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

Running head: Soil microbial biomass in grasslands

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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 if 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.

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 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.*, 2003a).

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, e.g. 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) (Supplementary Information 1). 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 done 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 lab 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 lab experiments) in plots of the Jena Experiment (Roscher *et al.*, 2004) with 8 and 16 plant species (8 replicates per plant diversity level). The median values of plant species per patch in 8-species plots was about 5, whereas in 16-species plots it was 7 species (Supplementary Information 2), 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 several long-term field studies.

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 (“lab” or “field”) as a random factor to account for the bias for the differences in the duration of lab 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) (Supplementary Information 3).

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 semi-parametric 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 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 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 (Supplementary Information 4).

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 lab 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).

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 invariant 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 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). Since 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 if 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 (Supplementary Information 5).

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 phylogentic 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).

GEC 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, i.e., 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, Lange et al. 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, e.g., 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 like 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, e.g by altering the balance between aerobic to 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 driver for 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 regultion of soil functions, including soil carbon sequestration.

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Figure legends

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 Supplementary Information 6.

Fig. 2: Effect sizes of GEC factors on soil microbial biomass (back transformed by taking exponentials of log response ratio) at three levels of plant diversity. 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.

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 lab 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 Supplementary Information 7.

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, i.e. neutral plant diversity effect on soil microbial biomass. The size of the circles indicate the approximate weight of the study from the mixed-effect model. Exact weights for each studies are provided in Supplementary Information 3.

Study	***Type of experiment***	***Location***	***Levels of plant diversity***	***GEC factors***	***Levels of global change agents***
Eisenhauer et al. 2012	Lab	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} y^{-1})
Ai et al. Unpubl.	Lab (B)	Nanjing, China	1, 2, and 3#	CO_2	CO_2 concentration (Ambient and +200 ppm)
Milcu et al. 2006	Lab	Darmstadt, Germany	1,4, and 8	Earthworm	Presence and absence of two earthworm species (*Lumbricus terrestris* and *Aporrectodea caliginosa*)
Milcu et al. 2011	Lab (B)	Silwood Park, Ascot, UK	1, 4, and 8	CO_2 and Earthworm	CO_2 concentration (Ambient = 400 ppm & 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 & Elevated = 600 ppm)
Ramirez et al. Unpubl.	Lab	Jena, Germany	1,2, and 4	Drought	50% reduction of 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 Experiement, 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 versus -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)

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 Supplementary Information 8. B = Block design,* Experimental design details are provided in Fry et al. (2013), # Functional diversity.

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

Table 2: Bootstrapped coefficient of interaction terms from the linear mixed models for interaction between plant species richness 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 Supplementary information 1.

Figure 1

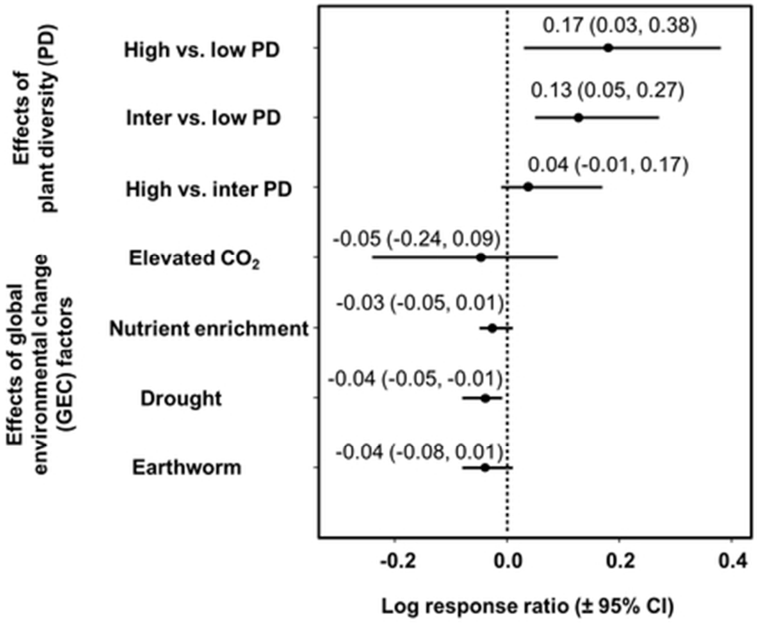

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Figure 2

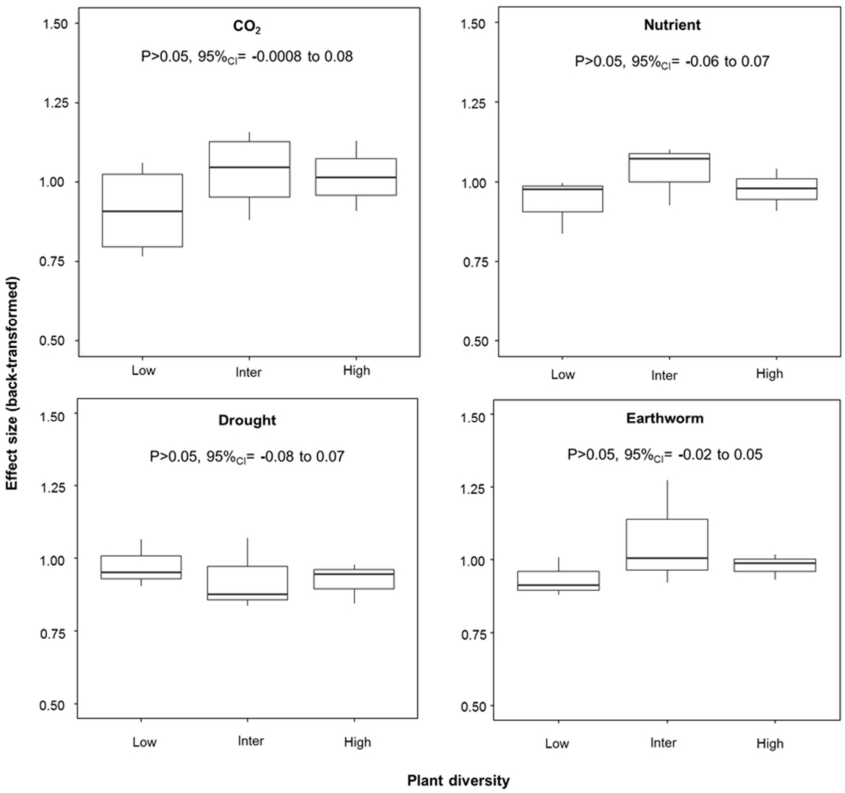

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Figure 3

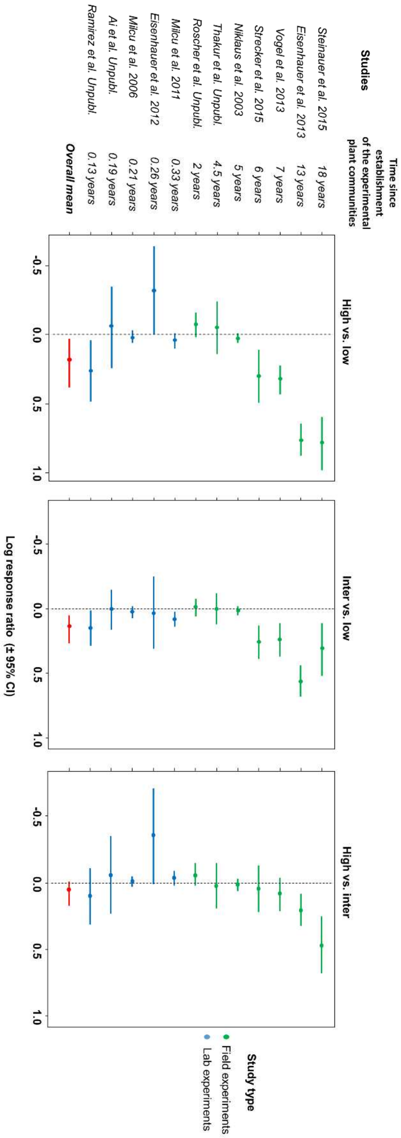

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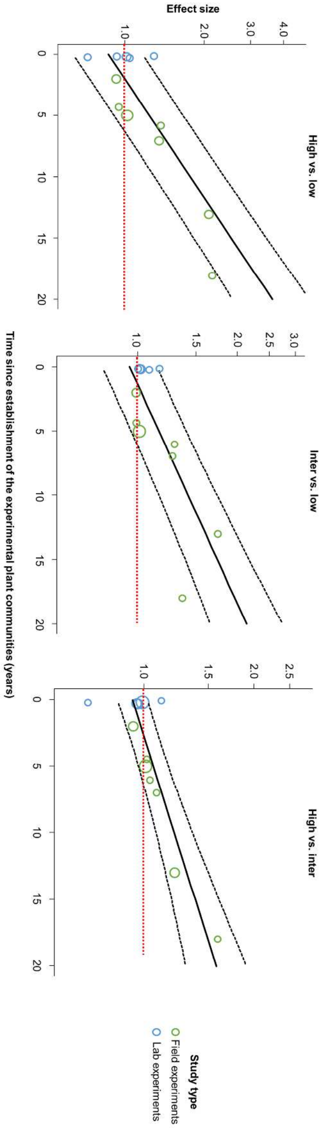
Figure 4



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Response to reviewers' comments

Reviewer: 1

Comments to the Author
This manuscript by Thakur et al. describes a meta-analysis of 12 studies of the impacts of plant diversity x 'global environmental change' factors on soil microbial biomass with the goal of assessing the consistency of GEC effects and their interactions with plant diversity across experiments. The authors' primary finding is that greater plant diversity increased microbial biomass, regardless of GEC factors, but there were no significant effects of GEC factors alone, with the exception of small effect of drought. Further, the authors found no significant interactions between plant diversity and GEC factors, implying that plant diversity has little capacity to mitigate the effects of GEC factors on microbial biomass.
I have a couple of questions regarding this manuscript.

Reply: We thank the reviewer for the very helpful comments on our manuscript.

1. Since the authors found very little effect of any of the GEC factors on microbial biomass overall, does this limit their ability to make conclusions about plant diversity interactions or the potential of plant diversity to 'mitigate' GEC factor impacts on microbial biomass?

Reply: Although we agree with the reviewer that effects of GEC factors were not very strong, the overall effect of drought was significant. Moreover, we found significant effects of GEC factors in three experiments (see SI 4). Thus, taken together, there was clear potential of plant diversity effects to buffer effects of GEC factors. Moreover, a lack of main GEC effects does not necessarily mean that they would not influence the plant diversity effects on microbial biomass. We therefore hypothesized that GEC factors could potentially alter plant diversity effects on microbial biomass, which was not supported by the present dataset.

2. The studies used for the 'nutrient enrichment' factor consist of different application of either N (BioCON) or N+P+K (Jena, Bad Lauchstadt). Can these be rightfully be considered equivalent factors, since the additional applications of P and K may have their own impacts on soil communities and their interactions with plant communities?

Reply: We thank the reviewer for raising this important point. We agree that N and P additions may differentially affect specific microbial functions, however, our results on microbial biomass show similar results for N addition vs. N+P+K addition, and we lack sufficient replicates to treat N and P separately. For these reasons we have chosen to keep our terminology and analysis general (SI 4).

I also think that the discussion section could be significantly clarified and improved (specific comments below).

Line 206: effect size of plant biodiversity on primary productivity?

Reply: Yes, we meant primary productivity. We made this clearer in the revised version of the manuscript.

Line 215-216: Shouldn't soil temperature and moisture effects be captured in your analysis?

Reply: Our analysis cannot incorporate soil temperature and moisture effects due to lack of data.

Line 216: Won't low diversity plant communities also fuel microbial growth?

Reply:Communities with low plant diversity could also fuel microbial growth, such as those containing some legume species (Eisenhauer et al. 2010), however several experimental studies have consistently shown that as plant diversity increases, the amount of soil nutrient resources increases (e.g., Spehn et al. 2000, Milcu et al. 2008), thus fuelling higher microbial growth than in low diversity plant communities.

Line 221: I don't follow this rationale. The little work that I've encountered has found little effect of plant diversity on specific associations between soil microbes and plant species (eg. Schlatter et al., 2015. Plant community richness and microbial interactions structure bacterial communities in soil.)

Reply: Actually, Schlatter et al. 2015 also point out the important role of plant community diversity in shaping the soil bacterial community. Indeed, the presence of species-specific associations between plant and soil microbes was a bit of speculation from our side. We rewrote the sentence as (lines 222-224):

"Moreover, diverse plant communities are expected to have higher root trait diversity and may also exude a diverse range of organic compounds into their rhizosphere, both of which can sustain higher microbial biomass (Hooper et al., 2000)."

Line 223: Again, shouldn't an effect of soil moisture be captured in your drought treatments in the meta-analysis?

Reply: Yes, the drought treatment will decrease soil moisture, and indeed, we found a significant negative (although weak) drought effect on soil microbial biomass. Studies have repeatedly shown strong effects of soil moisture on microbial biomass and some recent studies reported a positive link between plant diversity and soil moisture (lines 227-230). We therefore speculated that plant diversity effects on soil microbial biomass could be mediated via soil moisture. While, the drought treatment typically is applied for only a couple of weeks to months, variations in plant diversity may influence soil moisture over a longer period of time, e.g., throughout the whole growing season.

Lines 227-252: I find this paragraph quite difficult to read and it's difficult to distill the main point. Is this paragraph about resource availability for soil microbes? You skip around among GEC factors often without offering any clear linkages of previous findings to findings in your study.

Reply: We agree with the reviewer that this paragraph was difficult to read. We divided this paragraph into three parts and re-wrote some sentences to increase the clarity. The first paragraph (lines 232-242) now deals with GEC effects on soil resources that can potentially affect microbial biomass. The second paragraph (lines 244-254) explains why GEC factors showed weaker effects on soil microbial biomass. The third paragraph (lines 256-264) deals with potential direct effects of GEC on microbial biomass, such as via affecting microbial physiology.

Lines 228-230: Since Treseder et al., another meta-analysis, finds substantial effects of N deposition on microbial biomass, why isn't this seen in your study? What does N deposition have to do with resource availability for soil microbes?

Reply: Indeed, we missed an important meta-analysis that also showed a reduction of microbial biomass at higher N-deposition (Janssens et al. 2010, Nature Geoscience). We added this in the main text and included briefly why N-deposition may decrease microbial biomass carbon in the soil (lines 235-238). Further we added (lines 238-242):

"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 previous studies".

Line 236: Is moisture or carbon limiting in the soils in the studies you used? Are differences among studies in these controls on microbial biomass a reason why you found no consistent effect of GEC factors?

Reply: This is a very interesting point. We think the reviewer is right about soil resource limitations as a crucial factor determining how GEC may affect soil microbial biomass. Accordingly, we argued (lines 247-249) that GEC factors probably had minor effects on soil carbon concentrations in the studies we incorporated. Further, we added that plant diversity effects on microbial biomass could also operate via carbon availability in the soil (lines 249-254):

"This could also account for the for varying strength of plant diversity effects on microbial biomass in the studies considered, i.e., that plant diversity effects on soil microbial biomass were only significant where plant diversity also increased inputs of carbon to the soil (Eisenhauer et al. 2010). In addition, recent studies have shown that soil moisture and plant-derived organic matter inputs control soil microbial biomass and activity (Lange et al. 2014, Lange et al. 2015)."

Lines 239-242: I agree that a physiological response of microbes to drought can occur rapidly, but, as you highlighted in the introduction, you are utilizing many long-term field experiments. Shouldn't these capture longer-term effects of drought?

Reply: The drought experiments used in our study were all of short duration (<2 years), which is a very likely global change scenario. This is now explained in the manuscript (lines 260-261). Hence, we speculated that short-term physiological responses of soil microbial communities may be more likely than significant changes in soil microbial biomass.

Lines 249-251: Although interesting, this seems like a very large assumption that the authors have no evidence for, especially since the analysis includes a number of relatively short-term greenhouse experiments. I think that a separate paragraph discussing in more detail the possibilities of why you see no interactive effects would be more helpful.

Reply: We agree with the reviewer that this paragraph is speculative. We lack studies that have investigated the underlying mechanisms that may cause interactive effects between GEC and plant diversity on microbial biomass. We now carefully acknowledged the limitation of our speculation, stated the need of further studies, and provide further potential explanations, like shifts in soil microbial community composition (lines 268-276).

Lines 257-258: I think you should clarify what exactly you mean be associations between soil microbes and plant species. Do you mean associations between microbial biomass and plants? Species-specific microbial communities? Much of this paragraph seems redundant with previous parts of the discussion.

Reply: We have clarified that we meant microbial biomass in the sentence. The sentence reads (lines 286-289): **"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)."**

Reviewer: 2

Comments to the Author
Review: Thakur et al. Plant diversity drives soil microbial biomass carbon in grasslands irrespective of global change factors.

I found this to be an interesting paper that for the most part met its goal of determining the interactions between plant diversity and GEC factors. The finding that GEC factors are largely unaffected by plant diversity is relevant and important to our understanding of how GEC factors may impact microbial communities. Results are also applicable to climate change models that seek to incorporate more detailed data on plant diversity and microbial carbon dynamics.

Reply: We appreciate the positive comments and the helpful suggestions of the reviewer.

Some concerns:
Although you used a standard technique for measuring biomass (Substrate-induced O2 consumption), one concern in using this technique in a meta-analysis is how much variation there was from lab to lab in performing this measurement of microbial biomass. As noted in the Beck et al. (1997) comparison, there is high variability in measurements of soil microbial biomass using this technique. Furthermore, the basic assumption of this technique is that you have similar proportions of the microflora reacting to glucose addition. This may not be the case, as the proportion of aerobic (or facultative anaerobes operating aerobically) to anaerobic (or facultative anaerobes operating anaerobically) microbes isn't necessarily the same from soil to soil. In fact, some of your GEC factors, such as drought, could themselves shift the balance of aerobic to anaerobic microbial biomass. Beck et al. (1997) did note high variation in both low biomass and high biomass soils, which could explain some of the high variability between different sites and experiments (for example, since glucose is added on a dry weight basis, differences in mineral content of soils could lead to high variation in the amount of glucose per unit of microbial biomass). I accept that you need to use some standard technique for comparison, but can you rule out the possibility that your results, at least in part, may in fact indicate a shift in the ratio of aerobic microbes to anaerobic microbes?

Reply: This is a valid point noted by the reviewer. We agree that some GEC factors could potentially shift the microbial community in favour of anaerobes, which is not represented by the substrate-induced respiration method. Accounting to this comment, we added a sentence in the discussion (lines 276-279):

"**Some GEC factors, such as drought, could also shift soil microbial community composition, e.g by altering the balance between aerobic to 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."

I think your argument that cores have the same functional attributes of higher diversity plots because you would find a comparable number of plant species in a 10 cm diameter area in the plot is pretty weak. Cores that are removed from the field can have very different microbial attributes. Hosts that are physically distant from the 10 cm diameter circle of the core are still nonetheless influencing the microbial community through fungal linkages and common mycorrhizal networks. When these linkages are severed there can be large shifts in the microbial community, for example a shift from mycorrhizal fungi to saprotrophic fungi (we have noticed this in our lab, unpublished observations). Therefore the act of soil removal or coring will affect microbial community composition and function, and potentially biomass as well. This could well affect response to GEC factors. When you just analyze field studies, do you see the same patterns in your meta-analysis? Or is your power too low with the laboratory/ microcosm experiments removed? I think you need to convince readers that a major component of your observed effects isn't due to the difference between laboratory/microcosm and field experiments.

Reply: We agree with the reviewer that 10 cm diameter area within a field plot could be functionally different compared to a microcosm of 10 cm diameter with the same number of plant species. Please note that none of the studies used intact soil cores extracted from the field, but artificially assembled plant communities in the lab. Please also note that the implications of this study are not affected by the classification procedure of diversity levels as even the most conservative contrast between intermediate and high plant diversity was significant in several long-term field studies.

According to the reviewer's suggestion, we always incorporated the variance explained by study type for plant diversity effects, i.e., differentiating field and microcosm experiments. By contrast, the statistical power of our analyses would have been insufficient for calculating GEC effects on microbial biomass without incorporating microcosm experiments (see Methods, lines 96-97).

It is also interesting that you found an effect using study duration as a covariate. Measuring "time since establishment" of the study could just as easily be based upon a disturbance gradient, with laboratory and microcosm experiments representing highly disturbed and long-term established plots representing sites experiencing low levels of disturbance. It would be useful to follow up the relationship between time since establishment and level of disturbance, since models might gain power by incorporating levels of disturbance in their modelling of GEC impacts.

Reply: We agree with the reviewer, and the role of disturbance and soil legacy effects has been highlighted by some of the authors of this manuscript (Eisenhauer et al., 2012). However, due to lower number of GEC studies we lacked sufficient replication of lab and field studies to allow for the incorporation of 'level of disturbance' as random or fixed effect. We only incorporated study type (lab or field) as a random effect in all our models for estimating plant diversity effects (Lines 148-150).

Minor edits:
L28: add a space between "ecosystems" and "(Miltner…)".

Reply: Done.

L40: what is the “those” in “than those of plant diversity” referring to?

Reply: Changed to: “than effects of plant diversity”.

L52 and following: is it common to refer to “low diverse” and “high diverse” communities? When I read this I was expecting either “high diversity” or “highly diverse”, for example.

Reply: We agree with the reviewer and made changes as* high and low diversity *instead of* high and low diverse *throughout the manuscript.

L66: add a space after “microorganisms” and before the reference.

Reply: Done.

L73: add a space after “biomass” and before the reference.

Reply: Done.

L100: add a space after “disturbance” and before the reference.

Reply: Done.

L107: As previously noted, soil dry weight could be strongly influenced by differences in mineral content. Can you indicate how much variation in mineral content there was between soils?

Reply: Unfortunately, we do not have details of mineral contents of soils from the different studies. We, however, stressed soil resource availability as an important factor determining the effect of GEC on soil microbial biomass (lines 247-254).

L117-119: See my previous comment on the validity of extrapolating lab experiment species richness based upon field experiments. I’m not convinced this reflects the actual similarities between lab and field samples.

Reply: Answered above.

L122: add a space after “Experiment” and before the reference.

Reply: Done.

L130: Either the mean log response ratio “was” calculated, or the mean log response “ratios” were calculated.

Reply: Done.

L166: “significant positive effect size of plant diversity effects” is grammatically awkward. Can you reword to make this sentence clearer?

Reply: We rephrased the sentence as: “We found significant positive effects of plant diversity…”

L181: “greater”.

Reply: Done.

L221: is enhancing microbial associations “with” specific plants better?

Reply: Here, we meant that high diversity plant communities may provide a different variety of niches (in the form of different plant-derived inputs) for soil microbial communities (Hooper et al., 2000). To make this clearer, we removed “specific” and rewrote the sentence (line 224-226).

L225: add a space after “topsoil” and before the reference.

Reply: Done.

L232: add a space after “richness” and before the reference.

Reply: Done.

L258: add a space after “plants” and before the reference.

Reply: Done.

L263: “increase provide”? Please clarify.

Reply: Changed. It reads now as: “Increases in higher shoot and root biomass, in turn, provide..”

L274: add a space after “gradients” and before the reference.

Reply: Done.