Spatial and temporal variations in the microbiomes of different soil zones around clonal pedunculate oak trees (*Quercus robur* L.) out-planted as phytometers across grasslands in Europe

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Jean de Dieu Habiyaremye

geboren am 02.09.1978 in Rulindo (Rwanda)

Dekan: Prof. Dr. Marc Schönwiesner

Gutachter: Prof. Dr. François Buscot, Universität Leipzig

Prof. Dr. Philippe Vandenkoornhuyse, Université de Rennes I

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Jean de Dieu Habiyaremye

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Faculty of Life Science, Pharmacy and Psychology

University of Leipzig

Dissertation

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The present cumulative dissertation compiles investigations on the soil microbiomes associated to clonal pedunculate oak phytometer. This tree clone was generated by the project *TrophinOak-PhytOakmeter* of the Soil Ecology Department at the Helmholtz Centre for Environmental Research (UFZ) and out-planted in grassland, forest and urban field sites. This PhD research applied PCR-based Illumina MiSeq amplicon sequencing approach to analyze distribution patterns of bacterial and fungal communities in soils from different zones around roots of the pedunculate oak trees in grasslands of Europe at spatial and temporal scales.

After an introductory *Chapter 1* which describes the whole research work, the study in *Chapter 2* assessed concurrent impact of homogeneous climatic conditions and similar genetic identity of the host trees on bacterial and fungal communities in four grassland field sites of Central Germany. As it was expected, the findings indicated similar microbial diversity among the sites, but the community structure was site-specific for both bacteria and fungi. The results also demonstrated the capacity of the pedunculate oak tree phytometer to recruit beneficial microbial taxa from local microbial pools shortly after its

out-plant. This early recruitment of the microbial partners was assumed to be one of the mechanisms the tree used to acclimate to local conditions.

Chapter 3 explored variability of soil microbiome around soil zones of the pedunculate oak clone phytometer in four sites along a European North-South transect which is characterized by a wide range of climatic and soil physico-chemical parameters. Here, three categories of soil microbiomes were defined according to the soil zones around the host tree and the level of interactions between this tree and the soil microbiome. These are the tree root-free zone total microbiome, tree root zone total microbiome, and tree root zone affine microbiome. The latter includes the most actively tree-interacting soil bacteria and fungi. The results demonstrated an interplay among geographic, soil physico-chemical, and host tree parameters in shaping soil bacterial and fungal communities of the tree root zone, but the affine microbiome revealed an increased impact of tree-related parameters compared to the abiotic parameters, especially for fungi. The tree root zone affine microbial OTUs were revealed mostly common to all sites despite their spatial distance, which might be one element enabling broad latitudinal distribution of the oak.

Chapter 4 investigated temporal changes on the total microbiomes of the root and root-free soil zones of the clonal pedunculate oak phytometer along a vegetation period. The results showed a directional change over time for the bacterial communities. The fungal communities did not show such successional changes; they rather displayed a fine spatial scale partitioning closely linked to host plant individuals.

ZUSAMMENFASSUNG

Böden beherbergen eine große Vielfalt an Mikroorganismen, die von Bakterien und Pilzen dominiert werden. Diese Bodenmikroben, die zusammenfassend als Bodenmikrobiom bezeichnet werden, tragen wesentlich zur Biodiversität des Bodens bei und spielen eine wichtige Rolle bei essentiellen Bodenfunktionen (z.B. Bodenfruchtbarkeit, Pflanzenernährung, Abbau organischer Substanzen, Nährstoffkreisläufen und Bodenbildung). Viele Studien haben in den letzten Jahrzehnten die mikrobielle Gemeinschaft in Böden untersucht, um die treibenden Kräfte für die Bodendiversität zu entschlüsseln. Innerhalb dieser Doktorarbeit wurden nun räumliche und zeitliche Variationen des Bodenmikrobioms in Abhängigkeit verschiedener Standortspezifika wie dem lokalen Klima, der Bodenphysik und -chemie sowie den Wirtsbaumparametern untersucht.

Um Effekte intraspezifischer genetischer Variationen zu vermeiden, wurde der Stieleichenklon DF159 (*Quercus robur* L.) aus dem Projekt *TrophinOak-PhytOakmeter* des Departments Bodenökologie am Helmholtz-Zentrum für Umweltforschung (UFZ) als Phytometer-System verwendet. Im Projekt *PhytOakmeter*, von dem diese Arbeit ein Teil ist, wurden aus Mikrostecklingen von DF159 regenerierten Setzlingen auf Grünland-, Wald- und urbanen Feldstandorten in Mitteldeutschland und entlang eines europäischen Nord-Süd-Transekts ausgepflanzt. Das übergeordnete Ziel des Projektes ist es, zu analysieren, wie sich der Klon an regional unterschiedliche, klimatische Bedingungen und wechselnde Umweltbedingungen anpasst und, wie er sich dort verhält. Die Stieleiche wurde als Modellbaumart gewählt, weil sie in hochkomplexe und vielfältige

multitrophische Interaktionen, auch mit Bodenmikroorganismen, eingebunden ist. *Q. robur* zeigt ein endogenes, rhythmisches Wachstum mit abwechselnden Wachstumsschüben von Spross und Wurzel, die sich zwei- bis viermal innerhalb einer Vegetationsperiode wiederholen können. Diese abwechselnden Wachstumsschübe haben nachweislich Auswirkungen auf die biologischen Aktivitäten in den wurzelnahen Bodenzonen.

Basierend auf dem oben beschriebenen Hintergrund untersuchte die vorliegende Dissertation Veränderungen in den mikrobiellen Bodengemeinschaften um die Stieleichen-Phytometer in Grünlandstandorten auf zwei verschiedenen räumlichen Skalen: (1) auf der lokalen Skala wurden die Phytometer-assoziierten Bodenmikrobiome an vier Standorten, die sich innerhalb eines engen geographischen Raums mit ähnlichen klimatischen Bedingungen in Mitteldeutschland befinden, verglichen; und (2) auf der kontinentalen Skala wurde ein ähnlicher Vergleich zwischen Standorten entlang eines europäischen Nord-Süd-Transekts, der eine große Bandbreite an klimatischen und physikochemischen Bedingungen umfasst, durchgeführt. Auch die zeitliche Skala wurde berücksichtigt, indem die Variabilität des Mikrobioms innerhalb eines Jahres entlang einer Vegetationsperiode analysiert wurde. Bodenproben wurden nicht nur in der Baumwurzelzone (RZ – „root zone“) genommen, d.h. in der Bodenzone, die lebende Baumwurzeln enthält, sondern auch in der baumwurzelfreien Zone (RFZ – „root-free zone“), d.h. in der Bodenzone, die zwar frei von Baumwurzeln ist, aber innerhalb desselben Untersuchungsstandort liegt, um auch den lokalen mikrobiellen Pool zu erfassen. Für die molekularen Analysen wurden PCR-basierte Illumina MiSeq Amplikon-Sequenzierungen durchgeführt, um die bakterielle und pilzliche Diversität,

Gemeinschaftsstruktur und Funktionalität nach Zuordnung ihrer OTUs zu funktionellen Gruppen zu bewerten.

Zusätzlich zu *Kapitel 1*, das die gesamte Arbeit dieser Doktorarbeit vorstellt, werden die Ergebnisse in den *Kapiteln 2-4* präsentiert, von denen zwei Studien bereits in internationalen, von Experten begutachteten Zeitschriften veröffentlicht wurden, während ein weiteres Kapitel als Konferenzbeitrag publiziert wurde. Die Dissertation wird durch ein zusammenfassendes *Kapitel 5* abgeschlossen, das die Diskussion aller Veröffentlichungskapitel zusammen mit einem Ausblick integriert.

Kapitel 2, "Tree root zone microbiome: exploring the magnitude of environmental conditions and host tree impact", veröffentlicht in „Frontiers in Microbiology“, untersucht die jeweiligen Beiträge von abiotischen Umwelt- und Wirtsbaumparametern an vier Standorten, die durch homogene klimatische Bedingungen in Mitteldeutschland gekennzeichnet sind, zwei Jahre nach der Baumauspflanzung. Wir verglichen zunächst an jedem Feldstandort die Zusammensetzung der Bakterien- und Pilzgemeinschaften zwischen der RZ des Eichenklons, in diesem Kapitel PhytOakmeter genannt, und der RFZ des Baumes. Im weiteren Verlauf des Kapitels wird die Diversität und Struktur der mikrobiellen Gemeinschaften innerhalb der Baum-RZ zwischen den Standorten ausgewertet. Die Ergebnisse zeigten unterschiedliche mikrobielle Zusammensetzungen zwischen der Baum-RZ und der RFZ, wobei das mit der Baum-RZ assoziierte Mikrobiom zahlreiche Ektomykorrhizapilze der Gattungen *Hebeloma, Exophiala, Scleroderma, Tomentella, Trichophaea* und *Tuber* aufwies. Diese schnelle Rekrutierung speziell nützlicher mikrobieller Taxa aus dem lokalen mikrobiellen Pool scheint zu den standortspezifischen Akklimatisierungsstrategien der Bäume zu gehören. Der

Gesamtbeitrag der Bäume zur Gestaltung der mikrobiellen Bodengemeinschaften war jedoch geringer als der Einfluss der abiotischen Umweltparameter. Die Ergebnisse zeigten sowohl für die Bakterien als auch für die Pilze eine ähnliche mikrobielle Diversität innerhalb der Baum-RZ zwischen den Standorten. Ein Ergebnis, das auf die homogenen klimatischen Bedingungen innerhalb der Standorte und die gemeinsame genetische Identität der Wirtsbäume zurückgeführt werden kann. Im Gegensatz dazu war die Struktur der mikrobiellen Gemeinschaften standortspezifisch.

Kapitel 3, "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" veröffentlicht in „Environmental Microbiology“, untersucht ebenfalls den relativen Einfluss von Geografie, Bodenphysikochemie und dem Stieleichenklon auf die Variabilität des Bodenmikrobioms, jedoch auf einer größeren räumlichen Skala von Lapinjärvi (Finnland) bis Bordeaux (Südwestfrankreich), die durch eine breite Palette variabler Umweltbedingungen gekennzeichnet ist. Zusätzlich zum Baum-RFZ-Gesamtmikrobiom und dem Baum-RZ-Gesamtmikrobiom wird in diesem Kapitel ein neues Submikrobiom eingeführt, das Baum-RZ-affine Mikrobiom. Letzteres wurde als die Teilmenge der RZ-Bakterien und -Pilze definiert, die in dieser Zone im Vergleich zur Baum-RFZ signifikant angereichert sind. Die Ergebnisse zeigten ein Zusammenspiel zwischen abiotischen Umwelt- und Wirtsbaumparametern bei der Gestaltung der Bakterien- und Pilzgemeinschaften der Baum-RZ entlang des europäischen Transekts. Dabei konnte der abnehmende Einfluss von Geografie, Bodenphysikochemie und Wirtsbaum auf das Gesamtmikrobiom der Baum-RZ festgestellt werden. Für die Variabilität des RZ-affinen Mikrobioms allein nahm der Einfluss der abiotischen

Umweltparameter ab, während der Baumeinfluss stark zunahm, insbesondere auf die Pilze. Ein weiteres wichtiges Ergebnis war der hohe Anteil an Baum-RZ-affinen mikrobiellen OTUs, die an allen vier Standorten vorkamen. Dieses sogenannte „Kern"-Mikrobiom bezeichnet ubiquitäre Bakterien und Pilze mit signifikanter Affinität zum Wirtsbaum und ist in der Lage, mit den unterschiedlichen Umweltbedingungen entlang des Transekts zurechtzukommen. Möglicherweise spielen gerade diese Mikroben eine entscheidende Rolle bei der weiten Verbreitung von *Q. robur* in Europa. Interessanterweise konnten keine Mitglieder des RZ-affinen Mikrobioms, die nur an einem bestimmten Standort vorkommen, gefunden werden.

Kapitel 4, "Temporal changes and alternating host tree root and shoot growth affect soil microbiomes", veröffentlicht in der Online-Zeitschrift „Proceedings“ und als Konferenzbetrag im Rahmen der „1st International Electronic Conference on Microbiology“, analysierte die zeitliche Variabilität der Baum-RZ- und RFZ-Gesamtmikrobiome entlang einer Vegetationsperiode an zwei Standorten in Mitteldeutschland. Der Boden wurde zu verschiedenen Zeitpunkten beprobt, die mit dem abwechselnden Wurzel- und Sprosswachstum der Bäume und der Herbstseneszenz, die die Vegetationsperiode abschließt, zusammenfallen. Die Ergebnisse zeigen für die bakteriellen Gemeinschaften eine zeitliche Veränderung entlang der Vegetationsperiode. Die Pilzgemeinschaften zeigten jedoch keine derartigen zeitlichen Veränderungen; sie wiesen vielmehr eine feine räumliche Aufteilung, die eng an die individuellen Wirtspflanzen gekoppelt war, auf. Zusätzlich zum Effekt der zeitlichen Sukzession werden tiefergehende zukünftige Analysen des generierten Datensatzes, den Einfluss des abwechselnden Wurzel- und Sprosswachstums, das für das endogene rhythmische

Wachstum des Baumes charakteristisch ist, beleuchten. Diese weiterführenden Analysen werden z.B. das Baum-RZ-affine Mikrobiom und in einzelne mikrobielle Funktionsgruppen berücksichtigen.

Die in dieser Arbeit vorgestellten Ergebnisse belegen den schnellen Einfluss des Stieleichenklons auf das Bodenmikrobiom bereits zwei Jahre nach dem Auspflanzen des Baumes. Außerdem prägen geographische, bodenphysikochemische Faktoren und der Wirtsbaum in unterschiedlichem Ausmaß die bakteriellen und pilzlichen Gemeinschaften im Boden. Diese Arbeit zeigt unterschiedliche räumliche und zeitliche Reaktionen der bakteriellen und pilzlichen Bodengemeinschaften auf die variablen Umweltbedingungen.

Die Verwendung von Baumklon-Phytometern zur Untersuchung der baumbezogenen Parameter auf das Bodenmikrobiom hat sich als vielversprechendes Werkzeug erwiesen, um Hierarchien der verschiedenen abiotischen und biotischen Faktoren bei der Gestaltung des Bodenmikrobioms in Verbindung mit langlebigen Bäumen zu enträtseln. Schließlich stellt diese Arbeit einen ersten Schritt zur Etablierung einer langfristigen Überwachung der Dynamik von Bodenmikrobiomen in Verbindung mit Bäumen dar. Mit dieser Entschlüsselungsstrategie können Zusammenhänge zwischen langfristiger Akklimatisierung langlebiger Pflanzen, Mikroorganismen und sich verändernde Umgebungen perspektivisch verstanden werden.

SUMMARY

Soils harbor a huge diversity of microorganisms, which are dominated by bacteria and fungi. These soil microorganisms, collectively termed as the soil microbiome, are major contributors to soil biodiversity and play essential roles in soil functions (e.g. soil fertility and plant nutrition, organic matter degradation and nutrient cycling, and soil formation). Therefore, many studies in recent decades have explored soil microbial diversity in order to unravel driving forces of its variations. Hence, this thesis reports on spatial and temporal variations of the soil microbiome in response to site specificities, i.e. local climate as well as soil physico-chemistry, and host tree parameters.

To avoid effects of intraspecific genetic variations, the pedunculate oak clone DF159 (*Quercus robur* L.) generated by the project *TrophinOak-PhytOakmeter* of the Soil Ecology Department at the Helmholtz Centre for Environmental Research (UFZ) was used as phytometer system. In the *PhytOakmeter* project of which this thesis is a part, saplings regenerated from microcuttings of DF159 were out-planted in grassland, forest and urban field sites in Central Germany and along a European North-South transect. The overall goal of the project is to analyze how the clone adapts to and performs under different regional climatic contexts and changing environment conditions. Pedunculate oak was chosen as a model tree species because it is engaged in highly complex and diverse multitrophic interactions, including soil microorganisms. *Q. robur* displays an endogenous rhythmic growth with alternating growing flushes in shoot and root, which can be repeated two to four times along a vegetation period. These alternating flushes have been shown to impact on variations of biological activities in soil zones close to the tree roots.

Based on the above-described background, the current PhD study investigated changes in the soil microbial communities associated to the pedunculate oak phytometer out-planted in grassland sites at two different spatial scales: (1) the local scale by comparing the soil microbiomes associated to the phytometer in sites located within a close geographic space of Central Germany with similar climatic conditions; and (2) continental scale by making a similar comparison among sites along a European North-South transect, which encompasses a wide range of climatic and soil physico-chemical conditions. Moreover, temporal scale was considered, whereby the variability of the microbiomes intra-annually along a vegetation period was analyzed. Soil samples were taken not only in the tree root zone (RZ), i.e. soil zone containing living roots of the tree, but also in the tree root-free zone (RFZ), i.e. soil zone out of reach of any tree roots, but within the same field plot, to access also the local microbial pools. The analyses used a PCR-based Illumina MiSeq amplicon sequencing approach targeting bacteria and fungi, to assess their diversity, community structure and functionality after assignment of their OTUs to functional groups.

In addition to *Chapter 1*, which introduces the whole work of this PhD research, the findings are presented within *Chapters 2-4*, of which two studies were already published in international peer-reviewed journals, while another study was published as a conference paper. The thesis is closed by the synopsis *Chapter 5* that integrates discussion of all the publication chapters together with an outlook section.

Chapter 2 “Tree root zone microbiome: exploring the magnitude of environmental conditions and host tree impact” published in Frontiers in Microbiology investigates the relative contribution of abiotic environmental and host tree parameters among four sites

characterized by homogeneous climatic conditions in Central Germany, two years after the tree out-plant. We first compared at each field site the composition of the bacterial and fungal communities between the RZ of the oak clone, called PhytOakmeter in this chapter, and the tree RFZ. The chapter further evaluates the diversity and structure of the microbial communities within the tree RZ among the sites. The results revealed different microbial compositions between the tree RZ and RFZ, whereby the tree RZ-associated microbiome included numerous ectomycorrhizal fungi of the genera *Hebeloma*, *Exophiala*, *Scleroderma*, *Tomentella*, *Trichophaea*, and *Tuber*. This quick recruitment of specific beneficial microbial taxa from the local microbial pool seems to be among the tree strategies to acclimate to local site conditions. However, the overall tree contribution to shape soil microbial communities was lower than the impact of abiotic environmental parameters. The results revealed also a similar level of microbial diversity within the tree RZ among the sites for both the bacteria and fungi, an outcome attributed to the homogeneous climatic conditions within the sites and the common genetic identity of the host trees. In contrast, structure of the microbial communities was site-specific.

Chapter 3 “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” published in Environmental Microbiology, also examines the relative impact of geographic, soil physico-chemical, and pedunculate oak clone parameters on the variability of the soil microbiome, but at a larger spatial scale from Lapinjärvi (Finland) to Bordeaux (Southwestern France), which is characterized by a broad range of geographic and soil physico-chemical conditions. In addition to the tree RFZ total microbiome and the tree RZ total microbiome, this chapter introduces a new sub-

microbiome called tree RZ affine microbiome. The latter was defined as a subset of the RZ bacteria and fungi, significantly enriched in this zone compared to the tree RFZ. The results demonstrated an interplay among abiotic environmental and host tree parameters in shaping bacterial and fungal communities of the tree RZ along the European transect. These parameters showed a descending order of magnitude of their impact on the tree RZ total microbiome: geographic > soil physico-chemical > host tree parameters. However, for the variability of the RZ affine microbiome alone, the impact of the abiotic environmental parameters decreased, while the tree influence was strongly increased, particularly for fungi. Another important result was the highest proportion of the tree RZ affine microbial OTUs shared among all four sites, which was here designated as the tree "core" microbiome. These bacteria and fungi with significant affinity to the host tree, and shared by all the sites because of their ability to cope with diverging environmental conditions across the transect, may be playing a crucial role in supporting the wide distribution of *Q. robur* across Europe. Interestingly, we found no members of the RZ affine microbiome to be exclusive of only one particular site.

Chapter 4 "Temporal changes and alternating host tree root and shoot growth affect soil microbiomes" published in Proceedings as conference paper after "The 1st International Electronic Conference on Microbiology", considers a temporal scale, and here the variability of the tree RZ and RFZ total microbiomes was analyzed along a vegetation period in two sites of Central Germany. The soil was sampled at different time points coinciding with the tree alternating root and shoot growth, and the fall senescence that concludes the vegetation period. The results show a directional change over time along a vegetation period for the bacterial communities. However, the fungal communities did

not show such temporal changes; they rather displayed a fine spatial scale partitioning closely linked to host plant individuals. In addition to the effect of temporal succession, deeper analyses of the generated data set will enable us to specify the impact of the alternating root and shoot growth characteristic of the tree endogenous rhythmic growth in the near future. These further analyses will include for example zooming in the tree RZ affine microbiome and in individual microbial functional groups.

The results presented in this thesis evidence the quick impact of pedunculate oak tree clone on the soil microbiome within a two-year time span after the tree out-plant. Also, to different extents, geographic, soil physico-chemical, and host tree concurrently shape the soil bacterial and fungal communities. This thesis shows different spatial and temporal responses to the abiotic environmental and tree parameters between the soil bacterial and fungal communities.

The use of tree clonal phytometer to study the tree-related parameters on soil microbiomes was proved to be a promising tool, to unravel the hierarchy of different abiotic and biotic factors in shaping the soil microbiome associated to long live trees. Finally, this work represents a first step toward establishing a long term monitoring of the dynamics of soil microbiomes associated to trees, as a strategy to unravel how these microorganisms participate to the long term acclimation of these long live plants to diverse and changing environments.

INTRODUCTION

Soils harbor an immense biodiversity because they represent extremely heterogeneous and changing habitats both spatially and temporally, thus providing myriads of microniches, which enable habitat specialization or coexistence of species (Bardgett, 2002). Soil microorganisms, collectively termed the soil microbiome (Hartman and Tringe, 2019), and in particular bacteria and fungi are major contributors to soil biodiversity and perform critical roles in soil ecosystem functions and services (soil fertility and plant nutrition, organic matter degradation and nutrient cycling, and soil formation) (Bardgett, 2002; Hines et al., 2006; Vogel et al., 2009; Philippot et al., 2013; Saccá et al., 2017; Xue et al., 2018; He et al., 2020; Jansson and Hofmockel, 2020). For this reason, there is an increasing interest to characterize soil microbiomes and to unravel driving forces of their variations. The main factors that shape soil microbiomes are soil physico-chemical parameters (Lauber et al., 2008; Rousk et al., 2010; Wilpiszeski et al., 2019), geographic location (Gourmelon et al., 2016; Sun et al., 2017), and vegetation (Han et al., 2007; Wu et al., 2018; Tajik et al., 2020). These abiotic environmental and plant parameters act via complex interactions (Singh et al., 2009; de Vries et al., 2012), thus challenging the attempts to unravel their individual contribution. One of the possible approaches to cope with this challenge consists of generating a context in which at least one driving factor is constant or varies less. Thus, the use of genetically identical host plants, here designated as clonal phytometers, to analyze spatial and temporal variations in soil microbiome was the foundation idea of the research presented in this thesis.

1.1. Different dependence levels on the abiotic and biotic environmental parameters between bacterial and fungal communities

Bacterial and fungal communities respond differently to changes in abiotic and biotic environmental parameters because the two microbial groups differ broadly in growth rate, stress tolerance, and substrate utilization (Sun et al., 2017). Precisely, soil bacterial growth rate is much higher than that of soil fungi, which tend to have higher tolerance for dry soils and low temperatures (Rousk and Bååth, 2007; Kirchman, 2012). Also, fungi mainly degrade complex organic matter and are thus mediators of slower carbon cycling pathways in soil, while bacteria are degraders of simple organic molecules and typical regulators of the fast carbon cycling (Rinnan and Bååth, 2009). As consequence to the above mentioned differences, influence of changes in abiotic environmental conditions is overall higher for bacterial communities than for fungal communities (Singh et al., 2009; Rousk et al., 2010). Additionally, even though host plants impact both soil bacterial and fungal communities, especially via their organic carbon inputs to the soil through rhizodeposits and litter (Van Der Heijden et al., 2008; de Vries et al., 2012; Prescott and Grayston, 2013), their impact is higher on fungi than on bacteria (Sugiyama et al., 2008; Millard and Singh, 2010; Lange et al., 2014).

1.2. The pivotal role of trees in supporting various soil microorganisms versus other plant categories and effectiveness of grasslands to analyze a tree-associated microbiome

Plants growing in soil establish close associations with a large variety of soil microorganisms, which live in areas around, on, and inside plant roots (Hartman and Tringe, 2019). A wide range of those plant root-associated microorganisms is neutral to the plant but highly important in the biodegradation of C compounds that plants release into the soil, including the rhizodeposits (Raaijmakers et al., 2009). The plant root-

associated microbial community also harbors detrimental and beneficial microorganisms, the latter include for example nitrogen-fixing bacteria, plant growth-promoting bacteria and fungi, as well as endo- and ectomycorrhizal fungi known to improve fitness of their plant associates (Pozo et al., 2004; Raaijmakers et al., 2009; Hrynkiewicz and Baum, 2012; Lee et al., 2020). Plant rhizodeposits enriched in exudates are the major source of readily available organic nutrients (sugars, amino acids, and organic acids) to all the mentioned microbial groups (Loranger-Merciris et al., 2006; Rodríguez-Loinaz et al., 2008; Jones et al., 2009; Jacoby et al., 2017). These plant-derived soil nutrients vary among plant species and even among plant genotypes within the same species, reflecting a significant impact of plant identity on the soil microbial community (Broeckling et al., 2008; Berg and Smalla, 2009). Plant exudation is also positively correlated with and depends on plant biomass (Aulakh et al., 2001). Because trees are larger than herbaceous plants, they provide more and highly heterogeneous resources to the soil (Aulakh et al., 2001; Herz et al., 2018), supporting therefore multiple belowground trophic interactions (Hooper et al., 2000). Furthermore, as immobile organisms and long-lived plants, trees constantly restructure intra- and inter-annually the assembly of their root microbial partners as one of their strategies to persist in the face of a wide range of abiotic and biotic threats along their lifespan (Pennanen et al., 1999; Wallander et al., 2010; Kyaschenko et al., 2017; Averill et al., 2019). Thus, trees are very crucial in sustaining belowground biodiversity, and are suitable hosts to analyze the impact of a plant on spatial and temporal variations of the soil microbial communities.

In addition to production of the exudates which are readily decomposed by the soil microbes, trees act as long-term carbon pool to soil via litterfall (Dixon et al., 1994;

Vesterdal et al., 2012). Decomposition of litter is very slow because it proceeds through numerous cascading mechanisms (Krishna and Mohan, 2017), resulting in the gradual accumulation of organic matter especially in the forest soils (Isaac and Achuthan Nair, 2005). In this regards, the use of grasslands to investigate the impact of a specific tree on its roots-associated microbiome enables to avoid confounding effects resulting from the complex processes of litter decomposition.

1.3. DF159 oak phytometer for microbial host tree and soil sampling strategy

Oaks, which are common foundation tree species in temperate forests (Eaton et al., 2016), also grow as solitary trees in agricultural systems or grasslands (MacDougall et al., 2004; Löf et al., 2016; Bobiec et al., 2018; Parmain and Bouget, 2018). The trees are hosts for a wide variety of organisms, including microbes (Valencia-Cuevas and Tovar-Sánchez, 2015). Furthermore, growth of oaks via alternating root and shoot flushes (RF and SF) which are paralleled by shifts in resource allocations between the above and below plant parts, was shown to impact the biological soil activity since the early age of the trees (Herrmann et al., 2015; Ferlian et al., 2018).

This PhD research used the pedunculate oak clone DF159 (*Quercus robur* L.) out-planted as phytometer (Herrmann et al., 2016; Ferlian et al., 2018). The oak phytometer approach was developed by the TrophinOak-PhytOakmeter project of the Soil Ecology Department at the Helmholtz Centre for Environmental Research (UFZ) with an overall goal to understand how trees deal with multitrophic interactions and adapt to different climatic and environmental conditions (Herrmann et al., 2016). To achieve this, field plots were prepared in grassland, forest, and urban sites. The oak phytometer trees used in this PhD research were produced via *in vitro* propagation during winter 2012/2013 followed by a

two-step acclimatization in a greenhouse during summer 2013 and in an outfield nursery during summer 2014. In November 2014, the trees were out-planted in grassland field sites in Central Germany and along a European North-South transect. The choice of grasslands for the experimental set-up in this PhD research was motivated by two main reasons: (1) intolerance of young oaks to shade of closed woody vegetation (Bobiec et al., 2011) versus their favored growth in grasslands and other open or semi-open habitats (Jensen and Löf, 2017; Bobiec et al., 2018), and (2) avoiding the confounding effects from other trees and from complex litter and organic layers.

The general objective of this PhD research was to analyze spatial and temporal variations in the microbiomes of different soil zones around clonal pedunculate oak trees, and to reveal the respective impacts of the abiotic environmental and host tree parameters (**Figure1A**). Previously, numerous studies on plant root-associated microbial communities focused on the rhizosphere (Grayston et al., 1998; Fang et al., 2001; Hartmann et al., 2009; Haldar and Sengupta, 2015), which is defined as the narrow soil zone directly surrounding and often attached to plant roots (Hinsinger et al., 2009; Mendes et al., 2013). According to this definition, the rhizosphere microbial community is most directly controlled by the selective forces exerted by host plants (Kowalchuk et al., 2002), and the rhizosphere-focused studies do not give enough weight to the direct contribution of abiotic environmental factors in shaping the tree-associated soil microbiome. Therefore, investigating soil of the tree root zone (**Figure 1A**), i.e. soil containing living roots of the tree (Steven et al., 2014), by discarding the rhizosphere soil *senso stricto* enables to analyze more rationally the respective impacts of plant and environmental parameters in shaping the plant root microbiome (Weißbecker et al.,

2018). To distinguish between the impacts of tree-mediated recruitment and local environmental parameters on the microbiome, the tree root and root-free soil zones were sampled (**Figures 1B & C**) to compare their microbial composition and community structure.

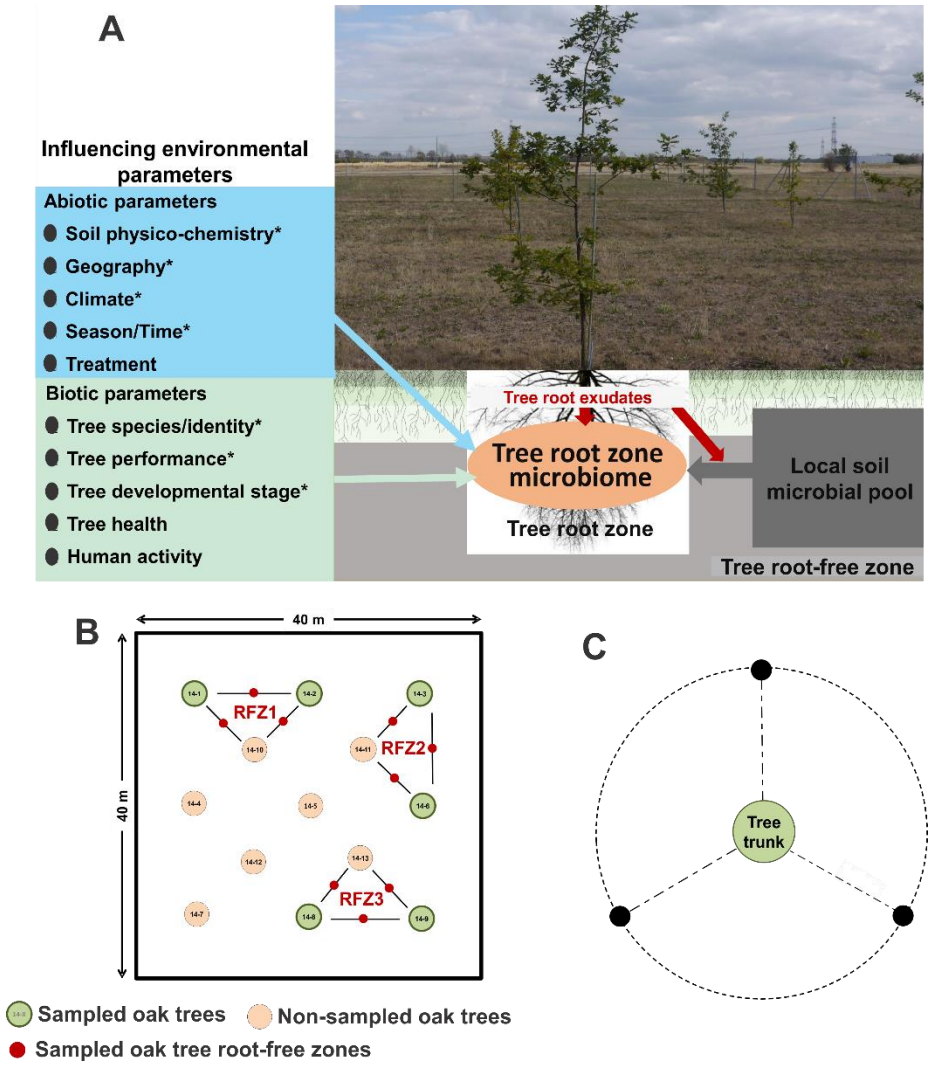

Figure 1. (**A**) Recruitment of soil microorganisms from the local microbial pool by a host tree using root exudates. Abiotic environmental and biotic parameters influencing a tree root zone microbiome. *****Parameters that are analyzed in this research. (**B**) Bordeaux site illustration to summarize soil sampling design in our field plots: green cycles indicate investigated oak phytometers, and red dots mark the sampling positions of the three subsamples that were taken and pooled to obtain the tree 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 phytometers.

1.4. Methods to characterize microbial communities

Because the majority of soil microorganisms cannot yet be cultured in the laboratory, culture-based methods are inadequate to analyze microbial communities in environmental samples. The use of high-throughput, culture-independent surveys which utilize soil-extracted microbial community DNA is therefore the preferred approach nowadays (Kent and Triplett, 2002). One of such approaches is the DNA metabarcoding, which is based on the amplification of the 16S rRNA gene and the internal transcribed spacer (ITS) region of the rDNA gene, the barcode regions of bacteria and fungi, respectively. With the Illumina's MiSeq platform, a large number of DNA sequences are generated and amplicon libraries from various samples can be pooled in one sequencing run (Caporaso et al., 2012). To allow this multiplexing of the samples, amplicons from each sample are indexed with barcoded PCR primers appended with a unique oligonucleotide sequence (Binladen et al., 2007). After sequencing, reads of the sequences are de-multiplexed and assigned back to their respective original samples. The sequencing results together with subsequent bioinformatics provide a list of the recovered microbial operational taxonomic units (OTUs) and attribute them to taxonomic and functional groups, while adequate statistical analyses give insight into microbial communities' changes across space, time, or in response to experimental treatments (Barberán et al., 2012).

1.5. Thesis outline

This PhD work aimed to analyze the spatial and temporal variations in the microbiomes of different soil zones around clonal pedunculate oak trees out-planted as phytometers across grasslands in Europe. After this introductory *Chapter 1*, the main findings

published in three independent manuscripts are documented in the *Chapters 2-4* of this thesis.

Manuscript 1 (Habiyaremye, J. D. D., Goldmann, K., Reitz, T., Herrmann, S., & Buscot, F. (2020). Tree root zone microbiome: exploring the magnitude of environmental conditions and host tree impact. *Frontiers in Microbiology*, *11*, 749) reported in *Chapter 2* used the mentioned clonal oak phytometer out-planted in field sites with similar climate but different soil physico-chemistry. This design with similar climatic variables enabled to assess the effect of different sites only based on their different soil physico-chemical properties and to capture the capability of the DF159 clone to trap microbial partners from different soils. The studied field sites were Harsleben, Pfeiffhausen, Greifenhagen, and Bad Lauchstädt, located in a close geographic space in Central Germany (**Figure 2A**).
In this *Chapter 2*, we were specifically guided by the following main questions:

1. Do the common genetic identity of the clonal oak trees and the homogeneity in climate conditions induce similar microbial diversity and community structure within the tree root zone among the sites?
2. Are some particular soil microbial taxa already enriched in the tree root zone two years after the out-planting of the trees?

Manuscript 2 (Habiyaremye, J. D. D., Herrmann, S., Reitz, T., Buscot, F., & Goldmann, K. (2021). 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. *Environmental Microbiology*) is reported in *Chapter 3*. Here, the study examined soil microbiomes in the oak phytometer root zone along a European North-South transect, from Finland to France, with four field sites in total: Lapinjärvi (Southern

Finland), Bad Lauchstädt (Central Germany), Fontain (Eastern France), and Bordeaux (Southern-West France) (**Figure 2B**). The sites are highly different from each other in terms of geographic location, climate, and soil physico-chemistry. As the tree root zone inherently contains a mix of actively tree-interacting microorganisms and those that diffuse passively from the surrounding tree root-free soil zone, the tree root zone microbiome was split into two sub-sets: the tree root zone total microbiome and the tree root zone affine microbiome. The latter is the part of the tree root zone total microbiome and only comprises bacteria and fungi significantly enriched in this zone compared to the tree root-free zone. The two root zone microbiomes were considered in addition to the tree root-free zone total microbiome, which represents the local microbial pools of the sites. Considering the three soil microbiomes around the clonal oak tree was adequate to rationally investigate the changes in soil microbiomes along a North-South gradient at a continental scale, and compare the host tree influence between the total microbiome inhabiting the root zone and the actively tree-interacting microbiome.

The following central questions guided *Chapter 3*:

3. Do the clonal oak trees shape an interacting soil microbiome from very different local soil communities, which might support their acclimation to a broad range of environmental conditions?
4. Does the clonal oak phytometer contribute more than the geographic and soil physico-chemical parameters in shaping the tree root soil zone microbiome across a larger spatial scale with gradient in these abiotic environmental conditions?

Manuscript 3 (Habiyaremye, J. D. D., Herrmann, S., Buscot, F., & Goldmann, K. (2021). Temporal changes and alternating host tree root and shoot growth affect soil

microbiomes. In *Multidisciplinary Digital Publishing Institute Proceedings* (Vol. 66, No. 1, p. 35) is reported in *Chapter 4*. Indeed, the tree alternation of root and shoot growth is paralleled with high and low concentrations of photoassimilates into roots during the RFs and SFs, respectively (Angay et al., 2014). According to the “push” hypothesis, the more C ‘pushed’ into the roots, the more C ought to be exuded from roots (Karst et al., 2016). The study was conducted on the clonal oak phytometer out-planted at Bad Lauchstädt and Harsleben grassland sites of Central Germany (**Figure 2A**). Soil sampling took place during 2018 and sampling times coincided with the tree alternating root and shoot flushes, which were determined based on the tree bud developmental stages (Herrmann et al., 2016). The tree rhythmic growth starts with a root flush (RF) followed by a shoot flush (SF) to make a complete rhythmic growth cycle. Over a vegetation period until autumnal leaf senescence, the tree can go through one growth cycle (i.e. one RF and one SF), two cycles (i.e. RF1, SF1, RF2, and SF2), or even more cycles, up to four, depending on environmental conditions. During the vegetation period 2018, the trees had two growth cycles, which induced five consecutive sampling times, i.e. at the end of RF1, SF1, RF2, SF2, and at the fall senescence which concludes the vegetation period.

The central question to answer in *Chapter 4* was:

5. Does the alternation of tree root and shoot flushes induce any changes on the expected temporal succession of the tree-associated microbial communities?

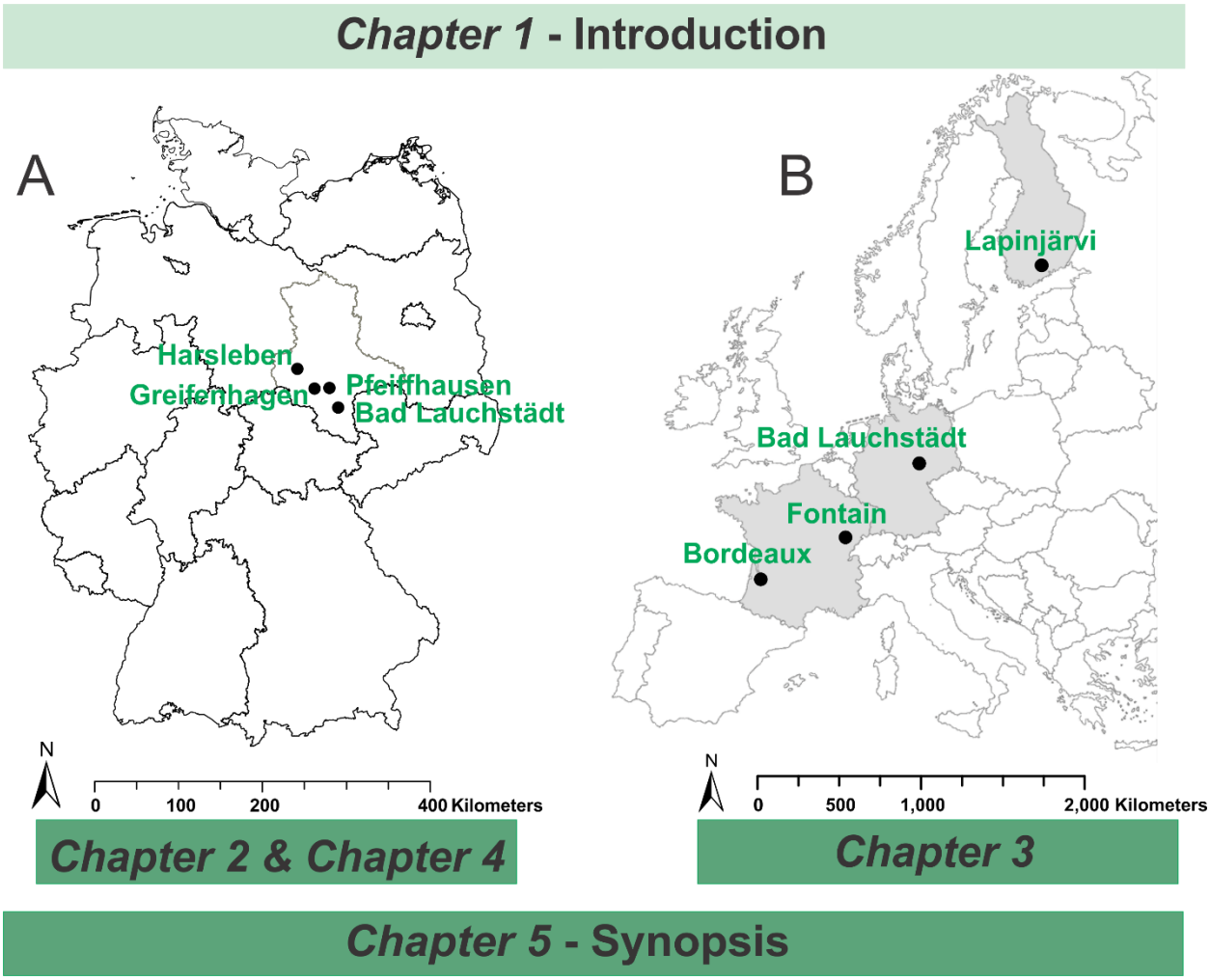

Figure 2. Study sites individually indicated by black dots. (**A**) Four field sites of Central Germany investigated in *Chapter 2*; two out of those sites, i.e. Bad Lauchstädt and Harsleben are also investigated in *Chapter 4*. (**B**) Four field sites along a European North-South transect investigated in *Chapter 3*: grey sections represent the study countries which are, from North to South, Finland, Germany, and France. *Chapter 1* introduces the whole work while *Chapter 5* connects results from the preceding chapters.

This thesis ends with a concluding synopsis - *Chapter 5* - which connects results from the preceding three chapters. The synopsis also compares microbial communities of the Bad Lauchstädt site between September 2016 and September 2018, i.e. two and four years after the trees out-plant, respectively. Overall, the results presented in this thesis demonstrated an interplay among geographic-climatic, soil hysico-chemical, and host

tree parameters in driving soil microbial communities, but the host tree influence increased from the total root zone inhabiting microbiome to the actively tree-interacting microbiome. The results also showed different spatial, temporal, and host plant-derived microbial patterns between the bacterial and fungal communities, which suggest different mechanisms shaping these two microbial groups.

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CHAPTER 2

Tree root zone microbiome: exploring the magnitude of environmental conditions and host tree impact

Jean de Dieu Habiyaremye, Kezia Goldmann, Thomas Reitz, Sylvie Herrmann, and François Buscot

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Tree Root Zone Microbiome: Exploring the Magnitude of Environmental Conditions and Host Tree Impact

Jean de Dieu Habiyaremye[1,2,3], Kezia Goldmann[1], Thomas Reitz[1,4], Sylvie Herrmann[1,4] and François Buscot[1,2,4]*

[1] Department of Soil Ecology, Helmholtz Centre for Environmental Research (UFZ), Halle, Germany, [2] Department of Biology II, Leipzig University, Leipzig, Germany, [3] Department of Mathematics, Science and Physical Education, University of Rwanda, Kigali, Rwanda, [4] German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, Leipzig, Germany

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***Correspondence:**
Jean de Dieu Habiyaremye
jean-de-dieu.habiyaremye@ufz.de

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Tree roots attract their associated microbial partners from the local soil community. Accordingly, tree root-associated microbial communities are shaped by both the host tree and local environmental variables. To rationally compare the magnitude of environmental conditions and host tree impact, the "PhytOakmeter" project planted clonal oak saplings (*Quercus robur* L., clone DF159) as phytometers into different field sites that are within a close geographic space across the Central German lowland region. The PhytOakmeters were produced via micro-propagation to maintain their genetic identity. The current study analyzed the microbial communities in the PhytOakmeter root zone vs. the tree root-free zone of soil two years after out-planting the trees. Soil DNA was extracted, 16S and ITS2 genes were respectively amplified for bacteria and fungi, and sequenced using Illumina MiSeq technology. The obtained microbial communities were analyzed in relation to soil chemistry and weather data as environmental conditions, and the host tree growth. Although microbial diversity in soils of the tree root zone was similar among the field sites, the community structure was site-specific. Likewise, within respective sites, the microbial diversity between PhytOakmeter root and root-free zones was comparable. The number of microbial species exclusive to either zone, however, was higher in the host tree root zone than in the tree root-free zone. PhytOakmeter "core" and "site-specific" microbiomes were identified and attributed to the host tree selection effect and/or to the ambient conditions of the sites, respectively. The identified PhytOakmeter root zone-associated microbiome predominantly included ectomycorrhizal fungi, yeasts and saprotrophs. Soil pH, soil organic matter, and soil temperature were significantly correlated with the microbial diversity and/or community structure. Although the host tree contributed to shape the soil microbial communities, its effect was surpassed by the impact of environmental factors. The current study helps to understand site-specific microbe recruitment processes by young host trees.

Keywords: PhytOakmeter, microbial recruitment, microbial diversity, environmental conditions, core and site-specific microbiomes

INTRODUCTION

The soil microbiome, the community of soil microorganisms and their genomes (Scher and Abramson, 2011), steers many ecological processes in soils and determines plant health (Aislabie et al., 2013) and productivity (Berg, 2009). Impacts of soil microorganisms on plants include increased nutrient availability and uptake (Lugtenberg et al., 2002; Morrissey et al., 2004), disease suppression (Mendes et al., 2011), as well as increased tolerance against abiotic (Zolla et al., 2013) and biotic stressors (Zamioudis and Pieterse, 2011). Microorganisms have abilities to rapidly adapt to changing environmental conditions (Gehring et al., 2017; Lau et al., 2017). Therefore, the "plant root microbiome" can be considered as "the powerhouse of plant adjustment to local conditions" (Vandenkoornhuyse et al., 2015).

The "plant root microbiome" originates from the local soil microbial community, and is shaped by the root exudate composition (Bais et al., 2006; Lareen et al., 2016). On the one hand, the composition of plant root-associated microbial communities across various ecosystems has been reported to highly depend on environmental parameters (Bulgarelli et al., 2012; Lundberg et al., 2012) such as climate and weather (Brockett et al., 2012; Lladó et al., 2018), but also on soil chemistry, especially pH and organic matter content (Zhou et al., 2002; Rousk et al., 2010; Lareen et al., 2016). However, in soils with similar edaphic parameters and climatic conditions, there can be significant local heterogeneity in the composition of soil bacterial and fungal communities even within the same region (Bokulich et al., 2014; Gourmelon et al., 2016). This may partly result from variations of unmeasured environmental parameters across the sampled field sites (Landesman et al., 2014) or from dispersal limitation among members of the microbial community (Bissett et al., 2010). On the other hand, the constituents of plant root exudates (sugars, vitamins, nucleotides, flavones, auxins, and stimulators), which differ between plant species and even among plant genotypes within a species (Broeckling et al., 2008), are also considered as important drivers structuring soil microbial communities proliferating in the plant root zone (Dotaniya and Meena, 2015). However, separating the effects of heterogeneity in environmental conditions within a region from those induced by variability of exudates between plant individuals is largely unexplored.

Oak, a foundation tree species, displays among the highest levels of below and aboveground biotic interactions in European forests (Plomion et al., 2018). More than 20 years ago, numerous investigations have been made on how oak trees harmonize their own development, biotic interaction and adaptation to the environment. These studies were through microcosm experiments using micro-cuttings of the oak clone DF159 (*Quercus robur* L.) with different analytic approaches including transcriptomics (Herrmann et al., 1998, 2015, 2016; Tarkka et al., 2013). More recently, clonal saplings regenerated from DF159 were planted in TERENO[1] field sites as "phytometers" (Herrmann et al., 2016; Ferlian et al., 2018), i.e., standardized plants transplanted into different environments to serve as environmental measuring "instruments" (Dietrich, 2013). The tree phytometer system using clone DF159 is called "PhytOakmeter" (Ferlian et al., 2018). A few years after outplant in the field, the PhytOakmeter saplings have been shown to exert an impact on the biological activity in their surrounding soil (Eisenhauer et al., 2018). Therefore, PhytOakmeter has the potential to help unraveling the tree-related factors that shape the root microbiome.

Previous investigations on soil microorganisms associated with plant roots focused on rhizospheric soil microbial communities (Grayston et al., 1998; Fang et al., 2001; Nunan et al., 2005; Hartmann et al., 2009; Haldar and Sengupta, 2015). However, as a shared environment between plant roots and microbes (Jacoby et al., 2017), the rhizosphere is most directly controlled by the selective forces exerted by host plants (Kowalchuk et al., 2002). Some studies reported an enhanced microbial species richness and diversity in the rhizosphere due to its enrichment in resources (Novello et al., 2017). However, there is an opposite view that, due to selection property of root exudates, the rhizosphere may comprise a strongly reduced proportion of the soil microorganisms (Philippot et al., 2013). In any case, rhizosphere-focused studies do not give enough weight to the contribution of environmental factors in shaping the microbiome of the root zone of soil. Therefore, investigating soil of the root zone by discarding the rhizosphere soil *senso stricto* enables to rationally analyze the respective impacts of plant and environment factors in shaping the plant root microbiome (Weißbecker et al., 2018).

Using PhytOakmeters planted in plots within the same central German region and under comparable climate conditions, the current study aimed to distinguish between the impacts of tree-mediated recruitment and local environmental factors on microbial diversity and community structure by comparing the tree root zone vs. the tree root-free zone of the soils. The study was performed using Illumina pair-end amplicon sequencing targeting the small subunit (SSU) of the 16S and the internal transcribed spacer (ITS) region of the 18S rDNA to gain bacteria and fungi, respectively. As result of the common genetic identity of the clonal saplings and of the homogeneity in climate conditions, we hypothesized a high similarity in microbial diversity and community structure within root zones of PhytOakmeters planted in Central German TERENO grassland field sites. Due to an extended rhizosphere mediated selection effect of the host tree, we expected a lower microbial diversity in the PhytOakmeter root zone than in the tree root-free zone within respective field sites. In comparison to the tree root-free zone, we expected to find higher abundance of some particular soil microbial taxa, due to creation in the PhytOakmeter root zone of a particular niche which selects specific microbial taxa.

MATERIALS AND METHODS

Field Sites and PhytOakmeter

The PhytOakmeter experiment was carried out in central Germany at four TERENO grassland field sites: Harsleben (51°51′43.43″ N, 11°04′58.73″ W, 138 m), Pfeiffhausen

[1]www.tereno.net

(51°37′47.68″ N, 11°42′19.95″ W, 137 m), Greifenhagen (51°37′20.80″ N, 11°24′59.62″ W, 292 m) and Bad Lauchstädt (51°23′29.65″ N, 11°52′32.14″ W, 119 m). Because of their geographic proximity, the PhytOakmeter field sites share comparable weather conditions (**Supplementary Table S1**). Due to the continental climate, flatness and position in the rain shadow of the Harz Mountains, this region is warm and dry with annual precipitations usually less than 500 mm (Wollschläger et al., 2016).

The DF159 oak tree saplings were produced via micropropagation which warrants their common genetic identity (Herrmann et al., 2016), and in November 2014, 2-year PhytOakmeter trees were outplanted in grassland sites. The distance between trees ranges from 6 to 10 m according to individual field plots. Beside the oaks, the entire soil surface of all field sites was covered by herbaceous plants as illustrated by Harsleben field site in **Supplementary Figure S1**. In September 2016, six core trees per site were randomly selected for this study. To determine tree performance and, later on, correlate it with soil microbial community structure, tree height at outplanting as well as tree percentage height increases in 2015 and 2016 were measured using a meter ruler. Moreover, number of shoot flushes produced by main stems of the core trees during the 2016 vegetation period were counted, and, as a proxy reflecting biomass production in each flush, five leaves were taken from every shoot flush of each tree. As core trees of all the sites had grown at least one shoot flush (SF1), we only considered the leaf biomass of the first shoot flushes during subsequent analyses.

Soil Sampling

In total, 38 soil samples were taken in September 2016: 24 samples in the tree root zone (6 trees per site × 4 sites = 24 soil samples) and 14 samples in the tree root-free zone that were used to analyze local soil microbial pools (4 samples per site in Harsleben and Pfeiffhausen, 3 samples per site in Greifenhagen and Bad Lauchstädt). At each field site, PhytOakmeter root zone and the tree root-free zone soil samples were taken within the same plot. Each soil sample consisted of three subsamples which were mixed to constitute a composite sample as illustrated by Harsleben plot sampling design in **Supplementary Figure S2**. All samples were collected using a 2 cm diameter soil auger to a 10 cm soil depth.

The soil samples were sieved using 2 mm mesh size to remove debris and homogenize the soil sample before being packed into sampling bags. From each sieved sample, one aliquot (±50 g) was kept for soil chemical analyses and another aliquot (±10 g) for molecular analyses, and both were stored at -20°C directly after sampling.

Soil Chemical Analysis

Sixteen soil chemical parameters were analyzed (**Table 1**). Soil pH was determined with a glass electrode after 1 h in a suspension 1:2.5 mixture of soil and 0.01 M $CaCl_2$ as in Moche et al. (2015) and Goldmann et al. (2015). Total soil carbon (TC) and nitrogen (TN) were determined in triplicate by dry combustion using a Vario EL III C/H/N analyzer (Elementar, Hanau, Germany). Due to negligible carbonate concentration of the soil samples (<2%), the obtained total carbon was taken to represent soil organic carbon, SOC (Francioli et al., 2016). To have an idea on the content of soluble soil organic matter, hot water extractable C (HWC) was measured as in Francioli et al. (2016) and N (HWN) as in Schulz et al. (2011). Cold water extraction of organic matter content was performed to measure the amount of labile and easily available organic carbon and nitrogen, representing the nutritional pool for these elements at the sampling time (Zsolnay, 1996). Cold water extractable carbon (CWC) and nitrogen (CWN) were then determined as in Schmidt et al. (2017). Mineral nitrogen contents (NH_4^+-N and NO_3^--N) were measured as in Francioli et al. (2016). Available P and K were extracted from soil with calcium acetate lactate (1:20 w/v, pH 4.2, 1.5 h) (Schüller, 1969). After filtration of the suspension (filter type: Whatman Schleicher and Schuell 595 1/5 Ø 270 mm), P and K were quantified in 1:10 diluted extracts 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).

DNA Extraction, Amplification, and Sequencing

Total microbial DNA was extracted from 0.4 g of each soil composite sample using the Power Soil DNA Isolation Kit (Qiagen, Hilden, Germany), following the manufacturer's instructions. The concentrations of DNA extracts were determined with a NanoDrop-8000 spectrophotometer (Thermo Fisher Scientific, Dreieich, Germany). DNA extracts were stored at −20°C, and adjusted to 10–15 ng/µl prior to PCR amplification. PCR genomic DNA amplicon libraries of the targeted microorganisms (bacteria and fungi) were produced from the genomic DNA templates. The bacterial 16S and fungal ITS2 within the rDNA region were amplified using a modified primer mix: P5_8N_515F + P5_7N_515F (forward) together with P7_2N_806R + P7_1N_806R (Caporaso et al., 2012; Moll et al., 2018) for the bacteria, and P5-5N-ITS4 (Gardes and Bruns, 1993; Leonhardt et al., 2019)/P7-4N-fITS7 (Ihrmark et al., 2012; Leonhardt et al., 2019) for the fungi, all containing the Illumina adapter sequences (see **Supplementary Table S2** for an overview of the utilized primer sequences according to Hendgen et al., 2018). All PCRs were conducted using the proofreading KAPA Hifi polymerase (Kapa Biosystems, Boston, MA, United States). Each PCR reaction was carried out in a total volume of 15 µl containing 1 µl template DNA, 0.3 µl forward primer, 0.3 µl reverse primer, 7.5 µl 2x KAPA HiFi HotStar ReadyMix, and 5.9 µl nuclease free water; under the following thermocycling steps. 16S rDNA amplification: initial denaturation at 95°C for 3 min, followed by 25 cycles of denaturation at 98°C for 20 sec, annealing at 55°C for 15 sec, elongation at 72°C for 15 s, followed by a final extension at 72°C for 5 min. Fungal ITS2 amplification: initial denaturation at 95°C for 3 min, followed by 30 cycles of denaturation at 98°C for 20 s, annealing at 56°C for 20 s, elongation at 72°C for 20 s, followed by a final extension at 72°C for 5 min. Every sample was amplified in three

TABLE 1 | Chemical parameters of the soil samples: pH, soil organic carbon (SOC), 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 (min.N), potassium (K), and phosphorous (P).

Parameter	Harsleben	Pfeiffhausen	Greifenhagen	Bad Lauchstädt
pH	7.6 (± 0.3)^a	7.5 (± 0.4)^a	7.5 (± 0.5)^a	6.3 (± 0.2)^b
SOC (%)	2.6 (± 0.4)^a	2.9 (± 0.4)^a	1.3 (± 0.2)^c	2.1 (± 0.1)^b
TN (%)	0.15 (± 0.03)^b	0.18 (± 0.01)^a	0.11 (± 0.03)^c	0.14 (± 0.01)^b
C/N	18.0 (± 4.5)^a	15.9 (± 1.2)^a	12.0 (± 4.2)^b	14.9 (± 0.4)^{ab}
CWC (mg/kg)	79.9 (± 14.8)^b	96.8 (± 13.7)^a	58.4 (± 10.6)^c	97.7 (± 14.4)^a
CWN (mg/kg)	5.3 (± 0.9)^c	7.7 (± 1.1)^a	5.7 (± 1.1)^b	7.6 (± 1.4)^a
CWC/CWN	15.4 (± 3.3)^a	12.6 (± 1.2)^b	10.5 (± 2.8)^c	13.3 (± 3.2)^{abc}
HWC (mg/kg)	1065.7 (± 166.6)^b	1437.0 (± 164.3)^a	627.8 (± 139.4)^c	616.8 (± 61.3)^c
HWN (mg/kg)	101.7 (± 19.9)^b	142.5 (± 18.3)^a	62.7 (± 14.7)^c	60.8 (± 7.3)^c
HWC/HWN	10.5 (± 0.6)	10.1 (± 0.6)	10.1 (± 0.4)	10.2 (± 0.8)
Soil moisture (%)	6.9 (± 1.1)^a	5.5 (± 1.4)^b	7.5 (0.7)^a	7.6 (0.6)^a
NH_4^+-N (mg/kg)	3.2 (± 0.5)^{ab}	3.7 (± 0.7)^a	2.5 (± 0.9)^b	2.6 (± 1.1)^b
NO_3^--N (mg/kg)	1.0 (± 0.8)	1.0 (± 0.5)	0.5 (± 0.4)	0.9 (± 1.4)
min.N (mg/kg)	4.2 (± 1.2)^a	4.6 (± 1.1)^a	3.1 (± 1.1)^b	3.1 (± 2.1)^{ab}
K (mg/kg)	156.1 (± 87.0)	153.9 (± 39.9)	199.3 (± 82.4)	148.2 (± 52.3)
P (mg/kg)	54.5 (± 47.3)^{ab}	51.8 (± 9.9)^a	33.1 (± 21.5)^b	24.0 (± 6.9)^b

Values represent means (± standard deviation). Different superscript letters after standard deviations in a row mean statistically different ($p < 0.05$) according a one-way ANOVA and Tukeys' HSD test.

replicates, resulting sample PCR products were checked by gel electrophoresis. The three replicates were pooled and cleaned-up using the Agencourt AMPure XP kit (Beckman Coulter, High Wycombe, United Kingdom). Subsequently, cleaned products were used as templates in an additional PCR, where Illumina Nextera XT indices and sequencing adaptors were attached according to the Illumina MiSeq protocol for amplicon preparation (Illumina Inc., San Diego, CA, United States). The amplifications followed these conditions: initial denaturation at 95°C for 3 min, 8 cycles of denaturation at 98°C for 30 s, annealing at 55°C for 30 s, followed by elongation at 72°C for 30 s, and a final extension at 72°C for 5 min. Resulting PCR products were purified again with AMPure beads. The libraries were then quantified by PicoGreen assays (Molecular Probes, Eugene, OR, United States) and pooled to provide equimolar representation. Fragment sizes and quality of DNA sequencing libraries were checked using an Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA, United States). The pool was used for paired-end sequencing of 2 × 300 bp with a MiSeq Reagent kit v3 on an Illumina MiSeq platform (Illumina Inc., San Diego, CA, United States) and was carried out at the Department of Soil Ecology of the Helmholtz-Centre for Environmental Research – UFZ in Halle (Saale), Germany.

Bioinformatics Analysis

The raw reads were de-multiplexed by the Illumina MiSeq Reporter software package v2.5.1.3 with default settings. Retained fastq files without Illumina adaptors, sequencing primers and indices were analyzed using the pipeline DeltaMP (v0.2)[2] by following the workflow presented in Schöps et al. (2018). In brief, soil-based Illumina sequences of 16S and ITS were processed and sequentially quality-filtered using mainly MOTHUR (v1.39.5-2, Schloss et al., 2009). Pair-end reads were merged with a minimum overlap of 20 bp using PandaSeq (v2.11, Masella et al., 2012). Sequences with any ambiguous base, more than four bp differences in the primer sequence, as well as homo-polymers with up to 20 bp differences were removed. Simultaneously, sequences, shorter than 50 or longer than 600 bp were discarded. Potential chimers were removed using UCHIME (Edgar et al., 2011) as implemented in MOTHUR (Schloss et al., 2009). Remaining sequences were pooled, de-replicated and sorted according to their abundance using OBITools (v1.2.11, Boyer et al., 2016). Unique sequences were clustered into operational taxonomic units (OTUs) with 97% sequence similarity using VSEARCH (v2.10.4, Rognes et al., 2016). By means of the Bayesian classifier as implemented in MOTHUR (Schloss et al., 2009), bacteria and fungi taxonomy was initially assigned using the SILVA reference database (v128, Quast et al., 2013) and UNITE (v8.0, Nilsson et al., 2018), respectively. The output was manually checked using Basic Local Alignment Search Tool (BLAST) of the National Center for Biotechnology (NCBI) (O'Leary et al., 2015). Plant derived 16S sequences assigned to chloroplasts or mitochondria were removed from the bacterial OTU table. Reads of samples were normalized at rarefaction depth of 96,167 and 26,578 reads per sample for bacteria and fungi, respectively, by using the function "rarefy_even_depth" from the phyloseq package 1.19.1 (McMurdie and Holmes, 2013) in R version 3.4.2 (R Development Core Team, 2017). The derived OTUs were assigned to their functional groups mainly based on FAPROTAX database (v1.1, Louca et al., 2016) and FUNGuild tool (v1.1, Nguyen et al., 2016) for bacteria and fungi, respectively. Raw sequences were deposited at the European Nucleotide Archive (ENA) and can be found under accession number PRJEB35688.

[2]https://github.com/lentendu/DeltaMP/

Statistical Analyses

The statistical analyses were carried out using R, v3.4.2 (R Development Core Team, 2017). The microbial Shannon diversity index (Shannon, 1948) was calculated using the diversity function of the vegan package (Oksanen et al., 2017), and results were visualized via overlaid boxplots and stripcharts using the ggplot2 package (Wickham, 2016). We used a two-way analysis of variance (ANOVA) to compare the microbial diversity of PhytOakmeter root and root-free zones within and among the field sites. We then used Tukey HSD test to determine at which sites the tree root zone and root-free zone revealed significant difference ($p < 0.05$). In the same way, significant differences in microbial Shannon diversity among the sites' tree root zones were analyzed. To explore how soil chemistry and weather parameters are correlated to the microbial Shannon diversity, multiple linear regression was done. We first removed auto-correlated parameters using the variance inflation factor (VIF < 5) (Akinwande et al., 2015), and the remaining parameters were differently combined into various models. The obtained regression models were then evaluated to choose the best approximating model by using Akaike's Information Criterion (AIC) (Johnson and Omland, 2004). Subsequently, to determine whether the field sites contained significantly different microbial communities, the analysis of similarities (ANOSIM) permutation test (999 permutations) was used together with a non-metric multidimensional scaling (NMDS) based on the Bray-Curtis dissimilarity matrices (Clarke, 1993). We then applied the envfit function of the vegan package (Oksanen et al., 2017) to analyze correlation between structure of soil microbial communities and soil chemical parameters. Goodness-of-fit statistics (R2) were calculated based on 999 permutations. NMDS was also used to compare microbial community structure between PhytOakmeter root and root-free zones within respective sites, and ANOSIM was as well applied to test the statistical significance. Moreover, the overlap analysis of bacterial and fungal OTUs among different locations was done using the online tool venny (Oliveros, 2007/2015). Using DEseq2 (v1.24.0) via phyloseq (McMurdie and Holmes, 2013; Love et al., 2014), we distinguished which genera significantly increased presence in PhytOakmeter root zone over the tree root-free zone ($p < 0.05$). The results were then plotted using the graphical library ggplot2 (Wickham, 2016). By using all the OTUs found within the host tree root zone, we performed variance partitioning (varpart function in vegan) to assess the relative contribution of the environmental parameters and the host tree performance in explaining variation of the bacterial and fungal communities.

RESULTS

Weather Data and Soil Chemical Parameters of the Field Sites

Details on weather data are summarized in **Supplementary Table S1**. The weather variables include precipitations as well as atmospheric and soil temperatures. There was no significant difference in any of the analyzed weather variables among the field sites.

The measured chemical parameters were mostly in similar ranges among the different field sites, even though some values differed significantly with, however, moderate difference amplitudes (**Table 1**). In particular, the soil of Greifenhagen and Bad Lauchstädt had lower values in SOC, hot and cold water extractable C and N.

The similarities among the soil parameters allowed repartition of the field sites into distinct groups. In this regard, concurrent similarity in pH and SOC grouped together Harsleben and Pfeiffhausen; C/N, HWC, and HWN put together Greifenhagen and Bad Lauchstädt; TN and C/N linked Bad Lauchstädt and Harsleben.

PhytOakmeter Growth Performance Among the Field Sites

PhytOakmeter growth parameters within the respective field sites are summarized in **Table 2**. The PhytOakmeters outplanted in the four field sites had similar initial height. Also, among the field sites, there was no difference in percentage increase of the tree height during 2015 and 2016 vegetation periods. The number of shoot flushes produced by the trees during 2016 was comparable among the sites, but the first shoot flushes were significantly longer in Bad Lauchstädt than in the other sites.

Overall Composition of Microbial Communities Among the Field Sites

For bacterial communities, a total of 5,092,013 reads representing 18,140 OTUs were obtained from the 38 samples from all four field sites. Removal of reads ascribed to chloroplasts and mitochondria gave a total of 5,066,965 reads corresponding to 17,890 OTUs, with a minimum of 96,167 and a maximum of 199,411 reads. Rarefaction to 96,167 reads per sample resulted in a total of 17,630 OTUs. For fungal community, the analysis availed a sum of 4,033 OTUs represented in a total of 1,545,424 reads; with minimum reads of 26,580 and a maximum of 56,794. Rarefaction to 26,578 reads per sample resulted in a sum of 3,970 OTUs. All rarefaction curves for both bacteria and fungi tended to approach the saturation plateau, an indication that the communities were almost exhaustively sampled and the data volume of sequenced reads was sufficient (see rarefaction curves in **Supplementary Figure S3**).

Overall, the rarefied bacterial OTUs were assigned to 42 different identifiable phyla, 126 classes, 169 orders, 319 families, and 582 genera. Bacterial communities were dominated by 13 phyla, with an individual relative abundance of at least 1%, all totaling up to 93% of the whole community. The five predominant phyla Proteobacteria, Actinobacteria, Planctomycetes, Acidobacteria and Chloroflexi covered more than 74% of the total community (**Figure 1A**). Unclassified OTUs at phylum level occupied 2.2%. All the bacteria phyla were similarly represented among all four field sites.

The rarefied fungal OTUs were classified into six different recognized phyla, 23 classes, 82 orders, 159 families, and 388 genera. The fungal phyla altogether were represented

TABLE 2 | Tested growth parameters on the investigated PhytOakmeters within respective field sites.

Tree parameters	Harsleben	Pfeiffhausen	Greifenhagen	Bad Lauchstädt
Height at the outplanting time (cm)	65.8 (± 16.5)	71.7 (± 8.8)	78.8 (± 2.5)	75.3 (± 5.9)
Height percentage increase in 2015	26.5 (± 15.2)	34.7 (± 27.6)	31.9 (± 11.9)	64.4 (± 39.3)
Height percentage increase in 2016	32.6 (± 34.2)	17.8 (± 17.6)	33.2 (± 12.5)	38.1 (± 31.4)
Mean SF number in 2016	1.8 (± 0.4)	1.5 (± 0.5)	2.0 (± 0.0)	2.0 (± 0.9)
Mean first SF length in 2016	11.4 (± 10.7)^{b}	10.0 (± 9.2)^{b}	7.6 (± 4.4)^{b}	27.3 (± 6.3)^{a}
Leaf dry weight (g)	0.6 (± 0.3)	0.8 (± 0.4)	1.0 (± 0.3)	0.9 (± 0.3)
Ratio leaf dry weight to fresh weight	0.5 (± 0.1)^{ab}	0.6 (± 0.1)^{a}	0.5 (± 0.0)^{a}	0.4 (± 0.0)^{b}

Values represent means of six selected trees (± standard deviation). Fresh and dry leaf weights represent total weights for five leaves of the main stem first shoot flush (SF1). Different superscript letters after standard deviations in a row mean statistically different ($p < 0.05$) according to a one-way ANOVA and Tukeys' HSD test.

in the following order: Ascomycota (56.0%), Basidiomycota (26.2%), Glomeromycota (10.5%), former Zygomycota (4.0%), and Chytridiomycota (3.0%), with 14.6% unclassified. The fungal phyla were shared and also similarly represented among all the four field sites (**Figure 1A**).

Microbial Shannon Diversity Associated With PhytOakmeter Root Zone, Field Sites and Environmental Parameters

Species diversity of both bacteria and fungi within PhytOakmeter root and root-free zones at each field site was determined by using the Shannon diversity index and results presented by boxplots (**Figure 1B**). The Shannon diversity values within the host tree root zones were similar among the sites for both bacteria and fungi. As well, species diversity of the host tree root-free zones was similar among the sites for both bacteria and fungi, except a significantly lower bacterial diversity value noticed at Pfeiffhausen. At each field site, the microbial species diversity values were also comparable between the host tree root and root-free zones. However, the species diversity of the host tree root zone tended to always be higher for the bacteria and, on the contrary, lower for the fungi.

As indicated by the lowest AIC values of the tested models (**Supplementary Table S3**), the best model to predict the microbial Shannon diversity included CWC, P, soil moisture and soil temperature for bacteria ($p < 0.001$ and adjusted $R^2 = 0.47$), while it included CWC and soil temperature for fungi ($p < 0.05$, adjusted $R^2 = 0.12$) (bold in **Supplementary Table S3**).

Structure of Microbial Communities Among the Field Sites

ANOSIM showed that the structure of soil microbial communities was significantly site-specific for both bacteria ($p < 0.001$, $R = 0.91$) and fungi ($p < 0.001$, $R = 0.82$). This was visually supported by NMDS plots in which samples were ordinated in separate clusters according to the respective field sites (**Figure 2**). The NMDS plot displayed that the soil microbial communities of Harsleben and Pfeiffhausen were close to each other especially for bacteria (**Figure 2**). The figure also shows the significant impacts of soil pH, SOC, C/N, and CWC on the microbial community structure for both bacteria and fungi, plus soil moisture for only bacteria.

When we separately plotted samples of the respective sites, we visually found start of separation between microbial communities of PhytOakmeter root and root-free zones in one site (Bad Lauchstädt) for the bacteria and in three sites (Harsleben, Pfeiffhausen, and Bad Lauchstädt) for the fungi (**Figure 3**), indicating a beginning of the host tree effect on microbial community structure. However, ANOSIM only confirmed this host tree effect on fungal community in the field sites of Pfeiffhausen ($p < 0.05$, $R = 0.37$) and Bad Lauchstädt ($p < 0.05$, $R = 0.57$).

Microbial Community Composition Within PhytOakmeter Root Zone in Comparison to the Tree Root-Free Zone

Composition of the soil microbial communities deduced from the OTUs overlap analysis between PhytOakmeter root zone and the tree root-free zone revealed significant differences (**Figure 4**). The highly abundant microbial OTUs tended to be generally shared between the two zones (55.7 and 51.2% for bacteria and fungi, respectively) while the least abundant tended to be uniquely detected within either zone. In this view, 29.6% bacterial OTUs and 32.7% fungal OTUs were exclusively detected within soil samples of the PhytOakmeter root zone, while 14.7% bacterial OTUs and 16.1% fungal OTUs were uniquely identified within the root free zone soil.

Further overlap analysis separated the microbial OTUs exclusive to the tree root zone into those commonly found in all the field sites and those exclusive to either site (**Figure 5**). The common ones were considered as the putative "core microbiome" of the rooting zone of the DF159 clone. The detected core microbiome consisted of 37 and 25 OTUs for bacteria and fungi, respectively (**Figure 5**). The number of PhytOakmeter site-specific microbial OTUs ranged from 369 (Pfeiffhausen) to 410 (Greifenhagen) for bacteria, and from 100 (Bad Lauchstädt) to 190 (Greifenhagen) for fungi, and was always much higher than the number of the "core" OTUs.

At the genus level, significant differences were also found between PhytOakmeter root and the tree root-free soil zones, as 27 bacterial and 48 fungal genera (including both the identified and unidentified) showed significant differential abundance between the two soil compartments (**Figure 6**, $p < 0.05$).

Specifically, **Figure 6** shows, for bacteria, higher abundance of six identifiable genera and lower abundance of seven

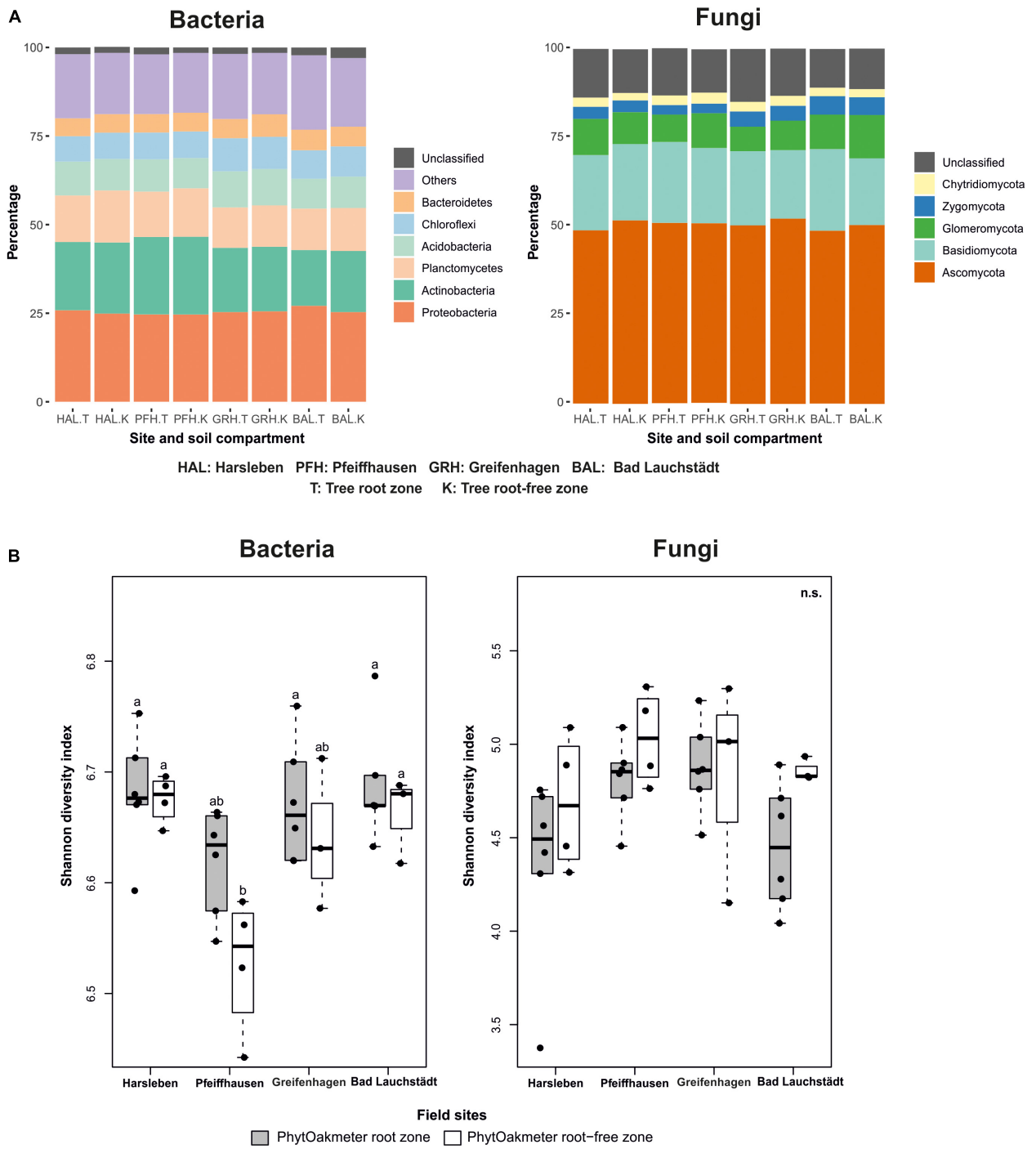

FIGURE 1 | (A) Distribution overview of bacterial and fungal phyla between PhytOakmeter root and root-free zones within and among the field sites, **(B)** Shannon diversity index for bacteria and fungi within soils from PhytOakmeter root zone and the tree root-free zone of the respective field sites. Different letters above boxplots indicate significant differences ($p < 0.05$) according to Tukey-HSD *post hoc* test. n.s., not significantly different.

recognizable genera in the PhytOakmeter root zone compared to the tree root-free zone. The bacterial genera highly abundant within PhytOakmeter root zone in comparison to the tree root-free zone included *Bryocella*, *Endobacter*, *Mucilaginibacter, Mycobacterium, Methylotenara,* and *Holophaga*. Always in comparison to the tree root-free zone, we clearly noticed higher abundance of 23 identifiable fungal genera in the PhytOakmeter root zone. These consisted of, among others, *Piriformospora*, *Typhula*, *Claviceps*, *Cyathus*, *Tomentella*, *Tuber*, *Trichophaea*, *Scleroderma*, *Exophiala,* and *Hebeloma*. Eight recognizable fungal

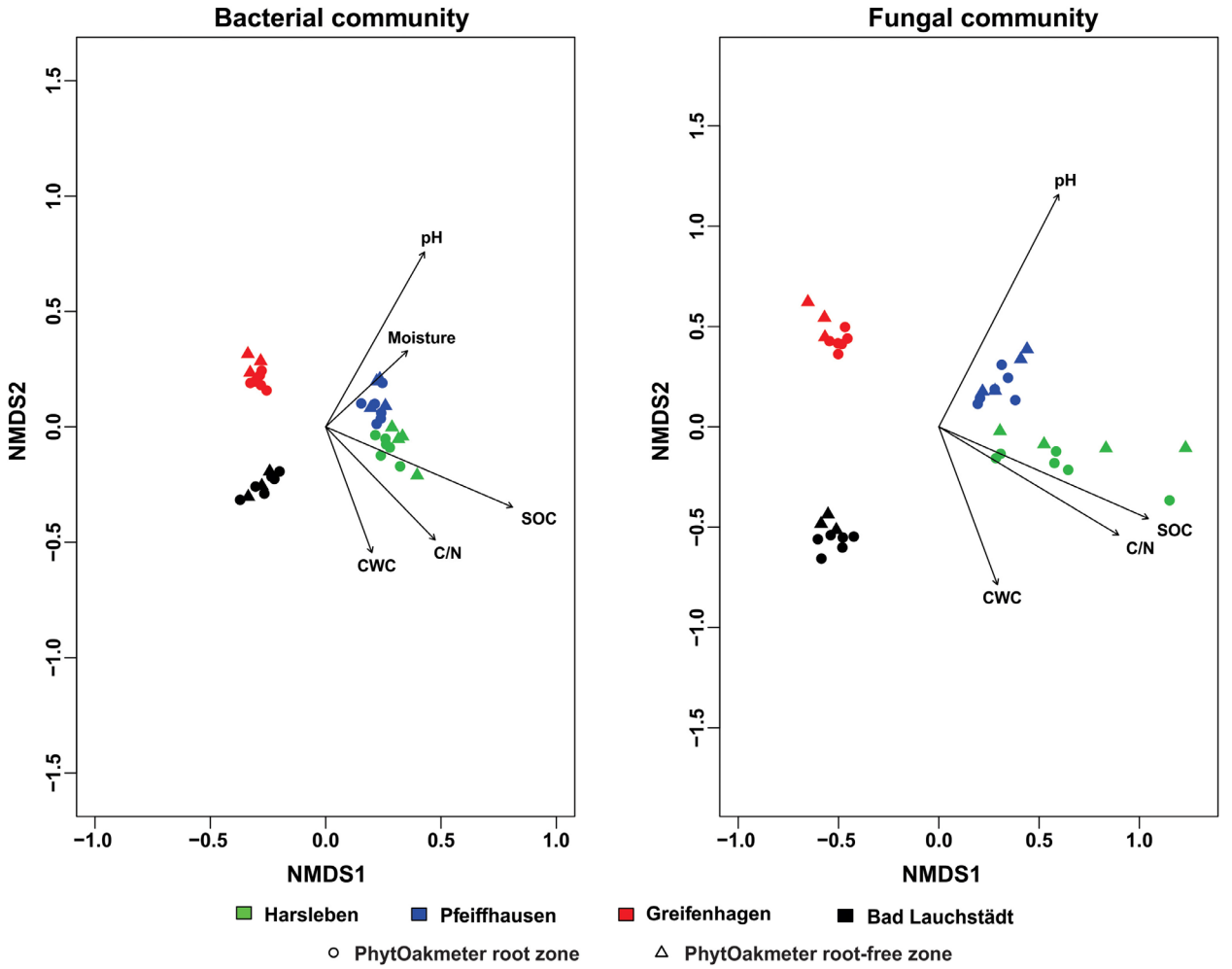

FIGURE 2 | Non-metric multidimensional scaling (NMDS) based on Bray-Curtis dissimilarity displaying bacterial (stress = 0.07) and fungal (stress = 0.09) communities' structure within field sites, and significantly correlated soil chemical parameters ($p < 0.05$).

genera showed higher abundance in the tree root-free zone. To summarize, more differentially abundant genera were in the PhytOakmeter root zone compared to the tree root-free zone. Furthermore, among the highly abundant microbial genera within PhytOakmeter root zone, we noticed more fungal than bacterial genera.

Compared Impacts of Soil Chemistry, Weather Parameters, and Host Tree Performance on Microbial Community Variation

Variance partitioning (**Figure 7**) showed that host tree performance traits alone could not explain any part of variation within the bacterial community while they accounted for 6.0% for the fungi. Similarly, the soil chemistry effect was only detectable for the fungi and explained 8.4%. Also, weather alone explained about 5.3% of the variance in bacteria and 9.7% in the fungi. The three types of factors had notably higher impacts when cumulating their single and combined effects derived from interactions with the other factors, whereby weather remained the strongest determinant followed by soil chemistry and, largely behind, tree performance. Even though this observation was similar in the two microbial groups, the explained variation was higher for bacteria than for fungi (**Figure 7**).

DISCUSSION

The current study revealed similar diversity levels of the microbiomes within PhytOakmeter root zone among the field sites and between the soil compartments (host tree root and root-free zones) within the individual sites. Our design was also adequate to detect specific changes in the community structure among the field sites. We also revealed different microbial composition between the PhytOakmeter root and root-free zones within respective sites. We were able to detect variations within the PhytOakmeter root zones amongst the sites and to separate

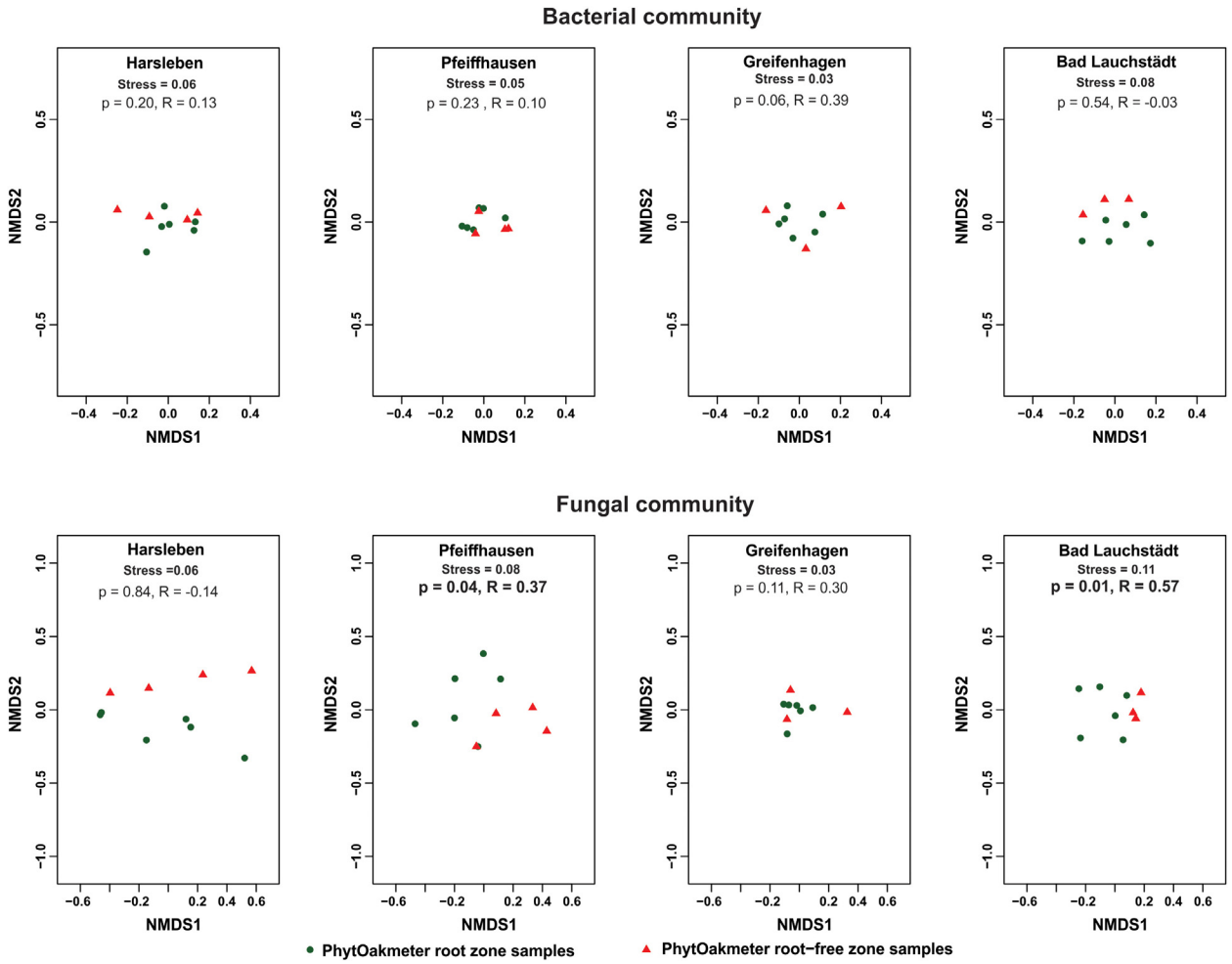

FIGURE 3 | Non-metric multidimensional scaling (NMDS) based on Bray-Curtis dissimilarity displaying bacterial and fungal communities' structure within respective field sites, and differentiating between the samples of PhytOakmeter root and root-free zones. p and statistic R values within respective sites are given by the analysis of similarities (ANOSIM) permutation test (999 permutations).

the change fraction explained by the host tree from the one accounted for by the environmental parameters.

Factors Equalizing the Microbial Diversity of PhytOakmeter Root and Root-Free Zones Within and Among the Field Sites

In our study, we partly confirmed our first hypothesis about microbial diversity levels in PhytOakmeter root zones among the field sites. However, we rejected the second hypothesis as we found no difference between the tree root and root-free zones within the individual sites. Despite small variations amongst the sites, this similar microbial diversity might mainly reflect comparable vegetation features and weather parameters among all the sites and between soil compartments (host tree root and root-free zones), which tended to equalize their microbiomes.

The first constant factor susceptible to homogenize the soil microbiomes of the field sites is the common genetic identity of the PhytOakmeters. As evidence to this PhytOakmeter clonal effect, microbial diversity within the tree root zones was similar among all the sites. Additionally and most importantly, bacterial diversity of the host tree root zone at Pfeiffhausen remained comparable to the tree root zones of the other sites in spite of its host tree root-free zone which was significantly different from most of its counterparts. According to previous reports, trees, especially through root exudates, provide specific carbon and energy sources to soil microorganisms. As a central source of nutrients, root exudates create therefore a niche for growth of microorganisms (Hassan et al., 2019), thus highly contributing to shaping the soil microbiome (Wieland et al., 2001; Garbeva et al., 2004; Nunan et al., 2005). Similar studies pointed out that variations in plant root exudates influence the diversity of the plant root microbiome (Grayston et al., 1998; El Zahar Haichar et al., 2008). As quantity and composition of root exudates are plant species-specific (Gransee and Wittenmayer, 2000; Gargallo-Garriga et al., 2018), each plant can shape its specific soil microbiome (Berg and Smalla, 2009). We can thus

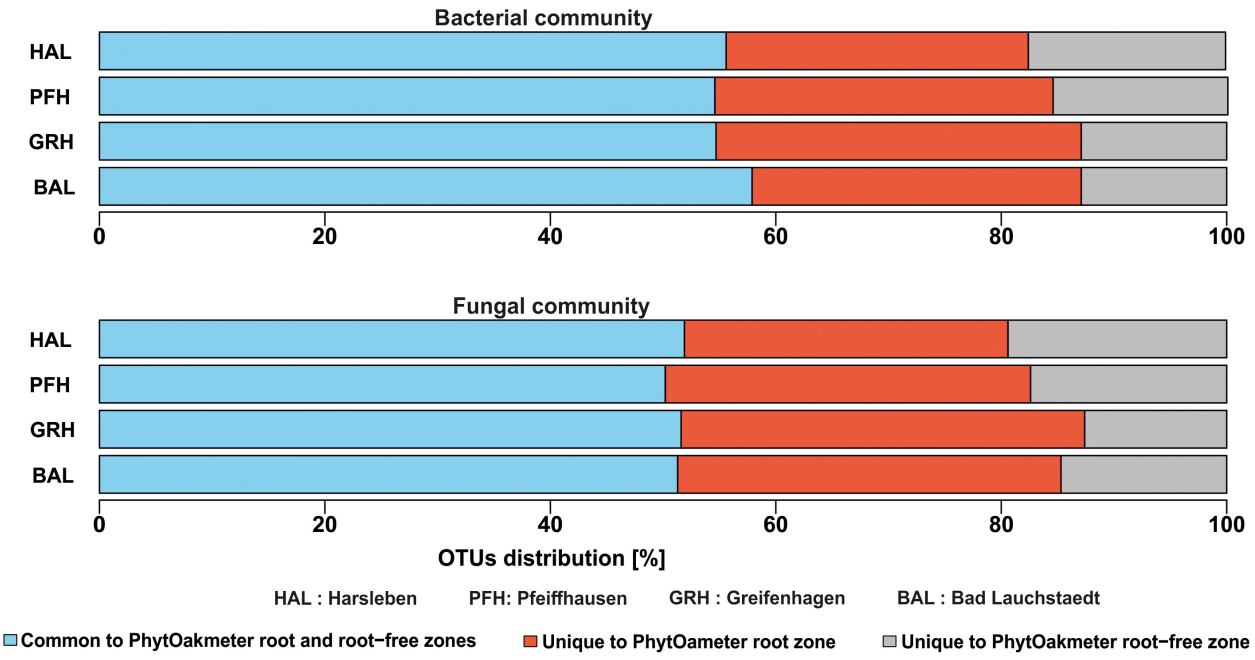

FIGURE 4 | Overlap of bacterial and fungal OTUs between PhytOakmeter root zone and the tree root-free zone.

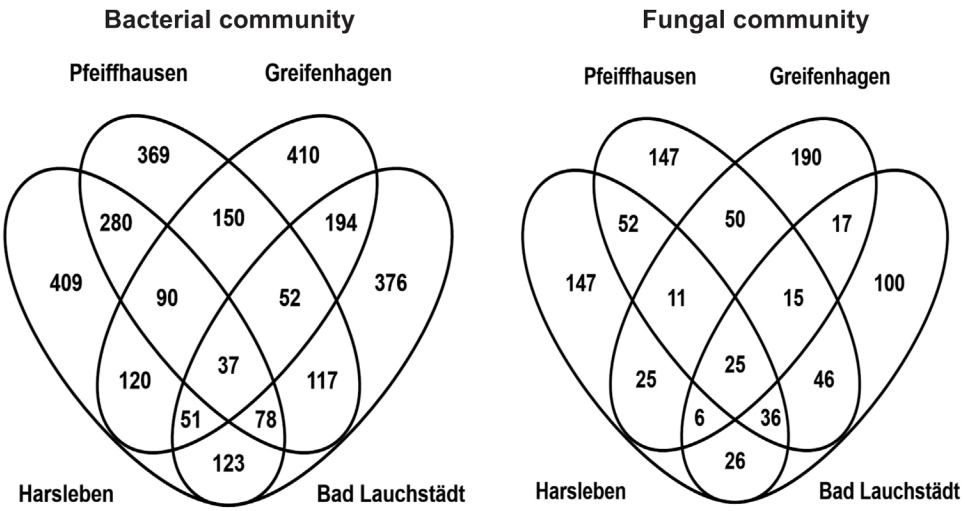

FIGURE 5 | Venn diagrams showing an overlap of OTUs exclusive to PhytOakmeter root zone among the field sites.

infer that genetically identical plants create within their root zones comparable microbial niches, resulting in similar diversity of their root-associated microbiomes.

Second, all the study sites share a similar climate with parallel weather variations. Temperature, the most important variable in defining the climate of a region, is one of the main factors influencing the occurrence, richness, stability, and activity of soil microorganisms (Borowik and Wyszkowska, 2016). Both atmospheric and soil temperatures were reported to impact on the soil microbiome (Alkorta et al., 2017). Atmospheric temperature has direct effect on soil temperature and indirectly affects host plant productivity as well as availability of carbon sources for microbial growth (Anderson, 1992; Bardgett et al., 1999). Also, both directly and indirectly, soil temperature significantly shapes the conditions for growth and development of microorganisms (Borowik and Wyszkowska, 2016). Directly, soil temperature influences microbial metabolism while the indirect effects are noticed via its impacts on plant productivity (Jefferies et al., 2010). Comparable atmospheric and soil temperatures amongst the study field sites may have also had an important contribution to the similar microbial diversity.

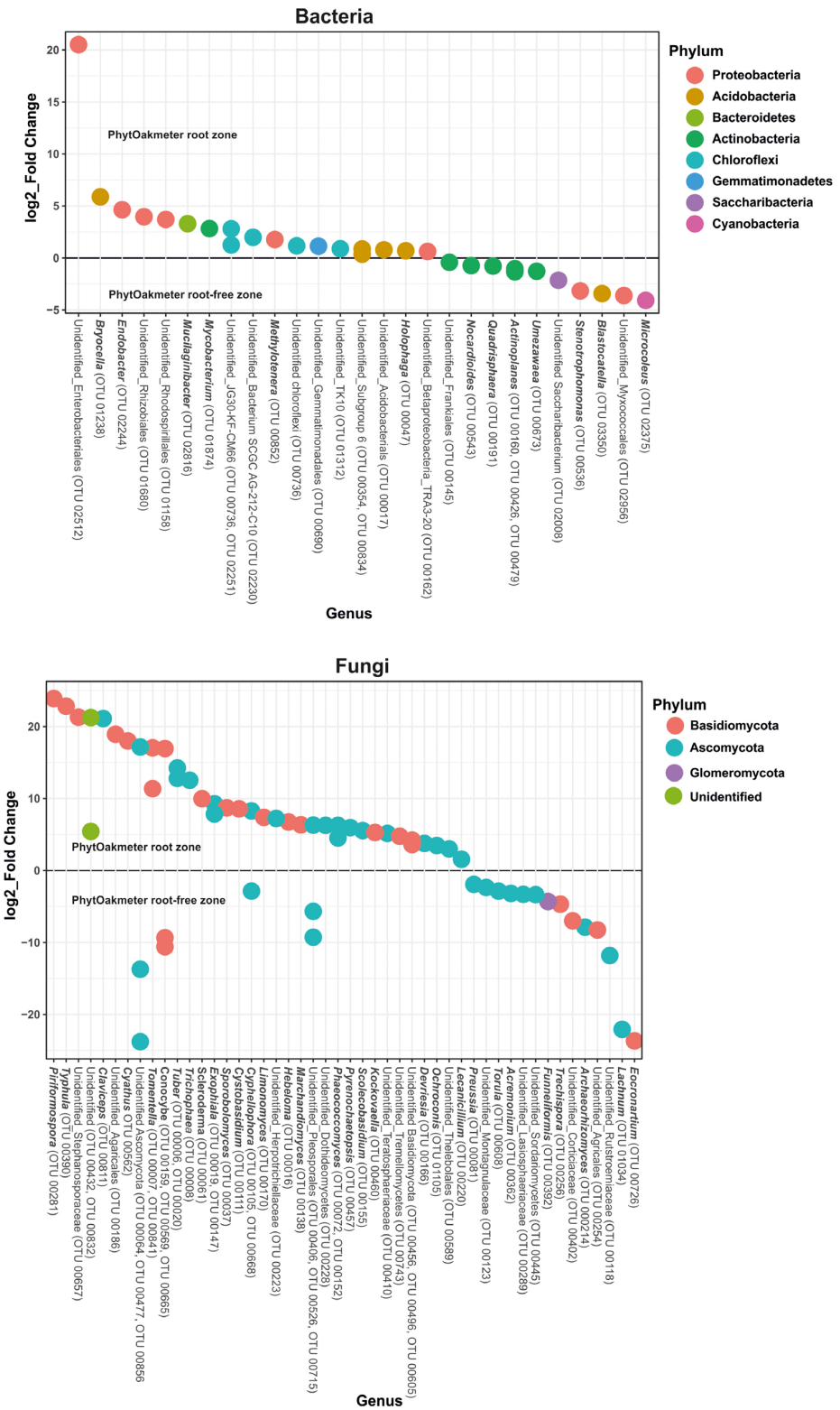

FIGURE 6 | Differential abundance test for bacterial and fungal genera using Phyloseq and DESeq2. The graphs represents log_2_fold change of the microbial genera with significantly different abundance ($p < 0.05$) in the PhytOakmeter root zone compared to the tree root-free zone. A positive value signifies higher abundance while a negative value means lower abundance of the respective genera within the PhytOakmeter root zone compared to the tree root-free zone.

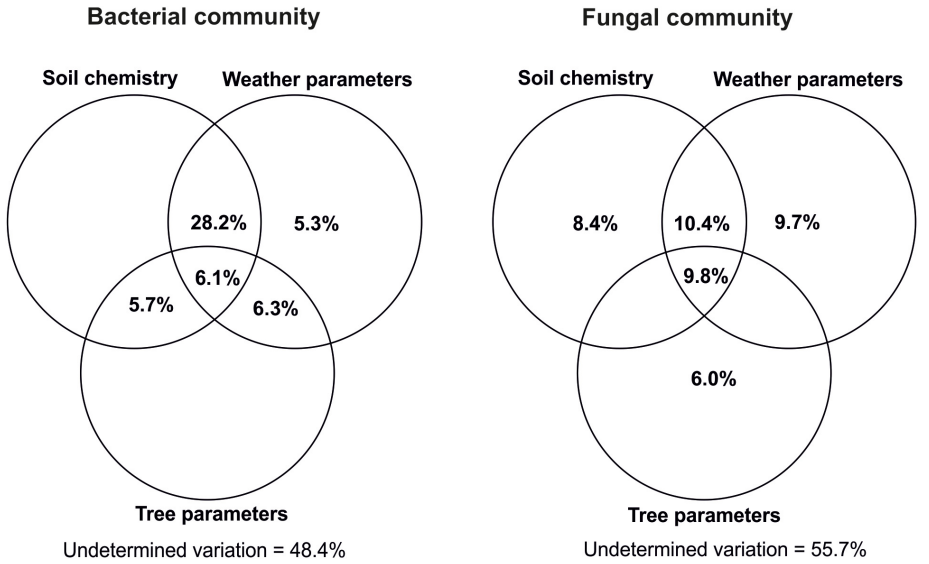

FIGURE 7 | Variance partitioning analysis of the respective impacts of soil chemistry, weather, and host tree growth parameters on variations within bacterial and fungal communities. Soil chemistry included pH and soil organic matter content (SOC, TN, C/N, CWC, CWN, CWC/CWN, HWC, and HWN). Weather data included annual precipitations as well as monthly mean atmospheric and soil temperatures in the period of January 2014–September 2016. Tree growth-related parameters were height at the outplanting time, height increases in 2015 and 2016, shoot flushes produced in 2016 vegetative period, height of 2016 first shoot flush (SF1) as well as fresh and dry matter weight of SF1 leaves produced in 2016. Each circle represents the portion of variation accounted by each factor. Shared variance is represented by the intersecting portions of the circles. Values ≤ 0 are not shown. The calculations were done by using all the OTUs found within the host tree root zone.

Lastly, all the sites are grassland. As roots of herbaceous plants highly impact soil microbial communities (Burke et al., 2009), herbaceous plant cover may have contributed a lot to the noticed similar microbial Shannon diversity between host tree root and root-free zones within individual sites. This assumption is supported by Christie et al. (1978) who reported that one plant root-associated microbiome can be influenced by neighboring plants. Therefore, herbaceous plant cover may have extended their effect to the PhytOakmeter root zone and, thus, contributed to homogenize microbial diversity between the host tree root and root-free zones at the individual grassland field sites.

Differences in Microbial Community Structure Among the Field Sites

As indicated by NMDS plots and ANOSIM, structure of the microbial communities was in fact revealed different from site to site in spite of their similar microbial diversity levels. With this, we rejected the second part of our first hypothesis which predicts high similarity in microbial community structure among the field sites. In general, the noticed difference might reflect the micro-heterogeneity of soil habitat (Buscot, 2005) among the sites in addition to their land use history. Besides, spatial isolation among the field sites may have also contributed to their differences in microbial community structure. According to various reports, spatial isolation leads to microbial species endemic to specific field sites (Zhou et al., 2002) and, therefore, to variations in soil microbial community, even within a single region (Bokulich et al., 2014; Zarraonaindia et al., 2015; Gourmelon et al., 2016).

Differences in soil pH and organic matter content can also be used to further explain the different microbial communities among the sites. This is supported by previous reports such as Eiland et al. (2001), Fierer and Jackson (2006), Medeiros et al. (2006), Zhalnina et al. (2015), and Xue et al. (2018). From this view, repartition of the sites into distinct groups, as shown by our NMDS plot analyses, can be explained relying on similarities in soil pH and organic matter content. Comparable pH, SOC, and C/N between Harsleben and Pfeiffhausen matched with the NMDS plot results where their soil microbiomes were found to be more similar. Comparable C/N and TN content between Bad Lauchstädt and Harsleben are also consistent with the similarity level of their respective microbial communities. In the same way, similar level of C/N, HWC, and HWN between Greifenhagen and Bad Lauchstädt relate to their comparable microbial community structure.

On the contrary, all the sites had the same microbial phyla with similar proportion. Proteobacteria and Ascomycota dominated the overall bacterial and fungal communities, respectively. High abundance of Proteobacteria was previously reported within numerous types of ecosystems, such as in grasslands (Singh et al., 2007), croplands (Tian and Gao, 2014), forest-grass ecosystems (Zeng et al., 2016), and natural hardwood forest soils (Lin et al., 2011). Ascomycota were reported dominant in soil fungal communities of semi-arid (Porras-Alfaro et al., 2011) and temperate (Prober et al., 2015; Chen et al., 2017) grasslands, oppositely to forest soils dominated by Basidiomycota (Goldmann et al., 2015; Terhonen et al., 2019).

Differences in Microbial Community Composition Between Soils of PhytOakmeter Root and the Root-Free Zones

Comparison between the PhytOakmeter root and root-free soil compartments confirmed our third initial hypothesis about higher abundance of some particular soil microbial taxa in the PhytOakmeter root zone. We found more microbial OTUs exclusive to the host tree root zone than the OTUs uniquely detected within the tree root-free zone. This indicates that, after two years of their field outplant, PhytOakmeter trees had already exerted significant effect on local microbial communities regardless of legacy effects of previously existing vegetation. This opposes Elgersma et al. (2011) who reported soil microbial structure to be not affected by the current vegetation two years after transplantation, rather largely determined by the legacy effect of the previous vegetation type. Examination of the PhytOakmeter root-associated microbial OTUs showed a PhytOakmeter "core" microbiome as well as a PhytOakmeter "site-specific" microbiome. Following the definition by Shade and Handelsman (2012), the PhytOakmeter "core" microbiome referred to bacterial and fungal OTUs exclusively found within the tree root zone in all the sites. Such a core microbiome has been estimated to likely play a key role in the plant soil systems among variable sites (Shade and Handelsman, 2012; Shakya et al., 2013). In the current study, however, all the PhytOakmeter "core" microbial OTUs were not identified for specific functions to the host tree itself, neither to the whole ecosystem. We also revealed PhytOakmeter site-specific microbial species, and this supported the view that plants recruit root-associated microorganisms from surrounding soil microbial reservoirs (Compant et al., 2019). The microbial recruitment by host plant roots was reported to depend on composition of the local microbial pool and microbial-host plant affinities designated as microbial host fidelity and preference (Bonito et al., 2014; Compant et al., 2019). In herbaceous plants, this process was shown to be promoted by nutrients and signaling molecules present in the plant exudates (Marschner et al., 2004; Prescott and Grayston, 2013; Jacoby et al., 2017). Similar processes were also observed for trees (Landeweert et al., 2001; Gahan and Schmalenberger, 2014). Metabolites exuded by the host tree serve to recruit and subsequently support or inhibit multiplication of particular microbial taxa within the tree root zone (Garbeva et al., 2004; Bais et al., 2006; Lareen et al., 2016). In line with these previous findings, our current study also revealed some highly abundant bacterial and fungal genera in the PhytOakmeter root zone compared to the tree root-free zone of soils.

Plant roots can attract beneficial microorganisms from surrounding soil, and those play important roles in plant performance especially by improving plant mineral nutrition. Even though there is still limited knowledge on which particular microbes are good partners for boosting plant nutrition, it has been postulated that plants have evolved specific recognition mechanisms to discriminate beneficial microorganisms from those that need to be repelled (Jacoby et al., 2017). In the current study, none of the differentially abundant bacterial genera between PhytOakmeter root and root-free zones could be identified for their potential function. Contrarily, we were able to annotate ecological functions to a certain number of the highly abundant fungal genera within the PhytOakmeter root zone. They included *Tomentella*, *Tuber*, *Trichophaea*, *Scleroderma*, *Exophiala,* and *Hebeloma* which are ectomycorrhizal (Tedersoo et al., 2010). The ectomycorrhizal fungi assist their associated plants to draw more nutrients and water from the soil as well as to increase the plant tolerance to different environmental stresses (Tedersoo et al., 2010). In recruiting the ectomycorrhizal fungal genera, the PhytOakmeter trees may have been targeting such an important contribution to the host plant health. Compared to the tree root-free zone, PhytOakmeter root zone was also enriched in yeast genera *Phaeococcomyces* (Butler et al., 2004), *Sporobolomyces* (Wang et al., 2015), *Cystobasidium* (Ramos-Garza et al., 2015; Yurkov et al., 2015), and *Cyphellophora* (Feng et al., 2014). Yeasts are essential in ecological processes involving mineralization of organic matter (Botha, 2011). The tree root zone incorporated as well *Marchandiomyces* whose several species are lignicolous (DePriest et al., 2005; Lawrey et al., 2008), and saprotrophic genera such as *Ochroconis* (Gams, 2015) and *Typhula* (Shiryaev and Kotiranta, 2007) which participate in breaking down of complex organic molecules. Our findings agree with the previously reported ectomycorrhizal status of oaks (Herrmann and Buscot, 2007) and the tree ability to interact with large microbial communities which assist in nutrients acquisition (Jumpponen and Jones, 2009; Tarkka et al., 2013). The tree root-associated microorganisms are well-known to serve in improving tree health and nutrition, preventing establishment of pathogens, and adapting to specific local environmental conditions (Uroz et al., 2016; Gehring et al., 2017; Lau et al., 2017).

Microbial Communities in the Host Tree Root Zone Are Shaped More by Environmental Parameters Than by the Host

Contribution of the environmental parameters to variations within bacterial and fungal communities of the PhytOakmerer root zone soil was found to be higher than contribution of the tree growth-related parameters. This finding might be due to two main reasons: (1) Host trees were still very young (only two years, i.e., two vegetation periods, in the field). Even more, the first vegetation period for trees after field release corresponds to a transplant shock. This period consists of acclimation to local soil environment and regeneration of the root system (Hargrave et al., 2002). After the transplant shock period, PhytOakmeters had practically only one single vegetation period to impact on surroundings and, apparently, this was not enough to exert a huge effect on local soil microbial community. The dependency of soil microbial community on the host tree age seems to be high. As previously proved, soil microbial communities associated with roots of perennial plant change in both richness and composition over the host's lifetime. After out-planting, the plants replace a common soil microbial community they were exposed to as saplings with local communities of their respective field sites. From there on, the host plants continue to shape

their respective root-associated microbial communities. These development dynamics were previously reported by Wagner et al. (2016) and Goldmann et al. (2020). (2) The soil was sampled in the tree root zone rather than rhizosphere where high tree effect on microbial community could be expected. As previously reported, the rhizosphere is known as a nutrient-rich compartment in the soil influenced by the plant. In the rhizosphere, carbon compounds, which serve as the main food and energy source for soil microbes, are continuously introduced via rhizodeposition and sloughed-off cells (Breidenbach et al., 2016). Sampling the host PhytOakmeter root zone rather than the rhizosphere led to dilute the host tree influence on the soil inhabiting microorganisms. However, even though tiny, the impact revealed at this young age of the trees is remarkable especially in the context of a temperate climate that does not promote rapid tree growth. Until now such quick effects of tree planting on soil microbial communities had been reported in the subtropics (Weißbecker et al., 2018).

CONCLUSION AND FUTURE PERSPECTIVES

In conclusion, there is a high similarity in microbial biodiversity among the field sites but their microbial community structure is different. Even though still young, the capability of PhytOakmeters to recruit a specific beneficial microbiome in their root zone from surrounding microbial reservoirs was evidenced. The study revealed concurrent impact of environmental parameters and the host PhytOakmeter in shaping soil microbiome of the host tree root zone, but the magnitude of environmental parameters was higher than the impact of the host tree. Since this finding is likely based on the age of the trees, a similar study with older host trees is needed. For this, further measures of soil properties, such as information on texture, might even explain more microbial variance. Ideally, the investigation of the root endophytic compartment and/or the rhizosphere would be beneficial to unravel the PhytOakmeter-microbe interaction further. Moreover, the analysis of PhytOakmeter effects on soil microbiome at a large-scale is also required to move toward a comprehensive understanding of the tree root microbiome assemblage, and to have a better overview on mutual impacts between host tree and environmental variables in shaping the tree root zone microbiome. Nevertheless, our presented approach is an important step toward more integrative studies using clonal trees, and provides an opportunity to perform long-term interaction biomonitoring.

DATA AVAILABILITY STATEMENT

The raw sequences generated for this study can be found in the European Nucleotide Archive (ENA). Accession number PRJEB35688.

AUTHOR CONTRIBUTIONS

FB and SH conceived the project and designed the sampling. SH performed the sampling. JH, SH, and TR performed the laboratory works. JH and KG analyzed and interpreted the results. JH and KG wrote the manuscript with input from SH, TR, and FB. All authors contributed to revisions of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2020.00749/full#supplementary-material

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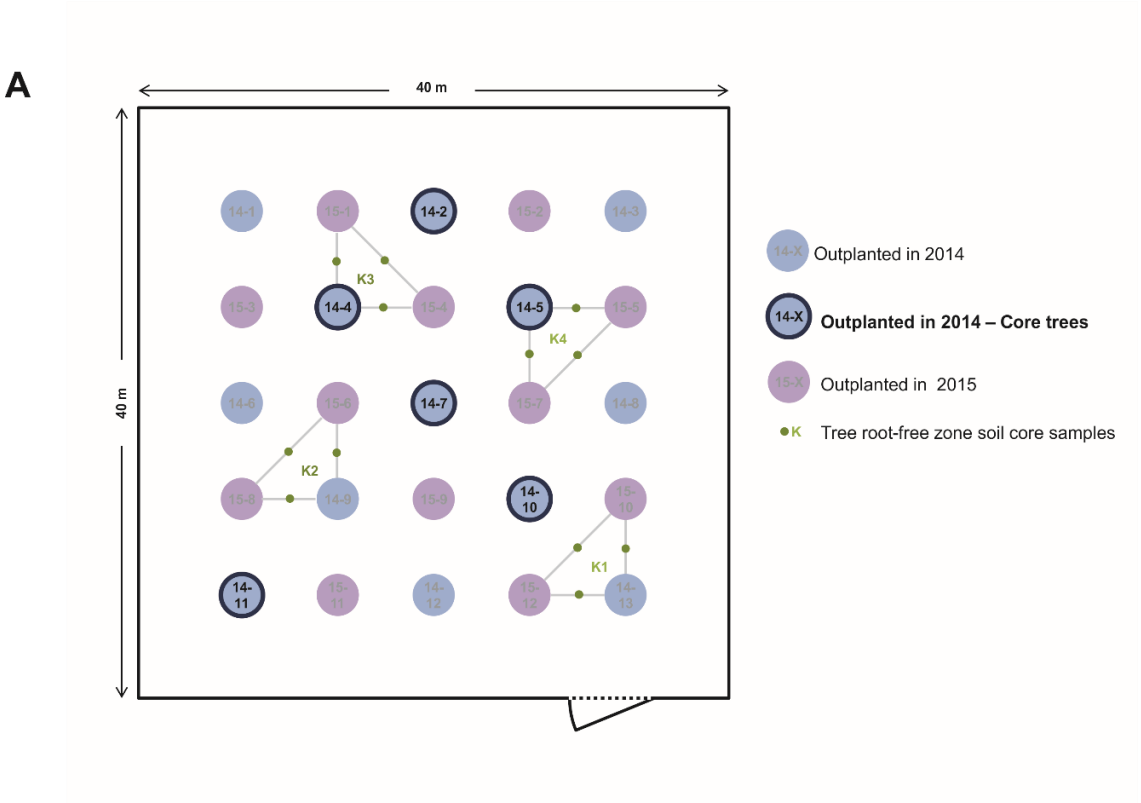
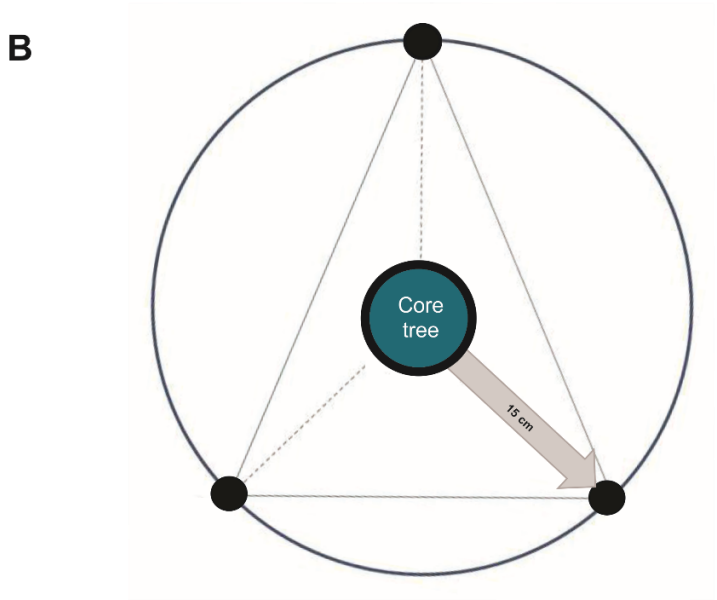
Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Supplementary material

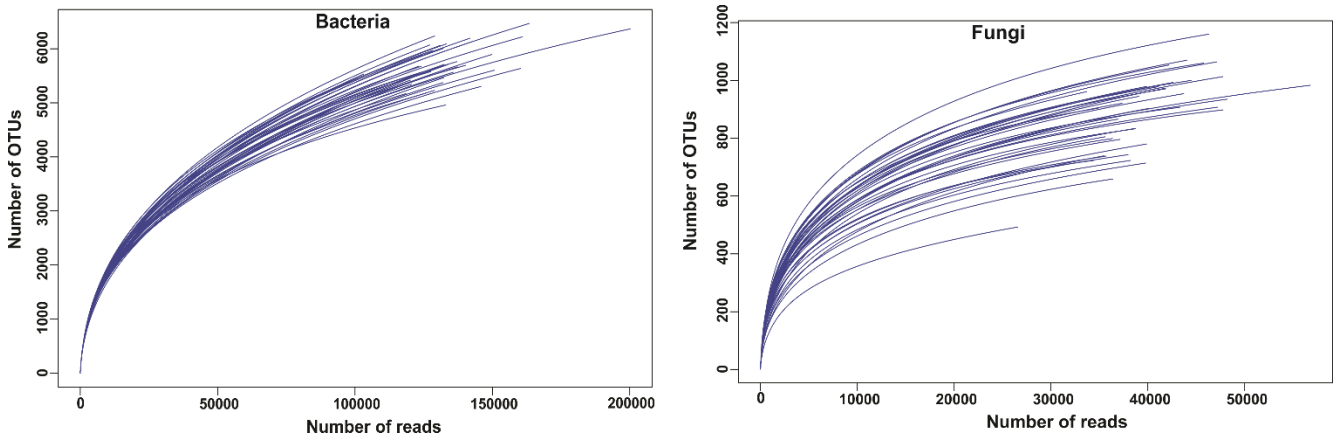
Supplementary figures

Supplementary Figure S1. Harsleben field site at sampling time in November 2016. Harsleben and the other field sites are grasslands with plot soil surface entirely covered by herbaceous plants. **(A)** General overview of the field plot. (**B**) Zoomed-in PhytOakmeter tree within the field plot.





Supplementary Figure S2. (A) Plot sampling design overview, case of Harsleben field site. In total, ten samples were taken (six samples were taken within root zone of six core trees and four samples were taken in the tree root-free zone). The three subsamples of each tree root-free zone were taken in positions illustrated by green dots, and pooled to respectively make composite samples K1, K2, K3 and K4. **(B)** Sampling positions within PhytOakmeter root zone. As indicated by three black balls, the three subsamples of every tree root zone were taken at 120° angle around a selected tree, 15 cm horizontal distance from the tree trunk, and pooled together.



Supplementary Figure S3. Individual rarefaction curves of bacterial and fungal OTUs at a 97% similarity level of all 38 soil samples.

Supplementary tables

Supplementary Table S1. Weather data among the study field sites. The parameters were measured at the weather stations of the Helmholtz Centre for Environmental Research-UFZ. Atmospheric temperatures were measured at 200 cm above soil surface, while soil temperatures represent an average of the upper 20 cm calculated from single measurements at 5, 10, and 20 cm soil depth. The here presented weather data encompass the period of January 2014 (year of host trees out-planting) to December 2016, but the ones used for further analysis go up to September 2016 (time of soil sampling). According to one-way ANOVA, no significant differences were observed amongst the sites' mean values.

Weather variables	Parameter	Harsleben	Pfeiffhausen	Greifenhagen	Bad Lauchstädt
Precipitation [mm]	Annual total 2014	555.2	540.5	612.4	452.6
	Annual total 2015	516.9	439.6	488.2	399.9
	Annual total 2016	365.9	333.2	407.4	437.2
	January-September 2016	274.8	248.8	298.5	337.1
	Grand total 2014-Sep 2016	1,346.9	1,228.9	1,399.1	1,189.6
Atmospheric temperature [°C]	Annual mean 2014(+SD)	11.1 (±5.9)	10.8 (±6.2)	10.1 (±6.0)	11.0 (±6.2)
	Annual mean 2015(+SD)	10.6 (±6.1)	10.3 (±6.2)	9.7 (±6.3)	10.7 (±6.5)
	Annual mean 2016(+SD)	12.3 (±7.2)	11.9 (±7.6)	11.0 (±7.5)	12.1 (±7.6)
	Overall mean (+SD)	11.2 (±6.2)	10.9 (±6.4)	10.2 (±6.3)	11.2 (±6.5)
	Maximum monthly mean	20.7	20.6	20.2	21.2
	Minimum monthly mean	1.7	0.5	0.2	1.0
Soil temperature [°C]	Annual mean 2014(+SD)	11.6 (±6.2)	11.4 (±6.5)	10.7 (±5.7)	11.9 (±6.9)
	Annual mean 2015(+SD)	11.1 (±6.5)	10.6 (±6.7)	10.0 (±6.0)	11.4 (±7.3)
	Annual mean 2016(+SD)	13.0 (±7.4)	12.3 (±7.5)	11.7 (±6.9)	13.1 (±8.0)
	Overall mean (+SD)	11.9 (±6.7)	11.4 (±6.9)	10.7 (±6.2)	12.1 (±7.2)
	Maximum monthly mean	21.3	20.9	19.5	22.3
	Minimum monthly mean	2.0	1.3	1.6	1.7

Data source*: Meteorological data, Helmholtz Centre for Environmental Research – UFZ*

Supplementary Table S2. Overview of the used bacterial 16S rDNA and fungal ITS2 primers (Hendgen et al., 2018). Abbreviations according to IUPAC Ambiguity Code: A - adenine, C - cytosine, G - guanine, T -tyrosine, N - “aNy” base, ie A, C, G or T/U (Johnson, 2010)

Primer name	Primer sequence 5’-3’
P5-8N-515F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNNNNNGTGCCAGCMGCCGCGGTAA
P5-7N-515F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNNNNGTGCCAGCMGCCGCGGTAA
P7-2N-806R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTNNGGACTACHVGGGTWTCTAAT
P7-1N-806R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTNGGACTACHVGGGTWTCTAAT
P5-5N-ITS4	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNNTCCTCCGCTTATTGATATGC
P7-4N-fITS7	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTNNNNGTGARTCATCGAATCTTTG

Supplementary Table S3: Results of linear model analysis testing the correlation between environmental parameters and the microbial Shannon diversity index results. We first removed auto-correlated parameters using the variance inflation factor (VIF < 5); then the remaining parameters were differently combined into various models and tested against the microbial Shannon diversity index results. The obtained regression models were then evaluated to choose the best approximating model by using Akaike's Information Criterion (AIC). Based on AIC values and significant correlation to the microbial Shannon diversity, the best model included CWC, P, soil moisture and soil temperature for bacteria ($p < 0.001$ and adjusted $R^2 = 0.47$), while it included CWC and soil temperature for fungi ($p < 0.05$, adjusted $R^2 = 0.12$).

	Bacteria			Fungi		
Model parameters/components	AIC	p	Adjusted R^2	AIC	p	Adjusted R^2
pH, TN, C/N, CWC, NO_3-N, total mineral N, K, P, soil moisture and soil temperature	-93.0	<0.01	0.44	53.6	0.08	0.22
TN, C/N, CWC, K, P, soil moisture and soil temperature	-105.1	<0.001	0.49	50.3	0.21	0.09
CWC, K, P, soil moisture and soil temperature	-111.9	<0.001	0.49	42.9	0.12	0.11
CWC, P, soil moisture and soil temperature	**-112.6**	**<0.001**	**0.47**	41.9	0.13	0.09
CWC, soil moisture and soil temperature	-111.3	<0.001	0.43	39.5	0.08	0.11
CWC and soil temperature	-111.9	<0.001	0.41	**37.4**	**0.04**	**0.12**
CWC, P and soil temperature	-112.9	<0.001	0.45	39.5	0.08	0.11

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CHAPTER 3

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

Jean de Dieu Habiyaremye, Sylvie Herrmann, Thomas Reitz, François Buscot, and Kezia Goldmann

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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

Jean de Dieu Habiyaremye,[1,2,3*] **Sylvie Herrmann**,[1,4] **Thomas Reitz**,[1,4] **François Buscot**[1,2,4] **and Kezia Goldmann**[1]

[1]*Department Soil Ecology, Helmholtz Centre for Environmental Research–UFZ, Halle, Germany.*
[2]*Institute of Biology, Leipzig University, Leipzig, Germany.*
[3]*Department of Mathematics, Science and Physical Education, University of Rwanda, Kigali, Rwanda.*
[4]*German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, Leipzig, Germany.*

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 saplings 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 affinity 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 parameters. 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.

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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, environmental heterogeneity potentially induces variations in the spatial distribution of soil microorganisms (Green *et al*., 2004; Green and Bohannan, 2006). Thereby, abiotic 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 microbial 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 (°C)	5.3(±0.7)^{c}	10.1(±0.7)^{b}	10.1(±0.6)^{b}	13.7(±0.5)^{a}
MAP (mm)	661(±91)^{d}	495(±83)^{c}	1142(±152)^{a}	793(±92)^{b}
Soil physico-chemistry				
pH_{CaCl2}	5.5(±0.1)^{d}	6.4(±0.2)^{c}	6.7(±0.2)^{b}	7.2(±0.1)^{a}
Moisture (%, wt./wt.)	20.0(±1.9)^{a}	6.0(±0.6)^{c}	14.6(±1.2)^{b}	6.7(±1.1)^{c}
TC (%)	2.7(±0.3)^{b}	2.1(0.2)^{c}	3.2(±0.3)^{a}	2.0(±0.1)^{c}
TN (%)	0.20(±0.02)^{b}	0.15(±0.01)^{c}	0.30(±0.03)^{a}	0.14(±0.02)^{c}
TC/TN	13.7(±1.2)^{a}	13.8(±0.8)^{a}	10.9(±0.3)^{b}	14.9(±2.1)^{a}
HWC (mg kg^{-1})	959(±135)^{a}	660(±73)^{b}	1069(±104)^{a}	745(±103)^{b}
HWN (mg kg^{-1})	60.6(±6.9)^{b}	53.8(±4.5)^{b}	84.0(±9.0)^{a}	74.9(±12.1)^{a}
HWC/HWN	15.8(±1.3)^{a}	12.2(±0.5)^{b}	12.7(±0.4)^{b}	10.0(±0.6)^{c}
CWC (mg kg^{-1})	159(±25)^{a}	113(±9)^{b}	172(±31)^{a}	110(±15)^{b}
CWN (mg kg^{-1})	11.8(±1.4)^{b}	10.9(±0.9)^{b}	17.9(±4.7)^{a}	21.6(±7.9)^{a}
CWC/CWN	13.5(±1.6)^{a}	10.5(±0.9)^{b}	9.9(±1.8)^{b}	5.7(±1.9)^{c}
NH_4^+-N (mg kg^{-1})	4.4(±0.8)^{a}	3.6(±1.3)^{a}	3.6(±0.6)^{a}	2.0(±0.8)^{b}
NO_3^--N (mg kg^{-1})	2.1(±0.6)^{c}	4.6(±1.6)^{b}	7.7(±6.3)^{b}	18.1(±13.9)^{a}
N_{min} (mg kg^{-1})	6.5(±1.2)^{b}	8.2(±2.2)^{b}	11.3(±6.8)^{ab}	20.1(±13.8)^{a}
K_{CAL} (mg kg^{-1})	212.2(±44.9)^{a}	116.4(±43.7)^{b}	4.5(±1.2)^{c}	178.1(±48.4)^{a}
P_{CAL} (mg kg^{-1})	72.3(±12.5)^{a}	24.8(±6.7)^{c}	12.2(±2.4)^{d}	36.5(±5.4)^{b}
Oak phytometer growth and performance				
Height at outplanting (cm)	62.8(±6.8)^{b}	75.3(±5.8)^{a}	64.8(±6.3)^{b}	57.0(±7.0)^{b}
Tree height in 2018 (cm)	142.2(±25.4)^{c}	240.5(±24.8)^{b}	285.8(±57.2)^{ab}	309.7(±49.2)^{a}
Tree height increase since outplanting (%)	129.0(±49.2)^{c}	219.1(±20.8)^{b}	348.0(±106.3)^{a}	451.5(±125.8)^{a}
Tree height increase in 2018 (%)	12.2(±5.5)^{b}	24.9(±17.5)^{ab}	15.3(±9.1)^{b}	44.6(±23.9)^{a}
LB with SF1	4.0(±0.0)	4.0(±0.0)	3.3(±0.8)	3.8(±0.4)
LB with SF2	0.2(±0.4)^{b}	1.0(±1.3)^{b}	3.0(±0.9)^{a}	3.8(±0.4)^{a}
LB with SF3	0.0^{b}	0.0^{b}	0.0^{b}	2.5(±1.4)^{a}
SF1 length (cm)	8.0(±1.8)^{b}	11.9(±2.5)^{a}	6.9(±4.0)^{b}	7.8(±5.5)^{ab}
LB total length (cm)	18.2 (±2.8)^{c}	47.0(±20.9)^{b}	106.7(±17.3)^{a}	83.6(±14.9)^{a}
LB % length increase in 2018	88.9 (±55.4)^{ab}	71.8(±44.2)^{ab}	37.9(±19.0)^{b}	82.2(±22.0)^{a}
Leaves' number on SF1 of LB	7.9(±2.5)^{b}	11.2(±1.4)^{a}	8.2(±1.8)^{b}	8.7(±2.0)^{b}
$LDMC_{SF1}$	0.44(±0.01)^{c}	0.51(±0.01)^{b}	0.50(±0.01) ^{b}	0.56(±0.03)^{a}
SLA_{SF1} (cm^2 mg^{-1})	9.9(±0.7)^{a}	8.1(±0.5)^{b}	7.4(±1.3)^{b}	7.7(±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 (±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

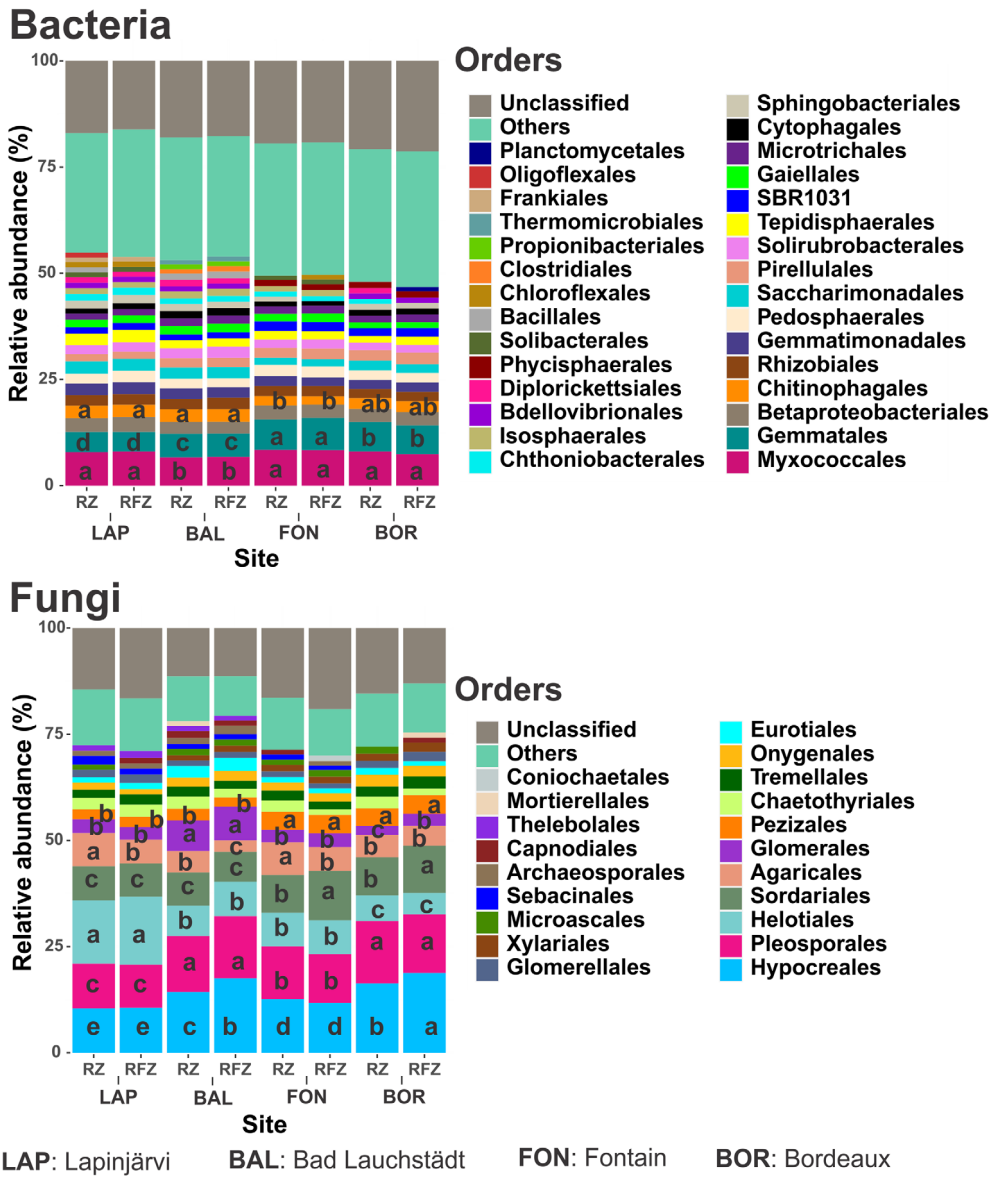

Fig 1. Compared distribution of soil bacterial and fungal orders between the tree root and root-free zones (RZ and RFZ respectively), and among field sites. Letters within the figures' rectangles indicate significant differences ($p < 0.05$) for one respective order, and this significant difference was 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 ($R = 0.52$, $p = 0.011$).

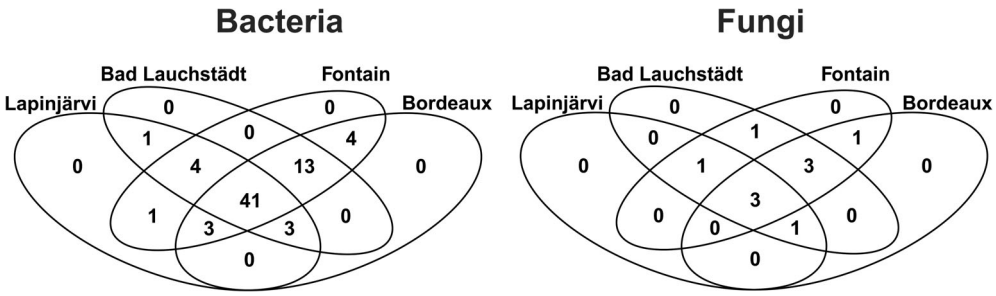

Fig 2. Overlap of the tree root zone affine microbial OTUs among the field sites.

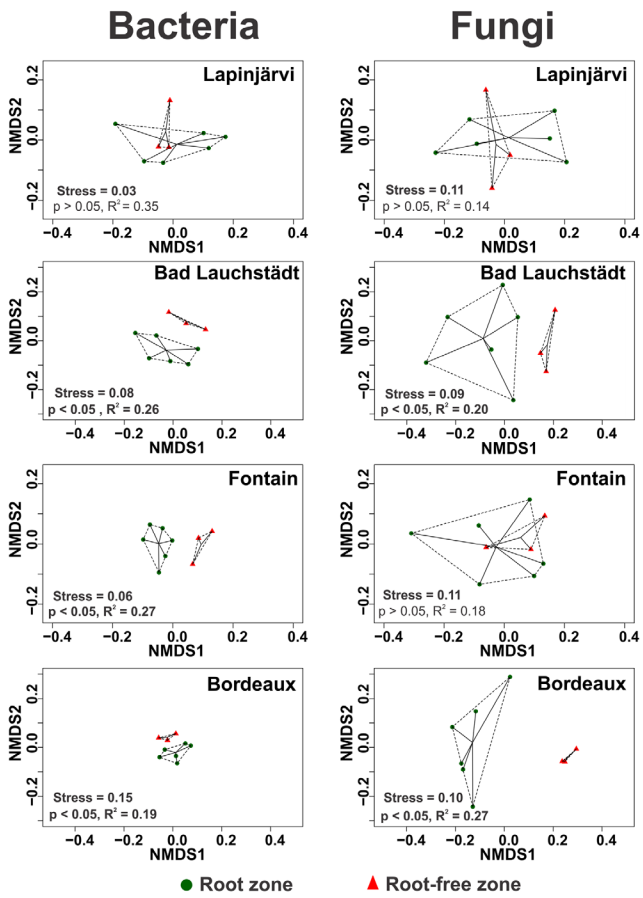

Fig 3. Non-metric multidimensional scaling (NMDS) based on Bray-Curtis dissimilarity displaying the soil bacterial and fungal communities: comparison between the tree root and root-free zones at individual field sites.

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

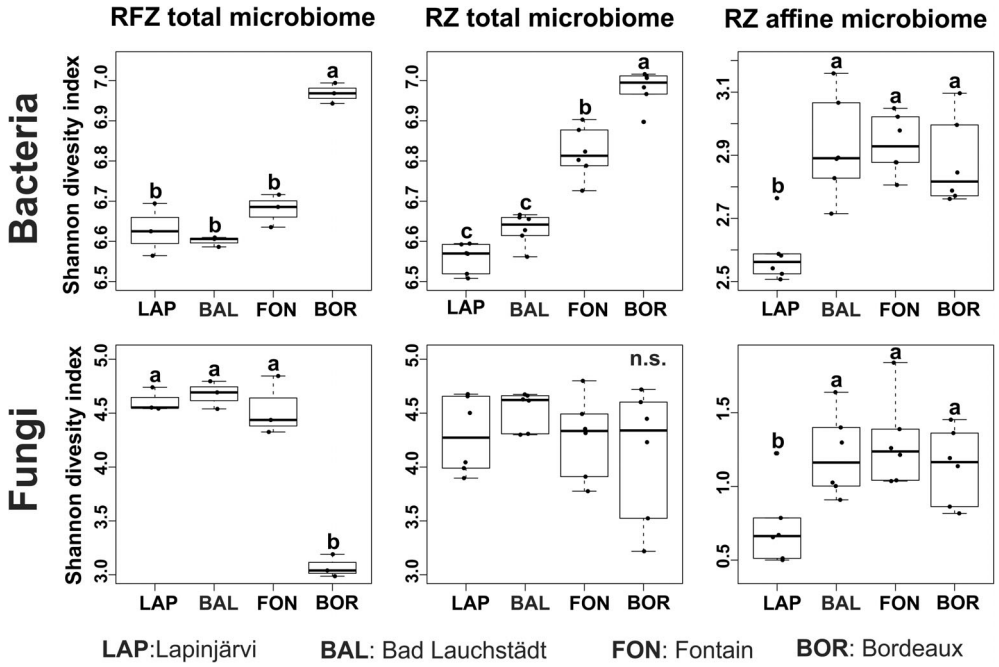

Fig 4. Soil microbial Shannon diversity along a European North–South transect. Cross comparison was done among three categories of the microbiome: (1) tree root-free zone total microbiome, (2) tree root zone total microbiome, and (3) tree root zone affine microbiome. The *y*-axes are not equally scaled 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.

	Tree RFZ total microbiome				Tree RZ total microbiome				Tree RZ affine microbiome			
	Bacteria		Fungi		Bacteria		Fungi		Bacteria		Fungi	
Site conditions	*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

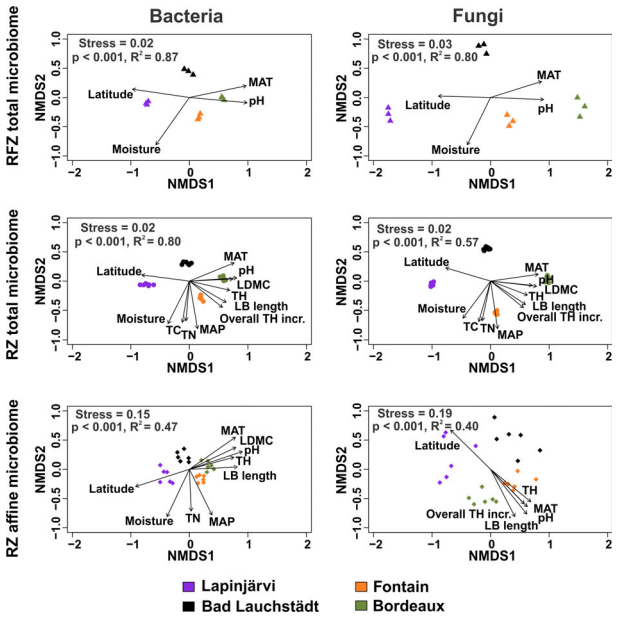

Fig 5. Non-metric multidimensional scaling (NMDS) based on Bray-Curtis dissimilarity displaying bacterial and fungal communities' structure among the field sites. Cross comparison was done among the tree root-free zone total microbiome, the tree root zone total microbiome, and the tree root zone affine 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).

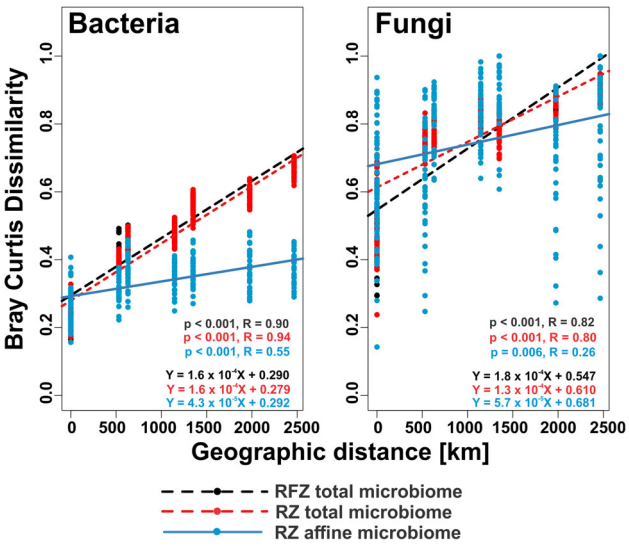

Fig 6. Correlation of Bray-Curtis dissimilarity with geographical distance among the study field sites for the soil bacterial and fungal communities. Cross comparison was done among the tree root-free zone total microbiome, tree root zone total microbiome, and the tree root zone affine microbiome.

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

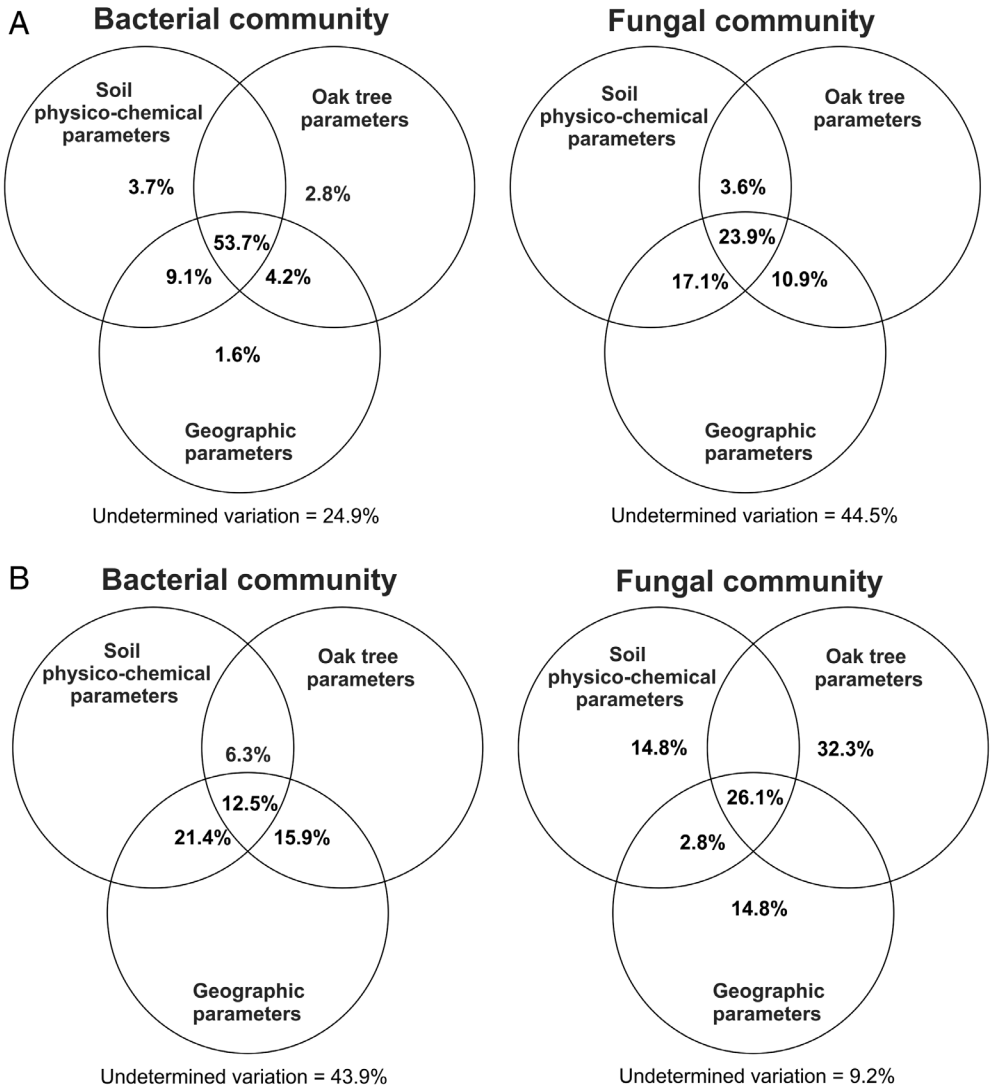

Fig 7. Variance partitioning analysis of the respective impacts of geographic, soil physico-chemical, and oak phytometer parameters on variations within the soil bacterial and fungal communities; A) Tree root zone total microbiome; B) Tree root zone affine microbiome; Geographic parameters included latitude, 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

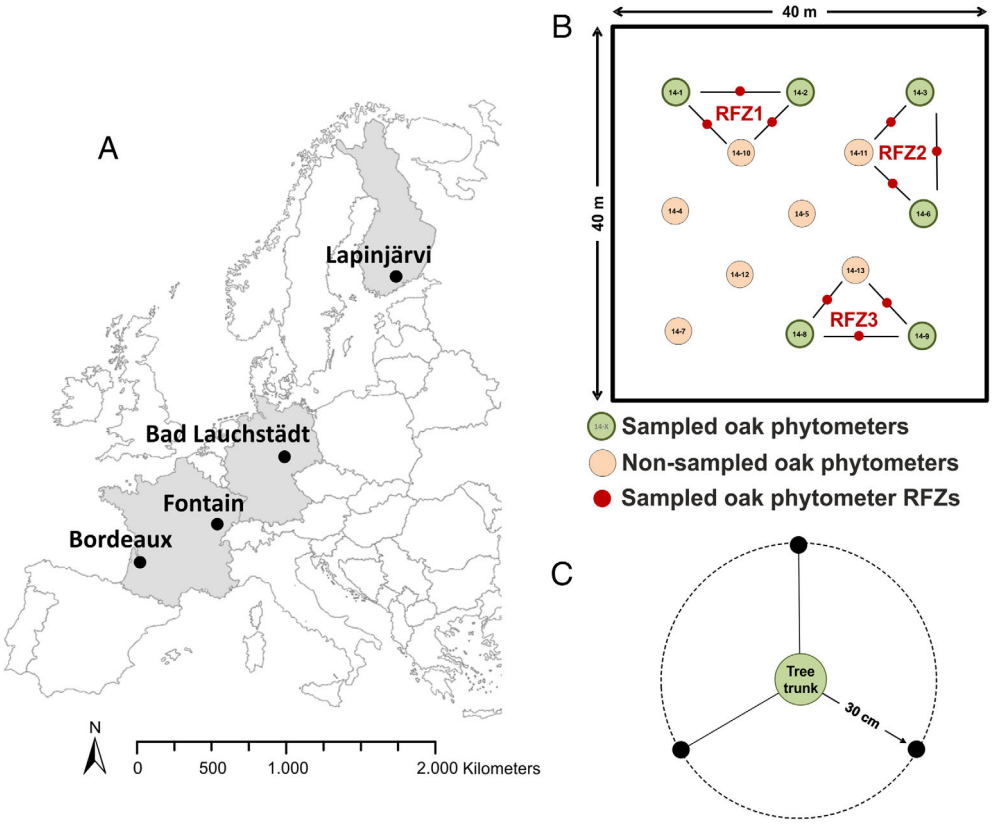

Fig 8. A) Study sites: grey sections represent the study countries, from North to South, Finland, Germany and France; black dots indicate the individual field sites; B) Bordeaux field plot design: green circles indicate investigated oak phytometer, and red dots mark the sampling positions of the three 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 outplanting 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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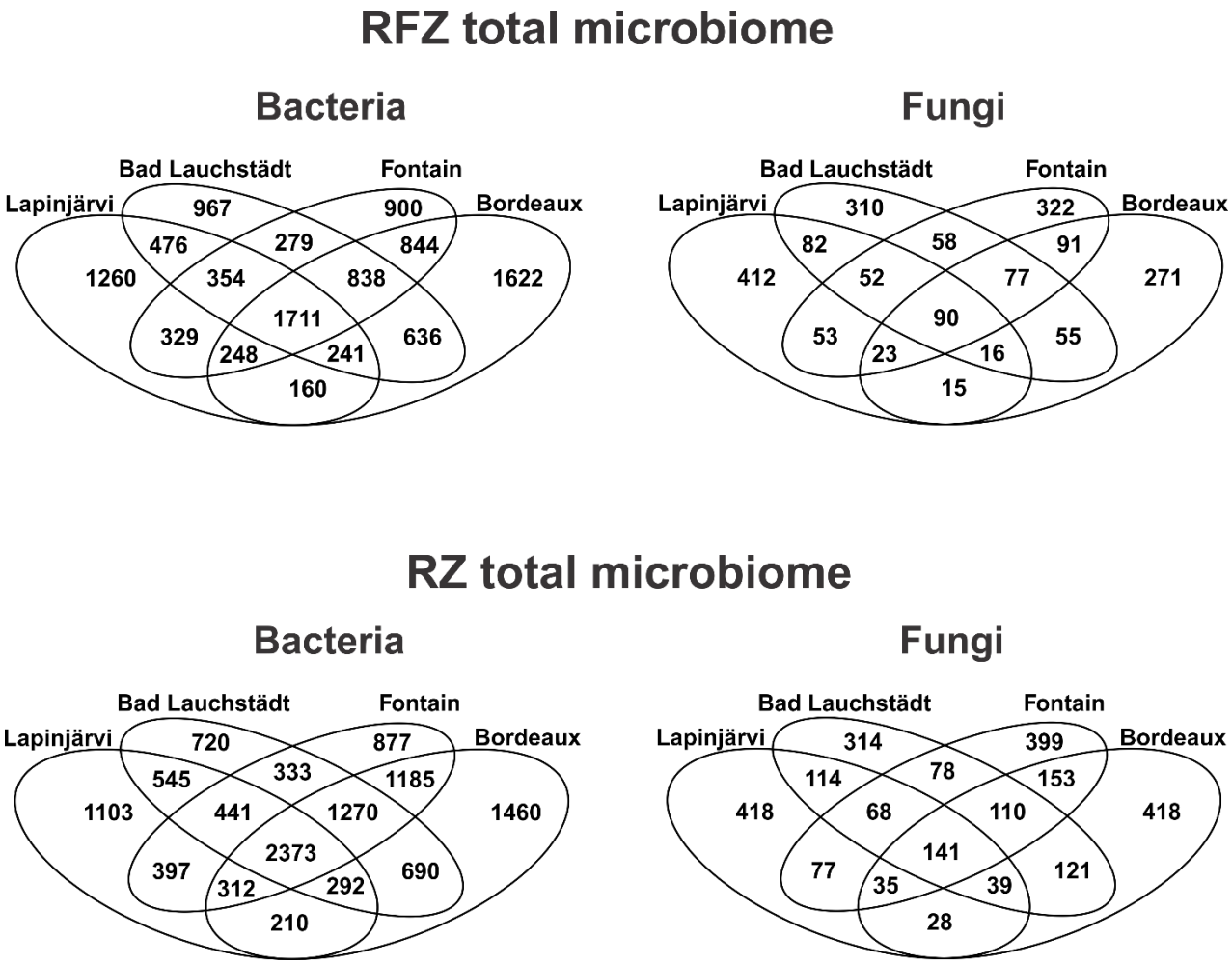
Supporting Information

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

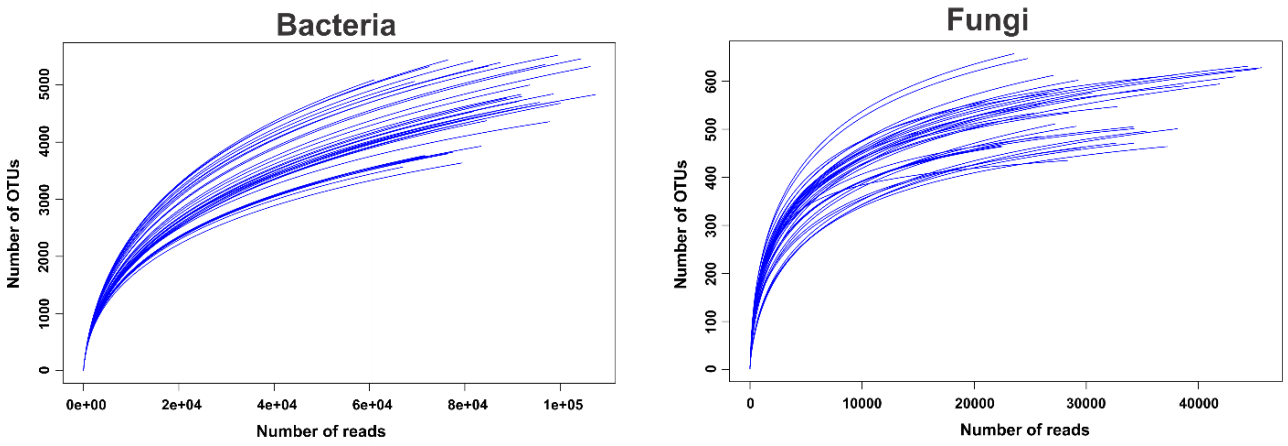
Appendix S1: Supplementary Information

Supplementary material

Supplementary figures



Supplementary Figure S1. Overlap of microbial OTUs of the tree root-free and root zones among the field sites.



Supplementary Figure S2. Individual rarefaction curves of bacterial and fungal OTUs at a 97% similarity level of all 36 soil samples.

Supplementary tables

Supplementary Table S1. Bray-Curtis dissimilarities between the communities of the oak phytometer root and root-free zones, as well as the significantly correlated oak tree parameters. Correlation was tested between the tree parameters and the mean Bray-Curtis dissimilarities of each tree root zone (from RZ1 to RZ6) versus all three root-free zones (from RFZ1 to RFZ3) of the same site.

		Bacteria				Fungi				TH by	TH incr.	
Sites	RZ	RFZ1	RFZ2	RFZ3	Mean	RFZ1	RFZ2	RFZ3	Mean	2018	in 2018	LDMC
	RZ1	0.238617	0.23742	0.240306	0.238781	0.44221	0.419094	0.423303	0.428202	140.0	4.03	6.25
	RZ2	0.183115	0.18118	0.254915	0.206403	0.414618	0.326229	0.414685	0.385177	111.5	3.33	6.25
	RZ3	0.187099	0.191018	0.257489	0.211869	0.419361	0.39845	0.454303	0.424038	101.5	3.10	12.25
	RZ4	0.237485	0.259522	0.267966	0.254991	0.471606	0.492384	0.479089	0.481026	142.5	3.30	9.25
	RZ5	0.215236	0.234272	0.25098	0.233496	0.490179	0.522982	0.505211	0.506124	109.0	3.58	6.00
LAP	RZ6	0.216957	0.242667	0.225992	0.228539	0.537413	0.560128	0.530198	0.54258	173.0	3.60	7.25
	RZ1	0.226779	0.249324	0.26413	0.246744	0.525788	0.54229	0.584246	0.550775	193.0	0.68	12.25
	RZ2	0.271049	0.272623	0.276673	0.273448	0.557256	0.538549	0.542424	0.546076	176.0	0.73	9.75
	RZ3	0.2427	0.250832	0.265031	0.252854	0.519241	0.536478	0.550508	0.535409	216.0	0.72	10.5
	RZ4	0.28515	0.267802	0.262588	0.271847	0.650454	0.600013	0.580372	0.61028	159.0	0.63	10.00
	RZ5	0.242618	0.229451	0.213317	0.228462	0.57957	0.532202	0.570551	0.560774	199.0	0.59	11.50
BAL	RZ6	0.234469	0.215449	0.202414	0.217444	0.535142	0.531734	0.491181	0.519352	235.0	1.01	13.50
	RZ1	0.250144	0.263015	0.237289	0.250149	0.404663	0.525922	0.371058	0.433881	230.5	13.39	10.75
	RZ2	0.250193	0.271508	0.243159	0.254953	0.432189	0.570283	0.499599	0.50069	318.5	11.88	8.75
	RZ3	0.215318	0.233485	0.218384	0.222395	0.447956	0.564204	0.460783	0.490981	283.5	8.75	9.33
	RZ4	0.242962	0.234633	0.214563	0.230719	0.464725	0.568413	0.436665	0.489934	257.0	11.20	6.00
	RZ5	0.219482	0.235715	0.22445	0.226549	0.475949	0.486371	0.494388	0.485569	200.0	10.48	7.66
FON	RZ6	0.209464	0.204594	0.183738	0.199265	0.498731	0.608231	0.453902	0.520288	287.5	11.49	6.50
	RZ1	0.284084	0.274328	0.278493	0.278968	0.597876	0.617117	0.610235	0.608409	236.0	10.16	10.75
	RZ2	0.262506	0.237584	0.268098	0.256063	0.483632	0.492718	0.430318	0.468889	167.0	8.57	11.67
	RZ3	0.279559	0.238551	0.26372	0.26061	0.63422	0.638295	0.633418	0.635311	185.0	8.51	8.25
	RZ4	0.272459	0.255554	0.291512	0.273175	0.761558	0.76383	0.748397	0.757928	209.0	9.67	7.00
	RZ5	0.288413	0.267442	0.265491	0.273782	0.589658	0.596473	0.598744	0.594958	179.0	6.69	7.75
BOR	RZ6	0.289938	0.252718	0.268655	0.270437	0.668894	0.676777	0.655064	0.666912	356.0	7.45	7.00

TH: Oak phytometer height in 2018, TH incr.: relative phytometer height increase, LDMC: leaf dry matter content, LAP: Lapinjärvi, BAL: Bad Lauchstädt, FON: Fontain, BOR: Bordeaux

Supplementary Table S2. Matrix of geographic distances (in km) among the field sites. The distances were computed by using the online tool GPS coordinates (https://gps-coordinates.org/distance-between-coordinates.php, accessed on 15 April 2020).

	Lapinjärvi	Bad Lauchstädt	Fontain	Bordeaux
Lapinjärvi	0			
Bad Lauchstädt	1,350.29	0		
Fontain	1,975.44	630.92	0	
Bordeaux	2,464.89	1,145.78	530.76	0

CHAPTER 4

Temporal changes and alternating host tree root and shoot growth affect soil microbiomes

Jean de Dieu Habiyaremye, Sylvie Herrmann, François Buscot, and Kezia Goldmann

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Temporal Changes and Alternating Host Tree Root and Shoot Growth Affect Soil Microbiomes †

Jean de Dieu Habiyaremye [1,2,3,*], Sylvie Herrmann [1,4], François Buscot [1,2,4] and Kezia Goldmann [1]

[1] Department of Soil Ecology, Helmholtz Centre for Environmental Research-UFZ, Theodor-Lieser-Str. 4, 06120 Halle (Saale), Germany; Sylvie.herrmann@ufz.de (S.H.); francois.buscot@ufz.de (F.B); kezia.goldmann@ufz.de (K.G.)
[2] Institute of Biology, Leipzig University, Johannisallee 21-23, 04103 Leipzig, Germany
[3] Department of Mathematics, Science and Physical Education, University of Rwanda, P.O. Box 55, Rwamagana, Rwanda
[4] German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, Deutscher Platz 5e, 04103 Leipzig, Germany
* Correspondence:jean-de-dieu.habiyaremye@ufz.de
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Abstract: Patterns of trees' endogenous rhythmic growth (ERG) and paralleled C allocation shift between root and shoot systems have been studied, but there is still a need to understand their impact in shaping soil microbiomes. Moreover, the impact of plants on soil microbial communities can be modulated or overweighed by time-induced plant and/or seasonal changes. Thus, we intended to analyze the structure of soil microbiomes as response to simultaneous alternated host tree root and shoot flushes and time-induced changes within one vegetation period at two sites in Central Germany. In this study, we utilized oak phytometers (*Quercus robur* L., clone DF159) as host trees, and made use of their ERG, whereby consecutive root and shoot flushes make a complete growth cycle. We studied two complete growth cycles during the same vegetation period, performed a non-destructive soil sampling and applied high-throughput amplicon sequencing of the bacterial 16S gene and the fungal ITS2 region. As C allocation shifts between the tree root and shoot, released root exudates and consequently the nutrient availability alternate for soil microorganisms. We therefore anticipated different microbial communities in the host tree root zone along the growth cycles until autumnal leaf senescence. In our results, the bacterial community exhibited a directional change over time along the vegetation period. In contrast, the fungal community appeared sample specific. Our findings enlarge the current understanding of the temporal microbial assembly in the host tree root zone.

Keywords: tree endogenous rhythmic growth; microbial community structure; bacteria; fungi; time-induced changes; tree root zone; *Quercus robur* L.

1. Introduction

Plant-root associated microorganisms feed primarily on plant rhizodeposits [1], of which the amount and dynamics are correlated with plant biomass [2]. Recently, a mesocosm experiment on annual dynamics of microbes associated with roots of grass and forb species found significant changes on the bacterial community over time, while the fungal community varied according to the host plant species [3]. However, this work did not consider the effects of plant development that occur over a vegetation period, and the sampling times were not determined according to phases of the plant development.

Oak trees (*Quercus robur* L.) are characterized by an endogenous rhythmic growth (ERG) with an alternation of root flush (RF) and shoot flush (SF), which are constitutive of one rhythmic growth cycle [4]. This alternation can be followed by using anatomical shoot bud developmental stages from A to D [5]: bud resting for stage A indicates the end of a SF; bud swelling for stage B corresponds to ongoing RF; bud outbusting for stage C indicates the end of a RF; and leaf expansion from the bud known as stage D corresponds to a new SF. Over a vegetation period and depending on environmental parameters, oak trees can have one growth cycle (one RF and one SF), two growth cycles (RF1, SF1, RF2 and SF2) or even more cycles, concluded by the autumnal leaf senescence. The tree ERG is paralleled with high and low concentrations of *photoassimilates* in roots during the root and shoot flushes, respectively [6]. According to the "push" hypothesis, the more C "pushed" into roots, the more C ought to be exuded from roots [7]. This should induce changes in the tree root associated microbial communities.

In the current research, we considered oak time-induced changes and the ERG as a single time entity, and analyzed their impact on the tree root zone microbial communities over one vegetation period. Due to bacteria rapid response to environmental changes [3], we hypothesized (1) temporal changes in bacterial communities. We also predicted (2) changes of the fungal communities over time, due to their dependence on host plants and tight attachment to recently assimilated C [8].

2. Materials and Methods

The study was carried out at two grassland field sites in Central Germany: Bad Lauchstädt (51°23′29.65″ N, 11°52′32.14″ W) and Harsleben (51°51′43.43″ N, 11°04′58.73″ W). The two sites have similar weather conditions due to their geographic proximity. Oak phytometers used for this study were outplanted at the sites in November 2014, and soil was sampled during the vegetation period 2018. Based on the tree bud development stages, we determined the times of RFs and SFs and respectively sampled at the ends of the tree RF1, SF1, RF2, SF2, and at the senescence to conclude the vegetation period.

Soil samples were taken in the tree root and root-free zones as described previously Habiyaremye, et al. [9], despite the upper 0–15 cm soil 30 cm from the tree trunk that were taken. Detailed descriptions of the molecular methods, from DNA extraction to Illumina-based sequencing were also published before [9]. Bioinformatics and processing of sequences data were conducted using the dadasnake pipeline (v0.5) [10], a DADA2 [11] implementation in snakemake [12].

Statistical analyses were performed using R v4.0.2 [13]. We performed a non-metric multidimensional scaling (NMDS) based on the Bray–Curtis dissimilarity matrices [14] to test divergences in the microbial communities over time. By using the function "adonis" of the vegan package v2.5-6 [15] we performed permutational analysis of variance (PERMANOVA) with 9999 permutations [16] to test the effect of sampling times for bacteria as well as sampling time and sampled position for the fungi. We further carried out Mantel correlation tests between a sampling time distance matrix (in days) and corresponding matrices of bacterial Bray–Curtis distances using 9999 permutations.

3. Results and Discussion

From both the NMDS plot and PERMANOVA results, bacterial community of the tree root and root-free zones changed between time points along a vegetation period ($p < 0.001$ for both the tree root and root-free zone) (Figure 1A). Mantel correlation test revealed positive linear correlation between distance in time and dissimilarities among the bacterial communities (Bad Lauchstädt: $R = 0.59$, $p < 0.001$ in the tree root zone, and $R = 0.67$, $p < 0.001$ in the tree root-free zone; Harsleben: $R = 0.65$, $p < 0.001$ in the tree root zone, and $R = 0.31$, $p = 0.008$ in the tree root-free zone) (Figure 1B).

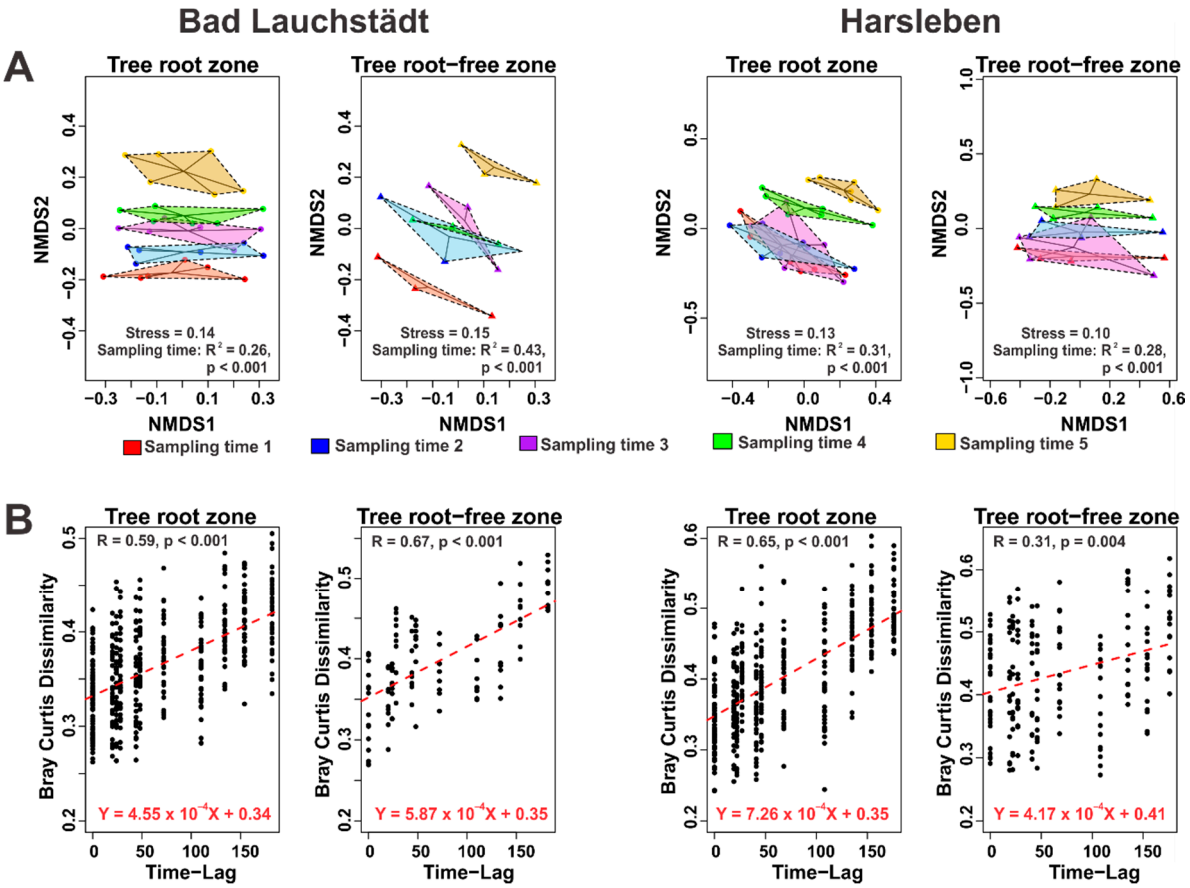

Figure 1. (**A**) Non-metric multidimensional scaling (NMDS) based on Bray–Curtis dissimilarity displaying bacterial community structure along with succession of sampling times. R^2 and p values are PERMANOVA results showing the effect of sampling time points, which coincided with ends of the tree RF1, SF1, RF2, SF2, and the senescence. (**B**) Correlation between distance in time along vegetation period and Bray–Curtis dissimilarity among the bacterial communities. R and p values are Mantel correlation test results. Time-Lag here means number of days in-between sampling times.

For fungal community of the tree root zone, sampling time points showed either no impact (Harsleben, PERMANOVA: $p > 0.05$) or less impact compared to sampled trees (Bad Lauchstädt, PERMANOVA: $R^2 = 0.13$ for sampling time and 0.50 for sampled trees, $p < 0.001$). Within the tree root-free zone, a fine-scale niche partitioning of the fungi was also noticed (Figure 2).

Our results confirmed the first hypothesis of temporal changes on the tree root zone bacterial community at both sites, Bad Lauchstädt and Harsleben. However, the second hypothesis of time-induced changes on the fungal community over the vegetation period was only confirmed at Bad Lauchstädt and rejected at Harsleben. Short-term variability in bacterial communities was also previously reported [17,18], while the majority of changes in soil fungal communities take place over longer time scales [17,18]. The rapid response of bacteria to environmental changes is a result of their relatively short generation times [17,18], versus the relatively long generation times for the fungi [3,17]. Referring to solely effects of plant time-induced changes, the bacterial community variations may have been a result of increasing size and productivity of the host plants, and subsequent changes in quantity and quality of the rhizodeposits over the vegetation period. This could explain the changes noticed in both the tree root and root-free zones.

The fungal community underwent time-induced succession in one site only. The community showed rather always fine spatial scale changes which may be explained by concurrent plant-soil interactions at fine spatial scales [19] and the fungi high dependence on host plants [8].

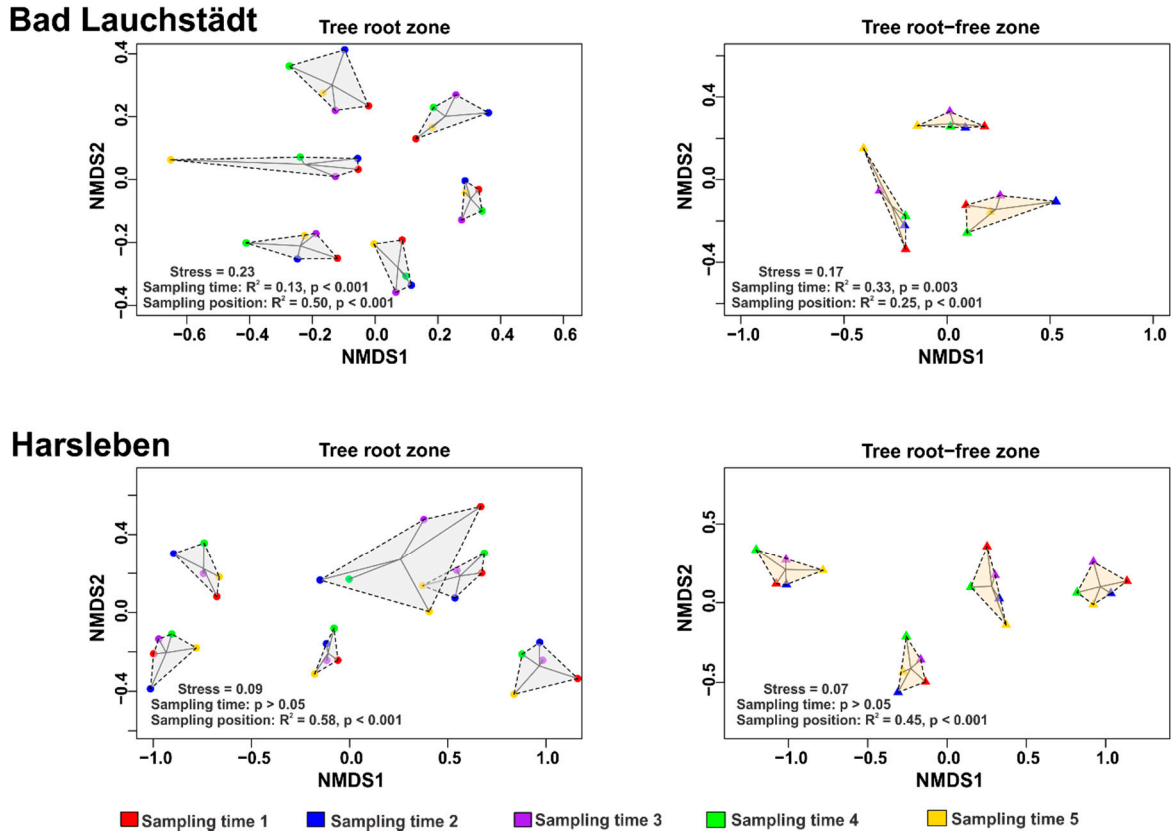

Figure 2. Non-metric multidimensional scaling (NMDS) based on Bray–Curtis dissimilarity displaying fine spatial scale partitioning of the fungal community. Sampling times coincided respectively with ends of the tree RF1, SF1, RF2, SF2, and the senescence.

4. Conclusions

Bacterial community structure in the root zone of the oak phytometers changed between time points along one vegetation period. On the contrary, the fungal community structure displayed fine spatial scale partitioning, closely linked to host plant individuals. The current research underlines the significance of repeated samplings over a vegetation period, but could not decouple respective impacts of time and ERG. Future studies should parallel rhythmically growing host trees with continuously growing trees, and track development of their root-associated microbial community over several consecutive vegetation periods to determine respective magnitude of time and host plant rhythmic growth.

Acknowledgments: We are thankful to the Soil Ecology Department of the Helmholtz Centre for Environmental Research (UFZ), especially the staff of the TrophinOak/PhytOakmeter project; to the German Academic Exchange Service (DAAD); and to the University of Rwanda.

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SYNOPSIS

This thesis reports on the spatial and temporal variability of soil microbial communities in different soil zones around trees of a pedunculate oak clone (*Quercus robur* L., DF159, Herrmann et al., 2016) out-planted as tree phytometers in grassland field sites across Europe. *Quercus robur* L. is widely distributed across Europe (Eaton et al., 2016), in forest, grassland and agricultural systems (MacDougall et al., 2004; Löf et al., 2016; Bobiec et al., 2018; Parmain and Bouget, 2018) and its strong interactions with soil bacteria and fungi were demonstrated (Herrmann and Buscot, 2007; Meaden et al., 2016).This tree species is also characterized by an endogenous rhythmic growth with alternation of root and shoot flushes (Herrmann et al., 2015), which impacts the biological soil activity since the tree early age (Eisenhauer et al., 2018). On this background, the use of a clone of *Q. robur* L. out-planted as a phytometer appeared to be an adequate model system to disentangle the pure and combined effects of geographic, soil physico-chemical, and host tree parameters in shaping soil microbial diversity and community structure.

Besides *Chapter 1* which introduced the whole work, *Chapters 2* and *3* individually dealt with changes of the soil microbiome at spatial scale while *Chapter 4* focused on a temporal scale. Spatially, sites of Central Germany characterized by comparable climatic conditions were investigated on the one hand in *Chapter 2*, while sites situated along a wide range of climatic conditions across Europe were considered on the other hand in *Chapter 3*. At temporal scale, investigation was done along a vegetation period, whereby soil sampling time points coincided with ending tree root and shoot flushes related to the endogenous rhythmic growth. We used the PCR-based Illumina MiSeq technology for 16S rRNA gene and the internal transcribed spacer (ITS) region of the rDNA gene to

investigate variability of the soil bacterial and fungal communities, respectively, in response to changes in abiotic environmental and host tree parameters.

As the plant root-associated microorganisms mostly originate from the surrounding soil, the potentially predominant drivers of their communities would be the edaphic parameters and geographic location attributes (Müller et al., 2016). However, the plant root-associated microorganisms are also dependent on plant rhizodeposits, which are composed of plant primary and secondary metabolites, underlining the strong relationship between root-associated microbial communities and the host plant (Müller et al., 2016; Chen et al., 2019). Meanwhile, previous researches were interested in investigating rhizosphere microbial communities, yet these are most directly controlled by the selective forces exerted by host plants (Kowalchuk et al., 2002). Thus, studies on diversity and structure of the rhizosphere inhabiting microbial communities do not give enough weight to direct contribution of the abiotic environmental parameters. Therefore, to rationally mediate between geographic-climatic, soil physico-chemical, and oak clone parameters and unravel their respective impact on the soil microbiome, we investigated and compared the microbiomes of the root and root-free soil zones around the trees. Here, the tree root soil zone means soil that contains living roots of the tree (Steven et al., 2014) whereas the tree root-free soil zone refers to soil from within the same plot, around the same trees but out of reach of any tree roots.

1.1. Main findings of this study

The main findings reported in this thesis are summarized here below:

i. DF159 pedunculate oak out-planted in the field started exerting an impact on the local soil microbial community since its early age. Two years after out-planting, the trees had already recruited symbiotic microbial partners including numerous species of ectomycorrhizal fungi and started shaping their root-associated microbial community as shown in *Chapter 2*. Indeed, this is a high achievement for such a short time, especially considering moderate size and consequent limited exudation potentials of the investigated trees. The early recruitment of the ectomycorrhizal fungi and other beneficial microorganisms might be one of the mechanisms that the pedunculate oak uses to quickly acclimate to local conditions.

ii. In particular, the soil bacterial and fungal communities of the tree root zone were shaped by interplay among geographic, soil physico-chemical, and host tree parameters. This was reported in *Chapter 2* on investigation among the sites with comparable climatic conditions in Central Germany. We also had the same observation in *Chapter 3* describing variability in soil microbial communities among the sites characterized by a broad range of climatic and soil physico-chemical conditions across a European North-South transect. The analyzed geographic parameters included mainly latitude, temperature, and precipitations, while the soil physico-chemical parameters included especially soil moisture, pH, as well as carbon and nitrogen content. Variability in these abiotic environmental parameters, especially along the European transect in *Chapter 3* triggered changes in the host tree growth and performance which were also reflected in the root-associated microbial communities. However, by comparing the tree root and root-free soil zones we also observed a potential of the trees to shape their most proximal soil microbiome. This effect is difficult to detect as it can be masked by the passive diffusion

of non-interacting tree independent microorganisms that diffuse passively from the tree root-free soil zone to the root soil zone. To eliminate this effect, in *Chapter 3* we considered a sub-community within the microbiome of the tree root zone, which is the affine microbiome, made of microorganisms enriched in this zone in comparison to the tree root-free zone. Within this root zone affine microbiome, a higher proportion of OTUs were identified at all sites of the European transect from Finland to South-Western France, while no OTUs was exclusive of any of the sites. This pinpoints the capacity of the trees to shape a core microbiome independently from the geographic, climatic and soil context.

iii. Bacterial and fungal communities responded differently to changes in the abiotic environmental and host tree parameters. These different patterns were mainly highlighted in *Chapter 3,* whereby the bacterial community responded more to the abiotic parameters, while the fungal community was more impacted by the host tree. Also, *Chapter 4* shows that the bacterial community structure exhibited a directional change over time along the vegetation period, while the fungal community structure appeared mainly closely linked to host plant individuals. Those findings suggest distinct mechanisms for shaping the two microbiomes.

Trees have the ability to build associations with specific microbial members from local community and harness their power to rapidly adjust to the ambient environmental conditions (Lau et al., 2017). The tree root-associated microorganisms also assist in improving the tree acquisition and absorption of nutrients from soil, and in preventing establishment of pathogens (Gehring et al., 2017; Lau et al., 2017). Meanwhile, dependence of the soil microbiome on the host plants and abiotic environmental

parameters was previously reported (Carrero-Colón et al., 2006; Fierer and Jackson, 2006; De Deyn et al., 2011; Brockett et al., 2012; Classen et al., 2015; Docherty et al., 2015), and this is what leads to variability of the microbial diversity and community structure across geographic space, time or both (Ladau and Eloe-Fadrosh, 2019). Higher dependence of the bacterial community on the abiotic environmental parameters was also previously reported (Millard and Singh, 2010; Lange et al., 2014), while the fungal community was rather more attached to the host plants (Chen et al., 2018; Roy et al., 2018; Wang et al., 2020).

To conclude on the different patterns between bacterial and fungal communities, we also took the opportunity that we could compare their variability at the site Bad Lauchstädt between September 2016 and September 2018 (i.e. respectively two and four years after trees out-planting) (**Figure 1**), by using the datasets of *Chapter 2* and *Chapter 3*, respectively. The Shannon diversity index decreased between the two time points in both the oak phytometer root and root-free soil zones for the bacteria, while for the fungi it increased significantly in the tree root-free zone (RFZ) and tended to increase in the tree root zone (RZ) (**Figure 1A**). According to **Figure 1B**, impact of sampling time on the microbial community structure was higher for the bacteria (perMANOVA: $p = 0.003$ $R^2 = 0.15$) than for the fungi (perMANOVA: $p = 0.006$, $R^2 = 0.11$). The host tree effect on microbial community structure was noticed at both sampling times for the fungi, while it was noticed only in September 2018 for the bacteria. Additionally, the rate of microbial community turnover ($Beta_{SIM}$, replacement of microbial OTUs) between September 2016 and September 2018 was overall significantly higher for fungi than for bacteria (**Figure 2**). Indeed, plants, especially long-lived trees, restructure their root-associated

microbiomes not only intra-annually as proved in *Chapter 4*, but also inter-annually (Pennanen et al., 1999;Wallander et al., 2010;Kyaschenko et al., 2017;Averill et al., 2019). This restructuring process started just after the tree out-planting and revealed continuous temporal dynamics, based on plant continuous needs to acclimate to changes in environmental conditions as well as on changing quality and quantity of rhizodeposits, which seem to induce more changes in the fungal than in the bacterial community. Furthermore, compared to the overall fungal community, the turnover rate of the ectomycorrhizal fungi (EcM) was lower (**Figure 2**). Also, the tree root zone EcM showed higher nestedness ($Beta_{SNE,}$ gain or loss of microbial OTUs between the time points) than the tree root free zone, indicating a continuing recruitment of these fungi along with the host tree age.

Different patterns of bacterial and fungal communities are due to fundamentally important differences between bacterial and fungal traits, including their different nutrient mode, morphology, physiology, generation time, temperature dependence, and carbon use efficiency (Six et al., 2006; Ullah and Dijkstra, 2019). As consequence, fungi depend more on their host plants (Chen et al., 2018; Roy et al., 2018; Wang et al., 2020) compared to bacteria, which are usually more affected by abiotic environmental parameters (Millard and Singh, 2010; Lange et al., 2014).

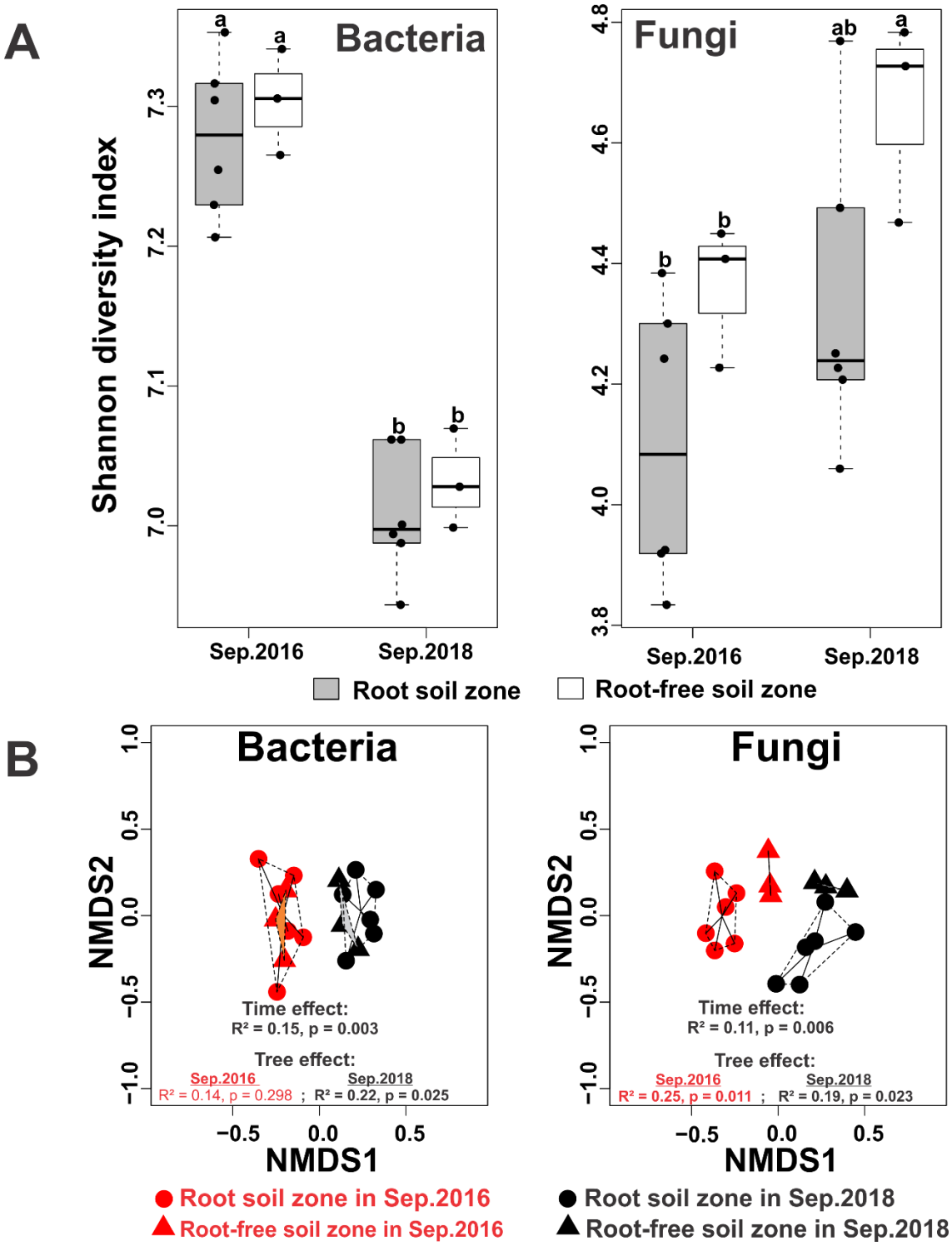

Figure 1. Variability of the soil microbiome at Bad Lauchstädt field site between September 2016 and September 2018. Comparison of microbial Shannon diversity index (**A**) and community structure (**B**) between the tree root and root-free soil zones, and between September 2016 and September 2018.

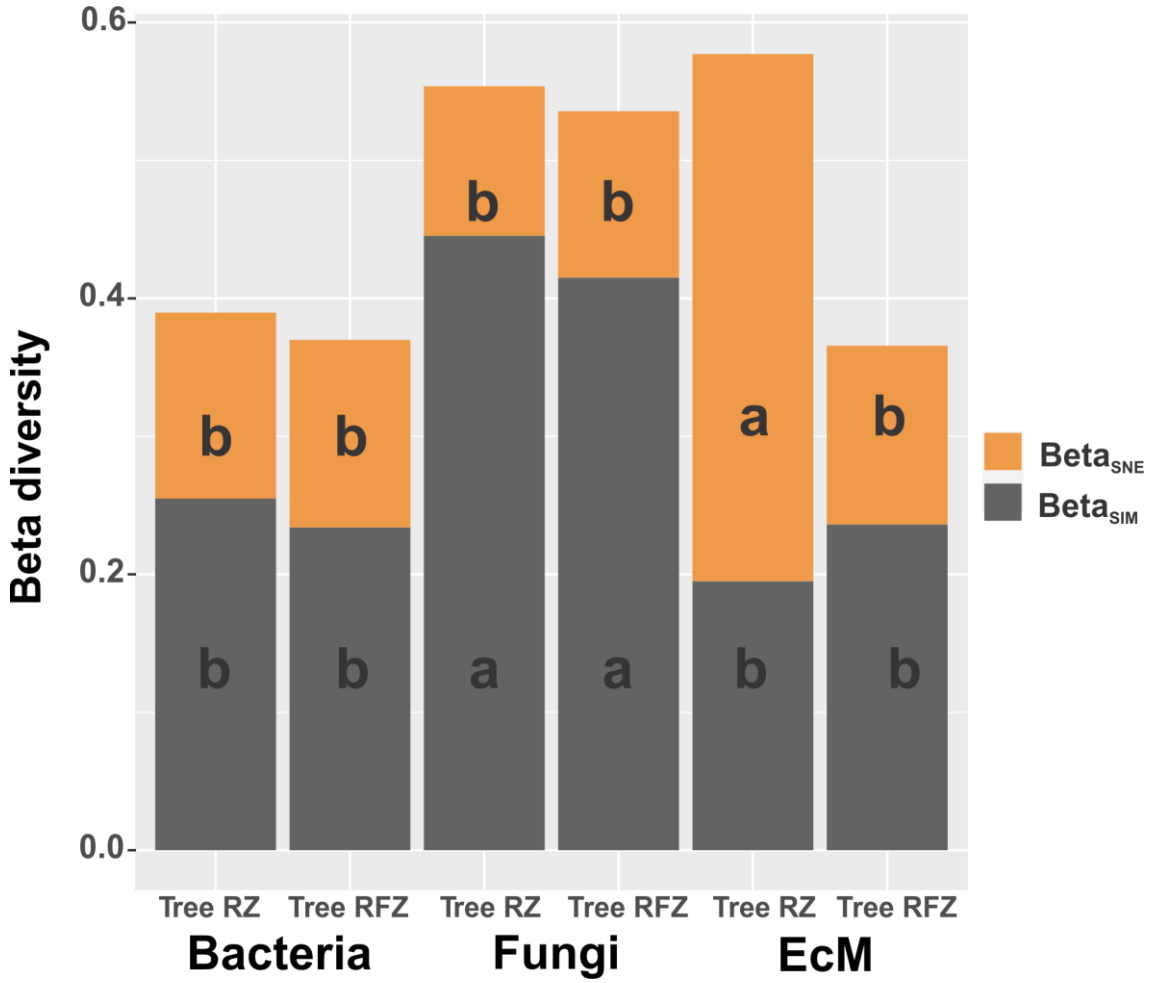

Figure 2. Overall beta diversity considering turnover ($Beta_{SIM}$), i.e. replacement of microbial OTUs, and nestedness ($Beta_{SNE}$), i.e. gain or loss of microbial OTUs from September 2016 to September 2018 at the site Bad Lauchstädt. Beta diversity, turnover and nestedness were calculated with R-package "betapart" (Baselga and Orme, 2012). RZ: root zone, RFZ: root-free zone, EcM: Ectomycorrhizal fungi.

Coming back to the central questions which directed our respective studies as mentioned within the introduction (*Chapter 1*) of this thesis, we found that:

1. Common genetic identity of the clonal pedunculate oak trees together with the homogeneous climate conditions induced similar microbial diversity within the tree root zone among the sites but the community structure was site-specific (*Chapter 2*)

2. Two years after out-plant of the clonal oak trees, the tree root zone was already enriched in particular microbial taxa, especially the beneficial microbial partners such as the ectomycorrhizal fungi (*Chapter 2*).
3. Even at a large spatial scale of European North-South transect with a wide range of the environmental variables, the clonal pedunculate oak trees were able to shape an interacting soil microbiome from very different local soil communities (*Chapter 3*). This tree-interacting microbiome is assumed to assist the tree establishment under the variable environmental conditions along the transect.
4. The clonal oak phytometer only contributed more than the investigated abiotic environmental parameters in shaping the tree root zone affine fungal community (*Chapter 3*). This highlights not only impact of trees on their most proximal soil microbiome but also the high attachment of the fungi on their host plants.
5. The results that are presented in *Chapter 4* of this thesis did not answer the original question about whether the impact of alternation of root and shoot flushes on the tree-associated microbiome can deviate the expected temporal succession of the tree-associated microbial communities. Further analyses are still going on to disentangle between influence of time and alternation of root and shoot flushes.

5.2. Contributions of this PhD research to soil biodiversity research

As one of its main contributions, this PhD research introduced the use of a tree clone phytometer to analyze plant impact on variations of soil microbial communities, especially along a wide range of environmental conditions. Phytometer plants have been used for a long time as environmental measuring “instruments” (Clements and Goldsmith, 1924;Gibson, 2015) to study the relationship between a plant species and its habitats or

different ecological contexts (Clements and Goldsmith, 1924; Antonovics and Primack, 1982; Antonovics et al., 1987). In this research, we used a clonal phytometer system, which warrants genotypic identity of all single plants. Such an approach is rare, particularly for phytometer trees (Kaldorf et al., 2004;Foulon et al., 2016). The main advantage of using a tree clone is to avoid intraspecific genetic variations among the phytometer trees while maintaining their locally adapted performances and consequent effect on soil microbial communities.

Working with such a clonal phytometer reinforced considerably the power of comparing the communities of the non-rooting and rooting soil zone, and to zoom into the root zone affine sub-community. This particularly enabled us to precise the impact of trees on buffering the distance decay of soil microbial communities (*Chapter 3*, Figure 6), which was already weakly detected by Goldmann et al. (2016) in their comparison of rooting and non-rooting soil around non clonal spruce trees. In this thesis, the tree root zone affine microbiome refers to soil bacterial and fungal species that demonstrate a significant preference to the tree root zone for their habitat. To identify members of this microbial sub-community, *Chapter 3* compared the community composition between the tree root and root-free soil zones by using the indicator species analysis. Contrarily to the outcome of the usual overlap analysis, the tree root zone affine microbiome has the advantage of not including rare species or OTUs. It rather consists of microbial species that are highly attached to the tree and fully exposed to its influence, besides the abiotic environmental parameters. Therefore, the approach is suitable to rationally tackle the tree impact on soil microbial community versus those of abiotic environmental variables. As our sampling

procedure only represents a minor disturbance, our design allows also long-term investigations and the phytometer trees can be further monitored in the coming years.

5.3. Technical potentials and limitations of the used molecular approach

This research used DNA metabarcoding coupled with high-throughput amplicon sequencing to assess the microbial diversity and community structure in the root soil zone of pedunculate oak clone out-planted in European grassland field sites. The choice of DNA metabarcoding was based on its greatest benefit to process multiple samples simultaneously without the need to isolate individuals first (Porter and Hajibabaei, 2018). In the DNA metabarcoding approach, DNA is extracted from a group of samples, amplified by using primers that target marker genes. These barcode genome regions are then sequenced using high-throughput sequencing, and identified against reference databases. DNA metabarcoding is therefore an efficient tool to access biodiversity and community composition including taxonomic classification, and has a great advantage in terms of high speed as well as relatively low cost (Comtet et al., 2015; Thomsen and Willerslev, 2015; Porter and Hajibabaei, 2018). Among the commonly used high-throughput sequencing platforms for DNA metabarcoding studies, Illumina is currently the most popular technique and the default choice due to its very low sequencing error rate, its low price, and the paired-end approach covering amplicons of up to ~550 bases in length (e.g. MiSeq 2 x 300) (Porter and Hajibabaei, 2018; Nilsson et al., 2019). Moreover, Illumina provides sequencing at greater depth for bacterial 16S and fungal ITS2 genes (Schmidt et al., 2013; Porter and Hajibabaei, 2018). In all studies of this thesis, Illumina MiSeq was used.

Multiple limitations associated with the DNA metabarcoding approach are related to the laboratory protocol routine and the reference databases. First, the use of a marker gene hinders to some extent taxonomic assignment at the species and strain levels and increases the risk for biases associated with the PCR because it does not allow differentiation between genomes with similar marker genes (Pérez-Cobas et al., 2020). Second limitation and the biggest concern due to its interference with PCR amplification of certain taxa is primer bias caused by variable primer-template mismatches (Piñol et al., 2015; Elbrecht et al., 2017). It is thus likely that not all taxa present in a sample can be properly detected with the DNA metabarcoding approach. Additionally, PCR and sequencing errors can lead to false-positive detection (Elbrecht et al., 2017), leading to incorrect taxonomic assignment and overestimation of the microbial diversity (Edgar, 2017). Also, taxonomic assignment based on reference databases limits DNA metabarcoding for ecosystem assessment as always demonstrated by a large number of unidentified OTUs because the taxa are not yet present in the reference databases (Elbrecht et al., 2017). Similarly, main goals of high-throughput sequencing include also functional assignment of the recovered microbial OTUs. However, DNA metabarcoding is challenged by insufficient guild data for many microbial groups (Nilsson et al., 2019). Thus, a higher proportion of the bacterial and fungal OTUs could not be assigned to well-defined guilds. To solve the above mentioned challenges, PacBio (Pacific Bioscience) sequencing technology, which analyzes completed genomes was introduced as a powerful way, but its use is still limited by its high cost (Song et al., 2019). To profile taxonomic composition and functional potential of the microbial communities at

reasonable cost, recent development has introduced shotgun high-throughput which targets a suite of genes (Quince et al., 2017).

Last, the DNA sequencing approaches used in this research also capture DNA from dead microbial cells (Emerson et al., 2017), free DNA from dead cells, and DNA adsorbed to soil particles (Nielsen et al., 2007). Therefore, the DNA metabarcoding provides information about the potential microbial community and is likely another source of overestimating the microbial diversity and abundance. To overcome this, future DNA-based microbial community analyses should be coupled with assessment of the microbial gene expression, like at the level of RNA (metatranscriptomics).

5.4. Considerations for future studies

Numerous studies conducted on temporal dynamics of soil microbial communities associated with plant roots indicated successional changes of bacterial and fungal communities at different time scales (Buckley and Schmidt, 2003; Kennedy et al., 2006;Lipson, 2007; Zhang et al., 2011). These findings were proved by the results reported in this thesis. However, connection between temporal variations in microbial assemblages and measurable changes in the host plant phenology is still limited (Chaparro et al., 2014). In this line, our ongoing study is applying deep analysis of the data set presented in *Chapter 4* to investigate the precise impact of rhythmic growth of the pedunculate oak clone, in particular its alternating root and shoot flushes along a vegetation period. The overall goal is to check if these repeatedly growth phases induce oscillations in the tree-associated microbial communities. The analyses are mainly focusing on the tree root zone affine microbiome and the individual microbial functional

guilds. Time constraints did not allow us to wait for the results and incorporate them in this thesis.

By using the clone of pedunculate oak phytometer, this PhD thesis improved the understanding of the diversity and structure of soil bacterial and fungal communities in response to changes in abiotic environmental and host tree growth parameters. Towards a full understanding of the contribution of biotic drivers to soil bacterial and fungal communities, future studies should also consider bacterial-fungal interactions and couple them with the host tree parameters. This is because, independently of edaphic parameters, bacteria and fungi contribute to shaping the structure of each other's community (Duponnois et al., 1993; von Alten et al., 1993; Requena et al., 1997; Dunstan et al., 1998; Singh et al., 2009). Moreover, bacterial-fungal interactions are not only beneficial to one or both of the interacting microbial partners, but also crucial for ecosystem functioning (Frey-Klett et al., 2011; Deveau et al., 2018).

Soil microbial communities vary with land-use types (Schöps et al., 2018; Xue et al., 2018; Plassart et al., 2019), and the conclusions of this thesis are based on the grassland systems. Therefore, similar researches on the other land-use types, such as forest and agricultural systems, are highly recommended. Furthermore, during this PhD research, core microbial OTUs of the pedunculate oak root soil zone were identified even across large spatial distance and assumed to be among strategies that the tree uses to support its wide distribution across Europe. To confirm this assumption, future research should compare along an environmental gradient the responses of the microbiomes vis-à-vis pedunculate oak and vis-à-vis another tree with a very small habitat range.

This PhD research revealed as well temporal changes on the tree root zone microbiome in two-year time span (between September 2016 and September 2018). This is because plants restructure the assembly of their root-associated microbiomes inter-annually (Pennanen et al., 1999; Wallander et al., 2010; Kyaschenko et al., 2017; Averill et al., 2019), beside the impact of changing abiotic environmental parameters over time. Because of this, the tree root zone microbiome turnover is likely a process which never ends. As the investigated trees remain in their field plots because sampling technique applied in this research inflicts minor disturbance, a long-term monitoring on variability of the soil microbiome associated to the investigated pedunculate oak clone is possible and highly recommended. The long-term monitoring will also answer the important question about whether the tree root core microbiome is stable or also exhibits turnover over time.

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CURRICULUM VITAE

Personal data

Name:	Habiyaremye, Jean de Dieu
Data of birth:	2nd September 1978 in Rulindo, Rwanda
Current address:	Unstrutstraße 19; 06122 Halle (Saale)
Phone:	+49 160 91076343
Email address:	jdhabiyaremye@gmail.com

Education

Since October 2017	PhD student in the group of Prof. Dr. François Buscot, Department of Soil Ecology, Helmholtz Centre for Environmental Research GmbH-UFZ, Halle (Saale)-Germany. Title of thesis: "*Spatial and temporal variations in the microbiomes of different soil zones around clonal pedunculate oak trees (Quercus robur L.) out-planted as phytometers across grasslands in Europe*".
November 2012-November 2014	Master student in Molecular Biology and Biotechnology at the Pan African University (PAU) and the Jomo Kenyatta University of Agriculture and Technology (JKUAT), Nairobi-Kenya. Title of thesis: "*Abundance, identification and effectiveness indigenous AMF species associated with agroforestry systems in the semi-arid and humid agroecologies of Rwanda*".
2000-2007	Bachelor student in Science with Education, Kigali Institute of Education, Kigali-Rwanda. Title of thesis: "*Analysis of Physico-chemical composition of Agashya passion fruit nectar produced by the Urwibutso company in Nyirangarama*".
1992-1999	Secondary Education in Latin-Sciences, Petit Séminaire Saint Vincent de Ndera, Kigali-Rwanda.

Work experience

Since 2015	Assistant Lecturer of Biology at the University of Rwanda, Kigali-Rwanda. Main duties and responsibilities: Teaching and Research.
2008-2012	Assistant Tutor of Biology at Kavumu College of Education, Muhanga-Rwanda Main duties and responsibilities: Teaching.

LIST OF PUBLICATION

Peer-reviewed publication

Habiyaremye, J.D. D., Herrmann, S., Reitz, T., Buscot, F, & Goldmann, K. 2021. 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. *Environmental Microbiology*. doi: 10.1111/1462-2920.15433.

Habiyaremye, J. D. D., Goldmann, K., Reitz, T., Herrmann, S., & Buscot, F. 2020. Tree root zone microbiome: exploring the magnitude of environmental conditions and host tree impact. *Frontiers in microbiology*, *11*, 749. doi: 10.3389/fmicb.2020.00749.

Conference proceedings

Conference paper

Habiyaremye, J. D. D., Herrmann, S., Buscot, F., & Goldmann, K. (2021). Temporal Changes and Alternating Host Tree Root and Shoot Growth Affect Soil Microbiomes. In *Multidisciplinary Digital Publishing Institute Proceedings* (Vol. 66, No. 1, p. 35).

Oral presentations

Habiyaremye, J.D. D., Herrmann, S., Reitz, T., Buscot, F, & Goldmann, K. 2020. Tree root-associated microbiomes across Europe: respective impacts of sites' specificities and an oak clone. 6th Joint Conference of the German Society for Hygiene and Microbiology (DGHM) & Association for General and Applied Microbiology (VAAM), Leipzig-Germany.

Habiyaremye, J.D. D., Goldmann, K., Herrmann, S., Reitz, T., & Buscot, F. 2019. Soil microbial community of the tree root zone is impacted by both host tree selection effect and site specificity. 2019. 49th Annual Meeting of the Ecological Society of Germany and Switzerland "Science meets practice", Münster-Germany

Poster presentations

Habiyaremye, J.D. D., Herrmann, S., Reitz, T., Buscot, F, & Goldmann, K. 2019. Soil properties and host tree drive the microbial community structure along a North-South transect across Europe. 4th Thunen Symposium on Soil Metagenomics. Braunschweig-Geramany.

STATUTORY DECLARATION

Hereby, I, Jean de Dieu Habiyaremye, affirm that I take note and accept the doctorate regulations of the Faculty of Life Science at the University of Leipzig from September 30th, 2019. I further affirm that the presented thesis was prepared autonomously without inadmissible help. All aids used in this thesis as well as scientific ideas which are quoted from or based on other sources were cited at the respective point.

All people who helped me to prepare the conception, to select and analyze the materials of this thesis as well as to improve the manuscripts are namely cited in the acknowledgments. With exception of the namely mentioned people no other persons were involved in the intellectual work. No PhD consultant service was employed. Third parties did not get money's worth for benefits that were conjunction with the content of this dissertation.

I declare that this dissertation has been neither presented nationally nor internationally in its entirety or in parts to any institution for the purpose of dissertation or other official or scientific examination and/or publishing.

Previously unsuccessful dissertations had not taken place.

The original document of the verification of the co-author parts are deposited in the office of the dean.

Halle (Saale), March 2021

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Jean de Dieu Habiyaremye

EIDESSTATTLICHE ERKLARUNG

Hiermit erkläre ich, Jean de Dieu Habiyaremye, eidesstattlich, dass mir die Promotionsordnung der Fakultät für Lebenswissenschaften der Universität Leipzig vom 30.09.2019 bekannt ist und von mir anerkannt wird.

Zudem versichere ich, dass die vorliegende Dissertation von mir selbständig und ohne unzulässige Hilfsmittel angefertigt worden ist. Sämtliche von mir verwendete Hilfsmittel sowie die aus fremden Quellen direkt oder indirekt übernommenen wissenschaftlichen Gedanken sind als solche in der vorliegenden Arbeit gekennzeichnet.

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Der Nachweis über die Anteile der (Co-)Autorenschaften sind im Original im Dekanat der Universität Leipzig hinterlegt.

Halle (Saale), März 2021

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Jean de Dieu Habiyaremye

Verification of the author parts

Title: **Tree root zone microbiome: Exploring the magnitude of environmental conditions and host tree impact**

Journal: Frontiers in Microbiology

Authors: Jean de Dieu Habiyaremye, Kezia Goldmann, Thomas Reitz, Sylvie Herrmann, and François Buscot

Rates of Jean de Dieu Habiyaremye (Author 1)
- Sample preparation
- Bioinformatics data processing
- Data analysis and interpretation
- Manuscript conception and writing

Rates of Kezia Goldmann (Author 2)
- Bioinformatics data processing
- Data analysis and interpretation
- Manuscript conception and writing

Rates of Thomas Reitz (Author 3)
- Sample preparation
- Manuscript revision

Rates of Sylvie Herrmann (Author 4)
- Project conception
- Sampling design
- Soil sampling
- Manuscript revision

Rates of François Buscot (Author 5)
- Project conception
- Sampling design
- Manuscript revision

Jean de Dieu Habiyaremye | Kezia Goldmann | Thomas Reitz

Sylvie Herrmann | François Buscot

Verification of the author parts

Title: **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**

Journal: Environmental Microbiology

Authors: Jean de Dieu Habiyaremye, Sylvie Herrmann, Thomas Reitz , François Buscot, and Kezia Goldmann

Rates of Jean de Dieu Habiyaremye (Author 1)
- Sampling design
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- Bioinformatics data processing
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Jean de Dieu Habiyaremye | Sylvie Herrmann | Thomas Reitz

François Buscot | Kezia Goldmann

Verification of the author parts

Title: **Temporal changes and alternating host tree root and shoot growth affect soil microbiomes**

Journal: Multidisciplinary Digital Publishing Institute (MDPI) conference proceeding

Authors: Jean de Dieu Habiyaremye, Sylvie Herrmann, François Buscot, and Kezia Goldmann

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- Sampling design
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