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Relationships between canopy complexity and germination microsites for *Phalaris arundinacea* L.

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Abstract Microsites that prevent seed germination are critical for slowing the invasion of native plant communities by aggressive, clonal species. A suitable model for study is the clonal grass, *Phalaris arundinacea*, which reproduces prolifically from seed and is spreading into wetlands across temperate North America. Knowing that light conditions control its seed germination in the laboratory and that light varies with canopy complexity in a Wisconsin fen, we tested multiple attributes of microsites under spatially and temporally dynamic canopies (namely, presence/absence of a matrix species, number of species in the canopy, plus indirect effects of three soil water levels) for their control of germination in microcosms. Our 6-species canopies + the matrix of *Glyceria striata* had the densest cover and reduced *P. arundinacea* germination to 1.9%, compared to 7.3% for 1-species canopies + the matrix. After selectively removing canopy components, germination increased to 36.1% for 6-species and 33.0% for 1-species canopies. Comparing canopies with each of the six species, germination declined in relation to increasing leaf width. Given moist soil, *P. arundinacea* germination microsites are determined by canopy complexity, which affects light penetration, which in turn determines germination rate.

Keywords Diversity · Fen · Gaps · Invasion · Invasibility

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Introduction

The lack of microsites for germination (sensu Harper 1977) has been proposed as a determining factor in plant population dynamics (Crawley 1990). Harper et al. (1965) proposed that microsites are both a function of the species germination requirements and the physical conditions at the soil level. Germination responds to soil properties (Harper et al. 1965), disturbance (Peart 1989; Owens et al. 1995; Sissons et al. 2000), canopy cover (Oswald and Neuenschwander 1993; Reader 1993; Facelli and Ladd 1996), and canopy removal (Barret and Silander 1992). Some studies have explained low germination rates as a combined effect of microsite limitation and seed dispersal (Eriksson and Erhlén 1992; Turnbull et al. 2000), but few have linked seed germination requirements to specific canopy attributes, such as species composition and species richness.

Seed dispersal into a suitable microsite is a first step in the invasion of native plant communities by aggressive exotic species. Although clonal species can invade via rhizome fragments, colonization is typically via seedling recruitment (Sackville Hamilton et al. 1987; Eriksson 1989, 1997; Cain 1990; Meyer and Schmidt 1999). The germination of one seed might be all that is necessary for a clone to establish and spread vegetatively. Thus, it is essential to know the critical attributes of germination microsites that reduce unwanted invasion events, both by clonal and non-clonal species.

Leading hypotheses on the invasibility of plant communities propose that more diverse plant communities are more resistant to invasions (Rejmanek 1989; Cronk 1995). This is a corollary to Elton's (1958) argument that "invasions and outbreaks most often happen on cultivated or planted land, that is, in habitats and communities very much simplified by man." The relationship between native and exotic species richness has been shown to be negative (Rejmanek 1989; Tilman 1997; Naeem et al. 2000) and positive (Knops et al. 1995; Wiser et al. 1998). Attempting to resolve conflicting patterns, Levine (2000) suggested that the relationship is scale-dependent

– negative at the microscale and positive within landscapes, with differential dispersal as the mechanism. However, information on the role of species richness in defining germination microsites is scarce, despite several studies of diversity effects on other ecosystem processes (Tilman and Downing 1994; Johnson et al. 1996; Naeem et al. 1996; Tilman et al. 1996; Hooper and Vitousek 1997). Also, there is the need to find the relevant scales and mechanisms that determine the invasibility of plant communities (Dukes 2001).

The increasing distribution of the grass, *Phalaris arundinacea*, is of major concern in wetlands of temperate North America (Galatowitsch et al. 1999); elsewhere, at least five species of the genus show weedy behavior (Villaseñor and Espinosa-García 1998). Although *P. arundinacea* is native to North America, European strains that were planted for cattle forage and stream bank erosion control have likely crossed with the native genotypes to create more aggressive clones (Hoffman and Kearns 1997; Merigliano and Lesica 1998). Its invasiveness is partly a consequence of high vegetative and phenological variability (Apfelbaum and Sams 1987) and broad tolerance for hydrological conditions (Conchou and Fustec 1988). The broad geographic range over which this species displaces natives and creates monotypic stands makes it an important target for study. Our accumulating knowledge of the germination requirements for *P. arundinacea*, together with field data on canopies where it is actively invading, makes this a model system for testing hypotheses about germination microsites and invasibility of plant communities.

Our previous laboratory experiments (Lindig-Cisneros and Zedler 2001) showed that *Phalaris arundinacea* germination begins in 7 days on moist substrates, reaches 80% in 12 days, and is limited by light quality, with high rates under white light (81.5%) and red light (69.3%) and low rates under far-red light (15.8%) and dark conditions (1.2%). These results led us to hypothesize that the light environment at the soil surface defines germination microsites for *P. arundinacea* and that the structure of the overhead canopy strongly affects germination rates.

Wetland plant canopies vary in the (1) presence of a matrix species, (2) species richness and (3) canopy growth. The latter two vary both spatially and temporally as water levels change. We tested the effect of each of these factors (alone and in combination) on *P. arundinacea* germination microsites in greenhouse experiments, using spatial and temporal scales that matched conditions in the field. Thus, we varied canopy complexity directly (± matrix species and low and high species richness) and indirectly (by growing plants in three water levels) in two independent experiments.

Materials and methods

Our reference wetland, Wingra Fen (43°03′N, 89°31′W), is located at the University of Wisconsin-Madison Arboretum. Like many wetlands, this 1-ha fen is adjacent to monotypic stands of *Phalaris arundinacea*. Seedlings of this invader were found within the fen in 1998 and 1999, although in reduced numbers where the fen canopy is more species rich (Lindig-Cisneros 2001).

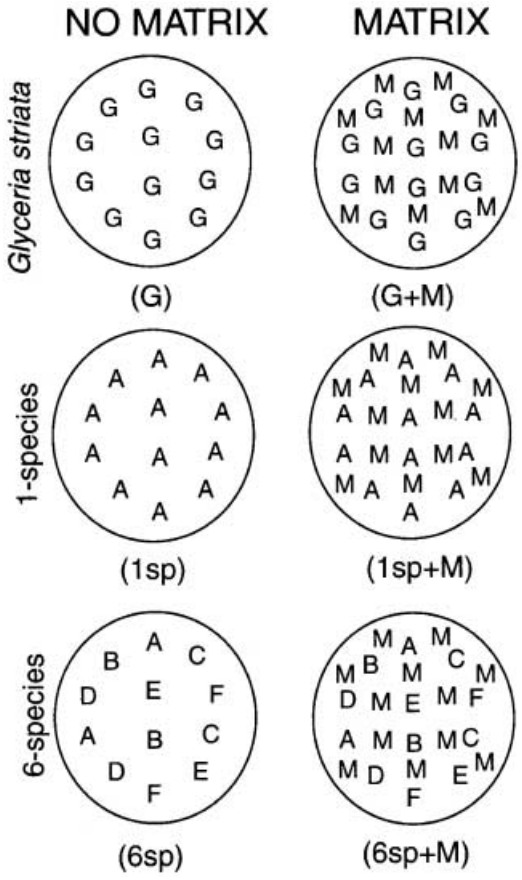

Fig. 1 Experimental design for the combination of species-richness treatments and presence/absence of the matrix species. For the canopy experiment, all six combinations were used. For the canopy and water level experiment, we used only those with the matrix species

As part of a field study (Lindig-Cisneros 2001) we sampled Wingra Fen in 1998 and 1999, and the perennial grass *Glyceria striata* had an average cover of 50% for all 1-m^2 sampling plots. This early season grass forms a dense canopy of vegetation up to 30 cm tall under the taller dominants, particularly *Aster firmus*, *Eupatorium* spp. and some *Carex* species. Thus we consider *G. striata* to be a matrix species, and we tested the effect of its presence/absence. Species richness averaged 1.25 species per dm^2 and ranged from 1 to 6 species. At the 1-m^2 scale, the range was from 4 to 9 species (Lindig-Cisneros 2001). Hence, for our 2.5-dm^2 microcosms, we added 1 or 6 species (combinations randomly chosen from a pool of 8). The 8 native species were those that had ≥10% cover at Wingra Fen (*Aster firmus*, *Eupatorium perfoliatum* and *Lycopus americanus*) or were known to occur there historically (*Eupatorium maculatum*, *Carex hystericina*, *Rudbeckia hirta*, *Lobelia siphilitica* and *Solidago ohiensis*; Curtis 1959).

Water levels at Wingra Fen varied from constantly waterlogged to moist throughout the growing season in some areas, while other areas had waterlogged soil in spring and relatively dry conditions in late summer. In a field experiment, standing water reduced *P. arundinacea* seedling establishment, while a variable hydroperiod allowed numerous seedlings to invade bare soil (Lindig-Cisneros 2001). These observations suggested three hydrological treatments, waterlogged, moist, and a temporal decline from waterlogged to moist conditions.

Two experiments were conducted, one "canopy" experiment, run twice to test the robustness of the results, and one "water level" experiment. Both experiments were conducted in a greenhouse at the University of Wisconsin-Madison under natural light supplemented by sodium lamps with a 14-h photoperiod. The response variable was germination of *Phalaris arundinacea* seeds. In all cases, seeds were placed under test canopies on soil. Germination is defined as emergence of the radicle in "complete" seeds (caryopsis with hull), and a seedling is a seed with a radicle. Percent light transmission was determined by measuring PAR above and below the canopy with a quantum meter (Model QMSS, Apogee Instruments). Canopy openness was measured using a 2-cm grid with 50 sampling points placed 60 cm above the microcosms. Points with soil visible through the canopy evaluated the openness of the canopy. Canopy openness is expressed as the number of points with visible soil/50.

We selected a commercial growing medium (Metro-Mix 360, Scotts), because homogenization and sterilization of soil from Wingra Fen degraded the humic peat. Native plants grew equally well in the commercial medium and in the field. The canopy experiment used 18-cm diameter, 20-cm deep microcosms. The water level experiment used microcosms of 16-cm diameter and 14-cm depth.

To create canopies of differing complexity, we planted 2-week-old seedlings as follows: 12 seedlings per microcosm for treatments with no matrix and 24 seedlings per microcosm for treatments with the matrix (Fig. 1). Thus, the *G. striata* treatment (G) had 12 *G. striata* seedlings while the *G. striata* with matrix treatment (G+M) had 24 *G. striata* seedlings, of which 12 represented the matrix and 12 were planted to test the effect of this species (Fig. 1). For the 1-species treatments for each of the 8 species listed above we added 12 seedlings, with and without the matrix (1sp+M and 1sp, respectively). The 6-species assemblages were randomly chosen from the pool of 8 (to avoid species composition effects), with and without the matrix (6sp+M and 6sp, respectively; Fig. 1).

Canopy experiment

The effects of (1) a matrix species and (2) species richness on *Phalaris arundinacea* germination were tested in a two factor experiment beginning 28 December 1999, and repeated on 30 March 2000. For each trial, canopies were planted as follows: for the treatments with no matrix, 8 G microcosms, 8 1sp microcosms (1 microcosm per species of our species pool) and 8 6sp microcosms; for the treatments with the matrix, 8 G+M microcosms, 8 1sp+M microcosms (1 microcosm per species of our species pool) and 8 6sp+M microcosms. Therefore, each trial consisted of 48 microcosms.

To avoid effects of native plant roots on *P. arundinacea* germination, seeds were placed in vials that were inserted into the soil. Preliminary tests showed that in microcosms with bare soil no differences in *P. arundinacea* germination rates occurred between seeds planted directly into the soil and seeds planted in vials. At week 6 of each trial, 30 seeds per microcosm were added in six 5-ml vials each containing 5 seeds. Vials contained the same growing medium as microcosms. The small volume of soil was adequate to test germination (growth was not assessed). The microcosms were watered with tap water to keep the soil wet but not waterlogged. The vials were watered with deionized water to prevent salt accumulation. At week 8 (2 weeks after adding seeds), *P. arundinacea* seedlings were counted.

When this experiment was finished, we conducted a follow-up experiment using the 1sp+M and 6sp+M microcosms to test the effect of reducing canopy complexity. Two removal treatments were applied; removal of the matrix (matrix removal) and removal of the non-matrix species (sp-removal). All non-matrix plants were removed, 12 plants of the same planted species for the 1sp+M microcosms or 12 plants of all 6 species planted in the 6sp+M microcosms. Replication in this follow-up experiment was equalized at the beginning of the canopy experiment by planting four microcosms per species-richness treatment (1sp+M and 6sp+M), treating them like all other microcosms. Hence, we had four replicates per trial for each species composition × removal treatment [control (no removal), matrix-removal and sp-removal]. Two weeks after the removal of canopy components, *P. arundinacea* seedlings were counted as the cumulative number before and after canopy removal.

Water level experiment

A two-factor experiment was set up on 28 March 1999, with 3 water levels and 3 levels of species richness. All microcosms had the *G. striata* matrix. Germination and early growth of *P. arundinacea* were assessed, so seeds were added to the soil (not vials). Twelve replicates of each treatment were planted as described for the previous experiment.

Three water level treatments mimicked conditions at Wingra Fen: (1) "waterlogged" = water table at –2 cm (relative to the soil surface), (2) "moist" = watered daily, and (3) "receding" = water table at –2 cm initially and lowered 0.5 cm/day for the next 20 days, followed by daily watering. Water levels in the waterlogged and receding treatments were maintained by placing microcosms in plastic trays (2 microcosms per canopy treatment, 6 microcosms/tray, 2 trays/water level). Microcosms in the moist treatment had no tray (a preliminary test indicated no effect of trays vs no trays). After the receding water level reached the bottom of the microcosms, those trays were perforated to allow drainage, as in the moist treatment.

Forty *P. arundinacea* seeds were added to each microcosm 6 weeks after setting up the experiment. Two weeks later, seedlings were counted. Because germination rates were low, the matrix (*G. striata)* plants were pruned 4 cm above the soil in all microcosms, while maintaining the species richness treatments. The experiment was terminated 6 weeks after pruning and seedlings were counted. Aboveground biomass was harvested and sorted by species, including *P. arundinacea* seedlings. Belowground biomass was sorted by depth from the soil surface (0–3, 3–6 and 6–12 cm), but not by species. All biomass samples were oven dried for 48 h at 70°C and weighed.

Statistical analyses

Two related germination response variables were analyzed in these experiments: presence of seedlings (proportion of microcosms with >1 seedling) and the proportion of seeds that germinated in a microcosm. We analyzed the presence of seedlings with GLM regression procedures for binomial data using the logit link function followed by ANOVA on the GLM regression results. *P* values were calculated for the χ^2 distribution. We analyzed the proportion germinating with fixed factor ANOVAs or multiple regression analyses. A fixed factor design was selected, because the species-richness treatments reflect minimum and maximum species richness at the dm^2 scale in our reference site, and the matrix species was planted at the median density of *G. striata* in our reference site.

The canopy experimental design had three factors: trial (first and second), species richness (three levels, *G. striata*, 1 species and 6 species) and matrix (presence/absence). Presence/absence of germinated seeds and germination rates per microcosm were the response variables in independent three factor ANOVAs. Each combination of trial × species richness × matrix had 8 replicates. The effect of individual species was analyzed from the canopy experiment using the 1sp and 1sp+M microcosms in a three factor ANOVA (trial × matrix × species identity). The follow-up experiment with canopy removals was analyzed with a three factor ANOVA (trial × species composition × sp-removal), and the response variable was total germination rates (the cumulative number of before and after sp-removal). Multiple comparisons, when appropriate, were done with Tukey HSD test (Day and Quinn 1989). Two ANCOVAs were carried out to test (a) canopy com-

Table 1 *Phalaris arundinacea* germination and canopy openness in the canopy experiment. (see Fig. 1 for treatment codes, *n* for all treatments =8 per trial) Germination rates and canopy openness are means and standard deviations

	No. of microcosms with at least 1 seedling		Germination rate (proportion of 30 seeds)		Canopy openness (proportion of 50 points that were bare)
Treatment	Trial 1	Trial 2	Trial 1	Trial 2	Both trials pooled
G+M	7	7	0.07±0.04	0.38±0.14	0.33±0.08
1sp+M	0	4	0	0.11±0.11	0.04±0.08
6sp+M	0	3	0.01 ±0.02	0.03±0.05	0.02±0.04
G	8	8	0.18±0.11	0.41±0.17	0.50±0.12
1sp	5	4	0.06±0.05	0.12±0.18	0.08±0.12
6sp	0	2	0	0.03±0.06	0.01±0.01

plexity effects after effects by canopy openness and (b) the proportion of broad-leaved species per microcosm as covariables.

The water level experiment is analogous to Winer et al. (1991) version of the split-plot experiment discussed in detail by Underwood (1997). We had two fully orthogonal treatments, water level and species richness. The trays are nested in each water level treatment and, because they are replicated, are orthogonal to species richness. Regression tree analysis was used to screen the effect of 11 explanatory variables: canopy openness, aboveground biomass per species (*G. striata* and each of the species in our species pool) and belowground biomass (0–3, 3–6 and 6–12 cm depths) on *P. arundinacea* germination. Regression tree analysis is a nonparametric technique (Breiman et al. 1984) that has been used for screening invasive species attributes (Reichard and Hamilton 1997), for life history analysis (De Rose and Pallard 1997), and habitat characteristics (Andersen et al. 2000). Regression tree analysis is very robust against outliers and correlations among variables. Tree models were selected by cross validation (De'Ath and Fabricius 2000). Regression trees are constructed by repeatedly splitting the data to create two mutually exclusive groups as homogeneous as possible defined by a simple rule based on a single explanatory variable. The splitting procedure is repeated until the response is partitioned into homogeneous groups maintaining a reasonably small tree. Therefore, each node of the tree represents a simple splitting rule and the terminal nodes the mean value of the response variable for each group.

Canopy openness and percent light transmission were analyzed using either ANOVA (if modeled as responses to the experimental treatments) or multiple regression analysis (when relationships with each other or germination rates were explored). For multiple regression results in addition to the statistics r^2, *F* and *P*, both the intercept coefficient (*b*) and the explanatory variable coefficient (*m*) are reported.

Germination rates and canopy openness (proportional data) were arc-sin square-root-transformed or log-transformed. Biomass data were log-transformed to comply with ANOVA and regression analysis assumptions when needed. We used the Tukey HSD test for comparisons of treatment means when appropriate. All statistical analyses were done with S-PLUS version 4.5 (Statistical Sciences 1999).

Results

Canopy experiment

The number of microcosms per treatment with at least 1 *Phalaris arundinacea* seedling was significantly lower under 6-species canopies (Table 1) than under less complex canopies (*P*=0.006). There were no statistically significant effects due to the matrix species (Table 2). The number of microcosms with at least one seedling was similar (*P*=0.41) for both trials, 20 microcosms (41.7%) for trial 1, and 28 microcosms (58.3%) for trial 2. However, germination rates per microcosm showed a significant difference between trials [*F* (1, 84)=32.99, *P*<0.0001; Table 3], with lower rates in the first trial than in the second trial (Table 1). The presence of the matrix did not affect germination rates. The effect of canopy complexity was significant [*F* (2, 84)=61.37, *P*<0.0001]. Germination rates decreased under more species-rich canopies; *G. striata* microcosms [both G and G+M had the highest germination rates, followed by 1-sp microcosms (1sp and 1sp+M), and 6-species microcosms (6sp and 6sp+M; Table 1)]. All pair-wise comparisons were significant.

Table 2 ANOVA table of the regression GLM analysis (binomial family, logit link) of presence/absence of *Phalaris arundinacea* seedlings per microcosm in the canopy experiment

	df	Deviance	Residual *df*	Residual deviation	*P* (χ^2)
Null			95	24	
Trial	1	0.67	94	23.3	0.41
Canopy complexity	2	10.19	92	13.14	0.006
Matrix	1	0.38	91	12.77	0.54

Germination responses differed by canopy species in the 1sp and 1sp+M microcosms. *Eupatorium perfoliatum* and *Rudbeckia hirta* prevented germination altogether in both trials (Fig. 2, Table 4). *Aster firmus* canopies had no seedlings in the first trial but several during the second trial, while *E. maculatum* canopies had the opposite pattern, i.e., several seedlings appeared in the first trial but none in the second. Overall, monocotyledon canopies (*G. striata* and *Carex hystericina*) allowed higher germination rates than dicotyledon canopies (*A. firmus*, *Lobelia siphilitica*, *Solidago ohioensis*, *Lycopus americanus* and *E. maculatum*). The lowest germination rates occurred under the broadest leaf dicotyledons (*E. perfoliatum* and *R. hirta*) and the 6-species canopies (Fig. 2).

Canopy openness did not differ between trials but was significantly lower (6.7%) under microcosms with the matrix [*F* (1, 91)=7.79, *P*<0.006, arc-sin square-root-transformed data] than in microcosms without the matrix. Can-

Table 3 ANOVA table for germination rates in the canopy experiment. Data were arc-sin square-root transformed

	df	Sum of squares Type III	Mean squares	*F* value	*P*
Trial	1	0.911	0.911	32.996	<0.0001
Canopy complexity (CC)	2	3.389	1.694	61.370	<0.0001
Matrix	1	0.074	0.074	2.677	0.105
Trial × CC	2	0.265	0.132	4.800	0.011
Trial × Matrix	1	0.083	0.083	3.019	0.086
CC × Matrix	2	0.079	0.039	1.443	0.242
Trial × CC × Matrix	2	0.054	0.027	0.976	0.381
Residuals	84	2.319	0.028		

Table 4 ANOVA table for germination rates in the one species treatments. Data were arc-sin square-root transformed

	df	Sum of squares Type III	Mean squares	*F* value	*P*
Trial	1	0.159	0.159	4.869	0.038
Matrix	2	0.015	0.015	0.473	0.498
Species	7	0.693	0.099	3.029	0.022
Residuals	22	0.719	0.033		

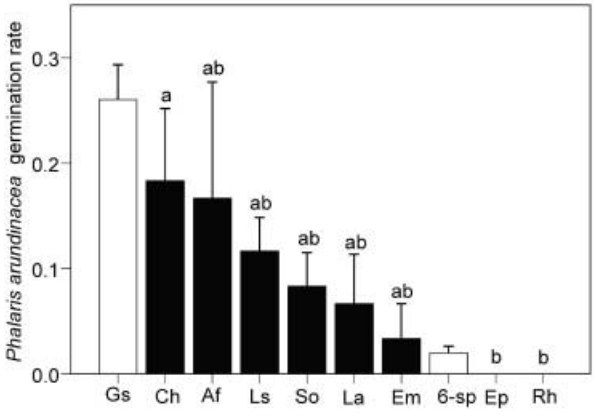

Fig. 2 *Phalaris arundinacea* germination rates by each of the 8 species of our species pool and under *Glyceria striata* and 6-species canopies for comparison (means and standard errors; *Gs = Glyceria striata, Ch = Carex hystericina, Af = Aster firmus, Ls = Lobelia siphilitica, So = Solidago ohioensis, La = Lycopus americanus, Em = Eupatorium maculatum, Ep = Eupatorium perfoliatum* and *Rh = Rudbeckia hirta*). Different letters represent significantly different treatments (Tukey HSD test). *Bars* for Gs and 6-sp are clear and not coded because they were not included in the analysis (see text)

opy openness also differed among species-richness treatments [$F(2, 91)=169.5$, $P<0.00001$, arc-sin square-root-transformed data], with more bare space under *G. striata* (41.4±13.6%), followed by 1-sp canopies (5.8±10.4%) and 6-sp canopies (1.4±2.9%). Percent PAR transmission was correlated with canopy openness [$b=26.74$, $m=50.66$, $r^2=0.37$; $F(1, 94)=54.43$, $P<0.000001$], and germination rates increased with percent PAR transmission [square-root transformed data, $b=-0.025$, $m=0.007$, $r^2=0.25$; $F(1, 94)=31.67$, $P<0.000001$].

Canopy openness explained most of the differences in *P. arundinacea* germination rates, and it varied with species richness and the identity of the species. Analyses of covariance showed a significant canopy complexity effect after accounting for the effect of canopy openness [$F(2, 111)=3.24$, $P=0.043$] or the proportion of broad-leaved species [$F(2, 111)=36.5$, $P<0.0001$].

When canopy complexity was reduced by removing non-matrix species, germination increased considerably. Cumulative germination rates in control microcosms (in which no removal occurred) were the lowest (0.12±0.15) followed by microcosms with removal of the matrix (0.32±0.22), and microcosms with removal of 1sp or 6sp species had the highest rate (0.59±0.19). Differences in germination rates were significant [arcsin square root transformed data $F(2, 43)=40.27$, $P<0.0001$] as well as all multiple comparisons as shown by Tukey test. The maximum germination rate (0.87) occurred in a 6-sp microcosm where removal occurred.

There was a significant trial effect [$F(1, 43)=32.80$, $P<0.0001$], since overall germination was lower in the first trial (0.24±0.26) than in the second trial (0.46±0.23). There was no effect of species richness (1sp+M=0.33±0.27; 6sp+M=0.36±0.27).

Water level experiment

The results of this experiment paralleled those above, despite differences in experimental design (only G+M, 1sp+M and 6sp+M microcosms were planted, microcosms were 20% smaller in this experiment and *P. arundinacea* seeds were planted directly on the soil). In all treatments, germination of *P. arundinacea* was greatly inhibited before pruning of the *G. striata* matrix.

Before pruning the matrix, only 18 seedlings were found in all 36 microcosms (1.25% of all seeds added to the experiment). Twelve seedlings were found in 5 G+M microcosms, 6 seedlings in 3 1sp+M microcosms and no

Table 5 Responses of *Phalaris arundinacea* germination, canopy openness and native species aboveground and belowground biomass to water level and canopy complexity treatments (mean and standard error) in the water level experiment

Treatment	Germination rates	Canopy openness	Aboveground biomass (g) Total	Belowground biomass (g)		
				Top 3-cm	3–6 cm	6–12 cm
Water regime						
Waterlogged	0.25±0.16	0.53±0.37	10.56±3.40	9.56±5.33	1.77±0.38	5.48±3.51
Receding	0.24±0.12	0.51±0.39	12.57±2.01	6.42±3.63	3.44±1.37	14.48±15.02
Moist	0.17±0.20	0.35±0.33	12.07±4.42	4.58±1.98	2.80±0.99	30.43±26.86
Canopy complexity						
G+M	0.39±0.12	0.86±0.14	9.21±1.87	5.23±4.11	2.3±1.11	10.30±5.78
1sp+M	0.17±0.13	0.35±0.34	12.90±4.06	7.46±3.91	2.94±1.59	18.95±27.28
6sp+M	0.11±0.07	0.18±0.08	13.08±2.67	7.82±4.77	2.77±0.74	21.14±21.42

Table 6 ANOVA table in a nested design for germination rates after pruning in the water level experiment

	df	Sum of squares	Mean squares	*F* value	*P*
Water level	2	0.043	0.022	1.357	0.282
Number of species (NOSP)	2	0.043	0.267	16.69	0.0001
Tray nested in water level	3	0.012	0.004	0.249	0.860
Water level × NOSP	4	0.041	0.010	0.638	0.642
(Tray nested in water level) × NOSP	6	0.028	0.005	0.292	0.933
Residuals	18	0.288	0.016		

seedlings appeared in 6sp+M microcosms. Germination rates differed among canopy complexity treatments. G+M microcosms had the highest germination rates (0.025±0.038) followed by 1sp+M microcosms (0.012±0.025) and in 6sp+M microcosms the germination rate was zero. Differences in germination rates are significant [F (2, 18)=4.95, P<0.019], but multiple comparisons showed significant differences only between G+M microcosms and 6sp+M microcosms.

After pruning, all but three 6sp+M microcosms had *P. arundinacea* seedlings. While the presence of seedlings in the microcosms among treatments was not statistically different, differences in germination rates (Table 5) among canopy complexity treatments were highly significant [F (2, 18)=16.69, P=0.0001]. G+M microcosms had higher germination rates (0.390±0.034) than the 1sp+M microcosms (0.167±0.039) and the 6sp+M microcosms (0.107±0.107). There was no effect of water level on germination rate, and there was no interaction (Table 6).

Before pruning, canopy openness was at or near 0%. The pruning treatment increased canopy openness to an average of 86%±14% for G+M; 35%±34% for 1sp+M and 18%±8% for 6sp+M. Differences among treatments were significant [F (2, 28)=36.65; P<0.0001]. All the microcosms with more than 15% canopy openness produced *P. arundinacea* seedlings. Canopy openness was correlated with percent PAR transmission [log transformed data, b=2.748, m=0.976, r^2=0.55; F (1, 34)=42.55, P=<0.000001], and percent PAR transmission was correlated with *P. arundinacea* germination rates (b=0.018, m=0.0074, r^2=0.36; F (1, 34)=18.86, P<0.00012].

Above- and belowground biomass of the native species showed marked responses to the experimental treatments (Table 5). Aboveground biomass was higher in the receding (12.57±2.01 g) and moist (12.07±4.42 g) microcosms than in the waterlogged microcosms (10.56± 3.40 g). There were also differences among canopy complexity treatments; G+M microcosms had the lowest aboveground biomass (9.21±1.87 g), followed by 1sp+M microcosms (12.90±4.06 g) and 6sp+M microcosms had the highest (13.08±2.67 g). Belowground biomass showed similar trends, receding and moist microcosms had overall higher belowground biomass in all three sections (top 3-cm, 3–6 cm and 6–12 cm) than waterlogged microcosms. Only waterlogged microcosms had most of their belowground biomass in the top 3 cm (56.9%).

The screening process of the regression tree analysis for *P. arundinacea* germination rates eliminated aboveground biomass per species, while retaining canopy openness and belowground biomass in the top 3 cm and 6–12 cm of soil as explanatory variables (Fig. 3). The first rule split the data in two groups defined by canopy openness greater or lower than 0.36. The homogeneous group with the highest mean germination rates (0.44) was defined by canopy openness of more than 0.90 and microcosms with less than 3.16 g of biomass in the top 3 cm of soil; these were mostly G+M microcosms of the moist treatment (Table 5). The homogeneous group with the lowest mean germination rates (0.04) was defined by canopy openness of less than 0.16 and microcosms with more than 30.76 g of biomass 6–12 cm below ground; these correspond mostly to 6sp+M microcosms (Table 5).

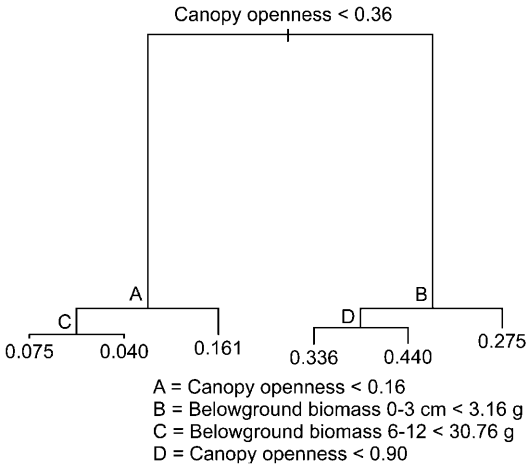

Fig. 3 Regression trees for germination rates in the water level experiment. Each split is defined by a simple rule that creates two homogeneous groups. Terminal nodes are mean germination rates per group (see text)

Discussion

A growing body of research is showing that species richness reduces invasibility. For example, Levine (2000) found that species-rich tussocks of *Carex nudata* were more resistant to invasion by *Cirsium arvense*, *Plantago major* and the grass *Agrostis stolonifera* than species-poor tussocks. Likewise, Naeem et al. (2000) and Symstad (2000) found that grassland communities were less invasible when more species were present. In these field settings, one can hypothesize the mechanisms responsible (e.g., presence of forbs, reduction of light), but these variables must be carefully controlled to test cause-effect relationships. By simulating field conditions in the greenhouse, at the relevant spatial scale, our experiments avoid the influence of confounding extrinsic factors (Naeem et al. 2000) and explain the link between *P. arundinacea* germination and plant canopy characteristics. Furthermore, our evaluation of canopy effects with species added through plantings, as well as subtracted through canopy removals, constitutes a rigorous test of species-richness and species-compositional effects.

P. arundinacea germination is strongly affected by light (Lindig-Cisneros and Zedler 2001); hence, we hypothesized that germination under canopies would be a function of the light environment, that the light environment would be a function of canopy openness, and that canopy openness would be a function of canopy complexity. We defined canopy complexity in terms of the presence/absence of a matrix species and the number and type of other species, with the more complex canopies having six species plus the matrix (*G. striata*, in this study).

Our results support this cause-effect model. The light environment at the soil surface was strongly correlated with canopy openness, and canopy openness was correlated with species richness and composition. Furthermore, although the experiments were performed in a greenhouse under artificial light, the higher germination rates in the second trial, performed during early spring with greater ambient light than in the first trial (during winter), support the overall finding that light increases *P. arundinacea* germination.

Effects of water level, root mass, and light

From our water-level experiment, we determined that inundation depth did not explain germination rates. In this experiment the number of microcosms with seedlings and germination rates was lower than in the canopy experiment, probably because of the higher planting density (the pots for this experiment were 20% smaller than the pots of the canopy experiment).

The main splits in the regression tree were defined by canopy openness, which correlated with percent light transmission. These results are consistent with our earlier finding that *P. arundinacea* does not germinate well under far-red light, which is high under dense canopies (Grant 1997). Root biomass defined two of the five splitting rules in the regression tree. It is possible that roots in the top 3 cm of soil had a direct effect on germination by increasing desiccation of the soil surface, particularly in the moist treatment. The split defined by root biomass 6–12 cm below the soil surface reflected overall higher biomass accumulation, i.e., a positive relationship between above- and belowground biomass.

Species-richness and species composition effects

In all our experiments (canopy experiment, subsequent species removals, and the water level experiment) species-richness levels affected germination more than the matrix species. Our matrix species, despite being the dominant species in our reference system, is a small species (when compared with the other natives) and it has slender leaves that do not create a closed canopy, all reasons that explain its low resistance to *P. arundinacea* germination. *G. striata* (G+M) was, on average, less resistant to invasion than other individual species (1sp treatments), which had more closed canopies than the matrix species. However, species differed in their ability to reduce germination. Species with large horizontal leaves (*E. perfoliatum* and *R. hirta*) shaded the soil more effectively than species with slender, vertical leaves (*Carex hystericina* and *G. striata)*. The 6-species assemblages reduced invasibility and germination rates more effectively than most treatments with one species or with *G. striata*. The 6-species assemblages were outperformed only by *E. perfoliatum* and *R. hirta* microcosms, which had the broadest leaves in our study. Although the results of adding species to the matrix are very clear, their robustness was further demonstrated by our follow-on test of species "subtraction." Removal of non-matrix species increased germination rates regardless of the spe-

cies removed. Removing the matrix had less effect than removing other species, both in 1-species and 6-species treatments. After removing non-matrix species, germination rates were as high as the viability of the seeds (>80%), as tested in the laboratory (Lindig-Cisneros 2001).

When either more species or broad-leaved species were present, less light penetrated to the soil. Hence, canopy complexity is a function of both species richness and species composition. This is consistent with the "idiosyncratic" hypothesis of Tilman et al. (1997) that more diverse plant communities have a higher probability of containing the species that most contribute to specific ecosystem functions, such as productivity (Tilman et al. 1997; Symstad et al. 1998) or, in our case, resistance to germination of an invasive species. Although some 1-species and 6-species microcosms produced no seedlings, only microcosms with *E. perfoliatum* and *R. hirta* consistently lacked seedlings in both trials of the canopy experiment. We conclude that species richness and species composition determined canopy openness, which in turn was the best descriptor of *P. arundinacea* germination microsites.

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