Plant diversity effects on soil food webs are stronger than those of elevated CO$_2$ and N deposition in a long-term grassland experiment

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Recent metaanalyses suggest biodiversity loss affects the functioning of ecosystems to a similar extent as other global environmental change agents. However, the abundance and functioning of soil organisms have been hypothesized to be much less responsive to such changes, particularly in plant diversity, than aboveground variables, although tests of this hypothesis are extremely rare. We examined the responses of soil food webs (soil microorganisms, nematodes, microarthropods) to 13-y manipulation of multiple environmental factors that are changing at global scales—specifically plant species richness, atmospheric CO$_2$, and N deposition—in a grassland experiment in Minnesota. Plant diversity was a strong driver of the structure and functioning of soil food webs through several bottom-up (resource control) effects, whereas CO$_2$ and N only had modest effects. We found few interactions between plant diversity and CO$_2$ and N, likely because of weak interactive effects of those factors on resource availability (e.g., root biomass). Plant diversity effects likely were large because high plant diversity promoted the accumulation of soil organic matter in the site's sandy, organic matter–poor soils. Plant diversity effects were not explained by the presence of certain plant functional groups. Our results underline the prime importance of plant diversity loss cascading to soil food webs (density and diversity of soil organisms) and functions. Because the present results suggest prevailing plant diversity effects and few interactions with other global change drivers, protecting plant diversity may be of high priority to maintain the biodiversity and functioning of soils in a changing world.

aboveground–belowground interactions | biodiversity–ecosystem functioning | global environmental change | soil microarthropods

Humankind is changing the composition and functioning of ecosystems via land use change and management, as well as by altering global biogeochemistry and climate (1–3). As a consequence, global biodiversity currently is declining significantly, with extinction rates exceeding those in the fossil records (2, 4). In addition to representing one dramatic consequence of anthropogenic global change, biodiversity loss (here meaning simplification at the patch scale) has been shown to itself be a major driver of ecosystem functioning (5, 6).

Two recent metaanalyses revealed that effects of biodiversity loss (mostly in primary producers) on ecosystem functioning, such as primary productivity, are of comparable magnitude to effects of many other global environmental change agents (7, 8). It is unclear, however, whether these patterns also apply to primary producer effects on the performance of higher trophic levels. We address this gap in understanding by examining the understudied responses of soil food webs and functions to a long-term and highly replicated manipulation of plant species richness, atmospheric CO$_2$ concentrations, and nitrogen (N) deposition (the BioCON experiment) in constructed grassland communities in central Minnesota (9). Specifically, we investigated main and interactive effects of experimental treatments on the structure and functioning of soil food webs, allowing us to study plant diversity effects on soil biota in four different environmental contexts.

Burning of fossil fuel has led to a substantial increase in atmospheric CO$_2$ concentrations, with accelerating impacts on global climate (3). Similarly, N inputs are increasing dramatically due to fertilization and fossil fuel burning (10, 11). Although many experiments assess the consequences of the above-mentioned global change agents in isolation, recent studies stress that multiple global change agents often interactively impact ecosystems (12–14), leading to repeated calls for multifactor experiments (7). In addition, there is a paucity of data on soil food webs and the key ecosystem functions they support in the few multifactor experiments that have been done (15, 16).

The role of plant diversity in shaping soil communities is highly disputed. In general, plant diversity is assumed to have no or weak effects on soil organisms due to the generalist behavior of many soil organisms (16); however, this conclusion is largely based on short-term plant diversity experiments (17). Recent long-term experiments (≥6 y) report significant positive plant diversity effects on the density and diversity of soil organisms (18, 19), as well as on the inputs of recent photosynthetic carbon that drive their functioning (20, 21). Thus, investigating the relevance of plant diversity effects on soil organisms and their potential dependency on other global change drivers in a long-term experiment may shed light on this controversy.

Previous studies of the BioCON experiment showed elevated CO$_2$ to mostly cause modest increases in the biomass of soil microorganisms (12), mycorrhizal hyphal length (22), and the abundance and diversity of soil microarthropods (12). These findings agree with a recent metaanalysis (15) and are most likely due to elevated soil moisture content (23), increased belowground translocation of assimilated carbon by plants (20, 24, 25), and increased above- and belowground litter input (9).

Effects of elevated N deposition on soil organisms have, however, been negative in the BioCON experiment, most likely due to decreased rhizodeposition (12), although previous studies in other experimental settings reported inconsistent N addition effects on soil microorganisms (26, 27).

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Moreover, there is insufficient knowledge of how different global change agents interactively impact soil communities (16). For instance, Reich et al. (9) found that elevated CO_2 and N deposition intensified positive effects of plant diversity on primary productivity, which may cascade to higher trophic levels. Indeed, Chung and colleagues (28) reported that the composition and functioning of soil microbial communities depended on complex interactions between plant diversity, CO_2, and N addition, and CO_2 and N interactively affected nematode community structure and diversity under trembling aspen (29) and wheat (30), respectively. By contrast, earthworm biomass increased significantly with increasing plant species richness, an effect that was not modified by elevated CO_2 levels in grassland plots (31), whereas a microcosm laboratory experiment found elevated CO_2 levels to increase earthworm biomass at the highest plant diversity level (32). There are clearly effects of global change drivers on soil organisms, but no consensus on whether and what generalities exist.

We studied soil microorganisms, nematodes, and microarthropods in the BioCON experiment to test the hypotheses that (i) plant diversity has positive effects on soil food webs due to increased availability of organic matter in soil, (ii) elevated CO_2 has positive effects on, and increases plant diversity effects on, soil food webs due to increased carbon inputs belowground (32), (iii) elevated N deposition negatively affects soil food webs (due to decreased rhizodeposition) but less so at higher diversity that ameliorates N effects on belowground inputs (11), and (iv) long-term plant diversity effects on soil food webs are of comparable absolute magnitude as CO_2 and N effects (7, 8).

Results

Overall, we found little support for our hypotheses regarding significant interactions between global change drivers, but we did observe numerous significant plant diversity effects on soil organisms and minor effects of CO_2 and N.

Hypothesis 1: Plant Diversity Has Positive Effects on Soil Food Webs. Plant diversity significantly increased a number of variables (Table 1; Table S1): soil microbial biomass (Fig. 1A), respiration (Fig. 1B), carbon use efficiency (inverse of metabolic quotient; Fig. 1C), bacterial feeding nematodes (Fig. 1D), nematode predators (Fig. 1E), nematode omnivores (Fig. 1F), Oribatida (Fig. 1G), Astigmata and Prostigmata (Fig. 1H), Thysanoptera (Fig. 1I), microfauna taxa richness, and mesofauna taxa richness (the latter two are summarized in Fig. 2). Gamasida was marginally significantly increased, whereas plant feeding nematodes, fungal feeding nematodes, and Collembola were not significantly affected by plant diversity.

Although with only three levels of richness, precise characterization of the shape of the response curve is impossible, the three levels do allow us to assess whether diversity effects saturate or peak at four species or also occur with increasing richness to nine species. With the exception of basal respiration, the density of nematode predators, and Oribatida, which showed a humped-shaped response to increasing plant species richness, response variables of soil biota increased with increasing plant species richness with a saturating (microbial biomass and C use efficiency, nematode omnivores, mesofauna taxa richness) or linear/exponential (bacterial feeding nematodes, Astigmata and Prostigmata, Thysanoptera, microfauna taxa richness) function (Table 1; Table S2; Figs. 1 and 2; Fig. S1; SI Methods).

Fitting the presence of the plant functional groups C3 grasses, C4 grasses, forbs, and legumes, as well as a more complex model with presence of C4 grasses, legumes, and the interaction between C4 grasses and legumes (with the interaction being significant in 5 of 11 cases) before plant species richness, in separate sequential analyses rendered only the effect on mesofauna taxa

Table 1. ANOVA table of F and P values on the effects of CO_2 (ambient and elevated), N (ambient and elevated), plant species richness (PSR; one, four, or nine species), and all possible interactions on soil microorganisms, microfauna, mesofauna, and biodiversity of soil organisms (taxon richness)

Soil biota	CO₂ F	CO₂ P		N F	N P		PSR F	PSR P		CO₂ × N F	CO₂ × N P	CO₂ × PSR F	CO₂ × PSR P	N × PSR F	N × PSR P	CO₂ × N × PSR F	CO₂ × N × PSR P
Microorganisms																	
Biomass	0.15	0.711		0.07	0.789		**171.14**	**<0.001**	S	1.07	0.303	**4.15**	**0.043**	0.07	0.799	2.78	0.097
Respiration	0.07	0.796		2.51	0.114		**46.24**	**<0.001**	H	0.43	0.513	3.11	0.080	0.21	0.647	1.75	0.188
Metabolic quotient	0.03	0.872		**4.75**	**0.031**	↓	**54.57**	**<0.001**	S	1.26	0.263	0.13	0.720	1.43	0.232	0.71	0.402
Microfauna (nematodes)																	
Plant feeders	0.34	0.582		0.22	0.641		1.82	0.179		0.80	0.371	<0.01	0.954	0.05	0.825	3.30	0.071
Bacterial feeders	0.02	0.895		0.11	0.741		**6.38**	**0.012**	L	0.53	0.467	0.56	0.454	0.05	0.825	0.19	0.664
Fungal feeders	0.68	0.444		**5.02**	**0.026**	↑	0.29	0.591		3.31	0.070	0.01	0.922	0.03	0.855	2.92	0.089
Predators*	0.29	0.612		**5.66**	**0.018**	↓	**16.19**	**<0.001**	H	0.41	0.523	1.29	0.258	0.78	0.380	1.54	0.217
Omnivores	0.26	0.633		0.58	0.447		**22.32**	**<0.001**	S	0.44	0.510	0.19	0.666	0.08	0.774	0.71	0.399
Mesofauna																	
Collembola	<0.01	0.967		0.01	0.907		0.89	0.346		0.64	0.425	1.01	0.317	0.18	0.676	2.18	0.142
Oribatida*	<0.01	0.956		0.22	0.637		**7.47**	**0.007**	H	0.01	0.924	0.48	0.488	0.02	0.889	0.02	0.882
Astigmata and Prostigmate	0.01	0.983		1.15	0.285		**9.85**	**0.002**	L	3.39	0.067	0.35	0.553	0.07	0.797	0.07	0.796
Gamasida	0.59	0.465		0.01	0.938		3.12	0.079		1.88	0.172	1.18	0.279	0.25	0.621	0.18	0.669
Thysanoptera	**9.59**	**0.010**	↓	2.06	0.153		**14.61**	**<0.001**	L	0.25	0.616	2.20	0.140	0.40	0.527	0.06	0.897
Soil biodiversity																	
Microfauna richness	0.59	0.445		0.05	0.822		**118.46**	**<0.001**	L	0.16	0.694	0.42	0.518	1.34	0.249	2.10	0.149
Mesofauna richness	**5.12**	**0.030**	↓	<0.01	0.964		**4.37**	**0.038**	S	3.20	0.075	1.65	0.201	1.65	0.201	0.99	0.322

Significant effects ($P \leq 0.05$) are given in bold. H, humped-shaped relationship between plant diversity and the response variable (best curve fit was determined by fitting linear, saturating, and humped-shaped response curves and reporting the fit with the highest R^2 value; see Table S2); L, linear or exponential increase in biomass or abundance with increasing plant species richness; S, saturating increase in abundance with increasing plant species richness; ↑, significantly higher mean at elevated levels of CO_2 and N; ↓, significantly lower mean.
*Plant species richness fitted as categorical factor.
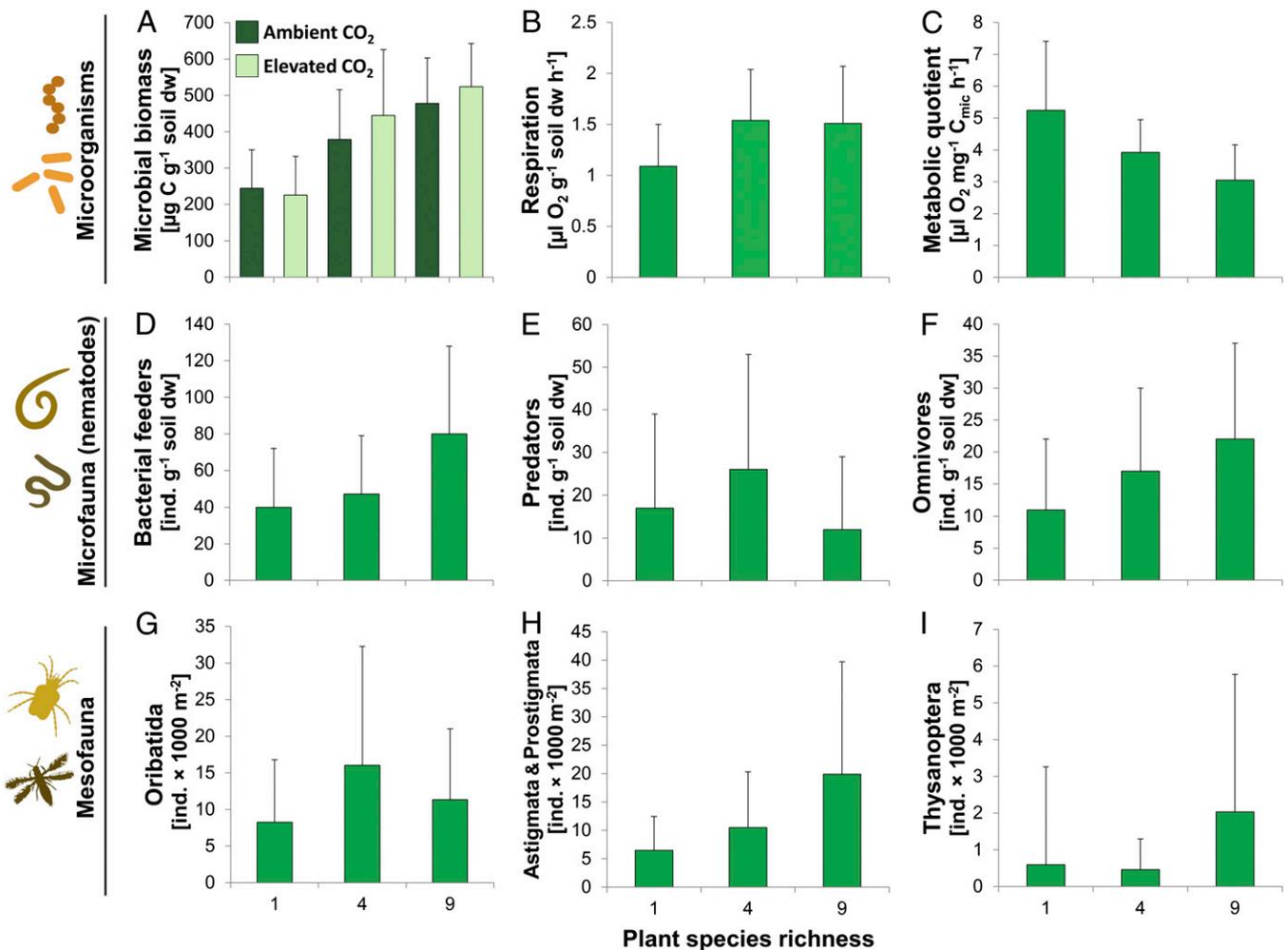
Fig. 1. (*A*) Effects of plant species richness and atmospheric CO_2 concentrations on soil microbial biomass C (plant species richness × CO_2 interaction: $F_{1,209} = 4.15$, $P = 0.043$). Effect of plant species richness on (*B*) basal respiration, (*C*) metabolic quotient, (*D*) bacterial feeding nematodes, (*E*) nematode predators, (*F*) nematode omnivores, (*G*) Oribatida, (*H*) Astigmata and Prostigmata, and (*I*) Thysanoptera. Means with SDs.

richness nonsignificant, whereas most other plant species effects remained highly significant (Tables S3 and S4). When the presence of forbs was included, all significant plant species richness effects on soil biota remained highly significant. By contrast,

Fig. 2. Effect of plant species richness on soil biodiversity, represented by the taxa richness of soil microfauna (nematodes) and soil mesofauna (Table 1). Means with SDs.
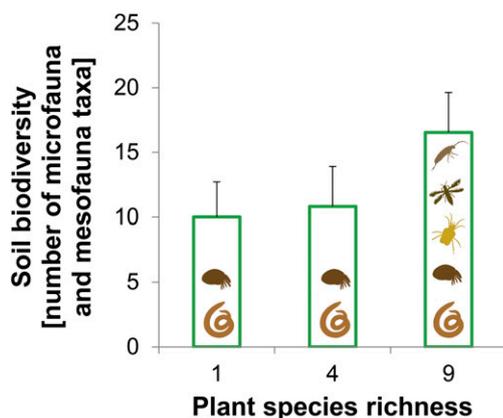
fitting plant species richness before the presence of plant functional groups rendered plant functional group effects nonsignificant in 29 of 34 cases (Table S5), indicating that plant species richness effects covered the variance explained by plant functional groups.

Hypothesis 2: Elevated CO_2 Has Positive Effects on Soil Food Webs and Increases Plant Diversity Effects on Soil Food Webs. In contrast to our second hypothesis, effects of elevated CO_2 were rare and included significant reductions in the density of Thysanoptera (−33%) and mesofauna taxa richness (−6%; Table 1). However, in line with our hypothesis, soil microbial biomass was affected by a significant interaction between plant diversity and elevated CO_2, with the plant diversity effect being significantly stronger at elevated CO_2 than at ambient conditions (Table 1; Fig. 1*A*). However, this interactive effect on soil microbial biomass did not cascade into higher trophic levels.

Hypothesis 3: Elevated N Deposition Negatively Affects Soil Food Webs, but Less So with Increasing Plant Diversity. In contrast to our third hypothesis, N effects on soil biota were minor, and there were no significant interactions between N and plant diversity (Table 1). N deposition effects included reduced soil microbial C use efficiency, i.e., the metabolic quotient was increased significantly (+10%; Table 1). Moreover, the density of

fungal feeding nematodes increased significantly at elevated N in comparison with ambient N (+62%), whereas the opposite was true for nematode predators (−33%; Table 1). We found no significant three-way interactions between CO_2, N, and plant diversity (Table 1).

Hypothesis 4: Long-Term Plant Diversity Effects on Soil Food Webs Are of Comparable Magnitude as CO_2 and N Effects. Overall, manipulation of plant diversity had much stronger effects on soil organisms than manipulation of CO_2 and N (Table 1). The evidence for this includes both the number of significantly affected response variables of soil biota and the magnitude of those responses. Plant diversity, CO_2, and N significantly altered 11 (73%), 2 (13%), and 3 (20%) variables, respectively, of 15 variables (Table 1).

Given these results relative to our four hypotheses, we focused on plant diversity effects in path analyses of the overall soil decomposer food webs and the soil nematode community. The path analysis approach revealed that plant species richness effects on soil decomposer food webs were due to several mechanisms (Table S6; Fig. 3A). A χ^2 test indicated that our hypothesized path analysis model cannot be rejected as a potential explanation of the observed covariance matrix [$\chi^2_7 = 2.43$, $P = 0.93$; Akaike information criteria (AIC) = 60.43]. Plant species richness significantly increased soil pH, soil N, root biomass, and soil water content, with indirect positive effects on soil microbial biomass and the density of microfauna and mesofauna detritivores. Although we were able to identify these important explanatory variables mediating plant diversity effects on decomposer food webs, direct paths leading from plant diversity to soil microbial biomass and micro- and mesofauna detritivores indicate that rhizodeposition (12, 20) or other unmeasured variables also had a significant effect on soil biota. The path analysis supported only bottom-up effects (from resources to consumers and from lower to higher trophic levels) of plant diversity on soil organisms (Fig. 3A); that is, top-down paths were not supported by the data (no paths from higher to lower trophic levels).

The path analysis of the soil nematode community suggests that bacteria are an influential structuring element of the soil food web at the BioCON site, with significant bottom-up effects on the density of nematode predators and omnivores (Table S6; Fig. 3B). Bottom-up effects of plant species richness on plant

feeding nematodes and fungal feeding nematodes did not propagate to higher trophic levels (Fig. 3B). A χ^2 test indicated that our hypothesized path analysis model cannot be rejected as a potential explanation of the observed covariance matrix ($\chi^2_9 = 11.54$, $P = 0.24$; AIC = 65.54).

Discussion

Human activities are causing environmental changes, including biodiversity, worldwide (1–4). Current research thus investigates the relative importance of these changes for the functioning of ecosystems (7, 8), as well as potential interactive effects of global change agents (9, 13, 14, 33). Results of the present study suggest that plant diversity effects are important for the structure and functioning of soil food webs at our experimental site, and within the context of our experiment, more so than changes in atmospheric CO_2 concentrations and N deposition.

In line with studies on plant biomass production (13) and earthworms (31), but in contrast to most previous studies in BioCON (9, 28), we found few significant interactions between other global change agents and plant diversity. Plant diversity effects were larger than the effects of CO_2 and N and—in most cases—did not interact with CO_2 and N in affecting soil food webs. However, interactive effects of plant diversity and elevated CO_2 on plant biomass production in the early years of the experiment (9) may have propagated to soil microbial biomass, with significantly stronger plant diversity effects on microbial biomass at elevated CO_2 than at ambient CO_2. However, the stronger effect of plant diversity on microbial biomass at elevated CO_2 was modest (one plant species: −7%; four species: +18%; nine species: +10% at elevated CO_2), which is why the interactive effect may not have cascaded significantly to higher trophic levels. Additionally, plant diversity effects on plant biomass production were up to 10 times larger than those of elevated CO_2 and N effects (9, 34), perhaps explaining why plant diversity effects propagated to belowground consumer levels.

Our results support the resource-driven perspective of plant diversity effects on consumers (18): plant diversity increased soil pH, soil N concentration, soil water content, and plant root biomass, which increased soil microbial biomass and the density of microfauna and mesofauna detritivores. Moreover, plant species richness may have fueled the soil decomposer food web by increasing levels of rhizodeposition, but this mechanism was
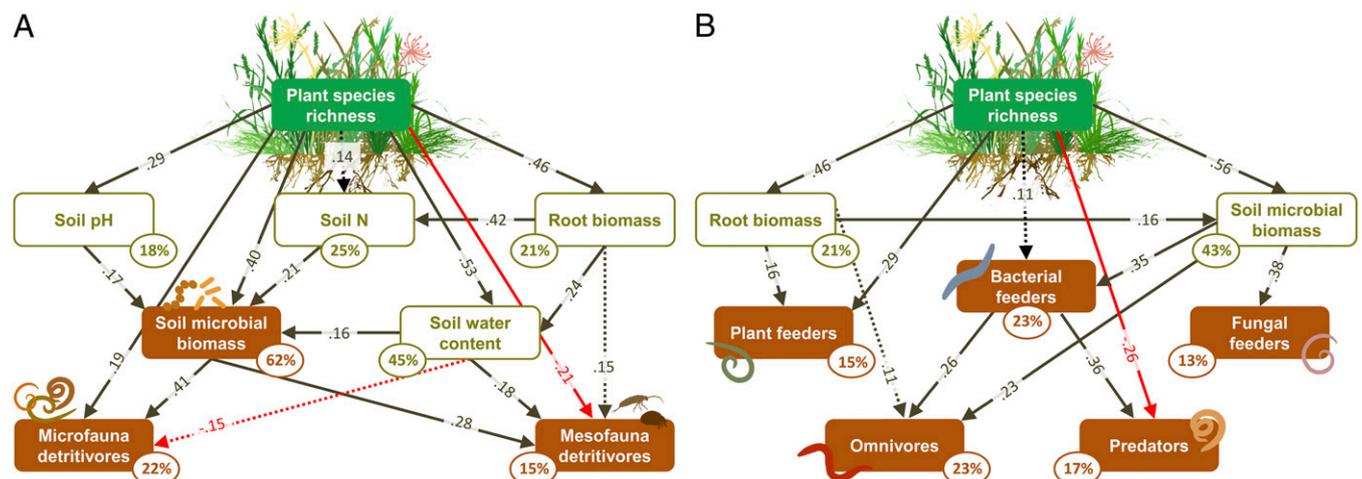
Fig. 3. Path analysis models of plant diversity effects on soil biota. (A) Causal influences of plant diversity (exogenous variable; green rectangle) on soil pH, soil N concentration, soil water content, root biomass productivity (endogenous explanatory variables; white rectangles), microbial biomass, and abundance of soil animals (endogenous variables; brown rectangles; $\chi^2_7 = 2.43$, $P = 0.93$). Numbers on arrows are standardized path coefficients. Solid line arrows indicate significant paths ($P < 0.05$); dotted lines indicate nonsignificant arrows (black = positive, red = negative). Percentages indicate the variance explained by the model. (B) Causal influences of plant diversity on the soil nematode community ($\chi^2_9 = 11.54$, $P = 0.24$).

only assessed indirectly by allowing plant diversity to exert direct effects (direct paths in path analyses) in addition to the other indirect plant diversity effects considered (35). Although we tested top-down effects of higher trophic levels on lower trophic levels, our path analyses only supported bottom-up paths. These findings are in contrast to those on aboveground consumers at the adjacent study site of the BioDIV experiment (36) and in a metaanalysis of fertilization (bottom-up) and predator removal (top-down) experiments (37), which both highlighted the relevance of top-down effects of predators on aboveground food web structure. Our results thus suggest that conservation approaches to maintain soil biodiversity should focus on protecting plant diversity.

In their recent book, Bardgett and Wardle (16) conclude that plant diversity has no or only weak effects on soil organisms, based on an evaluation of roughly 30 studies. Indeed, this conclusion represents current consensus very well, assuming that plant diversity effects are much less pronounced below- than aboveground (18). However, knowledge derived from previous short-term plant diversity experiments may not adequately elucidate long-term effects, because short-term effects may be both weaker than long-term effects and/or obscured by soil legacy effects of the former vegetation or the pretreatment of experimental soils (17). In fact, Eisenhauer and colleagues (17) showed that the proportion of tests of plant diversity effects on soil organisms that were significant increased with time, with significant effects occurring 4–6 y after the establishment of the experiments.

The results of the present study demonstrate that virtually all groups of soil biota were significantly affected by plant diversity and that plant diversity effects were stronger than effects of the prominent global change agents—elevated atmospheric CO_2 concentration and N deposition (17). This pattern did not apply only to density and diversity of soil organisms but also to their functioning, as indicated by significantly higher soil microbial carbon use efficiency with increasing plant species richness. Our findings support the conclusions of Tilman and colleagues (8), posing that loss of biodiversity may have at least as great an impact on plant productivity as other selected anthropogenic drivers of environmental change. The present study indicates that this finding may extend to plant diversity effects on soil food webs and functions. However, future experimental and synthesis work will be needed to evaluate how well our findings apply to other settings and contexts and should investigate the relative importance of additional global change drivers. For instance, plant diversity effects on soil biota may be particularly strong in the present study because the sandy soil at the field site is extremely poor in soil organic matter. Substantial increases of soil organic matter with increasing plant diversity, such as reported by Fornara and Tilman (38) in an adjacent plant diversity experiment, are likely to fuel soil food webs that are mainly based on decomposer species.

Notably, our plant species richness treatments span a significant portion of the range of native diversity (standardized by spatial scale) from species-rich native savanna to postagricultural successional grasslands at Cedar Creek (8, 39). Roughly half of the significantly influenced variables increased linearly or exponentially with plant species richness, contradicting the notion that effects of plant diversity on soil organisms generally saturate at low plant diversity levels. Also, plant diversity effects were not due to the presence of any one plant functional group, but plant diversity explained plant functional group effects. We therefore suggest considering plant diversity loss as a major driver of the biodiversity and functioning of soils.

Methods

Site and Experimental Design.
The present experiment was conducted within the BioCON experiment at the Cedar Creek Long Term Ecological Research (LTER) site in Minnesota (9) (see *SI Methods* for more details on methods). The region has a continental climate with cold winters (mean January temperature, −11 °C) and warm summers (July temperature, 22 °C) and

a mean annual precipitation of 660 mm (9). The soils are sands (Typic Udipsamment, Nymore series; 94.4% sand, 2.5% clay). The BioCON experiment simultaneously manipulates plant diversity, atmospheric CO_2 concentrations, and N deposition in experimental grassland plots under field conditions, using a well-replicated split-plot experiment comprising a full factorial combination of treatment levels in a completely randomized design (9). It was established in 1997 on a level, secondary successional grassland after removing prior vegetation (9), and experimental treatments had been continuously ongoing for 13 y at the time of the present study in summer 2010.

For the present experiment, we used experimental plots (each 2×2 m) with 1, 4, and 9 (but not 16) species, because of the laborious nature of soil organisms analysis. We focus on these plant diversity levels as they represent common small-scale plant species richness numbers in the study region and cover the range from disturbed grassland of anthropogenic origin to medium-high diversity native vegetation. Restored prairie on previously farmed sites had a mean of 3.5 species per m² and a range of 1–8 species per m² (40), whereas native savanna grasslands average 10 plant species per 0.5 m² at our site (41). Plots were planted with 1 (119 plots), 4 (51 plots), or 9 species (34 plots) randomly selected from a pool of 16 herbaceous species representing four functional groups (C3 grasses, C4 grasses, legumes, and nonleguminous forbs).

CO_2 treatments consist of ambient and elevated CO_2. Six circular areas (24 m diameter) were randomly assigned, three each to ambient and elevated CO_2 (+180 ppm, from early spring to late fall) using free-air CO_2 enrichment (FACE) technology (9). Nitrogen was added to the surface of half the plots in each ring as 4 g $N \cdot m^{-2} \cdot y^{-1}$ slow-release ammonium nitrate (NH_4NO_3) in equal fractions in early May, June, and July.

Samplings and Measurements.
In August 2010, we took soil samples to investigate treatment effects on soil biota. From each of the 204 plots, we took one 5- and three 2-cm-diameter soil samples (0–6 cm depth) using steel corers according to the size of the target organisms. Analyzing soil biota across the different size classes (microorganisms, microfauna, and microarthropods) is laborious, and we thus were unable to fully assess temporal and spatial heterogeneity. We aimed at addressing this issue by covering some spatial heterogeneity by taking three 2-cm-diameter soil samples per plot and by sampling a high number of replicates during the period of peak plant biomass. Moreover, investigating plant diversity effects in four $CO_2 \times N$ scenarios allowed us to study effects in different environmental contexts.

The 2-cm-diameter soil samples were pooled in plastic bags, carefully homogenized, and stored at 4 °C until processing. Large soil samples were kept intact and stored in plastic containers at 4 °C until processing. Soil from the 2-cm-diameter samples was subdivided into two portions of 15 g of soil (fresh weight) and used to measure soil microbial parameters and nematodes; 5-cm-diameter samples were extracted for soil microarthropods.

Microbial biomass C and respiration of 5 g soil (fresh weight; sieved at 2 mm) was measured using an O_2-microcompensation apparatus (42). Nematodes and microarthropods were extracted using standard protocols and were identified. To investigate how plant diversity and different global change agents influence soil biota, we measured several potential explanatory variables (see below).

Statistical Analyses.
General linear models (GLMs) were used to test the effects of CO_2, N, and plant species richness and interactions on soil organisms and functions (Table 1). To investigate whether plant diversity effects were solely due to the presence of plant functional groups and vice versa, we additionally performed sequential GLMs and fitted the presence of plant functional groups before plant species richness or the other way round. If this approach renders the effect of the second variable insignificant, plant diversity effects would arise solely because of variance explained by the first variable. By contrast, if effects of the second variable remain significant, those effects do not arise solely from the variable fitted first. To explore the shape of response between plant species richness and soil biota variables, we fitted either linear (plus exponential), saturating (log), or humped-shaped response curves and report the shape of response with the highest R^2 value (Table 1).

We used path analysis to investigate how plant diversity affects decomposer food webs and bacterial and fungal energy channels in soil (as indicated by soil nematode communities). The results of ANOVAs informed the initial path analysis model. The model fit was determined via χ^2 tests and AIC. We tested seven hypothetical pathways regarding how plant diversity affects soil biota; six of them were measured directly [soil water content, shoot, litter, and root biomass, quality of plant inputs (N concentration of plant tissue and soil), and soil pH]. For potential effects of rhizodeposits, we

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assumed that any effects of that type would be either covered by direct paths from plant diversity to endogenous variables or should have been captured by the model modification indices through other endogenous variables (35).

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