

Diversity effects on production in different light and fertility environments: an experiment with communities of annual plants

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Summary

1 Small-scale plant species richness may enhance local production if the local environment promotes resource partitioning (a form of complementarity) or causes some species to perform better next to interspecific neighbours (facilitation) than when grown in monoculture. Little is known, however, about how species richness and environmental factors such as soil fertility and light levels interact to affect ecosystem production.

2 To determine whether some environments promote a positive richness-production relationship, I performed an experiment using artificial plant communities, selected from a pool of six annuals and one perennial species, with different levels of species richness and composition. Each community was grown in either high or low soil fertility and either full sun or 50% shade.

3 Higher species richness significantly enhanced above-ground production. This effect did not depend on environmental treatment due to consistent sampling effects for a particularly productive and competitive species. However, overyielding, which measures the combined effect of complementarity and facilitation, did depend on environmental conditions and was enhanced by increased soil fertility in plots in full sunlight.

4 Relative yield analyses of species of different height and growth form, coupled with data on the performance of each species in monoculture, support the hypothesis that mixtures overyielded by means of light partitioning (rather than facilitation), an effect that was precluded under shaded conditions.

5 The results therefore suggest that higher annual plant species richness may enhance production when environmental conditions allow for resource partitioning.

Key-words: complementarity, facilitation, light partitioning, overyielding, sampling effect

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Introduction

Over the short-term, local plant species richness can have positive effects on above-ground production (Naeem *et al.* 1994; Tilman *et al.* 1996, 2001; Hector *et al.* 1999). What is less clear is how this effect of richness operates (Tilman *et al.* 1997; Huston *et al.* 2000; Fridley 2001; Mouquet *et al.* 2002), whether richness effects are sensitive to environmental parameters (Hector *et al.* 1999; Cardinale *et al.* 2000; Reich *et al.* 2001; Fridley 2002), and whether richness effects are of similar magnitude to the effects of environmental and compositional variables that are well-known determin-

ants of ecosystem production (Huston 1997; Tilman 1999; Grime 2001; Fridley 2002). Given that environmental control over the intensity and nature of plant competition has been widely documented (Keddy 1989; Goldberg 1990; Grime 2001), one might expect that plant mixture dynamics, and thus the effect of plant richness on mixture yield, should vary with environment (Austin *et al.* 1988; Cardinale *et al.* 2000; Fridley 2001; but see Hector *et al.* 1999). Facilitative interactions, in which species benefit from the presence of interspecific neighbours, can also drive richness-production relationships, but these should be especially dependent upon certain stressful environmental circumstances that limit the importance of competition (Mulder *et al.* 2001; Cardinale *et al.* 2002). These observations suggest that richness-production relationships might be under environmental control, yet

few experiments have tested this possibility (but see Hector *et al.* 1999; Reich *et al.* 2001; Fridley 2002).

One mechanism that could allow production to increase with richness is complementary use of resources, such as through light partitioning, in which differences in growth form between species allow for more complete harvesting of ambient sunlight (Naeem *et al.* 1994, 1995). While theoretically attractive, this mechanism has received little direct support and runs counter to the expectation that a few highly competitive species would usurp the light resource (Al-Mufti *et al.* 1977; Grime 1977; Goldberg 1990). Such dominance by one or a few species may cause 'sampling effects', where the likelihood of including these species increases with species richness (Aarssen 1997; Huston 1997; Tilman *et al.* 1997). However, partitioning of the light environment by species of different growth form is a common principle in the design of intercropping systems (Allen *et al.* 1976; Vandermeer 1989), and there may be environmental conditions that promote certain forms of light partitioning (Smith & Huston 1989). Soil fertility, for example, may actually promote light partitioning by accentuating growth form differences between species, thereby enhancing the structural complexity of the vegetation. Stratification of canopy layers promotes coexistence and increases production in forest vegetation (Connell & Lowman 1989; Parker 1995) and some herbaceous and grassland communities (Turitzin 1978; Mitchley 1988; Liira & Zobel 2000). If light partitioning is enhanced by fertility, richness effects may also increase along a fertility gradient, and are likely to be precluded by reducing overall light availability, thereby reducing the amount of light available for partitioning by subcanopy species.

To determine whether the influence of species richness on production is sensitive to environmental variation, I performed an experiment using mostly annual plant communities in which I manipulated species richness, soil fertility and light levels independently of each other. Specifically I sought to determine: (i) whether fertility and light availability change the relationship between richness and production; (ii) the relative importance of richness to production compared with that of environmental factors; (iii) the influence of

particular species, and whether this changes with environment; and (iv) how richness effects operate mechanistically. This last question, which was motivated by a previous study conducted at the same location, in which soil fertility enhanced plant mixture overyielding (i.e. an increase in yield in mixtures compared with that expected from the yield of monocultures) (Fridley 2002) was addressed by measuring relative yields in response to fertility and light treatments. The coupling of fertility and light treatments was specifically used to determine whether this overyielding was the result of facilitation-type mechanisms (Turner *et al.* 1966; Mulder *et al.* 2001), indicated by enhanced monoculture performance of some species in shade, or mechanisms related to light partitioning (Naeem *et al.* 1994, 1995), indicated by reduction of the overyielding–fertility interaction in high-fertility shaded plant mixtures compared with those in full sun.

Materials and methods

EXPERIMENTAL DESIGN AND CONSTRUCTION

Experimental plant communities were created within 252 raised-bed plots (50 × 50 cm, 15 cm deep) at Mason Farm Biological Reserve, North Carolina Botanical Garden, Chapel Hill, NC, USA. Plots were arranged in three replicate blocks. Seven taxonomically diverse plant species (Table 1) were chosen from a previous experiment (Fridley 2002) because they performed well under ambient growth conditions. Each of the seven species was grown at each of three richness levels, i.e. all seven unique monocultures, seven selected two-species 'bicultures' and all seven possible six-species 'polycultures' were constructed. Each composition was grown in four different 'environments' (21 compositions × four environments × three replicates = 252 plots). Biculture compositions were chosen to provide a range of differences in species sizes, with the constraint that each species had to be present in two biculture compositions (Table 1). Low and high levels of soil nutrient availability were crossed with full sun and shade treatments. A split-block design was used with the light treatments; each block was divided into halves and one

Table 1 Species in the experimental species pool listed in descending order of monoculture yield. Biculture pairings are the two species that coinhabited bicultures with the listed species. Biculture compositions were chosen to provide a range of differences in species sizes. 'Size' is the maximum monoculture yield of species in the full sun and high soil nutrient treatment. 'Germination' is the germination rate measured by the seed supplier. Species nomenclature follows Kartesz (1994)

Species	Abbreviation	Family	Life-history	Biculture pairings	Size (g)	Germination (%)
<i>Borago officinalis</i>	BOOF	Boraginaceae	Annual herb	CAOF, LIUS	287.5	88
<i>Amaranthus hypochondriacus</i>	AMHY	Amaranthaceae	Annual herb	ACMI, LIUS	285.7	95
<i>Fagopyrum esculentum</i>	FAES	Polygonaceae	Annual herb	CAOF, SAHO	215.0	92
<i>Linum usitatissimum</i>	LIUS	Linaceae	Annual herb	AMHY, BOOF	176.3	90
<i>Calendula officinalis</i>	CAOF	Asteraceae	Annual herb	BOOF, FAES	166.5	73
<i>Achillea millefolium</i>	ACMI	Asteraceae	Perennial herb	AMHY, SAHO	150.0	73
<i>Satureja hortensis</i>	SAHO	Lamiaceae	Annual herb	ACMI, FAES	127.6	84

half was shaded. Nutrient and composition treatments were arranged randomly within these light treatments for a block:light:plot nesting design.

Each plot was filled with a mixture of construction-grade sand (50%) and landscaping topsoil (50%). Soil of high fertility plots was enriched with 125 g of 'Osmocote' slow-release fertilizer (6-month NPK; The Scotts Co., Marysville, OH, USA), equivalent to 90 g N, 30 g P and 60 g K m⁻². Soil of low fertility plots was supplemented with a mixture of 300 g of hardwood sawdust and 300 g of table sugar, equivalent to a total carbon addition of 1.2 kg C m⁻². All soil additions were performed on 25 May. Carbon addition to soils generally reduces nutrient availability to plants but also has many other direct and indirect effects on soil fauna and microbial populations (Schmidt *et al.* 1997) that were not addressed in the present study.

Shaded plots were placed under a black air- and water-permeable 50% shade cloth ('Sudden Shade'; Dewitt Company, Sikeston, MO, USA). Shade cloths were arranged as one whole sheet per block placed about 2 m above the soil surface, hanging down and fastened to the ground on all sides. Shading treatments were not put in place until 28 June, after seedling establishment. Full sun plots were separated from shaded plots by at least 2 m. An automated sprinkler irrigation system, located beneath shade cloths where necessary, was used to water each plot daily. Species not sown into the plots were weeded at weekly intervals. All plots were sprayed with an organophosphate pesticide every few weeks, beginning on 16 June, to effectively deter herbivory of *Amaranthus hypochondriacus* by Japanese beetles (*Popillia japonica*). Where necessary, 1 m-high wire mesh was placed vertically around plot perimeters to keep *Fagopyrum esculentum* from falling into neighbouring plots.

I established plant communities of different composition and richness by sowing seed in two cohorts during the growing season. Total live seed mass was held constant between plots at 8 g per seed addition (16 g added total) in a replacement series design to account for differences in recruitment success between species of different seed sizes. The mass of seed added per plot per species was calculated by dividing seed mass (8 g) by the germination rate of the species (for monocultures), or by dividing the total seed mass by 2 (for bicultures) or 6 (for polycultures) and then dividing this value by each species' germination rate. Germination rates (listed in Table 1) were measured by the seed supplier (Johnny's Selected Seeds, Albion, ME, USA) prior to shipment. Species were divided into two groups for initial planting to assure approximately synchronous germination; *Achillea millefolium*, *Borago officinalis*, *Calendula officinalis* and *Linum usitatissimum* were initially sown on 30 May; *A. hypochondriacus*, *F. esculentum* and *Satureja hortensis* were initially sown on 8 June. A second cohort of 8 g of live seed of each species was sown on 9–11 July to create more realistic communities of neighbours of different growth stages (Fridley 2002).

Plots were harvested between 17–24 August after all species except *S. hortensis* had set seed. All above-ground biomass was dried to constant mass at 75 °C for 48 h and species within each plot were weighed to the nearest 0.1 g. Below-ground biomass was not harvested due to logistical difficulties associated with recovering fine roots of annual species. Above-ground biomass was therefore used as a surrogate for total production (a reasonable method for annual species; below-ground biomass of the perennial *A. millefolium* can be more decoupled from above-ground growth and thus its overall performance in different environments was measured with less accuracy).

DATA ANALYSIS

I used Type I sum-of-squares (SS) ANOVA modelling to determine the effect of main treatment factors (light, fertility, richness, composition), and interactions between these factors, on above-ground biomass. Composition was a categorical variable describing the list of seeded species for a particular community. The split-plot design required light treatments to be tested against light-within-block error; all other factors and interactions were tested with the within-plot (light treatment) residual error. Two different F contrasts were used to test the significance of species richness treatments: the within-plot residual SS and the species composition SS. The ANOVA ordering of light, nutrient and composition treatments did not affect SS calculations because these factors were perfectly balanced and orthogonal. However, because the effects of richness and the presence of particular species accounted for some proportion of the total SS explained by species composition, decomposing composition into various components required specific factor-ordering hypotheses. Because of this, a separate set of ANOVA contrasts was performed using only the SS for species composition and including species presence-absence (dummy variables) and richness terms in separate models. In this way, the significance of species richness as a determinant of production could be assessed against both compositional variation and the full plot residual variation (even though collinearity between compositional predictor variables may still be present in Type I-tests; see full discussion in Schmid *et al.* 2002). Above-ground biomass data were square-root transformed for all ANOVAs to achieve normally distributed residuals after model fitting.

A full mechanistic treatment of the relationship between richness and production must include statistical analysis of maximum effects of richness treatments on production, in addition to estimates of mean richness effects (Loreau 1998; Huston *et al.* 2000). To evaluate maximum yield responses in richness-production regressions, I used a quantile regression technique that fitted 90th-quantile regression lines to absolute yield data along richness gradients for each environmental treatment. Regression quantiles can be used to fit constraint lines in ecological relationships (Cade *et al.*

1999), and as used here they provide estimates of maximum possible yield at each richness level without undue influence from data outliers. The technique was implemented in S-PLUS 2000 (Professional Release 2, Mathsoft, Inc. 1999) using the 'rq' function from the 'quantreg' S software extension (available at StatLib; <http://lib.stat.cmu.edu/S/>). Confidence intervals of 90th-quantile regression slopes were calculated with a regression rank score inversion method (Koenker & d'Orey 1987) using a 0.05 confidence level. In addition, I examined the relationship between realized (final) species composition and above-ground biomass using non-metric multidimensional scaling (NMS) with PC-ORD (McCune & Mefford 1999). The ordination reported was a two-axis ordination of polyculture (six-species) realized composition using the Sorensen similarity measure, which uses species presence-absence data to calculate compositional similarity between two communities; these pairwise similarities are then represented in two-dimensional space by NMS (Legendre & Legendre 1998).

I examined mechanisms underlying richness-production relationships in different environments by employing relative yield analyses to estimate 'overyielding' (de Wit 1960; Trenbath 1974; Vandermeer 1989; Loreau 1998; Fridley 2001). Relative yield is species biomass in mixture divided by its monoculture biomass for the same block and fertility level; summed relative yields for all species in a mixture are that mixture's relative yield total (RYT). Because good monoculture data are extremely important in relative yield analyses (Vandermeer 1989), I replaced three *B. officinalis* values that were extremely low (< 1 g), probably due to seed predation, with the mean values of the other two replicates for these treatments to prevent them from spuriously inflating relative yield calculations. This procedure slightly over-inflates the degrees of freedom for hypothesis tests involving RYT but is preferable to total loss of RYT values for mixtures including *B. officinalis* in those three replicates (but see Vandermeer 1989). RYT values were log-transformed for normality, and seven extreme RYT outliers (> 6) were removed from all RYT analyses, leaving a total dataset of 161 values (biculture and polyculture mixtures). With log-transformation, expected RYT values (identical mixture and monoculture performance) are zero. Caution must be applied in interpreting any overyielding metric (Trenbath 1978; Garnier *et al.* 1997; Loreau 1998; Huston *et al.* 2000; Dukes 2001), especially using a replacement series design; an important assumption in the present context is that yield-density functions for species are similar in different environments (Austin & Austin 1980). The 'complementarity effect' of the more recently developed 'additive partition of biodiversity effects' (Loreau & Hector 2001) was not used due to its sensitivity to average monoculture yield, and thus environmental properties (Fridley 2002); it cannot be reliably compared between environmental treatments.

Table 2 Type I SS ANOVA for main treatment effects and interactions on above-ground production. Split-plot design means that the 'light' factor is contrasted with the among-light treatment (larger) error; all other F terms represent contrasts with (within-plot) residual error

Source	d.f.	SS	MS	F	P
Block	2	503.3	251.7		
Light	1	313.3	313.3	13.2	0.17
Fertility	1	754.8	754.8	99.8	< 0.001
Richness	2	402.5	201.3	26.6	< 0.001
Composition	20	668.0	33.4	4.4	< 0.001
Light:fertility	1	0.1	0.1	0.002	0.97
Fertility:richness	2	25.3	12.7	1.7	0.19
Light:richness	2	20.4	10.2	1.3	0.26
Light:fertility:richness	2	14.7	7.4	1.0	0.38
Residual	216	1634.0	7.6		

Results

OVERALL EFFECTS OF SPECIES RICHNESS AND ENVIRONMENTAL TREATMENTS ON PRODUCTION

In a full ANOVA model describing the effects of experimental treatments on plot production, soil fertility, richness and species composition significantly influenced production whereas light did not (Table 2). No significant interactions between light, fertility and richness treatments were evident, although the average effect of richness on production was slightly greater in full sun plots (Fig. 1). The absence of a significant richness-light interaction (and the lack of a significant main light effect) may stem partly from a loss in statistical power associated with using light as the blocking factor in the split-plot design (Snedecor & Cochran 1967). As estimated by 90th-quantile regressions, maximum yield did not increase significantly with species richness in any environment ($P > 0.05$), although slopes of these regressions were positive for all but the high fertility, shaded plots (Fig. 1).

When examined between all four environments, species richness explained at most 9% of the variation in production between plots, about half that explained by fertility (17%), and considerably less than that explained by species composition even after accounting for richness SS (Table 2). The SS attributed to sown species composition was decomposed into various non-independent components, including initial species richness, the presence or absence of each species alone, and the total SS accounted for all species listed in the same model (i.e. presence/absence terms for each species but no interspecific interactions included). Even when contrasted with total composition SS (instead of the residual SS in Table 2), richness significantly enhanced production ($P < 0.05$), indicating that the effects of richness operated above and beyond the effects of species composition. Nonetheless, a far greater percentage of composition SS could be explained by the presence of particular species, taken as a whole

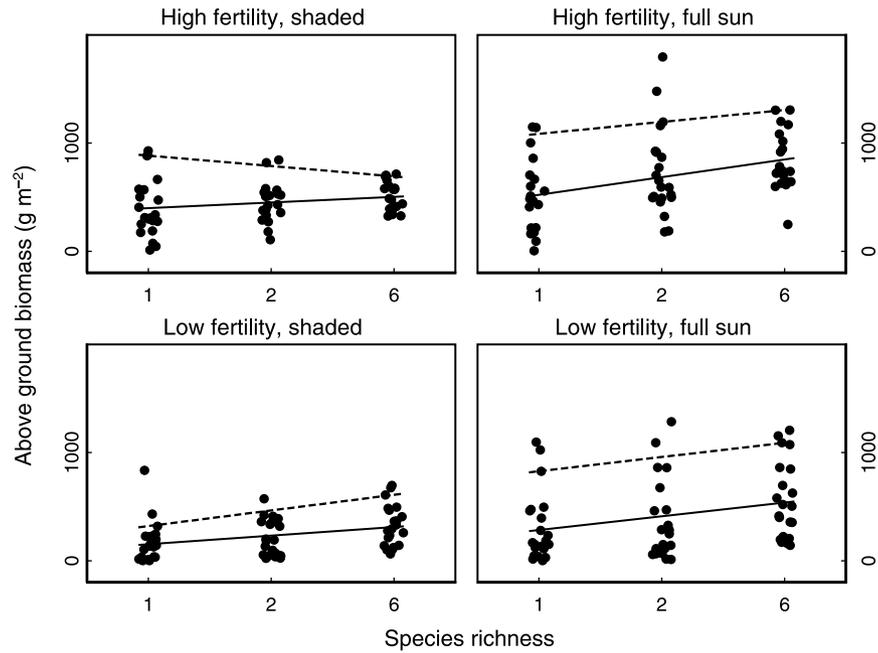


Fig. 1 The effect of species richness on above-ground biomass in each of the four unique environments created by low and high soil nutrient levels (fertility) and full sun and shaded light treatments. Values are staggered along the *x*-axis for clarity. Solid lines are least sum of squares regression lines of the mean; dashed lines are 90th-quantile regression envelopes (see Methods for details).

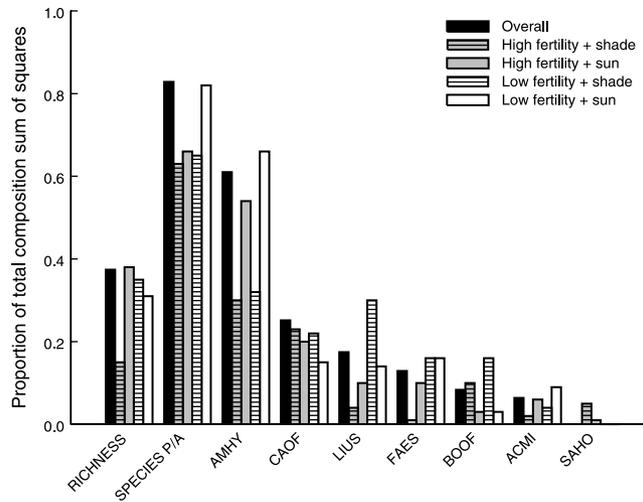


Fig. 2 Variation in above-ground biomass between plots explained by different components of species composition for overall data and within four environmental treatments, listed as proportion of total composition sum of squares explained by a component when listed in a single-factor ANOVA. ‘Species P/A’ is the SS accounted for by all species presence/absence terms included in the same model, i.e. without species interactions. Species terms describe the variation accounted for by the initial presence or absence of that species; abbreviations are listed in Table 1.

without interspecific interactions (83%) or decomposed into the presence or absence of individual species in the initial seed mixture (Fig. 2). This also means that, at a minimum, 17% of the overall variation in production related to species composition must be the result of interspecific interactions of some sort, whether pairwise or of higher order.

Rankings of the importance of factors associated with species composition did not change substantially between environmental treatments (Fig. 2). Exceptions were the importance of species richness (which fell

to 15% in high fertility shaded plots from an average importance of 37%) and the much reduced importance of the presence of *A. hypochondriacus* (AMHY) in both shaded treatments compared with full sun treatments. Small changes in the production of other species in different environments were also apparent, but they explained little total variation overall regardless of environment, except that of *L. usitatissimum* (LIUS) in low fertility shaded plots. In every environment, species interactions must be invoked to explain completely the effects of species composition (Species

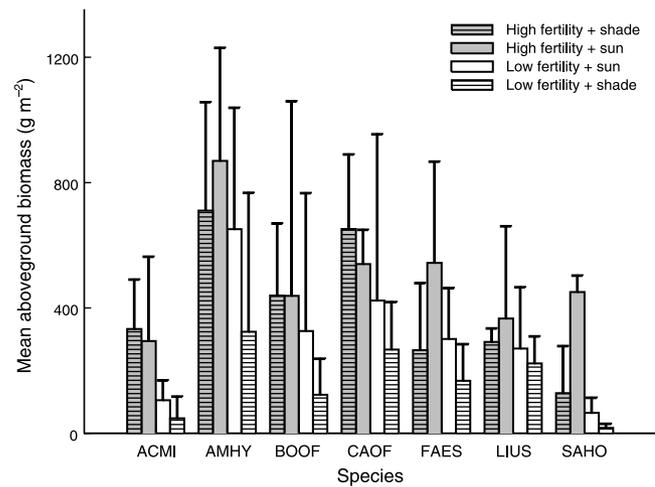


Fig. 3 Mean monoculture biomass (\pm SD) of each species in each of the four environmental treatments. Species abbreviations are listed in Table 1.

P/A, Fig. 2). This is because the total SS explained by all seven dummy variables describing the presence or absence of each species (listed in the same model) could not fully account for the total SS explained by composition for any environment.

SPECIES-SPECIFIC BEHAVIOURS AND SAMPLING EFFECTS

The presence or absence of *A. hypochondriacus* could itself explain 62% of the compositional variation in production (Fig. 2), indicating large sampling effects for this species in mixture. In addition, NMS ordination separated realized polyculture composition largely on the basis of *A. hypochondriacus* biomass, which was in turn strongly related to mixture total biomass (Pearson's $r = 0.63$; correlation coefficients between total biomass and *C. officinalis* and *B. officinalis* 0.46 and 0.32, respectively; that for all other species below 0.15 and down to 0.02 for *A. millefolium*). The performance of *A. hypochondriacus*, in turn, was influenced by environmental treatment, and the shaded, low fertility plots limited monoculture production of this species (Fig. 3). Polyculture composition as a whole, however, was not clearly related to environmental treatment in the NMS ordination. Also note that 'final' polyculture composition and richness estimates for a mostly annual community are ephemeral; a clear sorting of species composition by environmental treatment is less expected with a short-lived annual community than would be for more perennial communities that were studied for longer.

Like *A. hypochondriacus*, most species in monoculture performed best in full sun and high soil fertility, followed by high fertility and shade, low fertility and full sun, and low fertility and shade, although there was considerable within-species variation (Fig. 3). The two exceptions were *C. officinalis* and *A. millefolium*, which achieved their highest yields in nutrient-rich but shaded treatments. *S. hortensis* was apparently dependent upon

Table 3 (a) Type I SS ANOVA for effects of mixture richness (two or six species), environmental treatment, and their interaction on log-transformed relative yield total. (b) Two-tailed Wilcoxon tests of overyielding (log-RYTs) in each of the four environmental treatments for both two-species and six-species mixtures, with test statistic Z

(a)					
Source	d.f.	SS	MS	F	P
Block	2	0.7	0.4	1.1	0.34
Richness	1	2.1	2.1	5.9	0.02
Environment	3	1.9	0.6	1.9	0.14
Richness:environment	3	2.5	0.8	2.4	0.07
Residual	151	52.7	0.3		
(b)					
Environment	n	Z	P		
High fertility, shaded	41	1.41	0.16		
High fertility, full sun	42	4.29	< 0.001		
Low fertility, shaded	38	2.96	0.003		
Low fertility, full sun	40	2.10	0.04		

high levels of both light and nutrients, and in general performed poorly in mixture.

OVERYIELDING AND ENVIRONMENT

Overall, log-transformed relative yield totals were sensitive to mixture richness (two or six species) but not to environmental treatment or a richness–environment interaction (Table 3a). The richness effect was largely due to a significant difference between two- and six-species mixture RYTs in the low fertility, full sun treatment (Student's t , $P < 0.05$, $n_1 = 19$, $n_2 = 21$). The major effect of six-species mixtures on RYT was a reduction in variance compared with two-species mixtures (Fig. 4). This was probably due to the greater compositional similarity of six-species mixtures. Although the variance of RYTs was too great to detect significant differences in overyielding between environmental treatments, individual tests of overyielding within each environment revealed clear environmental effects

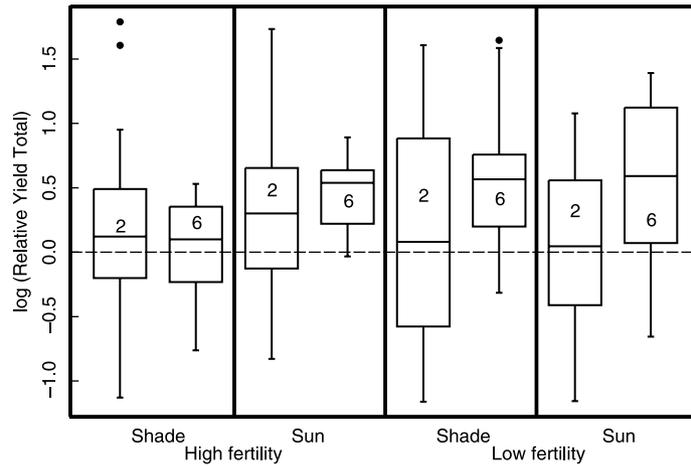


Fig. 4 Box plots of log-transformed relative yield total values for six-species and two-species mixtures, summarized by environmental treatment. Boxes indicate middle two data quartiles separated by median line. Vertical capped lines are the range of the data, with extreme outliers as dots. Dashed line indicates (expected) relative yield total of zero. Each distribution is from a sample size of 21, except that for two-species mixtures of high fertility and shade (20), low fertility and shade (17) and low fertility and full sun (19).

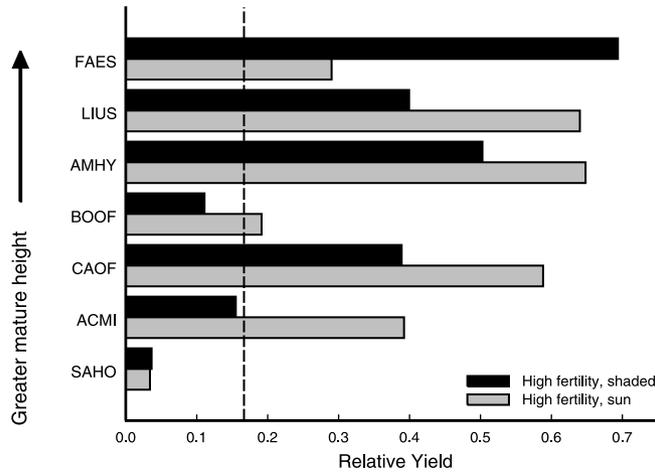


Fig. 5 Relative yields (mixture/monoculture yields) of species in full sun vs. shaded treatments of high soil fertility. Dashed line corresponds to the expected relative yield of 1/6 (mixture seed mass divided by monoculture seed mass). Species codes are listed in Table 1. Each species performed relatively better in full sun, except the tallest species, *F. esculentum*.

when RYT values for two- and six-species mixtures were analysed together. Mixtures grown under high fertility and full sun significantly overyielded, but this overyielding disappeared when high fertility plots were shaded (Table 3b). Mixtures in low fertility environments significantly overyielded in shade, but only marginally overyielded in full sun (Table 3b).

When relative yields were examined for each species individually, the effect of shade on overyielding in plots of high fertility was partially related to species size. Approximate relative heights of the seven species in mixture were:

FAES > LIUS > AMHY > BOOF > CAOF > ACMI > SAHO

For mixtures grown under high fertility, all species performed relatively better in full sun than in shade as measured by relative yield, with the notable exception of the tallest species, *F. esculentum* (Fig. 5; *S. hortensis*

relative yields were similar in sun and shade but contributed little to RYT). The relative yield of *C. officinalis*, the only species that significantly benefited from shade in monoculture, was a large contributor to mixture overyielding overall (Pearson's $r = 0.65$ between *C. officinalis* relative yield and mixture RYT) but could not solely account for the difference in overyielding between full sun and shade plots under high fertility. Although its relative yield contribution to mixture RYT in high fertility plots was greater in full sun (Pearson's $r = 0.56$) than in shade ($r = 0.27$), mixtures containing *C. officinalis* had relatively low RYT values overall ($\mu = 1.67$) compared with mixtures lacking *C. officinalis* ($\mu = 2.18$).

Discussion

When local plant species richness and composition were varied within four environments with different

nutrient and light levels, higher richness promoted a significant increase in production across all environments, consistent with the conclusions from a multisite study of European grasslands (Hector *et al.* 1999). The richness effect remained even after accounting for the effect of species composition, suggesting that the inevitable difference in species composition between richness treatments was not sufficient to explain the effect of plot richness on production. Furthermore, a model including dummy variables for the presence or absence of each species without species interactions could explain at most 83% of the production variation attributed to species composition, indicating that some form of species interaction contributed to the variation in production between plots. Relative yield analyses in different environments suggested that, particularly in plots of high soil fertility and full sun, some species performed better next to interspecific neighbours. In this way, species interactions allowed species richness to significantly enhance production, either by means of facilitation or light partitioning (see below). However, species richness explained only a small amount of variation in production even when confounding composition-related factors were ignored (Fig. 2).

Sampling effects, in which initially more diverse treatments have a higher probability of including particularly productive species, either because they are fast-growing and/or competitively superior species, were clearly important across environments (Fig. 2) and were the probable cause of a non-significant interaction between richness and environment. As in any experiment in which richness treatments are constructed from a fixed species pool, initial compositional similarity was far higher among six-species mixtures than one- and two-species plots (Fukami *et al.* 2001), although not identical; multivariate analysis of realized composition indicated that plot production and the performance of one species, *A. hypochondriacus*, were strongly correlated. As *A. hypochondriacus* was present in 86% (72 out of 84) of six-species mixtures, but only 28% of bicultures and 14% of monocultures, it is not surprising that sampling effects for this fast growing and competitive species were a primary cause of the observed relationship between richness and production. The importance of *A. hypochondriacus* attests to the sensitivity of the results of any richness-production experiment to the characteristics of the species chosen (Fridley 2002). On the other hand, lack of a statistical interaction between richness and environment may not have been the result of the consistent influence of *A. hypochondriacus* but of low statistical power, as the ANOVA model included four interaction terms with relatively low treatment replication (3). Moreover, because no species were present in all polycultures, results of the present study are influenced less by one species than those that include all species from the experimental pool in the most species-rich treatment.

LIGHT PARTITIONING VS. FACILITATION

Species in mixture performed better than expectation (i.e. overyielded) in three of the four environments when measured across all species mixtures. Overyielding in low fertility plots, especially those under shade, was unexpected and difficult to interpret in terms of the two commonly considered overyielding mechanisms, complementary resource use and facilitative interactions (Tilman *et al.* 1997; Hooper 1998; Dukes 2001; Fridley 2001; Loreau & Hector 2001; Mulder *et al.* 2001; Cardinale *et al.* 2002). One possibility is that the reduced performance of the typically dominant *A. hypochondriacus* in low fertility, shaded plots boosted species evenness and allowed for some resource complementarity (such as light partitioning) that would otherwise have been precluded. *A. hypochondriacus* did indeed have a slightly lower relative abundance in low fertility, shaded mixtures, although there was no evidence that environment influenced species evenness. Highly significant overyielding occurred in high-fertility, full-sun plots, replicating the results of a previous experiment conducted at the site with a similar experimental design (Fridley 2002). In the previous study it was not possible to determine whether fertility promoted overyielding by means of complementary or facilitative mechanisms. In the present study, additional mechanistic information is available in that a 50% shading treatment prevented overyielding in these same fertility treatments.

In this experiment, complementary resource use was unlikely for any resource other than light because the plots were relatively small and exhibited little soil heterogeneity. Facilitation of seedling recruitment of species intolerant of the relatively dry and hot conditions of the 50% sand substrate may have operated in plots where intact canopies were available from neighbouring species at the time the second seed cohort was establishing (Mulder *et al.* 2001); such canopy availability would have been promoted by fertility (Fridley 2002). On the other hand, because local extinction was not important in this short-duration study of mostly annual species, fertility may also have promoted light partitioning by accentuating size differences between species.

When high fertility plots were shaded, overyielding effects disappeared. This might have been a consequence of reduction in the vertical light gradient, precluding light partitioning, or better monoculture performance by some species under shade, supporting production enhancement by means of facilitative interactions between species. The evidence does not clearly support facilitation as a mechanism for overyielding. Only two species performed better in monoculture in shade (*A. millefolium* and *C. officinalis*, Fig. 3). Of these, the benefit of shade to *C. officinalis* was greater, and *C. officinalis* performed much better in most mixtures, as it was consistently a larger component of total yield and relative yield than *A. millefolium*. But *C. officinalis* was not the sole contributor to mixture

overyielding in any environment, and in fact mixtures that did not contain *C. officinalis* exhibited greater overyielding on average than those containing this species. Although it is impossible to rule out shade-mediated facilitation for *C. officinalis* in those mixtures where its relative yield exceeded expectation, it is clear that facilitation is not sufficient to explain most cases of observed mixture overyielding, whether for the high-fertility, full-sun treatment or otherwise.

The evidence for light partitioning in the high-fertility, full-sun treatment is more compelling, and consistent with the prediction of Naeem *et al.* (1994, 1995) that small-scale richness may promote production by allowing a greater packing of photosynthetic tissues. The light partitioning hypothesis can work only if the species in the lower height strata respond best to the full sun gradient; canopy species are presumably competing largely with themselves and should therefore only respond to differing intraspecific densities. As plots are shaded, the vertical light gradient is diminished and understorey species should perform relatively worse in mixture. The data support this scenario. Light partitioning may have occurred in the high fertility, full sun plots if species in the understorey (i.e. below *F. esculentum*) accounted for changes in relative yields between high fertility full sun and shaded treatments. Indeed, the relative yield of *F. esculentum* was markedly lower in the full sun treatment than under shade, while the relative yields of all species below *F. esculentum* increased markedly in full sun (Fig. 5). Thus, available evidence may indicate that soil fertility promotes light partitioning by accentuating differences in species' heights and growth forms, an effect that shading removes by taking away the full light gradient. Partial support for this hypothesis comes from studies of light partitioning in mostly perennial herbaceous and grassland communities (Turitzin 1978; Mitchley 1988; Hirose & Werger 1995; Liira & Zobel 2000). Little is known about light partitioning between annuals in natural communities, but this mechanism is often deemed important for the design of intercropping annual crops (Trenbath 1974; Allen *et al.* 1976; Marshall & Willey 1983).

The measurement of overyielding by means of relative yields gives insight into interspecific interactions in species mixtures but is far from a definitive test of overyielding. With relative yields, evidence of complementary resource use (or facilitation) is determined by positive average deviations from expected yields (based on monoculture performance) across all mixture species, rather than a 'transgressive overyielding' approach where complementarity or facilitation is indicated by higher mixture yields than those of the most productive monoculture (Garnier *et al.* 1997; Loreau 1998; Fridley 2001; Spaekova & Leps 2001). Transgressive overyielding is a more conservative estimate of complementary resource use (Huston *et al.* 2000; but see Fridley 2001; Hector *et al.* 2002), and in this study no transgressive overyielding was apparent

in any environment, as measured by 90th-quantile regressions (Fig. 1). Indeed, the most productive assemblages were bicultures in full sun and monocultures in shade. Thus, although six-species mixtures were *on average* more productive than two-species mixtures, any particular transition from mixtures of six species to two species may not be accompanied by lower production.

RELEVANCE TO 'NATURAL' VS. AGRICULTURAL ECOSYSTEMS

The possibility of light partitioning as a mechanism of production enhancement in sites of high fertility begs the question as to whether this should be expected to occur in highly fertile, natural old-field or grassland plant assemblages. The critical difference between most such natural assemblages and the experimental largely annual plant communities studied here is that, while the species richness of high fertility plots was still high at the conclusion of the experiment, this would not be expected to be stable in many natural systems. Indeed, species richness tends to decrease with added soil resources in both natural (Grime 1973; Grace 1999) and experimental systems (Kirchner 1977; Goldberg & Miller 1990; Gough *et al.* 2000). Species that dominate these species-poor communities (e.g. *Urtica dioica*) leave little room for light partitioning from other species because they are highly resistant to invasion, probably a result of high competitive ability for light and the ability to hoard and retain resources from year to year within perennating tissues (Grime 1977; Hutchings & Bradbury 1986). Had this experiment been performed with perennials and lasted several years, the interaction of overyielding and fertility might have disappeared due to local species extinction. Moreover, environmental treatments should influence root-shoot partitioning in addition to overall species performance (Gedroc *et al.* 1996; McConnaughay & Coleman 1998); root-shoot ratios were not measured in the present study but are likely to become an important component of longer-term studies with perennials in different environments.

The present study is especially relevant for crop assemblages where long-term realized values of species richness are of little concern due to crop harvest at the end of the growing season. This experiment suggests that soil nutrient enrichment, a concomitant benefit of intercropping practices in general (Vandermeer 1989), has a secondary benefit of increasing the intensity of complementary relationships between crop species. Indeed, overyielding in intercropping systems tends to increase as size differences between crop species increase; for example, some of the highest overyielding values in the intercropping literature come from agroforestry tree-shrub-forb systems (Ewel 1986). Soil fertility may act in the same way for a particular intercropping assemblage by accentuating the height differences between crops. However, the interaction of

species composition and environment in intercropping studies has apparently received little attention.

The significant interactions of biotic and environmental factors in this and a previous experiment should be a strong mandate to include environmental variation in future biodiversity experiments. Two important points are apparent when environmental variation is included. The first is that the effects of species richness are likely to be much less dramatic than the main environmental treatments that control production (Fridley 2002). The second is that the effect of species richness tends to change with environment. In a previous experiment, soil fertility enhanced the effect of richness on yield; this was also true in the present experiment, but became more complicated when another environmental factor (light) was manipulated independently of fertility. By concomitant manipulations of species composition, richness and environmental factors, researchers will be able to put the effects of species richness on production in proper context, and be able to identify the most likely environmental conditions under which richness effects will occur.

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