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DOI: <https://doi.org/10.1016/j.baae.2013.01.003>

Posted at the Zurich Open Repository and Archive, University of Zurich

ZORA URL: <https://doi.org/10.5167/uzh-81799>

Accepted Version

Originally published at:

Roscher, Christiane; Fergus, Alexander J F; Petermann, Jana S; Buchmann, Nina; Schmid, Bernhard; Schulze, Ernst-Detlef (2013). What happens to the sown species if a biodiversity experiment is not weeded? *Basic and Applied Ecology*, 14(3):187-198.

DOI: <https://doi.org/10.1016/j.baae.2013.01.003>

What happens to the sown species if a biodiversity experiment is not weeded?

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MS details: 300 words in abstract, 5833 words in full text, 5 figures, 1 table, Supplementary
Material

Abstract

Studies in experimental grasslands have extensively documented the effects of sown plant diversity on the colonization of new species, but the responses of the sown plant combinations themselves have rarely been investigated. We established experimental grasslands differing in species richness (1, 2, 4, 8, and 16) and functional group number and composition (1 to 4; legumes, grasses, small herbs, tall herbs), and we studied the changes in the abundance of sown species (= residents) in both weeded and non-weeded subplots over a period of five years after sowing. The accumulation of new species through spontaneous colonization in the non-weeded treatment did not affect the number of resident species, but had increasingly negative effects over time on the cover of resident species and their aboveground biomass production at community level. Temporal stability of resident populations was lower and year-to-year changes in resident species composition were larger in non-weeded than in weeded subplots. Compositional dissimilarity between weeded and non-weeded treatments increased through time. These negative effects of the colonization of new species on the abundances and stability of resident populations depended on resident species identity and not on additional variation between different functional groups. The colonization of new species did not change the number of resident species emerging from seeds, but reduced seedling densities of residents. Colonization did not affect the structure of resident communities as measured by species evenness, functional trait diversity and mean trait values suggesting that colonization can destabilize the species composition of residents in terms of abundance while leaving them unchanged in terms of functional characteristics. Generally, negative impacts of colonizing species on residents which accelerated through time decreased with an increasing number of sown species. Sowing more diverse grassland mixtures increases their predictability in terms of ecosystem characteristics, which is important for ecological restoration and sustainable agriculture.

47 **Keywords:** biodiversity, community assembly, functional trait composition, Jena Experiment,
48 productivity, stability
49

Introduction

Concerns about an accelerated loss of species diversity have stimulated an increasing interest in the potential impact of biodiversity on ecosystem processes (Hooper, Chapin, Ewel, Hector, Inchausti et al. 2005). Understanding the mechanisms that control community-level phenomena of assembly, compositional stability and resistance against invasion is essential to assess consequences of species loss. More diverse plant communities are hypothesized to have a greater resistance against invasion (Elton 1958). Increased resource capture by a diverse community, leaving fewer resources available for potential invaders, has been suggested as an explanation (Tilman 1982). Invading species themselves may affect ecosystem processes by modifying species interactions and altering the structure and composition of the established communities (Meiners, Pickett & Cadenasso 2001, Yurkonis, Meiners & Wacholder 2005). Invaders may affect the resident community by inhibiting germination and establishment of new individuals belonging to the resident community (Crawley, Brown, Heard & Edwards 1999). Invaders may also displace established individuals of resident species through resource competition or the development of antagonistic soil microbial feedbacks (Theoharides & Dukes 2007). The effects of invaders may depend on their identity and that of residents, however, effects at the species level may not necessarily translate into community processes (Yurkonis et al. 2005).

In spite of controversies over the interpretation of results about diversity–invasion resistance relationships obtained in observational and experimental studies (Fridley, Stachowicz, Naeem, Sax, Seabloom et al. 2007), similar mechanisms are supposed to explain the suppression of invaders of non-resident species by more diverse communities in natural and experimental systems. A number of biodiversity experiments have investigated the effects of species richness on the spontaneous colonization of new species at single or multiple points in time (e.g. Knops, Tilman, Haddad, Naeem, Mitchell et al. 1999, van Ruijven, de Deyn & Berendse 2003, Roscher, Beßler, Oelmann, Engels, Wilcke et al. 2009a). Less is known about

the response of the resident species themselves once a community is open to colonization by unsown species. Some studies reported a rapid loss of resident species in highly diverse mixtures in the first years after cessation of weeding (Pfisterer, Joshi, Schmid & Fischer 2004, Rixen, Huovinen, Huovinen, Stöckli & Schmid 2008) or when high-diversity mixtures were established on arable land (Lepš, Doležal, Bezemer, Brown, Hedlund et al. 2007). Such experiments did not include a weeded control and therefore lack the direct comparison of weeded vs. non-weeded artificially established communities. This distinction may have important implications for the evaluation of results obtained in numerous biodiversity experiments (Hooper et al. 2005), many of which have been criticized for immaturity of plant communities, their random species selection and the continuous manipulation required to maintain the designed species compositions (Wardle 2001, Lepš 2004). In addition, land-use changes are among the most important drivers of global biodiversity and intensification of land-use is thought to reduce the diversity and composition of biological communities (Schläpfer, Schmid & Seidl 1999). Therefore, developing strategies to maintain, create or reassemble communities resistant to biological invasion is a major challenge for ecological restoration (Funk, Cleland, Suding & Zavaleta 2008).

In an initial report of a study comparing a weeded and a non-weeded treatment in experimental grassland communities sown at different plant diversity levels (Jena Experiment; Roscher, Schumacher, Baade, Wilcke, Gleixner et al. 2004), we showed that the number and the abundance distribution of established resident species as well as their productivity were similar in both treatments in the first two years after sowing (Roscher, Temperton, Buchmann & Schulze 2009b). Here, we expand this study to look at the longer-term effects of colonizing species on the composition of resident species and their productivity over a 5-year time span. Based on the expectation that the impact of newly colonizing species accelerates through time in the non-weeded treatment by altering resource dynamics and species interactions we tested the following hypotheses: (1) Temporal stability

in population- and community-level characteristics of residents is lower in non-weeded communities than in weeded communities. (2) Extinctions or reductions in the abundances of resident species are more pronounced in non-weeded communities. (3) Cover and productivity of residents are lower in non-weeded communities. (4) Shifts in species abundance distributions reduce evenness and functional trait diversity of residents in non-weeded communities because subordinate species are at a greater risk for displacement by newly colonizing species. (5) Differences in population and community characteristics of residents between non-weeded and weeded communities decrease with increasing richness of resident species.

Material and Methods

Study site and experimental design

This study is part of a large biodiversity experiment (Jena Experiment; Roscher et al. 2004). The experimental site is situated in the floodplain of the river Saale at the northern edge of Jena (Jena-Löbstedt, Thuringia, Germany, 50°57'8" N, 11°37'16" E, 130 m a.s.l.). The area around Jena is characterized by a mean annual air temperature of 9.3 °C and mean annual precipitation of 587 mm (Kluge & Müller-Westermeier 2000). The soil of the experimental site is a Eutric Fluvisol developed from up to 2 m thick loamy fluvial sediments. Soil texture ranges from sandy loam near the river to silty clay with increasing distance from the river. A pool of 60 species common in Molinio-Arrhenatheretea grasslands (semi-natural Central European mesophilic grasslands, Ellenberg 1988) was chosen for the experiment. These species were categorized into four functional groups: grasses (16 species), small herbs (12 species), tall herbs (20 species), and legumes (12 species). In total, the Jena Experiment comprises 78 plots of a size of 20 × 20 m, which cover a gradient in species richness (1, 2, 4, 8, and 16) and functional group richness (1, 2, 3, and 4), the latter being near orthogonal to

species richness. Mixtures were created by random draws with replacement. Each species-richness level was established on 16 large plots, except for the 16-species-richness level which was established on 14 large plots (because the species number in the legume and the small herb functional groups was too low for pure 16-species mixtures). In addition, two replicated monocultures of each experimental species were established on smaller plots of 3.5×3.5 m. Plots were sown with a constant total density of 1000 germinable seeds per m^2 distributed equally among species in mixtures. The experimental plots were arranged in four blocks parallel to the riverside to account for the gradient in soil characteristics. Within each large plot, two subplots of 2.00×2.25 m size separated by 0.3 m between one another were established near to the plot margin (excluding the outer 0.5 m buffer) shortly after the biodiversity experiment was sown in 2002. One of these subplots was weeded regularly as the main experiment (April, July). The other subplot was never weeded after sowing. Plots were mown twice each year (early June, September) and mown biomass was removed. The weeded control subplot was maintained until 2007.

Data collection

Aboveground biomass was harvested twice per year at estimated peak standing biomass (late May, August) just prior to mowing during the study period (2003–2007). The vegetation was clipped at 3 cm aboveground in a randomly located rectangle of 0.2×0.5 m size in each subplot. Biomass was sorted into total resident (= sown) species, total colonizer species and detached dead plant material. Samples were weighed after being dried to a constant weight (70°C , 48 h). Total cover of resident and colonizer species was visually estimated to the nearest percentage before weeding (April, July) and again at estimated peak biomass (late May, August). Species cover was recorded twice per year directly before biomass harvest using a modified Londo scale (Londo 1976). Numerical values for species cover were coded as 0.5 (<1%), 3 (1–5%), 10 (6–15%), 20 (16–25%), 30 (26–35%), 40 (36–45%), 50 (46–55%),

60 (56–65%), 70 (66–75%), 80 (76–85%), and 90 (>85%). Seedlings (plant individuals with cotyledons) of resident species were counted three times in 2006 (April, July, October). Three quadrats (0.3×0.3 m) per subplot were randomly placed for each census. Although it is not possible with this procedure to completely exclude the emergence of additional seedlings between these three time points (underestimation of seedling densities) or the persistence of seedlings for a longer period in this stage (overestimation of seedling densities), seedling densities per m^2 were calculated for each subplot based on pooled data from all census points. Aboveground plant traits were measured in monocultures of each species in May and August 2004. These traits were plant height, specific leaf area (SLA), leaf nitrogen concentration (N_M) and shoot biomass:N ratios. Root characteristics (root depth, root type) were compiled as categorical variables from the literature (see Roscher et al. 2004 for details).

Data analyses

First, plant trait data and relative abundances of resident species were used to calculate community-weighted mean traits (CWMs; Garnier, Cortez, Billès, Navas, Roumet et al. 2004) and functional trait diversity (FD_Q) using Rao's quadratic diversity (Rao's Q; Rao 1982) for each subplot. Second, cover abundances of resident species were used to derive Shannon's evenness J' , which is known to give greater weight to rare species, and Simpson's index of evenness $E_{1/D}$, which gives more weight to abundant species (Smith & Wilson 1996). Third, cover values were used to compute Bray-Curtis distances (Bray & Curtis 1957) for resident species as a measure of compositional dissimilarity (1) between non-weeded vs. weeded subplot pairs per sampling date, and (2) per subplot to assess year-to-year compositional changes. All calculations were completed separately for data recorded in early summer (May) and late summer (August) and averaged to obtain mean values for each year (for details see Appendix A).

To evaluate effects of non-weeding vs. weeding, the log response ratio

$$\ln RR_X = \ln (X_{\text{non-weeded}}/X_{\text{weeded}})$$

(Hedges, Gurevitch & Curtis 1999) was computed between non-weeded vs. weeded subplots for all variables. Positive values of $\ln RR_X$ indicate that the studied variable increased in response to non-weeding, while negative $\ln RR_X$ -values indicate a decrease in response to non-weeding. The coefficient of variation (= CV) was calculated as a measure of temporal variability (McCann 2000) based on annual values of resident community characteristics and species abundances in weeded and non-weeded subplots (2003–2007).

Generalized linear models (type-I sum of squares) were used to test the effects of block, sown species richness (SR, log-linear term) and functional group number (FG, linear term) on variables measured at the plot level. In analyses of CVs, a split-plot term for non-weeded vs. weeded subplots, and in analyses of $\ln RR$, a term for repeated measures and their interactions with SR and FG were entered. In a series of alternative models, contrasts for the presence of each functional group (legumes, grasses, small herbs, tall herbs) were fitted after SR and FG. To account for the unbalanced occurrences of sown species in the experimental plots, mixed effects models were applied in analyses of CVs and $\ln RR$ of individual species. Block and plot identity were entered as random effects in a nested sequence. Starting from a constant null model, terms for sown species richness (SR, log-linear), species identity, $SR \times$ species identity, weeding treatment and time, plus their interactions with SR and species identity were added sequentially as fixed factors. In alternative models, species identity was replaced by terms for functional group identity or contrasts for each functional group. The maximum likelihood method was applied, and likelihood ratio tests (= L ratio) were used to assess the statistical significance of model improvement. Data analysis was performed with the statistical software R2.11.1 (R Development Core Team, <http://www.R-project.org>), the implemented packages *nlme* (Pinheiro, Bates, DebRoy, Sakar & R Core Team 2009) and *FD* (Laliberté & Shipley 2010).

Results

Resident species richness

The temporal variability (measured as CV) of resident species richness increased with the number of sown species (Appendix A: Table S1, Fig. 1A). Log response ratios in resident species richness ($\ln RR_{SR}$) became increasingly negative through time, suggesting an increasing loss of resident species in the non-weeded subplots (Fig. 1B). Species loss was accelerated by legume presence in the sown species combinations (increasingly negative $\ln RR_{SR}$ over time), while species loss was less pronounced in communities with small herbs. However, $\ln RR_{SR}$ was not different from zero across the study period, indicating no significant differences in resident species richness between weeded and non-weeded subplots.

Resident species abundance

The average temporal variability in resident species abundance increased with the number of sown species (Table S1, Fig. 1C). The temporal variability in resident species abundances was higher in non-weeded subplots, especially in communities with a lower number of sown species. Log response ratios of resident species abundances ($\ln RR_{Pop}$) were significantly different from zero in all study years (with exception of 2004, Fig. 1D), indicating lower abundances of resident species in non-weeded compared to weeded subplots. Generally, differences in species abundances between weeded and non-weeded subplots decreased with a higher number of sown species (less negative $\ln RR_{Pop}$). The deviation in species abundances in non-weeded from weeded subplots increased through time, particularly in communities with a lower number of sown species (more negative $\ln RR_{Pop}$, Table S1).

Analyses at the species-level showed that the temporal variability in species abundances depended on species and functional group identity (Table 1). Effects of weeding vs. non-weeding on species temporal variability was not significantly different among species (Table

1). Differences in species abundances in response to weeding and their changes through time (i.e., $\ln\text{RR}_{\text{Pop}}$ at species-level) were also dependent on species identity (Table 1). In total, only 5 out of 60 species had $\ln\text{RR}_{\text{Pop}}$ significantly < 0 , i.e. lower abundances in non-weeded subplots, across all species-richness levels in last study year (see Appendix A: Fig. S1). The $\ln\text{RR}_{\text{Pop}}$ was highly variable, but mostly negative, for other species.

Resident community cover and biomass production

Temporal variability in resident community cover and biomass production decreased with the number of sown species (Table S1, Fig. 1E, G). On average, temporal variability in resident community cover and biomass production was larger in non-weeded subplots, but differences between weeding treatments decreased with increasing numbers of sown species (Table S1). Log response ratios closer to zero in resident community cover ($\ln\text{RR}_{\text{Cov}}$) and biomass production ($\ln\text{RR}_{\text{Biom}}$) indicated decreased differences between weeding treatments with increasing number of sown species (Table S1, Fig. 1F, H). On average, $\ln\text{RR}_{\text{Cov}}$ and $\ln\text{RR}_{\text{Biom}}$ decreased through time, but the decline in $\ln\text{RR}_{\text{Cov}}$ was stronger when there were fewer sown species. From 2006 onwards, $\ln\text{RR}_{\text{Cov}}$ and $\ln\text{RR}_{\text{Biom}}$ were significantly lower than zero, indicating a lower community cover and biomass production in non-weeded compared to weeded subplots across all species-richness levels (Fig. 1F, H).

Number of germinating species and seedling density of resident species

The log response ratio of the number of germinating resident species ($\ln\text{RR}_{\text{SRgerm}}$) based on three censuses in 2006 was not significantly different from zero (test for overall mean $\neq 0$: $F_{1,72} = 0.33$, $p = 0.568$), suggesting that weeding treatments did not affect the number of germinating resident species (Fig. 2A) irrespective of sown species richness ($F_{1,72} = 0.90$, $p = 0.346$) or functional group number ($F_{1,72} = 0.05$, $p = 0.832$). The log response ratio of seedling densities of resident species ($\ln\text{RR}_{\text{Seed}}$) was significantly lower than zero (test for overall

mean $\neq 0$: $F_{1,72} = 9.78$, $p = 0.003$), indicating that less resident seedlings emerged in the non-weeded subplots (Fig. 2B). The reduction of seedling emergence in non-weeded compared to weeded subplots did not depend on sown species richness ($F_{1,72} = 0.94$, $p = 0.336$) or functional group number ($F_{1,72} = 0.10$, $p = 0.758$).

Compositional dissimilarity of resident species combinations

Compositional dissimilarity of resident species combinations (Bray-Curtis distances) between weeded and non-weeded subplots decreased with sown species richness ($F_{1,72} = 11.93$, $p = 0.001$), while functional group number or the presence of particular plant functional groups did not affect this compositional dissimilarity. Bray-Curtis distances between weeded and non-weeded treatments increased through time ($F_{1,75} = 64.58$, $p < 0.001$). This increase in dissimilarity was larger in species-poor compared to species-rich communities ($F_{1,75} = 23.61$, $p < 0.001$, Fig. 3).

Temporal variability in resident species composition (= CV of Bray-Curtis distances) decreased with increasing number of sown species, while functional group number, the presence of particular plant functional groups or weeding treatments did not affect temporal variability in composition (Appendix A: Table S2, Fig. 4A). Differences in temporal changes in terms of composition between weeded and non-weeded subplots decreased with increasing number of sown species (decreasing $\ln\text{RR}_{\text{Comp}}$, Table S2), but they became stronger through time (Table S2). The $\ln\text{RR}_{\text{Comp}}$ from 2006 to 2007 was significantly larger than zero, suggesting that temporal changes in non-weeded subplots exceeded those in weeded subplots across all species-richness levels (Fig. 4B).

Evenness of resident species combinations

The temporal variability of the Shannon evenness (J') decreased with increasing sown species richness, while the CV of the Simpson index of evenness ($E_{1/D}$) was not influenced by species

richness, indicating that species-rich communities had a more stable species abundance distribution of subordinate species (Table S2, Fig. 4C, D). The temporal variability of J' and $E_{1/D}$ did not differ between weeded and non-weeded subplots. Differences in J' between weeding treatments decreased at higher sown species-richness levels (increasing $\ln RR_{\text{Shan}}$, Table S2, Fig. 4D). In general, weeded and non-weeded subplots did not differ significantly in species evenness in the 5-year study (Fig. 4D, F), except for a higher $E_{1/D}$ across all species-richness levels in the non-weeded subplots in 2007 ($\ln RR_{\text{Simp}} > 0$; Fig. 4F).

Functional trait diversity and aggregated traits of resident species combinations

Temporal variability in single-trait as well as in multiple-trait functional diversity FD_Q decreased with an increasing number of sown species or functional groups, with the exception of FD_Q in root depth (Appendix A: Table S3, Fig. 4G). Temporal variability in community-weighted means of trait values (CWM) did not depend on sown species richness, but increasing functional group richness was coupled with increased temporal variability in community-weighted leaf nitrogen concentrations (N_M) and shoot biomass:N ratios (Table S3). Temporal variability in functional trait composition (CWM and FD_Q) was not influenced by weeding (Table S3). Log response ratios ($\ln RR_{\text{CWM}}$, $\ln RR_{\text{FDQ}}$) were not significantly different from zero (test for overall mean $\neq 0$: $p > 0.05$), indicating that functional trait composition of residents between weeded and non-weeded subplots across all species-richness levels did not differ (Fig. 4H). However, $\ln RR_{\text{FDQ}}$ in multiple traits, but also $\ln RR_{\text{FDQ}}$ in single traits such as N_M and shoot height, became increasingly negative at lower sown species richness, suggesting that non-weeding had negative effects on FD_Q (Table S3, Fig. 4H).

Cover and species number of colonizers

On average, colonizer cover and the number of colonizing species decreased with increasing

sown species richness (Appendix A: Table S4, Fig. 5). Weeding reduced colonizer cover and colonizer species number. Effects of weeding on colonizer cover did not depend on sown species richness, while colonizer species numbers were more successfully reduced through weeding at increasing species richness (Table S4). Overall, colonizer cover and species numbers increased with increasing time after sowing of the biodiversity experiment. Weeding did not prevent the establishment of a higher number of colonizer species through time, while the increase in colonizer cover through time was less pronounced when communities were weeded (Table S4).

Discussion

The goal of the present study was to test what would happen to the sown species over several years if a biodiversity experiment was not weeded. Typically, most plant biodiversity experiments weed unwanted species in order to maintain sown species-richness levels and community composition (for an exception, see Niklaus, Leadley, Schmid & Körner 2001). This is justified as the simulated extinction of all other species from a community (Schmid & Hector 2004). However, weeding may be seen as an undesirable disturbance or unusual management practice (Wardle 2001, Lepš 2004) generating doubt about whether similar results would be observed if biodiversity experiments were not weeded. Our study comparing weeded and non-weeded subplots in a grassland biodiversity experiment showed that newly colonizing species may reduce the temporal stability of resident population- and community-level characteristics (confirming hypothesis 1). While species and community cover as well as productivity were reduced in non-weeded communities (confirming hypotheses 2 and 3), the shift in abundance distribution had minor effects on community characteristics such as evenness and functional trait composition (rejecting hypothesis 4). In general, impacts of colonization by new species were moderate in communities with higher species richness,

while their negative effects became more severe in communities with lower species richness over several years (confirming hypothesis 5).

Negative effects of newly colonizing species on community structure and composition of resident species may involve direct competitive displacement as well as inhibition of establishment of new individuals. The competition-colonization model (Tilman 1994) is based on the assumption that resident species are excluded and the local diversity is reduced when competitive, dominant species are introduced. In our study, resident species numbers in weeded and non-weeded subplots were not different, but tended to diverge over the 5-year study period. However, a higher sown diversity increased the temporal variability of resident species numbers irrespective of weeding (Fig. 1A). This suggests that observations of increased species extinctions at higher species richness after cessation of weeding in studies lacking a continuously weeded control (Pfisterer et al. 2004, Rixen et al. 2008) may not necessarily have been caused by the colonization of new species but by a reduced compositional stability of communities with a higher number of less stable subordinate species (Foster, Smith, Dickson & Hildebrand 2002, Roscher, Weigelt, Proulx, Marquard, Schumacher et al. 2011). In addition, the pressure of colonizers might have been larger in previous experiments with smaller plots (Pfisterer et al. 2004, Rixen et al. 2008), while the non-weeded subplots in the large plots of Jena Experiment were surrounded by a weeded area with the same sown species combinations.

In contrast to negligible effects of weeding treatments on the numbers of resident species, average resident species cover was lower in non-weeded communities and compositional divergence between weeding treatments increased through time. As a consequence, non-weeded communities had on average a lower stability in biomass production and total plant cover of residents compared to weeded communities (Fig. 1E, G). However, temporal stability in productivity and cover of residents was positively related to sown plant diversity

irrespective of weeding, which is in line with a previous study by Bezemer & van der Putten (2007) on ex-arable land.

One possible explanation for the reduction of resident species abundances and community biomass is resource competition between residents and colonizers. The availability of light and soil resources have been shown to regulate the success of colonizing species in several experimental studies in grasslands (e.g. Davis, Grime & Thompson 2000, Roscher et al. 2009a). Grime (2006) predicted that traits associated with competition are more common in relatively undisturbed, productive environments, because species with traits associated with poor competitive ability are likely to be competitively excluded. Under nutrient-rich conditions fast growth is a prerequisite for high competitive ability. Specific leaf area and leaf nitrogen concentrations correlate positively with relative growth rates (e.g. van der Werf, van Nuenen, Visser & Lambers 1993). In addition, plant height is an important indicator for species competitive ability (Gaudet & Keddy 1988). In our study, the spontaneous colonization of new species had only minor impacts on trait composition of dominant species as shown by the non-significant differences in community-weighted means of traits related to competitive ability between weeded and non-weeded communities. Only at lower levels of sown plant diversity did colonizing species have negative impacts on the diversity of light- (plant height) and nitrogen-acquisition strategies of the resident species (more negative values of $\ln RR_{FDQ}$, Fig. 4H, Table S3), suggesting that the accumulation of more colonizers reduced niche diversification among residents.

Negative soil feedbacks, i.e. the accumulation of pathogens, parasites or herbivores of roots (Bever, Westover & Antonovics 1997), provide an alternative explanation for a declining performance of residents in our long-term experiment. Relative species abundances in plant communities may decline through reduced competitive ability when species are growing on the same soil for an extended time (Klironomos 2002, Petermann, Fergus, Turnbull & Schmid 2008). The composition of soil organisms is likely to differ between non-weeded and weeded

communities, where the removal of unwanted species causes soil disturbances and therefore could reduce the potential for negative soil feedbacks. A lower competitive ability of residents through antagonistic interactions with soil organisms would also favour the competitive displacement of residents through the accumulation of new colonizers in non-weeded communities. In addition, negative soil feedbacks could explain why reductions in the abundance of residents in non-weeded communities were common across nearly all experimental species and not restricted to subordinate species with lower ability for resource competition (Fig. S1).

Reduced establishment of new individuals of residents could further increase negative effects of colonizers in non-weeded communities in the long term. Higher seedling numbers of resident species in weeded communities (Fig. 2B) could be attributable to either a close relationship between reduced population sizes and propagule accumulation in non-weeded communities, a stimulation of germination caused by soil disturbance during weeding (Leck, Parker & Simpson 1989), or the limitation of favourable microsites for germination in non-weeded plant communities, which had a higher total plant cover (analysis not shown).

The colonization of new species apparently prevented single species from attaining extensive dominance. In the long term, the Simpson index of evenness, which gives greater weight to abundant species, was higher in non-weeded than in weeded communities (Fig. 4F). In contrast, at lower species richness, the Shannon evenness, which accords rare species greater weight, was lower in non-weeded communities (Fig. 4D). Therefore, those resident species that occurred in low abundances were acutely impeded by the colonization of new species in communities of lower sown species richness. This consolidates the decreasing functional trait diversity in non-weeded communities in our study and the non-random species extinction scenarios in natural ecosystems (Zavaleta & Hulvey 2004). Several removal experiments in natural grasslands and abandoned agricultural land have shown that the effects of removal of single species or functional groups on compensatory growth, the colonization of new species

and subsequent community structure is dependent on the identity of the removed and the remaining species (e.g. Munson & Lauenroth 2009, McLaren & Turkington 2011).

In summary, the observed changes in the abundance distribution of resident species and the compositional divergence between weeded and non-weeded communities suggest that the impact of colonizing species on resident species accelerates through time, particularly in communities with fewer initially sown species. It is well known from several studies in weeded experimental grasslands that their temporal stability increases with species richness (e.g. Tilman, Reich & Knops 2006, Roscher et al. 2011). Our study adds information showing that the patterns observed in weeded communities only apply at higher sown diversity when colonizing species are not removed through weeding. This is important for the evaluation of results obtained in biodiversity experiments and their implications for restoration and sustainable agriculture. Sowing more diverse grassland mixtures increases their predictability in terms of ecosystem characteristics such as productivity as well as their species and functional composition, which is critically important for interactions with organisms at higher trophic levels, which are usually more dependent on species composition than on intrinsic richness.

Acknowledgements

The Jena Experiment is funded by the German Science Foundation (FOR 456) with additional support from the Max Planck Society and the Friedrich Schiller University of Jena and coordinated by W.W. Weisser. B.S. was additionally supported by the Swiss National Science Foundation (310030E-131193). We thank V.M. Temperton for the provision of biomass data. We acknowledge U. Wehmeier, S. Junghans, S. Hengelhaupt, A. Oswald, U. Gerighausen and many student helpers for their support with the maintenance of the invasion experiment, harvest of biomass, measurement of plant traits and sample preparation for chemical analysis. I. Hilke carried out elemental analyses.

438

439 **Appendix A. Supplementary data**

440 Supplementary data associated with this article can be found, in the online version, at XXXX.

441

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Figure legends

Fig. 1. Temporal variation (2003–2007) of resident species numbers (A, B), resident species cover (C, D), resident community cover (E, F), resident community biomass (G, H). In the left panels, means of CVs (\pm SE) are plotted against sown species richness for weeded and non-weeded subplots (A, C, E, G). In the right panels, lnRR-values are shown representing log response ratios between non-weeded and weeded subplots. They are given as means (\pm SE) for each sown species-richness level per study year (B, D, F, H). Positive lnRRs indicate that values were larger in non-weeded subplots compared to weeded subplots, while negative lnRRs indicate the opposite. Significance of overall means $\neq 0$ across all species-richness levels was tested separately for each study year, where ns = non-significant, * $p \leq 0.050$, ** $p \leq 0.010$, *** $p \leq 0.001$.

Fig 2. The log response ratio (= lnRR) of the number of germinating resident species (A), and the log response ratio of seedling densities of resident species (B) based on three censuses in 2006 (April, July, October) plotted against sown species richness. Bars represent means per species-richness level (\pm SE, for symbols see Fig. 1). Positive lnRRs indicate that numbers of germinating species and resident seedling densities were higher in non-weeded than in weeded subplots, while negative lnRRs indicate the opposite.

Fig. 3. Compositional dissimilarity (Bray-Curtis distances) of resident species combinations between pairs of weeded and non-weeded subplots by species-richness level (mean \pm SE) and year. Values are based on two cover estimates (before the first (May) and second (August) mowing, respectively).

Fig. 4. Temporal variation (2003-2007) of year-to-year changes (= Bray-Curtis distances) in resident species composition (A, B), Shannon evenness J' of resident species combinations (C, D), Simpson index of evenness $E_{1/D}$ of resident species combinations (E, F), functional trait diversity FD_Q (G, H). In the left panels, means of CVs (\pm SE) are plotted against sown species richness for weeded and non-weeded subplots (A, C, E, G). In the right panels, lnRR-values are shown representing log response ratios between non-weeded and weeded subplots. They are given as means (\pm SE) for each sown species-richness level per study year (B, D, F, H). Positive lnRRs indicate that values were larger in non-weeded subplots compared to weeded subplots, while negative lnRRs indicate the opposite. Significance of overall means \neq 0 across all species-richness levels was tested separately for each study year, where ns = non-significant, * $p \leq 0.050$, ** $p \leq 0.010$, *** $p \leq 0.001$. For symbols see Fig. 1.

Fig. 5. Cover of colonizer species plotted against sown species richness (A) and year (B), and colonizer species numbers plotted against sown species richness (C) and year (D) in weeded subplots before weeding (mean values for data recorded before spring- and summer-weeding) and mowing (mean values for data recorded before early- and late-summer mowing) and non-weeded subplots before mowing. Values are means (\pm SE) per species-richness level across study years (2003-2007) in the left panels, and means (\pm SE) across all species-richness levels per study year in the right panels.

Table 1. Summary of mixed effects model analyses of coefficients of variation for resident species cover and log response ratios of resident species cover (lnRR comparing non-weeded vs. weeded subplots) based on a five-year study period (2003–2007)

	CV Species cover			ln RR Species cover	
	L ratio	p		L ratio	p
SR (log-linear)	19.92	<0.001 ↓	SR (log-linear)	29.05	<0.001 ↑
Species ID	806.33	<0.001	Species ID	89.86	0.006
Functional group ID	14.03	0.003	Functional group ID	4.10	0.251
Legume	6.25	0.012 ↑	Legume	0.25	0.617
Grass	0.29	0.208	Grass	2.10	0.147
Small herb	11.8	0.001 ↓	Small herb	0.03	0.867
Tall herb	0.2	0.657	Tall herb	3.22	0.073
Weeding Treatment (W)	4.51	0.034 ↑	Year	22.44	<0.001 ↓
SR x W	1.29	0.256	SR x Year	9.04	0.003
Species ID x W	19.69	1.000	Species ID x Year	78.06	0.049
Functional group ID x W	0.21	0.977	Functional group ID x Year	1.91	0.592
Legume x W	<0.01	0.955	Legume x Year	1.42	0.234
Grass x W	0.11	0.743	Grass x Year	0.06	0.801
Small herb x W	0.15	0.699	Small herb x Year	0.58	0.446
Tall herb x W	0.01	0.915	Tall herb x Year	0.44	0.509

Models were fitted by stepwise inclusion of fixed effects. Likelihood ratio tests were applied to assess model improvement (L ratio) and the statistical significance of the explanatory terms (p values). Significant effects are marked in bold. Arrows indicate increase (↑) or decrease (↓) of the variables with species richness, dependent on functional group identity or time. SR = sown species richness.

Figure 1

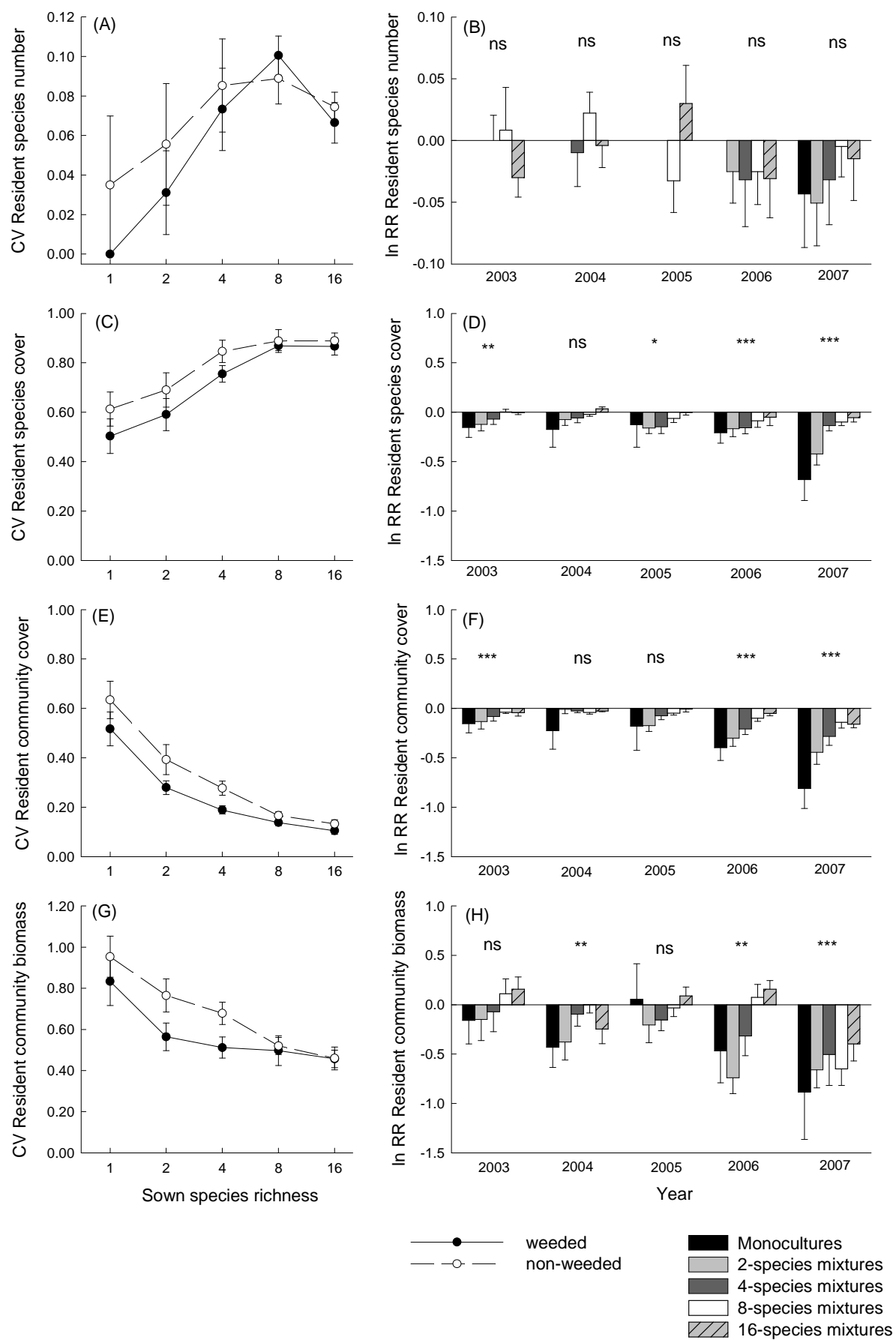


Figure 2

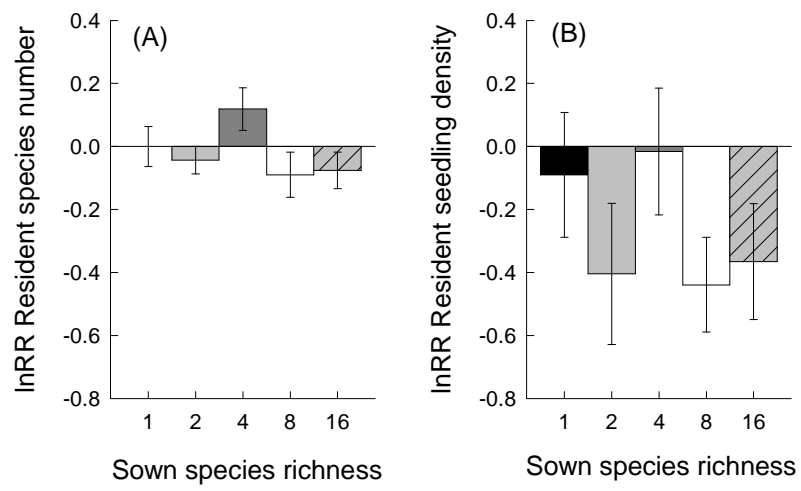


Figure 3

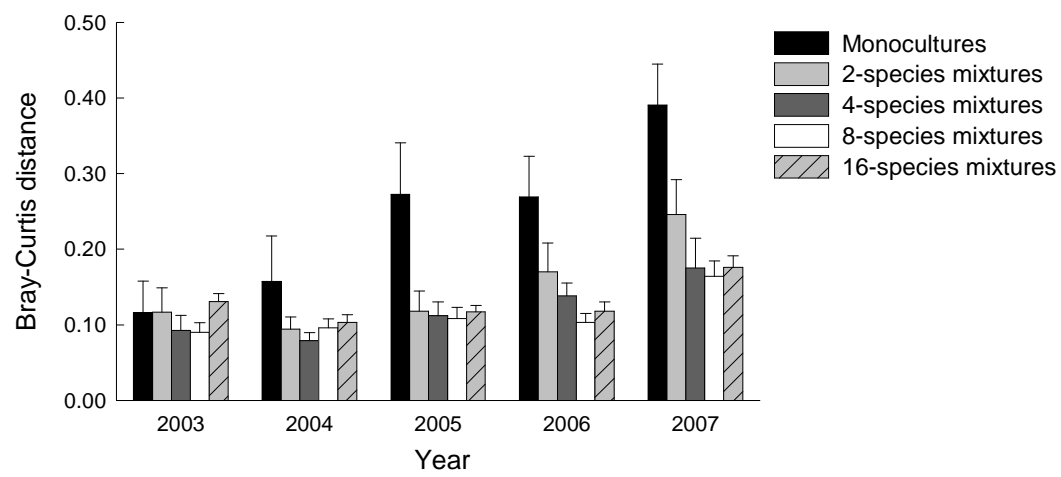


Figure 4

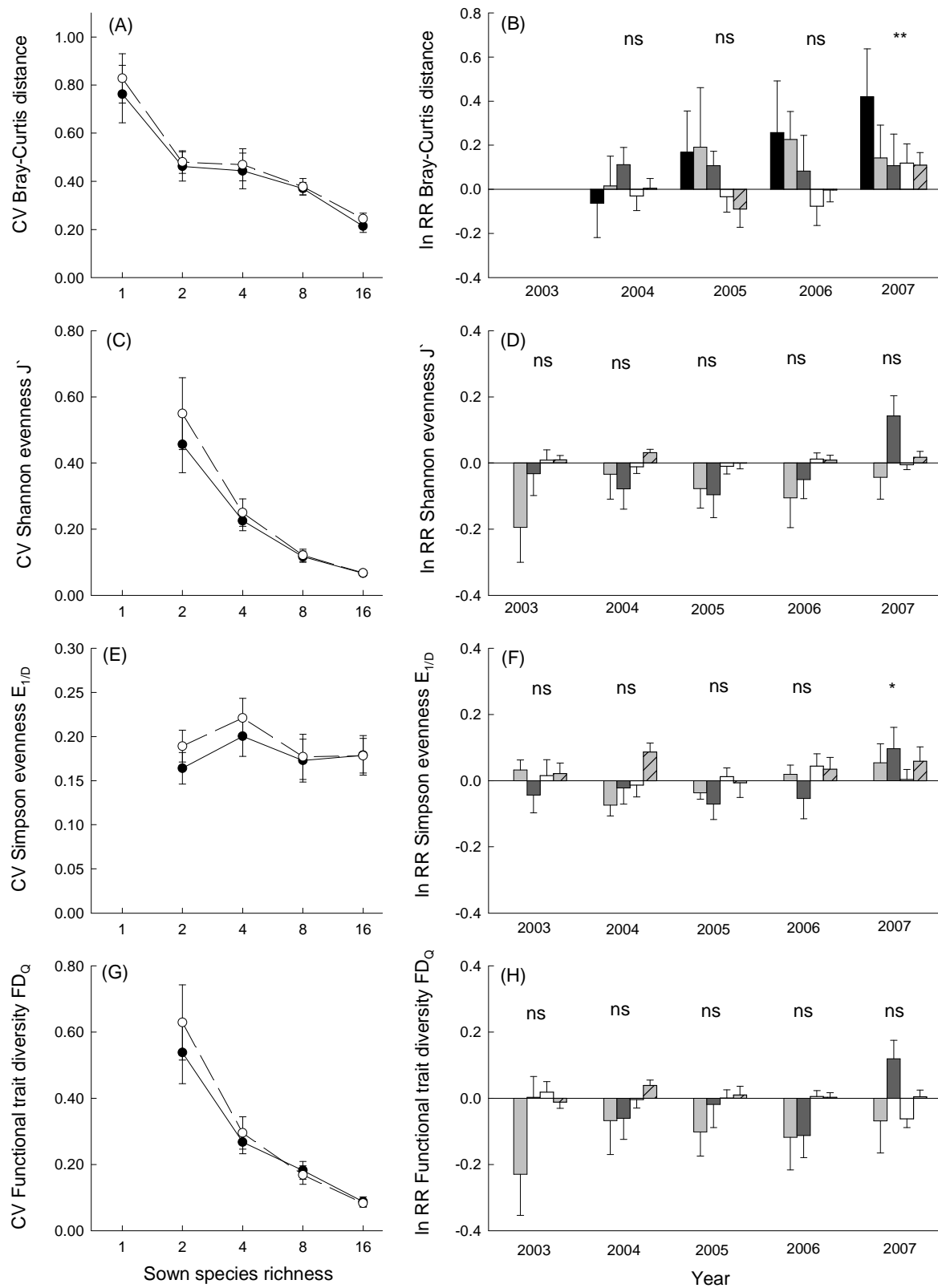


Figure 5

