittance C). A small quantum sensor with a gallium arsenide photodiode (Pontaillier 1990) was used, attached to a thin metal rod.

The index I_{\max} (after Trenbath 1976; Garnier *et al.* 1997) was used to analyse the effect of species richness on productivity in each mixture, and was calculated as:

$$I_{\max} = \left(\sum_{i=1}^n P_i \right) / \max(M_i) \quad \text{eqn 1}$$

with P_i biomass produced by species i in the mixture, and $\max(M_i)$ biomass produced by the component species that was most productive in monoculture. Applied to our experiment, $I_{\max} = P/\max(M)$, with P biomass of the eight neighbour plants surrounding a gap and $\max(M)$ biomass of the eight neighbour plants of the most productive monoculture among the component

species. When $I_{\max} > 1$, the mixture 'transgressively over-yields' the monocultures (transgressive indicates that mixture productivity is compared with the most productive monoculture, not with average monoculture productivity), which indicates positive complementarity (Garnier *et al.* 1997) and allows one to reject the sampling-effect hypothesis as the sole explanation of positive richness–biomass relationships (Spehn *et al.* 2000). The term complementarity refers to an effect arising from niche differentiation and/or facilitation, as distinguishing these effects is often difficult in practice (Loreau & Hector 2001). We used I_{\max} , which is a more stringent test for over-yielding than the often-used relative yield total (Loreau 1998; Fridley 2002), which only indicates over-yielding regardless of whether this is transgressive or not (Hooper & Dukes 2004). Note that if there are more than two species in a mixture, it is not possible to reject the sampling hypothesis as a partial explanation of diversity effects, even with I_{\max} (Spehn *et al.* 2000). Lack of transgressive over-yielding does not always rule out complementarity among species, because complementarity will increase yields above the maximum monoculture yield only if the resource base is expanded enough (Hooper & Dukes 2004).

Statistical analyses were performed with SPSS 10.0 (SPSS, Chicago, IL, USA). Invader leaf length was log-transformed and germination percentage arcsine transformed to improve normality.

Results

GERMINATION

For the three invader species, we examined if there was a relationship between percentage germination or germination time (days after seed insertion in the gaps), and species richness, percentage light transmittance or neighbour biomass. Percentage germination decreased significantly with species richness in *F. arundinacea* (linear regression, $y = 70.608 - 2.243x$, $P = 0.048$, $r^2 = 0.08$), while in *L. perenne* a negative relationship was found between germination percentage and biomass of neighbour plants (linear regression, $y = 106.355 - 50.984x$, $P = 0.016$, $r^2 = 0.12$). The influence of light on germination percentage was not significant, nor were there significant regressions for germination time ($P > 0.05$ for all species).

Seed mass (averaged per invader species) could not explain the observed variation in germination percentage (all invaded gaps; linear regression, $P > 0.05$), but had a significant effect on germination time, with longer germination times in larger seeds (linear regression, $y = 5.043 + 0.316x$, $P = 0.038$, $r^2 = 0.03$).

INVADER GROWTH

Because several invaders were smaller than the harvesting height, total leaf length per plant rather than

biomass was used as measure for invader growth. Invader leaf length at the end of the first growing season did not depend on the species richness of its neighbours, but increased significantly with increasing percentage photosynthetically active radiation (PAR) transmittance and was negatively related to the biomass of its neighbour plants at the end of the first growing season (linear regressions, Fig. 2). This pattern was observed for the three invader species (except for biomass in *L. perenne*), with percentage PAR transmittance O (measured when the plants around the gaps were recently mown, and a better predictor for leaf length in year 1 than percentage PAR transmittance C) being the best predictor in *F. arundinacea* and *L. perenne* ($r^2 = 0.21$ and $r^2 = 0.23$, respectively), and neighbour plant biomass in *P. trivialis* ($r^2 = 0.18$). In the second growing season, in contrast, invader leaf length declined significantly towards the higher richness levels (linear regression, Fig. 3). As in year 1, leaf length was positively associated with percentage PAR transmittance (in year 2 percentage PAR transmittance C, measured in more closed gaps, was a better predictor for leaf length than percentage PAR transmittance O), and negatively with biomass of the neighbour plants (measured in year 2), but this time the regression slopes were steeper (except for neighbour biomass in *P. trivialis*). In *F. arundinacea* and *L. perenne* the percentage of PAR penetrating in the closed gaps was the best predictor of leaf length in the second season ($r^2 = 0.24$ and $r^2 = 0.45$, respectively), while in *P. trivialis* leaf length was associated most strongly with species richness ($r^2 = 0.30$). For *P. trivialis*, the relative importance of biomass and light in affecting leaf length changed over time, with light becoming more important in year 2. Neighbour plant biomass and percentage PAR transmittance were correlated, with the strongest correlations between percentage PAR transmittance O and neighbour biomass year 1 (Pearson correlation: $n = 143$, $r = -0.458$, $P < 0.001$) and between percentage PAR transmittance C and neighbour biomass year 2 (Pearson correlation: $n = 143$, $r = -0.217$, $P = 0.009$).

To explain differences in richness effect on invader by year, we determined whether and how species richness modified percentage PAR transmittance, biomass of the neighbour plants or I_{\max} for both times of measurement. As community traits were not affected by invader identity (ANOVA with factors invader species and richness, invader effect for PAR O: $F_{2,131} = 0.236$, $P = 0.790$; for PAR C: $F_{2,131} = 0.406$, $P = 0.667$; for biomass year 1: $F_{2,131} = 0.786$, $P = 0.458$; for biomass year 2: $F_{2,131} = 1.202$, $P = 0.304$), correlations were calculated with all invaders combined. Species richness correlated negatively with percentage PAR transmittance and positively with the biomass of the plants surrounding the gaps in both harvesting years (Table 1). For I_{\max} , however, a different result was found between the two years. When I_{\max} was calculated with the biomass data of the first growing season, no relationship with species richness was observed, whereas I_{\max} increased

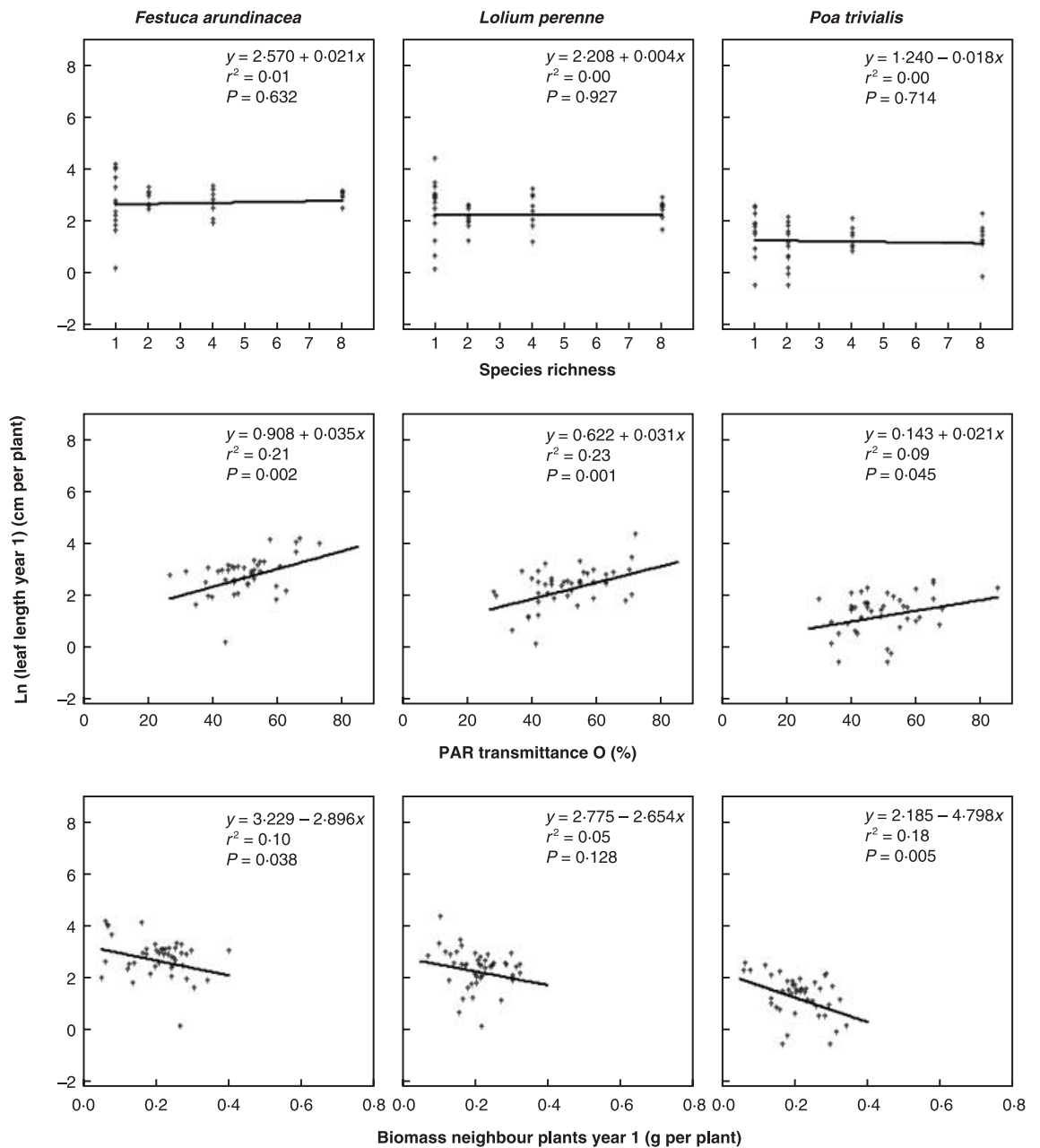


Fig. 2. Effect on invader performance of: species richness; percentage transmittance of photosynthetically active radiation (PAR transmittance O, measured when vegetation around gaps was recently mown and invaders were younger); and biomass of neighbour plants in year 1, expressed as total leaf length per plant at the end of the first growing season, for the three different invader species.

with richness in year 2. Regressions of invader leaf length on I_{\max} show that I_{\max} negatively affected invader growth in the second growing season, while leaf length in the first year was not associated with I_{\max} (Fig. 4). Values of I_{\max} were also higher in year 2 than in year 1 (ANOVA, factor = year, $F_{1,283} = 23.568$; $P < 0.001$), with transgressive over-yielding ($I_{\max} > 1$) occurring in 80% of the mixtures in year 2 vs. only in 55% of the mixtures in year 1. To summarize, in year 1 species richness did not affect the level of complementarity, and differences in complementarity did not affect invader growth. In contrast, in year 2 species richness did increase complementarity which, in turn, suppressed the invaders.

Invaders had a significantly higher leaf length in year 2 compared with year 1 (Figs 2 and 3; ANOVA with factors invader and year, for year: $F_{1,216} = 15.302$, $P < 0.001$). Especially in the monocultures and two-species mixtures, invader leaf length had increased. Community age also affected neighbour plant productivity, but not in the same way at all richness levels (Fig. 5). At the end of the first growing season, a wide range of monoculture productivity was observed, while the two-species mixtures all had very similar biomass. Also, the range was small in the four- and eight-species mixtures (Fig. 5a). However, in year 2 the monoculture biomasses converged (especially through elevated productivity of the

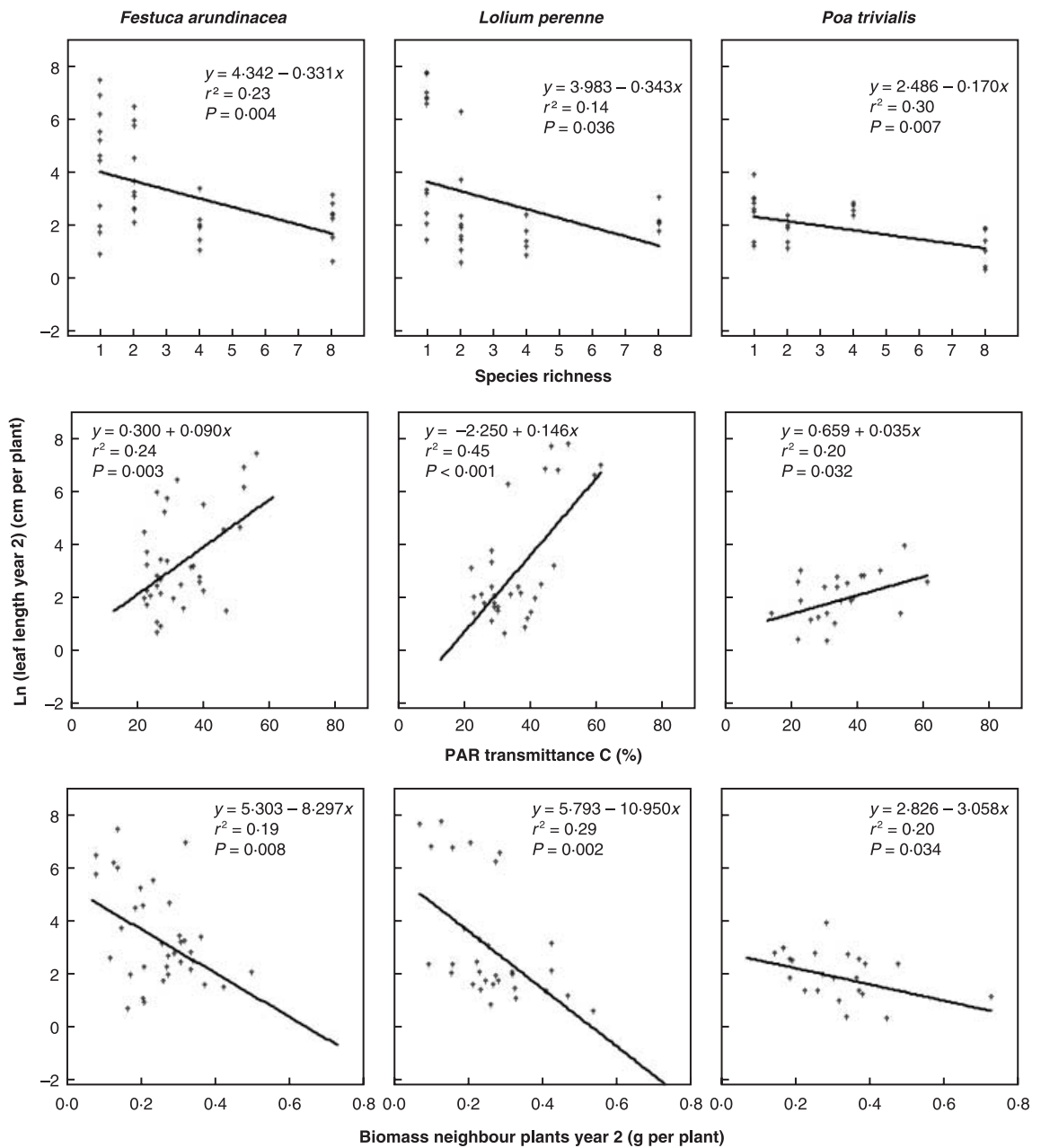


Fig. 3. Effect on invader performance of: species richness; percentage transmittance of photosynthetically active radiation (PAR transmittance C, measured after prolonged regrowth of neighbour plants); and biomass of neighbour plants in year 2, expressed as total leaf length per plant in the second growing season, for the three different invader species.

Table 1. Spearman's rank correlations between species richness of communities and percentage light transmittance in gaps, biomass of neighbour plants surrounding gaps, and I_{\max} (index for assessing degree of transgressive over-yielding)

	% PAR transmittance		Biomass neighbours		I_{\max}	
	O	C	Year 1	Year 2	Year 1	Year 2
Species richness						
Correlation coefficient	-0.273	-0.373	0.438	0.367	-0.124	0.514
<i>P</i>	0.001	<0.001	<0.001	<0.001	0.140	<0.001
<i>N</i>	143	143	143	143	143	143

Significant values ($P < 0.05$) in bold.

PAR transmittance was measured in more open gaps when the surrounding vegetation was recently mown (percentage PAR transmittance O), and after prolonged regrowth of neighbour plants in more closed gaps (percentage PAR transmittance C).

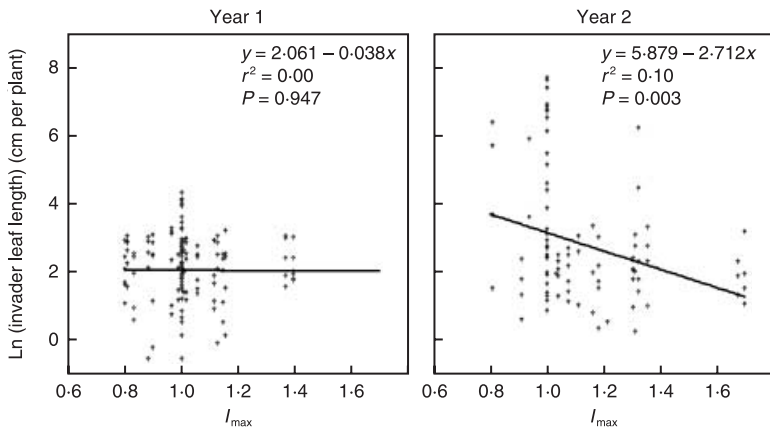


Fig. 4. Relationship between invader leaf length and I_{\max} (index indicating complementary resource use if $I_{\max} > 1$) in the first and second growing seasons with all richness levels combined.

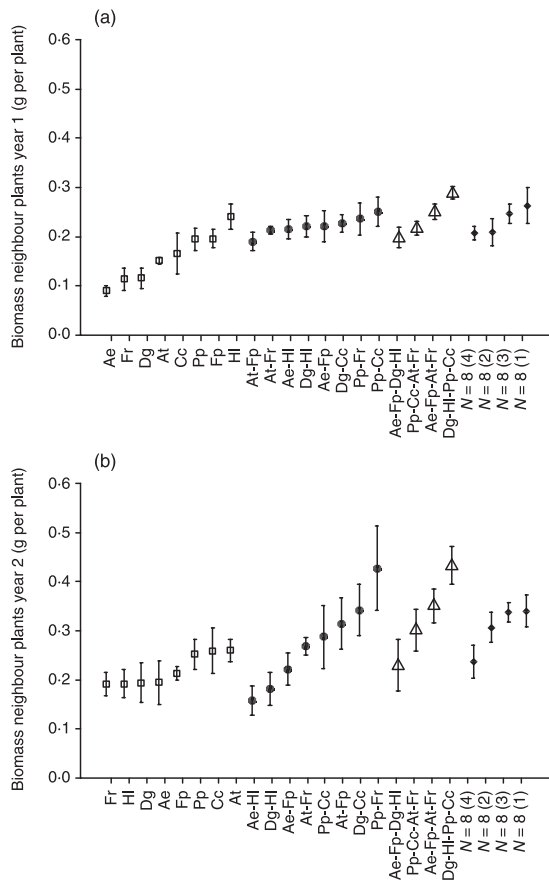


Fig. 5. Neighbour plant biomass in (a) first and (b) second growing season for different species compositions. □, Monocultures; ●, bicultures; △, four-species mixtures; ◆, eight-species mixtures. Ae, *Arrhenatherum elatius*; At, *Agrostis tenuis*; Cc, *Cynosurus cristatus*; Dg, *Dactylis glomerata*; Fp, *Festuca pratensis*; Fr, *Festuca rubra*; HI, *Holcus lanatus*; Pp, *Phleum pratense*. Symbols represent means of cumulative biomass of the eight neighbours for each composition ± 1 SE ($n = 6$).

mixtures that were least productive in year 1), while a wide range in productivity had developed in the mixtures (Fig. 5b). Whereas monoculture biomass increased only little in year 2, most of the two-, four- and eight-species mixtures became much more productive.

Further, a positive relationship between invader leaf length in all the gaps and invader seed mass (averaged per invader species) was found in both year 1 (linear regression, $y = 0.990 + 0.750x$, $P < 0.001$, $r^2 = 0.41$) and year 2 (linear regression, $y = 1.718 + 0.724x$, $P = 0.005$, $r^2 = 0.09$).

SURVIVAL

Survival of the invaders was highest in *F. arundinacea* (78%), while in *L. perenne* 65% and in *P. trivialis* only 53% of the plants survived until the second year. A logistic regression was used to examine whether invader survival until the second growing season was influenced by species richness, percentage PAR transmittance, neighbour biomass or invader leaf length. Neighbour biomass was a significant predictor of survival for the invader *F. arundinacea*, with more surviving individuals as neighbour biomass decreased (d.f. = 1, Wald = 5.350, $P = 0.021$). Survival of *L. perenne* could be predicted by means of its leaf length at the end of the first growing season, with a higher probability of survival when leaf length was higher (d.f. = 1, Wald = 6.487, $P = 0.011$). For *P. trivialis* no significant relationship was found.

Discussion

INVADER GERMINATION

Species richness of the communities did not influence germination time of the invader seeds, but was negatively related to the percentage germination of invader *F. arundinacea*. Germination of *L. perenne* decreased with increasing neighbour biomass (which was correlated with species richness). A negative relationship between germination and species richness was also found in the experiments of Levine (2000), who attributed it to greater species cover in the more diverse treatments, and those of Prieur-Richard *et al.* (2000), who postulated decreased shoot and root space and decreased resource availability in species-rich communities being responsible for poor invader establishment. In our experiment, the decrease in germination with increasing richness could not be attributed to light availability, but probably arose from covariation of species richness with other factors affecting seed germination, such as temperature in the gaps or the availability of water or chemical soil constituents (e.g. nitrate, Hilhorst & Karssen 2000). Higher richness might, by way of increased productivity, result in higher water and nutrient use, leaving less of these essential resources available for the invader to germinate and establish well. The fact that the three invader species reacted differently to richness and neighbour biomass suggests that the effects of richness on germination might be species-specific. Higher resident species cover might, in some cases, be beneficial in creating a suitable microclimate or by protecting seeds from extrinsic

influences such as being washed away by heavy rainfall or being eaten by birds.

INVADER SURVIVAL

Similarly to other studies, no direct relationship between species richness and invader survival was found (Lavorel, Prieur-Richard & Grigulis 1999; Prieur-Richard *et al.* 2000). However, in this study biomass of the neighbour plants significantly predicted survival of *F. arundinacea*. Decreased resource availability and root space for the invaders growing in more productive communities are possible explanations for lower survival in these cases (Aarssen & Epp 1990; cf. effects on germination). In *L. perenne*, leaf length of the individual invader plants at the end of the first season was a significant predictor for survival. A similar result was reported by Leishman (1999), who found a significant correlation between adult height and survival for species grown in competition with *Leontodon hispidus* neighbour plants, which is understandable as plant size is an important determinant of competitive interactions among individuals (Dalling & Hubbell 2002). Greater leaf length may help invaders escape from light competition with fast-growing established plants (Milbau *et al.* 2003), and thus may lead to increased survival.

INVADER GROWTH

Role of light transmittance and neighbour biomass

Our first question was whether invader growth was influenced by the species richness of the neighbour plants, the percentage light transmittance in the gaps, or neighbour plant biomass. A positive relationship with percentage PAR transmittance was observed for leaf length measured in both the first and second years, in all the invader species examined. Leaf length in the first growing season was associated most strongly with PAR transmittance O (measured when the vegetation around the gaps was recently mown), while invader leaf length in year 2 showed higher correlation with PAR transmittance C (measured after prolonged regrowth of neighbour plants, but still in year 1). This suggests that light availability played an important role in stimulating growth, in both younger and older invaders. The significant relationship between leaf length in year 1 and percentage light transmittance during the seedling stage indicates that seedling growth was an important determinant of plant size at the end of the first season. In the second year, however, realized growth appeared to depend more on the light conditions during the adult stage. The regression slopes were steeper in the second year than in the first, probably because the invaders did not yet have the opportunity to fully expand in year 1, in contrast to year 2 in which, especially, some of the invaders growing in the more open gaps became huge. This indicates that mowing, thus increasing available light, will more strongly promote

large/adult invaders because they can grow much more quickly due to their higher light interception and nutrient capture compared with smaller or younger plants. The exponential growth phase after cutting might therefore be important to focus on in future work. Also, other studies have demonstrated the importance of light in promoting invader growth and community invasibility (Knops *et al.* 1999; Naeem *et al.* 2000; Hector *et al.* 2001; Foster *et al.* 2002; Levine *et al.* 2002; Milbau *et al.* 2003).

Except for *L. perenne* in year 1, invader leaf length decreased significantly with increasing biomass of the eight neighbour plants surrounding each gap. Also, in other experiments standing biomass explained a significant part of the variation in community invasibility (Hector *et al.* 2001; Foster *et al.* 2002; Troumbis *et al.* 2002). Similarly to light transmittance, the regression slopes were steeper in year 2, mostly because leaf length had increased strongly in cases with unproductive neighbours. There are several reasons why higher biomass may lead to lower invader growth. First, high neighbour biomass may indicate high below-ground resource use, resulting in fewer available resources for the invaders. Second, because biomass and percentage PAR transmittance were negatively related, higher biomass might indirectly influence invader growth through its effect on light availability. Third, higher biomass will increase competition for space, above as well as below ground.

Role of species richness

In this section we discuss the effects of richness on invader growth, and how these effects were mediated by light, neighbour biomass and I_{\max} (questions 1 and 2). Although we selected only grass species as neighbour plants, we suggest that the use of more functional groups will not heavily change the results. Evidence from competition experiments has shown that functional differences among species may lead to strong complementarity because resource partitioning in space, time and resource type is greatest with species differing strongly in functional type (Hooper & Dukes 2004). Therefore we believe that using more functional groups will produce rather similar patterns, but that the effects may be stronger because of more complementarity between the species. Different results were found for the richness–invasibility relationship between the first and second years. At the end of the first growing season, invader leaf length was independent of the number of neighbour species, while in the second growing season it decreased with increasing species richness. This discrepancy might be due to a stronger diversity effect on resource availability as the invaded communities became older. The relatively similar correlation coefficients between richness and PAR, or between richness and biomass, for both times of measurement appear to contradict this hypothesis, but because of the overall higher values for neighbour

biomass in year 2 and the lower amounts of transmitted PAR C, a similar response of PAR or biomass to increasing richness in both years will have a stronger effect on the invaders in year 2. Moreover, the above hypothesis is strongly supported by the different relationships between richness and I_{\max} in both years. In year 1, I_{\max} was not related to species richness, whereas in year 2 a significant positive relationship was found. This suggests that invader growth in year 2 was partly suppressed by more complete resource use (e.g. nutrients) at the higher richness levels, which increased competition for limiting resources (Tilman, Lehman & Thomson 1997; Dukes 2001a, 2002) (answer to question 2). This was confirmed by the negative relationship between I_{\max} and invader leaf length in year 2. In year 1, on the other hand, over-yielding occurred in fewer mixtures than in year 2, and there was no relationship between species richness and I_{\max} , or between I_{\max} and invader leaf length, which explains the lack of a richness–invasibility relationship. Although some over-yielding occurred in the first year ($I_{\max} > 1$), it did not affect invader growth, suggesting that sufficient resources were available for the invaders. This might be due to the relatively low productivity of the neighbour plants in the first year, and indicates that transgressive over-yielding does not *per se* result in shortage of an essential resource.

The biomass data of the neighbour plants for each of the species mixtures show more clearly the differences between both years that led to the different values of I_{\max} . In year 1, biomass in the two-, four- and eight-species mixtures was fairly similar for all species combinations, and rarely exceeded the most productive monocultures. The observed gradient in monoculture productivity probably reflects slower- and faster-growing species, and the similar biomass for the mixtures indicates that the communities were not yet fully developed and species interactions (e.g. competition, facilitation, complementary resource use) were still limited. In year 2, however, neighbour plant biomass was much higher than in year 1, and the mixtures were much more productive than the monocultures, indicating interactions between the composing plant species. In addition, a range in productivities within the species-rich communities had developed which, together with the larger number of mixtures that were much more productive than the most productive of their component species, yielded the positive relationship between I_{\max} and species richness in year 2.

To summarize: in the second season increasing neighbourhood richness resulted in higher complementarity which, in turn, negatively affected invader leaf length. In the first year such a relationship had not yet developed, probably because the communities were still young and interactions were limited. The steeper regression slopes between leaf length and PAR or leaf length and biomass in the second growing season compared with the first made the decrease in leaf length with richness more pronounced.

Prediction and management

Invader seed mass significantly predicted invader growth. Having large seeds may be an advantage, especially in early seedling development, as this yields larger seedlings and thus better access to light and a more reliable water supply (Westoby, Leishman & Lord 1996; Hewitt 1998). However, later in seedling life, variation in growth rate becomes the major determinant of plant size (Leishman *et al.* 2000; Dalling & Hubbell 2002) which, in this study, was reflected in the weaker relationship between seed mass and leaf length in the second growing season.

The three invader species showed similar relationships with all the measured gap parameters (answer to question 4), but the relative importance of the parameters in explaining invader leaf length differed among species and between years. With both years and all species taken into account, species richness was the poorest predictor of invader leaf length, mostly because its relationship with invader performance was not consistent between years. The difficulty in predicting invasibility by means of species richness probably arises from the fact that the effects of richness on invader growth are indirect, and because the relationships between richness and the factors affecting the invaders depend on many variables. In this experiment, richness appeared to influence the invaders through its covariation with productivity and resource availability, and these relationships depended on community age (see previous section; Levine 2000; Naeem *et al.* 2000). Also, in other studies richness affected invaders indirectly, for example through influencing levels of extractable soil nitrate or light penetration (Knops *et al.* 1999; Hector *et al.* 2001). The relationship between richness and productivity can depend on many extrinsic factors, including resource supply, substrate heterogeneity, the size of local and regional species pools, and the scale at which the experiment is carried out (Crawley *et al.* 1999; Moore *et al.* 2001), which illustrates the difficulty of predicting the effect of increasing richness on invasibility. A model of Aarssen *et al.* (2003) showed that a wide variety of productivity–species richness relationships could be expected, depending on the successional stage (time since the last major disturbance) and the resource-supplying power (e.g. soil fertility) of the habitat. Also, over-yielding (which in our experiment negatively affected invader growth) was found to depend on environmental conditions and soil fertility (Dukes 2001b; Fridley 2003). Additionally, in this study the diversity–productivity relationship saturated at low richness levels, with no further increase in productivity beyond two- or four-species mixtures. This suggests that raising the richness of moderately diverse communities possibly has little effect on productivity (Wardle 2001) or invasibility. Invader establishment in grassland gaps might be easiest at very low richness levels, and become more difficult (but not impossible) in more species-rich mixtures. However, above a certain

species number, further increase in species richness might not increase resistance to invader establishment. Therefore we conjecture that attempts to reduce invasibility solely by increasing local species richness will not always guarantee success, and that establishing species-rich mixtures might reduce invasibility only when coinciding with high productivity and reduced availability of limiting resources.

Contrary to richness, percentage light transmittance and neighbour biomass were significant predictors of growth for all invader species in this experiment, in both young and older communities, with light availability explaining the largest proportion of the variation in leaf length, except for *P. trivialis* in year 1 (question 3). To derive strategies to control/prevent invasions (question 5), we therefore propose to concentrate on community productivity and light availability, and to a lesser extent on species richness. Because light availability, productivity and nutrient availability depend on each other, we must keep in mind that part of the variation explained by one of these variables must be attributed to other covarying variables. This suggests that the impact on invasibility of decreasing light availability or increasing neighbour productivity might be smaller or larger than expected, depending on the absolute amount of PAR reaching the communities, and on the nutrient and water availability in the ecosystem.

The results indicate that invader growth might be suppressed most in gaps with low light penetration and low availability of other resources (in our experiment, reflected in high neighbour biomass and high values of I_{\max}). Low light transmittance might be achieved by vegetation dominated by broad-leaved species or by high, dense vegetation (small gaps). Low resource availability requires low nutrient input and/or almost complete use of the available resources by the resident plants, which can be obtained through complementary resource use in a species-rich community. Reducing soil fertility by adding carbon is another possibility (Baer *et al.* 2004). Also, highly productive communities might limit invasions through increased competition for space and nutrients, but they mostly occur on nutrient-rich soils which, in turn, might benefit invasions. So moderately productive, species-rich communities, preferably with some broad-leaved species, and a nutrient input that is as low as possible, could offer the best defence against invasions.

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