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REPORT

Do species evenness and plant density influence the magnitude of selection and complementarity effects in annual plant species mixtures?

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Abstract

Plant species richness influences primary productivity via mechanisms that (1) favour species with particular traits (selection effect) and (2) promote niche differentiation between species (complementarity). Influences of species evenness, plant density and other properties of plant communities on productivity are poorly defined, but may depend on whether selection or complementarity prevails in species mixtures. We predicted that selection effects are insensitive to species evenness but increase with plant density, and that the converse is true for complementarity. To test predictions, we grew three species of annuals in monocultures and in three-species mixtures in which evenness of established plants was varied at each of three plant densities in a cultivated field in Texas, USA. Above-ground biomass was smaller in mixtures than expected from monocultures because of negative 'complementarity' and a negative selection effect. Neither selection nor complementarity varied with species evenness, but selection effects increased at the greatest plant density as predicted.

Keywords

Above-ground biomass, dominance, negative interactions, net biodiversity effect, niche differentiation, primary productivity, species diversity, species evenness, species richness.

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INTRODUCTION

Research on the role of plant species diversity in ecosystem function has intensified recently with the realization that Earth's biodiversity is declining (Loreau *et al.* 2001; Mouquet *et al.* 2002). As commonly defined, diversity is determined both by species richness – the number of plant species in a given area – and by species evenness – a measure of how equitably abundances are distributed among species. Recent studies have focused on species richness (Naeem *et al.* 1994; Tilman *et al.* 1996; Tilman *et al.* 1997a; Hooper 1998; Hector *et al.* 1999; Tilman *et al.* 2001). The potential importance of species evenness and of other aspects of plant communities for productivity and related ecosystem properties has largely been neglected (but see Wilsey & Potvin 2000; Nijs & Roy 2000; Wilsey & Polley 2002).

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This neglect of the role of species evenness and of other aspects of plant communities, like plant density, also may be complicating interpretation of some richness experiments (Doak *et al.* 1998; Schwartz *et al.* 2000). First, species in most richness experiments are planted at relative abundances that are more equitable than those typically encountered in natural communities (Schwartz *et al.* 2000). Greater evenness has been shown to enhance above-ground biomass production of some species mixtures (Wilsey & Potvin 2000), suggesting that richness effects may differ in communities with more realistic levels of species evenness. Second, richness treatments typically are established by seeding species at desired abundances, but seedling establishment usually is not quantified, which means that evenness and plant density are not rigorously controlled (Huston 1997). Consequently, any influence of varying richness on production or other ecosystem properties potentially is confounded in these experiments with effects of evenness or plant density.

Preliminary observations suggest that effects of richness and other aspects of plant communities are not

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independent. Species richness determines the range of trait variation present in a plant community (Tilman *et al.* 1997b; Loreau 2000). Evenness and plant density may influence the expression of this variation by defining its relative distribution among plants and the intensity of plant–plant interactions. The extent to which evenness and plant density influence productivity of mixtures thus may depend on the types of species interactions that result from trait variation.

Species richness is thought to influence productivity and other ecosystem processes via two general mechanisms: (1) facilitation or niche differentiation in resource use (complementarity) and (2) processes that favour plants with particular traits from among the range of phenotypic variation present (the selection effect; Tilman *et al.* 1997b; Loreau 1998a, 2000).

Complementarity may be either positive or negative in sign (Loreau & Hector 2001). Functional complementarity (positive in sign) results from resource partitioning or facilitation, promotes species co-existence, increases plant productivity (Loreau 2000; Tilman *et al.* 2001), and is mechanistically linked to plant–plant interactions (Tilman *et al.* 1997b). Complementary species suffer less from competition with interspecific neighbours than from competition with conspecific neighbours. Consequently, any increase in growth of one species is not offset by a similar decrease in production of other species in mixture (Loreau 1998a). By contrast, negative or antagonistic interactions that result from chemical or physical interference among plants reduce biomass in mixtures relative to that expected from monocultures (negative 'complementarity'). Selection processes favour species with extreme traits and may either enhance or reduce productivity of mixtures relative to average productivity of species monocultures (Loreau 2000). Competition has been implicated as the mechanism promoting selection (Tilman *et al.* 1997b), but differences in growth rate may favour some species over others independently of strong competitive interactions (Nijs & Roy 2000).

We predicted that expression of the selection effect is enhanced by greater plant density but is insensitive to species evenness, and that the converse is true for expression of both positive and negative complementarity. Facilitation and resource partitioning reduce the intensity of interspecific relative to intraspecific competition and increase the capture of available resources by plant mixtures, effects that may be relatively insensitive to density. Competition studies conducted with replacement series methodology, however, indicate that biomass of complementary mixtures sometimes depends on species relative abundances (e.g. Harper 1977), suggesting that expression of complementarity may be maximized in equitable mixtures. In contrast, expression of selection effects may depend on the intensity of interspecific interactions as determined by plant density. Indeed, Pacala & Tilman (2002) argue that density-dependent processes are required for full expression of the selection effect. At densities great enough to limit total production and resource acquisition (law of constant final yield), the advantage of the most rapidly growing species compounds with time as it captures an even-greater fraction of available resources. The selection effect may be relatively insensitive to variation in species evenness, unless intensities of interspecific compared with intraspecific interactions change significantly with species relative abundances. Although these competitive interactions may vary with species abundances (Law & Watkinson 1987), changes usually are minor.

As an initial test of predictions, we grew three species of annuals that co-occur in disturbed grasslands in central Texas (*Gaillardia pulchella* Foug., a C_3 forb, *Monarda citriodora* Cerv., a C_3 forb, and *Lolium perenne* L. – an annual or short-lived C_3 perennial grass in our area) in monocultures and in three-species mixtures at each of the three densities. Density was altered to vary the intensity of competitive interactions. Evenness (1 : 1 : 1 or 3 : 1 : 1 ratio among species) and the identity of the dominant species in 3 : 1 : 1 assemblages were varied at each density level in replicated mixtures in 1 m × 1 m field plots in central Texas, USA. Our objective was to determine whether species relative abundances (evenness and identity of the dominant species) and plant density influenced expression of complementarity and the selection effect in the three-species mixture studied. Because mixtures in this experiment all contained the same species, the 'sampling effect' associated with increasing richness in most diversity experiments (the greater probability of including species with extreme traits in species-rich than species-poor mixtures; Tilman *et al.* 1997b) was eliminated.

METHODS

Site characteristics

This experiment was conducted in Bell County, Texas, USA (31°05′N, 97°20′W) during 2000–2001. Soils at the study site are fine-silty, carbonatic, thermic Udorthentic Haplustolls. The surface 0.4 m of soil is composed mostly (55%) of clay.

An average of 66% of mean annual precipitation at the site (879 mm, 85 years record) falls during the 7-month period (December to June) included in this study. Precipitation during December 2000 to June 2001 was 76% of the 85-years mean (583 mm). Monthly mean temperature ranged between 8.0 and 26.9 °C in January and June 2001 and, for the 7-month period considered in this study, was similar to the 85-year average (range 9.0–27.0 °C).

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Experimental design and measurements

Five blocks (each 6 m × 16 m) were established in a cultivated field. Blocks were separated by 1.5 m walkways, and were arranged in two parallel rows along a north–south axis with three blocks in one row and the remaining two blocks in the second row. Two treatments were assigned to each block in a split plot design. Treatments included three plant density levels (low = 15 plants m^{-2}, medium = 45 plants m^{-2}, and high = 135 plants m^{-2}) and seven categories of plot type, including monocultures of each of three species of annuals, *G. pulchella*, *L. perenne*, and *M. citriodora*, and species mixtures in which relative abundances of the three annuals were varied. Species abundances in mixtures were completely equitable (maximum evenness; 1 : 1 : 1 ratio) or were distributed at a 3 : 1 : 1 ratio with each of the three species used as the dominant in one 3 : 1 : 1 mixture. Each block was divided into three rows to which density treatments were randomly assigned. The seven plot types (including monocultures and species mixtures) were then randomly assigned to 1 m × 1 m plots in each density treatment. Plots were separated by 1.5 m walkways.

The three species studied co-occur in disturbed ecosystems in central Texas, but differ in morphology and in timing of germination, factors that were expected to promote complementarity in resource use. The grass *Lolium* is shorter than the forbs *Gaillardia* and *Mondarda*, both of which grow to about 1 m in height, but *Lolium* typically germinates before the forbs. Although *Lolium* is a perennial, this widely distributed grass was chosen for study because it behaves as an annual in our region and is abundant in central Texas grasslands during winter and early spring.

All 105 of the 1 m^2 plots were hand-seeded on 21 November 2000 following tillage with a hand rake. Seeds were obtained from a local vendor (Native American Seed, Junction, TX, USA). Emerging seedlings subsequently were thinned to desired plant densities and species abundances. We sowed a total of 4.5, 1.5 and 0.5 g of seeds per 1 m^2 plot assigned to high density, medium density, and low density treatments, respectively. For plots assigned to species mixtures, we adjusted the proportional contribution of each species to total seed mass to match targeted relative abundances. Seedlings began to emerge in late December and were thinned during the following 3 months to adjust plant density and species abundances to assigned levels. One mixture plot at low density was eliminated because too few seedlings emerged. Plots were weeded monthly during the experiment, but weed seedlings were not quantified.

Volumetric soil water content to 0.15 m depth was measured weekly beginning in April 2001 using time domain reflectometry (TDR). Propagation time of electromagnetic waves through stainless steel probes installed in the centre of plots was measured with a MoisturePoint MP-917 instrument (Environmental Sensors Inc., Victoria, Canada). An empirical equation from Topp *et al.* (1980) was used to calculate volumetric water content of soil from these measurements. Calculations from the TDR technique were corrected to volumetric water content for the heavy-clay soil on which this study was conducted using a linear regression developed from direct measurements of soil water content ($r^2 = 0.66$, $n = 32$). Photosynthetic photon flux density (light) at the soil surface and above the plant canopy in each 1 m^2 plot was measured at midday on single clear days in May and in early July 2001 by placing a 1-m long probe containing silicon photodiodes (SunScan; Delta-T Devices Ltd, Cambridge, UK) diagonally across each plot (two measurements per plot).

We harvested above-ground biomass by species near the end of the growing season for the species studied (5–6 July 2001) by clipping each 1 m × 1 m plot to 1 cm height. To estimate below-ground biomass, we took one soil core (0.042 m diameter, 0.45 m depth) in the centre of each plot. Roots were washed from soil over a 2-mm sieve. All plant material was weighed after oven drying at 60 °C for 72 h.

Partitioning selection and complementarity

Several methods have been proposed to distinguish components of the biodiversity effect in species mixtures (Hector 1998; Loreau 1998b; Loreau & Hector 2001; Špaèková & Lepš 2001; Hector *et al.* 2002). We used the method described by Loreau & Hector (2001) to additively partition the net biodiversity effect in mixtures into a selection and complementarity effect. This approach provides a general and conceptually lucid method of assessing contributions of the two general mechanisms by which species richness is thought to influence productivity of species mixtures. The net biodiversity effect is the difference, summed across species, between observed and expected yields in mixtures, where the expected yield of each species in mixture is the product of biomass in monoculture and proportion of the species in the mixture. Complementarity is calculated by multiplying the number of species in mixture by the average, across species, of monoculture yields and the average, across species, of the difference between the observed relative yield (RY) in mixture and the expected RY in mixture (the proportion of each species in mixture), where observed RY is biomass per species in mixture divided by biomass of the species monoculture at the appropriate density. The selection effect is calculated by multiplying the number of species in mixture by the covariance between the monoculture yield and the difference between observed and expected relative yields of each species. Observed and expected yields used in calculations were derived from plots (monocultures, mixtures) with the same total density of plants. Expected yields

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were calculated using data from monocultures within the same block.

Calculations of complementarity and the selection effect based on above-ground biomass of mixtures alone may be misleading if component species differ greatly in root : shoot ratio or in the response of this ratio to interspecific interactions. To determine the potential bias introduced by partitioning the net biodiversity effect using above-ground biomass alone, we also calculated complementarity and the selection effect using an estimate of root biomass of each species in mixture. Root biomass was estimated by multiplying above-ground (shoot) biomass of each species in mixture by the root : shoot ratio of species monocultures. This estimate of root biomass per species then was constrained by the total biomass of roots in species mixtures as described by Hooper (1998).

Statistical analyses

Data on biomass and biodiversity effects were analysed with a split-plot analysis of variance (ANOVA), with block and density treatment as the main plot (block × density as the error term) and field plot type as the subplot. Differences among mean values within treatments were analysed with single degree of freedom contrasts. Neither the successive measurements of soil water content and light in plots nor the deviation of observed from expected relative yields of the three species in each mixture are independent. To accommodate correlations within each set of measurements, these variables were analysed with a repeated measures ANOVA. Data were log-transformed before analysis when required to satisfy assumptions of ANOVA. Variable means are presented for individual treatments (density, plot type) only when statistical interactions with other treatments were not significant ($P > 0.05$).

RESULTS

Biomass production

Root biomass was a relatively small fraction of total production for the annual species studied (mean of 25% of total biomass per plot). Excluding root biomass from analyses did not alter trends using above-ground production only. In the interest of clarity, therefore, we omit details of analysis which included data for roots.

Above-ground biomass per plant of each of the three annual species declined with each increase in density of 1 m × 1 m species monocultures (Fig. 1), suggesting that increasing density increased the intensity of competition. The absolute decline in biomass per plant was particularly great as density was increased from low to medium densities (15–45 plants m^{-2}). As density increased, however, the number of

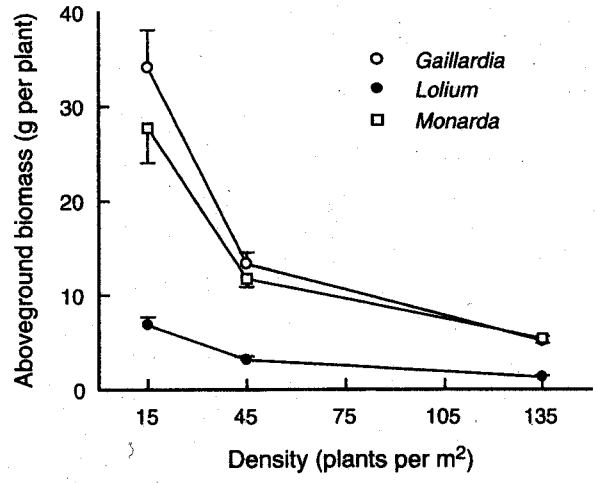

Figure 1 Mean values (±SE, $n = 5$) of above-ground biomass per plant for three annual species grown in monocultures at three different plant densities.

plants rose proportionally faster than biomass per plant declined, and above-ground biomass of 1 m^2 plots increased with each increase in density in monocultures alone ($P = 0.005$; mean = 343.5, 423.1, and 532.3 g m^{-2} at low, medium and high density), in mixtures alone ($P < 0.0001$; mean = 276.0, 383.5, and 408.5 g m^{-2} at low, medium and high density), and across monocultures and mixtures ($P = 0.0004$; mean = 304.9, 400.5, and 461.6 g m^{-2} at low, medium and high density). Across plot types, the increase in production was better described by a linear than quadratic function of density ($P = 0.13$ for quadratic term). Above-ground biomass differed with plot type ($P < 0.0001$), but the interaction between density and plot type was not significant ($P = 0.08$).

Biomass production differed substantially among monocultures (Fig. 2, Table 1). Above-ground biomass was greater by a factor of almost four in forb monocultures than in the grass monoculture. Biomass was greater in all species mixtures than in the least productive monoculture with the grass *Lolium*, but production was smaller in mixtures than in the higher-yielding monocultures of forbs. Biomass in most mixtures was smaller than the average biomass of species monocultures. Only in mixtures dominated by *Gaillardia* (419.7 g m^{-2}) did above-ground biomass approach the mean biomass for species monocultures (433.0 g m^{-2}). Biomass of mixtures depended on identity of the dominant species, but did not differ with evenness treatment. The mixture dominated by *Lolium* was the least productive of the three-species mixtures. Above-ground biomass did not differ significantly between completely equitable mixtures (mean ± SE = 367.8 ± 22.0 g m^{-2}) and those with a 3 : 1 : 1 ratio of species (mean ± SE = 352.1 ± 16.9 g m^{-2}).

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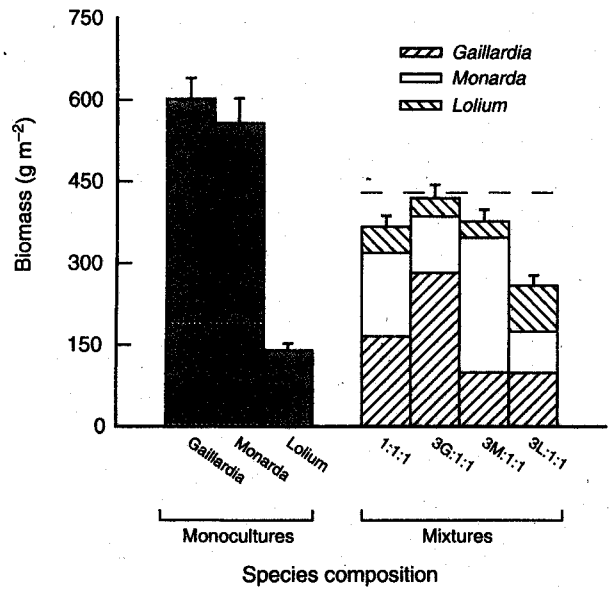

Figure 2 Above-ground biomass in monocultures with each of three annual species [*Gaillardia* (G), *Monarda* (M), *Lolium* (L)] and above-ground biomass by species in mixtures differing in relative abundances of the three annuals. Species abundances in mixtures were completely equitable (1 : 1 : 1 ratio) or were distributed at a 3 : 1 : 1 ratio with each of the three species as the dominant in one 3 : 1 : 1 mixture. Values are averages across density treatments. Error bars indicate 1 SEM of the total of above-ground biomass (n = 14–15). Mean biomass of monocultures is represented by the dashed line drawn over data from mixtures at 433.0 g m^{-2}.

Biodiversity effect

The net biodiversity effect was negative across all mixtures (mean = −77.6 g m^{-2}; t-test, $P < 0.001$), which meant that mixtures consistently underyielded compared with the expectation from monocultures (Table 2). Both components of this net effect were also negative (t-tests, $P < 0.001$). Across densities and mixture types, mixtures underyielded relative to expectation because of antagonistic or negative interactions among species (complementarity = −48.3 g m^{-2}) and because of a negative selection effect (selection effect = −29.3 g m^{-2}). Neither the net biodiversity effect nor negative interactions ('negative' complementarity) differed significantly among densities (Tables 2 and 3), among mixture types, or with evenness (linear contrasts, P = 0.37, 0.66). There was no statistical interaction between density and mixture type for the net effect or for complementarity (Table 3). The selection effect, by contrast, was more negative at high than medium and low densities (Tables 2 and 3), but did not differ with mixture type or between evenness treatments (linear contrast, P = 0.69). Neither complementarity nor the selection effect depended on identity of the dominant species in inequitable mixtures (linear contrasts, P = 0.34, 0.36).

Relative yields

Species differed in deviation of RY from expectation, but these differences depended on plant density (P = 0.007; Fig. 3). The difference between observed and expected RY of *Lolium* increased with increasing density of mixtures and was significantly greater than zero at the highest density (t-test; $P < 0.025$; 95% confidence interval for the mean = 0.008–0.138). Deviation of observed from expected RY was smaller (more negative) at high than medium and low densities for both *Gaillardia* and *Monarda*. For both species, the observed RY was significantly smaller than expected and RY deviation was significantly smaller than zero at the highest density (t-tests, $P < 0.01$; 95% confidence intervals for mean = −0.15 to −0.04 for *Gaillardia* and −0.15 to −0.02 for *Monarda*). Species differences in deviation of RY from expectation did not depend on mixture type (P = 0.17; data not shown).

Light and soil water

The temporal course of soil water content to 0.15 m depth varied among plot types (P = 0.0016; data not shown).

Table 1 Results of linear contrasts performed to compare above-ground biomass between plot types that included monocultures of each of three annual species (forbs *Gaillardia* and *Monarda* and the grass *Lolium*) and mixtures that differed in relative abundances of the three annuals (1 : 1 : 1 or 3 : 1 : 1 ratio of species; see Fig. 2 for means of above-ground biomass for each plot type)

Contrasts	MS	F-value	P-value
Monocultures			
Forbs vs. grass	1 932 100	316.20	<0.0001
Mixtures			
Grass monoculture vs. mixtures	560 086	91.66	<0.0001
Forb monocultures vs. mixtures	999 182	163.52	<0.0001
3 *Lolium* : 1 : 1 vs. other mixtures	187 539	30.69	<0.0001
1 : 1 : 1 vs. 3 : 1 : 1 mixtures	2774	0.45	0.50

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Table 2 Effects of plant density (15, 45 and 135 plants m^{-2} for low, medium and high densities, respectively; $n = 19–20$) and of mixture type ($n = 14–15$) on the net biodiversity effect and its components (complementarity, selection effect; expressed in g m^{-2}) in three-species mixtures differing in relative abundances of *Gaillardia* (G), *Monarda* (M) and *Lolium* (L). Species abundances in mixtures were completely equitable (1 : 1 : 1 ratio) or were distributed at a 3 : 1 : 1 ratio with each of the three species as the dominant in one 3 : 1 : 1 mixture

	Density			Mixture type			
	Low	Medium	High	1 : 1 : 1	3G:1 : 1	3M:1 : 1	3 L:1 : 1
Net effect	−68.9 (20.4)	−39.6 (11.6)	−123.8 (19.0)	−66.9 (17.6)	−80.6 (23.1)	−105.5 (29.9)	−56.6 (11.4)
Complementarity	−65.7 (18.3)	−18.2 (12.7)	−61.8 (17.5)	−43.2 (16.2)	−44.8 (16.4)	−69.9 (26.6)	−34.9 (16.0)
Selection effect	−3.2 (7.8)[b]	−21.4 (9.9)[b]	−62.0 (14.7)[a]	−23.7 (12.9)	−35.8 (16.4)	−35.6 (14.4)	−21.7 (14.3)

Mean values (±SE) for the selection effect do not differ significantly among density treatments if followed by the same letter. Mean values did not differ significantly among densities for the net effect or for complementarity and did not differ among mixture types for the net biodiversity effect or its components.

Table 3 Summary of results from split-plot analyses of treatment effects on the net biodiversity effect and its components in three-species mixtures with *Gaillardia*, *Monarda* and *Lolium*

	Net effect				Complementarity			Selection effect		
	d.f.	MS	*F*-value	*P*-value	MS	*F*-value	*P*-value	MS	*F*-value	*P*-value
Block	4	7412.1	0.61	0.67	9120.5	1.71	0.24	4104.5	1.50	0.29
Density	2	37 025.5	3.05	0.10	12 556.1	2.35	0.16	17 347.2	6.33	0.02
Block × density	8	12 156.2			5334.4			2741.6		
Mixture type	3	7109.2	1.71	0.18	3616.6	0.67	0.58	872.9	0.39	0.76
Mixture × density	6	6470.3	1.56	0.19	2855.0	0.53	0.78	3060.5	1.36	0.26
Residual	35	4145.5			5396.7			2245.7		

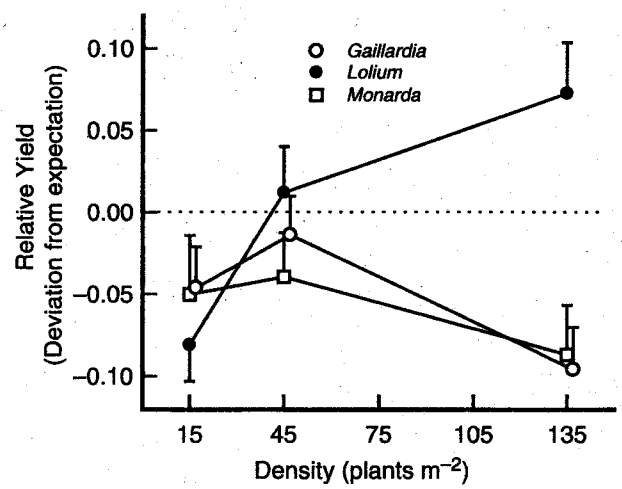

Figure 3 Means (±SE, $n = 19–20$) of the deviation from expectation of relative yields for three annual species grown in mixtures at different densities. Values are averages calculated using above-ground biomass across four mixture types that differed in evenness and identity of the dominant species.

Water content did not differ among plots in late April and early May of 2001. Thereafter, soil water was consistently greater in *Lolium* monocultures than in other plots, probably a reflection of reduced transpiration resulting from the earlier maturation of *Lolium* than *Gaillardia* and *Monarda*.

Treatment effects on light interception generally paralleled those on above-ground biomass. The proportion of incident light reaching the soil surface was lower at high (0.63) and medium (0.61) densities than at low density (0.75) on Day of Year (DOY) 142 in May (linear contrast, $P = 0.0009$). Effects of density on light interception disappeared by the end of the growing season (DOY 184; $P = 0.38$) when mean values of the proportion of light reaching the soil ranged between 0.41 and 0.46.

DISCUSSION

Mixtures with *Gaillardia*, *Monarda* and *Lolium* consistently underyielded compared with monocultures because of both negative or antagonistic interactions among species and selection effects that favoured the least productive species (the grass *Lolium*) at highest density. Neither antagonistic interactions nor selection effects were sensitive to species evenness in mixtures and neither component of the biodiversity effect depended on identity of the dominant species in mixture. Consistent with predictions, increasing density amplified selection effects that favoured *Lolium* in

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mixtures but did not affect expression of negative interactions in species mixtures.

Biodiversity effect

'Underyielding' of species mixtures, as indicated by a negative biodiversity effect, has been identified in a few earlier studies (Loreau & Hector 2001; Fridley 2002), but contrary to the pattern usually reported, both components of the biodiversity effect were negative in this three-species mixture. Loreau & Hector (2001), for example, found that the selection effect became increasingly negative as richness increased at one site (Portugal) in the European BIODEPTH experiment. Nevertheless, above-ground biomass increased with greater richness at this and other sites included in the experiment (Hector *et al.* 1999) because complementarity in resource use increased with richness (Loreau & Hector 2001; Hector *et al.* 2002). Hooper's (1998) experiment with functional groups of species produced evidence for negative selection, but this effect did not reduce relative yield total (RYT) of mixtures below 1. The least productive group of species that Hooper (1998) studied, early season annuals, competitively suppressed the most productive group, perennial bunchgrasses. The differing performances of annuals and perennials in mixtures were largely offsetting, however, and mixture yields were similar to the average of monoculture yields for component species.

Several mechanisms could contribute to a negative correlation between success in interspecific mixtures and biomass production. In this experiment, the explanation appears to involve differences among species in the timing of growth. *Lolium* germinated soon after seeding and completed much of its growth before being overtopped by taller forb species. By growing early in the season, *Lolium* may have pre-empted resources from the more productive forbs, although these resources were not identified.

Density and evenness effects

We predicted that expression of the selection effect would be amplified by greater plant density, implying that increases in density would increase the relative contribution of selection to the net biodiversity effect in mixtures. This expectation was supported. The 'Law of Constant Final Yield' holds that production becomes approximately independent of initial plant densities for densities that are sufficiently large (Harper 1977). That above-ground biomass increased with each increase in plant density in this experiment indicates that the densities employed were not sufficient to reach constant final yield and, by implication, full exploitation of available resources by *Gaillardia*, *Monarda* and *Lolium*. Consequently, density-dependent processes that favoured *Lolium* in mixtures and that contributed to the negative selection effect probably were not fully expressed (Pacala & Tilman 2002).

The substitutive design employed in this experiment, a design in which total density of mixtures is kept constant as one component of mixtures is replaced by another, has been criticized when used to study plant competition because (1) parameters derived to describe the dynamics of mixtures may be sensitive to total plant density and to differences in initial sizes of mixture components and (2) density of each component in mixture differs from that of its monoculture (Snaydon 1991; Gibson *et al.* 1999; Huston *et al.* 2000). These are valid criticisms of experiments designed to predict the outcome of competitive interactions, but are of more limited relevance for diversity experiments. A primary objective of most diversity experiments is to determine how species interactions influence biomass production or other properties of species mixtures, expressed per unit ground area, relative to performance of species monocultures of similar total density. Factors that may influence species interactions and that vary in nature, including plant density and the timing of germination and plant growth, must be included in experiments to predict biodiversity effects.

Above-ground biomass of mixtures in this study depended on identity of the dominant species, as reported by others (e.g. Troumbis *et al.* 2000; Lepš *et al.* 2001), but evenness alone had no effect on biomass production or on the net biodiversity effect or its components. Results are consistent with our expectation that evenness will have little or no impact on expression of the selection effect in species mixtures, but are contrary to our prediction that evenness will affect expression of negative complementarity. Deviation of observed from expected RY of *Lolium* increased at the highest density, an indication that effects on *Lolium* of competition or interference from interspecific neighbours diminished relative to effects of interactions with conspecifics as density increased. The question of whether components of the biodiversity effect were influenced by evenness largely reduces to the question of whether, for *Lolium*, the relative intensity of intraspecific compared with interspecific interactions depended on the frequency of interspecific vs. intraspecific contacts. In this experiment, it did not.

By contrast, Wilsey & Potvin (2000) found that below-ground and total biomass increased linearly with evenness in a study of three-species mixtures of old-field perennials. It is not clear why results of the two studies differ. Perhaps, effects of evenness depend on the sign and relative importances of complementarity and the selection effect in species mixtures, as predicted earlier. The selection effect and antagonistic interactions prevailed in our study. Wilsey & Potvin (2000), however, found that total biomass increased with greater evenness and as variance in plant

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heights increased, suggesting that evenness promoted species complementarity in light interception. Effects of evenness on biomass also may depend on the range of species relative abundances studied. The experiment reported by Wilsey & Potvin (2000) included plots in which evenness treatments and ratios of species abundances were more extreme (5 : 1 : 1 and 12 : 1 : 1) than studied here (3 : 1 : 1).

A negative biodiversity effect has been demonstrated in earlier diversity studies. This experiment, although, apparently is the first to demonstrate that both components of the biodiversity effect may be negative in communities of annuals. Our results also demonstrate that expression of the selection effect may be amplified at greater plant density but is insensitive to species evenness, at least over the range of densities and relatively high values of species evenness studied.

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