Evenness drives consistent diversity effects in intensive grassland systems across 28 European sites

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Summary

1 Ecological and agronomic research suggests that increased crop diversity in species-poor intensive systems may improve their provision of ecosystem services. Such general predictions can have critical importance for worldwide food production and agricultural practice but are largely untested at higher levels of diversity.

2 We propose new methodology for the design and analysis of experiments to quantify diversity-function relationships. Our methodology can quantify the relative strength of inter-specific interactions that contribute to a functional response, and can disentangle the separate contributions of species richness and relative abundance.

3 Applying our methodology to data from a common experiment at 28 European sites, we show that the above-ground biomass of four-species mixtures (two legumes and two grasses) in intensive grassland systems was consistently greater than that expected from monoculture performance, even at high productivity levels. The magnitude of this effect generally resulted in transgressive overyielding.

4 A combined analysis of first-year results across sites showed that the additional performance of mixtures was driven by the number and strength of pairwise inter-specific interactions and the evenness of the community. In general, all pairwise interactions

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contributed equally to the additional performance of mixtures; the grass-grass and legume-legume interactions were as strong as those between grasses and legumes.

5 The combined analysis across geographical and temporal scales in our study provides a generality of interpretation of our results that would not have been possible from individual site analyses or experimentation at a single site.

6 Our four-species agricultural grassland communities have proved a simple yet relevant model system for experimentation and development of methodology in diversity-function research. Our study establishes that principles derived from biodiversity research in extensive, semi-natural grassland systems are applicable in intensively managed grasslands with agricultural plant species.

Introduction

As human activities cause widespread threats to biodiversity, investigation of the potential effect of declining species diversity on ecosystem functions has emerged as an important scientific question (Costanza *et al.* 1997; Loreau *et al.* 2001; Hooper *et al.* 2005). Investigations of the relationship between diversity and ecosystem functioning have mostly used extensively managed grassland communities as a model system, and generally demonstrate that biomass production is reduced when plant species diversity declines (Tilman *et al.* 1996; Hector *et al.* 1999; Tilman *et al.* 2001; Hooper & Dukes 2004; Hooper *et al.* 2005; Roscher *et al.* 2005). Explanations of such patterns centre around hypotheses that more diverse communities better utilize available resources due to their greater occupation of niche space, and that they have a greater probability of containing positive inter-specific interactions (Trenbath 1974; Harper 1977; Vandermeer 1989; Hector *et al.* 1999). Selection effects (Huston 1997; Tilman *et al.* 1997; Loreau 2000) can also contribute, and these different explanations need not be mutually exclusive (Loreau 2000; Hooper *et al.* 2005).

Intensive grassland systems cover large land areas, perform important ecosystem functions such as biomass production and nutrient cycling and are often associated with high input of nutrients and the widespread use of monocultures in research and practice (but see Sanderson *et al.* 2004). Diversity-function research suggests that increased diversity in such systems could improve the provision of ecosystem services (Hooper *et al.* 2005). Such general predictions are largely untested due to the rarity of multi-species agronomic experiments (Vandermeer 1989; Federer 1999; Gibson *et al.* 1999). Whether ecological principles from diversity-function research in extensive semi-natural grassland communities (Hector *et al.* 1999; Tilman *et al.* 2001; Hooper *et al.* 2005) translate to intensively managed grasslands (Tilman 1999) requires investigation.

Diversity-function studies have generally manipulated the number (richness) or composition of species or functional groups in the community. However, relative abundance is also expected to be an important determinant of diversity-function relationships (Nijs & Roy 2000; Wilsey & Potvin 2000; Hooper *et al.* 2005). In diversity-function experiments the relative abundance of species has been almost universally used as a passive descriptor of a community (Stirling & Wilsey 2001; Mulder *et al.* 2004) but has rarely been investigated as an active determinant of ecosystem function (Schmid *et al.* 2002). The few results from experimental manipulations of relative abundance are ambiguous, sometimes indicating an effect on selected ecosystem functions (Wilsey & Potvin 2000; Smith & Knapp 2003; Smith *et al.* 2004), and sometimes not (Wilsey & Polley 2004). Where the effect of diversity depends on interspecific interactions (Trenbath 1974; Harper 1977; Loreau 2000), their total contribution depends on the number and the contribution of individual interactions. The number of interactions depends on species richness. The contribution of a particular interaction depends on the strength of the interaction and the relative abundances of the species involved (Sheehan *et al.* 2006). One would not expect a large contribution from two species that interact strongly but for which one or both have a very low relative abundance. At low evenness (e.g. close to a monoculture) all interactions will involve at least one species with very low relative abundance and hence the total contribution of interactions (the effect of diversity) will be low. The net effect of interactions may lead to overyielding (or underyielding), where the performance in mixture exceeds that expected from performance in monocultures and interactions may be so strong as to lead to transgressive overyielding (Trenbath 1974), where mixture performance exceeds that of the highest-yielding monoculture. To

assess the contribution of inter-specific interaction to ecosystem function requires an experimental and modelling framework within which the contributions of interactions can be separately estimated. To date such a framework has been lacking. We develop it in this paper.

We manipulated relative abundance to investigate the contribution of inter-specific interaction to diversity–function relationships in an experiment using four-species plant mixtures, repeated at 28 sites in Europe. We developed novel methods of design and analysis to address issues in diversity-function research. We address the following questions and discuss how our methods contribute to resolving issues in diversity-function research.

1. Can functional responses in mixed-species communities be decomposed into the sum of identity effects, and the effects of inter-specific interaction, and how does this relate to the richness and evenness of the community?

2. Are there patterns among the interaction effects that lead to a simplified description of the effect of diversity on a functional response?

3. Is there evidence of overyielding or transgressive overyielding in our four-species communities?

4. Are diversity effects consistent across large spatial scales?

Fig. 1 Location of European sites. The 28 sites are displayed according to the geographical species-groups. They are Mid-European (ME, ■), Northern-European (NE, ●), Moist-Mediterranean (MM, ▲), Dry-Mediterranean (DM, ◆) and the sites that used their own site-specific species (Other, ★).

Materials and methods

EXPERIMENT DESIGN

A common experiment with 30 plots was established at 28 sites in 17 countries in Europe (see Fig. 1 and Table S1 in Supplementary Material) in 2002 or 2003, giving 840 plots in total. At each site two legume and two grass species were sown. One of the grass and one of the legume species was fast establishing and the other was slow establishing. Five species-groups were used: north European (NE), mid-European (ME), dry Mediterranean (DM), moist Mediterranean (MM) (Table 1) and a fifth group (Other) consisted of three sites, each with its own species (Table S1). We used a simplex design (Cornell 2002; Ramseier et al. 2005) to define four monocultures and 11 mixtures of the four species (Fig. 2a). The 11 mixtures consisted of four mixtures dominated in turn

by each species (sown proportions of 70% of dominant and 10% of each of the other species), six mixtures dominated in turn by pairs of species (40% of each of two species and 10% of the other two) and the centroid community (25% of each species). The monocultures and mixtures were sown at two levels of overall sown abundance (Connolly et al. 2001) (low being 60% of high). Species proportions at sowing were based on proportions of seed mass considered appropriate for monocultures for each species at a site.

A common protocol was established for plot management. Experimental communities were randomly assigned to plots and managed by cutting (two to five cuts per annum as appropriate for local conditions). Annual application of nitrogen fertiliser ranged from 0 to 200 kg ha^{-1}. Minimum plot size was 2×3 m. For estimation of yield (total annual above-ground biomass including unsown species) a subplot (≥ 3 m^2) was cut to a height of 5 cm at each harvest. See Table S1 for other site information and management details.

Table 1 Composition of species-groups in the different geographical areas ME, NE, MM and DM

Species	Functional group	Mid-European (ME)	Northern European (NE)	Moist Mediterranean (MM)	Dry Mediterranean (DM)
1	Grass – Fast	*Lolium perenne*	*Phleum pratense*	*Lolium perenne*	*Lolium rigidum*
2	Grass – Slow	*Dactylis glomerata*	*Poa pratensis*	*Dactylis glomerata*	*Dactylis glomerata*
3	Legume – Fast	*Trifolium pratense*	*Trifolium pratense*	*Trifolium pratense*	*Medicago polymorpha*
4	Legume – Slow	*Trifolium repens*	*Trifolium repens*	*Medicago sativa*	*Medicago sativa*
Number of sites		15	5	3	2

Five species-groups were used: north European (NE), mid-European (ME), dry Mediterranean (DM), moist Mediterranean (MM) (Table 1) and a fifth group (Other) consisted of three sites, each with its own species (see Table S1).
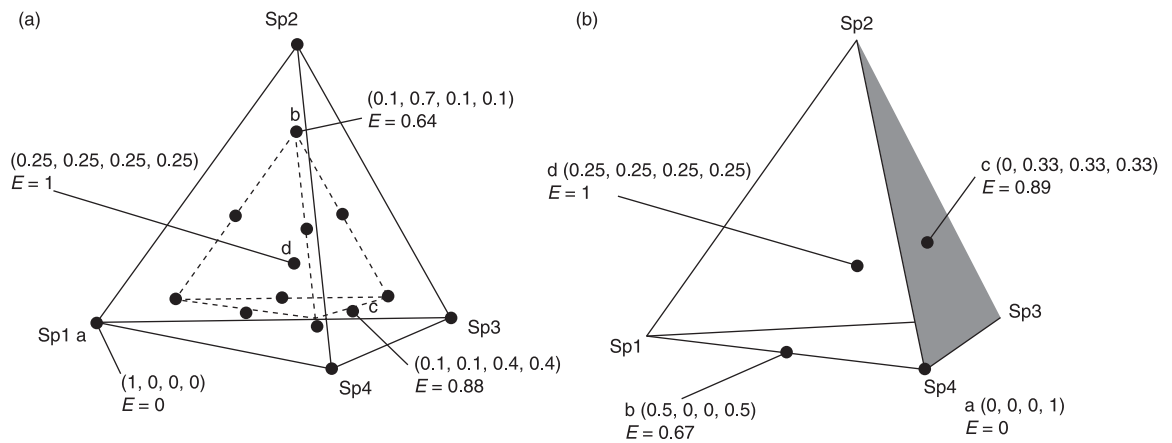
Fig. 2 Graphical representation of (a) the four-species simplex design and (b) the relationship between sown evenness (*E*) and richness in the simplex. (a) Each point in the tetrahedron represents a community with its position determined by its sown relative abundance pattern (P_1, P_2, P_3, P_4). Communities vary in sown evenness, with the most even community at the centroid, where all *s* species are equally represented. The communities at the vertices are the most uneven, containing 100% of a species (e.g. (1, 0, 0, 0) represents a monoculture of species 1). The 15 experimental communities in our design consisted of four monocultures (e.g. a), four mixtures dominated in turn by each species (e.g. b), six mixtures dominated in turn by pairs of species (e.g. c) and the centroid community (d). The sown evenness of the communities in the design varied from 0 to 0.64 and 0.88 to 1. The full design repeated this simplex at two levels of overall sown abundance. (b) Species evenness-richness relationships within the simplex. The four vertices contain 100% of each species in turn and so have species richness (R) of 1 and evenness of 0 (e.g. a). Where there are *t* species, *E* varies from nearly 0 to the maximum for that level of richness, where the *t* species are equally represented (the *t*-species centroid). The six lines joining the vertices contain all possible communities with species richness of 2 (e.g. the line between the vertices for Sp1 and Sp4 depicts all two-species communities containing species 1 and 4 and point b shows a community with maximum two-species evenness). The four faces contain all communities with species richness 3 (e.g. the shaded face depicts all three-species communities containing species 2, 3 and 4 and point c shows a community with maximum three-species evenness). In the interior of the tetrahedron, all communities contain all four species (with evenness maximum at point d).

DIVERSITY MODEL

We propose a diversity model for the analysis of yield that includes the effects of species identity, interactions based on species relative abundance and overall sown abundance. We define the diversity effect (*D*) as the difference between mixture performance and that expected from proportional combination of monocultures (Polley *et al.* 2003; Spehn *et al.* 2005). *D* is zero for a monoculture and must be close to zero for a community that is almost a monoculture. We first define a simple model based on a measure of sown community evenness that captures the effect of all pairwise interactions; this generalizes naturally to a wide range of models that describe alternative patterns of inter-specific interaction.

Evenness is a measure of the distribution of the relative abundance of species in a community. Where there are up to *s* species in a community and P_i is the sown relative abundance of the *i*th species, we define the sown evenness (*E*) of the community as $E = (2s/(s-1))\sum_{i<j}^{s} P_i P_j$, which lies between 0 for monocultures and 1 for a community in which all *s* species are equally represented (Fig. 2). The multiplier $2s/(s-1)$ is introduced to ensure that the values of *E* lie between 0 and 1. For a community strongly dominated by one species, at least one of the P_i values in each $P_i P_j$ pair will be small and so its evenness will be close to zero. As *D* and *E* are zero for monocultures the relationship between *D* and *E* must intercept the origin. Overall sown abundance (*M*) was coded −1 and 1 for low and high, respectively, with a mean of zero for the design. Applying the

evenness formula to the species proportions in our experimental design gives sown evenness values of 0, 0.64, 0.88 and 1 (Fig. 2a). We related yield (*y*) to species' sown relative abundance, total sown community abundance and evenness. The basic model for yield at a site was

$$y = \sum_{i=1}^{s} \beta_i P_i + \alpha M + \delta E + \varepsilon \qquad \text{(eqn 1)}$$

where β_i is the expected monoculture performance of the *i*th species at the average overall sown abundance, α is the effect of sown overall abundance, δ is the linear effect of evenness and ε is normally distributed with zero mean and constant variance. The coefficient β_i reflects the effect of species identity. (For a monoculture of the *i*th species $P_i = 1$, $P_j = 0$ for $j \neq i$ and $E = 0$. Its response is predicted as $\beta_i 1 + \alpha M$ which, at the mean value of *M* (i.e. zero), gives predicted monoculture yield for the *i*th species as β_i.) As the performance expected solely from monocultures is $\sum_{i=1}^{s} \beta_i P_i + \alpha M$, the diversity effect in model 1 is $D = \delta E$. For this model the diversity effect changes linearly with evenness and its maximum is δ when $E = 1$. From the definition of evenness all pairwise interactions among species in this model have the same coefficient ($\delta(2s/(s-1))$).

GENERALIZATIONS OF DIVERSITY MODEL

The diversity effect in model 1 generalizes to a rich class of alternative models based on alternative assumptions about the strength of pairwise species interactions. Pairwise interactions may all be different, leading to a

diversity effect $\sum_{i<j}^{s} \delta_{ij} P_i P_j (2s/(s-1))$ where δ_{ij} captures the strength of interaction between species i and j. There may be clear patterns among the δ_{ij} reflecting the traits of the species in the mixture (e.g. a functional group model that has a common coefficient for all pairwise interactions between species from different functional groups). Interactions may also involve more than two species or more complex functions (e.g. quadratic) in E. Many of these alternative models are hierarchical (Dobson 2002) to model 1 or to the model with separate coefficients for each interaction, which leads to straightforward statistical tests to select the most appropriate model. This modelling approach requires an experimental design that allows estimation of all the coefficients, those of likely alternative models, and the facility to test between them.

DISENTANGLING SPECIES RICHNESS AND RELATIVE ABUNDANCE

In general, the contribution of various levels of species richness to the functional response can be discerned by comparing the mean or maximum functional response at different levels of richness. The effect of variation in relative abundance can be isolated by exploring the range of variation in functional response across communities that have the same level of richness but vary in relative abundance.

The diversity effect defined in eqn 1 includes the effects of species richness and relative abundance. In model 1, the diversity effect, $D = \delta E$, is maximum when $E = 1$, which is only achieved at the s-species centroid (Fig. 2). For lower levels of species richness (t), the maximum diversity effect is reached at the t-species centroids (where $P_i = 1/t$ for t of the species and 0 for the other species, Fig. 2b) and is δE where $E = (2s/(s-1))\sum_{i<j}^{t} 1/t^2 = (t-1)s/((s-1)t)$. The maximum diversity effects for 2, 3 and 4 species are 0.67δ, 0.89δ and δ (Fig. 2). Comparing these D-values gives the effect of changing species richness. As E increases with species richness, model 1 implies that the diversity effect also increases with richness when δ is positive. Changing relative abundance within any level of richness (t) may also change the value of E (Fig. 2b) and hence the diversity effect. D varies from nearly 0 for t-species communities that are almost a monoculture to the maximum for a t-species centroid.

STATISTICAL ANALYSIS

Individual site analyses

We fitted model 1 to yield in the first complete year following establishment using multiple regression. Alternative models were fitted and tested against model 1. These included a model with separate coefficients for each pairwise interaction, functional group models for grass-legume and fast-slow establishing species, a model with both functional groupings and a model with a quadratic evenness term. Three-species interactions among the

relative abundances of the species were also tested. We fitted model 1 to yield from the second year for 16 of the sites.

Combined analysis

For the combined analysis of yield across sites we used a random-coefficients approach (Verbeke & Molenberghs 2000) to fit model 1 and also tested for the interaction between all model terms and species-group. We only included the three species-groups ME, NE and MM in this analysis and omitted the DM species-group as its yields were very much lower. We assumed that model coefficients for species identity, sown abundance and evenness were random across sites within species-group. In this combined analysis, the average value of each model coefficient is effectively tested against the variation in the coefficient across sites. A wide range of alternative models allowing for different assumptions about pairwise species interactions were tested against model 1 (Table S2). We used the structure for the variance-covariance matrix of the random coefficients shown in Appendix S1. Maximum likelihood (Littell *et al.* 2006) was used to fit and test between models and residual maximum likelihood (Littell *et al.* 2006) to test between random effect structures and to provide estimates of the variance-covariance matrix (Appendix S1).

Transgressive overyielding

Within each species-group we calculated the mean yields across sites for each of the 11 mixtures and each of the four monocultures. We used a permutation method to test for transgressive overyielding within each species-group (Appendix S2, Table S3).

Results

INDIVIDUAL SITE ANALYSES

Our data showed consistent positive effects of increasing plant evenness. Almost all analyses of individual sites showed a positive diversity effect that was linearly related to evenness, significantly so in 25 of 28 sites in the first year (Table 2). These diversity effects were observed across a very wide productivity gradient (Table 2). Analysis of second-year data at 16 sites using model 1 again showed a positive diversity effect at all sites, significant at 15 of them (Table 2). Model 1 usually best explained yield at individual sites. Generalizations of model 1 were also fitted to yield for the individual sites for the first year. Models involving more complex inter-specific interactions better explained yield at seven sites; the grass-legume functional group model was not better at any site.

COMBINED ANALYSIS

In the combined analysis across sites the diversity effect differed between species-groups. D was positively

Table 2 Estimates of the coefficient of evenness (δ) in model 1 for 28 sites in year 1 and 17 sites in year 2 (coefficients in bold indicate significance at ≤ 5%). This estimates the maximum diversity effect in mixtures in tonnes ha^{-1}. Also shown for each site for year 1 are species-group, mean mixture yield, and mean monoculture yield (mono). Multiple sites for a country are distinguished by letters in parentheses

Country	Species-group	Mean mixture yield (t ha^{-1})	Mean mono yield (t ha^{-1})	Coefficient of evenness δ ± SE (t ha^{-1})	
				Year 1	Year 2
Germany	ME	17.6	13.6	**4.84 ± 0.835**	
Ireland	ME	16.6	13.6	**3.75 ± 0.515**	**1.51 ± 0.641**
Lithuania (a)	ME	5.7	5.5	0.34 ± 0.545	**1.84 ± 0.543**
Lithuania (b)	ME	10.6	8.7	**2.44 ± 0.681**	
Lithuania (c)	ME	11.0	9.1	**2.17 ± 0.464**	
Netherlands	ME	11.5	8.4	**3.70 ± 1.069**	**5.44 ± 1.375**
Norway (a)	ME	13.7	7.8	**6.85 ± 0.602**	**4.08 ± 0.402**
Norway (b)	ME	11.7	9.9	**2.13 ± 0.36**	**3.44 ± 0.579**
Poland	ME	8.5	6.7	**1.77 ± 0.489**	**2.66 ± 0.610**
Spain (a)	ME	8.5	6.8	2.01 ± 1.217	
Sweden (a)	ME	10.4	7.9	**2.84 ± 0.582**	**5.75 ± 0.548**
Sweden (b)	ME	10.4	7.2	**3.72 ± 0.496**	
Switzerland	ME	15.5	10.4	**5.64 ± 0.689**	**7.46 ± 0.573**
Wales (a)	ME	10.4	6.7	**4.37 ± 0.583**	**2.21 ± 0.674**
Wales (b)	ME	10.5	6.8	**4.68 ± 0.655**	**4.05 ± 0.729**
Iceland (a)	NE	5.4	4.3	**1.13 ± 0.533**	0.49 ± 0.293
Iceland (b)	NE	2.3	1.3	**1.16 ± 0.262**	**1.25 ± 0.239**
Norway (c)	NE	11.3	7.1	**4.18 ± 0.481**	
Norway (d)	NE	10.2	8.1	**2.40 ± 0.465**	**2.04 ± 0.334**
Sweden (c)	NE	9.1	7.2	**2.38 ± 0.32**	
France	MM	9.5	8.1	1.71 ± 1.002	
Greece	MM	3.3	2.5	**0.92 ± 0.222**	
Italy (a)	MM	9.3	8.0	**1.50 ± 0.712**	
Italy (b)	DM	3.3	1.9	**1.62 ± 0.217**	**0.91 ± 0.428**
Spain (b)	DM	2.5	1.0	**1.70 ± 0.263**	**3.57 ± 0.684**
Belgium	Other	16.1	11.6	**5.18 ± 0.577**	**9.14 ± 0.789**
Denmark	Other	13.6	9.7	**4.57 ± 0.488**	
Finland	Other	9.3	7.0	**2.65 ± 0.355**	

linearly related to evenness for NE ($P = 0.002$, five sites) and MM (but not significantly, $P = 0.108$, three sites) and the maximum diversity effect was 2.26 and 1.38 t ha^{-1}, respectively, at $E = 1$ (Table 3). For ME (15 sites), the relationship between the diversity effect and evenness was quadratic ($D = \delta E + \delta_1 E^2$). The estimated maximum diversity effect for ME was 3.09 t ha^{-1} ($P < 0.001$) at $E = 0.97$ (Fig. 3). For ME the effect on yield in multi-species agronomic mixtures seemed less sensitive to modest changes across higher evenness levels (Table 3, Fig. 3), although to intercept the origin the diversity effect must decrease at lower evenness levels. There was a significant difference among identity effects (β_i) for each of the three species-groups, indicating that estimated performance in monoculture differed among species (Table 3).

Across sites, the annual use of nitrogen (N) fertiliser ranged between 0 and 200 kg ha^{-1} (Table S1). High N application was not incompatible with producing diversity effects, which were significant for sites ($n = 6$) with annual N application ≥ 150 kg ha^{-1} (Table 2, Table S2). To check the robustness of the models based on sown evenness, we fitted the models in Table 3 using established evenness at harvest 1 and obtained similar coefficient estimates (Table S4).

TRANSGRESSIVE OVERYIELDING

For ME, NE and DM (Fig. 4, Table S3) virtually all the mean yields of mixtures exceeded the mean yield of the highest-yielding monoculture, providing very strong evidence for transgressive overyielding. Estimates of average transgressive overyielding of 12%, 12% and 16% for ME, NE and DM, respectively, were computed by comparing the average mixture yield with the highest yielding monoculture; but the true overyielding effects are likely to be even larger (Appendix S2). Each of the 'Other' sites demonstrated transgressive overyielding ($P < 0.05$) but the MM species-group did not.

Discussion

In our study, the yield of four-species mixtures exceeded that expected from monoculture performances. This diversity effect was consistent across a wide geographical scale, adding generality to our finding. Our methodology partitions functional responses into the sum of the effects of species identity and the effects of inter-specific interactions. We demonstrate that even a modest increase in the diversity of intensive grassland systems can produce diversity benefits leading to

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Table 3 Estimates of the coefficients in the diversity model for three species-groups from the combined analysis of yield across sites, for the linear model where $D = \delta E$ and for the quadratic model where $D = \delta E + \delta_1 E^2$. The coefficient β_i is the expected monoculture performance of the ith species at the average overall sown abundance, α is the effect of sown overall abundance, δ is the linear effect of evenness and δ_1 is the quadratic effect of evenness

Species-group	Coefficient	Coefficient estimate (t ha⁻¹)	Standard error	Approximate d.f.*	Approx t	$P > t$
ME	β_1	7.78	0.709	26	10.98	< 0.001
	β_2	8.25	0.709	26	11.65	< 0.001
	β_3	10.21	0.792	29	12.89	< 0.001
	β_4	7.55	0.792	29	9.54	< 0.001
	α	0.30	0.136	23	2.20	0.039
	δ	6.32	0.783	308	8.07	< 0.001
	δ_1	−3.26	0.777	550	−4.20	< 0.001
NE	β_1	6.42	1.226	26	5.24	< 0.001
	β_2	4.74	1.227	26	3.87	0.01
	β_3	6.92	1.376	29	5.03	< 0.001
	β_4	4.72	1.371	29	3.44	0.002
	α	0.19	0.237	24	0.78	0.441
	δ	2.26	0.640	23	3.53	0.002
MM	β_1	5.13	1.583	26	3.24	0.003
	β_2	4.24	1.583	26	2.68	0.013
	β_3	8.06	1.770	29	4.55	< 0.001
	β_4	7.43	1.770	29	4.20	< 0.001
	α	0.08	0.304	23	0.27	0.792
	δ	1.38	0.825	23	1.67	0.108

*The approximate degrees of freedom (d.f.) are based on the Kenward-Roger approximation (Kenward & Roger 1997) and used in approximate t-tests for coefficients.
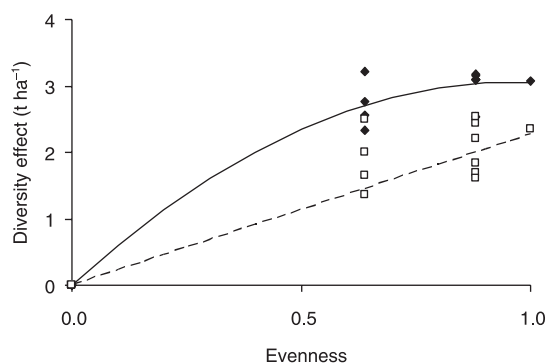

Fig. 3 Average diversity effects (◆ for ME and □ for NE) and predicted diversity effects (— for ME and --- for NE) for four levels of evenness. Average diversity effects for the 11 mixed communities for each species-group were computed as the mean yield for the community in the species-group minus the weighted average of the monoculture yields, weighted by the sown species proportions in the community.

transgressive overyielding. The data were best described by a model in which all two-species interactions contributed equally to the diversity effect; a simple model based on the evenness of the community captured the effects of these interactions. The experimental design based on the simplex allows for discrimination among alternative descriptions of the effects of species interactions, which may be more complex than those found here. Moreover, our methodology contributes to resolving issues in diversity-function research and is relevant for both agronomic and ecological investigations of diversity-function relationships.

In our combined analysis we found no evidence for a more complex diversity effect than one based on evenness, despite some evidence for alternatives at individual sites. The models presented in Tables 2 and 3 assume that all pairwise interactions between species contribute equally to the diversity effect; thus, the positive interaction between two grass species or two legume species was as strong as that between a grass and a legume. There is considerable evidence for positive interactions between grasses and legumes (Vandermeer 1989; Zanetti *et al*. 1997; Hector *et al*. 1999; Spehn *et al*. 2005). The mechanism for a mixing effect among grass or legume species is less clear but synergistic effects can occur in mixtures without legumes (Trenbath 1974; van Ruijven & Berendse 2003; Hooper & Dukes 2004). If these grass-grass and legume-legume effects in our study are due to temporal partitioning between fast- and slow-establishing species (Ofori & Stern 1987; Chesson *et al*. 2002), it is somewhat surprising that the strength of interaction between species that share both functional group differences (e.g. slow grass × fast legume) does not show an even greater positive effect (Table S2). To provide mechanistic explanations of observed responses and model terms will require information on site environments, the contribution of individual species to total yields, and temporal dynamics.

A positive diversity effect implies overyielding; but does it lead to transgressive overyielding? Our data show that on average within each species group, transgressive overyielding occurred in three of the four species-groups (Fig. 4) and at each of the 'Other' sites. The magnitude

Table 4 The effect of diversity on mean and percentage unsown species biomass. We present the average biomass (tonnes ha⁻¹) and percentage of unsown species, for monocultures and mixtures for each of four species-groups. The average biomass was calculated over all sites in each species-group. For the calculation of the average percentage, the average biomass from unsown species was calculated for each of the communities across all sites within the species-group and an average percentage computed for each community. These percentages were then averaged for monoculture and mixed communities

Species-group	Mean unsown species in Monoculture		Mean unsown species in Mixture	
	tonne ha⁻¹	%	tonne ha⁻¹	%
ME	1.28	15.2	0.61	5.3
NE	0.86	18.0	0.29	4.3
MM	1.38	17.7	0.95	10.1
DM	0.38	22.1	0.32	10.0
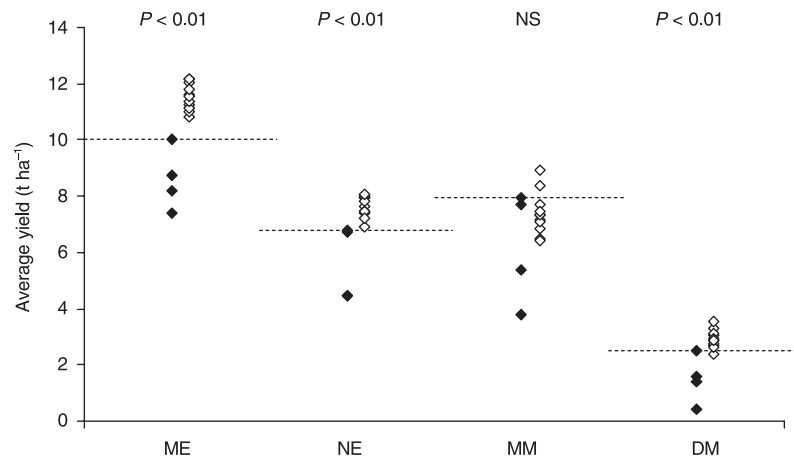
Fig. 4 Evidence for transgressive overyielding. For each species-group we show the mean yield averaged over sites for 11 mixture communities (◇) and four monocultures (◆). Details of the test of significance used are given in Appendix S2.

and generality of transgressive overyielding observed in our study strongly suggests that modest increases in agronomic species diversity can contribute to agricultural production in intensive grassland systems.

This study suggests that to obtain the practical benefit of the diversity effect in intensively managed agricultural grasslands, sowing rates and adaptive management can be used to maintain evenness at levels suggested by Fig. 3. Results for ME indicate that this range of evenness may be quite broad for this species-group (Fig. 3). Of course, yield is one of several important functional responses that are of practical relevance in intensive systems. Mixtures contained much lower proportions and biomass of unsown species than monocultures for all species-groups (Table 4), suggesting reduced invasibility of mixtures. Indeed, as our yield analysis includes unsown species, the diversity effect would be even greater if analysed in terms of sown species only. Other important functional responses will include the nutritional quality of the produced yield as well as the seasonal and multi-annual stability of yield production. Given that the diversity effect in these agricultural mixtures was related to evenness, the temporal persistence of the species in mixtures (to realize the diversity effect) is a significant issue, as are the effects of vegetation dynamics and abiotic factors in affecting persistence.

Designs based on the simplex allow us to estimate interaction effects, to discriminate between a range of possible models and to describe the diversity effect over the widest range of relative abundance and species richness. Models of the diversity effect that are based on our evenness index assume that the effect of diversity is due to pairwise interspecific interactions, all of which are equally strong. However, a model based on evenness may not best describe the diversity effect in other communities. Species may not interact equally, i.e. the strength of inter-specific interaction may differ (Sheehan *et al.* 2006). Patterns of species interaction may be associated with environmental conditions, with particular species traits (Hooper & Vitousek 1997; Hooper & Dukes 2004), and may involve more than two species.

Our methodology can be used to quantitatively disentangle the separate contributions of species richness and relative abundance to the diversity effect. In the broadest sense, the contribution of species richness to the diversity effect can be discerned by comparing mean or maximum diversity effects at different levels of richness. At each level of richness the community with the maximum diversity effect will depend on the relative strength of inter-specific interactions and may not occur at maximum evenness (the centroid). The effect of variation in relative abundance can be isolated by

exploring the diversity effect in communities that have the same level of richness but vary in relative abundance. This broad approach simplifies greatly when the diversity effect is a function of evenness (E) only. The relative effect of changing richness and evenness can be calculated from model 1 or the quadratic model in evenness. For model 1, increasing richness from two to four species increased maximum diversity effects from 0.67δ to δ. Within each level of richness, the effect of varying relative abundance ranges from 0 to 0.67δ for two species and 0 to δ for four-species mixtures. For the quadratic model fitted for ME sites, the effect of changing species richness was weak; the maximum estimated diversity effect for two and three species was only 9.7% and 0.6% lower, respectively, than that for the four-species community. By comparison, changing relative abundance had a far greater effect within two- and three-species mixtures. For two species the range of diversity effects associated with different patterns of relative abundance ranged from 0% to 90.3% of the maximum diversity effect achievable for four species. For three species the range was 0% to 99.4%. In both models the effect of changing relative abundance for a given level of richness was greater than the effect of changing richness (except from a monoculture to two species). The responses to richness in the quadratic model for ME sites suggests saturation (Hooper *et al.* 2005) of the diversity effect at four species, while model 1 suggests that diversity benefits increase with richness but at a diminishing rate.

Multi-site experiments increase statistical power to detect general relationships that may not be obvious from individual site analyses. Three of the individual site analyses did not show significant diversity effects (Table 2) and seven sites provided evidence of a more complex diversity effect than model 1. The combined analysis identifies effects that are generally consistent, despite variation across individual sites. This analysis generalizes interpretation (Verbeke & Molenberghs 2000); the average diversity effect coefficient(s) is the most reliable predictor of the magnitude of the diversity effect at any location in the absence of additional location-specific information. The formulation of general predictions from diversity-function research for large areas of intensive or natural systems therefore requires experiments to be conducted across multiple sites (Hector *et al.* 1999). Experiments with perennial species should be conducted over several years to check the persistence or evolution of effects (Hooper *et al.* 2005; Tilman *et al.* 2006). Our currently available data (Table 2) highlight the persistence of the effect of diversity over at least 2 years.

Acknowledgements

References

Chesson, P., Pacala, S.W. & Neuhauser, C. (2002) Environmental niches and ecosystem functioning. *The Functional Consequences of Biodiversity: Experimental Progress and Theoretical Extensions* (eds A.P. Kinzig, S.W. Pacala & G.D. Tilman), pp. 213–245. Princeton University Press, Princeton.

Connolly, J., Wayne, P. & Bazzaz, F.A. (2001) Interspecific competition in plants: how well do current methods answer fundamental questions? *American Naturalist*, **157**, 107–125.

Cornell, J.A. (2002) *Experiments with Mixtures: Designs, Models, and the Analysis of Mixture Data*, 3rd edn. Wiley, Chichester.

Costanza, R., d'Arge, R., de Groot, R., Farber, S., Grasso, M., Hannon, B. *et al.* (1997) The value of the world's ecosystem services and natural capital. *Nature*, **387**, 253–260.

Dobson, A.J. (2002) *An Introduction to Generalized Linear Models*, 2nd edn. Chapman & Hall/CRC, London.

Federer, W.T. (1999) *Statistical Design and Analysis for Intercropping Experiments, Volume II: Three or More Crops*. Springer, New York.

Gibson, D., Connolly, J., Hartnett, D. & Weidenhamer, J. (1999) Designs for greenhouse studies of interactions between plants. *Journal of Ecology*, **87**, 1–16.

Harper, J.L. (1977) *Population Biology of Plants*. Academic Press, London.

Hector, A., Schmid, B., Beierkuhnlein, C., Caldeira, M.C., Diemer, M., Dimitrakopoulos, P.G. *et al.* (1999) Plant diversity and productivity experiments in European grasslands. *Science*, **286**, 1123–1127.

Hooper, D.U., Chapin, F.S., Ewel, J.J., Hector, A., Inchausti, P., Lavorel, S. *et al.* (2005) Effects of biodiversity on ecosystem functioning: a consensus of current knowledge. *Ecological Monographs*, **75**, 3–35.

Hooper, D.U. & Dukes, J.S. (2004) Overyielding among plant functional groups in a long-term experiment. *Ecology Letters*, **7**, 95–105.

Hooper, D.U. & Vitousek, P.M. (1997) The effects of plant composition and diversity on ecosystem processes. *Science*, **277**, 1302–1305.

Huston, M. (1997) Hidden treatments in ecological experiments: re-evaluating the ecosystem function of biodiversity. *Oecologia*, **110**, 449–460.

Kenward, M.G. & Roger, J.H. (1997) Small sample inference for fixed effects from restricted maximum likelihood. *Biometrics*, **53**, 983–997.

Littell, R.C., Milliken, G.A., Stroup, W.W., Wolfinger, R.D. & Schabenberger, O. (2006) *SAS for Mixed Models*. SAS Press. SAS Institute Inc., Cary, NC.

Loreau, M. (2000) Biodiversity and ecosystem functioning: recent theoretical advances. *Oikos*, **91**, 3–17.

Loreau, M., Naeem, S., Inchausti, P., Bengtsson, J., Grime, J.P., Hector, A. *et al.* (2001) Biodiversity and ecosystem functioning: current knowledge and future challenges. *Science*, **294**, 804–808.

Mulder, C.P.H., Bazeley-White, E., Dimitrakopoulos, P.G., Hector, A., Scherer-Lorenzen, M. & Schmid, B. (2004) Species evenness and productivity in experimental plant communities. *Oikos*, **107**, 50–63.

Nijs, I. & Roy, J. (2000) How important are species richness, species evenness and interspecific differences to productivity? A mathematical model. *Oikos*, **88**, 57–66.

Ofori, F. & Stern, W.R. (1987) Cereal-legume intercropping systems. *Advances in Agronomy*, **41**, 41–90.

Polley, H.W., Wilsey, B.J. & Derner, J.D. (2003) Do species evenness and plant density influence the magnitude of selection and complementarity effects in annual plant species mixtures? *Ecology Letters*, **6**, 248–256.

Ramseier, D., Connolly, J. & Bazzaz, F. (2005) Carbon dioxide regime, species identity and influence of species initial abundance as determinants of change in stand biomass composition in five-species communities: an investigation using a simplex design and RGRD analysis. *Journal of Ecology*, **93**, 502–511.

Roscher, C., Temperton, V.M., Scherer-Lorenzen, M., Schmitz, M., Schumacher, J., Schmid, B. *et al.* (2005) Overyielding in experimental grassland communities – irrespective of species pool or spatial scale. *Ecology Letters*, **8**, 419–429.

van Ruijven, J. & Berendse, F. (2003) Positive effects of plant species diversity on productivity in the absence of legumes. *Ecology Letters*, **6**, 170–175.

Sanderson, M.A., Skinner, R.H., Barker, D.J., Edwards, G.R., Tracy, B.F. & Wedin, D.A. (2004) Plant species diversity and management of temperate forage and grazing land ecosystems. *Crop Science*, **44**, 1132–1144.

Schmid, B., Hector, A., Huston, M.A., Inchausti, P., Nijs, I., Leadley, P.W. *et al.* (2002) The design and analysis of biodiversity experiments. *Biodiversity and Ecosystem Functioning: Synthesis and Perspectives* (eds M. Loreau, S. Naeem & P. Inchausti), pp. 61–75. Oxford University Press, Oxford.

Sheehan, C., Kirwan, L., Connolly, J. & Bolger, T. (2006) The effects of earthworm functional group diversity on nitrogen dynamics in soils. *Soil Biology and Biochemistry*, **38**, 2629–2636.

Smith, M.D. & Knapp, A.K. (2003) Dominant species maintain ecosystem function with non-random species loss. *Ecology Letters*, **6** (6), 509–517.

Smith, M.D., Wilcox, J.C., Kelly, T. & Knapp, A.K. (2004) Dominance not richness determines invasibility of tall grass prairies. *Oikos*, **106**, 253–262.

Spehn, E.M., Hector, A., Joshi, J., Scherer-Lorenzen, M., Schmid, B., Bazeley-White, E. *et al.* (2005) Ecosystem effects of biodiversity manipulations in European grasslands. *Ecological Monographs*, **75**, 37–63.

Stirling, G. & Wilsey, B. (2001) Empirical relationships between species richness, evenness, and proportional diversity. *American Naturalist*, **158**, 286–299.

Tilman, D. (1999) Global environmental impacts of agricultural expansion: the need for sustainable and efficient practices. *Proceedings of the National Academy of Sciences of the United States of America*, **96**, 5995–6000.

Tilman, D., Lehman, C.L. & Thomson, K.T. (1997) Plant diversity and ecosystem productivity: theoretical considerations. *Proceedings of the National Academy of Sciences of the United States of America*, **94**, 1857–1861.

Tilman, D., Reich, P. & Knops, J. (2006) Biodiversity and ecosystem stability in a decade-long grassland experiment. *Nature*, **441**, 629–632.

Tilman, D., Reich, P.B., Knops, J., Wedin, D., Mielke, T. & Lehman, C. (2001) Diversity and productivity in a long-term grassland experiment. *Science*, **294**, 843–845.

Tilman, D., Wedin, D. & Knops, J. (1996) Productivity and sustainability influenced by biodiversity in grassland ecosystems. *Nature*, **379**, 718–720.

Trenbath, B.R. (1974) Biomass productivity of mixtures. *Advances in Agronomy*, **26**, 177–210.

Vandermeer, J.H. (1989) *The Ecology of Intercropping*. Cambridge University Press, Cambridge.

Verbeke, G. & Molenberghs, G. (2000) *Linear Mixed Models for Longitudinal Data*. Springer-Verlag, NY.

Wilsey, B.J. & Polley, H.W. (2004) Realistically low species evenness does not alter grassland species-richness-productivity relationships. *Ecology*, **85**, 2693–2700.

Wilsey, B.J. & Potvin, C. (2000) Biodiversity and ecosystem functioning: importance of species evenness in an old field. *Ecology*, **81**, 887–892.

Zanetti, S., Hartwig, U.A., Van Kessel, C., Lüscher, A., Hebeisen, T., Frehner, M. *et al.* (1997) Does nitrogen nutrition restrict the CO_2 response of fertile grassland lacking legumes? *Oecologia*, **112**, 17–25.

Supplementary material

The following supplementary material is available for this article:

Appendix S1 Covariance structure for the random coefficients for the final model across sites.

Appendix S2 Transgressive overyielding permutation test.

Table S1 Information on sites.

Table S2 Model selection in the combined analysis of yield across sites.

Table S3 Data and permutation test for transgressive overyielding.

Table S4 Combined analysis using established proportions.

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