PLANT DIVERSITY EFFECT ON SOIL CARBON DYNAMICS

By

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ABSTRACT

Soil organic carbon (SOC) is a valuable natural resource, supplying goods and services for human benefits, including mediating global climate change and securing food production and environmental quality. Biodiversity loss across multi-taxa is at an alarming rate globally. Recent advances have been made in our understanding of the negative impact of biodiversity loss on ecosystem production. The higher biomass production in species-rich communities is expected to enhance plant litter inputs to soils for SOC formation. Despite the critical importance of SOC and Rs in the global carbon and nutrient cycles, our understanding of the effects of plant diversity on SOC and soil respiration (Rs) remains equivocal. The purpose of this dissertation is to provide the first global-scale estimates of changes in Rs and SOC storage in response to global plant diversity loss, and to mechanistically understand the effects of plant mixtures on soil carbon dynamics.

In my first study, I examined the global effects of plant litter alterations on soil carbon release. By presenting a meta-analysis of 100 published studies to examine the responses of Rs to manipulated aboveground and belowground litter alterations. I found that aboveground litter addition increased Rs, while aboveground litter removal, root removal and litter + root removal reduced Rs, respectively. Estimated from the studies that simultaneously tested the responses of Rs to aboveground litter addition and removal and assuming negligible changes in root-derived Rs, "priming effect" on average accounted for 7.3% of Rs and increased over time. My meta-analysis indicates that priming effects should be considered in predicting Rs to climate change-induced increases in litterfall. This analysis also highlights the need to incorporate spatial climate gradient in projecting long-term Rs responses to litter alterations.

In my second study, I examined how plant diversity loss affects Rs. Here I present a meta-analysis using 446 paired observations from 95 published studies to evaluate the effects of plant and litter mixtures on Rs and its components. I found that total Rs and heterotrophic respiration were, on average, greater in plant mixtures than expected from those of monocultures. These mixture effects increased with increasing species richness in both plant and litter mixtures. While the positive effects of species mixtures remained similar over time for total soil respiration, they increased over time for heterotrophic respiration in plant mixtures but decreased in litter mixtures. My structural equation models suggested that the positive effects of species richness and stand age on total and heterotrophic respiration were driven by increased plant inputs and soil microbial biomass. My results suggest that plant diversity loss has ubiquitously negative impacts on soil respiration, one of the fundamental carbon-cycle processes sustaining terrestrial element cycling and ecosystem function.

In my third study, I examined how plant diversity affects SOC. By analyzing 1001 paired observations of plant mixtures and corresponding monocultures from 124 studies, I found that both SOC content and stock are higher in species mixtures than in monocultures. These positive mixture effects increase over time and are more pronounced in deeper soils. Our results indicate that converting 50% of global forests from mixtures to monocultures would release an average of 2.70 Pg C from soil annually over a period of 20 years: about 30% of global annual fossil-fuel emissions. This study highlights the importance of plant diversity preservation for the maintenance of soil carbon sequestration in discussions of global climate change policy.

Finally, my fourth study investigated whether the impacts of tree species mixtures on microbial biomass and composition were altered with variable water availability. This was accomplished by sampling soils from stands that were dominated by *Populus tremuloides* and

Pinus banksiana, respectively, and their relatively even mixtures under water reduction (-25%), ambient, and addition (+25%). Under ambient water conditions, soil microbial biomass was significantly lower in mixtures than expected from those of constituent monocultures. Water reduction increased species mixture effects on total and individual group microbial biomass from negative to neutral, while water addition only increased mixture effects on arbuscular mycorrhizal fungal and GP bacterial biomass. Further, microbial community compositions differed significantly with both overstory type and water alteration treatment. Our results suggested that higher species diversity is not always of benefit for soil microorganisms; however, mixed tree species have the potential to regulate ecosystem responses to climate change.

Our findings might provide us with the knowledge in developing effective forest and agricultural management and conservation strategies to meet international standards for carbon sequestration and mitigate the impacts of global environmental change. Moreover, our analysis provides insights to improve land surface models to better predict the consequences of global change on terrestrial carbon.

Keywords: meta-analysis, litter alteration, priming effect, double litter, soil respiration, plant diversity, experimental duration, heterotrophic respiration, soil carbon storage, plfa, microbial biomass, microbial composition

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NOTE

This is a manuscript-based thesis. The chapters were written to suit the submission requirements of the targeted journals. Formatting and reference styles may differ. Since individual chapters reflect the joint contributions of myself and my academic supervisor, I use "we" instead of "I" for individual manuscripts.

Chapters:

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5. Chen, X. & Chen, H.Y.H. (2019). Water availability regulates negative species mixture effects on soil microbial biomass. Submitted (Soil Biology and Biochemistry).

CHAPTER 1: GENERAL INTRODUCTION

Biodiversity loss across multi-taxa is at an alarming rate globally (Butchart *et al.* 2010). In recent decades, the biodiversity and ecosystem functioning relationship (BEF) has been a major ecological research focus to help understand the impact of global species extinction crisis on ecosystem functioning (Cardinale *et al.* 2012). The relationships between plant diversity and productivity have been well studied, and positive relationships have been observed across different ecosystem types (Ma & Chen 2016; Duffy *et al.* 2017). However, how plant diversity loss affects soil carbon dynamics remains uncertain. SOC is a valuable natural resource, supplying goods and services for human benefits, including mediating global climate change and securing food production and environmental quality (Lal 2004a, b). Global soils store three times more organic carbon than the atmosphere and vegetation combined (Ciais *et al.* 2013; Carvalhais *et al.* 2014), and thus slight changes in SOC can have a profound effect on the global carbon cycle. As the largest terrestrial carbon (C) efflux, Rs is a key ecosystem function that controls terrestrial energy balance and element cycling (Luo & Zhou 2006; Bond-Lamberty & Thomson 2010).

The higher biomass production in species-rich communities and the subsequent input of plant detritus to soil may have nonlinear effects on Rs and SOC via a commonly referred the “priming effect”, i.e., extra decomposition of extant, more stable soil organic matter (SOM) pools when microbes are stimulated by the energy released from the decomposition of fresh organic matter (Fontaine *et al.* 2003; Fontaine *et al.* 2007; Kuzyakov & Gavrichkova 2010). The priming effect could be quantitatively significant and presents a potent challenge to first-order kinetic models of soil C (Fontaine *et al.* 2007; Heimann & Reichstein 2008; Sayer *et al.* 2011). However, previous empirical studies have described priming as short-term and idiosyncratic (Dalenberg & Jager 1989; Leff *et al.* 2012; Cardinael *et al.* 2015; Liu *et al.* 2017a).

Soil microbial biomass plays a fundamental role in controlling both SOC release (microbial respiration) and sequestration (Miltner *et al.* 2012; Zhang *et al.* 2018). Biodiversity loss and climate change are two significant stressors on ecosystem productivity, element cycling, and other ecological processes (Cardinale 2012; Hisano et al. 2018). Both plant diversity and water availability are individually known to influence soil microorganisms (Hicks *et al.* 2018; Valencia *et al.* 2018; Chen *et al.* 2019). Although species-rich forests are thought to ensure more consistent productivity in contrast to monocultures in the context of global environmental change (Yachi & Loreau 1999; Ammer 2019; Hisano et al. 2019), our understanding of whether higher plant diversity could help mitigate the negative impacts of reduced water availability on Rs is surprisingly limited.

The objective of this dissertation was to further our understanding of patterns and mechanisms of plant species diversity effects on soil carbon dynamics. To achieve this goal, I first conducted a global meta-analysis to estimate litter priming effect and its temporal trend. Second, I examined the effects of plant and litter mixtures on Rs and its components and tested whether these effects might increase with species richness in mixtures and stand age or experimental duration. Third, I examined the effects of species mixture on SOC, soil microbial biomass carbon (SMBC), and SMBC/SOC in mineral soils, and tested whether these effects would increase with species richness in mixtures and stand age, and differ across a wide range of ecosystem types, mean annual temperatures, and annual aridity indexes at the study sites and across soil depths. Finally, I undertook an examination of whether water availability may regulate the effects of species mixture on soil microbial biomass and composition in boreal forests. By testing the plant diversity effect on soil microbial biomass, Rs, SOC and associated priming effect, our study will advance understanding beyond relationships between plant diversity and productivity, and gain new mechanistic insights for the diversity effects on soil carbon dynamics over different timescales and under altered environment background.

CHAPTER 2: GLOBAL EFFECTS OF PLANT LITTER ALTERATIONS ON SOIL CO_2 TO THE ATMOSPHERE

2.1 Abstract

Soil respiration (Rs) is the largest terrestrial carbon (C) efflux to the atmosphere and is predicted to increase drastically through global warming. However, the responses of Rs to global warming are complicated by the fact that terrestrial plant growth and the subsequent input of plant litter to soil are also altered by ongoing climate change and human activities. Despite a number of experiments established in various ecosystems around the world, it remains a challenge to predict the magnitude and direction of changes in Rs and its temperature sensitivity (Q_{10}) due to litter alteration. We present a meta-analysis of 100 published studies to examine the responses of Rs and Q_{10} to manipulated aboveground and belowground litter alterations. We found that 100% aboveground litter addition (double litter) increased Rs by 26.1% (95% confident intervals, 18.4% – 33.7%), while 100% aboveground litter removal, root removal and litter + root removal reduced Rs by 22.8% (18.5% – 27.1%), 34.1% (27.2% – 40.9%) and 43.4% (36.6% – 50.2%), respectively. Moreover, the effects of aboveground double litter and litter removal on Rs increased with experimental duration, but not those of root removal. Aboveground litter removal marginally increased Q_{10} by 6.2% (0.2% – 12.3%) because of the higher temperature sensitivity of stable C substrate than fresh litter. Estimated from the studies that simultaneously tested the responses of Rs to aboveground litter addition and removal and assuming negligible changes in root-derived Rs, "priming effect" on average accounted for 7.3% (0.6% – 14.0%) of Rs and increased over time. Across the global variation of terrestrial ecosystems, the effects of aboveground litter removal, root removal, litter + root

removal on Rs as well as the positive effect of litter removal on Q_{10} increased with water availability. Our meta-analysis indicates that priming effects should be considered in predicting Rs to climate change-induced increases in litterfall. Our analysis also highlights the need to incorporate spatial climate gradient in projecting long-term Rs responses to litter alterations.

2.2 Introduction

Terrestrial soils release approximately 98 ± 12 Pg carbon (C) into the atmosphere annually through soil respiration (Rs), which is the largest terrestrial C efflux (Bond-Lamberty & Thomson 2010). This C efflux is predicted to increase drastically through climate warming and consequently accelerate global climate change (Metcalfe 2017). However, the responses of Rs to global warming are complicated by the fact that terrestrial plant growth and the subsequent input of plant detritus to soil also change with ongoing climate change and human activities (Giardina *et al.* 2014; Ward *et al.* 2015; Xu *et al.* 2015). Extensive deforestation and cultivation have decreased plant litter inputs (Holmes *et al.* 2006), but elevated atmospheric CO_2 may increase plant litter inputs via enhanced plant growth and mortality (Hyvonen *et al.* 2007; van Groenigen *et al.* 2014; Brienen *et al.* 2015; Grace *et al.* 2016). Plant litters supply the main source of carbon into the soil and mediate physical changes between soil and atmosphere as a protective layer, thus playing a critical role in Rs (Sayer 2006; Sayer *et al.* 2011; Xu *et al.* 2013a).

Changes in plant litter inputs may have nonlinear effects on Rs (Sayer *et al.* 2011; van Groenigen *et al.* 2014). Plant litter addition increases Rs directly via increased substrate availability for decomposition, and indirectly via a commonly referred the "priming effect", i.e., extra decomposition of extant, more stable soil organic matter (SOM) pools when microbes are stimulated by the energy released from the decomposition of fresh organic matter

(Fontaine *et al.* 2003; Fontaine *et al.* 2007; Kuzyakov & Gavrichkova 2010) (Fig. 2-1). The priming effect could be quantitatively significant and presents a potent challenge to first-order kinetic models of soil C (Fontaine *et al.* 2007; Heimann & Reichstein 2008; Sayer *et al.* 2011). However, perspectives on the priming effect of plant litter on Rs are mixed with some empirical support (Sayer *et al.* 2007; Sayer *et al.* 2011; Han *et al.* 2015). Others have described priming as short-term and idiosyncratic (Dalenberg & Jager 1989; Leff *et al.* 2012; Cardinael *et al.* 2015; Liu *et al.* 2017a). To reconcile these divergent views, a quantitative synthesis of the results across multiple studies may assist in determining the role of the priming effect on Rs. We expected that Rs would increase with litter addition and decreased by litter removal, with a higher decrease with litter + root removal since a major proportion of organic C inputs to soil may be root-derived (Rasse *et al.* 2005; Schmidt *et al.* 2011) , and the increase of Rs by litter addition would outpace the reduction in Rs by litter removal due to litter priming effect.

The accurate estimation of the sensitivity of Rs to temperature is critical for estimating the consequences of a warmer global climate on soil carbon fluxes (Luo *et al.* 2001; Davidson & Janssens 2006). The temperature sensitivity of Rs is commonly quantified as Q_{10}, the rate of change in Rs for every 10 °C difference in soil temperature. Although Q_{10} values derived from field data are highly variable, partially due to the temporal and spatial variations in fresh C substrate availability, they increase with increasing recalcitrance, due to the higher net activation energy required for the decomposition of stable C substrate (Bosatta & Agren 1999). Several experimental and meta-analysis studies support the "C quality–temperature" hypothesis, i.e., Q_{10} and substrate quality are negatively related (Fierer *et al.* 2005; Wetterstedt *et al.* 2010; Liu *et al.* 2017b). Since fresh litter is of higher quality in term of decomposition than stable C substrate, we, therefore, hypothesized that the addition of litter would decrease Q_{10}, whereas litter removal would increase Q_{10}.

A number of experiments have been established in various ecosystems around the world to quantify the effects of litter alteration on Rs and Q_{10} (Fig. S2-1). Despite these efforts, it remains a challenge to predict the magnitude and direction of changes in Rs and Q_{10} to litter alteration due to the high spatiotemporal variability of Rs responses to litter alteration (Sayer 2006; Xu *et al.* 2013a). The impact of litter inputs on Rs may be time-dependent because the priming effect associated with repeated litter addition increased with time due to temporal changes in microbial C use (Qiao *et al.* 2014; Morrissey *et al.* 2017). The secondary effects of litter removal on Rs, such as soil nutrient depletion for soil microbial activity, may also increase over time as litterfall is the primary pathway for soil nutrient and energy cycling (Sayer 2006; Sayer & Tanner 2010a). We, therefore, hypothesized that the positive effect of litter addition and the negative effect of litter removal on Rs would also increase over time. As soil labile C availability associated with repeated litter addition may continue to increase, we expected that the negative effect of litter addition on Q_{10} would increasingly become more negative temporally. We expected that the positive effect of litter removal on Q_{10} would increase because the relative abundances of labile C to stable C would decrease over time without fresh litter inputs.

The responses of Rs to litter alterations may also vary depending on temperature and water availability since high temperatures could increase, whereas reduced water availability decreases both litter decomposition and the priming effect (Sayer *et al.* 2011; Zhu & Cheng 2011; Vogel *et al.* 2013; Martinez *et al.* 2014). Moreover, the responses of Q_{10} to litter alteration may also vary spatially. We hypothesized that, compared with cold and dry climates, warm and wet climates with higher litterfall production (Wu *et al.* 2011; Xu *et al.* 2013b) would decrease the responses of Q_{10} to litter addition, whereas removal of high litterfall inputs would increase the responses of Q_{10}, as predicted by the "C quality–temperature" hypothesis (Fierer *et al.* 2005; Wetterstedt *et al.* 2010; Liu *et al.* 2017b).

Two previous meta-analyses have discussed Rs under litter alterations. Xu et al. (2013a) assessed the response of Rs, as well as several other soil processes, to altered aboveground litter manipulation, and Subke et al. (2006) investigated the impact of root removal on Rs. Here we specifically tested how the responses of Rs and Q_{10} to litter addition, litter removal, root removal and litter + root removal responded to changes in experimental duration, water availability and mean annual temperature. We performed a meta-analysis of 2816 observations from 100 published studies that were conducted in forests, grasslands, and croplands. All these studies used double litter and complete litter removal as aboveground litter addition and litter removal treatments, respectively.

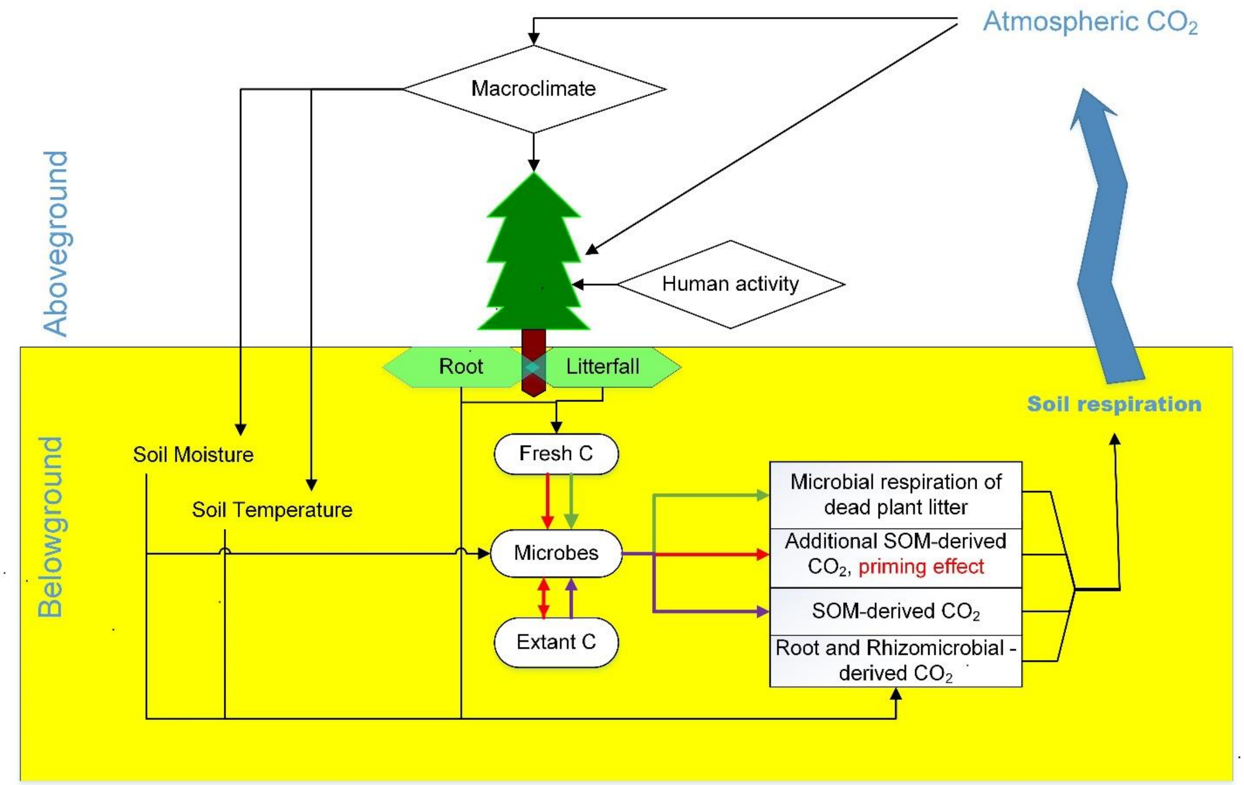


Figure 2-1: A conceptual diagram of the influence of global changes on the processes that control Rs. Rectangles are main biogenic sources of Rs; hexagons indicate plant litter inputs; ellipses are main soil carbon pools. Red, green and purple arrows represent different processes of soil microbial respiration.

2.3 Materials and Methods

2.3.1 Data collection

We searched peer-reviewed publications published before September 2017 that investigated the effects of plant litter-alteration on Rs using the ISI Web of Science and Google Scholar with the search terms: “soil respiration OR soil efflux OR soil CO_2 AND litter alteration OR litter removal OR residue management OR litter addition OR clipping.”

The following criteria were applied to select each study that: (*i*) was purposely designed to test the effects of litter alterations on Rs, (*ii*) had at least one pair of observations from the control and treatment with respective means and sample sizes, (*ii*) had same initial environmental condition, species composition, and soil property in the control and treatment plots, and (*iv*) was conducted in the field. In total, 100 published studies met these criteria (Table. S2-4) and distributed in all terrestrial biomes (Fig. S2-1). In all studies, double litter treatment represented the 100% aboveground litter addition, whereas the removal treatments included the complete removal of aboveground litter, roots, and aboveground litter + roots, respectively. Aboveground litter removal here included aboveground litterfall exclusion in both forests and croplands and clipping treatment in grasslands. Root removal included trenching and girdling experiments. These two methods yield quantitatively similar outcomes (Fig. S2-2).

For each study, we extracted the values, the number of replications, and standard deviation of Rs and Q_{10}, if reported. When an original study reported results graphically, we used SigmaScanPro version 5 (Systat Software Inc., Point Richmond, CA, USA) to extract data. We also extracted ecosystem types (forest, grassland, and cropland), mean annual temperature (MAT, °C), latitude, and longitude from the original or cited papers or WorldClim (http://WorldClim.org). Annual aridity index data were retrieved from CGIAR-CSI Global Aridity Index dataset (http://www.cgiar-csi.org/data/global-aridity-and-pet-database, Trabucco & Zomer 2009) by using the location information. The annual aridity index was calculated as the ratio of annual mean precipitation to mean annual potential evapotranspiration (UNEP 1997).

2.3.2 Data analysis

The natural log-transformed response ratio (ln*RR*) was used quantify the effects of litter alteration treatments following (Hedges *et al.* 1999):

$$\ln RR = \ln\left(\overline{X}_t / \overline{X}_c\right) \tag{1}$$

where $\overline{X_t}$ and $\overline{X_c}$ were means of the observed Rs or Q_{10} in treatment and control groups, respectively.

Effect size estimates and subsequent inferences in meta-analyses may be dependent on how individual observations are weighted (Ma & Chen 2016). In our dataset, sampling variance of Rs was not reported in ten of the 100 studies, and sampling variance of Q_{10} was not reported in 36 of the 45 studies. More importantly, weightings based on sampling variances could assign extreme importance to a few individual observations, and consequently, average ln*RR* would be primarily determined by a small number of studies (Pittelkow *et al.* 2015; Ma & Chen 2016). Similar to previous studies (Ma & Chen 2016), we used the number of replications for weighting:

$$W_n = (N_c \times N_t)/(N_c + N_t) \tag{2}$$

where W_n is the weight associated with each ln*RR* observation, N_c and N_t are the numbers of replications in the control and treatment, respectively.

To test our first and second hypotheses, we examined whether the overall ln*RR* (β_0) differed from zero and whether the ln*RR* was affected by experimental duration (ED, years) and using the following model:

$$\ln RR = \beta_0 + \beta_1 \cdot ED + \pi_{study} + \varepsilon \tag{3}$$

where β, π_{study} and ε are coefficient, the random effect factor of "study" and sampling error, respectively. The random effect explicitly accounts for autocorrelation among observations within each "study." We conducted the analysis using restricted maximum likelihood estimation with the *lme4* package (Bates *et al.* 2017). When ED in eqn. 3, are centered or scaled (minus mean and divided by one standard deviation), β_0 is the overall mean ln*RR* at the mean ED (Cohen *et al.* 2013). Since Q_{10} were estimated at different soil depths across studies, we added soil temperature measurement depth as a covariate in eqn. 3 when testing the average estimates and temporal trends of ln*RR* of Q_{10}.

To test our third hypothesis, we estimated litter priming effect using the experiments that simultaneously examined the responses of Rs to aboveground litter removal and double litter, following the method described by Crow et al. (2009). We calculated Rs attributable to the decomposition of aboveground litter (litter respiration) as $R_{litter} = R_{CK} - R_{LR}$, where R_{CK} and R_{LR} are Rs in the control and litter removal treatments, respectively. We assumed that litter respiration remained constant and then calculated the expected respiration ($R_{EXPECTED}$) for the double litter plots: $R_{EXPECTED} = R_{CK} + R_{litter}$. The priming effect (%) was then calculated as PE $= [(R_{LA} - R_{EXPECTED})/R_{LA}]\times 100$, where R_{LA} is measured Rs in double litter treatment. Similar to Rs, priming effect was tested using eqn. 3 to determine whether the overall priming effect differed from zero and whether the priming effect was affected by experimental duration (ED, years). Since the numbers of replications of litter addition and litter removal were the same in all experiments that simultaneously tested the responses of Rs to aboveground litter addition and removal, we used the same weighting described in eqn. 2. Moreover, since estimates for rhizosphere respiration have been observed to decrease (Sayer & Tanner 2010b) or not change (Lopez-Sangil *et al.* 2017) after aboveground litter addition, we assumed that extra CO_2 production following litter addition was largely derived from SOM heterotrophic respiration due to the priming effect (Xu *et al.* 2013a).

For our fourth hypothesis, we tested whether ln*RR* of Rs and Q_{10} changed with mean annual temperature (MAT) (or aridity index, AI) and vegetation type (if multiple types are available) with "study" as a random effect. Moreover, since substrate quality and water availability may affect Q_{10} (Craine *et al.* 2010), we examined the interaction effect of substrate quality and aridity index (AI) on the ln*RR* of Q_{10} with "study" as a random effect. We used four different litter types: grass, broadleaf, conifer, and mixed broadleaf and conifer trees, to represent litter quality. Grass litter is regarded as higher quality than those of trees due to its higher N/lignin (Kochy & Wilson 1997). Broadleaf litter tends to have higher quality and decompose faster than conifer litter because of its higher N/lignin and water-soluble extractive fractions (Prescott *et al.* 2000).

Our analysis indicated that many of our models validated the assumption of normality based on the Shapiro-Wilk's test on model residuals. We thus bootstrapped the fitted coefficients by 1000 iterations (Adams *et al.* 1997). The coefficients were significant from zero at $\alpha = 0.05$ if the bootstrapped 95% confidence intervals (CIs) did not cover zero. The difference between groups was significant if the 95% CIs of their coefficients did not overlap the other's mean. To facilitate interpretation, we transformed ln*RR* and its corresponding confidence intervals back to the percentage change by $(e^{\overline{\ln RR}} - 1) \times 100\%$. All statistical analyses were conducted in R (version 3.4.2., R Development Core Team, Vienna, Austria).

2.4 Results

Aboveground litter addition, i.e., double litter, increased Rs by 26.1%, on average (95% bootstrapped confidence interval, CI, 18.4% – 33.7%), while complete aboveground litter removal reduced Rs by 22.8% (CI, 18.5% – 27.1%) (Fig. 2-2a). Root removal and litter + root removal on average decreased Rs by 34.1% (CI, 27.2% – 40.9%) and 43.4% (CI, 36.6% – 50.2%), respectively, both of which are significantly higher than the effects of litter removal (*P*

= 0.002 and < 0.001, respectively), while the difference between the effects of root removal and litter + root removal on Rs was marginally significant (P =0.057) (Fig. 2-2a). The positive effect of double litter and the negative effect of litter removal on Rs increased with experimental duration, but the effects of root removal and litter +root removal did not (Fig. 2-2b). Aboveground litter removal marginally increased Q_{10} by 6.2% (CI, 0.2% – 12.3%), but double litter, root removal, litter + root removal did not significantly affect Q_{10} on average (Fig. 2-2c). The effects of litter alterations on Q_{10} did not change significantly with experimental duration (Fig. 2-2d).

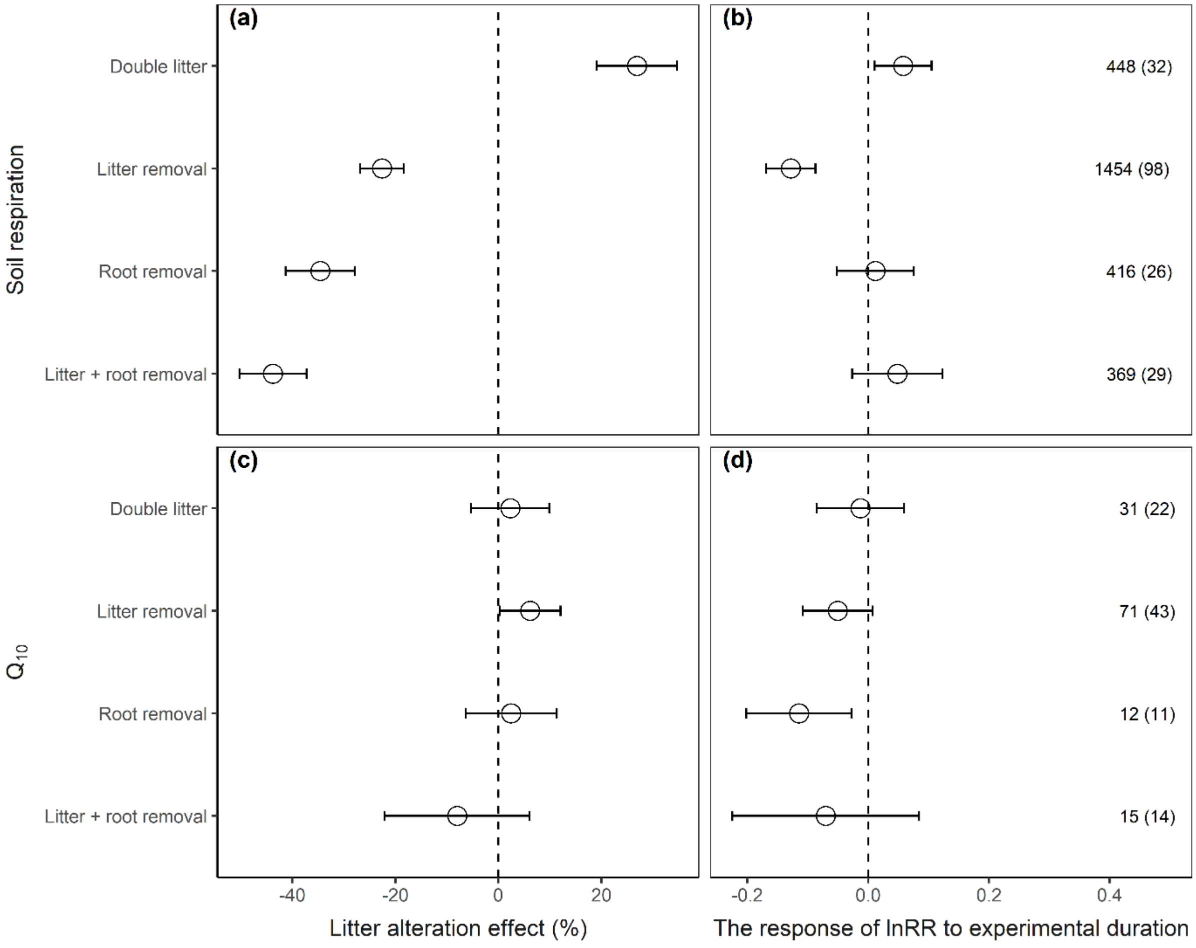


Figure 2-2: Average effects (%) of litter alterations on Rs and Q10 and their temporal trends associated with litter removal, double litter, root removal, litter + root removal. (a, c) Average effects of litter alterations. (b, d), The response slopes of log response ratio (lnRR) to experimental duration (scaled, see Methods). Values are bootstrapped mean and 95% confidence intervals. The numbers outside and inside the parentheses represent the numbers of observations and studies, respectively.

Based on the studies that simultaneously measured the effects of double litter and litter removal, the magnitude of the increase in Rs from double litter was on average 7.3% (CI, 0.6% – 14.0%) higher than that of the decrease in Rs from litter removal (Fig. 2-3). Moreover, the priming effect increased significantly with experimental duration (Fig. 2-3).

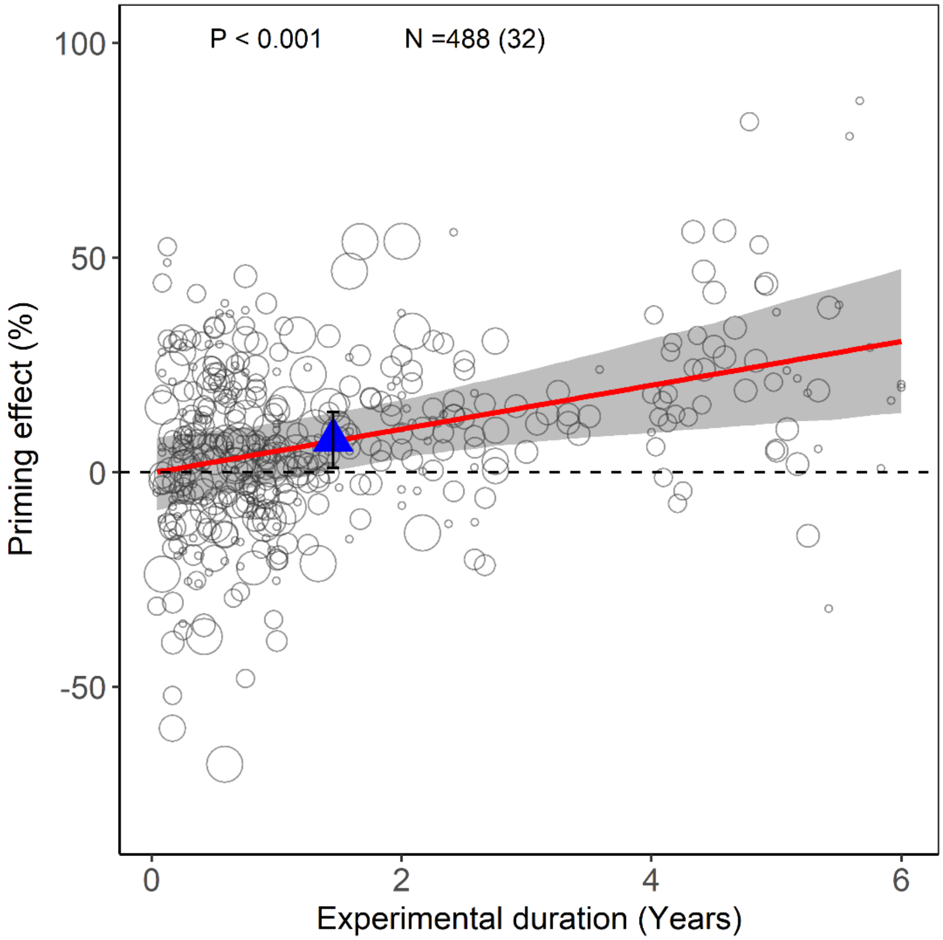


Figure 2-3: The average priming effect and priming effect in relation to experimental duration. Blue triangle and error bars show overall mean and its 95% confidence intervals. The size of

the bubble is the relative weight of each observation. The red line and grey shaded areas represent fitted regression and its bootstrapped 95% confidence intervals.

Across the global variation of mean annual temperature, the positive ln*RR* of Rs to double litter did not change significantly, nor did the negative effects of litter removal, root removal, and litter + root removal (Figs. S2-3a~d). Similarly, the ln*RRs* of Q_{10} did not change with mean annual temperature (Figs. S2-3e~h). With increasing aridity index, the positive ln*RR* of Rs to double litter did not change significantly, but the negative effects of litter removal, root removal, and litter + root removal became stronger (Figs. 2-4a~d). The negative effect of litter removal on Rs decreased more in grasslands and croplands than in forests with increasing aridity index (Fig. 2-4b; Table S2-2). The positive ln*RR* of Q_{10} became stronger with increasing aridity index, but the ln*RR* of Q_{10} to double litter, root removal and litter + root removal did not change significantly (Figs. 2-4e~h). The responses of ln*RRs* of Rs and Q_{10} to mean annual temperature or aridity index did not differ among ecosystem types, except a marginal interaction effect of aridity index and ecosystem type on Rs for litter removal (P = 0.039), as indicated by their interaction effects (Tables S2-1 and S2-2). The responses of ln*RRs* of Q_{10} to litter addition, litter removal, root removal and litter + root removal did not differ significantly by litter type, nor its interaction with aridity index (Tables S2-3).

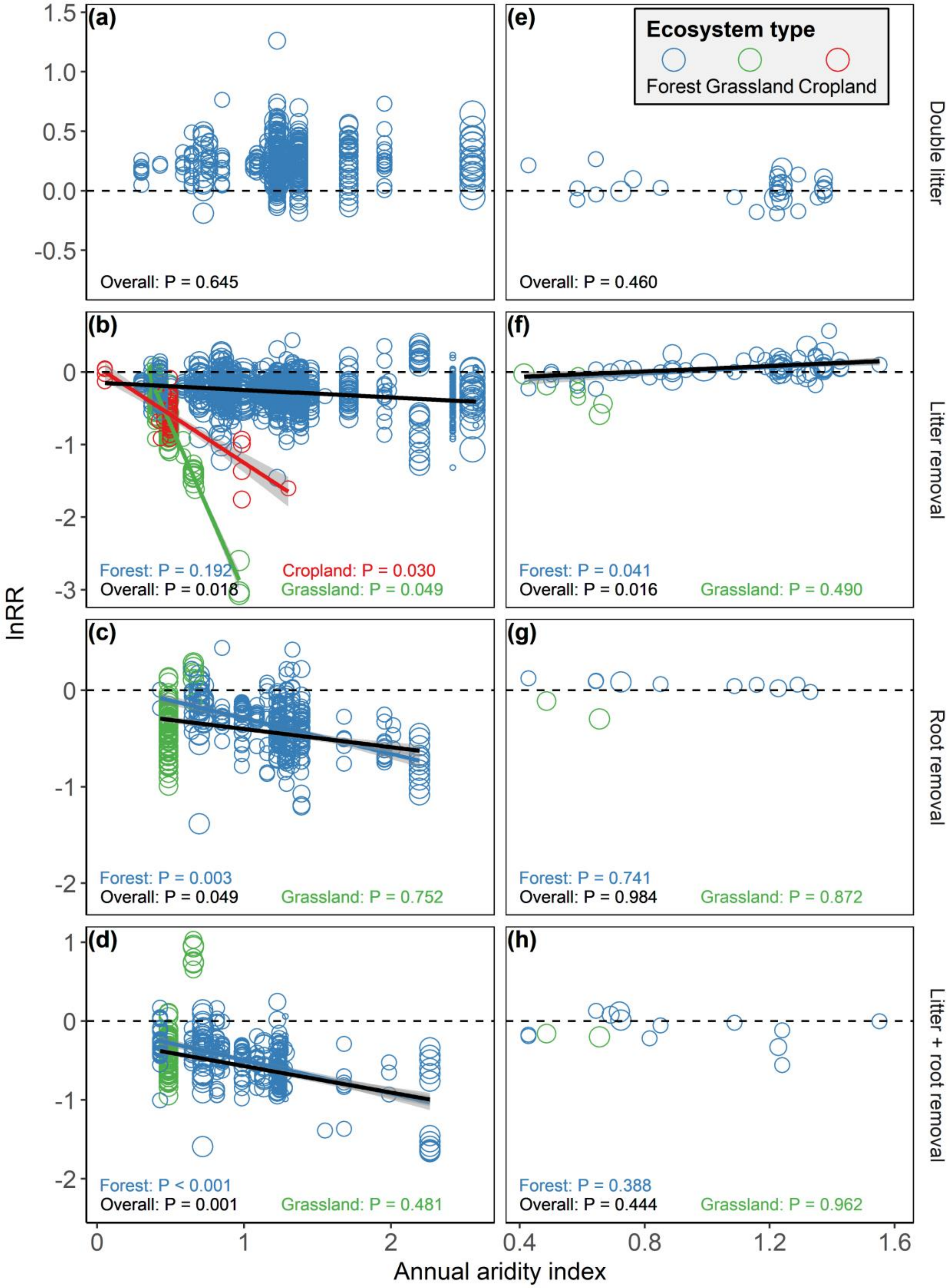

(a)
(b)
(c)
(d)
(e)
(f)
(g)
(h)
Ecosystem type
Forest Grassland Cropland
Overall: P = 0.645
Overall: P = 0.460
Forest: P = 0.192
Overall: P = 0.018
Cropland: P = 0.030
Grassland: P = 0.049
Forest: P = 0.041
Overall: P = 0.016
Grassland: P = 0.490
Forest: P = 0.003
Overall: P = 0.049
Grassland: P = 0.752
Forest: P = 0.741
Overall: P = 0.984
Grassland: P = 0.872
Forest: P < 0.001
Overall: P = 0.001
Grassland: P = 0.481
Forest: P = 0.388
Overall: P = 0.444
Grassland: P = 0.962
Double litter
Litter removal
Root removal
Litter + root removal
lnRR
Annual aridity index
1.5
1.0
0.5
0.0
-0.5
0
-1
-2
-3
1
0
1
2
0.4
0.8
1.2
1.6

Figure 2-4: Effects of annual aridity index and ecosystem type on lnRRs of Rs and Q10 associated with double litter (a, e), litter removal (b, f), root removal (c, g) and litter + root removal treatments (d, h). The size of the bubble is the relative weight of each observation. Black and colour lines represent overall and ecosystem type-specific responses, respectively, with their 95% confidence intervals shaded in grey.

2.5 Discussion

As expected, we found that 100% aboveground litter addition increased Rs, while 100% aboveground litter removal, root removal, and litter + root removal decreased Rs. Root removal and litter + root removal had a more significant effect on Rs than 100% litterfall removal, attributable to a substantial contribution of living roots and associated mycorrhizae to total soil respiration (Kuzyakov & Gavrichkova 2010) (Fig. 2-1). Our result indicated that ecosystem response to CO_2 fertilization (i.e., an increase in plant litter inputs to soil) may be primarily root-derived, to some extent, extending an emerging view in the last decade, which suggested that the primary source of organic C in soil usually comes from rhizodeposition (Rasse *et al.* 2005; Schmidt *et al.* 2011). However, only very few field experimental studies have focused yet on the effects of CO_2 fertilization (and increased rhizosphere inputs) on soil C dynamics (Lopez-Sangil *et al.* 2017).

Our analysis confirms the average litter alteration effects on Rs reported earlier (Subke *et al.* 2006; Xu *et al.* 2013a), although the magnitudes of the effects differ, which may partly result from the different numbers of observations (448 vs. 22 for litter addition (Xu *et al.* 2013a), 1454 vs. 22 for litter removal (Xu *et al.* 2013a), and 416 vs. 36 for root removal (Subke *et al.* 2006) for our analysis vs. previous analyses, respectively). Our findings offer several new insights. First, we found that litter alteration effects increased over time, suggesting that short-term experiments would underestimate litterfall alteration effects.

Second, in line with the "C quality–temperature" hypothesis, we found that litter removal marginally increased Q_{10}, whereas double litter, root removal, litter + root removal did not affect Q_{10}, provoking further investigations to unveil the underlying mechanisms. Third, we found a significant priming effect of aboveground litter addition, extending the findings shown previously in the rhizosphere area (Huo *et al.* 2017). Fourth, our analysis showed that the effects of litter removal on Rs and Q_{10} increased with water availability.

Our results showed both the magnitude of responses of Rs to double litter and litter removal increased with experimental duration. This positive impact of experimental duration on responses of Rs to double litter can be attributed to the increase of priming effect associated with experimental duration. The increasing priming effect of aboveground litterfall with experimental duration concurs with those from rhizosphere (Huo *et al.* 2017). One possible mechanism for this enhancement is that soil microbial community composition changes in concert with its C use (Morrissey *et al.* 2017). Microorganisms prefer to use fresh plant litter as a growth substrate at the beginning of fresh C addition, resulting in no or even negative priming effects; however, long-term C substrate addition would trigger the production of enzymes that are in favor of priming the native soil C (Morrissey *et al.* 2017). Alternatively, the initial weak response in priming effect could result from the disturbance of experimental establishment. Our results suggest that the priming effect of litterfall would be underestimated by short-term experiments (<1 year). The increase of the negative response of Rs to litter removal is attributable to the long-term negative effects of litter removal on soil nutrients and particulate organic matter (Sayer 2006; Sayer *et al.* 2012), resulting in a decline in soil biological activity (von Lutzow & Kogel-Knabner 2009). On the other hand, the lack of temporal trends of root removal and litter + root removal on Rs can be attributed to the termination of root-uptake nutrients (Kuzyakov 2006), which reduces the nutrient limitation for soil microbial activity.

We found weak support of our hypothesis, aboveground litter removal increased Q_{10}, attributable to the difference in substrate quality between fresh plant litter and more stable SOM (Craine *et al.* 2010). However, the enhancement of aboveground litter on Q_{10} is only marginally significant, indicating non-existence of positive effects, in some scenarios. It is possible that the positive response of Q_{10} to aboveground litter removal may be confounded by the "canceling effect" of decreased substrate availability and the variations associated with soil/climatic conditions (Davidson *et al.* 2006; Gershenson *et al.* 2009). The lack of the effects of root removal and litter + root removal on Q_{10} may partially result from that increased soil moisture associated with root removal (Kuzyakov 2006), which would lead to an oxygen availability deficit and mask the response of Rs to the increase in temperature (Blagodatskaya *et al.* 2014). The absence of response of Q_{10} to double litter could be attributable to that positive effect of increased substrate availability masked the negative effect of increased labile carbon substrate on Q_{10} (Yuste *et al.* 2004; Davidson & Janssens 2006; Gershenson *et al.* 2009). Based on Michaelis–Menten model for Rs, $Rs = (V_{max}[C])/(K_m+[C])$, where V_{max} is the maximal rate of enzymatic activity, and K_m is the half saturation constant that measures the affinity between the substrate and enzyme, and [C] is the substrate availability (concentration of the substrate at the active site of the enzyme) (Michaelis & Menten 1913; Davidson & Janssens 2006; Davidson *et al.* 2006), the increase of K_m with temperature would reduce the response of Rs to temperature. Since most soil microbial communities are C-limited, increased substrate availability could increase Q_{10} via reducing the "canceling effect" of K_m. Thus, we propose that future experiments should explicitly include both the availability and intrinsic temperature sensitivity of the added plant litter to evaluate these potential mechanisms (Davidson *et al.* 2012). We speculate that litterfall removal plots, which had the higher Q_{10}, be more sensitive to climate change under the scenarios of global warming.

Estimated from the studies that simultaneously tested the responses of Rs to aboveground litter addition and removal, "priming effect" on average accounted for 7.3% (0.6% – 14.0%) of Rs, and increased over time. This finding indicates that fresh litter input has a positive "priming effect" on Rs in natural soils, in agreement with the conclusion by van Groenigen *et al.* (2014), who reported that C substrate addition associated with CO_2 enrichment stimulates greater soil C release than expected from substrate availability. Importantly, the priming effect is highly dependent on experimental duration. It increased over time during the first years after litter input increases. Increased fresh organic matter inputs may stimulate the decomposition of extant SOM as the energy and nutrients released from the decomposition of fresh organic matter stimulates microbes, which increase production of extracellular enzymes that not only degrade more of the fresh plant litter inputs, but also a portion of the pre-existing stable SOM (Kuzyakov *et al.* 2000; Fontaine *et al.* 2003; Fontaine *et al.* 2007). These findings suggest that litterfall priming effect is likely to become a significant factor to be considered when estimating or modelling terrestrial CO_2 effluxes, as future increases in atmospheric CO_2 and warmer climates are likely to lead to the enhancement of plant litter from increased plant productivity (Hyvonen *et al.* 2007; van Groenigen *et al.* 2014; Brienen *et al.* 2015; Grace *et al.* 2016). Additional research will be required into the responses of Rs to a range of plant litter inputs since such knowledge is critical toward elucidating how the priming effect might best be incorporated into Earth systems models.

The reduction in Rs by litter removal, root removal, and litter + root removal did not change with mean annual temperature but increased with water availability. The increased negative effects of the removal of aboveground litter, roots or both on Rs with increasing water availability could be attributable to the higher sensitivity to water availability of leaf and root litter decomposition than that of SOM (Joos *et al.* 2010; Lopez-Sangil *et al.* 2013; Hinko-Najera *et al.* 2015) and to higher production of plant roots and litterfall in humid ecosystems than in arid systems (Wu *et al.* 2011) as well as inherent difference in litter quality among forest biomes (Olson 1963). Moreover, the reduction in Rs by litter removal decreased more rapidly with increasing water availability in grasslands and croplands than in forests. The difference in mean residence times of aboveground litter among vegetation types could have influenced these differential responses because compared with forests, more labile litter in grasslands and croplands could decompose more rapidly with increasing water availability, resulting in a more rapid decline in Rs after litter removal. The lack of observed water availability effect on the response of Rs to double litter is possibly a result of decreased priming effect under limited soil oxygen availability associated with high soil moisture (Luo *et al.* 2016; Wang *et al.* 2016) offsetting the increased contribution of Rs from the higher production of above- and below-ground plant litter in wetter locations. However, litterfall and root litter may exhibit lower sensitivity to temperature than SOM due to lower recalcitrance (Bosatta & Agren 1999). Since the lower temperature sensitivity of litterfall- and root-litter-derived Rs may offset the higher production of plant roots and litterfall in warmer environments (Wu *et al.* 2011), the response of Rs to litter alteration may not change with mean annual temperature.

Our results also revealed that the positive response of Q_{10} to litter removal increased with water availability. Since dry conditions would result in a substrate deficit due to limited diffusion of solutes in thin soil water films (Moyano *et al.* 2013), aboveground litter removal may exacerbate the substrate deficit through the reduction of substrate inputs and increased evapotranspiration, resulting in the reduction of Q_{10} in relatively arid areas in contrast to humid areas (Jassal *et al.* 2008). Furthermore, higher belowground production in humid areas (Wu *et al.* 2011; Xu *et al.* 2013b) might compensate for substrate loss of aboveground litter. We note that we did not find any trends in the response of Q_{10} to litter removal to water availability in grasslands, which is attributable to the lack of temperature dependence of Q_{10} in water limited ecosystems (Almagro *et al.* 2009; Leon *et al.* 2014). Our results confirmed the conclusion of Davidson et al. (2012) that environmental constraints of substrate supply under soil moisture extremes might prevent the intrinsic temperature sensitivity of Rs from being observed.

Our meta-analysis shows that increased litter inputs cause an increase in soil CO_2 efflux and that the magnitude of the priming effect may increase over time during the first years after litter input increases, indicating that the magnitude of estimated litterfall alteration on the priming effect may be underestimated by short-term experiments. In addition, the increase of Q_{10} after litter removal, especially in wet locations, indicates that increasing global deforestation and cultivation may accelerate Rs under ongoing global warming. Lastly, we show evidence that the effects of aboveground litter alterations on Rs increase over time and are amplified by increasing precipitation. Our analysis provides insights to improve land surface models to better predict the consequences of climate change on the largest terrestrial C efflux.

CHAPTER 3: PLANT DIVERSITY LOSS REDUCES SOIL RESPIRATION ACROSS TERRESTRIAL ECOSYSTEMS

3.1 Abstract

The rapid global biodiversity loss has led to the declines in ecosystem function. Despite the critical importance of soil respiration in the global carbon and nutrient cycles, how plant diversity loss affects Rs remains uncertain. Here we present a meta-analysis using 446 paired observations from 95 published studies to evaluate the effects of plant and litter mixtures on soil respiration and its components. We found that total soil respiration and heterotrophic respiration were, on average, greater in plant mixtures than expected from those of monocultures. These mixture effects increased with increasing species richness in both plant and litter mixtures. While the positive effects of species mixtures remained similar over time for total soil respiration, they increased over time for heterotrophic respiration in plant mixtures but decreased in litter mixtures. Despite the wide range of variations in mean annual temperature, annual aridity index, and ecosystem types, the plant mixture effects on total soil respiration and heterotrophic respiration did not change geographically, except for a more pronounced increase of total soil respiration in species mixtures with reduced water availability. Our structural equation models suggested that the positive effects of species richness and stand age on total and heterotrophic respiration were driven by increased plant inputs and soil microbial biomass. Our results suggest that plant diversity loss has ubiquitously negative impacts on soil respiration, one of the fundamental carbon-cycle processes sustaining terrestrial element cycling and ecosystem function.

3.2 Introduction

Soil respiration (Rs), which reflects plant and soil microbial metabolism, is a key ecosystem function that controls terrestrial energy balance and element cycling (Luo & Zhou 2006). As a component of Rs, the autotrophic respiration (Ra) of plants generates energy for water and nutrient acquisition, survival, growth, and defense, while heterotrophic respiration (Rh) from the activity of soil microorganisms regulates nutrient cycling (Ryan & Law 2005). Recently, there have been significant advances in our understanding of the negative impacts of plant diversity loss on ecosystem functions, including: net primary production (Liang *et al.* 2016; Duffy *et al.* 2017), carbon sequestration (Tilman *et al.* 2006), and nutrient cycling (Handa *et al.* 2014). However, how plant diversity loss affects Rs and its components remains uncertain.

Plants play a decisive role in regulating Rs since they are the principal pathways through which carbon enters the soil (Chen & Chen 2018). Biomass production increases with plant species diversity and trait heterogeneity due to complementary resource utilization among constituent species in species-rich ecosystems (Hooper *et al.* 2005; Zhang *et al.* 2012). The higher biomass production in species-rich ecosystems is expected to increase Rs primarily by increasing respiration in living roots and their associated rhizomicrobial and microbial communities (Kuzyakov & Gavrichkova 2010; Chen & Chen 2018). Higher plant diversity can also increase soil microbial respiration because of a higher quantity and multiplicity of plant-derived food resources that enter the soil as well as expanded niches for microbes associated with a higher microenvironment variability and habitat complexity (Hector *et al.* 2000; Hooper *et al.* 2000; Chapman & Newman 2010; Eisenhauer *et al.* 2010). Upon litter addition, diverse plant litter mixtures might accelerate the decomposition of organic matter via complementary resource use among microbes and detritivores, and active nitrogen transfer among different litters, resulting in greater Rs (Handa *et al.* 2014). Additionally, increased litter inputs in species-rich ecosystems may lead to more accumulation of soil carbon and nitrogen pools (Fornara & Tilman 2008; Lange *et al.* 2015), which can increase microbial respiration (Franzluebbers *et al.* 2001; Eisenhauer *et al.* 2013). However, despite these expected positive effects of plant mixture on Rs, previous empirical studies have reported positive (Eisenhauer *et al.* 2010; Vogel *et al.* 2013), negligible (Wardle *et al.* 2000), or even negative (Murphy *et al.* 2008) effects on Rs. Divergent empirical findings of plant diversity effects on Rs could also result from the level of species richness, experimental duration or stand age and background environment. A quantitative synthesis of results across multiple studies might assist in determining the overall effects of plant mixtures on Rs and identify sources of variation (Gurevitch *et al.* 2018).

Over the last decade, empirical work has also established that the effects of diversity on primary productivity increase over time (Cardinale *et al.* 2007; Reich *et al.* 2012; Zhang *et al.* 2012; Guerrero-Ramirez *et al.* 2017). Given the positive link between primary productivity and Rs, the increase in productivity over time may produce an associated temporal increase in Rs (Raich & Tufekcioglu 2000; Janssens *et al.* 2010). Studies that documented changes in Rs over brief timescales might have underestimated the effects of diversity loss on Rs, due to the delays in the responses of soil microbes to changes in plant diversity (Eisenhauer *et al.* 2010; Thakur *et al.* 2015). Additionally, temporal trends of species mixture effects on Rs may increase with species richness in mixtures since species-rich plots accumulate more nutrients, such as N, in soil organic matter over time, relative to species-poor plots, and these additional nutrients subsequently increase plant productivity (Fornara & Tilman 2009; Reich *et al.* 2012; Guerrero-Ramirez *et al.* 2017), and thus Rs.

The responses of Rs to plant mixtures may also vary contingent on the environment. The effects of plant species diversity and their temporal trends may differ among ecosystem types due to differences in the physiology, structure, and lifespan of their vegetation, as well as environmental conditions (Forrester & Bauhus 2016). Although the positive tree species mixture effects on productivity are suggested to be stronger in boreal than temperate forests (Paquette & Messier 2011), a global meta-analysis shows that positive mixture effects on productivity are consistent across forest biomes and between planted and natural forests (Zhang *et al.* 2012). In the meantime, the responses of Rs to plant inputs tend to decrease with reduced water availability induced by high temperatures, due to reduced litter decomposition (Zhu & Cheng 2011; Vogel *et al.* 2013; Chen & Chen 2018). We, therefore, expected that the effects of plant mixtures on Rs would differ among ecosystem types and change with temperature and water availability.

Here we conducted a global meta-analysis using data from 70 plant diversity studies and 25 litter diversity studies. We examined the effects of plant and litter mixtures on Rs and its components and tested whether these effects might increase with species richness in mixtures and stand age or experimental duration. We then examined if these responses varied across a wide range of ecosystem types, mean annual temperatures, and annual aridity indices. Moreover, we examined whether the effects of plant mixtures on plant inputs, soil microbial biomass, and soil characteristics (soil carbon and nitrogen content) would affect the response of Rs to plant mixtures.

3.3 Materials and Methods

3.3.1 Data collection

We examined peer-reviewed publications that were published prior to March 2018, which investigated the effects of plant diversity or litter diversity on Rs using the ISI Web of Science and Google Scholar with the search terms: “soil respiration OR soil efflux OR soil CO_2 AND diversity OR richness OR mixture OR pure OR polyculture OR monoculture OR overyielding OR litter diversity OR litter mixture.”

We employed the following criteria to select the studies: (*i*) they were purposely designed to test the effects of plant mixtures or litter mixtures on Rs, Ra, or Rh, (*ii*) they had at least one mixture treatment and corresponding monocultures, (*iii*) they had the same initial climatic and soil properties in the monoculture and mixture treatment plots. In total, 69 publications that investigated plant mixture effects on Rs or its components, and 23 publications that studied litter mixture effects on Rh, met these criteria (Fig. S3-1, Table S3-1). In two publications, several experiments, each with independent control, were conducted at different locations, which we considered to be different studies (Berger *et al.* 2010; Wang *et al.* 2014). This resulted in a total of 70 studies for plant mixtures and 25 studies for litter mixtures, respectively.

For each site, we extracted the values, the number of replications, and the standard deviation of Rs, Ra, and Rh, if reported. When an original study reported results graphically, we employed SigmaScanPro version 5 (Systat Software Inc., Point Richmond, CA, USA) to extract the data digitally. The Rs data included only *in situ* measurements, whereas the Rh data included both *in situ* and laboratory measurements; however, we did not include substrate-induced respiration studies as the addition of substrates tend to accelerate the proliferation of microbes that are adapted to particular substrates (Janssens *et al.* 2010). Across all studies, the field- and laboratory-based measurements of mixture effects on Rh did not differ significantly (Fig. S3-2). The selected studies employed a wide range of soil sampling depth; however, measurement depth did not affect the responses of Rh to plant mixtures (Fig. S3-3).

We also extracted species richness (SR, number of plant species), ecosystem types (i.e., forest, grassland, cropland, and pot), latitude, longitude, mean annual temperature (°C), stand age (years), as well as litterfall biomass, fine root biomass, soil microbial biomass, soil carbon content, and soil nitrogen content that were measured in the same year for the Rs from original or cited papers or cited data sources. For those studies conducted in the field (forest, grassland, and crop systems), annual aridity index data were retrieved from the CGIAR-CSI Global Aridity Index dataset (http://www.cgiar-csi.org/data/global-aridity-and-pet-database, Trabucco & Zomer 2009) through the use of location information (Supplementary dataset). The annual aridity index was calculated as the ratio of annual mean precipitation to mean annual potential evapotranspiration (UNEP 1997). Stand age was recorded as the number of years since a stand-replacing disturbance in forests, and the number of years between the initiation and measurements of the experiments in grasslands, croplands, and pots. The experiment length (years) was recorded as the number of years between the initiation and measurements in the litter diversity manipulation experiments. In the original studies, annual litterfall production was quantified as litterfall biomass for forests and aboveground biomass for grasslands and croplands, respectively. Soil microbial biomass was determined via substrate-induced respiration, fumigation-extraction method, or phospholipid fatty acid analysis (PLFA).

3.3.2 Data analysis

The natural log-transformed response ratio (ln*RR*) was employed to quantify the effects of plant mixtures or litter mixtures following Hedges et al. (1999):

$$\ln RR = \ln\left(\overline{X}_t / \overline{X}_c\right) = \ln \overline{X}_t - \ln \overline{X}_c \tag{1}$$

where $\overline{X}_t$ and $\overline{X}_c$ were the observed values of selected variables in the mixture, and the expected values of the mixture in each study, respectively. We calculated the $\overline{X}_c$ based on weighted values of the component species in monocultures following Loreau and Hector (2001):

$$\overline{X}_c = \sum (p_i \times m_i) \tag{2}$$

where P_i is the observed value of the selected variable of species *i* in monocultures or single-species litter treatments, and m_i is the proportion of species *i* density in mixed forests, grasslands, croplands, and pots, or the litter mass proportion of species *i* in the mixed litter treatments. When a study reported multiple types of mixtures (species richness levels) and experiment length (years), $\overline{X}_t$ and $\overline{X}_c$ were calculated separately for each mixture type and experimental year. This method accounted for the effects of species composition and plant/litter quality differences between the mixtures and monocultures corresponding to each mixture type and stand age within each original study. For two studies in natural forests where single-species stands were not available, monocultures were defined by having a single species that comprised ≥80% of the stand basal area, as in the original studies (Laganiere *et al.* 2012; Laganiere *et al.* 2015).

Estimates of effect sizes and subsequent inferences in meta-analyses could be dependent on how individual observations were weighted (Ma & Chen 2016). In our dataset (Supplementary Dataset 1), sampling variances were not reported in 22 of the 95 studies. Weightings based on sampling variances could assign extreme importance to only a few individual observations and, consequently, the average ln*RR* would be determined predominantly by a small number of studies (Adams *et al.* 1997; Ma & Chen 2016). Similar to previous studies (Pittelkow *et al.* 2015; Ma & Chen 2016), we instead employed the number of replications for weighting:

$$W_r = (N_c \times N_t)/(N_c + N_t) \tag{3}$$

where W_r is the weight associated with each ln*RR* observation, and N_c and N_t are the numbers of control and treatment replications, respectively.

Because only ten studies were available for Ra, we focused our tests on the ln*RRs* of Rs and Rh associated with plant and litter diversity. We employed the following model to determine the overall effect of species richness (SR), stand age, or experiment length (SA, years) and their interaction:

$$\ln RR = \beta_0 + \beta_1 \cdot \ln(SR) + \beta_2 \cdot \ln(SA) + \beta_3 \cdot \ln(SR) \times \ln(SA) + \pi_{study} + \varepsilon \tag{4}$$

where β, π_{study}, and ε are coefficients, the random effect factor of “Study”, and sampling error, respectively. The random effect explicitly accounted for autocorrelation among observations within each “Study”. We conducted the analysis using restricted maximum likelihood estimation with the *lme4 1.1-19* package (Bates *et al.* 2017). When continuous predictors, i.e., ln(*SR*) and ln(*SA*) in eqn. 4, are centered or scaled (minus mean and divided by one standard deviation), β_0 is the overall mean ln*RR* at the mean ln(*SR*) and ln(*SA*) (Cohen *et al.* 2013). To facilitate comparisons among Rs that had variable ln(*SR*) and ln(*SA*), we scaled these variables in our analysis.

We also compared the linear and logarithmic functions for species richness and stand age, and found that the logarithmic functions for species richness and stand age resulted in similar or lower Akaike information criterion (AIC) values (Table S2). To graphically illustrate whether the species richness effects on ln*RR* differed with stand age, we used the method described by Cohen et al. (2013) to estimate age effect at species richness values of 2, 3, 4, 8, 16, and 60, respectively, which were the most common SR levels in the original studies.

To examine whether ln*RR* changed geographically, we tested the effects of mean annual temperature, aridity index, or ecosystem type on ln*RR* by adding the terms of mean annual temperature, aridity index or ecosystem type to eqn. 4. We also examined the AIC values of the model with and without the interaction term of species richness × geographical factor (mean annual temperature, aridity index, or ecosystem type) and stand age × geographical factor. The models without the interaction term had the lowest AICs (Table S3). Since the models without the interaction term had the lowest AICs, we selected the model without the interaction between the geographical factor and ln(SR) or ln(SA) to avoid overfitting. The treatment effects were significant at $\alpha = 0.05$ if the 95% CIs did not cover zero. The mean effect sizes between groups were significantly different if their 95% confidence intervals did not overlap the other's mean. To facilitate interpretation, we transformed ln*RR* and its corresponding confidence intervals back to percentage change by $(e^{\overline{\ln RR}} - 1) \times 100\%$.

3.3.3 Structural equation modeling

To mechanistically understand the effects of plant mixtures on Rs and Rh, we first examined the ln*RR*s of litterfall biomass, fine root biomass, soil microbial biomass, soil carbon content, and soil nitrogen content rate in response to species richness, stand age, and their interaction using eqn. 4. Pearson correlation was used to examine the associations among the ln*RR*s of litterfall biomass, fine root biomass, soil microbial biomass, soil carbon content, soil nitrogen content, Rs, and Rh. Subsequently, we employed structural equation modeling (SEM) to examine the simultaneous effects of species richness, stand age, and their interaction on the ln*RR*s of Rs and Rh, both directly and indirectly, via the ln*RR* of plant litter inputs (litterfall and fine root biomass as a latent variable), while accounting for the effects of mean annual temperature and aridity index. Similar to Garcia-Palacios et al. (2015) and Zhang *et al.* (2018), we assessed direct effects of the ln*RR* of plant inputs on the ln*RR* of Rs, direct effects of species richness in mixtures (natural log transformed), stand age (natural log transformed), and the direct effects of mean annual temperature, and aridity index on the ln*RR* of plant inputs and the ln*RR* of Rs. As recommended (Grace 2006), we assessed the conceptual model (full model) vs. reduced models by goodness-of-fit statistics and used AIC to select among alternative models. We selected the final model with the lowest AIC value. We also evaluated the relationship between the ln*RR* of soil microbial biomass and the ln*RR* of Rh using the same method as above. We implemented SEMs using the '*piecewiseSEM* 1.2.1' package to account for the random effects of "Study" (Lefcheck 2016). All statistical analyses were performed in R 3.5.1 (R Core Team 2018).

3.4 Result

Plant mixtures, on average, increased Rs by 11.6% (95% confidence interval (CI), 7.6% – 15.6%) and Rh by 12.7% (7.5% – 17.9%) than expected from monocultures (Fig. 3-1a, c), whereas Ra did not increase significantly (49.7%, CI = -6.5% – 105.9%, $P = 0.165$, $n = 10$). Plant mixture effects for both Rs and Rh increased with species richness in mixtures (Fig. 3-1a, c, Table S3-4). While the plant mixture effects on Rs did not change with stand age, those on Rh increased significantly with stand age on average, with more pronounced increases in more diverse species mixtures (Fig. 3-1b, d, Table S3-4). The litter mixture effects on Rh were not significant on average, primarily due to the null effect of two species mixtures, but higher numbers of species led to significant positive effects on Rh (Fig. 3-1e, Table S3-4). The response of Rh to litter mixtures decreased significantly with experiment length on average, with more pronounced decreases in more diverse species mixtures (Fig. 3-1f, Table S3-4). The responses of Rs and Rh to plant mixtures did not change with mean annual temperature nor ecosystem type (croplands, grasslands, forests, and pots) (Table S3-5). However, with increasing aridity index (indicating more climate moisture availability), the positive response of Rs to plant mixtures became weaker, while that of Rh was not significantly altered (Fig. S3-4, Table S3-5).

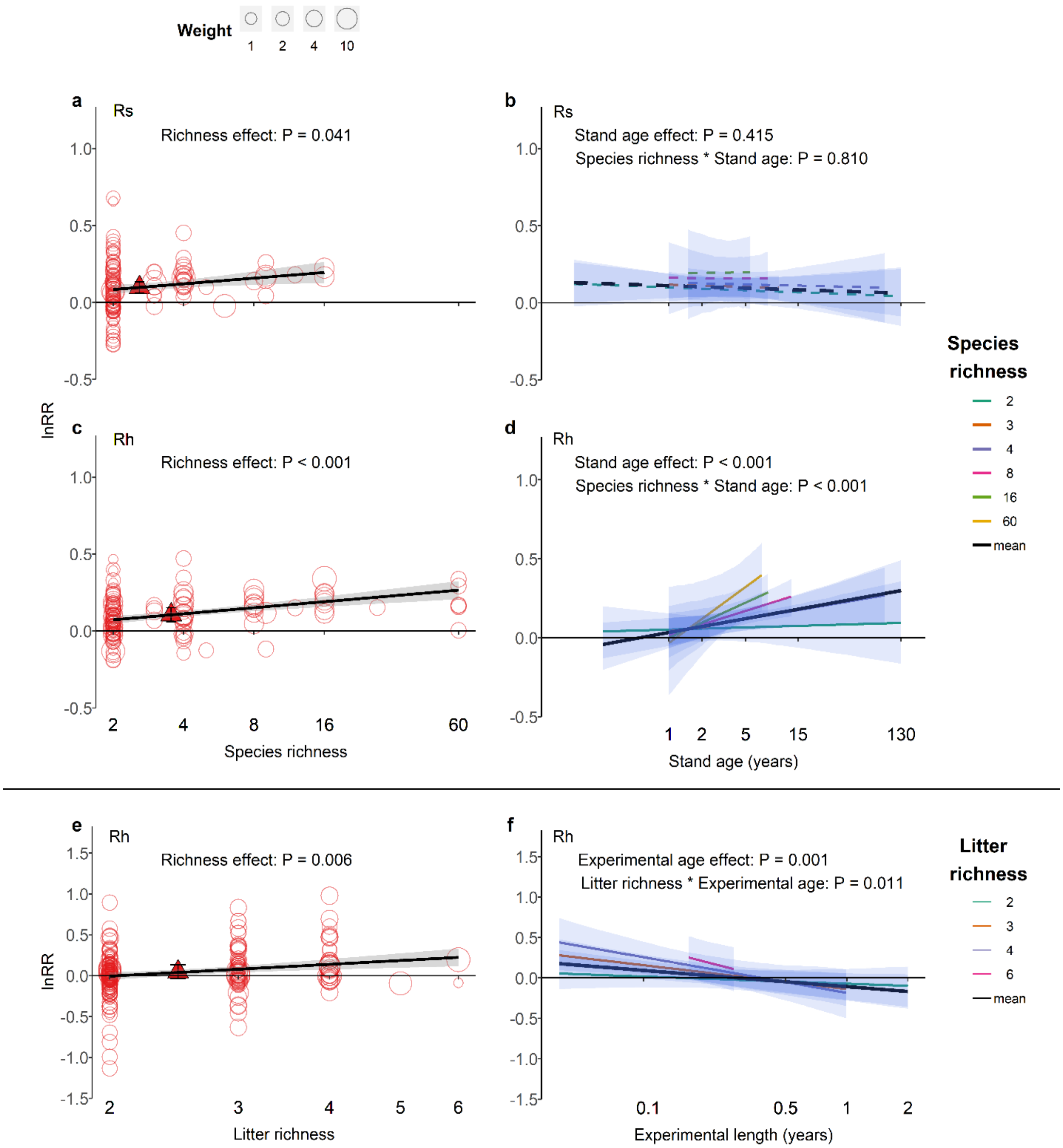


Figure 3-1: The natural log response ratio between species mixtures and monocultures (ln*RR*) in relation to the plant species richness in mixtures and their temporal trends associated with the species richness. a, b, The ln*RR*s of Rs and Rh in relation to the species richness in mixtures. c, d, Temporal trends of the ln*RR* of Rs and Rh corresponding to species richness levels. e, The ln*RR* of Rh in relation to litter species richness. f, Temporal trends of the ln*RR* of Rh corresponding to litter species richness. Red triangles and error bars represent the overall mean and its 95% confidence intervals. Black and colored lines represent the average and species richness -specific responses, respectively, with their bootstrapped 95% confidence intervals shaded in grey. Lines are bound by the range of the stand ages for each richness level.

The significance (*P*) is presented for each term tested. The sizes of the circles represent the relative weights of corresponding observations.

Based on the studies that reported both Rs and its contributors, plant mixtures on average increased litterfall biomass by 33.1% (22.0 – 44.2%), fine root biomass by 22.2% (10.9% - 33.4%), and soil microbial biomass by 12.4% (6.3 – 18.5%) over those expected from monocultures (Fig. 3-2a, c, e). The positive responses of litterfall biomass, fine root biomass, and soil microbial biomass to plant mixtures increased with the species richness in mixtures (Fig. 3-2a, c, e). The plant mixture effect on litterfall biomass did not change with stand age on average, but it increased in species-rich mixtures (Fig. 3-2b). The plant mixture effect on fine root biomass and soil microbial biomass increased significantly with stand age, with more pronounced increases in more diverse species mixtures (Fig. 3-2d, f). The plant mixture effect on soil carbon content increased with the species richness in mixtures and stand age, whereas those on soil nitrogen content did not differ with either species richness or stand age (Fig. S3-5). The responses of plant inputs (litterfall biomass and fine root biomass) and soil microbial biomass were positively correlated with those of Rh and Rs, while those of soil carbon content and nitrogen content were not (Fig. S3-6).

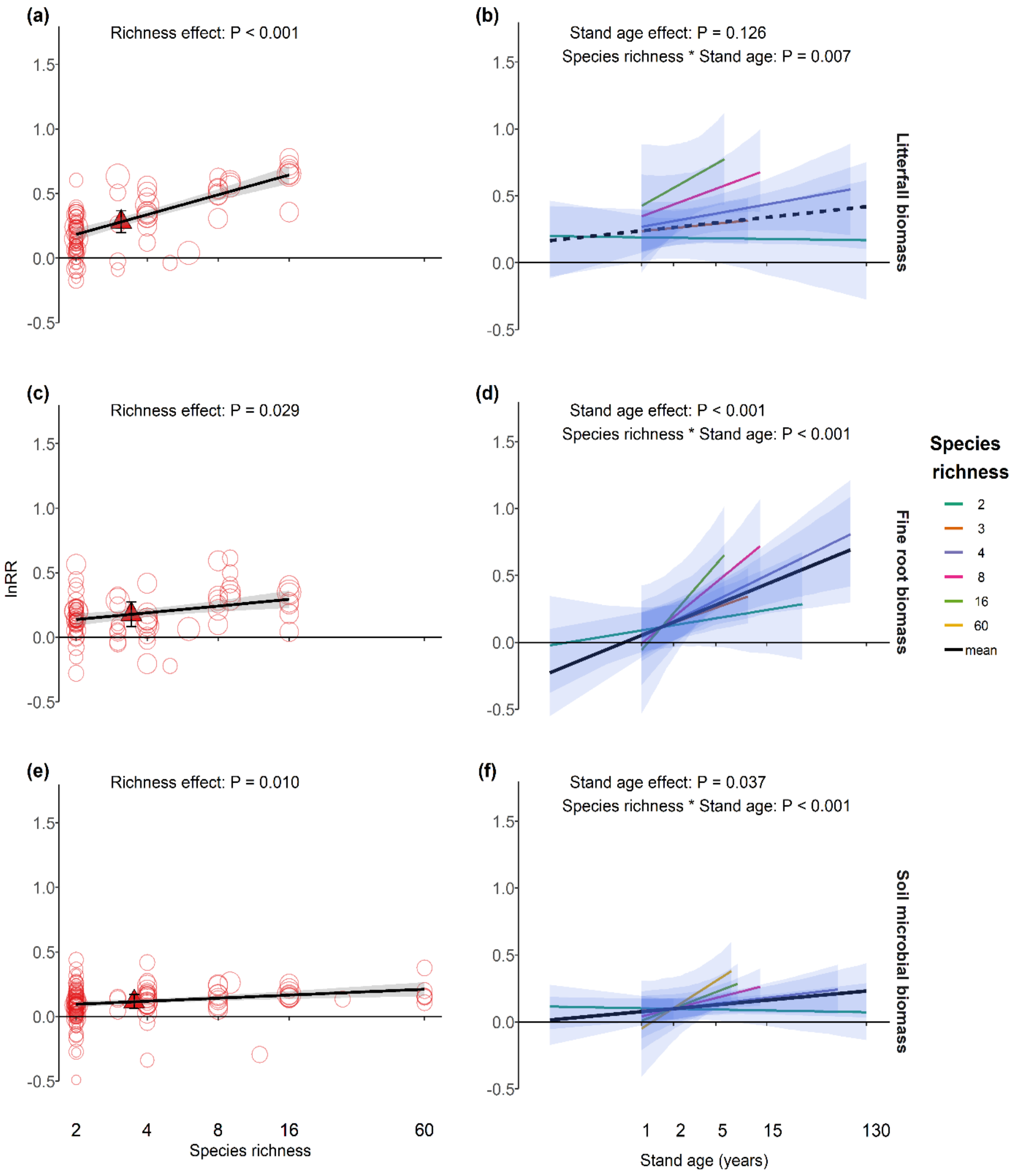
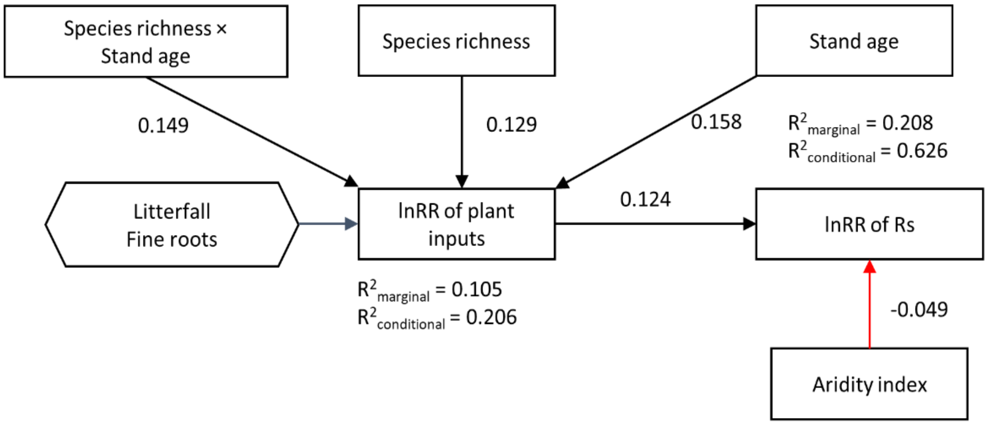


Figure 3-2. The natural log response ratios between species mixtures and monocultures (lnRR) for litterfall biomass, fine root biomass, and soil microbial biomass. a, c, e, The ln*RR* of litterfall biomass, fine root biomass, and soil microbial biomass in relation to the species richness in mixtures. b, d, f, Temporal trends of the ln*RR* corresponding to each level of species richness. Red triangles and error bars represent overall mean and its 95% confidence intervals. Black and colored lines represent the average and species richness-specific responses, respectively, with their bootstrapped 95% confidence intervals shaded in grey. Lines are bound by the range of the stand ages for each richness level. The significance (*P*) is

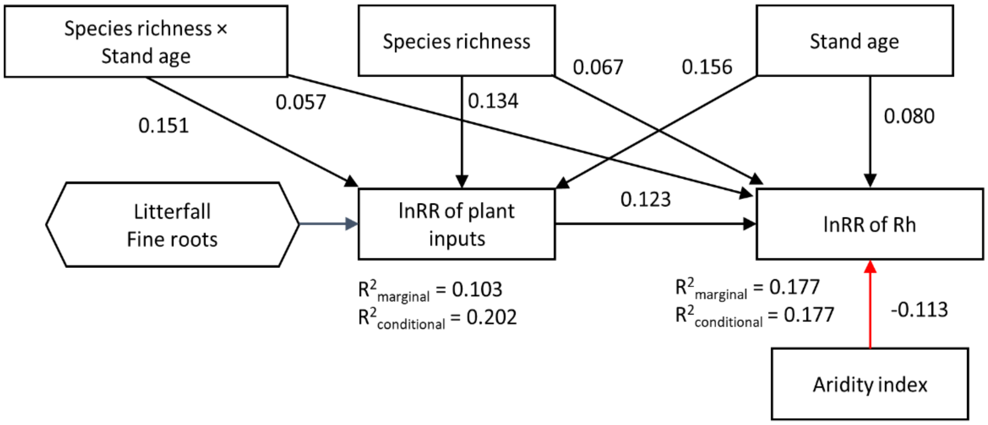
presented for each term tested. The sizes of the circles represent the relative weights of corresponding observations.

Our structural equation modelling results indicated that the responses of plant inputs to plant mixtures had a positive direct effect on that of Rs (Fig. 3-3a). The species richness in mixtures, stand age and their interactions had positive indirect effects on Rs via their positive direct effects on plant inputs (Fig. 3-3a). The positive effect of plant mixtures on Rs decreased with aridity index (Fig. 3-3a). The responses of Rh to species richness, stand age, their interactions, plant inputs and aridity index were similar to those of Rs except additional direct positive effects of the species richness in mixtures, stand age and their interactions on Rh (Fig. 3-3b). The alternative SEM with soil microbial biomass as the link between the species richness in mixtures, stand age, their interactions, and Rh showed positive effects of species richness, stand age, and their interactions on Rh via increased soil microbial biomass (Fig. 3-3c).

(a)


Species richness ×
Stand age
Species richness
Stand age
0.149
0.129
0.158
$R^2_{marginal} = 0.208$
$R^2_{conditional} = 0.626$
Litterfall
Fine roots
lnRR of plant
inputs
0.124
lnRR of Rs
$R^2_{marginal} = 0.105$
$R^2_{conditional} = 0.206$
-0.049
Aridity index

(b)


Species richness ×
Stand age
Species richness
0.067
0.156
Stand age
0.057
0.134
0.151
0.080
Litterfall
Fine roots
lnRR of plant
inputs
0.123
lnRR of Rh
$R^2_{marginal} = 0.103$
$R^2_{conditional} = 0.202$
$R^2_{marginal} = 0.177$
$R^2_{conditional} = 0.177$
-0.113
Aridity index

(c)

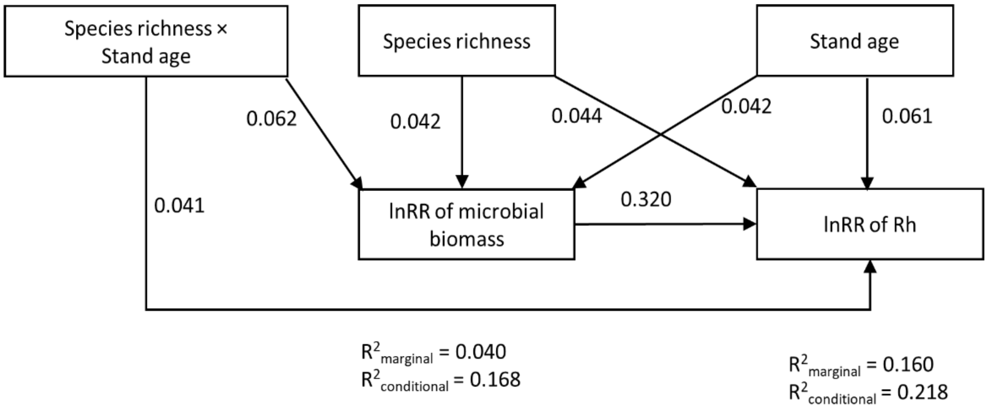

Species richness ×
Stand age
Species richness
Stand age
0.062
0.042
0.044
0.042
0.061
0.041
lnRR of microbial
biomass
0.320
lnRR of Rh
$R^2_{marginal} = 0.040$
$R^2_{conditional} = 0.168$
$R^2_{marginal} = 0.160$
$R^2_{conditional} = 0.218$

Figure 3-3. Structural equation model depicting the influence of the natural log response ratio (ln*RR*) of plant inputs and soil microbial biomass, species richness (natural log transformed), stand age (natural log transformed), and annual aridity index on the ln*RR*s of Rs and Rh. Structural equation model depicting A, the influence of the ln*RR* of plant inputs, species richness, stand age and annual aridity index on the lnRR of Rs (n = 74), B, the influence of the ln*RR* of plant inputs, species richness, stand age, and annual aridity index on the ln*RR* of Rh (n = 81) and C, the influence of the ln*RR* of soil microbial biomass, species richness, and stand age on the lnRR of Rh (n = 99). The ln*RR* of plant inputs is a latent variable of the ln*RR*s of litterfall biomass and fine roots. The single-headed arrow represents the directional influence of one variable on another. The number beside the arrow is corresponding standardized coefficient (r). All fitted coefficients are significant at $\alpha = 0.05$. R^2marginal and R^2conditional represent the level of deviance of the variable explained by all paths from the fixed effects, and both the fixed and random effects (“Study”), respectively.

3.5 Discussion

Our meta-analysis shows the first global-scale evidence that plant mixtures yield greater Rs and Rh than do monocultures. Mixture effects on Rh increased with plant species richness and stand age, extending our understanding of the critical role of species diversity beyond the positive biodiversity-ecosystem productivity relationships (Tilman *et al.* 1996; Zhang *et al.* 2012; Ma & Chen 2016). Our findings revealed several new insights into the ongoing debate regarding biodiversity-ecosystem functioning relationships. First, our study indicated that greater plant diversity leads to higher plant and soil microbial activity. Although the limited number of studies that have measured Ra showed an insignificantly positive trend of plant mixture effects on Ra, Species mixtures caused similar increases in Rs and Rh (+11.6% vs. +12.7%), suggesting similar species mixture effects on Ra and Rh since Ra accounts for nearly half of the total Rs (Subke *et al.* 2006). Second, we found that plant mixture effects on Rh increased with stand age, particularly in more diverse plant communities, which complemented the findings of previous meta-analyses wherein the effects of biodiversity on productivity and litter decomposition increased over time (Lecerf *et al.* 2011; Zhang *et al.* 2012). Third, our analysis suggested that the negative effects of plant diversity loss on Rs and

Rh were globally consistent, with a more pronounced reduction in Rs in drier climates. Fourth, we found that plant diversity regulated Rs and Rh through the changes in plant inputs and soil microbial biomass.

As expected, our analysis revealed higher Rs and Rh in species mixtures over monocultures, and that the mixture effect increased with the species richness in mixtures. Further, we found that the positive response of Rh to plant mixtures was strengthened over time, with more pronounced increases in more diverse species mixtures. This suggests that losses of even a very few species from diverse assemblages might cause significant declines in Rh. Given the current global scale decreases in species richness over longer timelines (Sala *et al.* 2000; Butchart *et al.* 2010), our findings indicate that soil biological activity will likely suffer progressive inhibition and continue to decrease.

Consistent with previous meta-analysis studies of litter decomposition rates, which showed negligible, or weak responses to litter mixtures (Lecerf *et al.* 2011; Hooper *et al.* 2012), we found that litter mixtures did not increase Rh on average, largely due to the null effect of two-species mixtures. However, Rh was significantly higher in litter mixtures of three or more species than in the average of one species litter. It is possible that high species diversity increased the probability of particular functional plant types in litter mixtures, which may have accelerated litter decomposition (Handa *et al.* 2014). We also found that the responses of Rh to litter mixtures decreased over time, particularly with more pronounced decreases in more diverse species mixtures. This decline might have been the result of a shift in the primary controlling factors of litter decomposition, from N content to lignin content, as degradation proceeded (Couteaux *et al.* 1995). Litter mixtures might have increased microbial access to N, due to N transfer between the litter of component species (Handa *et al.* 2014), accelerating the shift to lignin limitation and consequent decline in Rh over time.

Despite the wide range of variations in mean annual temperature, mean aridity index, and ecosystem types, the responses of Rs and Rh to plant mixtures did not change spatially, except for a marginally more pronounced increase of Rs in species mixtures with reduced water availability. The increased positive effects of plant mixtures on Rs in drier climates may be attributed to a hydraulic lift phenomenon (Zapater *et al.* 2011). Because species mixtures tend to increase resource acquisition both horizontally and vertically through fine roots (Ma & Chen 2017), deeper rooting species may transport available soil resident water to drier superficial soil layers via their root systems in diverse communities (Zapater *et al.* 2011) and accelerate Rs through increased water availability under dry conditions (Moyano *et al.* 2013).

Plant mixture effects on Rs and Rh were driven by positive associations between the increased biomass of litterfall, fine roots, and soil microbes rather than soil carbon and nitrogen content. Our analysis demonstrated that the species richness in mixtures, stand age and their interaction could indirectly drive an increase in Rs through alterations in plant inputs, while the species richness in mixtures, stand age and their interaction might directly or indirectly drive an increase in Rh through altering plant inputs and soil microbial biomass. Our finding of increased plant inputs in diverse communities could be driven by several mechanisms associated with complementary interactions including interspecific facilitation and/or competitive reduction (Forrester & Bauhus 2016). Further, previous studies have shown that the effects of species mixture on primary productivity increased over time (Cardinale *et al.* 2007; Zhang *et al.* 2012), particularly in more diverse systems (Fornara & Tilman 2009; Reich *et al.* 2012). This increase in aboveground productivity in more diverse systems may be driving the increase in Rs. In addition to directly increasing root- and dead litter-induced respiration (Kuzyakov & Gavrichkova 2010), the increase in plant litter inputs in mixtures might indirectly increase Rh via a "priming effect", i.e., extra decomposition of extant, more stable soil organic matter pools, when microbes are stimulated via the energy released from the decomposition of fresh organic matter (Fontaine *et al.* 2007). Species richness in mixtures, stand age, and their interactions could directly increase soil microbial respiration by increasing microenvironment variability and habitat complexity leading to a higher number of niches for microbes (Hooper *et al.* 2000; Cong *et al.* 2015).

In conclusion, our analysis demonstrated that species mixtures enhanced both total soil respiration and heterotrophic respiration, and these effects increased with the species richness in mixtures. While the positive mixture effects remained similar over time for total soil respiration, positive mixture effects on heterotrophic respiration increased over time, particularly in more diverse species mixtures, but decreased in litter mixtures over time. Our results indicate that the magnitude of estimated species and litter mixture effects on soil respiration may be underestimated when only narrow ranges of species richness and short experimental durations are considered. Our results raise concerns that global declines in plant species diversity could have ubiquitously negative global-scale impacts on soil carbon and nutrient cycling through decreases in soil resident biological activity, particularly in environments under water stress.

CHAPTER 4: PLANT DIVERSITY INCREASES SOIL CARBON: A GLOBAL META-ANALYSIS

4.1 Abstract

Soil organic carbon (SOC) is a valuable resource for mediating global climate change and securing food production. Despite an alarming rate of global plant diversity loss, uncertainties concerning the effects of plant diversity on SOC remain. Plant diversity not only stimulates litter inputs via increased productivity, thus enhancing SOC, it also stimulates microbial respiration, thus reducing SOC. By analyzing 1001 paired observations of plant mixtures and corresponding monocultures from 124 studies, here we show that both SOC content and stock are on average 5% and 8% higher in species mixtures than in monocultures. These positive mixture effects increase over time and are more pronounced in deeper soils. Microbial biomass carbon, an indicator of SOC release and formation, also increases, but its proportion in SOC is lower in mixtures. Moreover, these species mixture effects are consistent across forest, grassland, and cropland systems and independent of background climates. Our results indicate that converting 50% of global forests from mixtures to monocultures would release an average of 2.70 Pg C from soil annually over a period of 20 years: about 30% of global annual fossil-fuel emissions. Our study highlights the importance of plant diversity preservation for the maintenance of soil carbon sequestration in discussions of global climate change policy.

4.2 Introduction

Over the past 25 years, the alarming loss of global plant diversity has promoted the question of how changes in biodiversity will affect ecosystem function and their provision of goods and services to humanity (Tilman *et al.* 1996; Cardinale *et al.* 2012; van Groenigen *et al.* 2014; Newbold *et al.* 2015; Isbell *et al.* 2017). A compelling number of biodiversity experiments have shown many potential benefits of biodiversity across many terrestrial ecosystems worldwide (e.g. Tilman *et al.* 1996; Reich *et al.* 2012; Weisser *et al.* 2017; Grossman *et al.* 2018). Most biodiversity experiments are primarily focused on plant carbon sequestration; however, effects of plant diversity on soil carbon accumulation remain poorly understood (Duffy *et al.* 2017; Isbell *et al.* 2017; van der Plas 2019).

Soil organic carbon (SOC) is a valuable natural resource, supplying goods and services for human benefits, including mediating global climate change and securing food production and environmental quality (Lal 2004a, b). Global soils store three times more organic carbon than the atmosphere and vegetation combined (Carvalhais *et al.* 2014; Intergovernmental Panel on Climate 2014), and thus slight changes in SOC can have a profound effect on the global carbon cycle. Current rapid global changes and land use may switch soils from sinks to sources of atmospheric CO_2 (Lal 2003; van Groenigen *et al.* 2014; Crowther *et al.* 2016). However, our understanding of the effects of plant diversity on SOC remains equivocal (Isbell *et al.* 2017; van der Plas 2019): empirical studies have reported positive (Fornara & Tilman 2008; Chen *et al.* 2018), neutral (Dijkstra *et al.* 2005), and negative (Carol Adair *et al.* 2018) influences of plant species diversity on SOC.

Plant diversity can increase both gains and losses of SOC in mineral soils via contrasting mechanisms. In general, SOC changes are ultimately determined by the balance between carbon input via plant litter production and root exudates and output via microbial decomposition of plant inputs and existing soil organic matter (Amundson 2001). Plant species mixtures could affect the formation and accumulation of SOC via the decomposition and transformation of above and below-ground plant litters (Castellano *et al.* 2015; Cotrufo *et al.* 2015). SOC was thought to be derived primarily from recalcitrant plant litter; however, current evidence suggests that the products of microbial transformation of plant litter likely contribute more to the physicochemically stabilized SOC than recalcitrant plant litter (Mambelli *et al.* 2011; Cotrufo *et al.* 2013). On the one hand, hundreds of controlled experiments and observational studies have shown that plant diversity generally increases the carbon sequestration of live plants due to complementary resource utilization among constituent species in species-rich ecosystems (Duffy *et al.* 2017). Increased biomass production in species-rich communities can enhance plant litterfall and root inputs to soils (Ma & Chen 2016; Zheng *et al.* 2019), and thus increase SOC directly via increased recalcitrant plant components. Increased biomass production can indirectly increase SOC via accumulation of microbial necromass due to a greater amount of labile, 'high-quality' plant litters for soil microorganisms from increased aboveground litterfall and belowground fine root mortality and root exudates in species-rich plant communities (Tilman *et al.* 2001; Milcu *et al.* 2008; Eisenhauer *et al.* 2013; Lange *et al.* 2015; Khlifa *et al.* 2017; Ma & Chen 2018; Chen *et al.* 2019). These products produced during the degradation of litter become the main source of stabilized SOC by promoting aggregate formation and protection within the mineral soil matrix by physicochemical processes (Cotrufo *et al.* 2013; Castellano *et al.* 2015). On the other hand, increased quantity of plant inputs with plant diversity could also lead to an increase in SOC loss via what is commonly referred to as the "priming effect", i.e., extra decomposition of existing, more stable SOC when microbes are stimulated by the energy released from the decomposition of fresh organic matter (Fontaine *et al.* 2007; Sayer *et al.* 2011; Chen & Chen 2018).

Although soil microbial biomass carbon (SMBC) only comprised 1%~4% of the total SOC, it plays a fundamental role in controlling both SOC loss (microbial respiration) and sequestration (Janssens *et al.* 2010; Cotrufo *et al.* 2015; Lange *et al.* 2015; Zhang *et al.* 2018). The SMBC could increase with plant diversity because of the greater amount of carbon and nutrient resources available for soil microorganisms from increased aboveground litterfall and belowground fine root mortality in species-rich ecosystems (Eisenhauer *et al.* 2010; Lange *et al.* 2015; Steinauer *et al.* 2016; Chen *et al.* 2019). However, species-rich plant communities could increase N-use efficiency by producing biomass with C:N ratios higher than that found in monocultures (van Ruijven & Berendse 2005), and therefore provide low-quality substrate inputs (i.e., low litter nitrogen content) that are resistant to microbial decay and supporting less SMBC (Knops *et al.* 2007), resulting in the lower ratio of soil microbial biomass carbon to soil organic carbon (SMBC/SOC) (Liao & Boutton 2008). Nevertheless, similar to SOC, SMBC and SMBC/SOC have been reported to respond to plant diversity inconsistently (Dijkstra *et al.* 2005; Wen *et al.* 2014). These divergent empirical findings could result from variations in species richness, experiment duration, stand age, and background environments.

The effects of plant diversity on SOC and SMBC are anticipated to increase with species richness in mixtures due to feedback from the unique roles of individual species on primary productivity, soil resident microorganisms, and litter decomposition (Handa *et al.* 2014; Lange *et al.* 2015; Chen *et al.* 2018; Chen & Chen 2019). In addition, the evidence is also mounting that the diversity of functional groups is more relevant to ecosystem function than species richness (Reich *et al.* 2004; Wright *et al.* 2006; Dawud *et al.* 2017). High heterogeneity of plant functional traits may enhance the plant mixture effect on soil microorganisms and their induced SOC through plant litter with various chemical and physical traits (Wardle 2006; Reich 2014; Liu *et al.* 2018a). However, the negative effect of species mixtures on SMBC/SOC could be aggravated by increasing species richness due to decreased litter quality (Knops *et al.* 2007). Moreover, the effects of plant diversity on biomass production of both plant and microorganisms increase over time due to increasing interspecific complementarity (Reich *et al.* 2012; Chen *et al.* 2019). These temporal increases may be propagated to SOC via plant residue inputs and microbial necromass accumulation (Lange *et al.* 2015).

The responses of SOC and SMBC to plant diversity may also vary contingent on the background environment. This variation can arise due to differences in magnitude of diversity-productivity relationships between SOC and SMBC caused by vegetation dissimilarity in physiology, structure, and lifespan (Carol Adair *et al.* 2018; Chen *et al.* 2018). For example, grasslands and croplands are dominated by herbaceous plants and diversity studies tend to run for several lifetimes of their component species; while diversity studies often run only for a small fraction of the potential life span of most tree species in forests (Forrester & Bauhus 2016). As a result, mortality rates or species replacements are reduced in forest studies relative to grassland and cropland studies, and biodiversity-ecosystem functioning (BEF) relationships may be more stable in mature forest experiments than in grassland experiments (Weisser *et al.* 2017; Grossman *et al.* 2018). Across large spatial scales, positive effects of plant diversity on ecosystem multifunctionality tend to be stronger in drier climates than in more mesic ones (Ratcliffe *et al.* 2017). Furthermore, the effects of species diversity on SOC and SMBC may change with soil depth because of a stronger species diversity effect on root biomass in deeper soils (Mueller *et al.* 2013). A quantitative synthesis of results across multiple studies is needed, therefore, to assist in determining the overall effects of plant species diversity and identifying sources of variation (Gurevitch *et al.* 2018).

We conducted a meta-analysis using a global dataset of 1001 paired observations from 124 studies that included at least one species mixture treatment with corresponding monocultures (Fig. 4-1). By quantifying the complementarity effects of species mixtures compared to the expected response from corresponding monocultures (Loreau & Hector 2001) (see Methods), we examined the overall effects of species mixture on SOC content and stock, SMBC, and SMBC/SOC in mineral soils. Furthermore, we tested whether these effects would increase with species richness (or functional group richness) in mixtures and with stand age, and whether these effects differed across a wide range of ecosystem types, mean annual temperatures, and annual aridity indices at the study sites, and across soil depths.

4.3 Materials and Methods

4.3.1 Data collection

We examined peer-reviewed publications that were published prior to June 2019 which investigated the effects of plant diversity on SOC and SMBC using the ISI Web of Science and Google Scholar with the search term: "soil organic carbon OR microbial biomass carbon OR soil microbial quotient AND diversity OR richness OR mixture OR pure OR polyculture OR monoculture OR overyielding", and also searched references within these papers. The literature search was performed following guidelines from PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) (Moher *et al.* (2009); Fig. S4-1).

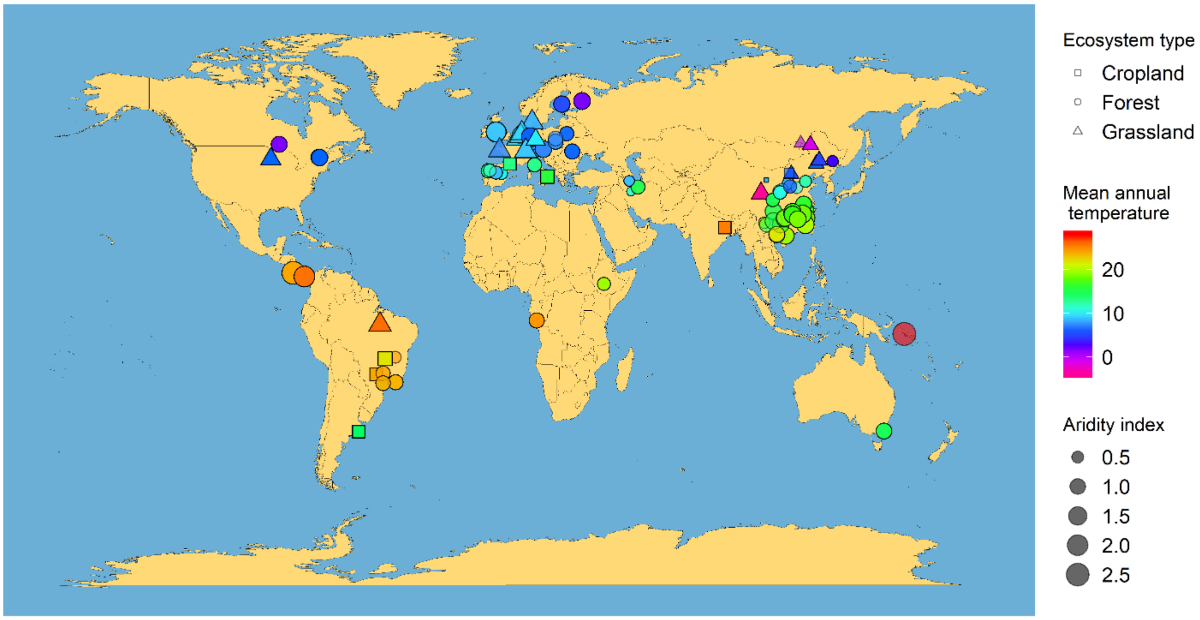


Figure 4-1. Global distribution of plant diversity experiments testing the effects of species mixtures on soil organic carbon and microbial biomass carbon, collected for this meta-analysis.

We employed the following criteria to select the studies: (*i*) they were purposely designed to test the effects of plant diversity on SOC content, SOC stock, and SMBC in mineral soils, (*ii*) they had at least one species mixture treatment and corresponding monocultures, (*iii*) they had the same initial climatic and soil properties in the monoculture and mixture treatment plots. In total, 121 publications met these criteria (Fig. 4-1, Table S4-1). The SOC stock is calculated from SOC content and soil bulk density of each layer, and represents the amount of carbon per area over a specified thickness of soil (Ellert *et al.* 2001). In eight publications, several experiments, each with independent controls, were conducted at different locations and were considered separate studies. When different publications included the same data, we recorded the data only once. When a publication included plant species mixtures of different numbers of species, we considered them distinct observations. This resulted in 94 studies for SOC content, 52 studies for SOC stock, 51 studies for SMBC, and 38 studies for SMBC/SOC.

For each site, we extracted the values, the number of replications, and the standard deviation of SOC, SMBC, and SMBC/SOC, if reported. Methods for determining SMBC included the substrate-induced respiration method (Anderson & Domsch 1978) and the fumigation-extraction method (Vance *et al.* 1987). When an original study reported results graphically, we used Plot Digitizer version 2.0 (Department of Physics at the University of South Alabama, Mobile, AL, USA) to digitally extract data from figures. We also extracted species richness (SR, number of plant species), functional group richness (FR, number of plant functional groups), soil depth, ecosystem types (e.g., forest, grassland, cropland), latitude, longitude, mean annual temperature (MAT, °C), and stand age from original or cited papers. Annual aridity index data were retrieved from the CGIAR-CSI Global Aridity Index dataset (Trabucco & Zomer 2009) through the use of location information. The annual aridity index was calculated as the ratio of annual mean precipitation to mean annual potential evapotranspiration (UNEP 1997). Stand age was recorded as the number of years since a stand-replacing disturbance in forests and the number of years between the initiation and measurements of the experiments in grasslands and croplands. Soil depth was recorded as the midpoint of each soil depth interval (Chen & Brassard 2013). The species proportions in plant mixtures were based on basal area or stem density in forests, coverage in croplands, and sowing seeds in grasslands. Plants in forests are classified into two functional groups (broadleaved and coniferous trees); those of grasslands are classified into four functional groups (C_3 grasses, C_4 grasses, forbs, and legumes); those of croplands are classified into two functional groups (grains and legumes).

4.3.2 Data analysis

The natural log-transformed response ratio (ln*RR*) was employed to quantify the effects of plant diversity following Hedges *et al.* (1999):

$$\ln RR = \ln\left(\bar{X}_t / \bar{X}_c\right) = \ln \bar{X}_t - \ln \bar{X}_c \tag{1}$$

where $\overline{X_t}$ and $\overline{X_c}$ were the observed values of a selected variable in the mixture and the expected value of the mixture in each study, respectively. We calculated the $\overline{X_c}$ based on weighted values of the component species in monocultures following Loreau and Hector (2001):

$$\overline{X_c} = \sum (p_i \times m_i) \tag{2}$$

where m_i is the observed value of the selected variable of the monoculture of species *i* and P_i is the proportion of species *i* density in the corresponding mixture. When a study reported multiple types of mixtures (species richness levels) and experimental years, $\overline{X_t}$ and $\overline{X_c}$ were calculated separately for each mixture type and experimental year. This method accounted for the effects of species composition (i.e., selection effect) among different levels of species richness in mixtures and stand age (Loreau & Hector 2001).

Estimates of effect sizes and subsequent inferences in the meta-analyses could be dependent on how individual observations were weighed (Ma & Chen 2016). In our dataset， sampling variances were not reported in 24 of the 117 publications, and importantly, weightings based on sampling variances could assign extreme importance to only a few individual observations. Consequently, the average ln*RR* would be determined predominantly by a small number of studies (Ma & Chen 2016). Similar to previous studies (Pittelkow *et al.* 2015), we employed the number of replications for weighting:

$$W_r = (N_c \times N_t)/(N_c + N_t) \tag{3}$$

where W_r is the weight associated with each ln*RR* observation, and N_c and N_t are the number of replications in monocultures and corresponding mixture, respectively.

We first used the following model to determine the effects of the species richness in mixture (SR), stand age (SA), soil depth (D), and environmental variables, (E, i.e., ecosystem type, mean annual temperature, or aridity index) and their interactions on SOC content, SOC stock, SMBC, and SMBC/SOC:

$$\begin{aligned}\ln RR &= \beta_0 + \beta_1 \cdot SR + \beta_2 \cdot SA + \beta_3 \cdot D + \beta_4 \cdot E + \beta_5 \cdot SR \times SA + \beta_6 \cdot SR \times D \\ &+ \beta_7 \cdot SR \times E + \beta_8 \cdot SA \times D + \beta_9 \cdot SA \times E + \beta_{10} \cdot D \times E + \beta_{11} \cdot SR \times SA \times D \\ &+ \beta_{12} \cdot SR \times SA \times E + \beta_{13} \cdot SR \times D \times E + \beta_{14} \cdot SA \times D \times E + \beta_{15} \cdot SR \times SA \times D \times E \\ &+ \pi_{study} + \varepsilon\end{aligned} \tag{4}$$

where β_i, π_{study}, and ε are coefficients for the random effect factor of "Study" and sampling error, respectively. The random effect explicitly accounted for autocorrelation among observations within each "Study". We conducted the analysis using the restricted maximum likelihood estimation with the *lme4* package (Bates *et al.* 2017). To validate the linearity assumption for the continuous predictors, we graphically plotted the ln*RR* vs. individual predictor pairs and compared the linear and logarithmic functions with the predictor of interest as the fixed effect and "study" as the random effect. We found that the logarithmic SA and SR resulted in lower or similar Akaike information criterion (AIC) values for all independent variables, while logarithmic D only resulted in lower AIC values for SMBC (Table S5-2). To prevent overfitting (Johnson & Omland 2004), we selected the most parsimonious model among all alternatives with the condition to keep species richness as it was intrinsic to the purpose of the study to assess the effect of the species richness in mixtures. The model selection was accomplished by using the '*dredge*' function of the *muMIn* package (Barton 2018). All terms associated with environmental variables (i.e., ecosystem type, mean annual temperature, and aridity index) (Eqn. 4) were excluded in the most parsimonious models. To further examine the effects of environmental variables, we conducted an analysis with the environment variable as the only fixed factor and "study" as the random factor. The analysis confirmed that there was no difference in the responses of SOC content and stock, SMBC, and SMBC/SOC to plant mixtures among experimental systems (Table S4-5). Finally, we employed the following models (5), (6), (7), and (8) to determine the overall effects from species richness, stand age, soil depth, and their interaction on SOC content ($\mathrm{SOC_{content}}$), SOC stock ($\mathrm{SOC_{stock}}$), SMBC, and SMBC/SOC:

$$\ln RR_{SOCcontent} = \beta_0 + \beta_1 \cdot \ln(SR) + \beta_2 \cdot \ln(SA) + \beta_3 \cdot D + \beta_4 \cdot \ln(SA) \times D + \pi_{study} + \varepsilon \tag{5}$$

$$\ln RR_{SOCstock} = \beta_0 + \beta_1 \cdot \ln(SR) + \beta_2 \cdot \ln(SA) + \beta_3 \cdot D + \beta_4 \cdot \ln(SA) \times D + \pi_{study} + \varepsilon \tag{6}$$

$$\ln RR_{SMBC} = \beta_0 + \beta_1 \cdot \ln(SR) + \beta_2 \cdot \ln(SA) + \beta_3 \cdot \ln(D) + \beta_4 \cdot \ln(SR) \times \ln(SA) + \pi_{study} + \varepsilon \tag{7}$$

$$\ln RR_{SMBC/SOC} = \beta_0 + \beta_1 \cdot \ln(SR) + \beta_2 \cdot D + \pi_{study} + \varepsilon \tag{8}$$

We scaled all continuous predictors (observed values minus mean and divided by one standard deviation). When continuous predictors are scaled, β_0 is the overall mean ln*RR* at the mean ln(SR), ln(SA), and D (or ln(D)) (Cohen *et al.* 2013). To graphically illustrate whether the effect of species richness and stand age on ln*RR* differed with soil depth, we calculated soil depth-dependent stand age and species richness effects using the method described by Cohen et al. (2013) at soil depths of 0 - 10, 10 - 20, 20 - 30, 30 - 40, and 40 - 50 cm, respectively, which were the most common soil depths sampled in the original studies. Similarly, we calculated species richness-dependent stand age effects at species richness values of 2, 4, 8, 16, and 60, which were the most common species richness values in the original studies. We also tested the effects of functional group richness on ln*RR* by replacing the terms of species richness in eqn. 5-8 with functional group richness.

In order to assess whether our results were biased by the relatively few data points with higher species richness (e.g. 60 species richness in the Jena experiment, Table S4-1), we conducted the same analysis for the dataset after removing observations with 60 species. We compared the estimates and species-richness trends for the datasets with and without 60 species richness level, and found that both datasets yielded qualitatively similar estimates and trends (Fig. S4-2). Thus, we reported the results for the whole dataset.

The treatment effects were significant at α = 0.05 if the 95% confidence intervals (Cis) of estimated ln*RR* did not cover zero. The mean effect sizes between groups were significantly different if their 95% CIs did not overlap the other's mean. To facilitate interpretation, we transformed the ln*RR* and its corresponding CIs back to a percentage using $(e^{\overline{\ln RR}} - 1) \times 100\%$. All statistical analyses were performed in R 3.5.2 (R Core Team 2018).

4.4 Results

Together, 1001 paired observations of plant mixtures and corresponding monocultures were derived from 124 studies, including 402 for SOC content, 229 for SOC stock, 254 for SMBC, and 116 for SMBC/SOC (Fig. 4-2). The geographical range of these studies spanned from 37.77 ºS to 62.6 ºN (Fig. 4-1), the mean annual temperature ranged from -3.9 to 28 º C, and the aridity index ranged from 0.10 to 2.56. A large number of studies on plant species diversity effects were conducted during the last few years, with 52% of the studies in this meta-analysis published between 2014 and 2019 (Table S4-1). Further, our meta-analysis covered 30 countries on five continents and encompassed most terrestrial ecosystem types, including 10 sites for croplands, 92 sites for forests, and 22 sites for grassland (Fig. 4-1, Table S4-1). These studies spanned a wide range of species richness in mixtures (2 - 60, mean ± S.D. = 3.7 ± 7.2), stand age (0.2 – 130 years, mean ± S.D. = 19.9 ± 27.3 years), and soil depth (1 - 100 cm, mean ± S.D. = 15.3 ± 16.4 cm). Among different ecosystems, grassland studies spanned the widest range of species richness in mixtures (2 - 60, mean ± S.D. = 8.2 ± 13.8), forest studies spanned a relatively narrow range of species richness in mixtures (2 - 12, mean ± S.D. = 2.4 ± 0.9), and cropland studies only had a species richness of 2 in mixtures (Table S4-1). Forest studies spanned the widest range of stand age (0.5 – 130 years, mean ± S.D. = 31.1 ± 30.6 years), while grassland and cropland studies spanned a relatively narrow range of stand age (grassland: 1 – 18 years mean ± S.D. = 4.4 ± 3.8 years; cropland: 0.2 – 7 years, mean ± S.D. = 2.4 ± 2.1 years) (Table S4-1). Cropland studies spanned the widest range of soil depth (2.5 – 100 cm, mean ± S.D. = 22.1 ± 24.1 cm), while grassland and forest studies spanned a relatively narrow range of soil depth (forest: 1 – 90 cm, mean ± S.D. = 15.8 ± 14.7 cm; grassland: 2.5 – 75 cm, mean ± S.D. = 9.0 ± 10.3 cm) (Table S4-1).

Across all experimental systems, plant species mixtures, on average, increased SOC content by 5.1% (95% confidence intervals, 1.5% – 8.7%) and SOC stock by 8.4% (3.2% – 13.7%) (Fig. 4-2). These positive species mixtures effects increased with species richness in mixtures, but this was only marginally significant (Table S4-3, Figs. 4-3a, b, $P = 0.060$ and $P =$

0.098 for SOC content and stock, respectively). Additionally, these positive species mixtures effects did not change with functional group richness (Table S4-4, $P = 0.330$ and $P = 0.756$ for SOC content and stock, respectively). The positive effect of species mixtures on SOC content and stock increased significantly with stand age (Figs. 4-3c, d, $P < 0.001$, $P = 0.016$, respectively), shifting from negative to positive over time. Importantly, the mixture effects on both SOC content and stock were dependent on soil depth (Table S4-3), with more pronounced species mixture effects in deep soils (Figs. 4-3c, d).

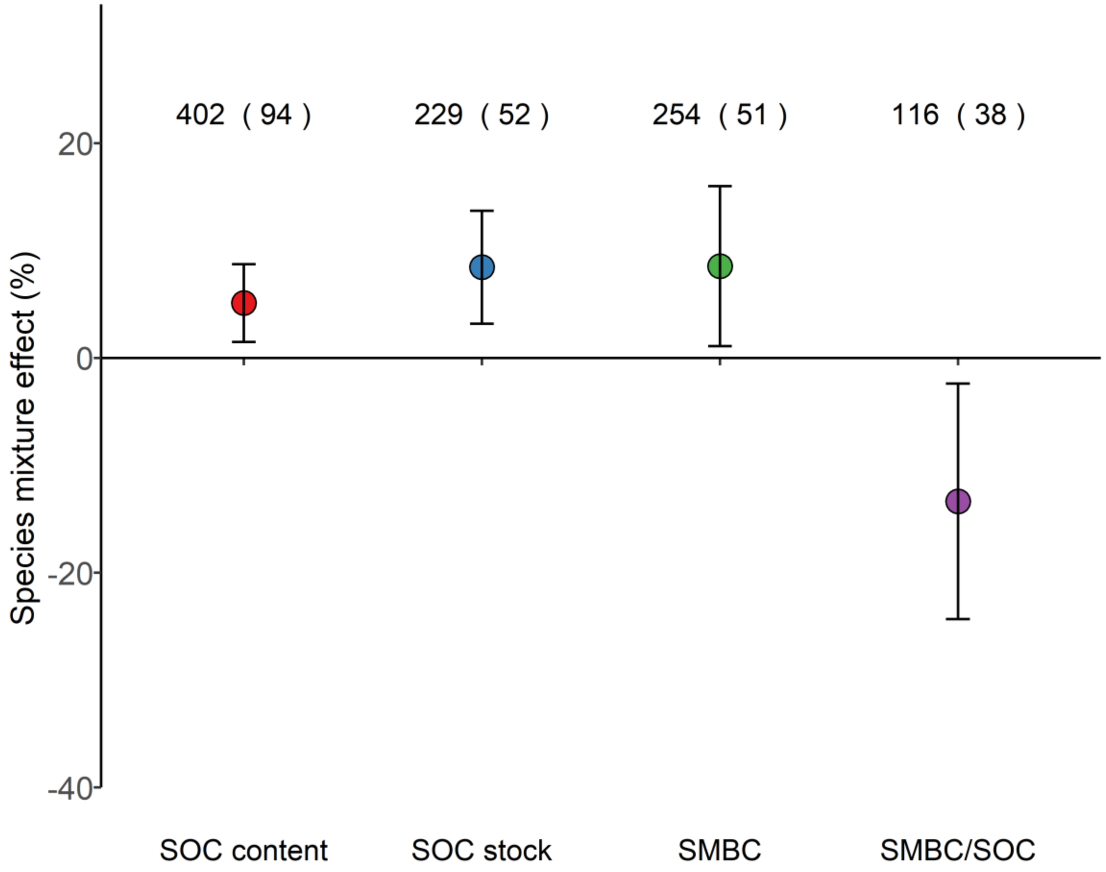


Figure 4-2. Comparison of soil organic carbon content (SOC content), soil organic carbon stock (SOC stock), soil microbial biomass carbon (SMBC), and the ratio of soil microbial biomass carbon to soil organic carbon (SMBC/SOC) in species mixtures versus monocultures across all studies**.** The average effect represents the increase or decrease (%) of a given attribute compared to the corresponding mean of constituent monocultures at the mean species richness, mean stand age, and mean soil depth in mixtures (see Methods). Values are mean ± 95% confidence intervals of the percentage effects between the species mixtures and monocultures. The number of observations is shown beside each attribute without parentheses with the number of studies in parentheses.

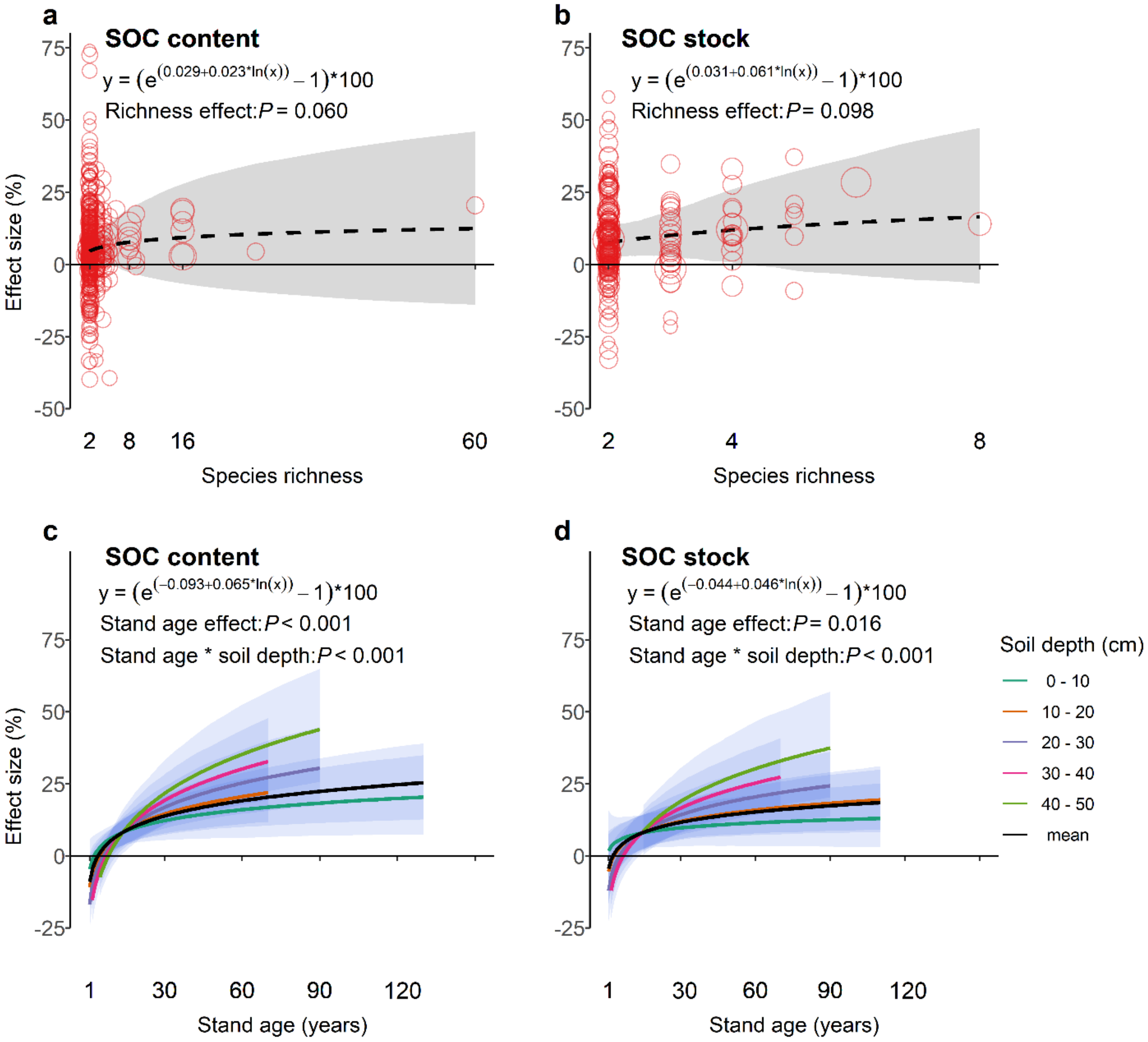


Figure 4-3. Comparison of soil organic carbon content and stock in species mixtures versus monocultures in relation to the species richness in mixtures and stand age**. a-b**, Comparison of soil organic carbon content and stock in species mixtures versus monocultures in relation to the species richness in mixtures. **c-d**, Comparison of soil organic carbon content and stock in species mixtures versus monocultures in relation to stand age on average (black line and fitted regression) and by soil depth intervals. The effects are quantified as the percent changes in mixtures compared to the corresponding mean value of constituent monocultures. Black and colored lines represent the average and soil depth-specific responses, respectively, with their bootstrapped 95% confidence intervals shaded in grey. The significance (*P*) is presented for each term tested. The sizes of the circles represent the relative weights of corresponding observations.

Plant species mixtures, on average, increased SMBC by 8.6% (1.1% – 16.0%) (Fig. 4-2), and the effects of species mixture on SMBC increased with species richness in mixtures and stand age, with more pronounced stand age-associated increases in more diverse species mixtures (Figs. 4-4a, b, *P* = 0.008, *P* = 0.021, and *P* < 0.001 for species richness in mixtures, stand age, and their interaction, respectively). However, studies that simultaneously measured SMBC and SOC showed that species mixtures decreased SMBC/SOC by 13.3% (-24.3% – -2.4%) relative to expected values from monocultures, indicating that the magnitude of increase in SMBC is less than the magnitude of increase in SOC (Fig. 4-2), and SMBC/SOC decreased in deep soils of plant mixtures (*P* < 0.001 for both SMBC and SMBC/SOC, Fig. S4-3). The effects of species mixtures on SMBC and SMBC/SOC did not change significantly with functional group richness (Table S4-4, *P* = 0.060 and *P* = 0.624 for SMBC and SMBC/SOC, respectively).

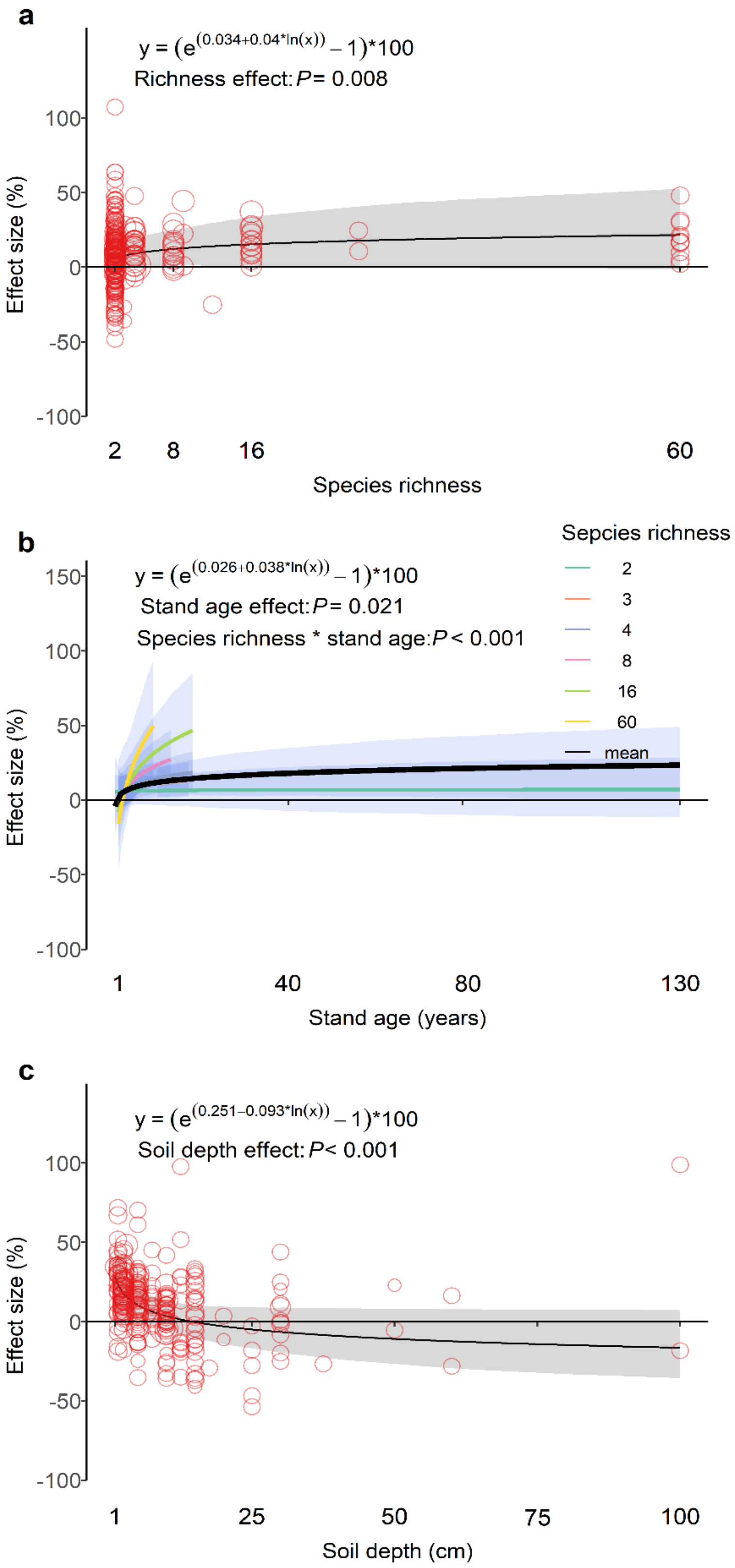

a
y = (e^(0.034+0.04*ln(x)) – 1)*100
Richness effect: P= 0.008
Effect size (%)
100
50
0
-50
-100
2
8
16
60
Species richness
b
Sepcies richness
2
3
4
8
16
60
mean
y = (e^(0.026+0.038*ln(x)) – 1)*100
Stand age effect: P= 0.021
Species richness * stand age: P< 0.001
Effect size (%)
150
100
50
0
-50
-100
1
40
80
130
Stand age (years)
c
y = (e^(0.251–0.093*ln(x)) – 1)*100
Soil depth effect: P< 0.001
Effect size (%)
100
50
0
-50
-100
1
25
50
75
100
Soil depth (cm)

Figure 4-4. Comparison of soil microbial biomass carbon in species mixtures versus monocultures in relation to the species richness in mixtures, stand age and soil depth. a, Comparison of soil microbial biomass carbon in species mixtures versus monocultures in relation to the species richness in mixtures. b**,** Comparison of soil microbial biomass carbon in species mixtures versus monocultures in relation to stand age on average (black line and fitted regression) and by the species richness level in mixtures**.** c, Comparison of soil microbial biomass carbon in species mixtures versus monocultures in relation to soil depth. The significance (*P*) is presented for each term tested. The sizes of the circles represent the relative weights of corresponding observations.

The responses of SOC content, SOC stock, SMBC, and SMBC/SOC to plant species mixtures did not change with ecosystem type (croplands, grasslands, and forests), mean annual temperature, or aridity index (Table S4-5), indicating globally consistent SOC and SMBC responses to plant diversity loss across ecosystem types and climatic gradients. Among different ecosystem types, plant species mixtures increased SOC content and stock in forests, but did not change them in grasslands and croplands, while plant species mixtures only increased SMBC in grasslands (Fig. 4-5). Plant species mixtures did not change SMBC/SOC across various ecosystem types except for forests, where SMBC/SOC was significantly lower in species mixtures than monocultures (Fig. 4-5).

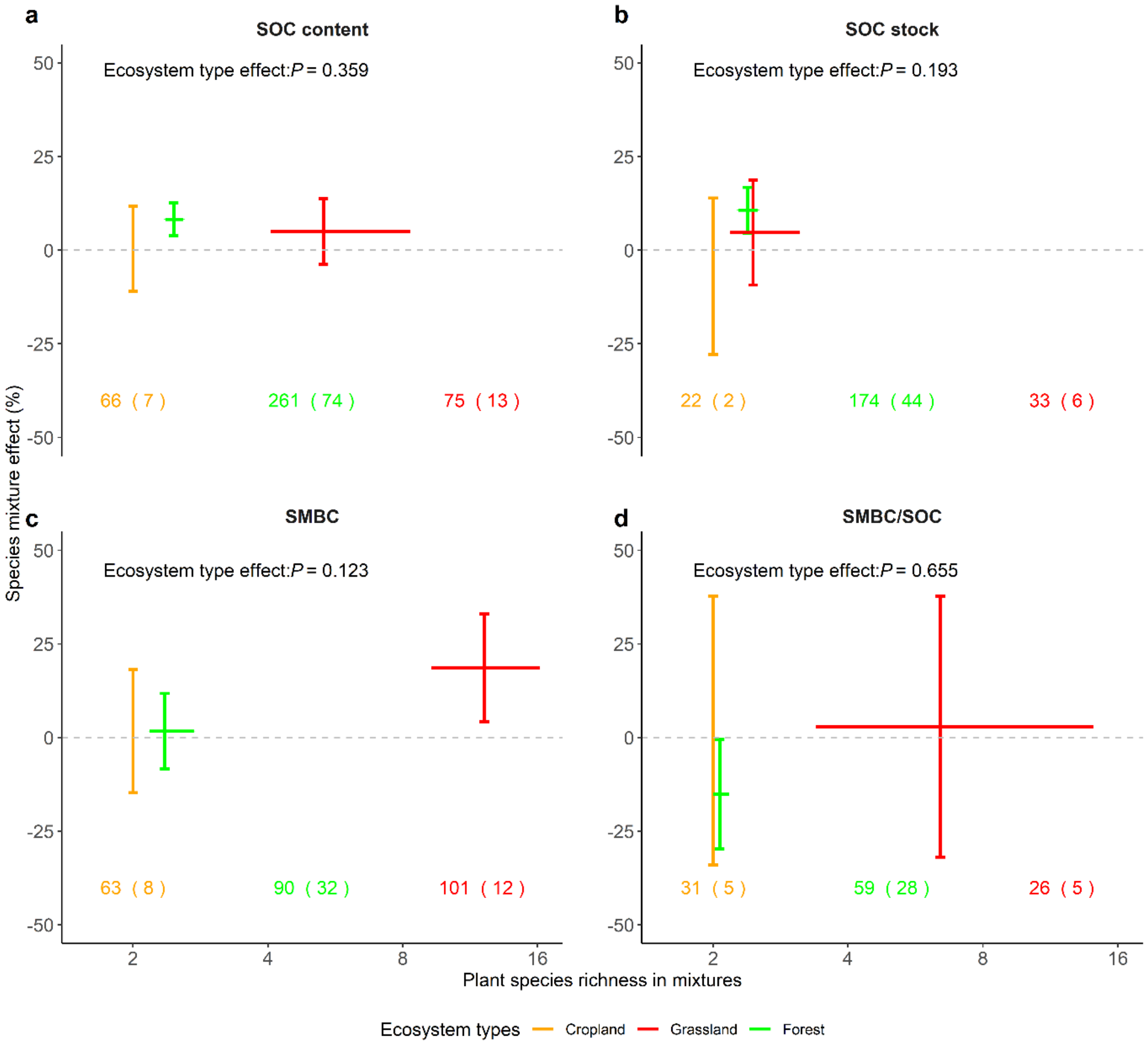


Figure 4-5. Comparison of soil organic carbon content (SOC content), soil organic carbon stock (SOC stock), soil microbial biomass carbon (SMBC), and the ratio of soil microbial biomass carbon to soil organic carbon (SMBC/SOC) in species mixtures versus monocultures among the three ecosystem types. Means and vertical and horizontal error bars represent means and 95% confidence intervals for species mixture effects and the species richness in mixtures, respectively. For each experimental system, the number of observations is shown without parentheses with the number of studies in parentheses. P value, derived from the linear mixed model with the experimental system as the fixed effect and study as the random effect, represents the significance of the difference in lnRRs among experimental systems. The figure shows that despite significant differences in the species richness in mixtures among experimental systems (as indicated by non-overlapping CIs), lnRR did not significantly differ for any of the studied attributes.

4.5 Discussion

4.5.1 Plant species mixture effects on SOC

Our meta-analysis shows the first global evidence that plant species mixtures yield greater SOC compared to monocultures. Further, these positive effects of species mixture were globally consistent, suggesting that the global conversion of mixed systems to monoculture plantations not only decreases biodiversity (Newbold *et al.* 2015) but also reduces SOC. This result extends our understanding of the importance of maintaining biodiversity to support ecosystem functioning (Reich *et al.* 2012; Zhang *et al.* 2012; Duffy *et al.* 2017).

We found weak support for our hypothesis that the positive effects of species mixtures on SOC content and stock would increase with species richness in mixtures, but the functional group richness in mixtures had little effect. The lack of a strong effect of species richness or functional group richness in mixtures on SOC could be attributable to decreases in litter quality with increases in species richness (Knops *et al.* 2007). Cotrufo et al. (2013) suggested that litter quality is the dominant controlling factor of microbial products, relative to input quantity, because the labile litter component could be utilized more efficiently by microbes because of increasing quality. The higher proportion of low-quality litter in plant species mixtures can decrease substrate use efficiency and lead to less microbial-derived SOC produced per unit of plant litter metabolized (Cotrufo *et al.* 2013; Castellano *et al.* 2015; Cyle *et al.* 2016). Therefore, the low-quality litter produced in species-rich plant communities could counteract the effect of increased litter inputs associated with increased species richness, causing marginal or no increases in total SOC. Alternatively, the size of SOC pools in species-rich plant communities reached an upper limit in soils and these soils could not retain new litter inputs in stable SOC, as soils have a finite capacity to store C within relatively stable pools in the mineral soil matrix (Castellano *et al.* 2015; Mitchell *et al.* 2016).

The positive species mixture effects on SOC content and stock increased with stand age, complementing the findings of previous meta-analyses wherein the effects of biodiversity on productivity increased over time (Cardinale *et al.* 2007; Zhang *et al.* 2012). It also suggests that duration needs to be sufficiently long (≥ 5 years based on Fig. 4-3) in order to properly estimate plant diversity effects on SOC content and stock. Short experimental durations could lead to neutral diversity-SOC relationships. Accumulation of soil C pools is a slow process, and perceptible changes in the stock itself may not be observable over the short-term (Liu *et al.* 2018b), let alone accurate estimates of the effects of outside factors.

Importantly, we found a larger increase in the mixture effect as stands aged for SOC content and stock in deeper soils, which is probably a consequence of a deeper fine root distribution in plant species mixtures compared to monocultures (Mueller *et al.* 2013; Ma & Chen 2017) and the delayed response of fine roots to changes in plant diversity (Ravenek *et al.* 2014) as well as a combination of different regulatory mechanisms for carbon dynamics in shallow and deep soils (Salome *et al.* 2010). Substrate accessibility by microbes and exoenzymes is the main controlling factor of SOC mineralization in the deep soil, while the supply of fresh litter inputs is the main regulatory mechanism of C dynamics in topsoil (Salome *et al.* 2010). Deep soils are more stable and less accessible by microbes due to the colder, more anoxic, and nutrient-limited environment compared with surface horizons, and thus more likely to preserve and enhance SOC (Salome *et al.* 2010; Dungait *et al.* 2012). Thus root inputs are the main source of SOC, compared with aboveground inputs (Jackson *et al.* 2017). The initial higher effects of species mixtures on SOC in topsoil relative to deep soils could be attributed to the quick response of aboveground biomass to plant diversity (Ravenek *et al.* 2014), which promotes litter-derived SOC formation in topsoil (Cotrufo *et al.* 2015). However, root inputs are approximately five times more efficiently stabilized as SOC than aboveground inputs (Jackson *et al.* 2017), as SOC in topsoils could still be accessed by soil microorganisms, and can degrade relatively rapidly, i.e. within years or decades, compared with deep SOC (Dungait *et al.* 2012). Our results indicate that the SOC response to plant mixtures could be mainly root-derived, supporting an emerging root-centric view of SOC formation that the primary source of SOC usually comes from rhizodeposition (Rasse *et al.* 2005; Schmidt *et al.* 2011; Sokol *et al.* 2019). We call for future field experimental studies to consider the role of root inputs in regulating diversity effects on SOC (Cong *et al.* 2014; Lange *et al.* 2015).

Since half of the global soil carbon is stored in deeper soil layers (below 30 cm) (Balesdent *et al.* 2018), our finding stresses that long-term diversity manipulation experiments need to consider the entire soil profile, and soil depth should be incorporated to better predict the consequences of diversity loss on soil carbon pools. The common practice of inferring plant diversity induced changes in soil processes from only shallow soil samples can lead to spurious estimates. At a minimum, we highlight the need to measure at a variety of soil depths to better predict the consequences of diversity loss on soil carbon pools, if not incorporate soil depth into modelling strategies.

We found that the effects of plant mixtures were consistent across a wide range of ecosystem types including forests, grasslands, and croplands. When analyzed by individual ecosystem types, we only found significant plant mixture effects in forests, which is likely a consequence of longer studied time scale (average 31 years) and greater sample sizes (92 sites) in forests. The lack of the mixture effects in croplands is largely attributable to the low species richness (2) in mixtures as well as small sample sizes (for example, only two studies for SOC stock). Shorter experimental duration likely resulted in the lack of the significant mixture effects in grassland, where plant diversity effects on SOC have been proved to increase over time (Fornara & Tilman 2008; Lange *et al.* 2015). Alternatively, the difference in mean residence times of litter inputs among vegetation types could also affect these differential responses to plant mixtures because compared with grassland and cropland, more recalcitrant litter in forests may result in more SOC accumulation in forests (Huang *et al.* 2011). Although plant mixture effects on both SOC did not change with temperature and aridity index in our study, further investigations are still needed to reveal whether species mixture effects on soil process change with climate conditions or intrinsic soil properties. Climate conditions (e.g. temperature and water availability) and soil texture could also affect both biodiversity-productivity relationships (Paquette & Messier 2011; Grossiord *et al.* 2014; Ratcliffe *et al.* 2017) and soil microbial composition (Wu *et al.* 2009; de Vries *et al.* 2012; Xu *et al.* 2018), and thereby affect soil carbon dynamics.

4.5.2 Plant species mixture effect on SMBC

As expected, our analysis revealed higher SMBC in species mixtures over monocultures, and that the mixture effect increased with species richness in mixtures. Further, we found that the positive response of SMBC to species mixtures was strengthened over time, with more pronounced increases in more diverse species mixtures. These findings extend our understanding of the positive diversity-productivity relationship from plant to microbial communities (Tilman & Lehman 2001; Liang *et al.* 2016) and also agree with the findings of previous meta-analyses wherein the effects of plant diversity on productivity and litter decomposition increased over time (Lecerf *et al.* 2011; Zhang *et al.* 2012). Increases in SMBC in species mixture may arise because species-rich plots accumulate more nutrients (e.g., nitrogen) in soil organic matter over time, relative to species-poor plots. These additional nutrients subsequently increase plant productivity (Fornara & Tilman 2009; Reich *et al.* 2012) and this effect may be propagated to the soil microbial community via plant litter inputs (Waldrop *et al.* 2017; Chen & Chen 2019). We also found that the plant species mixture effect on SMBC was restricted to the topsoil layer (≤10 cm) (Figs. 4-4c). The lack of species mixture effect on SMBC in depths > 10 cm is possibly a result of unfavourable conditions (e.g., lack of oxygen, energy-rich plant C) for microbes accumulation in deeper soils (Fontaine *et al.* 2007; Shahzad *et al.* 2018). Again, our study cautions against the common practice of inferring plant diversity driven induced changes in soil processes from shallow soil samples alone. Collectively, these results suggest a lasting effect of species mixture on soil microbes and active pool of SOC over time, especially in surface soil.

Our meta-analysis offers new insights into the variations of SMBC/ SOC ratio associated with plant species mixtures. Even though plant mixtures increased both SOC and SMBC, we found lower SMBC/SOC in species mixtures than monocultures, where these negative effects on SMBC/SOC increased with soil depth. This result indicates that total SOC benefits more from plant species mixtures than the active pool. This result suggests that there is less relative availability of easily decomposable carbon substrates, resulting in a larger proportion of stabilized carbon in the mineral soil in species mixtures than in monocultures (Anderson & Domsch 1989) and more so in deep soils. Since very few aboveground carbon inputs penetrate as deep as mineral soil (or more than 10 cm) due to the longer route (Sokol &

Bradford 2019), our results indicate that the magnitudinal increase in SOC and SMBC to plant mixture could be primarily root-derived.

4.5.3 Implications for future research and soil management strategies

Soils are fundamental to life on the planet but anthropogenic pressures on soil resources are reaching limits of sustainability (FAO and ITPS 2015). Soil resources are essential for the provision of services to humanity, including food production, water quality, and atmospheric greenhouse gas mitigation (Amundson *et al.* 2015). Soil organic carbon is a key resource and ecosystem function, strongly influencing all soil processes and functions (Wiesmeier *et al.* 2019). Due to anthropogenic land-use activities in the 20th century, such as the conversion of primary forests into croplands, global SOC has declined by 25% (Don *et al.* 2011), resulting in an increase of about 32% of human-induced greenhouse gas emissions (IPCC 2014b), thereby contributing to global climate warming and associated environmental problems. Additionally, global warming stimulates the loss of soil C into the atmosphere - especially in high-latitude areas with larger C stocks (Crowther *et al.* 2016) - while the increase in photosynthesis due to rising concentrations of CO_2 in the atmosphere only have limited positive effects on carbon storage (van Groenigen *et al.* 2014). Ecologists have long sought for sustainable management of the SOC pool to improve soil carbon sequestration (Stockmann *et al.* 2013) and therefore dampen rising atmospheric CO_2 concentrations, improve soil fertility, and sustain food production (Lal 2004a).

Given that global forests contain 1,204 Pg of SOC (Carvalhais *et al.* 2014) and that the species mixture effect on SOC stock increases over time (the equation of Fig. 4-3d based on the average soil depth of 17 cm for the entire dataset), converting 50% of the global forest area from tree species mixtures to monocultures would release 53.9 Pg C from soil over twenty years (on average 2.70 Pg C yr^{-1}). Our estimate of SOC loss with 50% of global forest area conversion from mixtures to monocultures is about 30% of global annual fossil-fuel emissions (9.0 Pg C yr^{-1} in 2011, ref (Intergovernmental Panel on Climate 2014)). Given that global average maximum root depth is about 104 cm (Schenk & Jackson 2002), the effect of plant diversity loss on global forest SOC could be even higher than our estimate because the average soil depth of our data was 17 cm and the effect of plant diversity loss on SOC increases more over time in deeper soils (Balesdent *et al.* 2018). On the other hand, global croplands contain 327 Pg of SOC (Carvalhais *et al.* 2014) and converting global monoculture cropping to intercropping would sequester 32.2 Pg C from the atmosphere to the soil over twenty years (on average 1.60 Pg C yr^{-1}), which could help to counteract about 17.7% of the global annual fossil-fuel emissions (Intergovernmental Panel on Climate 2014). Increasing SOC also improves other ecosystem functions and services, such as food production, soil nutrient storage, water holding capacity, aggregation, and pollutants retention (Lal 2004a; Kibblewhite *et al.* 2008; Guo *et al.* 2019).

As an important labile SOC fraction, SMBC plays a fundamental role in controlling soil element cycling and aboveground productivity (Friedel *et al.* 1996; Lange *et al.* 2015; Li *et al.* 2019). We found that plant species mixtures increased soil microbial biomass carbon across a diverse range of terrestrial ecosystems. Our results suggest that declines in soil microbial biomass carbon induced by lower plant diversity could have profound adverse effects on nutrient cycles and food and bioenergy production. Our analysis further revealed that these positive effects on SMBC increased with species richness in mixtures andstand age, suggesting that these adverse effects could amplify with further decreases in plant diversity and longer times post-disturbance.

Although plant mixture effects on both SOC and SMBC did not change with functional group richness in mixtures, we still want to emphasize the importance of diversity of functional traits when characterizing soil C responses to plant diversity. The lack of an effect of functional group richness could be attributable to our functional classification, which may underestimate the role of functional diversity (Wright *et al.* 2006). There is clear evidence that functional trait diversity (FD) of plant community (Paquette & Messier 2011; Gazol & Camarero 2016) and community-weighted-mean trait value (CWM) (Finegan *et al.* 2015; Tobner *et al.* 2016) play a critical role in determining the outcome of diversity effects on plant productivity. Upon increased plant productivity, diverse plant litter mixtures with high heterogeneity in chemical compounds could increase soil microbial biomass and their induced stable SOC due to expanded resource niches for microbes (Chapman & Newman 2010; Santonja *et al.* 2017). Furthermore, higher functional diversity of leaf and root physical traits, including specific leaf area and leaf dry matter content, can also introduce a stronger mixture effect on SOC and SMBC (Wardle *et al.* 2003; Pakeman *et al.* 2011), as physically diverse litter and root mixtures improve the microenvironment of microbial habitats by increasing soil aggregate stability and litter moisture and thus promote microbial biomass and SOC (Makkonen *et al.* 2013; Gould *et al.* 2016). Thus, more effort is needed to investigate how plant functional properties alter diversity effects on SOC.

Our meta-analysis is based on mostly controlled diversity experiments. Concerns about BEF experiments being raised are that experiments are too small in area and too short in timescale to be meaningfully representative of real-world ecosystems (Srivastava & Vellend

2005; Wardle & Palmer 2016). Positive BEF relationships have been suggested to be unlikely to occur or to be much weaker in nature compared with experiments (Grace *et al.* 2007; Wardle & Palmer 2016). However, recent meta-analyses have also demonstrated that positive mixture effects on above- and below-ground productivity and soil microbial biomass are consistent between natural systems and experiments (Zhang *et al.* 2012; Ma & Chen 2016; Chen *et al.* 2019). Furthermore, an observational study from forest, shrubland, and grassland sites across China also showed that species richness could substantially increase SOC, lending partial support to our results from the natural environment (Chen *et al.* 2018). Further qualitative and quantitative studies are needed to make a useful and clear comparison of the effect of plant diversity on SOC between natural systems and experiments.

Our meta-analysis might provide us with the knowledge to develop strategies to meet international standards for carbon sequestration and mitigate the impacts of global environmental change. For example, agricultural crop diversity can produce greater yields than current monocultures (Oelbermann *et al.* 2015) and also maintain soil fertility and mitigate climate change. Agroecosystem diversification should be encouraged to maintain SOC, even in the context of similar or slightly lower yields than that of the best sole cropping (McDaniel *et al.* 2014; Isbell *et al.* 2017). Further, the inclusion of plant diversity and other abiotic factors such as stand age and soil depth to earth system models, which may affect responses of SOC and SMBC to species mixture, can also provide beneficial information on mitigation strategies for rising atmospheric CO_2 concentrations and climate warming.

4.6 Conclusion

(1) Our analyses demonstrate that species mixtures enhance both total soil organic carbon and microbial biomass carbon, suggesting that the prevalence of global plantation and crop

monocultures threaten soil productivity and quality. Our results raise concerns that global declines in plant species diversity could have ubiquitously negative global-scale impacts on soil carbon and nutrient cycling.

(2) We further revealed that these positive species mixture effects on total soil organic carbon and microbial biomass carbon increase over time, but are strongly dependent on soil depth. Our results indicate that the magnitude of estimated species mixture effects on soil organic carbon and microbial biomass carbon may be underestimated when only short experimental durations and shallow soil samples are considered. Our analysis provides insights to improve land surface models to better predict the consequences of global change on the terrestrial carbon.

(3) Given the pressing need to limit global warming under 1.5°C (Allen *et al.* 2018), our findings suggest that conserving or promoting plant diversity in natural and managed systems is an integral part of achieving this goal.

CHAPTER 5: WATER AVAILABILITY REGULATES NEGATIVE SPECIES MIXTURE EFFECTS ON SOIL MICROBIAL BIOMASS

5.1 Abstract

Soil microorganisms are critical for the maintenance of terrestrial biodiversity and ecosystem functions. Both plant diversity and water availability are individually known to influence soil microorganisms; however, their interactive effects remain largely unknown. Here, we investigated whether the influences of tree species mixtures on microbial biomass and composition were modified with variable water availability. This was accomplished by sampling soils from stands that were dominated by Populus tremuloides and Pinus banksiana, respectively, and their relatively even mixtures under water reduction (-25%), ambient, and addition (+25%). Microbial community biomass and composition were determined by phospholipid fatty acid analysis. We found that water addition increased soil total microbial biomass and by individual groups, whereas water reduction had no effect. Under ambient water conditions, soil total microbial biomass, arbuscular mycorrhizal fungal, bacterial, gram-positive (GP) bacterial, and gram-negative (GN) bacterial biomass were significantly lower in mixtures than expected from the average of constituent monocultures. Saprotrophic fungal biomass and the ratios of fungal/bacterial and GN/GP bacteria did not vary significantly between mixed stands and the average of constituent monocultures. Water reduction increased the species mixture effects on total and individual group microbial biomass from negative to neutral, while water addition increased mixture effects, but only for arbuscular mycorrhizal fungal and GP bacterial biomass. Across all water treatments, soil total and individual group microbial biomass significantly increased with the abundance of broadleaved trees, but only

weakly with tree species richness. Further, microbial community compositions differed significantly with both overstory type and water alteration treatment. Microbial community compositions exhibited strong associations with tree species richness, soil moisture, soil pH, and litterfall production, whereas microbial biomass did not. Our results suggested that higher species diversity is not always beneficial for soil microorganisms; however, mixed tree species have the potential to regulate ecosystem responses to altered water availability.

5.2 Introduction

Forest soils harbour extremely rich microbial communities (Bahram et al. 2018), which are essential for many ecosystem functions that underpin a suite of services in terrestrial ecosystems (Lange *et al.* 2015; Li *et al.* 2019). Soil microbial biomass plays a major role in maintaining primary aboveground production, mediating greenhouse emissions, and controlling nutrient recycling (van der Heijen 2008; Lange *et al.* 2015; Walker *et al.* 2018; Li *et al.* 2019). Furthermore, shifts in relative microbial abundance and composition influence biogeochemical cycles in soil. For example, a higher fungal to bacterial biomass ratio (F/B) is associated with higher decomposition efficiency and greater soil carbon storage potential (Malik et al. 2016), while a higher ratio of gram-negative: gram-positive (GN/GP) bacteria indicates higher C availability in the soil (Fanin et al. 2019). Recent advances have been made in our understanding of the respective effects of water availability and plant diversity on soil microbial biomass and composition (Zhou *et al.* 2018; Chen *et al.* 2019). However, it remains unclear whether species mixture effects on soil microbial biomass and composition change with water availability.

Concurrent changes in terrestrial water availability and plant diversity, driven by anthropogenic activities, are expected to interact and alter soil microorganisms via changes in plant-derived litter inputs and the soil environment (Hicks *et al.* 2018; Valencia *et al.* 2018; Chen *et al.* 2019). Terrestrial water availability, driven by changes in precipitation and evapotranspiration, also affects soil microbial biomass, as the availability of soil water is essential for the growth and maintenance of both soil microorganisms and vegetation (Manzoni et al. 2012; Valencia et al. 2018). Decreased water availability is anticipated to reduce soil microbial biomass, whereas an increase has the opposite effect (Zhou et al. 2018). Meanwhile, the loss in biological diversity has been recognized as a critical threat to ecosystem functioning (Cardinale et al. 2012). For example, species mixtures can yield greater above- and belowground productivity in diverse ecosystem types, over monocultures (Ma & Chen 2016; Duffy et al. 2017). Recent meta-analyses of manipulated experiments revealed that plant mixtures could promote soil microbial biomass via a higher quantity and variability of inputted litters (e.g., foliar and root litter) that are returned to the soil, in contrast to monocultures (Thakur *et al.* 2015; Chen *et al.* 2019). Additionally, soils in diverse forests may have higher moisture and pH than those of monocultures (Zapater et al. 2011; Dawud et al. 2016), which could favour microbial growth (Rousk et al. 2010a; Rousk et al. 2010b). Furthermore, the effects of plant diversity may be contingent on how plant species in mixtures are phylogenetically and/or functionally dissimilar (Cadotte 2017). Phylogenetically dissimilar broadleaved and coniferous trees provide distinct and diverse qualities and quantities of food resources and living habitats for soil microorganisms, and their mixture effects may also depend on the proportion of species from either group in mixtures (Dawud et al. 2016; Tedersoo et al. 2016). Broadleaved forests tend to have higher soil microbial biomass, as their litters contain more labile compounds, higher nitrogen concentration, and less phenol than coniferous litters (Liu et al. 2012).

The compositions of microbial communities are also sensitive to changes in the configuration of plant species and water availability. Water reduction may enhance the soil F/B ratio, but decrease the GN/GP bacteria ratio, whereas water addition has the opposite effect, due to the extreme variability of water stress thresholds for different microbial groups (Schimel et al. 2007; Manzoni et al. 2012; Zhou et al. 2018). Fungi are expected to have a greater tolerance to water stress than bacteria due to their ability to accumulate osmoregulatory solutes and hyphal/mycelial growth forms (Manzoni et al. 2012). Within bacterial communities, GP bacteria have stronger and thicker cell walls; thus, they are inherently more tolerant to drought than GN bacteria (Schimel et al. 2007). An increased quantity of plant inputs associated with increasing plant diversity may enhance the soil F/B ratio, as fungi might benefit more from increased litter inputs than bacteria (Malik *et al.* 2016; Chen *et al.* 2019). Within bacterial communities, the GN/GP bacteria ratio may increase with plant diversity due to higher dependence on GN than GP bacteria for plant litter inputs (Fanin et al. 2019). Additionally, differences in aboveground litter chemistry, root exudate chemistry, and soil environment (e.g. soil moisture, pH) between different plant community types can also regulate the composition of soil microbial communities (Wan et al. 2015). For example, the relative abundance of bacteria to fungi would increase with soil pH (Rousk et al. 2010a), which has a strong positive association with plant diversity (Dawud et al. 2016).

The effects of mixed forests on soil microbial biomass and composition (e.g., F/B ratio and GN/GP bacteria ratio) might be enhanced by water reduction, as species-rich forests are widely expected to ensure more consistent productivity in contrast to monocultures in the context of global environmental change (Yachi & Loreau 1999; Ammer 2019; Hisano *et al.* 2019), despite other papers having suggested the reverse (Paquette *et al.* 2018). This effect may be propagated to soil microbial communities via plant litter inputs and improved microenvironments. Mixed forests may serve as a buffer against the negative impacts of reduced water availability on plant production, since (i) the performance of some species, which increases under drought conditions, may compensate for others with reduced performance; (ii) competition for water in mixtures may decrease due to complementary resource use and, (iii) denser canopies might facilitate the reduced loss of soil water (O'Brien et al. 2017; Ammer 2019). We also hypothesized that the effects of mixed forests on soil microbial biomass and composition could be enhanced by mild water addition, since they contain additional species with unique water requirements and water acquisition strategies; therefore, they more readily exploit increased water resources (Wright et al. 2015; Fischer et al. 2016). However, despite these expected positive effects of water alteration on the effects of mixed forests, previous grassland studies revealed a limited capacity for plant diversity to attenuate the effects of water availability alterations on soil microbial biomass or abundance (Thakur *et al.* 2015; Wagner *et al.* 2015; Valencia *et al.* 2018).

To the best of our knowledge, no previous study has evaluated whether water availability may regulate the effects of species mixture on soil microbial biomass and composition in the forest ecosystem. Here, we examined how soil microbial biomass and composition would respond to water addition and reduction in mixed forests compared to corresponding monocultures. We tested four hypotheses: (1) water reduction would decrease overall and individual microbial group biomass and GN/GP bacteria ratio, but increase the soil F/B ratio, whereas the addition of water would have the opposite effects; (2) mixed forests would have a higher total and individual group microbial biomass, F/B ratio, and GN/GP bacteria ratio, on average, than those in corresponding monocultures; alternatively, these microbial attributes increase with species richness, where positive mixed forest effects and species richness would be higher under both water reduction and addition conditions; (3) the proportion of broadleaf trees has a positive effect on microbial biomass and influences microbial composition; (4) the composition of microbial communities differs between overstory types and water treatments, and would be concurrently altered with changes in tree diversity and composition of tree species, plant-derived inputs, and soil environments.

5.3 Methods

5.3.1 Site description

This study was carried out in the central boreal forests of Canada, located north of Lake Superior and west of Lake Nipigon (49°27' N–49°38' N, 89°29' W–89°54' W). The study area falls within the Moist Mid-Boreal (MBX) ecoclimatic region and is characterized by warm summers and cold, snowy winters (Ecoregions Working Group 1989). The mean annual temperature is 2.5 °C and annual precipitation is 712 mm. The soils of the upland sites are relatively deep glacial tills of the Brunisolic order (Soil Classification Working Group et al. 1998). The study area has an extensive history of stand-replacing fire, with an average fire return interval of approximately 100 years (Senici et al. 2010). In young forests, the dominant overstory tree species included jack pine (*Pinus banksiana Lamb*.) and trembling aspen (*Populus tremuloides Michx*.). Common understorey shrub species in the area include mountain maple (*Acer spicatum Lam*.), alder (*Alnus spp*.), and beaked hazel (*Corylus cornuta Marsh*.).

5.3.2 Experimental design

In July 2016, we established the experiment in young stands (11 years old) from three overstory types: single-species stands dominated by *Populus tremuloides* Michx. (Populus), or *Pinus banksiana* Lamb. (Pinus), and their relatively even mixtures (Populus+Pinus) (Table 5-1). Each of the overstorey types was replicated in triplicate. The resulting nine stands were allocated with a distance of > 1 km between each stand, to reduce spatial autocorrelation. Within each stand, a circular plot (400 m^2) located at least 50 m from the forest edge was randomly established. Within each plot, we applied three split-plot level water availability treatments: ambient, 25% growing season (May to October) throughfall water reduction under tree overstory, and 25% throughfall water addition, which comprised the median changeability of expected water availability during the 21st century for Canada's boreal forests (IPCC 2014a). Each of the 27 treatment split plots consisted of an area of 6 × 6 m (36 m^2), with a mean tree density of 98 stems per plot (range = 33 to 416 stems). For each of the nine water reduction treatment plots, we constructed rain shelters under canopies that consisted of four shelters (3 × 3 m), which were held in place by metal stakes and wires. Each metal frame supported 4 U-shaped clear acrylic troughs (3 m long x 20 cm wide) that were spaced at 35 cm. The U-shaped troughs were oriented at a 10° angle, with the high end positioned at 1.8 m and the low end at 1.35 m above ground level (Fig. S5-1). The rain shelters funnelled water into eight 8-cm (inner diameter) polyvinyl chloride (PVC) pipes, each with six different-size holes (diameters: 0.64, 1.91, 3.18, 4.45, 5.72, and 6.99 cm), arranged at intervals of 46 cm, to distribute the collected water evenly over the adjacent water addition plot. A ≥ 4 m buffer zone was established between the treatment plots (Fig. S5-1).

Table 5-1. Characteristics (mean and 1 s.e.m., n = 3) of the study stands in Northwestern Ontario, Canada. Stand types are single-species Pinus banksiana dominated (Pinus), single-species Populus tremuloides dominated (Populus), and their mixtures (Populus+Pinus).

Stand type	*Populus*	*Pinus*	*Populus+Pinus*
Stand basal area (m^2 ha^{-1})	1.55 ± 0.35	0.93 ± 0.33	1.39 ± 0.24
Stand density (trees ha^{-1})	5933 ± 1790	11600 ± 4148	9200 ± 1301
Tree species richness	2.67 ± 0.33	2.67 ± 0.33	4.33 ± 0.33
Tree species composition (% of stand basal area)			
Pinus banksiana	3 ± 2	98 ± 1	48 ± 7
Populus tremuloides	92 ± 2	1 ± 1	28 ± 3
Betula papyrifera	3 ± 2	1 ± 1	15 ± 4
Other broadleaf species	2 ± 1	1 ± 0	4 ± 1
Forest floor depth (cm)	1.89 ± 0.75	2.76 ± 0.17	1.52 ± 0.35
Soil carbon concertration (0-15cm, g kg^{-1})	20.41 ± 1.65	14.24 ± 2.39	17.15 ± 3.86
Soil nitrogen concertration (0-15cm, g kg^{-1})	1.20 ± 0.12	0.93 ± 0.12	1.06 ± 0.21
Soil pH	5.22 ± 0.24	5.21 ± 0.29	5.21 ± 0.30

5.3.3 Field measurements

To explore how the changes in vegetation and soil environments between canopy types and water treatments were linked to variations in soil microbial composition, we identified all tree species and measured the diameter at breast height (DBH; 1.3 m above the root collar) of all trees in each split-plot, at the conclusion of the 2018 growing season. Within each split-plot, three 0.322 m^2 litterfall traps were randomly located to collect litterfall (Chen et al. 2017). We placed litter traps in May 2017 and collected litterfall every four weeks during the snow-free period, until late October 2018. The litterfall samples were dried at 65ºC in a convection oven until a constant mass was achieved, which was generally less than 48 hours. The total oven-dry biomass of annual litterfall production was calculated as Mg $ha^{-1}y^{-1}$ by summing all litterfall collections for the entire calendar year, from July 2017 to August 2018. We employed the minirhizotron method to monitor root length with a specific scanner (CI-600 Root Scanner, CID Inc., camas, WA, USA) (Mommer *et al.* 2015). Two tubes (Ø63.5 mm x 105-cm long) per treatment plot were installed in May 2017, and digital images were continuously obtained monthly by Rootsnap! Software (CID Inc., camas, WA, USA), from May 2018. We simultaneously estimated the "standing root length" for each subplot as the sum of individual root lengths in each image. The volumetric soil water content was measured biweekly, using a Decagon soil moisture sensor, at a depth of 5 cm below the soil surface during the growing season. We found that the soil water content during the growing season differed between water treatments, but not among overstory types (Fig. S5-2). Across all stand types, compared with ambient water conditions, water addition, on average, increased the soil moisture, while water reduction had no effect (Fig. S5-2).

5.3.4 Soil collection and pH analysis

Composite soil samples were acquired by mixing soil samples (0–10 cm) that were collected from five random points in each split-plot with a soil corer (Ø3.5 cm) in August 2018. The soil samples were immediately placed in polyethylene bags, stored in a cooler, and transported to the laboratory. Following sieving (<2 mm) and the removal of visible plant material, the soil pH was determined using a soil to water ratio suspension of 1:2·5. The remaining soil samples were stored in a refrigerator at −20°C until they were analyzed for microbial community biomass and composition. Soil microbial analyses assays were performed within two weeks of sample collection.

5.3.5 Phospholipid fatty acid (PLFA) analysis

The microbial PLFA composition was determined using 3.0 g freeze-dried soil samples, following the protocol described in Quideau et al. (2016). A surrogate standard (19:0; 1,2-dinonadecanoyl-sn-glycero-3-phosphocholine, Avanti Polar Lipids Inc, Alabaster, USA) was added prior to the initial extraction. An instrument standard (10:0Me; methyl decanoate, Aldrich, St. Louis, USA) was added prior to GC analysis. Fatty acid methyl ester analysis was conducted using an Agilent 6890 Series capillary gas chromatograph (GC; Agilent Technologies, Santa Clara, USA), which was fitted with a 25 m Ultra 2 column (Crosslinked 5 % PhMeSilicone), and a FID detector. The Sherlock Microbial Identification System Version 6.3 software (MIDI, Inc., Newark, USA), was employed to identify and quantify FAMES, and the microbial species were estimated using the MICSOIL3 method. The groups of lipid biomarkers used in the calculations of PLFA biomass and ratios are detailed in Table S5-1.

5.3.6 Data analysis

Initially, we tested the effects of overstory type (T) and water alteration treatment (W) on microbial biomass, F/B ratio, and GN/GP bacteria ratio using the following linear mixed-effect model, and post-hoc comparisons via the TukeyHSD test:

$$Y_{ijkl} = T_i + W_{j(k)} + T_i \times W_{j(k)} + \pi_k + \varepsilon_{l(ijk)} \quad (1)$$

where Y_{ijkl} is the soil microbial attribute of interest (total microbial biomass, saprotrophic fungal biomass, AM_fungal biomass, bacterial biomass, Gram-positive bacterial biomass, Gram-negative bacterial biomass, F/B ratio, or GN/GP bacteria ratio); T_i ($i = 1, 2, 3$) is the overstory type (broadleaf, conifer, and mixed wood); $W_{j(k)}$ is the water treatment (j = 25% water reduction, ambient, or 25% water addition) nested in each whole plot k ($k = 1, 2, \ldots 9$); π_k is the random effect of plot; $\varepsilon_{l(ijk)}$ is the sampling error. We conducted the analysis using the restricted maximum likelihood estimation with the package *lme4* (Bates *et al.* 2017).

To test the species mixture effect, the response ratio (*RR*) was employed to quantify the effects of species mixtures on soil microbial biomass, F/B ratio, and GN/GP bacteria ratio:

$$RR_{sm} = X_{observed}/X_{expected} \quad (2)$$

where $X_{observed}$ and $X_{expected}$ were the observed and expected values of total and individual group microbial biomass, F/B ratio, and GN/GP bacteria ratio in mixtures. We calculated the expected weighted values of the component species in monocultures following Loreau and Hector (2001):

$$X_{expected} = \sum (V_i \times p_i) \quad (3)$$

where V_i is the observed value of total and individual group microbial biomass, ratios of F/B and GN/GP bacteria in the monoculture of species *i*, and P_i is the proportion of species *i* basal area ($m^2\ ha^{-1}$) in the corresponding mixture per split-plot. To test whether the effects of species mixtures on microbial biomass and composition differed with water availability, we employed the following linear mixed-effect model:

$$RR_{ijk} = W_i + \pi_j + \varepsilon_{k(ij)} \tag{4}$$

where W_i is the water treatment (i = water addition, ambient, water reduction); π_j is the random effect of the overall plot; $\varepsilon_{k(ij)}$ is the sampling error. The mixed species effects were significant at $\alpha = 0.05$ if the 95% CIs of estimated *RR* did not cover 1. Values above or below 1 indicate and quantify over- and underyielding, respectively. The difference between groups was significant if 95% CIs of their coefficients did not overlap the other's mean.

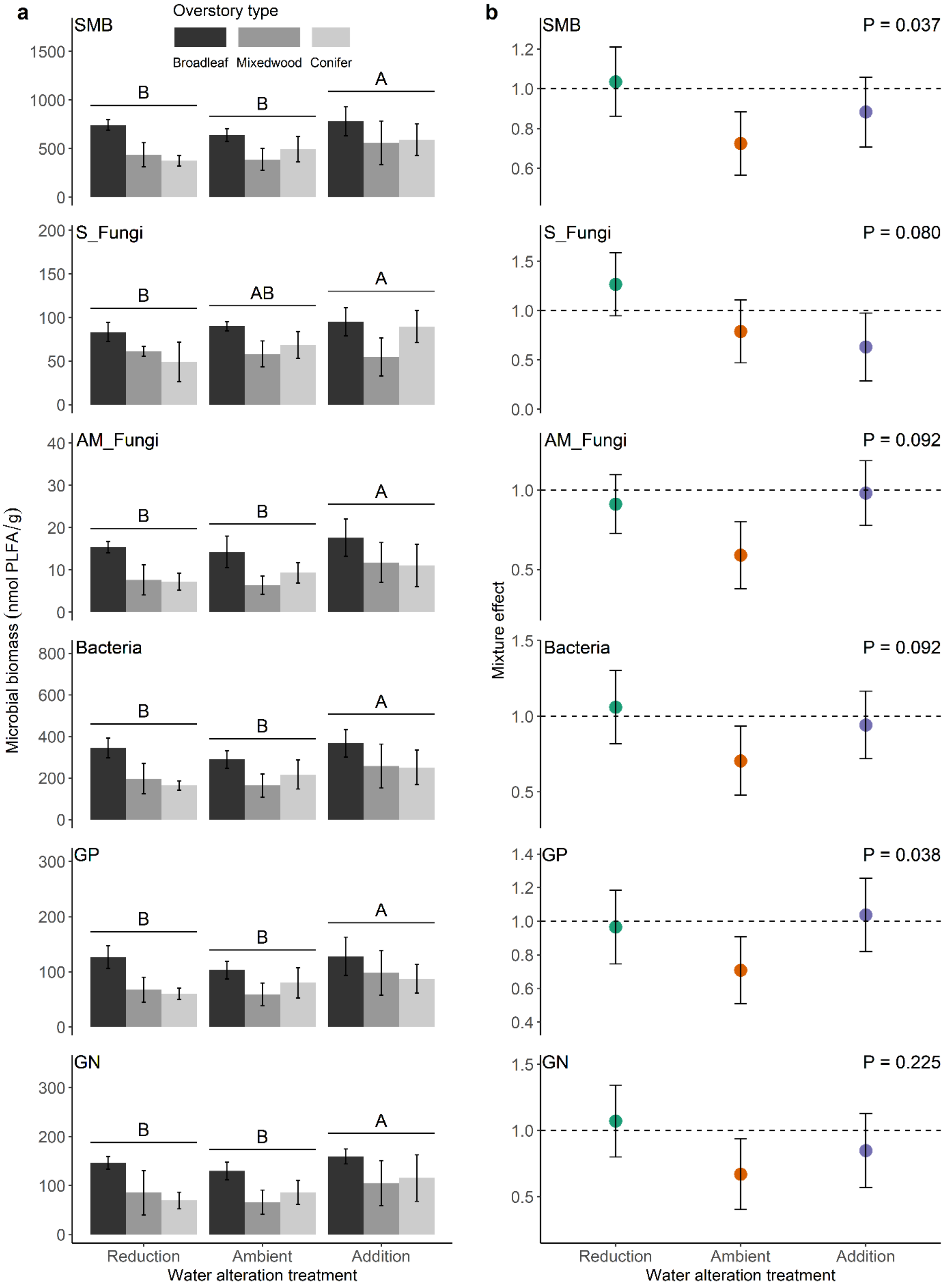
To simultaneously examine and identify species and diversity effects on soil microbial attributes, we also tested the effects of broadleaved tree proportions and tree species richness on soil microbial biomass, the F/B ratio, and GN/GP bacteria ratio, and whether the effects of tree species richness differed with water availability using the following linear mixed-effect model:

$$Y_{ijkl} = B_j + R_i + W_{k(l)} + B_j \times W_{k(l)} + R_i \times W_{k(l)} + \pi_l + \varepsilon_{m(ijkl)} \tag{5}$$

where Y_{ijkl} is the soil microbial attribute of interest and soil water content, soil pH, litterfall production, standing root length used; R_i is the tree species richness; B_i is the broadleaved tree proportion; $W_{k(l)}$ is the water alteration treatment (j = 25% water reduction, ambient, or 25% water addition) nested in each sample plot l (l =1, 2,…9); π_l is the random effect of plot; $\varepsilon_{m(ijkl)}$ is the sampling error.

To examine the effects of overstory type and water treatment on microbial community composition, we conducted permutational multivariate analysis of variance (perMANOVA) and included plot identity as a random factor in the model, implemented in the '*adonis*' function from the '*vegan*' package. The microbial community composition as a whole was analyzed using biomass data from major microbial groups of PLFAs, as well as individual PLFAs (Table S5-1). In perMANOVA, we employed the Bray-Curtis dissimilarity matrix to summarize species composition and used 999 permutations to determine statistical significance. We visualized the compositional data using nonmetric multidimensional scaling with the Bray-Curtis dissimilarity measure. To gain insights into the effects of mixed forests and variable water availability on microbial communities, we also tested the effects of overstory type (T), water treatment (W), and species mixture effects on soil water content, soil pH, litterfall production, and standing root length, using eqns. 1, 3, and 4, respectively. The Pearson correlation was used to examine the associations between soil water content, soil pH, litterfall production, standing root length, total microbial biomass, saprotrophic fungal biomass, AM_fungal biomass, bacterial biomass, Gram-positive bacterial biomass, Gram-negative bacterial biomass, F/B ratio, and GN/GP bacteria ratio. Subsequently, we examined the association between the PLFAs of soil microorganisms and overstory tree richness, broadleaved tree proportion, total stand basal area, annual litterfall productions, standing root length, and soil environment (soil water content, soil pH) across overstory types and water alteration treatments, and used the *envfit* function of the *vegan* package (Oksanen et al. 2017). Assumptions of normality and homogeneous variance were examined by Shapiro-Wilk's and Leven's tests, respectively. All statistical analyses were performed in R 3.5.3 (R Core Team 2018).

5.4 Results



a
Overstory type
Broadleaf
Mixedwood
Conifer
SMB
S_Fungi
AM_Fungi
Bacteria
GP
GN
Microbial biomass (nmol PLFA/g)
Reduction
Ambient
Addition
Water alteration treatment
B
B
A
AB
b
P = 0.037
P = 0.080
P = 0.092
P = 0.092
P = 0.038
P = 0.225
Mixture effect

Figure 5-1. The total and individual group microbial biomass in relation to water alteration treatments and the effects of species mixtures. a) Total soil microbial biomass, soil saprotrophic fungal biomass, AM_fungal biomass, bacterial biomass, Gram_positive bacterial biomass, and Gram_negative bacterial biomass for different overstory types in relation to water alteration treatments. b) Effects of species mixtures on total soil microbial biomass, soil saprotrophic fungal biomass, AM_fungal biomass, bacterial biomass, Gram_positive bacterial biomass, and Gram_negative bacterial biomass. The effects represent the response ratio of a given microbial attribute compared to the monocultures and mixtures (*see* Methods). Values are mean ± 95% confidence intervals. Different letters indicate a significant difference between water alteration treatments within the same overstory type category ($\alpha = 0.05$).

Total soil microbial biomass, saprotrophic fungal biomass, arbuscular mycorrhizal fungal biomass, bacterial biomass, GP bacterial biomass, and GN bacterial biomass differed between overstory types and water treatments, while the ratio of F/B and GN/GP bacteria did not (Table 5-2, Fig. 5-1a&2a). Across all water treatments, the overall and individual group microbial biomass were higher in the Populus stands than Pinus and Pinus+Populus stands, except for saprotrophic fungal biomass (Fig. 5-1a). Across all stand types, additional water, on average, increased the biomass of all microbial attributes, except for saprotrophic fungal biomass (Fig. 5-1a). The significant interactive effect of overstory type and water alteration treatment occurred only for saprotrophic fungal biomass (Table 5-2). Saprotrophic fungal biomass was higher under additional water in Pinus stands; however, water reduction had no effect, while it was similar between water addition, ambient, and reduction in the Pinus+Populus and Populus stands (Fig. 5-1a). In contrast to ambient water conditions, water reduction did not significantly change the total soil microbial biomass, saprotrophic fungal biomass, arbuscular mycorrhizal fungal biomass, bacterial biomass, GP bacterial biomass, or GN bacterial biomass (Fig. 5-1a).

Table 5-2. Effects (P values) of overstory type (T), water alteration treatments (W), and their interactions on soil microbial biomass, F/B ratio, GN/GP bacteria ratio, soil water content, soil pH, litterfall production, and standing root length.

Attribute	T			W			T × W		
	df	F	*P*	df	F	*P*	df	F	*P*
SMB	2,6	6.32	**0.033**	2,12	9.22	**0.004**	4,12	2.06	0.150
Fungi	2,6	6.12	**0.036**	2,12	5.21	**0.024**	4,12	4.07	**0.026**
AM_Fungi	2,6	10.05	**0.012**	2,12	5.01	**0.026**	4,12	0.57	0.691
Bacteria	2,6	6.44	**0.032**	2,12	7.84	**0.007**	4,12	1.67	0.221
GP	2,6	5.48	**0.044**	2,12	5.94	**0.016**	4,12	2.03	0.154
GN	2,6	5.75	**0.040**	2,12	10.07	**0.003**	4,12	1.36	0.306
F/B	2,6	0.89	0.459	2,12	1.70	0.224	4,12	2.46	0.102
GN/GP	2,6	0.47	0.648	2,12	0.26	0.777	4,12	1.36	0.304
Soil water content	2,6	0.87	0.465	2,12	3.96	**0.048**	4,12	1.09	0.404
Soil pH	2,6	0.01	0.991	2,12	0.34	0.722	4,12	1.56	0.247
Litterfall biomass	2,6	0.20	0.826	2,12	1.22	0.330	4,12	1.31	0.321
Standing root length	2,6	0.44	0.662	2,12	4.75	**0.030**	4,12	2.88	0.069

Note: Linear mixed-effects model fit tests used Satterthwaite approximations for denominator degrees of freedom (df). *P* is the significance of the model and $P < 0.05$ are highlighted in bold. SMB, Fungi, AM_Fungi, Bacteria, GP, GN, F/B, and GN/GP represent total soil microbial biomass, fungal biomass, AM_fungal biomass, bacterial biomass, Gram_positive bacteria biomass, Gram_negative bacteria biomass, fungal: bacterial ratios, and Gram_negative: Gram_positive bacterial ratios, respectively.

Total soil and individual group microbial biomass were significantly lower in mixtures than expected, from those of constituent monocultures in ambient water sites, except for saprotrophic fungal biomass, which was also lower in mixtures than expected from those of constituent monocultures, but not significant (Fig. 5-1b). However, compared with ambient water treatment, water reduction significantly increased species mixture effects on soil total microbial biomass and GP bacterial biomass ($P<0.05$), and marginally significantly increased those on saprotrophic fungal biomass, arbuscular mycorrhizal fungal biomass and bacterial biomass ($P<0.10$), from negative to neutral, whereas water addition increased species mixture effects on arbuscular mycorrhizal fungal biomass and GP bacterial biomass, from negative to neutral (Fig. 5-1b, Table S5-2). The ratios of F/B and GN/GP bacteria were not significantly different between the mixed stands and the average of constituent monocultures (Fig. 5-2b). Furthermore, neither the reduction or addition of water affected species mixture effects on the F/B and GN/GP bacteria ratios (Fig. 5-2b, Table S5-2).

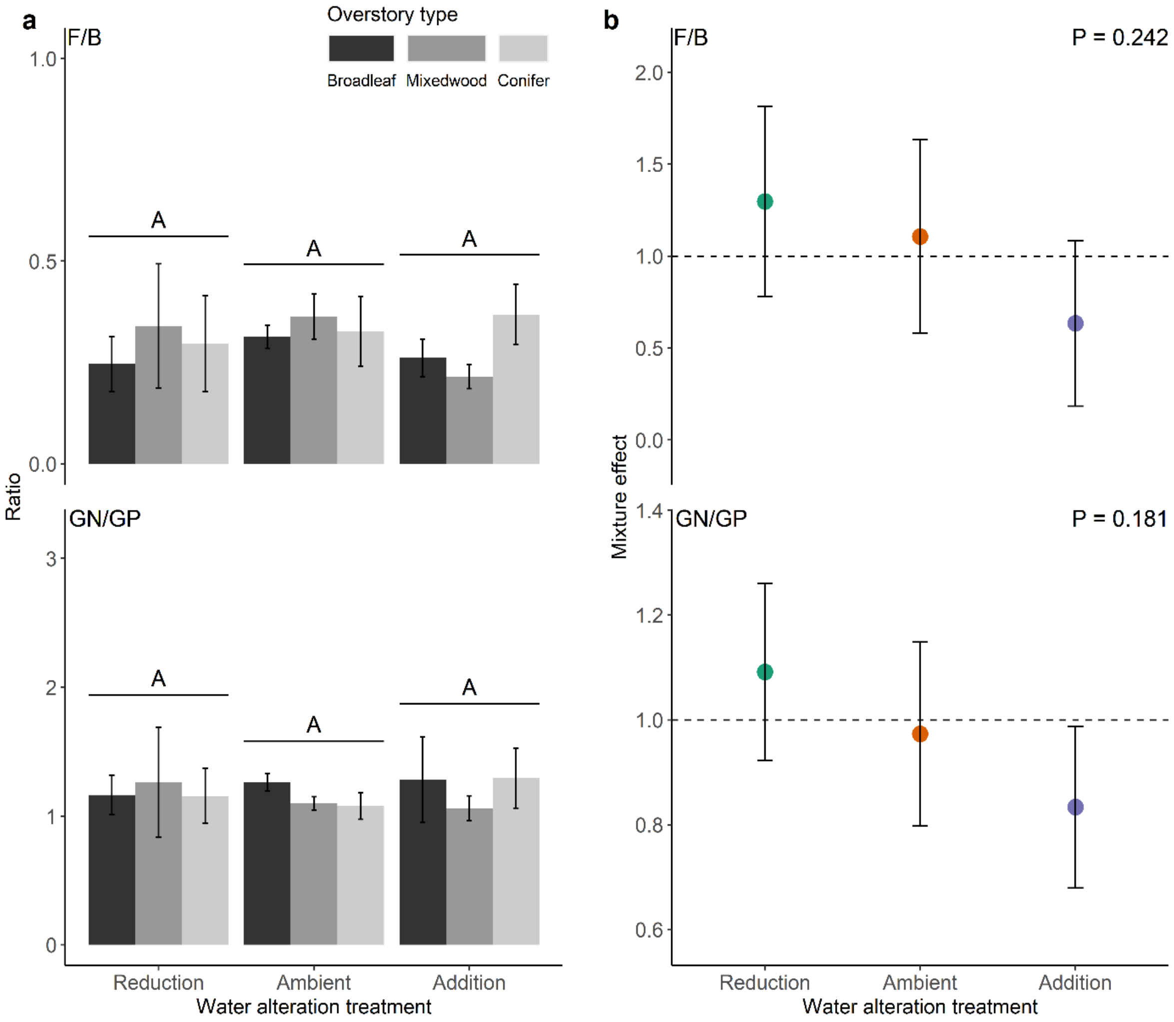


Figure 5-2. The ratios of fungal to bacterial biomass and gram-positive to gram-negative bacteria in relation to water alteration treatments and the effects of species mixtures. a) Ratios of fungal to bacterial biomass and gram-positive to gram-negative bacteria for different overstory types in relation to water alteration treatments. b) Effects of species mixtures on the ratios of fungal to bacterial biomass and gram-positive: gram-negative bacteria. The effects represent the response ratio of a given microbial attribute compared to the monocultures and the mixtures (*see* Methods). Values are mean ± 95% confidence intervals.

Compared with species richness, broadleaved tree proportions explained a greater proportion of the variation in microbial attributes, except for GN/GP (Table S5-3). Soil total microbial biomass, saprotrophic fungal biomass, arbuscular mycorrhizal fungal biomass, bacterial biomass, and GP bacterial biomass were not altered with species richness; however, they did significantly or marginally increase with the proportion of broadleaved trees (Fig. 5-3, Table S5-3). The GN bacterial biomass significantly increased with both species richness and the proportion of broadleaved trees (Fig. 5-3, Table S5-3). The GN/GP bacteria ratio was significantly increased with species richness, whereas the F/B ratio was not altered with species richness or the proportion of broadleaved trees (Fig. 5-4, Table S5-3). There was no significant interactive effect between species richness and water alteration for all microbial attributes (Table S5-3).

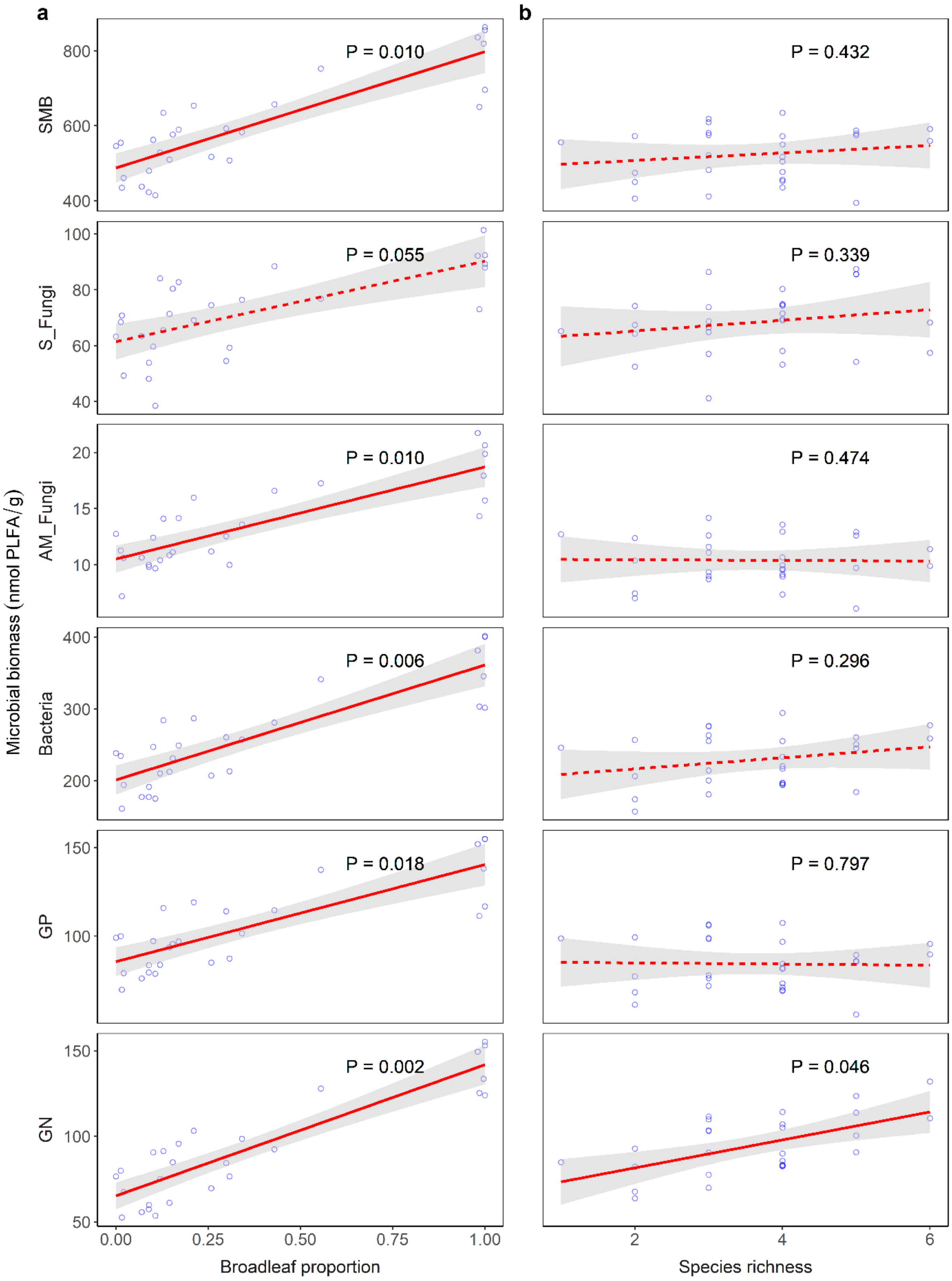

a
b
Microbial biomass (nmol PLFA/g)
SMB
400
600
800
P = 0.010
P = 0.432
S_Fungi
40
60
80
100
P = 0.055
P = 0.339
AM_Fungi
10
15
20
P = 0.010
P = 0.474
Bacteria
200
300
400
P = 0.006
P = 0.296
GP
100
150
P = 0.018
P = 0.797
GN
50
100
150
P = 0.002
P = 0.046
0.00
0.25
0.50
0.75
1.00
Broadleaf proportion
2
4
6
Species richness

Figure 5-3. The total and individual group microbial biomass in relation to tree species richness and proportion of broadleaved trees. a) Total soil microbial biomass, soil fungal biomass, AM_fungal biomass, bacterial biomass, Gram_positive bacterial biomass, and Gram_negative bacterial biomass in relation to overstory tree richness. b) Total soil microbial biomass, soil fungal biomass, AM_fungal biomass, bacterial biomass, Gram_positive bacterial biomass, and Gram_negative bacterial biomass for different overstory types as relates to the proportion of broadleaved trees**.** The red line and grey shaded areas represent the fitted regression and its bootstrapped 95% confidence intervals. The significance (*P*) is presented for each term tested.

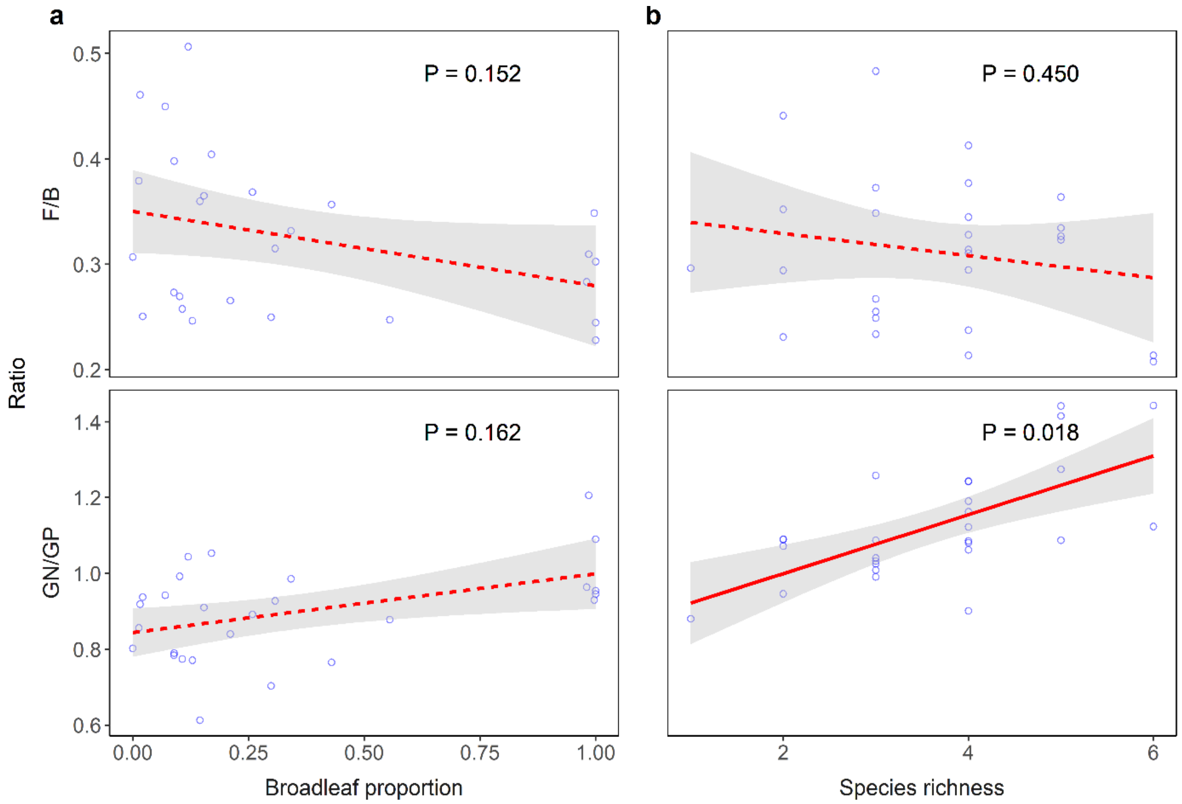


Figure 5-4. The ratios of fungal to bacterial biomass and gram-positive to gram-negative bacteria in relation to tree species richness and proportion of broadleaved trees. a) Ratios of fungal to bacterial biomass and gram-positive to gram-negative bacteria in relation to overstory tree richness**.** b) Ratios of fungal to bacterial biomass and gram-positive to gram-negative bacteria in relation to the proportion of broadleaved trees**.** The red line and grey shaded areas represent fitted regression and its bootstrapped 95% confidence intervals. The significance (*P*) is presented for each term tested.

Soil water content, pH, litterfall production, and standing root length were similar among overstory types, while soil water content and standing root length differed between water treatments; however, soil pH and litterfall production did not change with water treatments (Table 5-2). Across all stand types, water addition on average, increased soil water content, whereas water reduction did not affect soil water content, primarily due to the early stage null effect (May to July); however, water reduction led to significant negative effects on water content at the late stage (Figs. 5-5a, S5-1). On average, water reduction decreased, but water addition did not affect the standing root length (Fig. 5-5a). The soil water content was generally lower in mixtures than expected from those of constituent monocultures, with significant negative species mixture effect on the water reduction sites (Fig. 5-5b). Soil pH and litterfall production were not significantly different between the mixed stands and the average of constituent monocultures across all water treatments, and species mixture effects on these elements were not impacted by altered water availability (Fig. 5-5b). The stand root length was significantly ($P<0.05$) lower in mixtures than anticipated from those of constituent monocultures at ambient water sites; however, water reduction and addition significantly ($P<0.05$) increased the species mixture effects from negative to neutral and positive, respectively (Fig. 5-5b).

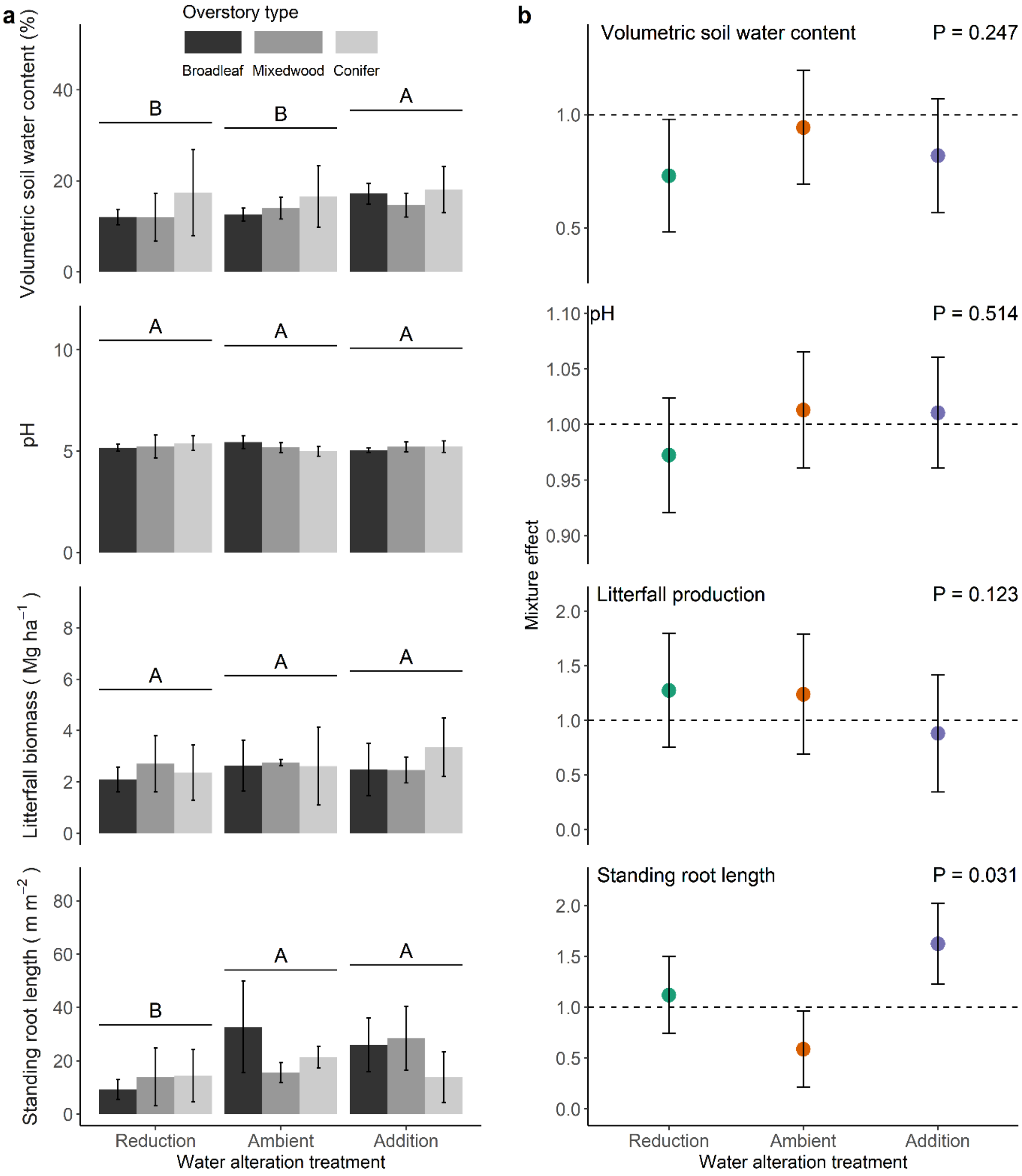


Figure 5-5. The soil properties and litter inputs in relation to water alteration treatments and the effects of species mixtures. a) Soil volumetric water content, soil pH, litterfall production and standing root length for different overstory types in relation to water alteration treatments. b) Effects of species mixtures on soil volumetric water content, soil pH, litterfall production, and standing root length. The effects represent the response ratio of a given microbial attribute compared to the monocultures and the mixtures (*see* Methods). Values are mean ± 95%

confidence intervals. Different letters indicate a significant difference between water alteration treatments within the same overstory types category ($\alpha = 0.05$).

Soil pH was positively correlated with the GN/GP ratio, whereas litterfall production was positively correlated with saprotrophic fungal biomass and the F/B ratio. Soil water content and standing root length were not correlated with any microbial attributes (Fig. S5-3). The perMANOVA analysis revealed that microbial community compositions differed significantly with both overstory type and water alteration treatment, for both individual PLFAs and microbial group levels ($P<0.05$) (Fig. 5-6). Between overstory types, the broadleaf was distinct from the others (Fig. 5-6). Among water alteration treatments, the addition of water was distinct from the other treatments (Fig. 5-6). The soil microbial community compositions revealed strong associations with species richness, soil moisture, soil pH, and litterfall production on individual PLFAs levels ($P<0.05$, Fig. 5-6a). At the microbial group level, soil microbial community compositions showed strong associations with species richness only ($P<0.05$, Fig. 5-6b).

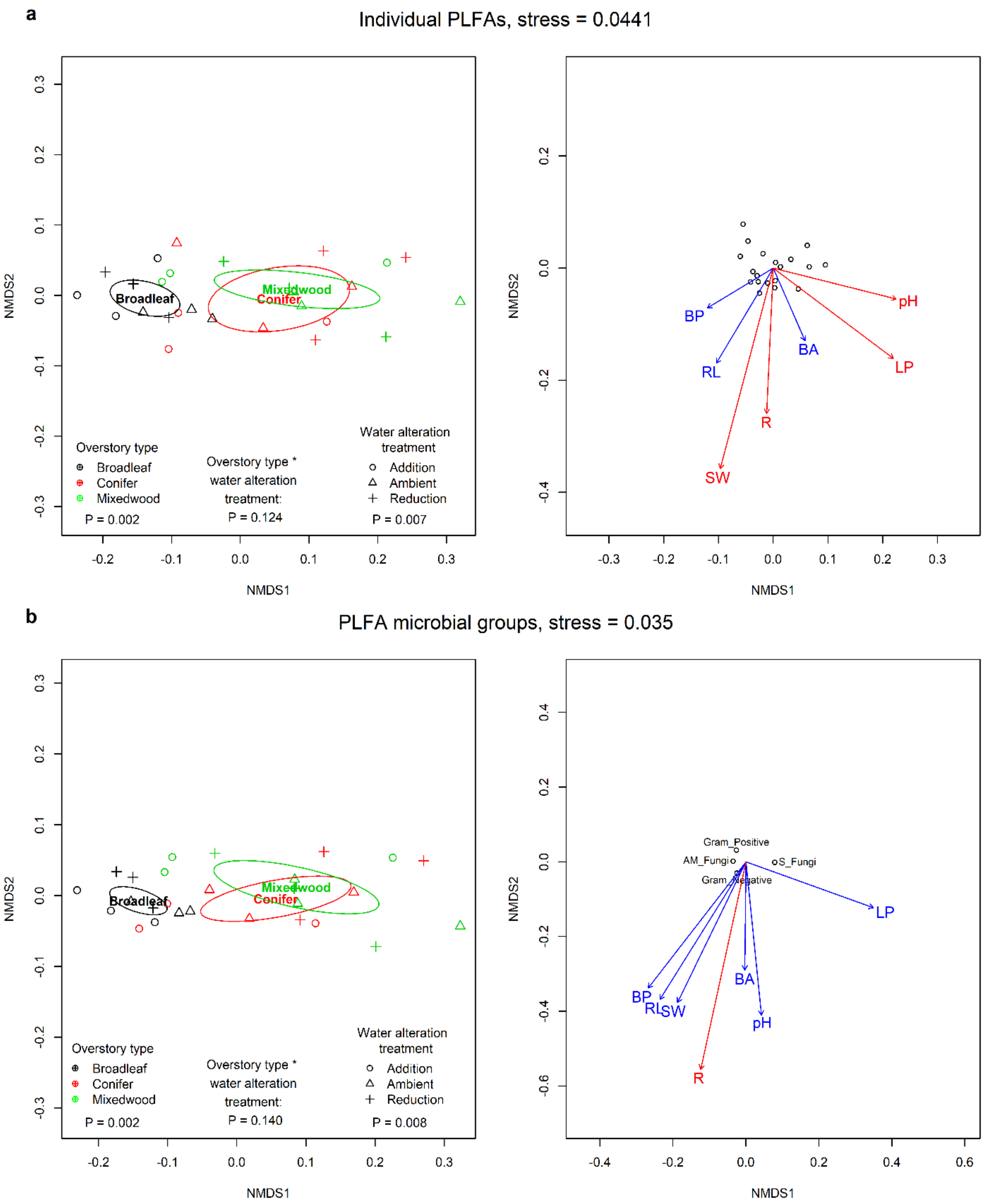


Figure 5-6. Nonmetric multidimensional scaling ordination of soil microbial communities as individual PLFAs (a) and microbial groups (b) for different overstory types and water alteration treatment combinations in relation to aboveground characteristics and soil moisture. Ellipses represent standard errors of the weighted averages of scores of corresponding to overstory types. Predictor variables included overstory tree richness (R), proportion of

broadleaved trees (BL), total stand basal area (BA), soil water content (SW), pH, annual litterfall production (LP), and standing root length (RL). The vector lengths represent correlation (r), and red and blue vectors indicate significance $P \leq 0.05$ and > 0.05, respectively.

5.5 Discussion

We demonstrated that both species mixture and water availability influenced soil microbial community biomass and composition. Our results provided experimental evidence that the effects of water availability on soil microbial biomass and composition were generally consistent across overstory types. Importantly, significant underyielding effects in total soil and individual group microbial biomass were observed, and these underyielding effects could be increased by water reduction and addition. Our results were in contrast to previous studies, which reported mostly positive relationships between plant diversity and microbial biomass (Chen *et al.* 2019), and also indicated the crucial role of water availability in shaping biodiversity and ecosystem functioning relationships. Additionally, although the broadleaved proportion was a more critical driver of soil microbial biomass, tree species richness explained more of the variation in microbial composition.

We found that water addition generally increased soil total and individual group microbial biomass, whereas water reduction had no impact, tracking the variation in soil water content, reinforcing the critical role of soil water availability in controlling soil microbial biomass (Manzoni et al. 2012; Zhou et al. 2018). The negligible effect of water reduction on soil moisture content might be partly attributable to the low proportion of the decrease in water availability (25%), and substantial water inputs from snowmelt in the spring. Additionally, plants may acclimatize to reduced water availability and decrease water uptake from the soil, as indicated by decreased standing root length under water reduction (Brunner et al. 2015) , especially for trembling aspen. Our analysis also revealed that neither water addition or reduction affected the F/B or GN/GP bacteria ratios, likely because the changes in soil moisture resulted from water treatments (-0.6% and +2.2% of soil water content for water reduction and addition, respectively) were too small to exceed the extreme soil water stress thresholds of any individual microbial groups. Beyond these thresholds, the biomass of less water stress tolerant microbial groups would decrease more drastically than those of higher water stress tolerant microbial groups; hence, the ratios of F/B and GN/GP bacteria may change (Manzoni et al. 2012).

In contrast to previous experimental studies and meta-analysis, where positive species mixture effects on microbial biomass were found (Thakur *et al.* 2015; Chen *et al.* 2019), our analysis revealed lower soil total and individual group microbial biomass in mixed forests over the average of corresponding monocultures. This underyielding of microbial biomass might have been the result of reduced root and root-exudation inputs, which were indicated by negative species mixture effects on standing root lengths. Species mixtures may increase the overall efficiency of water use, thereby reducing the investment required for roots (Canarini et al. 2016). As expected, we found that water reduction increased species mixture effects on soil total and individual group microbial biomass from negative to neutral. This shift was likely due to decreased water availability, which led mixed forests to allocate additional production partitioning belowground to support greater water and nutrient uptake required for higher ecosystem productivity, via more horizontal and vertical soil volume filling (Brassard et al. 2013; Ammer 2019). We found that water addition did not impact species mixture effect on total soil microbial biomass and individual microbial group biomass except arbuscular mycorrhizal fungi and GP bacteria, despite a positive species mixture effect on standing root lengths with water addition, which is possibly a result of the additional production partitioning belowground to exploit increased water resources (Wright et al. 2015; Fischer et al. 2016). This result may have been due to the short experimental duration of our study (two years) and the delayed response of microbes to changes in plant inputs (Chen & Chen 2018). Arbuscular mycorrhizal fungi establish symbiotic connections with plants where they receive a substantial fraction of readily available carbon sources in exchange for nutrients; which might facilitate a more rapid response to changes in root growth (Drigo et al. 2010). Typically, GP bacteria interact synergistically with arbuscular mycorrhizal fungi, which might benefit from the increase in arbuscular mycorrhizal fungi (Artursson et al. 2006). Our results revealed that the responses of microbial biomass to species mixture could be primarily root-derived, which reinforced the critical role of roots in the coupling of plant and microbial productivity (Paterson 2003; Lange *et al.* 2015).

We found that both F/B and GN/GP bacteria ratios were not significantly different between the mixed stands and the average of constituent monocultures, which was comparable with other studies in temperate forests (Khlifa *et al.* 2017). One possible interpretation might be that species mixtures affect microbial composition primarily by altering the abiotic soil environment (e.g., soil pH, moisture) and litterfall biomass, as indicated by correlation analysis. However, for our study, soil pH and moisture did not differ between mixed forests and monocultures (Rousk et al. 2010a; Lange et al. 2014; Leloup et al. 2018).

Across all water treatments, total soil and individual group microbial biomass increased significantly with the abundance of broadleaved trees, but only weakly with species richness. This was in agreement with previous forest diversity-manipulated experiments (Gunina et al. 2017), which indicated that tree species composition had a more potent effect than species number on microbial biomass. The GN/GP bacteria ratio increased significantly with species richness, which signified the increased availability of soil resident C with species richness (Fanin et al. 2019).

The microbial community of the mixed stands possessed more similarities to the conifer stands, over that of the broadleaved stands. Our results revealed that coniferous trees had a dominating effect on the composition of microbial communities; thus, they could be considered the keystone species with regard to plant-microbial interactions. Interestingly, we found that tree species richness was the main explanatory factor for microbial composition at both the individual PLFA and microbial group levels. This suggested that tree species richness plays a critical role in controlling soil microbial communities, as was previously observed in grassland diversity experiments, which was likely due to a diversity of litters and root exudates (Steinauer et al. 2016; Leloup et al. 2018). Similar to previous studies (Rousk et al. 2010b; Huang et al. 2014; Leloup et al. 2018), we observed that soil pH, moisture, and litterfall biomass affected microbial composition; however, they had a negligible or no impact on microbial biomass.

In conclusion, we found that the mild addition of water served to increase influence on microbial biomass, whereas mild water reduction did not. Additionally, our study revealed a lower soil microbial biomass in mixed forests over monocultures, where tree identity effects dominated over tree diversity effects on the microbial biomass. Further, we found that the effects of plant mixtures on microbial biomass increased following the modification of water availability, which indicated that interactions between plants and soil microbes have the potential to regulate ecosystem responses to climate change. Therefore, mixed forests may have the capacity to attenuate the impacts of changing water availability in boreal forests.

CHAPTER 6: GENERAL CONCLUSION

The findings of this dissertation show global-scale evidence that plant mixtures yield greater Rs and SOC than do monocultures, extending our understanding of the critical role of species diversity beyond the positive biodiversity-ecosystem productivity relationships (Tilman *et al.* 1996; Zhang *et al.* 2012; Ma & Chen 2016). Moreover, we found a significant priming effect of aboveground litter addition, extending the findings shown previously in the rhizosphere area (Huo et al., 2017). Furthermore, we found that the effects of plant mixtures on microbial biomass increased following the modification of water availability. A summary of the key findings of this dissertation are as follows:

1. Through the synthesis of 100 published studies, we show that the magnitude of the priming effect may increase over time during the first years after litter input increases, indicating that the magnitude of estimated litterfall alteration on the priming effect may be underestimated by short-term experiments. In addition, we found an increase of Q_{10} after litter removal, especially in wet locations, indicating that increasing global deforestation and cultivation may accelerate Rs under ongoing global warming. Furthermore, this study demonstrated that the effects of aboveground litter alterations on Rs increase over time and are amplified by increasing precipitation.
2. Our analysis demonstrated the positive relationships between plant diversity and Rs as well as heterotrophic respiration. Moreover, positive species mixture effects on heterotrophic respiration increased over time, particularly in more diverse species mixtures, indicating that the magnitude of estimated species mixture effects on soil respiration may be underestimated when only narrow ranges of species richness and short experimental durations are considered. Our results raise concerns that global

declines in plant species diversity could have ubiquitously negative global-scale impacts on soil carbon and nutrient cycling through decreases in soil resident biological activity, particularly in environments under water stress.

3. Our analyses showed global-scale evidence demonstrating that global declines in plant species diversity could have ubiquitously negative global-scale impacts on SOC. Our results indicate that converting 50% of global forests from mixtures to monocultures would release 2.47 Pg C from soil annually on average over 20 years: about 28% of global annual fossil-fuel emissions. Given the pressing need to limit global warming under 1.5°C, our findings suggest that promoting plant diversity could be an effective means to help achieve this goal.
4. In my species diversity and soil microbial biomass study, we found that a lower soil microbial biomass in mixed forests over monocultures, where tree identity effects dominated over tree diversity effects on the microbial biomass. Further, we found that the effects of plant mixtures on microbial biomass increased following the modification of water availability, which indicated that interactions between plants and soil microbes have the potential to regulate ecosystem responses to climate change. Therefore, mixed forests may have the capacity to modify the impacts of changing water availability in boreal forests.

Soils are fundamental to life on the planet but anthropogenic pressures on soil resources are reaching sustainable limits (FAO and ITPS 2015). Our findings will aid national and regional governmental agencies and the private sector in developing effective forest and agricultural management and conservation strategies to meet international standards for carbon sequestration and mitigate the impacts of global environmental change. Moreover, our analysis provides insights to improve land surface models to better predict the consequences of global change on terrestrial carbon.

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APPENDIX I: SUPPLEMENTAL INFORMATION FOR CHAPTER 2

Table S2-1. Effects of mean annual temperature (MAT) and ecosystem type (Eco) on ln*RRs* of Rs and Q_{10}.

Variable	Treatment	Source	df	F value	*P*
Rs	Double litter	Eco	na	na	na
		MAT	1, 29.6	0.557	0.462
		Eco × MAT	na	na	na
	Litter removal	Eco	2. 118.3	1.383	0.255
		MAT	1. 120.6	0.389	0.534
		Eco × MAT	2. 105.5	0.231	0.794
	Root removal	Eco	1. 23.1	0.912	0.349
		MAT	1. 18.6	0.01	0.922
		Eco × MAT	1. 18.6	0.304	0.588
	Litter + root removal	Eco	1. 17.0	0.12	0.733
		MAT	1. 15.8	< 0.001	0.999
		Eco × MAT	1. 15.8	0.989	0.335
Q_{10}	Double litter	Eco	na	na	na
		MAT	1, 20.3	0.041	0.842
		Eco × MAT	na	na	na
	Litter removal	Eco	2, 52.2	3.698	0.032
		MAT	1, 39.3	1.106	0.299
		Eco × MAT	1, 39.3	4.345	0.044
	Root removal	Eco	1, 6.8	0.695	0.433
		MAT	1, 6.9	0.266	0.622
		Eco × MAT	1, 6.9	0.769	0.410
	Litter + root removal	Eco	1, 9.1	0.113	0.745
		MAT	1, 9.1	0.053	0.822
		Eco × MAT	1, 9.1	0.100	0.758

Note: Linear mixed-effects model fit tests used Satterthwaite approximations for denominator degrees of freedom.

Table S2-2. Effects of annual aridity index (AI) and ecosystem type (Eco) on ln*RR* of Rs and Q_{10}.

Variable	Treatment	Source	df	F value	*P*
Rs	Double litter	Eco	na	na	na
		AI	1, 31.9	0.216	0.645
		Eco × AI	na	na	na
	Litter removal	Eco	2, 131.4	2.661	0.074
		AI	1, 107.3	8.854	0.004
		Eco × AI	2, 119.9	3.321	0.039
	Root removal	Eco	1, 18.9	< 0.001	0.984
		AI	1, 16.8	0.006	0.939
		Eco × AI	1, 16.8	0.316	0.581
	Litter + root removal	Eco	1, 17.0	0.212	0.651
		AI	1, 13.5	0.154	0.701
		Eco × AI	1, 13.5	1.110	0.311
Q_{10}	Double litter	Eco	na	na	na
		AI	1, 24.8	0.563	0.460
		Eco × AI	na	na	na
	Litter removal	Eco	2, 48.7	1.590	0.214
		AI	1, 39.4	0.186	0.669
		Eco × AI	1, 39.4	0.909	0.346
	Root removal	Eco	1, 6.8	0.118	0.742
		AI	1, 6.8	0.045	0.838
		Eco × AI	1, 6.8	0.014	0.908
	Litter + root removal	Eco	1, 6.5	0.027	0.874
		AI	1, 7.0	0.025	0.879
		Eco × AI	1, 7.0	0.004	0.953

Note: Linear mixed-effects model fit tests used Satterthwaite approximations for denominator degrees of freedom.

Table S2-3. Interaction effects of litter type and annual aridity index (AI) on ln*RR* of Q_{10}.

Treatment	Source	df	F value	*P*
Double litter	Litter type	2, 22.1	0.353	0.706
	AI	1, 23.6	0.230	0.636
	Litter type × AI	2, 22.6	1.556	0.233
Litter removal	Litter type	3, 56.2	1.722	0.173
	AI	1, 49.1	3.498	0.067
	Litter type × AI	3, 55.4	0.350	0.789
Root removal	Litter type	3, 3.2	1.482	0.371
	AI	1, 3.0	0.015	0.911
	Litter type × AI	2, 3.1	1.785	0.304
Litter + root removal	Litter type	3, 4.8	0.907	0.502
	AI	1, 4.9	0.106	0.758
	Litter type × AI	2, 5.0	0.006	0.994

Note: Linear mixed-effects model fit tests used Satterthwaite approximations for denominator degrees of freedom.

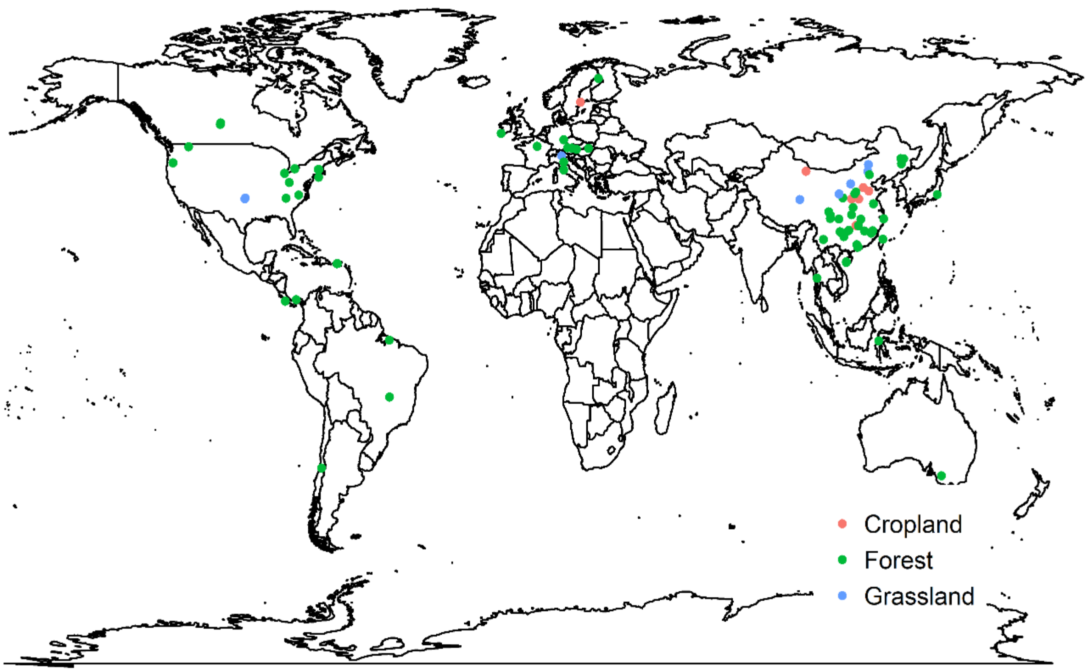

Figure S2-1. Global distribution of litter manipulation experiments selected for this meta-analysis.

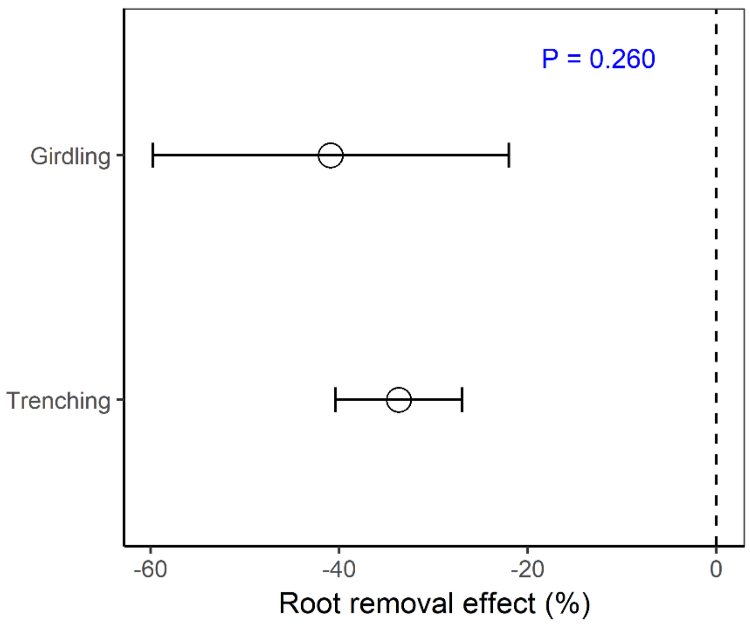

Figure S2-2. The root removal effect on soil respiration by girdling and trenching methods. The numbers outside and inside the parentheses represent the numbers of observations and studies, respectively.

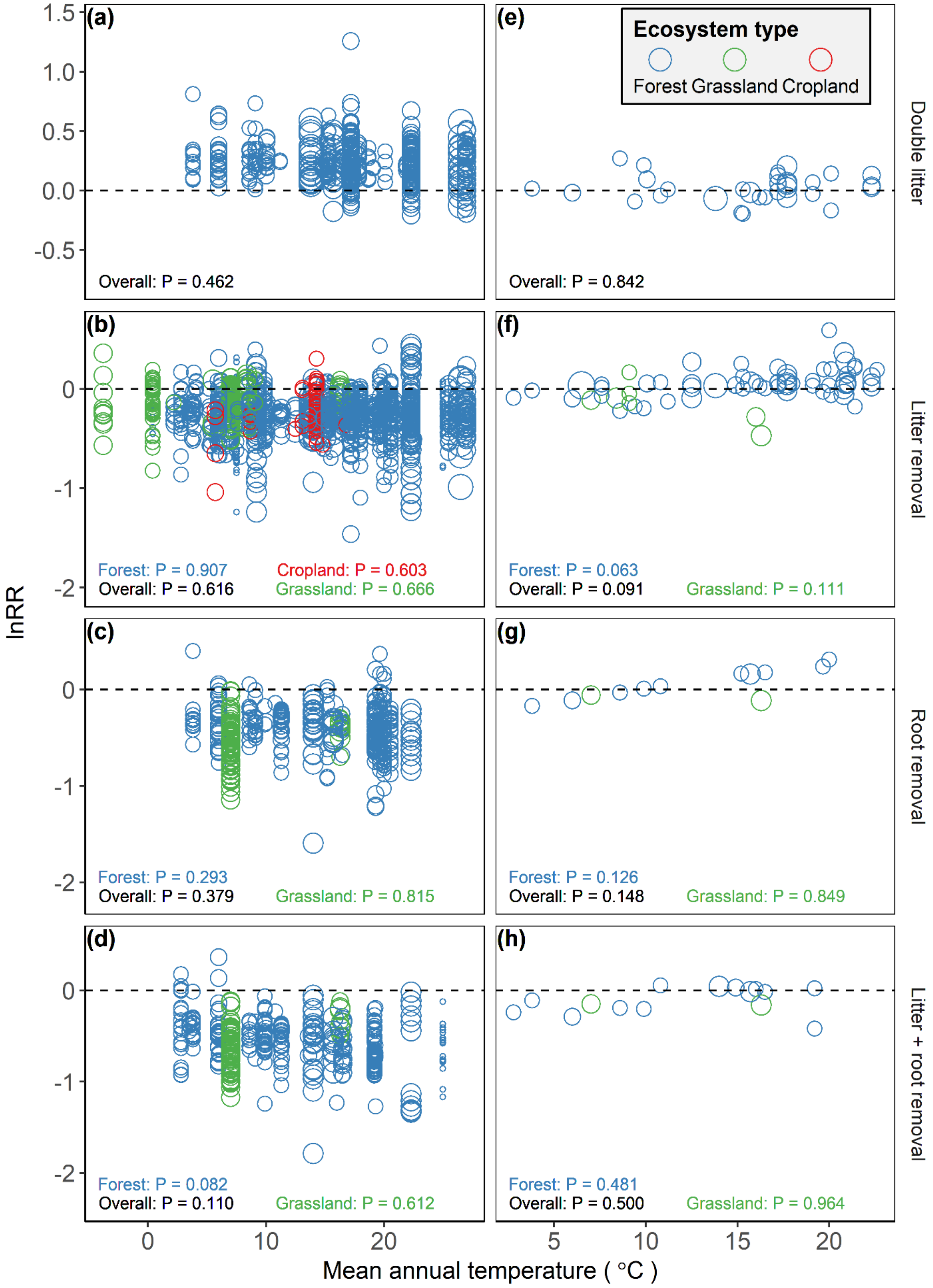
(a)
(b)
(c)
(d)
(e)
(f)
(g)
(h)
Ecosystem type
Forest
Grassland
Cropland
1.5
1.0
0.5
0.0
-0.5
0
-1
-2
lnRR
Overall: P = 0.462
Overall: P = 0.842
Forest: P = 0.907
Overall: P = 0.616
Cropland: P = 0.603
Grassland: P = 0.666
Forest: P = 0.063
Overall: P = 0.091
Grassland: P = 0.111
Forest: P = 0.293
Overall: P = 0.379
Grassland: P = 0.815
Forest: P = 0.126
Overall: P = 0.148
Grassland: P = 0.849
Forest: P = 0.082
Overall: P = 0.110
Grassland: P = 0.612
Forest: P = 0.481
Overall: P = 0.500
Grassland: P = 0.964
Double litter
Litter removal
Root removal
Litter + root removal
0
10
20
5
10
15
20
Mean annual temperature (°C)

Figure S2-3. Effects of mean annual temperature and ecosystem type on *lnRRs* of Rs and Q_{10} associated with double litter, litter removal, root removal and litter + root removal treatments. The size of the bubble is the relative weight of each observation.

APPENDIX II: SUPPLEMENTAL INFORMATION FOR CHAPTER 3

Table S3-1. The source for original studies that have examined plant species and litter diversity effects on soil respiration.

NO.	Publication
Plant diversity studies	
1.	Chai Q, Qin AZ, Gan YT, & Yu AZ (2014) Higher yield and lower carbon emission by intercropping maize with rape, pea, and wheat in arid irrigation areas. Agron Sustain Dev 34(2):535-543.
2.	Dyer L, Oelbermann M, & Echarte L (2012) Soil carbon dioxide and nitrous oxide emissions during the growing season from temperate maize-soybean intercrops. J Plant Nutr Soil Sc 175(3):394-400.
3.	Hu FL, et al. (2017) Integration of wheat-maize intercropping with conservation practices reduces CO2 emissions and enhances water use in dry areas. Soil Till Res 169:44-53.
4.	Latati M, et al. (2014) The intercropping cowpea-maize improves soil phosphorus availability and maize yields in an alkaline soil. Plant Soil 385(1-2):181-191.
5.	Liu C (2012) Effect of stubble and tillage management on soil carbon and nitrate under wheat/maize intercropping in Oases of the Shiyanghe River Basin. Doctor (Gansu Agricultural University, China).
6.	Scalise A, et al. (2015) Legume-barley intercropping stimulates soil N supply and crop yield in the succeeding durum wheat in a rotation under rainfed conditions. Soil Biol Biochem 89:150-161.
7.	Sharma RC & Banik P (2015) Baby Corn-Legumes Intercropping Systems: I. Yields, Resource Utilization Efficiency, and Soil Health. Agroecol Sust Food 39(1):41-61.
8.	Shen YW, et al. (2018) Greenhouse gas emissions from soil under maize-soybean intercrop in the North China Plain. Nutr Cycl Agroecosys 110(3):451-465.
9.	Souza MFP, da Silva MP, Arf O, & Cassiolato AMR (2013) Chemical and Biological Properties of Phosphorus-Fertilized Soil under Legume and Grass Cover (Cerrado Region, Brazil). Rev Bras Cienc Solo 37(6):1492-1501.
10.	Tortorella D, et al. (2013) Chemical and biological responses in a Mediterranean sandy clay loam soil under grain legume-barley intercropping. Agrochimica 57(1):1-21.
11.	Vachon K (2008) Soil carbon and nitrogen dynamics and greenhouse gas mitigation in intercrop agroecosystems in Balcarce, Argentina. Master (University of Waterloo, Canada).
12.	Adair EC, Reich PB, Hobbie SE, & Knops JMH (2009) Interactive Effects of Time, CO2, N, and Diversity on Total Belowground Carbon Allocation and Ecosystem Carbon Storage in a Grassland Community. Ecosystems 12(6):1037-1052.
13.	Alvarez G, Chaussod R, Loiseau P, & Delpy R (1998) Soil indicators of C and N transformations under pure and mixed grass-clover swards. Eur J Agron 9(2-3):157-172.

14. Craine JM, Wedin DA, & Reich PB (2001) The response of soil CO2 flux to changes in atmospheric CO2, nitrogen supply and plant diversity. Glob Change Biol 7(8):947-953.
15. De Boeck HJ, et al. (2007) How do climate warming and species richness affect CO2 fluxes in experimental grasslands? New Phytologist 175(3):512-522.
16. Dias ATC, van Ruijven J, & Berendse F (2010) Plant species richness regulates soil respiration through changes in productivity. Oecologia 163(3):805-813.
17. Eisenhauer N, et al. (2010) Plant diversity effects on soil microorganisms support the singular hypothesis. Ecology 91(2):485-496.
18. Eisenhauer N, et al. (2013) Plant diversity effects on soil food webs are stronger than those of elevated CO2 and N deposition in a long-term grassland experiment. P Natl Acad Sci USA 110(17):6889-6894.
19. Gong JR, et al. (2015) Effect of irrigation on the soil respiration of constructed grasslands in Inner Mongolia, China. Plant Soil 395(1-2):159-172.
20. Han Y, Zhang Z, Wang CH, Jiang FH, & Xia JY (2012) Effects of mowing and nitrogen addition on soil respiration in three patches in an oldfield grassland in Inner Mongolia. J Plant Ecol-Uk 5(2):219-228.
21. Johnson D, Phoenix GK, & Grime JP (2008) Plant community composition, not diversity, regulates soil respiration in grasslands. Biol Letters 4(4):345-348.
22. Malchair S, et al. (2010) Do climate warming and plant species richness affect potential nitrification, basal respiration and ammonia-oxidizing bacteria in experimental grasslands? Soil Biol Biochem 42(11):1944-1951.
23. Spehn EM, Joshi J, Schmid B, Alphei J, & Korner C (2000) Plant diversity effects on soil heterotrophic activity in experimental grassland ecosystems. Plant Soil 224(2):217-230.
24. Strecker T, Mace OG, Scheu S, & Eisenhauer N (2016) Functional composition of plant communities determines the spatial and temporal stability of soil microbial properties in a long-term plant diversity experiment. Oikos 125(12):1743-1754.
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Litter diversity studies

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Table S3-2. The values of Akaike information criterion for four alternative models (see Materials and Methods).

Model	*AIC* of Rs to PD	*AIC* of Rh to PD	*AIC* of Rh to LD
$\ln RR = \beta_0 + \beta_1 \times SR + \beta_2 \times SA + \beta_3 \times SR \times SA + \pi$	-56	-72	118
$\ln RR = \beta_0 + \beta_1 \times \log(SR) + \beta_2 \times SA + \beta_3 \times \log(SR) \times SA + \pi$	-56	-74	117
$\ln RR = \beta_0 + \beta_1 \times SR + \beta_2 \times \log(SA) + \beta_3 \times SR \times \log(SA) + \pi$	-55	-84	121
$\ln RR = \beta_0 + \beta_1 \times \log(SR) + \beta_2 \times \log(SA) + \beta_3 \times \log(SR) \times \log(SA) + \pi$	-56	-97	120

Note: SR, SA, and ET are species richness, stand age, and ecosystem type, respectively. PD and LD are plant diversity and litter diversity, respectively.

Table S3-3. The values of Akaike information criterion for four alternative models for geographical effects on Rs and Rh (see Materials and Methods).

Geographical factor	Model 1*	Model 2	Model 3	Model 4
Plant diversity on Rs				
Ecosystem type	-41	-32	-26	-18
MAT	-47	-42	-34	-30
Aridity index	-51	-48	-46	-43
Plant diversity on Rh				
Ecosystem type	-80	-71	-72	-62
MAT	-85	-80	-79	-79
Aridity index	-67	-62	-62	-57

Model 1: $\ln RR = \beta_0 + \beta_1 \cdot \ln(SR) + \beta_2 \cdot \ln(SA) + \beta_3 \cdot \ln(SR) \times \ln(SA) + \beta_4 \cdot GF + \pi_{study} + \varepsilon$

Model 2: $\ln RR = \beta_0 + \beta_1 \cdot \ln(SR) + \beta_2 \cdot \ln(SA) + \beta_3 \cdot \ln(SR) \times \ln(SA) + \beta_4 \cdot GF + \beta_5 \cdot \ln(SR) \times GF + \pi_{study} + \varepsilon$

Model 3: $\ln RR = \beta_0 + \beta_1 \cdot \ln(SR) + \beta_2 \cdot \ln(SA) + \beta_3 \cdot \ln(SR) \times \ln(SA) + \beta_4 \cdot GF + \beta_5 \cdot \ln(SA) \times GF + \pi_{study} + \varepsilon$

Model 4: $\ln RR = \beta_0 + \beta_1 \cdot \ln(SR) + \beta_2 \cdot \ln(SA) + \beta_3 \cdot \ln(SR) \times \ln(SA) + \beta_4 \cdot GF + \beta_5 \cdot \ln(SR) \times GF + \beta_6 \cdot \ln(SA) \times GF + \pi_{study} + \varepsilon$

GF is geographical factor.

Table S3-4. Effects of species richness and stand age on the natural log response ratios (ln*RR*) of Rs and Rh.

	Estimate	df	F value	*P*
Plant diversity on Rs				
ln(SR)	0.053	1, 66.1	4.35	0.041
ln(SA)	-0.010	1, 67.1	0.67	0.415
log(SR) × ln(SA)	0.007	1, 65.8	0.06	0.810
Plant diversity on Rh				
ln(SR)	0.056	1, 118.0	18.25	<0.001
ln(SA)	0.055	1, 74.5	15.14	<0.001
log(SR) × ln(SA)	0.062	1, 123.2	13.84	<0.001
Litter diversity on Rh				
ln(SR)	0.209	1, 207.5	7.71	0.006
ln(SA)	-0.087	1, 190.5	10.97	0.001
log(SR) × ln(SA)	-0.220	1, 210.9	6.42	0.012

Note: Linear mixed-effects model fit tests used Satterthwaite approximations for denominator degrees of freedom.
SR and SA are species richness and stand age, respectively.

Table S3-5. The effect (P values) of mean annual temperature (MAT), annual aridity index (AI) and ecosystem type on Natural log response ratios (ln*RR*) of Rs and Rh.

Attribute	MAT			AI			Ecosystem type		
	df	F value	P	df	F value	P	df	F value	P
Rs	1, 12	1.58	0.232	1, 10	6.17	**0.032**	3, 18	0.22	0.881
Rh	1, 24	0.07	0.792	1, 25	2.04	0.165	3, 19	0.07	0.977

Linear mixed effect models used Satterthwaite approximation for degrees of freedom (df).

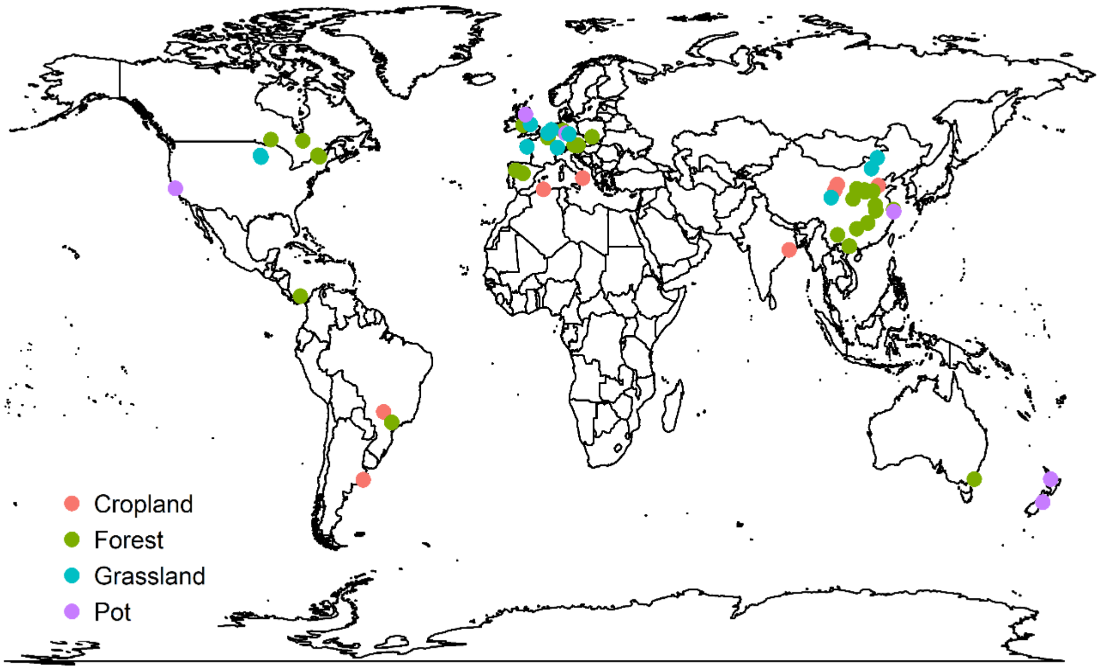

Figure S3-1. Global distribution of plant diversity experiments testing the effects of plant mixtures on soil respiration, collected for this meta-analysis.

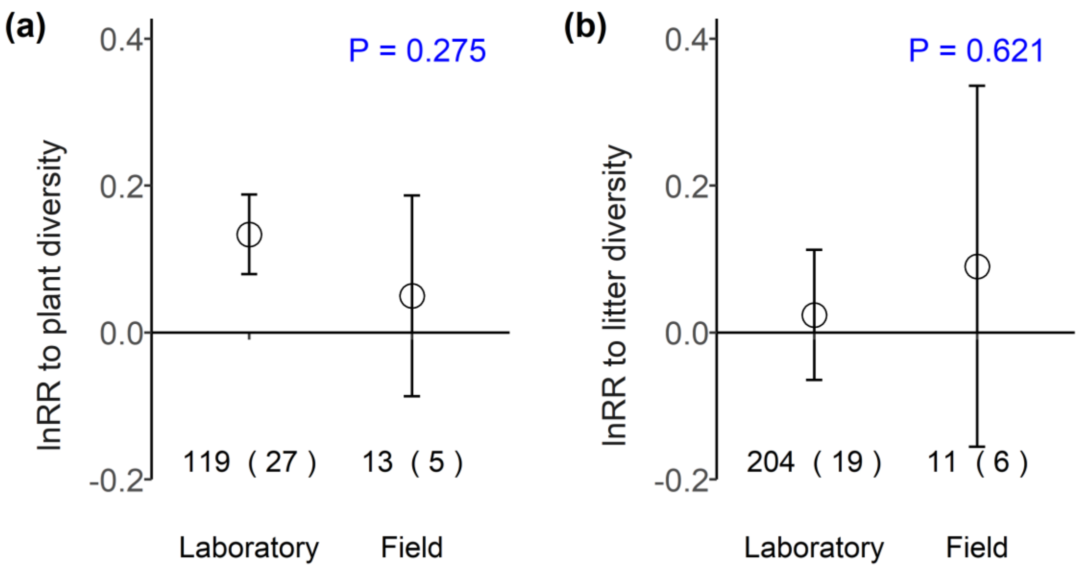

Figure S3-2. The natural log response ratio (ln*RR*) of Rh to plant diversity and litter diversity for the field- or laboratory-based measurements. The numbers outside and within the parentheses represent the number of observations and studies, respectively.

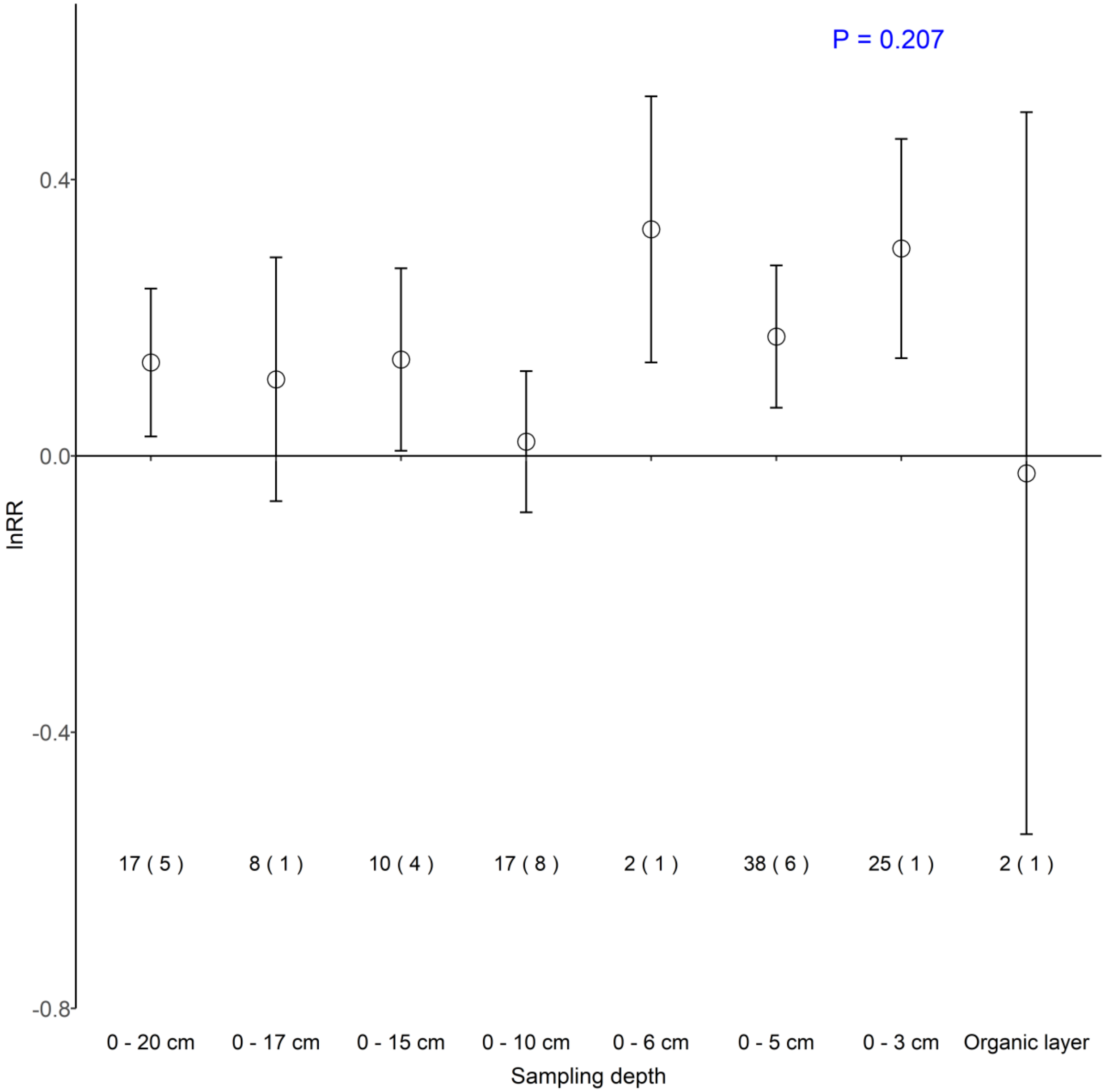

Figure S3-3. The natural log response ratio between species mixtures and monocultures (ln*RR*) of Rh in relation to different sampling depth measurements. The numbers outside and within the parentheses represent the number of observations and studies, respectively.

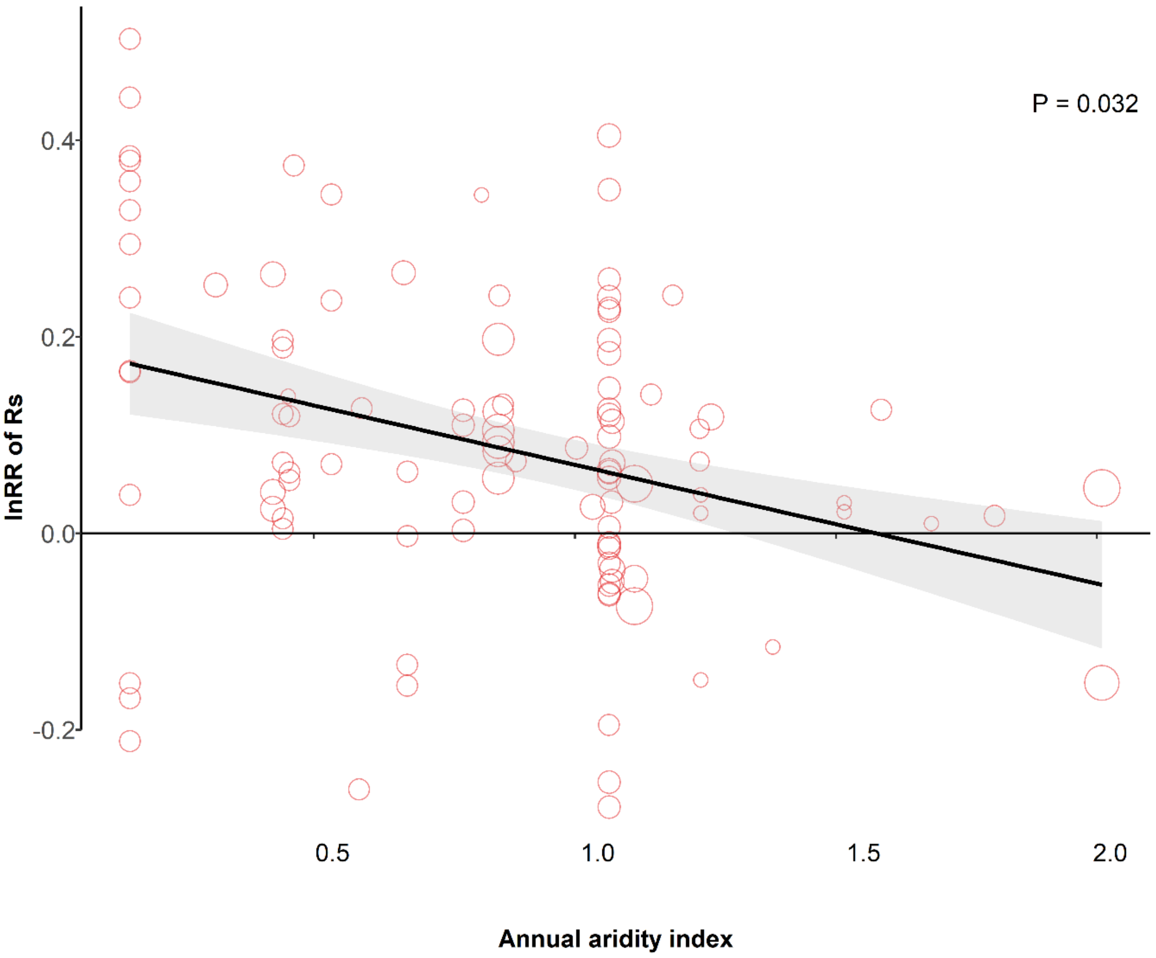

Figure S3-4. The natural log response ratio between species mixtures and monocultures (lnRR) of Rs in relation to annual aridity index. Fitted regression, its 95% confident intervals (shaded), and the corresponding significance (*P*) are presented. The sizes of the circles represent the relative weights of the corresponding observations.

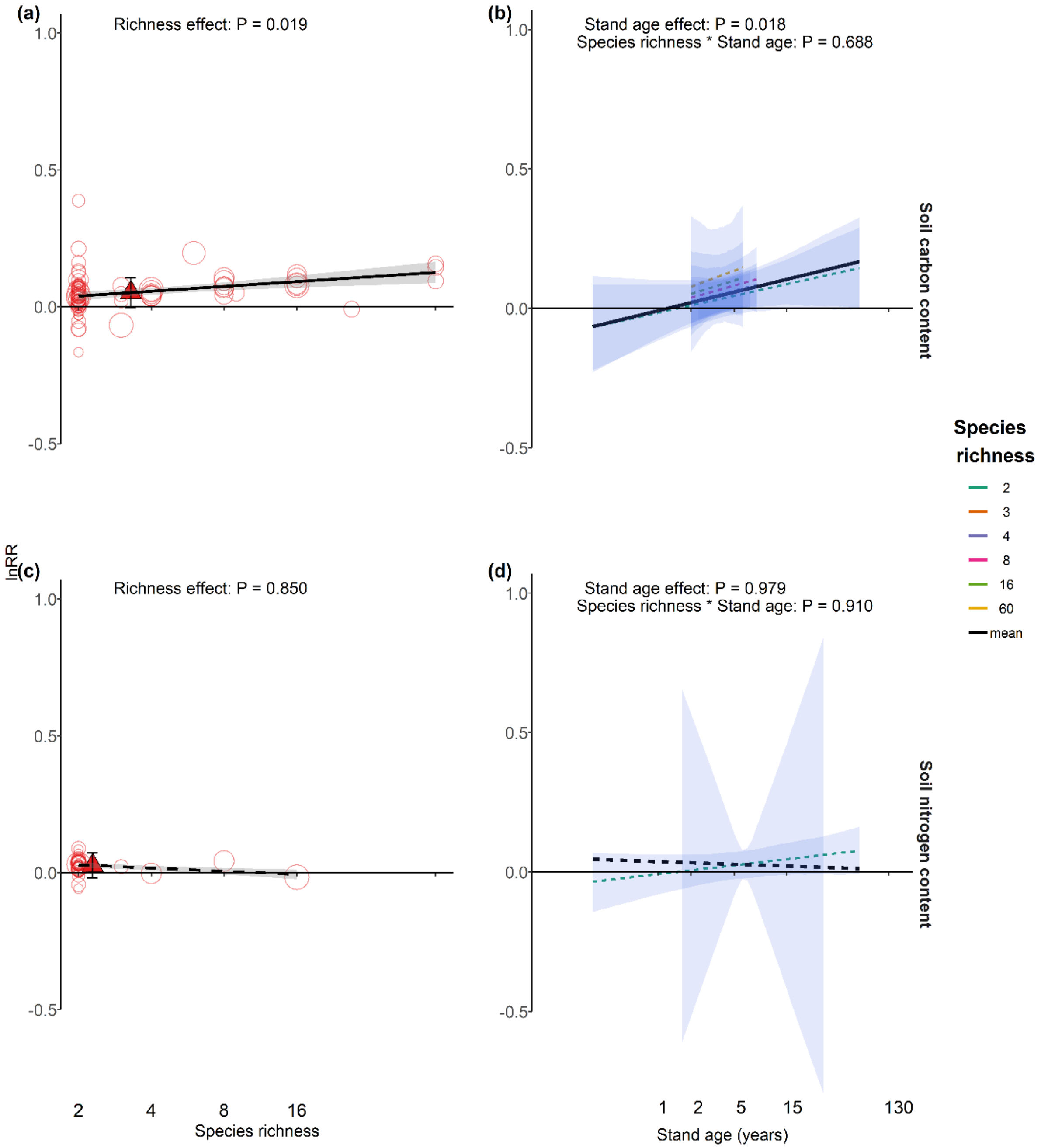

Figure S3-5. The natural log response ratio between species mixtures and monocultures (ln*RR*) in relation to plant species richness in mixtures and their temporal trends associated with species richness. a, c, The ln*RR*s of soil carbon content and soil nitrogen content in relation to the species richness in mixtures. b, d, Species richness gradient-dependent temporal trends of the ln*RR*s of soil carbon content (b) and soil nitrogen content (d) to plant diversity. Red triangles and error bars represent overall mean and its 95% confidence intervals. Black and colored lines represent overall and species, or litter richness gradient-specific responses, respectively, with their bootstrapped 95% confidence intervals shaded in grey. Lines are bound by the range of the stand ages for each richness category. The corresponding levels of significance (*P*) of the diversity effect are presented. The sizes of the circles represent the relative weights of the corresponding observations.

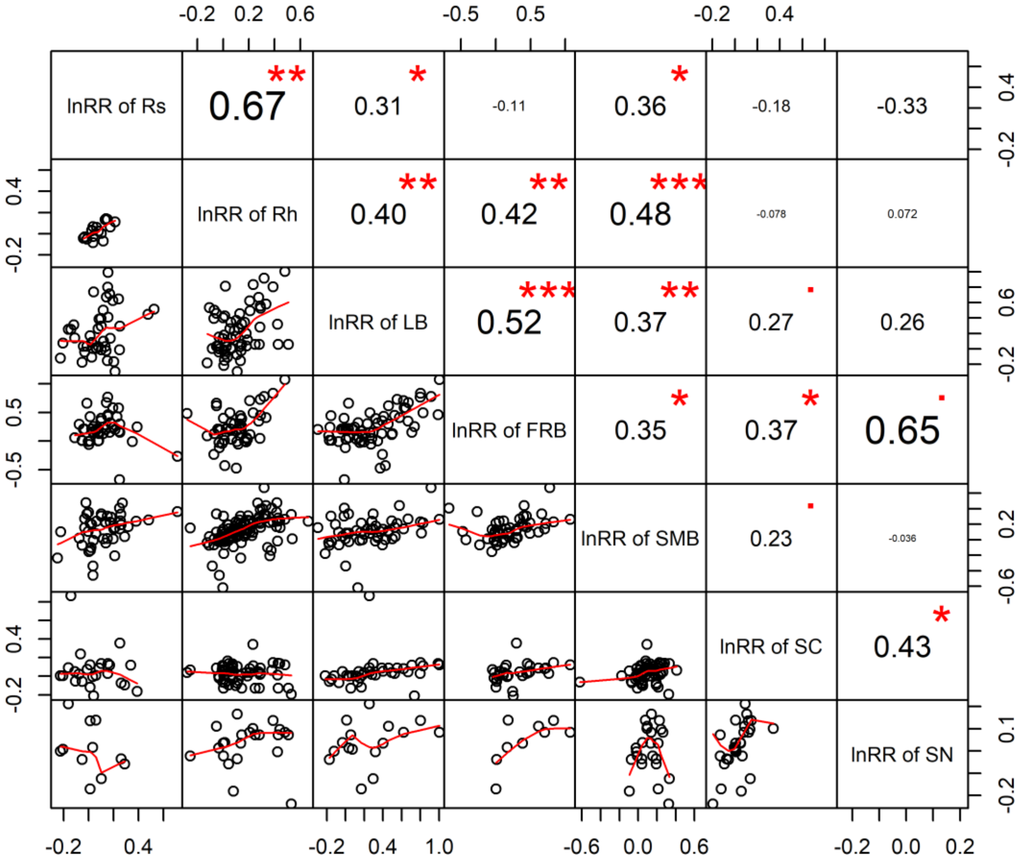

Figure S3-6. The Pearson correlation among ln*RRs* of Rs, Rh, litterfall biomass (LB), fine root biomass (FRB), soil microbial biomass (SMB), soil carbon content (SC) and soil nitrogen content (SN) to plant species mixture. ▪,·*, **, and *** indicate significance at P < 0.1, < 0.05, <0.01, and <0.001, respectively.

APPENDIX III: SUPPLEMENTAL INFORMATION FOR CHAPTER 4

Table S4-1. The source for original studies that have examined plant species effects on soil organic carbon (SOC) content and stock and soil microbial biomass carbon (SMBC).

Publication	Variable	Richness level	Stand age (year)	Soil depth (cm)
Cropland				
Cong, W. F. *et al.* Intercropping enhances soil carbon and nitrogen. *Global Change Biol* (2015).	SOCC, SOCS	2	7	0-100
Coser, T. R. *et al.* Soil microbiological properties and available nitrogen for corn in monoculture and intercropped with forage. *Pesquisa Agropecuaria Brasileira* (2016).	SOCC, SMBC	2	3	0-30
Dyer, L. *et al.* Soil carbon dioxide and nitrous oxide emissions during the growing season from temperate maize-soybean intercrops. *Journal of Plant Nutrition and Soil Science* (2012).	SOCC, SOCS	2	2	0-10
Oelbermann, M. *et al.* Changes in soil characteristics after six seasons of cereal–legume intercropping in the Southern Pampa. *Geoderma Regional* (2015).	SOCC, SOCS, SMBC	2	1, 5	0-40
Oelbermann, M. et al. Evaluating soil carbon and nitrogen dynamics in recently established maize-soyabean inter-cropping systems. European Journal of Soil Science (2011).	SMBC	2	0.5	0-120
Scalise, A. *et al.* Legume-barley intercropping stimulates soil N supply and crop yield in the succeeding durum wheat in a rotation under rainfed conditions. *Soil Biology & Biochemistry* (2015).	SOCC, SMBC	2	2	0-15
Sharma, R. C. *et al.* Baby Corn-Legumes Intercropping Systems: I. Yields, Resource Utilization Efficiency, and Soil Health. *Agroecology and Sustainable Food Systems* (2015).	SOCC, SMBC	2	2	0-20
Souza, M. F. P. *et al.* Chemical and biological properties of phosphorus-fertilized soil under legume and grass cover (Cerrado region, Brazil). *Revista Brasileira De Ciencia Do Solo* (2013).	SOCC, SMBC	2	1	0-20
Sun, Y. M. *et al.* Influence of intercropping and intercropping plus rhizobial inoculation on microbial activity and community composition in rhizosphere of alfalfa (Medicago sativa L.) and Siberian wild rye (Elymus sibiricus L.). *Fems Microbiology Ecology* (2009).	SMBC	2	1	0-15
Tang, X. Y. *et al.* Increase in microbial biomass and phosphorus availability in the rhizosphere of intercropped cereal and legumes under field conditions. *Soil Biology & Biochemistry* (2014).	SMBC	2	1	0-20
Tang, X. Y. *et al.* Phosphorus availability and microbial community in the rhizosphere of intercropped cereal and legume along a P-fertilizer gradient. *Plant and Soil* (2016).	SMBC	2	1	0-20
Tortorella, D. *et al.* Chemical and biological responses in a Mediterranean sandy clay loam soil under grain legume-barley intercropping. *Agrochimica* (2013).	SMBC	2	0.5	0-25
Vachon, K. *Soil carbon and nitrogen dynamics and greenhouse gas mitigation in intercrop agroecosystems in Balcarce, Argentina.* Master of Environmental Studies thesis, University of Waterloo, (2008).	SOCC, SOCS	2	1	0-10
Wang, Z. G. *et al.* Intercropping Enhances Productivity and Maintains the Most Soil Fertility Properties Relative to Sole Cropping. *Plos One* (2014).	SOCC	2	3	0-20
Forest				
Alberti, G. et al. Tree functional diversity influences belowground ecosystem functioning. Applied Soil Ecology (2017).	SOCC	3	12	0-30

Bagherzadeh, A. *et al.* Temperature dependence of nitrogen mineralization and microbial status in OH horizon of a temperate forest ecosystem. *Journal of Forestry Research* (2008).	SOCC, SMBC	2	110	0-14
Balieiro, F. D. *et al.* Soil carbon and nitrogen in pasture soil reforested with eucalyptus and guachapele. *Revista Brasileira De Ciencia Do Solo* (2008).	SOCC, SOCS	2	5	0-40
Berger, T. W. *et al.* Nutrient fluxes in pure and mixed stands of spruce (Picea abies) and beech (Fagus sylvatica). *Plant and Soil* (2009).	SOCS	2	61	0-50
Bini, D. *et al.* Eucalyptus grandis and Acacia mangium in monoculture and intercropped plantations: Evolution of soil and litter microbial and chemical attributes during early stages of plant development. *Applied Soil Ecology* (2013).	SOCC, SMBC	2	1	0-10
Brassard, B. W. *The Root Dynamics of Mixed- and Single-Species Stands in The Boreal Forest of Central and Eastern Canada.* Doctor of Philosophy thesis, Lakehead University, (2010).	SOCC	4	85	0-55
Cao, H. Research on nutrient status of pure Chinese fir, Masson pine, Schima superba and mixed forests. *Journal of Nanjing Forestry University* (1998).	SOCC	2	15	0-50
Chen, J. *et al.* Effects of five plantations on soil properties in subtropical red soil hilly region. *Journal of Northwest A&F University* (2013).	SOCC, SMBC	2	15	0-60
Chen, X. D. *et al.* Why does oriental arborvitae grow better when mixed with black locust: Insight on nutrient cycling? *Ecology and Evolution* (2018).	SOCC	2	10	0-20
Chodak, M. *et al.* Effect of texture and tree species on microbial properties of mine soils. *Applied Soil Ecology* (2010).	SOCC, SMBC	2	24	0-5
Chodak, M. *et al.* The effect of different tree species on the chemical and microbial properties of reclaimed mine soils. *Biology and Fertility of Soils* (2010).	SOCC, SMBC	2	23	0-5
Correa, E. *et al.* Effect of plant species on P cycle-related microorganisms associated with litter decomposition and P soil availability: implications for agroforestry management. *Iforest* (2016).	SOCC	2	1.5	0-20
Cremer, M. *et al.* Soil organic carbon and nitrogen stocks under pure and mixed stands of European beech, Douglas fir and Norway spruce. *Forest Ecology and Management* (2016).	SOCC, SOCS	2	60, 50, 90	0-60
Dawud, S. M. *et al.* Is Tree Species Diversity or Species Identity the More Important Driver of Soil Carbon Stocks, C/N Ratio, and pH? *Ecosystems* (2016).	SOCS	2, 3, 4, 5	42, 65, 91, 99, 101, 110	0-20
Diaz-Pines, E. *et al.* Does tree species composition control soil organic carbon pools in Mediterranean mountain forests? *Forest Ecology and Management* (2011).	SOCC, SOCS	2	70	0-50
Domisch, T. *et al.* Does species richness affect fine root biomass and production in young forest plantations? *Oecologia* (2015).	SOCC	2, 3, 4, 5	8, 14	0-40
Dong, H. *Soil Characteristics and Improvement of Long-term Plantation in the Yellow River Delta*. Shan Dong Agricultural University (2014).	SOCC, SOCS, SMBC	2	27	0-20
Dong, M. *et al.* Soil microbial biomass C, N and diversity characteristics in pure and mixed forest of *Pinus* and *Cinnamomun*. *Journal of Central South University of Forestry & Technology* (2017).	SOCC, SMBC	2	24	0-30
Duo, Y. *et al.* The Biomass Comparison of Soil Microbial Carbon and Nitrogen of 3 Kinds of Forest Types in Subtropics. *Chinese Agricultural Science Bulletin* (2012).	SOCC, SMBC	2	24	0-10

Fan, S. *et al.* Comparison of Soil Microbiology Characteristics in Five Subtropical Ecosystems. *Journal of Tropical and Subtropical Botany* (2016).	SOCC, SOCS, SMBC	2	15	0-60
Fataei, E. *et al.* Effects of Afforestation on Carbon Stocks in Fandoghloo Forest Area. *Pakistan Journal of Agricultural Sciences* (2018).	SOCS	2	20	0-50
Forrester, D. I. *et al.* Soil Organic Carbon is Increased in Mixed-Species Plantations of Eucalyptus and Nitrogen-Fixing Acacia. *Ecosystems* (2013).	SOCS	2	8.1	0-30
Gao, C. *et al.* Nutrient accumulation and cycling in pure and mixed plantations of Azadirachta indica and Acacia auriculiformis in a dry-hot valley, Yunnan Province, southwest China. *Chinese Journal of Applied Ecology*.	SOCC	2	10	0-20
Garau, G. et al. Effect of monospecific and mixed Mediterranean tree plantations on soil microbial community and biochemical functioning. Applied Soil Ecology (2019).	SOCC, SOCS	2	2	0-10
Gong, X. *et al.* Sub-tropic degraded red soil restoration: Is soil organic carbon build-up limited by nutrients supply. *Forest Ecology and Management* (2013).	SOCC	2	18	0-40
Gunina, A. *et al.* Response of soil microbial community to afforestation with pure and mixed species. *Plant and Soil* (2017).	SOCC, SOCS	2, 3	10	0-10
Hendriks, C. M. A. *et al.* Root density and root biomass in pure and mixed forest stands of Donglas-fir and Beech. *Netherlands Journal of Agricultural Science* (1995).	SOCC	2	60	0-25
Hu, B. *et al.* Comparison of nitrogen nutrition and soil carbon status of afforested stands established in degraded soil of the Loess Plateau, China. *Forest Ecology and Management* (2017).	SOCC, SOCS, SMBC	2	40	0-50
Huang, Y. *et al.* Changes in soil quality due to introduction of broad-leaf trees into clear-felled Chinese fir forest in the mid-subtropics of China. *Soil Use and Management* (2004).	SOCC, SMBC	2	20	0-10
Huang, Y. L. *et al.* Effects of a mixed plantation of Robinia pseudoacacia and Fraxinus velutina on soil bacterial structure and diversity in the Yellow River Delta. *Acta Ecologica Sinica* (2018).	SOCC	2	31	0-20
Hume, A. *et al.* Soil C:N:P dynamics during secondary succession following fire in the boreal forest of central Canada. *Forest Ecology and Management* (2016).	SOCC	3, 4, 5	7, 15, 33	0-30
Jiang, Y. M. *et al.* Soil soluble organic carbon and nitrogen pools under mono- and mixed species forest ecosystems in subtropical China. *Journal of Soils and Sediments* (2010).	SOCC, SOCS, SMBC	2, 3	17	0-20
Khlifa, R. *Effets de la diversité des arbres sur le fonctionnement de l'écosystème dans deux plantations de forêts tempérées.* Doctor of Philosophy thesis, Université Laval Québec Canada, (2016).	SOCC, SMBC	2, 4, 12	4	0-3
Koupar, S. A. M. *et al.* Effects of pure and mixed plantations of Populus deltoides with Alnus glotinosa on growth and soil properties: A case study of Foman Region, Iran. *African Journal of Agricultural Research* (2011).	SOCC	2	12	0-60
Lemma, B. Soil chemical properties and nutritional status of trees in pure and mixed-species stands in south Ethiopia. *Journal of Plant Nutrition and Soil Science* (2012).	SOCC	2	24	0-50
Liu, J. *et al.* Effects of tree species and soil properties on the composition and diversity of the soil bacterial community following afforestation. *Forest Ecology and Management* (2018).	SOCC, SMBC	2	24	0-20
Liu, M. Q. *et al.* Seasonal Dynamics of Soil Microbial Biomass and Its Significance to Indicate Soil Quality under Different Vegetations Restored on Degraded Red Soils. *ACTA PEDOLOGICA SINICA* (2003).	SMBC	2	10	0-20

Liu, Y. *et al.* Accumulation of soil organic C and N in planted forests fostered by tree species mixture. *Biogeosciences* (2017).	SOCS	2	10, 24, 45	0-40
Liu, Y. Q. *et al.* Effect of Rehabilitated Forest on Soil Microbial Characteristics of Severely Degraded Red Soil Region. *Journal of Fujian College of Forestry* (2003).	SMBC	2	10	0-40
Liuzhuo, M. *et al.* Soil Biological Characteristics in Different Kinds of Artificial Forests in the Semi-humid Zones of Loess Plateau. *Journal of Northwest Forestry University* (2009).	SMBC	2	38	0-40
Luo, D. *Characteristics of carbon and nitrogen in monoculture and mixed young stands of Erythrophleum fordii and Pinus massoniana in southern subtropical China.* Doctor of Philosophy thesis, Chinese Academy of Forestry, (2014).	SOCC, SOCS	2	7	0-100
Montagnini, F. Accumulation in above-ground biomass and soil storage of mineral nutrients in pure and mixed plantations in a humid tropical lowland. *Forest Ecology and Management* (2000).	SOCC	4	5	0-60
Nunes, L. *et al.* Growth, soil properties and foliage chemical analysis comparison between pure and mixed stands of Castanea saliva Mill. and Pseudotsuga menziesii (Mirb.) Franco, in Northern Portugal. *Forest Systems* (2011).	SOCC	2	28	0-60
Pan, H. *Soil carbon and nitrogen transformation process research of relationship between soil microbial community structure of Michelia macclurei, Pinus massoniana plantation.* Master thesis, Guangxi University, (2015).	SOCC	2	31	0-10
Pereira, A. P. A. *et al.* Mixed Eucalyptus plantations induce changes in microbial communities and increase biological functions in the soil and litter layers. *Forest Ecology and Management* (2019).	SOCC, SMBC	2	3	0-20
Pereira, E. L. *et al.* Microbial biomass and N mineralization in mixed plantations of broadleaves and nitrogen-fixing species. *Forest Systems* (2011).	SOCC, SOCS, SMBC	2	11	0-10
Potvin, C. *et al.* An ecosystem approach to biodiversity effects: Carbon pools in a tropical tree plantation. *Forest Ecology and Management* (2011).	SOCC, SOCS	3, 6	8	0-10
Rachid, C. T. C. C. *et al.* Mixed plantations can promote microbial integration and soil nitrate increases with changes in the N cycling genes. *Soil Biology & Biochemistry* (2013).	SOCC	2	4	0-15
Reverchon, F. *et al.* Tree Plantation Systems Influence Nitrogen Retention and the Abundance of Nitrogen Functional Genes in the Solomon Islands. *Frontiers in Microbiology* (2015).	SOCC	2	4	0-15
Rivest, D. *et al.* Tree communities rapidly alter soil microbial resistance and resilience to drought. *Functional Ecology* (2015).	SOCC, SMBC	2	4	0-20
Sayyad, E. *et al.* Comparison of growth, nutrition and soil properties of pure and mixed stands of Populus deltoides and Alnus subcordata. *Silva Fennica* (2006).	SOCC	2	7	0-15
Spohn, M. *et al.* Microbial respiration per unit biomass increases with carbon-to-nutrient ratios in forest soils. *Soil Biology and Biochemistry* (2015).	SOCC, SMBC	2	130	0-5
Su, X. *et al.* Effect of plant diversity on soil microbial community in the subtropical forest soil. *Chinese Journal of Ecology* (2018).	SOCC, SMBC	2, 4, 8	7	0-10
Sun, H. *et al.* Carbon Storage of 45-year-old Stands in Different Origins on the West Slope of Zhangguangcai Mountain. *Chinese Journal of Bulletin of Batanical Research* (2015).	SOCC, SOCS	2	45	0-60
Tan, G. *et al.* Content and Seasonal Change of Soil Labile Organic Carbon under Four Different Plantations in Degraded Red Soil Region. *Acta Agriculturae Universitatis Jiangxiensis* (2014).	SOCC, SOCS, SMBC	2	21	0-10
Tan, L. *et al.* Comparison of Soil Physical and Chemical Properties of Pure Castanopsis hystrix, Pure Pinus massoniana and Mixed-	SOCC, SOCS	2	28	0-60

species Tree Plantation in South Subtropical Area. *Journal of West China Forestry Science* (2014).				
Tang, G. Y. *et al.* Accelerated nutrient cycling via leaf litter, and not root interaction, increases growth of Eucalyptus in mixed-species plantations with Leucaena. *Forest Ecology and Management* (2013).	SOCC, SOCS	2	10	0-20
Tchichelle, S. V. *et al.* Differences in nitrogen cycling and soil mineralisation between a eucalypt plantation and a mixed eucalypt and Acacia mangium plantation on a sandy tropical soil. *Southern Forests* (2017).	SOCC, SOCS	2	7	0-25
Voigtlaender, M. *et al.* Introducing Acacia mangium trees in Eucalyptus grandis plantations: consequences for soil organic matter stocks and nitrogen mineralization. *Plant and Soil* (2012).	SOCC, SOCS	2	6	0-15
Wang, H. *et al.* C and N stocks under three plantation forest ecosystems of Chinese fir, Michelia macclurei and their mixture. *Frontiers of Forestry in China* (2007).	SOCS	2	23	0-100
Wang, H. *et al.* Effects of tree species mixture on soil organic carbon stocks and greenhouse gas fluxes in subtropical plantations in China. *Forest Ecology and Management* (2013).	SOCS	2	25	0-20
Wang, H. *et al.* Mixed-species plantation with Pinus massoniana and Castanopsis hystrix accelerates C loss in recalcitrant coniferous litter but slows C loss in labile broadleaf litter in southern China. *Forest Ecology and Management* (2018).	SOCC, SMBC	2	26	0-10
Wang, Q. Study on the growth efect of mixed forest with Cunninghamia lanceolatea, Pinus massoniana and Schima superba in mountanious region of south Fujian. *Journal of Fujian College of Forestry* (2012).	SOCC, SOCS	2, 3	15	0-60
Wang, Q. K. *et al.* Ecosystem carbon storage and soil organic carbon stability in pure and mixed stands of Cunninghamia lanceolata and Michelia macclurei. *Plant and Soil* (2013).	SOCC, SOCS	2	28	0-40
Wang, S. J. *et al.* Does tree species composition affect soil CO2 emission and soil organic carbon storage in plantations? *Trees-Structure and Function* (2016).	SOCS	2	33	0-5
Wen, L. *et al.* Soil microbial biomass carbon and nitrogen in pure and mixed stands of Pinus massoniana and Cinnamomum camphora differing in stand age. *Forest Ecology and Management* (2014).	SOCC, SMBC	2	10, 24, 45	0-40
Wu, P. *et al.* Effects of three different plantations on soil physicochemical and microbial characteristics in Krast region. *Journal of Nanjing Forestry University* (2015).	SOCC, SOCS, SMBC	2	10	0-10
Xia, Z.-C. *et al.* A broadleaf species enhances an autotoxic conifers growth through belowground chemical interactions. *Ecology* (2016).	SMBC	2	23	0-20
Yan, W. D. *et al.* Impacts of changed litter inputs on soil CO2 efflux in three forest types in central south China. *Chinese Science Bulletin* (2013).	SOCC, SOCS	2	27.5	0-10
Yan, W. D. *et al.* Soil CO2 Flux in Different Types of Forests Under a Subtropical Microclimatic Environment. *Pedosphere* (2014).	SOCC	2	24	0-10
Yang, Z. *et al.* Litter fall production and carbon return in Cunninghamia lanceolata, Schima superba, and their mixed plantations. *Chinese Journal of Applied Ecology* (2010).	SOCC	2	20	0-40
Zhang, H. *et al.* Recalcitrant carbon controls the magnitude of soil organic matter mineralization in temperate forests of northern China. *Forest Ecosystems* (2018).	SOCC, SMBC	2	70	0-20
Zhang, J. *et al.* Content and seasonal change in soil labile organic carbon under different forest covers. *Chinese Journal of Eco-Agriculture* (2009).	SOCC, SOCS, SMBC	2	24	0-10

Zhang, J. T. *et al.* Effects of mixed Hippophae rhamnoides on community and soil in planted forests in the Eastern Loess Plateau, China. *Ecological Engineering* (2007).	SOCC, SOCS	3, 5	21	0-60
Zhang, J. W. *et al.* Litter dynamics of Pinus massoniana and Michelia macclurei mixed forest and its effect on soil nutrients. *Chinese Journal of Applied Ecology* (1993).	SOCC	2	55	0-60
Zhang, L. *Study on Ecosystem Functions of Masson Pine mixedwoods with Different Mixed proportions* Doctor of Philosophy thesis, Anhui Agricultural University, (2013).	SOCC	2	30	0-30
Zhang, S. Y. *et al.* A Study on Microbial Biomass C，N Characteristics in Different Rehabilitating Forests on Degraded Red Soil. *Acta Agriculturae Universitatis Jiangxiensis* (2010).	SMBC	2	18	0-15
Zhao, R. D. *et al.* Effects of Plantation Restoration Approaches on Soil Enzyme Activities and Microbial Properties in Hilly Red Soil Region. *Soils* (2012).	SOCC, SMBC	2	14	0-40
Zhou, J. *et al.* Effects of Cunninghamia lanceolata-Betula luminifera Mixed Forests on Soil Microbial Biomass and Enzyme Activity. *Journal of Northwest A&F University* (2015).	SOCC, SMBC	2	6	0-40
Zhou, Z. Y. *et al.* Predicting soil respiration using carbon stock in roots, litter and soil organic matter in forests of Loess Plateau in China. *Soil Biology & Biochemistry* (2013).	SOCS	2	17	0-20
Grassland				
Alvarez, G. *et al.* Soil indicators of C and N transformations under pure and mixed grass-clover swards. *European Journal of Agronomy* (1998).	SMBC	2	4	0-20
Cong, W. F. *et al.* Forbs differentially affect soil microbial community composition and functions in unfertilized ryegrass-red clover leys. *Soil Biology & Biochemistry* (2018).	SOCC	2, 3	3	0-10
Cong, W. F. *et al.* Plant species richness promotes soil carbon and nitrogen stocks in grasslands without legumes. *Journal of Ecology* (2014).	SOCS	2, 4, 8	10	0-15
Cong, W. F. *et al.* Plant species richness leaves a legacy of enhanced root litter-induced decomposition in soil. *Soil Biology & Biochemistry* (2015).	SOCC	2	12	0-15
Dijkstra, F. A. *et al.* Divergent effects of elevated CO2, N fertilization, and plant diversity on soil C and N dynamics in a grassland field experiment. *Plant and Soil* (2005).	SOCC, SMBC	4	4	0-20
Eisenhauer, N. *et al.* Plant diversity effects on soil microorganisms support the singular hypothesis. *Ecology* (2010).	SMBC	2, 4, 8, 16, 60	6	0-5
Eisenhauer, N. *et al.* Plant diversity effects on soil food webs are stronger than those of elevated CO_2 and N deposition in a long-term grassland experiment. *Proceedings of the National Academy of Sciences* (2013).	SMBC	4, 9	13	0-6
Fischer, C. *et al.* Plant species diversity affects infiltration capacity in an experimental grassland through changes in soil properties. *Plant and Soil* (2015).	SOCC	2, 4, 8, 16, 60	10	0-5
Fornara, D. A. *et al.* Plant functional composition influences rates of soil carbon and nitrogen accumulation. *Journal of Ecology* (2008).	SOCC	2, 4, 8, 16	13	0-20
Gong, J. R. *et al.* Effect of irrigation on the soil respiration of constructed grasslands in Inner Mongolia, China. *Plant and Soil* (2015).	SMBC	2	1	0-60
Guenay, Y. *et al.* Transgressive overyielding of soil microbial biomass in a grassland plant diversity gradient. *Soil Biology & Biochemistry* (2013).	SMBC	3, 9	10	0-10
Malchair, S. *et al.* Do climate warming and plant species richness affect potential nitrification, basal respiration and ammonia-	SOCC	3, 9	2	0-10

oxidizing bacteria in experimental grasslands? *Soil Biology & Biochemistry* (2010).				
Qu, J. *Effects of Intercropping Oat and Common Vetch on Forage Yield and Ouality and Soil Characteristics.* Master thesis, InnerMongolia Agricultural University, (2017).	SOCC, SMBC	2	2	0-30
Reid, J. P. *et al.* Biodiversity, Nitrogen Deposition, and CO2 Affect Grassland Soil Carbon Cycling but not Storage. *Ecosystems* (2012).	SOCC	16	9	0-20
Spehn, E. M. *et al.* Plant diversity effects on soil heterotrophic activity in experimental grassland ecosystems. *Plant and Soil* (2000).	SOCC, SMBC	2, 4, 8, 27	3	0-10
Steinauer, K. *et al.* Convergence of soil microbial properties after plant colonization of an experimental plant diversity gradient. *Bmc Ecology* (2016).	SMBC	2, 4, 8, 16, 60	5	0-10
Steinauer, K. *et al.* Plant diversity effects on soil microbial functions and enzymes are stronger than warming in a grassland experiment. *Ecology* (2015).	SMBC	4, 16	18	0-20
Strecker, T. *et al.* Functional composition of plant communities determines the spatial and temporal stability of soil microbial properties in a long-term plant diversity experiment. *Oikos* (2016).	SMBC	2, 4, 8,16	9	0-5
Tai, J. *et al.* Effects of manual sowing methods of Medicago sativa and Bromus inermis on the distribution and content of soil organic carbon and nitrogen. *ACTA PRATACULTURAE SINICA (Chinese)* (2010).	SOCC, SOCS	2	2	0-40
Tang, X. *Effects of Water and Nitrogen Coupling on Pasture Establishment in HulunBuir.* Master thesis, Chinese Academy of Agricultural Sciences Dissertation, (2018).	SOCC, SMBC	2	1	0-10
van Eekeren, N. *et al.* Effect of individual grass species and grass species mixtures on soil quality as related to root biomass and grass yield. *Applied Soil Ecology* (2010).	SOCC	2	2	0-30
van Eekeren, N. *et al.* A mixture of grass and clover combines the positive effects of both plant species on selected soil biota. *Applied Soil Ecology* (2009).	SOCC, SOCS	2	1	0-10
Velasquez, E. *et al.* Soil macrofauna-mediated impacts of plant species composition on soil functioning in Amazonian pastures. *Applied Soil Ecology* (2012).	SOCC, SOCS	2	2	0-10
Vogel, A. *et al.* Plant diversity does not buffer drought effects on early-stage litter mass loss rates and microbial properties. *Global Change Biol* (2013).	SMBC	2, 4, 8, 16, 60	6	0-15
Weisser, W. W. *et al.* Biodiversity effects on ecosystem functioning in a 15-year grassland experiment: Patterns, mechanisms, and open questions. *Basic Appl Ecol* (2017).	SMBC	2, 4, 8, 16, 60	6	0-5
Wen, J. *Effects of alpine grassland degradation and establishment of tame grassland on soil respiration in the Three-River Source Region* Master of Ecology thesis, Chinese Academy of Sciences, (2012).	SOCS	2, 3	5	0-10

Table S4-2. The values of Akaike information criterion for the linear and log-linear species richness (S*R*), stand age (S*A*), and soil depth (D).

Model	*SOC content*	*SOC stock*	*SMBC*	*SMBC/SOC*
$\ln RR = \beta_0 + \beta_1 \times \text{SR} + \pi$	-217	-90	6	93
$\ln RR = \beta_0 + \beta_1 \times \ln(\text{SR}) + \pi$	**-216**	**-89**	**0**	**93**
$\ln RR = \beta_0 + \beta_1 \times \text{SA} + \pi$	-222	-90	9	90
$\ln RR = \beta_0 + \beta_1 \times \ln(\text{SA}) + \pi$	**-245**	**-95**	**1**	**91**
$\ln RR = \beta_0 + \beta_1 \times \text{D} + \pi$	**-224**	**-99**	9	**76**
$\ln RR = \beta_0 + \beta_1 \times \ln(\text{D}) + \pi$	-217	-90	**-6**	82

SR, SA, D, Eco, AI, MAT are species richness, stand age, soil depth, ecosystem type, aridity index, and mean annual temperature, respectively.

Table S4-3. Effects of species richness in mixtures (SR), stand age (SA), and soil depth (D) on the natural log response ratios (ln*RRs*) of SOC content, SOC stock, SMBC, and SMBC/SOC.

Source	Estimate	df	F value	*P*	R^2
Soil organic carbon content					
ln(SR)	0.023	1, 339	3.56	0.060	0.006
ln(SA)	0.065	1, 307	50.09	**<0.001**	0.079
D	-0.001	1, 369	3.87	0.051	0.006
ln(SA) × D	0.003	1, 349	37.21	**<0.001**	0.059
Soil organic carbon stock					
ln(SR)	0.060	1, 204	2.76	0.098	0.008
ln(SA)	0.046	1, 55	6.20	**0.016**	0.018
D	0.0002	1, 205	0.07	0.790	<0.001
ln(SA) × D	0.003	1, 193	47.14	**<0.001**	0.137
Microbial biomass carbon					
ln(SR)	0.040	1, 217	7.08	**0.008**	0.014
ln(SA)	0.038	1, 143	5.43	**0.021**	0.011
ln(D)	-0.093	1, 237	13.31	**<0.001**	0.026
ln(SR) × ln(SA)	0.076	1, 247	20.26	**<0.001**	0.040
The ratio of microbial biomass carbon to soil organic carbon					
ln(SR)	-0.009	1, 89	0.05	0.833	<0.001
D	-0.014	1, 102	16.92	**<0.001**	0.073

Note: Linear mixed-effects model fit tests used Satterthwaite approximations for denominator degrees of freedom (df). *P* and R^2 are significance of the model and explained variance by the model, respectively.

Table S4-4. The effect (P values) of functional group richness on Natural log response ratios (lnRR) of SOC content, SOC stock, SMBC, and SMBC/SOC.

Attribute	df	F value	P
SOC content	1, 386	0.95	0.330
SOC stock	1, 195	0.10	0.756
SMBC	1, 242	3.57	0.060
SMBC/SOC	1, 111	0.24	0.624

Linear mixed effect models used Satterthwaite approximation for degrees of freedom (df). *P* is significance of the model and explained variance by the model, respectively.

Table S4-5. The effect (P values) of mean annual temperature (MAT), annual aridity index (AI), and ecosystem type on Natural log response ratios (lnRR) of SOC content, SOC stock, SMBC, and SMBC/SOC.

Attribute	MAT			AI			Ecosystem type		
	df	F value	P	df	F value	P	df	F value	P
SOC content	1, 67	0.01	0.913	1, 59	0.49	0.489	2, 49	1.05	0.359
SOC stock	1, 39	0.03	0.870	1, 39	0.39	0.536	2, 32	1.73	0.193
SMBC	1, 38	2.61	0.114	1, 53	0.01	0.930	2, 33	2.23	0.123
SMBC/SOC	1, 34	0.05	0.818	1, 37	0.18	0.673	2, 28	0.43	0.655

Linear mixed effect models used Satterthwaite approximation for degrees of freedom (df). *P* is significance of the model and explained variance by the model, respectively.

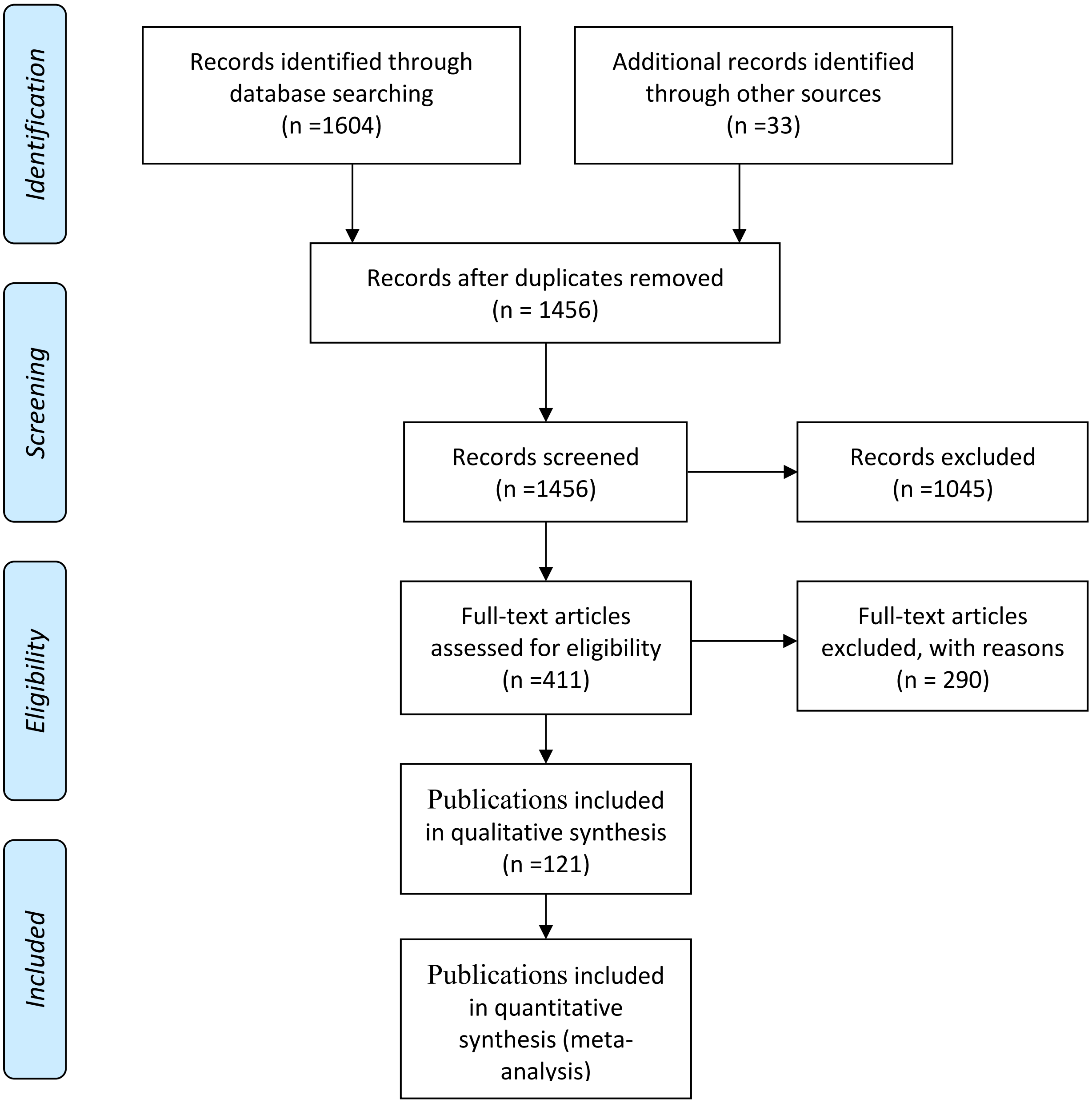

Figure S4-1. PRISMA diagram showing the process of locating studies included in this meta-analysis.

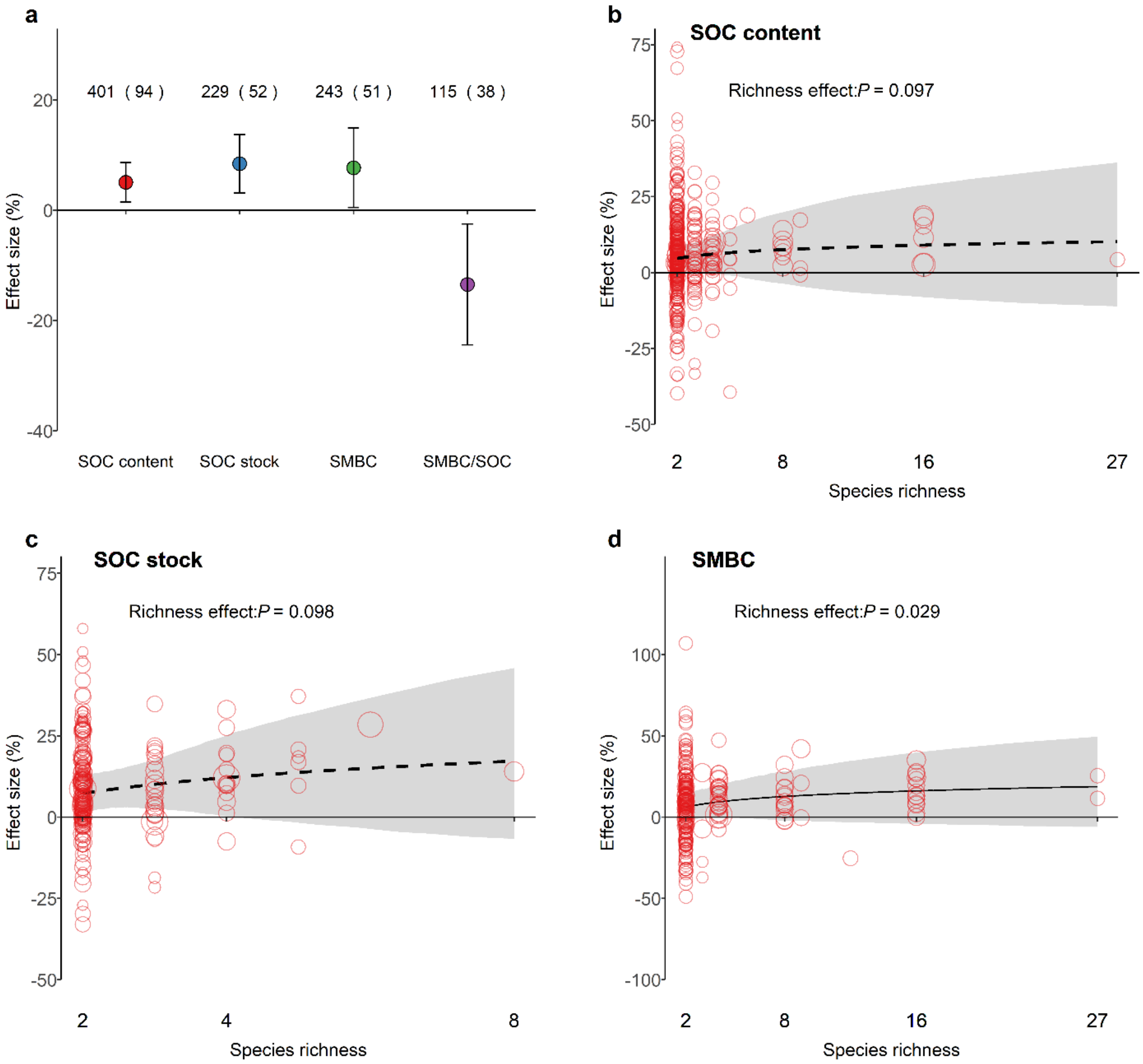

Figure S4-2. Comparison of soil organic carbon content (SOC content), soil organic carbon stock (SOC stock), soil microbial biomass carbon (SMBC), and the ratio of soil microbial biomass carbon to soil organic carbon (SMBC/SOC) in species mixtures versus monocultures and in relation to the species richness in mixtures across all studies without 60 species richness level. a, Comparison of SOC content, SOC stock, SMBC, and SMBC/SOC in species mixtures versus monocultures across all studies. c-d, Comparison of SOC content, SOC stock and SMBC in species mixtures versus monocultures in relation to the species richness in mixtures without 60 60 species richness level.

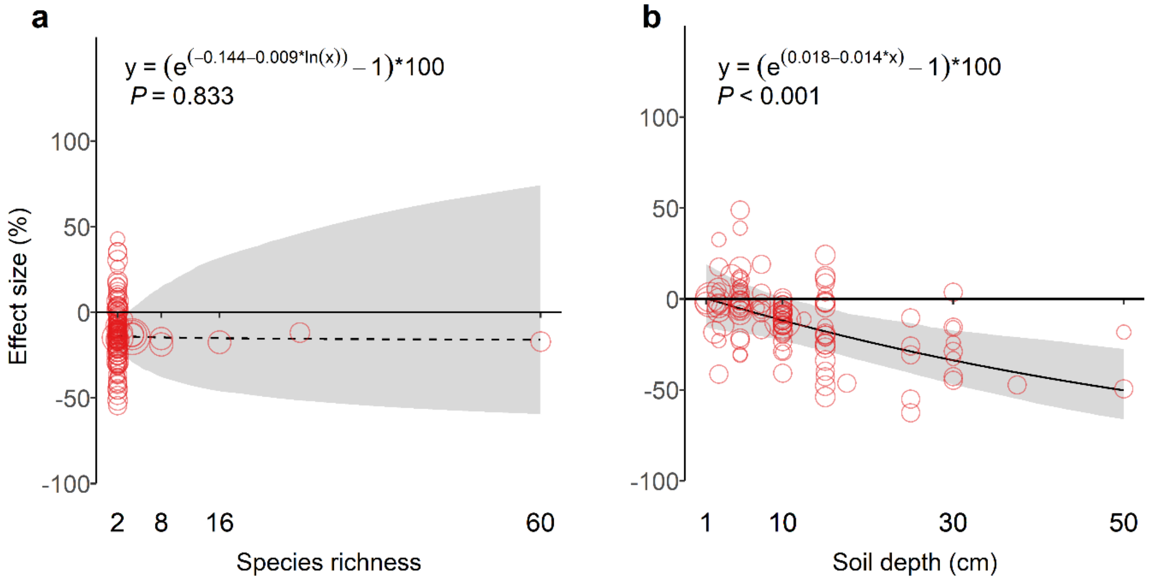

Figure S4-3. Comparison of the ratio of soil microbial biomass carbon to soil organic carbon (SMBC/SOC) in species mixtures versus monocultures in relation to the species richness in mixtures (a) and soil depth (b). The significance (P) is presented for each term tested. The sizes of the circles represent the relative weights of corresponding observations.

APPENDIX IV: SUPPLEMENTAL INFORMATION FOR CHAPTER 5

Table S5-1. Microbial PLFA biomarkers and metrics used.

Community Metric	PLFA Biomarker	References*
PLFA Biomass	Sum named and unnamed PLFAs (20 or less C atoms in length)	Zelles (1999)
Saprotrophic fungi	18:2ω6c, 18:1ω9c	Stahl and Klug (1996); Frostegard *et al.* (2011)
Arbuscular mycorrhizal (AM) fungi	16:1ω5c	Olsson *et al.* (1995)
Gram-positive bacteria	i15:0, a15:0, i16:0, a16:0, a17:0, and i17:0	Zelles (1997); Zogg *et al.* (1997)
Gram-negative bacteria	16:1ω7c, 16:1ω5c, 18:1ω7c, cy-17:0, and cy-19:0	Zelles (1997); Zogg *et al.* (1997)
Total Bacterial PLFAs	Gram-positive, gram-negative and 18:1ω5c, 15:0, 16:0 10-methyl, 17:0 10-methyl, 18:0 10-methyl	Smith *et al.* (2015)
Fungal ot bacterial ratio	18:2ω6c, 18:1ω9c /total bacterial PLFAs	Smith *et al.* (2015)
perMANOVA (individual PLFAs)	i15:0, a15:0, i16:0, a16:0, a17:0, i17:0,16:1ω7c, 16:1ω5c, 18:1ω7c, cy-17:0, cy-19:0, 18:1ω5c, 15:0, 16:0 10-methyl, 17:0 10-methyl, 18:0 10-methyl	Canarini *et al.* (2016)
perMANOVA (microbial groups)	Saprotrophic fungi, AM_fungi, Gram-positive bacteria, Gram-negative bacteria	Canarini *et al.* (2016)

Table S5-2. Effects (P values) of water alteration treatments on the response ratio of soil microbial biomass, F/B ratio, GN/GP bacteria ratio, soil water content, soil pH, litterfall production, and standing root length.

Attribute	W		
	df	F	*P*
SMB	2,4	8.39	**0.037**
Fungi	2,6	3.96	0.080
AM_Fungi	2,6	3.64	0.092
Bacteria	2,4	4.60	0.092
GP	2,4	8.31	**0.038**
GN	2,4	2.22	0.225
F/B	2,4	2.07	0.242
GN/GP	2,6	2.30	0.181
Soil water content	2,4	2.02	0.247
Soil pH	2,6	0.74	0.514
Litterfall biomass	2,4	3.69	0.124
Standing root length	2,6	6.50	0.032

Note: Linear mixed-effects model fit tests used Satterthwaite approximations for denominator degrees of freedom (df). *P* are significance of the model and $P < 0.05$ highlighted in bold. SMB, Fungi, AM_Fungi, Bacteria, GP, GN, F/B, and GN/GP are total soil microbial biomass, fungal biomass, AM_fungal biomass, bacterial biomass, Gram_positive bacteria biomass, Gram_negative bacteria biomass, fungal:bacterial ratios, and Gram_negative: Gram_positive bacterial ratios, respectively.

Table S5-3. Effects (P values) of overstory species richness (R), proportion of broadleaved trees (B), water alteration treatments (W), and interactions of overstory richness and water alteration treatments (R × W) on soil microbial biomass, F/B ratio, and GN/GP bacteria ratio.

Attribute	R				W				B				R × W			
	df	F	P	R^2	df	F	P	R^2	df	F	P	R^2	df	F	P	R^2
SMB	1,16	0.65	0.432	0.023	2,14	0.94	0.414	0.067	1,7	12.47	**0.010**	0.446	2,15	0.1	0.907	0.007
S_Fungi	1,14	0.98	0.339	0.044	2,15	0.55	0.586	0.050	1,7	5.30	0.055	0.236	2,15	0.13	0.881	0.011
AM_Fungi	1,13	0.54	0.474	0.017	2,15	1.34	0.291	0.083	1,7	13.07	**0.010**	0.403	2,15	0.36	0.705	0.022
Bacteria	1,14	1.18	0.296	0.037	2,15	0.86	0.443	0.054	1,7	15.32	**0.006**	0.478	2,15	0.11	0.897	0.007
GP	1,15	0.07	0.797	0.003	2,14	0.89	0.431	0.069	1,6	10.02	**0.018**	0.388	2,15	0.21	0.811	0.016
GN	1,15	4.78	**0.046**	0.106	2,15	1.07	0.368	0.048	1,7	23.15	**0.002**	0.514	2,15	0.39	0.681	0.017
F/B	1,9	0.62	0.450	0.032	2,16	0.09	0.912	0.009	1,6	2.66	0.152	0.134	2,17	0.002	0.998	<0.001
GP/GN	1,11	7.71	**0.018**	0.153	2,15	2.50	0.115	0.100	1,6	2.54	0.162	0.050	2,15	2.85	0.088	0.114

Note: Linear mixed-effects model fit tests used Satterthwaite approximations for denominator degrees of freedom (df). P and R^2 are the significance of the model and explained variance by the model, respectively. SMB, Fungi, AM_Fungi, Bacteria, GP, GN, F/B, and GN/GP are total soil microbial biomass, fungal biomass, arbuscular mycorrhizal fungal biomass, bacterial biomass, Gram_positive bacteria biomass, Gram_negative bacteria biomass, fungal: bacterial ratios, and Gram_negative: Gram_positive bacterial ratios, respectively.

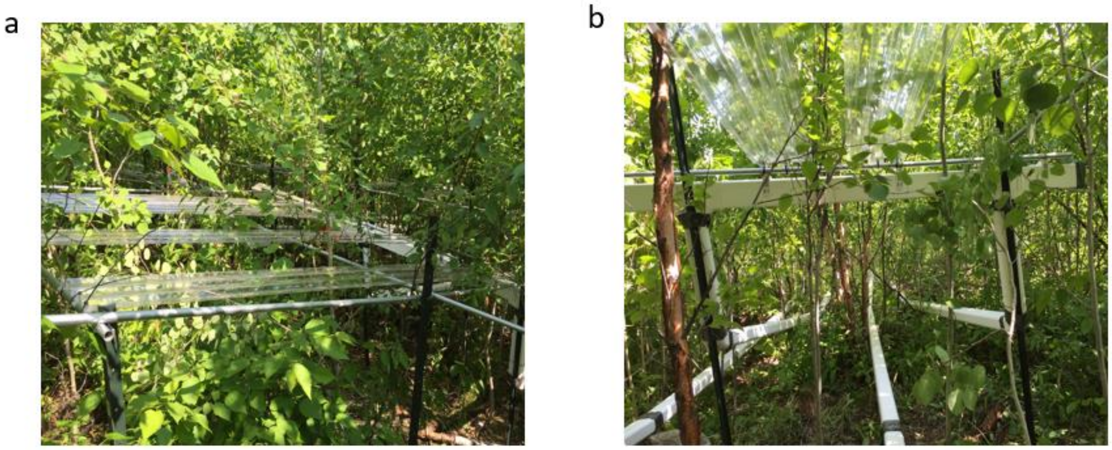

Fig S5-1. Water reduction shelters and water addition pipes employed at the forests.

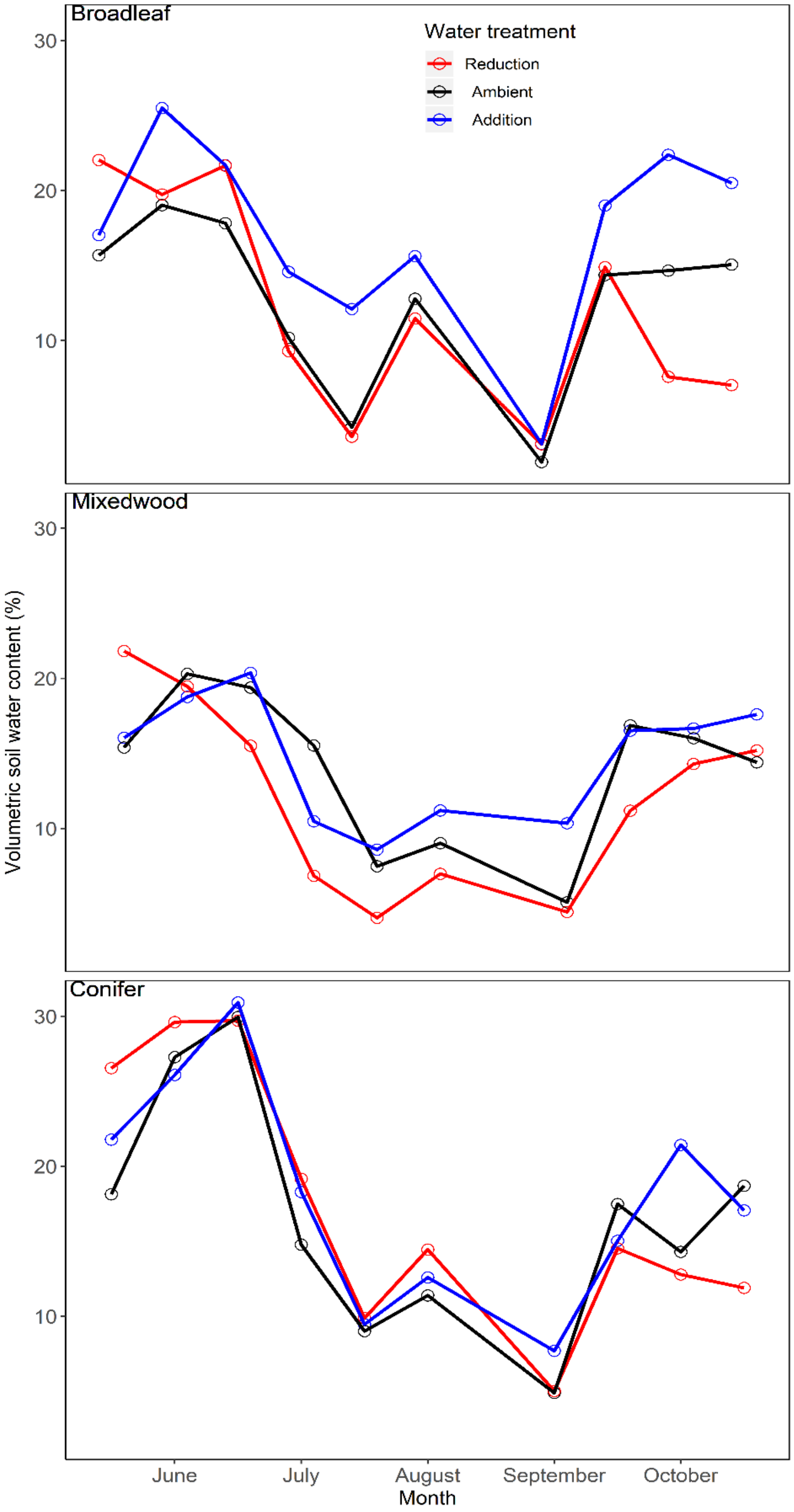

Fig S5-2. Seasonal variations in average soil water content (5 cm depth) during the growing season.

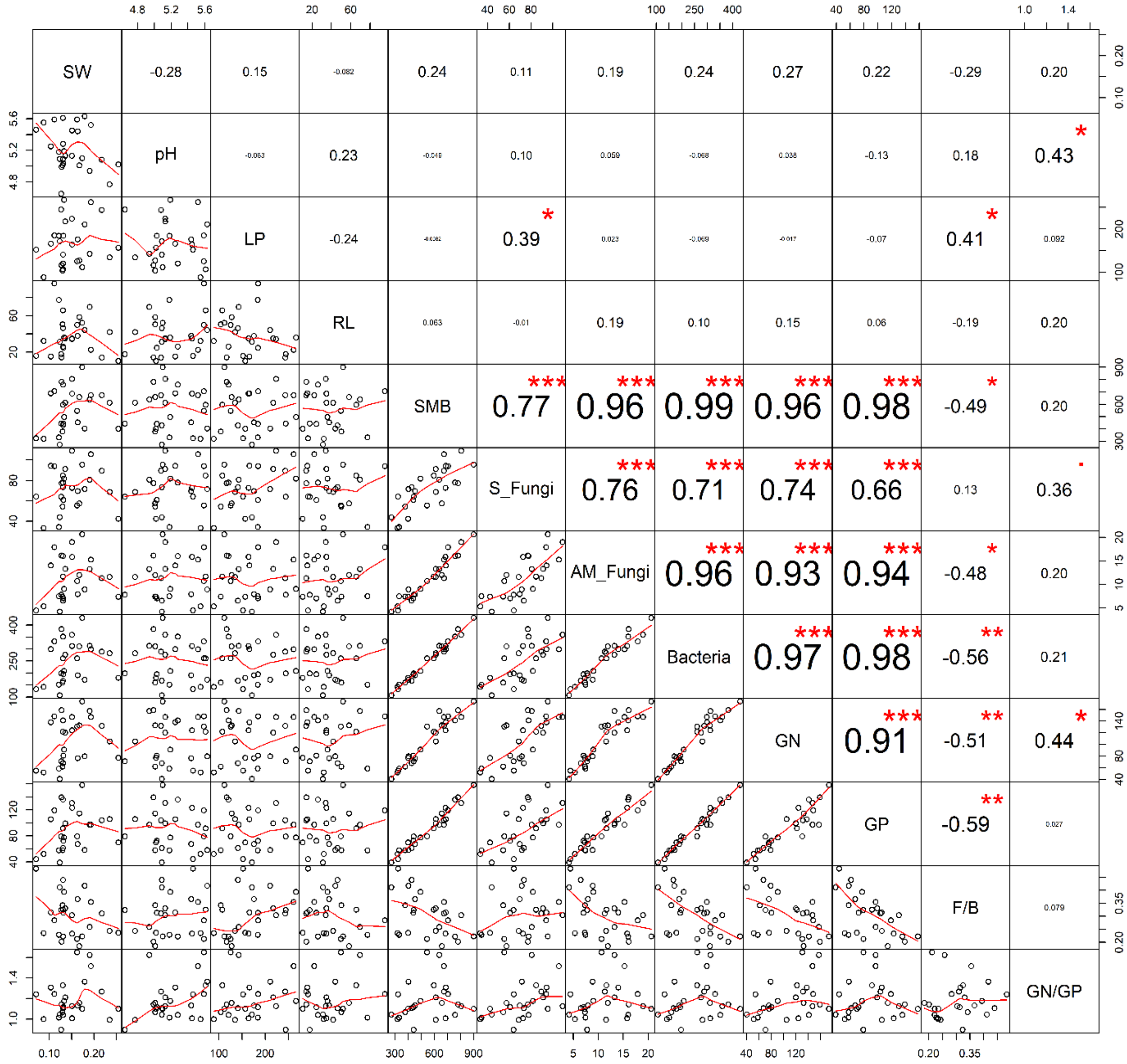

Fig. S5-3 The Pearson correlation between soil water content (SW), litterfall production (LP), soil pH, standing root length (RL), total microbial biomass (SMB), saprotrophic fungal biomass (S_fungi), AM_fungal biomass (AM_fungi), bacterial biomass, Gram-negative bacterial biomass (GN), Gram-positive bacterial biomass (GP), F/B ratio, and GN/GP bacteria ratio. ▪,·*, **, and *** indicate significance at P < 0.1, < 0.05, <0.01, and <0.001, respectively.