Plant diversity drives soil microbial biomass carbon in grasslands irrespective of global environmental change factors

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Abstract

Soil microbial biomass is a key determinant of carbon dynamics in the soil. Several studies have shown that soil microbial biomass significantly increases with plant species diversity, but it remains unclear whether plant species diversity can also stabilize soil microbial biomass in a changing environment. This question is particularly relevant as many global environmental change (GEC) factors, such as drought and nutrient enrichment, have been shown to reduce soil microbial biomass. Experiments with orthogonal manipulations of plant diversity and GEC factors can provide insights whether plant diversity can attenuate such detrimental effects on soil microbial biomass. Here, we present the analysis of 12 different studies with 14 unique orthogonal plant diversity × GEC manipulations in grasslands, where plant diversity and at least one GEC factor (elevated CO_2, nutrient enrichment, drought, earthworm presence, or warming) were manipulated. Our results show that higher plant diversity significantly enhances soil microbial biomass with the strongest effects in long-term field experiments. In contrast, GEC factors had inconsistent effects with only drought having a significant negative effect. Importantly, we report consistent non-significant effects for all 14 interactions between plant diversity and GEC factors, which indicates a limited potential of plant diversity to attenuate the effects of GEC factors on soil microbial biomass. We highlight that plant diversity is a major determinant of soil microbial biomass in experimental grasslands that can influence soil carbon dynamics irrespective of GEC.

Keywords: biodiversity loss, carbon dynamics, drought, long-term experiments, microbial activity, plant biomass

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Introduction

Soil microorganisms influence many ecosystem processes related to the maintenance of soil fertility (Yao *et al.*, 2000) and the regulation of biogeochemical cycles (Cleveland & Liptzin, 2007; Schimel & Schaeffer, 2012).

Moreover, the amount of soil microbial biomass carbon plays a major role in driving the balance between the release of soil carbon (respiration) and its sequestration in soil organic matter in terrestrial ecosystems (Miltner *et al.*, 2011; Lange *et al.*, 2015). Therefore, factors that alter the amount of soil microbial biomass are likely to change carbon dynamics in soil (Bardgett *et al.*, 2008).

Grasslands are a major reservoir of soil carbon (Ciais *et al.*, 2010), covering ~30% of the Earth's land

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surface and storing ~23% of the global terrestrial ecosystem carbon stock (Whittaker & Likens, 1975; Trumper *et al.*, 2009), which makes them a crucial model system to study drivers of soil microbial biomass. Some recent studies in experimental grasslands highlighted that plant diversity increases soil microbial biomass via driving inputs of organic matter and regulation of soil moisture (Zak *et al.*, 2003; Lange *et al.*, 2015), as diverse communities are more productive and their denser canopies cause a reduced loss of soil water (Eisenhauer *et al.*, 2013; Vogel *et al.*, 2013). Further, these studies showed weaker responses of soil microbial biomass to global environmental change (GEC) factors, such as N addition and summer drought, than to variation in plant diversity (Eisenhauer *et al.*, 2013; Vogel *et al.*, 2013), which parallels the response of plant biomass to plant diversity and GEC factors (Tilman *et al.*, 2012). Although high plant diversity may provide a more stable supply of resources for soil microorganisms (Milcu *et al.*, 2010) and could therefore buffer the destabilizing effects of other GEC factors (Zhang *et al.*, 2005; Treseder, 2008), the aforementioned case studies found limited support for this hypothesis (Eisenhauer *et al.*, 2013; Vogel *et al.*, 2013). Given that GEC factors are projected to intensify in their extent and magnitude over the next decades (Vitousek *et al.*, 1997; Field *et al.*, 2014) and that these factors also drive biodiversity loss, a comprehensive across-study analysis is needed to explore whether negative effects of plant diversity loss on microbial biomass are exacerbated by GEC factors.

Interactive effects between plant diversity and GEC factors on microbial biomass could be manifested via a wide range of mechanisms. For instance, high diversity plant communities can retain higher soil moisture than those with low diversity (Eisenhauer *et al.*, 2013; Lange *et al.*, 2014), which may dampen the detrimental effects of drought or warming on soil microbial biomass through reductions in soil water content (Serna-Chavez *et al.*, 2013). Furthermore, the different soil microbial communities that are generated by a plant diversity gradient (Zak *et al.*, 2003; Lange *et al.*, 2014) may respond differently to GEC factors (Bloor & Bardgett, 2012). For instance, the autochthonous soil microbial communities that are associated with highly diverse plant communities may respond less to nutrient pulses, such as those caused by fertilization and the presence of earthworms, than the zymogenous microbial communities found with low plant diversity communities (Eisenhauer *et al.*, 2010). Autochthonous microbial communities have higher carbon use efficiency, which implies lower soil respiration per unit of growth than zymogenous microbial communities

with lower carbon use efficiency (Manzoni *et al.*, 2012). The establishment of these more efficient autochthonous soil microbial communities in experimental high diversity plant communities takes several years (Eisenhauer *et al.*, 2010), which makes long-term studies indispensable in the quest to understand plant diversity effects on soil microorganisms (Eisenhauer *et al.*, 2012).

Plant diversity may also amplify the effects of GEC factors on soil microbial biomass. For instance, the effects of elevated atmospheric CO_2 concentrations and N inputs on plant biomass production were reported to be stronger in diverse plant communities due to their higher resource use efficiency (Reich *et al.*, 2001). As a consequence, this amplified plant biomass production can translate into greater organic matter inputs to the soil where plant diversity and elevated CO_2 or N levels can cascade to increased soil microbial biomass (Spehn *et al.*, 2000; Zak *et al.*, 2003).

Recent research has highlighted that the Earth system models used to project global carbon dynamics could be improved by incorporating information on soil microbial properties, including soil microbial biomass (Wieder *et al.*, 2013). While there is evidence that GEC factors influence soil microbial biomass (Blankinship *et al.*, 2011; Serna-Chavez *et al.*, 2013), and these changes are being incorporated into the new generation of Earth system models (Hurrell *et al.*, 2013), plant diversity and its interaction with GEC factors have rarely been implemented. This gap is largely due to a lack of generalization regarding these relationships, thus warranting a synthesis of studies where plant diversity has been orthogonally crossed with other GEC factors and soil microbial biomass has been measured. Accordingly, we analyzed the data from 12 different studies comprising both field and laboratory experiments with 14 unique plant diversity × GEC factor manipulations in grassland with the aim of examining the consistency of main and interactive effects of plant diversity and GEC factors on soil microbial biomass in experimental grasslands.

Materials and methods

Database

We compiled published and unpublished data from experiments that orthogonally manipulated grassland plant diversity and at least one GEC factor. We were able to include the following GEC factors in our study: atmospheric CO_2 concentrations ($n = 4$), nutrient enrichment ($n = 3$), drought ($n = 3$), earthworms ($n = 3$), and warming (n = 1). Warming was only used in calculating two-way interaction effects from

the mixed models (details below). All these GEC factors are recognized to strongly affect ecosystem structure and functioning (Tylianakis et al., 2008). Earthworms were included in this analysis as they represent invasive ecosystem engineers in many ecosystems with the potential to alter ecosystem structure and function (Hendrix et al., 2008), and their loss is also an important component of land-use change as many agricultural management practices reduce earthworm densities, for example, mechanical soil disturbance (Edwards & Bohlen, 1996) and pesticide application (Pelosi et al., 2013).

All studies compiled measured soil microbial biomass carbon using an O_2 microcompensation apparatus (Scheu, 1992) (Appendix S1). In total, data from 12 different experiments with 14 unique plant diversity × GEC manipulations were included in our analyses. Each study had at least three levels of plant species richness and two levels of GEC factor treatment. Among them, seven were field studies and five were experiments carried out in greenhouses or growth chambers (laboratory experiments). Soil sampled in all the studies were from the top layer of soil (5–10 cm deep), where microbial communities are most active due to high soil moisture (Griffiths et al., 2003). Details of all studies are provided in Table 1.

Effect size calculation

We calculated the natural log-response ratio as a metric of effect size for microbial biomass as $\log_{10} [(C_{mic})_{Trt}/(C_{mic})_{Con}]$, where $(C_{mic})_{Trt}$ and $(C_{mic})_{Con}$ are soil microbial biomass from treatment and control, respectively. For plant diversity effects on microbial biomass, three categories were differentiated: low, intermediate (inter), and high. This classification was performed to provide a conservative measure of the strength of plant diversity effects (Tilman et al., 2012) and to account for spatial differences in field vs. laboratory experiments. For instance, a laboratory experiment with 2 or 3 plant species per microcosm was considered equivalent to field experiments with 8 or 12 species per plot and accordingly used in the above classification scheme (see Table 1 for PSR levels used in different studies). This classification was validated by the determination of plant species richness in circular patches of 10 cm in diameter (to represent the diameter mostly used in microcosm laboratory experiments) in plots of the Jena Experiment (Roscher et al., 2004) with 8 and 16 plant species (eight replicates per plant diversity level). The median values of plant species per patch in 8-species plots were about five, whereas in 16-species plots, it was seven species (Figure S1), which is comparable to the intermediate and high diversity levels in microcosms, respectively. Please note that the implications of this study, however, are not affected by the classification procedure as even the most conservative contrast between intermediate and high plant diversity was significant in long-term field studies (Fig. 1).

The mean log-response ratios from different studies were calculated using random effect models [restricted maximum-likelihood estimator (REML)] with 10 000 bootstrapped 95% confidence intervals (bias-corrected estimates) based on the sample variances of log-response ratio using the metafor package (Viechtbauer, 2010) for R statistical software version 3.1.0 (R Development Core Team, 2014). Bootstrapping was carried out in the boot package (Canty & Ripley, 2014). Random effect models account for the variances within and between studies and are considered appropriate when different studies included in the meta-analysis differ from each other in terms of experimental design, location, or duration (Mengersen et al., 2013). The REML estimator was used due to its balance between unbiasedness and efficiency in getting maximum-likelihood estimates from random effect models, compared to other estimators (Viechtbauer, 2005).

Further, to account for the effects of time since establishment of the plant communities on the effect of plant diversity effects on microbial biomass, we used a mixed-effect model approach with experimental duration (expressed in years) as a covariate (commonly known as moderator in the meta-analysis literature) (Viechtbauer, 2010). In all random mixed-effect models, we used study type ('laboratory' or 'field') as a random factor to account for the bias for the differences in the duration of laboratory vs. field studies. Effect size estimates were weighted for each study based on the sum of the study variance and the estimate of random-effects variance (Viechtbauer, 2010) (Table S1).

Interaction effects

We quantified plant diversity and GEC factor interaction effects in two ways. First, we used a meta-analytic approach to quantify variation in the effect size of GEC factors at their ambient and treatment levels in three plant diversity contexts: low, inter, and high. That is, plant diversity (as a linear term with three levels) was used as a covariate to explain variations in effect size of GEC factors on soil microbial biomass. This analysis was performed in the metafor package, built for R statistical software version 3.1.0.

Second, we used linear mixed models to quantify two-way interaction terms and their statistical significance for each study separately. For studies with blocks (used to account for random effects; Table 1), linear mixed-effect models were used to estimate the interaction coefficients using lme4 package (Bates et al., 2013) for R statistical software version 3.02. We further estimated 95% confidence intervals for the interaction coefficients by applying the semiparametric bootstrapping method using bootmer function in lme4 (Bates et al., 2013).

Results

We found significant positive effects of plant diversity (high vs. low plant diversity and intermediate vs. low plant diversity) on soil microbial biomass (Fig. 1). In contrast, drought was the only GEC factor that had a significant negative effect on soil microbial biomass (Fig. 1). All other effects of GEC factors on soil microbial biomass were negative; however, their effect

Table 1 List of studies that were used for the analyses with details on the experimental setup. All these studies simultaneously manipulated plant diversity and at least one GEC factor. Full references of the published studies are provided in Appendix S2

Study	Type of experiment	Location	Levels of plant diversity	GEC factors	Levels of global change agents
Eisenhauer et al. (2012)	Laboratory	Minnesota, USA	1, 2, and 4	Earthworm	Presence and absence of *Lumbricus terrestris*
Eisenhauer et al. (2013)	Field (B)	BioCON Experiment, Minnesota, USA	1, 4, and 9	CO_2 and Nutrient	CO_2 concentration (ambient and +180 ppm); N concentration (ambient and +4 g N m^{-2} yr^{-1})
Ai et al. Unpubl.	Laboratory (B)	Nanjing, China	1, 2, and 3†	CO_2	CO_2 concentration (ambient and +200 ppm)
Milcu et al. (2006)	Laboratory	Darmstadt, Germany	1,4, and 8	Earthworm	Presence and absence of two earthworm species (*Lumbricus terrestris* and *Aporrectodea caliginosa*)
Milcu et al. (2011)	Laboratory (B)	Silwood Park, Ascot, UK	1, 4, and 8	CO_2 and Earthworm	CO_2 concentration (ambient = 400 ppm and elevated = 600 ppm); earthworm (presence and absence of *Lumbricus terrestris*)
Niklaus et al. (2007)	Field (B)	Northwestern Switzerland	5, 12, and 31	CO_2	CO_2 concentration (ambient = 356 ppm and elevated = 600 ppm)
Ramirez et al. Unpubl.	Laboratory	Jena, Germany	1,2, and 4	Drought	50% reduction in water in drought treatments compared to controls
Roscher et al. Unpubl.	Field (B)	Bad Lauchstädt, Germany	1, 2, and 4	Nutrient	Ambient and +NPK fertilizer as 120 : 52 : 100 (kg ha^{-1} yr^{-1}) (N as NO_3-N/NH_4-N equal proportions, P as P_2O_5-P, K as K_2O-K)
Steinauer et al. (2015)	Field (B)	BAC Experiment, Minnesota, USA	1, 4, and 16	Warming	Temperature (ambient, + 1.5, and + 3°C)
Strecker et al. (2015)	Field (B)	Jena Experiment, Jena, Germany	1, 8, and 16	Nutrient	Ambient and +NPK fertilizer as 100 : 43.6 : 83 (kg ha^{-1} yr^{-1}) (N as NO_3-N/NH_4-N equal proportions, P as P_2O_5-P, K as K_2O-K)
Thakur et al. Unpubl.*	Field (B)	DIRECT, Silwood Park, UK	1, 2, and 3†	Drought	Rainfall manipulation (ambient vs. −30% in summer)
Vogel et al. (2012)	Field (B)	Jena Experiment, Jena, Germany	1, 8, and 16	Drought	Ambient rainfall and drought manipulation using roof (−53.7 mm rainfall)

B, block design.
*Experimental design details are provided in Fry et al. (2013).
†Functional diversity.

size was not significantly different from zero (95% CI overlapping with zero; Fig. 1). Plant diversity did not explain the variations in effect size of any GEC factor on soil microbial biomass (Fig. 2). These results were supported by insignificant interaction effects among all 14 two-way interactions tested between plant diversity and GEC factors (Table 2). Further, few GEC factors showed a significant effect on microbial biomass (three
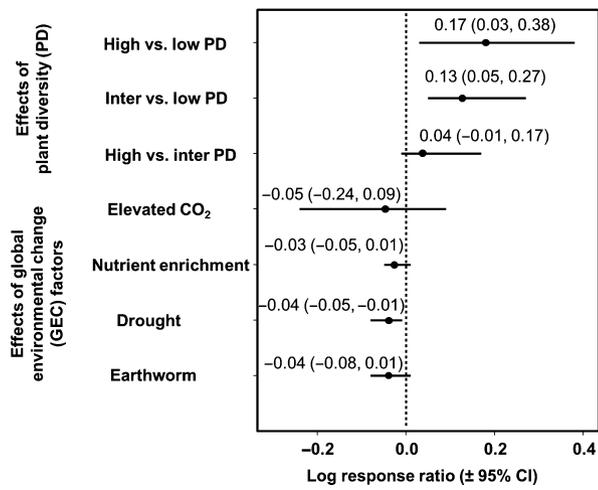
Fig. 1 Effect sizes (mean log-response ratio) of plant diversity and GEC factors on soil microbial biomass with bootstrapped 95% confidence intervals (CI). Effect sizes are significant only when confidence intervals do not overlap with zero. The values inside the brackets next to effect size values are low (2.5%) and high (97.5%) confidence intervals. The details of effect size and confidence intervals of GEC factors are provided in Table S3.

in total), all of them causing a decline, compared to consistently stronger and positive (except one negative out of five significant effects) effects of plant diversity, when studies were analyzed separately (Table S2).

We found plant diversity effects on soil microbial biomass were most pronounced in long-term field studies (Fig. 3). Plant diversity effects were greater in high vs. low and inter vs. low contrasts as compared to high vs. intermediate plant diversity contrasts. Large variability in effect sizes (grater size of confidence intervals) was common in short-term laboratory studies (Fig. 3). Further, when time since establishment of plant communities was used as a covariate, we found that it explained a significant fraction of the variance in effect size and was positively correlated with the effect size for high vs. low plant diversity comparisons (slope = 0.07, 2.5% CI = 0.06, 97.5% CI = 0.08). We found similar results for the variations in effect sizes of inter vs. low plant diversity (slope = 0.04, 2.5% CI = 0.03, 97.5% CI = 0.05) and high vs. intermediate plant diversity (slope = 0.02, 2.5% CI = 0.01, 97.5% CI = 0.03) (Fig. 4).
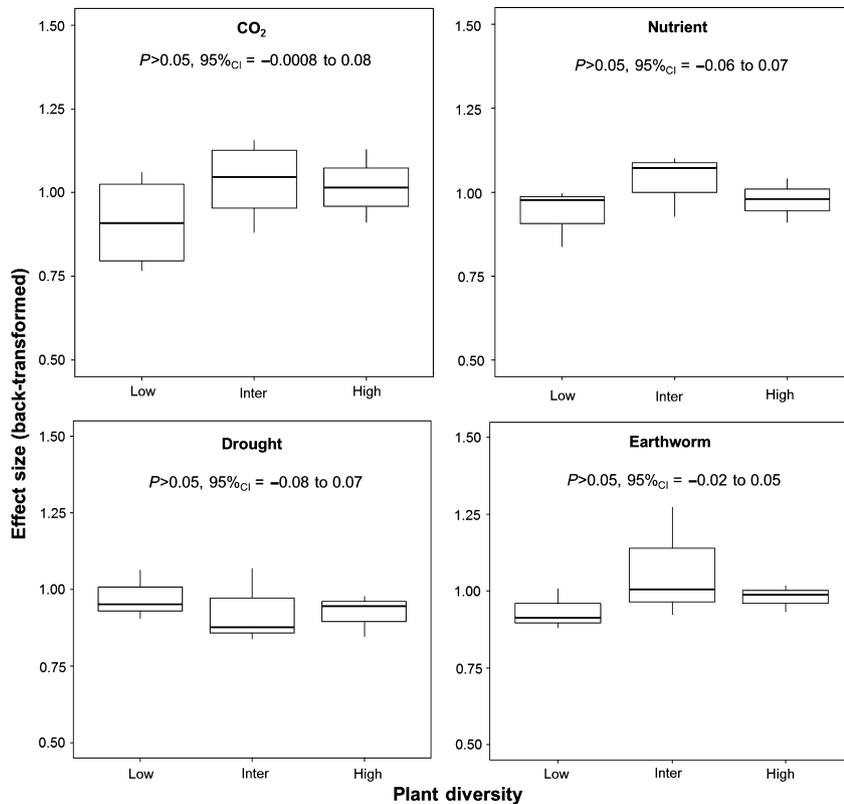

Fig. 2 Effects of GEC factors on microbial biomass at three levels of plant diversity (Effect sizes are reported as backtransformed log-response ratio). The statistics shown in the figure are calculated using plant diversity as a covariate to explain variations in effect sizes of the GEC factors shown in the figure.

Table 2 Bootstrapped coefficient of interaction terms from the linear mixed models for interactions between plant diversity and GEC factors on microbial biomass (log-transformed). Confidence intervals (CI) are 95% percentile bootstrapped. Slopes and their significance for the main effects (plant diversity and GEC factors) from the mixed-effect models are provided in Table S1

| Study	Interaction terms	Slope	Low CI (2.5%)	High CI (97.5%)	P-value
Eisenhauer et al. (2012)	Plant diversity × Earthworm	−0.0398	−0.1318	0.0834	0.49
Eisenhauer et al. (2013)	Plant diversity × CO_2	0.0270	−0.0142	0.0695	0.19
Eisenhauer et al. (2013)	Plant diversity × Nutrient	−0.0030	−0.0450	0.0390	0.88
Ai et al. Unpubl.	Plant diversity (functional) × CO_2	0.0889	−0.0432	0.2226	0.18
Milcu et al. (2006)	Plant diversity × Earthworm	0.0029	−0.0122	0.0191	0.39
Milcu et al. (2011)	Plant diversity × Earthworm	−0.0050	−0.0239	0.0135	0.56
Milcu et al. (2011)	Plant diversity × CO_2	0.0060	−0.0121	0.0256	0.54
Niklaus et al. (2003)	Plant diversity × CO_2	−0.0145	−0.0658	0.0388	0.53
Ramirez et al. Unpubl.	Plant diversity × Drought	0.0769	−0.0829	0.2598	0.36
Roscher et al. Unpubl.	Plant diversity × Nutrient	−0.0017	−0.0511	0.0492	0.97
Steinauer et al. (2015)	Plant diversity × Warming	0.0006	−0.0124	0.0136	0.68
Strecker et al. (2015)	Plant diversity × Nutrient	0.0075	−0.0052	0.0211	0.23
Thakur et al. Unpubl.	Plant diversity (functional) × Drought	−0.0210	−0.1704	0.1317	0.77
Vogel et al. (2013)	Plant diversity × Drought	0.0001	−0.0105	0.0116	0.95
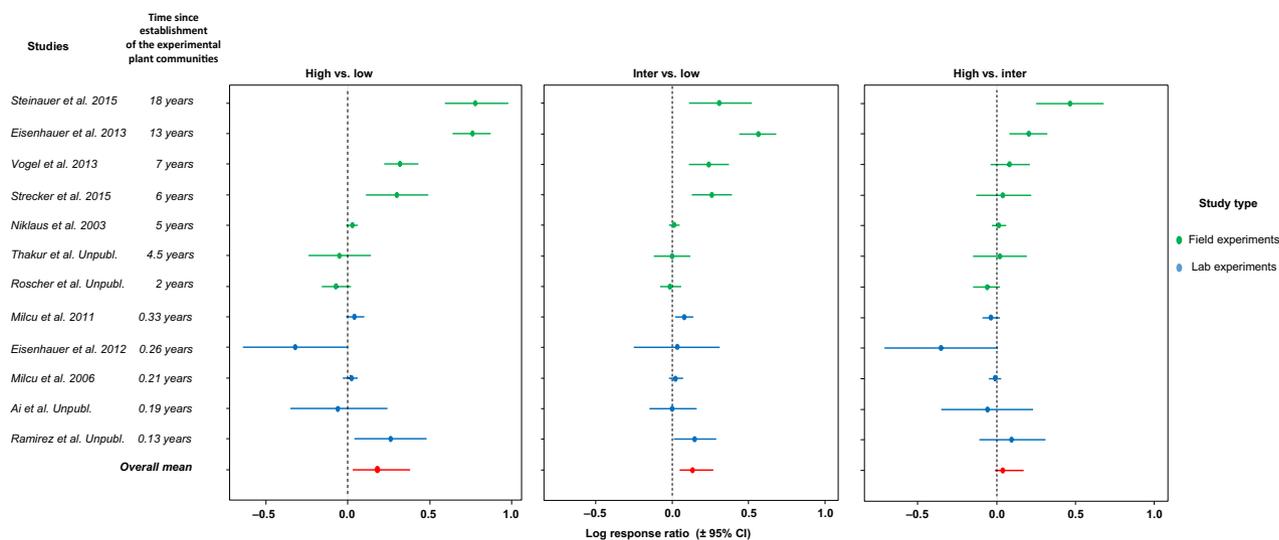
Fig. 3 Effect size (log-response ratio) of plant diversity effects on soil microbial biomass with 95% confidence intervals for 12 studies categorized as field and laboratory experiments. Studies are ordered in terms of their study duration from longer to shorter (given in years). The overall effect size in red color resembles the one provided in figure 1 for plant diversity effects. The details of effect size and confidence intervals are available in Table S4.

Discussion

Our results provide the first quantitative across-study evidence for strong plant diversity effects on soil microbial biomass in long-term field experiments, but also shows that plant diversity has a limited capacity to attenuate the effects of other GEC factors. Notably, we did not detect a single significant interaction effect between plant diversity and other GEC factors on soil microbial biomass (Fig. 2, Table 2), indicating that long-term plant diversity effects are strong but invari-

ant across global change contexts. Further, we observed a greater positive effect size of plant diversity effects on soil microbial biomass than effects of elevated atmospheric CO_2 concentrations, nutrient enrichment, drought, and earthworms from grassland experiments with orthogonal manipulations of plant diversity and GEC factors (Fig. 1).

The strong and positive plant diversity effects on soil microbial biomass could be due to several non-mutually exclusive mechanisms. First, a study comparing the sizes of the effects of various GEC factors on net
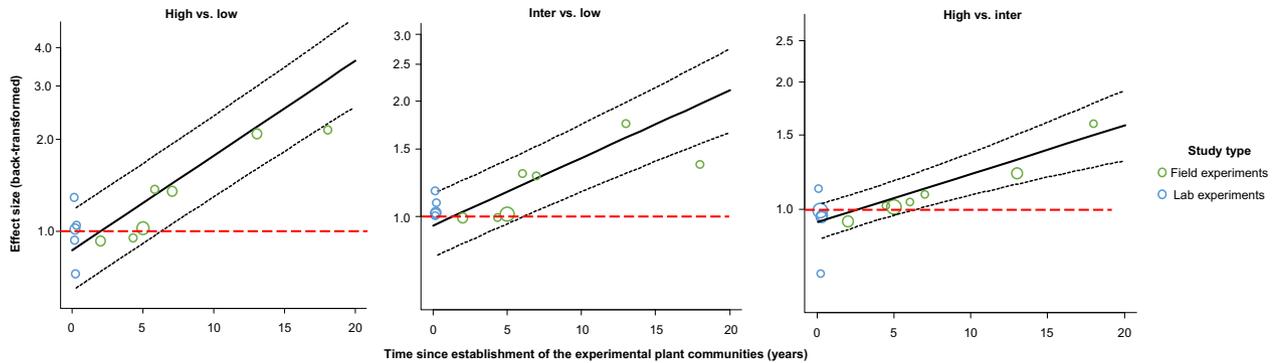
Fig. 4 Relation between the time since establishment of the plant community and the effect sizes of plant diversity on soil microbial biomass (back-transformed by taking exponentials of log-response ratio) from 12 studies. The dashed lines are ± 95% confidence intervals. The thick red line indicates an effect size of 1, that is, neutral plant diversity effect on soil microbial biomass. The size of the circles indicates the approximate weight of the study from the mixed-effect model. Exact weights for each studies are provided in Table S1.

primary productivity found that experimental manipulations of plant biodiversity had as great or greater an impact on net primary productivity as nitrogen addition and had greater effect sizes than elevated CO_2, fire, herbivory, and drought or water addition (Tilman *et al.*, 2012). As microbial biomass should increase with plant productivity within a given region (Zak *et al.*, 2003), the greater effect size of plant species diversity on plant productivity could contribute to the responses of microbial biomass that we observed. To test this hypothesis, we explored whether the positive relation between aboveground productivity and soil microbial biomass holds true in studies with a stronger plant diversity effect on soil microbial biomass (Fig. 3). Indeed, we found a positive association between aboveground productivity and microbial biomass with negligible effects of GEC factors modifying this relationship (Fig. S2).

Second, microbial biomass could be regulated via direct relationships between plant roots and microbial growth (Grayston & Wang, 1998; Bever *et al.*, 2012), along with indirect effects of plant diversity on soil microhabitat conditions, such as soil temperature (Spehn *et al.*, 2000) or moisture (Eisenhauer *et al.*, 2013). Diverse plant communities have been shown to fuel microbial growth in soil more than low diversity plant communities (Hooper *et al.*, 2000; Stephan *et al.*, 2000), and this may be due to higher amounts of rhizodeposits (Knops *et al.*, 2002; Lange *et al.*, 2015). Moreover, diverse plant communities are expected to have higher phylogenetic and root trait diversity, and this may in turn result in the exudation of a more diverse range of organic compounds into their rhizosphere, which can sustain higher microbial biomass (Hooper *et al.*, 2000). Positive plant diversity effects could also be mediated via changes in soil physio-chemical factors particularly

via soil moisture – a key abiotic factor regulating soil microbial biomass (Wardle, 1992) – that has been shown to positively correlate with high plant diversity in the topsoil (Eisenhauer *et al.*, 2013; Lange *et al.*, 2014).

Global environmental change factors can affect soil microbial biomass by altering resource availability in the soil. For instance, a meta-analysis showed that N enrichment decreased soil microbial biomass across ecosystems by inhibiting microbial growth and activity, mostly by reducing fungal biomass (Treseder, 2008). Another recent meta-analysis also found that N enrichment detrimentally affects soil microbial biomass due to a net decline in carbon acquisition by plant roots at high nitrogen availability, which in turn reduces carbon availability for soil microorganisms (Janssens *et al.*, 2010). Our study also showed an overall negative effect (although not significant) of nutrient enrichment on soil microbial biomass; thus, our results are in line with Treseder (2008) and Janssens *et al.* (2010). However, as our study focused on orthogonal manipulations of plant diversity and GEC drivers, we had lower replication than these other studies.

Studies in North American grasslands reported that elevated CO_2 and N enrichment only marginally increased plant biomass production when compared to the effect size of plant species richness (Reich *et al.*, 2001), with limited potential to have any cascading effect on soil microbial biomass (Eisenhauer *et al.*, 2013). We speculate that weak GEC effects on soil microbial biomass might have been possibly due to relatively minor alterations of carbon availability in the soil by GEC factors in our analysis. This could also be true for the varying strength of plant diversity effects on microbial biomass in the studies considered; that is, plant diversity effects on soil microbial biomass were only significant in cases where plant diversity also

increased soil carbon concentrations (Eisenhauer *et al.*, 2010). In addition, recent studies have shown that soil moisture and plant-derived organic matter inputs controlled soil microbial biomass and activities (Lange *et al.*, 2014, 2015).

Our results on drought showed a negative effect on soil microbial biomass, which is consistent with the idea that soil moisture is an important regulator of soil microbial communities (Wardle, 1992; Serna-Chavez *et al.*, 2013). Drought can affect microbial physiology even in the short term, while population and community level responses, which would be manifested in the microbial biomass, can be less pronounced (Schimel *et al.*, 2007). This could explain the relatively weak effect of drought on microbial biomass (all drought studies ran for <2 years). Further, the drought treatments were typically applied for short periods of only weeks to months, while variations in plant diversity may influence soil moisture over longer periods of time, for example, throughout the whole growing season.

The effects of GEC factors on soil microbial biomass could also depend on how strongly they influence biotic interactions between soil microbial communities and plants (Rouifed *et al.*, 2010; Bloor & Bardgett, 2012). GEC factors, such as drought, could intensify competition for nutrients between soil microorganisms and plants, if the microbial communities are poorly adapted to tolerate drought (Bloor & Bardgett, 2012), and diverse plant communities may be more efficient in their nutrient uptake (Hooper & Vitousek, 1998). Such a situation could cause an interactive effect between plant diversity and drought on microbial biomass (Bloor & Bardgett, 2012). The lack of interaction between GEC factors and plant diversity in our study indicates that microbial communities in high diversity plant communities were probably adapted to the manipulated GEC factors. However, this speculation needs further experimental investigation. Some GEC factors, such as drought, could also shift soil microbial community composition, for example, by altering the balance between aerobic and anaerobic microbial biomass (Fenner & Freeman, 2011). The latter is not represented by the substrate-induced respiration method used in the studies that entered our analysis, and so, it is possible that such changes went undetected. Future studies are required to investigate possible functional shifts in soil microbial communities in response to plant diversity and GEC factors and the implications of this for microbial biomass and soil carbon turnover.

Time since plant community establishment plays a crucial role for the effect of plant diversity on microbial biomass, with plant diversity effects often only becoming significant after a time lag of several years (Eisenhauer *et al.*, 2010). This implies that positive associations between soil microbial biomass and diverse plant communities need time to develop due to the slow accumulation of plant-derived carbon resources in the soil over time (Eisenhauer *et al.*, 2012; Kuzyakov & Xu, 2013). Additionally, root exudation, a major resource for soil microorganisms, peaks in grassland diversity experiments after several growing seasons (Harris, 2009). Species-rich plant communities increase complementary resource use with time (Cardinale *et al.*, 2007), and this subsequently increases plant diversity effects on both shoot and root biomass (Reich *et al.*, 2012). Increases in shoot and root biomass, in turn, provide higher resource availability for soil microorganisms, which is likely to increase soil microbial biomass (Spehn *et al.*, 2000; Zak *et al.*, 2000).

Our study highlights the importance of plant diversity as a key driver of soil microbial biomass, with particularly strong effects in long-term field experiments. As those long-term studies provide a more realistic picture of the significance of plant diversity effects (Eisenhauer *et al.*, 2012; Reich *et al.*, 2012), we expect changes in plant diversity to have important implications for soil carbon dynamics (Lange *et al.*, 2015). Although caution must be taken when transferring results of plant diversity experiments to relationships in natural communities, there is some evidence that relationships between plant diversity and soil microbial biomass also are significantly positive in plant removal experiments (Wardle *et al.*, 1999) and in natural plant diversity gradients (Eisenhauer *et al.*, 2011), thus implying that our results have significant implications for local changes in plant diversity in natural settings. As biodiversity is projected to decline in response to GEC factors (Isbell *et al.*, 2013), we also expect indirect effects of GEC on soil microbial biomass via alterations in plant diversity. Although plant diversity may not buffer effects of other GEC factors, it needs to be maintained to maximize soil microbial biomass, due to its importance in the regulation of soil functions, including soil carbon sequestration.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Microbial biomass measurement using O_2 device.

Figure S1. Plant species richness within 10 cm diameter from 8 – and 16 – species mixture plots of the Jena Experiment.

Table S1. Weight of the studies from the random mixed-effect model (duration of plant community establishment as a covariate, see methods) for high vs. low, inter vs. low and high vs. inter plant diversity effect size calculation.

Table S2. Slopes for the main effects of plant diversity and global environmental change (GEC) factors on microbial biomass carbon (log-transformed) from the linear mixed and mixed-effect (those with a randomized block design) models (two-way interactions) from 12 studies. Significant slopes (p-value<0.05) are given in bold.

Figure S2. Relation between plant shoot biomass and soil microbial biomass shown for three global environmental change factors in low, intermediate, and high plant diversity.

Table S3. Effect size (log response ratio) and confidence intervals (CI) of global environmental change (GEC) factors on soil microbial biomass carbon.

Table S4. Effect size (log response ratio) and confidence intervals (CI) of plant diversity effects on soil microbial biomass carbon for three diversity effect comparisons (high vs. low, intermediate vs. low, and high vs. intermediate).

Appendix S2. References.

Supplementary Information

Plant diversity drives soil microbial biomass carbon in grasslands irrespective of global environmental change factors

Madhav Prakash Thakur[1,2,*], Alexandru Milcu[3,4], Pete Manning[5], Pascal A. Niklaus[6], Christiane Roscher[7], Sally Power[8, 9], Peter B. Reich[9,10], Stefan Scheu[11], David Tilman[12], Fuxun Ai[13], Hongyan Guo[13], Rong Ji[13], Sarah Pierce [8], Nathaly Guerrero Ramirez[1,2], Annabell Nicola Richter[14], Katja Steinauer[1,2], Tanja Strecker[11], Anja Vogel[14], Nico Eisenhauer[1,2]

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Appendix S1. Microbial biomass measurement using O_2 device

All the studies included in our synthesis measured the biologically active microbial biomass carbon using an automated O_2 uptake method that involves addition of glucose as a carbon substrate. The details of the method are provided in Scheu (1992). Briefly, soil samples were sieved using a 2 mm sieve prior to the measurement. About 5 g of the sieved soil was added to an automated electrolytic microrespirometer, which were submersed in a water bath to maintain constant temperature (the standard temperature is ~20°C). Glucose was added as a substrate for microorganisms at a concentration of 4 mg/g dry mass dissolved in 400 µL deionized water. After the addition of glucose solution, the microbial respiratory response was recorded at hourly intervals using a computer. The mean of the lowest three readings from the first 10 h is considered as maximum initial respiratory response (MIRR; µL $O_2.h^{-1}.g$ soil dry mass $^{-1}$), which was multiplied with a correction factor of 38 to estimate microbial biomass C (Beck *et al.*, 1997).

Beck T, Joergensen R, Kandeler E, Makeschin F, Nuss E, Oberholzer HR, Scheu S (1997) An inter-laboratory comparison of ten different ways of measuring soil microbial biomass C. *Soil Biology and Biochemistry*, **29**, 1023–1032.

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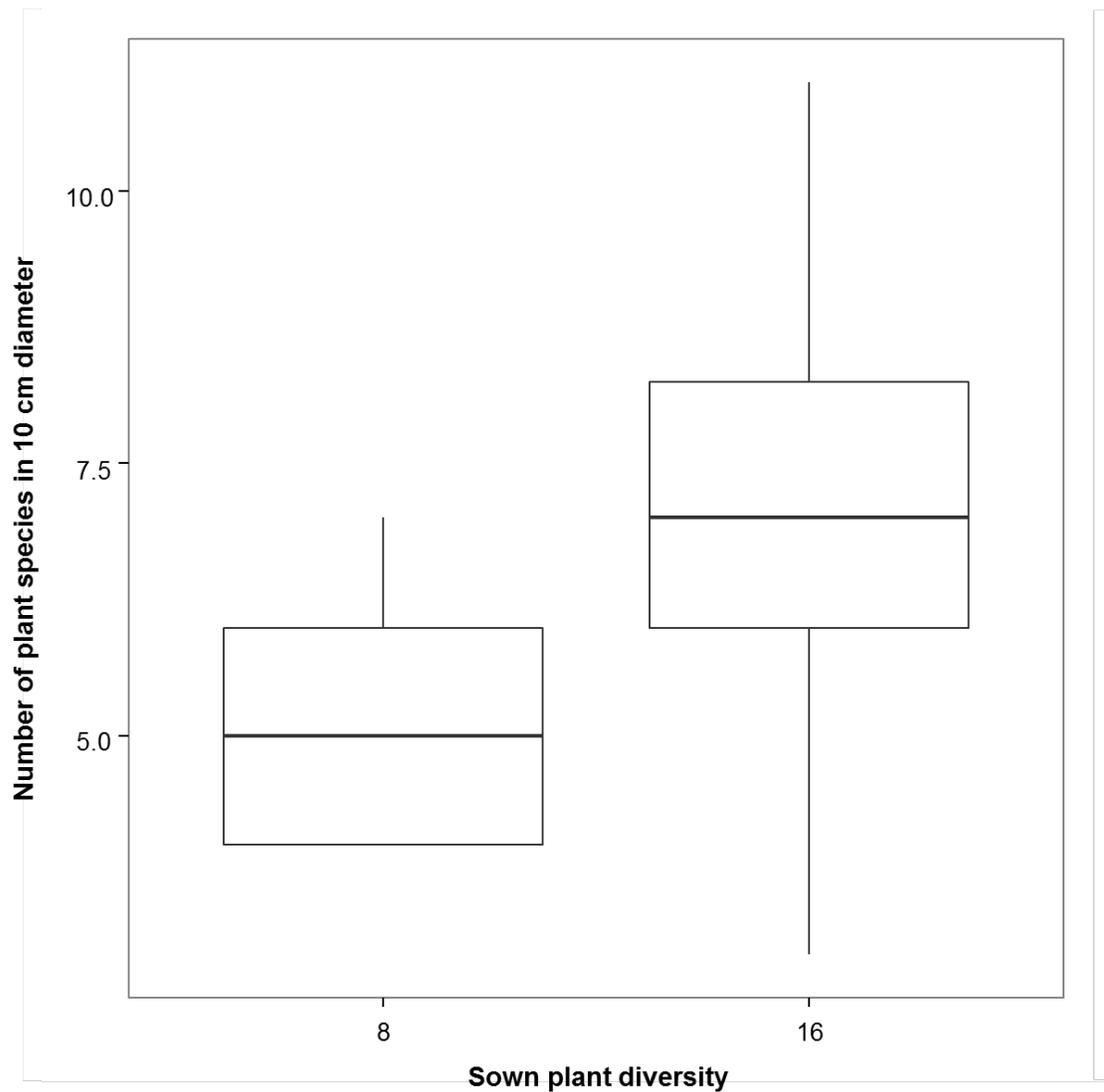
Figure S1. Plant species richness within 10 cm diameter from 8 – and 16 – species mixture plots of the Jena Experiment.

Study	High vs. low plant diversity	Inter vs. low plant diversity	High vs. inter plant diversity
	Weight (%)	Weight (%)	Weight (%)
Eisenhauer et al. 2012	0.93	1.00	0.71
Eisenhauer et al. 2013	6.98	5.60	6.14
Ai et al. Unpubl.	1.11	3.14	1.07
Milcu et al. 2006	19.51	16.16	22.55
Milcu et al. 2011	18.52	14.15	20.24
Niklaus et al. 2003	24.63	25.58	22.74
Ramirez et al. Unpubl.	1.95	3.84	2.05
Roscher et al. Unpubl.	10.43	14.34	11.12
Steinauer et al. 2014	2.47	1.92	1.95
Strecker et al. 2015	2.64	4.71	2.91
Thakur et al. Unpubl.	2.64	5.26	3.19
Vogel et al. 2013	8.19	4.31	5.33

Table S1. Weight of the studies from the random mixed-effect model (duration of plant community establishment as a covariate, see methods) for high vs. low, inter vs. low and high vs. inter plant diversity effect size calculation. The corresponding effect sizes are provided in the supplementary information 3. Weights are estimated as % based on the sum of the study variances together with the estimated random-effect variances.

| Study | Type of experiment | Plant diversity | | GEC factors | GEC factors | |
		Slope	p-value		Slope	p-value
Eisenhauer et al. 2012	Lab	**-0.110**	**<0.01**	Earthworm	0.063	0.85
Eisenhauer et al. 2013	Field	**0.092**	**<0.01**	CO_2	-0.070	0.93
Eisenhauer et al. 2013	Field	**0.110**	**<0.01**	Nutrient	0.009	0.99
Ai et al. Unpubl.	Lab	-0.040	0.91	**CO_2**	**-0.250**	**<0.05**
Milcu et al. 2006	Lab	0.003	0.19	**Earthworm**	**-0.087**	**<0.05**
Milcu et al. 2011	Lab	0.004	0.14	CO_2	0.022	0.14
Milcu et al. 2011	Lab	0.009	0.14	Earthworm	0.015	0.72
Ramirez et al. Unpubl.	Lab	0.06	0.22	Drought	-0.07	0.36
Niklaus et al. 2003	Field	0.011	0.87	CO2	0.049	0.50
Roscher et al. Unpubl.	Field	-0.023	0.06	**Nutrient**	**-0.053**	**<0.05**
Steinauer et al. 2015	Field	**0.020**	**<0.01**	Warming	-0.010	0.87
Strecker et al. 2015	Field	0.005	0.08	Nutrient	-0.057	0.91
Thakur et al. Unpubl.	Field	-0.007	0.6	Drought	0.030	0.91
Vogel et al. 2013	Field	**0.019**	**<0.05**	Drought	0.059	0.06

Table S2. Slopes for the main effects of plant diversity and global environmental change (GEC) factors on microbial biomass carbon (log-transformed) from the linear mixed and mixed-effect (those with a randomized block design) models (two-way interactions) from 12 studies. Significant slopes (p-value<0.05) are given in bold.
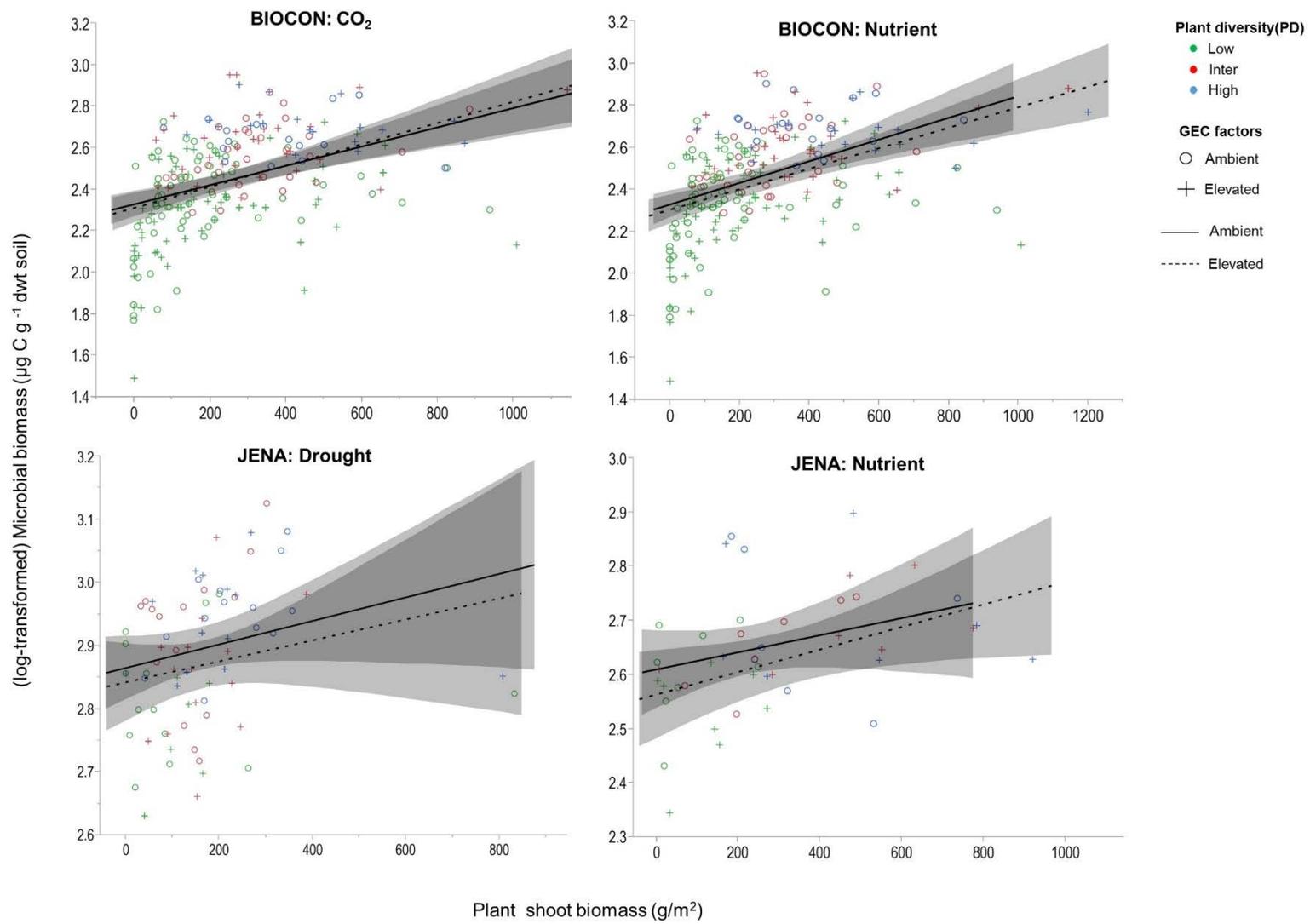
Plant shoot biomass (g/m²)

Figure S2. Relation between plant shoot biomass and soil microbial biomass shown for three global environmental change factors in low, intermediate, and high plant diversity. We show this relation from three studies incorporated in our meta-analysis: Eisenhauer et al. 2013 (BIOCON), Vogel et al. 2013 (JENA Drought), and Strecker et al. Unpubl. (JENA Nutrient). We found a significant positive relation between plant shoot biomass and microbial biomass from ANCOVA analyses with the model structure as Log (Microbial biomass) $\sim \beta_0 + \beta_1 *$ Shoot biomass $+ \beta_2 *$ GEC factor. We also assessed for multi-collinearity in the model and found all models to be valid (variance inflation factors<3). JENA (Drought): Shoot biomass (t-value=2.06, p-value=0.04), Drought (t-value= -1.06, p=0.29); BIOCON (CO_2): Shoot biomass (t-value=7.31, p-value<0.0001), CO_2 (t-value= -0.15, p=0.88); BIOCON (Nutrient): Shoot biomass (t-value=7.40, p-value<0.0001), Nutrient (t-value= -1.06, p=0.28). JENA (Nutrient): Shoot biomass (t-value=2.77, p-value<0.01), Nutrient (t-value= -1.01, p=0.31). The shaded region in the figure below are ± 95% confidence intervals.

Study	GEC factors	Effect Size	Low CI	High CI
Eisenhauer et al. 2012	Earthworm	-0.14	-0.39	0.11
Eisenhauer et al. 2013	CO_2	0.04	-0.09	0.18
Eisenhauer et al. 2013	Nutrient	0.01	-0.12	0.15
Ai et al. Unpubl.	**CO_2**	**-0.18**	**-0.3**	**-0.06**
Milcu et al. 2006	**Earthworm**	**-0.08**	**-0.12**	**-0.04**
Milcu et al. 2011	**CO_2**	**0.06**	**0.01**	**0.11**
Milcu et al. 2011	Earthworm	0.01	-0.04	0.06
Niklaus et al. 2003	CO_2	0.01	-0.09	0.11
Ramirez et al. Unpubl.	Drought	-0.03	-0.18	0.12
Roscher et al. Unpubl.	Nutrient	-0.05	-0.11	0.01
Steinauer et al. 2015	Warming	0.05	-0.2	0.3
Strecker et al. 2015	Nutrient	0.01	-0.1	0.11
Thakur et al. Unpubl.	Drought	-0.01	-0.12	0.1
Vogel et al. 2013	Drought	-0.05	-0.13	0.03

Table S3. Effect size (log response ratio) and confidence intervals (CI) of global environmental change (GEC) factors on soil microbial biomass carbon. Study with significant effect sizes (non-zero CIs) are given in bold

Study	High vs. low plant diversity			Inter vs. low plant diversity			High vs. inter plant diversity		
	Effect Size	Low CI	High CI	Effect Size	Low CI	High CI	Effect Size	Low CI	High CI
Eisenhauer et al. 2012	-0.32	-0.64	0	0.03	-0.25	0.31	-0.35	-0.71	0.01
Eisenhauer et al. 2013	**0.76**	**0.64**	**0.87**	**0.56**	**0.44**	**0.68**	**0.2**	**0.08**	**0.32**
Ai et al. Unpubl.	-0.06	-0.35	0.24	0	-0.15	0.16	-0.06	-0.35	0.23
Milcu et al. 2006	0.02	-0.03	0.06	0.02	-0.02	0.07	-0.01	-0.05	0.03
Milcu et al. 2011	0.04	-0.01	0.1	**0.08**	**0.02**	**0.14**	-0.04	-0.09	0.02
Niklaus et al. 2003	0.03	-0.01	0.06	0.01	-0.02	0.05	0.01	-0.03	0.06
Ramirez et al. Unpubl.	0.26	0.04	0.48	0.15	0.01	0.29	0.10	-0.11	0.31
Roscher et al. Unpubl.	-0.07	-0.16	0.02	-0.01	-0.08	0.06	-0.06	-0.15	0.02
Steinauer et al. 2014	**0.78**	**0.59**	**0.98**	**0.31**	**0.11**	**0.52**	**0.47**	**0.25**	**0.68**
Strecker et al. 2015	**0.3**	**0.11**	**0.49**	**0.26**	**0.13**	**0.39**	0.04	-0.13	0.22
Thakur et al. Unpubl.	-0.05	-0.24	0.14	0	-0.12	0.12	0.02	-0.15	0.19
Vogel et al. 2013	**0.32**	**0.22**	**0.43**	**0.24**	**0.11**	**0.37**	0.08	-0.04	0.21

Table S4. Effect size (log response ratio) and confidence intervals (CI) of plant diversity effects on soil microbial biomass carbon for three diversity effect comparisons (high vs. low, intermediate vs. low, and high vs. intermediate). The same data is visualized in figure 2 in the main text. Low CI indicates 2.5% and high CI indicate 97.5% confidence intervals. Significant effect sizes (non-zero CIs) are given in bold.

Appendix S2. Full references of the published studies included in the analysis (also see table 1 in the main text)

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Milcu A, Paul S, Lukac M (2011) Belowground interactive effects of elevated CO2, plant diversity and earthworms in grassland microcosms. *Basic and Applied Ecology*, **12**, 600–608.

Niklaus P, Alphei J, Kampichler C (2007) Interactive effects of plant species diversity and elevated CO2 on soil biota and nutrient cycling. *Ecology*, **88**, 3153–3163.

Steinauer K, Tilman D, Wragg PD, Cesarz S, Cowles JM, Pritsch, K, Reich, PB, Weisser, WW, Eisenhauer, N (2015) *Plant diversity effects on soil microbial functions and enzymes are stronger than warming in a grassland experiment*. *Ecology*, **96**, 99-112

Strecker T, Barnard RL, Niklaus P A., Scherer-Lorenzen M, Weigelt A, Scheu S, Eisenhauer N (2015) Effects of Plant Diversity, Functional Group Composition, and Fertilization on Soil Microbial Properties in Experimental Grassland. Plos One, 10, e0125678.

Vogel A, Eisenhauer N, Weigelt A, Scherer-Lorenzen M (2013) Plant diversity does not buffer drought effects on early-stage litter mass loss rates and microbial properties. *Global change biology*, **19**, 2795–803.