

Long-term invasibility patterns in Mediterranean grasslands of varying initial diversities

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This paper reports the findings of a long-term natural invasibility field study, in experimental Mediterranean grasslands of varying initial diversities and compositions. The study aims to explore the relationship between the initial planted species richness and composition, and the various invasibility components five years after the cessation of weeding of communities. During our experiment, the local species pools of communities with 1, 2, 4, 8, and 18 plant species were kept closed for three years and subsequently opened to natural invasion. Species richness, density and biomass of initial and invading species were monitored in the communities. Values of the Jaccard similarity index of all species in communities decreased over time and their turnover rate increased. External invader cohorts became more similar over time, with their similarity significantly reduced in high species richness plots. Invasibility components significantly declined across the initial species richness gradient. Invasion patterns across the initial richness gradient were constant during the time of the experiment as no significant ‘initial species richness x year’ interactions were observed. Initial species richness significantly affected external invader biomass. Canopy height eliminated the effect of initial species richness on external invader biomass. Invasion significantly declined with an increase in initial species dominance (i.e. their absolute and fractional biomass). Differences in initial species dominance across the experimental species-richness gradient fully explained the effect of initial species richness on invasibility. Consequently, species-rich communities supporting a taller and denser canopy and a higher initial species biomass than species-poor ones are less prone to invasions as they represent stronger competitive environments.

Key words: Biodiversity, dominance, field experiment, invasion, species richness.

INTRODUCTION

Community resistance to invasions is considered a function of diversity (Moore *et al.*, 2001) and a property of the environment (Davis *et al.*, 2000). It could also be the product of interspecific competition from previous established vegetation (Turnbull *et al.*, 2005). Several community properties, such as plant taxonomic identity (Crawley *et al.*, 1999), dominance (Smith *et al.*, 2004), fluctuating resource availability (Davis *et al.*, 2000) or the size of the species pool (Smith & Knapp, 2001) can influence a community’s resistance to invasion. Invasibility further depends on a variety

of features such as propagule pressure (Lockwood *et al.*, 2005), the local climate and the disturbance regime (Lonsdale, 1999), the ecosystem type and the presence and abundance of natural enemies, competitors and mutualists (Milbau *et al.*, 2005).

It has long been suggested that biodiversity, as a community characteristic, may reduce their vulnerability to invasions (Fargione & Tilman, 2005). Field experiments that focused on the diversity-invasibility hypothesis have been proven controversial, as both negative and positive relations have been reported (Loreau *et al.*, 2002). A series of studies support Elton’s hypothesis that locally diverse communities better resist invasions (e.g. Hector *et al.*, 2001; Loreau *et al.*, 2002; Troumbis *et al.*, 2002). Negative relations between diversity and invasions have also been found

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in natural systems but reported only on small scales (Stohlgren *et al.*, 1999). On broader scales, many observational studies have found that more diverse systems hold more diverse sets of invading species. This “invasion paradox” (Fridley *et al.*, 2007) and its conflicting results may arise from extrinsic factors that covary with the diversity of both natives and invaders (Levine *et al.*, 2002) such as the frequency and type of disturbances, resource availability, bare ground percentages, closeness to human populations and climate (Naeem *et al.*, 2000) or because diverse sites are experiencing higher levels of human-driven disturbances (Taylor & Irwin, 2004). A focal point for both conceptual and empirical studies is the way in which plant communities or species assemblages build their invasion resistance as a community attribute (Moore *et al.*, 2001). An equally important question is why some communities are more prone to invasions than others (Crawley, 1987).

At the Greek site of the BIODEPTH experiment and for the early establishment phase of invasion (i.e. before the cessation of weeding in fall 1998), a negative species richness-invasibility relationship had already been reported (Troumbis *et al.*, 2002; Dimitrakopoulos *et al.*, 2005). These short-term invasibility results refer to invader seedlings, as the invaders were not allowed to complete their biological cycle. Nevertheless, there is strong evidence that for regulating species distributions and invasion in grasslands this life-history phase is more important even than survivorship (Smith *et al.*, 2004).

This paper reports the findings of a long-term natural invasibility field study conducted in experimental Mediterranean herbaceous communities of varying initial diversities and compositions (Spehn *et al.*, 2005; Dimitrakopoulos *et al.*, 2006). Monitoring experiments for many years is important because the initial patterns may not last over time, especially if the focus is mainly on annual species. In this study, all reported results refer to invaders that established themselves, grew and matured in the plots. Hence, Crawley’s criterion (1987) for successful invasion (i.e. the increase in the invader population in the new site) can be tested. The vast majority of our species were native and already present in the surrounding grassland. In any case, as invasion theory applies equally well to both native and exotic invaders (Melbourne *et al.*, 2007), any species not planted in a plot is called ‘invader’ regardless of its origin (Kreyling *et al.*, 2008). Our aim is to explore the relationship between the initial planted species richness and the various in-

vasibility components (i.e. invader species number, density and above-ground biomass) five years after the cessation of weeding of experimental communities. Additionally, we attempt to identify possible community characteristics that shape communities’ resistance to invasion by species from the regional species pool (neighbouring plots and surrounding grassland).

MATERIALS AND METHODS

Experimental design and measurements

The experimental site was located in an abandoned arable field on the island of Lesbos, Greece. Plots were established according to the specifications of the BIODEPTH experiment (Troumbis *et al.*, 2002; Spehn *et al.*, 2005) of which constituted an integral part. The natural vegetation of the study site was floristically dominated by annual species (on average 27 ± 1 species/m²). In early winter 1996, we established sixty 4-m² artificial grassland communities of varying initial diversity (1, 2, 4, 8, 18 species), grouped and randomly allocated in two blocks to replicate particular species compositions in addition to richness. Functional diversity (one, two or three functional groups) was manipulated by controlling the initial composition of each plot in terms of the combination of three *ad hoc* defined plant functional groups: i) grasses, ii) non-legume forbs, including geophytes (henceforth called herbs), and iii) legumes. Seeds from a pool of 23 plant species (5 grasses, 12 herbs and 6 legumes, among them 18 annuals and 5 perennials) present in the surrounding natural grassland communities were collected and sown in the plots at a density of 2000 viable seeds per m², equally divided between species in each mixture. Before sowing, all natural vegetation was mechanically removed from the plots and the seed bank was killed with the use of methyl bromide [for a more detailed description, see Troumbis *et al.* (2002) and Dimitrakopoulos *et al.* (2005)].

By manually weeding, the local species pools of experimental resident communities were kept constant for three years until weeding ceased in fall of 1998. The richness of both initial and invading species was recorded in each plot in the years 2000, 2001 and 2003. In the same time period, the number of individuals was counted within the central 1 × 1 m square of each plot.

At the time of peak standing crop, the above-ground biomass per species was harvested in an area of 20 × 50 cm² in the centre of each plot, then dried at

80°C for 24 hrs and weighed (Spehn *et al.*, 2005). In addition, components of the vegetation structure of the communities such as canopy height, total plant cover, leaf area index (LAI) and leaf area density (LAD = LAI/height) were monitored throughout the whole experiment (Spehn *et al.*, 2005). Every species that did not belong to the initially sowing mixture of each community was considered as an ‘invader’ (see also Crawley *et al.*, 1999). Following Hector *et al.* (2001) and Fargione & Tilman (2005), two types of invader sets were used for the analyses: a) ‘all invaders’ defined as the species not planted in a specific plot and b) ‘externals’ defined as those species that did not belong to the initial species pool.

Data analyses

We analyzed our data with analysis of variance (ANOVA) applying multiple linear regression approaches (Schmid *et al.*, 2002). All models were run using R-language, v. 2.14.0 (R Development Core Team, 2011). Components of invasibility were examined for significant differences ($p < 0.05$) in relation to the following experimental factors: ‘year’, ‘block’, ‘initial species richness’ (numbers), ‘initial functional group richness’, and ‘initial species composition’ (types). At the start of the invasion experiment (i.e. 1998) initial (sown) species richness was highly correlated with the realized resident species richness (Pearson correlation coefficient = 0.992, $t = 53.95$, $df = 50$, $p < 0.001$), and the sown species richness gradient was maintained. Hence, initial species richness was used for analyses. In order to investigate the temporal variation of invasibility, the ‘year’ factor was added first in the models and its interactions with species richness and composition were examined (Spehn *et al.*, 2005). In addition, for the exploration of the independent effect of initial species richness on invasions, different components of community canopy structure (i.e. plant cover, canopy height, leaf area index, leaf area density) and of initial species dominance levels (i.e. absolute and fractional biomass of all initial species) were added separately as covariates into the ANOVAs.

Species occurrences were used to calculate extinction rates (ER) per plot between experimental years as $ER = 2 E / (S1 + S2)$, where E is the number of extinctions, and S1 and S2 are the species richness in the respective years. In the same way, immigration rates (IR) were computed using the number of immigrations (I). Finally, turnover rates (TR) were calcu-

lated as $TR = (E + I) / (S1 + S2)$ (Pfisterer *et al.*, 2004; Roscher *et al.*, 2009a). These calculations were performed separately for: i) all species (residents and invaders), ii) resident species, iii) all invaders, and iv) external invaders in each plot. In order to track the community assembly processes, presence/absence matrices of all species, residents, all invaders and external invaders per plot were produced for every experimental year. Thereafter, to detect potential convergences in species compositions, Jaccard similarities for each plot between the years were calculated.

For extinction, immigration and turnover rates as well as for Jaccard similarities, a ‘time’ factor with two levels (‘1’ for the 2000–2001 and ‘2’ for 2001–2003 comparison) was used in the ANOVA model.

RESULTS

Species presence

From the beginning of the experiment, 77 different species (13 grasses, 49 herbs and 15 legumes) were recorded in our plots: the 23 initially planted species that acted as internal invaders, and 54 newcomers that arrived from the surrounding grasslands and comprised the external invaders (8 grasses, 37 herbs and 9 legumes). In the last year of experimentation (2003), four of the initial species were no longer recorded: three herbs (*Malva sylvestris* L., *Anthemis cotula* L., *Hirschfeldia incana* (L.) Lagr.-Foss.), and one legume (*Medicago lupulina* L.). In the same year, the total species number in all plots was 55 (11 grasses, 34 herbs and 10 legumes) with a mean total species number (invading and initial species) of 17.6 species (± 0.6 SE) and a range of 9 to 25 species per plot (± 4.4 SD). As just one species (the sown *Hordeum geniculatum* All.) did not invade any plot, 54 of these species were invaders (both internal and external) with a mean invader species number of 14.8 (± 0.7 SE) and a range of 4 to 25 invader species per plot (± 5 SD). Out of these, 36 (or 65%) of plant communities’ species were external [mean species number 7.7 (± 0.4 SE) per plot] and 18 internal invaders [mean species number 7.1 (± 0.4 SE) per plot]. Ten of them were grasses (6 externals), 34 herbs (25 externals) and ten legumes (5 externals).

Community composition

Compositions of all species (initial and invaders) found in the same plot became significantly less similar over time ($F_{(1,21)} = 15.4$, $p < 0.001$). Resident spe-

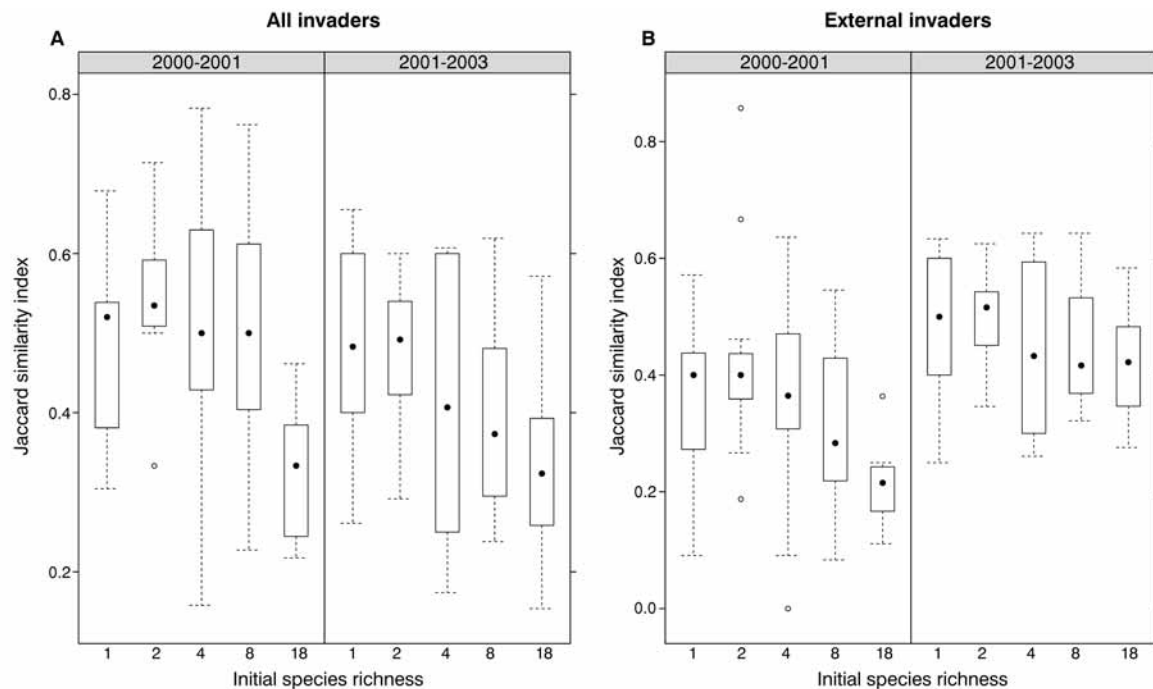


FIG. 1. Variation of the Jaccard similarity index of All invader (A) and External invader (B) cohorts between time intervals across the initial species richness gradient. Box plots show outliers (o), first and third quartile, min, max and median values of the data set.

cies cohorts, although less similar over time, illustrated a remarkably high Jaccard index, a fact that was not significantly affected by any of the experimental factors used. Invader species compositions became less similar over time ($F_{(1,21)} = 6.39, p = 0.02$), and their si-

milarity declined significantly with increasing initial species richness ($F_{(4,19)} = 4.26, p = 0.013$) (Fig. 1A). Conversely, for external invaders of each plot the Jaccard similarity index distribution was positively affected by time ($F_{(1,21)} = 28.7, p < 0.001$). Initial species

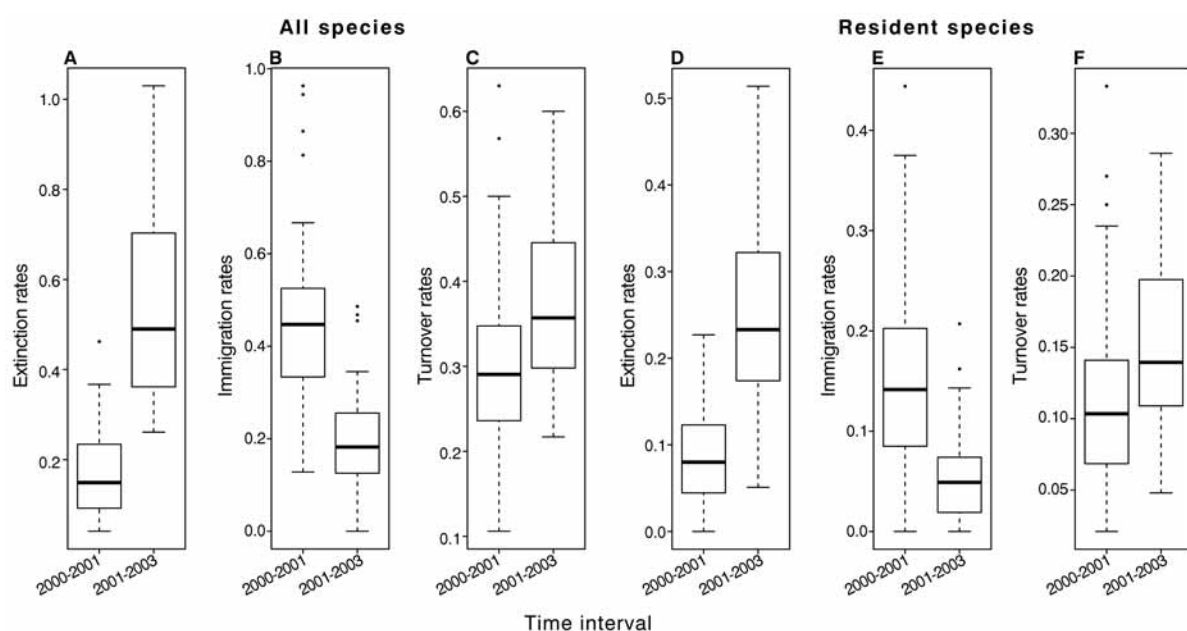


FIG. 2. Extinction, Immigration and Turnover rates distribution of All species and Resident species across Time intervals. Box plots show outliers (o), first and third quartile, min, max and median values of the data set.

richness negatively affected external invader similarity ($F_{(4,19)} = 3.92, p = 0.017$) (Fig. 1B).

The extinction rate of all species in each community was significantly different over time ($F_{(1,21)} = 113.15, p < 0.001$) (Fig. 2A), and its mean value across all plots was more than threefold higher for the second time interval compared with the first. This outcome emerged mainly due to a higher extinction rate for the resident species ($F_{(1,21)} = 69.31, p < 0.001$) (Fig. 2D), since all species' and external invader species' extinction was relatively constant over time ($p = 0.335$ and $p = 0.088$, respectively). Immigration rates for all species significantly declined over time ($F_{(1,21)} = 87.34, p < 0.001$) (Fig. 2B), due once again to significant temporal changes in resident species' immigration rates that also decreased over time ($F_{(1,21)} = 50.82, p < 0.001$) (Fig. 2E). The initial composition of the communities significantly affected all species' immigration rate ($F_{(19,21)} = 1.84, p = 0.044$), as well as resident species' immigration rate ($F_{(19,21)} = 2.36, p = 0.008$). The latter was found to significantly decline across the initial species richness gradient ($F_{(4,19)} = 2.64, p = 0.044$). The estimated all species' and resident species' turnover rates increased significantly over time ($F_{(1,21)} = 8.76, p = 0.005$ and $F_{(1,21)} = 6.95, p = 0.011$, respectively) (Fig. 2C, F).

Invasibility patterns

After five years of succession, the mean total species number per plot reached the initially designed maximum plot species richness (i.e. 18 species). Cumulative total species richness appeared to become unwavering. Our communities seemed to not be susceptible to further addition of external species and therefore may have reached a saturation plateau. Number, density and biomass of all invaders significantly declined with an increase in the initial species richness (Table 1). This negative species richness-invasibility relationship was constant for all experimental years (Fig. 3A, B, C). The initial composition of the experimental communities strongly affected invaders richness and density but not their biomass (Table 1). All components of invasion were determined by the experimental year because of their higher mean values during 2001. This trend was due to a relatively warmer winter and wetter germination and growth season in the specific experimental year (source: Hellenic National Meteorological Service). However, the negative species richness-invasibility relationship was still robust, since there was no significant 'year x ini-

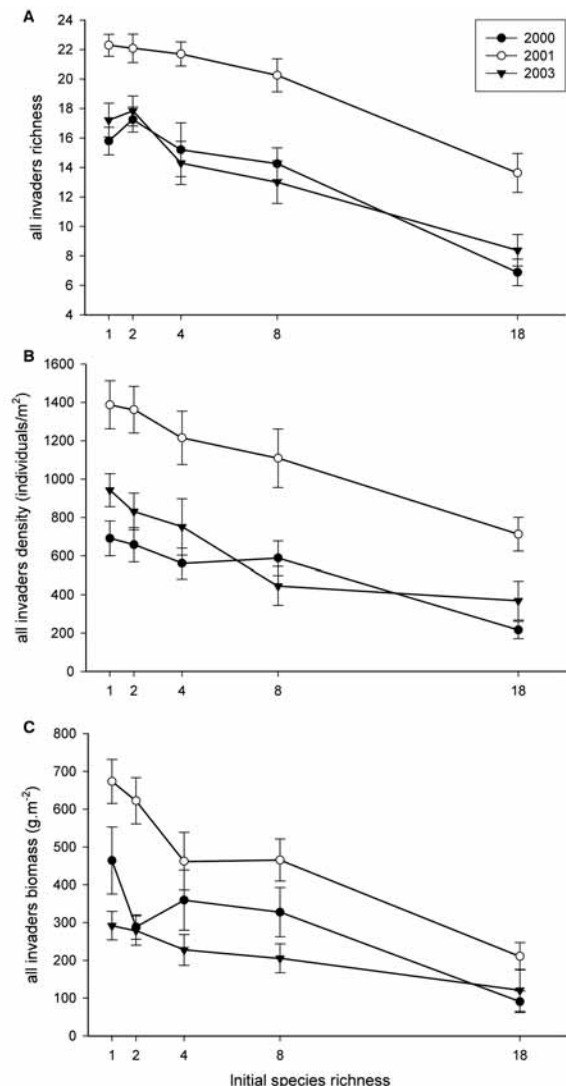


FIG. 3. Species richness (A), density (B) and aboveground biomass (C) of All invaders across the initial species richness gradient in 2000, 2001, and 2003. All results shown are means of original data \pm SE.

tial species richness' interaction. The year of experimentation significantly determined all the invasibility components of external invaders as well (Table 1). External invader biomass was significantly affected by initial species richness, their species numbers by the communities' initial composition and their densities by initial functional group richness (Table 1, Fig. 4).

When splitting all invaders dataset to annual and perennial invaders, and separately testing their performance against the experimental factors, no major changes in invasion patterns were observed. All components of annual and perennial species invasion were similarly determined by initial species richness (for annual all invaders $p < 0.001$, and for perennial ones $p < 0.005$), and differed between years without a

TABLE 1. Analyses of variance for species richness, density and biomass of all and external invaders

	Species Richness						Density						Aboveground Biomass						
	All invaders			External invaders			All invaders			External invaders			All invaders			External invaders			
	df	MS	F	p	MS	F	p	MS	F	p	MS	F	p	MS	F	p	MS	F	p
Year	2	617	49.66	<0.001	276.01	41.94	<0.001	5537572	50.48	<0.001	802310	16.81	<0.001	1060617	34.26	<0.001	463541	38.27	<0.001
Block	1	50	4.00	0.049	60.31	9.16	0.003	565206	5.15	0.026	720256	15.09	<0.001	60444	1.95	0.166	983	0.08	0.777
Initial species richness	4	393	31.66	<0.001	11.46	1.74	0.150	1483695	13.53	<0.001	73999	1.55	0.196	450853	14.56	<0.001	35820	2.96	0.025
Initial FG richness	2	13	1.03	0.364	3.95	0.60	0.552	7456	0.07	0.934	179903	3.77	0.028	10619	0.34	0.711	29317	2.42	0.096
Initial species composition	19	22	1.74	0.049	12.16	1.85	0.032	233849	2.13	0.011	30993	0.65	0.855	42957	1.39	0.159	11526	0.95	0.525
Year × block	2	31	2.50	0.089	8.78	1.33	0.270	332823	3.03	0.054	3233	0.07	0.935	255318	8.25	<0.001	23494	1.94	0.151
Year × species richness	8	7	0.53	0.831	7.72	1.17	0.327	59750	0.55	0.819	25064	0.53	0.834	46969	1.52	0.166	10135	0.84	0.573
Year × FG richness	4	2	0.19	0.942	0.68	0.10	0.981	49445	0.45	0.772	41954	0.88	0.481	24695	0.80	0.530	13657	1.13	0.350
Year × species composition	38	10	0.79	0.785	4.63	0.70	0.883	80341	0.73	0.853	16683	0.35	1.000	32134	1.04	0.435	10003	0.83	0.738
Errors	75	12			6.58			109695			47736			30960					12112

Listed are the degrees of freedom (df), mean squares (MS), F-ratio and the p-values. FG: functional group. Significant values ($p < 0.05$) are in bold

TABLE 2. Analyses of variance for species richness, density and biomass of all and external invaders with canopy height added as a covariate

	Species Richness						Density						Aboveground Biomass						
	All invaders			External invaders			All invaders			External invaders			All invaders			External invaders			
	df	MS	F	p	MS	F	p	MS	F	p	MS	F	p	MS	F	p	MS	F	p
Canopy height	1	430.3	34.22	<0.001	98.510	14.78	<0.001	1930565	17.96	<0.001	500024	10.34	0.002	95273	3.04	0.085	96503	8.10	0.006
Year	2	603.3	47.97	<0.001	268.230	40.23	<0.001	5412631	50.36	<0.001	769649	15.92	<0.001	1061286	33.87	<0.001	457476	38.41	<0.001
Block	1	43.4	3.45	0.067	57.140	8.57	0.005	521573	4.85	0.031	695136	14.38	<0.001	57013	1.82	0.181	609	0.05	0.822
Initial species richness	4	306.1	24.34	<0.001	3.700	0.56	0.696	1164277	10.83	<0.001	37101	0.77	0.550	427949	13.66	<0.001	22221	1.87	0.125
Initial FG richness	2	19.3	1.53	0.223	5.480	0.82	0.444	140	0.00	0.999	132616	2.74	0.071	9932	0.32	0.729	23019	1.93	0.152
Initial species composition	19	18.3	1.45	0.129	9.460	1.42	0.145	255549	2.38	0.004	22301	0.46	0.970	51807	1.65	0.065	10639	0.89	0.592
Year × block	2	31.1	2.47	0.091	8.610	1.29	0.281	157835	1.47	0.237	4923	0.10	0.903	177442	5.66	0.005	25985	2.18	0.120
Year × species richness	8	6.6	0.52	0.835	7.770	1.17	0.332	60262	0.56	0.807	25573	0.53	0.831	47042	1.50	0.171	10470	0.88	0.538
Year × FG richness	4	2.4	0.19	0.943	0.660	0.10	0.982	49950	0.47	0.761	42119	0.87	0.485	25093	0.80	0.529	13485	1.13	0.348
Year × species composition	38	9.8	0.78	0.796	4.620	0.69	0.891	76654	0.71	0.873	16465	0.34	1.000	31832	1.02	0.466	10530	0.88	0.656
Errors	74	12.6			6.670			107487			48352			31333			11909		

Listed are the degrees of freedom (df), mean squares (MS), F-ratio and the p-values. FG: functional group. Significant values ($p < 0.05$) are in bold

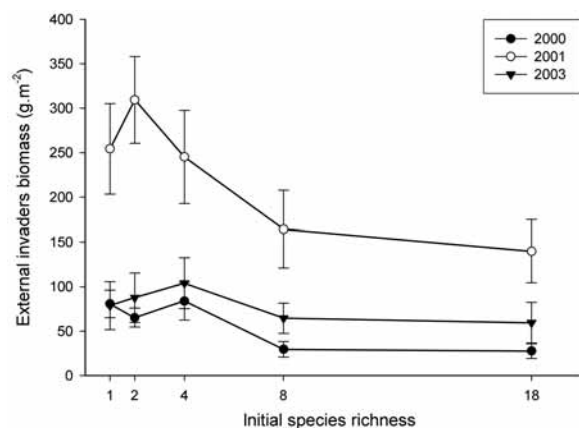


FIG. 4. Aboveground biomass of External invaders as a function of initial species richness for all years. All results shown are means of original data \pm SE.

significant ‘year \times initial species richness’ interaction effect. However, for the external invaders a clear distinction emerged between annuals and perennials. Any significant effect of initial species richness on external annual species invasion was no longer observed. On the contrary, initial species richness determined all components of external perennial species invasion (richness: $F_{(4,19)} = 2.67, p = 0.039$; density: $F_{(4,19)} = 5.86, p < 0.001$; biomass: $F_{(4,19)} = 3.55, p < 0.001$).

Invasibility and community architecture

Plant coverage was constantly over 95% across all experimental plots in every year. After weeding cessation, vegetation structure parameters significantly differed over the years (Cover: $F_{(2,38)} = 4.86, p = 0.01$; LAI: $F_{(2,38)} = 38.7, p < 0.001$; LAD: $F_{(2,38)} = 39.48, p < 0.001$, and Height: $F_{(2,38)} = 2.98, p = 0.057$). LAI and LAD increased with time, while mean canopy height decreased very slightly (Fig. 5). Height and LAI were positively related with initial species richness (Height: $F_{(4,38)} = 79.46, p < 0.001$; LAI: $F_{(4,38)} = 2.51, p = 0.049$), and significant differences among the initial compositions of species communities were found for height ($F_{(19,38)} = 66.65, p < 0.001$), LAI ($F_{(19,38)} = 3.34, p < 0.001$), and cover ($F_{(19,38)} = 2.2, p = 0.008$). When plant cover was included as a covariate in the initial ANOVA tables a significant effect was observed only for all invaders density ($F_{(1,74)} = 6.05, p = 0.016$), but initial species richness and composition remained significant predictors of invasion. External invaders were not affected by differences in plant cover. As a covariate, canopy height affected the number and density of all invaders (Table 2). For the external in-

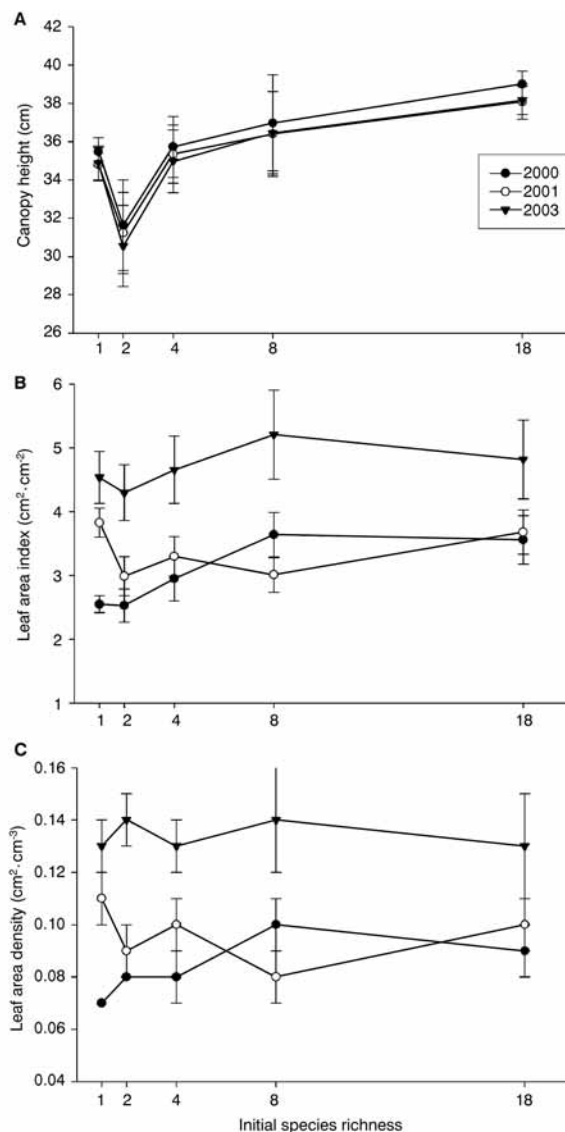


FIG. 5. Canopy height (A), Leaf Area Index (B) and Leaf Area Density (C) of communities across the initial species richness gradient in 2000, 2001, and 2003. All results shown are means of original data \pm SE.

vaders, canopy height had a significant effect on all three components of their invasibility (Table 2), eliminating the effect of initial species richness on external invaders biomass. Leaf Area Index significantly affected all invaders richness ($F_{(1,74)} = 55.06, p < 0.001$) and density ($F_{(1,74)} = 6.76, p = 0.011$), along with external invaders species number and individuals ($F_{(1,74)} = 27.28, p < 0.001$, and $F_{(1,74)} = 14.68, p < 0.001$, respectively). Leaf Area Density significantly affected overall invaders species number ($F_{(1,74)} = 23.32, p < 0.001$), and external invaders richness ($F_{(1,74)} = 12.12, p < 0.001$) and density ($F_{(1,74)} = 5.84, p = 0.018$).

TABLE 3. Analyses of variance for species richness, density and biomass of all and external invaders with initial species dominance added as a covariate

Species Richness										Density						Aboveground Biomass													
All invaders					External invaders					All invaders					External invaders					All invaders					External invaders				
df	MS	F	p		MS	F	p		MS	F	p		MS	F	p		MS	F	p		MS	F	p						
Initial species dominance	1	1407.2	114.03	<0.001	123.97	18.89	<0.001	12890466	157.39	<0.001	607472	14.92	<0.001	3608593	148.51	<0.001	348219	33.75	<0.001	427056	41.39	<0.001							
Year	2	535.2	43.37	<0.001	256.17	39.03	<0.001	4686186	57.22	<0.001	724049	17.78	<0.001	964796	39.71	<0.001	427056	41.39	<0.001										
Block	1	7.8	0.63	0.429	44.93	6.85	0.011	121889	1.49	0.226	597549	14.68	<0.001	486	0.02	0.888	1053	0.10	0.750										
Initial species richness	4	121.9	9.88	<0.001	3.16	0.48	0.749	36289	0.44	0.777	80636	1.98	0.106	13614	0.56	0.692	13443	1.30	0.277										
Initial FG richness	2	12.5	1.01	0.369	4.23	0.65	0.528	15774	0.19	0.825	186673	4.59	0.013	8492	0.35	0.706	32460	3.15	0.049										
Initial species composition	19	18.4	1.49	0.113	11.84	1.80	0.038	85344	1.04	0.427	36629	0.90	0.585	15074	0.62	0.879	12170	1.18	0.298										
Year x block	2	37.6	3.05	0.053	10.78	1.64	0.200	329516	4.02	0.022	5482	0.14	0.874	193997	7.98	<0.001	13146	1.27	0.286										
Year x species richness	8	5.4	0.44	0.894	7.24	1.10	0.371	68834	0.84	0.570	33638	0.83	0.582	45313	1.87	0.079	10862	1.05	0.405										
Year x FG richness	4	5.4	0.43	0.784	1.73	0.26	0.900	62688	0.77	0.551	20859	0.51	0.727	6549	0.27	0.897	6545	0.63	0.640										
Year x species composition	38	8.3	0.68	0.906	3.93	0.60	0.958	77649	0.95	0.562	19401	0.48	0.993	23144	0.95	0.556	9581	0.93	0.591										
Errors	74	12.3			6.56			81900			40713			24298			10317												

Listed are the degrees of freedom (df), mean squares (MS), F-ratio and the p-values. FG: functional group. Significant values ($p < 0.05$) are in bold

Invasibility and initial species dominance

Total community biomass production significantly differed over the years due to the high values noticed in 2001 ($F_{(2,38)} = 46.94, p < 0.001$). Initial species richness had no effect on total biomass both for all years ($p = 0.31$) and for each year separately (p -values > 0.2). Conversely, fractional biomass of the initial species that remained in the plots, i.e. the ratio of their biomass production to the total community production, was not affected by year but it was significantly higher in species-rich plots ($F_{(4,19)} = 41.8, p < 0.001$), and strongly affected by initial species composition ($F_{(19,38)} = 3.84, p < 0.001$). Resident species fractional biomass forms an index of their dominance levels within each community. When initial species dominance was included as covariate in the ANOVA tables all components of invasibility were negatively affected (Table 3). Initial species richness continued to have a significant effect on all invaders species richness (Table 3). For the external invaders, initial species fractional biomass negatively affected all of their invasibility components, excluding the significant effect of the initial species richness on their biomass (Table 3). The same pattern was observed when initial species' fractional biomass was replaced with their absolute biomass values in each plot, and tested against external invader biomass ($F_{(1,74)} = 8.94, p = 0.004$); the significant effect of initial species richness to external biomass was removed ($p = 0.237$).

DISCUSSION

Our results support Elton's hypothesis (Elton, 2000) that species-rich communities are more resistant to invasion than species-poor ones and affirm our early-establishment phase of invasion findings (Troumbis *et al.*, 2002; Dimitrakopoulos *et al.*, 2005). They also concur with the findings of several invasibility studies in grasslands that demonstrate the contribution of diversity in communities' invasion resistance (Knops *et al.*, 1999; Levine, 2000; Naeem *et al.*, 2000; Hector *et al.*, 2001; Lyons & Schwartz, 2001; Kennedy *et al.*, 2002; van Ruijven *et al.*, 2003; Pfisterer *et al.*, 2004; Fargione & Tilman, 2005; Roscher *et al.*, 2009b; Petermann *et al.*, 2010). When referring to all invaders, our negative diversity-invasibility relationship was constant for every experimental year and for every component of invasibility. In a comparable study for the Swiss BIODDEPTH site, Pfisterer *et al.* (2004) found the same invasibility trend for all invader and external invader richness. The observed significant ef-

fect of initial species richness on external invasion patterns was mainly driven by the response of perennial external invaders. Perennial species are thought to be stronger competitors than annuals in undisturbed low productivity Mediterranean grasslands (Pagnotta *et al.*, 1997; Troumbis *et al.*, 2000).

Even though the mean total species richness in 2003 approached the initially planted maximum diversity of 18 species, experimental communities continued to preserve a wide range of total species number (9-25 species). Two years after weeding cessation, Pfisterer *et al.* (2004) reported a rapid convergence of the actual mean species richness of communities to the same mean species number, although at a level lower than their initial maximum richness. Along with their species richness, our experimental communities remain distinct also in terms of their compositions, as their averaged Jaccard similarity indices declined with time. In the study of Pfisterer *et al.* (2004) species composition, although more similar, also remain distinct. The rate of immigration in the communities considerably decreased over time, and this decrease was independent from the initial species richness. This could imply less open spaces or opportunities for new species to colonise the communities. However, the decrease in the communities' immigration rate was mainly due to a lower rate of immigration of resident species and consequently, it was also dependent on initial species identities of the communities. Additionally, turnover rates in the communities increased and remained at high levels.

More resident species became extinct between 2001 and 2003, with relatively low turnover levels. Resident species assemblages did not significantly change over time in terms of their species identities, as on average high similarity values were found. Roscher *et al.* (2009a) have also reported comparable trends for resident species similarity, though at much lower rate values (e.g. ER = 0.033 ± 0.005 and TR = 0.044 ± 0.004). All invader species compositions became less similar over time and their similarity declined across the initial species richness gradient. A reverse trend emerged for external invaders species identities, as their taxonomic similarity increased over time. On the other hand, species-poor communities continued to hold higher taxonomic external invader diversity compared to the species-rich ones. This could reflect the rather restricted regional species pool from which they arrived. This pattern supports the findings of the early establishment phase of this experiment, where species-rich communities hold

fewer invaders but with greater floristic diversity (Troumbis *et al.*, 2002).

Initial species richness significantly affected only the biomass of external invaders. The fact that invasibility patterns grew much weaker when initially planted species acting as invaders were removed from the analyses reveals an essential methodological point. High diversity experimental plots have a lower internal invasion potential than those seeded with just one or two species, as they structurally contain more internal species. This inevitable statistical artefact of enclosing fewer internal invaders could lower their overall invasion rates. Such artefactual patterns seem to considerably contribute to all our invader results, and might be important factors in other analogous studies. In this manner, we focused our analyses on external invaders. Furthermore, external invaders are considered a better descriptor of invasion processes (Hector *et al.*, 2001; Fargione & Tilman, 2005), and their biomass production can be seen as a finer assessment of community resistance to invasion (Naeem *et al.*, 2000; Hector *et al.*, 2001; Fargione & Tilman, 2005). Differences in functional attributes between resident species and external invaders can shape invasion outcome, and their examination could improve the understanding of invasibility patterns at a community level.

Our results indicate that several components of community architecture, such as canopy height, LAI, and LAD, affected communities' invasion outcome. Bigger mean canopy height and higher LAI decreases the number and density of all invaders in the plots. Additionally, external invaders richness and density declined in the denser plots and their overall performance decreased with an increase in mean canopy height eliminating the effect of initial species richness. Thus, the effect of initial species richness on both externals and all invaders can be partly explained by changes in vegetation structure components and specifically in canopy height differences. Hector *et al.* (2001) reach the same conclusions for the effects of light transmittance and percent bare ground on weed biomass.

Naeem *et al.* (2000) associate the negative response of invasion to increasing diversity with higher interspecific competition for light and space. Knops *et al.* (1999), suggested that diversity impacts invasion via controlling resources, and reported strong positive relationships between light availability and total invaders biomass. Competition for light (canopy height, LAI) and space filling (LAD) inside plant communi-

ties directly reflect the amount of their photosynthetic activity and indirectly their productivity. Hence, moving from species-poor to species-rich plots, competition intensity by the initial species cohorts, expressed both by components of vegetation structure and by the performance of initial species, becomes stronger. As observed in our plots (Tables 2 and 3) and also proposed by Davis *et al.* (2000) the effect of this higher competitive environment on invasibility is negative to invasion success.

Competition has been suggested as an important mechanism on determining invasibility (Mitchell *et al.*, 2006; Davis & Pelsor, 2001). As the number of competitive species in a community increases, the negative effect of competition on invaders performance gets stronger (Levine *et al.*, 2004). Along with disturbance and resource availability, competition is one of the major parameters determining both species richness and invasion resistance of a community (Levine & D'Antonio, 1999). Davis *et al.* (2000) suggested that when competition is high, invasibility would be low irrespective of a community's diversity or productivity. In our experiment, initial species dominance, as an indicator of initial species competition that a successful invader has to overcome, partly explains the effect of initial species richness on community invasibility. In fact, it eliminates the direct effect of species richness on invasion components. We argue that higher levels of initial species dominance, in terms of their absolute or fractional biomass, lead to communities that are more invasion resistant.

This is in contrast with the results of Smith *et al.* (2004) who found that dominance of C4 grasses facilitates the establishment of invaders due to improved microclimatic conditions. However, it concurs with their general suggestion that for randomly constructing communities, dominant species could drive diversity-invasibility relationships. It is furthermore in agreement with other studies' results that propose a negative effect of dominance to invaders persistence (Robinson *et al.*, 1995; Burke & Grime, 1996; Smith & Knapp, 1999; Foster *et al.*, 2002). Negative correlations between biomass of resident species and biomass of colonising species have been reported in several experimental studies (Guo *et al.*, 2006; Lanta & Leps, 2008; Petermann *et al.*, 2010). A decrease in the biomass of colonising species with a log-linear increase of sown species richness has also been found in the never weeded treatment of the Jena Experiment (Roscher *et al.*, 2009a). Consequently, five years after the cessation of weeding, initial richness continues to

be a significant factor regulating invasibility, but it seems that the most essential factor is not the number of the remaining initial species but their contribution to the community productivity. We suggest that in communities where initial species have established and maintain robust populations, fill the space fully, exploit the resources more efficiently and are of the major community biomass producers, the chances for an invader to establish and survive are limited.

Invasion resistance in our grasslands continues to be a function of their species richness but ultimately what matters is how effectively these species take advantage of environment resources. In our experiment, the observed negative species richness-invasibility relationship derived completely from differences in initial species dominance and in canopy height of the communities across the experimental species richness gradient. Similar responses of invaders' growth to light and neighbour biomass have been reported by Milbau *et al.* (2005) and were attributed to higher below-ground resource use and competition for space in more productive communities. More initial species lead to less available niche space in the communities and to a higher functional variety of the occupied niches (Shea & Chesson, 2002), resulting in resource complementarity. This biological mechanism (Wardle, 2001) would decrease the available resources as the potential species combinations that could effectively utilize resources or reinforce communities' competitive ability (Fargione & Tilman, 2005) increase.

Five years after the cessation of weeding the initial species assemblages continued to regulate community assembly in our plots. A species-level priority effect mechanism, whereby initially sown species directly or indirectly affect the potential of later immigrants to establish themselves (Roscher *et al.*, 2009a), might occur. In conclusion, we propose that species-rich communities, supporting a taller and denser canopy and higher initial species' biomass, represent a more competitive environment for a potential invader in relation to species-poor ones and therefore would be less susceptible to invasions.

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