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Soil fertility increases with plant species diversity in a long-term biodiversity experiment

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Abstract Most explanations for the positive effect of plant species diversity on productivity have focused on the efficiency of resource use, implicitly assuming that resource supply is constant. To test this assumption, we grew seedlings of *Echinacea purpurea* in soil collected beneath 10-year-old, experimental plant communities containing one, two, four, eight, or 16 native grassland species. The results of this greenhouse bioassay challenge the assumption of constant resource supply; we found that bioassay seedlings grown in soil collected from experimental communities containing 16 plant species produced 70% more biomass than seedlings grown in soil collected beneath monocultures. This increase was likely attributable to greater soil N availability, which had increased in higher diversity communities over the 10-year-duration of the experiment. In a distinction akin to the selection/complementarity partition commonly made in studies of diversity and productivity, we further determined whether the additive effects of functional groups or the interactive effects of functional groups explained the increase in fertility with diversity. The increase in bioassay seedling biomass with diversity was largely explained by a concomitant increase in N-fixer, C4 grass, forb, and C3 grass biomass with diversity, suggesting that the additive effects of these four functional groups at higher diversity contributed to enhance N availability and retention. Nevertheless, diversity still explained a significant amount of the residual variation in bioassay seedling biomass after functional group biomass was included in a multiple regression, suggesting that interactions also increased fertility in diverse communities. Our results suggest a mechanism, the fertility effect, by which increased plant species diversity may increase community productivity over time by increasing the supply of nutrients via both greater inputs and greater retention.

Keywords Cedar Creek · Sampling effect · Legume

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Introduction

Many researchers have found a positive effect of plant species number (hereafter "diversity") on productivity (reviewed in Hooper et al. 2005; Kinzig et al. 2002; Loreau et al. 2002; Spehn et al. 2005), and most explanations for this effect have focused on either the greater presence of productive species or greater complementarity in the modes

and timing of resource consumption in diverse communities (Huston 1997; Loreau and Hector 2001; Tilman et al. 1997b, 2001). However, an additional mechanistic distinction can be made between the effect of diversity on resource consumption and on resource supply. A positive effect of diversity on resource supply, (i.e., a "fertility effect"), might be expected to amplify the observed positive effect of diversity on plant productivity through time, and, like effects of diversity on resource consumption, may be driven by the greater likelihood of including particular traits at higher diversity, the greater likelihood of interactions between particular traits at higher diversity, or a combination of these. However, there is limited evidence for increased soil fertility beneath species-rich plant communities (Balvanera et al. 2006; Zak et al. 2003) because most biodiversity experiments have not been conducted long enough for such an effect to manifest.

Composition and diversity may affect fertility through differential species effects on nutrient inputs. Plants that form associations with N-fixing bacteria (hereafter "N-fixers") may increase soil N availability. Like every functional group, N-fixers are more likely to be present in diverse communities (Huston 1997; Spehn et al. 2002; Tilman et al. 2001). Diverse plots may also promote microbial communities that mineralize a larger fraction of recalcitrant organic N (Zak et al. 2003), effectively increasing inputs of this growth-limiting nutrient, or they may support or attract greater consumer biomass and thus receive higher levels of labile inputs through frass or feces.

Composition and diversity may also affect fertility through differential species effects on nutrient retention. Importantly, the high root biomass of some grasses and the overall greater average root biomass of diverse plots may promote the retention of N by preventing leaching (Scherer-Lorenzen et al. 2003; Tilman et al. 1996). Additionally, interspecific differences may interact to reduce nutrient losses, such that diverse communities retain more nutrients. For example, species differ in their phenology, depth, and form (e.g., NO_3^- vs. NH_4^+) of nutrient uptake (McKane et al. 1990, 2002) such that diverse plots are expected to more completely capture nutrients through time and space and across different nutrient forms. Other interspecific differences may affect soil fertility: for instance, differences in stoichiometry (Reich and Oleksyn 2004) may affect rates of nutrient recycling (Wedin and Tilman 1990), and the effects of specialized enemies and mutualists (Bartelt-Ryser et al. 2005; Klironomos 2003; Knops et al. 1999; Mitchell et al. 2002) may indirectly affect nutrient inputs or retention by affecting species composition.

In a long-term biodiversity experiment established on low-N, sandy soil, Zak et al. (2003) demonstrated that some of the microbial processes that affect resource supply were positively affected by plant species diversity. For example, soil beneath high-diversity plant communities had greater microbial biomass and respiration than low-diversity communities, largely due to increased plant biomass (and hence litter) in the high-diversity communities (Zak et al. 2003). However, increased plant biomass could not completely explain an increase in N mineralization associated with higher diversity communities (Zak et al. 2003), indicating that plant diversity increases mineralization rates, and hence soil fertility, by mechanisms beyond those associated with increased biomass. Moreover, they and others have demonstrated that higher diversity communities in the same experiment have more N in plant pools than lower diversity communities, consistent with the idea that soil fertility increases with plant diversity (Fargione et al. 2007; Zak et al. 2003).

Here, using data from the same long-term field experiment, we: (1) test for a "fertility effect"—the positive effect of plant diversity on soil fertility (Zak et al. 2003)—using additional measures of fertility; and (2) determine whether the additive effects of species traits ("additive fertility effects"), the interaction of species traits ("interactive fertility effects"), or both are responsible. Because plant growth is an integrated measure of soil nutrient availability, we used a seedling bioassay to assess how plant diversity influences the fertility of soil beneath species-poor and species-rich plant communities. In addition, we include total soil N and N mineralization as additional measures of soil fertility in our analyses.

The mechanisms affecting productivity across diversity gradients are now routinely partitioned into selection and complementarity effects (e.g., Lanta and Leps 2007; Polley et al. 2007; Spehn et al. 2005). Unfortunately, it is not possible to partition the effects on soil fertility in the same way because, unlike a species' biomass in mixture, a species' contribution to soil fertility in mixture cannot be directly measured. Nevertheless, we present an analysis in the spirit of the selection/complementarity distinction by ascribing fertility effects to the additive effect of species traits (analogous to selection) and the interactive effect of species traits (analogous to complementarity).

We based our test on the assumptions that: (1) additive and interactive effects completely explain the overall effect of diversity on soil fertility, and (2) the additive effect of a functional group on soil fertility is linearly related to its biomass. It follows from these two assumptions that interactive effects on soil fertility would be revealed by significant effects of diversity after statistically controlling for functional group biomass. For example, both N-fixer biomass and C4 grass biomass increased with diversity in our experiment due to overyielding and their greater likelihood of being included in higher diversity plots (see "Results" and Fargione et al. 2007). If N-fixer biomass and N retention from C4 grasses were the only factors that affected soil

fertility, then the greater fertility of diverse plots would be completely explained by their greater N-fixer biomass and C4 grass biomass; these would represent additive fertility effects akin to selection. Alternatively, if there were an interaction between these or other functional groups on fertility akin to complementarity, then diversity should explain a significant amount of the residual variation in fertility after statistically controlling for functional group biomass because the opportunities for interactions would increase with diversity (Loreau and Hector 2001).

One caveat to our reasoning is that, in addition to being the cause of fertility, functional group biomass is also expected to respond to fertility (Gross and Cardinale 2007). Thus, it is possible that increases in fertility that were originally caused by the additive effects of one functional group may have contributed to increased biomass of a second functional group, which our test would ascribe to the additive effects of the second functional group. Similarly, it is possible that increases in fertility caused by interactions may have contributed to increased functional group biomass, which our test would ascribe to additive effects. As a result, our test may be liberal for detecting additive effects and conservative for detecting interactive effects, a characteristic shared with other tests of biodiversity effects (Loreau 1998).

Materials and methods

Biodiversity field experiment

Our biodiversity experiment is located at Cedar Creek Natural History Area (CCNHA) in east central Minnesota. It is situated on a glacial outwash plain with soil that is low in N and mainly composed of fine and medium-textured sand (Grigal et al. 1974; Tilman 1984). Experimental additions of N, P, K, Ca, and Mg have shown that N is the only limiting nutrient (Tilman 1984) during grassland succession. In the summer of 1993, a former pasture was treated with herbicide, burned, and bulldozed to remove the top 6–8 cm of A horizon soil to reduce the seed bank. Soil was then plowed and harrowed. In the spring of 1994, one hundred and sixty-eight 81-m^2 plots were seeded to contain one, two, four, eight, or 16 randomly selected perennial grassland species from a pool of four C4 grasses, four C3 grasses, four N-fixers, four forbs, and two woody species. Plots were weeded 2–4 times a summer to maintain the diversity gradient and burned every spring, before new growth occurred, to prevent aboveground litter accumulation. Further details on the experiment can be found in Tilman et al. (1997a, 2001) and at the CCNHA website (http://www.cedarcreek.umn.edu/research/exper/e120).

We analyzed total N in soil collected from each plot in 1994 (after field preparations were completed but before the experiment was seeded), 2004, and 2006. Each time, seven cores (2 cm diameter, 20 cm deep) were collected in each plot. The samples from each plot were pooled, dried, homogenized, and analyzed with a Costech 4010 total C&N analyzer (Costech Analytical Technologies, Valencia, Calif.). "Initial total soil N" is the average of two separate analyses on the 1994 soil. "Final total soil N" is the average of the 2004 and 2006 analyses. Where we use a measure of total soil N as a response variable, we use "change in total soil N" (final minus initial total soil N) to control for differences in initial soil N.

We used gross N mineralization data measured in root-free lab incubations from 113 of our experimental plots as reported by Zak et al. (2003). Net mineralization is the difference between gross mineralization and microbial immobilization. Microbial immobilization was not affected by the diversity gradient (Zak et al. 2003), and hence the increase in gross N mineralization across the diversity gradient indicates an increase in net N mineralization (Zak et al. 2003). To reflect this, we subsequently refer to gross N mineralization simply as "N mineralization". In our experiment, N mineralization and total N were significantly and positively correlated (Pearson $r = 0.438$, $P < 0.0001$).

Aboveground biomass was collected in 2003 in a 0.1-m × 6-m area in each plot; harvested tissue was sorted by species, dried, and weighed. The aboveground biomasses of species within each functional group were totaled within each plot for our analyses (e.g., the biomasses of all C4 grass species in a plot were summed to provide "C4 grass biomass" for that plot). Root biomass was measured in monocultures in 2003 by collecting, cleaning, and drying 24 root cores (5 cm diameter, 30 cm deep) per plot. We present the average monoculture root biomass by functional group.

Greenhouse seedling bioassay

In June 2004, we collected eight soil cores (2 cm diameter, 10 cm deep) from each plot (168 plots total) after removing residual litter from the soil surface. The soil from each plot was pooled, homogenized, sieved in a field-moist state, and placed in a pot (3.5 cm diameter, 20.5 cm deep with free drainage). The potted soil remained in a greenhouse unwatered until January 2005, when pre-germinated seeds of the native prairie perennial *Echinacea purpurea* were planted into each pot. We chose *E. purpurea* because no members of its genus are present at CCNHA, reducing the chance that host-specific, soil-borne biota might influence the results.

The seedlings were grown under full natural sunlight in a greenhouse, watered daily, and randomly repositioned weekly. After 9 weeks, seedlings were harvested, roots were washed, and aboveground and belowground biomass

was dried and weighed. Ten of the 168 seedlings failed to establish initially; these were replanted and harvested with a 3-week delay. There was no relationship ($P > 0.44$) between seedling failure and other variables, including diversity, initial or final total soil N, or functional group composition/presence/absence of the plots from which we obtained soil for this greenhouse experiment.

Analysis

Following Fargione et al. (2007) and others (Fornara and Tilman 2008; Tilman et al. 2006), we omitted low-diversity plots that contained woody species because, in contrast to our other functional groups, their aboveground biomass did not provide an estimate of annual productivity. Four monocultures and seven bicultures were removed from analyses, leaving 157 plots. Higher diversity plots had disproportionately low woody species densities (visual inspection of entire plots seldom revealed any individuals) and so remained in the analyses.

For analyses that separated plots by whether or not N-fixers were present in them, all 35 plots planted to 16 species were omitted because they all contained N-fixers by design. The fraction of plots containing N-fixers at other diversity levels were: one species 10/35, two species 14/28, four species 22/29, eight species 27/30. The predictor variable "N-fixer presence" is a nominal variable with two categories: "N-fixers present" and "N-fixers absent".

We used JMP (Version 6.0.3. SAS Institute, Cary, N.C., 1989–2006) to perform sequential, type I multiple regressions. In analyses where it was appropriate, initial total soil N was entered as the first predictor variable to statistically control for the effects of between-plot heterogeneity that existed before the long-term biodiversity experiment was established. For analyses that included the biomass of all four functional groups as predictor variables to test for additive fertility effects, we entered them in the order: N-fixer, C4 grass, forb, and C3 grass. N-fixers were entered first because they have the most obvious and direct influence on N supply. The order of non-N-fixing species was in descending order of average monoculture root biomass (see "Results") because of the demonstrated effect of root biomass on nutrient retention (Scherer-Lorenzen et al. 2003).

For all analyses, we comment on the statistical significance of the results when one-species plots were omitted. In all analyses, bioassay seedling biomass was square-root transformed to meet statistical assumptions and diversity was $\log_2$ transformed to improve fits. Prior to analysis, we standardized each predictor and response variable by subtracting its mean from all observed values and then dividing by its SD. This procedure provided parameter estimates in a common unit of 1 SD for each variable so that slopes could be meaningfully compared across predictors. All other statistics (e.g., *P*-values, R^2-values) were unaffected by standardization.

Results

Our three measures of soil fertility were bioassay seedling biomass, change in total soil N over 11 years, and N mineralization. All three increased significantly with the plant species number (hereafter "diversity") of the soil in which they were measured (three separate regressions: bioassay seedling biomass, $F_{1, 155} = 26.67$, $P < 0.0001$; change in total soil N, $F_{1, 155} = 11.25$, $P = 0.0010$; N mineralization, $F_{1, 104} = 33.57$, $P < 0.0001$; Fig. 1). When one-species plots were omitted from the regressions, all three regressions remained statistically significant with positive slope (not shown).

Plots in which N-fixers were present tended to produce a greater response in bioassay seedling biomass, total soil N, and N mineralization when compared with plots in which N-fixers were absent (see dashed lines Fig. 1 and statistics Table 1; analysis excluded all 35 plots that were planted to 16 species, which by design always contained N-fixers). Nevertheless, even after controlling for the presence of N-fixers, diversity remained a significant predictor of seedling biomass and N mineralization and a marginally non-significant predictor of final total soil N (again, excluding 16-species plots; Table 1).

The monoculture root biomasses of our four functional groups decreased in the order: C4 grasses, N-fixers, forbs, and C3 grasses (Table 2). The biomasses of N-fixers and C4 grasses were positively and significantly correlated with diversity (Table 2). This occurred, in part, because the likelihood that a given plot would contain members of a given functional group increased with diversity and, in part, because species tended to overyield at higher diversity (Tilman et al. 2001). The effects of N-fixer biomass and C4 grass biomass on bioassay seedling biomass, change in total soil N, and N mineralization were highly significant and had higher standardized estimates than either of the other two functional groups or diversity (Table 3). The effects of forb biomass and C3 grass biomass were less significant (Table 3), which remained true even when forb biomass and C3 grass biomass were placed before N-fixer biomass and C4 grass biomass in otherwise identical type I hierarchical multiple regressions ($0.0009 < P < 0.27$ for both forb biomass and C3 biomass entered earlier as compared with $0.0001 < P < 0.0031$ for both N-fixer biomass and C4 biomass entered later, not shown). Even after accounting for the biomass of all four functional groups, diversity was still a significant predictor of both bioassay seedling biomass and N mineralization, but not of final total soil N (Table 3). The standardized estimates of diversity on bioassay seedling biomass and N mineralization were greater than the standardized estimates of forbs or C3 grasses.

As expected, bioassay seedling biomass increased significantly with final total soil N and N mineralization in the field plots from which we gathered soil (Table 4). Nevertheless,

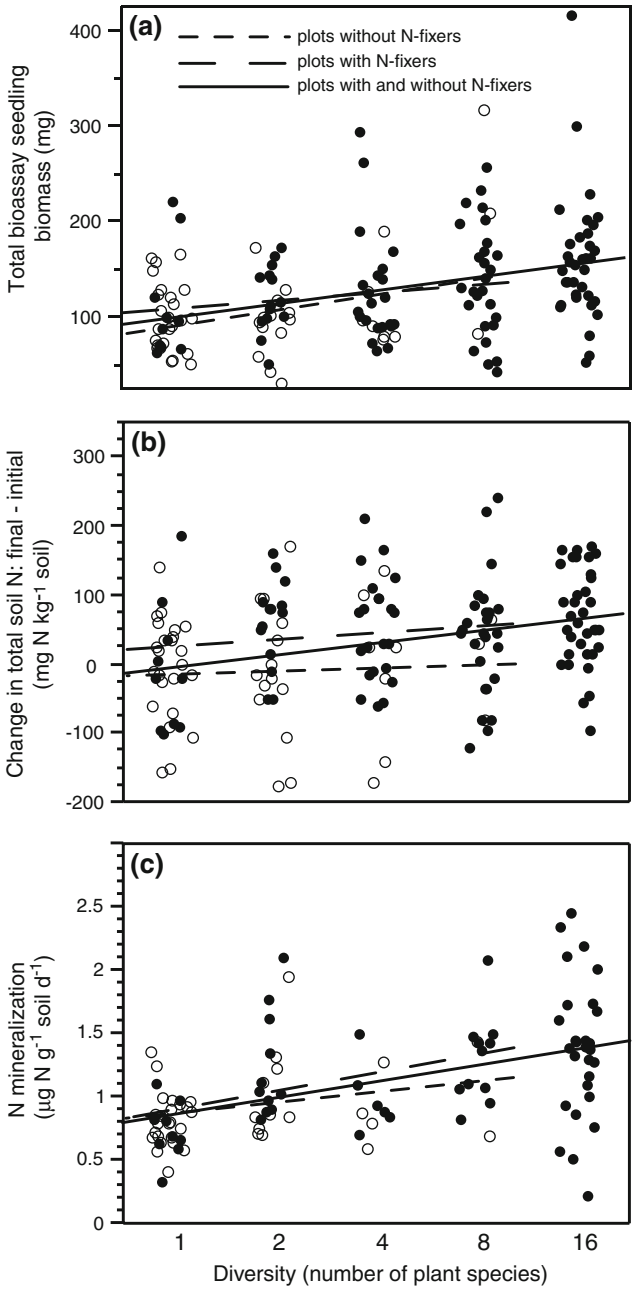

Fig. 1a–c Relationship between diversity and three measures of soil fertility. **a** Bioassay results from growing *Echinacea purpurea* seedlings in a greenhouse on soil gathered from 168 plots in a 10-year-old biodiversity experiment. **b** Change in total soil N over time, from measurements before the field experiment began (1994; initial soil N) and approximately 11 years later (final soil N). **c** N mineralization, measured from additional soil samples gathered from 113 plots (Zak et al. 2003). Data points are shifted along the *x*-axis for clarity. One outlier data point (1, −425; non-N-fixer) is not shown in **b**, but was included to generate the fit lines. *Open circles* represent plots without N-fixers; *closed circles* represent plots with N-fixers. 16-species plots were omitted from the fit of plots with N-fixers because there were no 16-species plots without N-fixers with which to contrast them

these measures, which also increased with diversity (Fig. 1b, c), did not fully capture the bioassay seedling response to diversity because diversity remained a significant predictor of bioassay seedling biomass even after controlling for final total soil N and N mineralization (Table 4).

Discussion

Although the development of soil fertility is a slow process (Poulton 1995), soil from high-diversity experimental plant communities was significantly more fertile than soil from low-diversity experimental plant communities after only 10 years. The increase was biologically significant: on average, bioassay seedling biomass increased by 70% across the diversity gradient (Fig. 1a). The increase was significantly dependent on final total soil N and N mineralization (Table 4), which were themselves dependent on diversity (Fig. 1b, c).

In a separate analysis, the observed increases in biomass of all four functional groups (N-fixers, C4 grasses, forbs, and C3 grasses) that occur at higher diversity (Table 2) also accounted for much of the variation in bioassay seedling biomass (Table 3), consistent with the hypothesis that the additive effects of these functional groups and their higher biomass in diverse plots was an important mechanism behind the increase in soil fertility with diversity. Nevertheless, diversity also remained a significant predictor of bioassay seedling biomass, even after statistically controlling for functional group biomass (Table 3). This result is consistent with the hypothesis that greater interactions between functional groups at higher diversity also contributed to the increase in soil fertility with diversity.

The effect of diversity on fertility via N

N supply may have been the only factor that affected our bioassay seedling growth response to diversity. However, if N supply were solely responsible, then neither final total soil N nor N mineralization fully reflected plant-available N because diversity remained a significant predictor of bioassay seedling biomass even after controlling for either of these measures (Table 4). This would not be surprising (Schimel and Bennett 2004) and was the initial motivation for our greenhouse study: plant available N is not fully reflected by total soil N because total soil N measures all forms of N in the soil, including microbial N and organic N. Plants have access to inorganic N in soil solution and perhaps some simple organic forms (Schimel and Bennett 2004), but these are a small fraction of the total soil N pool. Plant-available N is more fully reflected by measures of N mineralization, which quantify the microbial release of inorganic N. However, most methods of measuring N mineralization omit interactions between living roots and soil microbes that may facilitate higher rates of N mineralization.

Previous research on our long-term biodiversity experiment, as well as similar work at other sites, has shown that

Table 1 The effect of N-fixer presence on our three measures of fertility. Significant *P*-values shown in *bold*

Response	Variable	Standardized estimate	*F*	*P*	R^2
Bioassay seedling biomass ($mg^{1/2}$)	Initial total N[a]	0.254	9.40	**0.0027**	0.066
	N-fixer presence	0.230	8.27	**0.0048**	0.125
	Diversity[b]	0.276	5.73	**0.0183**	0.165
Final total soil N (mg N kg^{-1} soil)	Initial total N	0.471	48.99	**<0.0001**	0.272
	N-fixer presence	0.209	10.35	**0.0013**	0.329
	Diversity	0.160	2.92	0.09	0.345
N mineralization (μg N g^{-1} soil day^{-1})	Initial total N	0.032	0.12	0.73	0.001
	N-fixer presence	0.228	7.68	**0.0071**	0.085
	Diversity	0.388	10.54	**0.0018**	0.201

The three separate multiple regressions excluded all 35 of the 16 species plots, which by design all included N-fixing species. Variables were entered in the sequence shown (type I SS) with statistics for a given variable reflecting the inclusion of all prior variables in the regression. For each variable, numerator *df* = 1. Denominator *df* were 118, 118, and 73 for the three multiple regressions, respectively. The predictor variable *N-fixer presence* is a nominal variable with only two categories: "N-fixers present" and "N-fixers absent". When analyses were performed with one-species plots omitted, denominator *df* = 83 for the first two regressions and 40 for the last regression. Diversity was no longer a significant predictor in any of the regressions (although it was a marginally non-significant predictor of bioassay seedling biomass, $P = 0.0596$, not shown). N-fixer presence also became a marginally non-significant predictor of N mineralization ($P = 0.0723$, not shown)

[a] mg N kg^{-1} soil

[b] $\log_2$(treatment plant species number)

extractable N tends to decrease with diversity and/or root biomass during the growing season (Fargione and Tilman 2005; Scherer-Lorenzen et al. 2003; Spehn et al. 2005; Tilman et al. 1996), which may at first seem at odds with our results showing an increase in bioassay seedling biomass, total soil N, and N mineralization with diversity (Fig. 1). Nevertheless, they are consistent: higher diversity plant communities contain more soil organic matter and hence N (Fig. 1b) that is mineralized to a plant-available form at a faster rate (Fig. 1c), which is subsequently taken up with greater efficiency by higher root biomass (Table 2). Thus, the pool of extractable N is smaller (as cited above) and the total amount of N in plant tissues is greater at higher diversity (Fargione et al. 2007; Zak et al. 2003), when compared to low-diversity communities.

Soil fertility may have increased with diversity due to increased inputs of N. In addition to atmospheric N deposition, to which all plots were exposed equally and independently of diversity (ca. 0.6 g wet inorganic N m^{-2} $year^{-1}$, NADP 2007), additional N entered the soil via N-fixers (Mulder et al. 2002; Spehn et al. 2002), which, like any of the four functional groups, were more likely to be present in diverse plots (Huston 1997; Huston and McBride 2002; Spehn et al. 2002; Tilman et al. 1997a; Tilman et al. 2001). As expected, soil from plots without N-fixers had lower bioassay seedling biomass, lower increases in total soil N over time, and lower N mineralization than plots with N-fixers (Fig. 1, Table 1). These observations suggest that much (but not all) of the increase in these measures across the diversity gradient was due to the greater likelihood that higher diversity plots would contain N-fixers.

In addition to increased inputs, fertility may have increased with diversity due to increased N retention. Because root biomass increased with diversity in this field experiment (Tilman et al. 2001), diverse plots may have lost less N because higher root biomass led to greater N uptake and thus lower leaching losses (Scherer-Lorenzen et al. 2003; Tilman et al. 1996). The significant effect of C4 grasses, C3 grasses, and, to a lesser extent, forbs on bioassay seedling biomass, change in total soil N, and N mineralization (Table 3) may reflect concomitant increases in root biomass (Table 2) and thus N retention. However, diversity had a significant effect on bioassay seedling biomass and N mineralization (Table 3), even after controlling for the biomass of all four functional groups. The significant effect of diversity in these analyses suggests that interactions among species, in addition to the presence of particular functional traits, caused greater fertility. For instance, diverse plots were more likely to contain species that use resources in a complementary manner (Loreau and Hector 2001; van Ruijven and Berendse 2005), contributing to greater root biomass and nutrient retention throughout the growing season and across the soil profile (Scherer-Lorenzen et al. 2003; Tilman et al. 1996).

Additive and interactive effects on fertility

Additive and interactive effects both appeared to operate in the generation of soil fertility in higher diversity plots, suggesting that natural systems will benefit from both the presence of multiple functional groups and their interactions. The positive and highly significant effect of N-fixer biomass and

Table 2 Monoculture root biomass (g m^{-2}) by functional group with 95% confidence intervals and Pearson correlation coefficients[a] (*r*) between functional group biomass (g m^{-2}) and diversity with *P*-values. Significant *P*-values shown in *bold*

Variable	Root biomass	Aboveground biomass correlation with diversity
N-fixer	425 (270, 580)	0.38 (*P* < **0.0001**)
C4 grass	660 (465, 850)	0.56 (*P* < **0.0001**)
Forb	305 (155, 450)	0.14 (*P* = 0.08)
C3 grass	255 (100, 410)	0.03 (*P* = 0.68)

[a] For all correlations, *n* = 157. When correlations were calculated with one-species plots omitted, *n* = 122, and the statistical significance or non-significance of the correlations did not change (not shown). Only the sign of the C3 grass biomass correlation changed (but remained non-significant, not shown)

C4 grass biomass on our measures of fertility (Table 3), combined with their greater biomass in more diverse plots (Table 2), suggests that much of the positive effect of diversity on fertility (Fig. 1) was attributable to selection for these two functional groups. This is not surprising; N-fixers increased soil N and C4 grasses retained N within the system through high root biomass (Table 2) and efficient mineral N consumption (Tilman and Wedin 1991). The additive effects of forbs and C3 grasses were also evident (Table 3), though much less pronounced. Their positive effect may have been due to their effects on N retention; evidence suggests that forbs and C3 grasses differ from C4 grasses in the principal depth and timing of N uptake (McKane et al. 1990). Alternatively, the apparent positive additive effect of forbs and C3 grasses may have been due to a potential bias of our test: in addition to causing increased fertility, the biomass of these functional groups may have been responding to increased fertility caused by N-fixers, C4 grasses, or interactions. Unfortunately, we cannot differentiate among these possibilities.

We note that efficient resource consumption remains an important ecosystem function regardless of resource supply. Therefore, our results do not diminish the importance of additive and interactive effects with respect to resource consumption, which likely involve different relative contri-

Table 3 Test for additive fertility effects of our four functional groups (as revealed by the significant effect of their biomasses) and interactive fertility effects (as revealed by the significant effect of diversity) on our three measures of soil fertility. Significant *P*-values shown in *bold*

Response	Variable[a]	Standardized estimate	*F*	*P*	R^2
Bioassay seedling biomass ($mg^{1/2}$)	Initial total N[b]	0.235	11.82	**0.0008**	0.057
	N-fixer biomass[c]	0.219	10.22	**0.0017**	0.107
	C4 grass biomass[c]	0.318	16.90	**<0.0001**	0.189
	Forb biomass[c]	0.192	7.67	**0.0063**	0.226
	C3 biomass[c]	0.143	4.25	**0.0409**	0.246
	Diversity[d]	0.206	5.62	**0.0191**	0.274
Final total soil N (mg N kg^{-1} soil)	Initial total N	0.606	127.71	**<0.0001**	0.388
	N-fixer biomass	0.254	22.49	**<0.0001**	0.457
	C4 grass biomass	0.233	14.87	**0.0002**	0.502
	Forb biomass	0.070	1.69	0.20	0.507
	C3 biomass	0.180	11.10	**0.0011**	0.541
	Diversity	0.070	1.05	0.31	0.544
N mineralization (μg N g^{-1} soil day^{-1})	Initial total N	0.143	2.26	0.14	0.015
	N-fixer biomass	0.351	15.76	**0.0001**	0.123
	C4 grass biomass	0.386	17.51	**<0.0001**	0.243
	Forb biomass	0.078	0.98	0.32	0.250
	C3 biomass	0.159	3.76	0.0554	0.276
	Diversity	0.274	6.84	**0.0103**	0.322

[a] Variables were entered in the sequence shown (type I SS) with statistics for a given variable reflecting the inclusion of all prior variables in the regression. For each variable, numerator *df* = 1. Denominator *df* were 150, 150, and 99 for the three multiple regressions, respectively. We note that with one exception, the statistical significance or non-significance of the results did not change when we altered the order of the non-N-fixers to any of the five remaining permutations. When C3 biomass was moved to an earlier position, its significance in the bioassay seedling biomass regression became non-significant (0.08 < *P* < 0.19). When analyses were performed in the order shown with one-species plots omitted, denominator *df* = 115 for the first two regressions and 66 for the last regression. Nevertheless, the statistical significance or non-significance of only two predictors across all three regressions changed: C4 grass biomass and diversity became non-significant predictors of N mineralization (not shown)

[b] mg N kg^{-1} soil

[c] g m^{-2}

[d] $\log_2$(treatment plant species number)

Table 4 Three separate multiple regressions of the dependence of bioassay seedling biomass ($mg^{½}$) on measures of N availability

	Variable[a]	Standardized estimate	*F*	*P*	R^2
(1)	Final total soil N[b]	0.438	39.33	**<0.0001**	0.187
	Diversity[c]	0.285	16.46	**<0.0001**	0.266
(2)	N mineralization[d]	0.298	11.88	**0.0008**	0.095
	Diversity	0.277	9.79	**0.0023**	0.174
(3)	Final total soil N	0.494	29.87	**<0.0001**	0.212
	N mineralization	0.109	1.38	0.2428	0.222
	Diversity	0.233	7.71	**0.0065**	0.276

[a] Variables were entered in the sequence shown (type I SS) with R^2-values reflecting the inclusion of all prior variables. For each variable, numerator *df* = 1. Denominator *df* were 154, 103, and 102 for the three separate multiple regressions, respectively. When analyses were performed in the order shown with one-species plots omitted, denominator *df* were 119, 70, and 69 for the three separate multiple regressions, respectively. Nevertheless, the statistical significance or non-significance of the predictors did not change (not shown)

[b] mg N kg^{-1} soil

[c] $\log_2$(treatment plant species number)

[d] μg N g^{-1} soil day^{-1}

butions from different species or functional groups (Hector and Bagchi 2007).

Limitations of the present study: the possible effect of diversity on fertility via microbes

N-fixing bacteria were clearly implicated as important contributors to the observed fertility effect in our experiment. Although it was not our goal to test for it, other microbes may also have influenced soil fertility across the diversity gradient. Plant diversity might have affected mycorrhizal fungi diversity or abundances (Wolf et al. 2003; but see Waldrop et al. 2006) such that the soil we collected from lower diversity plots lacked mutualistic mycorrhizal fungi or contained parasitic mycorrhizal fungi (Klironomos 2003). Lata et al. (2003) demonstrated that after 3 months' growth in the absence of mycorrhizal fungi, the survival of *E. pallida*, a species in the same genus as our bioassay seedling species, was only 58% as compared to ~85% in the presence of different fungal inocula. This suggests that perhaps our bioassay seedling species, *E. purpurea*, also benefits from mycorrhizal fungi. Because only 6% of our seedlings did not initially survive; because survival was unrelated to diversity; and because the seedlings planted in their place survived without exception, it does not appear that essential fungal symbionts were lacking in our greenhouse study. However, we did not assay mycorrhizal infection and thus cannot reject the possibility that differences in mycorrhizal fungi composition, correlated with the diversity gradient, influenced fertility.

Additionally, synergies between N-fixing bacteria and differential mycorrhizal fungal communities across the diversity gradient may have enhanced N-fixation at higher diversity as a result of greater P uptake (Azcon et al. 1991). Soil from lower diversity plots might also have contained a greater abundance of soil pathogens (Knops et al. 1999; Mitchell et al. 2002). However, we specifically selected an assay seedling species whose genus was not present at our study site, reducing the chance that any of its host-specific pathogens would be present or, if present, would be correlated with the diversity gradient. Nevertheless, the indirect effects of diversity on plant growth as mediated by changes in microbial mutualists and pathogens warrant further study (van der Heijden et al. 2008).

Conclusion

Our results extend those of Zak et al. (2003), who demonstrated that rates of N mineralization increased with experimental plant species diversity. Bioassay seedling biomass and change in total soil N, two alternative measures of soil fertility, also increased significantly with diversity (Fig. 1). Together, the additive effects of functional groups at higher diversity, most notably N-fixers and C4 grasses, and greater interactions among functional groups at higher diversity drove this "fertility effect." To the extent that these same mechanisms operate in other systems, the contribution of fertility to the overall positive diversity-productivity relationship will increase over time. Thus, our study underscores the potential benefits of managing for diversity in natural or agricultural habitats and demonstrates that just as some species-poor habitats can lose fertility in a matter of years (Ewel et al. 1991; Templer et al. 2005), some species-rich habitats can gain fertility in a matter of years (Fig. 1; Templer et al. 2005).

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