

The spatial distribution of tree-tree interaction effects on soil microbial biomass and respiration

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Abstract

The capacity of forests to sequester carbon in both above- and belowground compartments is a crucial tool to mitigate rising atmospheric carbon concentrations. Belowground carbon storage in forests is strongly linked to soil microbial communities that are the key drivers of soil heterotrophic respiration, organic matter decomposition, and thus nutrient cycling. However, the relationships between tree diversity and soil microbial properties such as biomass and respiration remain unclear with inconsistent findings among studies. It is unknown so far how the spatial configuration and soil depth affects the relationship of tree richness and microbial properties. Here, we studied the spatial distribution of soil microbial properties in the context of a tree diversity experiment by measuring soil microbial biomass and respiration in subtropical forests (BEF-China experiment). We sampled soil cores at two depths at five locations along a spatial transect between the trees in mono- and heterospecific tree pairs of the native deciduous species *Liquidambar formosana* and *Sapindus saponaria*. Our analyses showed decreasing soil microbial biomass and respiration with increasing soil depth and distance from the tree in monospecific tree pairs. We calculated belowground overyielding of soil microbial biomass and respiration - which is a higher microbial biomass or respiration than expected from the monocultures - and analysed the distribution patterns along the transect. We found no general overyielding across all sampling positions and depths. Yet, we encountered a spatial pattern of microbial overyielding with a significant microbial overyielding close to *L. formosana* trees and microbial underyielding close to *S. saponaria* trees. We found similar spatial patterns across microbial properties and depths that only differed in their effect size. Our results highlight the importance of small-scale variations of tree-tree interaction effects on soil microbial communities and functions and are calling for better integration of within-plot variability to understand biodiversity-ecosystem functioning relationships.

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Abstract

The capacity of forests to sequester carbon in both above- and belowground compartments is a crucial tool to mitigate rising atmospheric carbon concentrations. Belowground carbon storage in forests is strongly linked to soil microbial communities that are the key drivers of soil heterotrophic respiration, organic matter decomposition, and thus nutrient cycling. However, the relationships between tree diversity and soil microbial properties such as biomass and respiration remain unclear with inconsistent findings among studies. It is unknown so far how the spatial configuration and soil depth affects the relationship of tree richness and microbial properties. Here, we studied the spatial distribution of soil microbial properties in the context of a tree diversity experiment by measuring soil microbial biomass and respiration in subtropical forests (BEF-China experiment). We sampled soil cores at two depths at five locations along a spatial transect between the trees in mono- and heterospecific tree pairs of the native deciduous species *Liquidambar formosana* and *Sapindus saponaria*. Our analyses showed decreasing soil microbial biomass and respiration with increasing soil depth and distance from the tree in monospecific tree pairs. We calculated belowground overyielding of soil microbial biomass and respiration - which is a higher microbial biomass or respiration than expected from the monocultures - and analysed the distribution patterns along the transect. We found no general overyielding across all sampling positions and depths. Yet, we encountered a spatial pattern of microbial overyielding with a significant microbial overyielding close to *L. formosana* trees and microbial underyielding close to *S. saponaria* trees. We found similar spatial patterns across microbial properties and depths that only differed in their effect size. Our results highlight the importance of small-scale variations of tree-tree interaction effects on soil microbial communities and functions and are calling for better integration of within-plot variability to understand biodiversity-ecosystem functioning relationships.

Key words: belowground overyielding, soil microbial biomass, tree-tree interaction, Biodiversity-Ecosystem Functioning

Introduction

Forest ecosystems are crucial for the planet's health and sustainability by supporting an extensive range of biodiversity and ecosystem services, including carbon storage, primary production, water and nutrient cycling (Bardgett and Wardle 2011; Van Der Heijden, Bardgett, and Van Straalen 2008; Wagg et al. 2014). The potential of carbon storage within a forest depends on the interactions with the environment and the dominant management practices (Erb et al. 2013). Especially carbon source-sink dynamics are significantly influenced by the interactions between soil microbes and understory plants (S. Xu et al. 2020).

Microbial-driven decomposition of organic matter and nutrient cycling is essential for maintaining ecosystem productivity in many different biomes (Delgado-Baquerizo et al. 2016; Gottschall et al. 2019; Gougoulias et al. 2014; Van Der Heijden et al. 2008). Microbes are the primary drivers of belowground carbon storage in forests (Schmidt et al. 2011). They transform organic carbon into stable soil organic matter through processes like aggregation or accumulation of microbial necromass (Buckeridge et al. 2020; Miltner et al. 2012; Wang et al. 2021). Thus, this stabilisation of the forest carbon pool provides tools to mitigate climate change (Bastin et al. 2019; Lewis et al. 2019). Understanding the drivers of belowground carbon storage and its relationship with biodiversity is crucial for effective forest management and carbon sequestration (Messier et al. 2022). In particular, soil microbial biomass and respiration could serve as a proxy for nutrient cycling and soil organic matter turnover (Crowther et al. 2019) and were shown to be correlated with soil carbon sequestration (Beugnon, Bu, et al. 2023; Lange et al. 2015). Therefore, these soil microbial properties together can provide important information on multiple soil ecosystem functions (Eisenhauer et al. 2018).

Microbial properties generally vary between soil layers due to lower resource availability (e.g. nutrients and oxygen) in the deeper soil layers leading to reduced microbial diversity and biomass (Goebes et al. 2019; Jobbágy and Jackson, 2000). However, rhizodeposition can increase microbial activity at deeper soil layers (Lopez et al. 2020), potentially leading to different drivers of microbial activity and biomass across soil layers (Blume et al. 2002; Loepmann et al. 2016).

Tree diversity was shown to enhance soil microbial diversity, abundance, and functioning, leading to improved nutrient cycling, organic matter decomposition, and carbon storage (Beugnon et al. 2021; Gamfeldt et al. 2013; Gottschall et al. 2019; Li et al. 2019; Pei et al. 2016); primarily due to higher diversity of substrates from litterfall and rhizodeposition as well as possible increased belowground interactions with tree species-specific soil microbes (Beugnon, Eisenhauer, et al. 2023; Huang et al. 2017). However, other studies showed that the tree diversity impact on soil microbial functions is non-significant, varies across functional groups such as bacteria and fungi (Cesarz et al. 2022; Rivest, Whalen, and Rivest 2019), or is less important than tree identity effects or abiotic conditions (Cesarz et al. 2022; Tedersoo et al. 2016; Yamamura, Schwendenmann, and Lear 2013). There are now empirical pieces of evidence that spatio-temporal dynamics along tree diversity gradients can drive soil microbial functions (Gottschall et al. 2022), which vary with the tree neighbourhood (Trogisch et al. 2021).

Forest soils' spatial structure and processes can become highly heterogeneous due to the spatial distribution of roots and root inputs. Soil respiration, for instance, was shown to be higher at the base of mountain birch trees compared to 150 cm away, indicating 'hot-spots' of soil microbial activity close to the tree (Parker et al. 2017). This spatial distribution of soil functions is crucial when considering interactions between trees or with the understory vegetation (Kuzuyakov and Blagodatskaya 2015; Mao et al. 2015). Microbial communities were found to be more active and diverse when surrounded by neighbouring trees than close to an isolated tree (Habiyaemye et al. 2020). Especially, the effects of tree-tree interactions are expected to be maximised in the interaction zone between the trees (Trogisch et al. 2021). This highlights the role of the neighbouring trees on the functioning of soil microbes in forest ecosystems, especially in the context of highly diverse forests. However, information on the spatial distribution of soil processes (e.g. soil respiration) at finer spatial scales is missing (Friggens et al. 2020).

In this study, we aimed to understand the effects of tree-tree interactions on soil microbial biomass and respiration and their spatial distribution. We set up small-scale transects in tree neighbourhoods in a Chinese subtropical forest experiment (BEF-China), where we tested the following hypotheses: **(H1)** In monospecific tree pairs, we expect decreasing microbial biomass and respiration with increasing distance from the trees and with increasing soil depth, due to lower resource availability in greater distances. **(H2)** Due to higher complementarity as well as quantity and diversity of resource inputs between hetero-specific tree pairs, we expect overall higher microbial biomass and respiration than in mono-specific pairs. **(H3)** We expect the interaction between trees to be maximised in the interaction zone between the two trees; thus, soil microbial biomass and respiration are highest in the topsoil in the middle of the transect between two adjacent trees.

Material and methods

Study site

The study site was located in south-east China near the city Xingangshan, Jiangxi province (29.12°N, 117.90°E) and is part of the BEF-China experiment (Bruehlheide et al. 2014). The experiment was planted in 2009, after a clear-cut of the previous commercial plantation of *Pinus massoniana* and *Cunninghamia lanceolata*, and it covers an area of 26.7 ha, ranging in altitude from 105 to 275 m. The region has a subtropical climate, with warm, humid summers and cool, dry winters. The local mean annual temperature is 16.7°C with an annual precipitation of 1,821 mm (Yang et al. 2013). The soils of this region are Cambisols and Cambisol derivatives, with Regosols on ridges and crests (Geißler et al. 2012; Scholten et al. 2017). The natural vegetation is characterised by species-rich, broad-leaved, subtropical forests dominated by evergreen and deciduous species such as *Castanopsis eyrei*, *Cyclobalanopsis glauca*, *Daphniphyllum oldhamii*, and *Lithocarpus glaber* (Bruehlheide et al. 2014; 2011).

Study design and field sampling

We selected two plots with the same species mixture of the deciduous tree species *Liquidambar formosana* and *Sapindus saponaria*. The selected tree species have significant and dissimilar effects on soil microbial properties (Beugnon et al. 2021). In each plot, we selected five replicates of both monospecific pairs (*L. formosana* – *L. formosana*, *S. saponaria* – *S. saponaria*) and of the heterospecific pair (*L. formosana* – *S. saponaria*). The litter layer was removed prior to sampling. To measure the spatial distribution of soil microbial biomass and respiration, we took five soil-cores on the transect line between each pair using 5 cm diameter soil-cores. To test for the effect of soil depth on soil microbial biomass and respiration, each soil-core was split into the depths of 0-5 cm and 5-10 cm and sieved through a 2 mm mesh. The soil samples were stored at -20°C. Altogether, 300 soil samples were collected from two plots, three combinations of trees replicated five times, five positions, and two depths (Fig. 1). Additionally, the tree diameter at breast height (DBH) was measured for each tree pair to calculate tree biomass, following (Beugnon, Bu, et al. 2023).

Soil microbial biomass and respiration measurements

We measured soil microbial biomass (Cmic) and respiration using 5 g of fresh soil on an automated O₂-micro-compensation apparatus (Scheu 1992). Soil microbial respiration was measured first, as the oxygen consumption per hour per dry weight of soil in μl (respiration given in $\mu\text{l O}_2 \text{ g}^{-1} \text{ dry weight h}^{-1}$). This reflects the active part of the soil microbial community at the sampling time. Afterwards, the microbial biomass (given in μg microbial carbon g^{-1} dry weight) was measured by adding glucose (8 mg per gram of dry soil) to the samples (substrate-induced respiration [SIR] method). This measurement represents the total metabolically active biomass of soil microorganisms of the sample.

Statistical analysis

A description of all the variables used in this study can be found in Supplementary Table S1. All data handling and statistical calculations were performed using the R statistical software version 4.2.2 (www.r-project.org), and R-scripts are provided on (<https://github.com/henriettechristel/Soil-microbes.-Tree-Interaction.git>), model fit and statistical assumptions can be found in Suppl. S2.

Spatial distribution of microbial biomass and respiration (H1)

To test the effects of distance to the closest tree and depth on the soil microbial biomass and respiration, we used linear mixed-effects models and normal distribution assumptions that included plot as a random effect, and distance and depth as fixed effects. The model was fitted on monospecific pairs and was used to predict the soil microbial properties over a distance to the closest tree from 0 to 90 cm and a depth from 0 to 10 cm (Suppl. S2).

Belowground overyielding between heterospecific tree pairs (H2) Belowground overyielding of soil microbial biomass and respiration was calculated as the difference between observed soil microbial properties between a heterospecific pair and what would be expected based on the weighted means of the monospecific pairs for a given position between the trees ($\text{overyielding}_{(\text{position} = i, \text{depth} = j)} = \text{observed}_{(i, j)} - ((\text{expected}_{L. formosana}_{(i, j)} + \text{expected}_{S. saponaria}_{(i, j)}) / 2)$), where "i" is the position between the trees and "j" is the depth. Positive results indicate soil microbial properties in mixed pairs are overyielding (i.e., producing more biomass or respiration than expected based on monospecific pairs), and negative results indicate soil microbial properties in mixed pairs are underyielding (i.e., producing less biomass or respiration than expected based on monospecific pairs). The expected values were predicted from the model fits from H1. We used belowground overyielding as a response variable to test for the effect of the heterospecific pair on the aggregated soil samples, as well as for the effect of depth using a linear mixed-effects model with plot as random effect and pair as fixed effect. To determine differences between soil depths, we used a Tukey HSD test based on an analysis of variance (ANOVA type 1).

Spatial distribution of belowground overyielding (H3) To test the effects of distance to the tree species and depth on the belowground overyielding of soil microbial biomass and respiration, we used linear mixed-effects models, which included plot as a random effect and distance in centimeters from the trees and depth as fixed

effects (Suppl. S2). We fixed the positions of the trees to *L. formosana* being tree 1 and *S. saponaria* being tree 2 in a mixed pair. Like this, the positioning of the trees was fixed within the data and could be analysed in terms of a spatial gradient.

All linear mixed-effect models were fitted, using the "lmer" function of the R package *lme4* (Bates et al. 2015). To define the quality of the model fits of all used linear mixed-effects models, the "check_model" function of the R package *performance* (Lüdtke et al. 2021) was used to investigate various model assumptions, such as normality of residuals, normality of random effects, linear relationship, homogeneity of variance, and multicollinearity (Briggs and Cheek 1986).

Results

The analyses showed on average a high variability in soil microbial biomass (mean \pm SD = 381.48 $\mu\text{g} \pm$ 137.02 $\mu\text{g Cmic g}^{-1}$ dry weight) and soil basal respiration ($1.77 \pm 0.93 \mu\text{l O}_2 \text{ g}^{-1}$ dry weight h^{-1}) among the samples from the investigated plots.

Spatial distribution of soil microbial biomass and respiration (H1)

Soil microbial biomass decreased significantly with increasing distance to the tree (estimate \pm SE = -1.60 ± 0.57 , $p = 0.006$) and with soil depth (-44.96 ± 8.20 , $p < 0.001$, Fig. 2). The interaction of distance and depth was not significant ($p = 0.064$). Likewise, soil microbial respiration decreased with increasing depth (-0.14 ± 0.06 , $p = 0.01$), but distance to the tree and the interaction of distance and depth had no significant effects ($p = 0.08$, $p = 0.315$, respectively, Fig. 2).

Spatial distribution of belowground overyielding (H2 - H3)

Our analyses showed no general belowground overyielding for microbial biomass or respiration between the mixed tree species pairs (Fig. 3A, D). An increasing depth reduced soil microbial biomass overyielding (-150.67 ± 42.17 , $p < 0.001$) but increased microbial respiration overyielding (0.71 ± 0.21 , $p = 0.001$, Fig. 3B and E, respectively).

We studied the spatial distribution of microbial properties overyielding by testing for the interacting effects of increasing distance to *L. formosana* (tree 1, i.e., closeness to tree 2) and depth. We found that an increased distance (from *L. formosana*) had a significant negative effect on the microbial biomass overyielding (-452.51 ± 34.90 , $p < 0.001$). The analysis showed overyielding close to *L. formosana* (i.e. tree 1), turning into underyielding close to *S. saponaria* (i.e. tree 2, Fig. 3C). This pattern was even stronger in the shallower soil (0-5 cm) compared to the deeper soil layer (5-10 cm), as indicated by a significant interaction effect between distance and depth (30.01 ± 10.07 , $p = 0.004$). We found similar results for the overyielding of microbial respiration: increased distance to *L. formosana* (tree 1) decreased the microbial respiration overyielding significantly (-0.77 ± 0.19 , $p < 0.001$) with overyielding close to *L. formosana*, and underyielding close to *S. saponaria* (Fig. 3F). Contrary to microbial biomass, this pattern was stronger in the deeper soil compared to the shallower soil layer (interaction estimate \pm SE = -0.38 ± 0.05 , $p < 0.001$).

Discussion

In this study, we tested the effect of tree-tree interactions and the spatial distribution of microbial biomass and respiration in subtropical forest soils. We did not find any significant tree-tree interaction effects on the aggregated soil samples at the species pair level. However, there were substantial variations in tree-tree interaction effects at the small spatial scale. In fact, effects on both soil microbial properties were spatially distributed, ranging from overyielding near one tree species to underyielding close to the other tree species.

Spatial distribution of soil microbial biomass and respiration (H1)

Soil microbial biomass decreased with increasing distance from the trees in monospecific tree pairs. This could be explained on the one hand by higher water availability due to stemflow near the tree base, which can leach and transport nutrients and microorganisms from the canopy layer to the soils (Bittar et al. 2018).

Soil moisture was shown to be important in many studies before (Cesarz et al. 2022; Schimel 2018; Serna-Chavez, Fierer, and van Bodegom 2013). High levels of soil moisture can increase soil enzyme activities, fluxes of soil nutrients, and oxygen availability (Brockett, Prescott, and Grayston 2012; Stark and Firestone 1995), and higher soil humidity can furthermore buffer possible negative changes in soil pH, suggesting it to be a key driver of soil microbial biomass (Cesarz et al. 2022). On the other hand it could also be explained by a higher rhizodeposition closer to the trees (Parker et al. 2017). Our findings would suggest the importance of forest density in modulating soil functioning.

As expected, we also found a negative effect of soil depth on both microbial properties. This is in line with previous findings where a lower amount of carbon and nutrients was found in deeper soil layers, as the main decomposition happens in the leaf litter cover and top soil layers (Goebes et al. 2019; Jobbágy and Jackson, n.d.; Prescott and Grayston 2013). Additionally, deeper soil layers often have a decreased amount of oxygen, soil water content, and contain less plant root biomass (Engelhardt et al. 2018; Fall et al. 2012; Serna-Chavez, Fierer, and van Bodegom 2013). Thus, the present results at the small scale are in line with previous findings at the larger scales, where soil organic carbon decreased with increasing soil depth and distance to trees (Rabearison et al. 2023). These similar results suggest that understanding interaction effects at small scales have the potential to be upscaled.

Spatial distribution of belowground overyielding (H2 - H3)

Our study showed no overyielding across all soil core positions and depth layers. However, we found significant differences between the soil layers for both soil microbial functions. Microbial biomass showed, on average, higher overyielding in the shallower soil (0-5 cm), whereas microbial respiration showed higher overyielding in the deeper soil (5-10 cm). The BEF-China experimental Site A was established in 2009 after a clear-cut of the previous plantation (Yang et al. 2013). Plant diversity effects on soil organic matter have been shown to become stronger in the topsoil layer over time (Lange et al. 2023). These findings from experimental grasslands could suggest that the soil microbial biomass in forests is strongly related to the organic matter content in the topsoil layer (Beugnon, Eisenhauer, et al. 2023) and that positive plant diversity effects could get stronger with stand age (Huang et al. 2018; Perles-Garcia et al. 2021). The increased microbial respiration in the deeper soils could suggest increased carbon sequestration by adding soil microbial necromass to the carbon pool (Buckeridge et al. 2020; Schmidt et al. 2011).

We expected heterospecific tree-tree interactions effects to be maximised in the middle between the planted trees. Contrary to our hypothesis (H3), we found an overyielding of soil microbial biomass and respiration close to *L. formosana* and underyielding close to *S. saponaria*, showing that microbes in mixed pairs perform better than expected close to *L. formosana* but less well than expected close to *S. saponaria*. The gradient from over- to underyielding of microbial respiration was less pronounced in the shallower soil than in the deeper soil. This could indicate that the presence of *S. saponaria* had a positive effect on microbes close to *L. formosana* and it is stronger in the deeper soil layer (5-10 cm), possibly through fine root exudates (Zheng, Wei, and Zhang 2017). Microbial respiration was less affected from the mixture than microbial biomass in the topsoil layer (0-5 cm). It was shown that the balsam of *L. formosana* contains acidic compounds, which were reported to be inhibitory for fungi (Chien et al. 2013). These could also be present in the leaf litter or root exudates (Öztürk et al. 2008) and inhibit microbial respiration more than microbial biomass. Together with a spatial distribution of litter in the heterospecific pairs (Beugnon, Eisenhauer, et al. 2023), this might lead to a small-scale change of soil pH. Soil pH was found to be a strong driver of microbial growth (Fierer and Jackson 2006), and additional pH measurements should be performed in future studies to better understand the opposing species identity effects of *S. saponaria* and *L. formosana*. It was shown that soil fungi and bacteria react differently to changes in soil pH: bacterial growth decreased with a more acidic pH, whereas fungal growth was shown to increase (Rousk, Brookes, and Bååth 2009). This might also explain the significant negative effect of *L. formosana* on microbial respiration in this experiment (Suppl. S2: Fig. S2). To better understand distribution patterns of microbial properties, belowground tree traits (e.g. specific root length, root diameter) should be taken into account. Recent studies could link them to carbon exudation and fine root density (Bergmann et al. 2020; Sun et al. 2021), as well as soil organic matter decomposition

(Adamczyk et al. 2019).

The positive tree-tree interaction effect of the heterospecific tree pair on soil microbial biomass and respiration shows that neighbourhood effects are acting at small spatial scales, which could explain the inconsistencies of BEF relationships reported in previous forest studies (Beugnon et al. 2021; Cesarz et al. 2022; Li et al. 2019; Pei et al. 2016). Our results stress the need to standardize sampling methods by considering small-scale interactions to understand the mechanisms behind tree-soil interactions. In addition, measurements of soil microbial properties across a wider range of species transects are now needed to better understand tree-tree interactions in space and their biological drivers.

Conclusion

In the present study, we were able to show in a subtropical tree diversity experiment (BEF-China) that soil microbial biomass and respiration show a fine spatial pattern in the tree-tree interaction zone, both vertically and horizontally. Whereas aggregated soil samples were not affected by tree-tree interactions, tree-tree interactions ranged from overyielding close to *Liquidambar formosana* to underyielding close to *Sapindus saponaria*. Our findings suggest that tree-tree interactions are driving soil functioning when zooming to the appropriate spatial scale. Therefore, in order to understand relationships between trees and soil processes, future research should focus on fine-scale spatial variability (Eisenhauer et al. 2023).

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Conflict of Interest

The authors declare no competing interests.

Author contributions

The authors contributed to the manuscript by (1) funding (NE, SC), (2) field sampling (HC, RB), (3) laboratory measurements (HC), (4) project conceptual framework (HC, RB, SC, NE), (5) statistical analyses (HC, RB, GH), (6) manuscript framing (HC, RB, NE, SC), (7) manuscript writing and revisions (HC, RB, NE, SC, GH, HB).

Data availability statement

All data and code are published through a public repository (<https://github.com/henriettechristel/Soil-microbes-Tree-Interaction.git>). Respective DOIs will be added once the paper is accepted.

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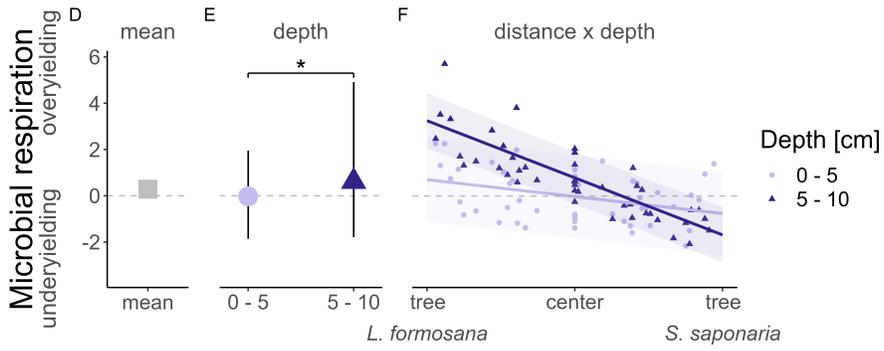
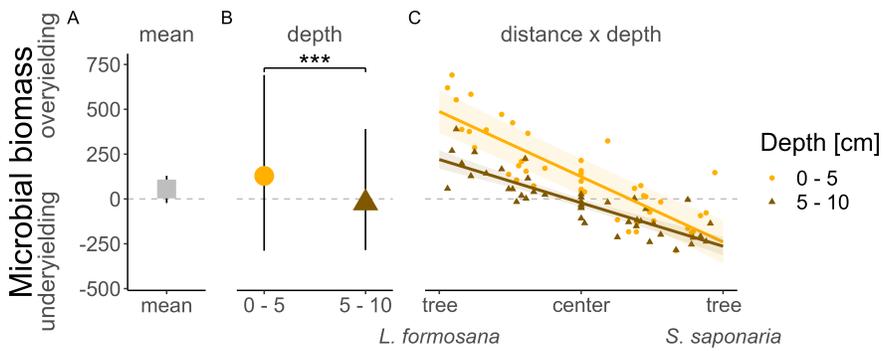
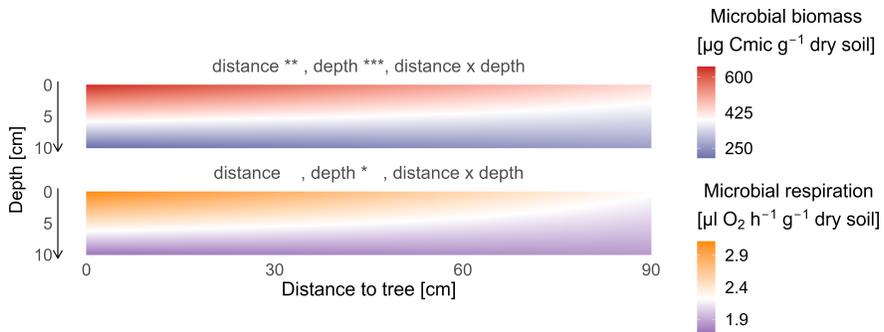
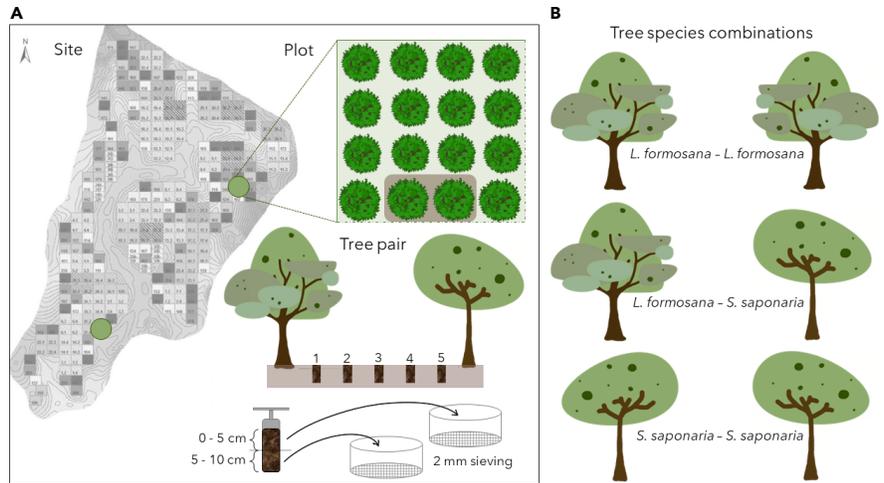
Figure captions

Figure 1: **Sampling Design.** Overview of the BEF-China experimental Site A (panel A) with the two sampled plots (green) representing two-species mixture plots. Plot tree planting grid pattern with a marked tree pair, here only a small section of the 20x20 trees plot was drawn. Positions of the five soil cores between the tree pair with the in-core division of the depths. Tree species combinations are shown in panel B.

Figure 2: **Distance to tree and depth effects on microbial biomass (top) and respiration (bottom) in monospecific tree pairs.** Effects are predicted from the model ($soil\ properties \sim depth * distance\ to\ tree$) with plot as random effect. Distance to tree reports the distance to the closest tree from monospecific tree pairs. Microbial biomass coloured blue (low) to red (high), microbial respiration coloured purple (low) to orange (high). The significance levels were standardised across the panels ($p < 0.05$: *, $p < 0.01$: **, $p < 0.001$: ***).

Figure 3: **Distance to tree and depth effects on microbial biomass (top) and respiration (bottom) overyielding in heterospecific tree pairs.** The mean value (grey square) of overyielding for microbial biomass (A) and respiration (D) across all depths and positions, for each depth (microbial biomass: B, yellow circle for 0-5 cm; brown triangle for 5-10 cm, and microbial respiration: E, light blue circle for 0-5 cm; dark blue triangle for 5-10 cm), and for each sampling point by depth (microbial biomass: C and respiration: F). Confidence intervals were predicted from models using the “ggpredict” function of the R package *ggeffects*. The significance levels were standardised across the panels ($p < 0.05$: *, $p < 0.01$: **, $p < 0.001$: ***).





Supplementary Material 1

The spatial distribution of tree-tree interaction effects on soil microbial biomass and respiration

Henriette Christel, Helge Bruelheide, Simone Cesarz, Nico Eisenhauer, Georg J. A. Hähn and Rémy Beugnon

Table S1: Description of variables

Variables	Code	Unit	Calculation
<i>Tree variables</i>			
Plot	plot	none	Treatment
Diameter at breast height	dbh	m	Measured
Basal Area	ba	m ²	$ba = \frac{(dbh)^2}{4\pi}$
Distance to closest tree	close_distance	cm	Measured
Diameter of closest tree	close_dbh	cm	Measured
Biomass of closest tree	close_biomass	kg _{carbon}	Estimated from ba
Closest tree species	close_species	none	Measured
Distance to tree 1	distance_T_1	cm	Measured
Distance to tree 2	distance_T_2	cm	Measured
Distance to each tree with soil core 3 as the center point	dist.T1.center	cm	Measured
Distance to closest <i>L. formosana</i>	dist.liqu	cm	Measured
Distance to closest <i>S. mukorossi</i>	dist.sapi	cm	Measured
Tree species combination of the tree pair	species_mix	none	Measured
<i>Soil microbial community</i>			
Soil microbial biomass	cmic	µg C _{microbial} g ⁻¹ dry weight	Measured
Soil microbial biomass overyielding	cmic_over		Calculated from observed cmic
Soil microbial respiration	bas_res	µl O ₂ g ⁻¹ dry weight h ⁻¹	Measured
Soil microbial respiration overyielding	bas_over		Calculated from observed bas_res
<i>Environmental variables</i>			
Soil depth	depth	cm	Measured
Root biomass	root_biomass	g m ⁻³	Measured from soil cores
Distance to closest understory plant	plant_distance	cm	Measured
Soil water content	rh	%	Measured

Supplementary Material 2

The spatial distribution of tree-tree interaction effects on soil microbial biomass and respiration

Henriette Christel, Helge Bruelheide, Simone Cesarz, Nico Eisenhauer,
Georg J. A. Hähn, and Remy Beugnon

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Figure S1: Correlation matrix

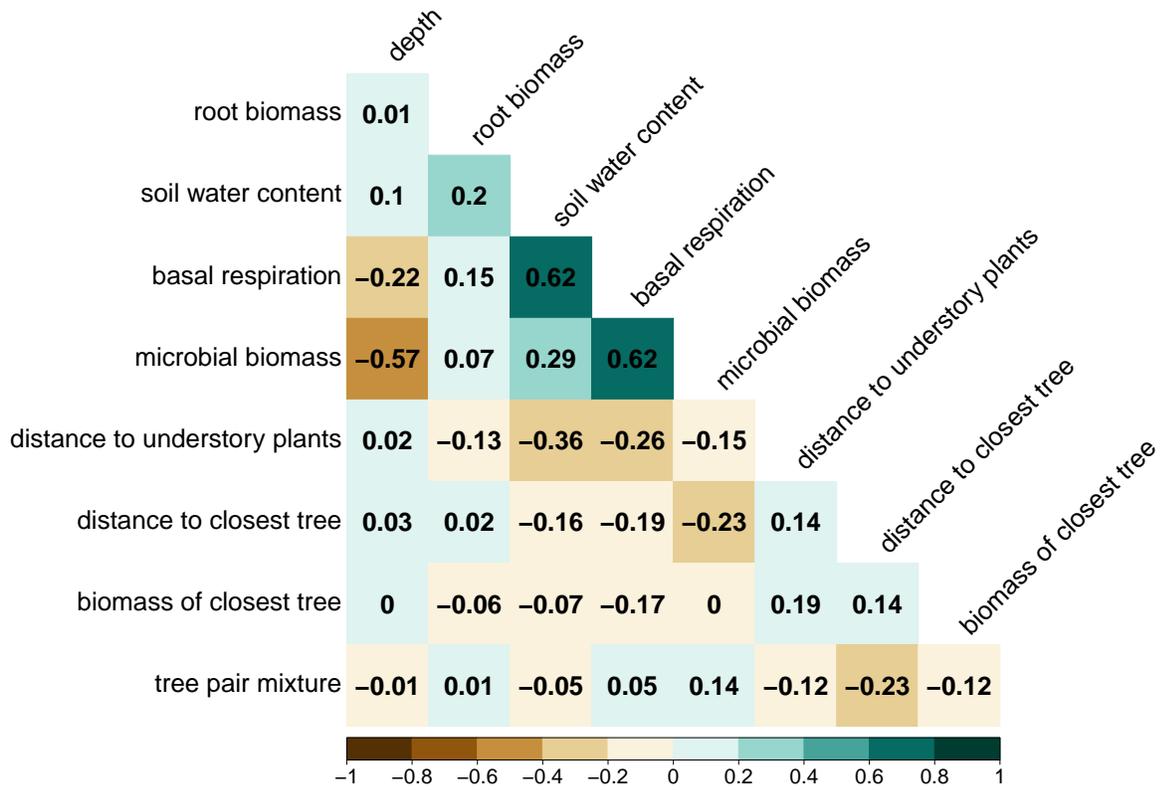


Figure S1: A correlation matrix of the variables used. Brown colour indicates negative correlation, green colour indicates positive correlation.

Section S2: Microbial respiration (H1)

Model summary

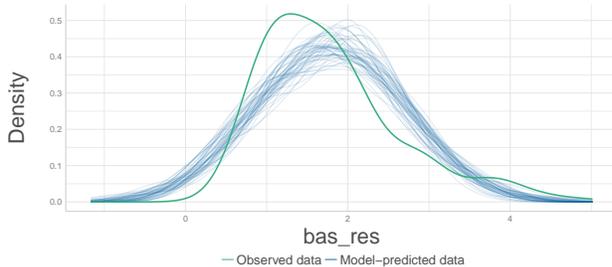
`lmer(formula = bas_res ~ close_distance * depth + (1|plot), data = df)`

	Estimate	Std. Error	t-value	p-value
(Intercept)	2.3262195	0.4764510	4.882389	0.0767479
close_distance	-0.0068739	0.0039270	-1.750412	0.0817317
depth	-0.1427127	0.0563661	-2.531891	0.0121907
close_distance:depth	0.0011358	0.0011281	1.006858	0.3153398

Model fit

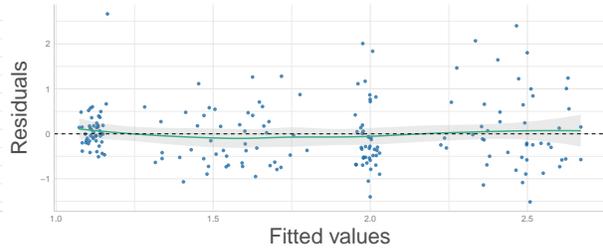
Posterior Predictive Check

Model-predicted lines should resemble observed data line



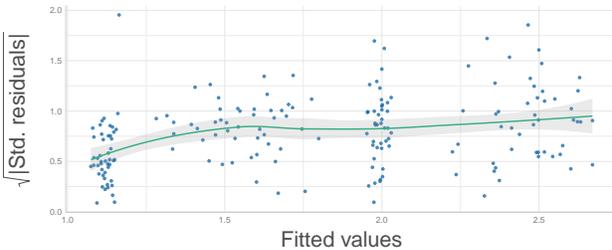
Linearity

Reference line should be flat and horizontal



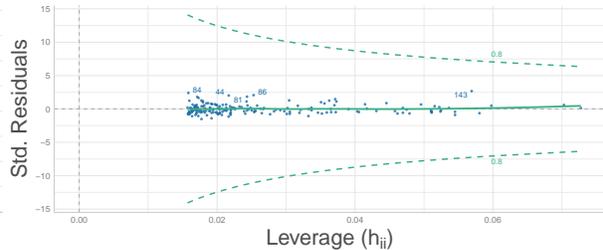
Homogeneity of Variance

Reference line should be flat and horizontal



Influential Observations

Points should be inside the contour lines



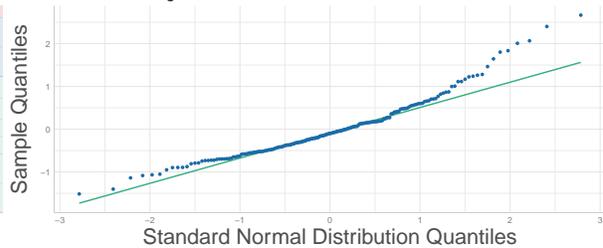
Collinearity

High collinearity (VIF) may inflate parameter uncertainty



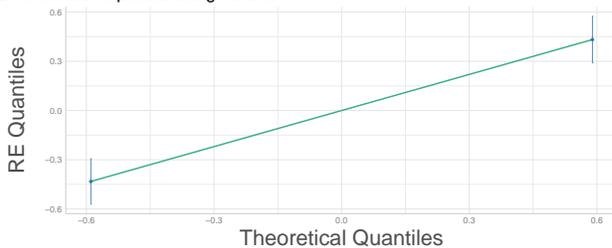
Normality of Residuals

Dots should fall along the line



Normality of Random Effects (plot)

Dots should be plotted along the line



Section S3: Microbial biomass (H1)

Model summary

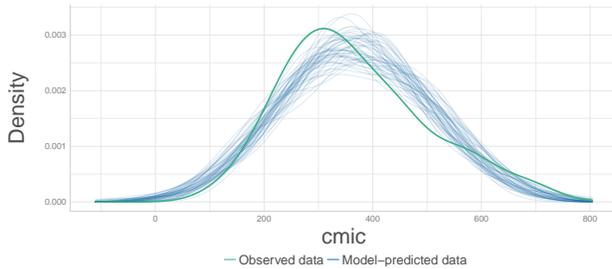
`lmer(formula = cmic ~ close_distance * depth + (1|plot), data = df)`

	Estimate	Std. Error	t-value	p-value
(Intercept)	515.8788176	36.9323184	13.968222	0.0000562
close_distance	-1.5954273	0.5709665	-2.794257	0.0057580
depth	-44.9601154	8.1995370	-5.483250	0.0000001
close_distance:depth	0.3061849	0.1640985	1.865861	0.0636699

Model fit

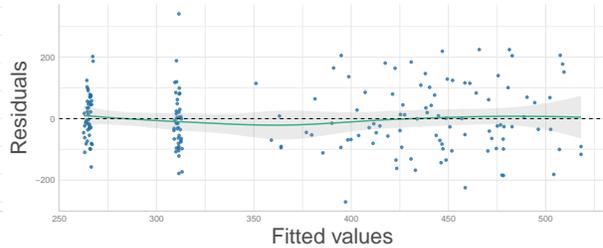
Posterior Predictive Check

Model-predicted lines should resemble observed data line



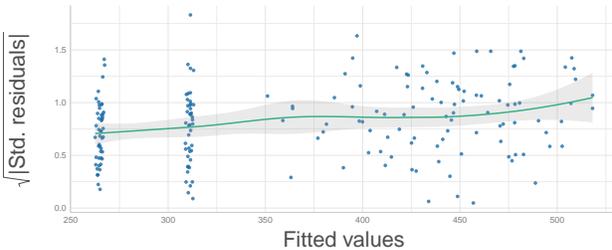
Linearity

Reference line should be flat and horizontal



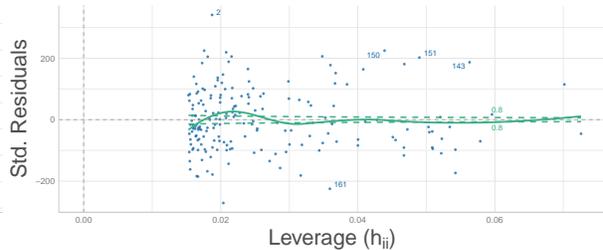
Homogeneity of Variance

Reference line should be flat and horizontal



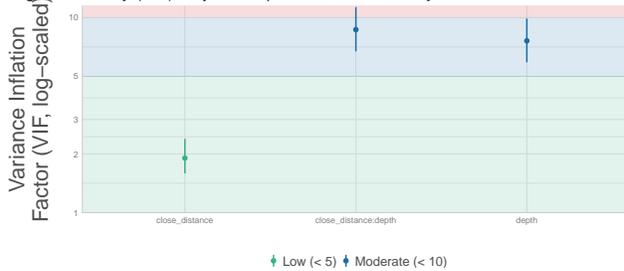
Influential Observations

Points should be inside the contour lines



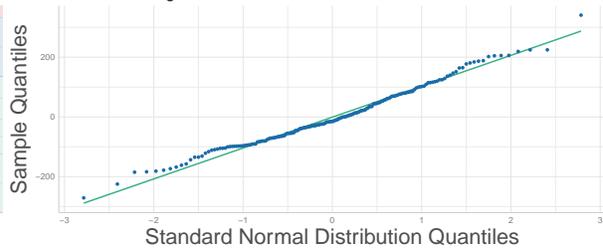
Collinearity

High collinearity (VIF) may inflate parameter uncertainty



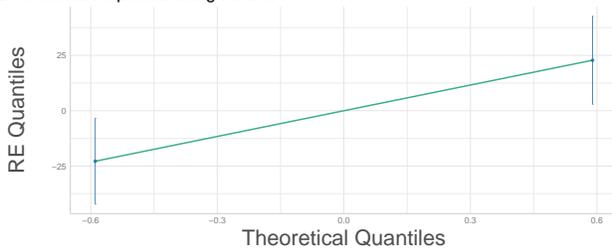
Normality of Residuals

Dots should fall along the line



Normality of Random Effects (plot)

Dots should be plotted along the line



Section S4: Overyielding microbial respiration (H2)

Model summary

`lmer(bas_over ~ depth + (1|plot), data = df)`

	Estimate	Std. Error	t-value	p-value
(Intercept)	-0.0142504	0.6264277	-0.0227487	0.9853692
depth	0.1416237	0.0428361	3.3061788	0.0013700

Figure 3 Tukey test - depth

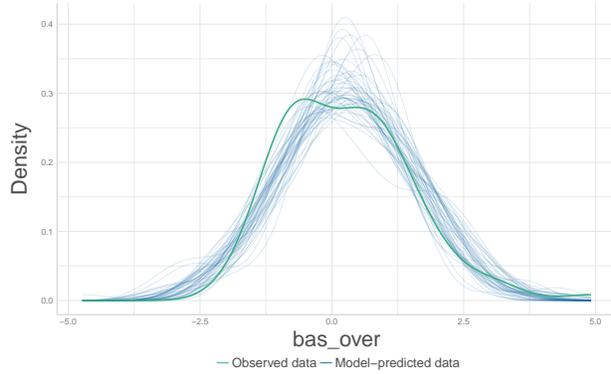
	Sum Sq.	Mean Sq.	f-value	p-value
depth %>% as.factor	9.283579	9.283579	6.564345	0.0120856
Residuals	125.867631	1.414243	NA	NA

	Diff	Lower	Upper	p-value
5-0	0.6391505	0.1434713	1.13483	0.0120856

Model fit

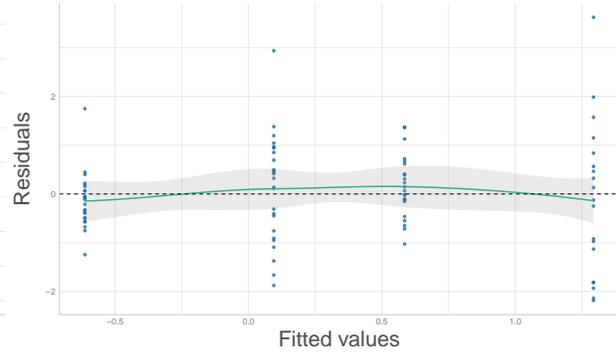
Posterior Predictive Check

Model-predicted lines should resemble observed data line



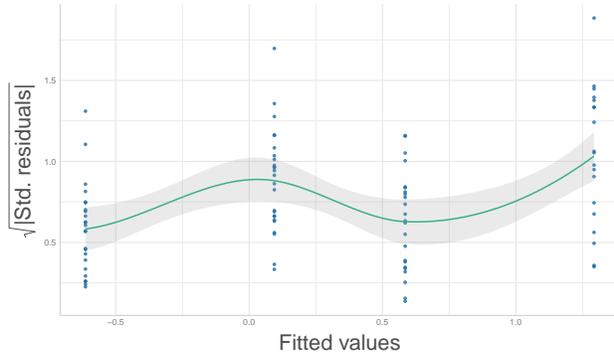
Linearity

Reference line should be flat and horizontal



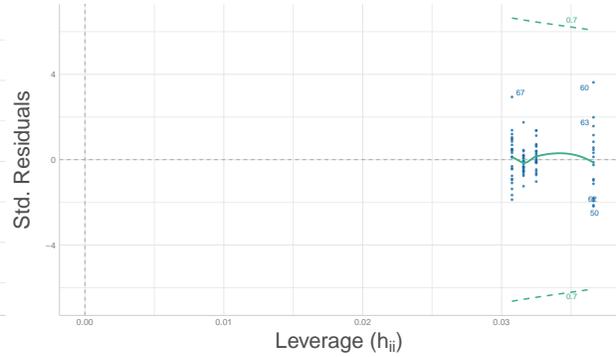
Homogeneity of Variance

Reference line should be flat and horizontal



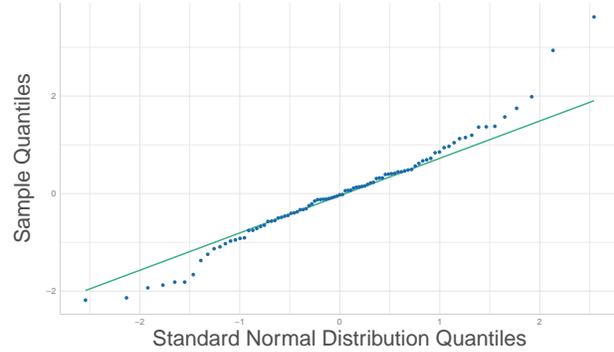
Influential Observations

Points should be inside the contour lines



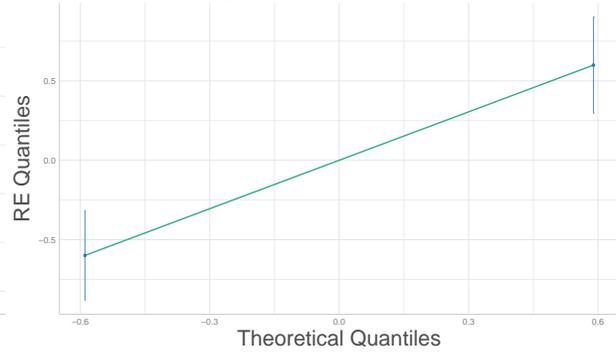
Normality of Residuals

Dots should fall along the line



Normality of Random Effects (plot)

Dots should be plotted along the line



Section S5: Overyielding microbial biomass (H2)

Model summary

`lmer(cmic_over ~ depth + (1|plot), data = df)`

	Estimate	Std. Error	t-value	p-value
(Intercept)	128.57173	29.616232	4.341259	0.0161693
depth	-30.13394	8.433534	-3.573110	0.0005736

Figure 3 Tukey test - depth

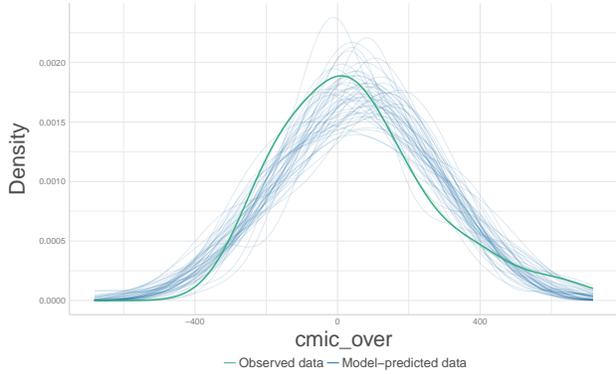
	Sum Sq.	Mean Sq.	f-value	p-value
depth %>% as.factor	516533.2	516533.20	12.77892	0.000569
Residuals	3597445.0	40420.73	NA	NA

	Diff	Lower	Upper	p-value
5-0	-150.7629	-234.5623	-66.96356	0.000569

Model fit

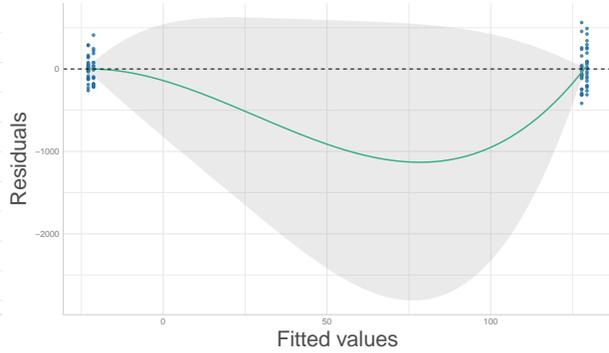
Posterior Predictive Check

Model-predicted lines should resemble observed data line



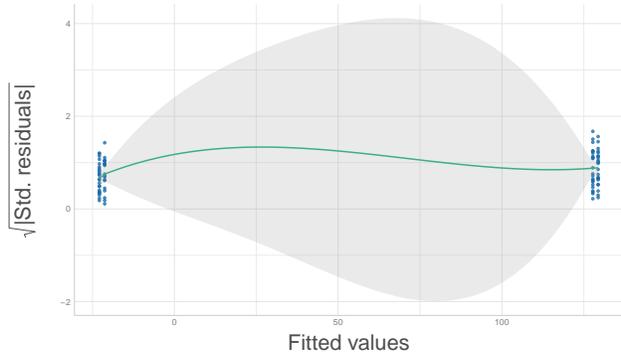
Linearity

Reference line should be flat and horizontal



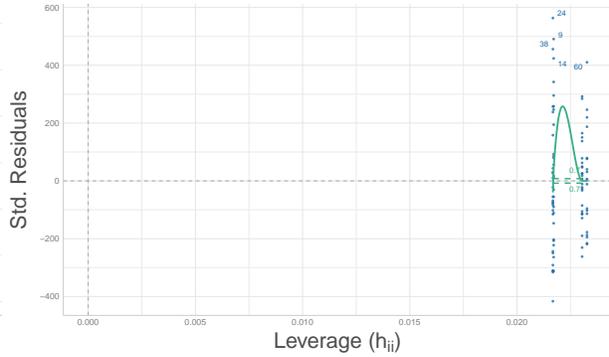
Homogeneity of Variance

Reference line should be flat and horizontal



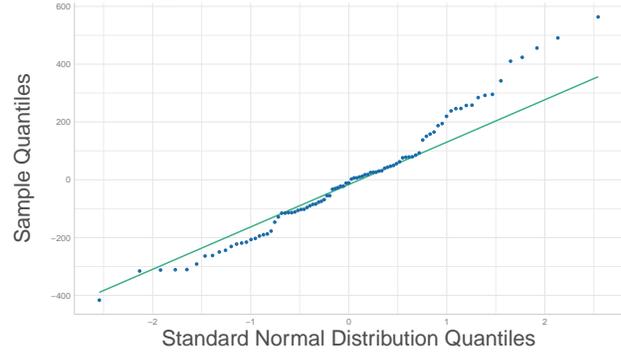
Influential Observations

Points should be inside the contour lines



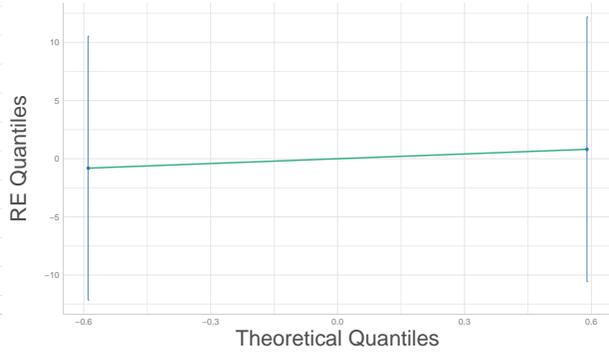
Normality of Residuals

Dots should fall along the line



Normality of Random Effects (plot)

Dots should be plotted along the line



Section S6: Spatial distribution of microbial respiration overyielding (H3)

Model summary

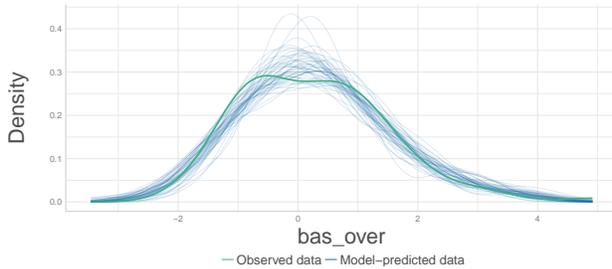
```
lmer(bas_over ~ dist.T1.center * depth + (1|plot), data = df)
```

	Estimate	Std. Error	t-value	p-value
(Intercept)	-0.0229950	0.6296411	-0.0365208	0.9766871
dist.T1.center	-0.7741122	0.1876610	-4.1250569	0.0000853
depth	0.1445341	0.0236760	6.1046629	0.0000000
dist.T1.center:depth	-0.3759578	0.0541292	-6.9455595	0.0000000

Model fit

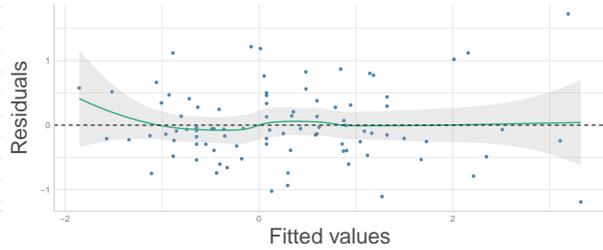
Posterior Predictive Check

Model-predicted lines should resemble observed data line



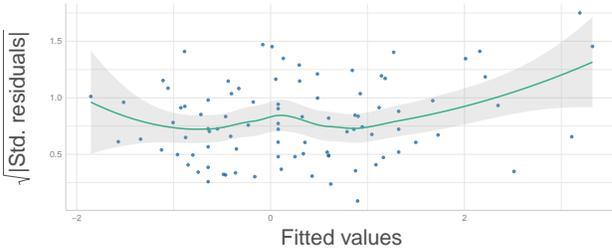
Linearity

Reference line should be flat and horizontal



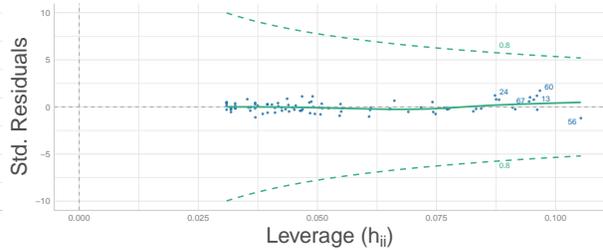
Homogeneity of Variance

Reference line should be flat and horizontal



Influential Observations

Points should be inside the contour lines



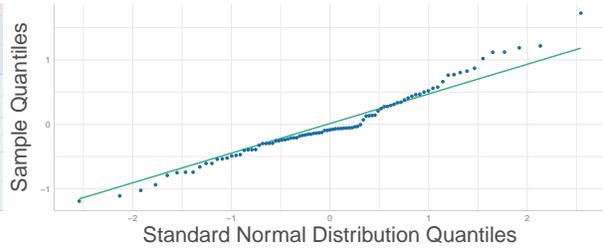
Collinearity

High collinearity (VIF) may inflate parameter uncertainty



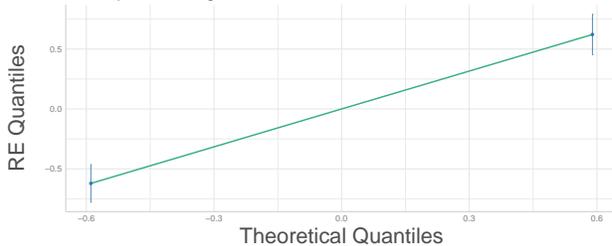
Normality of Residuals

Dots should fall along the line



Normality of Random Effects (plot)

Dots should be plotted along the line



Section S7: Spatial distribution of microbial biomass overyielding (H3)

Model summary

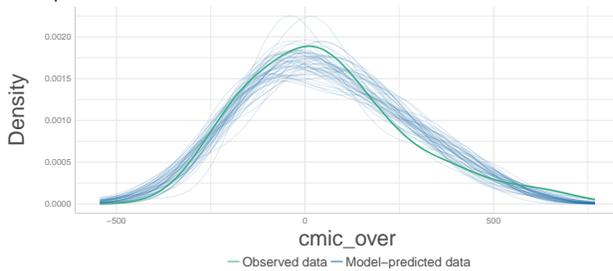
`lmer(cmic_over ~ dist.T1.center * depth + (1|plot), data = df)`

	Estimate	Std. Error	t-value	p-value
(Intercept)	123.62549	28.254908	4.375363	0.0930928
dist.T1.center	-452.50479	34.902000	-12.965010	0.0000000
depth	-28.51567	4.402464	-6.477206	0.0000000
dist.T1.center:depth	30.01283	10.067663	2.981112	0.0037344

Model fit

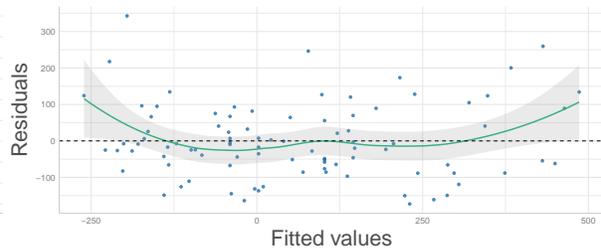
Posterior Predictive Check

Model-predicted lines should resemble observed data line



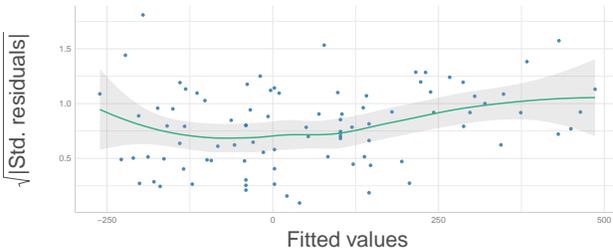
Linearity

Reference line should be flat and horizontal



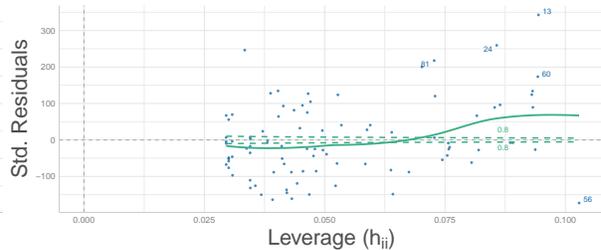
Homogeneity of Variance

Reference line should be flat and horizontal



Influential Observations

Points should be inside the contour lines



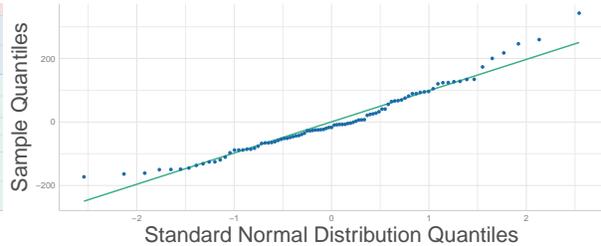
Collinearity

High collinearity (VIF) may inflate parameter uncertainty



Normality of Residuals

Dots should fall along the line



Normality of Random Effects (plot)

Dots should be plotted along the line

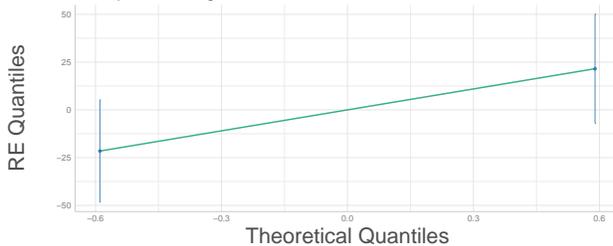


Figure S2: Abiotic and biotic drivers of soil microbial biomass and respiration

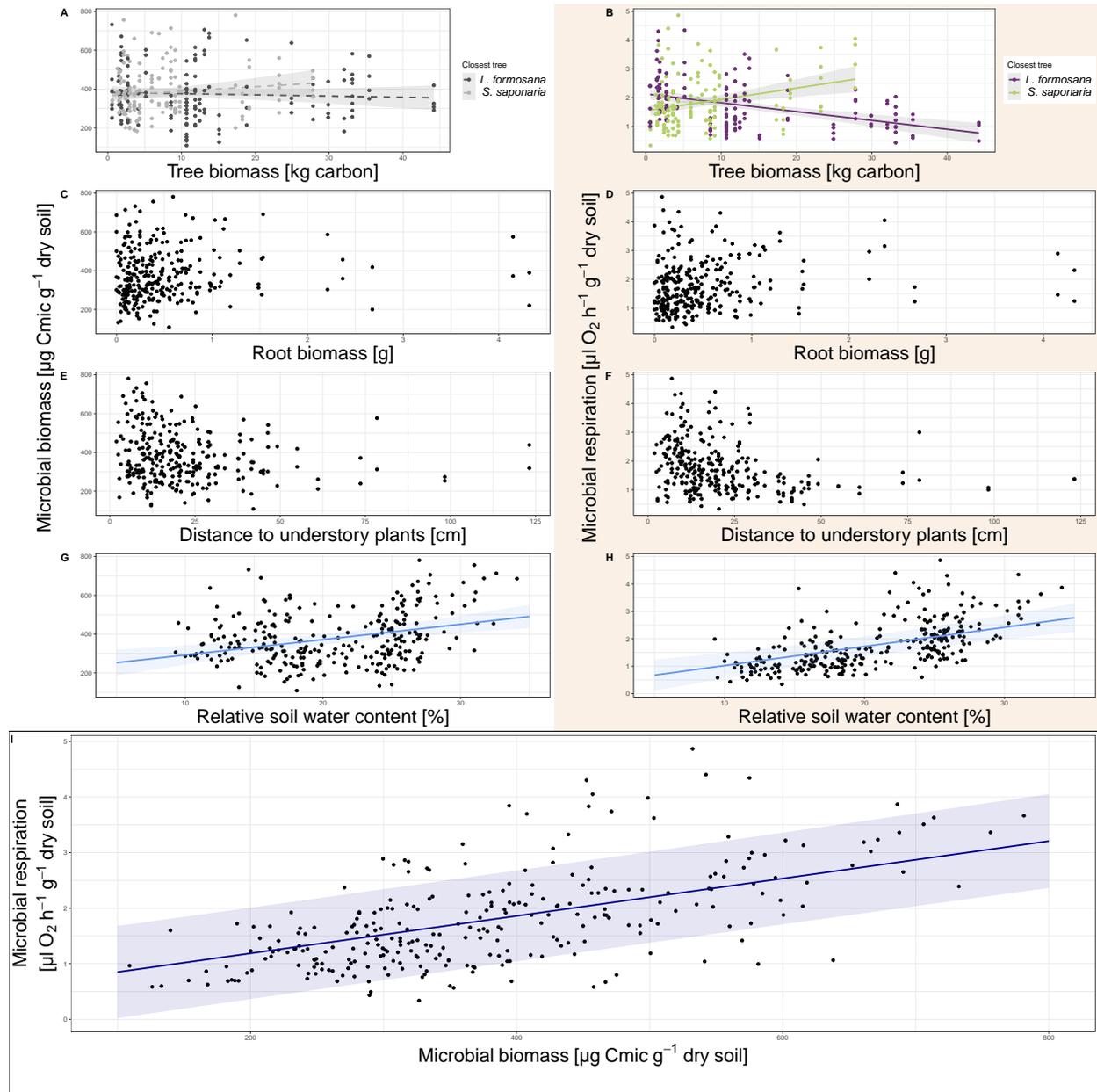


Figure S2: Overview of small scale drivers of microbial biomass and respiration. Effects of tree size and species identity on microbial biomass (A) and respiration (B). Effects of root biomass and distance to understory plants were non-significant on microbial biomass (C, E, respectively) or respiration (D, F, respectively). Positive effect of relative soil water content on microbial biomass (G) and respiration (H). Positive correlation of microbial biomass and respiration (I). Significant effects in colour.