



Chapter III - Abiotic and biotic drivers of scale-dependent tree trait effects on soil microbial biomass and soil carbon concentration

Rémy Beugnon^{C,1,2}, Wensheng Bu³, Helge Bruelheide^{4,1}, Andréa Davrinche^{4,1}, Jianqing Du⁵, Sylvia Haider^{4,1}, Matthias Kunz⁶, Goddert von Oheimb⁶, Maria D. Perles-Garcia^{6,1,4}, Mariem Saadani^{4,1}, Thomas Scholten⁷, Steffen Seitz⁷, Bala Singavarapu^{8,1,4}, Stefan Trogisch^{4,1}, Yanfen Wang^{5,9}, Tesfaye Wubet^{8,1}, Kai Xue^{5,9}, Bo Yang¹⁰, Simone Cesarz^{S,1,2} & Nico Eisenhauer^{S,1,2}.

^c: corresponding author, emails: <u>remy.beugnon@idiv.de</u>, ^S: senior authors

¹: German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, Puschstrasse 4, 04103 Leipzig, Germany

²: Institute of Biology, Leipzig University, Deutscher Platz 5e, 04103 Leipzig, Germany

³: College of Forestry, Jiangxi Agricultural University, Nanchang, 330045, China

⁴: Institute of Biology/Geobotany and Botanical Garden, Martin Luther University Halle-Wittenberg, Am Kirchtor 1, 06108 Halle, Germany

⁵: Yanshan Earth Critical Zone and Surface Fluxes Research Station, College of Resources and Environment, University of Chinese Academy of Sciences, 101408 Beijing, China

⁶: Institute of General Ecology and Environmental Protection, Technische Universität Dresden, Pienner Straße 7, 01737 Tharandt, Germany

⁷: Chair of Soil Science and Geomorphology, University of Tübingen, Rümelinstraße 19-23, 72070 Tübingen, Germany

⁸: UFZ-Helmholtz Centre for Environmental Research, Department of Community Ecology, Theodor-Lieser-Str. 4, D-06120 Halle (Saale), Germany

⁹: CAS Center for Excellence in Tibetan Plateau Earth Sciences, 100101 Beijing, China

¹⁰: Jiangxi Key Laboratory of Plant Resources and Biodiversity, Jingdezhen University, Jingdezhen, 333400, China

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Abstract

Forest ecosystems have been highlighted for their carbon fixation potential in both above- and belowground compartments, especially in species-rich forests. Soil microbial communities are strongly linked to soil carbon sequestration, and it is suggested that this link is mediated by the tree community, likely due to modifications of micro-environmental conditions (i.e. micro-climate, soil quality, and biotic conditions). We further expect that these relationships will depend on the scale considered, with local (i.e., at the level of a tree species pair, TSP) and neighborhood (i.e., the surrounding trees of a TSP) scale processes influencing soil conditions.

We studied soil carbon concentration and the microbial community composition of 180 TSPs along a gradient of tree species richness ranging from 1 to 16 per plot in the Chinese subtropical forest experiment (BEF-China). Tree productivity and different tree functional traits were measured at both the TSP level and neighborhood level. We tested the effects of tree productivity, functional trait identity and dissimilarity on soil carbon concentrations, and if these links were mediated by the soil microbial biomass and micro-environmental conditions.

Tree productivity, together with tree functional traits, modulated micro-environmental conditions with substantial consequences for soil microbial biomass. Especially, soil microbial biomass was modified by root morphological traits at both TSP and neighborhood levels. However, the effects of the root morphological traits on microbial biomass were highly scale-dependent, with a positive effect of root morphological traits at the TSP level but a negative effect at the neighborhood level. Moreover, our analyses showed a strong positive correlation between soil microbial biomass and soil carbon concentration. We found that soil carbon concentrations increased with historical carbon concentrations, themselves strongly affected by the plot topography. However, soil carbon concentrations decreased over time. Besides, soil carbon concentration increased with tree productivity and root morphological traits at the neighborhood level.

Altogether, these results imply that mechanistic studies on the drivers of microbial biomass and soil carbon sequestration need to consider the different spatial scales at which the underlying mechanisms act. Moreover, quantification of the different soil carbon pools is critical to the understanding of microbial community–soil carbon stock relationships.

Introduction

The rapid increase in atmospheric carbon is one of the main causes of climate change and becomes a major threat to life on Earth (IPCC 2013). 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 1993). In forests, soil carbon stocks are driven by the balance between soil carbon influx (e.g., due to photosynthesis) and efflux (e.g., due to soil respiration and erosion), but our understanding of their balance and the driving factors is still limited.

Forest diversity enhances forest productivity: tree biomass and litterfall quantity as well as root biomass and exudation (Eisenhauer *et al.* 2017; Huang *et al.* 2017; Huang *et al.* 2018; Xu *et al.* 2020; Zheng *et al.* 2019). Therefore, tree diversity is expected to increase carbon influxes in soil and consequently soil carbon concentration (Liu *et al.* 2018). Moreover, the kinetic energy of throughfall as a determinant of soil erosion under forest is influenced by neighborhood tree species richness (Goebes *et al.* 2015). The same holds true for interrill erosion. Thus, different tree morphologies have to be considered, when assessing soil erosion under forest, which can affect soil carbon concentrations and nutrient fluxes on small scales (Seitz *et al.* 2015). In addition, recent studies have started linking soil carbon concentration to tree roots (Adamczyk *et al.* 2019). Specifically, morphological traits were shown to control the release of both root carbon (i.e., either by desiccation or exudation) to the soil (Sun *et al.* 2020) and to drive soil organic matter decomposition (Adamczyk *et al.* 2019). For example, with a higher specific root length (SRL), root carbon exudation and desiccation increase due to a higher density of fine roots (Bergmann *et al.* 2020; Sun *et al.* 2020; Wen *et al.* 2019).

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Additionally, soil carbon concentrations have been linked to the mycorrhizal association of tree roots (Frey 2019), with trees associating with arbuscular mycorrhizal (AM) fungi having lower topsoil carbon concentrations, while tree stands with ectomycorrhizal (EM) fungi having higher soil carbon concentrations at large spatial scales (Averill *et al.* 2014; Averill and Hawkes 2016; Craig *et al.* 2018). These differential effects of the mycorrhizal association on soil carbon concentrations are expected to be driven by the difference in fungal metabolic pathways (Crowther *et al.* 2019). On top of that, fungal colonization increases with the increase of cortical tissues, themselves being positively correlated with root diameter (RD; Bergmann *et al.* 2020). Thus, root diameter should determine fungal association effects on soil carbon concentrations by modulating fungal colonization.

Tree-derived carbon substrates, such as litter and root exudates, are processed by soil biota. As microorganisms are the main consumers of soil organic matter, they should reduce soil carbon concentrations. However, recent studies highlighted that increased microbial activity can increase soil carbon concentrations by transferring higher amounts of microbial necromass to stable carbon pools (Buckeridge *et al.* 2020; Lange *et al.* 2015; Miltner *et al.* 2012; Schmidt *et al.* 2011; Trumbore 1993). Further, soil microbial community composition and its functioning are strongly influenced by the above-mentioned root traits (i.e. root functional trait identity) and thereby by the tree community composition due to species-specific traits and relations among these traits (Lareen *et al.* 2016; Pei *et al.* 2016). For example, root traits related to root biomass (e.g., RD, SRL) and to litter mass production may increase substrate availability for soil microorganisms with increasing species richness (Bardgett *et al.* 2014; Hooper *et al.* 2000). Besides, species-rich plant communities have also been shown to increase microbial biomass and diversity (Chapman *et al.* 2013; Eisenhauer *et al.* 2010; Lange *et al.* 2015) and, as a consequence, soil carbon concentrations (Li *et al.* 2019). For example, high litter diversity has been linked to an increase in microbial biomass (Thoms *et al.* 2010; Ushio *et al.* 2008). Further,

plant species richness has been shown to increase soil microbial biomass (Xu *et al.* 2020) and the relative proportion of fungi over bacteria by enhancing root biomass as well as the amount and diversity of root exudates (Eisenhauer *et al.* 2017). Moreover, the dissimilarity between root traits is expected to increase resource partitioning of soil microbial species, which should increase soil food web complexity (Kramer *et al.* 2016), and the overall microbial biomass, as shown in consumer communities (Eisenhauer *et al.* 2013; Scherber *et al.* 2010). However, the underlying mechanisms linking primary producers and the microbial community to soil carbon concentrations have rarely been investigated.

Next to root traits, environmental conditions such as climate, soil chemistry, and biotic interactions strongly influence microbial community abundance and composition (Gottschall et al. 2019). Recent 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). In particular, temperature and soil water content increase microbial biomass by increasing microbial activity and growth (Delgado-Baquerizo et al. 2016). Moreover, soil chemistry has been highlighted as a major driver of microbial community composition and functioning (Maaroufi and Long 2020). For instance, reduced water availability increases the osmotic pressure which, due to salt concentration and pH, constrains microbial biomass and alters community composition (Aciego Pietri and Brookes 2009; Delgado-Baquerizo et al. 2017; Wichern et al. 2006). Moreover, substrate limitation (e.g., high carbon to nitrogen ratio and/or carbon to phosphorus ratio) can reduce microbial biomass (Delgado-Baquerizo et al. 2017). Besides, a change from alkaline to neutral or acid soil pH coincides with qualitative differences in microbial habitats (Bernhard et al. 2018). Next to these abiotic parameters, a positive link between understory plant diversity and soil microbial biomass and activity was found in temperate forests (Eisenhauer et al. 2011), while empirical evidences remain inconsistent (Xu et al. 2020).

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Microbial community composition and processes are closely related to micro-environmental conditions, which are co-determined by tree community composition. Tree community effects on micro-climatic conditions can be manifold. For example, soil moisture can be affected by tree specific root length, as this trait affects the hydraulic lift (Burgess et al. 1998). Moreover, tree diversity can stabilize the micro-climate, as forests with a higher hydraulic diversity were shown to increase ecosystem resilience to drought (Anderegg et al. 2018). Additionally, species-rich forests were shown to have higher spatial complementarity in tree crowns and canopy closure (Kunz et al. 2019; Williams et al. 2017), and thereby a lower local temperature under the canopy (Frenne et al. 2021) with subsequent effects on soil microbial processes (Gottschall et al. 2019). Tree community composition can also modify soil chemistry, such as soil pH and nutrient availability (Reich et al. 2005), with significant consequences for the relative proportion of fungi over bacteria (Thoms et al. 2010; Rousk et al. 2010). Further, forest understory plant communities are connected to the 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 neither tree layer diversity (Germany et al. 2017), nor herb layer diversity (Both et al. 2011).

Forest ecosystems are horizontally structured, this is particularly important when it comes to species-rich forests. At a given location in the forest, the tree species composition can differ from the total species richness of the forest. As a consequence, sampling and observations are highly dependent on the scale considered (i.e., scale-dependency effect). Further, soil erosion can explain small scale changes like concurrently increasing carbon concentrations downslope, in hollows and valleys and that soil fertility is strongly influenced by topography (Scholten *et al.* 2017), as well as the transition from alkaline to acid soil pH (Slessarev *et al.* 2016). In order to take this scale-dependency into account, we considered two levels in this study: the local

level (i.e., between two neighboring trees) and the neighborhood level (i.e., the ten trees directly surrounding the two focal trees). We assume that the mechanisms driving soil functions and community composition are mediated by the tree community at both levels. For example, litter falling on the ground during litterfall may influence the neighborhood level, while root exudation into soils is expected to have local-level effects related to the closest trees (Walker *et al.* 2003).

In this study, we aim to mechanistically understand tree diversity, productivity, functional identity and dissimilarity effects on soil carbon concentration and its mediation by the soil microbial biomass and local environmental conditions (i.e. micro-climatic conditions, soil chemical quality, and biotic environment) across different spatial scales (Fig. III.1). We based our study on the BEF-China experiment and investigated two adjacent trees that will be called in the following a tree species pair (TSP). TSPs of a specific species combination were followed through plots with a species richness gradient ranging from 1 to 16. For each TSP, we measured soil chemical properties, soil microbial biomass, and environmental conditions to mechanistically describe and understand tree productivity and functional trait effects on soil carbon concentrations.

We assume tree diversity and productivity as well as functional trait identity and dissimilarity to drive soil carbon concentration (H1). In addition to that, tree diversity, productivity and functional identity and dissimilarity effects on soil carbon concentrations are expected to be mediated by soil microbial biomass (H2). Besides, we expected tree community effects on soil microbial biomass to be mediated by micro-environmental conditions (micro- climate, soil quality, and biotic environment; H3). Finally, we expected tree productivity and functional trait identity and dissimilarity effects on soil microbial biomass and soil carbon concentration to be scale-dependent (H4). All hypotheses described above must be seen with respect to the spatial scales. We expected that mechanisms related to root

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Fig. III.1: Conceptual framework of the study. Relation 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 micro-environmental conditions (micro-climate, soil quality, and biotic environment); and **H4** - tree productivity and functional trait identity and dissimilarity effects on soil microbial biomass are scale-dependent.

traits, such as root biomass inputs, are important at the TSP level. However, mechanisms related to the plot level, such as temperature or humidity, are likely to act at the neighborhood level. 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 (Fig. III.1).

Material and methods

Study site

The study site is located in south-east 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 clear-cut 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 rainfall of 1,821 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; Bruelheide *et al.* 2014).

Study design

We selected 24 combinations of tree species pairs (TSPs) and followed these TSPs across five plot species richness levels (1, 2, 4, 8, and 16 species). A TSP consists of two tree species next to each other. The neighbors of a TSP are defined as the ten trees directly adjacent in the planting grid (Suppl. III-S1.A-B). Each TSP was replicated three times in each richness level when available (see "broken stick design", Bruelheide *et al.* 2014), resulting in 180 TSPs in total (Suppl. III-S1.C-D).

Plot topography

A digital elevation model (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 m x 5 m. The plot mean slope, altitude, plan curvature (Curv. PL), and profile curvature (Curv. PR) were calculated from the DEM (Scholten *et al.* 2017).

Micro-climate modeling

The daily air temperature was recorded using 35 data loggers (HOBO® Pro v2, U23-001) installed at 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 Suppl. III-S2.A 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. effective number of layers index, "ENL", see below) and plot species richness (see Suppl. III-S2 for more details). Spatio-temporal trends for the whole experiment were estimated using Gaussian radial basis functions (functions auto_basis, eval_basis from the FRK package, see Suppl. III-S2.C 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 °C to 2 °C during our sampling period (Suppl. III-S2).

Field sampling

Our field measurements were performed from mid-August to the end of September 2018, before the litterfall season. To avoid spatio-temporal autocorrelation, each day another sampling area was randomly chosen. Between the two trees of each TSP, understory plant cover was estimated on a five-level factorial scale from 'no understory plant' to 'mainly understory plants'.

Starting from the center of the TSP, we extracted two soil cores with 5 cm diameter and 10 cm depth, 5 cm away from the center (Suppl. III-S1.B). 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 from these four soil cores and sieved with a 2 mm mesh size. Root fragments contained in the sieving residues were air-dried at 40°C for two days and weighed $(\pm 0.01 \text{ g})$, while the composite soil samples were stored at -20°C.

The litter cover between the two trees of each TSP was estimated on a five-level factorial scale from 'no-litter' to 'litter layer thicker than five centimeters'. Leaf litter was collected excluding green understory plant residuals, air-dried at 40°C for two days, and milled to powder. Carbon and nitrogen concentrations were measured by micro-combustion 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 two 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 auto-analyzer (SEAL Analytical GmbH, Norderstedt, Germany) using the Kjeldahl method (Bradstreet 1954). Soil total phosphorus (TP) was measured after wet digestion with H₂SO₄ and HClO₄ using a UV-VIS spectrophotometer (UV2700, SHIMADZU, Japan). Soil total organic carbon (TOC) was measured by 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 micro-combustion 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: $16:1\omega5$; $16:1\omega7$), arbuscular mycorrhizal fungi (20:1 ω 9), and saprophytic and ectomycorrhizal fungi (18:1 ω 9 and 18:2 ω 6,9, see Suppl. III-S3).

Tree functional traits

Tree biomass

Tree biomass was predicted for all TSPs and neighbors using tree basal area (BA) and speciesspecific allometric relationships estimated on the TSP trees. (1) Circumference at breast height (CBH) was measured in September 2018 for all TSPs and direct neighbors in order to calculate the basal area of these trees as $BA = \frac{(CBH)^2}{4\pi}$. (2) Tree height was measured for the TSP trees, and tree biomass was calculated following Huang *et al.* (2017). BA and TSP tree biomass were used to estimate species-specific allometric BA-biomass relationships (see Suppl. III-S4). (3) These species-specific allometric relationships were used to calculate the TSP biomass (i.e., sum of the two-tree biomass) and neighborhood biomass (i.e., sum of neighbors' biomass).

Leaf traits

For each tree species of the experiment, 10 samples consisting of 10 to 25 pooled fresh leaves were collected across all diversity levels from mid-August to October 2018 (Davrinche and Haider 2021). Each sample was dried at 80 °C for two days and milled 5 min at 26 shakes per second. Carbon and nitrogen concentrations were measured by micro-combustion 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 two 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 and Dames 2004; Wang and Qiu 2006).

Root functional trait variables

We considered three functional root traits that are related to soil processes (Bardgett *et al.* 2014): root diameter (RD), specific root length (SRL), and mycorrhizal tree association (i.e. AM or EM). For each TSP, two trait variables were calculated at both the TSP level and the neighborhood level. At the TSP level, we calculated trait community-weighted mean (CWM, Garnier *et al.* 2004) and trait functional richness (FRic) – defined as the range between the TSP trait values (Villéger *et al.* 2008) – of the above-mentioned root functional traits. At the neighborhood level, we calculated community-weighted means and functional dispersion (FDis) – defined as the weighted variance of the trait values within the neighborhood (Laliberté and 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

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; seePerles-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° x 305° field of view) with a spatial resolution of 6 mm at a distance of 10 m was acquired.

For each plot the Effective Number of Layers (ENL, Ehbrecht *et al.* 2016) 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 (~667 m²), each point cloud was clipped in a 20m square around the scan center (~400 m²). The point clouds were voxelized into a voxel grid of 5 cm voxels using 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: $ENL = 1 / \sum_{i=1}^{n} p_i^2$, were n refers to the number of slices, calculated as (height_{max} – height_{min}) / 50cm; and p_i is the proportion of filled voxels of the i_{th} slice.

A high ENL value indicates more evenly distributed layers, which can be an indication of higher crown complementarity and, thus, increased of canopy packing (Ehbrecht *et al.* 2016).

Litterfall measurement

From September to December 2018, the freshly fallen leaf litter between the two trees of each TSP was collected in a 1 m² litter trap (1 cm mesh). The collected litter was identified to species level, air-dried at 40 °C for two days, and weighed (\pm 0.01 g). Annual amounts of litter carbon (i.e. "C_{litterfall}") and nitrogen (i.e. "N_{litterfall}") deposited on the ground were calculated using species-specific leaf carbon and nitrogen contents and species-specific litter mass collected in the traps. We calculated the litterfall carbon to nitrogen ratio (CN_{litterfall}) from these measurements.

Statistical analyses

A description of all the variables used in this study can be found in Suppl. III-S5.A. 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 GitHub repository (i.e., <u>https://github.com/remybeugnon/Beugnon-et-al-2021_Soil-carbon-and-microbial-biomass-drivers</u>).

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 (Suppl. III-S6); highly correlated variables were excluded by our model selection algorithm. We first tested the effects of tree species richness on our productivity and structural variables (i.e., TSP biomass, neighborhood biomass, ENL, Clitterfall, and CNlitterfall) 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 (H1). 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 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 concentration 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: (i) the root-mean-squared error of approximation (RMSEA), (ii) the comparative fit index (CFI), and (iii) 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 (H2).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 micro-environmental conditions (H3). Microenvironmental conditions were described by (i) micro-climatic conditions, (ii) soil chemical quality conditions, and (iii) biotic conditions. Correlations between micro-environment variables were explored in Suppl. III-S7.A.

(i) Micro-climatic 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 Suppl. III-S7.B.1). The first axis of the PCA projection was negatively correlated with temperature variables (Suppl. III-S7.B.2.2). 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. (ii) To describe soil quality conditions, we used soil carbon to nitrogen ratio ('C:N'), and carbon to phosphorus ratio ('C:P'). (iii) Biotic conditions were described by using field measurements of understory plant cover, soil root biomass, litter cover, and leaf chemical traits (i.e., litter carbon and nitrogen contents).

For each micro-environmental 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. Explanatory variables were selected by a both-way step selection based on AIC. We used linear models and normal distribution assumptions to test the effects of micro-environmental 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 were 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*' (Suppl. III-S8).

Results

Local history and topography effects on soil carbon concentrations

On average, forest soil carbon concentrations slightly decreased over time (mean = -0.33 g yr-1, sd = 0.86 g yr-1), but we also observed high variability in the data (from -3.00 g yr-1 to +1.85 g yr-1, Fig. III.2.A). Soil carbon concentration measured in 2018 increased with historical soil carbon concentrations measured in 2010 before the experiment (estimate \pm sd = 0.263 \pm 0.077, Fig. III.2.D-F, Suppl. III-S9). As historical soil carbon concentrations were affected by local topography (slope: 0.175 \pm 0.038, plan curvature: 0.357 \pm 0.038, R² = 10%, Fig. III.2.B), topography indirectly affected soil carbon concentrations measured in 2018 by the modification of historical soil carbon concentrations (Fig. III.2.E-F).

Tree species richness effects on tree productivity

At the neighborhood level, plot tree species richness increased the different aspects of tree productivity: 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, Fig. III.2.C). However, we could not detect any effects of neither plot species richness nor TSP species richness on TSP biomass (Fig. III.2.C). These different aspects of forest productivity were correlated to each other (Pearson correlation: neighborhood biomass – ENL = 0.38, neighborhood biomass – "C litterfall" = 0.4, TSP biomass – "C litterfall" = 0.25, ENL – "C litterfall" = 0.61).



Fig. III.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 for a given estimated value. The drivers excluded during model selection have neither estimates nor confidence intervals. C. Tree species richness effect on tree productivity. For each response variable on the y-axis – TSP biomass, 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. N.B. ENL model controlled for topography effects. Tree species richness (D.) and 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% confidence interval for a given estimate value. Estimates and confidence intervals 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 confidence intervals. Six groups of explanatory variables were built: species richness variables (i.e. TSP species richness: "TSP sp. rich.", 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 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), TSP root trait indices (i.e. TSP' AM versus EM tree association: "TSP AM/EM", community weighted mean of root diameter and specific root length: "TSP RD" and "TSP SRL", functional dissimilarity of tree fungal association, root diameter, and specific root length: "TSP FRic AM/EM", "TSP FRic RD", and "TSP FRic SRL", respectively), aboveground productivity and traits (i.e. "TSP biomass", 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 non-significant 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 (\mathbb{R}^2 , in %) was added after the node name, see Suppl. III-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: ** and p-value < 0.001: ***).

Tree effects on soil carbon concentrations

Plot tree species richness did not affect soil carbon concentrations (Fig. III.2.C), but tree productivity, especially, forest vertical stratification (i.e., ENL), affected by tree species richness, increased soil carbon concentrations (0.249 ± 0.083 , Fig. III.2.D-F). In contrast, litterfall C:N ratio decreased soil carbon concentration (-0.200 ± 0.077 , Fig. III.2.D-F, Suppl. III-S9). Belowground, one root morphological trait, root diameter (RD), strongly influenced soil carbon concentration. At the neighborhood level, RD decreased soil carbon concentration (-0.286 ± 0.101), while at the TSP level, RD increased soil carbon concentration (0.206 ± 0.126). The latter became non-significant (i.e. p-value = 0.126) once taken together with the other variables in the SEM framework (Fig. III.2.F, Suppl. III-S9).

Tree effects on soil microbial biomass

Our analyses showed a positive effect of tree species richness on soil microbial biomass (0.202 \pm 0.079, R² = 3%, Fig. III.3.A). By considering tree functional traits and productivity, we got a better understanding of the variability in soil microbial biomass (R² = 14%, AIC_{sp. rich. based} model = 222 *vs.* AIC_{trait based model} = 210). We found that soil microbial biomass increased with tree productivity (i.e., ENL, 0.172 \pm 0.037) and was strongly affected by root morphological traits. At the neighborhood level, soil microbial biomass decreased with increasing RD (-0.359 \pm 0.100) and specific root length (SRL) functional dissimilarity (-0.216 \pm 0.102), while at the TSP level, soil microbial biomass increased with RD (0.308 \pm 0.116) and SRL (0.223 \pm 0.103, Fig. III.3.B). We did not observe any significant effect of tree mycorrhizal association on soil microbial biomass.

Relationship between soil microbial biomass and soil carbon concentration

We found a strong positive correlation between soil carbon concentration and soil microbial biomass (Pearson-correlation = 62.7%, p-value < 0.001, Fig. III.3.C). 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 (Fig. III.3.D). 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 taking 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 Fig. III.3.E. This SEM showed a strong positive effect of soil carbon concentration on microbial biomass (0.506 ± 0.145 , Fig.3.E), but a non-significant effect of soil microbial biomass on soil carbon concentration (p-value = 0.57, Suppl. III-S10). Additionally, root functional trait effects on soil microbial biomass remained strong (neighborhood root traits total effect = 0.285, TSP root traits total effect = 0.438, Fig. III.3.E, Suppl. III-S10), but the tree productivity effect on soil microbial biomass was mediated by soil carbon concentration (p-value = 0.103, Fig. III.3.E, Suppl. III-S10).

Tree effects on micro-environmental conditions

Tree species richness effects on micro-environmental conditions were limited to a negative effect on air temperature (-0.208 \pm 0.082, R² = 3%) and a positive effect on the amount of litter collected on the ground (0.168 \pm 0.080, R² = 2%, Fig. III.4.A). However, the trait-based model showed the major role of trees in controlling environmental conditions. Aboveground, forest vertical stratification (i.e., ENL) reduced air temperature (-0.406 \pm 0.078), plant cover, and amount of litter (-0.472 \pm 0.008 and -0.294 \pm 0.083, respectively), but also root biomass (-0.389 \pm 0.091), and litter C:N ratio (-0.306 \pm 0.089), while litterfall C:N ratio increased C:N ratio of the residual litter on the ground (0.233 \pm 0.077), but also decreased soil humidity (-0.247 \pm 0.077), soil nitrogen and phosphorus contents (-0.189 \pm 0.082 and -0.186 \pm 0.080), and plant cover (-0.305 \pm 0.085, Fig. III.4.B). Belowground, environmental conditions were mostly affected by the root morphological traits (RD and SRL). These effects were inconsistent with the scale considered (i.e. TSP *vs.* neighborhood levels, Fig. III.4.B). While SRL decreased soil



Fig. III.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% confidence interval for a given estimate value. Estimates and confidence intervals 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 confidence intervals. Four groups of explanatory variables were built: species richness variables (i.e. TSP species richness: "TSP sp. rich.", 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), TSP root trait indices (i.e. TSP' AM versus EM tree association: "TSP AM/EM", community weighted mean of root diameter and specific root length: "TSP RD" and "TSP SRL", functional dissimilarity of tree fungal association, root diameter, and specific root length: "TSP FRic AM/EM", "TSP FRic RD", and "TSP FRic SRL", respectively), aboveground productivity and traits (i.e. "TSP biomass", 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 (Fig. III.2.F.). F. 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 Fig. III.2.F.) 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 (\mathbb{R}^2 , in %) were added after the node name, see Suppl. III-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: ** and p-value < 0.001: ***).

humidity (-0.290 \pm 0.087), plant cover and amount of litter (-0.262 \pm 0.105 and -0.365 \pm 0.116, respectively) at TSP level, it increased soil nitrogen content (0.214 \pm 0.093) at the neighborhood level. Similarly, RD decreased plant cover and the amount of litter (-0.212 \pm 0.103 and -0.254 \pm 0.115, respectively) but increased soil phosphorus content (0.408 \pm 0.097). Moreover, root functional trait dissimilarity and richness also played a major role in controlling soil quality and biotic conditions at both TSP and neighborhood level (Fig. III.4.B). In addition,

plant cover was positively correlated to root biomass and amount of litter (Pearson correlation: plant cover ~ root biomass = 0.30, plant cover ~ amount of litter = 0.37, Suppl. III-S7).

Micro-environmental mediation of tree effects on microbial biomass

Microbial biomass was affected by micro-climate, soil quality, and biotic conditions (Fig. III.5.A). Both air temperature and soil humidity decreased soil microbial biomass (-0.379 \pm 0.072 and -0.221 \pm 0.066, respectively). In addition, soil microbial biomass increased with increasing soil nitrogen content (0.385 \pm 0.066) and increasing litter C:N ratio (0.240 \pm 0.068, Fig. III.5.A). By adding these drivers to the previous structural model, we explained up to 54% of the variability in soil microbial biomass (Fig. III.5.B). Microbial biomass was mostly affected by variations in soil carbon concentration (total effect: 0.562) and micro-environmental conditions (total effect: 0.610), which were themselves strongly mediated by tree productivity and functional traits (total effect: on soil carbon concentration = 0.733, on micro-environmental conditions = 2.308, Fig. III.5.B, Suppl. III-S11). In addition, our analyses revealed that soil carbon concentration was driven by tree productivity and functional traits at the neighborhood scale, while soil microbial biomass was driven by root functional traits at both investigated scales. The strongest effect on soil microbial biomass was exerted by variations in micro-environmental conditions, which were themselves strongly influenced by tree productivity and functional traits at both investigated scales. The strongest effect on soil microbial biomass was exerted by variations in micro-environmental conditions, which were themselves strongly influenced by tree productivity and functional traits at both investigated scales. The strongest effect on soil microbial biomass was exerted by variations in micro-environmental conditions, which were themselves strongly influenced by tree productivity and functional traits at both TSP and neighborhood scales (Fig. III.5.B).

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 at different local spatial scales of tree species pairs (TSPs) 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 micro-environmental conditions, such as

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micro-climate, soil quality, and biotic conditions. These changes in micro-environmental conditions had consequences for soil microbial biomass (e.g., an increase of temperature decreased soil microbial biomass). In addition, root functional traits modulated soil microbial biomass at both TSP and neighborhood levels. Soil microbial biomass was strongly correlated to 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 at the neighborhood level. Taken together, these findings for the first time show how tree diversity and productivity, and functional traits shape forest abiotic and biotic conditions and soil functioning, and how these effects are highly scale-dependent; these findings reconciling previous inconsistent findings and calling for a more thorough consideration of scale in soil ecological studies.

Tree diversity enhances productivity with consequences for environmental conditions

Our analyses confirmed previous results showing increased productivity with tree species richness (Huang *et al.* 2017; Huang *et al.* 2018; Kunz *et al.* 2019; Perles-Garcia *et al.* 2021). 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; Liu *et al.* 2020a; Xu *et al.* 2020). However, efforts are still needed to a finer quantification of belowground productivity, particularly so over time (Liu *et al.* 2020a). A major challenge is developing non-invasive quantification methods of belowground biomass (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 micro-environmental conditions: micro-climate, soil quality, and biotic conditions. Our results, by showing a negative effect of forest vertical stratification on

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A. Tree species richness effects on environmental conditions

Estimates

Fig. III.4: Tree species richness (A.), and tree productivity and functional traits effects (B.) on micro-environmental variables. For each driver on the y-axis, the dot represents the estimated effect of the driver on the micro-environmental variable, the line represents the 95% confidence interval for a given estimate value. Estimates and confidence intervals were drawn in dashed lines when the effect of the driver was non-significant (i.e. p-values > 0.05). The drivers excluded during model selection have neither estimates nor confidence intervals. Four groups of explanatory variables were built: species richness variables (i.e. TSP species richness: "TSP sp. rich.", 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), TSP root trait indices (i.e. TSP' AM versus EM tree association: "TSP AM/EM", community weighted mean of root diameter and specific root length: "TSP RD" and "TSP SRL", functional dissimilarity of tree fungal association, root diameter, and specific root length: "TSP FRic AM/EM", "TSP FRic RD", and "TSP FRic SRL", respectively), aboveground productivity and traits (i.e. forest vertical stratification: "ENL", "TSP biomass", 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.

temperature, confirmed previous findings emphasizing the role of forests as a heat buffer (Frenne *et al.* 2019). In the same line, we found negative effects of tree-specific root length 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 specific root length 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). Besides, we confirmed the role of trees in controlling soil nitrogen and phosphorus contents by modifying litter C:N ratio and root morphological traits related to desiccation and exudation (i.e., N and P-rich compounds, Bardgett *et al.* 2014; Sun *et al.* 2017).



Fig. III.5: Mediation of tree effects on soil microbial biomass by micro-environmental conditions. A. Effects of micro-environmental 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% confidence interval for a given estimated value. The drivers excluded during model selection have neither estimates nor confidence intervals. 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., Fig. III.3.E., and Fig. III.4.B.) 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 (\mathbb{R}^2 , in %) were added after the node name, see Suppl. III-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: ** and p-value < 0.001: ***).

Micro-environmental conditions and root morphological traits drive microbial biomass

We showed that three micro-environmental parameters drove soil microbial biomass: temperature, soil humidity, and litter C:N ratio. In contrast to our expectations, soil microbial biomass decreased with increasing air temperature. Notably, we sampled during summer with an average daily temperature of 27° C $\pm 3^{\circ}$ C and an average maximum daily temperature of 35° C $\pm 8^{\circ}$ C. These high temperatures may exceed the thermal niche of some microbial taxa and thus repress microbial growth (Barcenas-Moreno *et al.* 2009). Surprisingly, high soil humidity also reduced total soil microbial biomass as well as both fungal and bacterial biomass. This is in contrast with previous findings showing no effect or a positive effect of soil humidity on soil microbial biomass (Serna-Chavez *et al.* 2013; see Pei *et al.* 2017 for subtropical forests). However, the local precipitation regime in September (i.e., heavy rains interspersed by some dry spells) and the topography of the study site with valleys where water accumulates, may have favored anoxic conditions and repressed soil microbial biomass.

Soil microbial biomass and soil carbon concentration are strongly related

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; Lange *et al.* 2015). On the one hand, soil microbial growth is maintained and limited by soil organic carbon availability (see chapter 7, Bollag and 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). Soil microbial biomass and soil organic carbon are strongly related to each other (Serna-Chavez *et al.* 2013; Xu *et al.* 2013) due to the equilibrium between microbial growth and soil carbon consumption. However, in the present study, we could only verify the strong positive effect of soil microbes on soil carbon accumulation (Lange *et al.* 2015) was not significant. Measurements of the different soil carbon pools 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).

Soil carbon concentration dynamics in BEF-China

Our analyses showed a loss of soil carbon during the first ten years of the experiment. Site A of the BEF-China experiment was planted in 2009 after a clear-cut of the previous conifer plantation (Yang *et al.* 2013). Clear-cut 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 to 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 g yr-1 to 1.85

g yr-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 effects on soil carbon concentration were mostly mediated by the topography effects on historical soil carbon concentrations (Liu *et al.* 2020b; 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, integrating time in our studies of BEF relationships and considering soil history already proved useful to understand the slope of BEF relationships as well as its change over time (Guerrero-Ramírez *et al.* 2017; Vogel *et al.* 2019).

Neighborhood tree traits and productivity are driving soil carbon concentrations

Once controlling for topography and soil history effects, neighborhood trees influenced soil carbon concentrations, both through above- and belowground mechanisms. Aboveground, soil carbon concentration was increased by forest vertical stratification, which decreased litterfall C:N ratio, i.e. increasing litter quality. The positive effects of forest vertical stratification can be related to two independent mechanisms: on the one hand, the increase of 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). Moreover, the negative effect of litterfall C:N ratio suggests reduced nitrogen limitation may contribute to soil carbon stabilization, which emphasizes the central role of the biotic processes transforming the fresh litter to stable carbon forms (Buckeridge *et al.* 2020).

Belowground, root diameter increased soil carbon concentrations. Root morphological traits, such as RD, have been related to belowground biomass allocation and productivity (Bardgett *et al.* 2014) and 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

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plasticity (Sun *et al.* 2017). 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 non-invasive 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.

Scale-dependent effects of root functional traits

Our results highlighted the importance of the scale considered to explain root functional traits' effects on the micro-environment, soil microbial biomass, and soil carbon concentrations. While micro-climate and soil quality (including soil carbon concentration) were mostly driven at the neighborhood level, biotic conditions like understory plant cover were mainly affected by the TSP root functional traits. Besides, soil microbial biomass was affected by microenvironmental conditions but also by root functional traits acting at both scales. At the TSP level, root morphological traits (SRL and RD) increased microbial biomass, while at the neighborhood level, RD decreased microbial biomass. This spatial dependency of root traits such as RD could be explained by complementary mechanisms. At TSP level, microbial biomass may benefit from root productivity and exudation (Bardgett et al. 2014; Eisenhauer et al. 2017), while at the neighborhood level, RD may be related to tree resource use (e.g., water) and therefore to the competition for resources between trees and the microbial community (Bernhard et al. 2018; Burgess et al. 1998). Such spatial dependency of the processes could explain the inconsistent results found in previous soil microbiology studies (Cesarz et al. 2020; Pei et al. 2016) and emphasize the need to consider space in our measurements and analyses of soil ecosystem functioning (Eisenhauer et al. 2020; Ettema and Wardle 2002).

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