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Tree diversity regulates soil respiration through elevated tree growth in a microcosm experiment

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Abstract

Soil respiration is an essential component of carbon (C) cycling in terrestrial ecosystems. Despite increasing awareness of the significance of aboveground-belowground interactions, little is known about tree diversity effects on soil respiration and the underlying mechanisms. Here, we conducted a 105-days microcosm experiment in a climate chamber to test the effects of tree diversity (1, 2, and 4 species) on soil respiration. We expected tree diversity to affect soil respiration through changes in tree growth and surface litter decomposition (indicated by the litter mass loss). Our results show that soil respiration varied among the four focal tree species and increased with tree species richness. Path analysis revealed tree growth as the main mechanism driving soil respiration and explaining the tree diversity effect in this short-term experiment. Our results indicate that tree diversity and particular tree traits regulate C dynamics through balancing C storage (plant productivity) and C release (soil respiration).

Keywords

Aboveground-belowground interactions; biodiversity-ecosystem functioning; carbon dynamics; litter decomposition

Plant diversity enhances many ecosystem functions (Quijas et al., 2010), including plant biomass production (Hector et al., 1999; Reich et al., 2012; Liang et al., 2016) and subsequently plant inputs fueling soil processes through leaf litter, root litter, and rhizodeposits (Eisenhauer et al., 2010; Lange et al., 2015; Zak et al., 2003). As a consequence, previous studies reported positive relationships between plant diversity and soil microbial activity and respiration (Chung et al., 2007; Eisenhauer et al., 2013, 2010; Lange et al., 2015; Zak et al., 2003). Soil respiration is an important component of the C cycle, which is influenced, amongst other biotic and abiotic factors, by the decomposition of plant leaf litter, soil organic matter, and decomposers (Andrews, 2000; Robertson, 2000) with many poorly understood interactions among those factors.

Plant diversity effects on soil respiration may be due to multiple mechanisms. For instance, Dias et al. (2010) reported plant species richness effects on soil respiration through changes in plant productivity. Such effects can be due to elevated root growth and rhizodeposition, which represent important C sources for soil organisms (Eisenhauer and Reich, 2012; Pollierer et al., 2007). In addition, as plant species differ in their biochemical composition, changes in plant diversity likely alter the quality and quantity of leaf litter (Zak et al., 2003) and rhizodeposits (Philippot et al., 2013; Steinauer et al., 2016). As a consequence, an increasing number of studies indicates that mixed litter decomposes faster than single species leaf litter (Gartner and Cardon, 2004; Hättenschwiler et al., 2005).

Thus, tree diversity may affect soil respiration through multiple controls, which need to be considered to get a better mechanistic understanding of how changes in plant diversity can affect this important ecosystem process (Raich and Tufekcioglu, 2000). However, most of the previous studies on this topic were conducted in grasslands, and little is known about the relationship between the tree diversity and soil respiration. Here, we conducted a tree diversity experiment (with richness levels of 1, 2, and 4 species) in a climate chamber to explore how tree diversity affects soil respiration. As explanatory variables, we assessed aboveground tree biomass production as a measure of tree growth and surface litter mass loss as a measure of litter decomposition. We hypothesized (H1) increased tree biomass production with increasing tree diversity due to the complementarity among different species (Gross et al., 2007; Loreau and Hector, 2001; Warren et al., 2009; Liang et al., 2016). Further, we expected (H2) elevated surface litter mass loss with increasing tree diversity. As a consequence, we hypothesized to find (H3) increasing soil respiration with increasing tree diversity due to elevated tree biomass production and litter mass loss.

We set up microcosms consisting of plastic buckets (diameter 33.5 cm, height 31.5 cm) with small holes and a 1 mm mesh at the bottom to allow drainage of water. Each microcosm was filled with 6 kg dry soil (pH 8.1, carbon concentration 4.6%, nitrogen concentration 0.3%, C- to N- ratio 15.7). Soil was taken from the field site of the Jena Experiment (Roscher et al., 2004) in the floodplain of the Saale River at the northern edge of Jena (Thuringia, Germany) in January 2014. Before starting the experiment, soil samples were homogenized, sieved (2 mm) to remove larger roots, animals, and stones, and defaunated by two cycles of drying and rewetting at 55°C. We purchased four tree species from “Thüringen Forst” forestry office at Jena who ordered from a locale nursery garden.: *Fraxinus excelsior* L. (Ash), *Fagus sylvatica* L. (Beech), *Picea abies* L. (Spruce), *Pinus sylvestris* L. (Pine). These tree species were selected because they are functionally dissimilar, but all common to central Europe. Four tree individuals were transplanted into each microcosm, creating three tree diversity treatments (one, two, and four tree species). We aimed at transplanting saplings of similar size, however, different tree species varied in their initial size (Appendix 1) and growth rate. Plant monocultures had four individuals of one species, two species mixtures had two individuals of two species (planted in an alternate pattern), and four species mixtures had one individual per plant species. We placed a litter (10 g) layer on the soil surface to each microcosm with equal proportions of the same tree species as planted at diversity treatments (one tree species with 10 g of the same species’ litter, two tree species with 5 g of litter of each of the two tree species, and four tree species with 2.5 g of litter of

each of the four tree species). The litter was collected at the Bechstedt site of the BIOTREE experiment in central Germany (Scherer-Lorenzen et al., 2007) and dried at 60°C for 48 h.

The microcosms were placed in a temperature-controlled growth chamber at a day/night regime of 16/8 and $16/20 \pm 1^\circ\text{C}$. The microcosms were irrigated four times per week with tap water and the volume was increased from 100 ml to 150 ml over the course of the experiment due to increasing water demands by growing plants. All microcosms received the same amount of water allowing for treatment-induced differences in water availability. For soil respiration, we sealed a part of the surface (between the trees) of each microcosm using 5 ml 1N KOH to absorb soil CO₂ emissions as the measurement of soil respiration (Marhan et al., 2007; Kuzyakov et al., 2009). We exchanged the KOH solution every 7 days during the first month of the experiment, and afterwards we exchanged the KOH solution every 10 days until the end of the experiment to account for different levels of soil respiration. To minimize the difference to the natural conditions and to introduce across-replicate variability to increase reproducibility (Richter et al., 2010), we added 1 to 4 earthworms (*Lumbricus terrestris* L.) to each microcosm.

After 105 days, we destructively harvested the experiment. We carefully collected the remaining surface litter material and removed the soil attached to it to calculate surface litter mass loss. Then, we harvested the shoot material of each tree species separately by cutting shoots at the soil surface. Roots were washed from soil and collected with a 2 mm sieve. Shoot, root, and litter material were dried at 70°C for 1 week. Due to technical complications, we lost a substantial part of the roots samples, which did not allow us to test tree diversity effects on root biomass. However, as root biomass correlated significantly with tree shoot biomass ($R^2 = 0.55$, $P\text{-value} = 0.022$, $n = XX$), we use shoot biomass as proxy for tree growth in the following.

We performed one-way ANOVA to analyze the effects of tree diversity (three levels are one, 2, and 4 species) on tree aboveground biomass, surface litter mass loss, and soil respiration. Similarly, one-way ANOVA was performed to test the effects of tree identity on tree aboveground biomass, surface litter mass loss, and soil respiration. In addition to the ANOVA approach, we used structural equation modeling (SEM) to identify the main effect pathways influencing soil respiration. All analyses were performed using R software (version 3.1.1, The R Foundation for Statistical Computing, Vienna, Austria).

In line with our hypothesis, tree diversity significantly increased tree aboveground biomass ($F = 5.94$; $P\text{-value} = 0.019$), surface litter mass loss ($F = 8.37$; $P\text{-value} < 0.001$), and soil respiration ($F = 10.13$; $P\text{-value} = 0.003$). Tree aboveground biomass, surface litter mass loss, and soil respiration were all significantly higher in the treatment with four tree species than in those with one and two tree species (Fig. 1a-c). Our results also show significant tree identity effects on tree aboveground biomass ($F = 36.00$; $P\text{-value} < 0.001$), surface litter mass loss ($F = 4.75$; $P\text{-value} = 0.015$), and soil respiration ($F = 4.46$; $P\text{-value} = 0.023$). Tree aboveground biomass and total soil respiration were highest in the presence of ash and lowest in the presence of pine (Fig. 2a, 2c). Surface litter mass loss was highest in the presence of ash and lowest in the presence of beech (Fig. 2b).

However, our third hypothesis was only partly confirmed by SEM: the significant tree diversity effect on soil respiration was mainly caused by changes in tree aboveground biomass (reflecting tree growth), but changes in surface litter mass loss did not significantly contribute to elevated soil respiration (Table 1, Fig. 3).

Our results are consistent with previous studies in grassland biodiversity experiments reporting that plant species richness-induced increases in soil respiration are mainly due to elevated plant productivity (Craine et al., 2001; Hooper et al., 2005; Zak et al., 2003). Plant diversity effects on soil respiration likely are due to changes in C inputs to the soil (Janssens et al., 2001; Raich and Tufekcioglu, 2000; Steinauer et al., 2016). It has been indicated that aboveground biomass production is a powerful indicator of total root C allocation (root production and root respiration) (Raich and Nadelhoffer, 1989), and also grassland experiments showed similar increases in aboveground and belowground biomass in response to variations in plant diversity (Ravenek et al., 2014; Reich et al., 2012) and even used aboveground plant biomass as predictors of soil microbial biomass and respiration (Eisenhauer et al., 2010; Milcu et al., 2008).

Despite significant tree diversity effects on surface litter mass loss (Fig. 1b), this did not significantly contribute to soil respiration in our experiment. Elevated litter mass loss is likely, however, to contribute to soil respiration in the long-term, and we might underestimate the relevance of litter mass loss in our short-term experiment. In addition, we controlled the quantity of surface litter in our study (10 g per microcosm), while increased tree growth at high tree diversity (Liang et al., 2016) may increase the amount of surface litter material and soil respiration (Fang et al., 2015). However, previous studies have shown that root respiration plus belowground litter decomposition combined contribute 70%-80% of total soil respiration across a wide range of forests (Nadelhoffer and Raich, 1992; Raich and Nadelhoffer, 1989), supporting the limited contribution of aboveground litter decomposition to soil respiration, whereas other studies reported accumulative effects of surface litter decomposition over several years to contribute almost half of the total respiration (Bowden et al., 1992).

Generally, biomass production of trees reflects C storage, while soil respiration indicates C release from the system. Our results indicate that high tree diversity could fix more C by elevating tree growth (tree biomass) than low tree diversity, but could also release more C by promoting soil respiration. Further, significant identity effects of functionally dissimilar trees on aboveground biomass, litter mass loss, and soil respiration indicate the important role of traits related to tree growth and litter decomposability for the C balance. Indeed, it was demonstrated that plant diversity increases soil carbon storage in a long-term field experiment in grassland despite – or even because of – higher microbial respiration rates (Lange et al., 2015). Thus, C dynamics should be investigated in long-term field experiments as information on species composition, traits, and diversity effects of young tree stands would be very valuable for land managers that aim to maximize C storage.

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Figure 1.

Tree diversity effects (1, 2, and 4 species per microcosm) on (a) tree aboveground biomass (g), (b) litter mass loss (%), and (c) total soil respiration (g/m²). * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$; Means $\pm$ SE are shown. Bars with different letters vary significantly (Tukey's HSD test, $\alpha < 0.05$).

Figure 2.

Tree identity effects of *Fraxinus excelsior* L. (Ash), *Fagus sylvatica* L. (Beech), *Picea abies* L. (Spruce), *Pinus sylvestris* L. (Pine) along the experimental tree diversity gradient (1, 2, and 4 species per microcosm) on (a) aboveground tree biomass, (b) litter mass loss (%), and (c) total soil respiration (g/m²). * = $p < 0.05$; *** = $p < 0.001$; Means $\pm$ SE are shown. Bars with different letters vary significantly (Tukey's HSD test, $\alpha < 0.05$).

Figure 3.

Structural equation model of tree diversity effects on total soil respiration, aboveground tree biomass, and surface litter mass loss. Arrows indicate the direction of effects. Thick line arrows indicate significant (*= $P < 0.05$; ***= $P < 0.001$) and marginally significant ($P < 0.1$; without asterisks) standardized path coefficients, while the thin and dashed line arrows indicate non-significant ($P > 0.1$) standardized path coefficients. Numbers on arrows are standardized path coefficients, which represent the relative magnitude of each relationship.

R^2 values under the boxes represent the amount of variance variables explained by the model. Model fit statistics were $\text{fisher.c} = 5.81$, $\text{df} = 2$, $P\text{-value} = 0.055$.

Table 1
Results of structural equation model.

Variables	Estimate	SE	Z-value	P-value
<i>Soil respiration</i> ← <i>Tree diversity</i>	0.21	0.13	1.62	<i>0.099</i>
Soil respiration ← Aboveground tree biomass	0.59	0.12	4.92	0.000
Soil respiration ← Surface litter decomposition	0.02	0.13	0.16	0.874
Aboveground tree biomass ← Tree diversity	0.33	0.14	2.36	0.019
Surface litter decomposition ← Tree diversity	0.52	0.12	4.33	0.000

Notes: Arrows indicate the direction of effect. Given are the unstandardized path coefficients (estimates), standard error of regression weight (SE), the Z-value (Estimate/SE), and the level of significance for regression weight (*P*). Significant relationships ($P < 0.05$) are shown in bold, marginally significant relationships ($P < 0.1$) are given in italics.