# „tree species identity and tree-tree interaction effects on soil microbial biomass and basal respiration“

## Masterarbeit

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# 1. Introduction

All living organisms depend on the supply of essential elements, such as carbon (C), nitrogen (N) and phosphorus (P) (Gougoulias *et al.* 2014). A major part of the cycling of these elements depend on soils, such as forest soils (Lewis *et al.* 2019). Soil microorganisms play key roles in nutrient acquisition processes, carbon (Hogberg *et al.* 2001, Gougoulias *et al.* 2014) and nitrogen cycling (van der Heijden *et al.* 2008) and soil aggregation (Rillig & Mummey 2006). Soil microbial communities consist of two main groups who contribute differently to these ecosystem processes. Fungi act as obligate root symbionts, decomposers, pathogens of other organisms and are able to transform organic phosphorus to an inorganic state which can then be used by e.g. plants (Falkowski *et al.* 2008, Zhou *et al.* 2012). Soil bacteria fix atmospheric nitrogen, consume dead organic matter and disperse these degradation products as well as fungal and bacterial propagules in the soil (Wardle 2002, Adl & Gupta 2006, Waring *et al.* 2016). In general, fungi are more efficient at assimilating and storing nutrients than bacteria, due to their chemical composition of their cell walls. Which are composed of chitin and melanin polymers, making them very resistant to degradation. In comparison, bacterial membranes are made of phospholipids, which are energy-rich and degrade easily. This makes them a food source for a wide range of other microorganisms. The functioning of ecosystems depends on both fungi and bacteria as they are critical in the decomposition of organic matter and the cycling of nutrients in the soil. Microbial biomass and respiration can be used as indicators for nutrient cycling, soil organic matter turnover and secondary productivity (Crowther *et al.* 2019). It is therefore important to understand the drivers of soil microbial biomass and respiration as it can be a proxy for the functioning of the ecosystem.

On a global scale, microbial communities are largely controlled by abiotic conditions. In general, high soil moisture, neutral soil pH, a high soil organic carbon content as well as a low carbon to nitrogen ratio are the most important drivers. They all have been shown to increase soil microbial biomass and respiration directly (Fierer & Jackson 2006, Serna-Chavez *et al.* 2013, Maestre *et al.* 2015). It was shown that not only abundance, but also the diversity of soil bacteria and fungi were increased with higher soil moisture, due to the promotion of nutrient availability in the soils (Maestre *et al.* 2015). In contrast to these direct effects, soil microorganisms can also be indirectly affected by climatic conditions such as temperature. High temperatures may change the soil water content by evaporation and therefore change the soil microbial communities (Fierer *et al.* 2009, Serna-Chavez *et al.* 2013).

In terrestrial environments, nutrient-limitation is also considered a main driver of both plant and soil microbial community structure (Walker & Syers 1976, Vitousek & Howarth 1991). Mainly soil N and P availability were shown to limit soil microbial growth and activity (Castle *et al.* 2017). Tropical and subtropical forest soils are especially low in P, since it becomes increasingly sequestered with increased soil age, ongoing rock weathering and soil development as these ecosystems were not disturbed by glaciation (Walker & Syers 1976, Vitousek & Sanford 1986, Elser *et al.* 2007). Moreover, increasing N availability with N deposition may shift from N to P limitation in many forest ecosystems (Zeng and Wang 2015, Li *et al.* 2016, Camenzind *et al.* 2017). It was shown in a meta-analysis by Camenzind *et al.* (2017), that the addition of P had significantly positive effects on both soil microbial abundance and activity in tropical forest soils. This positive effect could be explained by the high demand of P in the high ribosome densities in microbial biomass (Hartman & Richardson 2013).

However, these effects of abiotic drivers can be altered by local biotic conditions and both could also interact to mediate soil microbial communities (De Deyn *et al.* 2008, Orwin *et al.* 2010, Yao *et al.* 2018). For example, plant species were shown to alter soil microbial communities by modifying pH (Wang *et al.* 2001, Thoms *et al.* 2010), soil moisture (Brussaard *et al.* 2007) or resource accessibility (Orwin *et al.* 2010). These resources can affect the abundance of soil microbial communities and their activity by the amount and quality of aboveground material (e.g. leaf litter) and belowground material (e.g. dead roots and root exudates) since a high heterogeneity of resources is associated with higher microbial biomass (Hooper *et al.* 2000, Wilkinson & Anderson 2001, Orwin *et al.* 2010. Sun *et al.* 2020). But plant species can also directly affect soil microorganisms (Scheibe *et al.* 2015, Pei *et al.* 2016, Habiyaremye *et al.* 2020) through their litter production, community composition, functional traits and interaction with root symbiotic organisms such as mycorrhizal fungi (Bauhus *et al.* 1998, Buckley & Schmidt 2002, Kao-Kniffin & Balser 2008, de Vries *et al.* 2012, Prescott & Grayston 2013). Especially, leaf litter traits of broadleaved and conifer tree species were shown to affect both the fungal and bacterial community differently (Chai *et al.* 2019) and were most pronounced in the top soil layer (Prescott & Grayston 2013). Also roots - especially those of trees - were reported to attract certain microbes from the local soil community, leading to an accumulation of soil-borne microbes in the rhizosphere (Singh *et al.* 2004, Broeckling *et al.* 2008, Berg & Smalla 2009, Habiyaremye *et al.* 2020). Differences in microbial communities in the rhizosphere were shown to be associated with tree species and also their mycorrhizal fungi associations (Prescott & Grayston 2013), which can be explained by the increased P and nutrients transfer by mycorrhizal fungi (Douglas 1995).

It was shown that plant species diversity positively affected soil microbial biomass and respiration in grasslands (Eisenhauer *et al.* 2010, Thoms *et al.* 2010, Thakur *et al.* 2015) with simultaneous effects on litter mineralization (Pei *et al.* 2016) and soil carbon storage (Lange *et al.* 2015). Plant diversity effects on microbial biomass increased with the species richness in mixtures, which stands in line with the pattern of the diversity-productivity relationship in the plant community (Liang *et al.* 2016). The reason for this could be the increased litter heterogeneity and higher quality of the resource input (Steinauer *et al.* 2016), as microbes benefit from different leaf litter chemicals and nutrients as well as root exudates (Hirsch *et al.* 2003). The increase of soil microbial biomass and respiration with an increased plant species diversity was shown to be consistent over many ecosystem types (Chen *et al.* 2019). However, most studies working on these effects take place in grasslands (Eisenhauer *et al.* 2010, Tedersoo *et al.* 2014, Thakur 2015) and only little is known about forest ecosystems.

Microbes have a great abundance in soils (Whitmann *et al.* 1998), and their large-scale distribution is well observed. At continental-scale, microbial diversity was unrelated to plant diversity or environmental factors such as temperature and latitude. Instead it was more dependent on soil pH (Fierer & Jackson 2006), whereas on landscape-scale microbial communities diversity was mainly determined by the local environmental factors such as climate and elevation (de Vries *et al.* 2012 and Lazzaro *et al.* 2015). On smaller scales, it was shown that soil depth is playing a major role, as with increasing depth the microbial biomass and basal respiration decrease e.g. in grasslands (Griffith *et al.* 2003 and Yao *et al.* 2018). This is caused by the decrease in plant root biomass, soil water content and nutrient availability (Engelhardt *et al.* 2018). Nutrients such as C are more present in the top soil layers where organic material is plentiful and gets decomposed (Prescott & Grayston 2013). As previous studies have shown, plants e.g. in meadows or grasslands (Eisenhauer *et al.* 2010, Tedersoo *et al.* 2014) can affect soil microbial properties, yet little is known about the effect of understory plant coverage in forests. Understory plants can increase the biodiversity within forests and might be able to change soil microbial properties locally.

A high microbial activity is important for the nutrient cycling in forests and it is necessary to understand the drivers of their spatial distribution in forest soils. To test for the effects of tree species diversity, identity, and tree-tree interactions, a dataset of a field experiment in a Chinese subtropical forest was analysed. The following hypothesis were tested:

H 1.1) There will be an increased microbial biomass and respiration between hetero-specific tree pairs compared to mono-specific tree pairs, due to a higher heterogeneity of nutrients between hetero-specific tree species pairs.

H 1.2) There will be increased microbial biomass and respiration with higher plot-level tree diversity.

To test for local environmental drivers of microbial properties, a transect experiment was set up in a Chinese subtropical forest. Here, potential abiotic and biotic drivers of microbial biomass and basal respiration were tested in neighbourhood controlled plots. Soil samples were analysed to test the following hypothesis:

H 2.1) There will be an increased microbial biomass and respiration between hetero-specific tree pairs compared to mono-specific tree pairs, due to a higher heterogeneity of nutrients between hetero-specific tree species pairs.

H 2.2) Tree species identity effects will be stronger closer to the trees, both at the vertical and horizontal scale.

H 2.3) Tree species identity effects will be stronger with larger size of the tree.

H 2.4) There will be increased microbial biomass and respiration with increased soil moisture. Higher biomass of trees increases tree identity effects, but is also expected to decrease soil humidity depending on the tree species. Therefore, we expect to find species-specific tree identity effects via changes in soil moisture on microbial biomass and respiration.

H 2.5) There will be increased microbial biomass and respiration with increased understory plant cover and root biomass.

To control for any potentially confounding effects of environmental conditions, a more controlled approach has to be taken, like a greenhouse experiment. It allows to specifically test tree species identity and tree-tree interaction effects that are independent of the neighbourhood. In addition, controlled experiments allow us to test the effect of the microbial community and soil nutrient availability, as soil communities can be removed via sterilizing the soil or by adding specific nutrients, respectively.

H 3.1) Under environmentally controlled conditions, there will be an increased microbial biomass and respiration between hetero-specific tree pairs compared to mono-specific tree pairs.

H 3.2) Adding a microbial inoculum to the soil will increase microbial biomass and respiration as well as the tree mixture effect on soil microbial properties.

H 3.3) The addition of phosphorus (P) will increase microbial biomass and respiration as well as the tree mixture effect on soil microbial properties due to enhanced tree inputs to the soil.

H 3.4) The addition of a microbial inoculum and P will synergistically increase microbial biomass and respiration as well as the tree mixture effect on soil microbial properties.

# 2. Materials and methods

## 2.1 Field Experiment

### *2.1.1 Study Site*

The study site is located in south-east China near the city Xingangshan in the Jiangxi province (29.12°N, 117.90°E). The experimental site is part of the Biodiversity Ecosystem Function-China experiment, which was established to assess the effects of tree species extinctions on ecosystem functioning (BEF-China; Bruelheide *et al.* 2014). The experimental site was planted in 2009, after a clear-cut of the previous commercial plantation of *Pinus massoniana* and *Cunninghamia lanceolata*. It covers an area of 26.7 ha, where the altitude is ranging from 105 to 275 m. The region has a subtropical climate, with warm, wet summers and cool, dry winters. The local mean annual temperature is 16.7 °C with an annual precipitation of 1821 mm (Yang *et al.* 2013). The soils of this region are Cambisols and Cambisol derivatives, with Regosols on ridges and crests (Geiβler *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* (Bruelheide *et al.* 2011, 2014).

### *2.1.2 Study Design and Soil Sampling*

To be able to better explain microbial community responses, this thesis investigates tree-tree interactions to mechanistically explain the positive BEF relationships found for forest ecosystems. Therefore, pairs of two trees were identified in plots ranging from 1, to 2, 4, 8, 16 and 24 tree species (*Fig.* 1b). These pairs are called tree-species pairs (TSP) in the following. Of the pool of 40 tree species used in the BEF-China experiment, twelve tree species were chosen to be sampled in both mono- and hetero-specific TSPs. According to the "Broken Stick Design" of the experiment (Bruelheide *et al.* 2014), each mono-specific TSP was replicated nine times (three times in species richness 1 and 2; once in 4, 8, and 16 or 24), when available. Hetero-specific TSPs were replicated six times (three times in species richness 2; once in 4, 8, 16 or 24), when available (Tab. 1). In total, 170 TSPs with 27 different combinations were sampled in 57 experimental plots. Between each TSP, four soil-cores were taken, two in the center and one between the center and each tree. They were taken at a depth of 0-10 cm excluding the litter layer and were pooled together to create a single bulk soil sample per pair (*Fig.* 1a). The samples were sieved in the field at 2 mm to remove large roots and stones. All samples were frozen at -20 °C until further analysis. Due to the random planting of the trees, the direct TSP neighbourhood might not represent the species richness level

of the plot. To be able to test for differences of neighbourhood trees and species richness levels, tree characteristics, such as height and diameter at breast height (dbh), were recorded for the trees of the TSPs and all ten directly surrounding neighbour-trees according to the planting grid (see Bruelheide *et al.* 2014). Tree biomass was then calculated following Huang *et al.* (2017).

### *2.1.3 Basal Respiration and Substrate Induced Respiration (SIR) Measurements*

Microbial biomass (Cmic) and basal respiration were measured using the substrate-induced respiration (SIR) method after Scheu (1992) using an automated $O_2$-micro-compensation apparatus. Approximately 10 g of the sieved soil samples were defrosted and acclimatised in closed plastic bags to room temperature (20 °C) for seven days. This ensured that the microbial community could adapt to a constant and

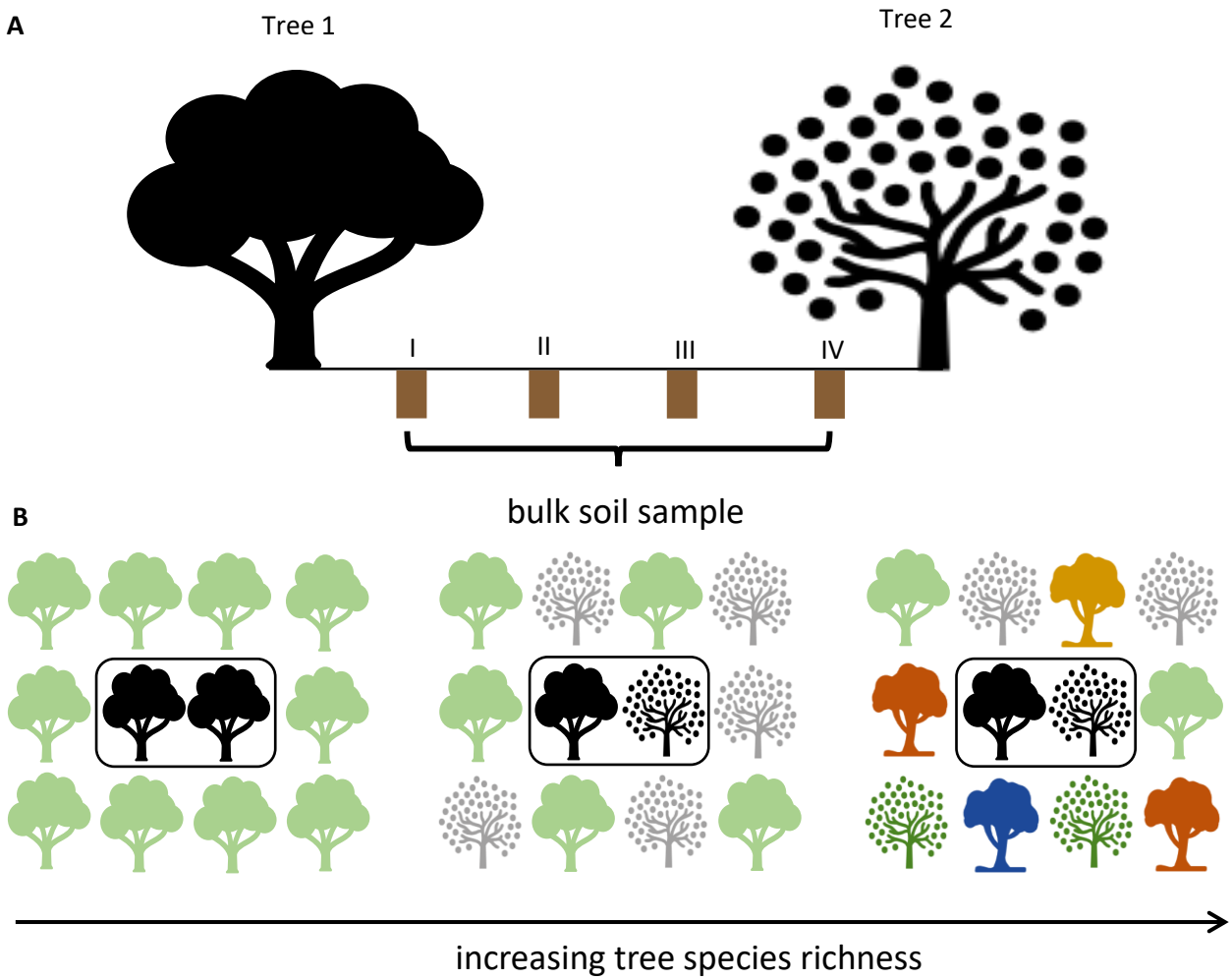


***Figure 1:*** *Examples of the sampling measurements in the field experiment. A) Scheme of the of the soil-core positions (I-IV) between the trees and the pooled bulk soil sample per TSP. B) TSPs (marked within the black box) were sampled across different diversity levels (1-24 species). Tree height and diameter at breast height (dbh) were collected for the TSPs and all ten direct neighbourhood trees (coloured).*

standardised temperature. A subsample of 6 g of soil was weighed into the apparatus' sampling pots using a microscale (scale error = 0.01 g). The apparatus was used following the protocol of Scheu (1992). The first run determined the basal respiration as the mean amount of oxygen consumption per hour without any addition of a substrate. This measurement ran for approximately 24 h and represented the active part of the microbial community at the time of sampling. In the second run, microbial biomass was measured as the respiratory response of microorganisms to glucose addition. A water solution of 0.008 g glucose per 1 g soil dry weight was added to each soil sample. The measurement ran for approximately 17 h and was used as the maximum initial respiratory response (MIRR). Soil moisture was measured from a subset of soil of approximately 20 g by drying it at 75 °C for 48 h. After the measurements with the O2-micro-compensation apparatus, all samples were dried at 75°C for 24 h, and the dry weight was determined using a micro scale (scale error = 0.01 g).

The oxygen consumption per h was calculated in dependency of the dry weight of soil in µl (0.83 µl corresponds to 0.58 µl $O_2$, factor 0.7; respiration given in µl $O_2$ $g^{-1}$ dry weight $h^{-1}$). The calculation of the microbial biomass contains the total metabolically active microflora. A differentiation between bacteria and fungi is not possible with this method. The measurable respiration in the SIR is called "maximum initial respiratory response" (MIRR). By using a calibration of this with the fumigation-incubation method (Jenkinson & Powlson 1976), the microbial carbon in microbial biomass can be calculated through the MIRR value. The unit of Cmic is (µg C / g dry weight) which equals to 38 * MIRR (µl $O_2$ / dry weight * h).

### *2.1.4 Statistical Analyses*

All analyses were performed using the statistics programme R Version 3.6.3 (R Foundation for Statistical Computing 2020).

To test for the effects of mono- and hetero-specific tree pairs and their interactions with the plot-level tree diversity, linear mixed-effects models were used. The tree pair and plot-level diversity were set as fixed factor and the plot was set as a random factor.

microbial properties ~ mono or hetero * diversity + (1|Plot)

To test for the effect of local environmental conditions on basal respiration and microbial biomass, root biomass, plot-level diversity and the proportions of the biomasses of surrounding neighbourhood trees were added to the model.

To test the effect of tree identity and tree-tree interaction effects on soil microbial properties, a generalised diversity-interaction model following Kirwan *et al.* (2009) was used. It investigates the properties of BEF relationships, based on identity effects, pairwise interactions and a coefficient that modifies the contribution of pairwise interactions to ecosystem function (Conolly *et al.* 2013). In contrast to a simple regression, the model analyses the importance of species identity and species interactions based on the general linear model:

$$y = \mathrm{ID} + \mathrm{DE} + \varepsilon \qquad \text{(equation 1)}$$

Here, the response variables (*y*) were either microbial biomass or basal respiration. The explanatory variables are both, species identity (ID) and species interaction (DE). Both variables are embedded in the model through functions of the initial proportions of species in the community (Kirwan *et al.* 2009).

$$y = \sum_{i=1}^{s} \beta_i p_i + \alpha M + \sum_{\substack{i,j=1 \\ i<j}}^{s} \delta_{ij} p_i p_j + \varepsilon \qquad \text{(equation 2)}$$

In the equation 2, the response variable (y) was modelled as a function of species identity ID = $\sum_{i=1}^{s} \beta_i p_i$ + α*M* and species interaction DE = $\sum_{\substack{i,j=1 \\ i<j}}^{s} \delta_{ij} p_i p_j$, where α reflects the effect of changing the total overall initial abundance (*M*). For each species *i* or *j* in *s*, $p_i$ is the proportion of the species in the mixture, and $\beta_i$ is the estimated performance of the species *i* in monoculture. The interaction of species might depend on the relative abundance of the species. Therefore, the term $\delta_{ij}$ defines the strength of interaction between species *i* and *j*.

An ANOVA with a post-hoc Tukey HSD test was applied to test for pairwise differences across the tree species.

Additionally, the "DImodels" package gave the possibility to also test for the effects of different functional groups. This is done by adding the proportion of the community made up of a functional group to the sum of all species proportions within this group (Kirwan *et al.* 2009). Therefore, it was tested whether there were any differences between broad-leaved and deciduous trees and mycorrhizal types (ectomycorrhizal fungi (EMF) and arbuscular mycorrhizal fungi (AMF) see Tab. S1).

To define the quality of the model fits, the "check_model" function of the "performance" package was used to investigate various model assumptions, such as normality of residuals, normality of random

effects, linear relationship, homogeneity of variance, and multicollinearity (Briggs & Cheek 1986) of all used models.

## 2.2 Transect Experiment

### *2.2.1 Study Design and Soil Sampling*

The experimental site is part of the Biodiversity Ecosystem Function-China experiment (BEF-China, Bruelheide *et al.* 2014), described in chapter 2.1.1. To test the effect of the neighbouring trees, while controlling for the species richness level, two plots with the same two-species mixture were chosen. Both plots contained the deciduous tree species *Liquidambar formosana* (Altingiaceae) and *Sapindus mukorossi*

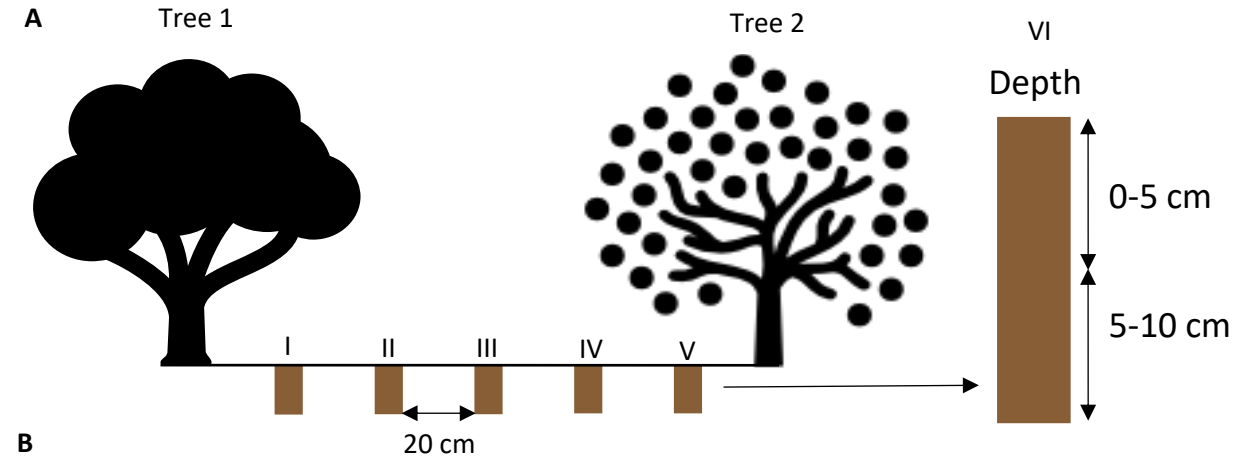


***Figure 2:*** *Sampling measurements in the transect experiment. A) Scheme of the array of the soil-core positions (I-V) between the trees and the division of the soil within the core (VI). B) Overview of the positioning of the soil-cores in the field with three soil-cores taken and two more still inside the soil next to the scale*

(Sapindaceae). These tree species were chosen due to previously-reported significant and dissimilar effects on soil microbial properties (Beugnon *et al.* 2021). This resulted in the following possible combinations for the tree species pairs (TSPs): two individuals of *L. formosana*, two individuals of *S. mukorossi*, and one individual of each of the two species. For each of the three tree combinations, five replicates were sampled in each of the two replicated plots. Prior to sampling the soil, the exact distance between the trees was measured, to ensure an equal distribution of the sampling positions. The mean distance between the trees was 1.4 m ± 0.4 m and varied from 1.07 m to 1.84 m.

Between each TSP, soil-cores with a diameter of 5 cm were taken in five positions (*Fig. 2A: I – V*). Position number three was always located in the centre between the two trees. Then, the remaining soil-cores were located in equal distances between the middle position and the trees on both sides. To test for the effect of depth on microbial biomass and respiration, each soil core was split in the field into the depths of 0-5 cm and 5-10 cm (*Fig. 2A: VI*). Each subsample was sieved at 2 mm to exclude large roots and stones and later freeze-dried in the field station at -20°C. The roots were collected in the sieves, dried, and weighted. Altogether, 300 soil samples were collected from two plots and three combinations of TSPs: 3 TSPs x 5 positions x 2 depths x 5 replicates x 2 plots.

### *2.2.2 Plant Measurements*

Additionally, the tree diameter at breast height (dbh) was measured for each TSP to calculate the biomass following Huang *et al.* (2017). For later analysis of understory plant coverage, first, a photo with a scale was taken from above with a camera (24 MP, f/1.7, 27mm) of each TSP (*Fig. 2B*). Afterwards, the distances from each soil-core to each of the understory plants and the two TSP trees were measured using the programme ImageJ. All distances were measured to the centres of the plants and trees.

### *2.2.3 Basal Respiration and Substrate Induced Respiration (SIR) Measurements*

Microbial biomass (Cmic) and basal respiration were measured using the substrate induced respiration (SIR) method following the same procedure as in chapter 2.1.3.

### *2.2.4 Statistical Analyses*

To test for the effects of mono- and hetero-specific tree pairs, linear mixed-effects models were used. The tree pair was set as a fixed factor and the plot was set as a random factor.

$$\text{microbial properties} \sim \text{mono or hetero} + (1|\text{Plot})$$

To test the effects of the three different tree pair combinations, an ANOVA with a post-hoc Tukey HSD test was applied to test for pairwise differences across the three TSP combinations.

A linear model was used to test the effects of distance to the closest tree, depth, soil water content, size of the tree species', root biomass, and distance to the closest understory plant. Explanatory variables were selected by a both-way step selection based on AIC. The drivers of soil microbial biomass and respiration were estimated from the final model. All significant variables of the model output (*p-value* < 0.05) were implemented in a Structural Equation Model (SEM). The SEM was fitted using the R "sem" function from the package "lavaan" (Rosseel *et al.* 2012)

To define the quality of the model fits, the "check_model" function of the "performance" package was used to investigate various model assumptions, such as normality of residuals, normality of random effects, linear relationship, homogeneity of variance, and multicollinearity (Briggs & Cheek 1986) of all used models.

## 2.3 Greenhouse Experiment

### *2.3.1 Study Design and Soil Sampling*

To test the effect of soil microbial community composition and P nutrition on soil microbial biomass and respiration, while controlling for climatic conditions, a greenhouse experiment was set up in the botanical garden of the Martin-Luther university Halle-Wittenberg in Halle (Saale). Eight species included in the BEF-China field experiment were chosen and planted in TSPs: *Choerospondias axillaris*, *Cyclobalanopsis glauca*, *Koelreuteria bipinnata*, *Quercus serrata*, *Quercus fabri*, *Rhus chinensis, Sapium sebiferum*, and *Schima superba*. The seeds of all species were collected in the Gutianshan Nature Reserve in China in 2018 and transferred to Germany. They all germinated under controlled conditions in a greenhouse chamber with a temperature of 20 °C and a relative humidity of 70-80 %.

The tree species were split into two sets of four species with *C. axillaris*, *K. bipinnata, Q. serrata*, and *S. sebiferum* in Set A and *C. glauca*, *Q. fabri*, *R. chinensis*, and *S. superba* in Set B (*Fig. 2*). Each set consisted of ten possible tree pairwise combinations: one mono-specific pair for each of the four species and six possible combinations of hetero-specific pairs, creating a total of 20 unique mixtures. The soil for the experiment consisted of two parts: part one was original Chinese soil containing all soil microorganisms from the natural habitat of the tree species (hereafter Inoculum). It was collected in 16 monoculture plots

in Site A of the BEF-China experiment and consisted of 64 subsamples. The second part was a mixture (3:1) of forest soils from the Brocken mountain and a forest in Petersberg near Halle (Saale), hereafter German soil. The German soil was sieved at 5 mm and then mixed with sand (1:1). Before being filled into the experimental tubes the soil was sterilised using a soil steam sterilizer ("Hombach-Erd-Dämpfanlage R15'') at 120°C for 20 min with a 10 min resting time. The tubes were about 1 m tall and had a diameter of 20 cm. They were designed to be opened and closed for later sampling. All tubes were sterilised with ethanol before 85% of the tubes were filled with the sterilised German soil-sand mixture. On top of the German soil, one half of the tubes was filled with 15% non-sterilised Chinese soil (Inoculum), the other half was filled with 15% sterilised Chinese soil (without Inoculum). Due to technical difficulties, the soils were not mixed together (see *Fig.* 3). To 60 tubes of each treatment (Inoculum or without Inoculum), P was added as a fertilizer, whereas the other 60 tubes of each treatment received a fertilizer with potassium (K), resulting in four different kinds of treatments for each set of species (*Fig. 3*). Fertilizers were added every third week. The tree seedlings were planted in pairs into the tubes with an age between 4 and 8 weeks. To protect the trees from pathogens and Sciaridae, approximately two centimetres of sterile sand were added on top of each tube after planting. Sciarids lay their eggs onto moist organic soils but not onto fast-drying sand. The tubes were placed in six different chambers of the greenhouse, with

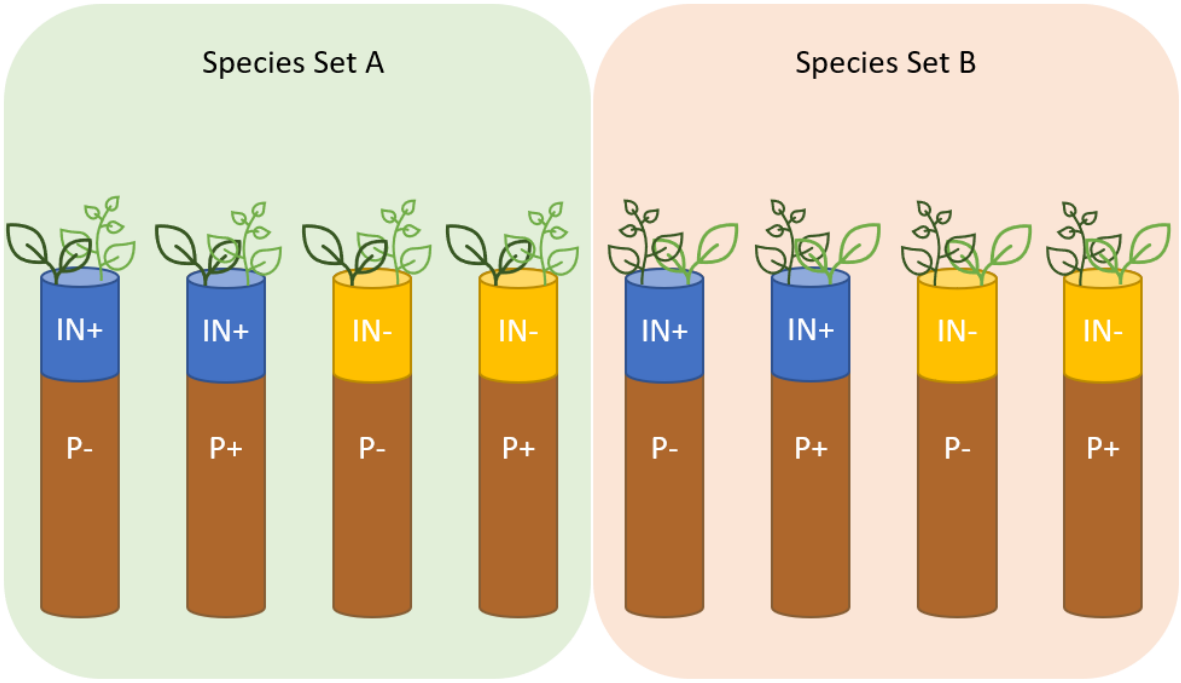


***Figure 3**: Set of species: Set A (green) with C. axillaris, K. bipinnata, Q. serrata, and S. sebiferum and Set B (orange) with C. glauca, Q. fabri, R. chinensis, and S. superba. Inoculum (IN+ in blue), without Inoculum (IN- in yellow), phosphorus fertilization (P+), no phosphorus fertilization (P-) and sterilised German soil-sand mixture (1:1) in brown in all tubes (height: 1 m, width: 20 cm)*

each chamber holding 40 tubes of a similar set. All tubes were harvested after a growing period of one year. Above- and belowground biomass, root and soil samples were sampled for each tube. Ten soil samples from ten different levels within the tube were pooled together to create the final soil sample per tube. All soil samples were frozen at -20 °C until further analysis.

### *2.3.2 Basal Respiration and Substrate Induced Respiration (SIR) Measurements*

Microbial biomass (Cmic) and basal respiration were measured using the substrate induced respiration (SIR) method following the same procedure as in chapter 2.1.3.

### *2.3.3 Statistical Analyses*

To test for the effects of mono- and hetero-specific tree pairs and their interactions with the phosphorus and Inoculation treatment, linear mixed-effects models were used. The tree pair, phosphorus and Inoculation (Ino) treatment were set as fixed factors and the greenhouse chamber was set as a random factor.

microbial properties ~ mono or hetero * P * Inoculum + (1|Chamber)

To test the effect of tree identity and tree-tree interaction effects on soil microbial properties, a generalised diversity-interaction model following Kirwan *et al.* (2009) was used (see chapter 2.1.4). To test additional effects of phosphorus addition and the presence of community-adapted microbes (Inoculation), both treatments were added to the "DImodels" function.

An ANOVA with a post-hoc Tukey HSD test was applied to test for pairwise differences across the tree species.

To define the quality of the model fits, the "check_model" function of the "performance" package was used to investigate various model assumptions, such as normality of residuals, normality of random effects, linear relationship, homogeneity of variance, and multicollinearity (Briggs & Cheek 1986) of all used models.

***Table 2:*** *Tree species pairs (TSP) combinations in the field experiment of BEF-China with replicate number per species richness (1 to 16 or 24 – depending on availability) and total number of samples per TSP. Pairs highlighted in grey are also represented in a controlled greenhouse experiment in Halle (Saale).*

| tree species 1 | tree species 2 | TSP type | species richness | | | | | total samples |
|---|---|---|---|---|---|---|---|---|
| | | | 1 | 2 | 4 | 8 | 16/24 | |
| *Castanea henryi* | *Castanea henryi* | mono | 3 | 3 | 1 | 1 | 1 | 9 |
| *Castanopsis sclerophylla* | *Castanopsis sclerophylla* | mono | 3 | 3 | 1 | 1 | 1 | 9 |
| *Choerospondias axillaris* | *Choerospondias axillaris* | mono | 3 | 3 | 1 | 1 | 1 | 9 |
| *Cyclobalanopsis glauca* | *Cyclobalanopsis glauca* | mono | 3 | 3 | 1 | 1 | 1 | 9 |
| *Liquidambar formosana* | *Liquidambar formosana* | mono | 3 | 3 | 1 | 1 | 1 | 9 |
| *Quercus serrata* | *Quercus serrata* | mono | 3 | 3 | 1 | 1 | 1 | 9 |
| *Sapindus mukorossi* | *Sapindus mukorossi* | mono | 3 | 3 | 1 | 2 | 1 | 10 |
| *Sapium sebiferum* | *Sapium sebiferum* | mono | 3 | 3 | 1 | 1 | 1 | 9 |
| *Lithocarpus glaber* | *Lithocarpus glaber* | mono | 3 | 3 | 0 | 1 | 1 | 8 |
| *Nyssa sinensis* | *Nyssa sinensis* | mono | 3 | 2 | 0 | 1 | 1 | 7 |
| *Koelreuteria bipinnata* | *Koelreuteria bipinnata* | mono | 2 | 3 | 1 | 1 | 1 | 8 |
| *Quercus fabri* | *Quercus fabri* | mono | 2 | 3 | 1 | 0 | 1 | 7 |
| *Choerospondias axillaris* | *Sapium sebiferum* | hetero | 0 | 4 | 1 | 1 | 1 | 7 |
| *Castanea henryi* | *Nyssa sinensis* | hetero | 0 | 3 | 1 | 1 | 1 | 6 |
| *Castanopsis sclerophylla* | *Quercus serrata* | hetero | 0 | 3 | 1 | 1 | 1 | 6 |
| *Cyclobalanopsis glauca* | *Quercus fabri* | hetero | 0 | 3 | 1 | 1 | 0 | 5 |
| *Liquidambar formosana* | *Sapindus mukorossi* | hetero | 0 | 3 | 1 | 0 | 0 | 4 |
| *Koelreuteria bipinnata* | *Lithocarpus glaber* | hetero | 0 | 3 | 0 | 1 | 0 | 4 |
| *Castanea henryi* | *Liquidambar formosana* | hetero | 0 | 0 | 1 | 1 | 1 | 3 |
| *Castanea henryi* | *Sapindus mukorossi* | hetero | 0 | 0 | 1 | 1 | 1 | 3 |
| *Castanopsis sclerophylla* | *Choerospondias axillaris* | hetero | 0 | 0 | 1 | 1 | 1 | 3 |
| *Castanopsis sclerophylla* | *Sapium sebiferum* | hetero | 0 | 0 | 1 | 1 | 1 | 3 |
| *Choerospondias axillaris* | *Quercus serrata* | hetero | 0 | 0 | 1 | 1 | 1 | 3 |
| *Liquidambar formosana* | *Nyssa sinensis* | hetero | 0 | 0 | 1 | 1 | 1 | 3 |
| *Quercus serrata* | *Sapium sebiferum* | hetero | 0 | 0 | 1 | 1 | 1 | 3 |
| *Sapindus mukorossi* | *Nyssa sinensis* | hetero | 0 | 0 | 1 | 1 | 1 | 3 |
| *Koelreuteria bipinnata* | *Quercus fabri* | hetero | 0 | 0 | 0 | 1 | 0 | 1 |

# 3. Results

## 3.1. tree diversity effects on microbial properties in subtropical forests

The analyses showed an overall very low microbial biomass and basal respiration ($\bar{x}$ = 344.83 and *SD* = 93.15, $\bar{x}$ = 1.61 and *SD* = 0.41, respectively).

Linear mixed models were used to test the effects of mono- and hetero-specific tree pairs on soil microbial biomass and basal respiration. The models showed no significant differences of mono- and hetero-specific tree pairs on neither microbial biomass nor basal respiration (*p*-value = 0.510, *p*-value = 0.724, respectively).

Following, we tested the effects of tree species identities and tree-tree interactions using a diversity-interaction model. The model revealed strong effects of all tested tree species on both microbial biomass

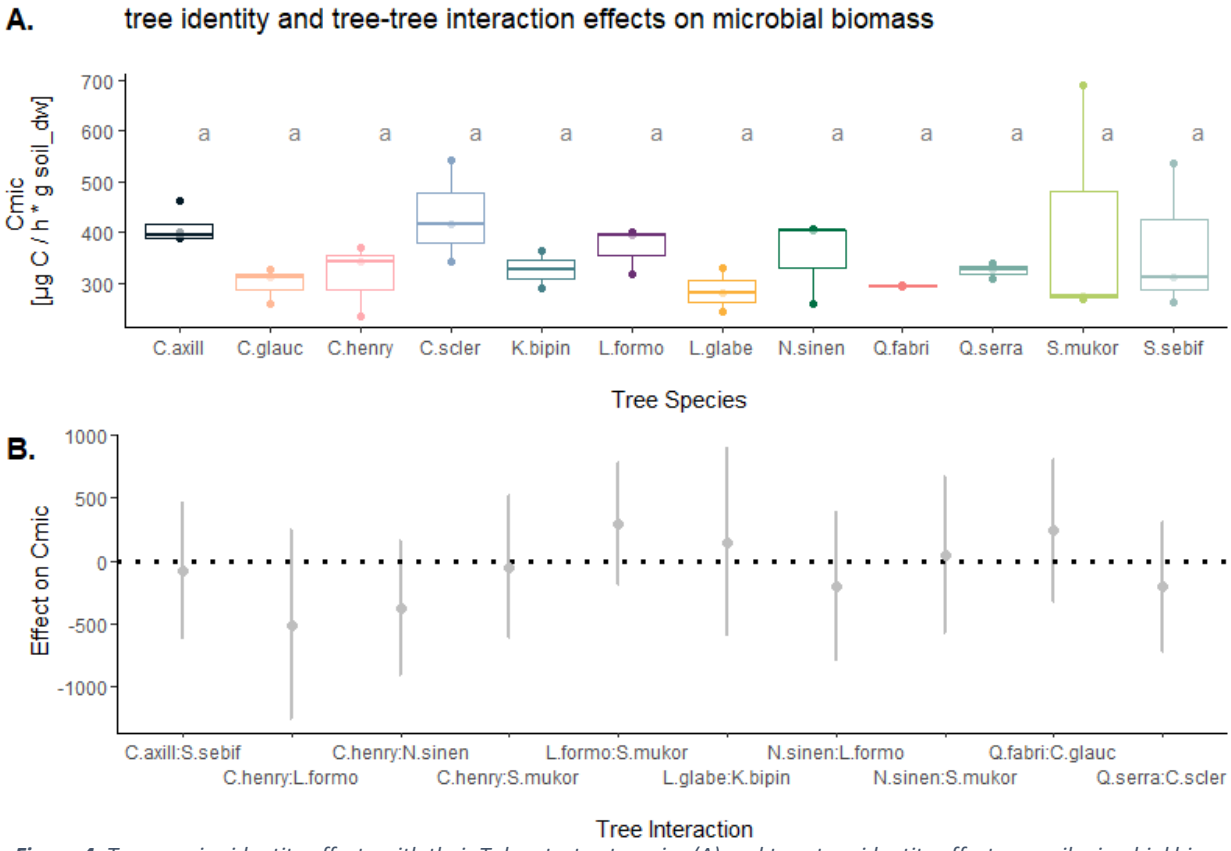


***Figure 4:*** *Tree species identity effects with their Tukey-test categories (A) and tree-tree identity effects on soil microbial biomass (B). Not significant effects are displayed In grey.*

(*p*-value < 0.001, *Tab. S2*) and basal respiration (*p*-value < 0.001, *Tab. S3*), but did not show any significant interaction effects between the tree species on the soil microbial properties (*Fig. 4B*, *Fig. 5B*).

In addition, a post-hoc Tukey test was used to test for differences between the tree species. There were no significant differences between the tree species, neither for microbial biomass (*Fig. 4A*) nor basal respiration (*Fig. 5A*) in monocultures.

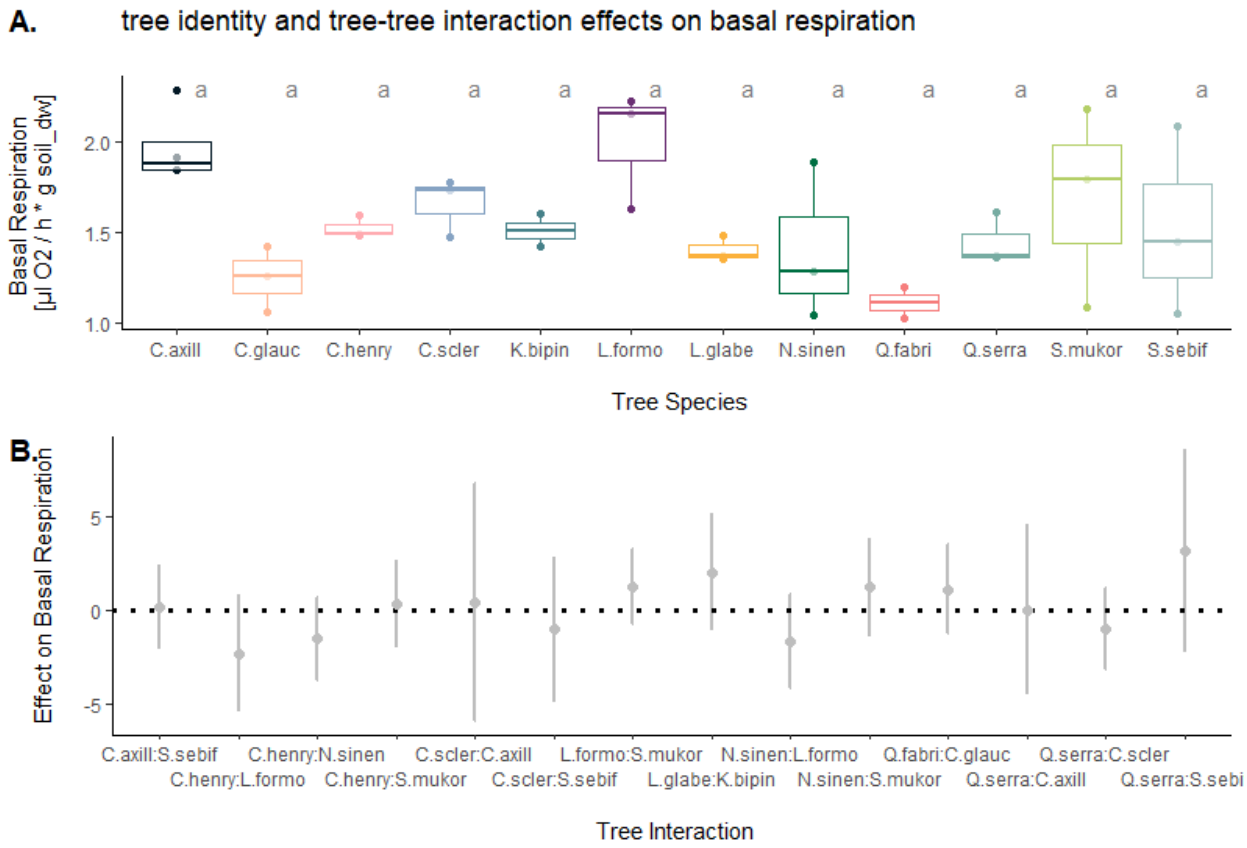


***Figure 5:*** *Tree species identity effects with their Tukey-test categories (A) and tree-tree identity effects on soil basal respiration (B). Not significant effects are displayed In grey.*

Finally, the plot-level diversity was added to the model to test for the effect of the surrounding neighbourhood. There was no effect of increased tree species richness on soil microbial biomass or basal respiration (*p*-value = 0.396, *p*-value = 0.226, respectively).

## 3.2 Spatial effects of tree diversity on microbial properties in subtropical forests

The analyses showed an overall very low microbial biomass and basal respiration ($\bar{x}$ = 381.48 and *SD* = 137.02, $\bar{x}$ = 1.77 and *SD* = 0.93, respectively). Additionally, we found a significantly higher microbial biomass and basal respiration in plot H31 compared to T17. This was correlated with the higher relative soil humidity in plot H31 ($\bar{x}$ = 25.92% and *SD* = 2.42) compared to T17 ($\bar{x}$ = 16.07 and *SD* = 3.16).

Linear mixed models were used to test the effects of mono- and hetero-specific tree pairs on soil microbial biomass and basal respiration. The models showed no significant differences of mono- and hetero-specific tree pairs on neither microbial biomass nor basal respiration (*p*-value = 0.815, *p*-value = 0.054, respectively). Following, we tested tree identity and interactions using mixed effects models with plot as a random factor. The analyses of the three TSP combinations (*L.formosana - L.formosana, L.formosana - S.mukorossi, S.mukorossi - S.mukorossi*) showed an significant effect of the hetero-specific pair on microbial biomass (*p*-value = 0.015) but not basal respiration (*p*-value = 0.498). In addition, a post-hoc Tukey test was used to test for differences between the three combinations. However, there were no significant differences for microbial biomass or basal respiration for the combined depths (*Fig. S1*). After that, we tested for the separated depths and found a positive effect of the hetero-specific pair *L. formosana – S. mukorossi* on soil microbial biomass and basal respiration in the depth 5-10 cm in the plot T17 (*p*-value = 0.002, *p*-value = 0.026, respectively, *Fig. 6*).

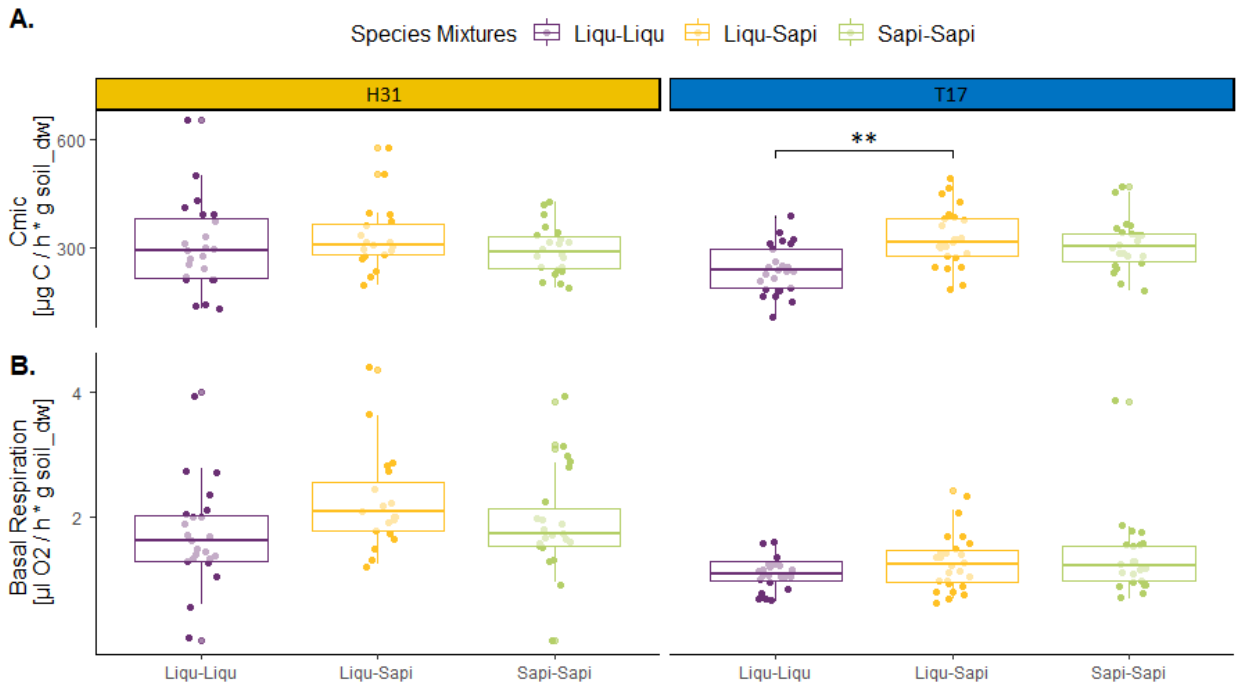


***Figure 6:*** *Differences of microbial biomass (A) and basal respiration (B) in the plots H31 (yellow) and T17 (blue) between the three following species mixtures: Liquidambar formosana – Liquidambar formosana (purple), Liquidambar formosana – Sapindus mukorossi (orange) and Sapindus mukorossi – Sapindus mukorossi (green) in the depth 5-10 cm. Tukey-test result for the significant difference between L. formosana – S. mukorossi and L. formosana – L.formosana*

Following, distance to the tree and the depth of soil samples were added to the model. Overall, our results showed that microbial biomass and basal respiration decreased with increasing distance to the tree and

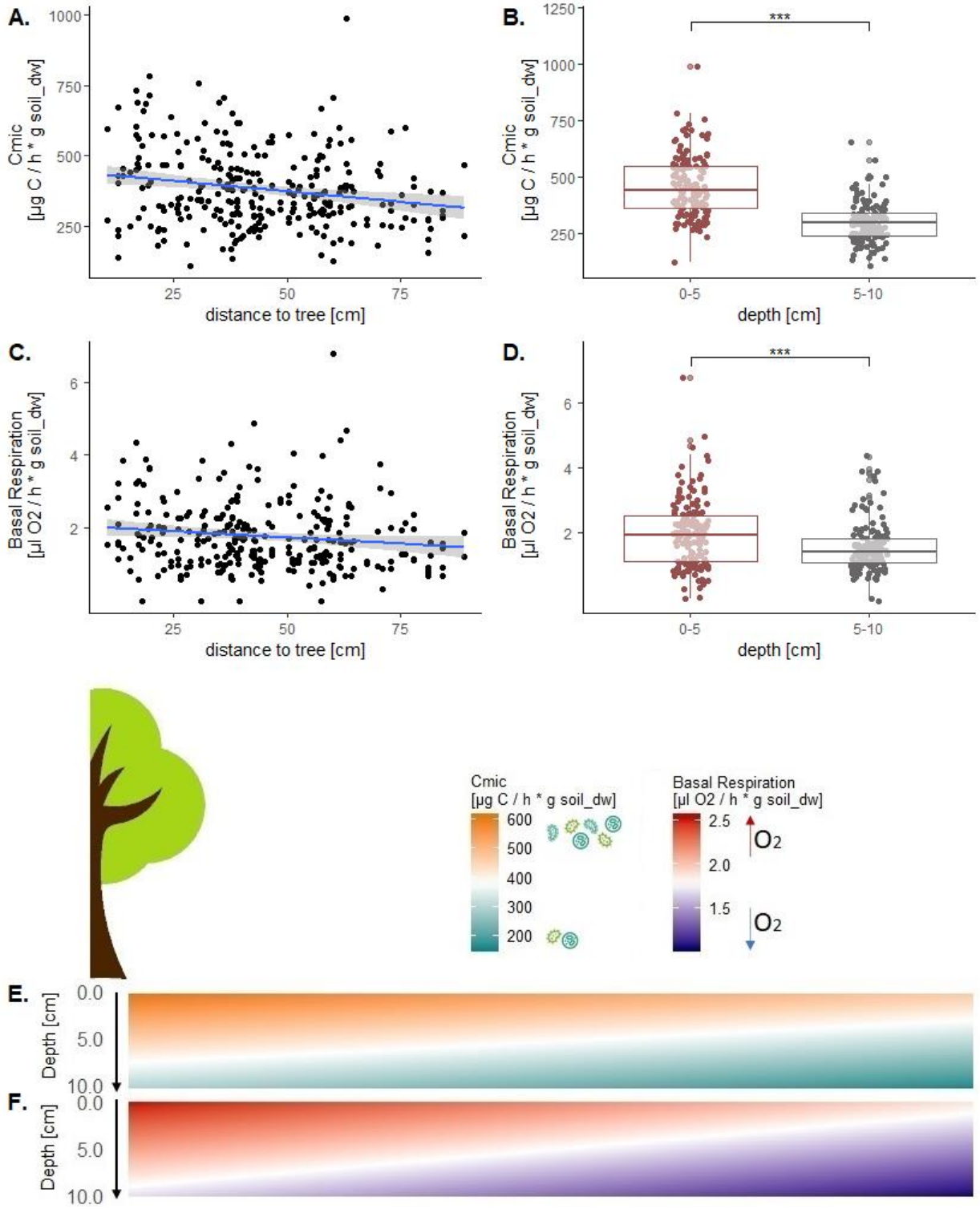


***Figure 7:*** *Spatial range of tree effects. Decreasing microbial biomass with increasing distance to the tree (A, p=0.004) and depth (B, p<0.001). Decreasing basal respiration with increasing depth (D, p<0.001), but no significant effect of increasing distance to the tree (C, p=0.609). Heatmap of predicted microbial biomass (E) and basal respiration (F)*

depth (*Fig. 7E and 7F*). The microbial biomass decreased significantly with increasing distance to the tree (*Estimate* = -17.743, *SE* = 6.22, *p*-value = 0.004, *Fig. 7A*) and depth (*Estimate* = -32.869, *SE* = 2.43, *p*-value < 0.001, *Fig. 7B*). Basal respiration also decreased with an increasing depth (*Estimate* = -0.107, *SE* = 0.01, *p*-value <0.001, *Fig. 7C*), but distance to the tree had no significant negative effect (*p*-value = 0.609, *Fig. 7D*).

Furthermore, we tested the effects of tree size, relative soil humidity, distance to understory plants and root biomass on soil microbial properties and added them to the model. The results showed that tree species effects were size dependent and increased with larger tree biomass when affecting basal respiration (*Fig. 8D*). It could be shown, that *S. mukorossi* increased basal respiration (*Estimate* = 21.496, *SE* = 8.97, *p*-value = 0.017), whereas *L. formosana* decreased the microbial activity (*Estimate* = -20.902, *SE* = 5.19, *p*-value < 0.001). In contrast, there was no significant effect of increased tree biomass on soil microbial biomass (*Fig. 8B*). Increases soil humidity was positively affecting both microbial biomass (*Estimate* = -20.902, *SE* = 5.19, *p*-value < 0.001, *Fig. 8A*) and basal respiration (*Estimate* = -20.902, *SE* = 5.19, *p*-value < 0.001, *Fig. 8C*). The analysis did not show any effect of root biomass or proximity to understory plants for either of the two soil microbial properties.

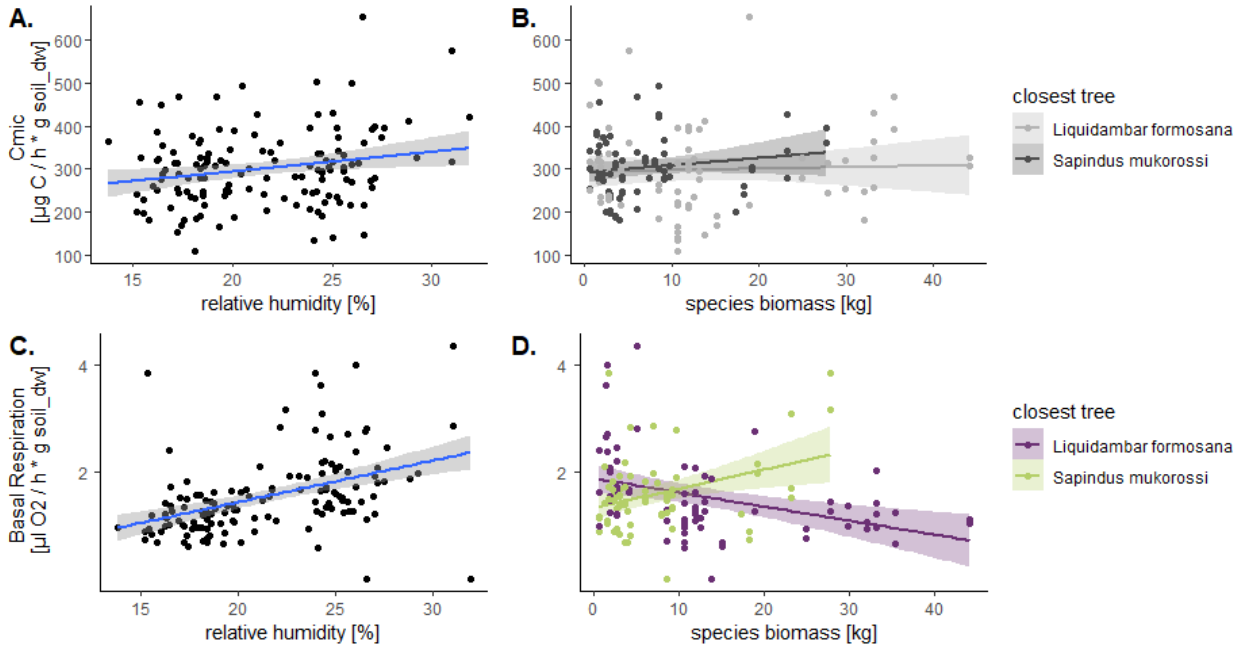


***Figure 8:*** *Positive effect of increased relative humidity on microbial biomass (A, p<0.001) and basal respiration (C, p<0.001). Size dependency of closest tree effects on basal respiration (D) with a positive effect of S. mukorossi (p=0.017) and a negative effect of L. formosana (p<0.001). No significant effect of closest tree on microbial biomass (B)*

Additionally, all significant drivers of soil microbial properties (*Fig. S2*) were added to a SEM to further investigate the environmental factors associated with the relationship between microbial biomass and basal respiration (*Fig. 9*). This analysis revealed that the plot was indirectly affecting the soil microbial properties due to the differences in soil moisture. Furthermore it showed a positive effect of soil microbial biomass on basal respiration. We also tested the effects of increased tree species biomasses on soil moisture to test for indirect effects on soil microbial properties. However, there were no effects of tree species biomass on soil humidity.

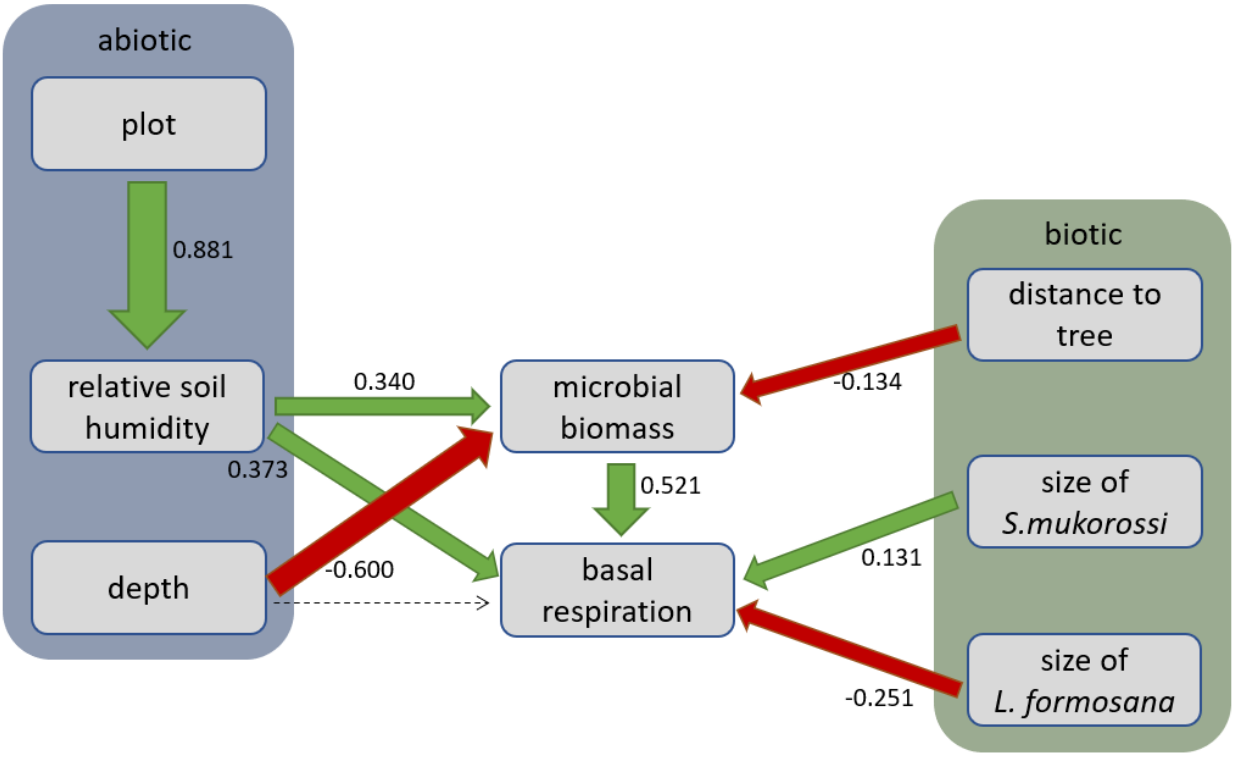


***Figure 9:*** *Structural equation model of the drivers of microbial biomass and basal respiration*

## 3.3 Tree diversity effects on microbial properties under controlled conditions

The analyses showed an overall very low microbial biomass and basal respiration in the greenhouse experiment ($\bar{x}$ = 59.73 and *SD* = 17.63, $\bar{x}$ = 0.627 and *SD* = 0.28, respectively).

Linear mixed models were used to test the effects of mono- and hetero-specific tree pairs on soil microbial biomass and basal respiration. The models showed significant negative effects of mono-specific tree pairs on both microbial biomass and basal respiration (*p*-value = 0.002, *p*-value = 0.001, respectively). The linear model showed no significant effect of the Inoculum and phosphorus treatment, nor their interaction on the soil microbial properties. However, it did show a positive interaction effect of mono-specific tree pairs and phosphorus addition on microbial biomass (*p*-value = 0.01625), but not basal respiration (*p*-value = 0.427216).

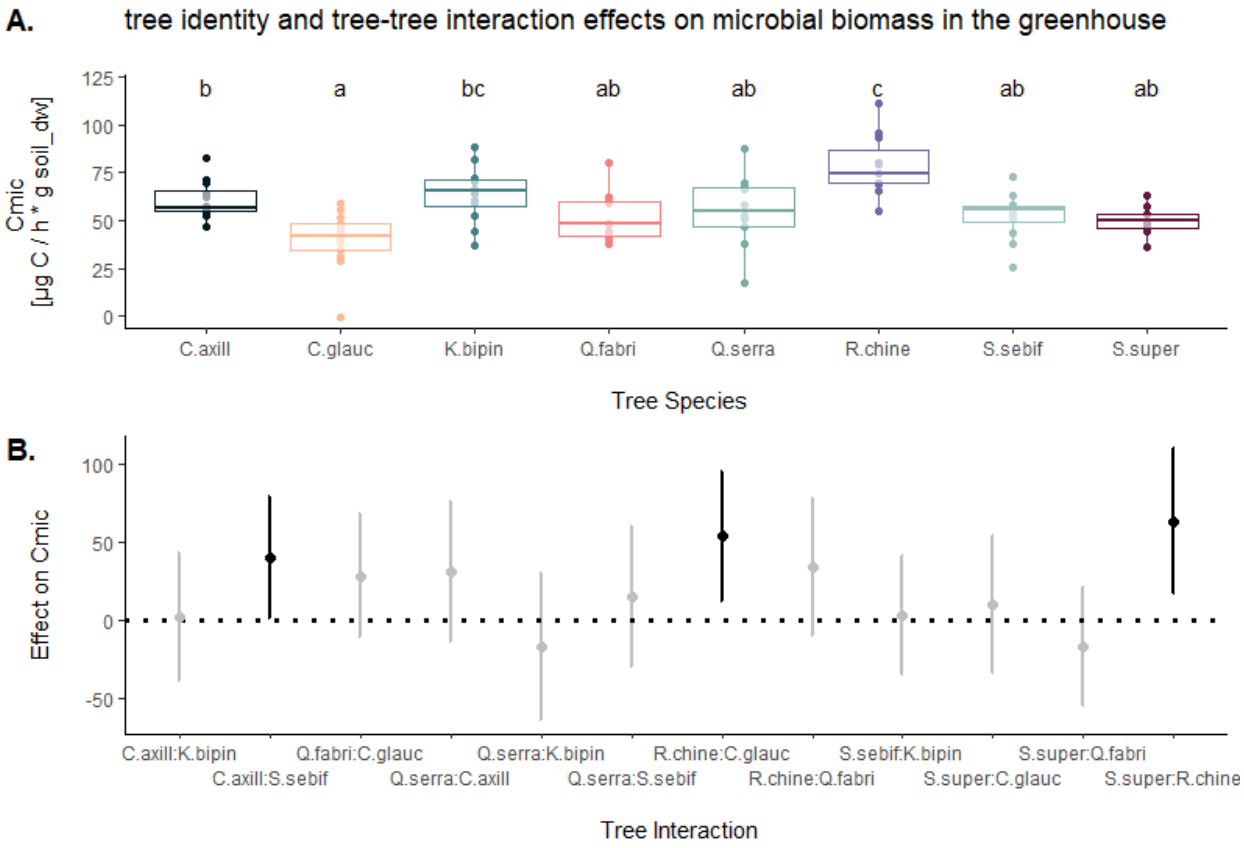


***Figure 10:*** *Tree species identity effects with their Tukey-test categories (A) and tree-tree identity effects on soil microbial biomass in the greenhouse (B). Significant effects are displayed in black, not significant effects are displayed In grey.*

Following, we tested the effects of tree species identities and tree-tree interactions using a diversity-interaction model. The model revealed strong effects of all tested tree species on both microbial biomass (*p*-value < 0.001, *Tab. S4*) and basal respiration (*p*-value < 0.001, *Tab. S5*). It also showed three significantly positive tree-tree interaction effects on microbial biomass (*Fig. 10B*), but none for basal respiration (*Fig. 11B*). This means that the hetero-specific tree pairs were significantly better than the monocultures of the

single species. The positive interactions were *S. superba – R. chinensis* (*p*-value = 0.00828), *R. chinensis – C. glauca* (*p*-value = 0.01205) and *C. axilliaris – S. sebiferum* (*p*-value = 0.04583). In addition, a post-hoc Tukey test was used to test for differences between the tree species. It showed that *R. chinensis* had a higher microbial biomass (*Fig. 10A*) than the other tree species in monocultures. However there were no significant differences between the tree species for basal respiration (*Fig. 11A*).

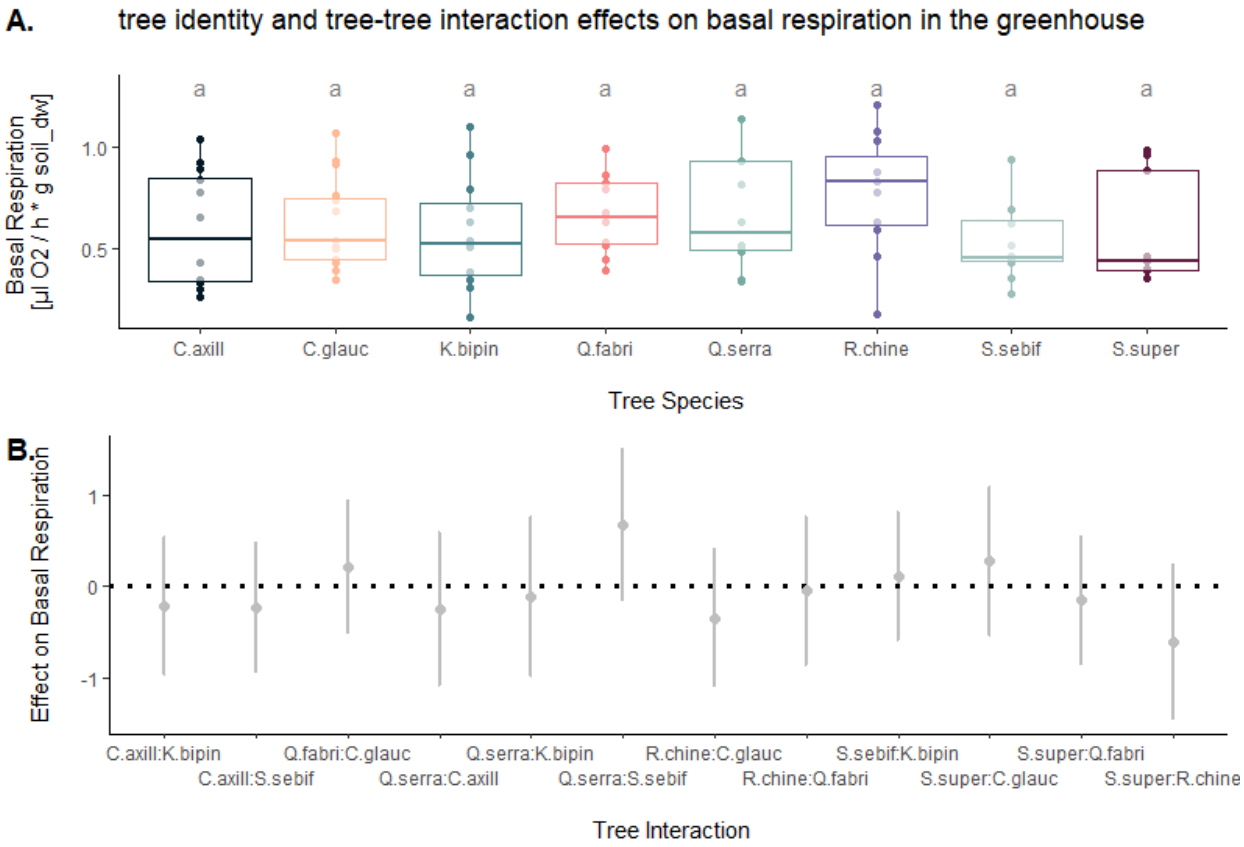


***Figure 11:*** *Tree species identity effects with their Tukey-test categories (A) and tree-tree identity effects on soil basal respiration in the greenhouse (B). Not significant effects are displayed In grey.*

Finally, the phosphorus and Inoculum treatment were added to the model to test for the effect of the microbial community and nutrient addition. The analysis showed a significant positive effect of the Inoculum treatment on microbial biomass (*p-value*= 0.034) and basal respiration (*p-value*= 0.0448). However, there was no significant effect of the phosphorus treatment on microbial biomass (*p*-value = 0.67094) or basal respiration (*p*-value = 0.1148).

# 4. Discussion

The goal of this study was to explain diversity effects on the small scale by focussing on tree identity and tree-tree interaction effects on soil microbial properties using three different experiments. The first two set ups were placed in a tree-diversity experiment (BEF-China) in a subtropical forest. In the first set-up, tree-tree interactions were investigated in a natural environment. In the second set up, specific tree-tree interactions were investigated in more detail with a focus on distance relationships. To complement the previous part of this study, a controlled greenhouse experiment was set up, focussing on the effects of microorganisms and P-limitation. It could be shown that over all experiments tree species identities were strong drivers of microbial properties. In contrast, tree-tree interactions and plot-level tree diversity had none or only weak effects. On smaller scales, the distance to the trees and tree size as well as soil depth and soil moisture were strong drivers of microbial biomass and respiration.

## 4.1 tree diversity effects on soil microbial properties

The first hypothesis expected a positive effects of hetero-specific tree pairs in comparison to mono-specific pairs on soil microbial properties. This could not be confirmed for the pooled soil samples of the first field set up. In contrast, the results of the neighbourhood controlled transect experiment showed a positive effect of the hetero-specific tree pair *Liquidambar formosana* – *Sapindus mukorossi* on soil microbial biomass. However, this was only true for one of the plots, in the depth of 5-10 cm and not for basal respiration. This can be explained by the much lower relative soil moisture of the plot. This stands in line with the findings of Cesarz *et al.* (2021), suggesting that biotic effects are context dependent and can be modulated by local abiotic conditions such as soil moisture. To better understand the local environmental impacts on soil microbial biomass and respiration, we also considered the results from the greenhouse experiment set-up.

The greenhouse experiment similarly showed that overall, hetero-specific tree pairs positively affected microbial biomass and basal respiration. However, the analyses also showed that the addition of phosphorus reversed the negative effect of mono-specific pairs. The interaction of P addition and mono-specific pairs had a positive effect on microbial biomass. The competition for nutrients is stronger between individuals of the same species than between different species due to the complementary resource use between different species (Ferlian et al. 2018). A possible explanation for the positive interaction of mono-specific pairs and P addition might be that the facilitation is increased by the P

treatment. Mono-specific pairs do not have to compete for limited nutrients and might benefit equally from correlated soil microbes. This finding for an experimental set-up stands in line with the findings from Burns & Strauss (2011), who showed that closely related plant species in potting soils, had more mutualistic relationships than distant relatives in these soils. They also showed that in field experiments the opposite effect occurred, which stands in line with our findings from the neighbourhood controlled transect experiment. The positive effect of phosphorus addition was also shown for other biomes such as grasslands (Ren *et al.* 2016). However, these studies found changes in effect directions over time, which demonstrates that long-term studies are needed to get more information about forest ecosystems. It also indicates that P-limitation becomes less important over time and that younger plants have a stronger demand for P. Therefore, the early stage of growth must be considered as the positive interaction effect of mono-specific pairs and P might change over time. Additionally, the close distance between the trees in the experimental tubes is important, as it increases the competition for nutrients and space for the young plants. This highlights the importance of experimental scales. Similar findings might have been observed in the field experiment for similar stand age and distances between the trees.

The second hypothesis expected higher microbial biomass and respiration with higher plot-level tree diversity. Contrary to our expectations, this could not be confirmed. This suggests that belowground communities are not solely linked to general plant diversity on the small scale but may rather be closely associated with the identity of species (Cesarz *et al.* 2021). Tree species can affect soil microbes in different ways, for example via water intake and a resulting change in soil moisture (Pei *et al.* 2016), changes in soil pH (Reich *et al.* 2005), through functional traits such as tannin or phenol contents in the leaf litter (Chai *et al.* 2019) or due to changes in the microclimate (Gottschall *et al.* 2019). It is also important to consider the functional diversity of the species within the plots, as species closely related to each other can share similar functional and physiological traits (Burns & Strauss 2011). Therefore they could provide e.g. more monogeneous leaf litter nutrients which would affect soil microbes as they are dependent on a larger diversity of resource types (Hooper *et al.* 2000). Since many previous studies showing the positive effect of plant diversity on microbial biomass took place in grasslands, these findings could mean that e.g. one tree species could have supported a greater heterogeneity of resources than several grass species (Wardle *et al.* 1999). Previous studies also showed no or only weak effects of tree diversity, highlighting that tree species identity might be the major driver of soil microbial properties (Pei *et al.* 2016, Khalifa *et al.* 2017). We must also consider that the design of the BEF-China experiment is based on a random planting, which means that the analysed TSPs seldomly had the same direct

neighbourhood composition. As Khalifa *et al.* (2017) showed, community-weighted means of individual tree traits were affecting soil microbial biomass and respiration strongly. This could mean that TSPs of the same plot could have been affected differently by the plot-level diversity. A solution for this might be to pool multiple soil samples for each plot to get an average effect of the diversity level.

## 4.2 tree identity and tree-tree interaction effects on soil microbial properties

This study showed clear differences between the effects of tree identities and tree-tree interactions. In the first field experiment set up, all twelve tree species identities were highly significant in affecting the soil microbial properties, whereas no tree-tree interaction effect could be found. This means that the tree-tree interactions did not perform significantly differently from the weighted average of the monoculture performances. All twelve tree species performed better in their monocultures than in any hetero-specific pair. This indicates that the tree species have associated soil microbes, which perform better in monocultures than in mixtures. As it was shown before, many plant taxa are colonized by specific microbial communities (Berg & Smalla 2009) e.g. *Betula pendula* stands were associated with the growth of gram-negative bacteria, whereas *Pinus sylvestris* stands favour gram-positive bacteria (Priha *et al.* 2001). To test whether these results of specifically associated microbes are also true for subtropical forests, community structures of soil microbes should be analysed. Following other study approaches (e.g. Pei *et al.* 2016, Beugnon & Du *et al.* 2021, Singavarapu *et al.* 2021), a phospholipid fatty acid (PLFA) analysis could bring important insights as it is an effective strategy for investigating soil microbial community composition (Frostegård *et al.* 2011). It could give more insights about the effects of tree species identities on soil microbes. I would expect a lower abundance and diversity of bacteria in more acidic soils and a higher abundance of fungi in evergreen dominated plots (Guo *et al.* 2016, Chai *et al.* 2019). It also could give additional information about the non-significant tree-tree interactions on microbial biomass and respiration. It is possible that hetero-specific pairs decrease the abundance and activity of specific tree identity associated microbes, but increase the overall diversity of the soil community.

However, here it needs to be taken into account, that the number of samples per tree species inmonocultures was very low (two to three samples per species) for the field measurements with large variances for some species such as *Sapindus mukorossi.* The variances of the samples can be explained by the environmental differences of the monoculture plots e.g. altitude, slope and light penetration. On average, two TSPs were sampled in two monocultures for each species. Further analyses focusing on the

different effects of tree identities and tree-tree interactions should include a higher number of samples and plot-specific abiotic factors to ensure a correct interpretation.

In the greenhouse experiment we also found strong effects of tree species identities. All eight tree species were significantly affecting soil microbial biomass and respiration, indicating specifically associated relationships between tree species and soil microbes. In contrast to the field, the analyses showed three significant tree-tree interaction effects. These positive interactions affected only soil microbial biomass but nor basal respiration. Possible explanations for the interaction effects could be the closer distance between the trees inside the experimental tubes and the resulting higher facilitation between some species. Another factor might be the controlled climatic conditions. Interestingly, our results showed that *R. chinensis* performed best and had the most positive effect on microbial biomass in the greenhouse experiment, whereas this species had to be removed from the field experiment as most individuals could not compete in mixtures and had died. This indicates that biotic drivers such as tree identity and tree-tree interactions are mediated by local abiotic conditions, which were controlled for in the greenhouse set-up. Additionally, the lack of herbivory and leaf litter input should be mentioned as a possible explanation for the different results between field and the greenhouse experiment.

However, for the greenhouse experiment it needs to be mentioned, that the above- and below-ground biomasses could not be taken into account since they were not yet weighed and could therefore not be part of the diversity-interaction model. It would be highly recommended to repeat the statistical analysis when the proportions of the trees can be added to the model, due to possible changes in the output. One would assume stronger effects of the bigger trees compared to the smaller one in the tube.

As previously mentioned, tree species can alter soil microbial communities in different ways (e.g. leaf litter, change soil pH and microclimate). This study tested the tree foliage (deciduous, evergreen) and the mycorrhizal type (EMF, AMF) of the tree species as possible predictors for tree species identity effects. Previous studies suggested that broad-leaved deciduous and evergreen species affect soil microbial abundance and activity differently due to their different leaf litter and root exudate compositions (Guo *et al.* 2016, Chai *et al.* 2019, Wang *et al.* 2021). It was also shown that different mycorrhizal associations can drive resource use complementarity and soil microbial community composition (Ferlian *et al.* 2018, Singavarapu *et al.* 2021). However, neither of the tested predictors had an effect on soil microbial biomass or respiration. It is therefore necessary to perform additional analyses, including other belowground tree traits such as root diameter (RD) or specific root length (SRL). SRL is highly dependent on fine roots, which

are functionally responsible for nutrient and water uptake (Ostonen *et al.* 2007) and are influenced by the rhizosphere microbial communities (Lõhmus *et al.* 2006). Fine roots are responsible for root exudates, which were - together with leaf litter inputs - shown to be driving factors for the effects of plant species on microbial communities (Wardle *et al.* 2004, Prescott & Grayston 2013, Eisenhauer *et al.* 2017), since plant species vary in quality and quantity of the resources they allocate belowground (Guillemot *et al.* 2020). These may change the types of substrates available for soil microbe nutrition and thus trigger changes in the rhizodeposition (Somers *et al.* 2004, Van der Krift *et al.* 2001).

## 4.3 small-scale drivers of soil microbial properties

The goal of the transect experiment was to determine small-scale drivers of soil microbial properties in a natural environment. Overall, an increased distance to the tree and depth reduced microbial biomass as well as basal respiration, whereas higher soil moisture increased both microbial properties. It could also be shown that specific tree species identity effects were stronger with larger size of the tree.

The negative correlation of an increased distance to the tree and microbial biomass was already shown by previous studies (Fall *et al.* 2012) and can possibly be explained by the higher amount of available nutrients in the soil close to the trees (Singh *et al.* 2004, Fall *et al.* 2012), or by the higher water availability due to throughfall and stemflow near the tree base.

We found furthermore a negative correlation of increased soil depth and microbial biomass. This can be explained since less nutrients are found in deeper soil layers, as the main decomposition happens in the leaf litter cover and top soil layers (Prescott & Grayston 2013). Additionally, deeper soils have a decreased amount of oxygen and soil water content and contain less plant root biomass (Fall *et al.* 2012, Serna-Chavez *et al.* 2013, Engelhardt *et al.* 2018).

Soil moisture was identified to have a strong positive relationship with both soil microbial properties. The high importance of soil water content was shown in many studies before (Serna-Chavez *et al.* 2013, Schimel 2018, Cesarz *et al.* 2021). This is consistent with the evidence from biomes other than subtropical forests, like grasslands or drylands (Fierer and Jackson 2006, Maestre *et al.* 2015). High levels of soil moisture were shown to increase soil microbial biomass and basal respiration due to positive effects on soil enzyme activities, fluxes of soil nutrients and oxygen availability (Stark and Firestone 1995, Brockett *et al.* 2012). A higher soil humidity can furthermore buffer possible negative changes in soil pH, suggesting

it to be the major driver (Cesarz *et al.* 2021). However, the positive effects of soil moisture can be altered by local biotic drivers such as tree species identity. Recent studies showed that tree species identities can alter soil characteristics such as pH and soil water content and explain variations in soil microbial abundance and activity through their morphological and leaf chemical traits (Thoms *et al.* 2010, Reich *et al.* 2015, Pei *et al.* 2016, Beugnon & Du *et al.* 2021). They can also alter the available soil water content due to their own water uptake, whereby some species can use water more efficiently due to their hydraulic traits (Brodribb 2009, Anderegg *et al.* 2018). More efficient species would affect soil microbes less in more drier periods, as they would use less water. Additionally, tree species can affect soil water content with an higher amount of organic matter on the ground, which reduces evaporation even better than a canopy structures (Magliano *et al.* 2017). However, we did not find any significant effects of the tested tree species on the soil water content. We expected a higher demand of water for larger trees as they have more leaves and a higher transpiration rate. In contrast to these expectations, we did not find an effect of the tree species size on the soil moisture. The SEM revealed, that the plot was indirectly affecting the soil microbial biomass and respiration by changes in soil moisture. This was probably due to differences in altitude and slope of the plots but also due to the higher amount of sand in the soil and the shallow soil layers. Many times the soil-corer reached stones or large roots at approximately 10 cm depth.

We expected understory plants to affect soil microbial biomass and respiration as they can change resource availability or microclimate locally. In contrast to the expectations, neither understory plant abundance nor root biomass had an effect on the soil microbial biomass and respiration. This stands in contrast to the findings of Liu *et al.* (2019) who showed that understory shrub biomass was positively correlated with basal respiration. They could also show, that an increased amount of belowground fine roots positively affected the soil microbial activity in forests.

We hypothesized to have stronger tree species identity effects with larger size of the trees. Indeed, we found that an increased tree size increased the species identity effect on soil microbial properties. *S. mukorossi* was found to have positive effects on soil microbial activity, whereas *L. formosana* was negatively correlated with basal respiration. It was shown by Chien *et al.* (2012) that the balsam of *L. formosana* contained acidic compounds which were found to be inhibitory for fungi. These compounds could also be present in the leaf litter or root exudates (Öztürka *et al.* 2008) leading to a small-scale change of soil pH. Since soil pH was found to be a strong driver of microbial growth (Fierer & Jackson 2006), additional pH measurements should be performed to identify the predictors of the contrary species identity effects of *S. mukorossi* and *L. formosana*. It was shown that soil fungi and bacteria react differently

to changes in soil pH: bacterial growth reduced with a more acidic pH, whereas fungi growth was shown to increase (Rousk *et al.* 2020). Changes in soil pH might also explain the significant negative effect of *L. formosana* on basal respiration in the transect experiment.

## 4.4 conclusion

The goal of this study was to explain diversity effects on microbial biomass and respiration on the small scale by focussing on tree identity and tree-tree interaction effects. It could be shown in different experimental settings, that over all, tree species identities were strong drivers of microbial properties, indicating a strong association to certain microorganisms. In contrast, tree-tree interactions and plot-level tree diversity had none or only weak effects. On smaller scales, an increased distance to the tree and depth reduced microbial biomass as well as basal respiration, whereas higher soil moisture increased both microbial properties. Latter was also shown to mediate local biotic drivers such as tree identity effects, as they were stronger under drier conditions. It could also be shown that specific tree species identity effects were stronger with larger size of the tree. However, we could not find predictors of the tree species identity effects, since neither leaf- nor mycorrhiza type had any significant effects.

Overall, we showed that microbial biomass and basal respiration were strongly affected by tree species identities but these effects were mediated by local abiotic conditions. Future studies could implement belowground tree trait data, pH measurements and microbial community structure analyses to find the predictors of tree species identity effects on soil microbial biomass and respiration.

# 5. Summary

Soil microorganisms play key roles in the recycling processes of essential elements such as carbon, nitrogen and phosphorus. They act as decomposers of organic matter, root symbionts and are able to fix atmospheric nitrogen and distribute nutrients across the soil. A majority of these processes happen in forest soils and it is crucial to understand the drivers of soil microbial activity as it is a proxy for the functioning of ecosystems. In subtropical forests, nutrient-limitation, pH and soil moisture are considered the main drivers of soil microbial abundance and activity. However, these effects of abiotic drivers can be altered by local biotic conditions and both could also interact to mediate soil microbial communities. Plant species, for example, can alter the soil pH, resource availability, the microclimate and also soil moisture through e.g. leaf litter contents or root exudates. To understand the drivers of soil microbial properties in subtropical forests, this study tested tree identity and tree-tree interaction effects on soil microbial biomass and respiration, using three different experimental set ups. The first two were placed in a subtropical tree-diversity experiment. In the first set-up, tree-tree interactions were investigated in a natural environment over a plot-level diversity gradient from monocultures to 24-species mixtures. In the second set up, specific tree-tree interactions were investigated in more detail in two-species mixtures with a focus on distance relationships. To complement the previous part of this study, a controlled greenhouse experiment was set up, focussing on the effects of microorganisms and phosphorus limitation. In all experimental set-ups, soil samples were taken and analysed using the substrate induced respiration (SIR) method to calculate the microbial biomass and basal respiration. It could be shown that over all three experiments, tree species identities were strong drivers of microbial properties, indicating strong associations between microbes and certain tree species. Additionally, it was shown that these effects were stronger with increased tree size. In contrast, tree-tree interactions and plot-level tree diversity had none or only weak effects. On smaller scales, an increased distance to the tree and depth reduced microbial biomass as well as basal respiration, whereas higher soil moisture increased both microbial properties.

## 5.1 Zusammenfassung

Alle lebenden Organismen benötigen essentielle Nährstoffe wie Kohlenstoff, Stickstoff und Phosphor zum Überleben. Bodenorganismen spielen eine wichtige Rolle in den Recyclingprozessen dieser Stoffe in dem sie unter anderem organisches Material zersetzen, als Symbionten mit Wurzeln agieren, atmosphärischen Stickstoff fixieren oder Nährstoffe im Boden verteilen. Die Mehrheit dieser Prozesse findet in Waldböden statt und es ist wichtig zu wissen, welche Faktoren die Aktivität der Bodenorganismen beeinflussen um die Funktionalität von Ökosystemen zu verstehen. In subtropischen Wäldern sind es vor allem Nährstofflimitierung, Boden-pH und -feuchtigkeit, welche die Biomasse und Aktivität der Bodenorgansimen beeinflussen. Diese abiotischen Faktoren können jedoch von lokalen biotischen Faktoren wie Pflanzenarten oder -gemeinschaften verändert werden. Beispielsweise können manche Pflanzen den Boden pH-Wert oder die Nährstoffverfügbarkeit durch Abbauprodukte des Laubes oder Wurzelexudate verändern. Um die treibenden Faktoren mikrobieller Biomasse und Aktivität in subtropischen Wäldern zu verstehen, wurden die Einflüsse von Baumarten und Baum – Baum Interaktionen getestet. Hierzu wurden Daten aus drei verschiedenen Experimenten analysiert. Das Erste untersuchte die Baum – Baum – Interaktionen innerhalb der natürlichen Umgebung und über einen Plotdiversitätsgradienten von Monokulturen bis 24-Arten-Mischungen hinweg. Das zweite Experiment untersuchte diese Beziehungen detailgenauer innerhalb einer 2-Arten Mischung unter Inbetrachtnahme von Distanzeinflüssen. Um die Ergebnisse aus dem Freiland zu komplementieren, wurden zusätzlich die Daten aus einem kontrollierten Gewächshausexperiment untersucht, welches den Fokus auf den Effekt von Mikroorganismen und Phosphorlimitierung setzte. Aus allen drei Experimenten wurden Bodenproben mithilfe der Substrat-Induktions-methode (SIR) analysiert um die Biomasse und Aktivität der Mikroorganismen festzustellen. Es konnte gezeigt werden, dass in allen drei Experimenten die Baumart den größten Effekt auf die mikrobielle Biomasse und Aktivität besaß, was darauf hinweist, dass es speziell an die Baumarten angepasste Mikroorganismen gibt. Diese finden die optimalen Bedingungen innerhalb der lokalen Veränderungen durch bestimmte Baumarten. Es konnte auch gezeigt werden, dass die Baumarten Effekte stärker waren je größer die Bäume waren. Im Gegensatz dazu, konnten keine, oder nur schwache, Effekte von Baum – Baum – Interaktionen oder den Plotdiversitätsleveln gefunden werden. Betrachtete man eine kleinere Skala, konnte festgestellt werden, dass eine größere Distanz zu den Bäumen, sowie eine größere Bodentiefe die mikrobielle Biomasse und Aktivität verringerte. Wohingegen eine erhöhte Bodenfeuchtigkeit beide Parameter steigerte.

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# 7. Statutory Declaration

## Eidesstattliche Erklärung

Hiermit erkläre ich, dass ich die vorliegende Arbeit mit dem Titel „tree identity and tree-tree interaction effects on soil microbial biomass and basal respiration“, selbstständig angefertigt habe und keine anderen als die von mir angegebenen Quellen und Hilfsmittel verwendet wurden.

Diese Arbeit hat weder in gleicher noch einer ähnlichen Form einer Prüfungskommission vorgelegen.

Ort, Datum Unterschrift

# 8. Acknowledgements

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Last but not least, I want to thank my family and friends for their constant support, proof-reading sessions and their occasional rants about “me wasting precious writing-time eating ice-cream”.

Thank you Georg, for everything.

# 9. Supporting Information

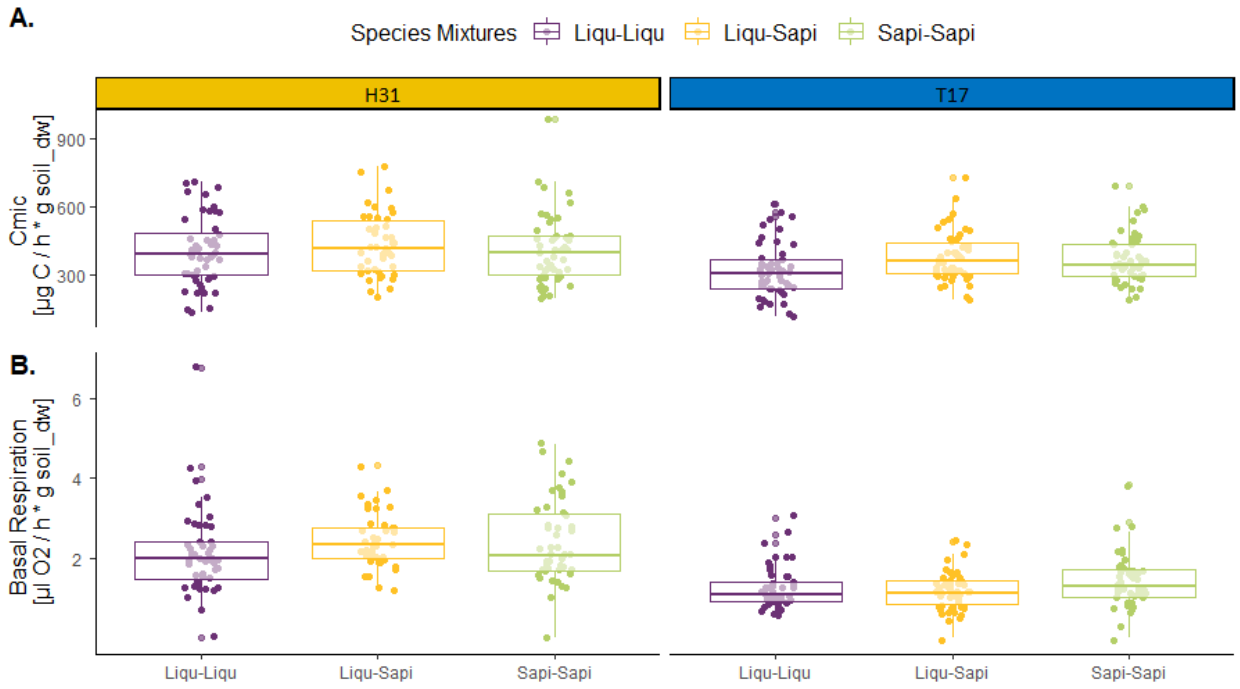


***Figure S1:*** *Differences of microbial biomass (A) and basal respiration (B) in the plots H31 (yellow) and T17 (blue) between the three following species mixtures: Liquidambar formosana – Liquidambar formosana (purple), Liquidambar formosana – Sapindus mukorossi (orange) and Sapindus mukorossi – Sapindus mukorossi (green) for the combined depth of 0-10 cm.*

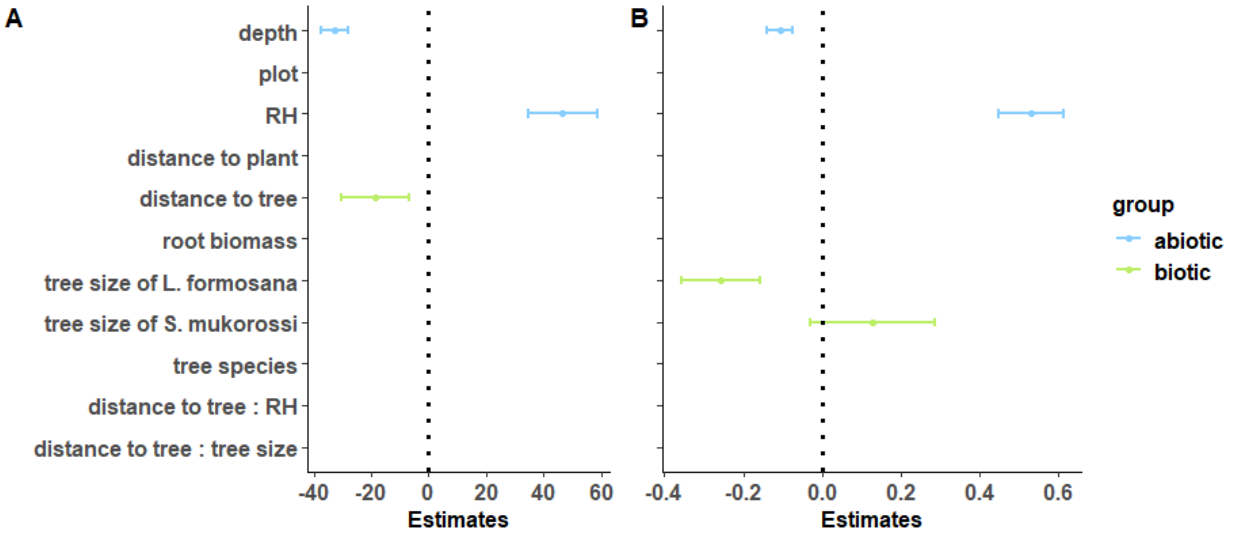


***Figure S2:*** *Forest-plot of all tested drivers of soil microbial biomass (A) and basal respiration (B). Shown are the estimates of the significant drivers*

***Table S1:*** *List of tree species building the TSP-combinations in the field experiment. Tree species used also in the greenhouse experiment marked with *, tree species used exclusively in the greenhouse experiment marked with ** and the tree species used for the transect experiment marked with °. Additional information about ectomycorrhizal-fungi (EMF) or arbuscular mycorrhizal-fungi (AMF) and leaf persistence: evergreen (E) or deciduous (D).*

| tree species | mycorrhizal type | leaf persistence |
|---|---|---|
| *Castanea henryi* | EMF | D |
| *Castanopsis sclerophylla* | EMF | E |
| *Choerospondias axillaris ** | AMF | D |
| *Cyclobalanopsis glauca ** | EMF | D |
| *Koelreuteria bipinnata ** | AMF | D |
| *Liquidambar formosana °* | AMF | D |
| *Lithocarpus glaber* | EMF | E |
| *Nyssa sinensis* | AMF | D |
| *Quercus fabri ** | EMF | D |
| *Quercus serrata ** | EMF | D |
| *Rhus chinensis ** | AMF | D |
| *Sapindus mukorossi °* | AMF | D |
| *Sapium sebiferum ** | AMF | D |
| *Schima superba ** | AMF | E |

***Table S2:*** *Model Output from the "DImodels" function for microbial biomass in the field set-up*

```
Coefficients:
                  Estimate Std. Error t value Pr(>|t|)
C.henry            336.322     29.142  11.541  < 2e-16 ***
N.sinen            358.705     35.637  10.066  < 2e-16 ***
L.formo            363.269     32.413  11.208  < 2e-16 ***
Q.fabri            309.375     34.485   8.971 2.39e-15 ***
L.glabe            284.289     34.170   8.320 9.23e-14 ***
Q.serra            358.105     28.189  12.704  < 2e-16 ***
C.scler            358.447     29.078  12.327  < 2e-16 ***
C.axill            325.704     26.708  12.195  < 2e-16 ***
S.sebif            378.415     31.344  12.073  < 2e-16 ***
K.bipin            317.112     32.164   9.859  < 2e-16 ***
S.mukor            331.157     28.931  11.446  < 2e-16 ***
C.glauc            339.564     31.554  10.761  < 2e-16 ***
Sp.rich              1.179      1.385   0.852    0.396
`C.henry:N.sinen` -363.437    272.504  -1.334    0.185
`C.henry:L.formo` -558.230    387.110  -1.442    0.152
`C.henry:S.mukor`  -72.694    289.648  -0.251    0.802
`N.sinen:L.formo` -226.538    306.030  -0.740    0.460
`N.sinen:S.mukor`    4.280    323.496   0.013    0.989
`L.formo:S.mukor`  303.543    246.324   1.232    0.220
`Q.fabri:K.bipin`  776.183   1245.473   0.623    0.534
`Q.fabri:C.glauc`  255.065    290.493   0.878    0.382
`L.glabe:K.bipin`  148.742    383.547   0.388    0.699
`Q.serra:C.scler` -207.438    264.268  -0.785    0.434
`Q.serra:C.axill`  434.407    574.664   0.756    0.451
`Q.serra:S.sebif`  178.457    671.902   0.266    0.791
`C.scler:C.axill`  636.627    779.254   0.817    0.415
`C.scler:S.sebif` -429.196    471.648  -0.910    0.364
`C.axill:S.sebif`  -79.872    277.517  -0.288    0.774
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

***Table S3:*** *Model Output from the "DImodels" function for basal respiration in the field set-up*

```
Coefficients:
                   Estimate Std. Error t value Pr(>|t|)
C.henry            1.590647   0.120832  13.164   <2e-16 ***
N.sinen            1.523470   0.147759  10.310   <2e-16 ***
L.formo            1.904823   0.134391  14.174   <2e-16 ***
Q.fabri            1.459106   0.142982  10.205   <2e-16 ***
L.glabe            1.401310   0.141678   9.891   <2e-16 ***
Q.serra            1.633787   0.116879  13.978   <2e-16 ***
C.scler            1.439947   0.120566  11.943   <2e-16 ***
C.axill            1.742618   0.110738  15.736   <2e-16 ***
S.sebif            1.707682   0.129960  13.140   <2e-16 ***
K.bipin            1.406410   0.133360  10.546   <2e-16 ***
S.mukor            1.427791   0.119957  11.903   <2e-16 ***
C.glauc            1.524348   0.130831  11.651   <2e-16 ***
Sp.rich            0.006979   0.005741   1.216   0.2263
`C.henry:N.sinen` -1.408994   1.129870  -1.247   0.2146
`C.henry:L.formo` -2.572058   1.605055  -1.602   0.1114
`C.henry:S.mukor`  0.196216   1.200955   0.163   0.8705
`N.sinen:L.formo` -1.821971   1.268880  -1.436   0.1534
`N.sinen:S.mukor`  0.971594   1.341298   0.724   0.4701
`L.formo:S.mukor`  1.319217   1.021322   1.292   0.1987
`Q.fabri:K.bipin`  9.086360   5.164048   1.760   0.0808 .
`Q.fabri:C.glauc`  1.193178   1.204459   0.991   0.3237
`L.glabe:K.bipin`  2.064551   1.590284   1.298   0.1965
`Q.serra:C.scler` -0.962403   1.095723  -0.878   0.3814
`Q.serra:C.axill` -0.713422   2.382705  -0.299   0.7651
`Q.serra:S.sebif`  2.650614   2.785878   0.951   0.3431
`C.scler:C.axill`  0.346141   3.230986   0.107   0.9148
`C.scler:S.sebif` -1.088080   1.955574  -0.556   0.5789
`C.axill:S.sebif`  0.189694   1.150656   0.165   0.8693
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

***Table S4:*** *Model Output from the "DImodels" function for microbial biomass in the greenhouse experiment*

Coefficients:

| | Estimate | Std. Error | t value | Pr(>\|t\|) | |
|---|---|---|---|---|---|
| S.super | 53.7346 | 7.3273 | 7.333 | 4.85e-12 | *** |
| R.chine | 82.8436 | 6.9531 | 11.915 | < 2e-16 | *** |
| Q.fabri | 56.1684 | 6.9180 | 8.119 | 4.01e-14 | *** |
| Q.serra | 56.0112 | 4.9911 | 11.222 | < 2e-16 | *** |
| C.axill | 61.5716 | 4.8986 | 12.569 | < 2e-16 | *** |
| S.sebif | 53.3966 | 4.8986 | 10.900 | < 2e-16 | *** |
| K.bipin | 64.9799 | 4.8986 | 13.265 | < 2e-16 | *** |
| C.glauc | 45.0017 | 6.6774 | 6.739 | 1.52e-10 | *** |
| Chamber | -0.7638 | 0.8096 | -0.943 | 0.34655 | |
| P_addno | -0.8303 | 1.9515 | -0.425 | 0.67094 | |
| Ino_addyes | 4.2274 | 1.9797 | 2.135 | 0.03390 | * |
| ‘S.super:R.chine‘ | 62.9026 | 23.5957 | 2.666 | 0.00828 | ** |
| ‘S.super:Q.fabri‘ | -16.8466 | 19.3405 | -0.871 | 0.38473 | |
| ‘S.super:C.glauc‘ | 9.9152 | 22.2448 | 0.446 | 0.65625 | |
| ‘R.chine:Q.fabri‘ | 33.7057 | 22.2298 | 1.516 | 0.13097 | |
| ‘R.chine:C.glauc‘ | 53.3743 | 21.0738 | 2.533 | 0.01205 | * |
| ‘Q.fabri:C.glauc‘ | 28.3333 | 20.2933 | 1.396 | 0.16414 | |
| ‘Q.serra:C.axill‘ | 30.9218 | 22.9552 | 1.347 | 0.17942 | |
| ‘Q.serra:S.sebif‘ | 14.9419 | 22.9281 | 0.652 | 0.51532 | |
| ‘Q.serra:K.bipin‘ | -17.0232 | 23.9330 | -0.711 | 0.47770 | |
| ‘C.axill:S.sebif‘ | 39.6773 | 19.7501 | 2.009 | 0.04583 | * |
| ‘C.axill:K.bipin‘ | 1.9833 | 20.7162 | 0.096 | 0.92382 | |
| ‘S.sebif:K.bipin‘ | 2.8605 | 19.3425 | 0.148 | 0.88258 | |

---

Signif. codes: 0 ’***’ 0.001 ’**’ 0.01 ’*’ 0.05 ’.’ 0.1 ’ ’ 1

***Table S5:*** *Model Output from the "DImodels" function for basal respiration in the greenhouse experiment*

```
Coefficients:
                  Estimate Std. Error t value Pr(>|t|)
S.super            0.80169    0.13933   5.754 3.07e-08 ***
R.chine            0.98865    0.13243   7.465 2.21e-12 ***
Q.fabri            0.88638    0.13185   6.723 1.67e-10 ***
Q.serra            0.80583    0.09118   8.838 4.05e-16 ***
C.axill            0.72725    0.09298   7.822 2.54e-13 ***
S.sebif            0.67808    0.09298   7.293 6.18e-12 ***
K.bipin            0.69991    0.09298   7.528 1.52e-12 ***
C.glauc            0.82595    0.12753   6.477 6.59e-10 ***
Chamber           -0.02122    0.01511  -1.404   0.1618
Ino_addno         -0.07461    0.03696  -2.019   0.0448 *
P_addyes          -0.05769    0.03643  -1.584   0.1148
`S.super:R.chine` -0.60897    0.44052  -1.382   0.1683
`S.super:Q.fabri` -0.15521    0.36107  -0.430   0.6677
`S.super:C.glauc`  0.27676    0.41530   0.666   0.5059
`R.chine:Q.fabri` -0.05233    0.41502  -0.126   0.8998
`R.chine:C.glauc` -0.34820    0.39343  -0.885   0.3772
`Q.fabri:C.glauc`  0.21119    0.37886   0.557   0.5778
`Q.serra:C.axill` -0.25062    0.42856  -0.585   0.5593
`Q.serra:S.sebif`  0.67212    0.42805   1.570   0.1179
`Q.serra:K.bipin` -0.11109    0.44681  -0.249   0.8039
`C.axill:S.sebif` -0.23490    0.36872  -0.637   0.5248
`C.axill:K.bipin` -0.21300    0.38676  -0.551   0.5824
`S.sebif:K.bipin`  0.10923    0.36111   0.302   0.7626
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```