Procedure for separating the selection effect from other effects in diversity–productivity relationship

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Abstract

In a greenhouse pot experiment we cultivated six meadow species in a replacement series design. The plants were grown at two sowing densities in monocultures and all possible species combinations. Our aim was to separate the selection effect from other diversity effects. This distinction is based on the notion that true overyielding is not a consequence of the selection effect. We suggest a hierarchical procedure, which is based on a repeated division of samples into the pots with the most productive species present and missing. Overyielding can be then demonstrated by a positive dependence of productivity on species richness in the subsets with the most productive species present. Although we found a strong dependence of biomass on species richness in the entire data set, the hierarchical method revealed no evidence of overyielding. Above-ground biomass in a monoculture was a good predictor of species success in a species mix.

Keywords

Competition, complementarity, overyielding, replacement series, sampling effect, chance effect, species richness.

INTRODUCTION

Several recent experimental studies have shown that in artificially constructed assemblages of species, productivity or ecosystem function, on average, usually increases with species richness (e.g. Naeem *et al.* 1995, 1996; Hector *et al.* 1999; Van der Putten *et al.* 2000; Lepš *et al.* 2001). These results have been hotly debated (Johnson *et al.* 1996; Grime 1998; Hodgson *et al.* 1998; Lawton *et al.* 1998; Hector *et al.* 1999; 2000; Naeem *et al.* 1999; Tilman 1999a,b; Huston *et al.* 2000; Kaiser 2000; Naeem 2000; Wardle *et al.* 2000). The debate has focused on the principle mechanisms that might cause this productivity increase, particularly on the separation of chance or sampling effects (Aarssen 1997; Huston 1997; Tilman *et al.* 1997a,b) from more "biological" effects, as complementarity (e.g. Loreau 1998a), or even facilitation. The chance effect is a basic probabilistic issue, i.e. with an increasing number of species the probability of including highly productive species (or species able "to do the job" under given conditions) in the assemblage increases. (Loreau (2000) recommended calling this effect "selection effect" to stress the fact that the "best" species have to prevail in the mixture; we will follow this recommendation in our paper.) Whether the selection effect is considered to be a legitimate "diversity effect" or not (see Lepš *et al.* 2001 for discussion),

the ability to distinguish it in experimental studies is extremely important. The interpretation of results and their importance for the functioning of real communities depends critically on mechanisms involved in this diversity–productivity relationship.

The selection effect probably plays a role in most experiments with manipulated diversity. Consequently, we are interested in a method that would unequivocally prove that more than a selection effect is involved in our results. Loreau (1998b) showed that overyielding (i.e. higher productivity of a species mix in comparison with a monoculture of the most productive of the constituent species) cannot be caused by the selection effect and, consequently, overyielding can be considered as proof that mechanisms beyond the selection effect are involved. This clearly shows that it is important to include monocultures of each species in the experimental design (see also Garnier *et al.* 1997). Recently, a method has been published for partitioning of selection and complementarity effects (Loreau & Hector 2001). This method, however, does not require the presence of overyielding to demonstrate the complementarity.

In the design of biodiversity experiments (i.e. experiments where diversity is experimentally manipulated and productivity or another functional characteristic is the response) a researcher faces a clear trade-off. For statistical power we

need to maximize the number of replications. For the ability to draw reasonable conclusions we need to maximize the number of species combinations (for an ideal scenario we need all possible species combinations, and in any case, all species in monocultures). To obtain realistic results we need a range of species richness, which means many species and many possible combinations. There are theoretical reasons why we can expect diversity effects to manifest themselves better under some natural environmental variability, both in space and time. This calls for long-term experiments under field conditions, with plots exceeding some minimal area. As shown by Vandermeer (1989), even for demonstrating overyielding for a mixture of only two species, it is desirable to use several sowing densities. Similarly, Rejmánek *et al.* (1989) have demonstrated the advantages of additive series experiments over replacement series (note that additive series experiments need more experimental units than replacement series). Taking all this into account, it is clear that it is hardly possible to conduct an experiment that fulfils all the above requirements. Consequently, one has to accept that the experiment will be found to be weak from other perspectives.

We conducted a pot experiment, thereby compromising on environmental spatial heterogeneity and temporal variability, and on the requirements of the realistic spatial extents of some plant assemblages. We used six species (three grasses and three forbs), planted in all possible combinations, and in two total densities. Our goal was to detect any diversity–productivity relationship and if such a relationship is found to distinguish the selection effect from any other possible diversity effects. (As we started from seeds, the biomass can be considered a good measure of productivity.)

METHODS

Experimental species

For the experiment we selected six species occurring commonly in our experimental wet meadow locality at Ohrazení, Czech Republic (Špačková *et al.* 1998; Lepš 1999). Thus, our experimental mixtures are likely to occur and be able to coexist under natural conditions. We chose three grasses (*Briza media*, *Holcus lanatus* and *Festuca rubra*) and three herbs (*Prunella vulgaris*, *Lychnis flos-cuculi* and *Lysimachia vulgaris*). All of the species are polycarpic perennials (annuals are rare and unimportant in our system). *Briza* and *Holcus* are broad-leaved grasses, *Festuca* is a narrow-leaved grass. *Lychnis* is a rosette hemicryptophyte (the rosette dies after flowering, but usually forms auxillary rosettes before). *Prunella* is a hemicryptophyte with creeping shoots, *Lysimachia* with erect shoots.

Most of the species chosen (both grasses and herbs) occur across a wide range of grassland habitats. *Festuca*

"prefers" vegetation in which competition is reduced to moderate intensities by mowing or low productivity. The distribution of *Holcus* is associated with mown and relatively fertile habitats, whereas the occurrence of *Briza* is more restricted to unfertilized and often species-rich grasslands. *Prunella* and *Lychnis* are absent from highly productive and relatively undisturbed areas, and *Lysimachia* frequently occurs in moister habitats and spreads vegetatively in unmown meadows.

Seeds are important for regeneration of *Holcus* and *Briza*. Seedlings of *Briza* are small and suffer heavy mortality during initial establishment. *Lychnis* reproduces generatively under competition stress; in less competitive conditions (gaps) formation of auxiliary rosettes is common. Rhizome growth plays an important role in the regeneration of *Festuca* and *Prunella*. *Lysimachia* creates long underground rhizomes with overwintering buds.

Experimental design

The greenhouse experiment was established in May 2000. The round pots (16 cm in diameter, 14 cm high) were filled with a 1 : 1 mixture of sand and common commercial soil substrate. Seeds of one to six species were sown at two densities, so that each pot would eventually contain six or 24 plants (the sowing densities were higher to compensate for lower germination rates). For the low sowing density (LD) every possible combination of two, three and four species was unreplicated, every five species mixture combination and monocultures were replicated three times, and pots containing all six species were replicated 14 times. For the high sowing density (HD) every combination of two, three and four species was replicated three times, monocultures and five species mixtures were replicated six times, and the mixture of all six species was replicated 28 times. In this way, we compensated for different numbers of species combinations for mixtures of various species richness. Thus, we had 350 pots in the beginning of the experiment. A few weeks after germination, seedlings were thinned (all the species at the same time) to eliminate differences in germination success between species and to obtain the required total number of plants in each pot (i.e. six for LD and 24 plants for HD) and balanced species ratios. Clearly, balanced species ratios were not possible for all combinations (e.g. the five species mixture). In these cases, we weeded the seedlings so that the number of individuals of each species would be as balanced as possible and total seedling numbers would remain stable. However, because of low germination, we were forced to change treatments in some of the pots, and to disregard some of them completely. We eventually ended up with 329 pots. From a total of 126 possible combinations of species identity, diversity and density, only four combinations were missing

(all of them in the LD treatment). During the experiment, the plants were grown under natural daylight regime in a greenhouse, and watered when needed. At the end of the growing season, above-ground parts of plants from all 329 pots were clipped, sorted, dried and weighed and soil from 221 pots was rinsed thoroughly, roots extracted, dried and weighed.

Data analysis

Ordinary methods of general linear models (i.e. regression, ANOVA, combinations) were used where appropriate. Original number of species in a pot was used as explanatory variable in the analyses. Positive relationships between species richness and biomass can be caused either by selection effects or other diversity effects (because the other effects are not distinguishable without further experiments, we subsume all the other effects under complementarity, similarly to Loreau & Hector 2001). Overyielding means that the biomass of species mixtures is higher than the biomass of the most productive monoculture. We use a hierarchical procedure to demonstrate overyielding (and thereby prove that more than selection is involved) as follows: when a positive richness–biomass relationship is found by regression in the complete data set, two separate regressions are subsequently performed: (1) for the subgroup of samples where the most productive species is present, and (2) for the subgroup where it is missing. (The most productive species is that with the highest biomass in the monoculture.) A positive relationship in the subgroups with the most productive species present might be considered to be a good indication of overyielding (and consequently we can deduce that more than selection is involved). A positive relationship in the subgroup without the most productive species can be caused either by selection or some other effect. However, we can again divide this group into two subgroups: the group containing the second most productive species, and the subgroup where the second most productive species is missing, and perform the separate regressions again. A positive relationship in the group with species present is considered to be a sign of overyielding, a positive relationship in the group with the species missing is a reason for repeating the procedure at a further level.

We compared the results based on hierarchical procedure with two existing methods. First, for each mixture, we calculated the overyielding index (*OI*) as follows:

$$OI = Y/\mathrm{MAX}(M_i)$$

where Y is a biomass of the mixture, and M_i is the biomass of ith species grown in monoculture. *OI* is the ratio of the biomass of the mixture and the biomass of the most productive species of the mixture. It is equivalent to the I_1

index of Garnier *et al.* (1997) and to the D_{max} of Loreau (1998b). For statistical analyses, log(*OI*) was used: if the biomass of each mixture would reach the biomass of its most productive species in monoculture, then the expectation of log(*OI*) is zero. As M_i, we used the average value of all the species monoculture replicates of corresponding density.

Furthermore, we used the recently published method of Loreau & Hector (2001) for partitioning of selection and complementarity effects (see Box 1 in Loreau & Hector 2001 for detailed explanations, here we closely follow their notation). Here, the net biodiversity effect is measured by ΔY. The ΔY is the difference between the observed biomass (yield) of the mixture Y and expected yield. The calculation of the expected yield is based on an assumption that the biomass of each species in the mixture is equal to its biomass in monoculture (M_i) multiplied by proportion of the species seeded. (In mixtures with equal representation of species this means simply M_i/N, where N is the number of species in the mixture). Similarly, as in the case of *OI*, average over all the replicates of corresponding density was used as a monoculture value. As shown by Loreau & Hector (2001), the net biodiversity effect can be partitioned as follows:

$$\Delta Y = N\overline{\Delta RY}\ \overline{M} + N\mathrm{cov}(\Delta RY, M)$$

where ΔRY_i is the deviation from expected relative yield of species i in the mixture, calculated as the difference between observed and expected relative yields. Observed relative yield of a species in the mixture is the ratio of its yield in the mixture and its yield in the monoculture. Expected relative yield is the proportion of the species planted. The first term in the equation measures the complementarity effect, the second the selection effect. Consequently, the complementarity is proportional to the average of ΔRY over all the species in the mixture, whereas selection is proportional to the covariance of ΔRY and yield of the species in a monoculture.

RESULTS

Both above-ground and below-ground biomass increase with the number of species (Fig. 1). The general linear model analysis (richness as continuous predictor, two density levels) has shown that the above-ground biomass increases with species richness ($P < 0.01$), and is independent of density, whereas the below-ground biomass also increases with species richness, and is significantly higher in the HD. No significant interaction between density and richness was found for any of the variables. Pronounced differences in the productivity of particular species in monocultures were found, with *Holcus* being the most productive species (Fig. 2). Above-ground biomass in monoculture is a good predictor of the success of a species
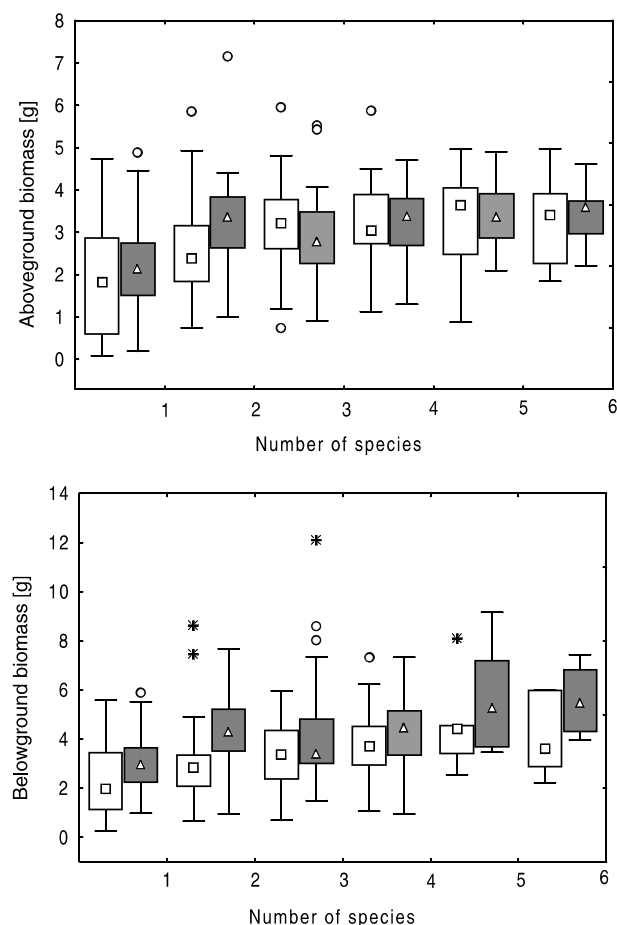
Figure 1 Above-ground and below-ground biomass in pots sown in monocultures and species mixtures with variable species richness. Empty boxes are LD and shaded boxes are HD. The box shows the interquartile range with median, whiskers reach to the non-outlier range (i.e. range of data within ⟨lower quartile − 1.5 × interquartile range; upper quartile + 1.5 × interquartile range⟩) (o) – outliers, * – extremes (more than 3 × interquartile range from the corresponding quartile). Biomass differences between various species richness values are significant for both above-ground and below-ground. These were significant both for the number of species being a quantitative predictor in regression or a categorical predictor in ANOVA, $P < 10^{-5}$ in both cases. The effect of density is significant for below-ground biomass only.

in a species mix (Fig. 3). (Only the full mixtures of all the six species are shown in Fig. 3, but a similar pattern can also be seen in the other mixtures.) However, the relationship between the biomass of a species in monoculture and in a mixture is not linear. Differences between species are much more pronounced in mixtures and the resulting relationship is convex. This suggests that biomass variation in different mixtures has a high species-specific component. Indeed, when using the presence of *Holcus* as the sole explanatory
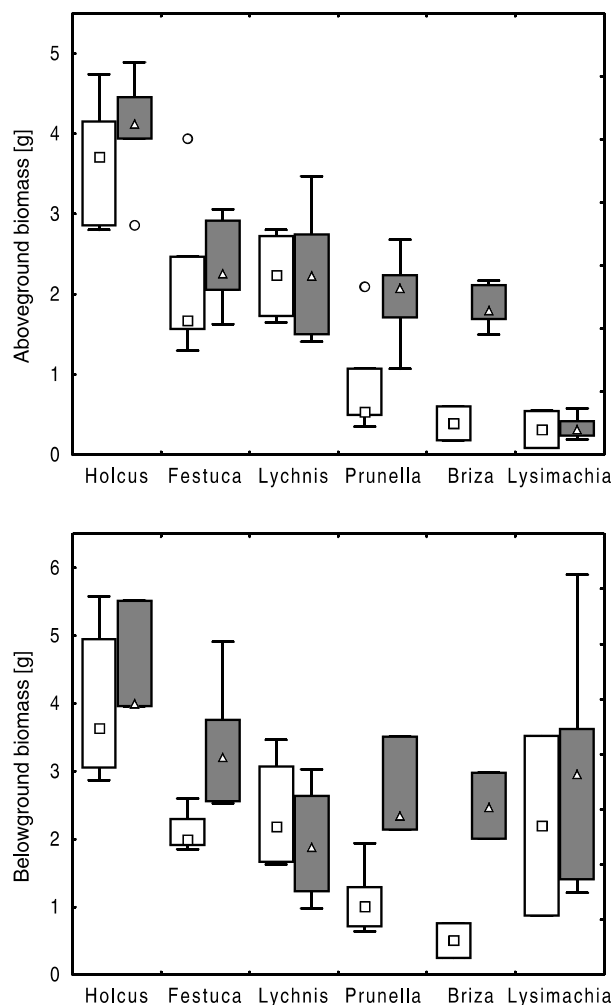
Figure 2 Above-ground and below-ground biomass per pot of the species in monocultures. Empty boxes are LD and shaded boxes are HD. The boxes show the interquartile range with median, whiskers reach to the non-outlier range (i.e. range of data within ⟨lower quartile − 1.5 × interquartile range; upper quartile + 1.5 × interquartile range⟩) (o) – outliers.

variable, 32.4% and 28.2% of the total variability in the above-ground biomass, and 26.0% and 24.7% of variability in below-ground biomass, LD and HD, respectively, is explained. In contrast, number of species as continuous predictor explains only 11.2% and 11.4% of variability in above-ground biomass, and 12.4% and 12.6% of variability in below-ground biomass, LD and HD, respectively.

The hierarchical procedure (Table 1) shows that there is no evidence for overyielding in our data set, either above- or below-ground. In the species mix subsets with the most productive species (*Holcus lanatus*) present, the slope of the regression for above-ground biomass is slightly negative and the slope for below-ground biomass is positive; however, none is significant. In all of the tests, the relationship was
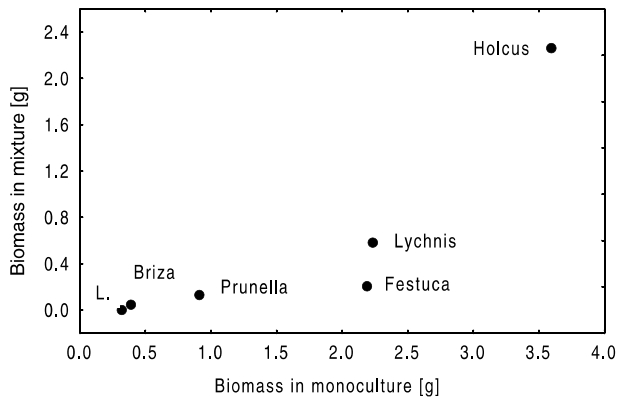
Figure 3 The relationship between the species biomass in monoculture and in mixture of all the six species. LD only shown. L. –*Lysimachia*.

significant only when the selection effect was not excluded (Fig. 4). For both densities, biomass increases significantly with the number of species in the absence of *Holcus*, but is nearly constant in pots with *Holcus* present. A similar pattern emerges at the next hierarchical level, in the subset without *Holcus*: biomass is constant when the second most productive species, *Festuca*, is present, but increases with species richness in pots without *Festuca*, and the pattern is repeated again at the next hierarchical level.

The analysis of log of the *OI* has shown that the grand mean does not differ from zero. The value is independent of sowing density, but there is slight but significant tendency of log(*OI*) to decrease with species richness (Fig. 5, Table 2). The richness–density interaction is not significant.

Using the method of Loreau & Hector (2001), all the three terms (i.e. net effect, complementarity and selection)

have highly significant positive grand means ($P < 0.001$ for all). Both the net effect and selection effect values increase with species richness and are slightly higher for LD. Complementarity effect value is independent of species richness, and also slightly higher for the LD (Table 2, Fig. 5). The richness by density interaction was not significant for any of them.

DISCUSSION

In our experiment, the trend of biomass increasing with the number of species was highly significant and very pronounced. However, the presence of a single species explained a larger proportion of biomass variability than the whole gradient of species richness. This confirms the prediction of Aarssen (2001) that the possible loss of productivity with loss of species depends on which species is lost and only marginally on how many species are lost. More generally, it confirms the view of Lepš *et al.* (1982) that life history strategies of constituent species are more important for ecosystem function than species richness.

For the complete data set, the slope of the relationship was 0.25 (Table 1), showing that adding each species increases the biomass by roughly 0.25 g. This is equal to a 10% increase over the original average biomass of the monocultures. Tilman's (1999a) "rule of thumb" (i.e. with a 50% diversity reduction productivity decreases by 10–20%) suggests that the slope of the dependence of log(productivity) on log(species richness) should range between 0.13 and 0.26. Due to our relatively narrow range of species richness there is no need to use the log-scale for species richness (see Hector *et al.* 1999). However, when we used double log regressions, resulting coefficients for the above-ground biomass at HD and LD, respectively, were 0.49 and
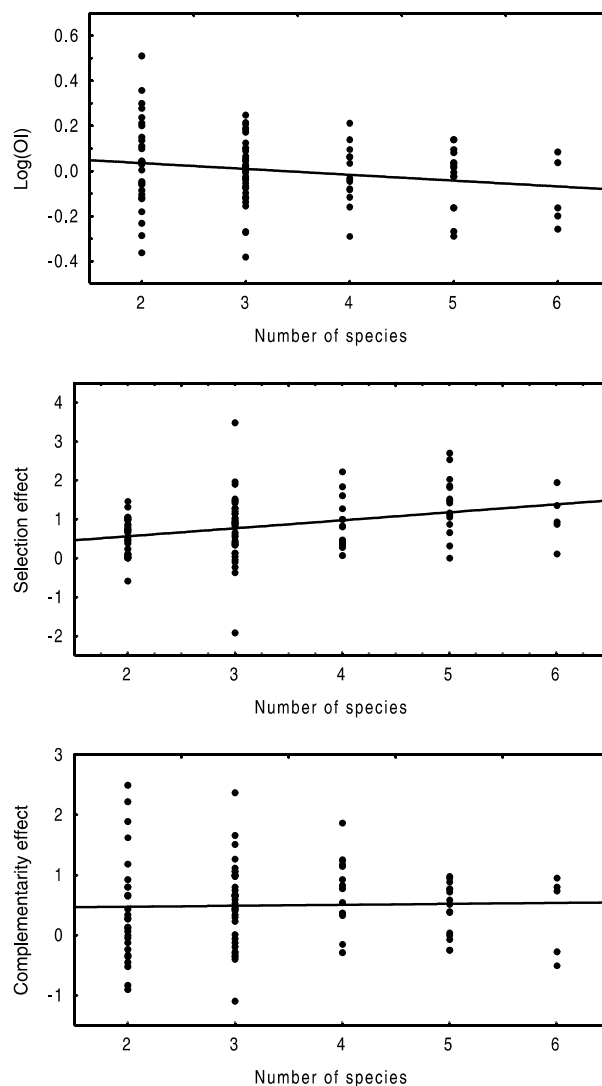
Table 1 Results of the regression analyses in the hierarchical procedure. Group – subset of units analysed defined by species presence or absence condition. Hol – *Holcus*, Fe − *Festuca*, Lych − *Lychnis*. For example, Hol−, Fe−, Lych + means a species group where *Holcus* and *Festuca* are absent, and *Lychnis* is present. In all of the regression analyses density was used as the other explanatory variable and the values correspond to partial regression coefficients (slope of the relationship after subtracting the effect of sowing density). Nonstandardized coefficients are presented so values correspond directly to average change of biomass when the number of species is increased by one. b – regression coefficient, SE of b – its standard error, d.f. – degrees of freedom for the test of b, *P* – significance of the test (H0: b = 0). 0.000 means $P < 0.0005$.

Group	Above-ground				Below-ground			
	b	SE of b	d.f.	*P*	b	SE of b	d.f.	*P*
All	0.246	0.038	326	0.000	0.446	0.080	218	0.000
Hol +	−0.060	0.046	176	0.194	0.129	0.116	108	0.267
Hol−	0.282	0.067	147	0.000	0.261	0.118	107	0.030
Hol−, Fe +	0.011	0.083	68	0.895	0.088	0.170	46	0.609
Hol−, Fe−	0.311	0.112	76	0.007	0.032	0.167	58	0.849
Hol−, Fe−, Lych +	−0.047	0.126	37	0.712	−0.097	0.249	27	0.700
Hol−, Fe−, Lych−	0.284	0.216	36	0.198	0.179	0.315	28	0.575
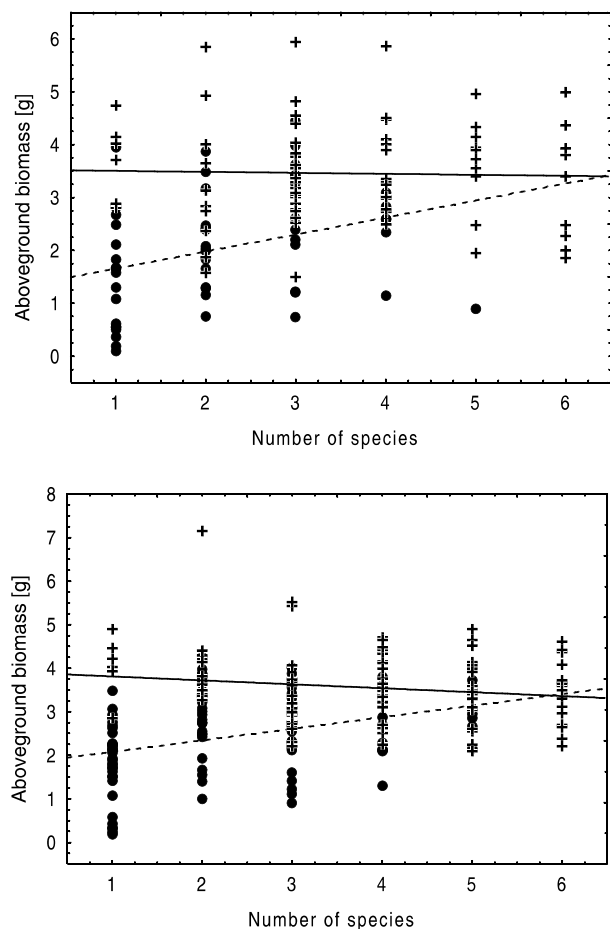
Figure 4 Relationship between number of species sown and above-ground biomass for pots with *Holcus* present (+, solid line) and *Holcus* absent (●, dotted line). Upper graph for LD and lower graph for HD.

Figure 5 Dependence of log(OI), selection effect and complementarity effect on the number of species in a mixture. The regression line is shown also for the complementarity effect, where the dependence is not significant, because it clearly shows that the grand mean is above zero.

0.36, suggesting a decrease of 30–40% by reducing diversity by half. Despite this, neither our hierarchical method, nor the *OI* was able to prove the presence of anything other than the selection effect. This corresponds to the pattern of changes in above-ground biomass where the non-outlier maximum is roughly constant over the whole range in species richness. The fact that the extremely high values are sometimes found at higher species richness is not in contradiction with the selection effect alone. The number of replications (pots) containing one of the two most productive species is higher in the mixture pots, and so is the chance of the appearance of an extreme value. However, the lowest biomass (the non-outlier minimum) increases consistently with species richness. This pattern is consistent with results of Lepš *et al.* (2001) and Hector *et al.* (1999). Below-ground biomass changes reveal a more complicated pattern and it seems that evidence of the complementarity effect could be possibly found here (the hierarchical

procedure does not show significant results, although the relationship is positive).

The inability to demonstrate overyielding is not proof that complementarity does not occur among the species. One of the reasons for this might be that in a replacement series the biomass of the most productive species is decreased because of its lower sowing density. However, this does not seem to be the case in our experiment. For the most productive species only small differences between HD and LD treatment were found in monoculture biomass. In the four species HD mixture the number of individuals of each of the species was the same as in the LD monoculture.

Table 2 Summary of the analyses of the log(OI), net effect, selection effect and complementarity effect.

	d.f.	log(OI)			Net effect			Selection			Complementarity		
		MS	F	P	MS	F	P	MS	F	P	MS	F	P
Number of species	1	0.1673	9.0022	0.0030	0.3002	12.5708	0.0005	12.1102	36.5251	0.0000	0.3119	0.5912	0.4427
Density	1	0.0095	0.5094	0.4760	0.1759	7.3635	0.0071	1.9465	5.8708	0.0161	2.5286	4.7933	0.0295
Error	261	0.0186			0.0239			0.3316			0.5275		

We can thus reasonably expect that the sowing density in a mixture should not be a limitation for detecting the overyielding effect, particularly in HD.

However, there might be an "artificial overyielding effect" when the final yield in a monoculture decreases with sowing density. This decrease has been observed at very high sowing densities (e.g. Wiley & Heath 1969). The decrease in sowing density (e.g. to half in a two species system) may result in overyielding, even if the biomass of the second species is close to zero. Note that this is a feature of the replacement series design and it is probably why Vandermeer (1989; p. 17) suggested that monocultures should be at their optimal density.

In accordance with the hierarchical procedure, the partitioning method of Loreau & Hector (2001) suggested increasing positive selection effect with increasing species richness and no changes in complementarity with species richness. However, contrary to the hierarchical analysis and OI, the partitioning method found a positive grand mean of complementarity, suggesting that there is some degree of complementarity in each mixture.

The basic difference between the method of Loreau & Hector (2001) and the other two methods lies in the fact that the latter consider overyielding as the evidence of complementarity. By contrast, the method of Loreau and Hector takes as the evidence of complementarity the fact that an average of deviations from expected relative yields over all the species in a mixture is positive. In Table 3 we have listed some examples of combinations that would lead to a positive complementarity effect according to Loreau & Hector (2001), but the yield of the mixture is below the yield of the most productive species. The positive value for complementarity might result from relative success of the competitor with lower biomass in a monoculture (as in mixtures A and B), in which case it might be associated with a negative selection effect. In this case, it is possible to get a positive complementarity effect, even when the yield of the mixture is below the average of corresponding monoculture yields (mixture A). Mixture C shows the case where both selection and complementarity are positive, while the yield of the mixture is still below the yield of the most productive species. In our data, the four

Table 3 Examples of some hypothetical two species mixtures, where the complementarity effect is positive. Explanations in the text.

	Mono-culture	Mixture A	Mixture B	Mixture C
Species 1	6	1.5	3	4
Species 2	2	1.9	1.9	1
Total yield		3.4	4.9	5
Net effect		−0.6	0.9	1
Complementarity		0.8	1.8	0.67
Selection		−1.4	−0.9	0.33

highest values of complementarity were associated with a negative selection effect; two of them resulted from a relative success of *Lychnis* in competition with *Holcus*. In those cases, the rosette plant, *Lychnis*, suppressed the grass *Holcus* (perhaps by space pre-emption), although *Holcus* attained higher biomass in the monoculture. However, because *Holcus* was not suppressed completely, and the relative gain of *Lychnis* was higher than the relative loss of *Holcus*, the positive complementarity effect was obtained. Although the rationale of the partitioning selection and complementarity effects according to Loreau & Hector (2001) is appealing, we still would prefer to view overyielding as a strong evidence of complementarity. As Loreau & Hector (2001) noted, their method is a generalization of the Relative Yield Total (RYT, see Hector 1998 for discussion of its use for diversity effects) approach, with similar strength and similar limitation; it is important to realize that this method differs from the overyielding approach, and that these two methods can provide contradictory results (see e.g. Vandermeer 1989; Garnier *et al.* 1997, or Loreau 1998b for the discussion). However, partitioning is superior to the other methods in its use of complete information on the species composition of a community, whereas the other methods take into account the total yield only.

We are well aware of the limitations of greenhouse pot experiments. Under natural conditions species richness might be important in buffering fluctuations over time or under spatial environmental heterogeneity (MacArthur

1955). Both of these factors are minimized in greenhouse pot experiments. Consequently, the lack of strong evidence for a mechanism beyond the selection effect in a pot experiment does not imply that only the selection effect is important under natural conditions. Indeed, the clear difference between our pot experiment and BIODEPTH results when analysed by the Loreau & Hector (2001) method suggests that complementarity plays more a important role in the field (but see Palmer & Chandler-Ezell 2001, who found complementarity in their pot experiment, and Kenkel *et al.* 2000, who found no diversity effect in the field). Our pot experiment also minimized the possibility of temporal resource partitioning, which might be important under natural conditions (Hooper 1998).

As the selection effect is an inevitable part of any experiment of this type, we can reasonably expect an increase of average biomass with increasing species richness, particularly when productivity differs among species (but see Kenkel *et al.* 2000). Consequently, to avoid trivial conclusions, the design of an experiment should always allow for the separation of the selection effect from other diversity effects. There are (at least) two different criteria for demonstration complementarity: those equivalent to RYT and overyielding. Of those, the second is a "more stringent condition that provides fully unambiguous evidence for a positive effect of species interactions on the yield of a mixture, whether by complementarity in resource use or direct facilitation" (Loreau 1998a). It seems that the first criterion is often met in the experimental studies (e.g. Loreau & Hector 2001), whereas the second is not (e.g. Lepš *et al.* 2001).

There are several statistical problems with the analyses. First, whether we should apply some Bonferroni correction in the hierarchical analysis, and second, what to consider as an independent observation. The application of Bonferroni correction is a controversial issue (Cabin & Mitchell 2000). Its use is a good protection against Type I error. On the other hand, it results in a weaker test. We adopted the "soft" approach to this problem, considering the Type I error probability separately for each step. However, even in this case, we were not able to demonstrate overyielding, despite a relatively large sample size.

Even more complicated is the second problem, i.e. what should be taken as an independent observation. We can expect that replications with the same species composition are more similar than replications of the same species richness, but different species composition. Consequently, in the strict sense, replications of the same species composition are not independent observations as we used them in our analyses. Moreover, even different combinations of species are not truly independent, if they share a species. This problem might be solved by using a large number of species; however, this would require a massive number of possible species combinations. (On the other

hand, having carefully balanced combinations with equal representation of the species and monocultures of all species, the hierarchical procedure still can be used.) As with the previous question, we adopted the "soft" approach to this problem, and considered each pot to be an independent observation. The optimal design depends on whether we are interested in the diversity effect within the set of species investigated (i.e. the species are considered to be a "fixed effect" factor), or whether the species are considered to be a random selection of a large species pool (i.e. the species are a "random effect" factor).

Another problem may be the selection of the most productive species. To select such species, we need a reliable estimate of species production in the monocultures (i.e. a sufficient number of monoculture replicates). When the number of monoculture replicates is insufficient it may occur that the most productive species has a low biomass in the monoculture due to random error. Then the selection effect might cause a positive relationship with richness in the group with the (second) most productive species present. The problem might be partially overcome by comparing the most productive species with the species having the most positive explanatory effect in the general linear model (in our case, *Holcus* had the highest explanatory effect, confirming that the selection was correct). This approach might also be used in data sets where some monocultures are missing.

The hierarchical method is superior to statistical analyses of both *OI* and Loreau & Hector (2001) in one aspect. Because the statistical analysis is carried out with the original data, the random errors of individual replications are independent. As the monoculture values are used in the calculation of either *OI* or particular terms in the partitioning, the random errors of the replications are dependent (e.g. if we underestimate the monoculture yield of a species, all the mixtures for which the species is the most productive one will have their *OI* overestimated; similar dependence among replicates' errors exists in the partitioning, because they are calculated using the same estimates of monoculture yields, containing random errors). This leads to the Type I error inflation, which could be very pronounced.

Another statistical problem is the fact that the data are often more heterogeneous at low species richness, which violates the homogeneity of variance assumption, and as the variance is not related in some simple way to the mean, the remedy is usually difficult. This problem seems to be more pronounced when original data are used; however, we consider it less dangerous than the interdependence of errors.

In our view, the present "rift over biodiversity" (Kaiser 2000) requires carefully designed experiments and clearly formulated questions. We suggest that there are basically three criteria of increasing stringency for evaluation of "biodiversity effect". They could be formulated as the

following questions: (1) does the productivity (or "function") of randomly assembled mixtures on average increase with species richness?, (2) does the average relative yield of the constituent species (and consequently also RYT) increase with species richness?, (3) is the productivity of a mixture higher than the productivity of the best of species poorer mixtures or monocultures of its constituent species? The answer of the second question requires species specific data. Consequently, when such data are not available (most of "ecosystem functions", such as nutrient retention and total community photosynthesis), only questions 1 and 3 can be answered. The first two questions correspond to the net biodiversity effect and complementarity of Loreau & Hector (2001) and can be tested using their method (condition 2 need not always be more stringent than condition 1, but usually is). The third question is based on the overyielding criterion and could be answered by the method proposed in this paper. It is the most stringent one (and consequently provides the weakest test). However, when the criterion is met, then we have "fully unambiguous evidence for a positive effect of species interactions" (Loreau 1998a). Moreover, only the third criterion demonstrates that the loss of species has a negative effect on productivity (or whatever function is measured), independent of the identity of the lost species. Similarly, only the overyielding criterion shows that the function of a diverse community cannot be substituted by a species-poor community, composed of a subset of its species.

By the use of our method we demonstrated that a positive productivity–diversity relationship, roughly comparable or higher than that reported in field studies, can be caused by the selection effect. However, we stress that our inability to demonstrate overyielding in a greenhouse is not proof that the effect is negligible under natural conditions, and that diversity might play a role on other spatial and temporal scales than those used in pot greenhouse experiments.

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