PRIFYSGOL
BANGOR
UNIVERSITY

**Tree diversity effects on soil microbial biomass and respiration are context dependent across forest diversity experiments**

Cesarz, Simone; Craven, Dylan; Auge, Harald; Bruelheide, Helge ; Castagneyrol, Bastien; Gutknecht, Jessica ; Hector, Andrew; Jactel, Hervé; Koricheva, J.; Messier, C.; Muys, B.; O'Brien, Michael J.; Paquette, A.; Ponette, Q.; Potvin, Catherine; Reich, P.; Scherer-Lorenzen, M.; Smith, Andy; Verheyen, K.; Eisenhauer, Nico

**Global Ecology and Biogeography**

Peer reviewed version

[Cyswllt i'r cyhoeddiad / Link to publication](#)

# Tree diversity effects on soil microbial biomass and respiration are context-dependent across forest diversity experiments

**Running title:** Drivers of soil microbial properties

## Abstract

### Aim

Soil microorganisms are essential for the functioning of terrestrial ecosystems. Although soil microbial communities and functions are linked to tree species composition and diversity, there has been no comprehensive study of how general or context-dependent these relationships are. Here, we examine tree diversity–soil microbial biomass and respiration relationships across environmental gradients using a global network of tree diversity experiments.

### Location

Boreal, temperate, subtropical, tropical forests

### Time Period

2013

### Major Taxa Studied

Soil microorganisms

## Methods

Soil samples collected from eleven tree diversity experiments were used to measure microbial respiration, biomass, and respiratory quotient using the substrate-induced respiration method. All samples were measured using the same analytical device, method, and procedure to reduce measurement bias. We used linear mixed-effects models and principal component analysis (PCA) to examine the effects of tree diversity (taxonomic and phylogenetic), environmental conditions, and interactions on soil microbial properties.

## Results

Abiotic drivers, mainly soil water content, but also soil carbon and soil pH, significantly increased soil microbial biomass and respiration. High soil water content reduced the importance of other abiotic drivers. Tree diversity had no effect on the soil microbial properties, but interactions with phylogenetic diversity indicated that diversity effects are context-dependent and stronger in drier soils. Similar results were found for soil carbon and soil pH.

## Main conclusions

Our results point to the importance of abiotic variables, and especially soil water content, for maintaining high levels of soil microbial functions and modulating the effects of other

environmental drivers. Planting tree species with diverse water-use strategies and structurally complex canopies and high leaf area may be crucial for maintaining high soil microbial biomass and respiration. Since higher phylogenetic distance alleviated unfavorable soil water conditions, reforestation efforts accounting for traits improving soil water content or choosing more phylogenetically distant species may assist in increasing soil microbial functions.

**Keywords:**

Aboveground-belowground interactions, Biodiversity-ecosystem functioning, Biodiversity loss, Context-dependency, Global change, Soil biota, Soil microbial functions, Soil microorganisms, Tree diversity, TreeDivNet

## Introduction

Soil microorganisms are the functional backbones of terrestrial ecosystems (van der Heijden *et al.*, 2008), as they underpin crucial ecosystem functions and services that humankind relies upon (Wall *et al.*, 2015). Given the critical role of soil microorganisms in carbon dynamics and soil feedback effects on climate, improving current understanding of the drivers of microbial biomass and activity is an essential step towards predicting global change impacts (Serna-Chavez *et al.*, 2013; Xu *et al.*, 2013, 2017; Chen *et al.*, 2019). Soil microbial biomass can serve as a proxy for nutrient cycling and soil enzyme dynamics, such as soil organic matter (SOM) turnover as well as for secondary productivity (Crowther *et al.*, 2019). In addition, in-situ measurements of microbial activity have been shown to correlate with rates

of soil C sequestration (Lange *et al.*, 2015). Together, microbial biomass and activity provide critical information on a range of important soil ecosystem functions.

Globally, abiotic factors are thought to be the main driver of soil microbial biomass and microbial activity (Serna-Chavez *et al.*, 2013; Xu *et al.*, 2017; Smith *et al.*, 2021; Wan *et al.*, 2021). Optimal soil water content (i.e., soil water holding capacity of around 60%), neutral soil pH, and high soil organic carbon content (here summarized as high soil quality) are among the most important factors that directly increase soil microbial biomass and activity (Schimel, 2018). In contrast, climatic conditions such as temperature may influence soil microbial biomass indirectly *via* evapotranspiration and changes in soil organic matter content ( Serna-Chavez *et al.*, 2013). These patterns become less clear when taking interactions among different drivers into account. For instance, the positive effects of high soil nutrient content may be constrained by stressful environments (Serna-Chavez *et al.*, 2013) or become even stronger (Guerrero-Ramírez *et al.*, 2017), highlighting the importance of context-dependent effects or microclimatic conditions regulated by the vegetation (Gottschall *et al.*, 2019). Moreover, the effects of abiotic drivers may further be modulated by local biotic conditions. For example, studies in grasslands and forests have demonstrated that plant diversity affects soil microbial community composition, activity, and biomass (Lange *et al.*, 2015; Chen *et al.*, 2019) with significant effects on ecosystem functions, such as soil carbon storage (Lange *et al.*, 2015), and soil N retention (Leimer *et al.*, 2016). However, global analyses of plant diversity effects on soil microbial communities have had limited scope, focusing either on soil communities but not on soil functions, or on grasslands only (Prober *et al.*, 2015; Thakur *et al.*, 2015; Tedersoo *et al.*, 2016). In addition, the magnitude and direction of plant diversity effects on soil microbial communities were inconsistent, probably due to strengthening of these effects with time (Thakur *et al.*, 2015) and different environmental contexts, such as different soil conditions (Guerrero-Ramírez *et al.*, 2017). So

far, plant diversity effects on soil microbial functions have been studied mostly in grasslands while little is known about tree diversity effects on soil microbial functions in forests (Chen *et al.*, 2019; Xu *et al.*, 2020). This is a major knowledge gap because there might be substantial differences between ecosystems in terms of soil microbial function and potential climate feedback effects on soil communities (Chen *et al.*, 2018).

Previous studies on tree diversity effects on soil microorganisms mainly compared monoculture stands with mixtures of two tree species in different environments, making it difficult to disentangle site conditions from tree diversity and tree identity effects (Klimek *et al.*, 2016; Liang *et al.*, 2016). One of the first studies using data from a tree diversity experiment with homogeneous abiotic conditions found soil microbial activity and biomass to increase with tree species richness in a saturating relationship, while soil microbial community composition did not vary significantly (Khlifa *et al.*, 2017). One of the potential mechanisms underlying a positive plant diversity effect on microorganisms is the increased input of diverse resources (Eisenhauer *et al.*, 2017). In line with the view that the quality of plant inputs is essential for soil microbial processes, the chemical composition of leaf litter determines nutrient mineralization, microbial respiration, and microbial biomass (Pei *et al.*, 2017), whereas species diversity *per se* was shown to have little effect (Meier & Bowman, 2008). This finding suggests that an increase in species richness may not increase soil microbial biomass and activity if not accompanied by a simultaneous increase in the functional dissimilarity of co-occurring species (Heemsbergen *et al.*, 2004). While much debated, research in grasslands suggests that functional diversity is of higher importance than species richness for soil microbial biomass and activity (e.g., Ebeling et al., 2014), while there is even less conclusive information for forest ecosystems (Scherer-Lorenzen *et al.*, 2007) using belowground traits (but see Bergmann *et al.*, 2020; Guerrero-Ramírez *et al.*, 2021). Unfortunately, access to and measuring the above- and belowground traits on the same

plants is often not possible for logistical reasons. To overcome this lack of data, phylogenetic diversity can be used as a proxy for functional diversity (Tucker *et al.*, 2018), which has been used successfully for aboveground ecosystem functions (Cadotte *et al.*, 2009).

Here, we present the first coordinated sampling and analysis of soil microbial properties across eleven tree diversity experiments distributed across four biomes. To explore potential tree diversity effects on three key soil microbial properties - soil microbial basal respiration, biomass, and carbon-use efficiency - we tested effects of tree species richness (the biodiversity measure most frequently manipulated in tree diversity experiments; Verheyen et al., 2016) and tree phylogenetic diversity. We expected that phylogenetically diverse experimental forests will provide more dissimilar resources and niches to soil microorganisms, thereby increasing soil ecosystem functioning. We investigated three hypotheses: (1) Both tree species richness and phylogenetic diversity are predicted to increase soil microbial processes, but phylogenetic diversity is expected to have stronger effects. (2) Abiotic drivers strongly influence soil microbial functions, because high soil carbon (soil C) concentration, high soil water content (SWC), and more neutral soil pH are hypothesized to increase the biomass, activity, and carbon use efficiency of soil microorganisms. (3) Interactions among abiotic and biotic drivers may influence soil microbial properties, given the context-dependency of biodiversity-ecosystem function relationships.

**Materials & Methods**

Soil samples were taken in 2013 from eleven tree diversity experiments that are part of the global network TreeDivNet (Verheyen et al., 2016; http://www.treedivnet.ugent.be/). Experiments are independent of each other with different experimental designs and plot configurations (Table 1). Experiments are distributed across four continents (Asia, Europe,

6

North, and South America) and four different biomes (boreal, temperate, tropical, subtropical, Olson et al., 2001), and differ in age, with the youngest experiments running for three years and the oldest for fourteen years as of 2013 (i.e., the year of the sampling campaign; Fig. 1; Table 1). In total, 106 tree species were included in this study (see Appendix S1 in Supporting Information as Table S1.1). Experiments had a mean ± SD number of diversity levels of 3.7 ± 1.0, with diversity levels ranging from monocultures to 18 tree species in sub/tropical regions. All experiments had an experimental gradient in tree species richness, with the exception of one (BIOTREE-FD, see Table 1) which manipulated functional diversity at a constant level of tree species richness.

**Soil sampling**

Soil samples were taken from a depth of 0 – 10 cm, excluding the litter layer using a soil corer. Temperate and boreal experiments were sampled in the summer season in the Northern hemisphere, while subtropical and tropical experiments were sampled in the wet summer season. All experiments were sampled between June and September 2013. Depending on the size of the experimental plot, different numbers of subsamples were taken per plot to create one composite sample. For plots <100 m², three subsamples were taken , while ten subsamples were taken for plots >100 m2. These subsamples were taken to capture the spatial heterogeneity of the plot and to represent as many different combinations of tree species as possible. Soil samples were always taken in the center of surrounding tree stems. Immediately after sampling, soil samples were stored at 5°C until sieving at 2 mm and then were stored at -20°C until and during shipping to minimize changes in microbial activity, biomass, and composition. Alternatively, if shipping at -20°C was not possible, samples defrosted during shipping and were measured shortly after arrival. Altogether, 1010 plots were sampled across the eleven tree diversity experiments (Table 1).

**Measurement of soil microbial properties**

Before the start of microbial measurements, samples were kept at +20°C for five days to unfreeze and to adapt the soil microbial community to a constant and standardized temperature. Three different soil microbial community properties were assessed using an automated $O_2$ micro-compensation system (Scheu, 1992). First, basal respiration ($\mu l\ O_2\ h^{-1}\ g^{-1}$ dry soil) was measured as the mean oxygen consumption per hour without the addition of any substrate. The mean oxygen consumption was measured for hours 15 to 20. Basal respiration reflects the active part of the soil microbial community at the time of sampling. Second, microbial biomass carbon was measured by substrate-induced respiration, i.e., the respiratory response of microorganisms to glucose and water addition. To saturate catabolic microbial enzymes, 8 mg glucose $g^{-1}$ soil dry weight was added as an aqueous solution to the soil samples. The lowest substrate-induced respiration of three contiguous hours within the first 10 h was taken as the maximum initial respiratory response (MIRR) – a period when microbial growth has not started. Microbial biomass ($\mu g\ C\ g^{-1}$ dry soil) was calculated as 38 $\times$ MIRR ($\mu l\ O_2\ h^{-1}\ g^{-1}$ dry soil) following Beck et al., (1997). By providing water and glucose, the maximum potential of the living microbial biomass is activated that is able to use glucose, whereas for basal respiration only a fraction of the entire community is active. Third, the microbial-specific respiratory quotient ($\mu l\ O_2\ mg^{-1}\ Cmic\ h^{-1}$) was calculated as the ratio of basal respiration and soil microbial biomass. The specific respiratory quotient is a measure of soil microbial carbon-use efficiency. Carbon-use efficiency is high when microbial biomass can be built up without high investment in basal respiration, which is indicated by a lower specific respiratory quotient. All measurements were conducted at +20°C in an air-conditioned laboratory using the same analytical devices (RMS Schuller, Darmstadt, Germany).

**Diversity metrics**

In addition to tree species richness, we aimed at testing a tree diversity metric that captures the functional diversity of each experimental forest plot. However, no comparable trait measurements were available from all experiments nor from the TRY database. Instead, we used phylogenetic diversity as a proxy for multi-trait functional diversity (Tucker *et al.*, 2018). Phylogenetic diversity indices have been shown to be powerful predictors of biodiversity-ecosystem functioning relationships (e.g., Craven et al., 2018) and are suggested to work when key functional traits are not available (Paquette *et al.*, 2015). We used the molecular phylogeny from previous studies (Zanne *et al.*, 2013; Pietsch *et al.*, 2014) as a backbone to build a phylogeny of all species within the tree diversity experiments, conservatively binding species into the backbone using dating information from congeners in the tree. We used the *comparative.comm* function in the R package *pez* to calculate a set of phylogenetic diversity indices, specifically MPD (mean phylogenetic diversity), MNTD (mean nearest taxonomic distance), and the standardized version of both to account for correlation with species richness (Pearse *et al.*, 2015). MNTD was found to correlate less (using Pearson correlation) with log species richness and, therefore, was used in all following analyses (see Appendix S2 in Supporting Information as Table S2.1). Taxonomic names of tree species were standardized using the website
http://tnrs.iplantcollaborative.org/index.html.

**Soil characteristics**

We included a set of explanatory variables to describe the experimental sites, which were shown to have an effect on soil microbial properties and reflect the designs and local conditions of the different experiments (Fig. S2.1). Gravimetric soil water content was measured as % $H_2O$ from fresh soil weight by drying the whole sample at 75°C for three days. Soil pH and soil C (%) were measured at the block level to obtain information about soil quality characteristics of each experiment. Therefore, equal proportions of dry soil were weighed from each sample to form a composite sample. The whole sample was ground, and a fraction of 10 g was used for pH measurements by adding 0.01 m $CaCl_2$. Soil C concentrations were analyzed by using the ground soil with an elemental analyzer (Vario EL Cube, Elementar). We further extracted clay (%), sand (%), and silt (%) content from the SoilGRIDS database (Hengl *et al.*, 2014).

**Environmental conditions**

For each experimental site, we extracted mean annual temperature (MAT), the seasonal variability of temperature (Season Temp.), annual precipitation (MAP), and the seasonal variability of precipitation (Season Prec.) from the WorldClim database (http://www.worldclim.org/current) with 2.5 arc-minutes resolution. Potential evapotranspiration (PET) and the aridity index (MAP/PET) were extracted from CGIAR-CSI (https://srtm.csi.cgiar.org/). In addition, we obtained the age of the experiment (years) and tree density (trees m⁻²) from publications associated with each experiment (Table 1) and the TreeDivNet website (http://www.treedivnet.ugent.be/). Biomes were assigned based on Olson et al., (2001).

**Data analysis**

Prior to analysis, all data were centered and standardized (i.e.; re-scaled to variance = 1) using the scale function from the base package in R, and the distributions of response variables were checked visually (Zuur *et al.*, 2010). We only included abiotic variables to our linear-mixed-effects models where block or plot-level data were available (i.e., soil water content, soil pH, and soil C), allowing us to test all possible two-way interactions. In addition, we included logarithmized tree species richness (log SR) and MNTD as fixed effects in our models. We tested if our models were overfitted by calculating the variation inflation factor (VIF) for each model, which were simplified removing interactions with VIF>3 (Montgomery *et al.*, 2012). Since only one interaction of soil pH and soil C for basal respiration and the respiratory quotient showed a VIF between 3.0 and 3.6, we used model comparison with anova() to check AICs. Both models, the reduced and the full model, did not differ by more than two units. Therefore, the full models were kept and are presented here.

The random effect structure accounted for the hierarchical data structure, with block nested within site and site nested within experiment. The Kenward-Rogers approximation was used to test for the significance of fixed effects and degrees of freedom. Marginal and conditional $R^2$ were calculated using the function *r.squaredGLMM* from the *MuMIn* package. Marginal $R^2$ represents the variance explained by the fixed effects, whereas conditional $R^2$ represents the variance explained by both fixed and random effects. Collinearity among explanatory variables in mixed-effects models was below r =|0.7| as suggested by Dormann et al., (2013) (Figure S2.1). We checked model assumptions of the most parsimonious models by fitting model residuals versus the results of fitted models. Basal respiration and the respiratory quotient were log-transformed to achieve the requirements of parametric statistical tests. Model fits of the mixed-effects models were used to plot estimates using the function

*plot_model* from the package *sjPlot*. Significant interactions were plotted using *ggpredict* from the package *ggeffects*.

Principal component analysis (PCA) was used to visualize how every single experiment is characterized by the explanatory variables and their relation to the dependent variables. Basal respiration, microbial biomass, and the respiratory quotient were treated as active variables as well as the explanatory variables available. PCA was computed using *prcomp* from the *stats* package. Visualization was done using the function *fviz_pca_biplot* from the factoextra package. In addition to linear mixed-effects models and the PCA we included a piecewise structural equation model (pSEM , Lefcheck, (2016)) to investigate causal relationships among variables as there is uncertainty about underlying mechanisms (see Appendix S3 as Fig. S3.2, Table S3.2). pSEM further allows to account for the nested structure of the underlying data. We accounted for correlated errors of all microbial properties, as well as for tree species richness and MNTD. Because of the mismatch in data resolution, we cannot test some potential effect pathways. For instance, we are unable to test if increasing sand content reduces soil C content, which – in turn – is negatively related to soil water content, - in turn – is positively related to soil microbial properties but with an overall negative effect on soil microbial biomass. Independent claims were all non-significant. All variables were scaled as mentioned above. All statistical analyses were performed in R (version 4.0.3) (R Core Team, 2016).

## Results

Mean soil basal respiration ($\pm$ SD) was $2.06 \pm 1.94$ µl $O_2$ h$^{-1}$ g soil dw$^{-1}$, with the lowest values in the FORBIO experiment in Belgium (min: $0.08$ µl $O_2$ h$^{-1}$ g soil dw$^{-1}$) and the highest values in the SATAKUNTA experiment in Finland (max: $15.26$ µl $O_2$ h$^{-1}$ g soil dw$^{-1}$;

see Appendix S4 as Fig S4.3). Similarly, we found the lowest soil microbial biomass values in the FORBIO experiment (min: 11.85 µg Cmic g soil dw$^{-1}$) and the highest values in the SATAKUNTA experiment (max: 2501.54 µg Cmic g soil dw$^{-1}$). Mean soil microbial biomass was 435.51 ± 325.03 µg Cmic g soil dw$^{-1}$. The respiratory quotient was lowest (i.e., highest carbon-use efficiency) in the BIOTREE-FD in Germany (min: 0.008 µl O$_2$ µg$^{-1}$ Cmic h$^{-1}$) and the highest respiratory quotient was measured in the ORPHEE experiment in France (max: 0.0395 µl O$_2$ µg$^{-1}$ Cmic h$^{-1}$). The grand mean across experiments for the respiratory quotient was 0.0052 ± 0.0031 µl O$_2$ µg$^{-1}$ Cmic h$^{-1}$. Mean soil water content was 17.2 ± 11.5%, and the driest soil was found in the IDENT Cloquet experiment in Minnesota, USA (min: <0.1%), whereas the highest values were measured in the experiment SATAKUNTA (max: 58.5%). Principal component analysis highlighted the strong relationship of basal respiration and microbial biomass to soil water content, experimental age, and soil C that correlated strongest with the first PCA axis and explained 59.3% of the variance (Fig. 2, Table S2.3). These three variables reached maximum values in the boreal experiment Satakunta. High microbial biomass values were also found in the Biotree-FD experiment that could be characterized by higher clay content and lower potential evapotranspiration. The second axis explained 36.2% of the variance and was mainly affected by the seasonality of temperature, soil pH, and tree density, variables that strongly increased the carbon use efficiency.


*Hypothesis 1: Tree diversity increases soil microbial properties*

Overall, tree species diversity and phylogenetic tree diversity did not significantly influence basal respiration, microbial biomass, or carbon use efficiency (Fig. 3; Table 2, Fig, S4.3, Table S4.4). Similarly, a detailed examination of each experimental forest revealed only one positive significant effect that was found in the ORPHEE experiment, where carbon-use

efficiency increased (i.e., the respiratory quotient decreased) with increasing MNTD (Fig. S4.3f, Table S4.4), whereas the other two significant effects showed negative relationships (Sardinilla Fig.S4.3a and BEF-China Fig.S4.3e, Table S4.4). Consequently, the $R^2$ of the models analyzing individual experimental forests were consistently low (Table S4.4), indicating that soil microbial properties are not well explained by tree diversity. Across all experimental forests, marginal $R^2$ of the linear mixed-effects models were 17% for basal respiration and for microbial biomass, and 38% for the respiratory quotient (Table 3). Conditional $R^2$ was roughly twice as high as marginal $R^2$.

*Hypothesis 2: High soil water content, soil C content, and soil pH increase soil microbial properties*

Soil water content increased all microbial properties significantly when all the experiments were considered together (Fig. 2, Table 2); this positive effect was seen in all but one (ORPHEE) experimental site (Fig. S4.4a). In contrast, soil C alone did not affect any of the microbial properties investigated in the linear mixed models nor in the piecewise SEM (Fig. 2, Table 2, Fig. S3.2, Table S3.2). Interestingly, the relationship between soil C, soil respiration, microbial biomass, and carbon use efficiency was not positive as expected, but negative for many of the experiments as mentioned above (Fig. S4.5). However, the very high C values in the boreal Satakunta experiment led to a generally positive effect that was removed in the linear mixed-effects models. Higher soil pH significantly increased microbial biomass and carbon use efficiency (i.e., a negative effect on the respiratory quotient) but not basal respiration (Figs. 3h,l).

*Hypothesis 3: Context dependency*

Although all three microbial properties were not affected by tree diversity itself, there was a significant effect of the interaction of MNTD and soil water content on all three properties,

and basal respiration was also affected by the interaction effect of MNTD and soil C. The interactive effects of tree species richness with abiotic factors were, however, not statistically significant (Fig. 3, Table 2). Generally, all soil microbial properties increased with increasing soil water content. At low soil water content, we detected significant positive effects of MNTD on soil microbial properties. In contrast, the effects of MNTD were not statistically significant at high levels of soil water content (Fig. 3b,f,j). High MNTD increased basal respiration and microbial biomass at low levels of soil water content but decreased carbon-use efficiency.

The significant interaction effect of MNTD and soil C for basal respiration showed a different pattern. Generally, the fitted model predicted basal respiration to be highest when soil C was low (Fig. 3c). Increasing MNTD increased basal respiration only at high soil C levels, whereas increasing MNTD decreased basal respiration at low soil C levels.

In addition to interactions with MNTD, we found additional interactions between the abiotic variables. A significant interaction for all three microbial properties was found for soil C and soil water content. At high soil water content, differences in soil C had less impact on all soil microbial properties (Figs. 3d,g,k). The increase in basal respiration and microbial biomass along the soil water gradient was steepest when soil C was high, and the reverse was true for the respiratory quotient.

Microbial biomass and carbon-use efficiency were significantly affected by the interaction of soil pH and soil water content (Figs. 3h,l). Effects of soil pH on soil microbial properties were negligible when soil water content was high.

**Discussion**

Leveraging a global network of experimental forests, we found that variation in soil microbial properties was mediated by abiotic factors to a greater extent than by biotic factors. Effects of tree diversity on soil microbial respiration were largely context-dependent, only emerging when soil water content was low.

Our first hypothesis posited that – independent of environmental context - taxonomic and phylogenetic diversity would increase soil microbial properties. Yet, we found that tree diversity did not have statistically significant effects on any soil microbial property. Recent studies in experimental plots found weak tree diversity effects on soil microorganisms, suggesting that tree species identity may be a more important driver of soil microorganisms and soil functions (e.g., Gottschall *et al.*, 2019; Khlifa *et al.*, 2017). Individual tree species can affect the structure of the litter layer by influencing microclimatic conditions that drive soil microbial functions (Gottschall *et al.*, 2019). Therefore, improved data on litter and root traits, as well as their influence on soil quality and microclimate, are need to improve our mechanistic understanding of tree identity effects on soil functions (Laliberté, 2017; Beugnon *et al.*, 2021). Using belowground traits, rather than aboveground traits, is essential to predict soil functions, as different mechanisms likely operate belowground. Unfortunately, no representative above- or belowground trait data were available for the eleven tree diversity experiments to investigate tree identity effects in greater detail. We were not able to use data from trait databases, since relevant traits were not available for many subtropical and tropical tree species. In addition, traits have been shown to be very plastic, even across local plant diversity gradients (Roscher *et al.*, 2018). This may complicate the investigation of tree diversity effects and calls for standardized plot-level measurements of traits in future work. Instead, we used phylogenetic diversity (MNTD) as a proxy for differences among tree species (Craven *et al.*, 2018). A notable drawback of using MNTD (or any other measure of phylogenetic diversity) is that it lacks a clear mechanistic link to soil microbial properties,

nor can it be used to explore mechanistic identity effects. A targeted trait approach paired with phylogenetic information may help to better understand underlying mechanisms. The growing network of global tree diversity experiments (Verheyen *et al.*, 2016; Paquette *et al.*, 2018), other similar networks (Borer *et al.*, 2014), and trait syntheses (Guerrero-Ramírez *et al.*, 2021) will allow for coordinated approaches and should aim to directly measure belowground traits to identify abiotic and biotic drivers of soil microbial functions.

We did not find any interactive effects of tree species richness and abiotic factors on soil microbial properties, confirming that tree species richness *per se* does not necessarily influence belowground ecosystem functions (Guerrero-Ramírez *et al.*, 2016) and/or that tree species richness did not interact with abiotic factors. Using phylogenetic diversity instead of tree species richness provided the advantage of having a more even data distribution. This is because the temperate and boreal experiments did not contribute to medium and high levels of tree species richness, while the limited number of tropical and subtropical experiments had higher levels of tree species diversity. The species pool of the sub- and tropical experimental forests had a more constrained phylogeny than that of the temperate experimental forests, possibly reflecting the absence of coniferous species and including multiple species of the same genera (Table S1.1). This may explain the weaker (or more variable) effects of MNTD in tropical experimental forests than in temperate or boreal experimental forests.

Our study confirmed that soil microorganisms are mainly influenced by abiotic drivers (hypothesis 2), which were also important in modulating tree diversity effects (hypothesis 3). Soil water content was the dominant abiotic driver, affecting all soil microbial functions and interacting significantly with all mentioned abiotic drivers. The strong impact of soil water content on soil microbial properties has been shown in many studies (see the review by Schimel, 2018) and could be more important than nutrient availability (Singh *et al.*, 2009).

We found that at high soil water content, changes in soil pH and soil C had minimal effects on the overall high values of soil microbial properties. For instance, positive effects of high temperature on soil biological activity can only be achieved when soil water is not limiting (Thakur *et al.*, 2018), and nutrient availability can be increased by higher soil moisture via increasing diffusion of soluble organic substrates (Schimel, 2018). This suggests that optimal soil water availability (i.e., between 50 – 70% of the field capacity, Manzoni et al., (2012)) can mitigate the unfavorable effects of other abiotic factors on soil ecosystem functioning. For a better mechanistic understanding, microclimatic parameters (e.g., soil humidity and temperature) should also be included, which can provide new insights (Gottschall *et al.*, 2019). Therefore, to maintain soil ecosystem functioning, especially when faced with more frequent dry periods due to global change, tree species or communities may be selected that directly use water more efficiently, have a higher diversity in hydraulic traits (Anderegg *et al.*, 2018), and/or have traits that indirectly maintain higher soil water levels, e.g., via higher leaf area, denser canopies, or leaf litter traits that build a thick litter layer (Gottschall *et al.*, 2019). In addition, further management practices, e.g., leaving leaf litter on the ground, applying mulch, planting a cover crop, may be needed to enhance soil water content, and thus, to increase soil functioning.

The present study shows that tree diversity effects, as captured by phylogenetic diversity in the present study, on soil microbial properties were statistically significant at low soil moisture levels, confirming earlier findings of an observational study across European forests (Ratcliffe *et al.*, 2017). In contrast, a recent study investigating the interaction with water availability and tree diversity on similar microbial properties did not find positive diversity effects at low soil water availability (Strukelj *et al.*, 2021). This study, however, only investigated context-dependency at two experimental sites. Using eleven tree diversity studies, our findings suggest that biodiversity may function as a buffer against harsh

environmental conditions and maintain ecosystem functioning under drought. As plant

diversity increases soil microbial diversity, diverse soil microbial communities can increase

the resilience of plants after drought (Prudent *et al.*, 2020). For instance, biodiversity may

enhance drought resistance due to strengthened biotic interactions, for instance, via

mycorrhiza (van der Heijden *et al.*, 2008), especially when mycorrhization rates increase with

increased tree diversity (Ferlian et al., 2021).

One mechanism by which tree diversity increases soil microbial properties is via enhanced

inputs of soil carbon since microorganisms are generally carbon limited (Soong *et al.*, 2020).

A recent global study investigating the effect of plant diversity (including 92 forest sites) on

carbon stocks and microbial biomass C found significant higher soil organic carbon in

mixtures compared to monocultures but did not detect a generally positive effect of

increasing species diversity on soil carbon stocks and microbial biomass C (Chen *et al.*,

2020). In the aforementioned study, carbon stocks increased with time, suggesting that more

time is needed for tree diversity effects to increase carbon stocks and likely cascading effects

on microbial functions. Therefore, the lack of a consistent diversity effect on soil carbon in

the present study could be due to the young age of most experimental forests (average 7.2 ±

3.9 years). This also suggests that belowground responses to tree diversity may take much

longer than aboveground responses (Guerrero-Ramírez *et al.*, 2017). Our dataset did not

allow testing for three-way interactions, but the PCA together with the interactions of soil

water suggest that high amounts of soil carbon and soil water increase soil microbial

respiration and biomass the most. These two variables are  strongly affected by soil texture at

one experimental site (Guenet *et al.*, 2011) but can also be affected by tree diversity, likely

over the long term. We were not able to include soil texture data in our linear-mixed effects

models, although other studies found strong effects (Xu *et al.*, 2017). A statistical analysis

(not presented) showed no effect of soil texture, likely reflecting the limited number of

experimental sites and the data source (SoilGRID database, Hengl *et al.*, (2014)). Consequently, the benefits of afforestation efforts in terms of soil ecosystem functioning will not be immediate and will likely take more than a decade to manifest. However, planting trees with specific traits combined with targeted management practices may promote this effect.

## Conclusion

Global analyses of biodiversity-ecosystem functioning relationships aim to identify general patterns, context-dependencies, and underlying mechanisms to predict and mitigate the consequences of biodiversity loss for human well-being. Our results indicate that tree diversity effects on soil microbial biomass and respiration in young plantations are generally weak but are strongest under dry soil conditions. Notably, the results of tree diversity experiments may have important practical implications, as many degraded ecosystems are in the process of being reforested, and recommendations regarding how to enhance the multifunctionality of these restored ecosystems are urgently needed to mitigate climate change. Because the potential impacts of high-diversity reforestation efforts will likely manifest over the long term, especially with regards to soil ecosystem functioning, we recommend management practices that maintain soil water content. Doing so will require addressing key gaps in biodiversity data, particularly belowground functional traits.. Exploring interactions between abiotic and biotic factors in driving soil microbial properties and carbon storage in future studies is pivotal in order to get a more mechanistic understanding of the driving forces of and management options for soil carbon storage.

# References

Anderegg, W.R.L., Konings, A.G., Trugman, A.T., Yu, K., Bowling, D.R., Gabbitas, R., Karp, D.S., Pacala, S., Sperry, J.S., Sulman, B.N. & Zenes, N. (2018) Hydraulic diversity of forests regulates ecosystem resilience during drought. *Nature*, **561**, 538–541.

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

Bergmann, A.J., Weigelt, A., Plas, F. Van Der, Laughlin, D.C., Kuyper, T.W., Guerrero-Ramírez, N.R., Valverde-Barrantes, O.J., Bruelheide, H., Freschet, G.T., Iversen, C.M., Kattge, J., McCormack, M.L., Meier, I.C., Rillig, M.C., Roumet, C., Semchenko, M., Sweeney, C.J., van Ruijven, J., York, L.M. & Mommer, L. (2020) The fungal collaboration gradient dominates the root economics space in plants. *Science Advances*, **6**, eaba3756.

Beugnon, R., Du, J., Cesarz, S., Jurburg, S.D., Pang, Z., Singavarapu, B., Wubet, T., Xue, K., Wang, Y. & Eisenhauer, N. (2021) Tree diversity and soil chemical properties drive the linkages between soil microbial community and ecosystem functioning. *ISME Communications*, **1**, 41.

Borer, E.T., Harpole, W.S., Adler, P.B., Lind, E.M., Orrock, J.L., Seabloom, E.W. & Smith, M.D. (2014) Finding generality in ecology: A model for globally distributed experiments. *Methods in Ecology and Evolution*, **5**, 65–73.

Cadotte, M.W., Cavender-Bares, J., Tilman, D. & Oakley, T.H. (2009) Using phylogenetic, functional and trait diversity to understand patterns of plant community productivity. *PLoS ONE*, **4**, 1–9.

Chen, C., Chen, H.Y.H., Chen, X. & Huang, Z. (2019) Meta-analysis shows positive effects of plant diversity on microbial biomass and respiration. *Nature Communications*, **10**, 1–10.

Chen, S., Wang, W., Xu, W., Wang, Y., Wan, H., Chen, D., Tang, Z., Tang, X., Zhou, G., Xie, Z., Zhou, D., Shangguan, Z., Huang, J., He, J.-S., Wang, Y., Sheng, J., Tang, L., Li, X., Dong, M., Wu, Y., Wang, Q., Wang, Z., Wu, J., Chapin, F.S. & Bai, Y. (2018) Plant diversity enhances productivity and soil carbon storage. *Proceedings of the National Academy of Sciences*, **115**, 4027–4032.

Chen, X., Chen, H.Y.H., Chen, C., Ma, Z., Searle, E.B., Yu, Z. & Huang, Z. (2020) Effects of plant diversity on soil carbon in diverse ecosystems: a global meta-analysis. *Biological Reviews*, **95**, 167–183.

Craven, D., Eisenhauer, N., Pearse, W.D., Hautier, Y., Isbell, F., Roscher, C., Bahn, M., Beierkuhnlein, C., Bönisch, G., Buchmann, N., Byun, C., Catford, J.A., Cerabolini, B.E.L., Cornelissen, J.H.C., Craine, J.M., De Luca, E., Ebeling, A., Griffin, J.N., Hector, A., Hines, J., Jentsch, A., Kattge, J., Kreyling, J., Lanta, V., Lemoine, N., Meyer, S.T., Minden, V., Onipchenko, V., Polley, H.W., Reich, P.B., van Ruijven, J., Schamp, B., Smith, M.D., Soudzilovskaia, N.A., Tilman, D., Weigelt, A., Wilsey, B. & Manning, P. (2018) Multiple facets of biodiversity drive the diversity–stability relationship. *Nature*

*Ecology and Evolution*, **2**, 1579–1587.

Crowther, T.W., van den Hoogen, J., Wan, J., Mayes, M.A., Keiser, A.D., Mo, L., Averill, C. & Maynard, D.S. (2019) The global soil community and its influence on biogeochemistry. *Science*, **365**.

Dormann, C.F., Elith, J., Bacher, S., Buchmann, C., Carl, G., Carré, G., Marquéz, J.R.G., Gruber, B., Lafourcade, B., Leitão, P.J., Münkemüller, T., Mcclean, C., Osborne, P.E., Reineking, B., Schröder, B., Skidmore, A.K., Zurell, D. & Lautenbach, S. (2013) Collinearity: A review of methods to deal with it and a simulation study evaluating their performance. *Ecography*, **36**, 27–46.

Ebeling, A., Pompe, S., Baade, J., Eisenhauer, N., Hillebrand, H., Proulx, R., Roscher, C., Schmid, B., Wirth, C. & Weisser, W.W. (2014) A trait-based experimental approach to understand the mechanisms underlying biodiversity–ecosystem functioning relationships. *Basic and Applied Ecology*, **15**, 229–240.

Eisenhauer, N., Lanoue, A., Strecker, T., Scheu, S., Steinauer, K., Thakur, M.P. & Mommer, L. (2017) Root biomass and exudates link plant diversity with soil bacterial and fungal biomass. *Scientific Reports*, **7**, 44641.

Fierer, N., Strickland, M.S., Liptzin, D., Bradford, M.A. & Cleveland, C.C. (2009) Global patterns in belowground communities. *Ecology Letters*, **12**, 1238–1249.

Gottschall, F., Davids, S., Newiger-Dous, T.E., Auge, H., Cesarz, S. & Eisenhauer, N. (2019) Tree species identity determines wood decomposition via microclimatic effects. *Ecology and Evolution*, **9**, 12113–12127.

Guenet, B., Leloup, J., Hartmann, C., Barot, S. & Abbadie, L. (2011) A new protocol for an artificial soil to analyse soil microbiological processes. *Applied Soil Ecology*, **48**, 243–246.

Guerrero-Ramírez, N.R., Craven, D., Messier, C., Potvin, C., Turner, B.L. & Handa, I.T. (2016) Root quality and decomposition environment, but not tree species richness, drive root decomposition in tropical forests. *Plant and Soil*.

Guerrero-Ramírez, N.R., Craven, D., Reich, P.B., Ewel, J.J., Isbell, F., Koricheva, J., Parrotta, J.A., Auge, H., Erickson, H.E., Forrester, D.I., Hector, A., Joshi, J., Montagnini, F., Palmborg, C., Piotto, D., Potvin, C., Roscher, C., Van Ruijven, J., Tilman, D., Wilsey, B. & Eisenhauer, N. (2017) Diversity-dependent temporal divergence of ecosystem functioning in experimental ecosystems. *Nature Ecology and Evolution*, **1**, 1639–1642.

Guerrero-Ramírez, N.R., Mommer, L., Freschet, G.T., Iversen, C.M., McCormack, M.L., Kattge, J., Poorter, H., van der Plas, F., Bergmann, J., Kuyper, T.W., York, L.M., Bruelheide, H., Laughlin, D.C., Meier, I.C., Roumet, C., Semchenko, M., Sweeney, C.J., van Ruijven, J., Valverde-Barrantes, O.J., Aubin, I., Catford, J.A., Manning, P., Martin, A., Milla, R., Minden, V., Pausas, J.G., Smith, S.W., Soudzilovskaia, N.A., Ammer, C., Butterfield, B., Craine, J., Cornelissen, J.H.C., de Vries, F.T., Isaac, M.E., Kramer, K., König, C., Lamb, E.G., Onipchenko, V.G., Peñuelas, J., Reich, P.B., Rillig, M.C., Sack, L., Shipley, B., Tedersoo, L., Valladares, F., van Bodegom, P., Weigelt, P., Wright, J.P.

& Weigelt, A. (2021) Global root traits (GRooT) database. *Global Ecology and Biogeography*, **30**, 25–37.

Heemsbergen, D.A., Berg, M.P., Loreau, M., van Hal, J.R., Faber, J.H. & Verhoef, H.A. (2004) Biodiversity effects on soil processes explained by interspecific functional dissimilarity. *Science*, **306**, 1019–1020.

van der Heijden, M.G.A., Bardgett, R.D. & van Straalen, N.M. (2008) The unseen majority: Soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecology Letters*, **11**, 296–310.

Hengl, T., De Jesus, J.M., MacMillan, R.A., Batjes, N.H., Heuvelink, G.B.M., Ribeiro, E., Samuel-Rosa, A., Kempen, B., Leenaars, J.G.B., Walsh, M.G. & Gonzalez, M.R. (2014) SoilGrids1km - Global soil information based on automated mapping. *PLoS ONE*, **9**.

Khlifa, R., Paquette, A., Messier, C., Reich, P.B. & Munson, A.D. (2017) Do temperate tree species diversity and identity influence soil microbial community function and composition? *Ecology and Evolution*, **7**, 7965–7974.

Klimek, B., Chodak, M., Jaźwa, M., Solak, A., Tarasek, A. & Niklińska, M. (2016) The relationship between soil bacteria substrate utilisation patterns and the vegetation structure in temperate forests. *European Journal of Forest Research*, **135**, 179–189.

Laliberté, E. (2017) Below-ground frontiers in trait-based plant ecology. *New Phytologist*, **213**, 1597–1603.

Lange, M., Eisenhauer, N., Sierra, C.A., Bessler, H., Engels, C., Griffiths, R.I., Mellado-Vázquez, P.G., Malik, A.A., Roy, J., Scheu, S., Steinbeiss, S., Thomson, B.C., Trumbore, S.E. & Gleixner, G. (2015) Plant diversity increases soil microbial activity and soil carbon storage. *Nature Communications*, **6**, 6707.

Lefcheck, J.S. (2016) piecewiseSEM: Piecewise structural equation modelling in r for ecology, evolution, and systematics. *Methods in Ecology and Evolution*, **7**, 573–579.

Leimer, S., Oelmann, Y., Eisenhauer, N., Milcu, A., Roscher, C., Scheu, S., Weigelt, A. & Wirth, C. (2016) Mechanisms behind plant diversity effects on inorganic and organic N leaching from temperate grassland. *Biogeochemistry*, **131**, 339–353.

Liang, J., Crowther, T.W., Picard, N., Wiser, S., Zhou, M., Alberti, G., Schulze, E., Mcguire, A.D., Bozzato, F., Pretzsch, H., Paquette, A., Hérault, B., Scherer-lorenzen, M., Barrett, C.B., Glick, H.B., Hengeveld, G.M., Nabuurs, G., Pfautsch, S., Viana, H., Vibrans, A.C., Ammer, C., Schall, P., Verbyla, D., Tchebakova, N., Fischer, M., Watson, J. V, Chen, H.Y.H., Lei, X., Schelhaas, M., Lu, H., Gianelle, D., Parfenova, E.I., Salas, C., Lee, E., Lee, B., Kim, H.S., Bruelheide, H., Coomes, D.A., Piotto, D., Sunderland, T., Schmid, B., Gourlet-fleury, S., Sonké, B., Tavani, R., Zhu, J., Brandl, S., Baraloto, C., Frizzera, L., Ba, R., Oleksyn, J., Peri, P.L., Gonmadje, C., Marthy, W., Brien, T.O., Martin, E.H., Marshall, A.R., Rovero, F., Bitariho, R., Niklaus, P.A., Alvarez-loayza, P., Chamuya, N., Valencia, R., Mortier, F., Wortel, V., Engone-obiang, N.L., Ferreira, L. V, Odeke, R.M. vasquez, Lewis, S.L. & Reich, P.B. (2016) Positive biodiversity-productivity relationship predominant in global forests. *Science*, **354**, aaf8957-1-aaf8957-12.

Manzoni, S., Schimel, J.P. & Porporato, A. (2012) Responses of soil microbial communities to water stress: results from a meta-analysis. *Ecology*, **93**, 930–938.

Meier, C.L. & Bowman, W. (2008) Links between plant litter chemistry, species diversity, and below-ground ecosystem function. *Proceedings of the National Academy of Sciences*, **105**, 19780–19785.

Montgomery, D.C., Peck, E.A. & Vining, G.G. (2012) *Introduction to Linear Regression Analysis*,.

Olson, D.M., Dinerstein, E., Wikramanayake, E.D., Burgess, N.D., Powell, G.V.N., Underwood, E.C., D'amico, J.A., Itoua, I., Strand, H.E., Morrison, J.C., Loucks, C.J., Allnutt, T.F., Ricketts, T.H., Kura, Y., Lamoreux, J.F., Wettengel, W.W., Hedao, P. & Kassem, K.R. (2001) Terrestrial Ecoregions of the World: A New Map of Life on Earth. *BioScience*, **51**, 933.

Paquette, A., Hector, A., Vanhellemont, M., Koricheva, J., Scherer-Lorenzen, M., Verheyen, K., Abdala-Roberts, L., Auge, H., Barsoum, N., Bauhus, J., Baum, C., Bruelheide, H., Castagneyrol, B., Cavender-Bares, J., Eisenhauer, N., Ferlian, O., Ganade, G., Godbold, D., Gravel, D., Hall, J., Hobbs, R., Hoelscher, D., Hulvey, K.B., Huxham, M., Jactel, H., Kreft, H., Liang, J., Mereu, S., Messier, C., Montgomery, R., Muys, B., Nock, C., Parker, J., Parker, W., Parra-Tabla, V., Perring, M.P., Ponette, Q., Potvin, C., Reich, P.B., Rewald, B., Sandén, H., Smith, A., Standish, R., Weih, M., Wollni, M. & Zemp, D.C. (2018) A million and more trees for science. *Nature Ecology and Evolution*, **2**, 763–766.

Paquette, A., Joly, S. & Messier, C. (2015) Explaining forest productivity using tree functional traits and phylogenetic information: two sides of the same coin over evolutionary scale? *Ecology and Evolution*, **5**, 1774–1783.

Pearse, W.D., Cadotte, M.W., Cavender-Bares, J., Ives, A.R., Tucker, C.M., Walker, S.C. & Helmus, M.R. (2015) Pez: Phylogenetics for the environmental sciences. *Bioinformatics*, **31**, 2888–2890.

Pei, Z., Leppert, K.N., Eichenberg, D., Bruelheide, H., Niklaus, P.A., Buscot, F. & Gutknecht, J.L.M. (2017) Leaf litter diversity alters microbial activity, microbial abundances, and nutrient cycling in a subtropical forest ecosystem. *Biogeochemistry*, **134**, 163–181.

Pietsch, K.A., Ogle, K., Cornelissen, J.H.C., Cornwell, W.K., Bönisch, G., Craine, J.M., Jackson, B.G., Kattge, J., Peltzer, D.A., Penuelas, J., Reich, P.B., Wardle, D.A., Weedon, J.T., Wright, I.J., Zanne, A.E. & Wirth, C. (2014) Global relationship of wood and leaf litter decomposability: the role of functional traits within and across plant organs. *Global Ecology and Biogeography*, n/a-n/a.

Prober, S.M., Leff, J.W., Bates, S.T., Borer, E.T., Firn, J., Harpole, W.S., Lind, E.M., Seabloom, E.W., Adler, P.B., Bakker, J.D., Cleland, E.E., DeCrappeo, N.M., DeLorenze, E., Hagenah, N., Hautier, Y., Hofmockel, K.S., Kirkman, K.P., Knops, J.M.H., La Pierre, K.J., MacDougall, A.S., McCulley, R.L., Mitchell, C.E., Risch, A.C., Schuetz, M., Stevens, C.J., Williams, R.J. & Fierer, N. (2015) Plant diversity predicts beta but not alpha diversity of soil microbes across grasslands worldwide. *Ecology*

*Letters*, **18**, 85–95.

Prudent, M., Dequiedt, S., Sorin, C., Girodet, S., Nowak, V., Duc, G., Salon, C. & Maron, P.A. (2020) The diversity of soil microbial communities matters when legumes face drought. *Plant Cell and Environment*, **43**, 1023–1035.

Ratcliffe, S., Wirth, C., Jucker, T., van der Plas, F., Scherer-Lorenzen, M., Verheyen, K., Allan, E., Benavides, R., Bruelheide, H., Ohse, B., Paquette, A., Ampoorter, E., Bastias, C.C., Bauhus, J., Bonal, D., Bouriaud, O., Bussotti, F., Carnol, M., Castagneyrol, B., Chećko, E., Dawud, S.M., Wandeler, H. De, Domisch, T., Finér, L., Fischer, M., Fotelli, M., Gessler, A., Granier, A., Grossiord, C., Guyot, V., Haase, J., Hättenschwiler, S., Jactel, H., Jaroszewicz, B., Joly, F.X., Kambach, S., Kolb, S., Koricheva, J., Liebersgesell, M., Milligan, H., Müller, S., Muys, B., Nguyen, D., Nock, C., Pollastrini, M., Purschke, O., Radoglou, K., Raulund-Rasmussen, K., Roger, F., Ruiz-Benito, P., Seidl, R., Selvi, F., Seiferling, I., Stenlid, J., Valladares, F., Vesterdal, L. & Baeten, L. (2017) Biodiversity and ecosystem functioning relations in European forests depend on environmental context. *Ecology Letters*, **20**, 1414–1426.

Roscher, C., Gubsch, M., Lipowsky, A., Schumacher, J., Weigelt, A., Buchmann, N., Schulze, E.D. & Schmid, B. (2018) Trait means, trait plasticity and trait differences to other species jointly explain species performances in grasslands of varying diversity. *Oikos*, **127**, 865.

Scherer-Lorenzen, M., Schulze, E., Don, A., Schumacher, J. & Weller, E. (2007) Exploring the functional significance of forest diversity: A new long-term experiment with temperate tree species (BIOTREE). *Perspectives in Plant Ecology, Evolution and Systematics*, **9**, 53–70.

Scheu, S. (1992) Automated measurement of the respiratory response of soil microcompartments: active microbial biomass in earthworm faces. *Soil Biology and Biochemistry*, **24**, 1113–1118.

Schimel, J.P. (2018) Life in Dry Soils: Effects of Drought on Soil Microbial Communities and Processes. *Annual Review of Ecology, Evolution, and Systematics*, **49**, 409–432.

Serna-Chavez, H.M., Fierer, N. & van Bodegom, P.M. (2013) Global drivers and patterns of microbial abundance in soil. *Global Ecology and Biogeography*, **22**, 1162–1172.

Singh, B.K., Dawson, L.A., Macdonald, C.A. & Buckland, S.M. (2009) Impact of biotic and abiotic interaction on soil microbial communities and functions: A field study. *Applied Soil Ecology*, **41**, 239–248.

Smith, L.C., Orgiazzi, A., Eisenhauer, N., Cesarz, S., Lochner, A., Jones, A., Bastida, F., Patoine, G., Reitz, T., Buscot, F., Rillig, M.C., Heintz-, A., Lehmann, A. & Guerra, C.A. (2021) Large- scale drivers of relationships between soil microbial properties and organic carbon across Europe. *Global Ecology and Biogeography*, 1–14.

Soong, J.L., Fuchslueger, L., Marañon-Jimenez, S., Torn, M.S., Janssens, I.A., Penuelas, J. & Richter, A. (2020) Microbial carbon limitation: The need for integrating microorganisms into our understanding of ecosystem carbon cycling. *Global Change Biology*, **26**, 1953– 1961.

Strukelj, M., Parker, W., Corcket, E., Augusto, L., Khlifa, R., Jactel, H. & Munson, A.D. (2021) Tree species richness and water availability interact to affect soil microbial processes. *Soil Biology and Biochemistry*, **155**.

Tedersoo, L., Bahram, M., Cajthaml, T., Põlme, S., Hiiesalu, I., Anslan, S., Harend, H., Buegger, F., Pritsch, K., Koricheva, J. & Abarenkov, K. (2016) Tree diversity and species identity effects on soil fungi, protists and animals are context dependent. *The ISME Journal*, **10**, 346–362.

Thakur, M.P., Milcu, A., Manning, P., Niklaus, P.A., Roscher, C., Power, S., Reich, P.B., Scheu, S., Tilman, D., Ai, F., Guo, H., Ji, R., Pierce, S., Ramirez, N.G., Richter, A.N., Steinauer, K., Strecker, T., Vogel, A. & Eisenhauer, N. (2015) Plant diversity drives soil microbial biomass carbon in grasslands irrespective of global environmental change factors. *Global Change Biology*, **21**, 4076–4085.

Thakur, M.P., Reich, P.B., Hobbie, S.E., Stefanski, A., Rich, R., Rice, K.E., Eddy, W.C. & Eisenhauer, N. (2018) Reduced feeding activity of soil detritivores under warmer and drier conditions. *Nature Climate Change*, **8**, 75–78.

Tucker, C.M., Davies, T.J., Cadotte, M.W. & Pearse, W.D. (2018) On the relationship between phylogenetic diversity and trait diversity. *Ecology*, **99**, 1473–1479.

Verheyen, K., Vanhellemont, M., Auge, H., Baeten, L., Baraloto, C., Barsoum, N., Bilodeau-Gauthier, S., Bruelheide, H., Castagneyrol, B., Godbold, D., Haase, J., Hector, A., Jactel, H., Koricheva, J., Loreau, M., Mereu, S., Messier, C., Muys, B., Nolet, P., Paquette, A., Parker, J., Perring, M., Ponette, Q., Potvin, C., Reich, P., Smith, A., Weih, M. & Scherer-Lorenzen, M. (2016) Contributions of a global network of tree diversity experiments to sustainable forest plantations. *Ambio*, **45**, 29–41.

Wall, D.H., Nielsen, U.N. & Six, J. (2015) Perspective Soil biodiversity and human health. *Nature*, **528**, 69–76.

Wan, X., Chen, X., Huang, Z. & Chen, H.Y.H. (2021) Global soil microbial biomass decreases with aridity and land-use intensification. *Global Ecology and Biogeography*, **30**, 1056–1069.

Xu, S., Eisenhauer, N., Ferlian, O., Zhang, J., Zhou, G., Lu, X., Liu, C. & Zhang, D. (2020) Species richness promotes ecosystem carbon storage: Evidence from biodiversity-ecosystem functioning experiments: Species richness promotes C storage. *Proceedings of the Royal Society B: Biological Sciences*, **287**.

Xu, X., Schimel, J.P., Janssens, I.A., Song, X., Song, C., Yu, G., Sinsabaugh, R.L., Tang, D., Zhang, X. & Thornton, P.E. (2017) Global pattern and controls of soil microbial metabolic quotient. *Ecological Monographs*, **87**, 429–441.

Xu, X., Thornton, P.E. & Post, W.M. (2013) A global analysis of soil microbial biomass carbon, nitrogen and phosphorus in terrestrial ecosystems. *Global Ecology and Biogeography*, **22**, 737–749.

Zanne, A.E., Tank, D.C., Cornwell, W.K., Eastman, J.M., Smith, S.A., Fitzjohn, R.G., McGlinn, D.J., O'Meara, B.C., Moles, A.T., Reich, P.B., Royer, D.L., Soltis, D.E., Stevens, P.F., Westoby, M., Wright, I.J., Aarssen, L., Bertin, R.I., Calaminus, A.,

Govaerts, R., Hemmings, F., Leishman, M.R., Oleksyn, J., Soltis, P.S., Swenson, N.G., Warman, L. & Beaulieu, J.M. (2013) Data from: Three keys to the radiation of angiosperms into freezing environments. *Dryad Digital Repository*, **https://do**.

Zuur, A.F., Ieno, E.N. & Elphick, C.S. (2010) A protocol for data exploration to avoid common statistical problems. *Methods in Ecology and Evolution*, **1**, 3–14.

## Data availability statement

The data that support the findings of this study are openly available in DRYAD at https://doi.org/10.5061/dryad.wm37pvmnn

## Biosketch

The research team is part of TreeDivNet a large network of tree diversity experiments investigating how different facets of tree diversity affects ecosystem functioning in major forest types around the world. http://www.treedivnet.ugent.be/

# Tables

**Table 1.** List of tree diversity experiments that contributed to the study (alphabetical order) to investigate abiotic and biotic drivers of soil microbial functions. All experiments differ in their plot architecture, as indicated by different numbers of diversity levels and the gradient of diversity. Further, experiments differ in experimental age (in years), number of sites and blocks, as well as in plot size, tree distance, tree density, and species pool. For the BEF-China and Sabah experiment, only a fraction of the whole diversity gradient was sampled (the respective missing richness levels are indicated by square brackets). The BIOTREE-FD experiment has only one species richness level (with four species per plot), but mixtures differ in their functional diversity (FD; indicated by a * in the Table). The number of plots only considers plots that entered the analysis, i.e., controls without trees and plots with missing measurements were excluded. The total number of existing plots is given in square brackets. A list of the full references is found in Appendix 1.

| Experi-ment | Country | Biome | Age (y) | Altitude (m) | Former land use | n sites | n blocks | n diversity levels | Species richness levels | Plot size (m²) | n plots | Minimal tree distance (m) | Tree density (trees m⁻²) | Reference |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Bangor | UK | temperate | 9 | 1 | forest | 1 | 2 | 3 | 1, 2, 3 | from 45 to 196 | 80 [92] | 1 | 1.0 | http://www.treedivnet.ugent.be/ExpBangor.html |
| BEF-China | China | subtropical | 4 | 190 | forest | 2 | NA | 5 | 1, 2, 4, 8, 16, [24] | 666.6 | 60 [566] | 1.29 | 0.6 | Bruelheide et al., 2014 |
| BIOTREE-FD | Germany | temperate | 10 | 400-415 | pasture | 1 | 4 | 4* | 4 (+FD) | 1700 | 24 [25] | 1 | 0.7 | Scherer-Lorenzen et al., 2007b |
| FORBIO | Belgium | temperate | 3 | 398, 56, 13 | forest, arable land | 3 | 6 | 4 | 1, 2, 3, 4 | 1764 | 126 [127] | 1.5 | 0.4 | Verheyen et al., 2013 |
| IDENT Auclair | Canada | temperate | 3 | 333 | pasture | 1 | 4 | 3 | 1, 2, 6 | 14.4 | 187 [192] | 0.4 | 8.2 | Tobner et al., 2013 |
| IDENT Cloquet | USA | temperate | 3 | 383 | forest | 1 | 4 | 3 | 1, 2, 6 | 14.4 | 190 [192] | 0.4 | 8.2 | Tobner et al., 2013 |
| Kreinitz | Germany | temperate | 8 | 115 | agricultural | 1 | 2 | 5 | 1, 2, 3, 5, 6 | 25 | 96 [98] | 0.8 | 1.2 | Hantsch et al., 2014 |
| ORPHEE | France | temperate | 5 | 60 | forest | 1 | 2 [8] | 5 | 1, 2, 3, 4, 5 | 400 | 61 [256] | 2 | 0.3 | Castagneyrol et al., 2013 |
| Sabah | Borneo | tropical | 11 | 102 | forest | 1 | NA [2] | 2 | 1, [4,] 16 | 40000 | 27 [124] | 3 | 0.003 | Hector et al., 2011 |
| Sardinilla | Panama | tropical | 10 | 70 | forest | 2 | 6 | 3 | 1, 2, 5, 9, 18 | 2025 | 46 [46] | 3 | 0.1 | Scherer-Lorenzen et al., 2007a |
| Satakunta | Finland | boreal | 14 | 35 | forest | 3 | NA | 4 | 1, 2, 3, 5 | 400 | 113 [163] | 1.5 | 0.4 | Vehviläinen & Koricheva, 2006 |
| **Mean± SD** | | | **7.2 ± 3.9** | **183.6 ± 163.8** | | | | **3.7 ± 1.0** | | **3937.5 ± 11381.2** | **total : 1010** | **1.6 ± 0.9** | **1.1 ± 1.2** | |

**Table 2:** ANOVA table of linear mixed effects models testing the effect of biotic and abiotic factors on three microbial properties in 11 tree diversity experiments. All variables were scaled. NumDF: numerator degrees of freedom, DenDF: denumerator degrees of freedom. SR = tree species richness, MNTD = mean nearest taxonomic distance, SWC = soil water content. Significant effects (P< 0.05) are highlighted in bold.

| Fixed factor | NumDF | Basal respiration | | | Microbial biomass | | | Metabolic quotient | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | DenDF | F value | Pr(>F) | DenDF | F value | Pr(>F) | DenDF | F value | Pr(>F) |
| scaled log SR | 1 | 950.92 | 0.49 | 0.485 | 946.76 | 0.28 | 0.594 | 855.34 | 0.01 | 0.937 |
| scaled MNTD | 1 | 948.32 | 0.96 | 0.328 | 944.48 | 0.41 | 0.521 | 964.10 | 2.02 | 0.156 |
| scaled log SWC | 1 | 897.22 | 120.46 | **<.001** | 969.62 | 108.42 | **<.001** | 328.67 | 6.26 | **0.013** |
| scaled soil C | 1 | 20.55 | 0.90 | 0.354 | 24.12 | 2.54 | 0.124 | 6.49 | 1.76 | 0.230 |
| scaled soil pH | 1 | 18.05 | 0.56 | 0.464 | 43.15 | 5.76 | **0.021** | 14.79 | 38.00 | **<.001** |
| scaled log SR : scaled log SWC | 1 | 954.09 | 3.37 | 0.067 | 948.70 | 0.96 | 0.327 | 714.17 | 1.24 | 0.265 |
| scaled log SR : scaled soil C | 1 | 945.70 | 0.36 | 0.550 | 945.07 | 0.40 | 0.526 | 956.39 | 1.66 | 0.198 |
| scaled log SR : scaled soil pH | 1 | 968.33 | 0.01 | 0.936 | 965.28 | 0.61 | 0.435 | 653.79 | 0.63 | 0.426 |
| scaled MNTD : scaled log SWC | 1 | 957.07 | 31.44 | **<.001** | 951.79 | 6.16 | **0.013** | 963.34 | 12.93 | **<.001** |
| scaled MNTD : scaled soil C | 1 | 949.95 | 7.04 | **0.008** | 945.72 | 1.49 | 0.223 | 961.91 | 2.17 | 0.141 |
| scaled MNTD : scaled soil pH | 1 | 953.69 | 0.19 | 0.663 | 951.73 | 0.02 | 0.891 | 960.43 | 0.11 | 0.740 |
| scaled log SWC : scaled soil C | 1 | 780.09 | 21.30 | **<.001** | 819.94 | 37.87 | **<.001** | 129.03 | 9.27 | **0.003** |
| scaled log SWC : scaled soil pH | 1 | 916.36 | 0.50 | 0.481 | 889.17 | 20.53 | **<.001** | 90.30 | 6.85 | **0.010** |
| scaled soil C : scaled soil pH | 1 | 14.98 | 2.34 | 0.147 | 16.70 | 0.12 | 0.729 | 2.97 | 1.57 | 0.300 |

**Table 3:** AICc and $R^2$ for mixed-effects models for three microbial properties as affected by tree diversity and abiotic factors in 11 tree diversity experiments. df: degrees of freedom.

| Response variable | df | AICc | $R^2$ fixed (marginal) | $R^2$ random (conditional) |
|---|---|---|---|---|
| Basal respiration | 19 | 1522.34 | 0.33 | 0.78 |
| Microbial biomass | 19 | 979.26 | 0.17 | 0.90 |
| Respiratory quotient | 19 | 2065.99 | 0.38 | 0.69 |

# Figures
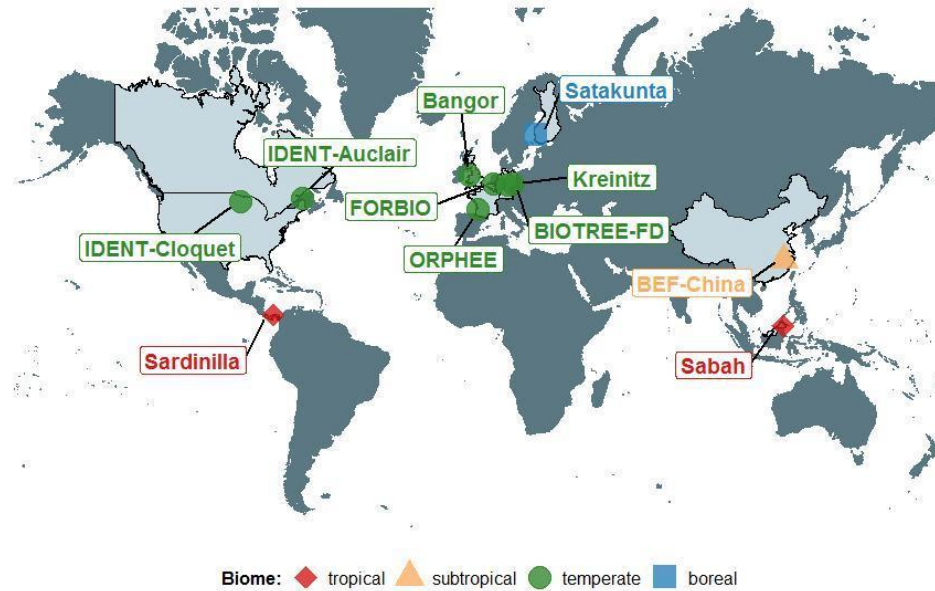


**Figure 1:** Locations of the eleven tree diversity experiments and assignments to biomes (Olsen et al., 2001). Details on the locations and experimental designs are shown in Table 1.
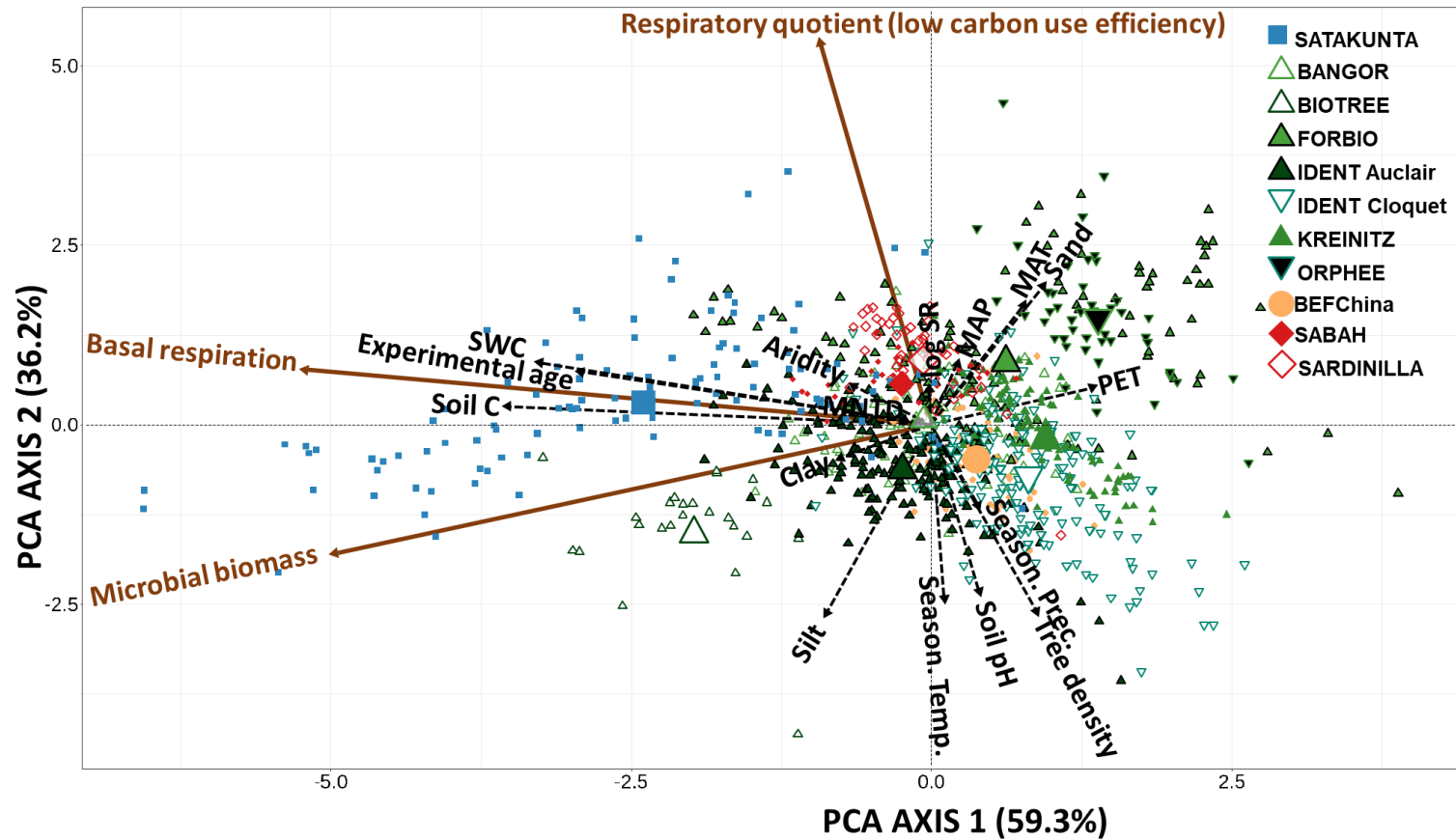
**Figure 2:** Principal component analysis with the three microbial properties in focus (given in brown: basal respiration, microbial biomass, and the respiratory quotient), as well as the abiotic variables soil water content (SWC), soil C, sand, silt, clay, seasonality of temperature (Season. Temp) and precipitation (Season. Prec), soil pH, potential evapotranspiration (PET), mean annual temperature (MAT), mean annual precipitation (MAP), aridity, and biotic variables experimental age, tree density, tree species richness (log SR) and mean nearest taxonomic distance (MNTD) in eleven tree diversity experiments in boreal (blue, n = 1), temperature (green, n = 7), subtropical (yellow, n = 1), and tropical (red, n = 2) biomes. All variables were scaled. Percent values in brackets give the variance explained by the different PCA axis. Large symbols represent the centroids of the samples for each experiment. Correlations between moderators and the principal components can be found in Table S2.
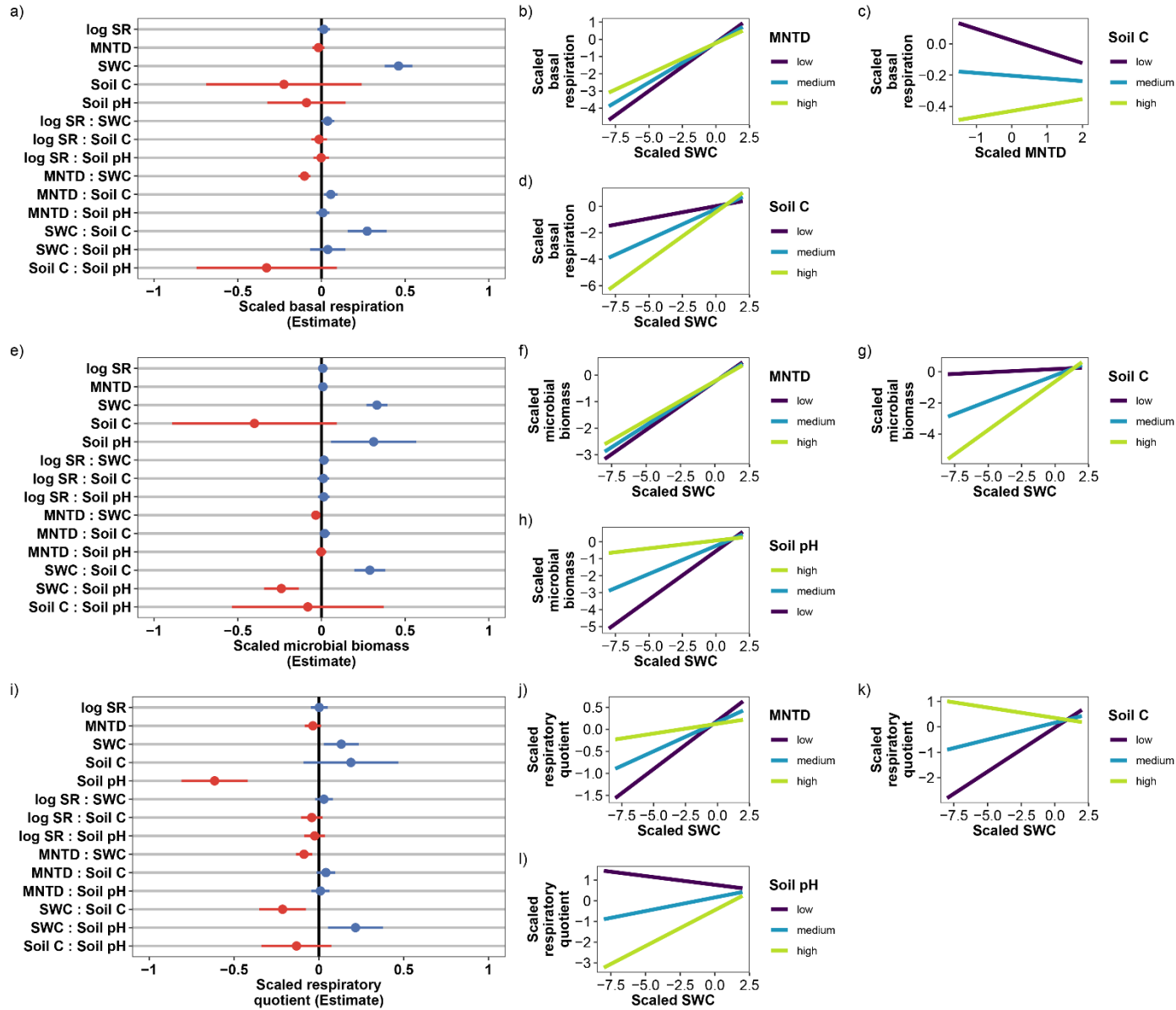
**Figure 3:** Coefficient estimates of linear mixed-effects models for three soil microbial properties (basal respiration, microbial biomass, and the respiratory quotient) as affected by two tree diversity metrics, namely tree species richness (SR) and mean nearest taxonomic distance (MNTD), and abiotic variables affecting soil microbial properties the most, i.e., soil water content (SWC), soil C, and soil pH. The blue color indicates a positive effect of the fixed factor on the response variable, whereas red indicates a negative effect. For significant interactions (not crossing the zero line), interaction plots were given as smaller panels, where one of the variables was categorized into low, medium, and high levels.

**Supplementary Information**

**Appendix S1 - List of tree species occurring in the tree diversity experiments studied**

**Appendix S2 - Correlations**

**Appendix S3 - piecewise SEM**

**Appendix S4 - Single experiments**

## Appendix S1: List of tree species occurring in the tree diversity experiments studied

**Table S1.1:** List of tree species occurring in the tree diversity experiments studied. * indicates that another tree species of the same genus was chosen to calculate phylogeny since no genetic information was available for the original tree species.

| Tree species name | Site (if applicable): x / Experiment: BANGOR | Site A / BEF CHINA | Site B / BEF CHINA | x / BIOTREE-FD | Gedinne / FORBIO | Hechtel / FORBIO | Zedelgem / FORBIO | Auclair / IDENT | Cloquet / IDENT | x / KREINITZ | x / ORPHEE | x / SABAH | Main plantation / SARDINILLA | High diversity plantation / SARDINILLA | Area 1 / SATAKUNTA | Area 2 / SATAKUNTA | Area 3 / SATAKUNTA | Overall species occurrence |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Acer campestre* | | | | 1 | | | | | | | | | | | | | | 1 |
| *Acer platanoides* | | | | 1 | | | | 1 | 1 | | | | | | | | | 3 |
| *Acer pseudoplatanus* | 1 | | | 1 | 1 | | | | | | | | | | | | | 3 |
| *Acer saccharum* | | | | | | | | 1 | 1 | | | | | | | | | 2 |
| *Ailanthus altissima* | | | 1 | | | | | | | | | | | | | | | 1 |
| *Albizia adinocephala* | | | | | | | | | | | | | | 1 | | | | 1 |
| *Alniphyllum fortunei* | | | 1 | | | | | | | | | | | | | | | 1 |
| *Alnus glutinosa* | 1 | | | | | | | | | | | | | | 1 | 1 | 1 | 4 |
| *Anacardium excelsum* | | | | | | | | | | | | | 1 | 1 | | | | 2 |
| *Astronium graveolens* | | | | | | | | | | | | | | 1 | | | | 1 |
| *Betula luminifera* | | | 1 | | | | | | | | | | | | | | | 1 |
| *Betula papyrifera* | | | | | | | | 1 | 1 | | | | | | | | | 2 |
| *Betula pendula* | 1 | | | 1 | | 1 | 1 | 1 | 1 | | 1 | | | | 1 | 1 | 1 | 10 |
| *Bursera simaruba* | | | | | | | | | | | | | | 1 | | | | 1 |
| *Calycophyllum candidissimum* | | | | | | | | | | | | | | 1 | | | | 1 |
| *Carpinus betulus* | | | | 1 | | | | | | | | | | | | | | 1 |
| *Castanea henryi* | | 1 | | | | | | | | | | | | | | | | 1 |
| *Castanea sativa* | 1 | | | | | | | | | | | | | | | | | 1 |
| *Castanopsis eyrei* | | 1 | | | | | | | | | | | | | | | | 1 |
| *Castanopsis fargesii* | | | 1 | | | | | | | | | | | | | | | 1 |
| *Castanopsis sclerophylla* | | 1 | | | | | | | | | | | | | | | | 1 |
| *Cedrela odorata* | | | | | | | | | | | | | 1 | 1 | | | | 2 |
| *Celtis biondii* | | | 1 | | | | | | | | | | | | | | | 1 |

Drivers of soil microbial functions

| Species | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Choerospondias axillaris* | | | 1 | | | | | | | | | | | | | | | 1 |
| *Colubrina glandulosa* | | | | | | | | | | | | | | 1 | | | | 1 |
| *Cordia alliodora* | | | | | | | | | | | | | 1 | 1 | | | | 2 |
| *Dalbergia retusa** | | | | | | | | | | | | | | 1 | | | | 1 |
| *Diphysa americana* | | | | | | | | | | | | | | 1 | | | | 1 |
| *Dipterocarpus conformis* | | | | | | | | | | | | 1 | | | | | | 1 |
| *Dipteryx oleifera* | | | | | | | | | | | | | | 1 | | | | 1 |
| *Dryobalanops lanceolata* | | | | | | | | | | | | 1 | | | | | | 1 |
| *Elaeocarpus chinensis* | | | | 1 | | | | | | | | | | | | | | 1 |
| *Elaeocarpus glabripetalus* | | | | 1 | | | | | | | | | | | | | | 1 |
| *Elaeocarpus japonicus* | | | | 1 | | | | | | | | | | | | | | 1 |
| *Enterolobium cyclocarpum* | | | | | | | | | | | | | | 1 | | | | 1 |
| *Erythrina fusca* | | | | | | | | | | | | | | 1 | | | | 1 |
| *Fagus sylvatica* | | 1 | | | 1 | 1 | | 1 | | 1 | | | | | | | | 5 |
| *Fraxinus excelsior* | | 1 | | | 1 | | | | | 1 | | | | | | | | 3 |
| *Gliricidia sepium* | | | | | | | | | | | | | | 1 | | | | 1 |
| *Guazuma ulmifolia* | | | | | | | | | | | | | | 1 | | | | 1 |
| *Hopea ferruginea** | | | | | | | | | | | | 1 | | | | | | 1 |
| *Hopea sangal** | | | | | | | | | | | | 1 | | | | | | 1 |
| *Hura crepitans* | | | | | | | | | | | | | 1 | 1 | | | | 2 |
| *Hymenaea courbaril* | | | | | | | | | | | | | | 1 | | | | 1 |
| *Idesia polycarpa* | | | | 1 | | | | | | | | | | | | | | 1 |
| *Inga punctata* | | | | | | | | | | | | | | 1 | | | | 1 |
| *Koelreuteria bipinnata* | | | 1 | | | | | | | | | | | | | | | 1 |
| *Larix decidua* | | | | | 1 | | | 1 | 1 | | | | | | | | | 3 |
| *Larix kaempferi* | | | | | | 1 | | | | | | | | | | | | 1 |
| *Larix laricina* | | | | | | | | 1 | 1 | | | | | | | | | 2 |
| *Larix sibirica* | | | | | | | | | | | | | | | 1 | 1 | 1 | 3 |
| *Larix x eurolepis** | | | | | | 1 | | | | | | | | | | | | 1 |
| *Liquidambar formosana* | | | 1 | | | | | | | | | | | | | | | 1 |
| *Lithocarpus glaber* | | | 1 | | | | | | | | | | | | | | | 1 |
| *Luehea seemannii* | | | | | | | | | | | | | 1 | 1 | | | | 2 |
| *Machilus grijsii* | | | | 1 | | | | | | | | | | | | | | 1 |
| *Machilus leptophylla* | | | | 1 | | | | | | | | | | | | | | 1 |
| *Machilus thunbergii* | | | | 1 | | | | | | | | | | | | | | 1 |
| *Magnolia yuyuanensis* | | | | 1 | | | | | | | | | | | | | | 1 |
| *Meliosma flexuosa* | | | | 1 | | | | | | | | | | | | | | 1 |
| *Nyssa sinensis* | | | 1 | | | | | | | | | | | | | | | 1 |
| *Ormosia macrocalyx* | | | | | | | | | | | | | | 1 | | | | 1 |
| *Pachira quinata* | | | | | | | | | | | | | | 1 | | | | 1 |
| *Parashorea malaanonan** | | | | | | | | | | | | 1 | | | | | | 1 |

Drivers of soil microbial functions

| Species | | | | | | | | | | | | | | | | | | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Parashorea tomentella** | | | | | | | | | | | | 1 | | | | | | 1 |
| *Phoebe bournei* | | | 1 | | | | | | | | | | | | | | | 1 |
| *Picea abies* | | | | | | | | 1 | 1 | 1 | | | | | 1 | 1 | 1 | 6 |
| *Picea glauca* | | | | | | | | 1 | 1 | | | | | | | | | 2 |
| *Pinus pinaster* | | | | | | | | | | | 1 | | | | | | | 1 |
| *Pinus strobus* | | | | | | | | 1 | 1 | | | | | | | | | 2 |
| *Pinus sylvestris* | | | | 1 | | 1 | 1 | 1 | 1 | 1 | | | | | 1 | 1 | 1 | 9 |
| *Populus tremula* | | | | 1 | | | | | | | | | | | | | | 1 |
| *Prunus avium* | | | | 1 | | | | | | | | | | | | | | 1 |
| *Pseudosamanea cubana** | | | | | | | | | | | | | | 1 | | | | 1 |
| *Pseudotsuga menziesii* | | | | | 1 | 1 | | | | | | | | | | | | 2 |
| *Quercus fabrei* | | 1 | | | | | | | | | | | | | | | | 1 |
| *Quercus glauca* | | 1 | | | | | | | | | | | | | | | | 1 |
| *Quercus ilex* | | | | | | | | | | | 1 | | | | | | | 1 |
| *Quercus myrsinifolia* | | 1 | | | | | | | | | | | | | | | | 1 |
| *Quercus petraea* | | | | 1 | 1 | 1 | | | | 1 | | | | | | | | 4 |
| *Quercus phillyreoides* | | | 1 | | | | | | | | | | | | | | | 1 |
| *Quercus pyrenaica* | | | | | | | | | | | 1 | | | | | | | 1 |
| *Quercus robur* | 1 | | | | | | 1 | 1 | 1 | | 1 | | | | | | | 5 |
| *Quercus rubra* | | | | | | | | 1 | 1 | | | | | | | | | 2 |
| *Quercus serrata* | | 1 | | | | | | | | | | | | | | | | 1 |
| *Rhus chinensis* | | 1 | | | | | | | | | | | | | | | | 1 |
| *Sapindus mukorossi* | | 1 | | | | | | | | | | | | | | | | 1 |
| *Sapium sebiferum* | | 1 | | | | | | | | | | | | | | | | 1 |
| *Schima superba* | | 1 | | | | | | | | | | | | | | | | 1 |
| *Shorea argentifolia** | | | | | | | | | | | | 1 | | | | | | 1 |
| *Shorea beccariana** | | | | | | | | | | | | 1 | | | | | | 1 |
| *Shorea faguetiana** | | | | | | | | | | | | 1 | | | | | | 1 |
| *Shorea gibbosa** | | | | | | | | | | | | 1 | | | | | | 1 |
| *Shorea johorensis** | | | | | | | | | | | | 1 | | | | | | 1 |
| *Shorea leprosula** | | | | | | | | | | | | 1 | | | | | | 1 |
| *Shorea macrophylla** | | | | | | | | | | | | 1 | | | | | | 1 |
| *Shorea macroptera** | | | | | | | | | | | | 1 | | | | | | 1 |
| *Shorea ovalis** | | | | | | | | | | | | 1 | | | | | | 1 |
| *Shorea parvifolia** | | | | | | | | | | | | 1 | | | | | | 1 |
| *Sorbus aucuparia* | | | | 1 | | | | | | | | | | | | | | 1 |
| *Sorbus torminalis* | | | | 1 | | | | | | | | | | | | | | 1 |
| *Spondias mombin* | | | | | | | | | | | | | | 1 | | | | 1 |
| *Tabebuia rosea* | | | | | | | | | | | | | 1 | 1 | | | | 2 |
| *Terminalia amazonia* | | | | | | | | | | | | | | 1 | | | | 1 |
| *Tilia cordata* | | | | 1 | | | 1 | | | 1 | | | | | | | | 3 |

38

| Ulmus glabra | | | | | 1 | | | | | | | | | | | | | | 1 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|

# Appendix S2 - Correlations

**Table S2.2:** Correlations of diversity metrics using Pearson correlation (cor). MPD: Mean phylogenetic diversity, MNTD: mean nearest taxon distance, log SR: log of species richness, SES indicates the standardized version of MPD and MNTD. The phylogenetic diversity metric that correlated less with log species richness is marked in bold and was used for further analyses.

| Row | Column | cor | p |
|---|---|---|---|
| SES.MPD | SES.MNTD | 1.00 | <0.001 |
| MPD | MNTD | 0.95 | 0.001 |
| MNTD | SES.MNTD | 0.71 | 0.183 |
| SES.MPD | MNTD | 0.70 | 0.184 |
| MPD | SES.MPD | 0.78 | 0.170 |
| MPD | SES.MNTD | 0.78 | 0.169 |
| log SR | MPD | 0.43 | 0.310 |
| **log SR** | **MNTD** | **0.25** | **0.295** |
| log SR | SES.MPD | -0.04 | 0.008 |
| log SR | SES.MNTD | -0.04 | 0.008 |

**Table S2.3.** Correlation between moderators (all scaled) and the principal components for the principal component analysis (PCA). The values for the first PCA axis (PC1) are sorted by their value/importance.

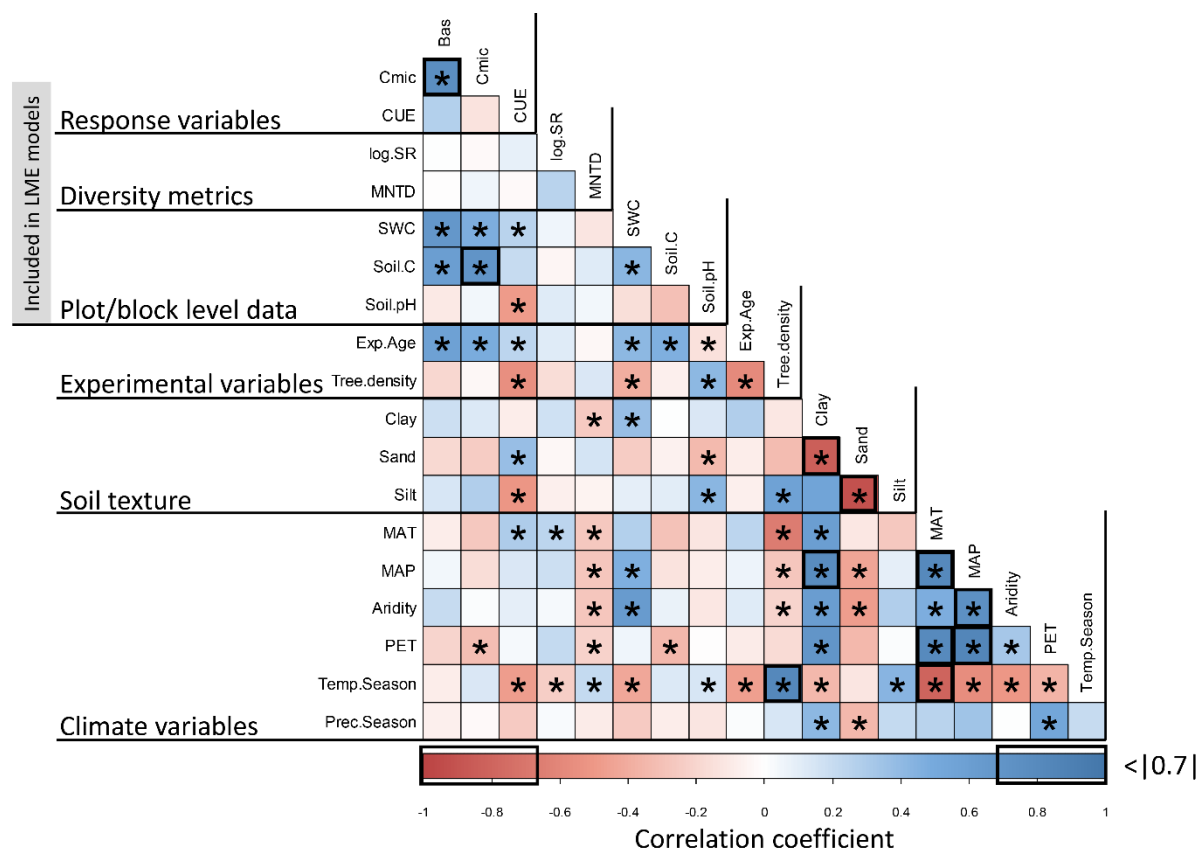| Moderator value | PC1 | PC2 |
| --- | --- | --- |
| Soil C | -0.71 | 0.05 |
| SWC | -0.65 | 0.17 |
| Experimental age | -0.58 | 0.15 |
| PET | 0.27 | 0.10 |
| Sand | 0.19 | 0.39 |
| Silt | -0.18 | -0.53 |
| Tree density | 0.17 | -0.52 |
| MAT | 0.16 | 0.33 |
| Clay | -0.15 | -0.09 |
| Aridity | -0.13 | 0.11 |
| Soil pH | 0.08 | -0.46 |
| Seasonality Precipitation | 0.08 | -0.23 |
| MAP | 0.04 | 0.18 |
| MNTD | -0.02 | -0.04 |
| Seasonality Temperature | 0.02 | -0.48 |
| log SR | 0.00 | 0.10 |

**Figure S2.1:** Correlation matrix of all variables that were used in the study. Response variables basal respiration (Bas), microbial biomass (Cmic), and carbon-use efficiency (CUE), the diversity metrics log species richness (log RS) and mean nearest taxonomic distance (MNTD), and the variables soil water content (SWC), soil C and soil pH were included in the linear-mixed effects models (LME). Correlation coefficients that exceed |0.7| were highlights by a black box. All variables were scaled. Correlation analyses are based on Pearson correlation. Asterisks indicate significant correlations at P<0.05, and colors indicate the direction of the effect with red highlighting negative correlations, blue positive correlations, and white neutral relationships. MAT: Mean annual temperature, MAP = mean annual precipitation, PET = Potential evapotranspiration. Temp.Season = seasonal variability of temperature, Prec.Season = seasonal variability of precipitation. Soil texture variables were extracted from SoilGRID and climate variables from the WorldClim database and CGIAR-CSI.

# Appendix S3 – piecewise SEM

Cesarz et al. Tree diversity effects on soil microbial biomass and respiration are context-dependent across forest diversity experiments

Table S2.2: Path coefficients of the piecewise SEM

Figure S2.1: Piecewise SEM

**Table S3.4:** Path coefficients of the piecewise SEM including the standardized values (scaled by standardized deviations). Significant paths are given in bold.

| Response | Predictor | Estimate | Std.Error | DF | Crit.Value | P.Value | Std.Estimate |
|---|---|---|---|---|---|---|---|
| SWC | Tree Density | -0.568 | 0.430 | 7 | -1.321 | 0.228 | -0.568 |
| SWC | PET | 0.000 | 0.001 | 7 | -0.149 | 0.886 | -0.042 |
| SWC | Sand | -0.309 | 0.336 | 7 | -0.921 | 0.388 | -0.309 |
| SWC | Soil pH | -0.007 | 0.128 | 19 | -0.055 | 0.956 | -0.007 |
| SWC | Soil C | -0.222 | 0.155 | 1994 | -1.430 | 0.169 | -0.222 |
| SWC | SR | -0.007 | 0.011 | 1941 | -0.670 | 0.503 | -0.007 |
| SWC | MNTD | -0.011 | 0.010 | 1 | -1.080 | 0.281 | -0.011 |
| Cmic | Tree Density | -0.096 | 0.272 | 7 | -0.352 | 0.735 | -0.096 |
| **Cmic** | **PET** | **-0.002** | **0.001** | **7** | **-3.179** | **0.016** | **-0.536** |
| Cmic | Sand | -0.415 | 0.184 | 7 | -2.252 | 0.059 | -0.415 |
| Cmic | Soil pH | 0.105 | 0.116 | 19 | 0.901 | 0.379 | 0.105 |
| Cmic | Soil C | 0.246 | 0.131 | 1994 | 1.886 | 0.075 | 0.246 |
| **Cmic** | **SWC** | **0.529** | **0.043** | **2** | **12.246** | **0.000** | **0.529** |
| Bas | Tree Density | -0.070 | 0.277 | 7 | -0.254 | 0.807 | -0.070 |
| Bas | PET | -0.002 | 0.001 | 7 | -2.145 | 0.069 | -0.386 |
| Bas | Sand | -0.336 | 0.212 | 7 | -1.584 | 0.157 | -0.336 |
| Bas | Soil pH | -0.147 | 0.122 | 19 | -1.199 | 0.245 | -0.147 |
| Bas | Soil C | 0.008 | 0.131 | 1994 | 0.064 | 0.950 | 0.008 |
| **Bas** | **SWC** | **0.659** | **0.051** | **2** | **12.979** | **0.000** | **0.659** |
| qO2 | Tree Density | -0.220 | 0.262 | 7 | -0.840 | 0.429 | -0.220 |
| qO2 | PET | 0.001 | 0.001 | 7 | 0.874 | 0.411 | 0.153 |
| qO2 | Sand | 0.298 | 0.213 | 7 | 1.397 | 0.205 | 0.298 |
| **qO2** | **Soil pH** | **-0.346** | **0.126** | **19** | **-2.743** | **0.013** | **-0.346** |
| qO2 | Soil C | 0.211 | 0.122 | 1994 | 1.723 | 0.101 | 0.211 |
| **qO2** | **SWC** | **0.173** | **0.068** | **2** | **2.555** | **0.011** | **0.173** |
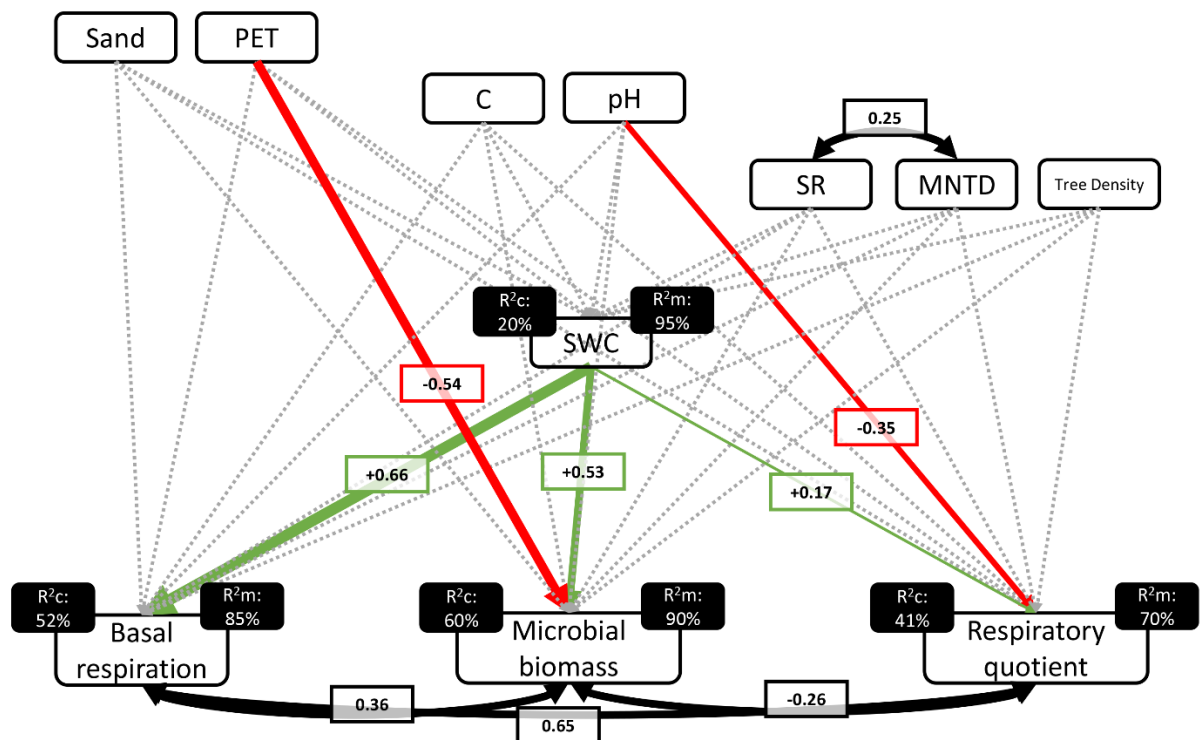
**Figure S3.2:** Piecewise Structural Equation Model (pSEM) of the effects of abiotic and biotic variables (SR, Tree species richness, MNTD: Mean nearest taxon Distance, Tree Density) on soil microbial properties (i.e., basal respiration, microbial biomass, and the respiratory quotient) across eleven tree diversity experiments. The selected variables reflect variables that represent climatic conditions (PET) and soil texture (sand content (%)). These variables were available at the experiment level. Further, experimental variables (tree density) and soil properties were included that were available at the plot level and were shown to have strong effects on soil microbial properties (SWC: soil water content, soil C, and soil pH). Due to high collinearity among climatic variables (MAT, MAP, Aridity, Seasonality), PET was selected as an integrative variable of all climatic variables. Further, sand was selected, as soil texture variables (sand, silt, clay) were generally highly correlated with each other. A detailed overview of the variables is provided in Figure S1. All variables were scaled. Double-headed errors indicate correlated errors that were accounted for. Solid green (positive) and solid red (negative) paths indicate significant effects, whereas grey dotted lines were non-significant. Numbers in boxes represent standardized coefficients. The size of the colored lines reflects the standardized coefficients. For readability, only significant coefficients were shown. An overview of all effects is given in Table pSEM. All variables were scaled. $R^2c$: conditional $R^2$. $R^2m$: marginal $R^2$. AIC: 99.89. Global goodness-of-fit: Fisher's C = 9.89 with P-value = 0.626 and on 12 degrees of freedom.

# Appendix S4 – Single experiments

Cesarz et al. Tree diversity effects on soil microbial biomass and respiration are context-dependent across forest diversity experiments

Table S4.4: $R^2$ and P values of simple linear regression models for each tree diversity experimental site for two diversity metric

Figure S4.3: The effect of log tree species richness and MNTD (Mean Nearest Taxonomic Difference) for three microbial properties in eleven tree diversity experiments

Figure S4.4: The effect of log soil water content on basal respiration and microbial biomass in eleven tree diversity experiments

Figure S4.5: The effect of soil carbon on basal respiration and microbial biomass in in eleven tree diversity experiments

**Table S4.4:** $R^2$ and P values of simple linear regression models for each tree diversity experimental site for two diversity metric (SR: species richness; MPD: mean phylogenetic distance) and three microbial properties (Bas: basal respiration, Cmic: Microbial biomass, $qO_2$: respiratory quotient). Experimental sites were listed seperately to account for different abiotic conditions. The BIOTREE-FD experiments only has a functional diversity gradient with a constant species richness level of 4.

| | SR | | | | | | MNTD | | | | | |
| | log.Bas | | Cmic | | log.qO$_2$ | | log.Bas | | Cmic | | log.qO$_2$ | |
| **Experiment (Site)** | **R2** | **p** | **R2** | **p** | **R2** | **p** | **R2** | **p** | **R2** | **p** | **R2** | **p** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Bangor | 0.03 | 0.115 | 0.02 | 0.229 | 0.00 | 0.714 | 0.00 | 1.00 | 0.01 | 0.44 | 0.01 | 0.34 |
| BEF-China (Site A) | 0.12 | 0.060 | 0.00 | 0.976 | **0.13** | **0.042** | 0.00 | 0.84 | 0.01 | 0.55 | 0.01 | 0.55 |
| BEF-China (Site B) | 0.02 | 0.524 | 0.02 | 0.453 | 0.00 | 0.901 | 0.02 | 0.47 | 0.00 | 0.96 | 0.02 | 0.50 |
| BIOTREE-FD | | | | | | | 0.09 | 0.15 | 0.00 | 0.95 | 0.09 | 0.16 |
| FORBIO (GE) | 0.00 | 0.852 | 0.00 | 1.000 | 0.00 | 0.678 | 0.02 | 0.34 | 0.01 | 0.49 | 0.01 | 0.56 |
| FORBIO (HE) | 0.03 | 0.280 | 0.00 | 0.820 | 0.03 | 0.272 | 0.02 | 0.44 | 0.00 | 0.99 | 0.00 | 0.83 |
| FORBIO (ZE) | 0.00 | 0.929 | 0.00 | 0.775 | 0.00 | 0.870 | 0.00 | 0.98 | 0.01 | 0.44 | 0.04 | 0.19 |
| IDENT (Auclair) | 0.00 | 0.948 | 0.00 | 0.978 | 0.00 | 0.988 | 0.00 | 0.44 | 0.00 | 0.83 | 0.01 | 0.23 |
| IDENT (Cloquet) | 0.00 | 0.912 | 0.00 | 0.488 | 0.00 | 0.487 | 0.01 | 0.17 | 0.00 | 0.59 | 0.01 | 0.31 |
| Kreinitz | 0.00 | 0.954 | 0.02 | 0.210 | 0.01 | 0.476 | 0.00 | 0.78 | 0.01 | 0.25 | 0.01 | 0.29 |
| ORPHEE | 0.05 | 0.104 | 0.01 | 0.535 | 0.04 | 0.193 | 0.04 | 0.15 | 0.00 | 0.73 | 0.09 | **0.04** |
| Sabah | 0.02 | 0.470 | 0.05 | 0.247 | 0.02 | 0.446 | 0.02 | 0.47 | 0.05 | 0.25 | 0.02 | 0.45 |
| Sardinilla | 0.11 | **0.023** | 0.00 | 0.702 | 0.08 | 0.052 | 0.03 | 0.26 | 0.00 | 0.79 | 0.04 | 0.20 |
| Satakunta (Area1) | 0.02 | 0.391 | 0.03 | 0.281 | 0.00 | 0.832 | 0.00 | 1.00 | 0.00 | 1.00 | 0.00 | 0.94 |
| Satakunta (Area2) | 0.02 | 0.417 | 0.00 | 0.936 | 0.02 | 0.454 | 0.01 | 0.59 | 0.05 | 0.16 | 0.02 | 0.43 |
| Satakunta (Area3) | 0.09 | 0.067 | 0.00 | 0.749 | 0.05 | 0.181 | 0.01 | 0.58 | 0.01 | 0.50 | 0.01 | 0.62 |
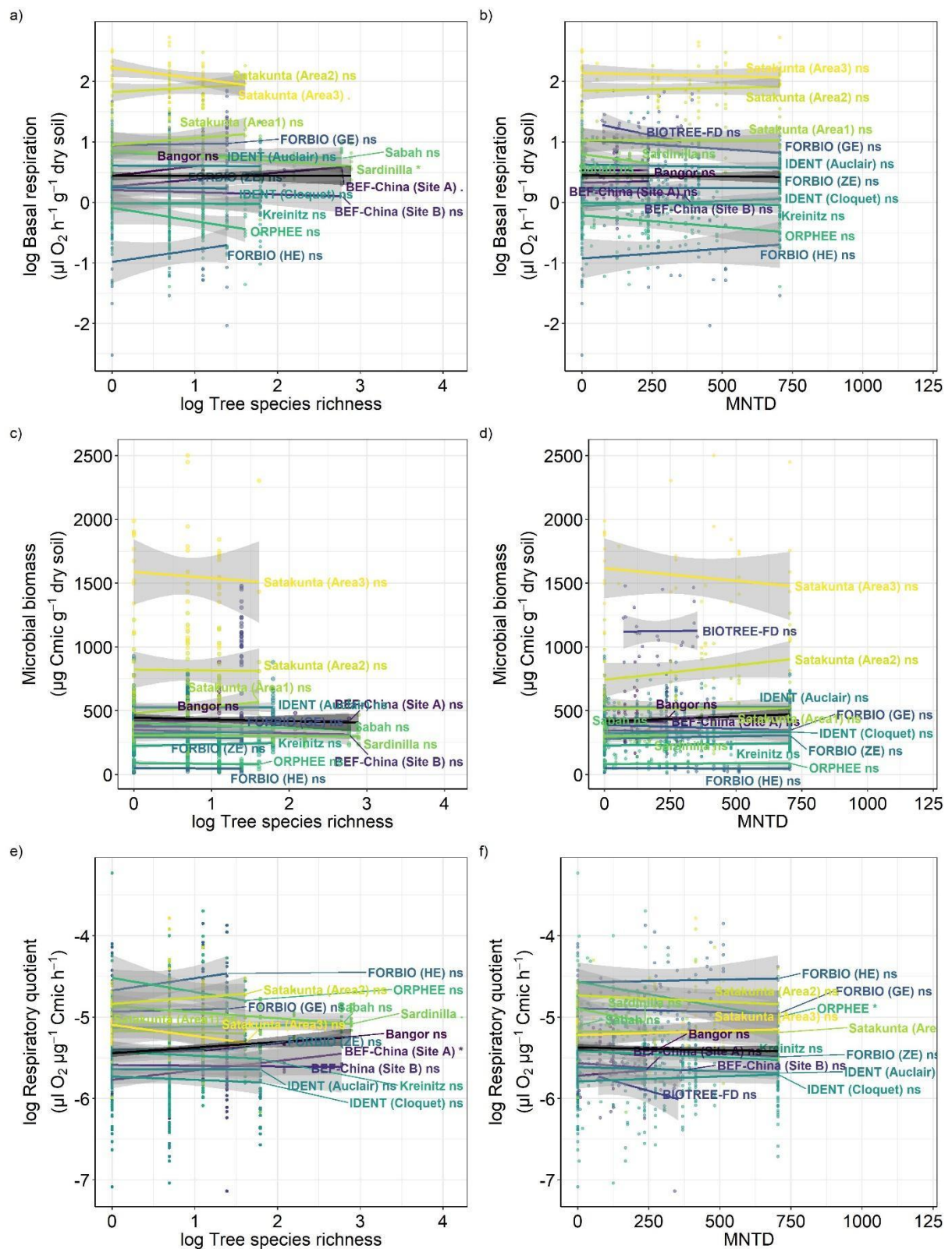
**Figure S4.3:** The effect of log tree species richness and MNTD (Mean Nearest Taxonomic

Difference) for three microbial properties in eleven tree diversity experiments. The black

lines give the overall mean of a linear regression, whereas colors highlight the single

experiments and, if applicable, specific sites within experiments are given in brackets.

Asterisks next to experiment names reflect the result of a linear regression with the

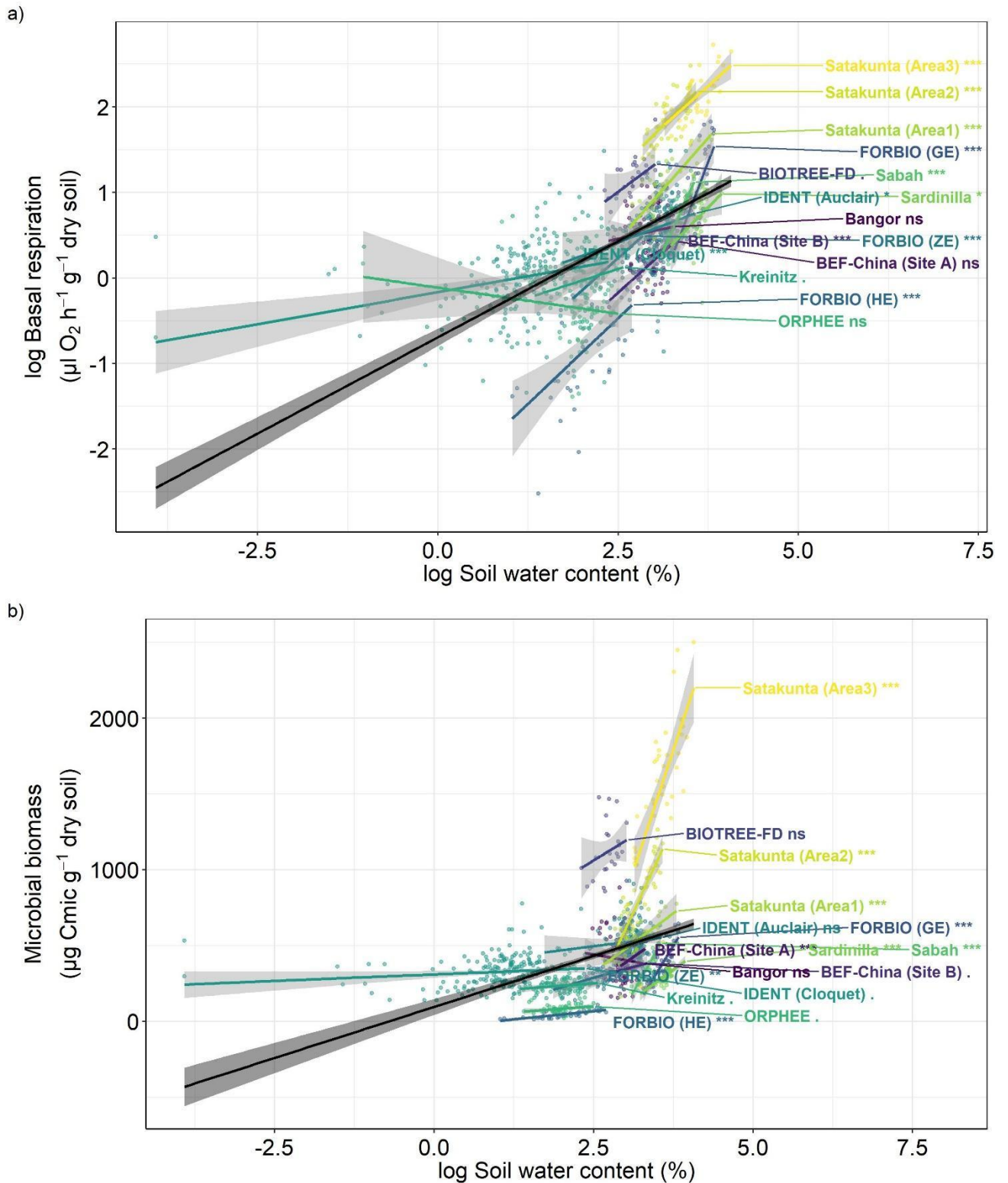significance levels and can be seen in Table S4.4:

```
***     P<0.001
**      P<0.01
*       P<0.05
.       P<0.1
ns      P>0.1
```
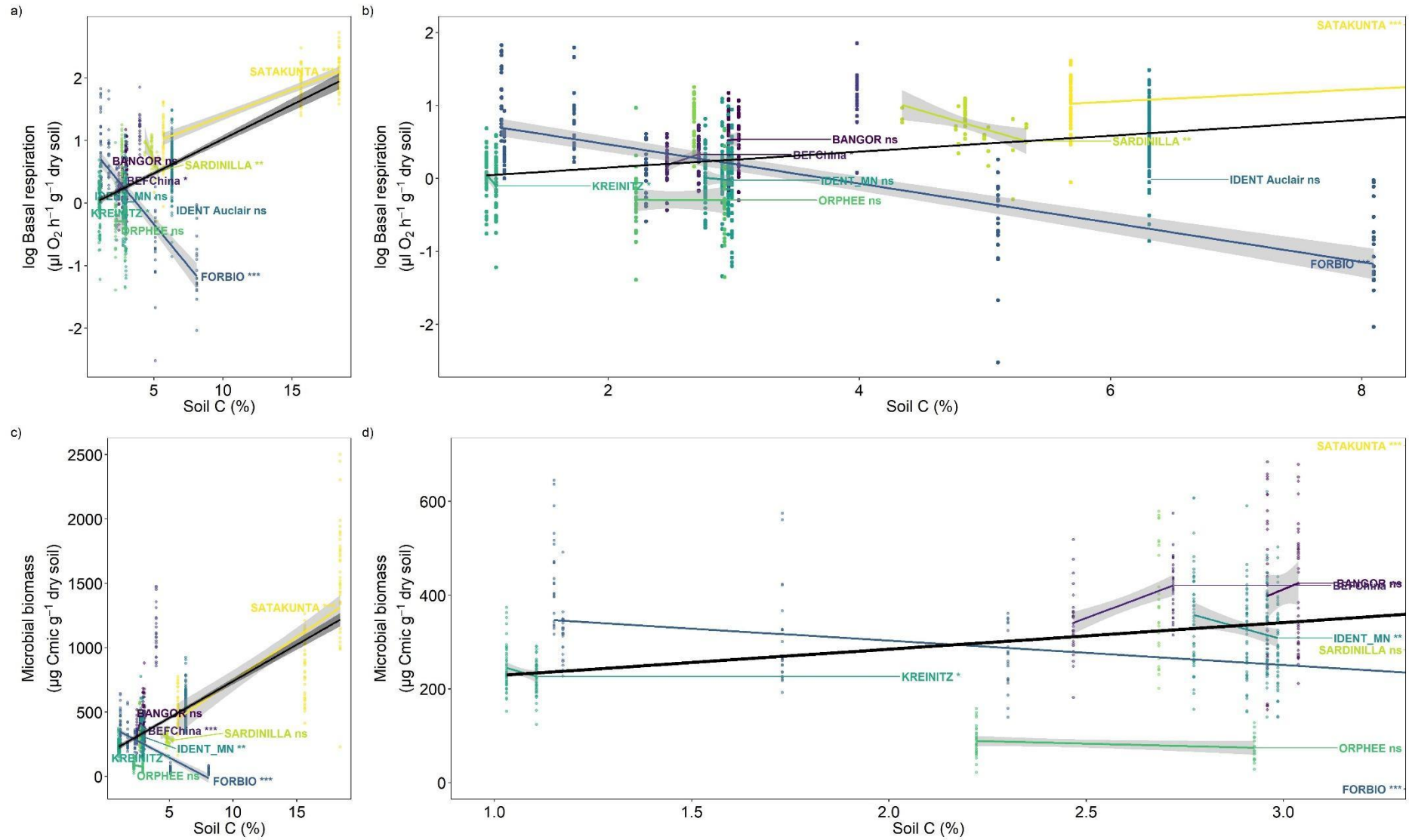
**Figure S4.4:** The effect of log soil water content (%) on a) basal respiration and b) microbial

biomass in eleven tree diversity experiments. The black lines give the overall mean of a linear

regression, whereas colors highlight the single experiments and, if applicable, specific sites

within experiments are given in brackets. Asterisks next to experiment names reflect the

result of a linear regression with the significance levels:

```
***     P<0.001
**      P<0.01
*       P<0.05
.       P<0.1
ns      P>0.1
```

**Figure S4.5:** The effect of soil carbon on a-b) basal respiration and c-d) microbial biomass in in eleven tree diversity experiments. The black

lines give the overall mean of a linear regression, whereas colors highlight the single experiments. The smaller plots provide an overview of all

experimental sites, whereas the large panels equalizes the application by focusing into the area with lower soil C, thus mainly excluding the

Satakunta experiment.. Asterisks next to experiment names reflect the result of a linear regression with the significance levels:

```
***     P<0.001
**      P<0.01
*       P<0.05
.       P<0.1
ns      P>0.1
```