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Plant diversity effects on soil microorganisms support the singular hypothesis

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Abstract. The global decline in biodiversity has generated concern over the consequences for ecosystem functioning and services. Although ecosystem functions driven by soil microorganisms such as plant productivity, decomposition, and nutrient cycling are of particular importance, interrelationships between plant diversity and soil microorganisms are poorly understood. We analyzed the response of soil microorganisms to variations in plant species richness (1–60) and plant functional group richness (1–4) in an experimental grassland system over a period of six years. Major abiotic and biotic factors were considered for exploring the mechanisms responsible for diversity effects. Further, microbial growth characteristics were assessed following the addition of macronutrients. Effects of plant diversity on soil microorganisms were most pronounced in the most diverse plant communities though differences only became established after a time lag of four years. Differences in microbial growth characteristics indicate successional changes from a disturbed (zymogeneous) to an established (autochthonous) microbial community four years after establishment of the experiment. Supporting the singular hypothesis for plant diversity, the results suggest that plant species are unique, each contributing to the functioning of the belowground system. The results reinforce the need for long-term biodiversity experiments to fully appreciate consequences of current biodiversity loss for ecosystem functioning.

Key words: above- and belowground interrelationships; biodiversity–ecosystem functioning relationship; Jena Experiment; microbial biomass; microbial nutrient limitation; microbial respiration; redundancy hypothesis.

Introduction

One of the most dramatic consequences of contemporary global change is the rapid decline of biodiversity in many ecosystems (Vitousek et al. 1997, Tilman 2000, Loreau et al. 2001). This unprecedented loss of biodiversity has generated concern over the consequences for ecosystem functioning and services (Sala et al. 2000, Loreau et al. 2001, Jenkins 2003, Millenium Ecosystem Assessment 2005), in particular those related to nutrient cycling which form vital agricultural provisioning services. Plants as producers acquire nutrients from inorganic sources that are supplied primarily by decomposers, whereas decomposers, mostly soil microorganisms, acquire carbon from organic resources that are supplied primarily by producers. Therefore, declining biodiversity has profound impacts on this producer–decomposer codependency that governs essential ecosystem processes (Naeem et al. 2000). Plant-derived inputs enter the belowground system via leaf and root litter, rhizodeposits, and exudates (Naeem et al. 2000, Wardle et al. 2004). Since plant species differ in their biochemical composition, changes in plant diversity likely alter the quantity and quality of these resources, thereby controlling the composition and functioning of soil microbial communities (Zak et al. 2003, Nilsson et al. 2008). Moreover, plant diversity impacts microclimatic conditions (Lorentzen et al. 2008) and these are major driving factors of microbial processes (Freiberg 1998), such as carbon and nitrogen cycling, thereby linking plant diversity and ecosystem functioning (Naeem et al. 2000, Zak et al. 2003).

Changes in plant diversity are known to affect aboveground ecosystem functioning (Tilman et al. 2001, Cardinale et al. 2007, Hector and Bagchi 2007),

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but it is increasingly recognized that it also alters the structure and functioning of belowground systems (Naeem et al. 2000, Zak et al. 2003, Wardle et al. 2004, Bardgett et al. 2005). Previous studies investigating the impact of plant diversity on soil microorganisms reported either positive (Bardgett and Shine 1999, Spehn et al. 2000, Zak et al. 2003, Liu et al. 2008, Milcu et al. 2008) or no effects (Gastine et al. 2003, Hedlund et al. 2003, Habekost et al. 2008). Most studies supported the redundancy hypothesis of plant diversity, assuming that more than one species performs a given role within an ecosystem (Walker 1992, Naeem and Li 1997), and not the singular hypothesis, assuming that each species contributes to ecosystem functioning (Naeem et al. 2002). In addition, results of a number of studies suggested particular significance of certain plant functional groups such as legumes, with the impact on soil microorganisms being primarily due to changes in plant productivity (Spehn et al. 2000, Zak et al. 2003, Milcu et al. 2008). Moreover, the existence of key plant functional groups argue for sampling effects of biodiversity, i.e., increased probability of presence of certain plant functional groups or species at high diversity levels (Huston 1997). In contrast, complementarity effects refer to positive species interactions and to differences among species in resource exploitation resulting in a linear relationship between diversity and ecosystem functioning (Loreau 2000, Naeem et al. 2002). Similarly, the singular hypothesis also suggests that loss or addition of species causes detectable changes in ecosystem functioning (Naeem et al. 2002); however, a linear relationship is not postulated since species might differ in their contribution to ecosystem functioning.

The recent review on effects of plant diversity on productivity by Cardinale et al. (2007) suggests that effects of diversity increase with time because of species complementarity. The authors concluded that due to the short duration of most biodiversity experiments the impact of species extinctions on ecosystem functioning likely has been underestimated. Since microbial communities respond to changes in land-use and plant diversity with a time lag (Hedlund et al. 2003, Bartelt-Ryser et al. 2005, Habekost et al. 2008, Kulmatiski and Beard 2008), plant diversity effects on soil microorganisms and processes mediated by them likely also have been underestimated.

Previous biodiversity–ecosystem functioning studies suffered from limitations such as singular measurements, lack of explanatory variables, and an inability to separate effects of plant species from that of functional groups (Spehn et al. 2000, Hedlund et al. 2003, Zak et al. 2003). Consequently, results have been disputed and little is known on how soil microorganisms respond to differences in plant species and functional group richness. To overcome these deficiencies, we investigated the functioning of microbial communities over a period of six years in a grassland experiment (the Jena Experiment), where plant species richness (1–60), functional group richness (1–4), and the presence of particular plant functional groups (grasses, legumes, small herbs, and tall herbs) were varied systematically. Furthermore, a wide range of ecosystem parameters were determined and considered as potential explanatory variables during statistical analyses and data interpretation. We hypothesized that (1) the effects of plant diversity on soil microorganisms increase with time, (2) plant diversity affects soil microorganisms mainly by increased plant productivity and the presence of key plant functional groups, and (3) plant functional group richness is more important for soil microorganisms than plant species richness.

Methods

Experimental setup

The study was conducted in the framework of the Jena Experiment, a large field experiment investigating the role of biodiversity for element cycling and trophic interactions in grassland communities (Roscher et al. 2004). The study site is located on the floodplain of the Saale River at the northern edge of Jena (Thuringia, Germany; Fig. 1). Mean annual air temperature 3 km south of the field site is 9.3°C and annual precipitation is 587 mm (Kluge and Müller-Westermeier 2000). The site had been used as an arable field for the last 40 years and the soil is an Eutric Fluvisol. The experiment was established in May 2002 and the studied system represents Central European mesophilic grassland traditionally used as hay meadow (Arrhenatherion community). A pool of 60 native plant species was used to establish a gradient of plant species (1, 2, 4, 8, 16, and 60) and plant functional group richness (1, 2, 3, and 4) in a total of 82 plots of 20×20 m (Table 1; Roscher et al. 2004). Further, bare ground plots were established without plants (0 species). Using above- and belowground morphological traits (growth form, canopy height, rooting depth and capacity for clonal growth), phenological traits (occupancy of seasonal niches, life cycle and seasonality of foliage) and N_2 fixation ability, plant species were aggregated into four plant functional groups: grasses (16 species), small herbs (12 species), tall herbs (20 species), and legumes (12 species). More details on the classification of plant functional groups are given in Roscher et al. (2004). Experimental plots were mown twice a year (June and September), as is typical for hay meadows, and weeded twice a year (April and July) to maintain the target species composition. Plots were assembled into four blocks following a gradient in soil characteristics, each block containing an equal number of plots of plant species and plant functional group richness levels. Further information on the design and setup of the Jena Experiment is given in Roscher et al. (2004).

Sampling

Soil samples were taken from all plots in May 2003, 2004, 2006, 2007, and 2008. At each sampling campaign,

FIG. 1. Photograph of the field site of the Jena Experiment taken in 2007 showing the main experimental plots (20 × 20 m) varying in plant species richness (1, 2, 4, 8, 16, and 60 species) and plant functional group richness (1, 2, 3, and 4 functional groups). The field site is located on the floodplain of the Saale river at the northern edge of Jena (Thuringia, Germany [visible in background]). Photo credit: A. Weigelt.

five soil samples were taken to a depth of 5 cm using a metal corer (diameter 5 cm), pooled and stored at 5°C. Before measurements, soil samples were homogenized, sieved (2 mm) to remove larger roots, animals, and stones (Anderson and Domsch 1978), and adjusted to a gravimetric soil water content of 25%.

Microorganisms

Basal respiration (BR) and microbial biomass C (C_{mic}) were measured using an O_2-microcompensation apparatus (Scheu 1992). The microbial respiratory response was measured at hourly intervals for 24 h at 22°C. Basal respiration (μL $O_2 \cdot h^{-1} \cdot g$ soil dry $mass^{-1}$) was determined without addition of substrate and measured as mean of the O_2 consumption rates of hours 14 to 24 after the start of the measurements. Substrate induced respiration was calculated from the respiratory response to D-glucose (Anderson and Domsch 1978). Glucose was added according to preliminary studies to saturate the catabolic enzymes of the microorganisms (4 mg/g dry mass solved in 400 μL deionized water). The mean of the lowest three readings within the first 10 h was taken as maximum initial respiratory response (MIRR; μL $O_2 \cdot h^{-1} \cdot g$ soil dry

TABLE 1. Design of the Jena Experiment.

Plant functional group richness	Plant species richness							Number of replicates
	0	1	2	4	8	16	60	
0	4							4
1		16	8	4	4	2		34
2			8	4	4	4		20
3				4	4	4		12
4				4	4	4	4	16
Number of replicates	4	16	16	16	16	14	4	86 plots

Notes: The table shows combinations of plant species richness and plant functional group richness and the number of replicates per diversity level. For more details on the experimental design, see Roscher et al. (2004).

mass^{-1}) and microbial biomass (μg C/g soil dry mass) was calculated as 38 × MIRR (Beck et al. 1997). The specific respiratory quotient (metabolic oxygen quotient, qO_2; μL O_2·mg C_{mic}^{-1}·h^{-1}) was calculated as a measure of the metabolic efficiency of the microbial community by dividing basal respiration by microbial biomass.

Covariates

In order to identify the mechanisms responsible for diversity effects on microbial biomass, basal respiration, and specific respiratory quotient in 2006, key abiotic and biotic factors affecting soil microorganisms were fitted as covariates. Fitted variables included the soil abiotic factors soil pH and gravimetric soil water content (%; upper 5 cm), the soil nutrient availability measures concentration of soil inorganic carbon (%; upper 5 cm), concentration of soil organic carbon (%; upper 5 cm), concentration of inorganic carbon in soil solution (mg/L; upper 20 cm), concentration of organic carbon in soil solution (mg/L; upper 20 cm), and total soil nitrogen concentration (%; upper 5 cm), and the plant productivity measures plant shoot biomass (g/m^2; community biomass, cut 3 cm above soil surface level), plant fine root biomass (g/m^2; root diameter < 2 mm; in 0–0.3 m soil depth), and plant large root biomass (g/m^2; root diameter > 2 mm; in 0–0.3 m soil depth) in 2006. These covariates were considered since soil microorganisms are known to strongly depend on soil abiotic factors and nutrient availability and since impacts of plant diversity on soil microbial functions have primarily been attributed to plant productivity (Spehn et al. 2000, Zak et al. 2003, Milcu et al. 2008).

Microbial nutrient limitations and growth

Microbial nutrient status was determined measuring the respiratory response of soil microorganisms to glucose and nutrient amendments for 24 h as described above. Different nutrients and their combinations were examined: (1) glucose (C; SIR), (2) glucose and nitrogen (CN; N as $(NH_4)_2SO_4$), (3) glucose and phosphorous (CP; P as K_2HPO_4), (4) glucose, nitrogen and phosphorous (CNP), and (5) glucose, nitrogen, phosphorous, and micronutrients (CNP∞; with ∞ including EDTA [0.50 μg/g soil dry mass], $ZnSO_4$ [2.19 μg/g soil dry mass], $FeSO_4$ [1.00 μg/g soil dry mass], $MnSO_4$ [0.31 μg/g soil dry mass], $CuSO_4$ [0.08 μg/g soil dry mass], $Co(NO_3)_2$ [0.05 μg/g soil dry mass], $Na_2B_4O_7$ [0.04 μg/g soil dry mass], $NiCl_2$ [0.26 μg/g soil dry mass]). The mass ratio of C, N, and P was 10:2:1 which is in the range of microbial tissue (Anderson and Domsch 1980). Nutrients were added as aqueous solutions (400 μL/g soil dry mass) and microbial growth was determined between the lowest reading within the first 3–6 h (MIRR) and the highest reading during the 20 h measurement (Scheu 1993). Accounting for the exponential growth of the soil microorganisms after nutrient addition, respiration rates were ln-transformed. Then, the slope of soil microbial growth was determined by linear regression. As a reference, soil microbial growth was determined for soil samples of two adjacent arable fields (monocultures) and two meadows (35 ± 3 plant species, 4 plant functional groups; E. Marquard, *personal communication*).

Statistical analyses

Data generally were log-transformed to improve normality and homoscedasticity of errors. Repeated-measures ANOVA as part of the general linear model (GLM, type I sum of squares) was used to analyze the effects of time (T), block (Bl), plant species richness (S), plant functional group richness (Fg), and presence/absence of grasses (Gr), small herbs (Sh), tall herbs (Th), and legumes (Leg) on microbial biomass, basal respiration, and specific respiratory quotient in May 2003, 2004, 2006, 2007, and 2008 in a hierarchical order. Further, more detailed analyses were performed for microbial biomass, basal respiration, and specific respiratory quotient in 2006 when microorganisms responded to variations in plant diversity. ANCOVA was used to identify the significance of abiotic and biotic factors including soil pH (cPH), gravimetric soil water content (cH_2O), plant shoot biomass (cBM), plant fine root biomass (cFR), plant large root biomass (cLR), concentration of soil inorganic carbon (cIC), concentration of soil organic carbon (cOC), concentration of inorganic carbon in soil solution (cIS), concentration of organic carbon in soil solution (cOS), and total soil nitrogen concentration (cNC).

MANOVA and protected ANOVAs were performed to analyze the effects of block, plant species richness, plant functional group richness, presence/absence of grasses, small herbs, tall herbs, and legumes on soil microbial growth after the addition of CN, CNP, and CNP∞ to soil samples in 2007. Effects of the addition of C and CP were not included since respiration rates decreased continuously after substrate addition. The respiratory response of microorganisms from two adjacent arable fields and meadows served as reference but were not included in the statistical analysis.

The *F* values given in the text and tables refer to those where the respective factor was fitted first (Schmid et al. 2002). Covariates were always fitted first, followed by block. Then, the effects of plant species richness and plant functional group richness were calculated, followed by presence/absence of certain plant functional groups. ANOVAs and comparisons of means (Tukey's hsd test, $\alpha < 0.05$) were performed using SAS version 9.1 (SAS Institute 2003). Means (and SE) presented in the text and figures were calculated using non-transformed data.

Results

Microorganisms

Basal respiration, microbial biomass, and specific respiratory quotient changed significantly with time (Table 2, Fig. 2). Basal respiration increased significant-

TABLE 2. Overall factor effects on microbial parameters.

Factor	df	BR	C_{mic}	qO_2
Between subjects				
Bl	3, 63	2.88*	16.21***	7.96***
S	5, 63	7.17***↑	3.42**↑	0.68
Fg	3, 63	4.51**↑	5.94**↑	0.87
Gr	1, 63	11.19**↑	0.54	12.75***↑
Sh	1, 63	1.16	1.95	0.26
Th	1, 63	1.93	0.36	0.31
Leg	1, 63	2.33	0.13	2.71
Within subjects				
T	3, 189	68.51***↓	49.20***↑	188.90***↓
T × Bl	9, 189	3.73***	3.81***	5.30***
T × S	15, 189	1.94*	1.88*	1.16
T × Fg	9, 189	1.55	1.52	1.42
T × Gr	3, 189	7.09***	2.55*	3.92**
T × Sh	3, 189	0.32	1.24	1.33
T × Th	3, 189	1.62	1.55	1.19
T × Leg	3, 189	3.31*	1.50	2.90*

Notes: These are *F* values from a MANOVA on the between- and within-subject factors of time (T), block (Bl), plant species richness (S), plant functional group richness (Fg), and presence/absence of grasses (Gr), small herbs (Sh), tall herbs (Th), and legumes (Leg) on basal respiration (BR), microbial biomass (C_{mic}), and specific respiratory quotient (qO_2) in May 2003, 2004, 2006, 2007, and 2008. Arrows indicate increases and decreases with increasing diversity level or in the presence of the respective factor.

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

ly from 2003 to 2004 but decreased considerably until 2006 (Fig. 2A). Thereafter, basal respiration increased from 2006 to 2007 but decreased again from 2007 to 2008. In contrast to basal respiration, microbial biomass was significantly higher in 2003 than in 2004 (Fig. 2B). Thereafter, microbial biomass increased significantly in 2006 and, following the trend, also in 2007. Microbial biomass did not differ between 2007 and 2008. Similar to basal respiration, the specific respiratory quotient increased significantly from 2003 to 2004 but decreased strongly until 2006 (Fig. 2C). Thereafter, the specific respiratory quotient increased slightly from 2006 to 2007 but decreased considerably from 2007 to 2008. Generally, abiotic soil parameters (block) strongly affected basal respiration, microbial biomass and specific respiratory quotient (Tables 2, 3); the block design of the experiment effectively excluded the respective variance from the statistical analyses.

Plant species richness significantly affected basal respiration in 2004, 2006, 2007, and 2008 (Table 3). While there was no consistent pattern in 2004 and 2007, basal respiration increased significantly with increasing plant species richness and plant functional group richness in 2006 and 2008 (Fig. 3A, B). Remarkably, fitting plant species richness after plant functional group richness did not eliminate significant effects in 2004, 2006, and 2007 (Table 3). Contrarily, fitting plant functional group richness after plant species richness eliminated significant effects (Table 3). Further, basal respiration increased in presence of grasses in 2006 (+26%), 2007 (+13%), and 2008 (+30%) but it slightly decreased in presence of legumes in 2006 (−2%; Table 3).

Microbial biomass was not affected by plant species richness in the first two years of measurement, however,

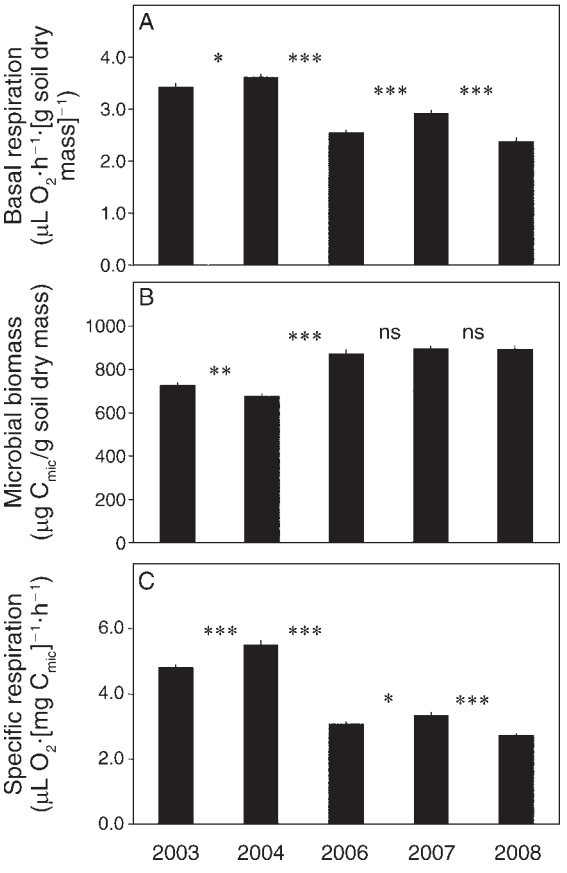

FIG. 2. Variations in (A) basal respiration, (B) microbial biomass, and (C) specific respiratory quotient as affected by time. C_{mic} is microbial biomass. Asterisks between bars indicate significant changes between years (contrasts between years).

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; ns, not significant ($P > 0.05$).

TABLE 3. Factor effects on microbial parameters in single years.

		2003			2004			2006		
Factor	df	BR	C_{mic}	qO_2	BR	C_{mic}	qO_2	BR	C_{mic}	qO_2
Bl	3, 63	0.46	1.83	2.25	3.17*	15.24***	10.64***	1.79	5.21**	6.30***
S	5, 63									
Before		1.39	1.33	0.15	2.96*↑	0.74	1.03	5.61***↑	2.83*↑	0.35
After		1.41	1.43	0.14	2.68*↑	0.70	1.40	3.23*↑	1.30	0.37
Fg	3, 63									
Before		0.44	0.28	0.05	0.44	1.45	0.97	3.76*↑	3.06*↑	1.13
After		0.46	0.43	0.02	0.05	1.27	1.35	0.51	0.52	1.31
Gr	1, 63	1.52	0.05	1.00	2.87	11.68**↓	2.24	15.76***↑	0.08	8.72**↑
Sh	1, 63	0.00	1.12	0.88	0.36	0.77	1.74	0.00	2.58	3.32
Th	1, 63	1.96	0.17	1.02	0.00	1.50	1.06	1.13	1.33	0.18
Leg	1, 63	2.88	0.03	3.16	0.26	5.16*↑	2.42	7.17**↓	0.76	1.50

Notes: Values are *F* values from protected ANOVAs for the effect of block (Bl), plant species richness (S; fitted before or after plant functional group richness), plant functional group richness (Fg; fitted before or after S), and presence/absence of grasses (Gr), small herbs (Sh), tall herbs (Th), and legumes (Leg) on basal respiration (BR), microbial biomass (C_{mic}), and specific respiratory quotient (qO_2) in May 2003, 2004, 2006, 2007, and 2008. Arrows indicate increases and decreases with increasing diversity level or in the presence of the respective factor.

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

in 2006, 2007, and 2008, microbial biomass increased significantly with increasing plant species richness (Table 3, Fig. 3C). Similarly, microbial biomass was not affected by plant functional group richness in 2003 and 2004, but increased significantly with increasing plant functional group richness in 2006, 2007, and 2008 (Table 3, Fig. 3D). In contrast to basal respiration, fitting plant species richness after plant functional group richness eliminated significant effects on microbial biomass (Table 3). Similarly, fitting plant functional group richness after plant species richness also eliminated significant effects of plant functional group richness on microbial biomass (Table 3). Further, microbial biomass decreased in 2004 in presence of grasses (−6%) but increased in presence of legumes (+11%; Table 3).

In 2007, the specific respiratory quotient decreased with increasing plant species richness (Fig. 3E) but was not affected in the other years. Further, the specific respiratory quotient was not affected by plant functional group richness (Fig. 3F). Remarkably, fitting plant species richness after plant functional group richness did not eliminate its significant effect on the specific respiratory quotient (Table 3). Moreover, the specific respiratory quotient was increased in presence of grasses in 2006 (+8%), 2007 (+4%) and 2008 (+17%; Table 3).

Covariates

To identify the mechanisms responsible for plant diversity effects on basal respiration, microbial biomass, and specific respiratory quotient in 2006, biotic and abiotic variables were fitted as covariates in separate analyses. Basal respiration was positively correlated with the biomass of fine and large roots, gravimetric soil water content and soil nitrogen concentration, nevertheless, fitting these variables did not eliminate the significant effects of plant species richness and presence of grasses and legumes on basal respiration (Table 4). In contrast, the effect of plant functional group richness on basal respiration disappeared (Table 4). Remarkably, fitting presence of grasses and legumes before plant species richness did also not eliminate the significant effect of plant species richness on basal respiration ($F_{5,28} = 3.97$, $P = 0.008$ and $F_{5,28} = 7.26$, $P < 0.001$, respectively). Although microbial biomass was negatively correlated with the concentration of soil inorganic carbon and positively correlated with the concentration of soil organic carbon and soil nitrogen, large root biomass, and the gravimetric soil water content, fitting these covariates did not eliminate the effect of plant species richness on microbial biomass (Table 4). Conversely, the effects of block (abiotic soil factors) and plant functional group richness disappeared. Further, the specific respiratory quotient was positively correlated with the concentration of soil inorganic carbon and negatively with the concentration of soil organic carbon and soil nitrogen; fitting these covariates eliminated the effects of block and presence of grasses on the specific respiratory quotient (Table 4).

Microbial nutrient limitations and growth

Addition of C and CP did not result in microbial growth, i.e., respiration rates remained constant or declined following maximum initial respiratory response. In contrast, addition of CN uniformly resulted in an increase in microbial growth (0.049 ± 0.002) suggesting that C supplemented microorganisms were limited by N in each of the treatments. Soil microbial growth after CNP addition uniformly exceeded that of the CN treatment (+30%, 0.064 ± 0.002, Tukey's hsd test, $\alpha < 0.05$) suggesting that CN supplemented microorganisms were generally limited by P. Soil microbial growth after the addition of CNP∞ (0.087 ± 0.003) also uniformly exceeded that of the CN (+78%,

TABLE 3. Extended.

2007			2008		
BR	C_{mic}	qO_2	BR	C_{mic}	qO_2
13.00***	15.91***	5.84**	1.78	14.04***	6.42***
2.83*↑	3.35**↑	2.37*↓	3.74**↑	3.81**↑	1.38
3.13*↑	2.02	2.29*↓	1.49	0.71	1.19
0.68	4.72**↑	1.73	4.49**↑	5.09**↑	2.19
1.44	2.42	2.03	0.74	2.44	1.88
12.47***↑	0.39	6.48*↑	8.61**↑	0.01	14.19***↑
0.76	0.42	0.10	1.81	0.15	0.02
1.03	0.10	0.42	0.73	2.55	2.38
2.66	0.23	2.76	2.23	0.05	3.10

Tukey's hsd test, $\alpha < 0.05$) and the CNP treatment (+36%, Tukey's hsd test, $\alpha < 0.05$) suggesting that other nutrients were limiting growth of CNP supplemented microorganisms. Soil microbial growth after nutrient addition depended strongly on soil abiotic characteristics (block) but the respective variance was excluded from statistical analyses (Table 5).

Soil microbial growth after the addition of CN, CNP, and CNP∞ decreased significantly with increasing plant species richness (Table 5; Fig. 4A). Soil microbial growth of soil of adjacent arable fields resembled that of one- and two-species mixtures. Further, soil microbial growth of soil of adjacent meadows was below but similar to that of 60-species mixtures. Moreover, soil microbial growth after the addition CN, CNP, and CNP∞ significantly decreased with increasing plant

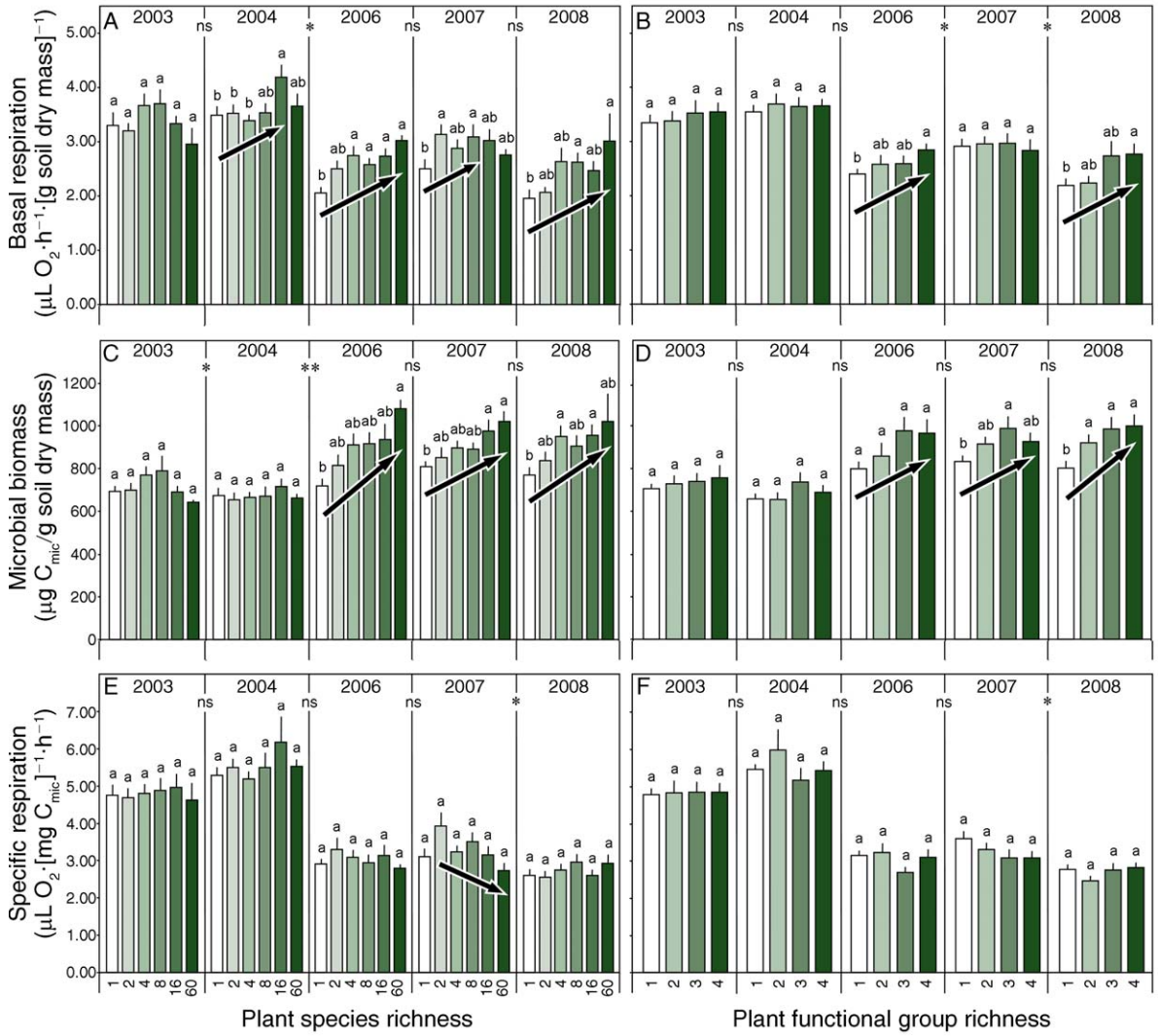

FIG. 3. Variations in (A, B) basal respiration, (C, D) microbial biomass, and (E, F) specific respiratory quotient as affected by (A, C, E) plant species richness and (B, D, F) number of plant functional groups in 2003, 2004, 2006, 2007, and 2008. Asterisks between years indicate significant changes of the respective plant diversity effect in time; see Fig. 2. Arrows indicate significant plant diversity effects. Values shown are means and SE. Bars with different letters vary significantly (Tukey's hsd test, $\alpha < 0.05$).

TABLE 4. Do covariates explain diversity effects?

	df	Basal respiration	Microbial biomass	Specific respiratory quotient
Covariates				
cBM	1, 27	1.22	1.85	0.51
cFR	1, 27	4.49*+	0.59	0.49
cH_2O	1, 27	39.48***+	20.05***+	0.15
cIC	1, 27	0.9	14.95***−	25.52***+
cLR	1, 27	9.43***+	4.74*+	0.03
cNC	1, 27	5.35*+	6.99*+	21.99***−
cOC	1, 27	3.38	5.62*+	16.23***−
cPH	1, 27	2.84	2.21	0.18
cIS	1, 27	0.93	3.34	1.8
cOS	1, 27	0.06	2.74	2.81
Factors				
Bl	3, 27	1.57	0.44	0.81
S	5, 27	4.53**↑	2.51*↑	1.01
Fg	3, 27	2.09	1.02	0.48
Gr	1, 27	5.60*↑	0.04	3.91
Sh	1, 27	0.05	1.57	2.46
Th	1, 27	0.88	0.67	0.05
Leg	1, 27	6.13*↓	0.16	1.93

Notes: Values are *F* values from ANCOVA for the effect of the covariates plant shoot biomass (cBM; g/m^2), plant fine root biomass (cFB; roots < 2 mm; g/m^2), gravimetric soil water content (%), concentration of soil inorganic carbon (cIC; %), plant large root biomass (cLB; roots > 2 mm; g/m^2), total soil nitrogen concentration (cNC; %), concentration of soil organic carbon (cOC; %), soil pH (cPH), concentration of inorganic carbon in soil solution (cIS; mg/L), and concentration of organic carbon in soil solution (cOS; mg/L) and the factors block (Bl), plant species richness (S), plant functional group richness (Fg), and presence/absence of grasses (Gr), small herbs (Sh), tall herbs (Th), and legumes (Leg) on basal respiration, microbial biomass, and specific respiratory quotient in 2006. Symbols are: +, positive correlation; −, negative correlation. Arrows indicate increases and decreases with increasing diversity level or in the presence of the respective factor.

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

functional group richness, with lowest values in mixtures containing three plant functional groups (Table 5; Fig. 4B). Again, soil microbial growth from soil of adjacent arable fields resembled that of mixtures with one plant functional group and soil microbial growth of adjacent meadows was lower than that of mixtures with three plant functional groups. Soil microbial growth after the addition of CN (−29%) and CNP∞ (−17%) was significantly lower in presence of legumes (Table 5). Further, soil microbial growth after the addition of CNP decreased slightly in presence of tall herbs (−2%; Table 5).

DISCUSSION

Do diversity effects increase with time?

Previous studies reported a time-lag in the response of soil microorganisms to changes in plant community composition and land-use management (Hedlund et al. 2003, Bartelt-Ryser et al. 2005, Habekost et al. 2008, Kulmatiski and Beard 2008). Consistent to these findings and hypothesis 1, plant diversity effects on soil microorganisms manifested only after a time lag of years after establishment of the Jena Experiment.

The initial effects of the presence of certain plant functional groups (grasses and legumes) on soil microorganisms occurred two years after the experiment was set up; however, both microbial biomass and basal respiration only increased with plant functional group richness four years after establishment. Similarly, microbial biomass and basal respiration increased with increasing plant species richness at four years and thereafter although basal respiration was already significantly increased in 16 species mixtures after two years.

As predominantly heterotrophic organisms, soil microorganisms essentially depend on plant-derived resources entering the belowground system (Naeem et al. 2000, Wardle et al. 2004). Presumably, after establishment of the experiment in 2002, dead plant materials and root exudates needed to accumulate before differences in plant community composition became manifest in specific microbial communities. Further, as indicated by specific microbial respiration, microorganisms became more energy efficient with time. Despite some

TABLE 5. Microbial growth after nutrient addition.

	MANOVA		Protected ANOVAs			
Factor	df	Roy's greatest root†	df	CN	CNP	CNP∞
Bl	3, 66	21.41***	3, 66	9.94***	9.17***	20.32***
S	5, 66	3.79**↓	6, 66	12.46***↓	7.41***↓	11.09***↓
Fg	3, 66	3.53*↓	4, 66	15.76***↓	9.86***↓	14.80***↓
Gr	1, 64	3.78*↓	1, 64	0.01	0.02	2.62
Sh	1, 64	1.61	1, 64	0.00	3.82	0.02
Th	1, 64	0.23	1, 64	1.89	4.02*↑	0.00
Leg	1, 64	3.87*↓	1, 64	8.37**↓	1.25	4.96*↓

Notes: The table shows *F* values from MANOVA and ANOVA for the effects of block (Bl), plant species richness (S), plant functional group richness (Fg), and presence/absence of grasses (Gr), small herbs (Sh), tall herbs (Th), and legumes (Leg) on the slope of microbial growth after addition of CN (carbon and nitrogen), CNP (carbon, nitrogen, and phosphorus), and CNP∞ (carbon, nitrogen, phosphorus, and micronutrients). Arrows indicate increases and decreases with increasing diversity level or in the presence of the respective factor.

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

† This is the largest of the roots of the product of the sum-of-squares matrix of the model and the sum-of-squares matrix of the error for the two linear regression functions. See SAS (2003).

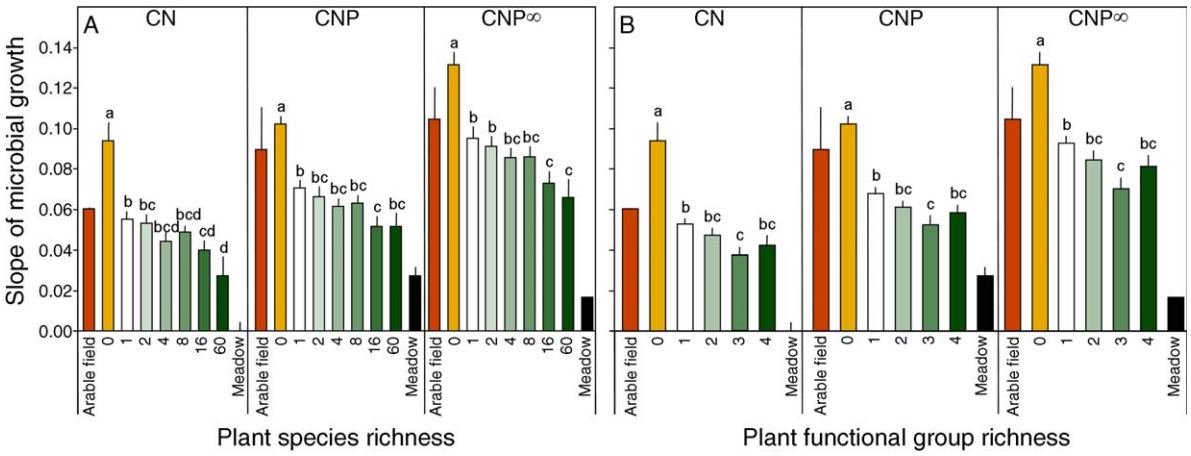

FIG. 4. Variations in microbial growth after the addition of CN (carbon and nitrogen), CNP (carbon, nitrogen, and phosphorus), and CNP∞ (carbon, nitrogen, phosphorus, and micronutrients) as affected by (A) plant species richness and (B) number of plant functional groups in 2007. Slopes of microbial growth of the soil of two arable fields and two adjacent meadows are given as reference but were not included in the statistical analysis. Values shown are means and SE. Bars with different letters differ significantly (Tukey's hsd test, $\alpha < 0.05$).

criticism (Wardle and Ghani 1995), the specific respiratory quotient generally is regarded as an indicator of change in microbial metabolism in response to disturbance (Anderson and Domsch 1985, Bardgett and Shine 1999). Based on Odum's theory of ecosystem succession (Odum 1969, 1985), this index declines both during succession and following recovery from disturbance, because equilibrium conditions are approached and the soil microbial community becomes more carbon efficient (Anderson and Domsch 1985, Wardle and Ghani 1995). Interestingly, simultaneous to the response of microbial biomass and basal respiration to plant diversity, the specific respiratory quotient decreased markedly between 2004 and 2006. This shift in microbial carbon use efficiency was likely to have been caused by successional changes from a disturbed (zymogeneous) to a more established (autochthonous) microbial community.

The establishment of the Jena Experiment started in autumn 2000 by harvesting, plowing, applying Glyphosate (Roundup; Monsanto, St. Louis, Missouri, USA) and harrowing the formerly agricultural field several times until spring 2002 (Roscher et al. 2004). As a consequence of these disturbances most soil animals including Collembola, Carabidae, Staphilinidae, and Myriapoda had low abundances (N. Eisenhauer, *unpublished data*) and also microbial biomass was low in 2003 and 2004. Thereafter, both recovery from the experimental pre-treatment and successional changes occurred towards autochthonous microorganisms with increased carbon use efficiency resulting in increased microbial biomass. As indicated by the decrease in specific microbial respiration with increasing plant species richness in 2007, we suggest that the shift from less efficient zymogeneous to more efficient autochthonous microorganisms was most pronounced at high plant diversity. Although this effect disappeared in 2008, our assumption is supported by the growth characteristics of carbon and nutrient supplemented microorganisms. In 2007, microbial growth decreased significantly with increasing plant species and functional group richness. Reference measurements on adjacent arable fields and meadows indicated an ongoing succession of soil microorganisms towards that of well-established meadows, with low diversity plots still resembling the community of arable systems whereas high diversity plots resembling the microbial community of meadows. Microbial functional characteristics, particularly low growth rates after temporary nutrient pulses, indicate that microbial community composition in high diverse plant communities changed towards a more stable "equilibrium" state (Tilman 2001, Hooper et al. 2005). In addition, the present study confirmed recent observations of land-use legacies (Bartelt-Ryser et al. 2005, Kulmatiski and Beard 2008) and time lags of plant community effects (Hedlund et al. 2003, Habekost et al. 2008) on soil microorganisms. Since soil abiotic and biotic parameters may take decades to equilibrate with unknown feedback effects on the parameters investigated, we suggest to continue following plant diversity effects on soil microorganisms further on in the Jena Experiment. This highlights the necessity of long-term biodiversity experiments for evaluating biodiversity–ecosystem functioning relationships.

What is important: plant productivity, key functional groups, redundancy, or singularity?

Previous studies suggested that plant diversity affects soil microbial communities mainly through increased plant productivity and the presence of key plant functional groups (Spehn et al. 2000, Habekost et al. 2008, Milcu et al. 2008). In order to test the effect of plant productivity on soil microorganisms, separate

analyses were performed fitting plant productivity parameters (shoot biomass, fine root biomass, large root biomass) as covariates. In contrast to our hypothesis 2, shoot biomass was not correlated and fine and large root biomass only weakly correlated with soil microbial biomass and respiration, and therefore did not affect the significance of plant species richness effects. Thus, the results support the conclusions of the recent laboratory experiment of Milcu et al. (2006) suggesting that the quality of rhizodeposits rather than plant productivity per se and the quantity of resources affects the functioning of microorganisms in soil.

Unexpectedly, the presence of grasses and legumes, which are regarded as key plant functional groups for soil microbial communities (Spehn et al. 2000, Hedlund et al. 2003, Milcu et al. 2008), had inconsistent effects on soil microbial functioning. Although grasses produce large amounts of fine roots thereby providing large amounts of root-derived resources and legumes increase nitrogen availability in soil (Oelmann et al. 2007, Temperton et al. 2007), the effects of grasses and legumes generally were of minor importance. Moreover, the significant increase in microbial biomass and basal respiration with increasing plant species richness was not due to the presence of particular plant functional groups; the effect did not disappear when presence of grasses and legumes were fitted before plant species richness. This indicates that plant diversity effects on the functioning of belowground systems likely were due to species complementarity rather than due to certain plant functional group characteristics.

Previously, the soil system has been considered to be affected mainly by the number and identity of plant functional groups rather than by plant species richness per se (Bardgett and Shine 1999, Spehn et al. 2000, Milcu et al. 2008, Scherer-Lorenzen 2008), which is conform to the redundancy hypothesis of biodiversity suggesting that only a small number of species are needed to maintain ecosystem functioning (Walker 1992, Naeem and Li 1997). In contrast, the singular hypothesis of biodiversity, assuming that each species contributes to ecosystem functioning, has received little experimental support (Naeem et al. 2002). The design of the Jena Experiment offers the possibility to disentangle effects of plant species richness and plant functional group richness (Roscher et al. 2004). In contrast to prevalent view and our hypothesis 3, results of the present study highlight the importance of plant species richness for soil microbial functioning. Microbial respiration, biomass, carbon use efficiency, and respiratory response to nutrients changed significantly with increasing plant species richness, whereas plant functional group richness and presence of certain key plant functional groups were of minor importance. Remarkably, including abiotic and biotic factors as covariates into the statistical analysis strengthened rather than reduced the significance of the plant species richness effect, whereas the effect of plant functional group richness was eliminated. In particular, although measures of soil nutrient availability and plant productivity were positively correlated with soil microbial parameters (significant correlations between BR and concentration of soil nitrogen, fine root biomass, and large root biomass and between C_{mic} and concentration of soil organic carbon, soil nitrogen, and large root biomass), this did not eliminate plant species richness effects indicating that the quality of resources was more important than the quantity. Moreover, fitting plant species richness after plant functional group richness did not eliminate significant effects of plant species richness on basal respiration in 2004, 2006, and 2007. On the contrary, the effect of plant functional group richness on basal respiration disappeared when fitted second. This indicates that diversity effects on soil microbial functioning were not only due to differences between plant functional groups but most likely due to impacts of single plant species. The results therefore suggest that plant litter materials and root exudates that govern the succession of microbial communities and their functioning are plant species specific rather than plant functional group specific. Since the biochemical composition of plant litter and root exudates differs among plant species, changes in plant diversity likely altered the quality of resources entering the decomposer system, thereby controlling microbial biomass, respiration, and community composition (Zak et al. 2003, Milcu et al. 2006, Nilsson et al. 2008). This is in stark contrast to the redundancy hypothesis and supports the singular hypothesis for soil microorganisms highlighting that species are unique and affect ecosystem functioning (Naeem et al. 2002).

Conclusions

The present study suggests for the first time that microbial community functioning is driven by plant species specific characters, i.e., responds conform to the singular hypothesis of biodiversity. This contrasts the dominant view that the functioning of decomposer systems is rather insensitive to changes in plant species and characterized by high redundancy among species. We conclude that the quality of litter and rhizodeposits rather than plant productivity, i.e., the amount of resources entering the decomposer system, affects the soil microbial community. As the decomposer system provides essential ecosystem services, such as litter decomposition and nutrient mineralization, plant–decomposer interrelationships need closer consideration for managing agricultural and forestry systems in a sustainable way. The results reinforce the need for long-term biodiversity experiments to fully appreciate the consequences of current biodiversity loss.

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