



HAL
open science

Abiotic and biotic drivers of tree trait effects on soil microbial biomass and soil carbon concentration

Rémy Beugnon, Wensheng Bu, Helge Bruelheide, Andréa Davrinche, Jianqing Du, Sylvia Haider, Matthias Kunz, Goddert von Oheimb, Maria Perles-Garcia, Mariem Saadani, et al.

► **To cite this version:**

Rémy Beugnon, Wensheng Bu, Helge Bruelheide, Andréa Davrinche, Jianqing Du, et al.. Abiotic and biotic drivers of tree trait effects on soil microbial biomass and soil carbon concentration. *Ecological monographs*, 2022, 93 (2), pp.e1563. 10.1002/ecm.1563 . hal-03930557

HAL Id: hal-03930557

<https://hal.science/hal-03930557v1>

Submitted on 5 Jun 2023

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.


















L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution 4.0 International License

ARTICLE

Abiotic and biotic drivers of tree trait effects on soil microbial biomass and soil carbon concentration

Rémy Beugnon^{1,2,3}  | Wensheng Bu⁴  | Helge Bruehlheide^{1,5}  |
 Andréa Davrinche^{1,5}  | Jianqing Du^{6,7} | Sylvia Haider^{1,5}  |
 Matthias Kunz⁸  | Goddert von Oheimb⁸  | Maria D. Perles-Garcia^{1,5}  |
 Mariem Saadani^{1,5}  | Thomas Scholten⁹  | Steffen Seitz⁹  |
 Bala Singavarapu^{1,5,10}  | Stefan Trogisch^{1,5}  | Yanfen Wang^{6,11} |
 Tesfaye Wubet^{1,10}  | Kai Xue^{6,11}  | Bo Yang¹² | Simone Cesarz^{1,13}  |
 Nico Eisenhauer^{1,13} 

¹German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, Leipzig, Germany

²Leipzig Institute for Meteorology, Universität Leipzig, Leipzig, Germany

³CEFE, University of Montpellier, CNRS, EPHE, IRD, Montpellier, France

⁴College of Forestry, Jiangxi Agricultural University, Nanchang, China

⁵Institute of Biology/Geobotany and Botanical Garden, Martin Luther University Halle-Wittenberg, Halle, Germany

⁶College of Resources and Environment, University of Chinese Academy of Sciences, Beijing, China

⁷Beijing Yanshan Earth Critical Zone National Research Station, University of Chinese Academy of Sciences, Beijing, China

⁸Institute of General Ecology and Environmental Protection, Technische Universität Dresden, Tharandt, Germany

⁹Institute of Geography, University of Tübingen, Tübingen, Germany

¹⁰Department of Community Ecology, UFZ-Helmholtz Centre for Environmental Research, Halle, Germany

¹¹State Key Laboratory of Tibetan Plateau Earth System, Environment and Resources (TPESER), Chinese Academy of Sciences, Beijing, China

¹²Jiangxi Key Laboratory of Plant Resources and Biodiversity, Jingdezhen University, Jingdezhen, China

¹³Institute of Biology, Leipzig University, Leipzig, Germany

Correspondence

Rémy Beugnon

Email: remy.beugnon@idiv.de

Funding information

Deutsche Forschungsgemeinschaft, Grant/Award Numbers: Ei 862/29-1, 202548816, DFG-FZT 118, 319936945/GRK2324; University of Chinese Academy Sciences

Handling Editor: Melissa Cregger

Abstract

Forests are ecosystems critical to understanding the global carbon budget, due to their carbon sequestration potential in both aboveground and belowground compartments, especially in species-rich forests. Soil carbon sequestration is strongly linked to soil microbial communities, and this link is mediated by the tree community, likely due to modifications of microenvironmental conditions (i.e., biotic conditions, soil properties, and microclimate). We studied soil carbon concentration and the soil microbial biomass of 180 local neighborhoods along a gradient of tree species richness ranging from 1 to 16 tree species per plot in a Chinese subtropical forest experiment (BEF-China). Tree productivity and different tree functional traits

Simone Cesarz and Nico Eisenhauer are co-last authors.

This is an open access article under the terms of the [Creative Commons Attribution](https://creativecommons.org/licenses/by/4.0/) License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2022 The Authors. *Ecological Monographs* published by Wiley Periodicals LLC on behalf of The Ecological Society of America.

were measured at the neighborhood level. We tested the effects of tree productivity, functional trait identity, and dissimilarity on soil carbon concentrations, and their mediation by the soil microbial biomass and microenvironmental conditions. Our analyses showed a strong positive correlation between soil microbial biomass and soil carbon concentrations. In addition, soil carbon concentration increased with tree productivity and tree root diameter, while it decreased with litterfall C:N content. Moreover, tree productivity and tree functional traits (e.g., fungal root association and litterfall C:N ratio) modulated microenvironmental conditions with substantial consequences for soil microbial biomass. We also showed that soil history and topography should be considered in future experiments and tree plantations, as soil carbon concentrations were higher at sites where historical (i.e., at the beginning of the experiment) carbon concentrations were high, themselves being strongly affected by the topography. Altogether, these results implied that the quantification of the different soil carbon pools is critical for understanding microbial community–soil carbon stock relationships and their dependence on tree diversity and microenvironmental conditions.

KEYWORDS

BEF-China, biotic conditions, microbial community, microclimate, microenvironment, productivity, root morphology, soil carbon stock, soil quality, subtropical forest, tree diversity

INTRODUCTION

The rapid increase in atmospheric carbon is one of the main causes of climate change and has become a major threat to life on Earth (IPBES, 2019). Atmospheric carbon concentrations can be reduced by both reducing carbon emissions and increasing carbon fixation. Forest ecosystems have been identified to be capable of mitigating increases in atmospheric carbon dioxide by capturing and fixing it aboveground and storing it both above and below the ground (Bastin et al., 2019; Lewis et al., 2019). Belowground carbon storage provides a high potential for atmospheric carbon control due to the long residence time of carbon in soil (Trumbore, 2009). However, our understanding of the balance between soil carbon influx (e.g., due to photosynthesis) and efflux (e.g., due to soil respiration and erosion) in forest systems is still limited.

Tree diversity can enhance carbon storage in forests (Huang et al., 2018; Liu et al., 2018) via increased productivity, such as tree biomass, litterfall quantity, root biomass, and exudation (Huang et al., 2017, 2018; Xu et al., 2020; Zheng et al., 2019), even though these processes might lead to higher soil respiration. Moreover, tree diversity should decrease soil erosion and thus soil carbon effluxes, by reducing the impact of precipitation on surface soil (Goebes et al., 2015; Seitz et al., 2016), for instance by increasing canopy closure (Perles-Garcia et al., 2021; Williams et al., 2017). Consequently, tree diversity is expected to enhance carbon

storage in forests by increasing the ratio of carbon influxes to carbon effluxes (Liu et al., 2018).

Recent studies linked soil carbon concentrations to tree roots (Adamczyk et al., 2019). Specifically, morphological traits, for example, root diameter (RD) and specific root length (SRL), were shown to control the release of root carbon to the soil by root turnover or exudation (Sun et al., 2020) and to drive soil organic matter decomposition (Adamczyk et al., 2019). For example, higher SRL increases root carbon exudation and root turnover due to a higher density of fine roots (Bergmann et al., 2020; Sun et al., 2020; Wen et al., 2019). Additionally, the mycorrhizal association of tree roots, for example, with arbuscular and ectomycorrhizal fungi, is a key driver of soil carbon storage (Averill et al., 2014; Averill & Hawkes, 2016; Craig et al., 2018; Crowther et al., 2019), as differences in mycorrhizal associations can lead to variations in fungal metabolic pathways due to different nutrient acquisition strategies (Bonfante & Genre, 2010; Crowther et al., 2019), and thus influence soil carbon dynamics. However, the effects of the mycorrhizal association on soil carbon dynamics still remain unclear (Frey, 2019). In addition, mycorrhizal colonization of roots increases with the increase of cortical tissues, themselves being positively correlated with RD (Bergmann et al., 2020). Thus, RD can determine soil carbon concentrations by modulating fungal colonization.

Soil microorganisms play a central role in soil carbon dynamics by processing soil carbon, thereby being the

main driver of carbon sequestration (Lange et al., 2015; Schmidt et al., 2011). As microorganisms are the main consumers of plant-derived and soil organic matter, one would expect them to reduce soil carbon concentrations. However, recent studies have highlighted that increased microbial activity could increase soil carbon concentrations by transferring higher amounts of soil organic matter to a stable pool of microbial necromass (Buckeridge et al., 2020; Lange et al., 2015; Miltner et al., 2012; Schmidt et al., 2011; Trumbore, 1993). The relative contribution of microbial derivatives in structuring soil organic carbon has been underestimated in the past (Kästner & Miltner, 2018; Simpson et al., 2007), whereas microbial residues are expected to represent 50%–80% of the total soil organic carbon (Liang & Balsler, 2011; Simpson et al., 2007).

Soil microorganisms and the functions provided are strongly influenced by the above-mentioned root traits and, thereby, the tree community composition due to species-specific associations (Lareen et al., 2016; Pei et al., 2016). Root biomass and litter production can further increase habitat space and substrate availability for soil microorganisms with increasing plant species richness (Bardgett et al., 2014; Hooper et al., 2000). Moreover, species-rich plant communities affect soil microorganisms and generally increase soil microbial biomass and diversity (Beugnon et al., 2021; Chapman et al., 2013; Eisenhauer et al., 2010; Lange et al., 2015). Thereby, the positive effect of high plant species diversity on soil microorganisms is suggested to increase soil carbon concentrations (Lange et al., 2015; Li et al., 2019; Liang & Balsler, 2011). For example, plant species diversity enhances soil microbial biomass and increases soil carbon by enhancing litter diversity (Thoms et al., 2010; Ushio et al., 2008), root biomass (Xu et al., 2020), and the amount and diversity of root exudates (Eisenhauer et al., 2017), and is due to the dissimilarity between root traits (Kramer et al., 2016).

Next to root traits, environmental conditions, such as biotic interactions, soil chemistry, and climate, influence soil microbial biomass and community composition (Bernhard et al., 2018; Beugnon et al., 2021; Cesarz et al., 2022; Delgado-Baquerizo et al., 2016; Gottschall et al., 2019). One example of biotic interactions is that understory plant diversity can increase soil microbial biomass and activity in temperate forests (Eisenhauer et al., 2011); however, empirical evidence remains inconsistent (Xu et al., 2020). Global studies have shown that climate and soil chemistry are the two main drivers of microbial biomass and composition in drylands (Delgado-Baquerizo et al., 2016), but also along large climate gradients from arid to humid (Bernhard et al., 2018). Temperature and soil water content increase microbial biomass by increasing microbial activity and growth (Delgado-Baquerizo et al., 2016). Moreover, reduced water availability increases the osmotic

pressure, which, due to salt concentration and pH, constrains microbial biomass and alters community composition, pointing to interactions among abiotic drivers (Aciego Pietri & Brookes, 2009; Delgado-Baquerizo et al., 2017; Wichern et al., 2006).

In forests, environmental conditions (i.e., biotic interactions, soil chemistry, and microclimate conditions) can be modulated by the tree community. For instance, forest understory plant communities are connected to tree community composition and diversity (Germany et al., 2017). Tree diversity, for example, has been identified to increase the cover of forbs, while the proportion of forest-specific understory species increased with canopy cover (Vockenhuber et al., 2011). However, herb layer productivity is not necessarily affected by tree layer diversity (Germany et al., 2017), or herb layer diversity (Both et al., 2011). Furthermore, tree community composition can also modify soil chemistry, such as soil pH and nutrient availability (Reich et al., 2005), with significant consequences for soil microbial community composition (Delgado-Baquerizo et al., 2017; Thoms et al., 2010). Likewise, soil moisture can be affected by tree SRL, as this trait affects the hydraulic lift (Burgess et al., 1998), with consequences for microbial communities (Cesarz et al., 2022). In addition, tree canopies provide a buffering layer between macroclimatic fluctuations and microclimatic fluctuations (de Frenne et al., 2019), as species-rich forests have a higher spatial complementarity in tree crowns and canopy closure (Kunz et al., 2019; Perles-Garcia et al., 2021; Williams et al., 2017). Thereby, tree diversity would stabilize the microclimate and enhance ecosystem functions (Gottschall et al., 2019).

In this study, we aim to mechanistically understand the effects of tree diversity, productivity, functional trait identity and dissimilarity on soil carbon concentration and its mediation by the soil microbial biomass and microenvironmental conditions (i.e., biotic interactions, soil chemical properties, and microclimatic conditions; Figure 1). We based our study on the BEF-China experiment and investigated 180 small-scale neighborhoods distributed with a species richness gradient ranging from 1 to 16. For each sample location, we measured soil carbon concentration, soil microbial biomass, and environmental conditions to mechanistically describe and understand tree diversity, productivity, and functional trait effects on soil carbon concentrations.

We hypothesized tree diversity, productivity, functional trait (e.g., SRL, RD, mycorrhizal status) identity, and dissimilarity between these root traits to drive soil carbon concentrations (Hypothesis 1; Figure 1). In addition, tree diversity, productivity, and functional identity; dissimilarity effects on soil carbon concentrations are mediated by soil microbial biomass (Hypothesis 2). We further hypothesized that tree community effects on soil microbial biomass were mediated by microenvironmental conditions

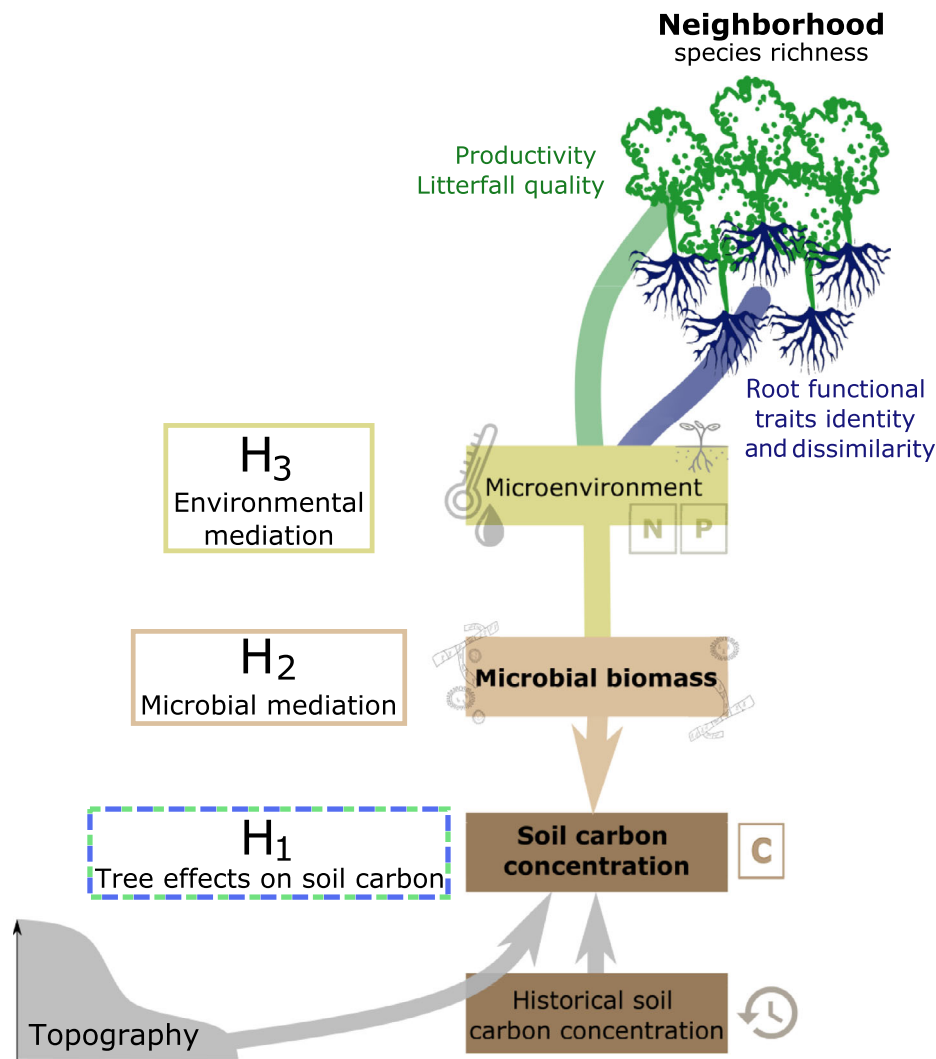


FIGURE 1 Conceptual framework of the study. Relationship between the different hypotheses tested in the study: H₁: tree productivity and functional trait identity and dissimilarity drive soil carbon concentration; H₂: tree productivity and functional identity and dissimilarity effects on soil carbon concentrations are expected to be mediated by soil microbial biomass; H₃: tree community effects on soil microbial biomass are mediated by microenvironmental conditions (microclimate, soil chemical properties, and biotic environment).

(biotic environment, soil chemical properties, and microclimate; Hypothesis 3). In order to control for soil history and topography effects on erosion and, therefore soil carbon concentration, we considered historical soil carbon concentration (measured before the onset of tree interactions) and plot topography (i.e., plot altitude, slope, and curvature) as covariates in our analyses (Figure 1).

MATERIAL AND METHODS

Study site

The study site is located in southeast China nearby the town of Xingangshan (Jiangxi Province, 29.08–29.11° N, 117.90–117.93° E). Our experimental site is part of the BEF-China experiment (site A; Bruelheide et al., 2014), and

it was planted in 2009 after a clearcut of the previous commercial plantation. The region is characterized by a subtropical climate with warm, rainy summers and cool, dry winters with a mean temperature of 16.7°C and a mean annual rainfall of 1821 mm (Yang et al., 2013). Soils in the region are Cambisols and Cambisol derivatives, with Regosol on ridges and crests (Geißler et al., 2012; Scholten et al., 2017). The natural vegetation consists of species-rich broad-leaved forests dominated by *Cyclobalanopsis glauca*, *Castanopsis eyrei*, *Daphniphyllum oldhamii*, and *Lithocarpus glaber* (Bruelheide et al., 2011, 2014).

Study design

We selected 180 small-scale sample locations across five species richness levels (1, 2, 4, 8, and 16 species) per plot,

according to the BEF-China planting design (Appendix S1). These small-scale locations are local neighborhoods of trees defined as the 12 trees directly adjacent in the planting grid with two central trees (Appendix S1: Figures S1 and S2; see Tree Species Pairs in Trogisch et al., 2021). Each local neighborhood was replicated three times in each richness level when available (see “broken stick” design; Bruelheide et al., 2014).

Plot topography

To control for the topography effect on soil carbon concentration, topography measures were calculated from a digital elevation model (DEM). The DEM was interpolated in 2015 from elevation measurements with a differential global positioning system (DGPS) using the ordinary kriging algorithm and a cell size of 5×5 m. Slope, altitude, plan curvature (Curv. PL), and profile curvature (Curv. PR) were calculated from the DEM at the plot level due to the low intraplot variability (Scholten et al., 2017).

Microclimate modeling

The daily air temperature was recorded using 35 data loggers (HOBO Pro v2, U23-001) installed at a 1-m height in the center of 35 plots across the experiment, while a meteorological station was set up in the central part of the experimental site (see Appendix S2: Figure S1 for more details; Bruelheide et al., 2014). To cover our full experimental area, the air temperature was modeled for all of our experimental plots using the available logger data. We modeled the temperature measurements of the 35 data loggers (i.e., daily minimum, mean, and maximum temperature) as a function of the meteorological station measurements (i.e., daily temperature, rainfall, and solar radiation), plot topography (i.e., latitude, longitude, altitude, orientation, slope, plot curvature, and mean annual solar radiation), forest vertical stratification (i.e., the effective number of layers index, “ENL,” see below) and plot species richness (see Appendix S2 for more details). Spatiotemporal trends for the whole experiment were estimated using Gaussian radial basis functions (functions “auto-basis,” “eval_basis” from the R package *FRK*, see Appendix S2: Section S1 and Wikle et al., 2019). Our model fits explained more than 90% of the loggers’ temperature measurement variability. The fitted models were used to predict daily minimum, mean, and maximum temperature for all experimental plots with a standard error from 0 to 2°C during our sampling period (see Appendix S2 for the complete procedure).

Field sampling

Our field measurements were performed from mid-August to the end of September 2018, before the main litterfall season (from September to December; Huang et al., 2017). To avoid spatiotemporal autocorrelation, each day another sampling area was randomly chosen. To test the effect of biotic conditions on soil microbial biomass and carbon concentrations, understory plant cover in each location was estimated on a five-level factorial scale from “no understory plant” to “mainly covered by understory plants.” Although this is a relatively coarse measurement, while being comparable with the Londo scale (Londo, 1976), it allows considering the influence of understory vegetation which was shown to be of importance (Vockenhuber et al., 2011). We encourage subsequent studies to assess the understory vegetation in a more detailed way.

Starting from the center of the two central trees of the local neighborhood, we extracted two soil cores with a 5 cm diameter and 10 cm depth, 5 cm away from the center (Appendix S1: Figure S2). Two additional cores of the same dimensions were taken 20 cm away from the center in the direction of each tree. A composite soil sample was built for soil analyses from these four soil cores and sieved with a 2 mm mesh. As a first measure of the biotic environment, root fragments contained in the sieving residues were air dried at 40°C for 2 days and weighed (± 0.01 g), while the composite soil samples were stored at -20°C .

To estimate the effect of biotic conditions and especially nutrient availability effect on soil microbial biomass and soil carbon concentration, the litter cover on the ground between the two central trees of each location was estimated on a five-level factorial scale from “no-litter” to “litter layer thicker than 5 cm.” Leaf litter was collected between the central trees from the ground, excluding green understory plant residuals, air dried at 40°C for 2 days, and milled to powder. Carbon and nitrogen concentrations were measured by microcombustion from a subsample of 4 mg (Elementar Vario El III analyzer, Elementar, Hanau, Germany).

Soil analyses

Soil moisture was measured from a subset of 25 g soil by drying the soil at 40°C for 2 days. A subsample was used to quantify soil pH in a 1:2.5 soil:water solution. Soil total nitrogen (TN) was determined on an autoanalyzer (SEAL Analytical GmbH, Norderstedt, Germany) using the Kjeldahl method (Bradstreet, 1954). Soil total phosphorus (TP) was measured after wet digestion with

H₂SO₄ and HClO₄ (Parkinson & Allen, 1975) using a UV-visible light spectrophotometer (UV2700, SHIMADZU, Japan). Soil total organic carbon (TOC) was measured using a TOC analyzer (Liqui TOC II; Elementar Analysensysteme GmbH, Hanau, Germany). TOC in 2010 was quantified in a previous study (Scholten et al., 2017) at the plot level using the microcombustion method (Elementar Vario El III analyzer, Elementar, Hanau, Germany).

Soil microbial biomass

Soil microbial biomass was measured using phospholipid fatty acid (PLFA) analysis. PLFAs were extracted from 5 g of frozen soil following Frostegård et al. (1991). Biomarkers were assigned to microbial functional groups according to Ruess and Chamberlain (2010) using markers to assign bacteria (Gram-positive bacteria: i15:0, a15:0, i16:0, i17:0; Gram-negative bacteria: cy17:0, cy19:0; general bacteria markers: 16:1ω5; 16:1ω7), arbuscular mycorrhizal fungi (20:1ω9), and saprophytic and ectomycorrhizal fungi (18:1ω9 and 18:2ω6,9; see Appendix S3).

Tree functional traits

Tree biomass

Tree biomass was predicted for all neighbor trees using tree basal area (BA) and species-specific allometric relationships estimated on the two central trees: (1) Circumference at breast height (CBH) was measured in September 2018 for all trees in order to calculate the BAs of these trees as $A = \frac{(CBH)^2}{4\pi}$. (2) Tree height was measured for the two central trees using a laser meter (4.9 ± 2.1 m, PLR 50C, Robert Bosch GmbH, Gerlingen, Germany), and tree biomass was calculated following Huang et al. (2018). BA and biomass of the central trees were used to estimate species-specific allometric BA–biomass relationships (see Appendix S4). (3) These species-specific allometric relationships were used to calculate the neighborhood biomass (i.e., the sum of the 12 surrounding trees' biomass).

Leaf traits

For each tree species of the experiment, 10 samples consisting of 10–25 pooled fresh leaves were collected across all diversity levels from mid-August to October 2018 (Davrinche & Haider, 2021). Each sample was dried at 80°C for 2 days and milled for 5 min at 26 shakes per second. Carbon and nitrogen concentrations were measured

by microcombustion from a subsample of 5 mg (Elementar Vario El III analyzer, Elementar, Hanau, Germany).

Root traits

Root functional traits were measured from BEF-China Site A from September to October 2013 using two to three tree individuals per species per diversity level. First-order roots were collected, cleaned, scanned, and analyzed by WinRHIZO (Regent Software, Canada). After measurements, roots were air dried at 60°C for 2 days and weighed. Average RD (in mm) and SRL (in m g⁻¹) were calculated from the measurements of each species at all species richness levels (Bu et al., 2017). The mycorrhizal status of the tree species was determined from the literature (Haug et al., 1994; Hawley & Dames, 2004; Wang & Qiu, 2006). The mycorrhizal status was assumed from the literature and confirmed by recent measurements in the same experiment (Singavarapu et al., 2021). However, intraspecific root functional trait variability can be high and may change over the course of an experiment, depending on the biotic context (e.g., Zuppinger-Dingley et al., 2014), which could not be considered in the present study.

Root functional trait variables

We considered three functional root traits that are related to soil processes (Bardgett et al., 2014): RD, SRL, and mycorrhizal tree association (i.e., AM or EM). For each location, trait identity and diversity were calculated at the neighborhood level. At the neighborhood level, we calculated community-weighted means (CWM; Garnier et al., 2004) and functional dispersion (FDis), defined as the weighted variance of the trait values within the neighborhood (Laliberté & Legendre, 2010). All measures were weighted using tree BA. Calculations were made using the “dbFD” function from the *FD* package in R (Laliberté et al., 2014).

Forest vertical stratification

To quantify the forest vertical stratification and estimate crown complementarity, we computed the ENL (Ehbrecht et al., 2016) using terrestrial laser scanning measurements. A high ENL value indicates more evenly distributed layers, which can be an indication of higher crown complementarity and, thus, an increase in canopy packing (Ehbrecht et al., 2016). A terrestrial laser scanning campaign took place in February–March of 2019 using a FARO Focus

S120 and a FARO Focus X130 laser scanner (FARO Europe, Korntal-Münchingen, Germany; for more information see Perles-Garcia et al., 2021). The scanner was set up on a tripod at 1.3 m height in the center of each plot and a fully three-dimensional point cloud ($360 \times 305^\circ$ field of view) with a spatial resolution of 6 mm at a distance of 10 m was acquired.

For each plot, the ENL was computed. First, the scans were filtered using a statistical outlier removal filter (SOR, $N = 10$, $SD = 3$) in CloudCompare 2.9.1 software. Taking into account the dimensions of each plot ($\sim 667 \text{ m}^2$), each point cloud was clipped in a 20-m^2 around the scan center ($\sim 400 \text{ m}^2$). The point clouds were voxelized into a voxel grid of 5 cm voxels using the R package *VoxR* (Lecigne et al., 2018). Then, they were grouped in vertical slices of 50 cm and, for each slice, we quantified the proportion of filled voxels. The ENL was the result of calculating the inverse Simpson Index: $ENL = 1 / \sum_{i=1}^n p_i^2$, where n refers to the number of slices, calculated as $(\text{height}_{\max} - \text{height}_{\min}) / 50 \text{ cm}$; and p_i is the proportion of filled voxels of the i th slice.

Leaf litterfall measurement

From September to December 2018, the freshly fallen leaf litter between the two central trees of each location was collected in a 1 m^2 litter trap (1 cm mesh). The collected litter was identified to species level, air dried at 40°C for 2 days, and weighed ($\pm 0.01 \text{ g}$). Annual amounts of litter carbon (i.e., “ $C_{\text{litterfall}}$ ”) and nitrogen (i.e., “ $N_{\text{litterfall}}$ ”) deposited on the ground were calculated using species-specific leaf carbon and nitrogen contents and species-specific table litter mass collected in the traps. We calculated the litterfall carbon to nitrogen ratio ($CN_{\text{litterfall}}$) from these measurements.

Statistical analyses

A description of all the variables used in this study can be found in Appendix S5: Table S1. All data handling and statistical calculations were performed using the R statistical software version 3.6.1. All R scripts used for this project can be found in our Zenodo release (<https://doi.org/10.5281/zenodo.7225739>).

In order to avoid any deviation due to scale differences between variables, all explanatory variables were centered and divided by two standard deviations for our analyses using the R “rescale” function from the *arm* package. Collinearity of root trait indices was inspected by Pearson’s correlation (Appendix S6); highly correlated variables were excluded by our both-way step selection algorithm favoring the simplest model (Venables & Ripley, 2002), that is,

the R “step” function from the *stat* package. We first tested the effects of tree species richness on our productivity and structural variables (i.e., neighborhood biomass, ENL, $C_{\text{litterfall}}$, and $CN_{\text{litterfall}}$) using linear models and normal distribution assumptions. Similarly, we used linear models to control for the effects of topography (plot slope, plan curvature, profile curvature, and altitude) on soil historical carbon concentration.

Drivers of soil carbon concentration (H_1)

We used linear models and normal distribution assumptions to test the effects of initial soil carbon concentration (i.e., $[C]_{2010}$), topography, tree productivity variables, litterfall carbon deposition, and C:N ratio, and root functional traits on soil carbon concentration (i.e., $[C]_{2018}$). Explanatory variables were selected by a both-way step selection based on the Akaike information criterion (AIC) (R “step” function from the *stats* package with back and forward selection). We estimated the drivers of soil carbon concentrations from the final model. All significant variables of the model output (p value < 0.05) were implemented with the effects of topography on soil historical C concentrations and, when applicable, with tree diversity effects on productivity in a structural equation model (SEM). Our SEM was fitted using the R “sem” function from the *lavaan* package (Rosseel, 2012). The quality of our model fit on the data was estimated using three complementary indices: (1) the root-mean-squared error of approximation (RMSEA), (2) the comparative fit index (CFI), and (3) the standardized root-mean-squared residuals (SRMR). A model fit was considered acceptable when $RMSEA < 0.10$, $CFI > 0.9$, and $SRMR < 0.08$.

Drivers of soil carbon concentration mediated by soil microbial biomass (H_2)

We used the same procedure to select drivers of microbial biomass. All selected drivers of microbial biomass were implemented in the above-described SEM structure. The relation between microbial biomass and soil carbon concentration (i.e., causal relation direction or correlation) was tested by comparing the models AIC.

Drivers of microbial biomass mediated by microenvironmental conditions (H_3)

Microenvironmental conditions were described by (1) biotic conditions, (2) soil chemical properties, and

(3) microclimatic conditions. Correlations between micro-environment variables are explored in Appendix S7: Figure S1: (1) Biotic conditions were described using field measurements of understory plant cover, soil root biomass, litter cover, and leaf chemical traits (i.e., litter carbon and nitrogen contents). (2) To describe soil chemical properties, we used a soil carbon to nitrogen ratio (“C:N”), and carbon to phosphorus ratio (“C:P”). (3) Microclimatic conditions were estimated using both soil humidity (RH) and air temperature.

The air temperature was used at the plot level on the day of sampling (minimum, average, and maximal temperature, “T.min,” “T.mean,” “T.max,” respectively) and during the week before sampling (minimum, average, and maximal temperature, “T.min.week,” “T.mean.week,” “T.max.week,” respectively, see Appendix S7: Figure S2). The first axis of the PCA projection was negatively correlated with temperature variables (Appendix S7: Figure S2). Given that the first PCA axis was negatively correlated with temperature indices and to simplify the presentation to the readers, we used the positive value of the vector for the first PCA axis as a proxy for air temperature variables in further analyses.

For each microenvironmental variable, we used linear models and normal distribution assumptions to test the effects of tree productivity, litterfall carbon deposition and C:N ratio, and root functional traits. However, only relationships with a strong biological reason described in the introduction were included in the analyses. We used linear models and normal distribution assumptions to test the effects of microenvironmental variables on soil microbial biomass. Explanatory variables were selected by a both-way step selection based on AIC. We estimated the drivers of microbial biomass from the final model. All variables selected and their relations to tree variables had been implemented in our previous SEM.

All the statistical assumptions of our linear models were tested using the “check_model” function from the R package *performance* (Appendix S8).

RESULTS

Local history and topography effects on soil carbon concentrations

On average, forest soil carbon concentrations were stable across years (mean \pm SD: -0.33 ± 0.86 g_{carbon} g_{soil}⁻¹ year⁻¹), but we observed high variability between the sampled locations (from -3.00 to $+1.85$ g_{carbon} g_{soil}⁻¹ year⁻¹, Figure 2A). Soil carbon concentrations measured in 2018

were higher at sites where historical soil carbon concentrations were high in 2010, before the experiment (estimate \pm SD: 0.265 ± 0.078 , Figure 2D–F, Appendix S9). As historical soil carbon concentrations were affected by local topography (slope: estimate \pm SD: 0.175 ± 0.038 , plan curvature: 0.357 ± 0.038 , $R^2 = 10\%$, Figure 2B), topography indirectly affected soil carbon concentrations measured in 2018 by the modification of historical soil carbon concentrations (Figure 2E,F).

Tree species richness effects on tree productivity

At the neighborhood level, plot tree species richness significantly increased the different aspects of tree productivity: neighborhood tree biomass (0.427 ± 0.073 , $R^2 = 18\%$), litterfall production (i.e., “C.litterfall,” 0.416 ± 0.078 , $R^2 = 17\%$), and forest vertical stratification (i.e., ENL, 0.248 ± 0.070 , $R^2 = 32\%$ when accounting for topography effects, Figure 2C). These different aspects of forest productivity were correlated to each other (Pearson correlation: neighborhood biomass—ENL = 0.38, neighborhood biomass—“C litterfall” = 0.4, “ENL”—“C litterfall” = 0.61, see Appendix S6).

Tree effects on soil carbon concentrations (H₁)

Plot tree species richness did not directly affect soil carbon concentrations (Figure 2D), but tree productivity, especially forest vertical stratification (i.e., ENL), increased soil carbon concentrations (0.256 ± 0.093 , Figure 2E) and increased with increasing tree species richness (Figure 2C). In contrast, increasing litterfall C:N ratio decreased soil carbon concentration (-0.187 ± 0.081 ; Figure 2D–F; Appendix S9). Belowground, increasing RD strongly decreased soil carbon concentration (-0.183 ± 0.084 ; Figure 2F; Appendix S9).

Tree effects on soil microbial biomass (H₂)

Our analyses showed a positive effect of tree species richness on soil microbial biomass (estimate \pm SD: 0.202 ± 0.079 , $R^2 = 3\%$; Figure 3A). By considering tree functional traits and productivity, we achieved a better understanding of the factors affecting soil microbial biomass ($R^2 = 11\%$, $AIC_{sp. \text{ rich. based model}} = 222$ vs. $AIC_{trait \text{ based model}} = 214$). Soil microbial biomass was affected by root morphological traits identity and

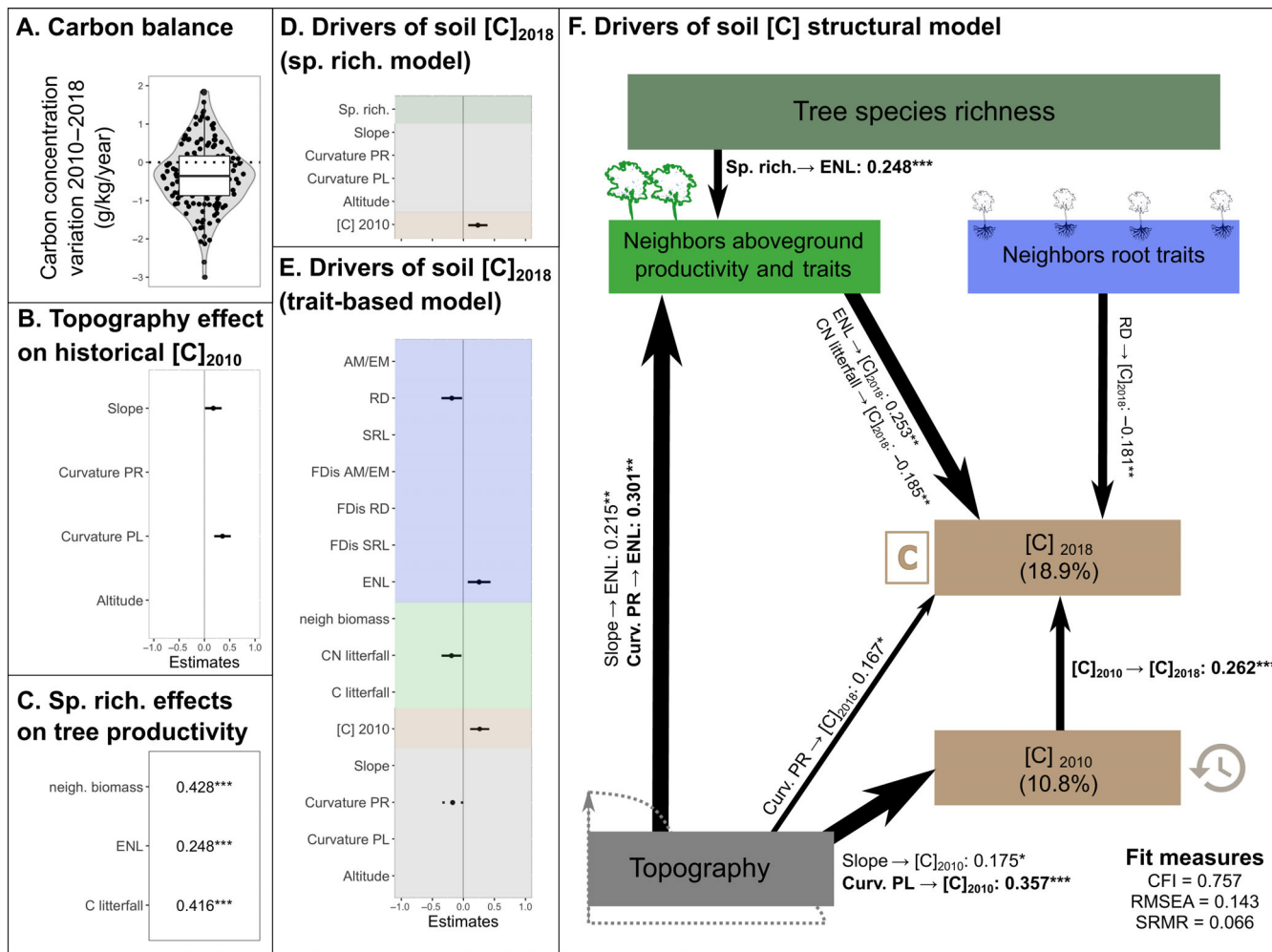


FIGURE 2 Tree diversity effects on tree productivity and consequences for soil carbon concentration, while controlling for soil history and topography effects. (A) Soil carbon balance between 2010 and 2018. (B) Topography effect on historical soil carbon concentrations. For each driver of soil historical carbon concentration on the y-axis (i.e., slope, plan curvature: “Curvature PL,” profile curvature: “Curvature PR,” altitude), the dot represents the estimated effect of the driver on historical soil carbon concentration, the line represents the 95% confidence interval (CI) for a given estimated value. The drivers excluded during model selection have neither estimates nor CI. (C) Tree species richness effect on tree productivity. For each response variable on the y-axis—neighborhood biomass (i.e., “neigh. biomass”), forest vertical stratification (i.e., “ENL”), and litterfall carbon deposition (i.e., “C litterfall”)—The standardized estimate of plot tree species richness (i.e., “Sp., Rich.”) was shown with the significance of the relationship. ENL model controlled for topography effects. Tree species richness (D), tree productivity, and functional traits effects (E) on soil carbon concentration (“Soil C 2018”) controlling for soil history (“Soil C 2010”) and topography effects (i.e., “Slope,” profile curvature: “Curvature PR,” plan curvature: “Curvature PL” and “Altitude”). For each driver on the y-axis, the dot represents the estimated effect of the driver on soil carbon concentrations; the line represents the 95% CI for a given estimate value. Estimates and CI were drawn in dashed lines when the effect of the driver on soil carbon concentration was nonsignificant (i.e., p values > 0.05). The drivers excluded during model selection have neither estimates nor CI. Six groups of explanatory variables were built: Species richness variables (i.e., plot species richness: “Sp. rich.”), soil history variables (i.e., “Soil C 2010”), plot topography (i.e., “Slope,” “Curvature PR,” “Curvature PR,” “Altitude”), neighborhood root trait indices (i.e., neighbors’ AM vs. EM tree association: “AM/EM,” community-weighted mean of root diameter and specific root length: “RD” and “SRL,” functional dissimilarity of tree fungal association, root diameter, and specific root length: “FDis AM/EM,” “FDis RD,” and “FDis SRL,” respectively), aboveground productivity and traits (i.e., neighbor biomass: “neigh biomass,” litterfall C:N ratio: “CN litterfall,” litterfall carbon deposition: “C litterfall”). (F) Structural equation model showing the relationships between topography (i.e., “Slope,” “Curv. PR,” and “Curv. PL”), soil history (i.e., “[C]₂₀₁₀”), tree species richness, tree aboveground productivity and functional traits (i.e., “ENL” and “CN.litterfall”) and root functional traits (i.e., “RD”), and soil carbon concentration (i.e., “[C]₂₀₁₈”). Each node represents a group of variables (selected from panels B–E), and each arrow summarizes all the significant effects between all the variables of two nodes. Arrow widths were sized by the sum of the standardized effect size of significant relations between all variables of the two nodes. When nonsignificant relations were found between any variables of two nodes, the arrows were drawn with dashed lines. The variance in soil carbon concentration explained by the model (R^2 , in %) was added after the node name; see Appendix S9 for detailed output. The significance levels were standardized across the panel (p value > 0.05: n.s., p value < 0.05: *, p value < 0.01: **, p value < 0.001: ***). AIC, Akaike information criterion; CFI, comparative fit index; ENL, effective number of layers; RMSEA, root-mean-squared error of approximation; SEM, Structural Equation Model; SRMR, standardized root-mean-squared residuals.

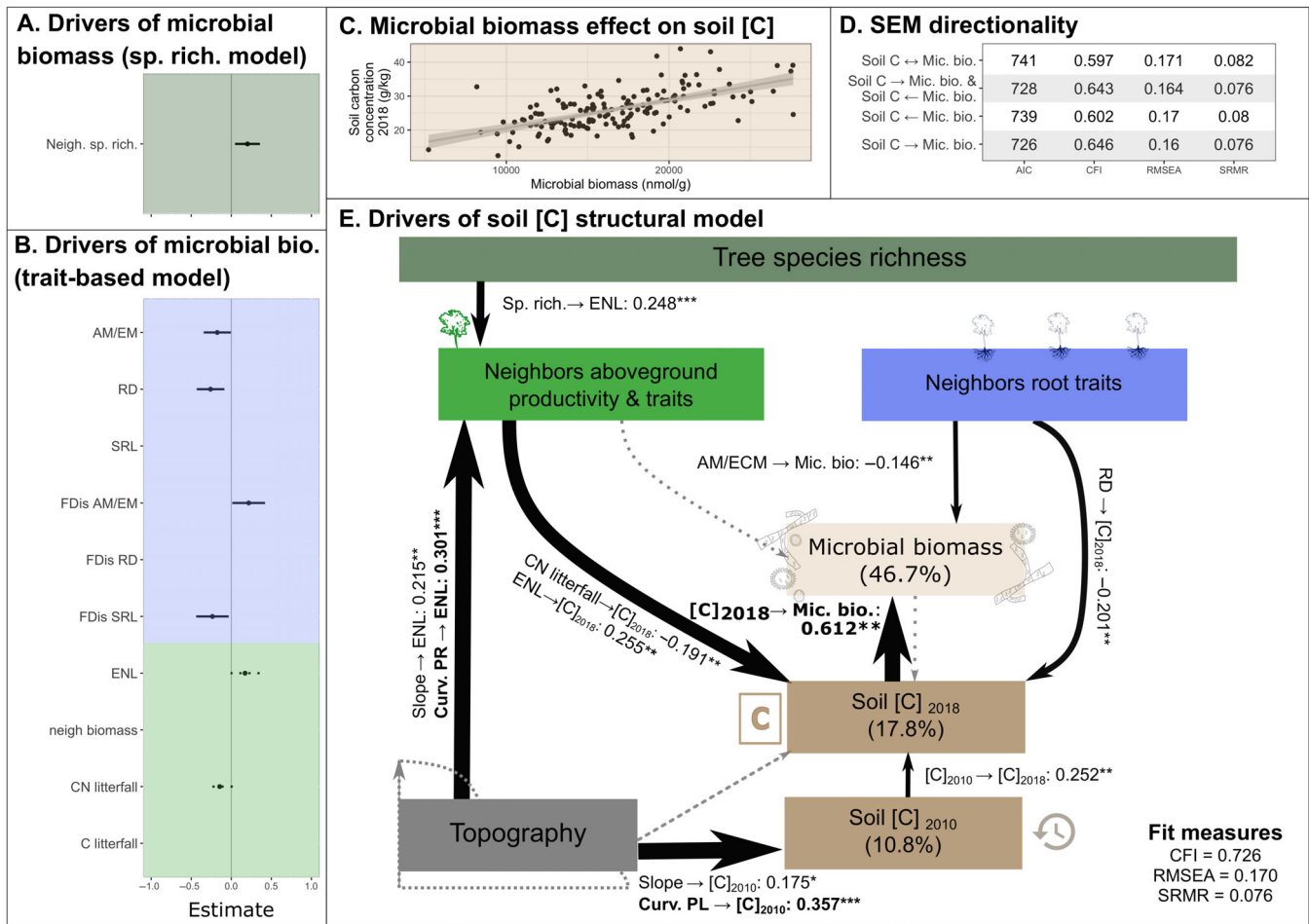


FIGURE 3 Biotic drivers of soil microbial biomass (A, B) and relationship with soil carbon concentrations (C–E). Tree species richness (A), and tree productivity and functional trait effects (B) on soil microbial biomass. For each driver on the y-axis, the dot represents the estimated effect of the driver on soil microbial biomass; the line represents the 95% CI for a given estimate value. Estimates and CI were drawn in dashed lines when the effect of the driver on soil microbial biomass was nonsignificant (i.e., p values > 0.05). The drivers excluded during model selection have neither estimates nor CI. Four groups of explanatory variables were built: Species richness variables (i.e., plot species richness: “Sp. rich.”), neighborhood root trait indices (i.e., neighbors’ AM vs. EM tree association: “AM/EM,” community-weighted mean of root diameter and specific root length: “RD” and “SRL,” functional dissimilarity of tree fungal association, root diameter, and specific root length: “FDis AM/EM,” “FDis RD,” and “FDis SRL,” respectively), aboveground productivity and traits (i.e., neighbor biomass: “neigh biomass,” litterfall C:N ratio: “CN litterfall,” litterfall carbon deposition: “C litterfall”). (C) Linear regression between soil carbon concentration and soil microbial biomass. (D) Directionality of the relationship between soil carbon concentration and soil microbial biomass tested in the SEM including the drivers of soil microbial biomass (A, B) and soil carbon concentration (Figure 2F). (E) Structural equation model showing the relationships between topography (i.e., “Slope,” profile curvature: “Curv. PR” and plan curvature: “Curv. PL”), soil history (i.e., “[C]₂₀₁₀”), tree species richness, tree aboveground productivity, and functional traits (i.e., “ENL” and “CN.litterfall”), root functional traits (i.e., “RD”), soil carbon concentration (i.e., “[C]₂₀₁₈”), and soil microbial biomass. Each node represents a group of variables (selected from (A, B) and Figure 2F) and each arrow summarizes all the significant effects between all the variables of two nodes. Arrow widths were sized by the sum of the standardized effect size of significant relations between all variables of the two nodes. When no significant relations were found between any variables of two nodes, the arrows were drawn with dashed lines. The variance in soil carbon concentration and microbial biomass explained by the model (R^2 , in %) were added after the node name, see Appendix S10 for detailed output. The significance levels were standardized across the panel (p value > 0.05: n.s., p value < 0.05: *, p value < 0.01: **, p value < 0.001: ***).

dissimilarity. Soil microbial biomass decreased with increasing RD (-0.259 ± 0.088) and decreased with increasing AM:ECM ratio (-0.176 ± 0.086). In addition, soil microbial biomass decreased with the

increasing functional dissimilarity of SRL (-0.235 ± 0.104) and increased with the increasing dissimilarity of tree fungal association (0.217 ± 0.104 ; Figure 3B).

Relationship between soil microbial biomass and soil carbon concentration (H₂)

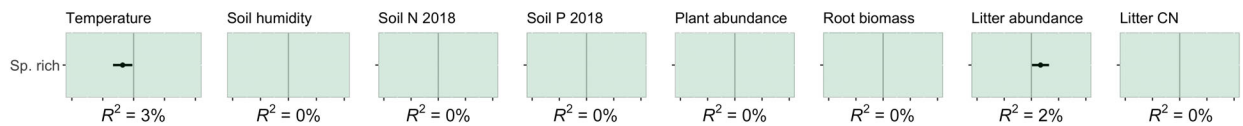
We found a strong positive correlation between soil carbon concentration and soil microbial biomass (Pearson correlation = 62.7%, *p* value < 0.001; Figure 3C). Taken together with the other drivers of soil carbon and microbial biomass, we tested the directionality of the relationship between soil carbon concentration and soil microbial biomass (Figure 3D). The AIC comparison between the models was in favor of the model with a causal effect from soil carbon concentration to soil microbial biomass and the model took into account both causal links (i.e., soil carbon concentration effect on microbial biomass and vice versa). The latter, being the most conservative model, is given in Figure 3E. This SEM showed a strong positive effect of soil carbon concentration on

microbial biomass (0.608 ± 0.059 ; Figure 3E), but a non-significant effect of soil microbial biomass on soil carbon concentration (*p* value = 0.72; Appendix S10). The tree productivity and root functional trait effects on soil microbial biomass were mostly mediated by soil carbon concentration (remaining direct root effect -0.147 ± 0.063 ; Figure 3E; Appendix S10).

Tree effects on microenvironmental conditions (H₃)

Tree species richness effects on microenvironmental conditions (i.e., biotic conditions, soil chemical properties, and microclimate) were limited to a negative effect on air temperature (-0.208 ± 0.082 , $R^2 = 3\%$) and a positive effect on the amount of litter collected on the ground (0.168 ± 0.080 ,

A. Tree species richness effects on environmental conditions



B. Functional trait effects on environmental conditions

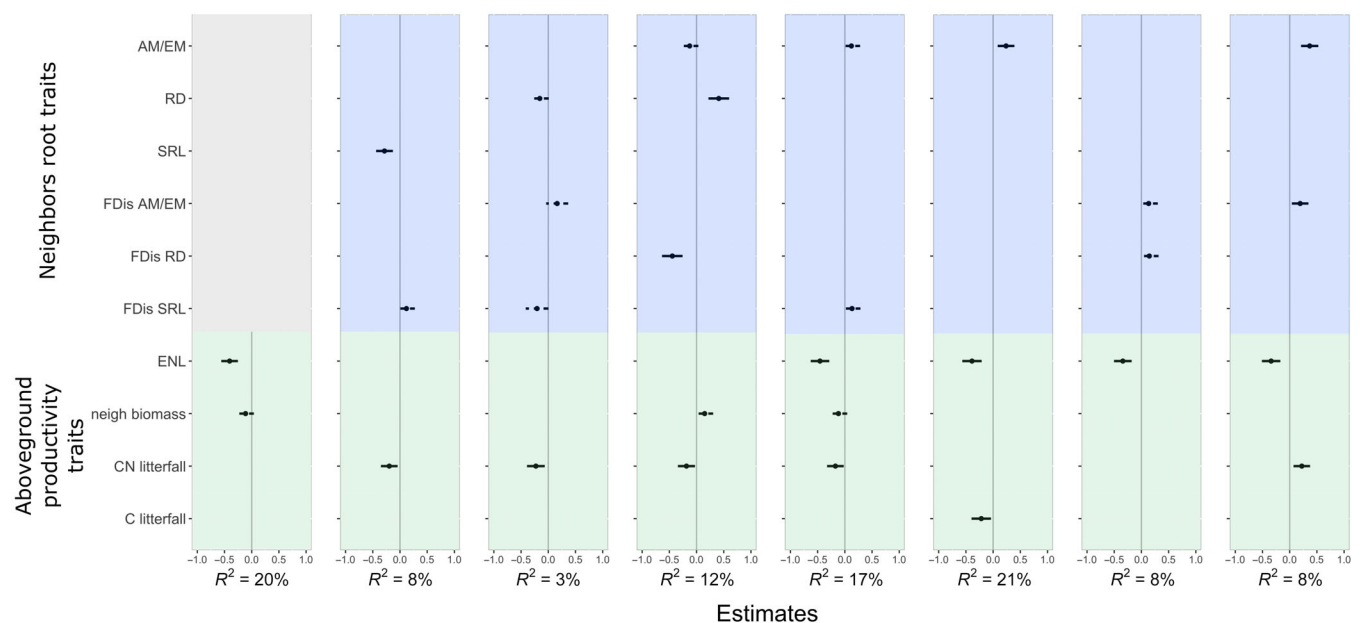


FIGURE 4 Tree species richness (A), and tree productivity and functional traits effects (B) on microenvironmental variables. For each driver on the y-axis, the dot represents the estimated effect of the driver on the microenvironmental variable, the line represents the 95% CI for a given estimate value. Estimates and CI were drawn in dashed lines when the effect of the driver was nonsignificant (i.e., *p* values > 0.05). The drivers excluded during model selection have neither estimates nor CI. Four groups of explanatory variables were built: Species richness variables (i.e., plot species richness: “Sp. rich.”), neighborhood root trait indices (i.e., neighbors’ AM versus EM tree association: “AM/EM,” community-weighted mean of root diameter and specific root length: “RD” and “SRL,” functional dissimilarity of tree fungal association, root diameter, and specific root length: “FDis AM/EM,” “FDis RD,” and “FDis SRL,” respectively), aboveground productivity and traits (i.e., forest vertical stratification: “ENL,” neighbors biomass: “neigh biomass,” litterfall C:N ratio: “CN litterfall,” litterfall carbon deposition: “C litterfall”). In the case of air temperature (i.e., “Temperature”), only tree aboveground productivity and functional traits were considered in the trait-basal model. ENL, effective number of layers.

$R^2 = 2\%$; Figure 4A). However, the trait-based model showed the major role of trees in controlling environmental conditions. Above ground, higher forest vertical stratification (i.e., ENL) reduced air temperature (-0.406 ± 0.078), understory plant abundance (-0.457 ± 0.009), root biomass (-0.389 ± 0.091), and amount of litter (-0.342 ± 0.083), but also litter C:N ratio (-0.342 ± 0.086). Increasing litterfall C:N ratio decreased soil humidity (-0.197 ± 0.078), soil nitrogen (-0.228 ± 0.083) and phosphorus contents (-0.186 ± 0.080), and plant abundance (-0.173 ± 0.078 , Figure 4B), while it increased C:N ratio of the residual litter on the ground (0.233 ± 0.077). Belowground, environmental conditions were affected by the root functional traits identity and diversity. While SRL decreased soil humidity (-0.285 ± 0.078), RD increased soil phosphorus contents (0.408 ± 0.097), and AM:ECM fungal association ratio increased root biomass and litter C:N ratio (0.276 ± 0.078 and 0.367 ± 0.081 , respectively). Moreover, root functional trait dissimilarity played a major role in controlling soil chemical properties and biotic conditions, AM:ECM fungal association dissimilarity increased the litter C:N ratio (0.192 ± 0.081), and fungal dissimilarity in RD reduced soil phosphorus content (-0.446 ± 0.096 ; Figure 4B). In addition, plant cover was positively correlated with root biomass and the amount of litter (Pearson correlation: plant cover—root biomass = 0.30, plant cover—amount of litter = 0.37; Appendix S7).

Microenvironmental mediation of tree effects on microbial biomass (H_3)

Microbial biomass was affected by the microenvironment (i.e., biotic conditions, soil chemical properties, and microclimate; Figure 5A). Soil microbial biomass decreased with increasing air temperature (-0.288 ± 0.054). In addition, soil microbial biomass increased with increasing litter C:N ratio (0.222 ± 0.055 ; Figure 5A; Appendix S8). By adding these drivers to the previous SEM, we explained up to 57% of the variability in soil microbial biomass (Figure 5B; vs. 47% without environmental drivers). Microbial biomass was mostly affected by variations in soil carbon concentration (total effect: 0.613) and microenvironmental conditions (total effect: 0.511), which were themselves strongly mediated by tree productivity and functional traits (total effect: on soil carbon concentration = 0.655, on microenvironmental conditions = 1.961; Figure 5B; Appendix S11).

DISCUSSION

The present study revealed strong effects of forest diversity, productivity, and functional traits on soil carbon

concentrations as well as the underlying biotic and abiotic drivers in a tree diversity experiment. In addition to the effects of topography, our analyses showed a strong positive effect of tree species richness on tree productivity (i.e., tree biomass, amount of litterfall, and forest vertical stratification). Tree productivity and tree functional traits modulated microenvironmental conditions, such as biotic conditions, soil chemical properties, and microclimate. These changes in microenvironmental conditions had significant consequences for soil microbial biomass (e.g., a decrease in temperature increased soil microbial biomass), in addition, root functional traits modulated soil microbial biomass. Soil microbial biomass was strongly correlated with soil carbon concentration, and our analyses found more support for a positive effect of soil carbon concentration on soil microbial biomass than vice versa. Moreover, soil carbon concentration increased with tree productivity and root morphological traits. Taken together, these findings showed how tree diversity, productivity, and functional traits shape forest abiotic and biotic conditions and soil functioning.

Soil carbon concentration dynamics in BEF-China (H_1)

Our analyses showed a slight loss of soil carbon during the first 10 years of the experiment in low diversity level plots. Site A of the BEF-China experiment was planted in 2009 after a clearcut of the previous conifer plantation (Yang et al., 2013). Clearcut harvestings are known to enhance soil carbon loss during the following decade (Li et al., 2019; Seedre et al., 2014). This is mainly caused by a massive input of deadwood into the soil acting as a primer of soil organic matter decomposition as well as by the removal of litterfall and exudation causing a shift in microbial physiology (Taylor et al., 2008). However, this average decrease of soil carbon concentrations was accompanied by a large range variability of plot-level values (ranging from -3.33 to 1.85 g year^{-1}), suggesting strong local drivers of soil carbon dynamics. First, we found a positive effect of soil historical carbon concentrations on current soil carbon concentrations. Second, we found that the topography had affected historical carbon concentrations with consequences for the current soil carbon concentration (Liu et al., 2020; Scholten et al., 2017). This result highlights the importance of soil history for in situ experiments and the need to consider historical variables in the analyses. Moreover, recent studies have shown the central role of soil history and temporal changes in BEF relationships to better understand ecosystem functions and their underlying drivers (Guerrero-Ramírez et al., 2017; Vogel et al., 2019).

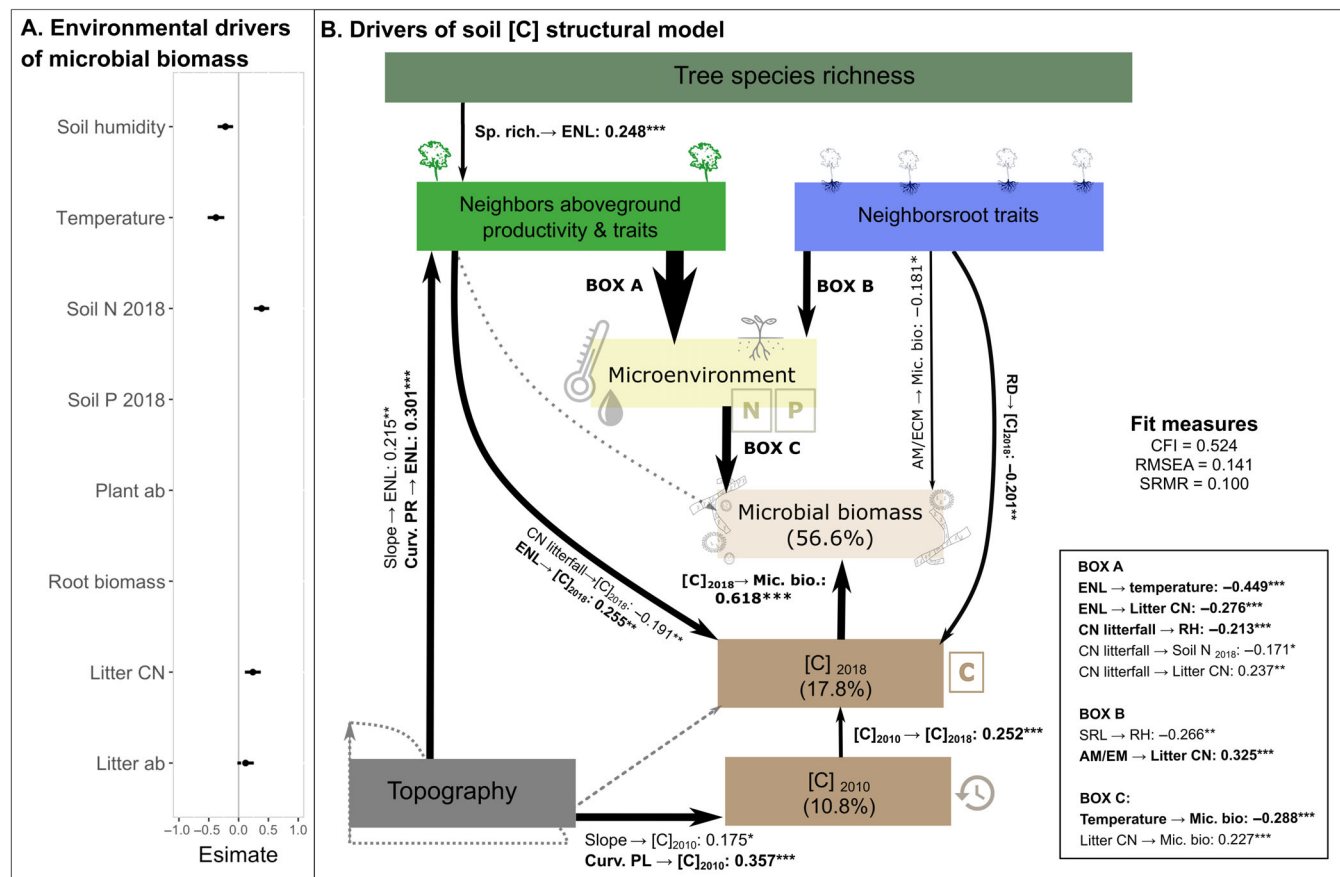


FIGURE 5 Mediation of tree effects on soil microbial biomass by microenvironmental conditions. (A) Effects of microenvironmental conditions on microbial biomass. For each driver of microbial biomass on the y-axis, the dot represents the estimated effect of the driver on microbial biomass, the line represents the 95% CI for a given estimated value. The drivers excluded during model selection have neither estimates nor CI. (B) Structural equation model showing the relationships between topography (i.e., “Slope,” profile curvature: “Curv. PR” and plan curvature: “Curv. PL”), soil history (i.e., “[C]₂₀₁₀”), tree species richness, tree aboveground productivity and functional traits (i.e., “ENL” and “CN.litterfall”) and root functional traits (i.e., “RD”), soil carbon concentration (i.e., “[C]₂₀₁₈”), soil microbial biomass, and microclimatic conditions (i.e., “temperature,” soil relative humidity: “RH,” soil nitrogen concentration: “Soil N 2018,” litter collected on the ground C:N ratio: “Litter CN”). Each node represents a group of variables (selected from (A), Figures 3E, and 4B) and each arrow summarizes all the significant effects between all the variables of two nodes. Arrow widths were sized by the sum of the standardized effect size of significant relations between all variables of the two nodes. When no significant relations were found between any variables of two nodes, the arrows are drawn with dashed lines. The variance in soil carbon concentration and microbial biomass explained by the model (R^2 , in %) were added after the node name; see Appendix S11 for detailed output. The significance levels were standardized across the panels (p value > 0.05: n.s., p value < 0.05: *, p value < 0.01: **, p value < 0.001: ***). CFI, comparative fit index; ENL, effective number of layers; RD, root diameter; RMSEA, root-mean-squared error of approximation; Sp. rich., plot species richness; SRL, specific root length; SRMR, standardized root-mean-squared residuals.

Therefore, future research should not only consider the mechanisms behind BEF relationships but also consider their temporal dynamics and evolution over time (Eisenhauer et al., 2019).

Neighborhood tree traits and productivity drive soil carbon concentrations (H₁)

Once controlling for topography and soil history effects, small-scale tree communities influenced soil carbon

concentrations, both through aboveground and belowground mechanisms. Aboveground, soil carbon concentration was increased by forest vertical stratification, which decreased litter C:N ratio, that is, increasing litter quality. This emphasizes the central role of the biotic processes in transforming fresh litter into stable carbon forms (Buckeridge et al., 2020). The positive effects of forest vertical stratification can be further related to two independent mechanisms: on the one hand, the increase in tree biomass production and, thereby, enhanced inputs to the soil (Liu et al., 2018); on the other hand, the reduction of erosion due

to the reduction of the kinetic energy of throughfall with higher crown complementarity (i.e., higher ENL; Goebes et al., 2015; Seitz et al., 2015).

Belowground, root morphological traits, such as RD, have been related to belowground biomass allocation and productivity (Bardgett et al., 2014) and were shown to increase soil carbon concentrations (Adamczyk et al., 2019). However, our measurements of root traits were based on species-specific values and did not consider trait plasticity (Sun et al., 2017; Zuppinger-Dingley et al., 2014). Tree diversity and forest productivity have been shown to influence fine root traits, such as RD (Sun et al., 2017). Our study again stresses the need for noninvasive methods and measurements of belowground productivity and root traits (Bu et al., 2017; Sun et al., 2017). Such measures will allow us to consider trait plasticity and disentangle productivity and physiological effects.

Soil microbial biomass and soil carbon concentration are strongly related (H₂)

Our analyses highlighted a robust positive correlation between soil microbial biomass and soil carbon concentrations. We expected feedback mechanisms between soil microbial biomass and soil organic carbon (Clemmensen et al., 2013; Kästner & Miltner, 2018; Lange et al., 2015). On the one hand, soil microbial growth is maintained and limited by soil organic carbon availability (Bollag & Stotzky, 1993). On the other hand, soil organic carbon is consumed and processed by soil microbes and is altered by their activity (Clemmensen et al., 2013; Schmidt et al., 2011). Therefore, soil microbial biomass and soil organic carbon are strongly related to each other across spatial scales (Serna-Chavez et al., 2013; Xu et al., 2013). However, in the present study, we could only verify the strong positive effect of soil carbon concentration on soil microbial biomass, while the potential feedback effect of soil microbes on soil carbon accumulation (Kästner & Miltner, 2018; Lange et al., 2015; Miltner et al., 2012) was not significant. Microbial necromass accumulation might become relevant on a longer time scale, thus repeated sampling of the experiment might help to better quantify microbial necromass accumulation and positive effects on soil carbon storage. Moreover, measurements of the different soil carbon pools (see Miltner et al., 2012; von Lützow et al., 2007) and more detailed assessments of soil microbial community structure and the activities of main groups therein would be needed to understand the fluxes of carbon between these carbon pools and the role of soil microbes as main consumers and producers of soil carbon (Goto et al., 1994; Liski et al., 2005).

Microenvironmental conditions and root morphological traits drive soil microbial biomass (H₂, H₃)

We highlighted the negative effect of AM-associated trees on soil microbial biomass, these observations in forest topsoils (0–10 cm) fall in line with previous studies showing higher concentrations of carbon and nitrogen in ECM associated forest topsoils in comparison with AM-associated forests (Craig et al., 2018). Therefore, microbial biomass and activity would be enhanced by higher nutrient availability (Beugnon et al., 2021; Cesarz et al., 2022; Smith et al., 2021). In addition, we showed that two microenvironmental parameters drove soil microbial biomass: temperature, and litter C:N ratio. In contrast to our expectations, soil microbial biomass decreased with increasing air temperature. Notably, we sampled during the summer with an average daily temperature of $27 \pm 3^\circ\text{C}$ and an average maximum daily temperature of $35 \pm 8^\circ\text{C}$. These high temperatures may exceed the thermal activity niche of some microbial taxa and thus repress microbial growth (Barcenas-Moreno et al., 2009). Further efforts are now needed to better grasp the temporal extent of the tree diversity effect on microenvironmental conditions, thus consequences for variables with slower dynamics such as soil carbon storage.

Tree diversity enhances productivity with consequences for microenvironmental conditions (H₃)

Our analyses confirmed previous results showing increased productivity with tree species richness (Fichtner et al., 2018; Huang et al., 2017, 2018; Kunz et al., 2019). Interestingly, our results highlighted that tree species richness simultaneously enhances tree biomass, litter production, and forest vertical stratification. This positive effect of tree species richness is also expected belowground (Liu et al., 2018, 2020; Xu et al., 2020). However, further efforts are needed to better quantify belowground productivity in space and time (Liu et al., 2020). A major challenge is the development of noninvasive quantification methods to measure belowground biomass and turnover (Clark et al., 2011; Metzner et al., 2014; Mooney et al., 2012).

Tree productivity combined with root functional traits allowed us to explore how tree effects are mediated by microenvironmental conditions: biotic conditions, soil chemical properties, and microclimate. Our results, by showing a negative effect of forest vertical stratification on temperature, confirmed previous findings emphasizing the role of forests as a heat buffer (de Frenne et al., 2019; Zhang et al., 2022). In the same line, we found negative

effects of tree SRL on soil water availability, which can be explained by increased water uptake with a denser root system (Zhang et al., 2020). This increase in water consumption, consequently decreasing soil water availability, would increase the competition for water between trees and understory plants and would explain the negative effects of SRL on understory productivity (i.e., plant cover and root biomass). In addition to the belowground competition, our results suggested an aboveground competition for light with negative effects of forest vertical stratification on understory productivity (Hakkenberg et al., 2020; Mueller et al., 2016). In addition, we confirmed the role of trees in controlling soil nitrogen and phosphorus contents by modifying the litter C:N ratio and root morphological traits related to desiccation and exudation (i.e., N-rich and P-rich compounds; Bardgett et al., 2014; Sun et al., 2020).

CONCLUSION

First, we highlighted that tree productivity and tree functional traits, modulated microenvironmental conditions with significant consequences for soil microbial biomass. Future studies would therefore gain to consider tree diversity mediation of microenvironmental conditions to better understand tree diversity effects on ecosystem functioning. Second, our analyses showed a strong positive correlation between soil microbial biomass and soil carbon concentration, suggesting a significant interplay between soil microbial communities and soil carbon stocks. Third, we found that soil carbon concentrations increased with historical carbon concentrations, with the latter being strongly affected by the plot topography. These results stress the need to consider topography and the historical baseline in order to understand soil carbon dynamics. To conclude, future mechanistic studies on the drivers of soil microbial biomass and carbon sequestration need to consider the local neighborhood in which the underlying mechanisms act. Moreover, the quantification of the different soil carbon pools is critical to the understanding of microbial community–soil carbon stock relationships.

AUTHOR CONTRIBUTIONS

The authors contributed to the manuscript by (1) funding (Nico Eisenhauer, Helge Bruelheide, Sylvia Haider, Goddert von Oheimb, Yanfen Wang, Tesfaye Wubet, Kai Xue, Simone Cesarz), (2) field sampling (Rémy Beugnon, Wensheng Bu, Andréa Davrinche, Sylvia Haider, Matthias Kunz, Maria D. Perles-Garcia, Mariem Saadani, Thomas Scholten, Steffen Seitz, Bala Singavarapu, Stefan Trogisch, Tesfaye Wubet, Bo Yang), (3) laboratory measurements (Rémy Beugnon, Wensheng Bu, Andréa Davrinche, Jianqing Du, Maria D. Perles-Garcia, Thomas

Scholten, Steffen Seitz, Bo Yang, Simone Cesarz), (4) project conceptual framework (Rémy Beugnon, Nico Eisenhauer, Helge Bruelheide, Jianqing Du, Simone Cesarz), (5) statistical analyses (Rémy Beugnon), (6) manuscript framing (Rémy Beugnon, Nico Eisenhauer, Simone Cesarz) (7) manuscript writing (Rémy Beugnon, Nico Eisenhauer, Simone Cesarz), (8) manuscript revisions (Helge Bruelheide, Andréa Davrinche, Jianqing Du, Sylvia Haider, Matthias Kunz, Goddert von Oheimb, Maria D. Perles-Garcia, Mariem Saadani, Thomas Scholten, Steffen Seitz, Bala Singavarapu, Stefan Trogisch, Yanfen Wang, Tesfaye Wubet, Kai Xue).

ACKNOWLEDGMENTS

We gratefully acknowledge funding by the Deutsche Forschungsgemeinschaft (DFG; German Research Foundation – 319936945/GRK2324) and the University of Chinese Academy Sciences (UCAS). We acknowledge the support of the TreeDi research group, especially many local helpers involved in collecting the samples. We also thank the laboratory members of the Experimental Interaction Ecology group for their support, especially Alfred Lochner, Anja Zeuner, Alla Kavtea, and Linnea Smith for their help during the laboratory measurements. The Experimental Interaction Ecology group is supported by the German Centre for Integrative Biodiversity Research (iDiv). We gratefully acknowledge the support of iDiv funded by the German Research Foundation (DFG-FZT 118, 202548816). Nico Eisenhauer acknowledges funding by the DFG (Ei 862/29-1). Open Access funding enabled and organized by Projekt DEAL.









CONFLICT OF INTEREST










The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data (Beugnon et al., 2022) are available in Dryad at <https://doi.org/10.5061/dryad.pvmcvdnqc>. All R scripts (remybeugnon, 2022) are available in Zenodo at <https://doi.org/10.5281/zenodo.7225739>.

ORCID

Rémy Beugnon  <https://orcid.org/0000-0003-2457-5688>
 Wensheng Bu  <https://orcid.org/0000-0002-5617-9789>
 Helge Bruelheide  <https://orcid.org/0000-0003-3135-0356>
 Andréa Davrinche  <https://orcid.org/0000-0003-0339-2997>
 Sylvia Haider  <https://orcid.org/0000-0002-2966-0534>
 Matthias Kunz  <https://orcid.org/0000-0002-0541-3424>
 Goddert von Oheimb  <https://orcid.org/0000-0001-7408-425X>
 Maria D. Perles-Garcia  <https://orcid.org/0000-0003-2475-4918>

Mariem Saadani  <https://orcid.org/0000-0002-9276-2568>
 Thomas Scholten  <https://orcid.org/0000-0002-4875-2602>
 Steffen Seitz  <https://orcid.org/0000-0003-4911-3906>
 Bala Singavarapu  <https://orcid.org/0000-0002-0147-895X>
 Stefan Trogisch  <https://orcid.org/0000-0002-1426-1012>
 Tesfaye Wubet  <https://orcid.org/0000-0001-8572-4486>
 Kai Xue  <https://orcid.org/0000-0002-5990-4448>
 Simone Cesarz  <https://orcid.org/0000-0003-2334-5119>
 Nico Eisenhauer  <https://orcid.org/0000-0002-0371-6720>

REFERENCES

- Aciego Pietri, J. C., and P. C. Brookes. 2009. "Substrate Inputs and PH as Factors Controlling Microbial Biomass, Activity and Community Structure in an Arable Soil." *Soil Biology and Biochemistry* 41(7): 1396–405. <https://doi.org/10.1016/j.soilbio.2009.03.017>.
- Adamczyk, B., O.-M. Sietiö, P. Straková, J. Prommer, B. Wild, M. Hagner, M. Pihlatie, H. Fritze, A. Richter, and J. Heinonsalo. 2019. "Plant Roots Increase Both Decomposition and Stable Organic Matter Formation in Boreal Forest Soil." *Nature Communications* 10(1): 3982. <https://doi.org/10.1038/s41467-019-11993-1>.
- Averill, C., and C. V. Hawkes. 2016. "Ectomycorrhizal Fungi Slow Soil Carbon Cycling." *Ecology Letters* 19(8): 937–47. <https://doi.org/10.1111/ele.12631>.
- Averill, C., B. L. Turner, and A. C. Finzi. 2014. "Mycorrhiza-Mediated Competition between Plants and Decomposers Drives Soil Carbon Storage." *Nature* 505(7484): 543–5. <https://doi.org/10.1038/nature12901>.
- Barcenas-Moreno, G., M. Gomez-Brandon, J. Rousk, and E. Bååth. 2009. "Adaptation of Soil Microbial Communities to Temperature: Comparison of Fungi and Bacteria in a Laboratory Experiment." *Global Change Biology* 15(12): 2950–7. <https://doi.org/10.1111/j.1365-2486.2009.01882.x>.
- Bardgett, R. D., L. Mommer, and F. T. de Vries. 2014. "Going Underground: Root Traits as Drivers of Ecosystem Processes." *Trends in Ecology & Evolution* 29(12): 692–9. <https://doi.org/10.1016/j.tree.2014.10.006>.
- Bastin, J.-F., Y. Finegold, C. Garcia, D. Mollicone, M. Rezende, D. Routh, C. M. Zohner, and T. W. Crowther. 2019. "The Global Tree Restoration Potential." *Science* 365(6448): 76–9. <https://doi.org/10.1126/science.aax0848>.
- Bergmann, J., A. Weigelt, F. van der Plas, D. C. Laughlin, T. W. Kuyper, N. Guerrero-Ramirez, O. J. Valverde-Barrantes, et al. 2020. "The Fungal Collaboration Gradient Dominates the Root Economics Space in Plants." *Sciences Advances* 6: 1–9.
- Bernhard, N., L.-M. Moskwa, K. Schmidt, R. A. Oeser, F. Aburto, M. Y. Bader, K. Baumann, et al. 2018. "Pedogenic and Microbial Interrelations to Regional Climate and Local Topography: New Insights from a Climate Gradient (Arid to Humid) along the Coastal Cordillera of Chile." *Catena* 170: 335–55. <https://doi.org/10.1016/j.catena.2018.06.018>.
- Beugnon, R., D. Jianqing, S. Cesarz, S. D. Jurburg, Z. Pang, B. Singavarapu, T. Wubet, K. Xue, Y. Wang, and N. Eisenhauer. 2021. "Tree Diversity and Soil Chemical Properties Drive the Linkages between Soil Microbial Community and Ecosystem Functioning." *ISME Communications* 1(1): 41. <https://doi.org/10.1038/s43705-021-00040-0>.
- Beugnon, R., B. Wensheng, H. Bruelheide, A. Davrinche, D. Jianqing, S. Haider, M. Kunz, et al. 2022. "Beugnon-et-al-2022_Soil-Carbon-and-Microbial-Biomass-Drivers: Data." Dryad. <https://doi.org/10.5061/dryad.pvmcvdnqc>.
- Bollag, J.-M., and G. E. Stotzky, eds. 1993. *Soil Biochemistry*. Boca Raton: CRC Press.
- Bonfante, P., and A. Genre. 2010. "Mechanisms Underlying Beneficial Plant–Fungus Interactions in Mycorrhizal Symbiosis." *Nature Communications* 1(1): 48. <https://doi.org/10.1038/ncomms1046>.
- Both, S., T. Fang, M. Böhnke, H. Bruelheide, C. Geißler, P. Kühn, T. Scholten, S. Trogisch, and A. Erfmeier. 2011. "Lack of Tree Layer Control on Herb Layer Characteristics in a Subtropical Forest, China." *Journal of Vegetation Science* 22(6): 1120–31. <https://doi.org/10.1111/j.1654-1103.2011.01324.x>.
- Bradstreet, R. B. 1954. "Determination of Nitro Nitrogen by Kjeldahl Method." *Analytical Chemistry* 26(1): 235–6.
- Bruelheide, H., M. Böhnke, S. Both, T. Fang, T. Assmann, M. Baruffol, J. Bauhus, et al. 2011. "Community Assembly during Secondary Forest Succession in a Chinese Subtropical Forest." *Ecological Monographs* 81(1): 25–41. <https://doi.org/10.1890/09-2172.1>.
- Bruelheide, H., K. Nadrowski, T. Assmann, J. Bauhus, S. Both, F. Buscot, X.-Y. Chen, et al. 2014. "Designing Forest Biodiversity Experiments: General Considerations Illustrated by a New Large Experiment in Subtropical China." *Methods in Ecology and Evolution* 5(1): 74–89. <https://doi.org/10.1111/2041-210X.12126>.
- Bu, W., B. Schmid, X. Liu, Y. Li, W. Härdtle, G. von Oheimb, Y. Liang, et al. 2017. "Interspecific and Intraspecific Variation in Specific Root Length Drives Aboveground Biodiversity Effects in Young Experimental Forest Stands." *Journal of Plant Ecology* 10(1): 158–69. <https://doi.org/10.1093/jpe/rtw096>.
- Buckeridge, K. M., K. E. Mason, N. P. McNamara, N. Ostle, J. Puissant, T. Goodall, R. I. Griffiths, A. W. Stott, and J. Whitaker. 2020. "Environmental and Microbial Controls on Microbial Necromass Recycling, an Important Precursor for Soil Carbon Stabilization." *Communications Earth & Environment* 1(1): 36. <https://doi.org/10.1038/s43247-020-00031-4>.
- Burgess, S. S. O., M. A. Adams, N. C. Turner, and C. K. Ong. 1998. "The Redistribution of Soil Water by Tree Root Systems." *Oecologia* 115(3): 306–11. <https://doi.org/10.1007/s004420050521>.
- Cesarz, S., D. Craven, H. Auge, H. Bruelheide, B. Castagnyrol, J. Gutknecht, A. Hector, et al. 2022. "Tree Diversity Effects on Soil Microbial Biomass and Respiration Are Context Dependent across Forest Diversity Experiments." *Global Ecology and Biogeography* 31(5): 872–85. <https://doi.org/10.1111/geb.13461>.
- Chapman, S. K., G. S. Newman, S. C. Hart, J. A. Schweitzer, and G. W. Koch. 2013. "Leaf Litter Mixtures Alter Microbial Community Development: Mechanisms for Non-Additive Effects in Litter Decomposition." *PLoS One* 8(4): e62671. <https://doi.org/10.1371/journal.pone.0062671>.

- Clark, R. T., R. B. MacCurdy, J. K. Jung, J. E. Shaff, S. R. McCouch, D. J. Aneshansley, and L. V. Kochian. 2011. "Three-Dimensional Root Phenotyping with a Novel Imaging and Software Platform." *Plant Physiology* 156(2): 455–65. <https://doi.org/10.1104/pp.110.169102>.
- Clemmensen, K. E., O. Ovaskainen, A. Dahlberg, A. Ekblad, H. Wallander, J. Stenlid, D. A. Finlay, D. A. Wardle, and B. D. Lindahl. 2013. "Roots and Associated Fungi Drive Long-Term Carbon Sequestration in Boreal Forest." *Science* 339(6127): 1615–8. <https://doi.org/10.1126/science.1232728>.
- Craig, M. E., B. L. Turner, C. Liang, K. Clay, D. J. Johnson, and R. P. Phillips. 2018. "Tree Mycorrhizal Type Predicts Within-Site Variability in the Storage and Distribution of Soil Organic Matter." *Global Change Biology* 24(8): 3317–30. <https://doi.org/10.1111/gcb.14132>.
- Crowther, T. W., J. van den Hoogen, J. Wan, M. A. Mayes, A. D. Keiser, L. Mo, C. Averill, and D. S. Maynard. 2019. "The Global Soil Community and its Influence on Biogeochemistry." *Science* 365(6455): eaav0550. <https://doi.org/10.1126/science.aav0550>.
- Davrinche, A., and S. Haider. 2021. "Intra-Specific Leaf Trait Responses to Species Richness at Two Different Local Scales." *Basic and Applied Ecology* 55: 20–32. <https://doi.org/10.1016/j.baae.2021.04.011>.
- de Frenne, P., F. Zellweger, F. Rodríguez-Sánchez, B. R. Scheffers, K. Hylander, M. Luoto, M. Vellend, K. Verheyen, and J. Lenoir. 2019. "Global Buffering of Temperatures under Forest Canopies." *Ecology and Evolution* 3: 744–9.
- Delgado-Baquerizo, M., F. T. Maestre, P. B. Reich, P. Trivedi, Y. Osanai, Y.-R. Liu, K. Hamonts, T. C. Jeffries, and B. K. Singh. 2016. "Carbon Content and Climate Variability Drive Global Soil Bacterial Diversity Patterns." *Ecological Monographs* 86(3): 373–90.
- Delgado-Baquerizo, M., P. B. Reich, A. N. Khachane, C. D. Campbell, N. Thomas, T. E. Freitag, W. A. Al-Soud, S. Sørensen, R. D. Bardgett, and B. K. Singh. 2017. "It Is Elemental: Soil Nutrient Stoichiometry Drives Bacterial Diversity." *Environmental Microbiology* 19(3): 1176–88. <https://doi.org/10.1111/1462-2920.13642>.
- Ehbrecht, M., P. Schall, J. Juchheim, C. Ammer, and D. Seidel. 2016. "Effective Number of Layers: A New Measure for Quantifying Three-Dimensional Stand Structure Based on Sampling with Terrestrial LiDAR." *Forest Ecology and Management* 380: 212–23. <https://doi.org/10.1016/j.foreco.2016.09.003>.
- Eisenhauer, N., H. Beßler, C. Engels, G. Gleixner, M. Habekost, A. Milcu, S. Partsch, et al. 2010. "Plant Diversity Effects on Soil Microorganisms Support the Singular Hypothesis." *Ecology* 91(2): 485–96. <https://doi.org/10.1890/08-2338.1>.
- Eisenhauer, N., M. Bonkowski, U. Brose, F. Buscot, W. Durka, A. Ebeling, M. Fischer, et al. 2019. "Biotic Interactions, Community Assembly, and Eco-Evolutionary Dynamics as Drivers of Long-Term Biodiversity-Ecosystem Functioning Relationships." *Research Ideas and Outcomes*: 5. <https://doi.org/10.3897/rio.5.e47042>.
- Eisenhauer, N., A. Lanoue, T. Strecker, S. Scheu, K. Steinauer, M. P. Thakur, and L. Mommer. 2017. "Root Biomass and Exudates Link Plant Diversity with Soil Bacterial and Fungal Biomass." *Scientific Reports* 7: 44641. <https://doi.org/10.1038/srep44641>.
- Eisenhauer, N., K. Yee, E. A. Johnson, M. Maraun, D. Parkinson, D. Straube, and S. Scheu. 2011. "Positive Relationship between Herbaceous Layer Diversity and the Performance of Soil Biota in a Temperate Forest." *Soil Biology and Biochemistry* 43(2): 462–5. <https://doi.org/10.1016/j.soilbio.2010.10.018>.
- Fichtner, A., W. Härdtle, H. Bruelheide, M. Kunz, Y. Li, and G. von Oheimb. 2018. "Neighbourhood Interactions Drive Overyielding in Mixed-Species Tree Communities." *Nature Communications* 9(1): 1144. <https://doi.org/10.1038/s41467-018-03529-w>.
- Frey, S. D. 2019. "Mycorrhizal Fungi as Mediators of Soil Organic Matter Dynamics." *Annual Review of Ecology, Evolution, and Systematics* 50(1): 237–59. <https://doi.org/10.1146/annurev-ecolsys-110617-062331>.
- Frostegård, Å., A. Tunlid, and E. Bååth. 1991. "Microbial Biomass Measured as Total Lipid Phosphate in Soils of Different Organic Content." *Journal of Microbiological Methods* 14(3): 151–63. [https://doi.org/10.1016/0167-7012\(91\)90018-L](https://doi.org/10.1016/0167-7012(91)90018-L).
- Garnier, E., J. Cortez, G. Billès, M.-L. Navas, C. Roumet, M. Debussche, G. Laurent, et al. 2004. "Plant Functional Markers Capture Ecosystem Properties during Secondary Succession." *Ecology* 85(9): 2630–7. <https://doi.org/10.1890/03-0799>.
- Geißler, C., P. Kühn, M. Böhnke, H. Bruelheide, X. Shi, and T. Scholten. 2012. "Splash Erosion Potential under Tree Canopies in Subtropical SE China." *Catena* 91: 85–93. <https://doi.org/10.1016/j.catena.2010.10.009>.
- Germany, M. S., H. Bruelheide, and A. Erfmeier. 2017. "Limited Tree Richness Effects on Herb Layer Composition, Richness and Productivity in Experimental Forest Stands." *Journal of Plant Ecology* 10(1): 190–200. <https://doi.org/10.1093/jpe/rtw109>.
- Goebes, P., S. Seitz, P. Kühn, Y. Li, P. A. Niklaus, G. von Oheimb, and T. Scholten. 2015. "Throughfall Kinetic Energy in Young Subtropical Forests: Investigation on Tree Species Richness Effects and Spatial Variability." *Agricultural and Forest Meteorology* 213: 148–59. <https://doi.org/10.1016/j.agrformet.2015.06.019>.
- Goto, N., A. Sakoda, and M. Suzuki. 1994. "Modelling of Soil Carbon Dynamics as a Part of Carbon Cycle in Terrestrial Ecosystems." *Ecological Modelling* 74(3–4): 183–204. [https://doi.org/10.1016/0304-3800\(94\)90119-8](https://doi.org/10.1016/0304-3800(94)90119-8).
- Gottschall, F., S. Davids, T. E. Newiger-Dous, H. Auge, S. Cesarz, and N. Eisenhauer. 2019. "Tree Species Identity Determines Wood Decomposition via Microclimatic Effects." *Ecology and Evolution* 9(21): 12113–27. <https://doi.org/10.1002/ece3.5665>.
- Guerrero-Ramírez, N. R., D. Craven, P. B. Reich, J. J. Ewel, F. Isbell, J. Koricheva, J. A. Parrotta, et al. 2017. "Diversity-dependent Temporal Divergence of Ecosystem Functioning in Experimental Ecosystems." *Nature Ecology & Evolution* 1(11): 1639–42. <https://doi.org/10.1038/s41559-017-0325-1>.
- Hakkenberg, C. R., R. K. Peet, T. R. Wentworth, K. Zhu, and M. P. Schafale. 2020. "Tree Canopy Cover Constrains the Fertility-Diversity Relationship in Plant Communities of the Southeastern United States." *Ecology* 101(10): e03119. <https://doi.org/10.1002/ecy.3119>.
- Haug, I., R. Weber, F. Oberwinkler, and J. Tschen. 1994. "The Mycorrhizal Status of Taiwanese Trees and the Description of some Ectomycorrhizal Types." *Trees* 8(5): 237–53. <https://doi.org/10.1007/BF00196628>.
- Hawley, G. L., and J. F. Dames. 2004. "Mycorrhizal Status of Indigenous Tree Species in a Forest Biome of the Eastern

- Cape, South Africa.” *South African Journal of Science* 100(11): 633–7.
- Hooper, D. U., D. E. Bignell, V. K. Brown, L. Brassard, J. Mark Dangerfield, D. H. Wall, D. A. Wardle, et al. 2000. “Interactions between Aboveground and Belowground Biodiversity in Terrestrial Ecosystems: Patterns, Mechanisms, and Feedbacks.” *BioScience* 50(12): 1049.
- Huang, Y., Y. Chen, N. Castro-Izaguirre, M. Baruffol, M. Brezzi, A. Lang, Y. Li, et al. 2018. “Impacts of Species Richness on Productivity in a Large-Scale Subtropical Forest Experiment.” *Science* 362(6410): 80–3. <https://doi.org/10.1126/science.aat6405>.
- Huang, Y., Y. Ma, K. Zhao, P. A. Niklaus, B. Schmid, and J.-S. He. 2017. “Positive Effects of Tree Species Diversity on Litterfall Quantity and Quality along a Secondary Successional Chronosequence in a Subtropical Forest.” *Journal of Plant Ecology* 10(1): 28–35. <https://doi.org/10.1093/jpe/rtw115>.
- IPBES. 2019. “Global Assessment Report on Biodiversity and Ecosystem Services of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services.” Zenodo. <https://doi.org/10.5281/ZENODO.3831673>.
- Kästner, M., and A. Miltner. 2018. “SOM and Microbes—What Is Left from Microbial Life.” In *The Future of Soil Carbon*, edited by C. Garcia, P. Nannipieri, and T. Hernandez, 125–63. Cambridge, MA: Elsevier. <https://doi.org/10.1016/B978-0-12-811687-6.00005-5>.
- Kramer, S., D. Dibbern, J. Moll, M. Huenninghaus, R. Koller, D. Krueger, S. Marhan, et al. 2016. “Resource Partitioning between Bacteria, Fungi, and Protists in the Detritosphere of an Agricultural Soil.” *Frontiers in Microbiology* 7: 1524. <https://doi.org/10.3389/fmicb.2016.01524>.
- Kunz, M., A. Fichtner, W. Härdtle, P. Raunonen, H. Bruelheide, and G. von Oheimb. 2019. “Neighbour Species Richness and Local Structural Variability Modulate Aboveground Allocation Patterns and Crown Morphology of Individual Trees.” *Ecology Letters* 22(12): 2130–40. <https://doi.org/10.1111/ele.13400>.
- Laliberté, E., P. Legendre, B. Shipley, and M. E. Laliberté. 2014. “Package FD: Measuring Functional Diversity from Multiple Traits, and Other Tools for Functional Ecology.” <https://citeseerx.ist.psu.edu/document?repid=rep1&type=pdf&doi=99009d911cb83b2bd2bc1ea6e7399b0d05e5cb63>.
- Laliberté, E., and P. Legendre. 2010. “A Distance-Based Framework for Measuring Functional Diversity from Multiple Traits.” *Ecology* 91(1): 299–305. <https://doi.org/10.1890/08-2244.1>.
- Lange, M., N. Eisenhauer, C. A. Sierra, H. Bessler, C. Engels, R. I. Griffiths, P. G. Mellado-Vázquez, et al. 2015. “Plant Diversity Increases Soil Microbial Activity and Soil Carbon Storage.” *Nature Communications* 6: 6707. <https://doi.org/10.1038/ncomms7707>.
- Lareen, A., F. Burton, and P. Schäfer. 2016. “Plant Root-Microbe Communication in Shaping Root Microbiomes.” *Plant Molecular Biology* 90(6): 575–87. <https://doi.org/10.1007/s11103-015-0417-8>.
- Lecigne, B., S. Delagrangé, and C. Messier. 2018. “Exploring Trees in Three Dimensions: VoxR, a Novel Voxel-Based R Package Dedicated to Analysing the Complex Arrangement of Tree Crowns.” *Annals of Botany* 121(4): 589–601. <https://doi.org/10.1093/aob/mcx095>.
- Lewis, S. L., C. E. Wheeler, E. T. A. Mitchard, and A. Koch. 2019. “Restoring Natural Forests Is the Best Way to Remove Atmospheric Carbon.” *Nature* 568(7750): 25–8. <https://doi.org/10.1038/d41586-019-01026-8>.
- Li, Y., H. Bruelheide, T. Scholten, B. Schmid, Z. Sun, N. Zhang, B. Wensheng, X. Liu, and K. Ma. 2019. “Early Positive Effects of Tree Species Richness on Soil Organic Carbon Accumulation in a Large-Scale Forest Biodiversity Experiment.” *Journal of Plant Ecology* 12(5): 882–93. <https://doi.org/10.1093/jpe/rtz026>.
- Liang, C., and T. C. Balser. 2011. “Microbial Production of Recalcitrant Organic Matter in Global Soils: Implications for Productivity and Climate Policy.” *Nature Reviews Microbiology* 9(1): 75–5. <https://doi.org/10.1038/nrmicro2386-c1>.
- Liski, J., T. Palosuo, M. Peltoniemi, and R. Sievänen. 2005. “Carbon and Decomposition Model Yasso for Forest Soils.” *Ecological Modelling* 189(1–2): 168–82. <https://doi.org/10.1016/j.ecolmodel.2005.03.005>.
- Liu, C., W. Xiang, B. Xie, S. Ouyang, Y. Zeng, P. Lei, and C. Peng. 2020. “Decoupling the Complementarity Effect and the Selection Effect on the Overyielding of Fine Root Production along a Tree Species Richness Gradient in Subtropical Forests.” *Ecosystems* 24: 613–27. <https://doi.org/10.1007/s10021-020-00538-z>.
- Liu, X., S. Trogisch, J.-S. He, P. A. Niklaus, H. Bruelheide, Z. Tang, A. Erfmeier, et al. 2018. “Tree Species Richness Increases Ecosystem Carbon Storage in Subtropical Forests.” *Proceedings. Biological Sciences* 285(1885): 20181240. <https://doi.org/10.1098/rspb.2018.1240>.
- Londo, G. 1976. “The Decimal Scale for Relevés of Permanent Quadrats.” *Vegetatio* 33(1): 61–4. <https://doi.org/10.1007/bf00055300>.
- Metzner, R., D. van Dusschoten, J. Bühler, U. Schurr, and S. Jahnke. 2014. “Belowground Plant Development Measured with Magnetic Resonance Imaging (MRI): Exploiting the Potential for Noninvasive Trait Quantification Using Sugar Beet as a Proxy.” *Frontiers in Plant Science* 5: 469. <https://doi.org/10.3389/fpls.2014.00469>.
- Miltner, A., P. Bombach, B. Schmidt-Brücken, and M. Kästner. 2012. “SOM Genesis: Microbial Biomass as a Significant Source.” *Biogeochemistry* 111(1–3): 41–55. <https://doi.org/10.1007/s10533-011-9658-z>.
- Mooney, S. J., T. P. Pridmore, J. Helliwell, and M. J. Bennett. 2012. “Developing X-Ray Computed Tomography to Noninvasively Image 3-D Root Systems Architecture in Soil.” *Plant and Soil* 352(1–2): 1–22. <https://doi.org/10.1007/s11104-011-1039-9>.
- Mueller, K. E., N. Eisenhauer, P. B. Reich, S. E. Hobbie, O. A. Chadwick, J. Chorover, T. Dobies, et al. 2016. “Light, Earthworms, and Soil Resources as Predictors of Diversity of 10 Soil Invertebrate Groups across Monocultures of 14 Tree Species.” *Soil Biology and Biochemistry* 92: 184–98. <https://doi.org/10.1016/j.soilbio.2015.10.010>.
- Parkinson, J. A., and S. E. Allen. 1975. “A Wet Oxidation Procedure Suitable for the Determination of Nitrogen and Mineral Nutrients in Biological Material.” *Communications in Soil Science and Plant Analysis* 6(1): 1–11. <https://doi.org/10.1080/00103627509366539>.
- Pei, Z., D. Eichenberg, H. Bruelheide, W. Kröber, P. Kühn, Y. Li, G. von Oheimb, et al. 2016. “Soil and Tree Species Traits Both Shape Soil Microbial Communities during

- Early Growth of Chinese Subtropical Forests.” *Soil Biology and Biochemistry* 96: 180–90. <https://doi.org/10.1016/j.soilbio.2016.02.004>.
- Perles-Garcia, M. D., M. Kunz, A. Fichtner, W. Härdtle, and G. von Oheimb. 2021. “Tree Species Richness Promotes an Early Increase of Stand Structural Complexity in Young Subtropical Plantations.” *Journal of Applied Ecology*. 58: 2305–14. <https://doi.org/10.1111/1365-2664.13973>.
- Reich, P. B., J. Oleksyn, J. Modrzynski, P. Mrozinski, S. E. Hobbie, D. M. Eissenstat, J. Chorover, O. A. Chadwick, C. M. Hale, and M. G. Tjoelker. 2005. “Linking Litter Calcium, Earthworms and Soil Properties: A Common Garden Test with 14 Tree Species.” *Ecology Letters* 8(8): 811–8. <https://doi.org/10.1111/j.1461-0248.2005.00779.x>.
- remybeugnon. 2022. “Remybeugnon/Beugnon-et-al-2021_Soil-Carbon-and-Microbial-Biomass-Drivers: Major Revision of the Analyses during Review Process (Manuscript-Update).” Zenodo. <https://doi.org/10.5281/zenodo.7225739>.
- Rosseel, Y. 2012. “lavaan: An R Package for Structural Equation Modeling and More. Version 0.5–12 (BETA).” *Journal of Statistical Software* 48(2): 1–36.
- Ruess, L., and P. M. Chamberlain. 2010. “The Fat that Matters: Soil Food Web Analysis Using Fatty Acids and their Carbon Stable Isotope Signature.” *Soil Biology and Biochemistry* 42(11): 1898–910. <https://doi.org/10.1016/j.soilbio.2010.07.020>.
- Schmidt, M. W. I., M. S. Torn, S. Abiven, T. Dittmar, G. Guggenberger, I. A. Janssens, M. Kleber, et al. 2011. “Persistence of Soil Organic Matter as an Ecosystem Property.” *Nature* 478(7367): 49–56. <https://doi.org/10.1038/nature10386>.
- Scholten, T., P. Goebes, P. Kühn, S. Seitz, T. Assmann, J. Bauhus, H. Bruelheide, et al. 2017. “On the Combined Effect of Soil Fertility and Topography on Tree Growth in Subtropical Forest Ecosystems—A Study from SE China.” *Journal of Plant Ecology* 10(1): 111–27. <https://doi.org/10.1093/jpe/rtw065>.
- Seedre, M., A. R. Taylor, B. W. Brassard, H. Y. H. Chen, and K. Jögiste. 2014. “Recovery of Ecosystem Carbon Stocks in Young Boreal Forests: A Comparison of Harvesting and Wildfire Disturbance.” *Ecosystems* 17(5): 851–63. <https://doi.org/10.1007/s10021-014-9763-7>.
- Seitz, S., P. Goebes, Z. Song, H. Bruelheide, W. Härdtle, P. Kühn, Y. Li, and T. Scholten. 2016. “Tree Species and Functional Traits but Not Species Richness Affect Interrill Erosion Processes in Young Subtropical Forests.” *Soil* 2(1): 49–61. <https://doi.org/10.5194/soil-2-49-2016>.
- Seitz, S., P. Goebes, P. Zumstein, T. Assmann, P. Kühn, P. A. Niklaus, A. Schuldt, and T. Scholten. 2015. “The Influence of Leaf Litter Diversity and Soil Fauna on Initial Soil Erosion in Subtropical Forests.” *Earth Surface Processes and Landforms* 40(11): 1439–47. <https://doi.org/10.1002/esp.3726>.
- Serna-Chavez, H. M., N. Fierer, and P. M. van Bodegom. 2013. “Global Drivers and Patterns of Microbial Abundance in Soil.” *Global Ecology and Biogeography* 22(10): 1162–72. <https://doi.org/10.1111/geb.12070>.
- Simpson, A. J., M. J. Simpson, E. Smith, and B. P. Kelleher. 2007. “Microbially Derived Inputs to Soil Organic Matter: Are Current Estimates Too Low?” *Environmental Science & Technology* 41(23): 8070–6. <https://doi.org/10.1021/es071217x>.
- Singavarapu, B., R. Beugnon, H. Bruelheide, S. Cesarz, J. du, N. Eisenhauer, L. D. Guo, et al. 2021. “Tree Mycorrhizal Type and Tree Diversity Shape the Forest Soil Microbiota.” *Environmental Microbiology*. 24: 4236–55. <https://doi.org/10.1111/1462-2920.15690>.
- Smith, L. C., A. Orgiazzi, N. Eisenhauer, S. Cesarz, A. Lochner, A. Jones, F. Bastida, et al. 2021. “Large-Scale Drivers of Relationships between Soil Microbial Properties and Organic Carbon across Europe.” *Global Ecology and Biogeography* 30(10): 2070–83. <https://doi.org/10.1111/geb.13371>.
- Sun, L., M. Ataka, M. Han, Y. Han, D. Gan, X. Tianle, Y. Guo, and B. Zhu. 2020. “Root Exudation as a Major Competitive Fine-Root Functional Trait of 18 Coexisting Species in a Subtropical Forest.” *The New Phytologist* 229: 259–71. <https://doi.org/10.1111/nph.16865>.
- Sun, Z., X. Liu, B. Schmid, H. Bruelheide, B. Wensheng, and K. Ma. 2017. “Positive Effects of Tree Species Richness on Fine-Root Production in a Subtropical Forest in SE-China.” *Journal of Plant Ecology* 10(1): 146–57. <https://doi.org/10.1093/jpe/rtw094>.
- Taylor, A. R., J. R. Wang, and W. A. Kurz. 2008. “Effects of Harvesting Intensity on Carbon Stocks in Eastern Canadian Red Spruce (*Picea Rubens*) Forests: An Exploratory Analysis Using the CBM-CFS3 Simulation Model.” *Forest Ecology and Management* 255(10): 3632–41. <https://doi.org/10.1016/j.foreco.2008.02.052>.
- Thoms, C., A. Gattinger, M. Jacob, F. M. Thomas, and G. Gleixner. 2010. “Direct and Indirect Effects of Tree Diversity Drive Soil Microbial Diversity in Temperate Deciduous Forest.” *Soil Biology and Biochemistry* 42(9): 1558–65. <https://doi.org/10.1016/j.soilbio.2010.05.030>.
- Trogisch, S., X. Liu, G. Rütten, K. Xue, J. Bauhus, U. Brose, W. Bu, et al. 2021. “The Significance of Tree-Tree Interactions for Forest Ecosystem Functioning.” *Basic and Applied Ecology*. 55: 33–52. <https://doi.org/10.1016/j.baae.2021.02.003>.
- Trumbore, S. 2009. “Radiocarbon and Soil Carbon Dynamics.” *Annual Review of Earth and Planetary Sciences* 37(1): 47–66. <https://doi.org/10.1146/annurev.earth.36.031207.124300>.
- Trumbore, S. E. 1993. “Comparison of Carbon Dynamics in Tropical and Temperate Soils Using Radiocarbon Measurements.” *Global Biogeochemical Cycles* 7(2): 275–90. <https://doi.org/10.1029/93gb00468>.
- Ushio, M., R. Wagai, T. C. Balser, and K. Kitayama. 2008. “Variations in the Soil Microbial Community Composition of a Tropical Montane Forest Ecosystem: Does Tree Species Matter?” *Soil Biology and Biochemistry* 40(10): 2699–702. <https://doi.org/10.1016/j.soilbio.2008.06.023>.
- Venables, W. N., and B. D. Ripley. 2002. *Modern Applied Statistics with S*. New York: Springer.
- Vockenhuber, E. A., C. Scherber, C. Langenbruch, M. Meißner, D. Seidel, and T. Tschardtke. 2011. “Tree Diversity and Environmental Context Predict Herb Species Richness and Cover in Germany’s Largest Connected Deciduous Forest.” *Perspectives in Plant Ecology, Evolution and Systematics* 13(2): 111–9. <https://doi.org/10.1016/j.ppees.2011.02.004>.
- Vogel, A., A. Ebeling, G. Gleixner, C. Roscher, S. Scheu, M. Ciobanu, E. Koller-France, et al. 2019. “A New Experimental Approach to Test Why Biodiversity Effects Strengthen as

- Ecosystems Age.” *Mechanisms Underlying the Relationship between Biodiversity and Ecosystem Function*: 221–64. <https://doi.org/10.1016/bs.aecr.2019.06.006>.
- von Lützow, M., I. Kögel-Knabner, K. Ekschmitt, H. Flessa, G. Guggenberger, E. Matzner, and B. Marschner. 2007. “SOM Fractionation Methods: Relevance to Functional Pools and to Stabilization Mechanisms.” *Soil Biology and Biochemistry* 39(9): 2183–207. <https://doi.org/10.1016/j.soilbio.2007.03.007>.
- Wang, B., and Y.-L. Qiu. 2006. “Phylogenetic Distribution and Evolution of Mycorrhizas in Land Plants.” *Mycorrhiza* 16(5): 299–363. <https://doi.org/10.1007/s00572-005-0033-6>.
- Wen, Z., H. Li, Q. Shen, X. Tang, C. Xiong, H. Li, J. Pang, M. H. Ryan, H. Lambers, and J. Shen. 2019. “Tradeoffs among Root Morphology, Exudation and Mycorrhizal Symbioses for Phosphorus-Acquisition Strategies of 16 Crop Species.” *New Phytologist* 223(2): 882–95. <https://doi.org/10.1111/nph.15833>.
- Wichern, J., F. Wichern, and R. G. Joergensen. 2006. “Impact of Salinity on Soil Microbial Communities and the Decomposition of Maize in Acidic Soils.” *Geoderma* 137(1–2): 100–8. <https://doi.org/10.1016/j.geoderma.2006.08.001>.
- Wikle, C. K., A. Zammit-Mangion, and N. A. C. Cressie. 2019. *Spatio-Temporal Statistics with R*. Chapman & Hall/CRC the R Series. Boca Raton, FL: CRC Press.
- Williams, L. J., A. Paquette, J. Cavender-Bares, C. Messier, and P. B. Reich. 2017. “Spatial Complementarity in Tree Crowns Explains Overyielding in Species Mixtures.” *Nature Ecology & Evolution* 1(4): 63. <https://doi.org/10.1038/s41559-016-0063>.
- Xu, S., N. Eisenhauer, O. Ferlian, J. Zhang, G. Zhou, L. Xiankai, C. Liu, and D. Zhang. 2020. “Species Richness Promotes Ecosystem Carbon Storage: Evidence from Biodiversity-Ecosystem Functioning Experiments.” *Proceedings. Biological Sciences* 287(1939): 20202063. <https://doi.org/10.1098/rspb.2020.2063>.
- Xu, X., P. E. Thornton, and W. M. Post. 2013. “A Global Analysis of Soil Microbial Biomass Carbon, Nitrogen and Phosphorus in Terrestrial Ecosystems.” *Global Ecology and Biogeography* 22(6): 737–49. <https://doi.org/10.1111/geb.12029>.
- Yang, X., J. Bauhus, S. Both, T. Fang, W. Härdtle, W. Kröber, K. Ma, et al. 2013. “Establishment Success in a Forest Biodiversity and Ecosystem Functioning Experiment in Subtropical China (BEF-China).” *European Journal of Forest Research* 132(4): 593–606. <https://doi.org/10.1007/s10342-013-0696-z>.
- Zhang, S., D. Landuyt, K. Verheyen, and P. De Frenne. 2022. “Tree Species Mixing Can Amplify Microclimate Offsets in Young Forest Plantations.” *Journal of Applied Ecology*, March 1365–2664: 14158. <https://doi.org/10.1111/1365-2664.14158>.
- Zhang, Z., Y. Liu, C. Brunel, and M. van Kleunen. 2020. “Evidence for Elton’s Diversity-Invasibility Hypothesis from Belowground.” *Ecology* 101: e03187. <https://doi.org/10.1002/ecy.3187>.
- Zheng, L., H. Y. H. Chen, and E. Yan. 2019. “Tree Species Diversity Promotes Litterfall Productivity Through Crown Complementarity in Subtropical Forests.” *Journal of Ecology* 107(4): 1852–61. <https://doi.org/10.1111/1365-2745.13142>.
- Zuppinger-Dingley, D., B. Schmid, J. S. Petermann, V. Yadav, G. B. De Deyn, and D. F. B. Flynn. 2014. “Selection for Niche Differentiation in Plant Communities Increases Biodiversity Effects.” *Nature* 515(7525): 108–11. <https://doi.org/10.1038/nature13869>.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Beugnon, Rémy, Wensheng Bu, Helge Bruelheide, Andréa Davrinche, Jianqing Du, Sylvia Haider, Matthias Kunz, et al. 2023. “Abiotic and Biotic Drivers of Tree Trait Effects on Soil Microbial Biomass and Soil Carbon Concentration.” *Ecological Monographs* 93(2): e1563. <https://doi.org/10.1002/ecm.1563>