Inorganic soil nitrogen under grassland plant communities of different species composition and diversity

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We measured aboveground plant biomass and soil inorganic nitrogen pools in a biodiversity experiment in northern Sweden, with plant species richness ranging from 1 to 12 species. In general, biomass increased and nitrate pools decreased with increasing species richness. Transgressive overyielding of mixed plant communities compared to the most productive of the corresponding monocultures occurred in communities with and without legumes. N₂-fixing legumes had a fertilizing function, while non-legumes had a N retaining function. Plant communities with only legumes had a positive correlation between biomass and soil nitrate content, whereas in plant communities without legumes they were negatively correlated. Both nitrate and ammonium soil pools in mixed non-legume communities were approximately equal to the lowest observed in the corresponding monocultures. In mixed legume/non-legume communities, no correlation was found for soil nitrate with either biomass or legume biomass as percentage of total biomass. The idea of complementarity among species in nitrogen acquisition was supported in both pure non-legume and mixed non-legume/legume communities. In the latter, however, facilitation through increased nitrogen availability and retention, was probably dominating. Our results suggest that diversity effects on biomass and soil N pools through resource use complementarity depend on the functional traits of species, especially N₂ fixation or high productivity.


Investigating how loss of species diversity affect different ecosystem processes has become a major scientific challenge. In 1995–1996, a number of coordinated field experiments were established in seven European countries within the BIODEPTH project (Biodiversity and Ecological Processes in Terrestrial and Herbaceous Ecosystems: experimental manipulations of plant communities) to answer this question (for an overview see Hector 2002 and Hector et al. 2002b). Those studies indicated that greater plant species richness, on average, resulted in a higher plant biomass (hereafter called biomass, Hector et al. 1999, Spehn et al. 2005). One reason for this could be a more complete utilization of resources in time and space, “the niche complementarity hypothesis” (Tilman et al. 1997, Loreau 1998a,b). Also facilitation, for example grasses benefiting from the N₂-fixation of neighbouring legumes, has been included in the term complementarity, because of the difficulty to
separate the different mechanisms (Loreau et al. 2002a). This may lead to the phenomenon that species-rich mixtures outperform their respective monocultures in biomass production – “overyielding” in the earlier botanical and agricultural literature (Hector 1998). This term has been split into two different concepts; “transgressive overyielding” where a mixture has a higher biomass production than all corresponding monocultures, or “non-transgressive overyielding” where the yield of a mixture is greater than the average of the species grown in monoculture (Hector et al. 2002a).

Few direct tests of belowground resource complementarity exist, but isotope techniques have shown that N fixed by legumes is taken up by co-occurring plants, thereby increasing total plant community N pools (Scherer-Lorenzen 1999, Mulder et al. 2002, Spehn et al. 2002). Some studies show little or no relationship between plant diversity and inorganic nitrogen in soil, but clear effects of species or functional group composition (Hooper and Vitousek 1998, Symstad et al. 1998). Other studies show diversity effects on both plant biomass and inorganic N in soil (Tilman et al. 1996, Niklaus et al. 2001). Only one study has compared inorganic N (in soil water) and plant biomass (Scherer-Lorenzen 1999, Mulder et al. 2002, Spehn et al. 2002a). In that study, no significant correlation between the two was found.

With the aim to understand some of the mechanisms leading to higher production in species rich communities, we performed a series of comparisons of plant production and soil nitrate and ammonium under monocultures and mixtures of twelve herbaceous species. One aim was to analyse if the increase in biomass in more diverse communities was, at least partly, due to more efficient depletion of inorganic N in soil. A second aim was to determine how the presence of particular species or functional groups influences inorganic N levels. We thus aimed to test the following hypotheses:

1) Soil inorganic N pools are negatively related to biomass production.
2) Mixed communities deplete soil inorganic nitrogen to a lower level than could be expected from the corresponding monocultures.

Material and methods
Experimental background
The field site was located at SLU (the Swedish University of Agricultural Sciences) in Umeå, Sweden (63°45N, 20°17E). The site is situated in the boreal zone with long and cold winters and frozen topsoil in winters. This paper presents plant biomass data from the years 1999 and 2000 (1999 also published in Mulder et al. 2002), and soil inorganic N data from 2000. In 1999 precipitation was close to the 1961–1990 average of 591 mm (575 mm) but it was much higher in 2000 (1024 mm). Mean temperatures in May–September were close to the long term average both years (10.3 and 9.9°C, respectively). The soil is a fine silty sand with little clay content (4.1% clay, 38.0% fine sand) and a pH_H2O of 6.0. We established 68 plots, measuring 2.2×5 m in early June 1996. Two plots were non-vegetated controls. The remaining 66 plots were sown with a total density of 2000 seeds m$^{-2}$, divided equally among the species in the plot. All plots were hand-weeded during the summers to prevent invasion of unwanted species. Plots were sown with 1, 2, 4, 8 or 12 species of vascular plants in 28 unique species mixtures. All species were sown in duplicated monoculture plots. Each mixture was duplicated, some were triplicated, and the twelve-species mixture had 6 replicates. The species used in the experiment were four grasses: Dactylis glomerata L., Festuca ovina L., Phalaris arundinacea L. and Phleum pratense L., four legumes: Lotus corniculatus L., Trifolium hybridum L., Trifolium pratense L. and Trifolium repens L., and four other herbaceous species (for simplicity hereafter called herbs): Achillea millefolium L., Leucanthemum vulgare (L.) Lam., Ranunculus acris L. and Rumex acetosa L. Borders with a width of 1.5 m were established between the plots and sown with Phleum pratense. Borders were mowed several times a year and the plots once, in late August. Clippings were removed. Fertilizer was last added in 1995, i.e. the year before the BIODEPTH experiment commenced. Further details of the experimental design are given in Mulder et al. (2002).

The experiment was located on three low ridges on the field in order to reduce ice damage from poor surface run-off in the otherwise flat field. Plots on the very top of the ridges were denoted block 1 and plots on the sides of the ridges, block 2. Most plots in block 2 were very seriously ice-damaged 1997 and 1998. Unwanted plant species had invaded many of these plots, in spite of weeding efforts, and because of this, the soil in many plots of block 2 was turned with a spade to a depth of approximately 10 cm before re-sowing in 1999. In block 1 the soil was only shallowly hoed at bare spots and raked otherwise to create germination sites for the seed. The latter treatment was applied to all re-sown plots in 2000. In 1999, all species were re-sown in all plots in the same amount and proportion as 1996. In 2000, as a precaution, even though only clovers seemed severely winter damaged, all species with a weak appearance in the end of May (in most cases all but P. arundinacea) were re-sown. Re-sowing of P. arundinacea was considered unnecessary, since it is spreading well by rhizomes and it was abundant in all plots where it should be present. The soil preparation and the subsequent re-sowing had no significant effects on either soil extractable nitrate or ammonium (nitrate: F_{1,65} = 0.289;
P = 0.593; ammonium: F_{1,65} = 2.032; P = 0.159), so we included all plots in our analyses of diversity effects.

Eight plots (the third replicates) were treated with insecticides in 1997 as an assessment of the effect of herbivory on the plants. In that year, the treatment resulted in an increase of biomass and in greater evenness of relative plant species abundances and it revealed a strong positive relationship between plant species richness and biomass (Mulder et al. 1999). However, the insecticide treatment had no continued effect on biomass in 1999 and 2000 (1999: F_{1,15} = 0.842; P = 0.374; 2000: F_{1,15} = 0.290; P = 0.599). Similarly, soil N status in 2000 was not affected by the treatment (nitrate: F_{1,15} = 0.94; P = 0.763; ammonium: F_{1,15} = 0.369; P = 0.554). Therefore, we also included these plots in the analysis of plant diversity effects.

**Biomass sampling**

Plant biomass was cut at 5 cm above the soil and sampled every year in mid-August within a frame of 20 × 50 cm, in the centre of each plot. Samples were sorted to species, dried at 60°C for at least 24 h and weighed. Weeds, if present in spite of weeding, were not included in the biomass calculations.

**Extraction of soil nitrate and ammonium**

Soil was sampled from 0–10 cm and 10–30 cm depth in all plant communities in October 2000. Six soil cores (29 mm diameter at 0–10 cm, 21 mm diameter at 10–30 cm) from each plot and each depth were pooled, roots were removed by hand and the samples were stored in 4°C for about one week, before freezing at −20°C. Thawed samples were extracted with 2M KCl during 2 h and the extracts were analysed for nitrate and ammonium using flow injection analysis (Tecator 5012 with detector 5042: Foss Tecator application notes ASN 50-06/91 and ASN 110-01/92). Data from both depths were used to calculate the inorganic N pools per hectare in the upper 30 cm of the soil. Soil bulk density was used to calculate the inorganic N pools per hectare in the upper 30 cm of the soil. Soil bulk density was used to calculate the inorganic N pools per hectare in the upper 30 cm of the soil. Soil bulk density was used to calculate the inorganic N pools per hectare in the upper 30 cm of the soil.

**Calculation of transgressive overyielding in plant biomass and under-depletion of soil N**

We used the proportional deviation index D_{mix} to compare observed and expected total biomass of a mixture (Hector et al. 2002a), calculated as follows:

\[
D_{mix} = \frac{O - E}{E}
\]

O is the observed total biomass of all sown species in a plot either in 1999 or 2000 and E is the biomass of the most productive of the corresponding monocultures that year (mean of two plots). A value of D_{mix} > 0 means the mixture is more productive than any of the species grown in monoculture (transgressive overyielding).

Similarly, we calculated the expected values of nitrate and ammonium pools under the plant communities in each plot. In order to test both if the mixed communities were depleting N more efficiently than any of the monocultures included, and if N depletion in mixtures was more efficient than could be expected from their botanical composition we calculated the expected soil N pools in two different ways:

- D_{min, NH_4/NO_3}: this is a parallel to D_{mix} for biomass. Here we put the lowest monoculture pool (mean of two plots) of nitrate or ammonium, found for any of the species in the mixture, as the expected value, and calculated the deviation index as above.

- D_{weight, NH_4/NO_3}: this index was calculated using the average of the corresponding monoculture pools (mean of two plots) of NH_4^+ or NO_3^- (P_i ...), weighted by the proportion of each species in the total biomass of each plot (B_{i-1} mean of the years 1999 and 2000), as the expected value:

\[
E_{weight} = B_{1}P_{1} + B_{2}P_{2} + \ldots + B_{i}P_{i}
\]

l = species 1, 2 = species 2 ... to species i

This test assumes that species with a high biomass have a dominating effect on ecosystem functions (Grime 1998). We believe this test is more adequate than D_{min, NH_4/NO_3}, since all plant species influence the model. The biomass the year before the sampling was included, since legume biomass can affect nitrate leaching the following year (Scherer-Lorenzen et al. 2003).

A value of D_{min/weight, NH_4/NO_3} < 0 indicates that mixtures are reducing soil ammonium or nitrate pools to lower levels than would be expected from the corresponding monocultures, suggesting complementary resource use (we call this phenomenon under-depletion, as a parallel to overyield). In contrast, equal competition for soil N would either give a value of 0 if there are compensatory tradeoffs among species, or even D_{weight, NH_4/NO_3} > 0, if species interfere or if species that are less efficient in depleting soil N dominate the community (Hooper and Vitousek 1998, Spehn et al. 2002).

Because all species used in mixtures were grown in monocultures, we were able to calculate D−values for all mixtures.

**Statistical analysis**

The BIODEPTH experiment manipulates two variables, species richness and functional group richness. Data
were analyzed with analysis of variance (ANOVA) using general linear model approaches to test for the effects of blocks, presence of legumes, species richness, number of functional groups, and identity of species mixture in a sequential fitting order (type I sum of squares; general linear models-procedure in SPSS 11.0.1, 2001; Schmid et al. 2002b, Scherer-Lorenzen et al. 2003). However, the hierarchical design required two error terms: the variance of the diversity levels was divided by the mean square of the individual mixtures. The variance among mixtures was then compared with the residual variance among plots. Thus, the problem of confounding effects of species identity and species number or number of functional groups was minimized (Schmid et al. 2002b). In addition, we split the factors species richness (log-scale) and number of functional groups into a test for linearity and deviation from linearity.

Functional group richness is correlated to both presence of legumes and species richness and most of the relevant variation is removed in the model described above. Because of that, and since most mixtures contained at least one grass species, the term functional group richness tests mainly for presence/absence of herbs. Therefore, we also reversed the fitting sequence of species richness and number of functional groups in our statistical model (for the rationale of this procedure see Schmid et al. 2002a).

We were particularly interested in the effect of the presence of legumes, which play a key-role in N dynamics of grasslands. Therefore, we always fitted the presence of legumes before the effects of diversity, i.e. ignoring their inevitable contribution towards numbers of species or functional groups (exploratory a posteriori ANOVA-model, Schmid et al. 2002a, Spehn et al. 2002). However, because of the unavoidable imbalance and co-linearity involved in biodiversity designs it is impossible to unequivocally separate the effects of legumes from other diversity effects with our design, where the effects of presence of legumes were embedded within species richness and functional group richness (Allison 1999). Therefore, fitting presence of legumes first removes parts of the effects of those aspects of diversity, and is an extremely conservative test of diversity effects (Loreau and Hector 2001).

We addressed whether the presence of particular species has large effects on ecosystem processes by calculating contrasts within mixtures between plots with or without particular species (see Stephan et al. 2000 for an exemplary skeleton ANOVA table). However, because our experiment was not specifically designed to test for species-specific effects, the statistical analysis was unbalanced. For instance, the legumes *Trifolium repens* and *T. hybridum* always occurred together in mixtures. We thus interpret these exploratory post-hoc tests with care. In addition, it is important to note that a significant contrast for a particular species does not necessarily imply that it is this species that contributes most to the measured ecosystem variable (Schmid et al. 2002b). For example, legumes may stimulate the productivity of co-occurring species, but may directly contribute to the overall biomass production of the community only to a small extent.

**Table 1.** Results of the ANOVA on biomass in the years 1999 and 2000, and on the pools of soil nitrate and ammonium in October 2000. (A) Fitting species richness prior to the number of functional groups, (B) Fitting the number of functional groups prior to species richness (other values are the same as in A). Marginally significant P-values (0.1 > P > 0.05) are in italics, significant P-values (P < 0.05) are in bold.

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<th></th>
<th></th>
<th>Biomass2000</th>
<th></th>
<th></th>
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<th>Soil NH₄⁺</th>
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<td>1.2</td>
<td>0.544</td>
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linearly with species richness, whereas in plots without legumes or with legumes only, no significant increase of biomass with increasing diversity could be detected (Fig. 1).

The residual effect of number of functional groups on biomass was not significant after the presence of legumes and species richness were taken into account in our sequential model (Table 1A). After reversing the diversity terms in the statistical model, the number of functional groups had a significant effect on biomass in 1999, and a marginally significant effect in 2000 (Table 1B). The residual effect of species richness was marginally significant in 2000.

Soil nitrate was significantly affected by legume presence, species richness and functional group richness (Table 1). The mixture term (variations in plant identities within diversity levels) was also highly significant and explained 21% of the variation. In general, topsoil nitrate pools decreased log-linearly with increasing species richness. However, if analyzed for communities with legumes only, without legumes or with mixed legumes and non-legumes separately, no effect of species richness was detectable (Fig. 2a). Communities with legumes only had even higher nitrate values than bare ground plots. Reversing the diversity terms in the statistical model showed that number of functional groups had a highly significant negative linear effect on nitrate pools (Table 1B, Fig. 2b). For soil ammonium, the only significant term was presence of legumes, which had a positive effect on the ammonium pool size (Table 1, Fig. 2c–d). Biomass and soil inorganic N pools were not correlated when all plots were analysed together. However, a more detailed analysis showed a highly significant negative correlation between biomass and soil nitrate in plots without legumes. In contrast, pure legume plots showed a significant positive correlation between biomass and soil nitrate. No correlation was found in communities containing both legume and non-legume species (Fig. 3).

Since the presence of legumes had such a pronounced effect, we analysed whether the proportion of legumes in the biomass within a mixture influenced soil nitrate and ammonium pools. Only pure legume plots had higher nitrate values than the other plots with legumes; there was no significant correlation between legume proportion either 1999 or 2000 and nitrate or ammonium pools when pure legume plots were omitted (p =0.801 and p =0.088 respectively; biomass from 2000).

**Species identity effects**

Since the species composition of the communities determined ecosystem processes to a large extent (high shares of % ss or highly significant mixture term in our ANOVA model, Table 1), we looked more closely upon the effect of individual species on biomass and on pools of inorganic N in the soil.

The biomass of monocultures differed strongly among species and years (Fig. 4). Some species that performed poorly in 1999 increased their biomass in 2000 dramatically (e.g. the legume *Lotus corniculatus* by a factor of 50). On the other hand, some species, e.g. *Phleum pratense*, declined from 1999 to 2000. The grass *Phalaris arundinacea* yielded highest biomass in both years. With the exception of *Lotus corniculatus* in 1999, legumes always had a high monoculture biomass.

Differences among years were also evident in the analysis of the effects of presence of particular species in a mixture (Table 2). Only *Phalaris arundinacea* had a significant positive effect on community biomass in both years. This tall clonal grass has been increasingly
dominant ever since the start of the experiment in all the plots where it was present. Interestingly, presence of legume species within a mixture did only sometimes have positive effects on community biomass (Trifolium pratense 1999 and T. repens 2000). Species with significant positive effects on community biomass did not always perform well in monoculture. For example, the grass Festuca ovina had a positive effect in mixture in 1999, although its monoculture biomass was among the lowest of all species.

Both ammonium and nitrate pools under the monocultures differed clearly among the twelve species (Fig. 5). Soil under monocultures of legumes had large nitrate pools and variable but never very small ammonium pools. Leucanthemum vulgare and Phalaris arundinacea had small ammonium pools and nitrate was almost absent. Soil under other herbs and grasses had small nitrate pools while ammonium pool sizes were more variable.

Trifolium repens and T. hybridum had significant positive species effects on soil nitrate pools, whereas P. arundinacea had a marginally significant negative effect (Table 2). Because of opposing patterns in non-legume and legume plots, there were no significant species effects on ammonium pools. For example, non-legume plots with P. arundinacea always were low in ammonium, while the opposite was true for plots with P. arundinacea containing legumes.

**Overyielding and under-depletion of nitrogen**

Biomass in the mixtures was often higher than biomass in the best performing corresponding monoculture. $D_{\text{mix}}$ was significantly greater than zero in 1999 ($p=0.045$), and the difference was marginally significant in 2000 ($p=0.075$). There was a significant legume effect both years (1999: $p=0.026$, 2000: $p=0.008$) and a block effect in 1999 ($p=0.001$). In 1999, most mixed communities with legumes were transgressively overyielding, similar to most mixed non-legume communities in 2000 (Fig. 6).

When species with the smallest soil inorganic nitrogen pools were used to predict ammonium and nitrate
pools (D_{\text{min}}\text{NH}_4/\text{NO}_3), the predictions almost always underestimated both pools in plots with legumes, i.e. the deviation index values were higher than zero. Increasing species richness lead to an increase in the D_{\text{min}}\text{NH}_4/\text{NO}_3 values (p = 0.011 for D_{\text{min}}\text{NO}_3 and p = 0.029 for D_{\text{min}}\text{NH}_4). However, there was also a significant effect of legume presence (p ≤ 0.001 for D_{\text{min}}\text{NO}_3 and p = 0.005 for D_{\text{min}}\text{NH}_4) because non-legume plots had deviations close to zero.

The biomass-weighted index D_{\text{weight}}\text{NO}_3 was not significantly different from zero across all communities, but the expected values had large deviations from the measured nitrate pools with some mixtures showing under-depletion (values < 0), whereas others did not (values > 0; Fig. 7). There was a significant mixture effect (p = 0.004) in D_{\text{weight}}\text{NO}_3 indicating that species identity was important. D_{\text{weight}}\text{NH}_4 was significantly higher than zero (p = 0.027) and there was also a significant legume effect (p = 0.020). Thus, ammonium pools in communities with legumes were larger than expected based on species composition. Communities without legumes, on the other hand, showed under-depletion in both nitrate and ammonium pools in the biomass-weighted measure (p = 0.036, n = 9, for D_{\text{weight}}\text{NO}_3, and p = 0.030, n = 9, for D_{\text{weight}}\text{NH}_4, one sample t-test of difference from zero SYSTAT 10.2).

**Discussion**

In our experiment, five growing seasons after establishment, higher plant species richness and functional diversity resulted in higher biomass and lower soil nitrate pools (Fig. 1, 2). The positive diversity-productivity relation in numerous other experimental studies (reviewed by Kinzig et al. 2002, Loreau et al. 2002b) supports the view that diversity effects are not transient, but persistent (Tilman et al. 2001). Several studies also found increases of soil nitrate pools with the loss of plant species diversity or functional group diversity (Tilman et al 1996, 2001).

**Table 2. Direction (“sign”) and significance of effects of particular species on community biomass production in 1999 and 2000, and on soil nitrate and ammonium pools. Marginally significant P-values (0.1 > P > 0.05) are in italics, significant P-values (P < 0.05) are in bold.**

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<thead>
<tr>
<th>Taxon</th>
<th>Biomass\text{1999}</th>
<th>Biomass\text{2000}</th>
<th>NO3</th>
<th>NH4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>sign</td>
<td>P</td>
<td>sign</td>
<td>P</td>
</tr>
<tr>
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<td>0.043</td>
<td>NS</td>
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</tr>
<tr>
<td>Festuca ovina</td>
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<td>0.022</td>
<td>+</td>
<td>0.046</td>
</tr>
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<td>+</td>
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<tr>
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<td>NS</td>
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<td>Lotus corniculatus</td>
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<td>NS</td>
<td>NS</td>
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<td>Trifolium repens</td>
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<td>+</td>
<td>&lt; 0.001</td>
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</table>

In this study, biomass correlated positively with nitrate content in soil for communities with legumes; this effect was opposite for plant communities without legumes and did not occur in mixed legume/non-legume communities (Fig. 3). Below, we discuss the underlying mechanisms separately for these three types of communities, although sample sizes are different and diversity gradients are narrow for pure legume (only 1 and 2 species) and pure non-legume communities (1 to 4 species).

Pure legume communities and species effects of legumes

Under pure legumes (monocultures and one 2-species mixture), a doubling of biomass approximately corresponded to a doubling in soil nitrate pools. This correlation between legume biomass and soil N pool size supports Grime’s “mass ratio hypothesis” (Grime 1998), which suggests that the effects of species on a certain ecosystem process should largely be predictable from their contribution to the total biomass. It is important to note that this result contradicts our initial hypotheses predicting lower N-pools with increasing biomass, obviously because legumes depend less on soil resources for their N supply.

Even though legumes had pronounced positive effects on community biomass, only two legume species had significant effects in the species-specific analysis. *Trifolium pratense* had a significantly positive effect on community biomass the fourth year of the experiment (1999) and *T. repens* the fifth year (2000). The species specific analysis of the second year biomass across all BIODEPTh-sites in Europe, showed that presence of *T. pratense* had a very strong positive effect on biomass
Together with *T. hybridum*, *T. repens* also had positive effects on soil nitrate pools. These two were always grown together in polycultures, so it is not possible to tease their effects apart. *Trifolium repens*, the species with highest nitrate pools in monoculture, is a very effective N$_2$ fixer in temperate grasslands (Carlsson and Huss-Danell 2003) and its litter decomposes very fast resulting in high net mineralization of N (Marstorp and Kirchmann 1991). Since perennial forage legumes depend heavily on fixation of atmospheric N$_2$ (Carlsson and Huss-Danell 2003), they invest little in N acquisition from the soil (Craine et al. 2002). *Trifolium pratense* took up soil ammonium slower than the grasses *Phleum pratense* and *Festuca ovina*, and the herb *Achillea millefolium* (Jumpponen et al. 2002). The combination of high N input and low root uptake likely explain the high levels of soil inorganic nitrogen in legume-dominated communities. Substantial nitrate leaching from pure legume communities or low diversity grass-clover mixtures have been shown in several agricultural studies (Low 1973, Alvarez et al. 1998, Loiseau et al. 2001) and also within the German BIODEPTH-experiment (Scherer-Lorenzen et al. 2003).

**Plant communities without legumes, and species effects of non-legumes**

In plant communities without symbiotic N$_2$-fixation, community biomass was negatively correlated with soil nitrate (Fig. 3), confirming our first hypothesis: the greater the biomass, the more soil resources are utilized to maintain it. However, mean biomass was not significantly higher in more diverse non-legume communities (Fig. 1). One likely reason for this is the sampling effect: our 4-species mixture included only species with low monoculture yield. Thus, a simple visual inspection of Fig. 1 comparing monoculture yield with yield in mixtures may be misleading. In contrast, the direct comparison of mixture vs monoculture yield using the proportional deviation index revealed transgressive overyielding for the year 2000 (Fig. 6), which unambiguously shows that mixtures perform better than the corresponding monocultures (Loreau 1998b). Our test of under-depletion of soil N-pools under mixed plant species using the biomass-weighted index (Fig. 7), also show that the pools of soil nutrients were usually lower than the levels predicted by the species composition, supporting our second hypothesis. We thus conclude that an increase in biomass with increasing species richness is at least partly due to belowground complementary resource use. This result is in line with the work of van Ruijven and Berendse (2003), which showed positive diversity effects on productivity in the absence of legumes. Our non-legume communities were probably N-limited as a result of removal of aboveground biomass for five consecutive years without fertilisation. This was also reflected in low tissue N concentrations (range 3.9–20 mg N g$^{-1}$ DM). Low N-concentrations in litter could lead to prominent microbial immobilisation thus keeping inorganic N low in most non-legume plots.

The clonal grass *P. arundinacea*, which had the highest biomass and the lowest soil nitrate levels in monoculture (Fig. 4, 5), had positive effects on biomass and nitrate retention in mixtures (Table 2). This grass has become increasingly abundant during the course of the experiment and it has a high capacity to deplete the soil of both nitrate and ammonium (Fig. 5). The capacity for high biomass production and low demand for NPK-fertilisation, caused by low nutrient concentrations in harvested biomass (Hadders and Olsson 1997), has made this species suitable for removing nitrogen from sewage water (Quin 1979). Many non-legumes had positive species-specific effects on biomass. Similarly, species with small monoculture biomass had significant positive effects in the analysis (e.g.
F. ovina 1999 and P. pratense 2000). Interestingly, no species affected community biomass negatively. This could indicate that none of these species had adverse effects (for example allelopathic effects) on ecosystem productivity under these experimental conditions.

**Interactions between legumes and non-legumes**

Legumes had a clear fertilising effect in our plant communities. This was manifested by increased biomass and increased nitrate pools in mixed legume/non-legume communities, compared to communities with grasses and non-legume herbs only. Biomass in many mixed legume/non-legume communities was higher than the highest yielding monoculture in the mixture (Fig. 6). This indicates complementarity between species even though many mixed communities did not transgressively overyield. Overyielding was probably mainly due to facilitation through enhanced N availability, since grasses and herbs in these communities have been shown to use N fixed by legumes (Mulder et al. 2002).

Community biomass and soil nitrate did not correlate in mixed legume/non-legume communities (Fig. 3), although community biomass increased with increasing species richness. This is in contrast to our first hypothesis. Presumably, the N needed for this increasing biomass production was supplied through N$_2$ fixation by legumes, so that soil pools did not get depleted. In addition, biomass in mixed legume/non-legume communities depended on winter survival of legumes. In several years, like 1997 and 2000, the legumes survived well in some plots and poorly in others. This generated a large variation between and within plots, also reflected in high variation in $D_{\text{weight}}$NO$_3$ and $D_{\text{weight}}$NH$_4$. Increased N uptake through complementarity between non-legumes might have been obscured by enhanced and variable N mineralization rates, originating from legume litter. Another explanation to the lack of correlation between biomass and soil nitrate could be the strong microbial control of soil N availability in grasslands (Knops et al. 2002). In legume/non-legume communities, the higher availability of N, compared to non-legume communities could cause other nutrients like carbon or phosphorous to become limiting for the microbes in some of the communities, and inorganic N levels thus might be less predictable. Microbial processes should be relatively more important in the fall, when the soil was sampled, since most plants have low rates of nutrient uptake then. Species composition of the mixed legume/non-legume communities explained some of the variation in nitrate content. High diversity plots (four species and more) with P. arundinacea had significantly lower nitrate contents than plots without this species (two sample t-test with separate variances, p = 0.003).

Ammonium contents were higher than expected in the most diverse plots (Fig. 7b) using the biomass proportion of the different species. Since ammonium is adsorbed to soil particles and thus is less mobile than nitrate, the risk of ecosystem N loss is not as high as when nitrate pools would be enhanced. Thus larger ammonium pools could be seen as an enhancement of overall fertility created by functional diversity among plant species.

In summary, for mixtures of legumes and non-legumes, positive interactions resulted in increased biomass and reduced soil nitrate levels, compared to legume monocultures. Plant species without the capacity to symbiotically fix nitrogen are thus important because they are able to use the surplus of N from legume residues, thereby reducing the risk of nitrate leaching (Scherer-Lorenzen 1999). The positive role of grasses and herbs in N retention has also been recognised in agricultural catch crop and intercropping studies (Alvenaes and Marstorp 1993, Wallgren and Linden 1994, Clotuche et al. 1998, Loiseau et al. 2001, Zagal et al. 2001).

**Conclusions**

Our results support the view that biodiversity can influence ecosystem functioning, especially processes that are under direct plant control, e.g. primary production or nutrient retention. It is also evident that there is no magic effect of “numbers of species per se”, but that any diversity effects are due to differences in functional traits between species and species interactions (Diaz and Cabido 2001, Hector 2002). Confirming other studies, legumes played a key-role in our system. However, our novel comparisons of inorganic N pools in mixed communities to pools in the corresponding monocultures showed that, also in non-legume communities, complementary N utilisation was likely. Also, most often, more biomass was present in mixed communities than in the best corresponding monoculture. Thus, our initial hypotheses were supported for non-legume communities. Under-depletion of N was not seen in plots with both legumes and non-legumes, but positive interactions between legumes and non-legumes caused transgressively overyielding in many communities. In addition, non-legumes significantly reduced soil nitrate pools in legume/non-legume mixtures, compared to communities composed entirely of legumes, underlining their importance for nitrate retention in the system.

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