Balance between geographic, soil, and host tree parameters to shape soil microbiomes associated to clonal oak varies across soil zones along a European North–South transect

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Summary

**Tree root-associated microbiomes are shaped by
geographic, soil physico-chemical, and host tree
parameters. However, their respective impacts on
microbiome variations in soils across larger spatial
scales remain weakly studied. We out-planted sap-
lings of oak clone DF159 (*Quercus robur* L.) as
phytometer in four grassland field sites along a
European North–South transect. After four years, we
first compared the soil microbiomes of the tree root
zone (RZ) and the tree root-free zone (RFZ). Then, we
separately considered the total microbiomes of both
zones, besides the microbiome with significant affin-
ity to the RZ and compared their variability along the
transect. Variations within the microbiome of the tree
RFZ were shaped by geographic and soil physico-
chemical changes, whereby bacteria responded more
than fungi. Variations within both microbiomes of the
tree RZ depended on the host tree and abiotic param-
eters. Based on perMANOVA and Mantel correlation
tests, impacts of site specificities and geographic
distance strongly decreased for the tree RZ affine
microbiome. This pattern was more pronounced for
fungi than bacteria. Shaping the microbiome of the**

**soil zones in root proximity might be a mechanism
mediating the acclimation of oaks to a wide range of
environmental conditions across geographic regions.**

Introduction

Two decades ago, soil microbial taxa were assumed to
be ubiquitously distributed (Finlay, 2002). But soon after,
the importance of environmental filtering in shaping soil
microbial communities was highlighted (Green and
Bohannan, 2006; Martiny *et al*., 2011; Tedersoo
et al., 2014; Deakin *et al*., 2018). Accordingly, environ-
mental heterogeneity potentially induces variations in the
spatial distribution of soil microorganisms (Green
et al., 2004; Green and Bohannan, 2006). Thereby, abi-
otic soil parameters are known as the major drivers of soil
microbial communities, and they act within individual soil
aggregates (Trivedi *et al*., 2017; Wilpiszeski *et al*., 2019)
up to broad spatial scales (Fierer and Jackson, 2006;
Lauber *et al*., 2008; Jesus *et al*., 2009; Rousk
et al., 2010). Climate also significantly impacts soil micro-
bial communities at regional and continental scales
(Fierer *et al*., 2009). Likewise, soil microbial communities
vary with land-use types (Schöps *et al*., 2018; Xue
et al., 2018; Plassart *et al*., 2019) and vegetation (Carney
and Matson, 2006). Such biotic filtering is strongly linked
to the fact that plant roots establish close associations
with specific groups of soil microorganisms, especially
those with plant-beneficial properties (Hartman and
Tringe, 2019), for instance, the ones involved in plant
nutrition as well as resistance to abiotic and biotic
stresses (Lugtenberg *et al*., 2002; Vandenkoornhuyse
et al., 2015).

The 'plant–soil microbe' interaction starts when plants
recruit microbial partners from local soil communities
(Hartman and Tringe, 2019) using signal molecules or
rhizodeposits, which include exudates, sloughed-off root
cells or tissues and mucilage (Berg and Smalla, 2009;
Jones *et al*., 2009; Dennis *et al*., 2010). Rhizodeposits,
especially root exudates represent a readily available
carbon source for soil microorganisms (van Hees

et al., 2005). Consequently, the plant root environment is potentially enriched in saprotrophic microorganisms due to this nutrient source (Baldrian and Kohout, 2017). As composition and quantity of root exudates differ among plant species and even between plant genotypes (Broeckling *et al.*, 2008), plant identity is also a strong driver of the soil microbial communities in the vicinity of roots (Somers *et al.*, 2004; Dotaniya and Meena, 2015; Prada-Salcedo *et al.*, 2020; Prada-Salcedo *et al.*, 2021). The exudate quality and quantity depend on the photosynthesis level, which does not only vary according to plant identity but is also related to local parameters including climate and soil properties (Haichar *et al.*, 2008; Yamaguchi *et al.*, 2019).

Plant species with wide geographic distributions acclimate and adapt to local conditions, and thereby impact specifically their soil microbiome (Savolainen *et al.*, 2013). These plant-driven changes in soil microbial communities are confoundable with those directly resulting from local abiotic factors. Phytometers, i.e. plants homogenous in age and genetic origin planted in sites under variable environmental conditions (Clements and Goldsmith, 1924), can bypass such confounding effects (Schöps *et al.*, 2020). Tree-based approaches to investigate soil bacterial and fungal communities previously used poplar clones (Gamalero *et al.*, 2012; Foulon *et al.*, 2016; Karliński *et al.*, 2020). However, phytometers remain underused in ecology research (Dietrich *et al.*, 2013) and not exploited in studies trying to unravel the concurrent and congruent effects of geographic location, soil physico-chemistry and host plant traits on soil microbial communities across large spatial scales (de Souza *et al.*, 2015). Besides, large-scale studies on the respective strength of these three sources of soil microbial community variability rarely consider differences between the plant rooted and the non-rooted soil zones (Goldmann *et al.*, 2016).

Here we present a study on variations of bacterial and fungal soil communities along a European North–South transect by comparing systematically the root zone (RZ) and root-free zone (RFZ) soil of clonal oak trees (*Quercus robur* L., clone DF159, Herrmann *et al.* (2016)). In 2014, saplings were out-planted as phytometer in different grassland field sites. *Quercus* spp. are foundation tree species in European forests with a broad geographic distribution (Plomion *et al.*, 2018). Besides in forests, *Quercus* spp. also grow as solitary trees in agricultural systems or grasslands, and their contribution to regenerate cultural landscape is high (MacDougall *et al.*, 2004; Löf *et al.*, 2016; Bobiec *et al.*, 2018; Parmain and Bouget, 2018). Thereby, oak trees establish strong interactions with soil bacteria and fungi (Herrmann and Buscot, 2007; Jumpponen and Jones, 2009; Meaden *et al.*, 2016; Lasa *et al.*, 2019). For instance, DF159 oak

phytometers recruit specific microbial partners from local soil microbial pools (Habiyaremye *et al.*, 2020a). The characteristic rhythmic growth of clone DF159 paralleled by shifts in resource allocations between the above and below-ground plant parts (Herrmann *et al.*, 2015) was shown to have an impact on the biological soil activity (Eisenhauer *et al.*, 2018) and to induce changes in the root-associated microbiome (Habiyaremye *et al.*, 2020b). Therefore, this clonal phytometer system appeared suitable to analyse the balance between tree-related and abiotic environmental parameters in driving soil microbial communities along a broad European geographical transect. We analysed soil microbial variability at two different scales: at the plot scale, we analysed the oak phytometer microbiomes, i.e. the microbial communities of the tree RZ versus its RFZ. Furthermore, along the European transect, we compared these different microbiomes among the investigated sites. The RZ microbial community is directly impacted by not only the plant but also by local abiotic conditions. Therefore, a specific tree effect is better captured by considering the RZ affine microorganisms separately. This subset of the RZ microbiome refers to bacteria and fungi, significantly enriched in this zone compared with the RFZ. Hence, our analyses individually considered three groups of soil microbiomes: (i) the tree RFZ total microbiome, (ii) the tree RZ total microbiome, and (iii) the tree RZ affine microbiome.

To characterize these soil microbiomes, we performed high-throughput amplicon sequencing of the bacterial 16S rRNA and fungal ITS2 rDNA. The microbial communities were analysed in relation to geographic, soil physico-chemical, and host tree parameters. Due to creation of a particular niche in the oak RZ, which promotes the enrichment of specific microbial taxa, we hypothesized (i) different microbial community compositions between the tree RZ and RFZ. Due to the general increase of biodiversity towards the Equator and concomitant enhanced oak performance at lower latitudes, we predicted within the tree RZ (ii) a southward increase of microbial Shannon diversity and different microbial communities among the studied sites. As root exudates are an important resource for root-associated microorganisms, we anticipated within the tree RZ soil (iii) a higher impact of parameters related to the oak phytometer than those of geographic and soil physico-chemical parameters, in particular for the RZ affine communities.

Results

Overview on soil physico-chemical and oak phytometer parameters among the field sites

We observed variability in all the analysed soil physico-chemical parameters among the field sites. Concretely,

pH consistently changed from acidic soil at the northernmost site Lapinjärvi in Finland to neutral soil at the southernmost site Bordeaux in France. Soil nitrate content and total mineral nitrogen showed a steady southwards increase as well. For the other soil parameters we measured, site-to-site variations were not consistent (see Table 1).

Regarding tree parameters, we found significantly taller trees at lower latitude sites (Table 1). For example, by the end of the vegetation period 2018, the trees were more than two times taller and branches more than four times longer at Bordeaux than at Lapinjärvi. Additionally, oak phytometers at Lapinjärvi had higher specific leaf area (SLA) but lower leaf dry matter content (LDMC) than the trees at the other sites, indicating a short leaf lifespan coupled with low photosynthesis rate. However, during 2018, some growth parameters at Fontain in Eastern France did not follow this general latitudinal performance gradient. At this site, the relative yearly elongation of the tree trunks and lateral branches (LB), and LDMC were similar or by trend even lower than at more northern sites during the vegetation period 2018 (Table 1).

Results of the Spearman rank correlation tests of the tree growth with soil physico-chemical parameters, as well as geographic location and attributes among the sites are shown in Table 2. Specifically, site-to-site

Table 1. Geographic location and attributes of the field sites, soil physico-chemical, and oak phytometer parameters among the sites.

Parameter	Lapinjärvi	Bad Lauchstädt	Fontain	Bordeaux
Geography and climate				
Latitude (N)	60.61590	51.39133	47.18503	44.58046
Longitude (W)	26.14303	11.87556	6.029146	0.279746
Elevation (m)	29	119	351	8
MAT ($^\circ$C)	5.3($\pm$0.7)[c]	10.1($\pm$0.7)[b]	10.1($\pm$0.6)[b]	13.7($\pm$0.5)[a]
MAP (mm)	661($\pm$91)[d]	495($\pm$83)[c]	1142($\pm$152)[a]	793($\pm$92)[b]
Soil physico-chemistry				
pH_{CaCl2}	5.5($\pm$0.1)[d]	6.4($\pm$0.2)[c]	6.7($\pm$0.2)[b]	7.2($\pm$0.1)[a]
Moisture (%, wt./wt.)	20.0($\pm$1.9)[a]	6.0($\pm$0.6)[c]	14.6($\pm$1.2)[b]	6.7($\pm$1.1)[c]
TC (%)	2.7($\pm$0.3)[b]	2.1(0.2)[c]	3.2($\pm$0.3)[a]	2.0($\pm$0.1)[c]
TN (%)	0.20($\pm$0.02)[b]	0.15($\pm$0.01)[c]	0.30($\pm$0.03)[a]	0.14($\pm$0.02)[c]
TC/TN	13.7($\pm$1.2)[a]	13.8($\pm$0.8)[a]	10.9($\pm$0.3)[b]	14.9($\pm$2.1)[a]
HWC (mg kg^{-1})	959($\pm$135)[a]	660($\pm$73)[b]	1069($\pm$104)[a]	745($\pm$103)[b]
HWN (mg kg^{-1})	60.6($\pm$6.9)[b]	53.8($\pm$4.5)[b]	84.0($\pm$9.0)[a]	74.9($\pm$12.1)[a]
HWC/HWN	15.8($\pm$1.3)[a]	12.2($\pm$0.5)[b]	12.7($\pm$0.4)[b]	10.0($\pm$0.6)[c]
CWC (mg kg^{-1})	159($\pm$25)[a]	113($\pm$9)[b]	172($\pm$31)[a]	110($\pm$15)[b]
CWN (mg kg^{-1})	11.8($\pm$1.4)[b]	10.9($\pm$0.9)[b]	17.9($\pm$4.7)[a]	21.6($\pm$7.9)[a]
CWC/CWN	13.5($\pm$1.6)[a]	10.5($\pm$0.9)[b]	9.9($\pm$1.8)[b]	5.7($\pm$1.9)[c]
NH_4^+-N (mg kg^{-1})	4.4($\pm$0.8)[a]	3.6($\pm$1.3)[a]	3.6($\pm$0.6)[a]	2.0($\pm$0.8)[b]
NO_3^--N (mg kg^{-1})	2.1($\pm$0.6)[c]	4.6($\pm$1.6)[b]	7.7($\pm$6.3)[b]	18.1($\pm$13.9)[a]
N_{min} (mg kg^{-1})	6.5($\pm$1.2)[b]	8.2($\pm$2.2)[b]	11.3($\pm$6.8)[ab]	20.1($\pm$13.8)[a]
K_{CAL} (mg kg^{-1})	212.2($\pm$44.9)[a]	116.4($\pm$43.7)[b]	4.5($\pm$1.2)[c]	178.1($\pm$48.4)[a]
P_{CAL} (mg kg^{-1})	72.3($\pm$12.5)[a]	24.8($\pm$6.7)[c]	12.2($\pm$2.4)[d]	36.5($\pm$5.4)[b]
Oak phytometer growth and performance				
Height at outplanting (cm)	62.8($\pm$6.8)[b]	75.3($\pm$5.8)[a]	64.8($\pm$6.3)[b]	57.0($\pm$7.0)[b]
Tree height in 2018 (cm)	142.2($\pm$25.4)[c]	240.5($\pm$24.8)[b]	285.8($\pm$57.2)[ab]	309.7($\pm$49.2)[a]
Tree height increase since outplanting (%)	129.0($\pm$49.2)[c]	219.1($\pm$20.8)[b]	348.0($\pm$106.3)[a]	451.5($\pm$125.8)[a]
Tree height increase in 2018 (%)	12.2($\pm$5.5)[b]	24.9($\pm$17.5)[ab]	15.3($\pm$9.1)[b]	44.6($\pm$23.9)[a]
LB with SF1	4.0($\pm$0.0)	4.0($\pm$0.0)	3.3($\pm$0.8)	3.8($\pm$0.4)
LB with SF2	0.2($\pm$0.4)[b]	1.0($\pm$1.3)[b]	3.0($\pm$0.9)[a]	3.8($\pm$0.4)[a]
LB with SF3	0.0[b]	0.0[b]	0.0[b]	2.5($\pm$1.4)[a]
SF1 length (cm)	8.0($\pm$1.8)[b]	11.9($\pm$2.5)[a]	6.9($\pm$4.0)[b]	7.8($\pm$5.5)[ab]
LB total length (cm)	18.2 ($\pm$2.8)[c]	47.0($\pm$20.9)[b]	106.7($\pm$17.3)[a]	83.6($\pm$14.9)[a]
LB % length increase in 2018	88.9 ($\pm$55.4)[ab]	71.8($\pm$44.2)[ab]	37.9($\pm$19.0)[b]	82.2($\pm$22.0)[a]
Leaves' number on SF1 of LB	7.9($\pm$2.5)[b]	11.2($\pm$1.4)[a]	8.2($\pm$1.8)[b]	8.7($\pm$2.0)[b]
$LDMC_{SF1}$	0.44($\pm$0.01)[c]	0.51($\pm$0.01)[b]	0.50($\pm$0.01) [b]	0.56($\pm$0.03)[a]
SLA_{SF1} (cm^2 mg^{-1})	9.9($\pm$0.7)[a]	8.1($\pm$0.5)[b]	7.4($\pm$1.3)[b]	7.7($\pm$0.7)[b]

Geographic coordinates (latitude, longitude, and elevation) were provided by Google Earth. MAT (monthly average temperature, from January 2000 to December 2019) and MAP (Mean annual precipitations, from January 2000 to December 2019) were calculated using meteorological data retrieved from CRU TS (Climatic Research Unit gridded Time Series) v4.0.4 (Harris et al., 2020).
Physico-chemical parameters of the soil samples: pH, total carbon (TC), total soil nitrogen (TN), carbon-to-nitrogen ratio (C/N), cold-water extractable carbon (CWC) and nitrogen (CWN), CWC-to-CWN ratio (CWC/CWN), hot water extractable carbon (HWC) and N (HWN), HWC-to-HWN ratio (HWC/HWN), soil moisture, ammonium and nitrate-bound nitrogen (NH_4^+-N and NO_3^--N), total mineral nitrogen (N_{min}), plant-available potassium (K_{CAL}), and phosphorous (P_{CAL}). LB represents the first four lateral branches; SF1, SF2 and SF3 mean the first, second and third shoot flushes during the vegetation period 2018. LDMC means leaf dry matter content and SLA is the specific leaf area. Mean ($\pm$standard deviation), display of ANOVA (with Tukey-HSD post-hoc test) results. Different superscript letters after standard deviations mean statistically different ($p < 0.05$) in a row.

Table 2. Spearman rank correlation test results of the site conditions (soil physico-chemical and geographic parameters) with the oak phytometer growth parameters.

Site conditions	Tree height in Sept. 2018		% Tree height increase since out-planting		% Height increase during the vegetation period 2018		LB with SF2		LB with SF3		LB total length		Length of SF1 of LB		LDMC$_{SF1}$		SLA$_{SF1}$	
	R	p-value	R	p-value	R	p-value	R	p-value	R	p-value	R	p-value	R	p-value	R	p-value	R	p-value
Soil physico-chemical parameters																		
pH	**0.71**	**<0.001**	**0.74**	**<0.001**	**0.51**	**0.012**	**0.74**	**<0.001**	**0.67**	**<0.001**	**0.69**	**<0.001**	−0.27	0.20	**0.76**	**<0.001**	**−054**	**0.007**
Moisture	**−0.47**	**0.02**	**−0.49**	**0.02**	**−0.50**	**0.015**	−0.41	0.05	**−0.42**	**0.04**	**−0.47**	**0.02**	−0.24	0.26	−049	**0.02**	**0.45**	**0.03**
TC	−0.24	0.26	−020	0.34	**−0.46**	**0.022**	−0.15	0.48	**−0.55**	**0.005**	−0.12	0.58	−0.26	0.22	−0.18	0.41	0.13	0.54
TN	−022	0.31	−0.15	0.47	**−0.56**	**0.004**	−0.14	0.52	**−0.46**	**0.02**	−0.15	0.49	−0.39	0.06	−0.10	0.67	0.04	0.86
TC/TN	−0.05	0.81	−0.01	0.65	**0.42**	**0.041**	0.003	0.99	0.36	0.08	−0.08	0.71	0.23	0.28	−0.18	0.41	0.17	0.42
N$_{min}$	**0.74**	**<0.001**	**0.66**	**<0.001**	0.14	0.52	**0.55**	**0.005**	**0.48**	**0.002**	**0.48**	**0.02**	−0.02	0.94	**0.61**	**0.002**	**−0.49**	**0.015**
Geographic and climatic parameters																		
Latitude	**−0.83**	**<0.001**	**−0.86**	**<0.001**	**0.46**	**0.025**	**−0.87**	**<0.001**	**−0.68**	**<0.001**	**−0.82**	**<0.001**	0.19	0.38	**−0.84**	**<0.001**	**0.67**	**<0.001**
MAP	**0.46**	**0.023**	**0.54**	**<0.01**	0.016	0.94	**0.64**	**<0.001**	0.23	0.28	**0.55**	**0.005**	**−0.42**	**0.042**	**0.52**	**0.001**	−0.39	0.06

LB represents the first four lateral branches; SF1, SF2, and SF3 mean the first, second, and third shoot flushes during the vegetation period 2018. LDMC means leaf dry matter content and SLA is the specific leaf area. TC and TN represent total carbon and nitrogen respectively, while N$_{min}$ represents the total mineral nitrogen. MAP indicates the mean annual precipitation in the period of September 2014 to August 2018. Significant correlations ($p < 0.05$) are highlighted in bold.

variability in soil pH, moisture, and total mineral nitrogen content was significantly correlated with most of the tree parameters. The same analysis also revealed significant correlations of the tree growth with latitude and mean annual precipitation (MAP) for geography-related parameters.

Microbiome variations between the tree RZ and RFZ along the European transect

Across all samples, we obtained a total of 3 087 776 high-quality 16S rRNA gene sequences. The sequences were clustered into 12 770 bacterial operational taxonomic units (OTUs), and rarefaction to a minimum of 60 989 sequences per sample to normalize sequencing depth among all samples resulted in a total of 12 638 bacterial OTUs. For fungi, we gained a total of 1 112 637 ITS2 rDNA sequences, which were clustered into 2867 fungal OTUs. Rarefaction to a minimum of 14 968 sequences per sample resulted in a total of 2809 fungal OTUs.

Proteobacteria (25.8%), Planctomycetes (16.7%) and Actinobacteria (11.0%) predominated the recovered bacterial phyla, while the fungi were dominated by Ascomycota (69.8%), Basidiomycota (17.8%) and Glomeromycota (5.4%). An overview of the taxonomic composition at the order level showed variabilities of the relative abundance among the sites but only very few differences between the root and RFZs of the individual sites (Fig. 1).

To determine the soil microbial OTUs with preference to oak RZ designated as the RZ affine bacterial and fungal OTUs or RZ affine microbiome, we applied an indicator species analysis. This analysis showed a total of 209 soil bacterial OTUs with significant habitat preference ($p < 0.05$) between the tree RZ and RFZ, out of which 70 OTUs (i.e. 33.5%) were found in the RZ, while 139 OTUs (i.e. 66.5%) were found in the RFZ. Similarly, we found a total of 40 soil fungal OTUs with significant preference ($p < 0.05$) to either zone, out of which 10 OTUs (i.e. 25.0%) were preferentially associated to the RZ and 30 OTUs (i.e. 75.0%) to the RFZ. Some of the tree RZ affine bacterial OTUs could be identified at the genus level and belong to the genera *Arenimonas*, *Candidatus Solibacter*, *Caulobacter*, *Conexibacter*, *Gemmatimonas*, *Haliangium*, *Methylobacterium*, *Microbacterium*, *Mucilaginibacter*, *Nitrospira*, *Peredibacter*, *Pirellula*, *Reyranella*, and *Sphingobium*. Some tree RZ affine fungal OTUs were also identified at the genus level and assigned to the genera *Ascobolus*, *Cyphellophora*, *Hebeloma*, *Myrmecridium*, *Podospora*, *Purpureocillium*, *Sarocladium*, and *Scleroderma*.

According to overlap analysis of the soil microbial OTUs among the sites, the highest proportion of OTUs shared among all four sites was found in the RZ affine
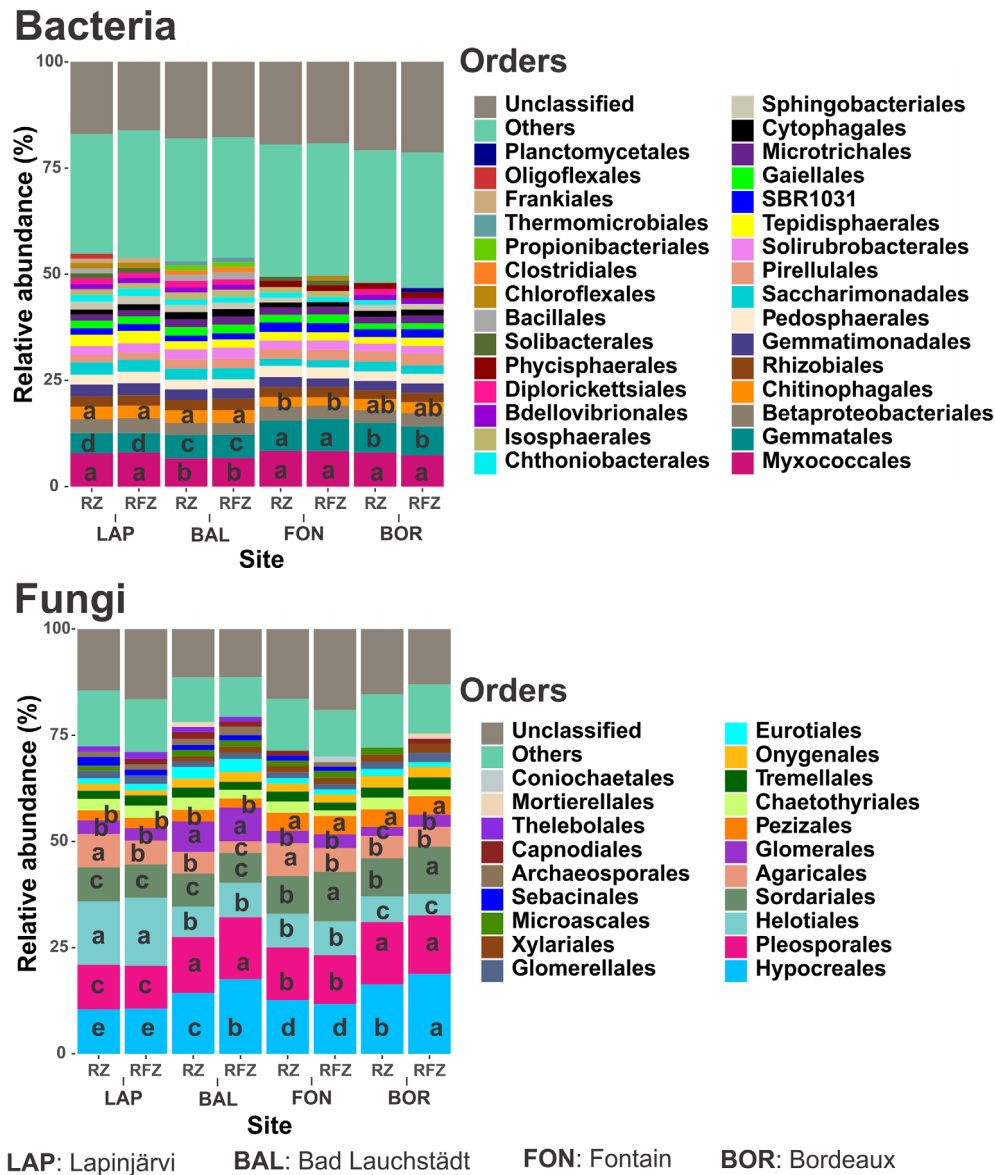
Bacteria

Fungi

LAP: Lapinjärvi   **BAL**: Bad Lauchstädt   **FON**: Fontain   **BOR**: Bordeaux

Fig 1. Compared distribution of soil bacterial and fungal orders between the tree root and root-free zones (RZ and RFZ respectively), and among field sites. Letters within the figures' rectangles indicate significant differences ($p < 0.05$) for one respective order, and this significant difference was only shown for the seven most abundant bacterial and fungal orders.

microbial communities, in which we observed no site-specific OTU (Fig. 2). For the tree RFZ total microbiome and the RZ total microbiome, however, we noticed site-specific microbial OTUs, which even outnumbered the core OTUs for the fungi (Fig. S1).

According to non-metric multidimensional scaling (NMDS) and permutational multivariate analysis of variance (perMANOVA), soil microbial communities in the tree RZ and RFZ at the northernmost site Lapinjärvi were similar for both the bacteria and fungi. The two soil zones had different bacterial communities at Bad

Lauchstädt, Fontain and Bordeaux, and different fungal communities at Bad Lauchstädt and Bordeaux (Fig. 3). Overall, Bray-Curtis dissimilarities between the soil microbiomes of the RZ and RFZ (Table S1) were positively correlated with the total tree height in 2018 for both, bacteria ($R = 0.48$, $p = 0.017$) and fungi ($R = 0.43$, $p = 0.037$). Moreover, the bacterial community dissimilarities additionally correlated with the percentage of tree height increase in 2018 ($R = 0.68$, $p < 0.001$), while dissimilarity of the fungal communities correlated with LDMC ($R = 0.52$, $p = 0.011$).
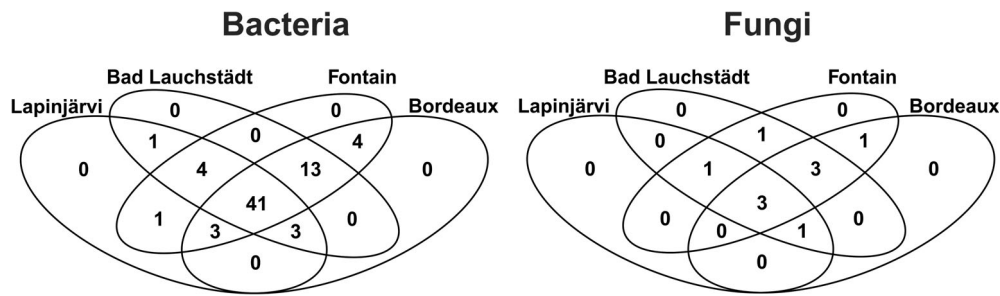
Bacteria

Fungi

Fig 2. Overlap of the tree root zone affine microbial OTUs among the field sites.
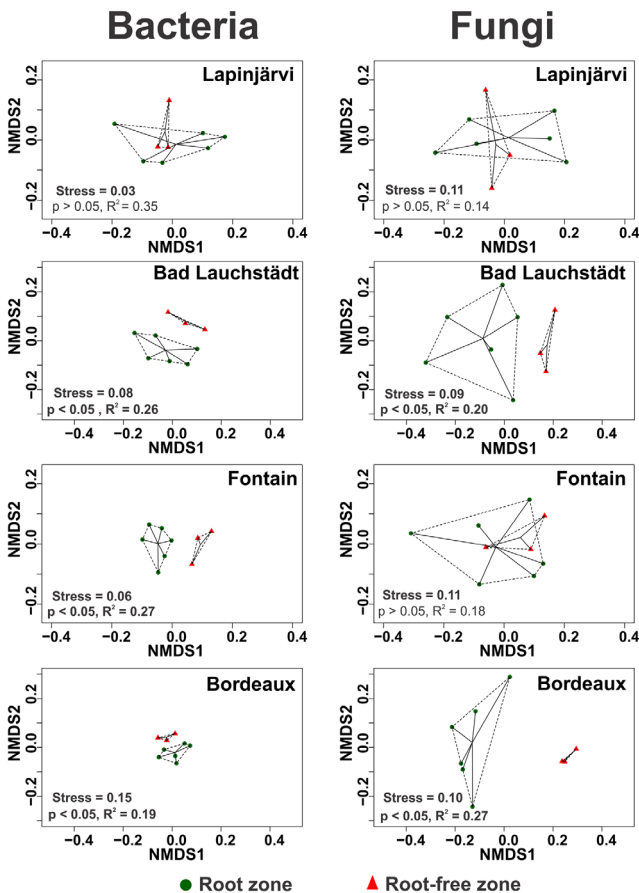
Bacteria

Fungi

Fig 3. Non-metric multidimensional scaling (NMDS) based on Bray-Curtis dissimilarity displaying the soil bacterial and fungal communities: comparison between the tree root and root-free zones at individual field sites.

Respective impacts of geographic, soil physico-chemical, and host tree parameters on the soil microbiomes associated to the oak phytometer along the European transect

Analysis of the Shannon diversity (Fig. 4) revealed a similar pattern of increasing diversity of the total bacterial microbiomes with decreasing latitude in the tree RZ

(R = -0.94, p = 0.004) and RFZ (R = -0.76, p < 0.001). Fungal Shannon diversity was comparable among all sites for the RZ total microbiome, while it was significantly lower at Bordeaux than at the other sites for the RFZ total microbiome. For the RZ affine bacterial and fungal microbiomes, the Shannon diversity was similar among Bad Lauchstädt, Fontain and Bordeaux but significantly lower at Lapinjärvi. According to the results from the Spearman rank correlation test (Table 3), soil pH and total mineral nitrogen content correlated with bacterial and fungal diversity of the tree RFZ. For the RZ total microbiomes, the fungal Shannon diversity correlated with none of the soil physico-chemical parameters, while for bacteria, it correlated with pH, moisture, and total mineral nitrogen. For the RZ affine microbiome, only soil pH and moisture correlated with the bacterial and fungal Shannon diversity.

As indicated by NMDS results (Fig. 5), structure of the microbial communities was different among the field sites of the European North–South transect. This site effect was demonstrated for all microbiome groups and confirmed by perMANOVA (bacterial community: p < 0.001, R^2 = 0.87 for the tree RFZ total microbiome; p < 0.001, R^2 = 0.80 for the tree RZ total microbiome; and p < 0.001, R^2 = 0.47 for the tree RZ affine microbiome; fungal community: p < 0.001, R^2 = 0.80 for the tree RFZ total microbiome; p < 0.001, R^2 = 0.57 for the RZ total microbiome and p < 0.001, R^2 = 0.40 for the RZ affine microbiome). Noteworthy, for both, bacteria and fungi, the magnitude of site effects decreased from the tree RFZ total microbiomes (highest R^2 values), over the RZ total microbiomes, to the RZ affine microbiomes (smallest R^2 values). Figure 5 also shows the strength and direction of geographic (latitude, monthly average temperature (MAT), and MAP), soil physico-chemical (pH, moisture, TC, and TN), and oak phytometer parameters (tree height, LB length, and LDMC), which significantly impacted the structure of the microbial communities along the European transect. With Mantel correlation tests to evaluate the impact of geographic distance, a positive correlation was observed for the three
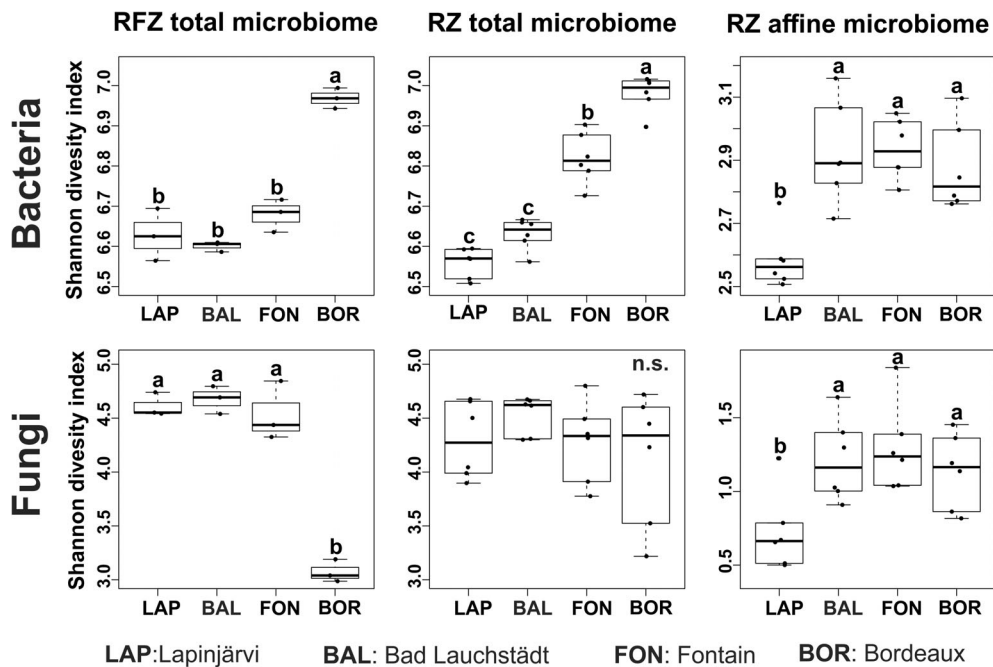
Fig 4. Soil microbial Shannon diversity along a European North–South transect. Cross comparison was done among three categories of the microbiome: (1) tree root-free zone total microbiome, (2) tree root zone total microbiome, and (3) tree root zone affine microbiome. The *y*-axes are not equally scaled for the root affine microbiomes. Different letters in each panel indicate significant differences ($p < 0.05$) according to Tukey-HSD post-hoc test; n.s. means no significant difference.

Table 3. Spearman rank correlation test results between the site conditions (soil physico-chemical and geographic parameters) and the microbial Shannon diversity within the oak RFZ and RZ soil.

Site conditions	Tree RFZ total microbiome				Tree RZ total microbiome				Tree RZ affine microbiome			
	Bacteria		Fungi		Bacteria		Fungi		Bacteria		Fungi	
	R	*p*-value	*R*	*p*-value	*R*	*p*-value	*R*	*p*-value	*R*	*p*-value	*R*	*p*-value
Soil physico-chemistry												
pH	**0.71**	**0.01**	**−0.72**	**0.01**	**0.89**	**<0.001**	−0.03	0.91	**0.51**	**0.01**	**0.43**	**0.04**
Moisture	0.04	0.96	0.14	0.67	**−0.46**	**0.03**	0.01	0.98	**−0.47**	**0.02**	**−0.56**	**0.005**
TC	−0.26	0.42	0.47	0.13	−0.27	0.20	0.04	0.87	−0.07	0.76	−0.07	0.74
TN	−0.08	0.80	0.3	0.34	−0.27	0.21	0.07	0.76	−0.17	0.44	−0.07	0.76
TC/TN	−0.23	0.47	0.09	0.78	−0.01	0.96	0.01	0.99	−0.07	0.74	−0.12	0.58
N_{min}	**0.66**	**0.02**	**−0.88**	**<0.001**	**0.49**	**0.02**	−0.01	0.96	0.25	0.23	0.19	0.38
Geographic and climatic parameters												
Latitude	**−0.76**	**0.004**	**0.67**	**0.02**	**−0.94**	**<0.001**	0.15	0.48	**−0.47**	**0.02**	**−0.43**	**0.04**
MAP	**0.63**	**0.03**	−0.37	0.24	**0.61**	**0.002**	−0.24	0.27	0.17	0.42	0.22	0.31

TC and TN represent total carbon and nitrogen respectively, while N_{min} represents the total mineral nitrogen. MAP indicates the mean annual precipitation in the period of September 2014 to August 2018. Significant correlations ($p < 0.05$) are highlighted in bold.

considered microbiomes (Fig. 6), indicating that more distant sites harboured more distinct microbial communities. For bacterial communities, the similarly high correlations were observed for the tree RZ and RFZ total microbiomes, while a lower correlation was observed for the RZ affine microbiome. For the fungi, the observed correlation was highest for the tree RFZ total microbiome followed by RZ total microbiome and the RZ affine microbiome.

Hierarchical impacts of geographic, soil physico-chemical, and oak phytometer parameters on microbial community variations

Without considering interactions, the tested soil physico-chemical, tree, and geographic parameters explained 3.7%, 2.8% and 1.6% of variations in the tree RZ total bacterial microbiome respectively (Fig. 7A), while none of these parameter groups showed pure impacts on the tree
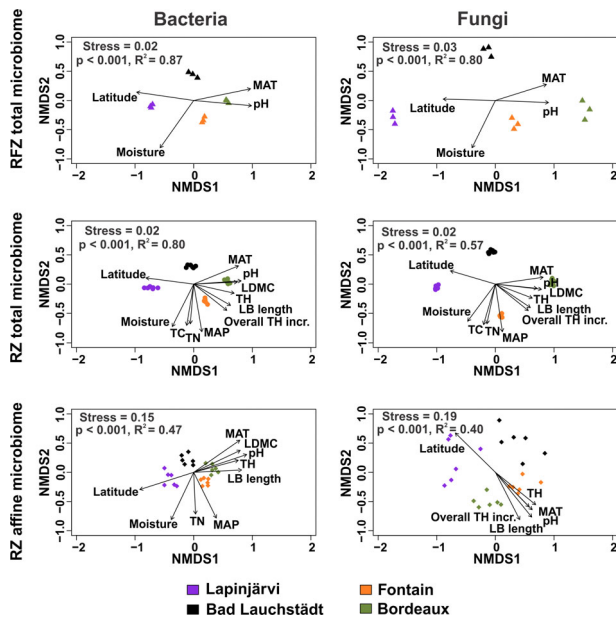
Fig 5. Non-metric multidimensional scaling (NMDS) based on Bray-Curtis dissimilarity displaying bacterial and fungal communities' structure among the field sites. Cross comparison was done among the tree root-free zone total microbiome, the tree root zone total microbiome, and the tree root zone affine microbiome. *p*-values and R^2 are results of perMANOVA. Significantly correlated abiotic environmental and oak phytometer parameters ($p \leq 0.05$). Abbreviations: TC (total carbon), TN (total nitrogen), MAT (monthly average temperature, from September 2014 to August 2018), MAP (mean annual precipitation, from September 2014 to August 2018), LB (lateral branches-the first four branches on each targeted oak tree), LDMC (leaf dry matter content of the first shoot flush of lateral branches), TH (main tree trunk height), and TH incr. (total height increase).

Fig 6. Correlation of Bray-Curtis dissimilarity with geographical distance among the study field sites for the soil bacterial and fungal communities. Cross comparison was done among the tree root-free zone total microbiome, tree root zone total microbiome, and the tree root zone affine microbiome.

RZ total fungal microbiome. When cumulating the pure and combined impacts derived from interactions with other sources of variability, we found for the RZ total microbiomes a descending order of magnitude: geographic (68.6% of bacterial, 51.9% of fungal variations); soil physico-chemical (66.5% of bacterial, 44.6% of fungal variations); oak tree parameters (60.7% of bacterial, 38.4% of fungal variations). Overall, the tested parameters could explain 75.1% and 55.5% of variations in the total bacterial and fungal communities of the RZ respectively (Fig. 7A). For the RZ affine microbiomes, we observed no pure impact of the tested sources of the variability for the bacteria, while for the fungi, we had 32.3% purely explained by the tree parameters and 14.8% individually explained by soil physico-chemical and geographic parameters. Considering their pure and combined impacts altogether for the RZ affine bacteria, geographic parameters remained the main driver of community variability (49.8%), followed by soil physico-chemical parameters (40.2%), and the tree parameters (34.7%). For the RZ affine fungi, the tree parameters
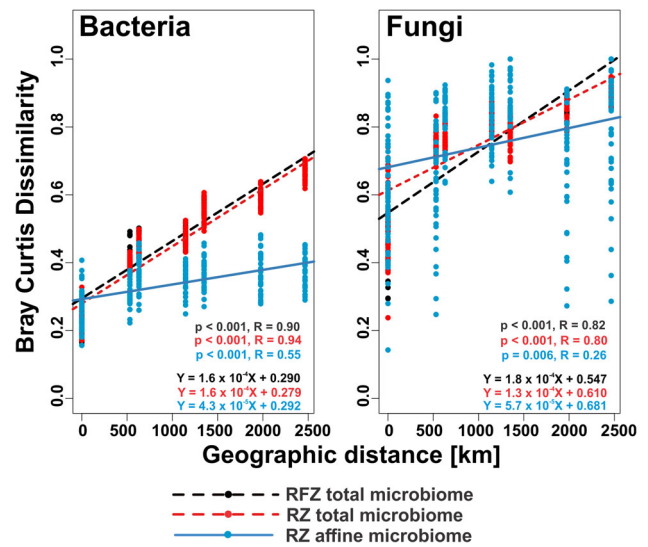
explained the highest variations (58.1%), followed by the geographic and soil physico-chemical parameters with equal explained variations (43.7% per each). Overall, 56.1% and 90.8% of variations in the respective RZ affine bacterial and fungal communities could be explained by the tested parameters (Fig. 7B).

Discussion

The current study revealed different soil microbial community structures in the RZ and RFZ of clonal oak trees out-planted as phytometer in four sites along a European North–South transect. Because microbiomes of the tree RZ and RFZ partially overlap due to their proximity, we sharpened the comparison between the respective impacts of the tree and abiotic environment parameters by considering the RZ affine microbiomes. We defined these RZ affine microbiomes as sub-communities of the soil bacteria and fungi significantly enriched in the RZ compared with the tree RFZ. Indeed, while we observed different site-specific patterns between the bacteria and fungi Shannon diversity along the transect when considering the total microbiomes of the tree RZ and RFZ, these patterns were highly similar when zooming into the RZ affine bacterial and fungal microbiomes. The total and affine bacterial and fungal communities of the RZ were impacted by the interplay among the considered geographic, soil physico-chemical, and tree parameters. However, the RZ affine microbiomes showed a decreased impact on the abiotic environmental
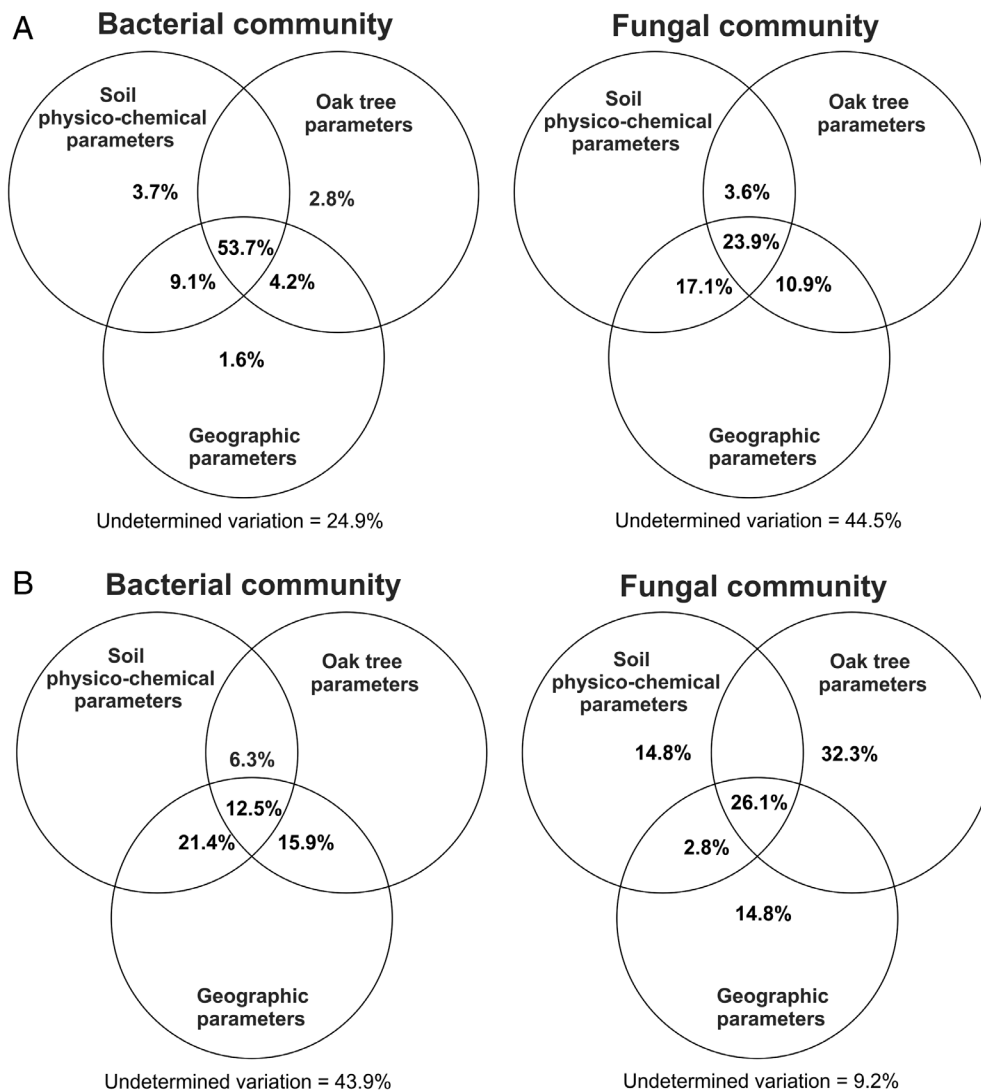
A

Bacterial community

Soil physico-chemical parameters 3.7%

Oak tree parameters 2.8%

53.7%

9.1%   4.2%

1.6%

Geographic parameters

Undetermined variation = 24.9%

Fungal community

Soil physico-chemical parameters 3.6%

Oak tree parameters

23.9%

17.1%   10.9%

Geographic parameters

Undetermined variation = 44.5%

B

Bacterial community

Soil physico-chemical parameters

Oak tree parameters

6.3%

12.5%

21.4%   15.9%

Geographic parameters

Undetermined variation = 43.9%

Fungal community

Soil physico-chemical parameters 14.8%

Oak tree parameters 32.3%

26.1%

2.8%

14.8%

Geographic parameters

Undetermined variation = 9.2%

Fig 7. Variance partitioning analysis of the respective impacts of geographic, soil physico-chemical, and oak phytometer parameters on variations within the soil bacterial and fungal communities; A) Tree root zone total microbiome; B) Tree root zone affine microbiome; Geographic parameters included latitude, elevation and $MAP_{Sep\ 2014–Aug\ 2018}$. Soil physico-chemical parameters included pH, moisture, CWN, N_{min}, TC, TN and TC/TN. Oak phytometer parameters were the main trunk height at sampling time in September 2018; tree trunk height increase during vegetation period of 2018; length of lateral branches; number of lateral branches with first, second and third shoot flushes (SF1, SF2 and SF3); length of SF1 of the lateral branches; leaf dry matter content and specific leaf area of the first shoot flush of lateral branches ($LDMC_{SF1}$ and SLA_{SF1} respectively). Each circle represents the ratio of variation accounted for by each category. Shared variance is represented by the intersecting portions of the circles.

parameters, while the tree influence was strongly increased, particularly for fungi.

Oak phytometer growth and performance versus site specificities along a European North–South transect and implication to the root-associated microbiome

Spanned sampling sites along the European North–South transect differed in climate and soil physico-chemistry. This had an impact on the growth and performance

of the oaks. As previously demonstrated, the warmer climate at lower latitudes accelerates the decomposition of organic matter to enhance the availability of nutrients for the trees, whereas soils of colder regions at higher latitudes often accumulate undecomposed organic matter (Vancampenhout *et al.*, 2009). Moreover, better tree growth was previously noticed under nearly neutral soil pH (6.5–7.5), since the mineral nutrients are available within this pH range (Pausas and Austin, 2001; Soti *et al.*, 2015). This direct effect of soil pH on the soil nutrient availability is coupled with the activity of soil

microorganisms, responsible for nutrient transformations (Rorison, 1980; Alam *et al*., 1999; De Boer and Kowalchuk, 2001; Nicol *et al*., 2008). Thus, good tree growth and performance as we noticed at our lower latitude sites like Bordeaux versus minor growth at the higher latitude site Lapinjärvi coincided with their respective climatic conditions and soil pH.

Increased tree biomass implies an increased amount of root exudates (Aulakh *et al*., 2001), which strongly impacts the root-associated microbiomes (Haichar *et al*., 2008). Thus, the observed variations in the oak tree growth and performance along the European transect were expected to impact microbial communities of the RZ among the studied sites.

Microbial community composition of the oak RZ versus RFZ and the tree effect on structure of the soil microbial community

Even though the majority of soil bacterial and fungal taxa of the RZ were also detected in the tree RFZ, some genera and OTUs showed preference to either zone as revealed by their detection frequency. Some of the particular taxa enriched in the RZ are saprotrophic bacteria and fungi, and symbiotrophic fungi. The identified RZ affine bacteria included members of the *Nitrospira*, a genus including important nitrifiers in soil (Daims and Wagner, 2018), as well as *Caulobacter* spp. and *Microbacterium* spp., which can degrade complex polysaccharides and potentially promote the growth of their host plants (Madhaiyan *et al*., 2010; Berrios and Ely, 2020). For the RZ affine fungi, we detected the ectomycorrhizal fungi *Hebeloma* spp. and *Scleroderma* spp. (Tedersoo *et al*., 2010; Tedersoo and Smith, 2013); the saprotrophs *Purpureocillium* spp. (Luangsa-ard *et al*., 2011) and *Ascobolus* spp. (Melo *et al*., 2014); and the yeast *Cyphellophora* sp. (Feng *et al*., 2014). As trees release higher amounts of exudates in comparison to herbaceous plants (Aulakh *et al*., 2001; Herz *et al*., 2018), enrichment of the listed microbial functional guilds in the RZ is consistent with their high dependence on rhizodeposits as their main source of carbon and nutrients (de Boer *et al*., 2015; Baldrian and Kohout, 2017).

Effect of the trees on soil microbial community was also demonstrated by our NMDS analyses of the microbial community structure between the tree RZ and RFZ within the individual field sites. Lack of separation between the two zones, which we noticed at Lapinjärvi for both bacterial and fungal communities, might result from the reduced tree performance with minor growth and low LDMC at this northernmost site of the transect. Since LDMC can serve as a proxy for photosynthesis (Shipley and Vu, 2002), low values often suggest a reduced rhizodeposition. Similarly, the minor tree growth

and reduced LDMC during the sampling year 2018 at Fontain may have resulted in decreased assimilate supply to the tree roots, negatively affecting the quality and quantity of C available in the tree RZ for fungi, which tightly depend on recently assimilated plant C (Denef *et al*., 2009; Fuchslueger *et al*., 2014). Based on our data, we could not identify the reason behind the reduced tree growth and performance at Fontain in 2018, which is in contrast with the otherwise good performance at this site. But together with the pattern in Finland at the margin of the oak distribution zone in Europe, the reduced tree performance in 2018 in Fontain validated both, our operating with a clonal phytometer system and our first hypothesis of different microbial community compositions in soils of the tree RZ and RFZ.

Relative contribution of the abiotic environmental parameters

In the current study, pure and cumulative impact of geographic and soil physico-chemical parameters was observed on both, soil microbial diversity and community structure. Variations in those abiotic environmental parameters resulted in site specificities along the transect and generally displayed higher effects on soil bacteria than on fungi. This strong site effect on soil bacterial diversity and community structure seems to be mainly linked to the high dependence of bacteria on soil pH and climate parameters, as previously demonstrated by other studies (Fierer and Jackson, 2006; Lauber *et al*., 2009; Griffiths *et al*., 2011). In our study, the fungal community structure was also impacted by soil pH, corroborating the report from Bahram *et al*. (2018). Furthermore, as a result of consistently increasing differences in soil pH and climate conditions along our European North–South transect, the greater the geographic distance among the sites, the more dissimilar microbial communities are. A significant positive correlation between geographic distance and dissimilarities among the microbial communities, also called distance decay, was previously reported for bacteria (Wang *et al*., 2015) and fungi (Shi *et al*., 2014; Goldmann *et al*., 2016). In our study, however, we revealed different spatial patterns between the bacterial and fungal communities, which suggests distinct mechanisms for shaping the two microbiomes.

Soil total carbon, total nitrogen, and moisture were also among the strongest parameters that determined the microbial community structure along the transect. These findings are in line with studies that revealed impacts of soil organic matter and water content on soil microbial communities at local and global scales (Wardle, 2002). As soil microorganisms feed on organic substrates, soil microbial community structure depends on the amount and type of organic substrate available in the soil

(Rodríguez-Zaragoza *et al.*, 2008; Mohammadi *et al.*, 2011). Furthermore, soil organic substrates result from plant primary production, which is climate-related (Haichar *et al.*, 2008; Yamaguchi *et al.*, 2019). In this line, reports at regional and continental scales showed that climate parameters have more impact on soil microbiomes than soil physico-chemical parameters (Tedersoo *et al.*, 2012; Bardgett and van der Putten, 2014). Potentially, also the divergent land-use history of our study sites, e.g. previously arable land or frequently flooded, might have impacted the found soil microbial patterns, as previously reported (Suleiman *et al.*, 2013; Bauer *et al.*, 2017; Goss-Souza *et al.*, 2017).

According to our results, part of our second hypothesis about a southward increase of the microbial Shannon diversity was confirmed for bacteria but rejected for the tree RZ total fungal microbiome. The second part of this hypothesis about dissimilar microbial communities of the RZ among the studied sites was confirmed for both bacteria and fungi.

Relative contribution of the oak phytometer

In comparison to the tree RZ and RFZ total microbiomes, the RZ affine microbiome was considerably less impacted by site specificities and geographic distance. This is mostly linked to the close connection of the RZ affine microbiome to the host tree. Our results suggest that this host stabilizing effect, which was previously described for rhizosphere microbial communities (Costa *et al.*, 2006; Raaijmakers *et al.*, 2009; Novello *et al.*, 2017), is more relevant for the fungi than for the bacteria. This, in turn, likely results from the higher dependence of fungi on their host plants (Uroz *et al.*, 2016; Chen *et al.*, 2018; Roy *et al.*, 2018; Wang *et al.*, 2020) compared with that of bacteria, which are usually more affected by abiotic environmental parameters (Millard and Singh, 2010; Lange *et al.*, 2014; Uroz *et al.*, 2016). Our third hypothesis about the contribution of the trees in explaining the highest microbial variations across the European transect was therefore only confirmed for the RZ affine fungi.

Overlap analysis of the bacterial and fungal OTUs affine to the tree RZ among the field sites revealed a microbiome fraction, which can be considered as the 'core microbiome' of the oak clone DF159. In our case, and according to the definition of Shade and Handelsman (2012) and Toju *et al.* (2018), the tree core microbiome refers to the bacterial and fungal OTUs enriched in the RZ because of their affinity to the host tree, and generalists in all the sites because of their ability to cope with diverging environmental conditions along the transect. The tree core microbiome contained mainly the bacterial genera *Arenimonas*, *Caulobacter*, *Conexibacter*, *Gemmatimonas*, *Haliangium*, *Methylobacterium*, *Pirellula*, and *Sphingobium*, and the fungal genera *Podospora* and *Sarocladium*. As the core plant microbiome comprises important microbial taxa, supporting plant fitness (Lemanceau *et al.*, 2017; Compant *et al.*, 2019), it can be assumed that the oak phytometer core microbiome assisted the trees to establish along the transect. The interplay of this core microbiome with site-specific microbes, promoting the tree adaptation to individual sites, may explain the wide distribution of *Q. robur* across Europe (Plomion *et al.*, 2018).

Conclusion and future perspectives

In the current study, we demonstrated that the soil microbiome associated to the tree roots is responsive to an interplay of geographic, soil physico-chemical, and host tree parameters. We revealed that the relative contribution of these abiotic and host tree parameters varies between bacteria and fungi, and that host tree impact is reinforced when zooming on the microbiome enriched in the proximity of roots. In our analyses, we considered the sources of microbial community variability as completely independent from each other without interactions. Indeed, the abiotic and host tree parameters affect soil microbial communities via highly complex interactions. Our results indicated a high dependence of tree parameters on climatic or soil conditions, and the latter is also reversely impacted by host trees. However, the use of a phytometer approach enabled us to exclude influences of intraspecific genetic tree variations, while maintaining locally adapted tree performances and their effect on soil microbial communities. Last, the tree RZ affine microbial OTUs, which were revealed mostly common to all sites despite their spatial distance might be one element enabling broad latitudinal distribution of the oak.

Even if our study was conducted in grasslands, many of the tree root-associated microbial taxa, especially ectomycorrhizal fungi, had been previously identified in forest ecosystems. However, conclusions about the variability of soil microbial communities along a European transect in other ecosystems cannot be drawn from the presented results. Therefore, and towards a full understanding of the impact of trees on their root-associated microorganisms under field conditions, similar studies under other land-use systems are required.

Methodology

Description of the host trees, sites and soil sampling

This study used phytometers of the pedunculate oak clone DF159 (*Quercus robur* L.), which were generated via micro-propagation to retain their common genetic

identity (Herrmann *et al*., 2016; Ferlian *et al*., 2018), and inoculated in the Petri dishes with the ectomycorrhizal fungus *Piloderma croceum* to increase their survival rate (Herrmann and Buscot, 2007). From Petri dishes to pots in the greenhouse, only tree saplings were picked, leaving out the substrate, and there was no new inoculation with ectomycorrhizal fungi in pots. In November 2014, DF159 trees were out-planted at grassland field sites due to better growth of young oaks in open or semi-open habitats oppositely to their shade intolerance (Jensen and Löf, 2017; Bobiec *et al*., 2018). In this regard, 13 oak saplings were out-planted in each of the four grassland field sites along a European North–South transect. From North Europe to South, the sites were Lapinjärvi (Southern Finland), Bad Lauchstädt (Central Germany), Fontain (Eastern France), and Bordeaux (Southern France) (Fig. 8A). Because of their geographic position and distance from each other, the sites are characterized by different weather conditions (Table 1). Additionally, the history of the sites was also different. For example, Lapinjärvi was a pure grassland and had not been exploited before; Bad Lauchstädt was used for

agricultural activities before the time of the tree out-planting; while Fontain and Bordeaux are frequently inundated. The oak saplings were propagated during winter 2012/2013 followed by a two-step acclimatization in a greenhouse during summer 2013, an outfield nursery during summer 2014, and out-planting in November 2014. In each field site, six of the trees, which had developed at least four LB were selected to conduct this study. The total height of the trees' main trunk was measured at the soil sampling time in September 2018, and their percentage height increase since out-planting and during the vegetation period 2018 was calculated. Also, the total length of the first four main LB and their length increase during the vegetation period 2018 were determined. Shoot flushes (SFs) produced by the four branches over the vegetation period 2018 were counted. Because all branches of the selected trees produced at least an initial SF designated as first shoot flush (SF1), its length was also measured and leaves number counted to compare the tree performance during 2018 among the sites. For the same purpose, five of the SF1 leaves were harvested to measure their area as well as fresh and dry weight
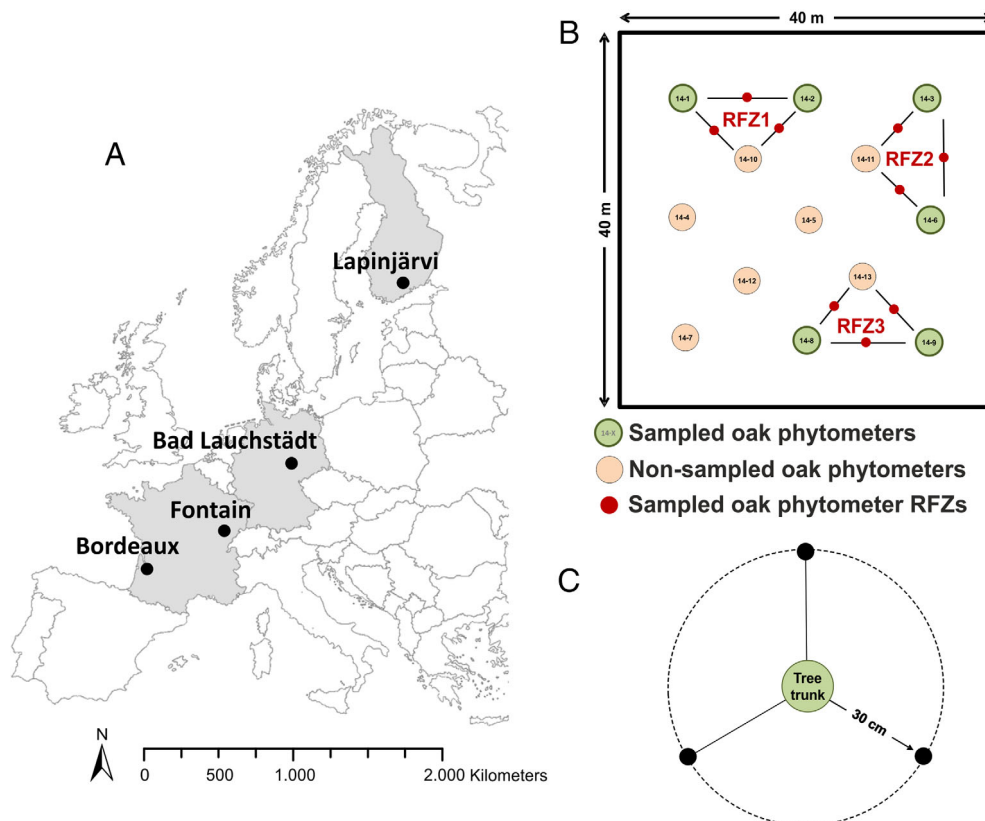

Fig 8. A) Study sites: grey sections represent the study countries, from North to South, Finland, Germany and France; black dots indicate the individual field sites; B) Bordeaux field plot design: green circles indicate investigated oak phytometer, and red dots mark the sampling positions of the three subsamples that were taken and pooled to obtain the oak phytometer root-free soil samples (RFZ1, RFZ2 and RFZ3); C) Sampling positions, i.e. three subsamples illustrated as black dots around the trunk of investigated oak phytometer.

(FW and DW respectively). From these SF1 data, we also calculated specific leaf area (SLA_{SF1}, the ratio between the one-sided area of a fresh leaf and its DW) and leaf dry matter content ($LDMC_{SF1}$, the ratio leaf DW-to-FW) as important traits in determining the tree relative performance and as a proxy of the photosynthesis rate (Poorter and Garnier, 1999; Shipley and Vu, 2002; Poorter and Bongers, 2006).

Six soil samples were collected in the oak tree RZ at every site plus three samples from the tree RFZ within the same plot (see Bordeaux field plot design in Fig. 8B). The soil of RZ includes both, rhizosphere soil and non-rhizosphere soil located around the active tree roots, and is therefore expected to accommodate microbial communities strongly shaped by the respective tree (Burns et al., 2015). Studying the tree RZ soil allowed us to distinguish between the respective impacts of the host tree and local environmental conditions in shaping the soil microbial community (Weißbecker et al., 2018; Habiyaremye et al., 2020a). We also sampled the tree RFZ soil to analyse communities of the local soil microbial pools. Based on the criterion of the presence of living plant roots to define the RZ (Steven et al., 2014), we conducted a pre-sampling to examine and estimate the distance from the tree trunk and soil depth which contain a great amount of the tree terminal rootlets. This soil sampling test was done at Bad Lauchstädt, which represents nearly the centre of the transect (Table S2), and resulted in sampling 30 cm from the tree trunk to 15 cm soil depth. Each soil sample consisted of three pooled subsamples taken with a 2 cm diameter soil auger. The tree RZ subsamples were taken around the tree trunk (Fig. 8C), whereas samples of the tree RFZ were collected in between three neighbouring trees at the same distance from a tree to another (Fig. 8B). A total of 36 soil samples (6 trees × 4 sites = 24 RZ soil samples) + (3 RFZ× 4 sites = 12 RFZ soil samples) were individually sieved (2 mm mesh size) to homogenize the soil and to remove roots and large organic debris. Each composite soil sample was divided into two aliquots. One aliquot (15 g) was kept for soil microbial DNA analysis and the other aliquot (50 g) for characterization of soil physicochemical properties. All samples were cooled within ice boxes immediately after sampling, taken to the laboratory and stored at -20°C until the start of laboratory analysis.

Physico-chemical analyses of the soil samples

As described previously (Goldmann et al., 2015; Moche et al., 2015), soil pH was determined with a glass electrode in a 1:2.5 soil/0.01 M $CaCl_2$ suspension after 1 h. Gravimetric soil moisture was determined using a fully automated moisture analyser (DBS60-3, KERN & SOHN GmbH, Balingen, Germany). Soil total nitrogen content

(TN) and total carbon content (TC) were determined in triplicate by dry combustion with a Vario elemental analyser (EL III, Elementar, Hanau, Germany). The carbon to nitrogen (C/N) ratio was then calculated based on TC and TN. To determine the potentially bioavailable soil organic C and N for microbial utilization, hot water extractable C and N (HWC and HWN respectively) were measured (Ghani et al., 2003; Schulz et al., 2011; Francioli et al., 2016). Additionally, the amount of labile organic C and N, which are readily decomposable by soil microorganisms according to Zsolnay (1996) and Zakharova et al. (2015) were determined in the form of cold water-extractable C (CWC) and N (CWN) as described in Schmidt et al. (2017). As in Francioli et al. (2016), we determined mineral nitrogen contents (NH_4^+-N and NO_3^--N whose sum gave total mineral nitrogen content, N_{min}) as well. Plant-available phosphorous (P) and potassium (K) content were extracted from the soil with calcium acetate lactate (1:20 wt./vol., pH 4.2, 1.5 h) as in Schüller (1969) and, after filtration of the suspension (filter type: Whatman Schleicher and Schuell 595 1/5 diameter 270 mm), quantified in extracts (diluted 1:10) by inductively coupled plasma optical emission at emission lines 766.49 nm (K) and 178.287 nm (P) using a SPECTRO ARCOS spectrometer (Spectro Analytical Instruments GmbH, Kleve, Germany).

Soil microbial DNA extraction, PCR amplification and Illumina-based sequencing

The total microbial DNA of each soil sample was extracted from 0.4 g using the Power Soil DNA Isolation Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. After determining the concentrations of DNA extracts using a NanoDrop-8000 spectrophotometer (Thermo Fisher Scientific, Dreieich, Germany), the DNA extracts were stored at -20°C. Before PCR amplification, the DNA extracts were adjusted to 10–15 ng μl^{-1}. The microbial genomic DNA was used as a template to produce PCR DNA amplicon libraries for bacteria and fungi. Bacterial 16S rRNA genes were amplified using a primer mix: P5-8N-515F + P5-7N-515F together with P7-2N-806R + P7-1N-806R (Caporaso et al., 2012; Moll et al., 2018), while P5-5N-ITS4 + P5-6N-ITS4 (Gardes and Bruns, 1993; Leonhardt et al., 2019)/P7-3N-fITS7 + P7-4N-fITS7 (Ihrmark et al., 2012; Leonhardt et al., 2019) were used to amplify fungal ITS2 rDNA, with the Illumina adapter sequences in all the primers.

We used the proofreading KAPA Hifi polymerase (Kapa Biosystems, Boston, MA, USA) in all the PCR reactions. PCR amplification, quality check-up by gel electrophoresis, cleaning up of the PCR products, attachment of Illumina Nextera XT indices and sequencing adaptors, index PCR amplification, libraries' quantification

and sequencing were done as described in Habiyaremye *et al*. (2020b). Illumina MiSeq sequencing was performed at the Department of Soil Ecology of the Helmholtz-Centre for Environmental Research-UFZ in Halle (Saale), Germany.

Sequences analysis

The generated raw sequences for this study can be found in the European Nucleotide Archive, under accession number PRJEB39387. Sequences analysis and processing were conducted following the DeltaMP pipeline (v0.2, https://github.com/lentendu/DeltaMP) as in Schöps *et al*. (2018). Prior to clustering, 16S and ITS2 sequences were quality-filtered. Using uparse of PandaSeq algorithm (Masella *et al*., 2012; Edgar, 2013), pair-end reads were merged with a minimum 20 bp for both 16S and ITS2 while the maximum was 440 and 450 bp for 16S and ITS2 respectively. No ambiguous sequence was allowed, and primer sequences with more than 4 bp differences were discarded. Homo-polymers of 20 bp differences at maximum were also removed. At the same time, we discarded sequences shorter than 200 bp and longer than 300 bp sequence length. Using UCHIME (Edgar *et al*., 2011), chimeras were also identified and eliminated as implemented in MOTHUR (Schloss *et al*., 2009). The remaining high-quality sequences with a 97% similarity level were clustered into OTUs using VSEARCH [v2.10.4, (Rognes *et al*., 2016)]. We based on the Bayesian classifier as implemented in MOTHUR (Schloss *et al*., 2009) to assign taxonomy, and this was done using the SILVA reference database [v128, (Quast *et al*., 2013)] and UNITE [v8.0, (Nilsson *et al*., 2018)] for bacteria and fungi respectively. 16S sequences ascribed to chloroplasts or mitochondria were discarded from the bacterial OTU table. To get rid of bias due to sampling size, 60 989 and 14 968 sequences were randomly selected in each sample for bacteria and fungi respectively, and retained for the downstream analysis. This normalization of the samples was done using the function 'rarefy_even_depth' from the phyloseq package v1.19.1 (McMurdie and Holmes, 2013) in R v4.0.2 (R Development Core Team, 2020). As reflected by the rarefaction curves (Fig. S2), the sequencing depth was adequate to fully cover the microbial communities.

Statistical analyses

Data analysis was performed using R v4.0.2 (R Development Core Team, 2020). In all our analyses we used a significance threshold of $p < 0.05$. Initially, the examination embraced two groups of explanatory parameters: (i) abiotic environmental parameters including soil physico-chemistry (pH, soil organic and mineral matter and soil moisture) and geographic position-related parameters of the sites (latitude, longitude, elevation, MAT and MAPs), and (ii) oak phytometer-related parameters (total height of the main trunk at sampling time in September 2018, percentage height increase since out-planting and during the vegetation period 2018; total length of the LB, their length increase and number of SFs in 2018; length of SF1 of the LB and its leaves number, specific leaf area-SLA$_{SF1}$ and leaf dry matter content-LDMC$_{SF1}$). The parameters were compared among the field sites using one-way analysis of variance (ANOVA) with Tukey-HSD post-hoc test. We also performed Spearman's rank correlation test to examine the relationship between the abiotic environmental parameters and tree growth. After, we analysed the oak tree effect by comparing between microbiomes of the tree RZ and RFZ. We took the sites altogether and applied the indicator species analysis to detect microbial OTUs with preference to the tree RZ or RFZ by using the multipatt function implemented in indicspaces package v1.7.9 (Cáceres and Legendre, 2009). From this, we extracted the bacterial and fungal OTUs with significant preference to the RZ, which we designated as the RZ affine microbiome. As well, we applied NMDS based on the Bray-Curtis dissimilarity matrices (Kruskal, 1964; Clarke, 1993) and perMANOVA with 9999 permutations (Anderson, 2001) to test dissimilarities between the microbial communities structure of the tree RZ and RFZ within individual sites. By using the distance function of the analogue package v0.17.5 (Simpson *et al*., 2020), we calculated the mean Bray-Curtis distances of each RZ microbial community with the communities of sampled RFZs of the same site and analysed their relation with the tree parameters by using Spearman rank correlation test.

For most of the subsequent analyses, we separately considered the total microbiomes of the tree RFZ and RZ as well as the RZ affine microbiome, retrieved from the overall dataset based on the described indicator species analysis. We tested the individual variability of these three microbiomes along the European transect. We first generated Venn diagrams to visualize the shared and unique bacterial and fungal OTUs among the study sites using R package VennDiagram (V1.6.20). After, we calculated the Shannon diversity index (Shannon, 1948) using the diversity function of the vegan package v2.5-6 (Oksanen *et al*., 2019) and applied Tukey-HSD post-hoc test to compare the Shannon diversity among sites and to reveal significant differences. We then related the microbial Shannon diversity values to the abiotic environmental parameters along the European transect. Subsequently, perMANOVA with 9999 permutations and an NMDS based on Bray-Curtis dissimilarity matrices were used to test divergences in the microbial communities' structures among the sites. The envfit function of the

vegan package (Oksanen *et al.*, 2019) was used to assess the effect of geographic, soil physicochemical and oak phytometer parameters, and included highly significant parameters in the NMDS plots. We further tested correlations between microbial dissimilarities and increasing geographical distance among field sites. We set a distance of 0 km for samples from the same field site and used the online tool GPS coordinates (https://gps-coordinates.org/distance-between-coordinates.php) to compute the distances among the field sites and to construct a geographical distance matrix (in km) (Table S3). We then carried out Mantel tests between the matrix of geographical distances and corresponding matrices of microbial Bray-Curtis distances. Simultaneously, we implemented a variation partitioning analysis using varpart function in vegan (Oksanen *et al.*, 2019) to compare the relative contribution of the geographic, soil physicochemical, and host tree parameters in explaining the noticed variations within both the tree RZ total microbiome and the RZ affine microbiome.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Appendix S1: Supplementary Information