DNA-based analysis of bark associated fungal, algal, and bacterial communities – contributions to understanding the unknown biodiversity of forests

Dissertation zur Erlangung des Doktorgrades der Naturwissenschaften

vorgelegt beim Fachbereich Biowissenschaften
der Johann Wolfgang Goethe-Universität
in Frankfurt am Main

von

Lukas Dreyling

aus Duderstadt

Frankfurt (2023)

(D 30)

Vom Fachbereich Biowissenschaften der

Johann Wolfgang Goethe-Universität als Dissertation angenommen.

Dekan: Prof. Dr. Sven Klimpel

Institut für Ökologie, Evolution und Diversität

Johann Wolfgang Goethe-Universität

D-60438 Frankfurt am Main

Gutachter: Prof. Dr. Imke Schmitt

Institut für Ökologie, Evolution und Diversität

Johann Wolfgang Goethe-Universität

D-60438 Frankfurt am Main

Prof. Dr. Claus Bässler

Arbeitsgruppe Ökologie der Pilze

Universität Bayreuth

D-95447 Bayreuth

Datum der Disputation:

This thesis is based on the following publications and manuscripts:

Dreyling, L., Schmitt, I. & Dal Grande, F. (2022): Tree size drives diversity and community structure of microbial communities on the bark of beech (*Fagus sylvatica*). *Frontiers in Forests and Global Change* 5:858382. doi: 10.3389/ffgc.2022.858382

Dreyling, L., Penone, C., Schenk, N., Schmitt, I. & Dal Grande, F.: Biotic interactions outweigh abiotic factors as drivers of bark microbial communities in Central European forests.

Hofmann, B., Dreyling, L., Dal Grande, F., Otte, J. & Schmitt, I. (2023): Habitat and tree species identity shape aboveground and belowground fungal communities in central European forests. *Frontiers in Microbiology* 14:1067906. doi: 10.3389/fmicb.2023.1067906

Dreyling, L., Boch, S., Lumbsch, T. & Schmitt, I.: Surveying lichen diversity in forests: A comparison of expert mapping and simple eDNA metabarcoding.

Table of Contents

1.	Abstract	1
2.	Introduction	5
	2.1. Forests and their microbiome	5
	2.1.1. The role of bark surfaces within forests	5
	2.1.2. Characteristics of the bark surface microbiome	6
	2.2. Environmental DNA (eDNA)	7
	2.2.1. Concept and applications	7
	2.2.2. Leveraging eDNA to study community ecology	8
	2.3. Drivers of epiphytic microbial communities	10
	2.3.1. Environmental factors	10
	2.3.2. Mutual biotic influences	11
	2.4. Knowledge Gap	12
3.	Thesis Structure and Research Questions	13
	3.1. Structure and Methods	13
	3.1.1. Structure Overview	13
	3.1.2. Study System	16
	3.1.3. Sampling design	17
	3.1.4. Metabarcoding of multiple organismal groups	17
	3.2. (Q1) What is the diversity of the microbial community on the surface of tree bal	rk?
	How do microbial communities respond to tree size and forest management?	18
	3.3. (Q2) What drives the diversity of the bark surface microbiome? Do biotic	
	interactions play a greater role than abiotic influences?	19
	3.4. (Q3) How does the tree species shape fungal micro-communities? What are the	ne
	differences between bark and soil fungal communities and do they share diversity?	19
	3.5. (Q4) Is eDNA metabarcoding a useful tool for surveying epiphytic lichen	
	biodiversity? Is it a reliable standalone tool?	20
4.	Main Results and Discussion	21
	4.1. (Q1) Regional diversity and interactions of the bark surface microbiome of	
	differently sized trees	21

4.	.2. (Q2) Biotic and abiotic drivers of the bark surface microbiome	23
4.	.3. (Q3) Differences between soil and bark surface microbiome associated with	
СО	niferous and deciduous trees	26
4.	.4. (Q4) Suitability of eDNA metabarcoding as a tool in surveys of lichen diversity.	27
4.	.5. General Discussion	29
4.	.6. Critical Evaluation of eDNA metabarcoding	32
5. Sı	ummary and Outlook	36
Zusar	mmenfassung	38
Refer	rences	44
Gloss	sary	61
A. Ap	ppendix – Publications	62
Α.	1. Tree size drives diversity and community structure of microbial communities on	the
ba	ark of beech (<i>Fagus sylvatica</i>)	62
A.2	2. Biotic interactions outweigh abiotic factors as drivers of bark microbial	
CO	ommunities in Central European forests	88
Α.3	3. Habitat and tree species identity shape aboveground and belowground fungal	
CO	ommunities in central European forests	138
A.4	4. Surveying lichen biodiversity in forests: A comparison of expert mapping and	
sin	mple eDNA metabarcoding	157
Ackno	owledgments	179
Curric	culum Vitae	181

L	is	t o	f I	Fi	αı	ır	es
_						'	

Figure	1: Overview	figure showing	the structure	of this thesis	16
--------	-------------	----------------	---------------	----------------	----

1. Abstract

Methods using environmental DNA to explore and analyze biodiversity from previously unexplored habitats and ecosystems have become increasingly popular in recent years. This is particularly due to the potential reduction in necessary taxonomic expertise, the opportunity to assess microorganismal communities, and decreased time investments required to cover large spatial extents. In forests, the surface of tree bark is an important habitat for epiphytic diversity. Because of the large surface area rich in microniches, the seasonal stability of the substrate, and the longevity of trees, tree bark surfaces provide an ideal habitat for many species. In fact, bark surfaces are known to support a diverse community of macro- and microorganisms, despite the challenging environmental conditions, mainly related to nutrient scarcity and low water availability. Many of the bark-associated organisms contribute considerably to forest functions such as nutrient cycling. Yet, we lack a comprehensive understanding of their communities and the environmental drivers behind the community assembly. These missing links hinder the exploration of the forest microbiome as a whole and limits our understanding of functions of a large forest habitat and its connections to other forest microbiomes. Using eDNA metabarcoding we can profile the microbial communities and identify drivers of community assembly. With a holistic approach encompassing samples of major taxonomic groups (e.g. bacteria, fungi, and green algae), as well as simultaneous collections from multiple forest habitats we can contribute to closing these gaps and increase our knowledge of the forest microbiome.

My dissertation is set within the framework of the Biodiversity Exploratories and was conducted in four parts: I. the establishment of an eDNA metabarcoding workflow to reveal the local diversity of the bark surface microbiome; II. the upscaling of the method to large geographic and environmental gradients to uncover the drivers of the microbiome; III. the integration of soil and bark samples to investigate compositional differences in two important forest habitats; IV. the evaluation of eDNA metabarcoding as a tool for biodiversity assessments of lichen diversity in forests.

In the first part, I developed a simple, cost-effective and fast sampling strategy to acquire eDNA samples from the bark of trees in forest ecosystems. Using readily available medical-specimen-collection swabs I sampled bark surfaces of individual trees

in Central German forests and used metabarcoding to amplify marker genes of green algae, fungi and bacteria. From the sequencing reads I calculated the first diversity estimates of the major organismal groups of bark surface microbiomes from Central European forests. Bacteria were the most diverse group, followed by fungi and algae. Overall the diversity was highly influenced by the size of the tree (as a proxy for age) and the significantly lower diversity of older trees suggests a process leading to less diverse microbial communities over time. Furthermore, I could show through microbial networks that the three organismal groups are strongly connected and co-occurrence patterns reveal a connecting nature of bacterial taxa in the bark microbiome. Overall the methodology produced reliable results, allowing for an expanded sampling in the second part.

In the second part of the dissertation, I expanded the sampling based on the results of part one. I collected bark surface samples from the three regions of the Biodiversity Exploratories covering large spatial and environmental gradients representative for Central European forests. The collection included composite samples from 150 plots and over 750 trees. Utilizing measurements of climatic and forest structure variables provided by the Biodiversity Exploratories, as well as my own community data, I identified the biotic and abiotic drivers behind alpha and beta diversity of the bark surface microbiome. I applied linear modeling to study responses of alpha diversity, as well as Generalized Dissimilarity Modeling to reveal the non-linear responses of beta diversity. While the abiotic conditions greatly influenced the alpha diversity, the community composition was determined by mutual biotic interactions between the taxonomic groups. This suggest an influence of the abiotic environment on the number of taxa occupying the niche, but not which taxa occur. The strong effect of mutual biotic associations underline the potential for close interactions in the bark microbiome, which could be related to beneficial exchanges.

In the third part, I studied the differences between the bark surface as an unexplored and the soil as an example of a well characterized forest microbiome. Using only the fungal part of the large sampling campaign and soil samples obtained from the same plots at the same time, I assessed the commonalities and differences of the micro-communities of these distinct forest niches. Furthermore, I included two

coniferous and one deciduous tree species to examine, if the effect of tree species, previously shown for soil microbiomes, also holds true for the bark surface. I showed that the two forest niches (bark surface and soil) contain a significantly different community, but the few taxa shared between them account for a large proportion of sequencing reads. The effect of tree species on the bark surface microbiome was small but significant. Furthermore, co-occurrence networks revealed taxa potentially connecting the two microbiomes, thus indicating a connecting function of bark surfaces.

In the last part of my dissertation, I used eDNA in a more applied way as a tool in biodiversity assessments of lichenized fungi. I compared the results from eDNA metabarcoding to an expert floristic mapping conducted in the same plots in 2007/2008. I assigned functional guilds to the fungal taxa obtained in the large sampling campaign and used a subset that was assigned as lichenized fungi. Only 25% of the total number of species were shared between the two methods, and both identified a particular set of species. In the floristic study many inconspicuous taxa were potentially overlooked or not distinguishable, while the biggest challenge of the eDNA were incomplete databases. However, eDNA can be a useful complimentary tool for assessments of lichen biodiversity, in particular for the identification of taxa that are small and easily overlooked, or hard to distinguish morphologically.

In conclusion, I showed that eDNA metabarcoding is a valuable tool to reveal the unknown diversity of microorganisms in forest ecosystems. In particular, my results advance our understanding of the bark surface microbiome, an underexplored habitat within forests. The tightly linked interactions of the three major microbial groups underline that studies need to take holistic approaches across multiple taxonomic groups to deepen our understanding of processes governing the assembly of microbiomes. Furthermore, the differences between forest micro-niches clearly indicate the need to include more than one habitat if we want to understand the functions and connections in the forest microbiome. I provided baseline diversity data for bark-associated micro-communities in Central European forests, identified abiotic and biotic drivers of these communities, and explored the use of eDNA for assessment of a selected group of organisms, the lichenized fungi. Results from my dissertation may serve as a foundation to compare the microbiomes of Central European to other forest

Abstract

types, inform hypotheses addressing the functions of forest microbiomes, and provide data for setting up monitoring programs for particular bacterial, fungal or algal groups. The massive diversity data collected may also contribute to closing the gap in our understanding of macro-organisms and micro-organisms with respect to diversity distributions and patterns of richness, and serve as a baseline for predictions of biodiversity responses under future anthropogenic change.

2. Introduction

2.1. Forests and their microbiome

Forest biomes are some of the largest ecosystems in the world, with an estimated number of over three trillion trees (Crowther et al., 2015). Forests cover approximately one third of the global landmass, with European forests making up around 25% of the total forested area (FAO, 2020). Not surprisingly, a large percentage of global biodiversity can be found within these forest ecosystems (Crowther et al., 2015). Trees are keystone species in forests, and are at the base of many important resource cycles, such as nutrients, water and particularly carbon sequestration. Furthermore, they create a large spatial heterogeneity and structure the forest into a variety of habitats, such as soil, leaves (phyllosphere), deadwood and bark (Baldrian, 2017). These different habitats are in turn connected and regulated by the trees themselves (Wardle et al., 2004).

Soils and leaves are two of the most comprehensively studied habitats in regard to microbial activity and their ecological functions within forests (Baldrian, 2017). An important function largely driven by soil microorganisms is the decomposition of organic matter (Baldrian, 2017). In addition to photoassimilates released into the soil by roots, the compounds resulting from decomposition can be fixed for long-term storage in the soil by fungal mycelium (Clemmensen et al., 2013). Another function is the provision of growth-limiting nutrients, such as phosphorus, which is largely supplied through mycorrhizal fungi, and atmospheric nitrogen fixed by bacteria (Van Der Heijden et al., 2008) that can also be found in the phyllosphere (Bashir et al., 2022). Aside from the provision of nutrients, phyllosphere microorganisms are involved in several other key functions, such as defence against plant pathogens (Bashir et al., 2022). Many of these ecological processes are not yet well understood, and since they often occur across multiple forest habitats, it is not possible to completely understand their functioning until we study forest microbiomes holistically (Baldrian, 2017).

2.1.1. The role of bark surfaces within forests

Although bark surfaces provide a large surface area of 0.9 ha per ha of forest (Baldrian, 2017), they have been understudied to date. Compared to below-ground forest habitats,

taxa of bark surfaces experience unique and challenging environmental conditions. These include low water availability, nutrient scarcity and occurrence of compounds inhibiting microbial growth and temperature (Buck et al., 1998; Baldrian, 2017). Like other aboveground habitats, e.g., the phyllosphere, it is also subjected to desiccation by wind and increased ultra-violet (UV) radiation (Lindow and Brandl, 2003; Vorholt, 2012). Additionally, bark is particularly poor in carbon, since many of the available compounds are able to withstand microbial decomposition (Buck et al., 1998; Baldrian, 2017). Despite these challenges, bark surfaces have been shown to carry a high microbial biomass, especially if lichens are present (Baldrian, 2017).

Tree trunks and their associated bark act as connecting habitats in forest ecosystems by linking the phyllosphere to the soil, particularly the rhizosphere (Baldrian, 2017). Perhaps the most important channel of connection is stemflow, which transports microorganisms from the phyllosphere along the bark to the soil (Bittar et al., 2018; Ptatscheck et al., 2018; Magyar et al., 2021). In addition, the atmosphere has been suggested to act as a transient habitat important for dispersal in forests (Baldrian, 2017), and bark surfaces can act as a source and sink for microorganisms dispersed by wind. We currently lack a clear understanding of how the bark and its microorganisms interact with their surrounding habitats. However, bark has been proposed as a reservoir for microbial diversity (Aschenbrenner et al., 2017; Arrigoni et al., 2018; Kobayashi and Aoyagi, 2019) because of its seasonal stability and the multitude of micro-niches available due to its structure (Beck et al., 2014; Leff et al., 2015).

2.1.2. Characteristics of the bark surface microbiome

Throughout this thesis, I use the term microbiome as defined by (Berg et al., 2020):

"The microbiome is defined as a characteristic microbial community occupying a reasonable well-defined habitat which has distinct physio-chemical properties. The microbiome not only refers to the microorganisms involved but also encompass their theatre of activity, which results in the formation of specific ecological niches."

This definition thus not only covers the microorganisms themselves, but also their environment, potential interactions and functions in regard to each other and their

potential host organisms. The term microbiome is often used to refer only to bacterial communities, but in this dissertation I use it to refer to the larger microbial diversity that includes multiple prokaryotic and eukaryotic groups.

Only a few studies have been conducted on bark surface communities. These studies have identified lichens (and their symbionts), yeasts, and cyanobacteria as the characteristic taxa (Bhadra et al., 2008; Beck et al., 2014; Grube et al., 2015; Baldrian, 2017). Much of the research on bark surface communities has been focused on lichens, which are complex associations of algae, fungi and bacteria (Grimm et al., 2021). Additionally, previous studies on microorganisms include morphological and culturedependent evidence of algae, cyanobacteria, fungi and a host of bacteria from tropical (Akinsoji, 1991) and temperate (Magyar and Révay, 2008) forests. Recent studies have suggested that these microbial communities on bark are distinct from those found in the phyllo- and rhizosphere (Martins et al., 2013; Lambais et al., 2014; Arrigoni et al., 2018). Additionally, there is evidence for seasonal variation in the community composition of the bark surface microbiome (Beck et al., 2014). Other studies have shown that the bark microbiome supports the forest food web and supplies food to many organisms such as molluscs, mites, lice and other micro-arthropods (Beyer and Saari, 1978; Andre, 1985; Erdmann et al., 2007; Baz, 2008). We currently lack knowledge of how the bark microbiome components interact with each other and how the bark microbiome integrates into the larger forest microbiome, especially when taxa occur in several forest habitats simultaneously (e.g., (Yang et al., 2022)).

2.2. Environmental DNA (eDNA)

2.2.1. Concept and applications

In the early 2000s the identification of species using DNA barcoding became feasible at larger scales, and this process has since been remarkably simplified by the main-stream emergence of High-Throughput Sequencing (HTS) (Taberlet et al., 2012). As a result, environmental DNA (eDNA) methods have gained popularity in a variety of scientific contexts, with the number of papers applying eDNA concepts rising exponentially (Jiang and Yang, 2017) to more than 50 papers each year between 2016 and 2020 (Beng and Corlett, 2020). In contrast to bulk specimen samples, eDNA does not include only a pre-selected community, but all DNA found in samples originating

from an environmental source (Taberlet et al., 2012). Identification of species contained in this mixed sample largely relies on the metabarcoding of marker genes, such as 16S for bacteria, COI for animals and ITS for plants and fungi (Taberlet et al., 2012; Bálint et al., 2016). In this method, universal primers are added during PCR to amplify a broad taxonomic variety while using tags to enable the sequencing of many samples during a single sequencing run (e.g., (Zinger et al., 2019)). Originally arising in soil and marine sciences, eDNA has gained the largest prominence in studies of microbial ecology and especially bacteria (Yoccoz, 2012).

Environmental DNA has been applied to many different subdisciplines in ecology, and has been forecast to make conventional methods of species diversity assessment obsolete (Taberlet et al., 2012; Yoccoz, 2012; Fediajevaite et al., 2021). Some studies where eDNA metabarcoding has been successfully implemented include the study of prey-predator interactions and animal diets (e.g., (Rayé et al., 2011; Shehzad et al., 2012)) as well as the most comprehensive study of microbiomes to date, the Earth Microbiome Project (Thompson et al., 2017). Further uses of eDNA metabarcoding include species monitoring, surveys of complete communities and the detection of rare or elusive species (Yoccoz, 2012). Furthermore, eDNA is also a promising technique for future assessments of an ecosystem's ecological state, and can potentially even lead to estimations of species abundance and densities (Yoccoz, 2012). The fast advance in methods and comparatively easy and quick sampling protocols have shown eDNA metabarcoding's potential for replacing conventional time-consuming expert surveys like vegetation mapping, fish trawling, insect trapping or acoustic surveys (Fediajevaite et al., 2021).

2.2.2. Leveraging eDNA to study community ecology

Using DNA extracted from environmental sources to study community ecology has many advantages. In conventional studies, species identification often presents a bottle neck, particularly at the large spatial and taxonomic scales feasible in eDNA metabarcoding experiments. In fact, many groups require immense taxonomic knowledge and are challenging even for experts (Giordani et al., 2009; Vondrák et al., 2016). Metabarcoding on the other hand allows the identification of diverse organisms without *a priori* taxonomic knowledge (Deiner et al., 2017; Fediajevaite et al., 2021).

Additionally, eDNA surveys hold the promise of avoiding biases depending on the person conducting the survey, as it does not rely heavily on prior experience (Giordani et al., 2009; Deiner et al., 2017; Wright et al., 2019; Fediajevaite et al., 2021). Furthermore, eDNA samples can also pick up traces of rare or cryptic species that are generally missed in classical survey methods (e.g. (Deiner et al., 2017)). Due to its simple protocols, eDNA metabarcoding has become a fast and cost-effective method that will likely become more affordable in the future as sequencing costs decrease and accuracy increases (Yoccoz, 2012). Currently, the high resolution of Next-Generation-Sequencing (NGS) already enables us to identify even small amounts of genetic variation, including Amplicon Sequence Variants (ASV), and to assign them to a species, thanks to the support of expansive open-source and open-data communities (e.g., (Abarenkov et al., 2023)).

The output of an eDNA metabarcoding workflow is comparable to that of a traditional survey, and normally includes information on which species occur in a sample and (approximately) how often (e.g., (Deiner et al., 2017)). Thus, microbial ecology borrows many analytical approaches from macroecology (Prosser et al., 2007). Usually, changes in diversity or community composition are investigated by comparing groups of samples, e.g., through ordinations and Permutational Analysis of Variance (Anderson, 2001; Anderson and Walsh, 2013), Differential Abundance Analysis (Fernandes et al., 2013) or Mantel tests (Mantel, 1967). Although the use of classic alpha diversity measures for microbiome research requires cautious interpretation (Bálint et al., 2016; Willis, 2019), it still provides useful information for studying environmental effects on microbiomes.

Measuring biodiversity is an inherently multifaceted concept, among others including multiple dimensions of taxonomic diversity (Chao et al., 2014; Naeem et al., 2016). Therefore, an approach that captures these multiple dimensions is necessary (Penone et al., 2018). A useful approach are the so-called Hill numbers (Hill, 1973; Chao et al., 2014). They measure not only the diversity of the full community, but also those of the "typical" and "dominant" taxa, by increasing the weighting of species abundance (or count, in the case of sequencing reads) each time the Hill number increases (Chao et al., 2014). Using these indices, we can apply statistical methods

such as linear regression or Generalized Dissimilarity Modeling (GDM) (Ferrier et al., 2007; Mokany et al., 2022), along continuous gradients instead of more or less arbitrarily-imposed group structures, as is done in "traditional" methods. Generalized Dissimilarity Modeling in particular is more flexible and can also model non-linear relationships, which are commonly found in ecology (Ferrier, 2002; Ferrier et al., 2004, 2007; Mokany et al., 2022).

One of the fundamental questions in microbiome research is how and which taxa interact with each other. In recent years, many potential methods surrounding microbial interaction networks have been proposed (e.g., SPIEC-EASI (Kurtz et al., 2015), SparCC (Friedman and Alm, 2012)). Microbial interaction networks are a computational method that allows us to infer connections of microorganisms from co-occurrence data (e.g., (Barberán et al., 2012)), that for example can be obtained from population level metabarcoding (Kurtz et al., 2015). Furthermore, microbial interaction networks enable the identification of highly influential taxa in the microbiome, so-called hub taxa, based on their connections to other taxa (Agler et al., 2016).

2.3. Drivers of epiphytic microbial communities

2.3.1. Environmental factors

To understand how bark microbial communities integrate and interact with other forest microbiomes, we need to examine why these communities change, as well as what is driving these changes (Baldrian, 2017). To date, there have been remarkably few studies on bark communities, making extrapolation from other microbiomes necessary to hypothesize potential drivers. The majority of studies related to epiphytic microbial communities have only considered the phyllosphere (e.g., (Bálint et al., 2013; Meyer et al., 2022; Zhu et al., 2022). In this dissertation, I expand the field to another epiphytic forest niche and target the three major organismal groups present on the surface of tree bark: green algae, fungi and bacteria.

Several environmental variables have been demonstrated, primarily in phyllosphere research, to influence microbial diversity and community composition. In tropical forests, a decline of algal biomass and diversity in the phyllosphere was associated with reduced water availability on leaf surfaces (Liu et al., 2023). Similarly,

the richness of fungal and bacterial micro-communities in the grape vine phyllosphere showed an increase with higher precipitation (Perazzolli et al., 2014). In addition, moisture content is known to regulate nitrogen fixation by diazatroph bacteria (Rousk et al., 2015), and thus directly influences microbiome function in forests. In one of the few studies examining bark surface microorganisms, temperature was suggested to be as important for microalgae as water availability (Neustupa and Štifterová, 2013), with higher temperature and precipitation leading to higher diversity. Mirroring the findings for water availability, increased fungal and bacterial diversity in the phyllosphere were also linked to higher temperatures (Perazzolli et al., 2014). According to (Baldrian, 2017), increased exposure to sunlight, and therefore UV radiation, could be an additional driving factor of bark surface micro-communities. Indeed, an increased UV exposure has been linked to changes in community composition, but not size, for bacterial phyllosphere communities (Jacobs and Sundin, 2001).

Bark microbiomes are also closely linked to their host. For example, the microorganismal communities in orchards have been shown to differ substantially between host materials of different ages, i.e., the age of the plant (Arrigoni et al., 2018, 2020). Furthermore, we know from below-ground microbiome communities like the rhizosphere that many microbial taxa, e.g., mycorrhizal fungi, only associate with specific tree species (Lang et al., 2011). Comparable relationships were also found for fungal, bacterial and protist communities of the phyllosphere, litter and soil (Redford et al., 2010; Prescott and Grayston, 2013; Urbanová et al., 2015; Tedersoo et al., 2016). Even differences in host genotype resulted in significantly different community structures (Bálint et al., 2013). Macroorganisms, such as lichens and bryophytes, have been shown to respond to similar drivers as the micro-communities. For example, the diversity of epiphytic species in forests was highly correlated with the occurrence of large and old trees (Aude and Poulsen, 2000; Boch et al., 2013; Nascimbene et al., 2013).

2.3.2. Mutual biotic influences

Interactions of microbial groups, with each other as well as with their environment, are common in forest ecosystems and can take many forms, ranging from interactions with their host to the provision of resources. One of the longest-studied association are lichens (Schwedener, 1886), macro-ecological holobionts (or "micro-ecosystems") in which fungi, algae and bacteria assemble and interact. The fungus provides structure and protection for algae and bacteria (Hawksworth and Grube, 2020; Krespach et al., 2020), and the algae in turn provide photoassimilates to the lichen consortium. Additionally, bacteria found in lichen have been linked to provision of growth inducing hormones (Grube et al., 2015) and may contribute to the nitrogen cycle of whole ecosystems (Rousk et al., 2015).

Outside of the lichen holobiont, free-living microbial communities of these three groups potentially engage in similar interactions. Algae primarily provide photosynthetic products, and although they are the primary producers in these communities (Durán et al., 2022), they rely on external nitrogen supplies that are commonly provided by mutualistic bacteria (Gonzalez and Bashan, 2000). While many bacteria engage in these beneficial interactions, other bacteria are harmful, especially to algae, for example causing cell death through the induction of certain compounds (Wang et al., 2010; Krespach et al., 2020). Fungal protection mechanisms can potentially prevent these harmful interactions (Krespach et al., 2020), and occasionally lichen-like formations of fungi and algae occur spontaneously under non-beneficial conditions (Hom and Murray, 2014). The structures provided by fungi, e.g., hyphae, have also been proposed to act as "highways" for bacterial dispersal (Kohlmeier et al., 2005). Fungal-bacterial interactions are also visible through intense competition for nutrients and parasitism (Deveau et al., 2018). Since all three organismal groups occur in close spatial association on the bark of trees, it is highly likely that they mutually affect each other's diversity. It is therefore essential to include the interactions between microorganisms if we want to understand the microbiome (Uroz et al., 2016).

2.4. Knowledge Gap

Bark surfaces are a large and important habitat for epiphytic diversity in forests, and support various communities of macro- and microorganisms. Despite their importance we lack a comprehensive understanding about the processes shaping these communities, in particular with respect to the microbial diversity. However, studying microbial communities in these environments is not trivial and conventional methods, such as cultivation, are usually not able to cover the full microbiome diversity. Therefore,

studying microbiomes nowadays requires the application of modern, genetically informed, methods of community ecology. To date, only few studies have investigated the communities on the bark of trees, often focusing on commercial systems related to fruit production or on single taxon groups, such as bacteria. However, we know from previous studies that green algae, fungi and bacteria occur simultaneously in these habitats. Taxa from these three groups are known to engage in multiple interactions, for example in the lichen holobiont. However, their combined communities have not been investigated until now. Samples of environmental DNA can help to reveal the diversity of both macro- and microorganismal communities on the bark surface. Nevertheless, a comprehensive assessment of eDNA methods applied to samples of bark surfaces is currently lacking.

3. Thesis Structure and Research Questions

3.1. Structure and Methods

3.1.1. Structure Overview

This thesis is structured into four questions, each representing one manuscript.

- I. (Q1) What is the diversity of the microbial community on the surface of tree bark? How do microbial communities respond to tree size and forest management?
- II. (Q2) What drives the diversity of the bark surface microbiome? Do biotic interactions play a greater role than abiotic influences?
- III. (Q3) How does the tree species shape fungal micro-communities? What are the differences between bark and soil fungal communities and do they share diversity?
- IV. (Q4) Is eDNA metabarcoding a useful tool for surveying epiphytic lichen biodiversity? Is it a reliable stand-alone tool?

Figure 1 provides an overview about the content of this dissertation, which is described in more detail below. Two questions (Q1, Q2) examine the three major organismal groups of the bark surface microbiome: green algae, fungi and bacteria. The other two questions (Q3, Q4) are focused on fungal diversity.

In a first step (Q1) I study the regional diversity of microorganisms on bark surfaces. I clarify the diversity and interactions of the microbiome, and how it responds to forest management and tree size (Fig. 1, Q1). Based on the results of Q1, I carried out a larger sampling campaign (Figure 1, Data Collection), during which I sampled bark surfaces of over 750 trees and soil in 150 plots across Germany. These samples were used to extract DNA and amplify ITS and 16S marker genes from green algae, fungi and bacteria, resulting in approximately 2,700 technical replicates. The sequencing reads of these replications form the basis of the remaining manuscripts (Q2 – Q4). In Q2, I investigate the changes of microbial alpha and beta diversity along environmental gradients. These include climatic conditions like temperature and precipitation, as well as the mutual biotic influences of each organismal group (Fig. 1, Q2).

In the second half of the thesis, I use a subset of the dataset acquired in the larger sampling campaign, to study the community of fungi (Q3) and lichens (Q4). In Q3, I examine the differences in diversity and community composition between the bark and soil microbiome of deciduous and coniferous trees (Fig. 1, Q3). Lastly, in Q4, I investigate if the eDNA from bark surface samples can be used to survey lichen species within forests. I use a subset of fungal reads from the eDNA dataset of the large sampling campaign, that were assigned to lichenized fungi, and compare the results to an expert floristic survey (Fig. 1, Q4). Using this approach, I explore whether eDNA can replace expert knowledge when surveying lichen communities.

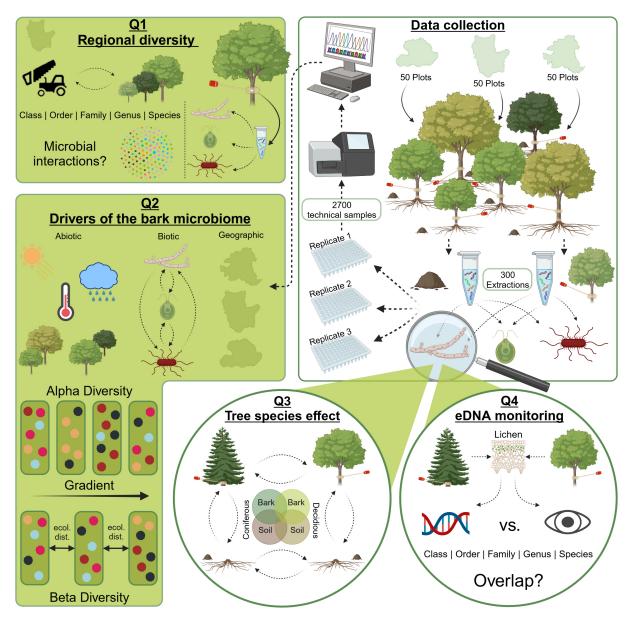


Figure 1: Overview figure showing the structure of this thesis. Included are a representation of the four questions (Q1-Q4) relating to the four manuscripts as well as an overview of the sampling procedure, lab work and bioinformatic processing. The questions are: I. What is the diversity of the microbial community on the surface of tree bark? How do microbial communities respond to tree size and forest management? II. What drives the diversity of the bark surface microbiome? Do biotic interactions play a greater role than abiotic influences? III. How does the tree species shape fungal micro-communities? What are the differences between bark and soil fungal communities and do they share diversity? IV. Is eDNA metabarcoding a useful tool for surveying epiphytic lichen biodiversity? Is it a reliable stand-alone tool? Figure created with BioRender.com.

3.1.2. Study System

The four manuscripts included in this dissertation are set within the framework of the Biodiversity Exploratories (Fischer et al., 2010) which were established in 2006. The main objective of the Biodiversity Exploratories is to provide a platform for long-term biodiversity research (Fischer et al., 2010). Research is carried out in circa 250 projects including, but not limited to, research on above- and belowground relations, studies on widely differing spatial scales, socioecologic questions, as well as the biodiversity of varied taxonomic groups from macro- to microorganisms. All projects are unified by common research aims, that target questions related to anthropogenic land use and how it affects diversity and ecosystem processes, as well as the relationships between different parts of biodiversity and how they influence ecosystem dynamics and services (see www.biodiversity-exploratories.de).

To enable comprehensive analysis of these topics, it is necessary to supply a wide range of sampling opportunities offering both a wide spatial and land use gradient. To address this need, the "Biodiversity Exploratories" encompass 300 "Experimental Plots" (EP) located in three regions across Germany. These regions display a spatial cross-section ranging from the South-West (Swabian Alb) to the North-East (Schorfheide-Chorin), with an intermediate region in the center of Germany (Hainich-Dün). Of the 300 EPs, 150 are located in grasslands of varying land use, such as pastures and meadows. The remaining 150 are within forests with varying degrees of human management, ranging from national park core zones to commercial timber plantations (Fischer et al., 2010). Each region contains 50 grassland and 50 forest plots.

The results presented in this dissertation are the product of a comprehensive sampling effort across all 150 forest EPs in all three regions of the "Biodiversity Exploratories". We carried out an initial sampling campaign in October 2020 using a subset of plots located within the central region (Hainich-Dün), allowing me to establish a baseline of biodiversity of the previously unexplored bark surface microbiome (Manuscript 1; Appendix A1). Following the results of our first study, a second sampling took place in May 2021 that was expanded to include samples from all three regions (Manuscript 2, 3 and 4; Appendix A2-A4). In addition to the sampling of bark surface

communities, we also included soil samples collected at the same time in our analyses (Manuscript 3; Appendix A3).

3.1.3. Sampling design

The EPs cover an area of 100 × 100 m, including a subplot of 20 × 20 m established for the joint Soil Sampling Campaign (SSC) of the Biodiversity Exploratories. We used these subplots to constrain the sampling space of the bark surface samples, thus obtaining the microbial diversity of spatially close trees. Combining the samples of individual trees, either during bioinformatic processing or physically in composite samples, provides a representative snapshot of plot-level diversity. Furthermore, sampling in the same area as the SSC facilitates comparisons between bark surface and soil communities.

To characterize the microbial diversity of the sites, we collected individual tree and plot-level samples of the bark surface micro-community within the forest EPs (Figure 1). We sampled a total of six trees across three size classes per plot, two small (5 – 15 cm diameter at a height of 150 cm), two medium (15 – 30 cm diameter) and two large trees (> 30 cm). We used nylon-flocked medical collection swabs to collect the bark surface biofilm in a 3 cm wide band around the complete tree trunk, while purposefully including holes, cracks and lichen thalli but avoiding larger patches of bryophytes so as to not confound fungal and algal DNA amplification. This sampling method proved to be reliable, easy, fast, cost-effective and replicable, allowing me to cover a large spatial extent.

3.1.4. Metabarcoding of multiple organismal groups

A comprehensive diversity profiling of the bark surface microbiome requires an equally broad and intensive metabarcoding approach. To obtain taxa of the three main microbial groups of the bark surface microbiome, we chose a DNA extraction kit that is suitable for algae, fungi and bacteria (Quick-DNA Fecal/Soil Microbe Microprep, Zymo Research GmbH, Freiburg, Germany). The extracted DNA fragments were amplified using well-established universal primer pairs of the common marker regions ITS2 (algae and fungi) and 16S V3-V4 (bacteria). Using these universal (degenerate) primers allows for a

broad taxonomic coverage, representing the true diversity as closely as possible, and facilitates comparisons with future studies.

To obtain reliable diversity estimates and sequencing results, a robust combination of sampling design, laboratory procedure and bioinformatic processing is crucial. We employed a strict protocol in order to minimize errors during PCR and reduce ambiguities and contamination during the processing of sequencing reads. In the laboratory, this included individual, separate treatment of the three organismal groups after DNA extraction, fine-tuning of the PCR to maximize yield and quality, PCR negatives and multiplex controls, amplification in triplicates and PCR-free library preparation to minimize PCR bias. The bioinformatic processing was performed with state-of-the-art tools for sample inference and taxonomic assignment against suitable databases (*DADA2* pipeline, (Callahan et al., 2016)) and furthermore included identification of potential contaminant sequences (*decontam*, (Davis et al., 2018)) and post clustering curation of similar sequences (*LULU*, (Frøslev et al., 2017)).

3.2. (Q1) What is the diversity of the microbial community on the surface of tree bark? How do microbial communities respond to tree size and forest management?

In order to model the relationships between microorganisms and nature from unknown forest micro-habitats, it is crucial to first obtain a baseline of the unknown diversity on the bark surface of trees. In this first manuscript, we focused on establishing a sampling method using samples from a local subset of 16 plots in the central region (Hainich-Dün, Thuringia, Germany) of the Biodiversity Exploratories. We collected samples from six individual trees of *Fagus sylvatica* per plot that were individually sequenced and processed to enable comparison of single trees as well as plot-level communities. We sampled trees from three different size classes (see 1.1.3. Sampling design) on plots along a gradient of forest management intensity, ranging from highly-managed stands to the core zone of a national park. The manuscript addresses the following questions:

- I Which microbial taxa occur on the bark of beech trees (*Fagus sylvatica*), the dominant tree species in Central European forests?
- II Which taxa are the most important based on co-occurrence patterns?

III Do responses to anthropogenic influence and tree size (as a proxy for tree age) differ between organism groups?

3.3. (Q2) What drives the diversity of the bark surface microbiome? Do biotic interactions play a greater role than abiotic influences?

After establishing a first baseline assessment of the diversity in the bark surface microbiome, we aimed to understand which environmental factors drive microbial diversity and community composition patterns on the bark surface in a larger context. To achieve this, we sampled in all three regions within the Biodiversity Exploratories, which considerably increased the spatial extent of the subsequent analysis. This was possible by combining six trees across the three size classes into one composite sample per EP, totaling 150 plot-level samples. This allowed our study to cover a large geographic gradient across Germany. We always sampled from the dominant tree species in the plot based on a previous forest assessment by Schall and Ammer (2018). Mainly samples were obtained from three tree species: European beech (Fagus sylvatica), Scots pine (Pinus silvestris) and Norway spruce (Picea abies), which represent the most important Central European trees from both potentially natural forests and commercial timber plantations. In this manuscript we addressed the following questions:

- I Which environmental variables drive the community composition and alphadiversity of the bark surface microbiome?
- II Are the strong connections between taxa (shown in Manuscript 1) more important for community assembly and diversity than the influence of abiotic variables?

3.4. (Q3) How does the tree species shape fungal microcommunities? What are the differences between bark and soil fungal communities and do they share diversity?

Our knowledge of the taxonomic diversity in the bark surface microbiome allows us to compare the communities of these previously-unexplored habitats to other habitats of the forest microbiome. With regards to microbial diversity, the most studied and best characterized forest habitats are soils (Baldrian, 2017). Using a subset of the data obtained in the large sampling campaign, containing both soil and bark surface samples from 150 forest EPs, we compare the soil and bark surface microbiome of the same forest area. We chose to include samples from two regions (South-West and North-East), both of which contain coniferous (spruce and pine, respectively) as well as broadleaved (beech) trees. These tree species have pronounced differences in bark surface structure as well as chemistry, e.g., pH levels. In this manuscript we address the following questions:

- I What differences and commonalities do we observe between bark surface and soil microbial communities?
- II Can tree species identity account for differences in bark surface diversity and community composition?

3.5. (Q4) Is eDNA metabarcoding a useful tool for surveying epiphytic lichen biodiversity? Is it a reliable standalone tool?

Environmental DNA is commonly used to survey the occurrence of species in an environment (e.g., (Taberlet et al., 2012; Yoccoz, 2012; Deiner et al., 2017)). In traditional vegetation surveys, the identification of lichens requires considerable expertise, and many species can be easily overlooked or misidentified (Vondrák et al., 2016). In this manuscript, we use a subset of the fungal taxa obtained from eDNA metabarcoding that were assigned as "Lichenized" by the FUNGuild algorithm (Nguyen et al., 2016). We compare the obtained species list with that of a previous floristic survey carried out in 2007/2008 (Boch et al., 2013) in the same plots. We examine the quality and coverage of both methods and ask the following questions:

- I Which species of lichenized fungi can be identified from environmental samples via eDNA metabarcoding?
- II How does the set of species compare to an expert floristic survey?

4. Main Results and Discussion

In my dissertation I investigated the diversity of the tree bark surface, an understudied forest habitat. The aim of this dissertation was to reveal the previously-unknown diversity of the natural bark surface microbiome and its responses to environmental drivers by using a fast and simple eDNA metabarcoding approach. Furthermore, I compared the microbiome of the bark surface to the microbial community of their adjacent forest soils, an example of a well-characterized microbial habitat. Finally, I investigated whether eDNA samples can be used to survey the epiphytic lichen community, a group typically identifiable only by taxonomic experts. In the following paragraphs, I first look at my four questions individually and summarize their main results. Subsequently, I discuss the respective findings, connect them and place them into a wider context.

4.1. (Q1) Regional diversity and interactions of the bark surface microbiome of differently sized trees

Previous studies of aboveground microbiomes, like the phyllosphere, have shown a strong link between diversity and host characteristics (Lindow and Brandl, 2003; Vorholt, 2012). For example, some of the studies investigating aboveground microbiomes show that substrate age affects the microbiome community (Arrigoni et al., 2018, 2020). Anthropogenic influence, for example through forest management, strongly correlates with the presence or absence of old-growth trees and thus the availability of mature substrates (Aude and Poulsen, 2000). Currently, the responses of the bark microbiome to these variables are largely unknown.

I used eDNA metabarcoding on samples obtained from bark surfaces of beech trees to profile the three main organismal groups of the bark surface microbiome. In this manuscript I established a workflow consisting of a cost-effective and easy sampling technique as well as a processing and analysis pipeline. I shed light on the taxa occurring on natural bark surface communities and provide regional-level diversity estimates for several forests within the Central Region of the Biodiversity Exploratories. Furthermore, I identified which taxa are important for the microbiome community and how they interact both within their own and across other microorganismal groups.

Lastly, I examined how tree size, as a proxy for tree age, and the intensity of forest management affects the bark surface microbiome.

Because the bark surface microbiome is largely unexplored, theories to describe and predict responses to environmental conditions must be borrowed from the study of macro-epiphytic organisms. Studies of epiphytic lichens and bryophytes have shown that their diversity and community assembly processes largely depend on tree age (Fritz et al., 2008, 2009) and the availability of large and old-growth forest structures (Aude and Poulsen, 2000; Boch et al., 2013; Nascimbene et al., 2013). In this study, we found that tree size (as a proxy for age) greatly influenced the beech bark microbiome. Algal and fungal diversity was lower in larger trees, possibly indicating filtering of pioneer communities occupying the smaller, i.e., younger trees, for example because they are outcompeted by other organisms. The differences are particularly visible when comparing large and small trees, but not when comparing large and small to medium trees, suggesting a gradual process. We observed differences in community composition when comparing the size groups, and differential abundance analysis revealed several differentially abundant taxa, especially when comparing large and small trees.

In our study, forest management had a negligible but significant effect on community assembly of the bark surface microbiome. However, a previous study of macro-ecological taxa undertaken in the same region reported a much stronger effect of management on diversity (Boch et al., 2013). This suggests that our classification into "high" and "low" management intensity may be too coarse to make the effect visible. Additionally, the forest management in the study region is rather uniform, leading to similar forest structures (Schall et al., 2020).

Taxa are usually highly interconnected within the bark surface microbiome. In our study, co-occurrence networks show dense associations of taxa that cluster into distinct modules of strong association. This may indicate a high level of niche specialization, in particular for fungi and algae, which coincides with a stronger effect of tree size for these two groups. Many of the interconnected taxa in the networks were previously known as important elements of sub-aerial and phyllosphere communities. These include important groups of free-living green algae (Rindi, 2007; Štifterová and

Neustupa, 2015; Škaloud et al., 2016; Zhu et al., 2018), as well as lichens and their photobionts (Sanders and Masumoto, 2021). The important bacteria have often been found in association with macro-epiphytes (Yamamura et al., 2011; Ma et al., 2017). Interestingly, two important bacterial genera within the networks, *Acidiphilium* and *Methylocella*, have different metabolic demands (Dedysh and Dunfield, 2011; Hiraishi and Imhoff, 2015), with *Acidiphilium* metabolizing simple sugars and *Methylocella* obtaining carbon from atmospheric methane. Their co-occurrence may also point towards metabolic niche differentiation on the bark surface.

Many of the ecologically relevant fungal taxa are either unknown or unassignable with current databases. Up to 40% of fungal reads are from unknown origin, and many of the most connected taxa in the co-occurrence networks could not be assigned at the genus level, which is comparable to previous bark microbiome studies from orchards (Arrigoni et al., 2020). This "unknown" diversity suggests a high amount of previously undescribed diversity for taxa on the bark surface, but could also be an artefact of incomplete databases or occurrence of species that have yet to be sequenced. Considering that the majority of previous studies has investigated other microbial habitats, it is a reasonable assumption that we find numerous unique taxa that have not been studied before. A considerable portion of this "unknown diversity" is made up of Dothideomycetes, a highly diverse class of Ascomycota. Several species of this class are found in the lichen holobiont (Smith et al., 2020) and are known from the beech phyllosphere (Unterseher et al., 2016). These results underline the fact that our knowledge of the bark as a habitat for microbial diversity is currently insufficient, despite composing a large part of the forest microbiome. With this study, we provide some initial findings that can help to fill in these gaps in our knowledge of the bark microbiome.

4.2. (Q2) Biotic and abiotic drivers of the bark surface microbiome

Based on the results of Q1, we expanded the sampling to a considerably larger number of sites across Germany. Informed by the diversity differences between differently-sized trees, we took plot-level composite samples that were evenly distributed between small, medium and large trees. In total, the obtained samples represented forests in three regions, and more than 750 trees from 150 forest plots in the Biodiversity Exploratories,

which are representative for Central Europe (Fischer et al., 2010). We used Hill Numbers to characterize the multiple levels of alpha and beta diversity in the bark surface microbiome, and examined how diversity responds to environmental factors and biotic interactions. Based on results from phyllosphere studies and the characteristics of the bark habitat, we hypothesized that bark surface communities would be influenced by similar variables to those influencing the phyllosphere microbiome. These mainly include geographic distance, (micro-)climatic conditions, and species interactions. To investigate the influence of these variables we applied advanced statistical methods such as Generalized Dissimilarity Modeling which allows us to model non-linear relationships (Ferrier, 2002; Ferrier et al., 2004, 2007; Mokany et al., 2022).

In previous studies of aboveground microbiomes, geographic distance was often reported as a factor leading to of distinct communities (Neustupa and Štifterová, 2013; Perazzolli et al., 2014). Although ASV richness in this study usually decreased from South-West to North-East Germany, the abundance patterns across regions were comparable. Consequently, the effect of geographic distance on beta diversity was negligible in this study, similar to what has been reported for the bark microbiome of avocado trees (Aguirre-von-Wobeser et al., 2021). However, geographic distance was of considerable importance for the alpha diversity when considered together with environmental conditions, suggesting an interconnected effect.

Communities of aboveground habitats are influenced by many environmental variables, such as humidity (e.g., (Liu et al., 2023)), temperature (e.g., (Perazzolli et al., 2014)) or UV radiation (Jacobs and Sundin, 2001). In our study, environmental variables were of great importance for alpha diversity, while having a minimal effect on beta diversity. Interestingly, the effect of light availability on algae was small and non-significant, despite their photosynthetic requirements. Additionally, the effects of abiotic variables on fungi and bacteria were often contradictory. Studies of soil microbiomes have shown similar effects, for example drought responses differed between bacteria and fungi (de Vries et al., 2018) and even between different fungal guilds (Castaño et al., 2018). Comparable results have also been reported for the phyllosphere (Perazzolli et al., 2014) and rhizosphere microbiomes (Bazany et al., 2022). Interestingly, the opposite responses of bacteria and fungi to increases in temperature, where fungal

communities homogenize and bacterial communities become increasingly dissimilar, mirrors results from the soil microbiome (de Vries et al., 2018).

Microbial communities of bacteria, fungi and green algae are known to engage in various interactions with each other (e.g. (Ramanan et al., 2016; Deveau et al., 2018)). Thus, the large influence of mutual biotic interactions detected in this work is not unexpected. The community composition of each organismal group in particular was significantly influenced by the composition of the other two groups. In our study, the bacterial influence was by far the most important. Bacteria are known to engage in harmful interactions like pathogenicity (Deveau et al., 2018; Krespach et al., 2020), but are also important factors in the provision of nutrients (Gonzalez and Bashan, 2000), and commonly colonize the minute space surrounding algal or fungal cells (Warmink and van Elsas, 2008; Durán et al., 2022). The influence of fungi on algae has been long known from the lichen symbiosis (Schwedener, 1886; Hawksworth and Grube, 2020), and similar associations can also form spontaneously under suboptimal conditions (Hom and Murray, 2014). In addition, fungi strongly influence bacterial communities, which could be related to the provision of transport paths (Kohlmeier et al., 2005), protective structures (Venkatesh et al., 2022) or even carbon from cell walls (Ballhausen and de Boer, 2016).

The effect curves of the biotic interactions suggest that changes in community compositions occur in tight links, and that organismal groups vary simultaneously and at comparable speed. These results may indicate that distinct sets of algae, fungi and bacteria frequently associate with each other. Preferential associations are not uncommon in plant-microbiome relationships (e.g., (Hawkes et al., 2020)) and have been observed in bark communities before. For example, Arrigoni et al. (2018) describe a "stable equilibrium" of pathogenic and beneficial bacteria and fungi on the bark of fruit trees.

Our results indicate that the richness and evenness of the bark surface microbiome is constrained by the abiotic environment. However, the interaction with other taxa plays a significant role in determining the composition and dominance of the species present. In, fact the effects of mutual biotic interactions are usually the largest

influence changing the composition of the bark surface microbiome. Lastly, the geographic distance between plots was usually of small importance.

4.3. (Q3) Differences between soil and bark surface microbiome associated with coniferous and deciduous trees

Trees and fungi interact with each other in many different ways. For example, trees provide a greatly structured habitat, with a large number of diverse micro-niches. Due to variations in various inherent traits of the bark, such as physical structure and chemistry, tree species is likely an important driver of bark fungal community composition. In this manuscript, we investigated the fungal community from two regions and in two forest habitats, soil and bark. Additionally, we included two coniferous (*Picea abies* and *Pinus sylvestris*) and one deciduous (*Fagus sylvatica*) tree species. We assessed the differences and similarities in diversity and community *composition* of the soil and bark surface microbiomes in relation to the tree species. Furthermore, we studied the interactions within the combined microbiome of the above- and belowground habitats.

Previous studies have often focused on individual tree compartments (e.g., on the phyllosphere (e.g., (Unterseher et al., 2016)) or rhizosphere (e.g., (Prescott and Grayston, 2013)), and studies of more than one forest compartment are still comparatively rare (Yang et al., 2022). From these studies, we know that different forest compartments also feature distinct microbiome compositions (Lambais et al., 2014; Leff et al., 2015; Durand et al., 2017; Yang et al., 2022). A similar structure is visible from the ordinations in our study, and interaction networks reveal that taxa are more strongly connected within their habitat. In fact, very few taxa are shared between habitats (343 or approximately 4% of ASVs), although these taxa on average account for 62% of the total sequencing reads. In addition to the distinct community composition, soil communities were on average approximately five times more diverse than bark surface communities, in terms of ASV richness. This could be directly related to lower nutrient availability as well as chemical and physical stressors on the bark (Buck et al., 1998; Baldrian, 2017).

Despite the apparent differences in composition, the co-occurrence networks revealed a clearly distinguishable ecological module potentially connecting the soil and

bark surface communities. Indeed, fungi have been suggested to provide such connections in the forest microbiome (Baldrian, 2017). This may indicate that the bark surface serves as a connecting habitat for the forest microbiome. One potential path is stemflow, which has been proposed as a transport vector of microbial taxa from the phyllosphere to the soil (Magyar et al., 2021).

Chemical and structural characteristics of the bark may create large differences in the effect of the coniferous and deciduous tree species on their fungal communities (Alfredsen et al., 2008; Oka et al., 2021). For example, pine and fir bark is more acidic than beech bark (Prasetya and Roffael, 1990; Fritz and Heilmann-Clausen, 2010), and deciduous litter is less acidic than coniferous (Burgess-Conforti et al., 2019). Contrary to other studies (Prescott and Grayston, 2013; Urbanová et al., 2015), tree species was of unexpectedly low importance in our study, explaining only a small proportion of the diversity variance. Nonetheless, the community composition and ASV richness of coniferous and deciduous trees differs significantly in both regions. This effect is particularly pronounced in the soil samples. A previous study conducted in the Biodiversity Exploratories showed comparable differences in the composition of soil microbiomes, depending on the tree species in the vicinity (Goldmann et al., 2015). These distinct compositions could be explained by the occurrence of specialized litter degraders (Barbi et al., 2016), or by the presence of mycorrhiza specific to these tree species (Goldmann et al., 2015; Nacke et al., 2016).

4.4. (Q4) Suitability of eDNA metabarcoding as a tool in surveys of lichen diversity

Environmental DNA metabarcoding provides the promise of easy and fast species identification using the complete DNA present in an environmental sample (Taberlet et al., 2012; Deiner et al., 2017). This promise is especially intriguing when it comes to surveying organisms that are morphologically difficult to identify. Lichens provide one such example where species identification is often challenging even for taxonomic experts (Vondrák et al., 2016), with species identification remaining highly dependent on the level of training (Giordani et al., 2009). To date, there have been few studies conducted to assess lichen diversity using eDNA metabarcoding. Only a small number of studies have attempted to identify species from bulk specimen collections (Wright et

al., 2019; Henrie et al., 2022). Based on a floristic survey by Boch et al. (2013), it is known that the Biodiversity Exploratories contain a diverse community of lichens. In this study, we used the full set of fungal reads acquired from eDNA metabarcoding of bark surfaces to identify lichenized fungi in the same plots and to compare the results with the floristic survey.

eDNA metabarcoding studies usually include unspecific primers that amplify many variable target taxa (Taberlet et al., 2012). By using broad primers amplifying the fungal ITS2 gene marker (White et al., 1990; Ihrmark et al., 2012) we amplified 99 lichen species. On average, they corresponded to 25% of the total fungal reads in the three study regions. Nonetheless, three species of lichenized fungi are among the five relatively most abundant taxa in the dataset, *Scoliciosporum umbrinum*, *Lepraria incana* and *Phlyctis argena*, with the latter two also being found in the floristic survey.

Generally, we find that both methods preferentially detected sets of different taxa with little overlap. Indeed, only 40 of the 154 species found in total, or 26% percent, were jointly identified by both methods. Some of the species detected in the floristic survey, such as *Pseudevernia furfuracea*, are typically found on branches or in tree crowns (Kranner et al., 2003). It is therefore unsurprising that they cannot be identified using our sampling approach. Additionally, the UNITE database does not include ITS sequences of several taxa identified via floristic methods, which therefore cannot be assigned from the fungal sequencing reads. On the other hand, eDNA metabarcoding allowed us to identify a broader diversity in inconspicuous genera, such as *Bacidina* (Czarnota and Guzow-Krzemińska, 2018), which could not be distinguished or were overlooked in the floristic survey. In addition, it is also important to note that 13 years lay between the floristic and the eDNA metabarcoding surveys. In the meantime, acidofrequent species, like *Straminella conizaeoides*, have been under decline due to a reduction in acidic air pollution (Farkas et al., 2022).

It is clear that both methods have unique challenges that must be overcome. The main drawback of floristic studies is the large time investment and the need for expert knowledge (Giordani et al., 2009; Vondrák et al., 2016), while the eDNA metabarcoding suffers from poorly-resolved databases. One potential solution is the development of better (and/or lichen-targeted) reference databases that can improve the results

obtained in the taxonomic assignment. Very recently, Vondrák et al. (2023) have spearheaded the development of such a database. A different approach could be the use of bulk collections of voucher specimens for targeted community metabarcoding. A previous study has shown that the overlap between samples of minimally-trained personnel and a lichen expert was substantial (Wright et al., 2019). Therefore, the need for taxonomic expertise could be reduced with a comparatively lower investment in training. Due to its simple sampling protocol, the eDNA metabarcoding can also be extended to other surfaces, which could potentially reveal additional species. Nevertheless, in our opinion eDNA cannot currently replace floristic surveys by experts. However, they can serve as a complimentary tool, for example, to identify and monitor inconspicuous taxa, or taxa that feature intraspecific genetic diversity.

4.5. General Discussion

Characterizing unknown biodiversity requires considerable effort. Due to the limited number of studies on the microbial life found on the bark surface, it is almost impossible to establish clear hypotheses *a priori*. Thus, the theoretical background of this dissertation relies substantially on findings from spatially-close forest microbiomes like the phyllosphere and rhizosphere. In addition, much of microbial ecological theory is still under development, although it has made significant progress over the last decade (Prosser et al., 2007; Bálint et al., 2016). Consequently, many methods borrow theory from macro-ecological backgrounds, although it is debatable if they can be applied in the same manner to microbial contexts (Prosser et al., 2007; Ritter et al., 2019; Willis, 2019).

Changes in microbial processes are generally not easy to address, particularly because of fast generation times and the associated constant turnover, which greatly surpasses that of long-lived organisms like trees (e.g., (Uhr et al., 2019)). Contrary to the popular belief that all microorganisms are ubiquitous and that microbial communities are selected for by the environment (see (O'Malley, 2008)), studies have shown that many microbial taxa are actually limited in their dispersal range, which clearly influences their biogeographic distributions (Choudoir et al., 2018; Walters et al., 2022). Furthermore, they often engage with each other in many different and often unclear interactions (e.g. (Ramanan et al., 2016; Deveau et al., 2018; Muggia and Grube, 2018;

Grimm et al., 2021)). Nonetheless, many studies only focus on single taxonomic groups, most prominently fungi (e.g., (Petrolli et al., 2021)) and bacteria (e.g., (Aschenbrenner et al., 2017)), and disregard these interactions.

The strong connections of green algae, fungi and bacteria found in the included studies emphasize the importance of their interactions in the bark microbiome. In fact, studies have suggested that some of the interactions and associations between these groups are shaped by the same ecological principles as the plant-microbiome interactions (Durán et al., 2022). Furthermore, microorganisms can manipulate and mitigate the impact of environmental conditions (Landeweert et al., 2001; Pauwels et al., 2023), which has previously been shown to alter plant-microbiome interactions (e.g., (Afkhami et al., 2014)). In our study the weak responses to abiotic effects, in addition to the strong interactions between taxonomic groups, may indicate that similar mitigation mechanisms occur in the bark microbiome.

Aside from deterministic processes, such as environmental filtering and interactions, there is increasing evidence that many microbial processes are underpinned by stochastic effects (Zhou and Ning, 2017; Dove et al., 2021; Skouroliakou et al., 2022). These processes include dispersal and diversification events as well as ecological drift (Zhou and Ning, 2017). They can involve birth and death events, the evolution or extinction of species, dispersal limitation or so-called priority effects in colonization events, among others (Zhou and Ning, 2017). However, not all of these processes are purely random, and likely occur simultaneously with deterministic processes (Zhou and Ning, 2017). In the majority of the studies included in this thesis, a considerable portion of the variance in microbial diversity could not be explained by the included deterministic drivers. Therefore, it is possible that stochastic processes are also of high importance for the forest microbiome.

Unfortunately, many of the stochastic processes that play a role in forest microbiomes are currently unknown. In a study of deadwood microbiome assembly, Hagge et al. (2019) found that bark increases the importance of these processes. One potential process may involve previously-unknown priority effects of the bark surface microbiome of the living tree, i.e., the microbial community found on the living bark surface shaped the deadwood community. Furthermore, microbial dispersal is currently

poorly understood (Choudoir et al., 2018; Walters et al., 2022). Dispersal is another stochastic process considerably influencing microbial taxa (Zhou and Ning, 2017). In forests, the bark surface might be an important dispersal path that connects forest habitats (Baldrian, 2017). Microbial taxa can, for example, be transported from the phyllosphere to soils via stemflow (Magyar et al., 2021) or bilaterally by wind (Walters et al., 2022). In addition, bark has been proposed as a reservoir of microbial diversity (Aschenbrenner et al., 2017), that could potentially influence assembly processes, for example in the renewing phyllosphere in spring. Both dispersal and reservoir effects indicate that the bark surface community may have a role in shaping the stochastic microbiome assembly in other habitats, such as soil, phyllosphere or deadwood.

In my dissertation, I clearly show the importance of an integrative approach to analyzing microbial communities in forests. Baldrian (2017), calls for the inclusion of multiple habitats, which I further extended to the inclusion of multiple taxonomic groups. The considerable overlap between the soil and bark surface microbiome, as well as the significant interactions between the three major microbial groups, strongly underlines this need for a more integrative approach. Using eDNA as the basis for these genetic analyses enables us to quickly and cost effectively acquire species compositions not only of micro-communities, but also for macroorganisms like lichens. By storing the extracted DNA, this research could even be extended to other groups of interest for forests, like insects or mammals, which were beyond the scope of this dissertation.

My results highlight that bark surfaces are an important but underestimated habitat for microbial diversity. However, further research is needed to better understand the processes and functions within the forest microbiome. Especially the considerable number of undescribed or unassignable fungal taxa, which account for up to 50% of the relative abundance in some samples, underlines how understudied bark surfaces are. Considering that current estimates suggest that only 10% of fungi have been described to date (Hawksworth and Lücking, 2017), bark surfaces offer an opportunity to uncover a large amount of new diversity. Future studies would greatly benefit from the identification of these taxa, for example by using a combined approach of (meta-)genomics and cultivation. Without knowledge of these taxa, understanding the

functions of the bark microbiome and its connection within the larger forest microbiome will be challenging (e.g. (Aschenbrenner et al., 2017; Baldrian, 2017)).

I provide a comprehensive assessment (and an associated dataset) of the bark surface microbiome that is representative of forests in Central Europe (Fischer). Furthermore, I shed light on the important environmental drivers of these communities. Although generalizations, for example to other climate zones, cannot be directly drawn, previous studies of macro-ecological groups have shown that similar drivers are important in the tropics as well (Penone et al., 2018; Fornoff et al., 2021).

This baseline of diversity and the key drivers identified in these studies are highly valuable for future research projects, especially when it comes to understanding forest microbiome processes. For future users, especially in the field of microbial ecology, archival and provision of the data is invaluable (Nekrutenko and Taylor, 2012; Sandve et al., 2013; Deiner et al., 2017). Consequently, all my code and data, intermediate and raw, is available through GitHub (https://github.com/LukDrey), the Biodiversity Exploratories Information System (BExIS; https://www.bexis.uni-jena.de/) and the Short Read Archive (SRA; https://www.ncbi.nlm.nih.gov/sra). The corresponding accession numbers are included in the manuscripts.

4.6. Critical Evaluation of eDNA metabarcoding

Despite the many advantages, eDNA methods also suffer from unique challenges and drawbacks. Firstly, the assignment of species from sequencing variants is often not trivial (Kõljalg et al., 2013; Bálint et al., 2016). Secondly, many steps of the pipeline, from eDNA sampling to the generation of a final taxonomic table, can potentially introduce bias or errors (e.g., (Taberlet et al., 2012; Deiner et al., 2017; Fediajevaite et al., 2021)). Thus, considerable knowledge and care is required, even if species identification does not rely on taxonomic expertise, with the expertise required being shifted to other areas such as laboratory work or bioinformatic processing.

In the laboratory, potential areas of error include the choice of DNA extraction method, selecting a fitting but broad enough taxonomic primer pair, PCR amplification and the choice of sequencing method and chemistry (Deiner et al., 2017). Furthermore, DNA degrades quickly under normal environmental conditions and might already be

degraded at the time of sampling, thus limiting the choice of marker genes to shorter genetic regions (Willerslev et al., 2004; Hansen et al., 2006). Here I chose a broad extraction kit due to its coverage of both eukaryotic and bacterial organisms. I used two markers commonly used in microbiome studies, and amplified them using well-established, taxonomically broad primer pairs. Additionally, I used Illumina Sequencing due to its value as a robust and proven technology for microbiome studies, its low rate of sequencing errors, and the many bioinformatic tools that are available for this data type. In addition to robust and proven lab methodology, I also included several measures to address potential contamination issues, namely the inclusion of extraction blanks, PCR negative controls, and so-called multiplex controls to assess levels of tag jump during sequencing (Schnell et al., 2015).

The output of sequencing runs needs to be examined and interpreted carefully. Due to the technical constraints of the machine, samples are inherently of a compositional nature (Gloor et al., 2017). Because the sequencing machine has a fixed limit of reads it can provide, it is not possible to determine if all DNA molecules have been sequenced (Gloor et al., 2017). Due to these limitations, classical alpha diversity measures face several problems in microbiome studies, since they heavily depend on the abundance, or counts in the case of NGS, of the taxa in the community and on an exhaustive sampling (Willis, 2019). Therefore, alpha diversity tends to increase with higher sampling intensity, or in this case, with higher sequencing depth (Willis, 2019). Because of these two main concerns, some form of bias correction is needed, e.g. by using compositionally aware methods and transformation (Gloor et al., 2017) or by correcting for sequencing depth in the analysis (Bálint et al., 2015). I used both methods in this dissertation.

Bioinformatic approaches are necessary to process the resulting reads obtained from the sequencing machine. When conducting an eDNA metabarcoding study, researchers have to choose between a multitude of tools, programs and pipelines that can be of varied accuracy and precision (Deiner et al., 2017). The results can vary considerably between tools, especially in terms of the number of taxa obtained (Prodan et al., 2020). I chose *DADA2* (Callahan et al., 2016) because of its increased sensitivity and resolution when compared to other common methods (Prodan et al., 2020). Despite

DADA2 originating as a tool for classifying 16S amplicon reads, it also performs well for the classification of ITS amplicons (Pauvert et al., 2019). Therefore, the same pipeline could be used for all included organismal groups in order to obtain robust comparisons.

Depending on the chosen tool, the obtained taxonomic unit also changes. Studies commonly cluster sequencing reads either into Operational Taxonomic Units (OTU) (Schloss and Handelsman, 2005; Schloss et al., 2009) based on a similarity threshold, e.g., 97% (Westcott and Schloss, 2015), and more recently into Exact Sequence Variants (ESV) or Amplicon Sequence Variants (ASV), which resolve sequencing reads into taxa at the single nucleotide level (Callahan et al., 2017). There is ongoing debate on the use of ASVs, especially for fungi, due to the variable length of the ITS marker (Kauserud, 2023). However, no consensus on which method is superior in regard to diversity estimation has been reached to date, with valid arguments for both (Joos et al., 2020; Cholet et al., 2022; Tedersoo et al., 2022; Kauserud, 2023). In this thesis I have used ASVs because they offer higher resolution without using arbitrary cutoffs. However, I adopted best-practice tools for post-clustering curation (*LULU*, (Frøslev et al., 2017)) and decontamination (*decontam*, (Davis et al., 2018)) to reduce the risk of overestimating diversity.

Moreover, the taxonomic assignment of a taxon relies heavily on the quality of the database (Balvočiūtė and Huson, 2017). Many of the most commonly used databases like GenBank of the National Center for Biotechnology Information (NCBI) are community driven, i.e., researchers submit their results to the database (Benson et al., 2013). The accuracy of these databases has been questioned in relation to metabarcoding (e.g. (Elbrecht et al., 2017)), but they are generally of high accuracy considering the vast amount of data stored in them (Leray et al., 2019). Expertly-curated databases are rarely available and are often focused on single organism groups (e.g., SILVA for bacteria (Quast et al., 2012) or UNITE for fungi (Abarenkov et al., 2023). However, gaps in coverage are still prominent and are visible for example when assigning lichen taxonomy. Fortunately, there is an ongoing community effort to obtain reference databases at finer taxonomic levels (e.g., Martin7 for European lichen (Vondrák et al., 2023)), which will be beneficial for future studies.

Given the considerable amount of unexplained variance left in the identification of microbiome drivers, it may be worthwhile to include additional variables beyond stochastic processes in future studies. Unfortunately, it is often not possible to define all environmental variables *a priori* or to measure them during the sampling. This is especially difficult for unknown habitats such as the bark surface, and may leave unanswered questions, for example, on whether all relevant environmental drivers have been captured by our analysis.

5. Summary and Outlook

With my thesis I contributed to understanding the diversity of Central European forests by revealing the unknown fungal, algal and bacterial diversity associated with the tree bark surface through the application of environmental metabarcoding techniques. In the first two parts I established a simple, fast and reliable protocol for eDNA sampling, processing and analysis. Subsequently, I assessed the diversity and drivers of the bark surface microbiome. In the third part, I used the fungal portion of the bark surface microbiome in a focused comparison of an unknown (bark surface) and well characterized (soil) forest habitat. Lastly, I evaluated if eDNA can serve as a standalone tool in biodiversity assessments of lichen forming fungi.

My results clearly highlight the unknown nature of the bark surface microbiome. In particular, the large "unknown" diversity of fungal taxa evident in all three studies of fungal micro-communities warrants the attention of future research. Potential areas to explore include isolation and cultivation approaches to potentially reveal the large hidden diversity of the bark surface. Furthermore, I stress the importance of considering multiple dimensions when studying microbial communities. Integrative sampling of the main microbial groups enabled me to study not only the effects of environmental conditions on individual groups but also showcased the strong interactions between them. Microbiome studies can clearly benefit from the inclusion of multiple taxonomic groups and might even obfuscate the real underlying drivers of micro-communities when only single taxonomic groups are included.

The data generated in my thesis can be a basis to generate hypotheses about responses of microbial forest taxa. For example, the data can be used in modeling approaches that try to predict responses of the microbiome under changing climatic conditions. This could be useful to predict the future of the forest microbiome and its stability, and to identify strategies to mitigate potential impacts (Baldrian et al., 2023). Datasets like this have already been used to predict soil health (Wilhelm et al., 2022), or are necessary to validate predictions of microbiome compositions based on environmental variables (García-Jiménez et al., 2021).

Many opportunities to study the diversity of bark surface microbiomes were beyond the scope of this dissertation. Possibilities for new research projects include: I. the individual sampling of micro-niches on the tree bark, such as cracks or tree holes to develop a finer spatial understanding of the microbiome, II. the repeated sampling throughout the year to assess seasonal community dynamics, III. the effects of tree specific variation in chemical variables, for example in bark pH or molecules linked to anti-microbial bark properties like suberin, IV. the inclusion of (meta-)genomic and/or (meta-)transcriptomic analyses to gain an understanding of the functional profile of the bark microbiome and how it contributes to the function of the forest microbiome. Lastly, future studies could take the eDNA biodiversity assessment beyond the description of diversity. Recently developed methods allow the use of unclassified taxa in predictions of ecological states (Keck et al., 2023), thus circumventing the apparent database issue for eDNA surveys of lichens.

Zusammenfassung

Wälder sind eines der wichtigsten Ökosysteme der Erde und bedecken etwa ein Drittel der Erdoberfläche. Ungefähr 25% dieser Waldflächen befinden sich auf dem europäischen Kontinent und beherbergen einen großen Teil der biologischen Vielfalt in Europa. Wälder, und insbesondere Bäume als Schlüsselarten dieses Ökosystems, leisten einen wichtigen Beitrag zu den globalen Wasser- und Nährstoffkreisläufen und gelten als größter Speicher von aus der Atmosphäre fixiertem Kohlenstoff. Durch ihre Wuchsform strukturieren Bäume den Wald in eine Vielzahl von verschiedenen Lebensräumen, wie beispielsweise Baumkronen, Wurzeln oder auch den Stamm. All diese Lebensräume werden von einer Vielzahl an Mikroorganismen besiedelt, welche für die Nahrungsnetze und Stoffkreisläufe von hoher Bedeutung sind. Insbesondere Abbauprozesse von organischem Material durch Bakterien und Pilze, aber auch die Fixierung von wichtigen Nährstoffen wie Phosphor durch Mykorrhizapilze sind hierbei hervorzuheben.

Baumstämme sind ein wichtiger Lebensraum in Wäldern, und bieten aufgrund der saisonalen Stabilität und Struktur der Borkenoberfläche viele kleine Nischen zur Trotz Besiedlung durch eine Vielzahl von Organismen. der Lebensbedingungen durch eine geringe Verfügbarkeit von Nährstoffen und Wasser, sowie eine hohe UV-Strahlung, wurden auf der Borke bereits eine hohe Vielfalt verschiedener Gruppen wie Flechten und Moose beschrieben. Nichtsdestotrotz stellt die Borke einen bisher vernachlässigten Lebensraum dar, mit deren Artenvielfalt sich nur wenige Studien befasst haben. Hierbei wurden Flechten und ihre Symbionten, sowie Hefepilze und Cyanobakterien als die charakteristischen Organismen des Habitats Borke identifiziert. In anderen Studien aus tropischen und temperaten Wäldern wurden außerdem Gemeinschaften von Grünalgen, Pilzen und einer Vielzahl an Bakterien auf der Borke gefunden und die Borkenoberfläche als ein potentielles Reservoir für mikrobielle Diversität beschrieben. Zum jetzigen Zeitpunkt fehlt eine umfassende Betrachtung der Artenvielfalt auf der Oberfläche der Borke, insbesondere mit Hinblick auf die mikrobielle Diversität von Grünalgen, Pilzen und Bakterien.

Besonders die mikrobielle Diversität lässt sich mit konventionellen Methoden nur schwer beschreiben und eine Unterscheidung zwischen Arten ist häufig nur durch

Mikroskopie oder chemische Tests möglich. Besonders die hohe Anzahl an Organismen macht eine Beschreibung größerer Lebensräume nahezu unmöglich. Abhilfe können hierbei molekulare Methoden schaffen, wie zum Beispiel die Probennahme von sogenannter "environmental DNA", also der Entnahme von DNA aus der Umwelt (auch eDNA genannt). Mittels moderner Techniken der Hochdurchsatz-Sequenzierung können so der Großteil der an einem Ort vorkommenden Arten identifiziert werden.

Die Zielsetzung dieser Dissertation war es daher, eine Methode zur Beschreibung der Artenvielfalt auf der Borkenoberfläche mittels eDNA zu entwickeln und zu identifizieren durch welche Umweltprozesse die Gemeinschaften der Borkenoberfläche beeinflusst werden. Die Arbeit gliedert sich in vier Teile: I. die Etablierung einer Methode zur Probennahme und Beschreibung der Diversität des Borkenmikrobioms, d.h. aller Mikroorganismen auf der Borkenoberfläche. II. die Erweiterung der Probennahme auf große geographische Areale und Umweltgradienten um zu identifizieren welche Prozesse die mikrobiellen Artgemeinschaften beeinflussen, III. die gemeinsame Betrachtung von Borken- und Bodenmikrobiomen um die Unterschiede in der Diversität von zwei wichtigen Waldlebensräumen zu untersuchen. Und IV. die Evaluierung von Umwelt-DNA als Methode zur Kartierung von Flechten als einer der wichtigsten Gruppe auf Borkenoberflächen.

Ökologische Studien beschäftigen sich häufig mit der Reaktion von Artgemeinschaften auf verschiedene Umwelteinflüsse. Für die mikroorganismischen Gemeinschaften auf der Borke wurden diese Reaktionen bisher wenig untersucht und sind daher weitestgehend unbekannt. Deshalb ist es nötig die Vorgänge in anderen Baumlebensräumen zu betrachten, um Hypothesen über die Prozesse auf der Borkenoberfläche aufzustellen. Die überwiegende Mehrheit der Studien zu oberirdischen Baumhabitaten beschäftigt sich mit der (mikrobiellen) Diversität in Baumkronen, der sogenannten Phyllosphäre. In diesem Habitat wurden bereits mehre Umwelteinflüsse als treibende Kräfte hinter Veränderungen der mikrobiellen Gemeinschaften beschrieben. Zum Beispiel bedingte eine bessere Wasserverfügbarkeit, sowie eine höhere Umgebungstemperatur eine höhere Diversität von Algen, Pilzen und Bakterien und UV-Strahlung war ein wichtiger Einflussfaktor für die Artzusammensetzung der bakteriellen Gemeinschaft. Des Weiteren hat das Alter des Wirtsorganismus einen großen Einfluss, sowohl auf die Diversität von Mikro- als auch von Makroorganismen.

Zusätzlich zu den Einflüssen der Umwelt beeinflussen sich Mikroorganismen durch verschiedenste Interaktionen auch gegenseitig. Die Flechtensymbiose ist eine der ältesten bekannten Interaktion zwischen mehreren Mikroorganismen und wird heutzutage als "Mikro-Ökosystem" bestehend aus mehreren Pilzen, Algen und Bakterien gesehen, welche die beiden Hauptpartner der Symbiose ergänzen. Auch außerhalb der Flechte kommen diese Artgemeinschaften auf der Borkenoberfläche vor, und interagieren dabei auf vielerlei Art und Weise. Aufgrund ihrer Photosyntheseaktivität nehmen Algen in diesen Gemeinschaften die Rolle der Primärproduzenten ein und werden von Bakterien und Pilzen mit Nährstoffen wie Stickstoff oder Phosphor versorgt, die sie nicht selbst bereitstellen können. Aufgrund dieser vermuteten Interaktionen ist es wichtig, nicht nur einzelne Organismengruppen zu betrachten, sondern bei der Untersuchung einen holistischen Ansatz mit mehreren taxonomischen Gruppen zu wählen.

Um diese abiotischen und biotischen Faktoren und ihren Einfluss auf die Artgemeinschaften der Borkenoberfläche zu untersuchen ist es essentiell, die mikrobiellen Gemeinschaften direkt in Wäldern zu beproben. Hierfür bietet die Forschungsplattform der Biodiversitäts-Exploratorien exzellente Rahmenbedingungen. Sie ermöglichen die Probennahme in einem großen geographischen Kontext und stellen dafür etwa 150 Versuchsflächen in Wäldern in ganz Deutschland bereit, welche repräsentativ für mitteleuropäische Wälder sind. Im Rahmen dieser Dissertation wurden etwa 250 Umwelt-DNA Proben aus diesen 150 Versuchsflächen genommen und die hier gefundene Diversität von Algen, Pilzen und Bakterien untersucht. Mithilfe von Tupfern, welche normalerweise zur medizinischen Probennahme eingesetzt werden, wurden die Mikroorganismen auf der Borkenoberfläche aufgesammelt und anschließend chemisch fixiert.

Dank moderner Methoden der Hochdurchsatz-Sequenzierung wurde die Untersuchung der mikrobiellen Diversität hochgradig vereinfacht. Durch Meta-Barcoding Ansätze lassen sich nicht nur eine große Anzahl an Arten aus einer einzelnen Probe, sondern auch eine hohe Zahl an verschiedenen Proben gleichzeitig sequenzieren. Die

Identifizierung von verschiedenen Arten erfolgt hierbei durch verschiedene kurze DNA Fragmente, sogenannter Marker-Gene, welche eine Art eindeutig kennzeichnen. Durch zusätzliche Basen an den zur Sequenzierung eingesetzten Primer, lassen sich die verschiedenen DNA Sequenzen später bioinformatisch einer Probe zuordnen.

Im Labor wurde die gesamte in den fixierten Proben enthaltene DNA extrahiert und Marker-Gene der Algen (ITS2), Pilze (ITS2) und Bakterien (16S) in jeweils drei Replikaten pro Artengruppen mittels PCR vervielfältigt. Das Labor-Protokoll beinhaltete dabei Extraktionskontrollen, PCR-Negativkontrollen und Multiplex-Kontrollen um potentielle Kontaminierung auszuschließen bzw. zu kontrollieren. Die insgesamt mehr als 3000 technischen Replikate wurden anschließend via Illumina-Sequenzierung sequenziert. Die resultierenden DNA Sequenzen wurden auf ihre Qualität überprüft, den Proben zugeordnet und in sogenannte "Amplicon-Sequencing-Variants" unterteilt, welche die Sequenzen anhand von Unterschieden in einzelnen Basenpaaren in potentielle "Arten" unterteilt. Anschließend wurde durch eine weitere Kuratierung, basierend auf der Abundanz und dem gleichzeitigen Auftreten von Sequenzen sichergestellt, dass die Diversität in den folgenden Analysen nicht verzerrt wird.

Auf der Basis der generierten Datensätze habe ich die Veränderungen der Diversität und Artzusammensetzung der mikrobiellen Gemeinschaften der Borkenoberfläche und ihre Verbindungen zu anderen Waldmikrobiomen untersucht. Insgesamt habe ich mehr als 200 verschiedene Algen, mehr als 1500 Pilze und knapp 2000 Bakterien gefunden. Die Hauptergebnisse meiner Dissertation sind:

- 1. Ein erheblicher Teil des borkenassoziierten Mikrobioms mitteleuropäischer Wälder ist unbekannt (Manuskripte 1, 2 und 3). Insbesondere in der Gruppe der Pilze konnten viele der genetisch unterscheidbaren Organismen keiner Art zugewiesen werden. Dies trifft unter anderem auch auf viele Organismen zu, die mithilfe mikrobieller Netzwerke als wichtige Arten für das Borkenmikrobiom identifiziert werden konnten. Hieraus lässt sich schließen das die Borke potentiell ein Lebensraum für viele bislang unbekannte Arten ist, welche aber eine wichtige ökologische Rolle in der mikrobiellen Gemeinschaft übernehmen.
- 2. Wichtige Prozesse die die Diversität und Artzusammensetzung der borkenassoziierten mikrobiellen Gemeinschaft beeinflussen sind das Alter der

Wirtsbäume (Manuskript 1), einige abiotische Faktoren wie Temperatur, Luftfeuchtigkeit und Lichteinfall (Manuskript 2), starke Interaktionen zwischen den drei taxonomischen Gruppen (Manuskript 2), sowie die in geringem Maße die Baumart (Manuskript 3) und die geographische Distanz (Manuskript 2).

Die abiotischen Faktoren haben hierbei vor allem einen starken Einfluss auf die Anzahl der Arten in der Gemeinschaft und nur einen geringen Effekt auf deren Zusammensetzung. Von besonderem Interesse ist, dass Pilze und Bakterien häufig gegensätzliche Reaktionen zu abiotischen Einflüssen zeigen, was bereits für unter Trockenstress stehende mikrobielle Gemeinschaften in Böden gezeigt wurde. Im Gegensatz zu den abiotischen Faktoren haben die biotischen Interaktionen einen starken Einfluss auf die Artzusammensetzung des Borkenmikrobioms, welcher auch durch die starken Vernetzungen zwischen den Organismen gezeigt werden konnte. Besonders stark ist hier der Einfluss der Bakterien, welche möglicherweise durch Nährstoffaustausch mit den Algen und Pilzen interagieren. Insgesamt lässt sich sagen, dass die Artenzahl des Mikrobioms durch die Umwelt limitiert wird, während Interaktionen die Artzusammensetzung bestimmen und als treibender Prozess hinter den Veränderungen der mikrobiellen Gemeinschaft wirken.

- der Mikrobiome des Waldbodens 3. Die Artzusammensetzung und der Borkenoberfläche sind signifikant unterschiedlich (Manuskript 3). Nur etwa 4% der gefundenen Organismen kamen in beiden Mikrobiomen vor, aber machten im Durchschnitt mehr als 60% der relativen Abundanz aus. In mikrobiellen Netzwerken konnte gezeigt werden, dass die Organismen zwar hauptsächlich innerhalb ihres Lebensraums interagieren, es aber auch eine klar erkennbare Verbindung von Organismen zwischen Borke und Boden gibt. Diese Verbindung unterstreicht, dass Baumstämme als verbindende Elemente der Lebensräume in Wäldern dienen können. beispielsweise durch den Transport von Mikroorganismen der Baumkrone zum Boden durch den Stammabfluss.
- 4. Die eDNA-Methode ist zum jetzigen Zeitpunkt nicht als eigenständige Methode für die Flechtenkartierung geeignet (Manuskript 4). Zwar konnten mithilfe der eDNA etwa 100 verschiedene Flechtenarten gefunden werden, aber nur 40

dieser Arten wurden auch in einer 2007/2008 von Experten durchgeführten floristischen Kartierung auf den gleichen Versuchsflächen gefunden. Allerdings konnte durch die eDNA eine höhere Diversität bei morphologisch nur schwer bestimmbaren Genera gefunden werden. Die momentan größte Hürde für den Einsatz von eDNA zur Bestimmung von Flechten waren nicht vorhandene Referenzsequenzen in den gängigen Datenbanken, zum Beispiel waren einige der häufigsten Arten der floristischen Kartierung nicht in den Datenbanken vertreten. Aktuell kann die eDNA deshalb nur als ergänzende Methode zur Kartierung durch Experten eingesetzt werden um beispielsweise morphologisch kryptische Arten zu unterscheiden.

Insgesamt trägt diese Arbeit zu einem besseren Verständnis der Diversität von Artgemeinschaften auf der Borke von Bäumen in mitteleuropäischen Wäldern bei und zeigt, wie diese Gemeinschaften von ihrer Umwelt beeinflusst werden. Erstmals wurde gezeigt ,dass die Interaktionen zwischen den Organismen einen stärkeren Einfluss auf die Artzusammensetzung des Borkenmikrobioms haben als die abiotische Umwelt, weshalb eine ganzheitliche Betrachtung mehrerer taxonomischer Gruppen unabdingbar ist. Zusätzlich zeigt der Unterschied, aber auch insbesondere die Verbindungen zwischen den verschiedenen Mikrobiomen der Waldlebensräume, dass es für zukünftige Studien von großer Bedeutung ist, mehrere Lebensräume gleichzeitig zu untersuchen um die Verbindungen und Prozesse des gesamten Waldmikrobioms besser zu verstehen. Die Nutzung der Methode eDNA Meta-Barcoding ist hierbei, trotz einiger methodischer Herausforderungen wie beispielsweise Lücken in den Referenzdatenbanken und der benötigten bioinformatischen Expertise, ein geeignetes Mittel um die mikrobielle Diversität in bisher unerforschten Habitaten wie der Borkenoberfläche in Wäldern zu erfassen und zu beschreiben. Meine Dissertation kann außerdem als Grundlage für weitere Studien dienen, um beispielsweise andere Mikrobiome der mitteleuropäischen Wälder zu vergleichen, die Funktionen des Waldmikrobioms weiter zu untersuchen oder auch um ausgewählte Bakterien, Pilze und Algen und die Entwicklung ihrer Diversität über längere Zeiträume zu beobachten. Der umfangreiche Datensatz der mikrobiellen Diversität in mitteleuropäischen Wäldern kann außerdem die Basis für weitergehende prädiktive Modellierung bilden, zum Beispiel um die Reaktionen des Mikrobioms auf den fortschreitende Klimawandel vorherzusagen.

References

- Abarenkov, K., Nilsson, R. H., Larsson, K.-H., Taylor, A. F. S., May, T. W., Frøslev, T. G., et al. (2023). The UNITE database for molecular identification and taxonomic communication of fungi and other eukaryotes: sequences, taxa and classifications reconsidered. *Nucleic Acids Research*, gkad1039. doi: 10.1093/nar/gkad1039.
- Afkhami, M. E., McIntyre, P. J., and Strauss, S. Y. (2014). Mutualist-mediated effects on species' range limits across large geographic scales. *Ecology Letters* 17, 1265–1273. doi: 10.1111/ele.12332.
- Agler, M. T., Ruhe, J., Kroll, S., Morhenn, C., Kim, S.-T., Weigel, D., et al. (2016). Microbial Hub Taxa Link Host and Abiotic Factors to Plant Microbiome Variation. *PLOS Biology* 14, e1002352–e1002352. doi: 10.1371/journal.pbio.1002352.
- Aguirre-von-Wobeser, E., Alonso-Sánchez, A., Méndez-Bravo, A., Villanueva Espino, L. A., and Reverchon, F. (2021). Barks from avocado trees of different geographic locations have consistent microbial communities. *Archives of Microbiology* 203, 4593–4607. doi: 10.1007/s00203-021-02449-6.
- Akinsoji, A. (1991). Studies on epiphytic flora of a tropical rain forest in Southwestern Nigeria. *Vegetatio* 92, 181–185. doi: 10.1007/BF00036038.
- Alfredsen, G., Solheim, H., and Slimestad, R. (2008). Antifungal effect of bark extracts from some European tree species. *Eur J Forest Res* 127, 387–393. doi: 10.1007/s10342-008-0222-x.
- Anderson, M. J. (2001). Permutation tests for univariate or multivariate analysis of variance and regression. *Can. J. Fish. Aquat. Sci.* 58, 626–639. doi: 10.1139/f01-004.
- Anderson, M. J., and Walsh, D. C. I. (2013). PERMANOVA, ANOSIM, and the Mantel test in the face of heterogeneous dispersions: What null hypothesis are you testing? *Ecological Monographs* 83, 557–574. doi: 10.1890/12-2010.1.
- Andre, H. M. (1985). Associations between corticolous microarthropod communities and epiphytic cover on bark. *Ecography* 8, 113–119. doi: 10.1111/j.1600-0587.1985.tb01161.x.
- Arrigoni, E., Albanese, D., Longa, C. M. O., Angeli, D., Donati, C., Ioriatti, C., et al. (2020). Tissue age, orchard location and disease management influence the composition of fungal and bacterial communities present on the bark of apple trees. *Environmental Microbiology* 22, 2080–2093. doi: 10.1111/1462-2920.14963.
- Arrigoni, E., Antonielli, L., Pindo, M., Pertot, I., and Perazzolli, M. (2018). Tissue age and plant genotype affect the microbiota of apple and pear bark. *Microbiological Research* 211, 57–68. doi: 10.1016/i.micres.2018.04.002.

- Aschenbrenner, I. A., Cernava, T., Erlacher, A., Berg, G., and Grube, M. (2017). Differential sharing and distinct co-occurrence networks among spatially close bacterial microbiota of bark, mosses and lichens. *Mol Ecol* 26, 2826–2838. doi: 10.1111/mec.14070.
- Aude, E., and Poulsen, R. S. (2000). Influence of management on the species composition of epiphytic cryptogams in Danish Fagus forests. *Applied Vegetation Science* 3, 81–88. doi: 10.2307/1478921.
- Baldrian, P. (2017). Forest microbiome: diversity, complexity and dynamics. *FEMS Microbiology Reviews* 41, 109–130. doi: 10.1093/femsre/fuw040.
- Baldrian, P., López-Mondéjar, R., and Kohout, P. (2023). Forest microbiome and global change. *Nat Rev Microbiol* 21, 487–501. doi: 10.1038/s41579-023-00876-4.
- Bálint, M., Bahram, M., Eren, A. M., Faust, K., Fuhrman, J. A., Lindahl, B., et al. (2016). Millions of reads, thousands of taxa: microbial community structure and associations analyzed via marker genes. *FEMS Microbiology Reviews* 40, 686–700. doi: 10.1093/femsre/fuw017.
- Bálint, M., Bartha, L., O'Hara, R. B., Olson, M. S., Otte, J., Pfenninger, M., et al. (2015). Relocation, high-latitude warming and host genetic identity shape the foliar fungal microbiome of poplars. *Molecular Ecology* 24, 235–248. doi: 10.1111/mec.13018.
- Bálint, M., Tiffin, P., Hallström, B., O'Hara, R. B., Olson, M. S., Fankhauser, J. D., et al. (2013). Host Genotype Shapes the Foliar Fungal Microbiome of Balsam Poplar (Populus balsamifera). *PLOS ONE* 8, e53987. doi: 10.1371/journal.pone.0053987.
- Ballhausen, M.-B., and de Boer, W. (2016). The sapro-rhizosphere: Carbon flow from saprotrophic fungi into fungus-feeding bacteria. *Soil Biology and Biochemistry* 102, 14–17. doi: 10.1016/j.soilbio.2016.06.014.
- Balvočiūtė, M., and Huson, D. H. (2017). SILVA, RDP, Greengenes, NCBI and OTT—how do these taxonomies compare? *BMC Genomics* 18, 114. doi: 10.1186/s12864-017-3501-4.
- Barberán, A., Bates, S. T., Casamayor, E. O., and Fierer, N. (2012). Using network analysis to explore co-occurrence patterns in soil microbial communities. *ISME J* 6, 343–351. doi: 10.1038/ismej.2011.119.
- Barbi, F., Prudent, E., Vallon, L., Buée, M., Dubost, A., Legout, A., et al. (2016). Tree species select diverse soil fungal communities expressing different sets of lignocellulolytic enzyme-encoding genes. *Soil Biology and Biochemistry* 100, 149–159. doi: 10.1016/j.soilbio.2016.06.008.
- Bashir, I., War, A. F., Rafiq, I., Reshi, Z. A., Rashid, I., and Shouche, Y. S. (2022). Phyllosphere microbiome: Diversity and functions. *Microbiological Research* 254, 126888. doi: 10.1016/j.micres.2021.126888.

- Baz, A. (2008). "Bark-Lice, Book-Lice or Psocids (Psocoptera)," in *Encyclopedia of Entomology* (Dordrecht: Springer Netherlands), 381–399. doi: 10.1007/978-1-4020-6359-6 236.
- Bazany, K. E., Wang, J., Delgado-Baquerizo, M., Singh, B. K., and Trivedi, P. (2022). Water deficit affects inter-kingdom microbial connections in plant rhizosphere. *Environmental Microbiology* 24, 3722–3734. doi: 10.1111/1462-2920.16031.
- Beck, A., Peršoh, D., and Rambold, G. (2014). First evidence for seasonal fluctuations in lichen- and bark-colonising fungal communities. *Folia Microbiologica* 59, 155–157. doi: 10.1007/s12223-013-0278-y.
- Beng, K. C., and Corlett, R. T. (2020). Applications of environmental DNA (eDNA) in ecology and conservation: opportunities, challenges and prospects. *Biodivers Conserv* 29, 2089–2121. doi: 10.1007/s10531-020-01980-0.
- Benson, D. A., Cavanaugh, M., Clark, K., Karsch-Mizrachi, I., Lipman, D. J., Ostell, J., et al. (2013). GenBank. *Nucleic Acids Res* 41, D36-42. doi: 10.1093/nar/gks1195.
- Berg, G., Rybakova, D., Fischer, D., Cernava, T., Vergès, M.-C. C., Charles, T., et al. (2020). Microbiome definition re-visited: old concepts and new challenges. *Microbiome* 8, 103–103. doi: 10.1186/s40168-020-00875-0.
- Beyer, W. N., and Saari, D. M. (1978). Activity and Ecological Distribution of the Slug, Arion subfuscus (Draparnaud) (Stylommatophora, Arionidae). *American Midland Naturalist* 100, 359–367. doi: 10.2307/2424835.
- Bhadra, B., Rao, R. S., Singh, P. K., Sarkar, P. K., and Shivaji, S. (2008). Yeasts and Yeast-Like Fungi Associated with Tree Bark: Diversity and Identification of Yeasts Producing Extracellular Endoxylanases. *Curr Microbiol* 56, 489–494. doi: 10.1007/s00284-008-9108-x.
- Bittar, T. B., Pound, P., Whitetree, A., Moore, L. D., and Van Stan II, J. T. (2018). Estimation of Throughfall and Stemflow Bacterial Flux in a Subtropical Oak-Cedar Forest. *Geophysical Research Letters* 45, 1410–1418. doi: 10.1002/2017GL075827.
- Boch, S., Prati, D., Hessenmöller, D., Schulze, E.-D., and Fischer, M. (2013). Richness of Lichen Species, Especially of Threatened Ones, Is Promoted by Management Methods Furthering Stand Continuity. *PLOS ONE* 8, e55461. doi: 10.1371/journal.pone.0055461.
- Buck, J. W., Lachance, M.-A., and Traquair, J. A. (1998). Mycoflora of peach bark: population dynamics and composition. *Can. J. Bot.* 76, 345–354. doi: 10.1139/b98-009.
- Burgess-Conforti, J. R., Moore, P. A., Owens, P. R., Miller, D. M., Ashworth, A. J., Hays, P. D., et al. (2019). Are soils beneath coniferous tree stands more acidic than

- soils beneath deciduous tree stands? *Environ Sci Pollut Res* 26, 14920–14929. doi: 10.1007/s11356-019-04883-y.
- Callahan, B. J., McMurdie, P. J., and Holmes, S. P. (2017). Exact sequence variants should replace operational taxonomic units in marker-gene data analysis. *ISME J* 11, 2639–2643. doi: 10.1038/ismej.2017.119.
- Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., and Holmes, S. P. (2016). DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods* 13, 581–583. doi: 10.1038/nmeth.3869.
- Castaño, C., Lindahl, B. D., Alday, J. G., Hagenbo, A., Martínez de Aragón, J., Parladé, J., et al. (2018). Soil microclimate changes affect soil fungal communities in a Mediterranean pine forest. *New Phytologist* 220, 1211–1221. doi: 10.1111/nph.15205.
- Chao, A., Chiu, C.-H., and Jost, L. (2014). Unifying Species Diversity, Phylogenetic Diversity, Functional Diversity, and Related Similarity and Differentiation Measures Through Hill Numbers. *Annual Review of Ecology, Evolution, and Systematics* 45, 297–324. doi: 10.1146/annurev-ecolsys-120213-091540.
- Cholet, F., Lisik, A., Agogué, H., Ijaz, U. Z., Pineau, P., Lachaussée, N., et al. (2022). Ecological Observations Based on Functional Gene Sequencing Are Sensitive to the Amplicon Processing Method. *mSphere* 7, e00324-22. doi: 10.1128/msphere.00324-22.
- Choudoir, M. J., Barberán, A., Menninger, H. L., Dunn, R. R., and Fierer, N. (2018). Variation in range size and dispersal capabilities of microbial taxa. *Ecology* 99, 322–334. doi: 10.1002/ecy.2094.
- Clemmensen, K. E., Bahr, A., Ovaskainen, O., Dahlberg, A., Ekblad, A., Wallander, H., et al. (2013). Roots and Associated Fungi Drive Long-Term Carbon Sequestration in Boreal Forest. *Science* 339, 1615–1618. doi: 10.1126/science.1231923.
- Crowther, T. W., Glick, H. B., Covey, K. R., Bettigole, C., Maynard, D. S., Thomas, S. M., et al. (2015). Mapping tree density at a global scale. *Nature* 525, 201–205. doi: 10.1038/nature14967.
- Czarnota, P., and Guzow-Krzemińska, B. (2018). Bacidina mendax sp. nov., a new widespread species in Central Europe, together with a new combination within the genus Bacidina. *The Lichenologist* 50, 43–57. doi: 10.1017/S0024282917000627.
- Davis, N. M., Proctor, D. M., Holmes, S. P., Relman, D. A., and Callahan, B. J. (2018). Simple statistical identification and removal of contaminant sequences in markergene and metagenomics data. *Microbiome* 6, 226–226. doi: 10.1186/s40168-018-0605-2.

- de Vries, F. T., Griffiths, R. I., Bailey, M., Craig, H., Girlanda, M., Gweon, H. S., et al. (2018). Soil bacterial networks are less stable under drought than fungal networks. *Nature Communications* 9, 3033–3033. doi: 10.1038/s41467-018-05516-7.
- Dedysh, S. N., and Dunfield, P. F. (2011). "Facultative and Obligate Methanotrophs," in *Methods in Methane Metabolism*, eds. A. C. Rosenzweig and S. W. Ragsdale, 31–44. doi: 10.1016/B978-0-12-386905-0.00003-6.
- Deiner, K., Bik, H. M., Mächler, E., Seymour, M., Lacoursière-Roussel, A., Altermatt, F., et al. (2017). Environmental DNA metabarcoding: Transforming how we survey animal and plant communities. *Molecular Ecology* 26, 5872–5895. doi: 10.1111/mec.14350.
- Deveau, A., Bonito, G., Uehling, J., Paoletti, M., Becker, M., Bindschedler, S., et al. (2018). Bacterial–fungal interactions: ecology, mechanisms and challenges. *FEMS Microbiology Reviews* 42, 335–352. doi: 10.1093/femsre/fuy008.
- Dove, N. C., Veach, A. M., Muchero, W., Wahl, T., Stegen, J. C., Schadt, C. W., et al. (2021). Assembly of the Populus Microbiome Is Temporally Dynamic and Determined by Selective and Stochastic Factors. *mSphere* 6, 10.1128/msphere.01316-20. doi: 10.1128/msphere.01316-20.
- Durán, P., Flores-Uribe, J., Wippel, K., Zhang, P., Guan, R., Melkonian, B., et al. (2022). Shared features and reciprocal complementation of the Chlamydomonas and Arabidopsis microbiota. *Nature Communications* 13, 406–406. doi: 10.1038/s41467-022-28055-8.
- Durand, A., Maillard, F., Foulon, J., Gweon, H. S., Valot, B., and Chalot, M. (2017). Environmental Metabarcoding Reveals Contrasting Belowground and Aboveground Fungal Communities from Poplar at a Hg Phytomanagement Site. *Microb Ecol* 74, 795–809. doi: 10.1007/s00248-017-0984-0.
- Elbrecht, V., Vamos, E. E., Meissner, K., Aroviita, J., and Leese, F. (2017). Assessing strengths and weaknesses of DNA metabarcoding-based macroinvertebrate identification for routine stream monitoring. *Methods Ecol Evol* 8, 1265–1275. doi: 10.1111/2041-210X.12789.
- Erdmann, G., Otte, V., Langel, R., Scheu, S., and Maraun, M. (2007). The trophic structure of bark-living oribatid mite communities analysed with stable isotopes (15N, 13C) indicates strong niche differentiation. *Experimental and Applied Acarology* 41, 1–10. doi: 10.1007/s10493-007-9060-7.
- FAO (2020). Global Forest Resources Assessment 2020: Main report. Rome doi: 10.4060/ca9825en.
- Farkas, E., Varga, N., Veres, K., Matus, G., Sinigla, M., and Lőkös, L. (2022).

 Distribution Types of Lichens in Hungary That Indicate Changing Environmental Conditions. *Journal of Fungi* 8, 600. doi: 10.3390/jof8060600.

- Fediajevaite, J., Priestley, V., Arnold, R., and Savolainen, V. (2021). Meta-analysis shows that environmental DNA outperforms traditional surveys, but warrants better reporting standards. *Ecology and Evolution* 11, 4803–4815. doi: 10.1002/ece3.7382.
- Fernandes, A. D., Macklaim, J. M., Linn, T. G., Reid, G., and Gloor, G. B. (2013). ANOVA-Like Differential Expression (ALDEx) Analysis for Mixed Population RNA-Seq. *PLoS ONE* 8, e67019–e67019. doi: 10.1371/journal.pone.0067019.
- Ferrier, S. (2002). Mapping Spatial Pattern in Biodiversity for Regional Conservation Planning: Where to from Here? *Systematic Biology* 51, 331–363. doi: 10.1080/10635150252899806.
- Ferrier, S., Manion, G., Elith, J., and Richardson, K. (2007). Using generalized dissimilarity modelling to analyse and predict patterns of beta diversity in regional biodiversity assessment. *Diversity and Distributions* 13, 252–264. doi: 10.1111/j.1472-4642.2007.00341.x.
- Ferrier, S., Powell, G. V. N., Richardson, K. S., Manion, G., Overton, J. M., Allnutt, T. F., et al. (2004). Mapping More of Terrestrial Biodiversity for Global Conservation Assessment. *BioScience* 54, 1101–1109. doi: 10.1641/0006-3568(2004)054[1101:MMOTBF]2.0.CO;2.
- Fischer, M., Bossdorf, O., Gockel, S., Hänsel, F., Hemp, A., Hessenmöller, D., et al. (2010). Implementing large-scale and long-term functional biodiversity research: The Biodiversity Exploratories. *Basic and Applied Ecology* 11, 473–485. doi: 10.1016/j.baae.2010.07.009.
- Fornoff, F., Staab, M., Zhu, C.-D., and Klein, A.-M. (2021). Multi-trophic communities reestablish with canopy cover and microclimate in a subtropical forest biodiversity experiment. *Oecologia* 196, 289–301. doi: 10.1007/s00442-021-04921-y.
- Friedman, J., and Alm, E. J. (2012). Inferring Correlation Networks from Genomic Survey Data. *PLOS Computational Biology* 8, e1002687. doi: 10.1371/journal.pcbi.1002687.
- Fritz, Ö., Gustafsson, L., and Larsson, K. (2008). Does forest continuity matter in conservation? A study of epiphytic lichens and bryophytes in beech forests of southern Sweden. *Biological Conservation* 141, 655–668. doi: 10.1016/j.biocon.2007.12.006.
- Fritz, Ö., and Heilmann-Clausen, J. (2010). Rot holes create key microhabitats for epiphytic lichens and bryophytes on beech (Fagus sylvatica). *Biological Conservation* 143, 1008–1016. doi: 10.1016/j.biocon.2010.01.016.
- Fritz, Ö., Niklasson, M., and Churski, M. (2009). Tree age is a key factor for the conservation of epiphytic lichens and bryophytes in beech forests. *Applied Vegetation Science* 12, 93–106. doi: 10.1111/j.1654-109X.2009.01007.x.

- Frøslev, T. G., Kjøller, R., Bruun, H. H., Ejrnæs, R., Brunbjerg, A. K., Pietroni, C., et al. (2017). Algorithm for post-clustering curation of DNA amplicon data yields reliable biodiversity estimates. *Nature Communications* 8, 1188–1188. doi: 10.1038/s41467-017-01312-x.
- García-Jiménez, B., Muñoz, J., Cabello, S., Medina, J., and Wilkinson, M. D. (2021). Predicting microbiomes through a deep latent space. *Bioinformatics* 37, 1444–1451. doi: 10.1093/bioinformatics/btaa971.
- Giordani, P., Brunialti, G., Benesperi, R., Rizzi, G., Frati, L., and Modenesi, P. (2009). Rapid biodiversity assessment in lichen diversity surveys: implications for quality assurance. *J. Environ. Monit.* 11, 730–735. doi: 10.1039/B818173J.
- Gloor, G. B., Macklaim, J. M., Pawlowsky-Glahn, V., and Egozcue, J. J. (2017). Microbiome Datasets Are Compositional: And This Is Not Optional. *Frontiers in Microbiology* 8. doi: 10.3389/fmicb.2017.02224.
- Goldmann, K., Schöning, I., Buscot, F., and Wubet, T. (2015). Forest Management Type Influences Diversity and Community Composition of Soil Fungi across Temperate Forest Ecosystems. *Frontiers in Microbiology* 6. Available at: https://www.frontiersin.org/articles/10.3389/fmicb.2015.01300 [Accessed December 20, 2023].
- Gonzalez, L. E., and Bashan, Y. (2000). Increased Growth of the Microalga Chlorella vulgaris when Coimmobilized and Cocultured in Alginate Beads with the Plant-Growth-Promoting Bacterium Azospirillum brasilense. *Applied and Environmental Microbiology* 66, 1527–1531. doi: 10.1128/AEM.66.4.1527-1531.2000.
- Grimm, M., Grube, M., Schiefelbein, U., Zühlke, D., Bernhardt, J., and Riedel, K. (2021). The Lichens' Microbiota, Still a Mystery? *Frontiers in Microbiology* 12. doi: 10.3389/fmicb.2021.623839.
- Grube, M., Cernava, T., Soh, J., Fuchs, S., Aschenbrenner, I., Lassek, C., et al. (2015). Exploring functional contexts of symbiotic sustain within lichen-associated bacteria by comparative omics. *The ISME Journal* 9, 412–424. doi: 10.1038/ismej.2014.138.
- Hagge, J., Bässler, C., Gruppe, A., Hoppe, B., Kellner, H., Krah, F.-S., et al. (2019). Bark coverage shifts assembly processes of microbial decomposer communities in dead wood. *Proceedings of the Royal Society B: Biological Sciences* 286, 20191744–20191744. doi: 10.1098/rspb.2019.1744.
- Hansen, A. J., Mitchell, D. L., Wiuf, C., Paniker, L., Brand, T. B., Binladen, J., et al. (2006). Crosslinks Rather Than Strand Breaks Determine Access to Ancient DNA Sequences From Frozen Sediments. *Genetics* 173, 1175–1179. doi: 10.1534/genetics.106.057349.

- Hawkes, C. V., Bull, J. J., and Lau, J. A. (2020). Symbiosis and stress: how plant microbiomes affect host evolution. *Philosophical Transactions of the Royal Society B: Biological Sciences* 375, 20190590. doi: 10.1098/rstb.2019.0590.
- Hawksworth, D. L., and Grube, M. (2020). Lichens redefined as complex ecosystems. *New Phytologist* 227, 1281–1283. doi: 10.1111/nph.16630.
- Hawksworth, D. L., and Lücking, R. (2017). Fungal Diversity Revisited: 2.2 to 3.8 Million Species. *Microbiology Spectrum* 5, 5.4.10-5.4.10. doi: 10.1128/microbiolspec.FUNK-0052-2016.
- Henrie, J. R., Thomson, B. M., Yungfleisch, A. A., Kerr, M., and Leavitt, S. D. (2022). Characterizing Crustose Lichen Communities—DNA Metabarcoding Reveals More than Meets the Eye. *Diversity* 14, 766. doi: 10.3390/d14090766.
- Hill, M. O. (1973). Diversity and Evenness: A Unifying Notation and Its Consequences. *Ecology* 54, 427–432. doi: 10.2307/1934352.
- Hiraishi, A., and Imhoff, J. F. (2015). "Acidiphilium," in *Bergey's Manual of Systematics of Archaea and Bacteria*, eds. M. E. Trujillo, S. Dedysh, P. DeVos, B. Hedlund, P. Kämpfer, F. A. Rainey, et al. (Wiley), 1–14. doi: 10.1002/9781118960608.gbm00877.
- Hom, E. F. Y., and Murray, A. W. (2014). Niche engineering demonstrates a latent capacity for fungal-algal mutualism. *Science* 345, 94–98. doi: 10.1126/science.1253320.
- Ihrmark, K., Bödeker, I. T. M., Cruz-Martinez, K., Friberg, H., Kubartova, A., Schenck, J., et al. (2012). New primers to amplify the fungal ITS2 region evaluation by 454-sequencing of artificial and natural communities. *FEMS Microbiology Ecology* 82, 666–677. doi: 10.1111/j.1574-6941.2012.01437.x.
- Jacobs, J. L., and Sundin, G. W. (2001). Effect of Solar UV-B Radiation on a Phyllosphere Bacterial Community. *Applied and Environmental Microbiology* 67, 5488–5496. doi: 10.1128/AEM.67.12.5488-5496.2001.
- Jiang, L., and Yang, Y. (2017). Visualization of international environmental DNA research. *Current Science* 112, 1659–1664.
- Joos, L., Beirinckx, S., Haegeman, A., Debode, J., Vandecasteele, B., Baeyen, S., et al. (2020). Daring to be differential: metabarcoding analysis of soil and plant-related microbial communities using amplicon sequence variants and operational taxonomical units. *BMC Genomics* 21, 733. doi: 10.1186/s12864-020-07126-4.
- Kauserud, H. (2023). ITS alchemy: On the use of ITS as a DNA marker in fungal ecology. *Fungal Ecology* 65, 101274. doi: 10.1016/j.funeco.2023.101274.
- Keck, F., Brantschen, J., and Altermatt, F. (2023). A combination of machine-learning and eDNA reveals the genetic signature of environmental change at the landscape levels. *Molecular Ecology* 32, 4791–4800. doi: 10.1111/mec.17073.

- Kobayashi, K., and Aoyagi, H. (2019). Microbial community structure analysis in Acer palmatum bark and isolation of novel bacteria IAD-21 of the candidate division FBP. *PeerJ* 7, e7876–e7876. doi: 10.7717/peerj.7876.
- Kohlmeier, S., Smits, T. H. M., Ford, R. M., Keel, C., Harms, H., and Wick, L. Y. (2005). Taking the Fungal Highway: Mobilization of Pollutant-Degrading Bacteria by Fungi. *Environmental Science & Technology* 39, 4640–4646. doi: 10.1021/es047979z.
- Kõljalg, U., Nilsson, R. H., Abarenkov, K., Tedersoo, L., Taylor, A. F. S., Bahram, M., et al. (2013). Towards a unified paradigm for sequence-based identification of fungi. *Molecular Ecology* 22, 5271–5277. doi: 10.1111/mec.12481.
- Kranner, I., Zorn, M., Turk, B., Wornik, S., Beckett, R. P., and Batič, F. (2003). Biochemical traits of lichens differing in relative desiccation tolerance. *New Phytologist* 160, 167–176. doi: 10.1046/j.1469-8137.2003.00852.x.
- Krespach, M. K. C., García-Altares, M., Flak, M., Hanno Schoeler, Scherlach, K., Netzker, T., et al. (2020). Lichen-like association of Chlamydomonas reinhardtii and Aspergillus nidulans protects algal cells from bacteria. *The ISME Journal* 14, 2794–2805. doi: 10.1038/s41396-020-0731-2.
- Kurtz, Z. D., Müller, C. L., Miraldi, E. R., Littman, D. R., Blaser, M. J., and Bonneau, R. A. (2015). Sparse and Compositionally Robust Inference of Microbial Ecological Networks. *PLoS Computational Biology* 11, 1–25. doi: 10.1371/journal.pcbi.1004226.
- Lambais, M. R., Lucheta, A. R., and Crowley, D. E. (2014). Bacterial Community Assemblages Associated with the Phyllosphere, Dermosphere, and Rhizosphere of Tree Species of the Atlantic Forest are Host Taxon Dependent. *Microbial Ecology* 68, 567–574. doi: 10.1007/s00248-014-0433-2.
- Landeweert, R., Hoffland, E., Finlay, R. D., Kuyper, T. W., and van Breemen, N. (2001). Linking plants to rocks: ectomycorrhizal fungi mobilize nutrients from minerals. *Trends in Ecology & Evolution* 16, 248–254. doi: 10.1016/S0169-5347(01)02122-X.
- Lang, C., Seven, J., and Polle, A. (2011). Host preferences and differential contributions of deciduous tree species shape mycorrhizal species richness in a mixed Central European forest. *Mycorrhiza* 21, 297–308. doi: 10.1007/s00572-010-0338-y.
- Leff, J. W., Del Tredici, P., Friedman, W. E., and Fierer, N. (2015). Spatial structuring of bacterial communities within individual Ginkgo biloba trees. *Environmental Microbiology* 17, 2352–2361. doi: 10.1111/1462-2920.12695.
- Leray, M., Knowlton, N., Ho, S.-L., Nguyen, B. N., and Machida, R. J. (2019). GenBank is a reliable resource for 21st century biodiversity research. *Proc. Natl. Acad. Sci. U.S.A.* 116, 22651–22656. doi: 10.1073/pnas.1911714116.

- Lindow, S. E., and Brandl, M. T. (2003). Microbiology of the Phyllosphere. *Applied and Environmental Microbiology* 69, 1875–1883. doi: 10.1128/AEM.69.4.1875-1883.2003.
- Liu, B.-W., Li, S.-Y., Zhu, H., and Liu, G.-X. (2023). Phyllosphere eukaryotic microalgal communities in rainforests: Drivers and diversity. *Plant Diversity* 45, 45–53. doi: 10.1016/j.pld.2022.08.006.
- Ma, J., Tang, J. Y., Wang, S., Chen, Z. L., Li, X. D., and Li, Y. H. (2017). Illumina sequencing of bacterial 16S rDNA and 16S rRNA reveals seasonal and species-specific variation in bacterial communities in four moss species. *Applied Microbiology and Biotechnology* 101, 6739–6753. doi: 10.1007/s00253-017-8391-5.
- Magyar, D., and Révay, Á. (2008). Trinacrium tothii spec. nov. (Hyphomycetes) from the cortex of living tree. *Nova Hedwigia* 87, 513–519.
- Magyar, D., Van Stan, J. T., and Sridhar, K. R. (2021). Hypothesis and Theory: Fungal Spores in Stemflow and Potential Bark Sources. *Frontiers in Forests and Global Change* 4. doi: 10.3389/ffgc.2021.623758.
- Mantel, N. (1967). The Detection of Disease Clustering and a Generalized Regression Approach. *Cancer Research* 27, 209–220.
- Martins, G., Lauga, B., Miot-Sertier, C., Mercier, A., Lonvaud, A., Soulas, M.-L., et al. (2013). Characterization of Epiphytic Bacterial Communities from Grapes, Leaves, Bark and Soil of Grapevine Plants Grown, and Their Relations. *PLoS ONE* 8, e73013–e73013. doi: 10.1371/journal.pone.0073013.
- Meyer, K. M., Porch, R., Muscettola, I. E., Vasconcelos, A. L. S., Sherman, J. K., Metcalf, C. J. E., et al. (2022). Plant neighborhood shapes diversity and reduces interspecific variation of the phyllosphere microbiome. *ISME J* 16, 1376–1387. doi: 10.1038/s41396-021-01184-6.
- Mokany, K., Ware, C., Woolley, S. N. C., Ferrier, S., and Fitzpatrick, M. C. (2022). A working guide to harnessing generalized dissimilarity modelling for biodiversity analysis and conservation assessment. *Global Ecology and Biogeography* 31, 802–821. doi: 10.1111/geb.13459.
- Muggia, L., and Grube, M. (2018). Fungal Diversity in Lichens: From Extremotolerance to Interactions with Algae. *Life* 8, 15. doi: 10.3390/life8020015.
- Nacke, H., Goldmann, K., Schöning, I., Pfeiffer, B., Kaiser, K., Castillo-Villamizar, G. A., et al. (2016). Fine Spatial Scale Variation of Soil Microbial Communities under European Beech and Norway Spruce. Frontiers in Microbiology 7. Available at: https://www.frontiersin.org/articles/10.3389/fmicb.2016.02067 [Accessed December 20, 2023].

- Naeem, S., Prager, C., Weeks, B., Varga, A., Flynn, D. F. B., Griffin, K., et al. (2016). Biodiversity as a multidimensional construct: a review, framework and case study of herbivory's impact on plant biodiversity. *Proceedings of the Royal Society B: Biological Sciences* 283, 20153005. doi: 10.1098/rspb.2015.3005.
- Nascimbene, J., Thor, G., and Nimis, P. L. (2013). Effects of forest management on epiphytic lichens in temperate deciduous forests of Europe A review. *Forest Ecology and Management* 298, 27–38. doi: 10.1016/j.foreco.2013.03.008.
- Nekrutenko, A., and Taylor, J. (2012). Next-generation sequencing data interpretation: enhancing reproducibility and accessibility. *Nat Rev Genet* 13, 667–672. doi: 10.1038/nrg3305.
- Neustupa, J., and Štifterová, A. (2013). Distribution patterns of subaerial corticolous microalgae in two European regions. *Plant Ecology and Evolution* 146, 279–289. doi: 10.5091/plecevo.2013.862.
- Nguyen, N. H., Song, Z., Bates, S. T., Branco, S., Tedersoo, L., Menke, J., et al. (2016). FUNGuild: An open annotation tool for parsing fungal community datasets by ecological guild. *Fungal Ecology* 20, 241–248. doi: 10.1016/j.funeco.2015.06.006.
- Oka, A., Takahashi, J., Endoh, Y., and Seino, T. (2021). Bark Effects on Stemflow Chemistry in a Japanese Temperate Forest I. The Role of Bark Surface Morphology. *Frontiers in Forests and Global Change* 4. Available at: https://www.frontiersin.org/articles/10.3389/ffgc.2021.654375 [Accessed December 14, 2023].
- O'Malley, M. A. (2008). 'Everything is everywhere: but the environment selects': ubiquitous distribution and ecological determinism in microbial biogeography. Studies in History and Philosophy of Science Part C: Studies in History and Philosophy of Biological and Biomedical Sciences 39, 314–325. doi: 10.1016/j.shpsc.2008.06.005.
- Pauvert, C., Buée, M., Laval, V., Edel-Hermann, V., Fauchery, L., Gautier, A., et al. (2019). Bioinformatics matters: The accuracy of plant and soil fungal community data is highly dependent on the metabarcoding pipeline. *Fungal Ecology* 41, 23–33. doi: 10.1016/j.funeco.2019.03.005.
- Pauwels, R., Graefe, J., and Bitterlich, M. (2023). An arbuscular mycorrhizal fungus alters soil water retention and hydraulic conductivity in a soil texture specific way. *Mycorrhiza* 33, 165–179. doi: 10.1007/s00572-023-01106-8.
- Penone, C., Allan, E., Soliveres, S., Felipe-Lucia, M. R., Gossner, M. M., Seibold, S., et al. (2018). Specialisation and diversity of multiple trophic groups are promoted by different forest features. *Ecology Letters* 22, 170–180. doi: 10.1111/ele.13182.
- Perazzolli, M., Antonielli, L., Storari, M., Puopolo, G., Pancher, M., Giovannini, O., et al. (2014). Resilience of the Natural Phyllosphere Microbiota of the Grapevine to

- Chemical and Biological Pesticides. *Applied and Environmental Microbiology* 80, 3585–3596. doi: 10.1128/AEM.00415-14.
- Petrolli, R., Augusto Vieira, C., Jakalski, M., Bocayuva, M. F., Vallé, C., Cruz, E. D. S., et al. (2021). A fine-scale spatial analysis of fungal communities on tropical tree bark unveils the epiphytic rhizosphere in orchids. *New Phytologist* 231, 2002–2014. doi: 10.1111/nph.17459.
- Prasetya, B., and Roffael, E. (1990). Zur Acidität der Rinde einiger Nadelbaumarten. Holz als Roh- und Werkstoff 48, 429–435. doi: 10.1007/BF02627626.
- Prescott, C. E., and Grayston, S. J. (2013). Tree species influence on microbial communities in litter and soil: Current knowledge and research needs. *Forest Ecology and Management* 309, 19–27. doi: 10.1016/j.foreco.2013.02.034.
- Prodan, A., Tremaroli, V., Brolin, H., Zwinderman, A. H., Nieuwdorp, M., and Levin, E. (2020). Comparing bioinformatic pipelines for microbial 16S rRNA amplicon sequencing. *PLOS ONE* 15, e0227434. doi: 10.1371/journal.pone.0227434.
- Prosser, J. I., Bohannan, B. J. M., Curtis, T. P., Ellis, R. J., Firestone, M. K., Freckleton, R. P., et al. (2007). The role of ecological theory in microbial ecology. *Nat Rev Microbiol* 5, 384–392. doi: 10.1038/nrmicro1643.
- Ptatscheck, C., Milne, P. C., and Traunspurger, W. (2018). Is stemflow a vector for the transport of small metazoans from tree surfaces down to soil? *BMC Ecol* 18, 43. doi: 10.1186/s12898-018-0198-4.
- Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., et al. (2012). The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Research* 41, D590–D596. doi: 10.1093/nar/gks1219.
- Ramanan, R., Kim, B.-H., Cho, D.-H., Oh, H.-M., and Kim, H.-S. (2016). Algae–bacteria interactions: Evolution, ecology and emerging applications. *Biotechnology Advances* 34, 14–29. doi: 10.1016/j.biotechadv.2015.12.003.
- Rayé, G., Miquel, C., Coissac, E., Redjadj, C., Loison, A., and Taberlet, P. (2011). New insights on diet variability revealed by DNA barcoding and high-throughput pyrosequencing: chamois diet in autumn as a case study. *Ecological Research* 26, 265–276. doi: 10.1007/s11284-010-0780-5.
- Redford, A. J., Bowers, R. M., Knight, R., Linhart, Y., and Fierer, N. (2010). The ecology of the phyllosphere: geographic and phylogenetic variability in the distribution of bacteria on tree leaves: Biogeography of phyllosphere bacterial communities. *Environmental Microbiology* 12, 2885–2893. doi: 10.1111/j.1462-2920.2010.02258.x.
- Rindi, F. (2007). "Diversity, Distribution and Ecology of Green Algae and Cyanobacteria in Urban Habitats," in *Algae and Cyanobacteria in Extreme Environments*, ed. J.

- Seckbach (Dordrecht: Springer Netherlands), 619–638. doi: 10.1007/978-1-4020-6112-7 34.
- Ritter, C. D., Faurby, S., Bennett, D. J., Naka, L. N., ter Steege, H., Zizka, A., et al. (2019). The pitfalls of biodiversity proxies: Differences in richness patterns of birds, trees and understudied diversity across Amazonia. *Sci Rep* 9, 19205. doi: 10.1038/s41598-019-55490-3.
- Rousk, K., Sorensen, P. L., Lett, S., and Michelsen, A. (2015). Across-Habitat Comparison of Diazotroph Activity in the Subarctic. *Microb Ecol* 69, 778–787. doi: 10.1007/s00248-014-0534-y.
- Sanders, W. B., and Masumoto, H. (2021). Lichen algae: the photosynthetic partners in lichen symbioses. *The Lichenologist* 53, 347–393. doi: 10.1017/S0024282921000335.
- Sandve, G. K., Nekrutenko, A., Taylor, J., and Hovig, E. (2013). Ten Simple Rules for Reproducible Computational Research. *PLOS Computational Biology* 9, e1003285. doi: 10.1371/journal.pcbi.1003285.
- Schall, P., and Ammer, C. (2018). Stand composition based on 2nd forest inventory (abundance, basal area, crown projection area and volume) on all forest EPs, 2014 2018. Version 2. Biodiversity Exploratories Information System. Dataset. Available at: https://www.bexis.uni-jena.de/ddm/data/Showdata/22907.
- Schall, P., Heinrichs, S., Ammer, C., Ayasse, M., Boch, | Steffen, Buscot, F., et al. (2020). Can multi-taxa diversity in European beech forest landscapes be increased by combining different management systems? *J Appl Ecol* 57, 1363–1375. doi: 10.1111/1365-2664.13635.
- Schloss, P. D., and Handelsman, J. (2005). Introducing DOTUR, a Computer Program for Defining Operational Taxonomic Units and Estimating Species Richness. *Applied and Environmental Microbiology* 71, 1501–1506. doi: 10.1128/AEM.71.3.1501-1506.2005.
- Schloss, P. D., Westcott, S. L., Ryabin, T., Hall, J. R., Hartmann, M., Hollister, E. B., et al. (2009). Introducing mothur: Open-Source, Platform-Independent, Community-Supported Software for Describing and Comparing Microbial Communities. *Applied and Environmental Microbiology* 75, 7537–7541. doi: 10.1128/AEM.01541-09.
- Schnell, I. B., Bohmann, K., and Gilbert, M. T. P. (2015). Tag jumps illuminated reducing sequence-to-sample misidentifications in metabarcoding studies. *Molecular Ecology Resources* 15, 1289–1303. doi: 10.1111/1755-0998.12402.
- Schwedener, S. (1886). Ueber die Beziehungen zwischen Algen und Flechtengonidien. Botanische Zeitung (Berlin), 289–292.

- Shehzad, W., Riaz, T., Nawaz, M. A., Miquel, C., Poillot, C., Shah, S. A., et al. (2012). Carnivore diet analysis based on next-generation sequencing: application to the leopard cat (Prionailurus bengalensis) in Pakistan. *Molecular Ecology* 21, 1951–1965. doi: 10.1111/j.1365-294X.2011.05424.x.
- Škaloud, P., Friedl, T., Hallmann, C., Beck, A., and Dal Grande, F. (2016). Taxonomic revision and species delimitation of coccoid green algae currently assigned to the genus Dictyochloropsis (Trebouxiophyceae, Chlorophyta). *Journal of phycology* 52, 599–617. doi: 10.1111/jpy.12422.
- Skouroliakou, D.-I., Breton, E., Irion, S., Artigas, L. F., and Christaki, U. (2022). Stochastic and Deterministic Processes Regulate Phytoplankton Assemblages in a Temperate Coastal Ecosystem. *Microbiology Spectrum* 10, e02427-22. doi: 10.1128/spectrum.02427-22.
- Smith, H. B., Dal Grande, F., Muggia, L., Keuler, R., Divakar, P. K., Grewe, F., et al. (2020). Metagenomic data reveal diverse fungal and algal communities associated with the lichen symbiosis. *Symbiosis* 82, 133–147. doi: 10.1007/s13199-020-00699-4.
- Štifterová, A., and Neustupa, J. (2015). Community structure of corticolous microalgae within a single forest stand: evaluating the effects of bark surface pH and tree species. *Fottea* 15, 113–122. doi: 10.5507/fot.2015.013.
- Taberlet, P., Coissac, E., Pompanon, F., Brochmann, C., and Willerslev, E. (2012). Towards next-generation biodiversity assessment using DNA metabarcoding. *Molecular Ecology* 21, 2045–2050. doi: 10.1111/j.1365-294X.2012.05470.x.
- Tedersoo, L., Bahram, M., Cajthaml, T., Põlme, S., Hiiesalu, I., Anslan, S., et al. (2016). Tree diversity and species identity effects on soil fungi, protists and animals are context dependent. ISME J 10, 346–362. doi: 10.1038/ismej.2015.116.
- Tedersoo, L., Bahram, M., Zinger, L., Nilsson, R. H., Kennedy, P. G., Yang, T., et al. (2022). Best practices in metabarcoding of fungi: From experimental design to results. *Molecular Ecology* 31, 2769–2795. doi: 10.1111/mec.16460.
- Thompson, L. R., Sanders, J. G., McDonald, D., Amir, A., Ladau, J., Locey, K. J., et al. (2017). A communal catalogue reveals Earth's multiscale microbial diversity. *Nature* 551, 457–463. doi: 10.1038/nature24621.
- Uhr, G. T., Dohnalová, L., and Thaiss, C. A. (2019). The Dimension of Time in Host-Microbiome Interactions. *mSystems* 4, 10.1128/msystems.00216-18. doi: 10.1128/msystems.00216-18.
- Unterseher, M., Siddique, A. B., Brachmann, A., and Peršoh, D. (2016). Diversity and Composition of the Leaf Mycobiome of Beech (Fagus sylvatica) Are Affected by Local Habitat Conditions and Leaf Biochemistry. *PLOS ONE* 11, e0152878–e0152878. doi: 10.1371/journal.pone.0152878.

- Urbanová, M., Šnajdr, J., and Baldrian, P. (2015). Composition of fungal and bacterial communities in forest litter and soil is largely determined by dominant trees. *Soil Biology and Biochemistry* 84, 53–64. doi: 10.1016/j.soilbio.2015.02.011.
- Uroz, S., Buée, M., Deveau, A., Mieszkin, S., and Martin, F. (2016). Ecology of the forest microbiome: Highlights of temperate and boreal ecosystems. *Soil Biology and Biochemistry* 103, 471–488. doi: 10.1016/j.soilbio.2016.09.006.
- Van Der Heijden, M. G. A., Bardgett, R. D., and Van Straalen, N. M. (2008). The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecology Letters* 11, 296–310. doi: 10.1111/j.1461-0248.2007.01139.x.
- Venkatesh, N., Greco, C., Drott, M. T., Koss, M. J., Ludwikoski, I., Keller, N. M., et al. (2022). Bacterial hitchhikers derive benefits from fungal housing. *Current Biology* 32, 1523-1533.e6. doi: 10.1016/j.cub.2022.02.017.
- Vondrák, J., Malíček, J., Palice, Z., Coppins, B., Kukwa, M., Czarnota, P., et al. (2016). Methods for obtaining more complete species lists in surveys of lichen biodiversity. *Nordic Journal of Botany* 34, 619–626. doi: 10.1111/njb.01053.
- Vondrák, J., Svoboda, S., Košnar, J., Malíček, J., Šoun, J., Frolov, I., et al. (2023). Martin7: a reference database of DNA barcodes for European epiphytic lichens and its taxonomic implications. *Preslia* 95, 311–345. doi: 10.23855/preslia.2023.311.
- Vorholt, J. A. (2012). Microbial life in the phyllosphere. *Nature Reviews Microbiology* 10, 828–840. doi: 10.1038/nrmicro2910.
- Walters, K. E., Capocchi, J. K., Albright, M. B. N., Hao, Z., Brodie, E. L., and Martiny, J. B. H. (2022). Routes and rates of bacterial dispersal impact surface soil microbiome composition and functioning. *The ISME Journal* 16, 2295–2304. doi: 10.1038/s41396-022-01269-w.
- Wang, X., Li, Z., Su, J., Tian, Y., Ning, X., Hong, H., et al. (2010). Lysis of a red-tide causing alga, Alexandrium tamarense, caused by bacteria from its phycosphere. *Biological Control* 52, 123–130. doi: 10.1016/j.biocontrol.2009.10.004.
- Wardle, D. A., Bardgett, R. D., Klironomos, J. N., Setälä, H., van der Putten, W. H., and Wall, D. H. (2004). Ecological Linkages Between Aboveground and Belowground Biota. *Science* 304, 1629–1633. doi: 10.1126/science.1094875.
- Warmink, J. A., and van Elsas, J. D. (2008). Selection of bacterial populations in the mycosphere of Laccaria proxima: is type III secretion involved? *The ISME Journal* 2, 887–900. doi: 10.1038/ismej.2008.41.
- Westcott, S. L., and Schloss, P. D. (2015). De novo clustering methods outperform reference-based methods for assigning 16S rRNA gene sequences to operational taxonomic units. *PeerJ* 3, e1487. doi: 10.7717/peerj.1487.

- White, T. J., Bruns, T., Lee, S., and Taylor, J. (1990). "Amplification and direct sequencing of fungal ribosomal rna genes for phylogenetics," in *PCR Protocols*, eds. M. Innis, D. H. Gelfland, J. J. Sninsky, and T. J. White (San Diego, CA: Academic Press), 315–322. doi: 10.1016/b978-0-12-372180-8.50042-1.
- Wilhelm, R. C., van Es, H. M., and Buckley, D. H. (2022). Predicting measures of soil health using the microbiome and supervised machine learning. *Soil Biology and Biochemistry* 164, 108472. doi: 10.1016/j.soilbio.2021.108472.
- Willerslev, E., Hansen, A. J., and Poinar, H. N. (2004). Isolation of nucleic acids and cultures from fossil ice and permafrost. *Trends in Ecology & Evolution* 19, 141–147. doi: 10.1016/j.tree.2003.11.010.
- Willis, A. D. (2019). Rarefaction, Alpha Diversity, and Statistics. Frontiers in Microbiology 10. Available at: https://www.frontiersin.org/articles/10.3389/fmicb.2019.02407 [Accessed December 20, 2023].
- Wright, B., L., St. C., Larry, and D., L., Steven (2019). Is targeted community DNA metabarcoding suitable for biodiversity inventories of lichen-forming fungi? *Ecological Indicators* 98, 812–820. doi: 10.1016/j.ecolind.2018.11.061.
- Yamamura, H., Ashizawa, H., Nakagawa, Y., Hamada, M., Ishida, Y., Otoguro, M., et al. (2011). Actinomycetospora iriomotensis sp. nov., a novel actinomycete isolated from a lichen sample. *The Journal of Antibiotics* 64, 289–292. doi: 10.1038/ja.2011.15.
- Yang, H., Yang, Z., Wang, Q.-C., Wang, Y.-L., Hu, H.-W., He, J.-Z., et al. (2022). Compartment and Plant Identity Shape Tree Mycobiome in a Subtropical Forest. *Microbiol Spectr* 10, e01347-22. doi: 10.1128/spectrum.01347-22.
- Yoccoz, N. G. (2012). The future of environmental DNA in ecology. *Molecular Ecology* 21, 2031–2038. doi: 10.1111/j.1365-294X.2012.05505.x.
- Zhou, J., and Ning, D. (2017). Stochastic Community Assembly: Does It Matter in Microbial Ecology? *Microbiology and Molecular Biology Reviews* 81, 10.1128/mmbr.00002-17. doi: 10.1128/mmbr.00002-17.
- Zhu, H., Li, S., Hu, Z., and Liu, G. (2018). Molecular characterization of eukaryotic algal communities in the tropical phyllosphere based on real-time sequencing of the 18S rDNA gene. *BMC Plant Biology* 18, 365–365. doi: 10.1186/s12870-018-1588-7.
- Zhu, Y.-G., Xiong, C., Wei, Z., Chen, Q.-L., Ma, B., Zhou, S.-Y.-D., et al. (2022). Impacts of global change on the phyllosphere microbiome. *New Phytologist* 234, 1977–1986. doi: 10.1111/nph.17928.

References

Zinger, L., Bonin, A., Alsos, I. G., Bálint, M., Bik, H., Boyer, F., et al. (2019). DNA metabarcoding—Need for robust experimental designs to draw sound ecological conclusions. *Molecular Ecology* 28, 1857–1862. doi: 10.1111/mec.15060.

Glossary

ASV amplicon sequencing variant

BExIS Biodiversity Exploratories Information System

BLAST Basic Local Alignment Search Tool

DNA deoxyribonucleic acid

eDNA environmental deoxyribonucleic acid

EP experimental plot

ESV exact sequencing variant

GDM Generalized Dissimilarity Modeling

HTS High-Throughput-Sequencing

NCBI National Center for Biotechnology Information

NGS Next-Generation-Sequencing

OTU operational taxonomic unit

SRA Short Read Archive

SSC soil sampling campaign

UV ultraviolet

A. Appendix – Publications

A.1. Tree size drives diversity and community structure of microbial communities on the bark of beech (*Fagus sylvatica*)

Declaration of author contributions to the publication:

<u>Tree size drives diversity and Community Structure of Microbial Communities on the Bark of Beech (Fagus sylvatica)</u>

Status: accepted (08.05.2022)

Name of journal: Frontiers in Forests and Global Change

doi: 10.3389/ffgc.2022.858382

Contributing authors: <u>Lukas Dreyling (LD)</u>, Imke Schmitt (IS), Francesco Dal

Grande (FDG)

What are the contributions of the doctoral candidate and his co-authors?

(1) Concept and design

Doctoral candidate (LD): 30% Co-author IS: 35% Co-author FDG: 35%

(2) Conducting tests and experiments

Doctoral candidate (LD): 80% (sample collection from the field, DNA isolation, PCR)

Co-author IS: 10% (sample collection from the field)
Co-author FDG: 10% (sample collection from the field)

(3) Compilation of data sets and figures

Doctoral candidate (LD): 100% (processing of sequencing reads, ASV inference,

taxonomic assignment, preparation of all figures and tables)

(4) Analysis and interpretation of data

Doctoral candidate (LD): 60% All Data analysis (alpha diversity & community

composition, species interaction networks, differential

abundance analysis)

Co-author IS: 15% (data interpretation)
Co-author FDG: 25% (data interpretation)

(5) Drafting of manuscript

Doctoral candidate (LD): 50% Co-author IS: 20% Co-author FDG: 30%

I hereby certify that the inf	formation above is correct.
Date and place	Signature doctoral candidate
Date and place	Signature supervisor
Date and place	If required, signature of corresponding author



frontiers Frontiers in Forests and Global Change

ORIGINAL RESEARCH published: 16 May 2022 doi: 10.3389/ffgc.2022.858382



Tree Size Drives Diversity and **Community Structure of Microbial** Communities on the Bark of Beech (Fagus sylvatica)

Lukas Dreyling^{1,2*}, Imke Schmitt^{1,2,3} and Francesco Dal Grande^{2,3,4*}

¹ Institute of Ecology, Evolution and Diversity, Goethe-University Frankfurt, Frankfurt, Germany, ² Senckenberg Biodiversity and Climate Research Centre (SBiK-F), Frankfurt, Germany, 3 LOEWE Centre for Translational Biodiversity Genomics (TBG), Frankfurt, Germany, ⁴ Department of Biology, University of Padova, Padua, Italy

OPEN ACCESS

Edited by:

Paolo Giordani, University of Genoa, Italy

Reviewed by:

Patricia Moya, University of Valencia, Spain Andreas Beck, Botanische Staatssammlung München, Germany

*Correspondence:

Lukas Drevlina lukas.dreyling@senckenberg.de Francesco Dal Grande francesco.dalgrande@unipd.it

Specialty section:

This article was submitted to Temperate and Boreal Forests. a section of the journal Frontiers in Forests and Global Change

Received: 19 January 2022 Accepted: 08 April 2022 Published: 16 May 2022

Citation:

Dreyling L, Schmitt I and Dal Grande F (2022) Tree Size Drives Diversity and Community Structure of Microbial Communities on the Bark of Beech (Fagus sylvatica). Front. For. Glob. Change 5:858382. doi: 10.3389/ffgc.2022.858382

Tree bark constitutes an ideal habitat for microbial communities, because it is a stable substrate, rich in micro-niches. Bacteria, fungi, and terrestrial microalgae together form microbial communities, which in turn support more bark-associated organisms, such as mosses, lichens, and invertebrates, thus contributing to forest biodiversity. We have a limited understanding of the diversity and biotic interactions of the bark-associated microbiome, as investigations have mainly focused on agriculturally relevant systems and on single taxonomic groups. Here we implemented a multi-kingdom metabarcoding approach to analyze diversity and community structure of the green algal, bacterial, and fungal components of the bark-associated microbial communities of beech, the most common broadleaved tree of Central European forests. We identified the most abundant taxa, hub taxa, and co-occurring taxa. We found that tree size (as a proxy for age) is an important driver of community assembly, suggesting that environmental filtering leads to less diverse fungal and algal communities over time. Conversely, forest management intensity had negligible effects on microbial communities on bark. Our study suggests the presence of undescribed, yet ecologically meaningful taxa, especially in the fungi, and highlights the importance of bark surfaces as a reservoir of microbial diversity. Our results constitute a first, essential step toward an integrated framework for understanding microbial community assembly processes on bark surfaces, an understudied habitat and neglected component of terrestrial biodiversity. Finally, we propose a cost-effective sampling strategy to study bark-associated microbial communities across large spatial or environmental scales.

Keywords: algae, bacteria, biofilm, community ecology, dermosphere, forest management, fungi, metabarcoding

INTRODUCTION

The aboveground surfaces of plants are ideal substrates for microbial colonization. The bark surface [or dermosphere; Lambais et al. (2014)], in particular, is one of such important aboveground substrates in forests. The bark provides a range of microhabitats that promote colonization of microbial communities with varied ecologies (Whitmore, 1963). On the one hand, microsites such

Frontiers in Forests and Global Change | www.frontiersin.org

May 2022 | Volume 5 | Article 858382

as holes, cracks, and lenticels retain humidity and nutrients, thus constituting stable microhabitats suitable for slow-growing, stress-sensitive microbes. On the other hand, the exposed surfaces of the bark may harbor more stress-resistant microbial communities that can cope with environmental challenges (Vorholt, 2012; Aguirre-von-Wobeser et al., 2021), such as low nutrient availability, increased exposure to light, fluctuating moisture conditions and desiccation (Lindow and Brandl, 2003; Vorholt, 2012; Leff et al., 2015), and presence of compounds that are resistant to microbial degradation (e.g., suberin), or that directly inhibit microbial growth (Baldrian, 2017).

Compared to other aboveground components, such as leaves, branches or fruits, that undergo seasonal and diurnal changes (Vitulo et al., 2019), bark represents a stable, long-lived substrate that supports microbial colonization (Leff et al., 2015). Further, the bark surface is often screened from excessive precipitationand/or UV radiation by the tree canopy and changes slowly during development over several years (Whitmore, 1963). A number of studies have investigated the bark-associated microbial diversity, especially for fungi and bacteria, in various systems, e.g., grapevine plants (Martins et al., 2013; Arrigoni et al., 2018), bark beetle-infested spruce (Strid et al., 2014), Ginkgo (Leff et al., 2015) and avocado trees (Aguirre-von-Wobeser et al., 2021). These studies report that the tree bark supports microbial communities that are often distinct from spatially-close substrates like leaves and roots (Martins et al., 2013; Leff et al., 2015; Arrigoni et al., 2018), indicating niche differentiation and a clearly structured habitat (Aguirre-von-Wobeser et al., 2021). Furthermore, the dermosphere constitutes a reservoir for microbial diversity (Arrigoni et al., 2018; Hagge et al., 2019; Kobayashi and Aoyagi, 2019), potentially harboring undiscovered specialist taxa (Aschenbrenner et al., 2017), and taxa that facilitate the colonization of other epiphytes, including lichens (Aschenbrenner et al., 2017). The microbial communities on tree bark, and the biofilm which they form, can indeed be considered the basis of a food web that supports photosynthetic epiphytes (e.g., mosses and lichens), as well as a diverse microfauna (Andre, 1985). With an estimated more than 3 trillion trees in the world (Crowther et al., 2015), bark communities could thus be particularly important reservoirs of biological diversity. However, bark is a poorly explored habitat with respect to microbial diversity and community structure, compared to other substrates such as the phyllosphere and rhizosphere.

Biological and environmental factors driving diversity and community assembly in bark-associated epiphytes have been linked to forestry management, e.g., management intensity (Boch et al., 2021), forest homogeneity (Lamit et al., 2015), deadwood abundance (Boch et al., 2021), and tree age. For the latter, higher epiphyte diversities have been linked to the availability of large, old-growth trees (Aude and Poulsen, 2000; Nascimbene et al., 2013; Boch et al., 2021), probably because of higher niche partitioning in older trees (Łubek et al., 2020). At smaller spatial scales, abiotic drivers of bark-associated diversity and community structure include ultraviolet radiation, water shortages and correlated desiccation, and poor nutrient availability (Lindow and Brandl, 2003; Vorholt, 2012; Leff et al., 2015), while biotic drivers include host traits, such as maturity of the substrate

and host genotype (Arrigoni et al., 2018, 2020). Community composition is therefore, to some degree, host specific. A few studies showed that the trends observed for macroepiphytes (e.g., bryophytes, lichens) or components of the phyllosphere also appear in bark-associated microbes (e.g., Vorholt, 2012; Arrigoni et al., 2020). However, our understanding of the factors shaping the different components of the highly diverse bark-associated microbial communities is still limited. Most of the studies focus on non-natural, commercially driven ecosystems like orchards or vineyards (Martins et al., 2013; Arrigoni et al., 2018) and are often conducted over small spatial scales with small sample sizes (e.g., Leff et al., 2015). Lastly, the focus often lies on only a single group of microorganisms, with bacteria and fungi far outweighing terrestrial algae (Aschenbrenner et al., 2017; Petrolli et al., 2021). Integrative sampling of major microbial contributors over regional or potentially even global scales can help identifying not only the diversity of microorganisms but also potential cooperative and competitive interactions among them. Revealing the diversity and structure of these rather unique microbial communities is essential to predict their responses in a changing environment. Furthermore, considering the importance of fungi, bacteria and algae to ecosystem nutrient and energy budgets in terrestrial habitats, gaining information on the bark-associated microbial communities and their dynamics is essential and directly relevant for ecosystem service assessment.

In this study we present one of the first integrated investigations of the bark microbiome in temperate forests. Here we use the term microbiome following the definition by Berg et al. (2020). Specifically, we study the three main components of the bark microbiome, i.e., green algae, bacteria and fungi. We sampled bark surfaces in forests under different management regimes, ranging from highly-managed stands to relatively undisturbed sites in the core zone of a national park. We used metabarcoding to analyze microbial diversity, community structure and species interactions from the tree to the landscape level. Specifically, we asked the following questions: (i) What is the microbial diversity found on the bark of the most common broadleaved tree in central Europe (Fagus sylvatica)?, (ii) Which species co-occur and who are the main players in the identified ecological modules?, (iii) Which factors, i.e., management intensity and tree-size classes (as a proxy for tree age), affect the bark-associated microbiome, both at tree and landscape level?

The comparison of diversities among trees of different size classes within a spatially-explicit framework allowed us to test for the effects of sampling design on the estimation of microbial diversity. This information is essential for further, larger scale sampling campaigns.

MATERIALS AND METHODS

Study Site and Sampling

Sampling sites are situated within the central European region of Hainich-Dün (Thuringia, Germany), one of the three regions of the Biodiversity Exploratories (Fischer et al., 2010). The Hainich-Dün region is characterized by soils stemming from calcareous

bedrock, an annual rainfall between 500–800 mm, and a mean temperature of 6.5–8°C at an elevation of 285–550 m above sea level (Fischer et al., 2010).

Sample collection took place in autumn between the 13th and 15th of October 2020. We chose a subset of 16 out of the established 50 experimental plots (Fischer et al., 2010), sampling a subplot of 20 m \times 20 m within the 100 m \times 100 m experimental plots. These plots were chosen to represent two regimes of land-use intensity, namely a high and a low intensity forest management (eight plots each), according to the Forest Management Index (ForMI, high > 1, low < 1). This is an index combining measures of harvested stem volume, occurrence of non-natural species and deadwood stemming from harvest (Kahl and Bauhus, 2014). The plots had an average stand density of 485 trees/ha (min = 152 trees/ha, max = 1,830 trees/ha). We defined three size classes: large [i.e., > 30 cm diameter at breast height (DBH)], medium (15-30 cm DBH) and small (5-15 cm DBH). We sampled two trees per size class, resulting in a total of 96 samples. When one tree-size class was not available (three plots), we sampled more trees of the other size classes depending on which was highly abundant in the direct vicinity as judged in the field. Within each plot we recorded the spatial position of the trees relative to each other by measuring distance (m) and azimuth (degrees) from the nearest sampled tree.

We collected microbial bark surface communities using individually wrapped sterile nylon-flocked medical swabs with a 30 mm breakpoint, typically used for medical specimen collection (FLOQSwabsTM, Copan, Brescia, Italy). The breakpoint mechanism minimizes the possibility of contamination when transferring the swab into the Eppendorf tube. Prior to collection the bark was moisturized with deionized water to mobilize the surface biofilms. Then the tree was swabbed at approximately 150 cm height in a 3 cm-wide band around the trunk, rolling the swab and moving it up and down while applying gentle pressure. While swabbing, we took care to include smooth surfaces as well as cracks and crevices in the bark, to ensure a good representation of micro-habitats. If present, large patches (>10 cm) of bryophytic epiphytes were excluded. Conspicuous, larger lichen thalli were not present in the swabbed areas, however, small lichen thalli/propagules were included during the swabbing. The swab head was broken off into Eppendorf tubes pre-filled with 750 µl Nucleic Acid Preservation (NAP) buffer (Camacho-Sanchez et al., 2013). Tubes were immediately placed in styrofoam boxes with ice and the samples were subsequently stored at 4°C until DNA extraction.

DNA Extraction

Prior to DNA extraction we added 750 μ l ice-cold phosphate-buffered saline (PBS) into the Eppendorf tube and centrifuged the sample for 15 min at 6,000 \times g as recommended by Menke et al. (2017). The supernatant was then discarded without disturbing the swab head or pellet. DNA was extracted using the Quick-DNA Fecal/Soil Microbe Microprep kit (Zymo Research Europe GmbH, Freiburg, Germany). Initial tissue lysis was achieved through mechanical disruption by bead beating, using the beads included in the extraction kit. We modified the kit protocol by directly adding the beads and bead-beating buffer into the tube containing the pellet and swab and shaking for a total of 6 min

(SpeedMill PLUS, Analytik Jena, Jena, Germany). In the later steps we followed the manufacturer's protocol, using DNAsefree water as elution buffer. We included six extraction blanks as contamination controls, that were sequenced as well. Extraction blanks consisted of one unused swab, unpacked and transferred to the NAP buffer in the field and subsequently treated in the same way as regular samples. DNA extracts were frozen at $-20^{\circ}\mathrm{C}$ until PCR.

PCR Amplification and High-Throughput Sequencing

Algal, bacterial and fungal fractions of the extracted microbial DNA were amplified, using universal primers for the ITS2 region for fungi and algae, and the 16S hyper-variable region V3–V4 for bacteria (Table 1).

All samples were amplified in duplicate with forward and reverse primers individually tagged with octamers allowing for a double index multiplexing approach. Each duplicate contained eight PCR negative controls (i.e., master mix without sample), that were sequenced as well, meaning that a total of 110 \times 2 samples were obtained after PCR. Additionally we included 16 "Multiplex Controls" (i.e., empty wells) to allow detection of potential primer jump during sequencing (Schnell et al., 2015). We set up 15 µl PCR reactions containing 5 ng of DNA, 7.5 µl of MyTaqTM HS Mix, 2x (Bioline GmbH, Luckenwalde, Germany), 0.6 µl 10 µM of each primer, and 4.3 µl DNAse free water. Cycling conditions differed in cycle number and annealing temperature among organismal groups. Conditions were as follows: an initial denaturation at 95°C for 1 min, followed by 30 (algae, bacteria) or 35 (fungi) cycles of denaturation at 95°C for 15 s, annealing a 54°C (algae), 59°C (bacteria) or 56°C (fungi) for 15 s and elongation at 72°C for 10 s, with a final extension at 72°C for 1 min. The number of PCR cycles was determined prior to sampling, using initial test PCRs with material obtained in the same manner. The algal and bacterial amplicons reached a homogenous PCR amplification across all samples after 30 cycles, while the fungal amplification required 35 cycles. Samples were

Direction	Name	Sequence	Sources
Algae			
Forward	ITS-Cha3	CAACTCTCRRCAACGGATA	Cheng et al., 2016
Reverse	ITS u4	RGTTTCTTTTCCTCCGCTTA	Cheng et al., 2016
Bacteria			
Forward	341F (modified)	CCTACGGGWGGCWGCAG	Muyzer et al., 1993; Vieira et al., 2020
Reverse	785R	GACTACHVGGGTATCTAATCC	Herlemann et al., 2011
Fungi			
Forward	FITS7 (modified)	GTGARTCATCGAATCTTTG	Ihrmark et al., 2012
Reverse	ITS 4 (modified)	TCCTCCGCTTATTGATATGC	White et al., 1990

Frontiers in Forests and Global Change | www.frontiersin.org

May 2022 | Volume 5 | Article 858382

randomly distributed over two 96-well plates, with both replicates following the same placement scheme.

The amplicons were individually cleaned using magnetic beads (MagSI-NGS^{Prep} Plus, magtivio B.V., Geelen, Netherlands) and DNA concentration was quantified with fluorescence measurement using the Qubit dsDNA HS assay (Thermo Fisher Scientific, MA, United States) as specified by the manufacturer. The replicates were equimolarly pooled within the respective organismal groups, creating a total of three pools for sequencing. The pooled amplicons were send for library preparation and sequencing to Fasteris SA (Plan-les-Ouates, Switzerland). Libraries were prepared for each pool according to the Fasteris MetaFast protocol¹, in order to avoid PCR for library preparation and thus minimizing additional PCR bias and chimera creation. The samples were sequenced on an Illumina MiSeq (Illumina Inc., San Diego, CA, United States) with 2 × 300 bp pairedend reads.

Bioinformatics

Adapter-trimmed reads trimmed with Trimmomatic (Bolger et al., 2014) were supplied by the sequencing provider. We demultiplexed the reads using Cutadapt v3.3 (Martin, 2011) following the demultiplexing combinatorial dual-indexes section of the manual. The error rate was set to 0.15, allowing no insertions or deletions, and discarding reads shorter than 50 bp. Commands were run a second time with the octamer tags in the reverse order to account for amplicons in mixed orientation resulting from PCR-free library preparation. The resulting files were merged to obtain one R1 and one R2 file per replicate. Reads were checked for remaining primer sequences, which were removed using Cutadapt, if present.

The demultiplexed reads were further processed with the DADA2 pipeline (Callahan et al., 2016). Filtering and trimming operations used DADA2 default parameters, except for setting a truncation length [truncLen = c(250,260)] for bacteria, but not for algae and fungi since the length of the ITS2 region can vary between taxa (Schoch et al., 2014). Furthermore, the maximum error rates were relaxed to maxEE = c(5,5) for bacteria and maxEE = c(6,6) for algae and fungi. After de-noising and sample inference, pairs were merged within each replicate, chimeras were removed and one amplicon sequence variant (ASV) table was constructed per replicate. To account for the mixed orientation of the libraries we checked the tables for reverse complement sequences, reversed them and added their counts to the respective complement sequence using DADA2s rc() function. Finally, the replicates were merged by summing up the read counts.

For taxonomic assignment the sequences were matched against publicly available databases, namely UNITE general release 8.2 (Abarenkov et al., 2020) for fungal reads and SILVA 138.1 SSU Ref NR 99 (Quast et al., 2012) for bacteria. Since no similar database is currently available for green algae we used the program SEED2 v2.1.2 (Větrovský et al., 2018) to conduct a BLASTn search against GenBank (Clark et al., 2016, last accessed 30.03.2022).

We then checked the reads for potential contamination with the *decontam* package (Davis et al., 2018), using the combined prevalence and frequency approach. For all organismal groups *decontam* only showed low numbers (algae = 0, bacteria = 8, fungi = 4) of potential contaminant ASVs, which were discarded. The *decontam*-filtered ASV tables were curated using the LULU algorithm (Frøslev et al., 2017) to merge highly similar ASVs and obtain more reliable diversity metrics. Taxonomic information for all ASVs can be found in **Supplementary Table 1**.

Diversity and Community Structure Analyses

All analyses were conducted in R (R Core Team, 2021, version 4.0.4) through RStudio (RStudio Team, 2021). ASV tables, taxonomic information and accompanying metadata were combined using the *phyloseq* R package (McMurdie and Holmes, 2013) to ease analyses. Figures were created with *ggplot2* (Wickham, 2016) and *gridExtra* (Auguie, 2017). Samples were not rarefied, as recommended by McMurdie and Holmes (2014) and instead treated as compositional count data (Gloor et al., 2017). Scripts of all analyses are available on GitHub at https://github.com/LukDrey/beech_micro_communities.

Intra-Group Diversities

We calculated the Shannon Index (Shannon, 1948) as a measure of alpha diversity, using the function <code>estimate_richness()</code> on the full untransformed ASV table as obtained from DADA2 and LULU. Differences in Shannon diversity between tree sizes and management category were tested with an Analysis of Variance (ANOVA) with the function <code>aov()</code> and verified <code>via</code> a Tukey Honest Significant Differences test (Tukey HSD). Furthermore, we tested whether the Shannon diversity for trees within a plot was spatially autocorrelated. For this purpose, we computed Moran's I (method from Gittleman and Kot, 1990) as a measure of spatial autocorrelation with the function <code>Moran.I</code> from the <code>ape</code> R package (Paradis and Schliep, 2019).

To create the community barplots we aggregated taxa at the order rank and subset the datasets to the 25 relatively most abundant taxa with $get_top_taxa()$ (Teunisse, 2017). The resulting subsets were transformed to reflect their compositional nature by transform() and plotted using $plot_composition()$, both from the microbiome R package (Lahti and Shetty, 2017).

Inter-Group Differences

Before comparing differences in community composition of differently sized trees and management regimes, each full dataset was transformed based on centred log-ratios (CLR) with transform(). After the transformation we conducted a principal component analysis (PCA) on the clr-transformed datasets using the phyloseq function ordinate() ("RDA" method) which for clr-transformed data is the same as PCA. In the ordination plots we show the first two Principal Components (PC), with the axes scaled to the proportion of variance the PC explains, as recommended by Nguyen and Holmes (2019).

To test if groups showed similar within-group variance, we used the <code>betadisper()</code> function and verified the results with

 $^{^1}https://www.fasteris.com/en-us/NGS/DNA-sequencing/Metabarcoding/Metagenomics-16S-18S-ITS-or-custom-PCR-amplicons$

the accompanying permutation test permutest() from the vegan package (Oksanen et al., 2020). To test for differences in community composition between tree sizes and management intensity we performed Permutational Analysis of Variance (PERMANOVA) with a distance matrix based on Aitchison's distance (method = "euclidean" with the phyloseq function distance() for clr-transformed data). The PERMANOVA was computed using the vegan function adonis2() examining marginal effects of tree size and management intensity together.

Species Interactions

The interaction networks were generated with the SPIEC-EASI method (Kurtz et al., 2015), a robust method for the sparse and compositional nature of microbiome datasets implemented in the R package SpiecEasi. Before network inference the ASV tables were subset to contain only ASVs contributing at least one percent of the total reads to ease both visualization and computational load. The main SPIEC-EASI algorithm was set to use the meinshausen-bühlmann's neighborhood selection (Meinshausen and Bühlmann, 2006) and Bounded StARS model selection (Müller et al., 2016) on 50 subsamples (rep.num = 50), with lambda.min.ratio = 0.1, nlambda = 100, pulsar.select = TRUE and seed = 10010. We calculated one network per organismal group, as well as one containing all three groups together.

The obtained models were refit, turned into *igraph* (Csardi and Nepusz, 2006) objects and loaded in *Gephi* v0.9.2 (Bastian et al., 2009) for visualization. Modularity and betweenness centrality (for visualization purposes) were computed with *Gephis* internal algorithms (Brandes, 2001; Blondel et al., 2008). The graph layouts were constructed using the Fruchterman-Reingold algorithm (Fruchterman and Reingold, 1991). For each network, hub taxa were calculated based on vertex betweenness centrality using the *igraph* function *betweeness()* with default parameters, except setting directed = FALSE. The top five hub taxa, based on vertex betweenness centrality, were extracted.

Differential Abundance Analysis

Differential abundance analysis was conducted using ALDEx2 (Fernandes et al., 2013, 2014; Gloor et al., 2016). We compared abundances of two groups, i.e., high/low management intensity, large/small, large/medium and medium/small trees, for each organismal group. ALDEx2 generates Monte Carlo samples (N=128), drawn from the Dirichlet distribution for each individual sample, and tests differences between specified groups through Wilcoxon rank-sum tests. ALDEx2 is a robust choice for compositional datasets because the data is clr-transformed internally. Taxa were declared differentially abundant if they showed a Benjamini-Hochberg corrected p-value < 0.05.

RESULTS

Intra-Group Diversities

In total we obtained on average 59,324 reads per sample for algae (min = 22,913, max = 99,255), 58,259 reads for bacteria (min = 24,830, max = 124,263) and 45,403 reads for fungi

(min = 18,510, max = 163,736). The extraction blanks had on average 888 reads for algae (min = 283, max = 1,679), 12,836 for bacteria (min = 462, max = 19,723), and 34,211 for fungi (min = 6171, max = 109,074), while the PCR negative controls contained on average 1,152 reads for algae (min = 272, max = 3,745), 625 reads for bacteria (min = 196, max = 2,076), and 3,241 for fungi (min = 196, max = 15,752). The negative controls were discarded from the analysis after controlling for possible contaminant sequences with *decontam* (Davis et al., 2018). From these reads, we retrieved 216 algal, 1,742 bacterial and 992 fungal ASVs.

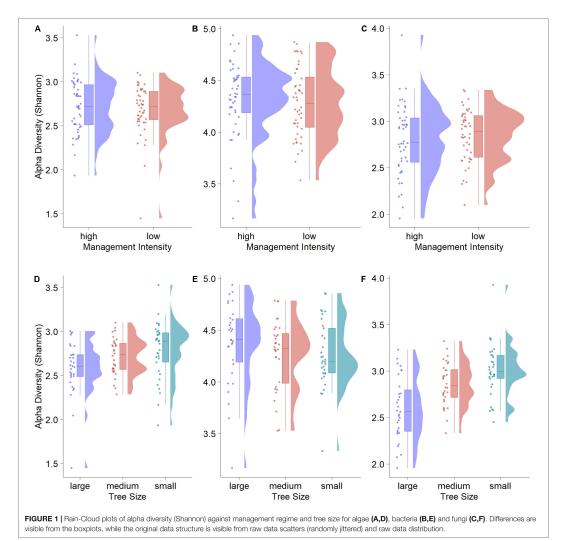
Overall algae and fungi displayed similar Shannon alpha diversity, while bacteria showed a slightly higher diversity (Figure 1). Neither algae, fungi nor bacteria exhibited statistically significant differences in alpha diversity when comparing low and high management intensity plots (Figures 1A–C). Considering differences between tree sizes, smaller trees displayed higher alpha diversity for algae and fungi (Figures 1D,F). Overall, bacterial diversities were more uniform, but displayed higher median Shannon diversity values for larger trees (Figure 1E).

This trend was corroborated by the results of an ANOVA comparing the three tree-size classes. For algae, we found a significant overall effect of tree size (F=4.163, p<0.05), that was driven by a significant difference between large and small trees (Tukey HSD p-value <0.05). No significant differences were found between large/medium and medium/small trees. Tree size had a significant effect overall (F=17.33, p<0.001) in fungi, with significant differences between large and medium (p<0.01), as well as large and small trees (p<0.001). For bacteria we found no significant overall effects.

Spatial autocorrelation tests showed that only in four of 48 cases (three organismal groups \times 16 plots) the null-hypothesis of no spatial correlation could be rejected (Table 2). This indicates that the effect of spatial autocorrelation within plots is negligible. Trees belonging to the same plot showed very similar Shannon alpha diversity values. One exception is plot HEW8 where alpha diversity was significantly spatially autocorrelated for both algae and bacteria.

Bark microbial communities were similar among trees, with only minor differences in rare orders for all three organismal groups. For algae, the predominant orders were Trebouxiales and Chlorellales, with Trebouxiales contributing more than 50% of the reads in many plots (Figure 2A). Rare orders displayed a relatively high inter-plot variability, with Prasiolales being almost absent for the plot HEW5. Compared to algae, bacteria were more homogeneous, with the same four orders-Rhizobiales, Sphingomonadales, Acetobacterales and Cytophagales—displaying comparably high relative abundances in all plots (Figure 2B). We found a higher rare-order diversity in bacteria and fungi compared to algae. Capnodiales was by far the most abundant fungal order, and dominant in all plots (Figure 2C). Compared to bacteria and algae, a higher proportion of fungal reads could not be assigned at the order rank. These unassigned reads contributed more than 25% of the total reads in some samples. The three relatively most abundant ASVs in the algal dataset belong to the genera Symbiochloris (12%), Apatoccocus (12%) and Desmococcus (8%), and in the

Exploring the Beech Bark Microbiome



bacterial dataset to *Acidiphilium* (10%), 1174-901-12 (4%) and two nodes was through ten et al. (4%). Only one of the three most abundant fungal four and a modularity score of the score

Intra-Group Interactions

to Ascomycota (7%).

We inferred ASV interaction networks for all three microbial groups (**Figure 3**). Each ASV entering the networks contributed at least 1% of the total reads, resulting in 129 ASVs for the algae, 624 for bacteria and 289 for fungi. The algal network (**Figure 3A**) had a diameter of 10 (i.e., the longest shortest path between

ASVs could be assigned to a genus—namely *Scoliciosporum* (4%)—while one was assigned to Capnodiales (26%) and one only

two nodes was through ten edges), an average path length of four and a modularity score of 0.575. Modularity scores > 0.4 indicate strong modularity (Newman, 2006). The diameter for the fungal network (**Figure 3C**) was 7, with an average path length of \sim 3.2 and a modularity slightly lower than the algae at 0.434. The bacterial network (**Figure 3B**) was denser and more interconnected with a diameter of 5, an average path length of \sim 2.7 and a modularity of 0.335.

The algal network could be subdivided into nine different modules, five of which consisting of more than 10 ASVs (see **Table 3**). There were also 8 ASVs that did not interact with any other taxon in the network. The algal module with the highest

TABLE 2 | Overview of Moran's I (MI) values for all three organismal groups in the studied plots

		Algae				Bacteria				Fungi		
Plot ID	MI (observed)	MI (expected)	sd	P value	MI (observed)	MI (expected)	sd	P value	MI (observed)	MI (expected)	sd	P value
HEW11	-0.19	-0.2	0.17	0.95 (n.s.)	-0.09	-0.2	0.12	0.32 (n.s.)	0	-0.2	0.24	0.41 (n.s.)
HEW20	-0.41	-0.2	0.16	0.17 (n.s.)	-0.15	-0.2	0.14	0.75 (n.s.)	-0.43	-0.2	0.16	0.13 (n.s.)
HEW26	-0.26	-0.2	0.24	0.8 (n.s.)	-0.55	-0.2	0.21	0.09 (n.s.)	-0.24	-0.2	0.23	0.86 (n.s.)
HEW27	-0.46	-0.2	0.2	0.19 (n.s.)	-0.08	-0.2	0.19	0.53 (n.s.)	-0.27	-0.2	0.24	0.77 (n.s.)
HEW28	0.32	-0.2	0.3	0.09 (n.s.)	-0.31	-0.2	0.22	0.60 (n.s.)	-0.39	-0.2	0.31	0.53 (n.s.)
HEW31	-0.09	-0.2	0.11	0.31 (n.s.)	-0.35	-0.2	0.16	0.33 (n.s.)	0.02	-0.2	0.14	0.12 (n.s.)
HEW32	0.04	-0.2	0.13	0.06 (n.s.)	-0.19	-0.2	0.09	0.92 (n.s.)	0.05	-0.2	0.15	0.09 (n.s.)
HEW33	-0.22	-0.2	0.25	0.93 (n.s.)	-0.21	-0.2	0.08	0.95 (n.s.)	0.04	-0.2	0.29	0.41 (n.s.)
HEW34	-0.3	-0.2	0.16	0.52 (n.s.)	-0.3	-0.2	0.19	0.61 (n.s.)	-0.35	-0.2	0.2	0.47 (n.s.)
HEW35	-0.35	-0.2	0.14	0.29 (n.s.)	-0.14	-0.2	0.15	0.68 (n.s.)	0.09	-0.2	0.13	0.03 (*)
HEW36	-0.31	-0.2	0.12	0.39 (n.s.)	-0.19	-0.2	0.11	0.90 (n.s.)	-0.12	-0.2	0.09	0.37 (n.s.)
HEW37	0.01	-0.2	0.11	0.07 (n.s.)	-0.2	-0.2	0.09	0.95 (n.s.)	-0.27	-0.2	0.11	0.53 (n.s.)
HEW43	-0.31	-0.2	0.15	0.48 (n.s.)	-0.34	-0.2	0.09	0.13 (n.s.)	-0.34	-0.2	0.08	0.08 (n.s.)
HEW49	-0.15	-0.2	0.1	0.58 (n.s.)	-0.38	-0.2	0.11	0.11 (n.s.)	-0.13	-0.2	0.08	0.44 (n.s.)
HEW5	-0.06	-0.2	0.09	0.09 (n.s.)	-0.29	-0.2	0.1	0.40 (n.s.)	-0.01	-0.2	0.09	0.04 (*)
HEW8	0.31	-0.2	0.22	0.02 (*)	0.39	-0.2	0.26	0.02 (*)	0.25	-0.2	0.28	0.11 (n.s.)

An observed higher significant p-value (< 0.05) vs. expected indicates positive autocorrelation, whereas lower MI values indicate negative autocorrelation.

number of nodes was module 2 (gold color, **Figure 3A**) with the most abundant ASV belonging to the genus *Desmococcus* (relative abundance = \sim 47% in the module, **Table 3**).

The bacterial network consisted of seven modules, all of which included more than 10 ASVs and no taxa with no connections. In this case, module 6 (purple color in **Figure 3B**) was the module with the highest count of taxa, with an ASV assigned to the genus *Abditibacterium* being the predominant strain (12% of reads in the module, **Table 3**).

For fungi, the network clustered into nine different modules, all containing more than ten ASVs and no unconnected taxa. Also in this case, module two (purple color, **Figure 3C**) was the module with the highest number of taxa for the fungal network, with an ASV belonging to the order Capnodiales—not assignable more specifically (**Table 3**)—with the highest relative abundance in the module (14%).

Network structure was examined by identifying nodes with the highest number of shortest paths going through them (betweenness centrality), indicating taxa that are important for the connectivity of the network. We defined so called hub taxa as the five taxa with the highest betweenness centrality (Table 4). In the algal network these hub taxa belonged to two orders, Chlorellales and Trebouxiales, and three different genera. Three ASVs were assigned to the genus Apatoccocus, and one to Trebouxia and Symbiochloris, respectively. Bacterial hub taxa showed a higher diversity than the algae with hub taxa belonging to five different orders. The genera include Tundrisphaera, Actinomycetospora and Oligoflexus. One of the ASVs was assigned to the group 1174-901-12, a group of uncultured bacterial strains within the order Rhizobiales and one to the family Chitinophagaceae. Many of the fungal hub taxa were not assigned at the genus rank, with the exception of two ASVs belonging to the genera *Tremella* and *Aureobasidium*. Two more ASVs were assigned to the order Capnodiales while one was only assigned at the phylum rank.

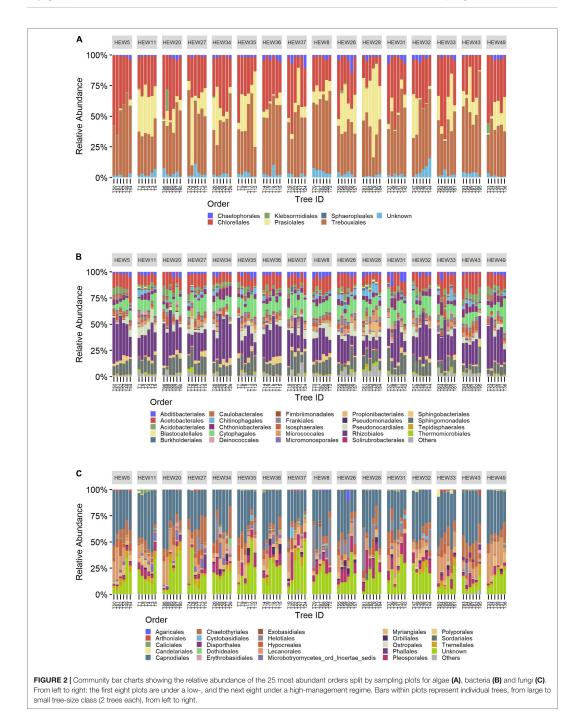
Inter-Group Interactions

The combined network of algae, bacteria and fungi (**Figure 4**) displayed a diameter of 4, an average path length of \sim 2.6 and a modularity of 0.259, making this network more densely connected than the bacterial network. Out of the eight modules, modules two (29%) and three (26%) accounted for more than half of the total reads.

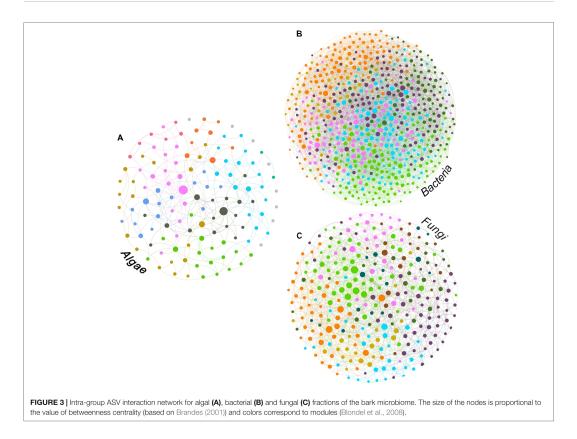
The top orders for algae, bacteria and fungi in the relatively most abundant module (module 2, pink color in **Figure 4B**) were Trebouxiales, Sphingomonadales and Capnodiales, respectively. Algae and fungi accounted for up to 36 and 26% of the module reads, respectively, while Sphingomonadales only contributed 5% of the reads. Trebouxiales contributed 87% of the algal reads, Sphingomonadales 30% of bacterial reads, and Capnodiales 77% of the fungal reads. In total, algae contributed 42%, bacteria 19%, and fungi 34% of the reads assigned to module 2.

In the second most abundant module (module 3, blue color in Figure 4B). Chlorellales were the most important algal order, Rhizobiales the most important bacterial order, while the most important fungal order was again Capnodiales. Chlorellales contributed 18%, Rhizobiales 16% and Capnodiales only 5% of the module reads. Chlorellales accounted for 43% of the algal reads, Rhizobiales 48% of the bacterial reads, and Capnodiales 34% of the fungal reads. Overall, module three consisted of 42% algae, 34% bacteria and 10% fungi.

The hub taxa of the combined network (**Table 4**) contained members of two microbial groups, specifically three bacteria and two fungi. Four ASVs could be assigned down to genus rank, while one fungal ASV—a hub taxon present also in the fungal network—could only be assigned to order rank (Capnodiales).



Exploring the Beech Bark Microbiome



The other fungus—also a fungal hub taxon—belonged to the genus *Aureobasidium*, while the three bacterial ASVs belonged to the genus *Edaphobaculum*, the uncultured group LD29 within the order Chthoniobacterales, and the genus *Kineococcus*.

Drivers of Changes in Community Composition

To investigate differences in community composition we plotted the results of the PCA (**Figure 5**). The differences between the high and low management intensity are not readily visible by looking at the clusters of the two groups. Yet, there are significant differences between the groups as revealed by the PERMANOVA results (algae: p < 0.05, bacteria: p < 0.01, fungi: p < 0.001). The dispersion permutation test was significant (algae: p < 0.05, bacteria: p < 0.05, fungi: p < 0.05) indicating a heterogeneous dispersion within groups. If on one hand this might suggest that the results are not reliable, Anderson and Walsh (2013) showed that PERMANOVA is not sensitive against heterogeneity, compared to other analyses. Management intensity explained only ~2% of the variance in the dataset, suggesting a subtle yet significant effect.

The tree sizes clustered in a much clearer structure, with less overlap of the 95% confidence interval ellipses. The PERMANOVA analysis confirmed this observation, with highly significant results for all microbial groups (p < 0.001 for all), while the permutation test indicated homogenous dispersion within the bacteria and fungi (p > 0.05), but not the algae (p < 0.05).

Further examination of the community structure showed that the differences in community composition were also visible through significantly differentially abundant taxa (Supplementary Table 2). The difference between management intensities was little, with only four differentially abundant fungal ASVs. The tree-size classes, however, showed a different signal and confirmed the stronger differences indicated by the PERMANOVA results. Between large and medium trees we found nine algae, four bacteria and 10 fungal taxa that showed significant abundance differences. A larger difference in abundances could be seen between large and small trees, with 19 algae, 43 bacteria and 34 fungi that were differentially abundant. Almost no difference could be observed between communities of medium and small trees with only three algal and one bacterial ASVs with significant changes in abundance.

72

Exploring the Beech Bark Microbiome

TABLE 3 | Taxonomic assignment of the most abundant ASVs and their relative abundance for modules with more than 10 ASVs.

ASV ID	#	Kingdom	Phylum	Class	Order	Family	Genus	Rel. abund.
Algae								
ASV 4	2	Viridiplantae	Chlorophyta	Trebouxiophyceae	Prasiolales	Stichococcaceae	Desmococcus	0.47
ASV 1	4	Viridiplantae	Chlorophyta	Trebouxiophyceae	Trebouxiales	Trebouxiaceae	Symbiochloris	0.8
ASV 7	5	Viridiplantae	Chlorophyta	Trebouxiophyceae	Trebouxiales	Trebouxiaceae	Trebouxia	0.4
ASV 2	7	Viridiplantae	Chlorophyta	Trebouxiophyceae	Chlorellales	Chlorellaceae	Apatococcus	0.38
ASV 33	9	Viridiplantae	Chlorophyta	Trebouxiophyceae	Prasiolales	Stichococcaceae	Diplosphaera	0.44
Bacteria								
ASV 2	0	Bacteria	Proteobacteria	Alphaproteobacteria	Acetobacterales	Acetobacteraceae	Acidiphilium	0.47
ASV 8	1	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Beijerinckiaceae	Methylocella	0.13
ASV 210	2	Bacteria	Abditibacteriota	Abditibacteria	Abditibacteriales	Abditibacteriaceae	Abditibacterium	0.08
ASV 54	3	Bacteria	Proteobacteria	Alphaproteobacteria	Acetobacterales	Acetobacteraceae	Acidiphilium	0.11
ASV 38	4	Bacteria	Proteobacteria	Alphaproteobacteria	Acetobacterales	Acetobacteraceae	Acidiphilium	0.11
ASV 6	5	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Beijerinckiaceae	1174-901-12	0.15
ASV 46	6	Bacteria	Abditibacteriota	Abditibacteria	Abditibacteriales	Abditibacteriaceae	Abditibacterium	0.13
Fungi								
ASV 11	0	Fungi	Ascomycota	Lecanoromycetes	Lecanorales	Lecanoraceae	Scoliciosporum	0.19
ASV 23	1	Fungi	Ascomycota	Eurotiomycetes	Chaetothyriales	Herpotrichiellaceae	Capronia	0.58
ASV 51	2	Fungi	Ascomycota	Dothideomycetes	Capnodiales			0.14
ASV 32	3	Fungi	Ascomycota	Dothideomycetes	Capnodiales	Mycosphaerellaceae		0.25
ASV 43	4	Fungi	Ascomycota	Dothideomycetes	Capnodiales			0.39
ASV 4	5	Fungi	Ascomycota					0.36
ASV 6	6	Fungi	Ascomycota	Leotiomycetes	Helotiales			0.3
ASV 1	7	Fungi	Ascomycota	Dothideomycetes	Capnodiales			0.88
ASV 61	8	Fungi	Ascomycota					0.18

TABLE 4 | Taxonomic information for the hub taxa of the respective network, identified based on their betweenness centrality.

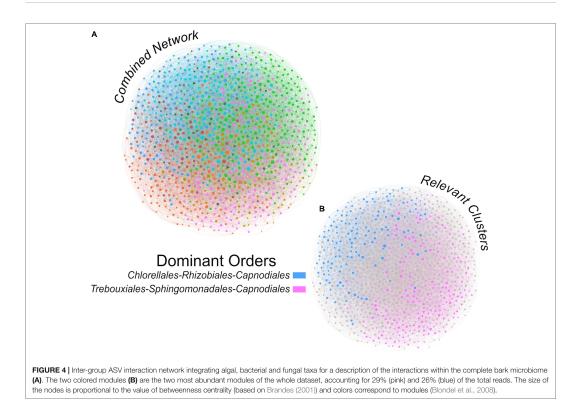
ASV ID	Kingdom	Phylum	Class	Order	Family	Genus
Algae						
ASV 10	Viridiplantae	Chlorophyta	Trebouxiophyceae	Chlorellales	Chlorellaceae	Apatococcus
ASV 11	Viridiplantae	Chlorophyta	Trebouxiophyceae	Chlorellales	Chlorellaceae	Apatococcus
ASV 13	Viridiplantae	Chlorophyta	Trebouxiophyceae	Trebouxiales	Trebouxiaceae	Symbiochloris
ASV 22	Viridiplantae	Chlorophyta	Trebouxiophyceae	Trebouxiales	Trebouxiaceae	Trebouxia
ASV 8	Viridiplantae	Chlorophyta	Trebouxiophyceae	Chlorellales	Chlorellaceae	Apatococcus
Bacteria						
ASV 162	Bacteria	Planctomycetota	Planctomycetes	Isosphaerales	Isosphaeraceae	Tundrisphaera
ASV 19	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Beijerinckiaceae	1174-901-12
ASV 372	Bacteria	Bacteroidota	Bacteroidia	Chitinophagales	Chitinophagaceae	
ASV 79	Bacteria	Actinobacteriota	Actinobacteria	Pseudonocardiales	Pseudonocardiaceae	Actinomycetospora
ASV 855	Bacteria	Bdellovibrionota	Oligoflexia	Oligoflexales	Oligoflexaceae	Oligoflexus
Fungi						
ASV 16	Fungi	Ascomycota	Dothideomycetes	Capnodiales		
ASV 18	Fungi	Basidiomycota	Tremellomycetes	Tremellales	Tremellaceae	Tremella
ASV 4	Fungi	Ascomycota				
ASV 56	Fungi	Ascomycota	Dothideomycetes	Dothideales	Aureobasidiaceae	Aureobasidium
ASV 65	Fungi	Ascomycota	Dothideomycetes	Capnodiales		
Combined						
ASV 56	Fungi	Ascomycota	Dothideomycetes	Dothideales	Aureobasidiaceae	Aureobasidium
ASV 65	Fungi	Ascomycota	Dothideomycetes	Capnodiales		
ASV 116	Bacteria	Verrucomicrobiota	Verrucomicrobiae	Chthoniobacterales	Chthoniobacteraceae	LD29
ASV 395	Bacteria	Bacteroidota	Bacteroidia	Chitinophagales	Chitinophagaceae	Edaphobaculum
ASV 187	Bacteria	Actinobacteriota	Actinobacteria	Kineosporiales	Kineosporiaceae	Kineococcus

DISCUSSION

In this study we used a multi-kingdom metabarcoding approach to investigate the microbiome (algae, bacteria, fungi) on the

bark of beech from sites with different forest management intensity in the Hainich-Dün region, Thuringia, Germany. We provide a first characterization of aboveground bark-associated microbial communities in beech forests, as well as an

Exploring the Beech Bark Microbiome



account of microbial inter-kingdom interactions. Additionally, by testing how community diversity and structure of the different organismal groups change in relation to land-use intensity and tree size, we identify potential drivers of community assembly and provide sampling recommendations for studying the bark microbiome at broader spatial scales.

Diversity of the Bark-Associated Beech Microbiome

Our results show that the bark of beech harbors highly diverse algal, bacterial and fungal communities.

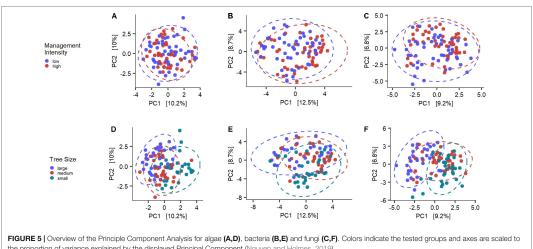
The modules and hub-taxa of the algal interaction networks are mainly represented by species of the families Trebouxiophyceae and Chlorellaceae, in particular by genera commonly found in subaerial environments and already detected in forest settings (Štifterová and Neustupa, 2015). Algae of the genus *Apatococcus*, a "flagship" taxon of above-ground ecosystems (Rindi, 2007), are abundant in the modules and interconnected members of the algal microbiome. Additionally, *Apatococcus* is a known photobiont of *Scoliciosporum* (Sanders and Masumoto, 2021), a very common fungal genus in our dataset. Another abundant alga is *Desmococcus* sp., which typically forms visible powdery, greenish layers on the bark of

trees in association with *Apatococcus* and other subaerial green algae (Rindi, 2007). Both are part of what is possibly the most tolerant subaerial algal community, being able to thrive even in urban areas (Barkman, 1958). Furthermore, our results confirm *Symbiochloris* as an important component of the dermo- and phyllosphere (Škaloud et al., 2016; Zhu et al., 2018), as well as other Trebouxiales, an order consisting of free-living as well as lichen-forming green algae (Sanders and Masumoto, 2021). One of the algal hub taxa comes from the genus *Trebouxia*, the most common lichen forming alga (Sanders and Masumoto, 2021).

Compared to the algae and fungi, the important taxa of the bacterial network modules are far more diverse. Similarly to Aschenbrenner et al. (2017), the bacterial community is dominated by the class Alphaproteobacteria, in particular Rhizobiales and Acetobacteriales. Among the Rhizobiales we detected *Methylocella* sp., a facultative methanotroph adapted to various nutrients (acetate, pyruvate, succinate, malate, and ethanol) (Dedysh and Dunfield, 2011), and 1174-901-12, previously described as an early colonizer of aerial environments (Romani et al., 2019) and a known member of the phyllosphere (Ares et al., 2021). In the case of Acetobacterales, *Acidiphilium* is among the taxa contributing the most to the modules. The genus consists of aerobic bacteria with photosynthetic pigments, with a pH range that matches well to the pH of beech bark

74

Exploring the Beech Bark Microbiome Dreyling et al.



the proportion of variance explained by the displayed Principal Component (Nauven and Holmes, 2019)

(~4.4 according to Asplund et al. (2015)) and that do not overlap in metabolic demands with Methylocella (Hiraishi and Imhoff, 2015). Another well represented class in the modules are the Abditibacteria, especially the genus Abditibacterium whose representatives are well adapted to low-nutrient conditions and have been reported on tree bark before (Tahon et al., 2018; Kobayashi and Aoyagi, 2019). Among the bacterial hub taxa we find genera from classes already reported for treeassociated microbiomes, such as Oligoflexus in bryophytes (Ma et al., 2017), Actinomycetospora and 1174-901-12 on tree bark and lichens (Yamamura et al., 2011; Ares et al., 2021), and Tundrisphaera, previously only isolated from lichen-dominated tundra soils (Kulichevskaya et al., 2017). One ASV belongs to Chitinophagaceae, a family associated with the degradation of fungal cell-walls (Carrión et al., 2019). As found for the algae, some of the most abundant genera are important components of the bacterial networks. Contrary to Aschenbrenner et al. (2017), we did not find major contributions from the genera Burkholderia and Pseudomonas, possibly indicating that these genera are specific to the bark of sycamore maple (Acer pseudoplatanus).

The investigation of the important fungal diversity was somewhat hindered by low taxonomic resolution with many ASVs that could not be assigned past phylum rank. This may result from a lack of resolution in public fungal databases combined with the presence of several unknown taxa in our dataset. Important members of the fungal networks in the beech bark community are the so-called black yeasts, e.g., Capronia and Aureobasidium, which are known to occur on tree bark and leaves (Untereiner and Malloch, 1999; Andrews et al., 2002), but also decaying wood (Cooke, 1959) and on other fungi or lichens as secondary saprobionts (Untereiner and Malloch, 1999). Other important network components belong to the genus Tremella, known mycoparasites (Zugmaier et al., 1994). Among the lichen-forming fungi, one of the most common fungi in our dataset belongs to the genus Scoliciosporum, a genus of crustose lichens that was already reported on beech bark (e.g., Dymytrova, 2011). The biggest contributors at the order rank are members of the Capnodiales (Dothideomycetes), whose species have been shown to associate with the lichen microbiome (Smith et al., 2020) and are abundant in the beech phyllosphere as well (Unterseher et al., 2016). Yet, more research into these orders is needed as they are taxonomically and ecologically highly diverse and include a large diversity of life forms, from lichenized, to mycoparasytic, epi-, ecto-, endophytic, as well as saprobiontic species. In contrast to Unterseher et al. (2016) we could not find a larger contribution of Helotiales, suggesting a specialization of this fungal order to the phyllosphere. The most abundant ASVs of two fungal modules, as well as one of the most abundant ASV overall, could not be assigned further than Ascomycota, highlighting that important components of the fungal bark microbiome remain undescribed. Sampling in different seasons may reveal an even higher—described and undescribed—fungal diversity on tree trunks (Beck et al., 2014). In conclusion, more research is needed in order to confirm the role of the bark habitat as a reservoir of novel fungal diversity. This could be done by combining genetics and culture-based approaches.

Biotic Interactions and Inter-Kingdom Synergies in the Bark Microbiome

The higher modularity scores of the fungal and especially algal networks may indicate higher specialization or niche differentiation in these groups (Augustyn et al., 2016). In contrast, bacteria are less clearly divided into ecological modules, which potentially indicates closer interactions between all taxa as there seems to be no split into specialized groups. Further analyses based on a broader dataset are needed to exclude that the observed patterns are an artifact of the overall higher diversity found in bacteria.

The results from the combined, inter-kingdom co-occurrence analysis indicate that algal and fungal specialists might be connected through a common set of bacteria. It is tempting to speculate that the interactions between Rhizobiales and Chlorellales (mostly represented by members of the genus Apatococcus) observed in the main ecological cluster in our dataset are of symbiotic nature, as Rhizobiales are well-known beneficial partners in plant-microbe interactions and common associates of lichens (Erlacher et al., 2015; Grube et al., 2015). Positive interactions among Sphingomonadales, Trebouxiales, and Capnodiales—all known occupants of bark substrates—characterize the second most important cluster. The bacterial genus Sphingomonas is very common in above-ground forests habitats (Vorholt, 2012), exhibiting facultative photosynthesis.

Finally, we identified the most highly connected taxa (hubs), i.e., taxa that are crucial for the stability of the ecological network (Banerjee et al., 2018). For bacteria, the hub taxa belong to the genera LD29 (Verrucomicrobiota), Edaphobaculum (Bacteroidetes) and Kineococcus (Actinobacteria). Little is known about their ecology, with LD29 particularly abundant in lichen thalli (Aschenbrenner et al., 2017), Edaphobaculum previously found in soils where it contributes to the creation of biofilms (Keuschnig et al., 2021), and Kineococcus isolated from soil samples as well as the rhizosphere (Normand and Benson, 2015). As for the fungi, both of the inter-kingdom hub taxa are also found as hub taxa in the fungal network. One of them belongs to the genus Aureobasidium, common on leaves of apple trees (Andrews et al., 2002) but also linked to the decay of bark (Cooke, 1959). The other could not be assigned below the order rank and is a member of the Capnodiales.

Bark Microbiome Responds to Tree Size, but Not to Intensity of Forest Management

The intensity of the forest management regime has virtually no effect on microbial community diversity and structure in our study area. This might be a result of a forest management plan that avoids clear cuts and carefully selects trees to harvest, which leads to a uniform forest structure in the study area (Schall et al., 2020). Based on a broader sampling including this and other two large forest areas in Germany, Boch et al. (2021) showed that an increase in forest management intensity is linked to reduced lichen species richness. A larger sampling effort covering a broader gradient of land-use intensity is therefore required to test whether the response of the bark-associated microbiome differs from that of the macroepiphytes.

We found significant differences in diversity and composition of the bark microbiome according to different tree-size classes. The lower microbial diversity found on larger (older) trees for algae and fungi is probably the result of environmental filtering on highly heterogeneous pioneer communities over time. This is particularly evident when comparing large and small trees, thus suggesting slow succession of these microbiomes toward final community composition. Our results from the spatial autocorrelation analysis underpin random assembly of the microbial bark community at the local (plot) level, with a high heterogeneity between trees. Lastly, a

finer-scale comparison of micro-habitats, i.e., different exposures, bark crevices/cracks/holes, would be of great interest for disentangling micro-scale interactions that our approach cannot reliably identify.

Conclusions and Sampling Recommendations

In this pioneering study we provide novel insights into the diversity, spatial context, and biotic interactions that characterize the beech bark microbiome in Central European forests. We showed that there are predictable community shifts depending on tree age. These represent the first steps toward proposing a framework of community assembly on forest tree bark, a ubiquitous, ecologically relevant, yet overlooked component of terrestrial habitats.

Taken together, our results show that a single tree does not adequately characterize the bark-associated microbial community at plot level. To capture most of the microbial diversity, considering the spatial randomness shown by the spatial autocorrelation analysis, we recommend sampling using a spatially random approach with a balanced representation of the main tree-size classes present in the plot. Samples taken from multiple trees can then be combined into a composite sample. The use of composite samples ensures relatively low costs for obtaining adequate sequencing depths while maximizing the spatial range of the study and the number of plots, allowing for easy upscaling to large areas and/or environmental gradients. This data can then be used to assess the effects of factors, e.g., related to forest structure, such as stand density and canopy openness.

DATA AVAILABILITY STATEMENT

The raw sequences are deposited in the NCBI SRA repository, accession numbers SRR18461106, SRR18461107, and SRR18461108. All scripts and additional data necessary to replicate the analysis are available at https://github.com/LukDrey/beech_micro_communities. The data on the Forest Management Index are available at https://www.bexis.uni-jena.de/underAccessionnumber16466.

AUTHOR CONTRIBUTIONS

FD and IS secured the funding. FD and LD devised the sampling and analytical methods, conducted the fieldwork and sampling, and wrote the manuscript with contributions from IS. LD performed the laboratory work and analysis. All authors contributed to the article and approved the submitted version.

FUNDING

The work has been funded through the DFG Priority Program 1374 "Biodiversity-Exploratories" (SCHM 1711/8-1 and GR 5437/4-1). Field work permits were issued by the responsible state environmental offices of Thüringen.

ACKNOWLEDGMENTS

We thank the managers of the Hainich-Dün Exploratory, Anna K. Franke, and all former managers for their work in maintaining the plot and project infrastructure; Victoria Grießmeier for giving support through the central office, Andreas Ostrowski for managing the central data base, and Markus Fischer, Eduard Linsenmair, Dominik Hessenmöller, Daniel Prati, Ingo Schöning, François Buscot, Ernst-Detlef Schulze, Wolfgang W. Weisser and the late Elisabeth Kalko for their role in setting up the Biodiversity Exploratories project. We thank the administration

of the Hainich national park, as well as all landowners for the excellent collaboration. Additionally, we thank Ulrich Pruschitzki for his valuable help in the forest and Jürgen Otte for helpful comments on laboratory procedures and technical assistance.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/ffgc.2022. 858382/full#supplementary-material

REFERENCES

- Abarenkov, K., Zirk, A., Piirmann, T., Pöhönen, R., Ivanov, F., Nilsson, R. H., et al. (2020). Unite General Fasta Release For Fungi. doi: 10.15156/BIO/78 6368
- Aguirre-von-Wobeser, E., Alonso-Sánchez, A., Méndez-Bravo, A., Villanueva Espino, L. A., and Reverchon, F. (2021). Barks from avocado trees of different geographic locations have consistent microbial communities. Arch. Microbiol. 203, 4593–4607. doi: 10.1007/s00203-021-02449-6
- Anderson, M. J., and Walsh, D. C. I. (2013). PERMANOVA, ANOSIM, and the Mantel test in the face of heterogeneous dispersions: what null hypothesis are you testing? *Ecol. Monogr.* 83, 557–574. doi: 10.1890/12-2010.1
- Andre, H. M. (1985). Associations between corticolous microarthropod communities and epiphytic cover on bark. *Ecography* 8, 113–119. doi: 10.1111/j.1600-0587.1985.tb01161.x
- Andrews, J. H., Spear, R. N., and Nordheim, E. V. (2002). Population biology of Aureobasidium pullulans on apple leaf surfaces. Can. J. Microbiol. 48, 500–513. doi: 10.1139/w02-044
- Ares, A., Pereira, J., Garcia, E., Costa, J., and Tiago, I. (2021). The leaf bacterial microbiota of female and male kiwifruit plants in distinct seasons: assessing the impact of *Pseudomonas syringae* pv. actinidiae. *Phytobiomes J.* 5, 275–287. doi: 10.1094/PBIOMES-09-20-0070-R
- Arrigoni, E., Albanese, D., Longa, C. M. O., Angeli, D., Donati, C., Ioriatti, C., et al. (2020). Tissue age, orchard location and disease management influence the composition of fungal and bacterial communities present on the bark of apple trees. Environ. Microbiol. 22, 2080–2093. doi: 10.1111/1462-2920. 14963
- Arrigoni, E., Antonielli, L., Pindo, M., Pertot, I., and Perazzolli, M. (2018). Tissue age and plant genotype affect the microbiota of apple and pear bark. *Microbiol. Res.* 211, 57–68. doi: 10.1016/j.micres.2018.04.002
- Aschenbrenner, I. A., Cernava, T., Erlacher, A., Berg, G., and Grube, M. (2017). Differential sharing and distinct co-occurrence networks among spatially close bacterial microbiota of bark, mosses and lichens. Mol. Ecol. 26, 2826–2838. doi: 10.1111/mec.14070
- Asplund, J., Ohlson, M., and Gauslaa, Y. (2015). Tree species shape the elemental composition in the lichen *Hypogymnia physodes* transplanted to pairs of spruce and beech trunks. *Fungal Ecol.* 16, 1–5. doi: 10.1016/J.FUNECO.2015.03.006
- Aude, E., and Poulsen, R. S. (2000). Influence of management on the species composition of epiphytic cryptogams in Danish Fagus forests. Appl. Veg. Sci. 3, 81–88. doi: 10.2307/1478921
- Auguie, B. (2017). gridExtra: Miscellaneous Functions for "Grid" Graphics. Available online at: https://cran.r-project.org/package=gridExtra (accesed January 19, 2021)
- Augustyn, W. J., Anderson, B., and Ellis, A. G. (2016). Experimental evidence for fundamental, and not realized, niche partitioning in a plant-herbivore community interaction network. J. Anim. Ecol. 85, 994–1003. doi: 10.1111/ 1365-2656.12536
- Baldrian, P. (2017). Forest microbiome: diversity, complexity and dynamics. FEMS Microbiol. Rev. 40, 109–130. doi: 10.1093/femsre/fuw040
- Banerjee, S., Schlaeppi, K., and van der Heijden, M. G. A. (2018). Keystone taxa as drivers of microbiome structure and functioning. Nat. Rev. Microbiol. 16, 567–576. doi: 10.1038/s41579-018-0024-1

- Barkman, J. J. (1958). Phytosociology And Ecology Of Cryptogamic Epiphytes, Including A Taxonomic Survey And Description Of Their Vegetation Units In Europe. Assen: Van Gorcum.
- Bastian, M., Heymann, S., and Jacomy, M. (2009). Gephi: An Open Source Software for Exploring and Manipulating Networks. Available online at: https://gephi.org/ (accessed January 19, 2022).
- Beck, A., Peršoh, D., and Rambold, G. (2014). First evidence for seasonal fluctuations in lichen- and bark-colonising fungal communities. Folia Microbiol. (Praha) 59, 155–157. doi: 10.1007/s12223-013-0278-v
- Berg, G., Rybakova, D., Fischer, D., Cernava, T., Verges, M.-C. C., Charles, T., et al. (2020). Microbiome definition re-visited: old concepts and new challenges. Microbiome 8:103. doi: 10.1186/s40168-020-00875-0
- Blondel, V. D., Guillaume, J.-L., Lambiotte, R., and Lefebvre, E. (2008). Fast unfolding of communities in large networks. J. Stat. Mech. Theory Exp. 2008:10008. doi: 10.1088/1742-5468/2008/10/p10008
- Boch, S., Saiz, H., Allan, E., Schall, P., Prati, D., Schulze, E.-D., et al. (2021). Direct and indirect effects of management intensity and environmental factors on the functional diversity of lichens in central european forests. *Microorganisms* 9:463. doi: 10.3390/microorganisms9020463
- Bolger, A. M., Lohse, M., and Usadel, B. (2014). Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* 30, 2114–2120. doi: 10. 1093/bioinformatics/btu170
- Brandes, U. (2001). A faster algorithm for betweenness centrality. *J. Math. Sociol.* 25, 163–177. doi: 10.1080/0022250X.2001.9990249
- Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., and Holmes, S. P. (2016). DADA2: high-resolution sample inference from *Illumina amplicon* data. Nat. Methods 13, 581–583. doi: 10.1038/nmeth.3869
- Camacho-Sanchez, M., Burraco, P., Gomez-Mestre, I., and Leonard, J. A. (2013). Preservation of RNA and DNA from mammal samples under field conditions. Mol. Ecol. Resour. 13, 663–673. doi: 10.1111/1755-0998.12108
- Carrión, V. J., Perez-Jaramillo, J., Cordovez, V., Tracanna, V., de Hollander, M., Ruiz-Buck, D., et al. (2019). Pathogen-induced activation of disease-suppressive functions in the endophytic root microbiome. *Science* 366, 606–612. doi: 10. 1126/science.aaw9285
- Cheng, T., Xu, C., Lei, L., Li, C., Zhang, Y., and Zhou, S. (2016). Barcoding the kingdom Plantae: new PCR primers for ITS regions of plants with improved universality and specificity. Mol. Ecol. Resour. 16, 138–149. doi: 10.1111/1755-0998.12438
- Clark, K., Karsch-Mizrachi, I., Lipman, D. J., Ostell, J., and Sayers, E. W. (2016). GenBank. *Nucleic Acids Res.* 44, D67–D72. doi: 10.1093/nar/gkv1276
- Cooke, W. B. (1959). An ecological life history of Aureobasidium pullulans (de Bary) Arnaud. Mycopathol. Mycol. Appl. 12, 1–45. doi: 10.1007/BF02118435
- Crowther, T. W., Glick, H. B., Covey, K. R., Bettigole, C., Maynard, D. S., Thomas, S. M., et al. (2015). Mapping tree density at a global scale. *Nature* 525, 201–205. doi: 10.1038/nature14967
- Csardi, G., and Nepusz, T. (2006). The Igraph Software Package For Complex Network Research. Interjournal Complex Sy, 1695. Available online at: https://igraph.org (accesed January 19, 2022).
- Davis, N. M., Proctor, D. M., Holmes, S. P., Relman, D. A., and Callahan, B. J. (2018). Simple statistical identification and removal of contaminant sequences in marker-gene and metagenomics data. *Microbiome* 6:226. doi: 10.1186/ s40168-018-0605-2

- Dedysh, S. N., and Dunfield, P. F. (2011). "Facultative and obligate methanotrophs," in Methods in Methane Metabolism, eds A. C. Rosenzweig and S. W. Ragsdale (Cambridge, MA: Elsevier/Academic Press), 31–44. doi: 10.1016/B978-0-12-386905.0 00003-6
- Dymytrova, L. V. (2011). Notes on the genus Scoliciosporum (Lecanorales, Ascomycota) in Ukraine. Polish Bot. J. 56, 61–75.
- Erlacher, A., Cernava, T., Cardinale, M., Soh, J., Sensen, C. W., Grube, M., et al. (2015). Rhizobiales as functional and endosymbiontic members in the lichen symbiosis of *Lobaria pulmonaria L. Front. Microbiol.* 6:53. doi: 10.3389/fmicb. 2015.00053
- Fernandes, A. D., Macklaim, J. M., Linn, T. G., Reid, G., and Gloor, G. B. (2013). ANOVA-like differential expression (ALDEx) analysis for mixed population RNA-Seq. PLoS One 8:e67019. doi: 10.1371/journal.pone.0067019
- Fernandes, A. D., Reid, J. N., Macklaim, J. M., McMurrough, T. A., Edgell, D. R., and Gloor, G. B. (2014). Unifying the analysis of high-throughput sequencing datasets: characterizing RNA-seq, 16S rRNA gene sequencing and selective growth experiments by compositional data analysis. *Microbiome* 2:15. doi: 10. 1186/2049-2618-2-15
- Fischer, M., Bossdorf, O., Gockel, S., Hänsel, F., Hemp, A., Hessenmöller, D., et al. (2010). Implementing large-scale and long-term functional biodiversity research: the biodiversity exploratories. *Basic Appl. Ecol.* 11, 473–485. doi: 10. 1016/j.baae.2010.07.009
- Frøslev, T. G., Kjøller, R., Bruun, H. H., Ejrnæs, R., Brunbjerg, A. K., Pietroni, C., et al. (2017). Algorithm for post-clustering curation of DNA amplicon data yields reliable biodiversity estimates. *Nat. Commun.* 8:1188. doi: 10.1038/s41467-017-01312-x
- Fruchterman, T. M. J., and Reingold, E. M. (1991). Graph drawing by forcedirected placement. Softw. Pract. Exp. 21, 1129–1164. doi: 10.1002/spe. 4380211102
- Gittleman, J. L., and Kot, M. (1990). Adaptation: statistics and a null model for estimating phylogenetic effects. Syst. Zool. 39:227. doi: 10.2307/2992183
- Gloor, G. B., Macklaim, J. M., and Fernandes, A. D. (2016). Displaying variation in large datasets: plotting a visual summary of effect sizes. J. Comput. Graph. Stat. 25, 971–979. doi: 10.1080/10618600.2015.1131161
- Gloor, G. B., Macklaim, J. M., Pawlowsky-Glahn, V., and Egozcue, J. J. (2017). Microbiome datasets are compositional: and this is not optional. Front. Microbiol. 8:2224. doi: 10.3389/fmicb.2017.02224
- Grube, M., Cernava, T., Soh, J., Fuchs, S., Aschenbrenner, I., Lassek, C., et al. (2015).
 Exploring functional contexts of symbiotic sustain within lichen-associated bacteria by comparative omics. ISME J. 9, 412–424. doi: 10.1038/ismej.201
- Hagge, J., Bässler, C., Gruppe, A., Hoppe, B., Kellner, H., Krah, F.-S., et al. (2019). Bark coverage shifts assembly processes of microbial decomposer communities in dead wood. Proc. R. Soc. B Biol. Sci. 286:20191744. doi: 10.1098/rspb.2019.
- Herlemann, D. P., Labrenz, M., Jürgens, K., Bertilsson, S., Waniek, J. J., and Andersson, A. F. (2011). Transitions in bacterial communities along the 2000 km salinity gradient of the Baltic Sea. ISME J. 5, 1571–1579. doi: 10.1038/ismej. 2011.41
- Hiraishi, A., and Imhoff, J. F. (2015). "Acidiphilium," in Bergey's Manual of Systematics of Archaea and Bacteria, eds M. E. Trujillo, S. Dedysh, P. DeVos, B. Hedlund, P. Kämpfer, F. A. Rainey, et al. (Hoboken, NJ: Wiley), 1–14. doi:10.1002/9781118960608.gbm00877
- Ihrmark, K., Bödeker, I. T. M., Cruz-Martinez, K., Friberg, H., Kubartova, A., Schenck, J., et al. (2012). New primers to amplify the fungal ITS2 region - evaluation by 454-sequencing of artificial and natural communities. FEMS Microbiol. Ecol. 82, 666–677. doi: 10.1111/j.1574-6941.2012.01437.x
- Kahl, T., and Bauhus, J. (2014). An index of forest management intensity based on assessment of harvested tree volume, tree species composition and dead wood origin. Nat. Conserv. 7, 15–27. doi: 10.3897/natureconservation.7.7281
- Keuschnig, C., Martins, J. M. F., Navel, A., Simonet, P., and Larose, C. (2021). Micro-aggregation of a pristine grassland soil selects for bacterial and fungal communities and changes in nitrogen cycling potentials. bioRxiv [Preprint] bioRxiv: 2021.10.13.464334, doi: 10.1101/2021.10.13.464334
- Kobayashi, K., and Aoyagi, H. (2019). Microbial community structure analysis in Acer palmatum bark and isolation of novel bacteria IAD-21 of the candidate division FBP. PeerJ 7:e7876. doi: 10.7717/peerj.7876

- Kulichevskaya, I. S., Ivanova, A. A., Detkova, E. N., Rijpstra, W. I. C., Sinninghe Damsté, J. S., and Dedysh, S. N. (2017). *Tundrisphaera lichenicola* gen. nov., sp. nov., a psychrotolerant representative of the family *Isosphaeraceae* from lichen-dominated tundra soils. *Int. J. Syst. Evol. Microbiol.* 67, 3583–3589. doi: 10.1099/jisem.0.002172
- Kurtz, Z. D., Müller, C. L., Miraldi, E. R., Littman, D. R., Blaser, M. J., and Bonneau, R. A. (2015). Sparse and compositionally robust inference of microbial ecological networks. *PLoS Comput. Biol.* 11:e1004226. doi: 10.1371/journal.pcbi.1004226
- Lahti, L., and Shetty, S. (2017). Microbiome Tools For Microbiome Analysis In R. Available online at: https://github.com/microbiome/microbiome (accesed January 19, 2022).
- Lambais, M. R., Lucheta, A. R., and Crowley, D. E. (2014). Bacterial community assemblages associated with the phyllosphere, dermosphere, and rhizosphere of tree species of the atlantic forest are host taxon dependent. *Microb. Ecol.* 68, 567–574. doi: 10.1007/s00248-014-0433-2
- Lamit, L. J., Lau, M. K., Næsborg, R. R., Wojtowicz, T., Whitham, T. G., and Gehring, C. A. (2015). Genotype variation in bark texture drives lichen community assembly across multiple environments. *Ecology* 96, 960–971. doi: 10.1890/14-1007.1
- Leff, J. W., Del Tredici, P., Friedman, W. E., and Fierer, N. (2015). Spatial structuring of bacterial communities within individual *Ginkgo biloba* trees. *Environ. Microbiol.* 17, 2352–2361. doi: 10.1111/1462-2920.12695
- Lindow, S. E., and Brandl, M. T. (2003). Microbiology of the phyllosphere. Appl. Environ. Microbiol. 69, 1875–1883. doi: 10.1128/AEM.69.4.1875-1883.2003
 Łubek, A., Kukwa, M., Jaroszewicz, B., and Czortek, P. (2020). Identifying
- Łubek, A., Kukwa, M., Jaroszewicz, B., and Czortek, P. (2020). Identifying mechanisms shaping lichen functional diversity in a primeval forest. For. Ecol. Manage. 475:118434. doi: 10.1016/j.foreco.2020.118434
- Ma, J., Tang, J. Y., Wang, S., Chen, Z. L., Li, X. D., and Li, Y. H. (2017). Illumina sequencing of bacterial 165 rDNA and 165 rRNA reveals seasonal and speciesspecific variation in bacterial communities in four moss species. Appl. Microbiol. Biotechnol. 101, 6739–6753. doi: 10.1007/s00253-017-8391-5
- Martin, M. (2011). Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet J. 17:10. doi: 10.14806/ej.17.1.200
- Martins, G., Lauga, B., Miot-Sertier, C., Mercier, A., Lonvaud, A., Soulas, M.-L., et al. (2013). Characterization of epiphytic bacterial communities from grapes, leaves, bark and soil of grapevine plants grown, and their relations. PLoS One 8:e73013. doi: 10.1371/journal.pone.0073013
- McMurdie, P. J., and Holmes, S. (2013). phyloseq: an r package for reproducible interactive analysis and graphics of microbiome census data. *PLoS One* 8:e61217. doi: 10.1371/journal.pone.0061217
- McMurdie, P. J., and Holmes, S. (2014). Waste Not, want not: why rarefying microbiome data is inadmissible. PLoS Comput. Biol. 10:e1003531. doi: 10.1371/ journal.pcbi.1003531
- Meinshausen, N., and Bühlmann, P. (2006). High-dimensional graphs and variable selection with the Lasso. *Ann. Stat.* 34, 1436–1462. doi: 10.1214/009053606000000281
- Menke, S., Gillingham, M. A. F., Wilhelm, K., and Sommer, S. (2017). Home-made cost effective preservation buffer is a better alternative to commercial preservation methods for microbiome research. Front. Microbiol. 8:102. doi: 10.3389/fmich.2017.00102
- Müller, C. L., Bonneau, R., and Kurtz, Z. (2016). Generalized Stability Approach for Regularized Graphical Models. Available online at: http://arxiv.org/abs/1605. 07072 (accessed January 19, 2022).
- Muyzer, G., de Waal, E. C., and Uitterlinden, A. G. (1993). Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. Appl. Environ. Microbiol. 59, 695–700. doi: 10.1128/aem.59.3.695-700.1993
- Nascimbene, J., Thor, G., and Nimis, P. L. (2013). Effects of forest management on epiphytic lichens in temperate deciduous forests of Europe a review. For. Ecol. Manage. 298, 27–38. doi: 10.1016/j.foreco.2013.03.008
- Newman, M. E. J. (2006). Modularity and community structure in networks. *Proc Natl. Acad. Sci. U.S.A.* 103, 8577–8582. doi: 10.1073/pnas.0601602103
- Nguyen, L. H., and Holmes, S. (2019). Ten quick tips for effective dimensionality reduction. *PLoS Comput. Biol.* 15:e1006907. doi: 10.1371/journal.pcbi.1006907 Normand, P., and Benson, D. R. (2015). "Kineococcus," in *Bergey's Manual of*
- Normand, P., and Benson, D. R. (2015). "Kineococcus," in Bergey's Manual of Systematics of Archaea and Bacteria, eds M. E. Trujillo, S. Dedysh, P. DeVos,

- B. Hedlund, P. Kämpfer, F. A. Rainey, et al. (Hoboken, NJ: Wiley), 1–5. doi: 10.1002/9781118960608.gbm00053
- Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., et al. (2020). *vegan: Community Ecology Package*. Available online at: https://cran.r-project.org/package=vegan (accessed lanuary 19, 2022).
- //cran.r-project.org/package=vegan (accesed January 19, 2022).

 Paradis, E., and Schliep, K. (2019). ape 5.0: an environment for modern phylogenetics and evolutionary analyses in R. *Bioinformatics* 35, 526–528. doi: 10.1093/bioinformatics/btv633
- Petrolli, R., Augusto Vieira, C., Jakalski, M., Bocayuva, M. F., Vallé, C., Cruz, E. D. S., et al. (2021). A fine-scale spatial analysis of fungal communities on tropical tree bark unveils the epiphytic rhizosphere in orchids. New Phytol. 231, 2002–2014. doi: 10.1111/nph.17459
- Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., et al. (2012). The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Res.* 41, D590–D596. doi: 10.1093/nar/gks 1219
- R Core Team (2021). R: A Language and Environment for Statistical Computing.

 Available online at: https://www.r-project.org/ (accesed January 19, 2022).
- Rindi, F. (2007). "Diversity, distribution and ecology of green algae and cyanobacteria in urban habitats," in Algae and Cyanobacteria in Extreme Environments, ed. J. Seckbach (Dordrecht: Springer Netherlands), 619–638. doi: 10.1007/978-1-4020-6112-7_34
- Romani, M., Carrion, C., Fernandez, F., Intertaglia, L., Pecqueur, D., Lebaron, P., et al. (2019). High bacterial diversity in pioneer biofilms colonizing ceramic roof tiles. *Int. Biodeterior. Biodegradation* 144:104745. doi: 10.1016/j.ibiod.2019. 104745
- RStudio Team (2021). RStudio: Integrated Development Environment for R. Available online at: http://www.rstudio.com/ (accesed January 19, 2022). Sanders, W. B., and Masumoto, H. (2021). Lichen algae: the photosynthetic
- Sanders, W. B., and Masumoto, H. (2021). Lichen algae: the photosynthetic partners in lichen symbioses. *Lichenology* 53, 347–393.
- Schall, P., Heinrichs, S., Ammer, C., Ayasse, M., Boch, S., Buscot, F., et al. (2020). Can multi-taxa diversity in European beech forest landscapes be increased by combining different management systems? *J. Appl. Ecol.* 57, 1363–1375. doi: 10.1111/1365-2664.13635
- Schnell, I. B., Bohmann, K., and Gilbert, M. T. P. (2015). Tag jumps illuminated reducing sequence-to-sample misidentifications in metabarcoding studies. *Mol. Ecol. Resour.* 15, 1289–1303. doi: 10.1111/1755-0998.12402
- Schoch, C. L., Robbertse, B., Robert, V., Vu, D., Cardinali, G., Irinyi, L., et al. (2014). Finding needles in haystacks: linking scientific names, reference specimens and molecular data for Fungi. *Database* 2014:bau061. doi: 10.1093/database/bau061
- Shannon, C. E. (1948). A mathematical theory of communication. *Bell Syst. Tech. J.* 27, 379–423. doi: 10.1002/j.1538-7305.1948.tb01338.x
- Škaloud, P., Friedl, T., Hallmann, C., Beck, A., and Dal Grande, F. (2016). Taxonomic revision and species delimitation of coccoid green algae currently assigned to the genus *Dictyochloropsis* (Trebouxiophyceae, Chlorophyta). *J. Phycol.* 52, 599–617. doi: 10.1111/jpy.12422
- Smith, H. B., Dal Grande, F., Muggia, L., Keuler, R., Divakar, P. K., Grewe, F., et al. (2020). Metagenomic data reveal diverse fungal and algal communities associated with the lichen symbiosis. Symbiosis 82, 133–147. doi: 10.1007/s13199-020-00699-4
- Štifterová, A., and Neustupa, J. (2015). Community structure of corticolous microalgae within a single forest stand: evaluating the effects of bark surface pH and tree species. Fottea 15, 113–122. doi: 10.5507/fot.2015.013
- Strid, Y., Schroeder, M., Lindahl, B., Ihrmark, K., and Stenlid, J. (2014). Bark beetles have a decisive impact on fungal communities in Norway spruce stem sections. *Fungal Ecol.* 7, 47–58. doi: 10.1016/j.funeco.2013.09.003
- Tahon, G., Tytgat, B., Lebbe, L., Carlier, A., and Willems, A. (2018). Abditibacterium utsteinense sp. nov., the first cultivated member of candidate phylum FBP,

- isolated from ice-free Antarctic soil samples. Syst. Appl. Microbiol. 41, 279–290. doi: 10.1016/j.syapm.2018.01.009
- Teunisse, G. M. (2017). Fantaxtic Fantaxtic Plots For Phyloseq Objects!. Available online at: https://github.com/gmteunisse/Fantaxtic (accesed January 19, 2022).
- Untereiner, W. A., and Malloch, D. (1999). Patterns of substrate utilization in species of Capronia and allied black yeasts: ecological and taxonomic implications. Mycologia 91, 417–427. doi: 10.1080/00275514.1999.12061035
- Unterseher, M., Siddique, A. B., Brachmann, A., and Peršoh, D. (2016). Diversity and composition of the leaf mycobiome of beech (Fagus sylvatica) are affected by local habitat conditions and leaf biochemistry. PLoS One 11:e0152878. doi: 10.1371/journal.pone.0152878
- Větrovský, T., Baldrian, P., and Morais, D. (2018). SEED 2: A user-friendly platform for amplicon high-throughput sequencing data analyses. Bioinformatics 34, 2292–2294. doi: 10.1093/bioinformatics/btv071
- Vieira, Ś., Sikorski, J., Dietz, S., Herz, K., Schrumpf, M., Bruelheide, H., et al. (2020). Drivers of the composition of active rhizosphere bacterial communities in temperate grasslands. *ISME J.* 14, 463–475. doi: 10.1038/s41396-019-0543-4
- Vitulo, N., Lemos, W. J. F., Calgaro, M., Confalone, M., Felis, G. E., Zapparoli, G., et al. (2019). Bark and grape microbiome of vitis vinifera: influence of geographic patterns and agronomic management on bacterial diversity. Front. Microbiol. 9:3203. doi: 10.3389/fmicb.2018.03203
- Vorholt, J. A. (2012). Microbial life in the phyllosphere. Nat. Rev. Microbiol. 10, 828–840. doi: 10.1038/nrmicro2910
- White, T. J., Bruns, T., Lee, S., and Taylor, J. (1990). "Amplification and direct sequencing of fungal ribosomal rna genes for phylogenetics," in PCR Protocols, eds M. Innis, D. H. Gelfland, J. J. Sninsky, and T. J. White (San Diego, CA: Academic Press), 315–322. doi: 10.1016/b978-0-12-372180-8.50042-1
- Whitmore, T. C. (1963). Studies in systematic bark morphology. IV. The bark of beech, oak and sweet chestnut. New Phytol. 62, 161–169. doi: 10.1111/j.1469-8137.1963.tb06323.x
- Wickham, H. (2016). ggplot2: Elegant Graphics for Data Analysis. New York, NY: Springer-Verlag.
- Yamamura, H., Ashizawa, H., Nakagawa, Y., Hamada, M., Ishida, Y., Otoguro, M., et al. (2011). Actinomycetospora iriomotensis sp. nov., a novel actinomycete isolated from a lichen sample. J. Antibiot. (Tokyo) 64, 289–292. doi: 10.1038/ja. 2011.15
- Zhu, H., Li, S., Hu, Z., and Liu, G. (2018). Molecular characterization of eukaryotic algal communities in the tropical phyllosphere based on real-time sequencing of the 18s rDNA gene. *BMC Plant Biol.* 18:365. doi: 10.1186/s12870-018-1588-7 Zugmaier, W., Bauer, R., and Oberwinkler, F. (1994). Mycoparasitism of some *Tremella* species. *Mycologia* 86, 49–56. doi: 10.1080/00275514.1994.12026373

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.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Copyright © 2022 Dreyling, Schmitt and Dal Grande. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

Supplementary material to

Dreyling, L., Schmitt, I. & Dal Grande, F. (2022): Tree size drives diversity and community structure of microbial communities on the bark of beech (*Fagus sylvatica*). *Frontiers in Forests and Global Change* 5:858382. doi: 10.3389/ffgc.2022.858382

Table S1: Taxonomy table and ITS2 and 16S sequences of all identified ASVs.¹

Table S2: Results of the differential abundance analysis for tests between management categories and tree sizes.

¹ This supplementary can be found online accompanying the published paper at https://doi.org/10.3389/ffgc.2022.858382.

Table S2

ASV	Kingdom	Phylum	Class	Order	Family	Genus	P Value
	nent Intensity	•					
Fungi ASV 47	Fungi	Ascomycota	Eurotiomycetes	Chaetothyriales	Herpotrichiellaceae	Veronaea	0.0141
ASV 56	Fungi	Ascomycota	Dothideomycetes	Dothideales	Aureobasidiaceae	Aureobasidium	0.0192
ASV 161	Fungi	Ascomycota	Eurotiomycetes	Chaetothyriales	Trichomeriaceae	Knufia	0.0417
ASV 16	Fungi	Ascomycota	Dothideomycetes	Capnodiales			0.0473
Large – N Algae	Aedium Trees	3					
ASV 32	Eukaryota	Streptophyta	Klebsormidiophyceae	Klebsormidiales	Klebsormidiaceae	Interfilum	0.0003
ASV 10	Eukaryota	Chlorophyta	Trebouxiophyceae	Chlorellales	Chlorellaceae	Apatococcus	0.0003
ASV 7	Eukaryota	Chlorophyta	Trebouxiophyceae	Trebouxiales	Trebouxiaceae	Trebouxia	0.0004
ASV 8	Eukaryota	Chlorophyta	Trebouxiophyceae	Chlorellales	Chlorellaceae	Apatococcus	0.0008
ASV 12	Eukaryota	Chlorophyta	Trebouxiophyceae	Trebouxiales	Trebouxiaceae	Trebouxia	0.0099
ASV 29	Eukaryota	Chlorophyta	Trebouxiophyceae	Trebouxiales	Trebouxiaceae	Symbiochloris	0.0213
ASV 27	Eukaryota	Chlorophyta	Trebouxiophyceae	Trebouxiales	Trebouxiaceae	Dictyochloropsis	0.0243
ASV 21	Eukaryota	Chlorophyta	Trebouxiophyceae	Trebouxiales	Trebouxiaceae	Dictyochloropsis	0.0248
ASV 14	Eukaryota	Chlorophyta	Trebouxiophyceae	Trebouxiales	Trebouxiaceae	Trebouxia	0.0449
Bacteria							
ASV 18	Bacteria	Bacteroidota	Bacteroidia	Sphingobacteriales	Sphingobacteriaceae	Pedobacter	0.0310
ASV 85	Bacteria	Bacteroidota	Bacteroidia	Cytophagales	Hymenobacteraceae	Hymenobacter	0.0337
ASV 23	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Beijerinckiaceae	1174-901-12	0.0359
ASV 258	Bacteria	Verrucomicrobiota	Verrucomicrobiae	Chthoniobacterales	Chthoniobacteraceae	LD29	0.0412

Dreyling et al. (2022), Frontiers in Forests and Global Change

Fungi							
ASV 4	Fungi	Ascomycota					0.0153
ASV 21	Fungi	Ascomycota					0.0159
ASV 18	Fungi	Basidiomycota	Tremellomycetes	Tremellales	Tremellaceae	Tremella	0.0206
ASV 32	Fungi	Ascomycota	Dothideomycetes	Capnodiales	Mycosphaerellaceae		0.0214
ASV 33	Fungi	Ascomycota	Dothideomycetes	Capnodiales	Mycosphaerellaceae	Mycosphaerella	0.0269
ASV 226	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Didymosphaeriaceae	Pseudopithomyces	0.0282
ASV 63	Fungi	Ascomycota	Sordariomycetes	Hypocreales			0.0308
ASV 146	Fungi	Ascomycota	Eurotiomycetes	Chaetothyriales	Herpotrichiellaceae		0.0338
ASV 96	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Didymellaceae		0.0407
ASV 28	Fungi	Ascomycota	Dothideomycetes				0.0485
Large – Sn Algae	nall Trees						
ASV 10	Eukaryota	Chlorophyta	Trebouxiophyceae	Chlorellales	Chlorellaceae	Apatococcus	0.0000
ASV 29	Eukaryota	Chlorophyta	Trebouxiophyceae	Trebouxiales	Trebouxiaceae	Symbiochloris	0.0000
ASV 32	Eukaryota	Streptophyta	Klebsormidiophyceae	Klebsormidiales	Klebsormidiaceae	Interfilum	0.0000
ASV 12	Eukaryota	Chlorophyta	Trebouxiophyceae	Trebouxiales	Trebouxiaceae	Trebouxia	0.0000
ASV 5	Eukaryota	Chlorophyta	Trebouxiophyceae	Trebouxiales	Trebouxiaceae	Trebouxia	0.0000
ASV 7	Eukaryota	Chlorophyta	Trebouxiophyceae	Trebouxiales	Trebouxiaceae	Trebouxia	0.0000
ASV 43	Eukaryota	Chlorophyta	Trebouxiophyceae	Trebouxiales	Trebouxiaceae	Symbiochloris	0.0000
ASV 21	Eukaryota	Chlorophyta	Trebouxiophyceae	Trebouxiales	Trebouxiaceae	Dictyochloropsis	0.0000
ASV 27	Eukaryota	Chlorophyta	Trebouxiophyceae	Trebouxiales	Trebouxiaceae	Dictyochloropsis	0.0000
ASV 8	Eukaryota	Chlorophyta	Trebouxiophyceae	Chlorellales	Chlorellaceae	Apatococcus	0.0001

ASV 2	Eukaryota	Chlorophyta	Trebouxiophyceae	Chlorellales	Chlorellaceae	Apatococcus	0.0038
ASV 107	Eukaryota	Chlorophyta	Chlorophyceae	Sphaeropleales	Radiococcaceae	Gloeocystis	0.0059
ASV 42	Eukaryota	Chlorophyta	Trebouxiophyceae	Trebouxiales	Trebouxiaceae	Dictyochloropsis	0.0078
ASV 60	Eukaryota	Chlorophyta	Trebouxiophyceae	Trebouxiales	Trebouxiaceae	Dictyochloropsis	0.0093
ASV 72	Eukaryota	Chlorophyta	Trebouxiophyceae	Trebouxiales	Trebouxiaceae	Symbiochloris	0.0111
ASV 48	Eukaryota	Chlorophyta	Trebouxiophyceae	Trebouxiales	Trebouxiaceae	Trebouxia	0.0129
ASV 71	Eukaryota	Chlorophyta	Trebouxiophyceae	Trebouxiales	Trebouxiaceae	Symbiochloris	0.0260
ASV 16	Eukaryota	Chlorophyta	Trebouxiophyceae	Trebouxiales	Trebouxiaceae	Asterochloris	0.0327
ASV 1	Eukaryota	Chlorophyta	Trebouxiophyceae	Trebouxiales	Trebouxiaceae	Symbiochloris	0.0362
Bacteria							
ASV 56	Bacteria	Bacteroidota	Bacteroidia	Sphingobacteriales	Sphingobacteriaceae	Mucilaginibacter	0.0000
ASV 96	Bacteria	Bacteroidota	Bacteroidia	Cytophagales	Hymenobacteraceae	Hymenobacter	0.0000
ASV 48	Bacteria	Actinobacteriota	Actinobacteria	Pseudonocardiales	Pseudonocardiaceae	Actinomycetospora	0.0003
ASV 147	Bacteria	Proteobacteria	Alphaproteobacteria	Acetobacterales	Acetobacteraceae	Acidiphilium	0.0003
ASV 209	Bacteria	Armatimonadota	Fimbriimonadia	Fimbriimonadales	Fimbriimonadaceae		0.0005
ASV 99	Bacteria	Actinobacteriota	Actinobacteria	Frankiales	Frankiaceae	Jatrophihabitans	0.0008
ASV 19	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Beijerinckiaceae	1174-901-12	0.0008
ASV 85	Bacteria	Bacteroidota	Bacteroidia	Cytophagales	Hymenobacteraceae	Hymenobacter	0.0009
ASV 226	Bacteria	Actinobacteriota	Actinobacteria	Micrococcales	Microbacteriaceae	Amnibacterium	0.0009
ASV 39	Bacteria	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae		0.0011
ASV 286	Bacteria	Bacteroidota	Bacteroidia	Cytophagales	Spirosomaceae	Huanghella	0.0013
ASV 101	Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingomonas	0.0015

Dreyling et al. (2022), Frontiers in Forests and Global Change

ASV 169	Bacteria	Armatimonadota	Fimbriimonadia	Fimbriimonadales	Fimbriimonadaceae		0.0017
ASV 134	Bacteria	Bacteroidota	Bacteroidia	Cytophagales	Hymenobacteraceae	Hymenobacter	0.0018
ASV 93	Bacteria	Proteobacteria	Gammaproteobacteria	Burkholderiales	Burkholderiaceae	Limnobacter	0.0019
ASV 18	Bacteria	Bacteroidota	Bacteroidia	Sphingobacteriales	Sphingobacteriaceae	Pedobacter	0.0019
ASV 308	Bacteria	Armatimonadota	Fimbriimonadia	Fimbriimonadales	Fimbriimonadaceae		0.0031
ASV 59	Bacteria	Acidobacteriota	Acidobacteriae	Acidobacteriales	Acidobacteriaceae (Subgroup 1)	Granulicella	0.0047
ASV 100	Bacteria	Acidobacteriota	Acidobacteriae	Acidobacteriales	Acidobacteriaceae (Subgroup 1)	Granulicella	0.0055
ASV 477	Bacteria	Bacteroidota	Bacteroidia	Cytophagales	Hymenobacteraceae	Hymenobacter	0.0060
ASV 34	Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Novosphingobium	0.0085
ASV 112	Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingomonas	0.0093
ASV 13	Bacteria	Actinobacteriota	Actinobacteria	Pseudonocardiales	Pseudonocardiaceae	Actinomycetospora	0.0093
ASV 8	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Beijerinckiaceae	Methylocella	0.0110
ASV 88	Bacteria	Abditibacteriota	Abditibacteria	Abditibacteriales	Abditibacteriaceae	Abditibacterium	0.0114
ASV 86	Bacteria	Actinobacteriota	Actinobacteria	Frankiales	Frankiaceae	Jatrophihabitans	0.0121
ASV 65	Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae		0.0157
ASV 297	Bacteria	Bacteroidota	Bacteroidia	Cytophagales	Hymenobacteraceae	Hymenobacter	0.0158
ASV 119	Bacteria	Verrucomicrobiota	Verrucomicrobiae	Chthoniobacterales	Chthoniobacteraceae	LD29	0.0168
ASV 258	Bacteria	Verrucomicrobiota	Verrucomicrobiae	Chthoniobacterales	Chthoniobacteraceae	LD29	0.0173
ASV 654	Bacteria	Bacteroidota	Bacteroidia	Cytophagales	Spirosomaceae	Fibrella	0.0183
ASV 22	Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingomonas	0.0224
ASV 193	Bacteria	Abditibacteriota	Δhditihacteria	Abditibacteriales	Abditibacteriaceae	Abditibacterium	0.0250

ASV 822	Bacteria	Bacteroidota	Bacteroidia	Cytophagales	Hymenobacteraceae	Hymenobacter	0.0268
ASV 314	Bacteria	Proteobacteria	Gammaproteobacteria	Burkholderiales	Comamonadaceae		0.0274
ASV 79	Bacteria	Actinobacteriota	Actinobacteria	Pseudonocardiales	Pseudonocardiaceae	Actinomycetospora	0.0275
ASV 750	Bacteria	Actinobacteriota	Thermoleophilia	Solirubrobacterales	Solirubrobacteraceae	Conexibacter	0.0283
ASV 6	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Beijerinckiaceae	1174-901-12	0.0320
ASV 158	Bacteria	Bacteroidota	Bacteroidia	Cytophagales	Hymenobacteraceae	Hymenobacter	0.0343
ASV 163	Bacteria	Verrucomicrobiota	Verrucomicrobiae	Chthoniobacterales	Chthoniobacteraceae	LD29	0.0352
ASV 77	Bacteria	Actinobacteriota	Actinobacteria	Micromonosporales	Micromonosporaceae	Actinoplanes	0.0375
ASV 142	Bacteria	Actinobacteriota	Actinobacteria	Frankiales	Frankiaceae	Jatrophihabitans	0.0428
ASV 555	Bacteria	Patescibacteria	Saccharimonadia	Saccharimonadales			0.0428
ASV 262	Bacteria	Bacteroidota	Bacteroidia	Cytophagales	Hymenobacteraceae	Hymenobacter	0.0481
ASV 247	Bacteria	Verrucomicrobiota	Verrucomicrobiae	Chthoniobacterales	Chthoniobacteraceae	LD29	0.0497
Fungi							
ASV 33	Fungi	Ascomycota	Dothideomycetes	Capnodiales	Mycosphaerellaceae	Mycosphaerella	0.0000
ASV 226	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Didymosphaeriaceae	Pseudopithomyces	0.0000
ASV 21	Fungi	Ascomycota					0.0000
ASV 58	Fungi	Ascomycota					0.0000
ASV 1	Fungi	Ascomycota	Dothideomycetes	Capnodiales			0.0001
ASV 28	Fungi	Ascomycota	Dothideomycetes				0.0001
ASV 13	Fungi	Ascomycota	Dothideomycetes				0.0002
ASV 56	Fungi	Ascomycota	Dothideomycetes	Dothideales	Aureobasidiaceae	Aureobasidium	0.0004
ASV 162	Fungi	Ascomycota					0.0006

Dreyling et al. (2022), Frontiers in Forests and Global Change

ASV 63	Fungi	Ascomycota	Sordariomycetes	Hypocreales			0.0006
ASV 146	Fungi	Ascomycota	Eurotiomycetes	Chaetothyriales	Herpotrichiellaceae		0.0015
ASV 11	Fungi	Ascomycota	Lecanoromycetes	Lecanorales	Lecanoraceae	Scoliciosporum	0.0015
ASV 4	Fungi	Ascomycota					0.0023
ASV 124	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Pleosporaceae	Alternaria	0.0028
ASV 96	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Didymellaceae		0.0030
ASV 143	Fungi	Ascomycota	Eurotiomycetes	Chaetothyriales	Herpotrichiellaceae	Veronaea	0.0031
ASV 144	Fungi	Ascomycota	Eurotiomycetes	Chaetothyriales	Trichomeriaceae	Knufia	0.0042
ASV 46	Fungi	Ascomycota	Orbiliomycetes	Orbiliales	Orbiliaceae		0.0045
ASV 232	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Pleosporaceae	Alternaria	0.0047
ASV 18	Fungi	Basidiomycota	Tremellomycetes	Tremellales	Tremellaceae	Tremella	0.0048
ASV 12	Fungi	Ascomycota	Dothideomycetes				0.0056
ASV 84	Fungi	Ascomycota	Dothideomycetes	Capnodiales	Cladosporiaceae	Cladosporium	0.0079
ASV 173	Fungi	Ascomycota	Eurotiomycetes	Chaetothyriales	Herpotrichiellaceae	Rhinocladiella	0.0081
ASV 163	Fungi	Ascomycota	Lecanoromycetes	Lecanorales	Micareaceae	Micarea	0.0091
ASV 184	Fungi	Ascomycota	Lecanoromycetes	Ostropales	Coenogoniaceae	Coenogonium	0.0186
ASV 15	Fungi	Ascomycota	Lecanoromycetes	Lecanorales	Lecanoraceae	Scoliciosporum	0.0304
ASV 289	Fungi	Ascomycota	Sordariomycetes	Sordariales	Sordariales_fam_Incertae_sedis	Rhodoveronaea	0.0307
ASV 127	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Didymosphaeriaceae	Montagnula	0.0334
ASV 39	Fungi	Ascomycota	Dothideomycetes	Capnodiales			0.0376
ASV 237	Fungi	Ascomycota	Dothideomycetes	Capnodiales	Mycosphaerellaceae	Ramularia	0.0392

ASV 189	Fungi	Basidiomycota	Agaricomycetes	Agaricales	Strophariaceae	Hypholoma	0.0429
ASV 8	Fungi	Ascomycota	Eurotiomycetes	Chaetothyriales			0.0442
ASV 23	Fungi	Ascomycota	Eurotiomycetes	Chaetothyriales	Herpotrichiellaceae	Capronia	0.0463
ASV 149	Fungi	Ascomycota	Lecanoromycetes	Lecanorales	Micareaceae	Micarea	0.0466
ASV 54	Fungi	Basidiomycota	Cystobasidiomycetes	Cystobasidiales	Cystobasidiaceae		0.0470
ASV 209	Fungi	Ascomycota	Lecanoromycetes	Lecanorales	Micareaceae	Micarea	0.0475
Medium – Small Trees							
Algae							
ASV 10	Eukaryota	Chlorophyta	Trebouxiophyceae	Chlorellales	Chlorellaceae	Apatococcus	0.0015
ASV 43	Eukaryota	Chlorophyta	Trebouxiophyceae	Trebouxiales	Trebouxiaceae	Symbiochloris	0.0270
ASV 29	Eukaryota	Chlorophyta	Trebouxiophyceae	Trebouxiales	Trebouxiaceae	Symbiochloris	0.0293
Bacteria							
ASV 56	Bacteria	Bacteroidota	Bacteroidia	Sphingobacteriales	Sphingobacteriaceae	Mucilaginibacter	0.047

A.2. Biotic interactions outweigh abiotic factors as drivers of bark microbial communities in Central European forests

Declaration of author contributions to the manuscript:

Biotic interactions outweigh abiotic factors as drivers of bark microbial communities in Central European forests

Status: in revisions

Contributing authors: <u>Lukas Dreyling (LD)</u>, Caterina Penone (CP), Noëlle

Schenk (NS), Imke Schmitt (IS), Francesco Dal Grande

(FDG)

What are the contributions of the doctoral candidate and his co-authors?

(1) Concept and design

Doctoral candidate (LD): 20%
Co-author CP: 10%
Co-author NS: 10%
Co-author IS: 30%
Co-author FDG: 30%

(2) Conducting tests and experiments

Doctoral candidate (LD): 80% (sample collection from the field, PCR)
Co-author FDG: 10% (sample collection from the field)
Co-author IS: 10% (sample collection from the field)

(3) Compilation of data sets and figures

Doctoral candidate (LD): 100% (processing of sequencing reads, ASV inference,

taxonomic assignment, preparation of all figures and tables)

(4) Analysis and interpretation of data

Doctoral candidate (LD): 60% (linear models (lm) of alpha diversity, Generalized

Dissimilarity Modelling (GDM) of beta diversity, variance

partitioning, data interpretation)

Co-author CP: 10% (constructing Im and GDM, data interpretation)
Co-author NS: 10% (constructing Im and GDM, data interpretation)

Co-author IS: 10% (data interpretation)
Co-author FDG: 10% (data interpretation)

(5) Drafting of manuscript

Doctoral candidate (LD): 70%
Co-author CP: 5%
Co-author NS: 5%
Co-author IS: 15%
Co-author FDG: 5%

I hereby certify that the information above is correct.					
Date and place	Signature doctoral candidate				
Date and place	Signature supervisor				
Date and place	If required, signature of corresponding author				

Biotic interactions outweigh abiotic factors

2 as drivers of bark microbial communities

3 in Central European forests

Lukas Dreyling^{1,2}, Caterina Penone³, Noëlle Schenk³, Imke Schmitt^{1,2}, Francesco Dal Grande^{1,4,5} 5 1: Senckenberg Biodiversity and Climate Research Centre (SBiK-F), Frankfurt am Main, Germany; 2: Goethe University Frankfurt, Institute of Ecology, Evolution and Diversity, Frankfurt am Main, Germany; 8 3: Institute of Plant Sciences, University of Bern, Bern, Switzerland; 4: Department of Biology, University of Padova, Padua, Italy 10 11 5: National Biodiversity Future Center (NBFC), Palermo, Italy 12 Corresponding author: lukas.dreyling@senckenberg.de 14 Running Title: Drivers of bark surface microbial communities 15 16 17 **Competing Interests** 18 The authors declare no competing interests. 19 Keywords 20 21 Microbial Ecology; Microbiome; Forest; Algae; Bacteria; Fungi; Bark Surface; Community 22 Ecology; Metabarcoding; environmental DNA

Abstract

23

24

25

26

27

28

29

30

31

32

33

34

35

36

37

38

39

40

41

Bark surfaces are extensive areas within forest ecosystems, that provide an ideal habitat for microbial communities, through their longevity and seasonal stability. Here we provide a comprehensive account of the bark surface microbiome of living trees in Central European forests, and identify drivers of diversity and community composition. We examine algal, fungal and bacterial communities and their interactions using metabarcoding on samples from over 750 trees collected in the Biodiversity Exploratories in northern, central and southern Germany. We show that mutual biotic influence is more important than the abiotic environment with regard to community composition, whereas abiotic conditions and geography are more important for alpha diversity. Important abiotic factors are the relative humidity and light availability, which decrease the algal and bacterial alpha diversity, but strongly increase fungal alpha diversity. In addition, temperature is important in shaping the microbial community, with higher temperature leading to homogeneous communities of dominant fungi, but high turnover in bacterial communities. Changes in the community dissimilarity of one organismal group occurs in close relation to changes in the other two, suggesting that there are close interactions between the three major groups of the bark surface microbial communities, which may be linked to beneficial exchange. To understand the functioning of the forest microbiome as a whole, we need to further investigate the functionality of interactions within the bark surface microbiome and combine these results with findings from other forest habitats such as soil or canopy.

Introduction

42

Forest ecosystems harbour a great diversity of microbial life in a variety of forest compartments, 43 such as soil, dead wood, leaf surfaces, or bark surfaces [1]. Communities of microorganisms 44 45 perform important functions in forests, including nutrient cycling and fixation, and symbiotic relationships with plants [2–4]. It is therefore crucial to understand the causes (and consequences) 46 of microbial diversity changes in forests, i.e., understand their biotic and abiotic drivers. Ideally, we 47 would know the complete microbial spectrum in all forest compartments, a task not nearly 48 49 accomplished yet [1]. Here we contribute to closing this knowledge gap by focusing on a large but 50 neglected forest compartment, the bark surface of living trees, and assessing the diversities, as well 51 as biotic and abiotic drivers of bacterial, fungal, and algal bark surface communities. 52 Bark surfaces constitute one of the largest forest compartments [1]. They offer a multitude of micro niches for microbial colonization [5] and sustain diverse bacterial, fungal and algal 53 communities [6-10] despite challenging environmental conditions such as low nutrient and water 54 55 availability [6, 7, 11]. Microbial communities on bark are at the base of the forest food web, 56 supporting animals, such as molluscs, mites, and lice [12–15] and macro-epiphytes such as mosses and lichens [16, 17]. According to Aschenbrenner et al. (2017) [16], these communities could also 57 represent reservoirs of microbial taxa potentially "feeding" other forest compartments, e.g., via 58 transmission by stemflow from the phyllosphere to soil [18]. 59 60 Natural microbial communities engage in a wide variety of important interactions, ranging 61 from the provision of nutrients [19] to parasitism [20]. Algae are primary producers, supplying these communities with photosynthetic products (e.g., [21]), but lack the ability to fix nitrogen which in 62 turn is often provided by mutualistic bacteria [22]. Other bacteria have been shown to be harmful to 63 algae, e.g., by producing cell death inducing compounds [23, 24]. Fungi on the other hand are 64 65 known to protect algae, for example from such harmful bacteria [24] but also from environmental 66 stressors such as UV radiation [25]. They often provide a structural component for colonization 3

through their filamentous nature, e.g., in lichens [26] or mycorrhiza [19]. In addition, the fungal 67 68 mycelium has been proposed as a "transport path" for bacterial dispersal [27]. However, fungi and bacteria also engage in competition for nutrients [19]. Considering these types of interactions, it is 69 70 highly likely that algae, fungi and bacteria occurring in close spatial association on tree bark jointly 71 influence each other's diversity and community composition (e.g., [28]). 72 Abiotic conditions, which affect aboveground microbial communities of trees, have mostly 73 been analysed with a focus on phyllosphere communities. For example, Liu et al. (2023) [29] found 74 that lower biomass and species richness of phyllosphere algae in tropical forests is likely related to 75 lower moisture retention on the leaf surface. Similar patterns can be observed for fungi and bacteria, 76 e.g., in the phyllosphere of grapes, where the richness of fungi and bacteria increased with higher 77 temperatures and rainfall [30]. Another important abiotic factor is exposure to UV radiation in 78 aboveground habitats, which has been shown to alter bacterial community composition, but not 79 population size, in the phyllosphere [31]. Based on these studies, we hypothesize that the bark 80 surface microbiome, similarly to the phyllosphere, is affected by fluctuations in temperature, water 81 availability and UV radiation. For example, a previous study on bark microalgae from 82 Mediterranean forests showed a higher abundance and diversity compared to those of temperate 83 forests, likely due to differences in temperature and precipitation [32]. However, additional factors 84 may contribute to shaping the unique communities on bark surfaces [7], for example the age of the 85 host tree [10, 33]. 86 Interestingly, studies from soil microbial communities revealed that organismal groups occurring in the same habitat tend to exhibit different and sometimes opposite responses to abiotic 87 88 changes. De Vries et al. [34] showed that bacterial networks are destabilized under drought 89 conditions, while the effect on fungi was negligible. Even within organismal groups, responses can 90 differ, as has been shown for drought responses of free living and mycorrhizal fungi [35]. To

4

Dreyling et al. (2023), submitted

91	understand how abiotic conditions affect the microbiome we thus need to include the full microbial
92	spectrum captured at the same scales and time points.
93	Only few studies about natural bark micro-communities exist (e.g., [7, 8, 35]), and although
94	they often included only single organismal groups [16, 36, 37], we already know parts of the
95	diversity in bark surface communities [10]. However, to gain insights into how the whole
96	community of microorganisms responds to present environmental change, and to model future
97	changes, we need a comprehensive overview of the drivers behind community structure [1, 10].
98	Since diversity is multifaceted we need to go beyond alpha diversity and consider multiple diversity
99	dimensions [38] to fully understand which biotic and abiotic factors shape community responses.
100	Additionally, rare and common species might respond differently to the same drivers [39] and beta
101	diversity can reveal homogenisation patterns [40].
102	In this study we aim to elucidate how the environment structures multi-kingdom micro-
103	organismal communities in one of the largest above-ground habitats of the terrestrial realm, the bark
104	surface of forest trees. We sampled micro-communities from the bark of living trees, in 133 plots
105	(over 750 trees) along a south-west to north-east gradient across Germany and assessed the relative
106	contribution of abiotic (e.g., climate and forest features) and biotic (i.e., co-occurrences) factors in
107	predicting the metabarcoding-derived diversity of three major microbial domains, i.e. terrestrial
108	green algae, fungi and bacteria. Specifically, we aimed at answering the following questions:
109	1) What is the alpha diversity and community composition associated with bark surfaces in Central
110	European temperate forests?
111	2) What are the drivers of alpha diversity and composition of the bark surface microbial
112	community? And specifically, what is the relative importance of biotic and abiotic factors?
113	
111	

Material and Methods

116 Study Design

115

We collected samples in May 2021 from the full set of 150 forest plots established by the 117 118 Biodiversity Exploratories in three regions across Germany [41]. We defined a 20 m × 20 m subplot 119 of the original 100 × 100 m plot and collected a composite sample of bark surface swabs from six 120 trees per plot. Prior to sampling, we determined the most abundant tree species for each plot, based 121 on a forest inventory [42]. All six trees sampled in each plot belonged to the predominant species. 122 Some plots were excluded prior to the analysis, either because the dominant tree species did not 123 occur in enough plots necessary for robust statistics, the plot was clear cut before sampling or 124 because the extraction did not yield enough DNA. The dataset used for analysis contained 133 plots 125 of the original 150 sampling plots. The dominant tree species in the final subset of 133 plots were 126 beech (Fagus sylvatica), pine (Pinus sylvestris) or spruce (Picea abies). Following a previous study 127 [10], the composite sample included two small (5-15 cm diameter at 150 cm height), two medium 128 (15 – 30 cm) and two large (> 30 cm) trees. When no equal sampling was possible, we chose the 129 size class that best represented the surrounding forest (36 of 133 plots, ~27%). 130 The sampling technique is described in detail in [10] with the only difference that all swabs 131 from one plot were pooled together. In brief: we sampled the microbial community of the bark surface by swabbing with nylon-flocked swabs (FLOQSwabsTM, Copan, Brescia, Italy) once around 132 133 the stem at 150 cm height. The swabs were fixed in nucleic acid preservation (NAP) buffer [43] and 134 stored at 4 °C until DNA extraction. We included three extraction blanks (one per region) of six 135 swabs exposed to ambient air. These were processed as if they were a biological sample. 136 **DNA** extraction 137 As described in [44], samples stabilized in DNA preservation buffers need extra processing before extraction. To allow liberation of all material, including bacterial cells, we added an equal amount 138 139 of phosphate buffered saline (PBS) to the tube containing the swabs in NAP buffer. Afterwards, we 6

140	moved the contents to a 50 ml tube (to allow movement) and vortexed the swabs for 30 s to
141	dislodge material. We transferred 1.5 ml of the suspension to a 2 ml tube, centrifuged it at $6000 \times g$
142	for 15 min. and discarded the supernatant. We used an extraction kit (Quick-DNA Fecal/Soil
143	Microbe Microprep, Zymo Research Europe GmbH, Freiburg, Germany). Modifying the protocol,
144	we added the beads and buffer directly to the centrifugation pellet. Samples were shaken for a total
145	of six minutes (SpeedMill PLUS, Analytik Jena, Jena, Germany). All subsequent steps followed the
146	manufacturer's protocol. DNA extracts were stored at -20 °C.
147	PCR Amplification and High-Throughput Sequencing
148	We amplified algal, fungal and bacterial DNA with universal primer pairs, targeting the ITS2 (ITS-
149	Cha3 (CAACTCTCRRCAACGGATA) [45] and ITSu4 (RGTTTCTTTTCCTCCGCTTA) [45] for
150	algae; FITS7 (GTGARTCATCGAATCTTTG) [46] and ITS 4 (TCCTCCGCTTATTGATATGC)
151	[47] for fungi) and 16S V3-V4 (341F (CCTACGGGWGGCWGCAG) [48, 49] and 785R
152	(GACTACHVGGGTATCTAATCC) [50] for bacteria) regions. We used double-index multiplexing
153	with both primers being tagged by an octamer, allowing us to amplify all samples in triplicate. Each
154	technical replicate included eight PCR negative controls as well as 23 empty wells as so-called
155	"multiplex controls" to detect potential primer jump [51]. PCR reactions with a volume of 15 μl
156	contained 5 ng of DNA, 7.5 µl of MyTaqTM HS Mix, 2x (Bioline GmbH, Luckenwalde, Germany),
157	$0.6~\mu l$ 10 mM of each primer, and $4.3~\mu l$ DNAse free water. All samples were randomly placed on
158	two 96-well plates, sharing the placement scheme between replicates. The cycling conditions
159	differed between the organismal groups (Tab. 1).
160	After PCR, we cleaned each sample with magnetic beads (MagSI-NGSPREP Plus, magtivio
161	B.V., Geelen, Netherlands) and measured DNA concentration using the Qubit dsDNA HS assay
162	with a Qubit 3.0 (Thermo Fisher Scientific, MA, United States). Triplicates were pooled
163	equimolarly and sent to Fasteris SA (Plan-les-Ouates, Switzerland) for library preparation

164	(MetaFast protocol) and sequencing. Amplicons were sequenced with 2 \times 300 bp paired-end read
165	on an Illumina MiSeq machine (Illumina Inc., San Diego, CA, United States).
166	Bioinformatics
167	We trimmed the primers and demultiplexed the reads using Cutadapt (version 3.3., [52]). We used
168	DADA2 (version 1.12.1, [53]) for filtering and trimming, denoising and sample inference to obtain
169	Amplicon Sequencing Variants (ASVs). For fungi and bacteria, we used DADA2 assignTaxonomy(
170	and the publicly available databases UNITE general fasta release 9.0 [54], including eukaryotic ITS
171	as outgroups, and SILVA 138.1 SSU Ref NR 99 [55]. For algae, no such database exists and we
172	used the NCBI nt database (generated on 25.04.2022) with a local call to BLASTn. Afterwards we
173	used the taxonomizr R package (version 0.8.0, [56]) to assign taxonomy. BLAST hits from
174	uncultured or environmental origin, and below 95% identity were excluded. Reads were checked
175	for contaminant sequences using decontam (version 1.16.0, [57]). The resulting ASV table was
176	curated with the LULU algorithm (version 0.1.0, [58]) which is a tool for post-clustering curation
177	based on co-occurrence of similar sequences and merges potential parent and child sequences.
178	Analyses
179	We used R (version 4.2.2, [59]) together with RStudio (version 2022.12.0.353, [60]) to perform all
180	the analyses. Data were combined with <i>phyloseq</i> (version 1.40.0, [61]). All graphics were generated
181	with ggplot2 (version 3.4.0, [62]) and ggpubr (version 0.5.0, [63]). To visualize community
182	composition, we created relative abundance barplots of the 25 most abundant orders with the
183	microbiome [64], fantaxtic [65] and microViz [66] packages. To avoid a loss of data, samples were
184	not rarefied [67]. All analysis scripts are available at Zenodo [68] under doi:
185	10.5281/zenodo.10200121.
186	Diversity
187	In order to capture multiple dimensions of diversity, we calculated Hill numbers [69, 70] (or
188	effective species (in this case ASV) number) for both alpha and beta diversity using the hillR
	8

189 package (version 0.5.1, [71]). In general, the weight given to the abundance (counts) of a taxon 190 increases with Hill number. According to the definition by Chao et al. (2014) [70], Hill numbers measure the diversity of "all" (q = 0), of "typical" (q = 1) and "dominant" (q = 2) species (ASVs). 191 192 In the following sections we use these terms when referring to the according q values. 193 We chose the first three levels of q = 0, 1 and 2 to have a direct comparison to widely used 194 indices. For alpha diversity these correspond to species (ASV) richness (q = 0), Shannon entropy (q 195 = 1) and inverse Simpson index (q = 2). Since beta diversity is inherently a comparison between 196 two spatially separate populations [72] we calculated Sørensen-type similarity between pairwise 197 communities as the CqN measure [73, 74]. To mirror the metrics used for alpha diversity, we 198 calculated measures of Sørensen dissimilarity (q = 0), dissimilarity of the Horn index (q = 1) and 199 dissimilarity of the Morisita-Horn index (q = 2). **Environmental influence on diversity** 200 201 To assess the effect of the environment on the microbial bark communities we chose a set of 202 explanatory variables, based on prior hypotheses of how they might influence the community. Table 203 2 gives an overview and explains what the variables represent and how they were measured. All 204 variables were scaled to standardize effect sizes and make them comparable. An overview of 205 estimates can be found in Supplementary Table 3 and Tab. 4. We tested for significant differences in 206 the tree dependent variables between the tree species using a multivariate analysis of variance 207 (MANOVA). Based on the results (Pillais trace = 0.86, F = 19.33, p < 0.001) and biological 208 interpretation, we hypothesize that the host tree species (where the sample was collected) and tree 209 dependent variables (see Tab. 2) represent the same processes. Thus, we excluded the host tree 210 species from the analysis. 211 We used multiple linear regression models to study the responses of alpha diversity to 212 abiotic and biotic factors. All models were specified as follows: 213 lm(biotic Y ~ region + relative humidity + temperature + average DBH + canopy openness

9

Appendix – Publications

214	+ gini coefficient + stand density + ratio of dominant trees
215	+ forest area + biotic1 + biotic2 + offset(library size))
216	Where biotic 1 and 2 represent the alpha diversities of the two other groups (e.g., when bacteria are
217	the response variable, fungi and algae are biotic 1 and 2). Biotic influences were always modelled
218	on the same diversity level, e.g., the response of algal q0 to changes in bacterial and fungal q0. The
219	linear models included an offset term to account for the variation in library size between the
220	samples.
221	To correct for multiple testing, we corrected the p-values for type 1 errors with a Benjamini-
222	Hochberg correction and a threshold of $p \le 0.2$. Combining this relaxed threshold and correction
223	allows us to detect effects on this unknown system we would otherwise miss, while still being
224	cautiously optimistic that the effect is not a false positive.
225	To model changes in beta diversity we used Generalized Dissimilarity Modelling (GDM,
226	[75–78]) fit through the <i>gdm</i> package [79]. GDM models pairwise dissimilarities between plots
227	taking non-linear relationships into account, e.g., rates of change can be more rapid at some points
228	along a gradient. This allows us to observe patterns of non-linearity common in ecology [78]. GDM
229	follows a similar structure as Generalized Linear Models, but instead of taking individual
230	explanatory values and assessing their effect on the response variable (here the beta diversity) it
231	models the absolute difference between a pair of values, ordered along the explanatory variable's
232	gradient. For example: if Plot1 has an average temperature of 5 °C and Plot2 of 9 °C, then the GDM
233	takes the difference of 4 $^{\circ}\text{C}$ to (non-linearly) model how dissimilar the microbial communities of
234	the two plots are, at the gradient between 5 °C and 9 °C. The explanatory variables mirrored the
235	linear models of the alpha diversity analysis but included the geographic distance between plots
236	instead of the region identity. The variation in library size between samples was incorporated into
237	the GDM as a weighting factor, putting less weight on larger differences. P-Values for GDMs are
238	calculated based on a permutation procedure (n = 100 permutations) and were also corrected for

239 multiple testing using the Benjamini-Hochberg correction with a threshold of p < 0.2. An overview 240 of predictor importance can be found in Table 4. 241 Results 242 **Diversity** 243 244 We found a total of 131 ASVs for algae, 1750 for fungi, and 1263 for bacteria. The highest ASV 245 richness at the regional level occurred within fungi, while the lowest number of ASVs was found 246 for the terrestrial green algae (Tab. 3). Generally, we observed that ASV richness decreases from 247 south-west (Swabian Alb) to north-east (Schorfheide-Chorin) Germany (Tab. 3). 248 The most abundant algal orders were Trebouxiales, Chlorellales and Prasiolales (Fig. 1). The 249 bacterial portion was primarily composed by taxa from the order Rhizobiales followed by 250 Acetobacterales, which became dominant on some plots in the north-east (Fig. 1) where pine was 251 the dominant tree species. For fungi we found a different pattern, with a large proportion of reads 252 not assignable at the order level, predominantly stemming from unassigned Dothideomycetes. 253 There were no dominating orders, with Lecanorales, Capnodiales and Chaetothyriales showing the 254 highest relative abundance in the assignable portion of reads (especially in the north-east). Generally, abundance patterns were similar across regions and plots, with the exception of pine-255 256 dominated plots. **Drivers of Diversity** 257 Alpha Diversity 258 259 All three organismal groups responded significantly (p < 0.2 after Benjamini-Hochberg correction) 260 to biotic factors on at least one diversity level. Both algal and bacterial "all species" diversity (q = 261 0) significantly (both p < 0.01) increased with higher fungal ASV richness (Fig. 2), while there was 262 no effect of algal ASV richness on bacteria and vice versa. Fungal ASV richness increased 263 significantly with algal and bacterial ASV richness (algae p = 0.056, bacteria p = 0.001). Dominant

Appendix - Publications

algae (q = 2) positively influenced bacterial diversity (p = 0.078, Fig. 2). The mutual influence of dominant fungi and algae was negative, although not significant.

An important abiotic factor was canopy openness (proxy for light availability; Tab. 2), which influenced both fungal and bacterial diversity significantly. While fungal diversity increased with higher canopy openness (q0 p = 0.078, q1 p = 0.063, q2 p = 0.057), bacterial diversity of all and common species (ASVs) significantly decreased (q0 p = 0.005, q1 p = 0.143) (Fig. 2). A negative direction of algal diversity (q = 0 to 2) could be observed with increasing canopy openness. Relative humidity increased fungal diversity significantly, while decreasing directions were found for the diversity of all and typical bacteria while dominant bacteria increased. All levels of diversity for algae increased with higher humidity. A table of all effects, including p-values can be found in Supplementary Table 3.

The variance (adjusted R2) explained by the linear models ranged between 17 and 48 % (Fig. 3) and was the lowest for algal alpha diversity. The pure variance explained by abiotic factors was often higher than that of biotic factors on the alpha diversity level (Fig. 3), except when assessing diversity of "all" species. Abiotic factors also explained more variance when considered in combination with geographic factors (Fig. 3 a +g), especially for models of fungal and bacterial alpha diversity.

Beta Diversity

The beta diversity of all three organismal groups responded significantly (algae all p < 0.05, except effect of dominant fungi p < 0.1; fungi all p < 0.05, except "all" and dominant algae p < 0.1; bacteria all p < 0.05) to changes in the diversity of the respective microbial partners (Fig. 4), e.g.., if two plots differed in their bacterial community they also differed in their algal and fungal composition. Most of the effect curves followed an exponential shape sloping upwards with increasing community dissimilarity, meaning the effects were strongest at high β -diversity and indicating a concurrent change of community composition. Assessing predictor importance

(deviation in variance explained when a given predictor is permuted), bacteria were the most important biotic predictor on all three levels of fungal and algal beta diversity (Tab 4), followed by fungi which were slightly less important for algae and bacteria at all levels. Dominant bacteria were the most important biotic predictor for fungi, while algae were the most important biotic predictor of β -diversity of dominant bacteria.

Of the abiotic factors we tested, temperature and canopy openness were the two most important predictors of community dissimilarity (Tab 4), but their importance was usually lower than that of the biotic factors, except for typical and dominant fungi, as well as dominant bacteria. Differences in temperature significantly influenced the communities for most measures (Fig. 4, algae q0 = p < 0.2, bacteria and fungi all p < 0.05), but not the typical and dominant algal communities. For fungi, we found high community dissimilarity at lower temperature which saturated into homogeneous communities at higher temperatures (Fig. 4). Bacterial communities generally showed strong changes in dissimilarity at higher temperature, but beta diversity remained unchanged at low temperature (Fig 4). Changes in canopy openness and the associated increase in light availability significantly influenced the communities of both fungi and bacteria at all levels of beta diversity (Fig. 4, fungi q0 + q1 = p < 0.2, q2 = p < 0.1; bacteria q0 = p < 0.1, q1 + q2 = p < 0.10.05). Bacterial communities displayed more rapid turnover with small increases in light availability in closed forests than in open ones (Fig. 4). The patterns of algal diversity were similar to those of bacteria and, although not significant, canopy openness usually was the most important abiotic predictor (Tab. 4; small effect size). Fungi responded differently to canopy openness. While the response of the full community was almost linear with shallow slope at increasing openness, the typical and dominant taxa showed a rapidly changing composition with high rates of change in more open conditions which is potentially connected to the strong increase in alpha diversity.

The variance of beta diversity explained by the GDMs was between 37 and 75% and again was lowest in the models of algal diversity. In contrast to the alpha diversity, biotic factors

13

289

290

291

292

293

294

295

296

297

298

299

300

301

302

303

304

305

306

307

308

309

310

311

312

Appendix - Publications

explained more variance than abiotic factors in all three organismal groups, except for dominant fungi and bacteria (Fig. 3). A similar amount of variance was explained by combined effects of biotic and abiotic effects. Geographic distance explained only small amounts of variance.

317

318

Discussion

Fungi are the richest group found on bark surfaces with many unknown taxa 319 320 We studied the three main micro-organismal groups of the bark surface, and found that communities 321 of fungi contained 90 ASVs per sample on average; 4.5 times more than algae (20 ASVs) and 1.3 322 times more than bacteria (70 ASVs). Compared to other forest habitats like soil, the microbial bark 323 surface community is more unknown, especially for fungi. A study with soil samples from the same 324 plots, sampled approximately at the same time, found that only two percent of the relative 325 abundance came from fungal ASVs that were not assignable past the order rank [80], while in our 326 study it was up to 50 %, much of it from the ubiquitous class Dothideomycetes [81, 82]. The 327 diversity harboured by bark surfaces, and especially its unknown portion, underlines the importance 328 of further research on the bark microbiome. Of particular interest is the identification and 329 potentially isolation of unknown fungi, considering that bark surfaces have been proposed as 330 microbial reservoirs [16] that potentially contain an array of pathogenic and/or beneficial taxa 331 relevant to plant health, as previously shown for bacterial epiphytes on grapevines [35]. 332 Furthermore, it is likely that the reservoir effects of bark shape assembly processes in other forest 333 compartments, e.g. by enabling early colonization of the phyllosphere in spring [8, 16] through the 334 bark's seasonal stability [83], or dispersal to soils via stemflow [18]. Additionally, it has been shown 335 that bark is of great importance for the composition and diversity of deadwood microbiomes. In a 336 study by Hagge et al. [84] bark coverage increased the importance of stochastic assembly 337 mechanisms, one of which could be "priority effects" of the original community found in the bark 338 surface reservoir.

Abiotic conditions strongly impact alpha, but not beta diversity

To assess how the different groups within the bark surface microbiome respond to changes in their environment, we tested abiotic variables associated with climatic conditions and habitat connectedness. From previous studies of phyllosphere microbiomes, we expected that variables directly influencing individual organisms (e.g., humidity, temperature, light) would have the largest impacts on the communities. We found that the alpha diversity of the bark surface microbiome is strongly affected by abiotic factors, while abiotic factors (except temperature) were less important for community dissimilarity (Fig. 3 + 4, Tab. 4). This indicates that while the size of the community may be limited by certain environmental conditions, which regulate ASV richness and how evenly common and rare taxa occur, they only weakly affect which taxa are present in the microbiome.

Since abiotic conditions are known to influence different components of micro-organismal communities differentially, e.g., bacterial soil communities responding more strongly to drought than fungal communities [34] and free-living fungi being more susceptible to drought than mycorrhizal fungi [85], we expected different responses of algae, bacteria and fungi, especially to climatic conditions. When considering the three organismal groups, we found the strongest difference for humidity (alpha diversity) and temperature (beta diversity). While humidity strongly increased fungal alpha diversity, it had no significant effects in the other two organismal groups. However, bacterial diversity of "all" and "typical" species (ASVs) decreased with relative humidity. In contrast, temperature had no significant effect on alpha diversity at all. These contrasting effect directions for humidity are known from phyllosphere microbiomes [30], as well as from rhizosphere communities [86]. Strong differences even within organismal groups (e.g., [30, 85]) underline the need for caution when generalizing these patterns.

At the beta diversity level, temperature had the strongest effect on fungal communities and on dominant bacteria. Similar to the findings of de Vries et al [34], we found that fungal

Appendix - Publications

communities were more similar at the high end of the temperature range, while the (typical and dominant) bacterial, as well as algal, communities showed higher turnover with increases in temperature. The exponential curve for algae and bacteria might indicate that these communities reach a "tipping point" after which rapid change sets in, accompanied by high turnover along the temperature gradient [78].

Light is expected to affect both photosynthetic organisms and microbial alpha and beta diversity [84, 87]. In the current study a higher availability of light lead to an increase of fungal, but a decrease of algal and bacterial diversity (Fig 2), suggesting that bark surface algae and bacteria are adapted to low light conditions and potentially damaged by too much UV radiation [31, 88]. For beta diversity the slopes reached a plateau at higher light levels for both algae and bacteria, indicating homogeneous communities at higher light conditions. This suggests that the algal and bacterial communities become less rich but are potentially adapted to higher light conditions, for example through the synthesis of carotenoid compounds [89]. Although many fungi employ strategies to limit damage from UV radiation, making them potentially more resistant to high light stress [90], we detected high rates of change under high light conditions for fungi. The high turnover, especially in the dominant fungal taxa, potentially indicates that fungal communities of open canopy forests are specialized towards high light availability.

Biotic interactions determine community composition

Interactions between algae, bacteria and fungi have been shown to take many forms, from competition over parasitism to well described symbioses like lichens [17, 19, 20, 24]. Thus, we expected close connections between the three organismal groups in the bark surface microbiome. Indeed, we could observe that changes in the community composition were highly depending on changes in composition of the other groups. This suggests that there are certain fractions of algal, fungal and bacterial communities that are favourably associating with one another. Similarly,

Arrigoni et al (2018) [8] described a state of stable equilibrium between pathogenic and beneficial bacteria and fungi on the bark of fruit trees.

While biotic effects were less important for alpha diversity, they were in almost all cases far more important for beta diversity than abiotic effects and explained the most variance (Fig. 3). Fiore-Donno and colleagues [91] recently reported similar patterns within the alpine soil microbiome, where biotic interactions outweighed edaphic and topographic influences. Within the biotic factors, bacterial beta diversity was the most important. This might be due to the high pathogenic potential of bacteria affecting both algae and fungi [19, 24], but also due to supplying nutrients like nitrogen, especially considering the high impact on algal communities for which growth promotion through bacterial co-occurrence has been reported [22, 92]. Furthermore, heterotrophic bacteria commonly colonize the phyco- and mycosphere, the space surrounding algal and fungal cells [21, 93]. In these niches, they exchange compounds like photoassimilates and engage in other beneficial interactions with the host, mirroring the plant rhizosphere [21]. Bacteria, on the other hand are strongly influenced by the fungal communities, potentially due to the fungal mycelium providing opportunities for transport [27] but also protective structures under nonfavourable conditions [94], in addition to the provision of carbon from cell wall material [95]. Since close interactions between fungi and algae have been known for a long time, most notably from the lichen symbiosis [96], the strong fungal influence on algae was expected and was only slightly less than that of bacteria. A study by Hom and Murray [97] showed that mutualistic interactions of algae and fungi can also form spontaneously under low nutrient conditions, which are an inherent characteristic of the bark surface habitat.

It is important to note that a large proportion of the variance in the beta diversity models is jointly explained by abiotic and biotic conditions, and thus we cannot exclude indirect effects of abiotic conditions on a certain group through changes in another, e.g., increasing temperature could lead to changes in fungi which in turn changes bacterial and algal communities. However, we also

17

389

390

391

392

393

394

395

396

397

398

399

400

401

402

403

404

405

406

407

408

409

410

411

412

Appendix – Publications

414	need to consider that the microbiome can alter and mediate abiotic conditions such as nutrient or
415	water availability [98, 99] as has been shown for microbiome-plant relationships (e.g., [100]).
416	Regions differ mostly in their alpha but not beta diversity
417	Geographical distance is often associated with differences in environmental conditions. Previous
418	studies of macro-organisms, like plants [101] and arthropods [102], in the Biodiversity
419	Exploratories found significant differences between the three study regions that can be explained by
420	nutrient availability, substrate differences and land-use intensity. For the bark microbiome, we
421	expected a similar pattern due to some differences in tree species (pine in the north-east, spruce in
1 22	the south-west) and the corresponding differences in the direct abiotic environment. Similarly, we
423	found significant differences for alpha diversity between the regions with a diversity decrease from
124	the south-west to the north-east region. Region explained much of the variation in alpha diversity,
425	but the high variation explained jointly with abiotic conditions suggests that there may be abiotic
426	differences between regions not considered in our study, e.g., wind as a dispersal vector [103]. In
1 27	previous studies of subaerial algae [32] and phyllosphere microbiomes [30] geographic location was
128	a main influencer of microbial diversity leading to distinct communities. However, predictors were
129	not as finely differentiated as in our study. We found that the geographic effect on community
430	composition is negligible (Tab. 4), despite significant impacts on algae (Supplementary Figure S2),
431	mirroring the results of Aguirre-von-Wobeser et al. [9] for bacteria and fungi on avocado bark.
432	Caveats
433	While our sample sites are representative of Central European forests [41] generalisations extending
134	to other forest types, climate zones or continents should be drawn with caution. Since the bark
435	microbiome is still highly unknown at the global level we want to underline the need for further
436	studies to make these comparisons possible. However, because similar forest features affect the
1 37	diversity of multiple trophic groups in both temperate and tropical forests (e.g. [38, 104]) we
438	hypothesize that this might also be the case for the bark microbiome. Additionally, we were not able
	18

439 to capture all possible forest parameters that might contribute to the community assembly, as 440 evident from the percentage of unexplained variance. The tree species included in this study (Fagus 441 sylvatica, Picea abies and Pinus sylvestris) vary in additional features like bark texture, pH or 442 chemical composition, opening up other possibilities for niche differentiation even at the level of 443 individual trees. Furthermore, there may also be a seasonal influence on the microbiome (e.g. 444 [105]). Future studies would greatly benefit from including not only micro-niche parameters, but 445 also spatio-temporal data, to clarify the driving mechanisms further. 446 Beyond the addition of further deterministic factors, the unexplained variance also warrants 447 consideration of other, non-deterministic, assembly mechanisms at play. Zhou and Ning [106] state 448 that deterministic and stochastic processes are both of great importance for microbial communities, 449 and act in the same temporal space. Ecological stochasticity, including processes such as ecological 450 drift, diversification, death and "birth" but also random colonization events, are certainly also 451 influencing the bark microbiome. These processes are highly likely to shape the community 452 composition and manipulate the connection of the bark surface microbiome with other forest 453 compartments, e.g. through dispersal or "priority effects". Indeed, bark has already been shown to 454 increase the importance of stochastic processes for community dynamics in deadwood 455 microbiomes, especially in the early colonization stage [84]. 456 Another important aspect is of technical nature: the use of ASVs over OTUs. It is an ongoing debate which approach is more meaningful for obtaining diversity estimates, especially for 457 458 fungi. No consensus has been reached to date, with recent studies making compelling statements for either choice [107-110]. We decided to use ASVs because of the higher accuracy without imposing 459 460 arbitrary thresholds. To account for possible slight variations even within individuals and species, 461 especially when considering ITS as a marker, we employed best-practice tools like the LULU 462 algorithm [58]. Diversity estimates of any metabarcoding study, in our opinion, should be 463 interpreted with care and taken as the diversity of sequence variants.

Appendix - Publications

Conclusions

464

465

466

467

468

469

470

471

472

473

474

475

476

In this study we provide the first comprehensive assessment of the bark surface microbiome and its drivers. Our results can inform future hypothesis-driven research such as predictive modelling to assess the responses of the forest microbiome to future environmental conditions under climate change. We show that while abiotic factors influence the microbial communities, biotic interactions are usually more important, especially for community composition. Our study highlights the importance of integrating research on a diverse array of organisms if we want to understand the processes governing microbiome assembly. Combining our findings with results from other forest compartments will allow us to assess which taxa are shared between microbial habitats in forests and study how connections as well as dispersal, e.g., through stemflow, function among them. Lastly, future studies will benefit from the addition of functional information, e.g., through metatranscriptomics or -genomics, since the nature of the interactions remains hidden and difficult to identify.

477

478

Data availability

479 The raw reads have been deposited in the NCBI BioProject database

480 (https://www.ncbi.nlm.nih.gov/bioproject/) under BioProject accession number PRJNA932736,

481 SRA numbers SRR23371988 – SRR23371990. Species list, ASV tables and metadata can be found

482 in the Biodiversity Exploratories Information System (BExIS) (https://www.bexis.uni-jena.de/)

483 under Dataset IDs 31506, 31508 – 31512. Additional datasets used in, but not generated for this

484 study, are also available from BExIS under the accession numbers stated in Tab. 2. Dataset 19007

485 refers to the climate data which can be exported via the public climate data tool at

486 https://www.bexis.uni-jena.de/). The full script as well as intermediate data needed to replicate the

487 analysis are available at Zenodo [68] under doi: 10.5281/zenodo.10200121.

488

Competing Interests

490 The authors declare no competing interests.

492 References

- Baldrian P. Forest microbiome: diversity, complexity and dynamics. FEMS Microbiology Reviews 2017; 41: 109–130.
- 2. Schröter D, Wolters V, De Ruiter PC. C and N mineralisation in the decomposer food webs of a European forest transect. Oikos 2003; 102: 294–308.
- Clemmensen KE, Finlay RD, Dahlberg A, Stenlid J, Wardle DA, Lindahl BD. Carbon sequestration is related to mycorrhizal fungal community shifts during long-term succession in boreal forests. New Phytologist 2015; 205: 1525–1536.
- Uroz S, Buée M, Deveau A, Mieszkin S, Martin F. Ecology of the forest microbiome: Highlights of temperate and boreal ecosystems. Soil Biology and Biochemistry 2016; 103: 471–488.
- 5. Whitmore TC. Studies in systematic bark morphology. IV. The bark of beech, oak and sweet chestnut. New Phytologist 1963; 62: 161–169.
- 6. Vorholt JA. Microbial life in the phyllosphere. Nature Reviews Microbiology 2012; 10: 828–840.
- 7. Leff JW, Del Tredici P, Friedman WE, Fierer N. Spatial structuring of bacterial communities within individual G inkgo biloba trees. Environmental Microbiology 2015; 17: 2352–2361.
- 8. Arrigoni E, Antonielli L, Pindo M, Pertot I, Perazzolli M. Tissue age and plant genotype affect the microbiota of apple and pear bark. Microbiological Research 2018; 211: 57–68.
- Aguirre-von-Wobeser E, Alonso-Sánchez A, Méndez-Bravo A, Villanueva Espino LA,
 Reverchon F. Barks from avocado trees of different geographic locations have consistent microbial communities. Archives of Microbiology 2021; 203: 4593–4607.
- Dreyling L, Schmitt I, Dal Grande F. Tree Size Drives Diversity and Community Structure of Microbial Communities on the Bark of Beech (Fagus sylvatica). Frontiers in Forests and Global Change 2022; 5.
- Lindow SE, Brandl MT. Microbiology of the Phyllosphere. Applied and Environmental Microbiology 2003; 69: 1875–1883.

- Beyer WN, Saari DM. Activity and Ecological Distribution of the Slug, Arion subfuscus (Draparnaud) (Stylommatophora, Arionidae). American Midland Naturalist 1978; 100: 359–367.
- Erdmann G, Otte V, Langel R, Scheu S, Maraun M. The trophic structure of bark-living oribatid mite communities analysed with stable isotopes (15N, 13C) indicates strong niche differentiation. Experimental and Applied Acarology 2007; 41: 1–10.
- Baz A. Bark-Lice, Book-Lice or Psocids (Psocoptera). Encyclopedia of Entomology. 2008.
 Springer Netherlands, Dordrecht, pp 381–399.
- Anderson OR. Microbial Communities Associated with Tree Bark Foliose Lichens: A
 Perspective on their Microecology. Journal of Eukaryotic Microbiology 2014; 61: 364–370.
- Aschenbrenner IA, Cernava T, Erlacher A, Berg G, Grube M. Differential sharing and distinct co-occurrence networks among spatially close bacterial microbiota of bark, mosses and lichens. Molecular Ecology 2017; 26: 2826–2838.
- 17. Grimm M, Grube M, Schiefelbein U, Zühlke D, Bernhardt J, Riedel K. The Lichens' Microbiota, Still a Mystery? Frontiers in Microbiology 2021; 12.
- 18. Magyar D, Van Stan JT, Sridhar KR. Hypothesis and Theory: Fungal Spores in Stemflow and Potential Bark Sources. Frontiers in Forests and Global Change 2021; 4.
- Deveau A, Bonito G, Uehling J, Paoletti M, Becker M, Bindschedler S, et al. Bacterial– fungal interactions: ecology, mechanisms and challenges. FEMS Microbiology Reviews 2018; 42: 335–352.
- 20. Ramanan R, Kim B-H, Cho D-H, Oh H-M, Kim H-S. Algae–bacteria interactions: Evolution, ecology and emerging applications. Biotechnology Advances 2016; 34: 14–29.
- Durán P, Flores-Uribe J, Wippel K, Zhang P, Guan R, Melkonian B, et al. Shared features and reciprocal complementation of the Chlamydomonas and Arabidopsis microbiota. Nature Communications 2022; 13: 406–406.
- Gonzalez LE, Bashan Y. Increased Growth of the Microalga Chlorella vulgaris when Coimmobilized and Cocultured in Alginate Beads with the Plant-Growth-Promoting Bacterium Azospirillum brasilense. Applied and Environmental Microbiology 2000; 66: 1527–1531.

- Wang X, Li Z, Su J, Tian Y, Ning X, Hong H, et al. Lysis of a red-tide causing alga,
 Alexandrium tamarense, caused by bacteria from its phycosphere. Biological Control 2010;
 52: 123–130.
- Krespach MKC, García-Altares M, Flak M, Hanno Schoeler, Scherlach K, Netzker T, et al. Lichen-like association of Chlamydomonas reinhardtii and Aspergillus nidulans protects algal cells from bacteria. The ISME Journal 2020; 14: 2794–2805.
- Nybakken L, Solhaug KA, Bilger W, Gauslaa Y. The lichens Xanthoria elegans and Cetraria islandica maintain a high protection against UV-B radiation in Arctic habitats. Oecologia 2004; 140: 211–216.
- 26. Hawksworth DL, Grube M. Lichens redefined as complex ecosystems. New Phytologist 2020; 227: 1281–1283.
- Kohlmeier S, Smits THM, Ford RM, Keel C, Harms H, Wick LY. Taking the Fungal Highway: Mobilization of Pollutant-Degrading Bacteria by Fungi. Environmental Science & Technology 2005; 39: 4640–4646.
- 28. Agler MT, Ruhe J, Kroll S, Morhenn C, Kim S-T, Weigel D, et al. Microbial Hub Taxa Link Host and Abiotic Factors to Plant Microbiome Variation. PLOS Biology 2016; 14: e1002352–e1002352.
- 29. Liu B-W, Li S-Y, Zhu H, Liu G-X. Phyllosphere eukaryotic microalgal communities in rainforests: Drivers and diversity. Plant Diversity 2023; 45: 45–53.
- Perazzolli M, Antonielli L, Storari M, Puopolo G, Pancher M, Giovannini O, et al. Resilience
 of the Natural Phyllosphere Microbiota of the Grapevine to Chemical and Biological
 Pesticides. Applied and Environmental Microbiology 2014; 80: 3585–3596.
- 31. Jacobs JL, Sundin GW. Effect of Solar UV-B Radiation on a Phyllosphere Bacterial Community. Applied and Environmental Microbiology 2001; 67: 5488–5496.
- Neustupa J, Štifterová A. Distribution patterns of subaerial corticolous microalgae in two European regions. Plant Ecology and Evolution 2013; 146: 279–289.
- 33. Arrigoni E, Albanese D, Longa CMO, Angeli D, Donati C, Ioriatti C, et al. Tissue age, orchard location and disease management influence the composition of fungal and bacterial

- communities present on the bark of apple trees. Environmental Microbiology 2020; 22: 2080–2093.
- de Vries FT, Griffiths RI, Bailey M, Craig H, Girlanda M, Gweon HS, et al. Soil bacterial networks are less stable under drought than fungal networks. Nature Communications 2018; 9: 3033–3033.
- 35. Martins G, Lauga B, Miot-Sertier C, Mercier A, Lonvaud A, Soulas M-L, et al.

 Characterization of Epiphytic Bacterial Communities from Grapes, Leaves, Bark and Soil of Grapevine Plants Grown, and Their Relations. PLoS ONE 2013; 8: e73013–e73013.
- Kobayashi K, Aoyagi H. Microbial community structure analysis in Acer palmatum bark and isolation of novel bacteria IAD-21 of the candidate division FBP. PeerJ 2019; 7: e7876– e7876.
- 37. Petrolli R, Augusto Vieira C, Jakalski M, Bocayuva MF, Vallé C, Cruz EDS, et al. A fine-scale spatial analysis of fungal communities on tropical tree bark unveils the epiphytic rhizosphere in orchids. New Phytologist 2021; 231: 2002–2014.
- 38. Penone C, Allan E, Soliveres S, Felipe-Lucia MR, Gossner MM, Seibold S, et al. Specialisation and diversity of multiple trophic groups are promoted by different forest features. Ecology Letters 2018; 22: 170–180.
- Liang Y, Xiao X, Nuccio EE, Yuan M, Zhang N, Xue K, et al. Differentiation strategies of soil rare and abundant microbial taxa in response to changing climatic regimes.
 Environmental Microbiology 2020; 22: 1327–1340.
- Gossner MM, Lewinsohn TM, Kahl T, Grassein F, Boch S, Prati D, et al. Land-use intensification causes multitrophic homogenization of grassland communities. Nature 2016; 540: 266–269.
- 41. Fischer M, Bossdorf O, Gockel S, Hänsel F, Hemp A, Hessenmöller D, et al. Implementing large-scale and long-term functional biodiversity research: The Biodiversity Exploratories. Basic and Applied Ecology 2010; 11: 473–485.
- 42. Schall P, Ammer C. Stand composition based on 2nd forest inventory (abundance, basal area, crown projection area and volume) on all forest EPs, 2014 2018. Version 2. Biodiversity Exploratories Information System. Dataset. 2018.

- 43. Camacho-Sanchez M, Burraco P, Gomez-Mestre I, Leonard JA. Preservation of RNA and DNA from mammal samples under field conditions. Molecular Ecology Resources 2013; 13: 663–673.
- 44. Menke S, Gillingham MAF, Wilhelm K, Sommer S. Home-Made Cost Effective Preservation Buffer Is a Better Alternative to Commercial Preservation Methods for Microbiome Research. Frontiers in Microbiology 2017; 8: 102–102.
- 45. Cheng T, Xu C, Lei L, Li C, Zhang Y, Zhou S. Barcoding the kingdom Plantae: new PCR primers for ITS regions of plants with improved universality and specificity. Molecular Ecology Resources 2016; 16: 138–149.
- 46. Ihrmark K, Bödeker ITM, Cruz-Martinez K, Friberg H, Kubartova A, Schenck J, et al. New primers to amplify the fungal ITS2 region evaluation by 454-sequencing of artificial and natural communities. FEMS Microbiology Ecology 2012; 82: 666–677.
- 47. White TJ, Bruns T, Lee S, Taylor J. Amplification and direct sequencing of fungal ribosomal rna genes for phylogenetics. In: Innis M, Gelfland DH, Sninsky JJ, White TJ (eds). PCR Protocols. 1990. Academic Press, San Diego, CA, pp 315–322.
- 48. Muyzer G, de Waal EC, Uitterlinden AG. Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. Applied and environmental microbiology 1993; 59: 695–700.
- Vieira S, Sikorski J, Dietz S, Herz K, Schrumpf M, Bruelheide H, et al. Drivers of the composition of active rhizosphere bacterial communities in temperate grasslands. The ISME Journal 2020; 14: 463–475.
- Herlemann DP, Labrenz M, Jürgens K, Bertilsson S, Waniek JJ, Andersson AF. Transitions in bacterial communities along the 2000 km salinity gradient of the Baltic Sea. The ISME Journal 2011; 5: 1571–1579.
- Schnell IB, Bohmann K, Gilbert MTP. Tag jumps illuminated reducing sequence-to-sample misidentifications in metabarcoding studies. Molecular Ecology Resources 2015; 15: 1289– 1303.
- 52. Martin M. Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet.journal 2011; 17: 10–10.

- Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP. DADA2: Highresolution sample inference from Illumina amplicon data. Nature Methods 2016; 13: 581– 583.
- 54. Abarenkov K, Zirk A, Piirmann T, Pöhönen R, Ivanov F, Nilsson RH, et al. UNITE general FASTA release for eukaryotes. Version 27.10.2022. UNITE Community 2022.
- 55. Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, et al. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Research 2012; 41: D590–D596.
- 56. Sherrill-Mix S. taxonomizr: Functions to Work with NCBI Accessions and Taxonomy. 2022.
- Davis NM, Proctor DM, Holmes SP, Relman DA, Callahan BJ. Simple statistical identification and removal of contaminant sequences in marker-gene and metagenomics data. Microbiome 2018; 6: 226–226.
- 58. Frøslev TG, Kjøller R, Bruun HH, Ejrnæs R, Brunbjerg AK, Pietroni C, et al. Algorithm for post-clustering curation of DNA amplicon data yields reliable biodiversity estimates. Nature Communications 2017; 8: 1188–1188.
- 59. R Core Team. R: A Language and Environment for Statistical Computing. 2022.
- RStudio Team. RStudio: Integrated Development Environment for R. 2021. Boston, MA, USA.
- 61. McMurdie PJ, Holmes S. phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. PLoS ONE 2013; 8: e61217–e61217.
- 62. Wickham H. ggplot2: Elegant Graphics for Data Analysis. 2016. Springer-Verlag, New York.
- 63. Kassambara A. ggpubr: 'ggplot2' Based Publication Ready Plots. 2020.
- 64. Lahti L, Shetty S. microbiome Tools for microbiome analysis in R. 2017.
- 65. Teunisse GM. Fantaxtic Fantaxtic plots for phyloseq objects! 2017.
- 66. Barnett DJM, Arts ICW, Penders J. microViz: an R package for microbiome data visualization and statistics. Journal of Open Source Software 2021; 6: 3201.
- 67. McMurdie PJ, Holmes S. Waste Not, Want Not: Why Rarefying Microbiome Data Is Inadmissible. PLoS Computational Biology 2014; 10: e1003531–e1003531.

- Dreyling L, Penone C, Schenk N, Schmitt I, Dal Grande F. Biotic interactions outweigh
 abiotic factors as drivers of bark microbial communities in Central European forests. Dataset
 & Code: Version 1.0.0. 2023.
- Hill MO. Diversity and Evenness: A Unifying Notation and Its Consequences. Ecology 1973;
 427–432.
- Chao A, Chiu C-H, Jost L. Unifying Species Diversity, Phylogenetic Diversity, Functional Diversity, and Related Similarity and Differentiation Measures Through Hill Numbers.
 Annual Review of Ecology, Evolution, and Systematics 2014; 45: 297–324.
- 71. Li D. hillR: taxonomic, functional, and phylogenetic diversity and similarity through Hill Numbers. Journal of Open Source Software 2018; 3: 1041–1041.
- Whittaker RH. Vegetation of the Siskiyou Mountains, Oregon and California. Ecological Monographs 1960; 30: 279–338.
- 73. Chao A, Chiu C-H, Hsieh TC. Proposing a resolution to debates on diversity partitioning. Ecology 2012; 93: 2037–2051.
- 74. Alberdi A, Gilbert MTP. A guide to the application of Hill numbers to DNA-based diversity analyses. Molecular Ecology Resources 2019; 19: 804–817.
- 75. Ferrier S. Mapping Spatial Pattern in Biodiversity for Regional Conservation Planning: Where to from Here? Systematic Biology 2002; 51: 331–363.
- Ferrier S, Powell GVN, Richardson KS, Manion G, Overton JM, Allnutt TF, et al. Mapping More of Terrestrial Biodiversity for Global Conservation Assessment. BioScience 2004; 54: 1101–1109.
- 77. Ferrier S, Manion G, Elith J, Richardson K. Using generalized dissimilarity modelling to analyse and predict patterns of beta diversity in regional biodiversity assessment. Diversity and Distributions 2007; 13: 252–264.
- 78. Mokany K, Ware C, Woolley SNC, Ferrier S, Fitzpatrick MC. A working guide to harnessing generalized dissimilarity modelling for biodiversity analysis and conservation assessment. Global Ecology and Biogeography 2022; 31: 802–821.
- 79. Fitzpatrick M, Mokany K, Manion G, Nieto-Lugilde D, Ferrier S. gdm: Generalized Dissimilarity Modeling. 2022.

- Hofmann B, Dreyling L, Dal Grande F, Otte J, Schmitt I. Habitat and tree species identity shape aboveground and belowground fungal communities in central European forests.
 Frontiers in Microbiology 2023; 14.
- 81. Coleine C, Stajich JE, Zucconi L, Onofri S, Pombubpa N, Egidi E, et al. Antarctic Cryptoendolithic Fungal Communities Are Highly Adapted and Dominated by Lecanoromycetes and Dothideomycetes. Frontiers in Microbiology 2018; 9.
- 82. Krah F, March-Salas M. eDNA metabarcoding reveals high soil fungal diversity and variation in community composition among Spanish cliffs. Ecology and Evolution 2022; 12.
- 83. Vitulo N, Lemos WJF, Calgaro M, Confalone M, Felis GE, Zapparoli G, et al. Bark and Grape Microbiome of Vitis vinifera: Influence of Geographic Patterns and Agronomic Management on Bacterial Diversity. Frontiers in Microbiology 2019; 9.
- 84. Hagge J, Bässler C, Gruppe A, Hoppe B, Kellner H, Krah F-S, et al. Bark coverage shifts assembly processes of microbial decomposer communities in dead wood. Proceedings of the Royal Society B: Biological Sciences 2019; 286: 20191744–20191744.
- 85. Castaño C, Lindahl BD, Alday JG, Hagenbo A, Martínez de Aragón J, Parladé J, et al. Soil microclimate changes affect soil fungal communities in a Mediterranean pine forest. New Phytologist 2018; 220: 1211–1221.
- 86. Bazany KE, Wang J, Delgado-Baquerizo M, Singh BK, Trivedi P. Water deficit affects interkingdom microbial connections in plant rhizosphere. Environmental Microbiology 2022; 24: 3722–3734.
- 87. Delgado-Baquerizo M, Eldridge DJ. Cross-Biome Drivers of Soil Bacterial Alpha Diversity on a Worldwide Scale. Ecosystems 2019; 22: 1220–1231.
- 88. Holzinger A, Lütz C. Algae and UV irradiation: Effects on ultrastructure and related metabolic functions. Micron 2006; 37: 190–207.
- Mutschlechner M, Walter A, Colleselli L, Griesbeck C, Schöbel H. Enhancing carotenogenesis in terrestrial microalgae by UV-A light stress. Journal of Applied Phycology 2022; 34: 1943–1955.

- 90. Wong HJ, Mohamad-Fauzi N, Rizman-Idid M, Convey P, Alias SA. Protective mechanisms and responses of micro-fungi towards ultraviolet-induced cellular damage. Polar Science 2019; 20: 19–34.
- 91. Fiore-Donno AM, Freudenthal J, Dahl MB, Rixen C, Urich T, Bonkowski M. Biotic interactions explain seasonal dynamics of the alpine soil microbiome. bioRxiv 2023; 2023.04.17.537150-2023.04.17.537150.
- 92. Aburai N, Tsukagoshi T, Sekiguchi S, Arakawa H, Imamura Y, Abe K. Mutual supply of carbon and nitrogen sources in the co-culture of aerial microalgae and nitrogen-fixing bacteria. Algal Research 2023; 70: 103001–103001.
- 93. Warmink JA, van Elsas JD. Selection of bacterial populations in the mycosphere of Laccaria proxima: is type III secretion involved? The ISME Journal 2008; 2: 887–900.
- 94. Venkatesh N, Greco C, Drott MT, Koss MJ, Ludwikoski I, Keller NM, et al. Bacterial hitchhikers derive benefits from fungal housing. Current Biology 2022; 32: 1523-1533.e6.
- 95. Ballhausen M-B, de Boer W. The sapro-rhizosphere: Carbon flow from saprotrophic fungi into fungus-feeding bacteria. Soil Biology and Biochemistry 2016; 102: 14–17.
- 96. Schwedener S. Ueber die Beziehungen zwischen Algen und Flechtengonidien. Botanische Zeitung (Berlin) 1886; 289–292.
- 97. Hom EFY, Murray AW. Niche engineering demonstrates a latent capacity for fungal-algal mutualism. Science 2014; 345: 94–98.
- 98. Landeweert R, Hoffland E, Finlay RD, Kuyper TW, van Breemen N. Linking plants to rocks: ectomycorrhizal fungi mobilize nutrients from minerals. Trends in Ecology & Evolution 2001; 16: 248–254.
- Pauwels R, Graefe J, Bitterlich M. An arbuscular mycorrhizal fungus alters soil water retention and hydraulic conductivity in a soil texture specific way. Mycorrhiza 2023; 33: 165–179.
- 100. Afkhami ME, McIntyre PJ, Strauss SY. Mutualist-mediated effects on species' range limits across large geographic scales. Ecology Letters 2014; 17: 1265–1273.
- 101. Klaus VH, Hölzel N, Boch S, Müller J, Socher AS, Prati D, et al. Direct and indirect associations between plant species richness and productivity in grasslands: regional 30

- differences preclude simple generalization of productivity-biodiversity relationships. Preslia 2013; 85: 97–112.
- 102. Simons NK, Gossner MM, Lewinsohn TM, Boch S, Lange M, Müller J, et al. Resource-Mediated Indirect Effects of Grassland Management on Arthropod Diversity. PLoS ONE 2014; 9: e107033–e107033.
- 103. Walters KE, Capocchi JK, Albright MBN, Hao Z, Brodie EL, Martiny JBH. Routes and rates of bacterial dispersal impact surface soil microbiome composition and functioning. The ISME Journal 2022; 16: 2295–2304.
- 104. Fornoff F, Staab M, Zhu C-D, Klein A-M. Multi-trophic communities re-establish with canopy cover and microclimate in a subtropical forest biodiversity experiment. Oecologia 2021; 196: 289–301.
- 105. Beck A, Peršoh D, Rambold G. First evidence for seasonal fluctuations in lichen- and bark-colonising fungal communities. Folia Microbiologica 2014; 59: 155–157.
- 106. Zhou J, Ning D. Stochastic Community Assembly: Does It Matter in Microbial Ecology? Microbiology and Molecular Biology Reviews 2017; 81: 10.1128/mmbr.00002-17.
- 107. Joos L, Beirinckx S, Haegeman A, Debode J, Vandecasteele B, Baeyen S, et al. Daring to be differential: metabarcoding analysis of soil and plant-related microbial communities using amplicon sequence variants and operational taxonomical units. BMC Genomics 2020; 21: 733.
- 108. Cholet F, Lisik A, Agogué H, Ijaz UZ, Pineau P, Lachaussée N, et al. Ecological Observations Based on Functional Gene Sequencing Are Sensitive to the Amplicon Processing Method. mSphere 2022; 7: e00324-22.
- 109. Tedersoo L, Bahram M, Zinger L, Nilsson RH, Kennedy PG, Yang T, et al. Best practices in metabarcoding of fungi: From experimental design to results. Molecular Ecology 2022; 31: 2769–2795.
- 110. Kauserud H. ITS alchemy: On the use of ITS as a DNA marker in fungal ecology. Fungal Ecology 2023; 65: 101274.

Acknowledgements

493

494 We thank the managers of the three Exploratories, Max Müller, Julia Bass, Robert Künast, Anna K. Franke, Franca Marian and all former managers for their work in maintaining the plot and project 495 496 infrastructure; Victoria Grießmeier for giving support through the central office, Andreas Ostrowski 497 for managing the central data base, and Markus Fischer, Eduard Linsenmair, Dominik 498 Hessenmöller, Daniel Prati, Ingo Schöning, François Buscot, Ernst-Detlef Schulze, Wolfgang W. 499 Weisser and the late Elisabeth Kalko for their role in setting up the Biodiversity Exploratories project. We thank the administration of the Hainich national park, the UNESCO Biosphere Reserve 500 501 Swabian Alb and the UNESCO Biosphere Reserve Schorfheide-Chorin as well as all land owners for the excellent collaboration. The work has been (partly) funded by the DFG Priority Program 502 1374 "Biodiversity- Exploratories" (SCHM 1711/8-1). Field work permits were issued by the 503 504 responsible state environmental offices of Baden-Württemberg, Thüringen, and Brandenburg.

505 **Figure Captions** 506 Figure 1: Community composition of algal, fungal, and bacterial communities associated with bark 507 surfaces in three study regions. Given is the relative abundance of orders per plot. Each bar 508 represents one plot. Only the 25 most abundant orders per organismal group are included. 509 510 Figure 2: Drivers of algal, fungal, and bacterial communities associated with bark surfaces. Graphs 511 indicate the effects of biotic and abiotic variables on three α -diversity measures (hill number q = 0, 512 1 and 2) of all three organismal groups. These are the results of linear models. Axes are scaled. Asterisks indicate Benjamini-Hochberg corrected p-values (* = < 0.2, ** = < 0.1, *** = < 0.05). For 513 514 an explanation of the variables see Tab. 2. 515 516 Figure 3: Contribution of different drivers to total observed variance in alpha and beta diversities. 517 Abiotic variables (a), biotic variables (b), geographic distance (g), and combinations of these drivers 518 explain part of the overall variance. Negative variance in the linear models is explained by the two 519 factors having opposing effects on the response. 520 521 Figure 4: Drivers of algal, fungal, and bacterial communities associated with bark surfaces. Graphs 522 indicate the effects of biotic and abiotic variables on three β -diversity measures (hill number q = 0, 1 and 2) of all three organismal groups. These are the results of Generalized Dissimilarity 523 524 Modeling. The higher the curves maximum is, the larger the effect of the explanatory variable. The 525 form and slope of the curve indicates how rapid changes in β-diversity are in relation to outside 526 influences. Asterisks indicate Benjamini-Hochberg corrected p-values (* = < 0.2, ** = <0.1, *** =

< 0.05). For an explanation of the variables see Tab. 2.

Table captions

- 529 Table 1: Cycling conditions for all three organismal groups. Differences are highlighted in bold. A =
- 530 Algae, F = Fungi, B = Bacteria.
- 531 Table 2: Drivers of community composition tested in this study. Given are the tested variables, their
- 532 measurements, descriptions, and data sources, as well as the direction of the effects obtained from
- 533 linear models of β-diversity. Only the direction of significant (p < 0.2) effects from the Generalized
- 534 Dissimilarity Models are shown. A = Algae, F = Fungi and B = Bacteria. q = 0, 1, 2 correspond to
- 535 the Hill number. All datasets can be found in the "Biodiversity Exploratories Information System
- 536 (BExIS)" at https://www.bexis.uni-jena.de/.
- Table 3: Number of ASVs per organism group in total, and split by study region.
- 538 Table 4: Importance of the tested variables for the prediction of algal (A), fungal (F), and bacterial
- 539 (B) beta diversity on all three levels of q. The most important predictors are highlighted in bold;
- 540 asterisks indicate significance of predictors after Benjamini-Hochberg correction in the Generalized
- 541 Dissimilarity Modeling. * p < 0.2, ** p < 0.1 and *** p < 0.05

Figure 1

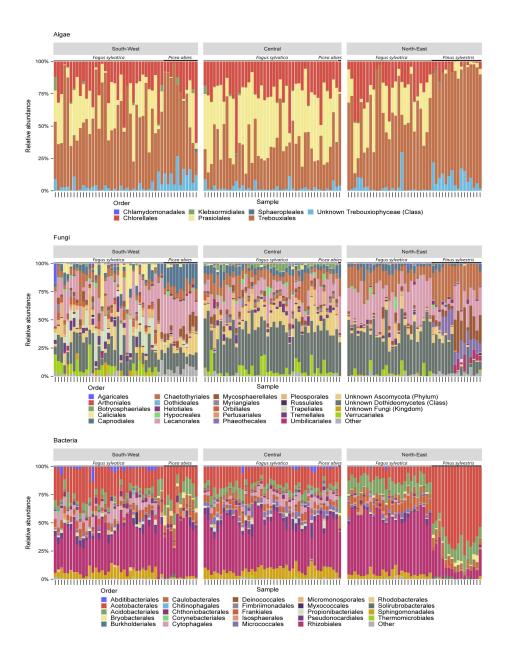


Figure 2

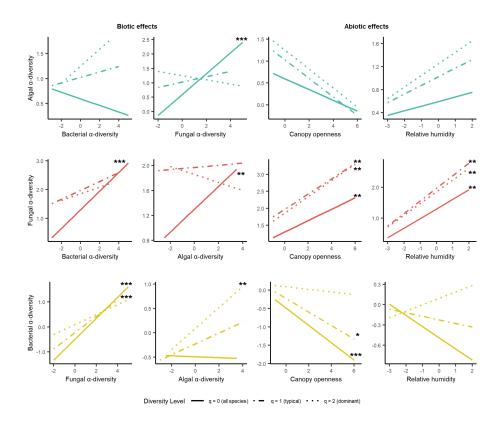


Figure 3

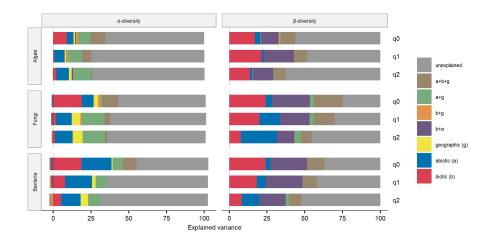


Figure 4

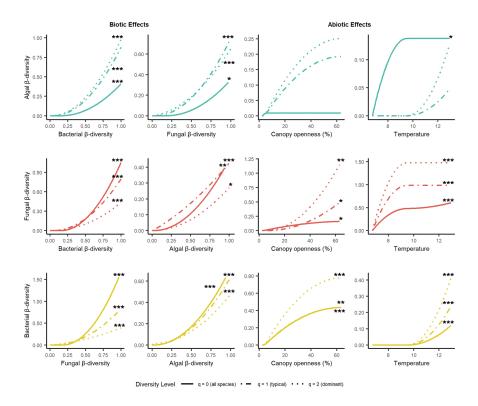


Table 1

Phase	Temperature	Duration	Number of cycles
Initial Denaturation	95 °C	60 s	1
Denaturation	95 °C	15 s	A: 30
Annealing	A: 54 °C, F: 56 °C, B: 59 °C	15 s	F: 35
Elongation	72 °C	10 s	B: 30
Final Extension	72 °C	60 s	1

Table 2

Variable	Measurement	Proxy for	Source		Effect direction (β-diversity)								
					q = 0			q = 1			q = 2		
				A	F	В	A	F	В	A	F	В	
Abiotic factors:													
Relative humidity	Relative humidity at 2m above ground measured using a Rotronic HC-S3 probe. Averaged over the two weeks before and the week of sampling.	Water availability from the air.	BExIS dataset ID 19007 accessible through the public climate data search	1				1			1		
Temperature	Air temperature in °C at 2m above ground measured using a Rotronic HC-S3 probe. Averaged over the two weeks before and the week of sampling.	Growing conditions.	BExIS dataset ID 19007 accessible through the public climate data search	1	1	1		1	1		1	1	
Average DBH	Average diameter at breast height of all trees in the plot. Tree dependent variable.	Average age of the trees.	BExIS dataset ID 22766										
Canopy openness	Percentage of pixels classified as "Sky" after LIDAR scanning. Tree dependent variable.	Light availability.	BExIS dataset ID 27828		1	1		1	1		1	1	
Gini coefficient	Mean heterogeneity of the tree size in the plot. Tree dependent variable.	Mixture of old and young trees.	BExIS dataset ID 22766			1			1			1	
Stand density	Based on basal area (m²/ha). Measures the area of the plot is covered by tree trunks. Tree dependent variable.	aConnectedness of the potential habitat.	BEXIS dataset ID 22766						1			1	
Ratio of dominant trees	Percentage of the most abundant tree species in the plot. Tree dependent variable.	Availability of suitable host trees for sampled communities.	Based on BExIS dataset ID 21426						\downarrow				
Forest area	Percentage of forest in a 2000 m ² buffer around the plot.	Connection at the landscape scale.	BExIS dataset ID 15929								1		
Geographic factors	•												
Region (LM) and Geographic distance (GDM) Biotic factors	Distance between the plots.	Regional differences in available recruitment pool.	BExIS dataset ID 1000										
Algal diversity	Measured as Hill number $q = 0, 1 \& 2$.	Biotic interactions.	Calculated from BEXIS dataset ID 31511		1	1		1	1		1	1	
Fungal diversity	Measured as Hill number $q = 0, 1 \& 2$.	Biotic interactions.	Calculated from BExIS dataset ID 31510	1		1	1		1	1		1	
Bacterial diversity	Measured as Hill number $q = 0, 1 \& 2$.	Biotic interactions.	Calculated from BEXIS dataset ID 31512	1	1		1	1		1	1		

Table 3

Organism Group	Total # of ASVs	Swabian Alb	Hainich-Dün	Schorfheide-Chorin			
		(South-West)	(Central)	(North-East)			
Algae	131	99	100	76			
Fungi	1750	1123	775	763			
Bacteria	1263	787	666	541			

Table 4

	Predictor importance for β-diversity									
Variable	Q = 0			Q = 1			Q =2			
	A	F	В	A	F	В	A	F	В	
Biotic Factors										
Algal diversity	X	1.47**	2.73***	X	3.42***	6.69***	X	2.79*	7.31***	
Fungal diversity	3.30***	X	19.64***	8.67***	X	9.68***	10.28***	X	4.41***	
Bacterial diversity	7.95**	17.52***	X	11.66***	10.32***	X	16.50***	5.20***	X	
Abiotic Factors										
Geographic	0.15*	0	0	0.04	0.01	0	0.36***	0	0	
Relative humidity	2.57*	0.02	0	0	1.03**	0.03	0	1.91**	0.32	
Temperature	1.89*	6.51***	0.85***	0.13	12.85***	4.31***	0.55*	27.15***	11.79***	
Average DBH	0.23	0.06	0	0.23	0.03	0	0.55	0.03	0.09	
Canopy openness	0.04	0.46*	2.51**	0.54	1.33*	2.40***	0.61	4.31**	6.82***	
Gini coefficient	0.64	0.26	0.58*	0.41	0.25	1.02***	0.48	0.13	1.71***	
Stand density	0	0.04	0.20	0.22	0.08	1.04*	0.38	0.10	3.15***	
Ratio of dominant trees	0.78	0.07	0.13	0.28	0.03	0.51*	0.33	0.13	0.51	
Forest area	0.66	0.19	0.05	0	0.33	0.36	0	1.40*	0.52	

Supplementary material to

Dreyling, L., Penone, C., Schenk, N., Schmitt, I. & Dal Grande, F.: Biotic interactions outweigh abiotic factors as drivers of bark microbial communities in Central European forests. (submitted)

- Figure S1: Additional tested drivers of algal, fungal, and bacterial communities associated with bark surfaces. Graphs indicate the effects of biotic and abiotic variables on three α -diversity measures (hill number q=0, 1 and 2) of all three organismal groups. These are the results of linear models. Axes are scaled. Asterisks indicate Benjamini-Hochberg corrected p-values (* = < 0.2, ** = <0.1, *** = < 0.05). For an explanation of the variables see Tab. 2.
- Figure S2: Additional tested drivers of algal, fungal, and bacterial communities associated with bark surfaces. Graphs indicate the effects of biotic and abiotic variables on three β -diversity measures (hill number q = 0, 1 and 2) of all three organismal groups. These are the results of Generalized Dissimilarity Modeling. The higher the curves maximum is, the larger the effect of the explanatory variable. The form and slope of the curve indicates how rapid changes in β -diversity are in relation to outside influences. Asterisks indicate Benjamini-Hochberg corrected p-values (* = < 0.2, ** = <0.1, *** = < 0.05). For an explanation of the variables see Tab.
- Table S1: ASV numbers tracked through our assignment and data cleaning pipeline.

 Our sequencing runs contained DNA from soil and bark samples that thus needed to be jointly processed until they could be split after curation and filtering.
- Table S2: A table of the samples and their associated values of the environmental variables measured in the plots and tested in this study.
- Table S3: Model estimates, standard errors, p-values and adjusted p-values for each organismal group and diversity level.

Figure S1

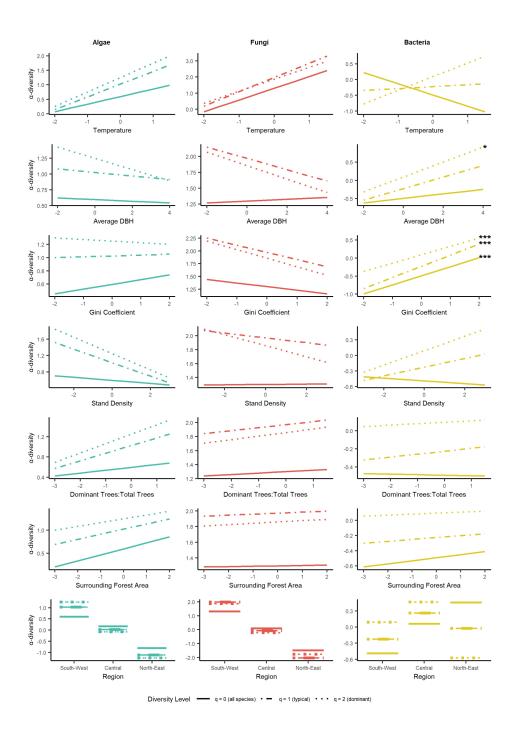


Figure S2

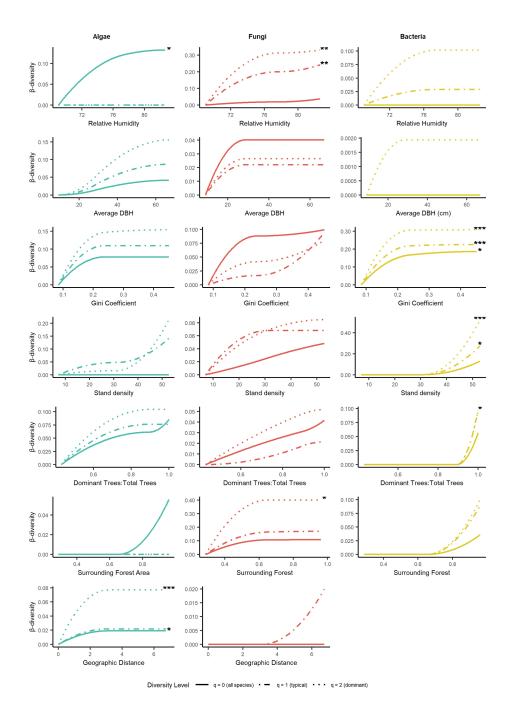


Table S1

	Algae	Fungi	Bacteria
ASV table processing			
Raw ASVs	12,911	18,300	9,692
After contaminant removal	12,896	18,280	9,684
After LULU curation	8,091	11,981	4,352
Taxonomy table processing			
Before filtering	12,340	18,300	9,692
After filtering uncultured assignments	4,994	Not applicable	Not applicable
After filtering at 95% similarity	3,153	Not applicable	Not applicable
After filtering for organism group	966	16,385	7,131
Combining the tables			
After combining curated ASV table and taxonomy table	536	9,645	3,019
Taxa in bark samples (included in this study)	131	1,750	1,263
Taxa in soil samples (excluded from this study)	514	8,680	1,815

Table S2

						tary_table2						
Sample_ID exploratory Sample_A1_B Swabian Alb	dominant_tree Picea abies	74.54	7.61	27.34	9.89	0.16	44.35	_of_dominant_trees fores 0.93	0.5	4150	28504	13002
Sample_A1_B Swabian Alb Sample_A1_B Swabian Alb Sample_A11_B Swabian Alb Sample_A12_B Swabian Alb Sample_A13_B Swabian Alb	Picea abies Picea abies Picea abies	78.85 76.11 79.29	6.81 7.39 6.94 7.27	23.8 37.68	14.5 10.43	0.19 0.13 0.15	45.19 51.93	0.93 0.98 0.98	0.67	13330 20971 17644	14282 22004	9981 9586 8406
Sample_A12_B Swabian Alb Sample_A13_B Swabian Alb	Picea abies	78.31	6.94 7.27	37.68 39.6 39.71	10.43 17.32 11.63	0.18	51.93 42.37 52.82	0.87	0.58 0.49 0.65	24099	22004 17308 18434	16003
	Picea abies Fagus sylvatica	80.56 76.22	6.74	39.38	17.86	0.25 0.15	41.37	0.8 0.59	0.59	23214 10395	14911	12725 13395
Sampte_A15_B Swabian Alb Sampte_A16_B Swabian Alb Sampte_A17_B Swabian Alb Sampte_A18_B Swabian Alb	Fagus sylvatica Fagus sylvatica Fagus sylvatica	70.01 71.49 74.12	8.23 7.59 7.68	12.09 15.16	2.68 2.7 15.02	0.23 0.21 0.29	19.5 34.73 23.36	0.98 0.98 0.71	0.56	22105 19298 20852	16159 17603 12480	15828 16804 6634
	Picea abies	77.92	7.68 6.89 7.99	12.09 15.16 37.74 34.06		0.19		0.97	0.56 0.74 0.52 0.6		9770	
Sample_A20_B Swabian Alb Sample_A21_B Swabian Alb	Fagus sylvatica Fagus sylvatica	74.06 71.47	7.99 8.23	41.14 27.14	5.41 3.78	0.2 0.4 0.2 0.44 0.21 0.14	28.57 26.36	0.8 0.47		29113 6927	23632 10539	11645 7215
Sampte_A21_B Swabian Alb Sampte_A22_B Swabian Alb Sampte_A23_B Swabian Alb Sampte_A24_B Swabian Alb	Fagus sylvatica Fagus sylvatica Fagus sylvatica	72.06 70.41	8.23 7.95 7.97 8.42 8.54	37.16 24.8 12.61	3.78 9.28 10.35	0.2	26.36 26.83 22.6	0.89 0.65	0.55 0.57 0.47 0.57	34726 22317	14839 17020	7215 12025 13864
	Fagus sylvatica	70.06 70	8.42 8.54	12.61 9.79	2.67 2.42	0.21	28.61 17.99	0.43 0.77	0.57	22464 18975	17359 16277	12387 13428
Sample_A26_B Swabian Alb Sample_A27_B Swabian Alb	Fagus sylvatica	72.51	7.89	13.19	12.20	0.24	12.60	0.75		12020	15002	9726
Sample_A26_B Swabian Alb Sample_A27_B Swabian Alb Sample_A28_B Swabian Alb Sample_A28_B Swabian Alb Sample_A3_B Swabian Alb	Fagus sylvatica Fagus sylvatica Fagus sylvatica Picea abies	70.87 70.8 73.52 79.44	8.29 7.7 7.2	23.81 32.4 31.6	2.63 5.81 10.68	0.19 0.28 0.19	23.35 29.59 35.28 43.26	0.6 0.79 0.58	0.43 0.54 0.62 0.55	19922 44252 25324 15349	12007 15338 24630	11204 14783 16098
		79.44 75.34	7.2 7.51	31.6 22.35	15.95 9.77	0.17	43.26 27.53	0.94 0.87	0.55	15349 25560	20423 24017	12337 13400
Cample A21 B Carehian Alb	Fagus sylvatica Picea abies	76.96	7.99	20.44	20.52	0.14	42.50	1	0.66	26679	25104	12055
Sample_A32_B Swabian Alb Sample_A33_B Swabian Alb Sample_A34_B Swabian Alb	Picea abies Picea abies Picea abies	75.84 73.98 75.55	7.44 7.38 7.48 8.44	24.02 25.05 29.33 10.74	15.36 19.91 16.57	0.18 0.17 0.13	30.75 37.63	0.91 0.95	0.54 0.47 0.41	13356 22572 8811	14971 20226 14197	11847 13862 10279
Sample_A35_B Swabian Alb			8.44 7.62	10.74 15.47	2.57 8.67	0.17 0.38	39 13.06 18.26	0.5 0.51	0.57	16658 19388	12474 7587	12553 11766
Cample A27 B Cambian Alb	Fagus sylvatica Fagus sylvatica Fagus sylvatica Fagus sylvatica	74.84	0.70	12.12	2.42		14.02	0.65	0.51	24220	15000	16069 12039
Sample_A38_B Swabian Alb Sample_A39_B Swabian Alb Sample_A4_B Swabian Alb	Fagus sylvatica Fagus sylvatica	71.96 71.19 72.46	8.17 8.19 7.82	12.01 17.24 13.73	5.02 2.89	0.19 0.26 0.18	20.63 29.82 32.48	0.92 0.98 0.92	0.54 0.68 0.71	23978 9793 10922	6988 17038 13689	11147
Sample_A40_B Swabian Alb Sample_A41_B Swabian Alb			8.44	35.41 33.25	2.77 5.75 3.82	0.22	29.72 31.74	0.92 0.87 0.91	0.62	5884 26951	13484 14463	11261 7996 12268
Comple A42 D. Combine Alb	Fagus sylvatica Fagus sylvatica Fagus sylvatica Fagus sylvatica Fagus sylvatica Fagus sylvatica	71.13 70.14 71.15 72.06	7.83 8.14 7.83 7.98 8.16	25.02	2.6	0.28 0.19 0.33 0.24	25.07	0.02	0.61 0.38 0.61 0.44	6771	10200	14242
Sample_A45_B Swabian Alb Sample_A46_B Swabian Alb	Fagus sylvatica	71.15	7.83	12.01 23.36 26.48	2.42 5.31	0.33	19.18 26.79 34.78	0.52 0.84 0.74	0.61	16516 13197	19271 14942 15848	16779 11032
Sample_A47_B Swabian Alb Sample_A48_B Swabian Alb Sample_A5_B Swabian Alb			8.16 8.24	26.48 37.14 46.91	5.17 16.15 3.62	0.19	28.61 21.76	0.74 0.66 0.98	0.61	18149 11621 23697	12325 21362	16726 7812 12337
Sample_A45_B Swabian Alb Sample_A50_B Swabian Alb Sample_A6_B Swabian Alb Sample_A7_B Swabian Alb Sample_A7_B Swabian Alb Sample_A8_B Swabian Alb	Fagus sylvatica Fagus sylvatica Fagus sylvatica Fagus sylvatica Fagus sylvatica	72.79 69.87	8.24 8.23	46.91 34.43	3.62 3.01	0.13	21.76 31.73	0.85	0.48	10403	21362 21631	12337 17711
Sample_A50_B Swabian Alb Sample_A6_B Swabian Alb Sample_A7_B Swabian Alb	Fagus sylvatica Fagus sylvatica	73.02 72.87 71.69	8.23 7.99 8.02	34.43 28.44 30.41	3.01 4.53 14.69	0.41 0.26 0.39	31.73 27.44 37.74	0.88	0.64 0.59 0.55	22029 17114	21631 21553 11790	17711 13541 17433
			8.1 8.29	36	5.11	0.29	42.05	0.69 0.83	0.67 0.51	16838 31218	17879 35348	16213 11281
Sample_H1_B Hainich-Dün Sample_H10_B Hainich-Dün Sample_H11_B Hainich-Dün	Picea abies Fagus sylvatica	78.48 80.32 76.87	9.09 10.19	44.28 27.17	14.69 2.77 2.91	0.19	48.21 38.44	0.75 0.89	0.84	27957 21194 23794	10941 17522	11563 13198
Sample H14 B Hainich-Dün	Fagus sylvatica Picea abies Fagus sylvatica Fagus sylvatica Fagus sylvatica	76.87 78.45 76.34	9.09 10.19 9.57 9.07	44.28 27.17 23.07 10.72	2.67	0.19 0.38 0.4 0.26 0.18	48.21 38.44 38.93 10.12	0.75 0.89 0.84 0.77	0.91 0.87 0.43	11827	17522 17085 13747	13198 29493 12989
Sample_H15_B Hainich-Dün Sample_H16_B Hainich-Dün		76.34	9.98	10.34	2.53 2.63		30.53	0.59	0.49 0.96 0.77	29822 7739	11595 4999	9874
Sample_H17_B Hainich-Dün Sample_H18_B Hainich-Dün Sample_H19_B Hainich-Dün	Fagus sylvatica Fagus sylvatica Fagus sylvatica Fagus sylvatica	77.98 77.41 78.78	8.86 9.7 9.1	12.51 15.05 34.11 34.16	2.68 3.62 11.57	0.21 0.23 0.22 0.2	23.04 31.63 31.12	0.94 0.64 0.88	0.77 0.68 0.61	4902 6956	15285 6161 14310	16949 9614 12936
Sample_H20_B Hainich-Dun		78.61	9.18	34.11 34.16	5.15	0.22	34.96	0.9	0.63	6956 20760 10595	13303	14734
Sample_H21_B Hainich-Dün Sample H22 B Hainich-Dün	Fagus sylvatica Fagus sylvatica	78.74 79.38	9.31 9.29	35.55 EE 70	5.44 11.16	0.24	31.87 28.74	0.97	0.7	14144	18695 15126	15305 9966
Sample_H23_B Hainich-Dün Sample_H24_B Hainich-Dün	Fagus sylvatica Fagus sylvatica Fagus sylvatica	78.27 81.21 78.91	8.89 9.75 9.18	10.24 34.75	9.44 2.67	0.23	11.18 30.11 30.78	0.67 0.94 0.99	0.81	9509 12928 12042	9684 6818	11638 10723 15805
Sample_H22_B Hainich-Dün Sample_H23_B Hainich-Dün Sample_H24_B Hainich-Dün Sample_H26_B Hainich-Dün Sample_H27_B Hainich-Dün	Fagus sylvatica Fagus sylvatica	78.91 80.36	9.18 9.4	10.24 34.75 35.2 29.1	9.98 3.1	0.23 0.42 0.35 0.37	30.78 25.89	0.99 0.98	0.81 0.61 0.56 0.72	12042 15359	11398 12496	15805 8603
	Enque mécatica	79.48	9.15	31.15	2.9	0.34	25.95	0.96	0.77	12888	7563	9790
Sample_H29_B Hainich-Dün Sample_H30_B Hainich-Dün Sample_H31_B Hainich-Dün Sample_H32_B Hainich-Dün	Fagus sylvatica Fagus sylvatica Fagus sylvatica	76.89 77	9.8 9.85 9.41	23.88 24.26 21.3	16.19 5.25 5.46	0.36 0.39 0.42	27.98 27.22	0.98 0.83	0.87 0.84 0.93	6972 13925 14664	14082 7193	10076 10150
	Fagus sylvatica	78.19 80.76					22.33	0.68	0.51		15350 7068	10523 10957
Cample H24 P. Hainich Don	Fagus sylvatica Fagus sylvatica Fagus sylvatica Fagus sylvatica Fagus sylvatica	77.50	9.6 9.48 9.77 9.74	24.92	3.02	0.07	20.76	0.92	0.00	9229	4977	7221
Sample_H35_B Hainich-Dün Sample_H36_B Hainich-Dün Sample_H37_B Hainich-Dün	Fagus sylvatica	79.1 78.09 76.35 77.47	9.77	22.58 20.31 33.38	3.03 7.35	0.37 0.38 0.36 0.4	37.46 34.42 38.53	0.64 0.94	0.79 0.7 0.88 0.78	9116 20686 13315	15761 13651	16157 11736
Sample_H30_B Harrich Don	Earnie sylvatica	77.47 76.53	9.34	28.85 29.63	5.76 2.82	0.4	39.38 36.59	0.91 0.85	0.78	12004 16811	12529 9858	13026 11014
Sample H4 B Hainigh Don	Fagus sylvatica Fagus sylvatica Fagus sylvatica	70.72	9.54 9.95 10.23 10.31	0.16	2.02	0.12	7.09	0.93	0.71	4625	7152 10343	8390
Sample_H4D_B Hainich-Dün Sample_H41_B Hainich-Dün Sample_H42_B Hainich-Dün	Fagus sylvatica Fagus sylvatica	76.57 77.49 79.72	10.23	29.72 26.99 35.06	4.25 2.73 3.4	0.3 0.39 0.33	34.14 36.1	0.67 0.54 0.98	0.55 0.54 0.68	19033 15161 12805	12017 6211	13743 7969
	Fagus sylvatica	78.5	8.99 9.14	10.08	5.73 2.58	0.15	12.56 28.41	0.73 0.96	0.7	15503 9911	18315 5879	20393 8196
Sample_H46_B Hainich-Dün Sample_H47_B Hainich-Dün Sample_H48_B Hainich-Dün Sample_H49_B Hainich-Dün	Fagus sylvatica Fagus sylvatica Fagus sylvatica Fagus sylvatica Fagus sylvatica	75.56 76.97 78.2	0.42	30.57 34.82 35.86 29.37	10.56	0.19 0.26 0.36 0.33	20.77	0.07	0.60	19749	19070	16467
Sample_H48_B Hainich-Dün Sample_H49_B Hainich-Dün	Fagus sylvatica	78.2 75.52	10.19 10.1 10.1	35.86	4.01 6.11 6.82	0.36	38.82 28.55 26.89	0.94 0.97 0.93	0.81	14411 21640 13606	8963 15430 15720	9831 12079 13520
Sample_H5_B Hainich-Dün Sample_H50_B Hainich-Dün		76.87 76.0E	9.25 10.1	25.5 22.68	5.09 2.74	0.26	30.29 30.76	0.97 0.97	0.69 0.75 0.9	4683 9722	13737 5700	8494 7762
Sample_H6_B Hainich-Dün Sample_H7_B Hainich-Dün Sample_H8_B Hainich-Dün	Fagus sylvatica Fagus sylvatica Fagus sylvatica Fagus sylvatica	82.61 74.67	0.40	39.76 27.13 25.23	10.47	0.16 0.37 0.37	20 54	0.96 0.9 0.95	0.82 0.79 0.67	15020	20550	14665
Sample_H8_B Hainich-Dün Sample_H9_B Hainich-Dün	Fagus sylvatica Fagus sylvatica	82.61 74.67 79.82 77.88	10.01 8.68 9.73	25.23 28.27	14.86 2.7 7.1	0.37	29.89 29.62 27.15	0.95 0.98	0.67	14411 16967 28419	12306 25588 69100	12768 18886 30175
		78.4	12.56	20.6	11.87	0.13	34.64	0.98	0.65	18678	11736	11329
Sample S10 B Schorfheide-C Sample S11 B Schorfheide-C Sample S12 B Schorfheide-C Sample S13 B Schorfheide-C	horin Pinus sylvestris horin Pinus sylvestris horin Pinus sylvestris horin Pinus sylvestris horin Pinus sylvestris horin Pinus sylvestris	76.21 77.7 77.2 76.38 77.51	12.4 12.69 12.92	13.36 15.85 21.18	18.28 11.8 11.41	0.15 0.18 0.14 0.11	42.51 34.69 50.49	i	0.55 0.73 0.4	21799 22644 37248	12963 12581 16649	11043 10601 15458 21052
Sample_S13_B Schorfheide-C Sample_S14_B Schorfheide-C	horin Pinus sylvestris	76.38 77.51	12.92 12.66	21.18 25.24	11.41 18.99	0.14	50.49 31.05	0.99	0.4	37248 34585	16649 20129	15458
		77.83		21.91	15.65		36.06	1 0.86	0.77	40367	19065	
Sample_S18_B Schorfheide-C Sample_S17_B Schorfheide-C Sample_S18_B Schorfheide-C Sample_S19_B Schorfheide-C	horin Pinus sylvestris horin Pinus sylvestris horin Pinus sylvestris horin Pinus sylvestris	80.57 78.01	12.57 12.8 12.7 12.76 12.78	39.63 32.2 28.87	36.21 22.35 16.28 33.43	0.13 0.12 0.25 0.15 0.09	35.61 36.14 22.97	0.71 0.86	0.4 0.61 0.95 0.5	71602 25111	16291 12756	10183 4845 15654 7848
Sample_S19_B Schorfheide-C Sample_S2_B Schorfheide-C	horin Pinus sylvestris horin Pinus sylvestris	76.94 79.57	12.78	33.32 21.16	33.43 10.47	0.09	22.97 42.54	0.98 0.82	0.5	28233 10236	17622 13744	7848 15284
Sample_S2_B Schorlheide-C	borio Dinus rubunetrie	78.18 78.08	12 50	34.04 35.59	42.56 29.04	0.12	24.02	0.94	0.50	37700 35665	11468 21232	8248 16224
Sample S20 B Schorfheide-C Sample S21 B Schorfheide-C Sample S23 B Schorfheide-C Sample S27 B Schorfheide-C	horin Pinus sylvestris horin Fagus sylvatica horin Fagus sylvatica	81.19 80.93	12.69 12.57 12.77	26.8 32.93	2.78 9.8	0.16 0.33 0.32 0.29	30.7 32.44 33.71	0.76 0.59 0.66	0.83 0.68 0.75 0.43	25800 22473	11668 16724	13970 12821
Sample_S29_B Schorfheide-C	norin Fagus sylvatica horin Fagus sylvatica horin Pinus sylvestris	77.6	12.85	32.93 24.4 33.79	21.6	0.32	28.35 37.51	0.84	0.43	14962	14258	13227
Sample_S3_B Schorfheide-C Sample_S30_B Schorfheide-C	horin Pinus sylvestris horin Fagus sylvatica	79.86 76.2	12.44 12.65	29.77	19.82 6.11	0.12 0.27	20.2	0.94 0.74	0.9 0.45	16870 14139	8805 14280 18961	7780 9621
Sample S30 B Schorfheide-C Sample S31 B Schorfheide-C Sample S32 B Schorfheide-C Sample S33 B Schorfheide-C	horin Fagus sylvatica horin Fagus sylvatica horin Pinus sylvestris horin Fagus sylvatica	81.45 75.68	12.76 12.94 12.63	33.45 42.27 25.1	11.02 63.08	0.25 0.14 0.29	32.08 17.33 37	0.5 0.62 0.72	0.93	8168 19876 24417	18961 10054 19357	10852 7461
		81.45 75.68 79.38 77.18	12.76	26.33	3.63 4	0.28	26.71	0.89	0.93 0.43 0.72 0.69	8880	8329	10852 7461 15459 11591
Sample_S35_B Schorfheide-C Sample_S36_B Schorfheide-C	horin Fagus sylvatica horin Fagus sylvatica horin Fagus sylvatica horin Fagus sylvatica horin Fagus sylvatica	80.72	12.52 12.68	36.13 34.5	13.86 2.5	0.3 0.18	24.2 29.05	0.96 0.99		19291 14998	15361 7013	10726
Sample_S36_B Schorfheide-C Sample_S37_B Schorfheide-C Sample_S38_B Schorfheide-C Sample_S39_B Schorfheide-C	norin Fagus sylvatica horin Fagus sylvatica	79.23 80.43 82.45	12.66 12.66 12.34	41.56 47.14	8.59 8.85	0.22 0.2 0.32	31.78 24.37 18.77	0.83 0.52 0.64	0.75 0.7 0.68 0.63	6794 18319	13904 8880 11757	8719 7037
		81.18	12.4	39.88 27.26	5.68 2.71	0.26	38.64	0.73	0.67	20784 12785	12136	7638 8562
Sample_S40_B Schorfheide-C Sample_S41_B Schorfheide-C	horin Fagus sylvatica horin Fagus sylvatica horin Fagus sylvatica horin Fagus sylvatica	81.51 81.87	12.43 12.16	33.95 28.75	10.17 3.46	0.41	20.26 22.19	0.72 0.55	0.69 0.85	9181 16509	14444 14657	8093 8003
Sample_S41_B Schorfheide-C Sample_S42_B Schorfheide-C Sample_S44_B Schorfheide-C Sample_S45_B Schorfheide-C	horin Fagus sylvatica horin Fagus sylvatica	81.87 80.9 79.2	12.45 12.16 12.66 12.49 12.49	28.75 18.58 28.83 50.9	12.66 7.35	0.38 0.45 0.43	22.19 19.81 21.55	0.99 0.66	0.85 0.87 0.67	16509 14714 32351	4875 16692	8003 9490 12414
Sample_545_B Schormeide-C	horin Fagus sylvatica	77.67			2.57	0.17	35.57	0.97 0.95	0.91	17425 8634	8687 3386	6586
Sample_S47_B Schorfheide-C Sample_S48_B Schorfheide-C	horin Fagus sylvatica horin Fagus sylvatica	79.15 77.98	12.44 12.58 12.81	49.87 24.19	5.83 2.68 9.89	0.22 0.43 0.27	34.82 31.6	0.95 0.47 0.97	0.64	16011 27734 14467	7097 13398	11153 13030 14198
Sample_S46_B Schorfheide-C Sample_S48_B Schorfheide-C Sample_S49_B Schorfheide-C Sample_S5B Schorfheide-C Sample_S50_B Schorfheide-C	horin Fagus sylvatica horin Fagus sylvatica horin Fagus sylvatica horin Fagus sylvatica	77.98 78.85 79.14	12.81 12.86 12.56	24.19 38.5 43.84	38.93	0.27 0.38 0.21	31.6 23.08 25.72	0.93	0.89 0.84 0.73	30368	13398 14692 17427	10338
Sample_S50_B Schorfheide-C Sample_S7_B Schorfheide-C		78.08 78.39	12.49	48.5 52.91	15.63 5.64	0.16	29.24 37.51	0.97 0.96	0.63	19644 24713	21682	13131 16046
Sample_S8_B Schorfheide-C Sample_S9_B Schorfheide-C	horin Fagus sylvatica horin Fagus sylvatica horin Fagus sylvatica	79.18 77.55	12.32 12.45	48.98 42.95	3.09 2.72	0.3 0.21	39.77 42.97	0.9 0.95	0.76 0.86	15631 22800	11266 14441	16257 11392

Table S3

level	variable	estimate standa	rd_error p_v	/al p_val_adj	supplementary_table
algae_q0	exploratoryHainich	-0.426	0.625 0.	497 0.735	
algae_q0 algae_q0	exploratorySchorfheide	-1.404 0.08	1.385 0. 0.145 0.	313 0.603 582 0.793	
algae_q0	rH_200 Ta_200	0.08		603 0.798	
algae_q0	stand_density_basal_area	-0.037	0.082 0.	651 0.837	
algae_q0	DBH_avg	-0.013		878 0.928	
algae_q0 algae_q0	d_gini canopy_openness_2019	0.072 -0.123		417 0.697 162 0.39	
algae_q0	dom_tot_ratio	0.057	0.081 0.	488 0.732	
algae q0	RA forest	0.131	0.088 0.	142 0.364	
algae_q0	bac_q0	-0.066	0.1 0.	509 0.743	
algae_q0	fun_q0	0.364	0.098	0 0.005	
algae_q1 algae_q1	exploratoryHainich exploratorySchorfheide	-1.007 -2.141	0.78 0. 1.698 0	199 0.448 0.21 0.463	
algae_q1	rH_200	0.15	0.176 0.	397 0.68	
algae g1	Ta_200	0.439	0.605	0.715	
algae_q1	stand_density_basal_area	-0.165	0.1 0.	101 0.294	
algae_q1	DBH_avg	-0.028		782 0.908 901 0.928	
algae_q1 algae_q1	d_gini canopy_openness_2019	0.013 -0.211		901 0.928 048 0.199	
algae_q1	dom_tot_ratio	0.151	0.099 0.	129 0.34	
algae_q1	RA_forest	0.11	0.106 0.	302 0.594	
algae_q1	bac_q1	0.055	0.101 0.	588 0.793	
algae_q1	fun_q1 exploratoryHainich	0.092 -1.335		459 0.709 093 0.278	
algae_q2 algae_q2	exploratoryHamich exploratorySchorfheide	-2.499	1.711 0.	147 0.368	
algae_q2	rH 200	0.2	0.178 0.	263 0.546	
algae_q2	rH_200 Ta_200	0.497	0.614	0.697	
algae_q2	stand_density_basal_area	-0.199	0.102 0.	053 0.213	
algae_q2 algae_q2	DBH_avg d gini	-0.089 -0.023		393 0.68 826 0.928	
algae_q2 algae_q2	d_gini canopy_openness_2019	-0.023 -0.217	0.107 0.	826 0.928 045 0.197	
algae q2	dom tot ratio	0.185	0.1 0.	0.244	
algae_q2	RA_forest	0.083	0.108 0.	447 0.709	
algae_q2	bac_q2	0.17	0.099 0.	088 0.273	
algae_q2	fun_q2	-0.072		564 0.782 227 0.491	
bacteria_q0 bacteria_q0	exploratoryHainich exploratorySchorfheide	0.554 0.951	0.456 0. 1.014 (0.491	
bacteria_q0	rH 200	-0.166	0.104 0.		
bacteria_q0	rH_200 Ta_200	-0.356	0.368 0.	335 0.613	
bacteria_q0	stand_density_basal_area	-0.026	0.06	0.842	
bacteria_q0	DBH_avg	0.061	0.061 0.		
bacteria_q0 bacteria_q0	d_gini	0.252 -0.236	0.062 0.061	0 0.003	
bacteria_q0 bacteria_q0	canopy_openness_2019 dom tot ratio	-0.236	0.061		
bacteria q0	RA_forest	0.04	0.065 0.		
bacteria_q0	fun_q0	0.418	0.067	0 0	
bacteria_q0	alg_q0	-0.01	0.063 0.	879 0.928	
bacteria_q1	exploratoryHainich	0.488		451 0.709	
bacteria_q1 bacteria_q1	exploratorySchorfheide rH 200	-0.201 -0.053		886 0.928 714 0.861	
bacteria q1	Ta_200	0.061	0.498 0.	902 0.928	
bacteria_q1	stand_density_basal_area	0.084	0.081 0.	299 0.594	
bacteria_q1	DBH_avg	0.159	0.083 0.	058 0.225	
bacteria_q1	d_gini	0.31	0.082 0.085 0.	0 0.005 032 0.143	
bacteria_q1 bacteria_q1	canopy_openness_2019 dom_tot_ratio	-0.184 0.032	0.085 0.	0.32 0.143 0.69 0.846	
bacteria_q1	RA_forest	0.025	0.087 0.	774 0.908	
bacteria_q1	fun q1	0.326	0.099 0.	0.021	
bacteria_q1	alg q1	0.112	0.077 0.	151 0.371	
bacteria_q2	exploratoryHainich	0.373	0.727 0.	609 0.798	
bacteria_q2 bacteria_q2	exploratorySchorfheide rH_200	-0.669 0.095		0.67 0.842 556 0.782	
bacteria_q2	Ta_200	0.437	0.556 0.	434 0.709	
bacteria q2	stand density basal area	0.139	0.091 0.	128 0.34	
bacteria_q2	DBH_avg	0.204		0.143	
bacteria_q2	d gini	0.232	0.093 0.	0.078	
bacteria_q2	canopy_openness_2019 dom_tot_ratio	-0.035	0.097 0.	717 0.861 861 0.928	
bacteria_q2 bacteria_q2	dom_tot_ratio RA_forest	0.016 0.013		861 0.928 891 0.928	
bacteria q2	fun n2	0.199		0.926	
bacteria_q2	fun_q2 alg_q2	0.215	0.086 0.	014 0.078	
fungi_q0	exploratoryHainich	-1.213	0.503 0.	0.085	
fungi_q0	exploratorySchorfheide	-2.779	1.12 0.	0.078	
fungi_q0	rH_200 Ta_200	0.311 0.728		008 0.063 078 0.26	
fungi_q0 fungi_q0	stand_density_basal_area	0.728		975 0.975	
fungi q0	DBH_avg	0.014		834 0.928	
fungi_q0	d_gini	-0.071	0.072 0.	325 0.605	
fungi_q0	canopy openness 2019	0.169	0.068 0.	0.078	
fungi_q0	dom_tot_ratio	0.02	0.066 (0.76 0.902 947 0.956	
fungi_q0 fungi_q0	RA_forest bac_q0	0.005	0.072 0.	947 0.956 0 0.001	
fungi_q0	alg_q0	0.183	0.066 0.	006 0.056	
fungi_q1	exploratoryHainich	-2.049	0.634 0.	0.021	
fungi_q1	exploratorySchorfheide	-4	1.404 0.	0.055	
fungi_q1	rH_200	0.407	0.145 0.	0.055	
fungi_q1 fungi_q1	Ta_200 stand_density_basal_area	0.885 -0.035		087 0.273 681 0.846	
fungi_q1 fungi_q1	DBH_avg	-0.035	0.085 0.	303 0.594	
fungi_q1	d gini	-0.14	0.09 0.	122 0.337	
fungi_q1	canopy openness 2019	0.225	0.085 0.	0.063	
fungi_q1	dom_tot_ratio	0.043	0.084 0.	613 0.798	
fungi_q1	RA_forest	0.013		889 0.928	
fungi_q1 fungi_q1	bac_q1 alg_q1	0.155 0.017		0.244 833 0.928	
fungi_q1 fungi_q2	aig_q1 exploratoryHainich	-2.075	0.081 0.	0.928	
fungi_q2	exploratorySchorfheide	-3.624		015 0.078	
fungi_q2	rH_200	0.381 0.741	0.151 0.	0.078	
fungi_q2	Ta_200	0.741	0.536 0.	169 0.397	
	stand_density_basal_area	-0.08		375 0.664	
fungi_q2	DBH_avg	-0.106	0.09 0.	243 0.514 0.07 0.244	
fungi_q2				0.244	
fungi_q2 fungi_q2	d_gini	-0.168		007 005	
fungi_q2 fungi_q2 fungi_q2	canopy_openness_2019	0.242 0.051	0.088 0.	0.057	
fungi_q2 fungi_q2 fungi_q2 fungi_q2 fungi q2	canopy_openness_2019 dom_tot_ratio RA_forest	0.242 0.051 0.017	0.088 0. 0.088 0. 0.095 0.	007 0.057 564 0.782 858 0.928	
fungi_q2 fungi_q2 fungi_q2	canopy_openness_2019	0.242 0.051	0.088 0. 0.088 0. 0.095 0.	007 0.057 564 0.782 858 0.928 0.19 0.437	

Page 1

A.3. Habitat and tree species identity shape aboveground and belowground fungal communities in central European forests

Declaration of author contributions to the publication:

Habitat and tree species identity shape aboveground and belowground fungal communities in central European forests

Status: accepted (07.02.2023)

Name of journal: Frontiers in Microbiology (doi: 10.3389/fmicb.2023.1067906)

Contributing authors: Benjamin Hofmann (BH), <u>Lukas Dreyling (LD)</u>, Francesco

Dal Grande (FDG), Jürgen Otte (JO), Imke Schmitt (IS)

What are the contributions of the doctoral candidate and his co-authors?

(1) Concept and design

Doctoral candidate (LD): 40% Co-author BH: 10% Co-author IS: 50%

(2) Conducting tests and experiments

Doctoral candidate (LD): 50% (sample collection from the field, PCR) 20% (sample collection from the field, PCR) Co-author BH:

Co-author FDG: 5% (sample collection from the field)

Co-author JO: 20% (DNA isolation)

5% (sample collection from the field) Co-author IS:

(3) Compilation of data sets and figures

Doctoral candidate (LD): 70% (processing of sequencing reads, ASV inference,

taxonomic assignment, preparation of figures and tables)

Co-author BH: 30% (preparation of figures and tables)

(4) Analysis and interpretation of data

Doctoral candidate (LD): 35% (code review, variance partitioning, interpretation) Co-author BH:

40% (alpha diversity analysis, NMDS, interaction networks,

interpretation)

Co-author FDG: 5% (data interpretation) 20% (data interpretation) Co-author IS:

(5) Drafting of manuscript

Doctoral candidate (LD): 15% Co-author BH: 35% 5% Co-author FDG: Co-author IS: 45%

Appendix – Publications

I hereby certify that the information above is correct.							
Date and place	Signature doctoral candidate						
Date and place	Signature supervisor						
Date and place	If required, signature of corresponding author						



TYPE Original Research
PUBLISHED 06 March 2023
DOI 10.3389/fmicb.2023.1067906



OPEN ACCESS

EDITED BY
Kezia Goldmann,
Helmholtz Association of
German Research Centres (HZ),
Germany

REVIEWED BY
Rodica Pena,
University of Reading,
United Kingdom
Tijana Martinović,
Institute of Microbiology (ASCR),
Czechia

*CORRESPONDENCE
Imke Schmitt

imke.schmitt@senckenberg.de

PECIALTY SECTION

This article was submitted to Microbe and Virus Interactions With Plants, a section of the journal Frontiers in Microbiology

RECEIVED 12 October 2022 ACCEPTED 07 February 2023 PUBLISHED 06 March 2023

CITATION

Hofmann B, Dreyling L, Dal Grande F, Otte J and Schmitt I (2023) Habitat and tree species identity shape aboveground and belowground fungal communities in central European forests. Front. Microbiol. 14:1067906. doi: 10.3389/fmicb.2023.1067906

COPYRIGHT

© 2023 Hofmann, Dreyling, Dal Grande, Otte and Schmitt. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

Habitat and tree species identity shape aboveground and belowground fungal communities in central European forests

Benjamin Hofmann^{1,2}, Lukas Dreyling^{1,2}, Francesco Dal Grande^{2,3,4}, Jürgen Otte² and Imke Schmitt^{1,2}*

¹Institute of Ecology, Diversity and Evolution, Goethe University Frankfurt, Frankfurt, Germany, ²Senckenberg Biodiversity and Climate Research Centre (SBiK-F), Frankfurt, Germany, ³Department of Biology, University of Padova, Padua, Italy, ⁴National Biodiversity Future Center (NBFC), Palermo, Italy

Introduction: Trees interact with fungi in mutualistic, saprotrophic, and pathogenic relationships. With their extensive aboveground and belowground structures, trees provide diverse habitats for fungi. Thus, tree species identity is an important driver of fungal community composition in forests.

Methods: Here we investigate how forest habitat (bark surface vs. soil) and tree species identity (deciduous vs. coniferous) affect fungal communities in two Central European forests. We assess differences and interactions between fungal communities associated with bark surfaces and soil, in forest plots dominated either by *Fagus sylvatica*, *Picea abies*, or *Pinus sylvestris* in two study regions in southwestern and northeastern Germany.

Results: ITS metabarcoding yielded 3,357 fungal amplicon sequence variants (ASVs) in the northern and 6,088 in the southern region. Overall, soil communities were 4.7 times more diverse than bark communities. Habitat type explained 48–69% of the variation in alpha diversity, while tree species identity explained >1–3%. NMDS ordinations showed that habitat type and host tree species structured the fungal communities. Overall, few fungal taxa were shared between habitats, or between tree species, but the shared taxa were highly abundant. Network analyses, based on co-occurrence patterns, indicate that aboveground and belowground communities form distinct subnetworks.

Discussion: Our study suggests that habitat (bark versus soil) and tree species identity are important factors structuring fungal communities in temperate European forests. The aboveground (bark-associated) fungal community is currently poorly known, including a high proportion of reads assigned to "unknown Ascomycota" or "unknown Dothideomycetes." The role of bark as a habitat and reservoir of unique fungal diversity in forests has been underestimated.

KEYWORDS

biodiversity, fungal diversity, metabarcoding, tree microbiome, rhytidome

1. Introduction

Tree-fungus interactions are fundamental for the functioning of forest ecosystems. Fungi form mutualistic, pathogenic, and saprotrophic interactions with forest trees. For example, mycorrhizal fungi improve nutrient and water uptake by forest trees (Gerdemann, 1968), and endophytic fungi associated with leaves can promote tree growth (Doty, 2011), or prevent infection by pathogens (Herre et al., 2007). Pathogenic fungi cause severe diseases

Frontiers in Microbiology 01 frontiersin.org

of forest trees, such as beech bark disease (Cale et al., 2017), while soilborne saprotrophic fungi are essential for litter decomposition, energy flow and nutrient cycling in forest ecosystems (Baldrian et al., 2012). Since trees are keystone species in forests - forming extensive above- and belowground structures - they provide ample and diverse habitats for fungal communities. Ecological niches provided by trees are, e.g., the phyllosphere, rhytidome (bark surface), and rhizosphere, including the soil surrounding the root. Fungi are able to occur in several of these habitats, connect and exploit them (Baldrian, 2017). To obtain a more complete picture of the interaction of trees with their fungal communities in forests, we need more information on fungal communities simultaneously associating with different tree niches (e.g., Yang et al., 2022a). This will help us understand the fungal linkages between above- and belowground habitats, or between living and dead tree tissues. One confounding factor to such analyses is that some ecological niches in forests, e.g., the bark surface, are little known, especially with respect to fungal diversity (Dreyling et al., 2022). The comparison between the bark surface - the largest, seasonally stable aboveground structure - and the soil is one step toward understanding the "ecosystem microbiome" (Baldrian, 2017) of a temperate forest.

The rhytidome, or "dermosphere" is defined as the outer bark surface of living trees (Lambais et al., 2014), and constitutes a terrestrial habitat with an estimated surface area of 0.9 ha/ha in woodlands (Baldrian, 2017). Despite its vastness, the bark microecosystem of forests remains largely unknown with respect to fungal diversity. Bark provides mechanical and chemical defense to the tree, for example against herbivores and pathogens, and thus drives a process of selection with regards to fungi and other microbiota that that are able to exploit the habitat (Lambais et al., 2014). It consists of different biomolecules like starch, sugars and xvlem exudates as well as cellulose, hemicellulose, lignin, and xylan (Martins et al., 2013). In general, the rhytidome is characterized by nutrient scarcity, since the described biomolecules cannot be easily exploited by microbes as carbon sources (Buck et al., 1998). Other characteristics of the bark surface habitat are desiccation, the secretion of mycotoxic substances like tannins or terpenes (Buck et al., 1998), or exposure to solar radiation (Baldrian, 2017). Nevertheless, tree bark provides microhabitats in form of cracks and cavities with favorable conditions for the survival of microorganisms and the attachment and germination of fungal spores (Buck et al., 1998; Magyar, 2008). Arrigoni et al. (2018) described the bark of fruit trees as a likely reservoir of potentially pathogenic or beneficial fungi and bacteria.

Tree species identity is known to be an important driver of tree-associated fungal communities. Forest trees actively recruit and select mycorrhizal fungi, resulting in species-specific ectomycorrhizal communities (e.g., Lang et al., 2011). Deadwood from different tree species features specific fungal communities (e.g., Krah et al., 2018; Yang et al., 2022b), with broadleaved dead trees generally exhibiting higher diversity compared to coniferous dead trees (Rajala et al., 2010; Purhonen et al., 2020). The type of litter produced by different tree species influences the community composition of soil fungi and bacteria (Urbanová et al., 2015) and the diversity of genes involved in lignin degradation (Barbi et al., 2016). Concerning fungi of the phyllosphere, tree species identity (Kembel and Mueller, 2014) and tree genotype (Bálint et al., 2013) shape community composition. In some cases, tree species identity is a stronger driver of fungal

communities than abiotic or other environmental variables (Krah et al., 2018; Saitta et al., 2018).

In this work, we analyze diversity, composition, and structure of fungal communities associated with bark surfaces and soil in temperate Central European forests, focusing on three common tree species (Fagus sylvatica, Picea abies, Pinus sylvestris). Specifically, we address the following questions: 1. How does the dominant tree species in a forest plot influence bark-associated and soil-associated fungal communities? 2. What are the compositional differences between bark and soil fungal communities? 3. Which fungal taxa are shared between the aboveground and belowground communities?

2. Materials and methods

2.1. Study regions

We collected environmental samples of bark surface- and soilassociated fungi in two study regions in Germany, which are part of the Biodiversity Exploratories¹: Swabian Alb in the southwest and Schorfheide-Chorin in the northeast. In each region 50 forest plots $(100\,\text{m}\times100\,\text{m})$ were selected, which represent typical Central European temperate forest vegetation of various management intensities (Fischer et al., 2010), Regional differences between the sampling regions are summarized in Table 1. The dominant (most abundant) tree species on a forest plot was determined based on a stand composition assessment of all trees with a diameter at breast height (DBH) greater than 7 cm (Schall and Ammer, 2018). The most frequent dominant tree species in both study regions was European beech (Fagus sylvatica), followed by Norway spruce (Picea abies) in Swabian Alb and Scots pine (Pinus sylvestris) in Schorfheide-Chorin (Schall and Ammer, 2018). Thus, we sampled beech and the dominant coniferous tree species, respectively, in each study region. Bark and soil samples were taken from the same $20 \,\mathrm{m} \times 20 \,\mathrm{m}$ subplots, in a total of 94,100 m × 100 m plots, during the month of May in 2021. For each plot we generated one composite sample for soil (from 14 subsamples) and one composite sample for tree bark (from 6 subsamples).

2.2. Sample collection

We obtained soil samples from the 2021 Biodiversity Exploratories Soil Sampling Campaign. In brief, 14 soil samples were collected per plot from seven equidistant sampling points along two 40 m transects. Soil was sampled with a split-tube auger (diameter 50 mm) from a soil horizon of 0–10 cm after the removal of twigs, litter, and the organic soil layer. The fourteen subsamples per plot were mixed and sieved to <2 mm particle size for Schorfheide-Chorin and <4 mm particle size for Swabian Alb. For each plot, 5 g of the composite sample were stored at $-20^{\circ}\mathrm{C}$ until further processing. Given this sampling design, it cannot be ruled out that the soil samples are influenced by plants other than the dominant tree species. However, the *Picea* and *Pinus* forests we sampled were nearly monocultures, and the *Fagus* forests did not feature extensive below-canopy vegetation. Furthermore,

¹ http://www.biodiversity-exploratories.de/en/

TABLE 1 Differences between the study regions Swabian Alb and Schorfheide-Chorin (Fischer et al., 2010).

Study region	Swabian Alb	Schorfheide-Chorin
Area	422 km²	1,300 km ²
Geology	Calcareous bedrock, karst	Young glacial landscape
Altitude a.s.l.	460-860 m	3-140 m
Annual mean temperature	6.0-7.0°C	8.0-8.5°C
Annual mean precipitation	700-1,000 mm	500-600 mm
# of sampled forest plots	50	44
Dominant deciduous tree species	Fagus sylvatica (37 plots)	Fagus sylvatica (28 plots)
Dominant coniferous tree species	Picea abies (13 plots)	Pinus sylvestris (16 plots)
Average (min / max) proportion of dominant tree species per plot	80% (43%/100%)	84% (47%/100%)

Dominant tree species are the most abundant species per plot based on a forest assessment (Schall and Ammer, 2018).

we sampled from the mineral horizon, so that potential influences from mosses and small herbaceous plants with shallow roots (e.g., geophytes) are less likely. Lastly, we included a large number of plots with the same dominant tree species, so that potential effects of other under-canopy species are expected to be leveled out.

To analyze the rhytidome, we collected six subsamples per plot from the trunk bark, 150 cm above ground, from six spatially random individuals of the dominant tree species. Following Dreyling et al. (2022), we selected two small trees (DBH 5–15 cm), two medium size trees (DBH 15–30 cm) and two large trees (DBH > 30 cm), to account for community variation between age groups. After mobilizing the biofilm *via* spray-moistening with sterile water, we used sterile nylon swabs (FLOQSwabsTM, Copan, Brescia, Italy) to collect the sample. These swabs were moved around the trunk in a 3 cm wide band (Dreyling et al., 2022). The six swab tips of each plot were pooled in a 15 ml tube containing 5 ml of Nucleic Acid Preservation (NAP) buffer (Camacho-Sanchez et al., 2013), kept on ice in the field and subsequently stored at 4°C until DNA extraction. We took one blank sample per study region. For this we exposed six swabs to ambient air and processed them in the same fashion as the environmental samples.

2.3. DNA extraction

We extracted DNA from 150 mg of soil from the composite sample, using the Quick-DNA Fecal/Soil Microbe Microprep Kit (Zymo Research Europe GmbH, Freiburg, Germany) as specified in the manufacturer's protocol. Cell structures were disrupted by mechanical bead beating for a duration of 6 min (SpeedMill PLUS, Analytik Jena, Jena, Germany). Bark samples were processed with the same extraction kit. However, an additional pre-processing is required to liberate the biological material from the swabs (Menke et al., 2017). Thus, each composite sample was diluted with 5 ml of ice-cold phosphate buffered saline (PBS), transferred to a 50 ml tube (to allow movement) and vortexed for 30 s (Menke et al., 2017). Afterward, 1.5 ml of the resulting suspension were transferred to a 1.5 ml tube and centrifuged at 6000×g for 15 min (Menke et al., 2017). The supernatant was removed and beads and buffer from the extraction kit were directly added to the remaining sediment. Consecutive steps followed the same treatment as the soil samples. DNA quantity was checked via photometry (Implen NanoPhotometerTM, Pearl Implen GmbH, München, Germany).

2.4. PCR amplification and high-throughput sequencing

The DNA extracted from the environmental samples was amplified with primer pairs targeting for the internal transcribed spacer 2 (ITS2) region. We selected the forward primer fITS 7 (GTGARTCATCGAATCTTTG) (Ihrmark et al., 2012) and the reverse primer ITS 4 (TCCTCCGCTTATTGATATGC) (White et al., 1990). Each sample was amplified in triplicate following a double index multiplexing scheme for each replicate, with individual octamer barcodes attached to both, forward and reverse primers. Each PCR run contained eight PCR negative controls (i.e., no sample added to the reaction) and the two extraction blanks, adding up to a total of 104×3 samples after PCR. To assess potential primer jump at sequencing (Schnell et al., 2015) during the bioinformatic processing, we included 23 empty wells as so called "Multiplex Controls." Each reaction (total volume 15 µl) contained 5 ng of DNA, 7.5 µl hot-start polymerase (MyTaqTM HS Mix 2X, Meridian Bioscience Inc., Cincinnati, United States), $0.6\,\mu l$ with $10\,\mu M$ of each primer and $4.3\,\mu l$ DNAse free water (Dreyling et al., 2022). The initial denaturation was executed at 95°C for 60 s, followed by 35 cycles of denaturation at 95°C for 15 s, annealing at 56°C for 15 s and extension at 72°C for 10 s, as well as one final extension step at 72°C for 60 s (Dreyling et al., 2022).

The PCR products were cleaned by magnetic bead purification (MagSi-NGSPREP Plus®, magtivio BV, Nuth, Netherlands) according to the manufacturer's protocol. The final DNA concentration was determined by fluorometric measurement, using the Qubit dsDNA HS assay on a Qubit® 3.0 Fluorometer (both Thermo Fisher Scientific Inc., Waltham, Massachusetts, United States). Afterward, the cleaned PCR products were pooled equimolarly and sent to Fasteris SA (Planles-Ouates, Switzerland) for library preparation and sequencing. To avoid bias and chimera formation from additional PCR during the library creation, we chose the Fasteris MetaFast protocol. The amplicons were sequenced on an Illumina MiSeq (Illumina Inc., San Diego, California, United States) with 2×300 bp reads.

2.5. Bioinformatics

Reads were supplied by Fasteris SA with adapters removed [Trimmomatic (Bolger et al., 2014)]. We demultiplexed the

sequencing reads using Cutadapt v3.3 (Martin, 2011) in two runs, once with barcodes in forward and once in reverse complement orientation, with the recommended settings for dual-indexes (error rate 0.15, zero indels allowed, minimum sequence length 50 bp). This is required due to the mixed orientation libraries obtained from the PCR-free library construction. Any remaining primer sequences were removed with Cutadapt v3.3.

Amplicon Sequence Variants (ASVs) were determined following the DADA2 pipeline for ITS amplicons (Callahan et al., 2016). The filtering and trimming was applied with default parameters (maxN=0. truncQ=2, minLen=50, rm.phix=TRUE, compress=TRUE, multithread=TRUE), except for the number of expected errors maxEE, which was set to c(6,6) to take into account the mixed orientation of the sequencing library. Following the denoising and sample inference steps, read-pairs were merged within each triplicate and chimeras were removed from the dataset, resulting in one ASV table per triplicate. Because of the mixed orientation, we checked for reverse complement reads with DADA2s rc() function (Callahan et al., 2016) and added them to the original ASV table. In a last step, the three replicates were merged into a single table, which was matched against the UNITE database (version 9.0, including eukaryotic ITSs as outgroups; Abarenkov et al., 2022) by the DADA2 assignTaxonomy() function (Callahan et al., 2016) with minBoot=50 and $tryRC = TRUE. \ The \ ASV \ table \ was \ checked \ for \ potential \ contaminants$ with the decontam algorithm (Davis et al., 2018), set to both prevalence and frequency, and further curated using the LULU algorithm (Frøslev et al., 2017) with default parameters, grouping ASVs through patterns of similarity and sequence co-occurrence.

2.6. Data analysis

We analyzed diversity and composition of fungal ASVs in R (R Core Team, 2021) with the R Studio Environment (RStudio Team, 2021). Taxonomy tables, ASV tables and sample data were combined in phyloseq objects, which are specifically designed for the analysis of microbiome data (McMurdie and Holmes, 2013). Figures were generated in R with functions from the ggplot2 package (Wickham, 2016) and arranged using the ggpubr package (Kassambara, 2020), if not stated otherwise. Samples were not rarefied but viewed as count data (McMurdie and Holmes, 2014; Gloor et al., 2017). Prior to the community analyses, we split the dataset by study region to exclude any influence of variance in the regional conditions, such as climate, soil properties or elevation (Table 1). The number of samples per region and dominant tree species within the region can be found in Table 1.

2.6.1. Alpha diversity

We visualized ASV richness in relation to the number of reads per sample as rarefaction curves, generated with the *ggrare*() function from the ranacapa (v0.1.0) package (Kandlikar et al., 2018). We calculated alpha diversity [Shannon Index (Shannon, 1948)] of fungal communities with the *estimate_richness*() function (McMurdie and Holmes, 2013). We determined whether fungi were specific to a particular habitat using the *ps_venn*() function of the MicEco package (Russel, 2021).

2.6.2. Taxonomic community composition

To display the taxonomic community composition at order level, phyloseq objects were subset to the 25 relatively most abundant taxa with the *top_taxa()* function of the fantaxtic package (Teunisse, 2017).

Barplots were generated using the function *plot_composition()* from the microbiome package (Lahti and Shetty, 2017). To explore influences of different habitats and host tree species, the plot for each study region was split into these two categories.

2.6.3. Beta diversity

The effect of host tree species and habitat on fungal community composition was assessed with a non-metric multidimensional scaling approach (NMDS). To generate the NMDS, we used the function ordinate() set to "NMDS" and using Bray-Curtis dissimilarity. We visualized the result with plot_ordination() (McMurdie and Holmes, 2013). The variation between the groups included in the NMDS was reviewed with a Permutational Analysis of Variance (PERMANOVA) using the adonis() function (Stevens, 2019) from the vegan package (Oksanen et al., 2020). The variation within the groups was tested with the betadisper() function and verified with a permutation test with the function permutest() (Oksanen et al., 2020).

2.6.4. Variance partitioning

To assess how much of the variation in the data was explained by habitat and tree species we used variance partitioning analyses for both alpha and beta diversity. To partition the variance for alpha diversity we used the *varPart()* function of the modEvA package (Barbosa et al., 2013) with three linear models, containing (I) both explanatory variables, (II) only habitat and (III) only tree species. For beta diversity the variance was assessed based on the ASV table using the function *varpart()* from the vegan package (Oksanen et al., 2020). This function uses redundancy analyses to assess the amount of variance explained by the individual fractions of each explanatory variable and their overlap. Both methods used adjusted R² values (Table 2).

2.6.5. Co-occurrence networks

Co-occurrence networks of the fungal ASVs were generated for each study region with the SPIEC-EASI algorithm, which is based on co-occurrence and relative abundance (Kurtz et al., 2015). To reduce the number of nodes for better visibility, only ASVs that constitute more than $1\,\%$ of the reads were included. The applied parameters for the algorithm were lambda.min.ration=0.01, nlambda=70, pulsar.select=TRUE, seed=10,010 on 50 subsamples (rep.num=50), method="mb" (Meinshausen and Bühlmann, 2006), and sel.criterion="bstars" (Müller et al., 2016). The graphics for the networks were created in Gephi v0.9.2 (Bastian et al., 2009) with the Fruchterman-Reingold Layout (Fruchterman and Reingold, 1991) and considered modularity (Brandes, 2001) as well as betweenness centrality (Brandes, 2001; Blondel et al., 2008). The modules determined via Gephi v0.9.2 were assigned to different habitats by aligning the ASVs to the habitat types (i.e., tree species and above- / belowground). We calculated hub-taxa for each network, which we defined as the five nodes in the network with the highest betweenness centrality (i.e., the highest number of shortest paths; Freeman, 1977) with the betweenness() function from the igraph package (Csardi and Nepusz, 2006).

3. Results

3.1. Alpha diversity

The metabarcoding approach yielded an average of 39,245 reads for the Swabian Alb, and an average of 43,607 reads for

TABLE 2 Top five hub-taxa (ASVs) from co-occurrence networks.

ASV_ID	Phylum	Class	Order	Family	Genus	Species
(Swabian Alb	p)					
ASV_1676	Basidiomycota	Agaricomycetes	Agaricales	Inocybaceae	NA	NA
ASV_257	Ascomycota	Dothideomycetes	Capnodiales	Cladosporiacea	Cladosporium	NA
ASV_258	Ascomycota	Dothideomycetes	Pleosporales	Nigrogranaceae	Nigrograna	NA
ASV_28	Basidiomycota	Agaricomycetes	Phallales	Phallaceae	Phallus	Impudicus
ASV_790	Ascomycota	Dothideomycetes	Pleosporales	Cucurbitariaceae	Neocucurbitaria	Quercina
(Schorfheide	e-Chorin)					
ASV_1227	Ascomycota	Leotiomycetes	Helotiales	Leotiaceae	NA	NA
ASV_257	Ascomycota	Dothideomycetes	Capnodiales	Cladosporiacea	Cladosporium	NA
ASV_27	Ascomycota	Lecanoromycetes	Trapeliales	Phlyctidaceae	Phlyctis	Argena
ASV_28	Basidiomycota	Agaricomycetes	Phallales	Phallaceae	Phallus	Impudicus
ASV_839	Basidiomycota	Agaricomycetes	Thelephorales	Thelephoraceae	Amaurodon	NA

Hub-taxa were defined as taxa with the highest number of shortest connections to other ASVs (betweenness centrality).

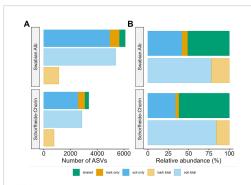
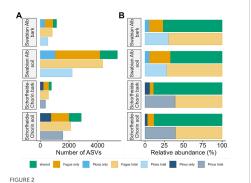


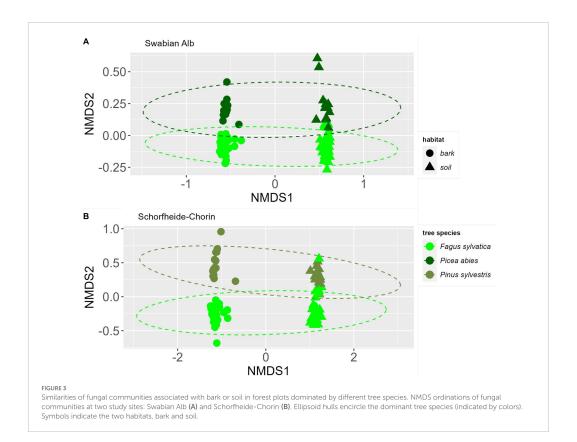
FIGURE 1
Fungal diversity associated with two forest habitats: bark surface and soil. Diversity is presented as total number of amplicon sequence variants (ASVs) (A), and relative abundance of ASVs (B) in soil, on bark, and in both habitats. Data are presented for two regions, Swabian Alb and Schorfheide Chorin.

Schorfheide-Chorin. The resulting number of ASVs in soil samples (7,033) was about 4.6 times higher than the number of ASVs in bark samples (1,497), suggesting a strong effect of habitat on fungal diversity. This effect is highlighted by a significant difference in Shannon diversity between the habitats within each region (Wilcoxon test: p < 0.001 for both). The habitat effect is also visible in the rarefaction curves (Supplementary material S1). The curves reach saturation plateaus, suggesting a low number of undiscovered ASVs and sufficient sequencing depth. Bark samples yielded fewer reads than soil samples, regardless of tree species identity and study region, while the read distribution bark/soil is similar in both study regions (Figure 1). The number of taxa shared between soil and bark surface was low (on average 343), but the abundance of these taxa was high (on average 62% of the reads; Figure 1 and Supplementary material S2). Considering each tree species, the number of associated fungal ASVs was generally higher in the



Fungal diversity associated with different tree species, and different habitats. Diversity is presented as total number of amplicon sequence variants (ASVs) (A), and relative abundance of ASVs (B). In each of the two study regions, Swabian Alb and Schorfheide Chorin, we compared fungal communities associated with a deciduous and a coniferous tree species.

Swabian Alb ($Picea\ abies=2,602,\ Fagus\ sylvatica=4,872$) than in Schorfheide-Chorin (Pinus sylvestris = 1,833,sylvatica = 2,499). Additionally, fungal diversity was significantly higher in the Swabian Alb (6,088 ASVs) than in Schorfheide-Chorin (3,357 ASVs; Wilcoxon test, Shannon diversity: p < 0.001), indicating an effect of geographic location. Fagus sylvatica had a higher number of associated fungal ASVs than the coniferous tree species in soil and on bark surfaces in each of the two study regions (Figure 2A). However, Shannon diversities between tree species within a study region were not significantly different (Wilcoxon test p < 0.001). The number of taxa shared between the coniferous and deciduous tree species was low (on average 1,018 ASVs in soil, and 195 ASVs on bark), but the relative abundance of these taxa was high (on average 78% of the reads in soil, and 83% on bark) (Figure 2B).



3.2. Beta diversity

The NMDS ordinations indicate a strong effect of habitat (bark surface vs. soil) on the fungal community composition (Figure 3). In addition, the fungal community is affected by the dominant tree species identity. The communities show a significant difference between dominant tree species in Swabian Alb and Schorfheide-Chorin, which is confirmed by PERMANOVA tests (p < 0.001) for both study regions. Permutation tests of the homogeneous dispersion within the groups (by tree species) confirm that there is no difference in the inner group distribution of the samples (p>0.05) for both study regions.

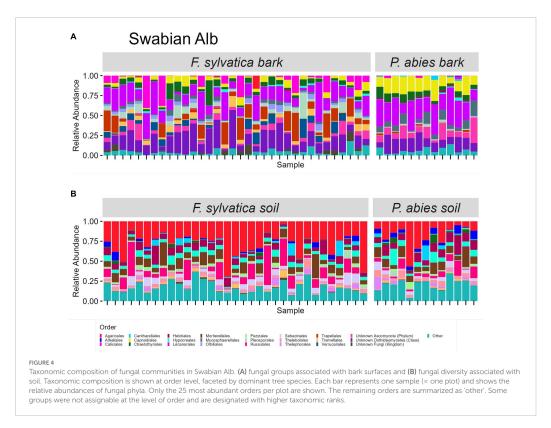
3.3. Taxonomic community composition

The taxonomic composition of fungal communities at order level differs according to habitat (Figures 4, 5). The most abundant orders in soil (in descending order) are Agaricales, Mortierellales, Russulales in Swabian Alb, and Russulales, Helotiales, Agaricales in Schorfheide-Chorin. On bark surfaces, the most abundant orders, which could be assigned to this taxonomic level, are Lecanorales, Capnodiales, Trapeliales (Swabian Alb), and Lecanorales, Chaetothyriales, Mycosphaerellales (Schorfheide-Chorin). We note, however, that taxonomic assignment yielded 'unknown Dothideomycetes' as the

second largest group of bark surface communities in both study regions (Figures 4, 5).

Bark surfaces generally contain a high proportion of reads (32–34%) unassignable at the level of order, here designated as 'unknown Dothideomycetes,' unknown Ascomycota,' unknown Fungi,' and 'unknown Rozellomycota'. The number of ASVs unassignable to order is 179 (16%) in Swabian Alb, and 114 (15%) in Schorfheide Chorin. Taxonomically unassigned diversity is largely lacking in the soil habitat (Figures 4, 5). Bark surfaces furthermore contain a high proportion of lichenized fungi: 39% of the reads in Swabian Alb, and 23% in Schorfheide Chorin. Swabian Alb features the lichen-forming orders Lecanorales, Trapeliales, Caliciales, and Verrucariales, whereas Schorfheide Chorin mostly contains Lecanorales.

Tree species identity affects the abundance of some orders in soil and on bark surfaces. This effect is different in the two study regions. For example, in bark samples from Swabian Alb the lichen-forming orders Caliciales and Trapeliales are more abundant on the deciduous tree species (Fagus sylvatica), whereas the non-lichenized Capnodiales and Mycosphaerellales are more abundant on the coniferous tree species (Ficea abies; Figure 4A). In bark samples from Schorfheide-Chorin the lichenized Lecanorales are more abundant on the deciduous tree species (Fagus sylvatica), whereas Chaetothyriales, Phaeothecales, and Mycosphaerellales are more abundant on the coniferous tree species (Finus sylvestris; Figure 5A). In soil samples from Swabian Alb,



Atheliales are more abundant below the coniferous tree species (Figure 4B). In Schorfheide-Chorin, Russulales are more abundant in soil below the deciduous tree species, whereas Archaeorhizomycetales, Helotiales and Atheliales are more abundant in soil below the coniferous tree species (Figure 5B).

3.4. Variance partitioning

More than 50% of the variance in alpha diversity can be explained by habitat and tree species, while the majority of variance in beta diversity is unexplained (Figure 6). However, in both cases the habitat explains more variance than the dominant tree species. For the alpha diversity models 48% (Swabian Alb) and 68% (Schorfheide-Chorin) of the variance can be explained by the habitat. Although the overall explained variance is less for beta diversity, we observe a similar allocation between habitat and dominant tree species where habitat explains approximately three times (Swabian Alb) and nine times (Schorfheide-Chorin) more variance than the dominant tree species.

3.5. Analysis of co-occurrence networks

We constructed co-occurrence networks for each of the two study regions (Figure 7). Only ASVs that contributed at least 1 % of the total reads entered the network calculations, which resulted in 938 ASVs for

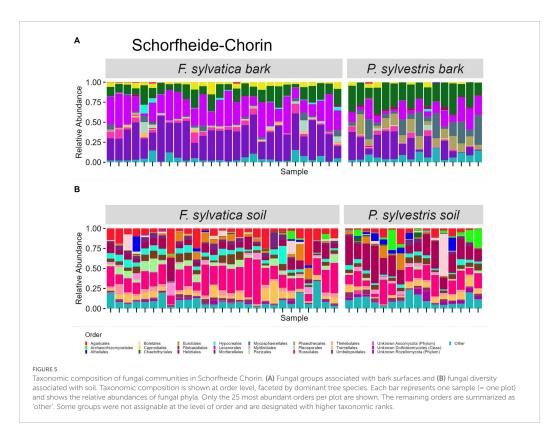
the Swabian Alb and 588 ASVs for Schorfheide-Chorin. The network for Swabian Alb is characterized by a diameter of 6 with an average path length of 2.8 and the network for Schorfheide-Chorin by a diameter of 6 and average path length of 3.1. Both networks have a modularity close or higher than 0.4 (Swabian Alb: 0.382; Schorfheide-Chorin: 0.454). indicating a strong division of the networks into modules (subnetworks) (Newman, 2006), and cluster into four modules. One module (green) contains mostly bark-associated fungi and is clearly distinct from the other three modules (Figure 7). The pink module contains mainly ASVs that occur in the soil of Fagus sylvatica-dominated plots, while the orange module contains mainly ASVs that occur under soil associated with both coniferous tree species. ASVs in the blue module are found in association with either tree species and either habitat in Schorfheide-Chorin and with soil of both tree species in the Swabian Alb (Figure 7). In general, the networks are similar for both study regions, especially showing a clear distinction between soil and bark.

4. Discussion

4.1. Geographic location affects fungal diversities, but not overall community patterns

Abiotic factors, such as climatic conditions or soil properties, influence fungal communities associated with trees (e.g., Goldmann

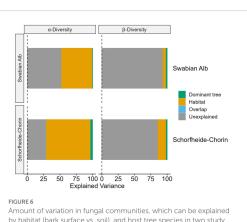
Frontiers in Microbiology 07 frontiers in.org



et al., 2016; Glassman et al., 2017). Here we study two regions in Germany with different climatic and edaphic conditions and report a strong effect of geographic location on fungal diversities associated with bark and soils. The southern sampling region, Swabian Alb, is significantly more diverse than the northern region, Schorfheide-Chorin (Figure 1). This effect is also visible when considering the same host tree species, *Fagus sylvatica*, in both study regions (Figure 2). Reasons for the lower fungal diversity in the northern region may be lower precipitation and overall humidity, as well as lower nutrient availability, due to sandy soils (Fischer et al., 2010).

The two study regions differ in abiotic conditions, dominant coniferous tree species, and overall fungal diversities. Yet, some general patterns of fungal community composition emerge across all sampling sites: 1. Fungal bark communities are less diverse than soil communities, regardless of host tree species; 2. Forest habitat explains more of the fungal variation than host tree species identity. 3. The number of shared fungal taxa – between habitats and between tree species – is small, but the relative abundance of these taxa is high. 4. Fungal networks indicate a separation of bark and soil communities. Fungal bark communities are less diverse and less known than soil communities.

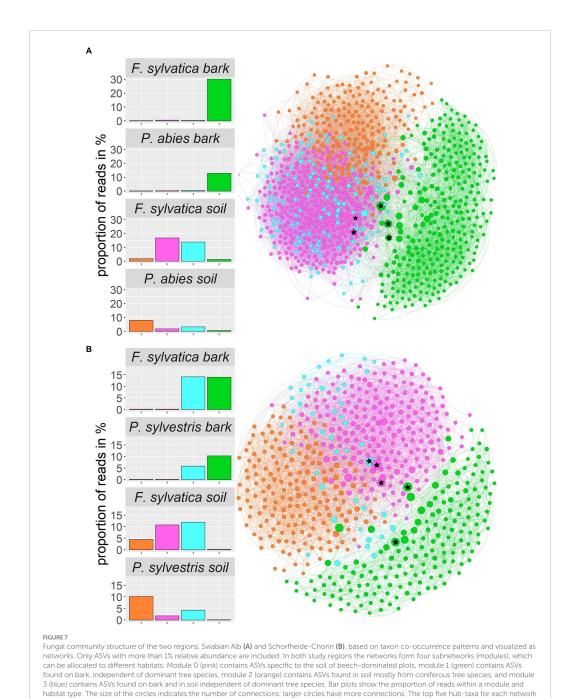
Forest trees form aboveground and belowground ecological niches that are habitat for diverse fungal communities. It has been suggested that fungi often exploit more than one of these habitats, and serve as connector elements between habitats (Baldrian, 2017), but empirical studies including more than one tree-associated niche are still rare (but



see Yang et al., 2022a), and some habitats are poorly known altogether, e.g., the bark surface (Dreyling et al., 2022). Here we contribute to the topic by providing an assessment of fungal communities on bark

regions, Swabian Alb and Schorfheide Chorin. These are results from

a variance partitioning analysis. Values are given for alpha and beta



surfaces of living trees and in soil below these trees, across different tree species in temperate European forests. As a general pattern we observe that bark communities are less diverse than soil communities,

are indicated by asterisks in the circles.

regardless of tree species identity. This may be due to lower nutrient availability, and the presence of physical and chemical stressors in bark habitats (Buck et al., 1998; Baldrian, 2017). In contrast, soil contains

Frontiers in Microbiology 09 frontiersin.org

ample carbon and nutrient sources, as well as various micro niches, supporting diverse fungal communities (Baldrian, 2017).

A striking result of this work is the high proportion of barkassociated fungal reads, which are not assignable at the level of order. This is consistent with an earlier study in the Hainich-Dün Exploratory in central Germany, which included only beech forests (Dreyling et al., 2022). In the present study we report that approximately 16% of the ASVs, corresponding to 33% relative abundance, on bark, cannot be assigned at order level. The fraction of "unknown Dothideomycetes" is particularly prominent across bark surfaces of all tree species in all study regions (Figures 4, 5). In contrast, less than 5% (relative abundance) of the fungi found in soil are unassignable at order level. These results, found in both study regions, and across all tree species, highlight gaps in our knowledge of bark-associated fungal communities in temperate forests. Bien and Damm (2020) found a considerable number of fungi that are not assignable at species or genus level in the wood of fruit trees. Other studies of fungal bark communities focused so far on certain groups, like yeasts (Bhadra et al., 2008), lichens (Aschenbrenner et al., 2017), or endophytic fungi (Pellitier et al., 2019), and did not consider the entire community of fungi that occur on the substrate. The undescribed fungal diversity on tree bark underlines our poor knowledge of aboveground plantmicrobiome relationships (e.g., Aschenbrenner et al., 2017), and fungal diversity in general, with less than an estimated 10% of fungi scientifically described (Hawksworth and Lücking, 2017)

Community composition differs between bark and soil communities: Bark is dominated by ascomycete orders, whereas soil is dominated by basidiomycete orders. The dominance of Basidiomycota in forest soils (including ectomycorrhizal fungi) has been known since the earliest metabarcoding studies (e.g., Buée et al., 2009). The dominance of Ascomycota on bark was shown for apple and pear trees by Arrigoni et al. (2018).

Tree bark is a typical habitat for lichenized fungi, and as expected, a large proportion of bark-associated reads belong to lichen-forming fungal orders. Tree species identity drives part of the lichen flora in Swabian Alb, where Caliciales and Trapeliales are much more prominent on beech than on spruce, adding to the higher overall diversity of lichens on beech. This is in line with a floristic study, which showed that deciduous forests in Swabian Alb are more diverse than coniferous forests with respect to lichenized fungi (Boch et al., 2013). Most tree trunks sampled in the present study were not visibly covered with lichens directly at the sampling sites. However, we likely collected lichen propagules like soredia, thallus fragments, or spores, which adhered to the bark at the sampling site, and which may have been transported there by stemflow or wind (Magyar et al., 2021). The presence of lichens may be connected to the large amount of unassigned fungal reads on bark surfaces: The lichens themselves constitute an ecological niche associated with a particularly high proportion of unknown fungal species, compared to other fungal habitats worldwide (Baldrian et al., 2022).

4.2. Forest habitat explains more of the fungal variation than host tree species identity

Aboveground and belowground habitats provided by trees are characterized by different fungal communities (e.g., Durand et al., 2017). Here we confirm that much of the variance in alpha diversity is

explained by habitat, however, a small portion is explained by tree species identity (Figure 6). The number of fungal taxa shared between aboveground and belowground habitats is low, but the abundance of these taxa is high.

Tree species identity has been described as an important driver of fungal community composition in some forest habitats, such as litter (Prescott and Grayston, 2013; Urbanová et al., 2015). In the present study the bulk of the fungal community did not respond to tree species identity. Different tree species share more than 75% of the fungal reads in the soil habitat, and more than 80% in the bark habitat (Figure 2). However, in both sampling regions, a small portion (less than 30%) of fungal reads was found either on the coniferous or the deciduous tree species, in both habitats, bark, and soil (Figure 2B).

Bark traits of the host trees may account for differences of the associated fungal communities. They include physical texture, water storage capacity, mineral content, pH, chemical composition, and stability. The bark surfaces of most Fagus sylvatica trees sampled in this study were smooth and unbroken, but older trees occasionally featured deep crevices. Bark surfaces of Picea abies were rough and scaly with many cracks, while those of Pinus sylvestris consisted of flaking corky ridges, separated by vertical furrows. The bark of Picea abies and Pinus sylvestris is slightly more acidic (pH 4–5) (Prasetya and Roffael, 1990) than the bark of Fagus sylvatica (pH 4.9–7.0) (Fritz and Heilmann-Clausen, 2010). Furthermore, beech bark contains phenolic substances with antibiotic properties (Tănase et al., 2018).

Differences in light availability at the tree trunks in coniferous versus deciduous forests may influence the local community of light-dependent fungi, such as lichenized fungi. Tree species identity and bark traits are known to influence fungal communities on decaying bark and wood (Yang et al., 2022b). It is presently not known whether fungal communities of living bark affect the communities that assemble on dead bark, e.g., through priority effects, or by switching to a saprotrophic lifestyle (Rai and Agarkar, 2016).

A previous study in the Biodiversity Exploratories found that the fungal soil community differed significantly between beechdominated and coniferous tree-dominated stands (Goldmann et al., 2015). In the present study the tree species effect is slightly more visible in fungal communities in soil than on bark. Possible reasons for this could be the presence of specialized fungal degraders associated with different litter types (Barbi et al., 2016), or the presence of tree species-specific ectomycorrhizal communities (Goldmann et al., 2015; Nacke et al., 2016).

4.3. Ecological networks indicate largely separated above- and belowground fungal communities, with some connecting elements

Aboveground-belowground species interactions affect ecosystem properties, especially at local scales (Deyn and Van Der Putten Wim, 2005). In the forest ecosystem, fungi have been suggested to be connecting elements between different habitats (Baldrian, 2017), yet we know little about the interaction of above- and belowground fungal communities in temperate forests. Fungal communities associated with different tree compartments differ, as it has been shown for poplar trees (Durand et al., 2017), and phyllosphere versus root and soil fungi associated with forest trees in subtropical forests (Yang et al., 2022a). In the present study we use co-occurrence networks to better understand the differences and linkages

between aboveground (bark surface associated) and belowground (soil associated) fungal communities. Networks comprising all fungi of one study region are strikingly similar for the northern and southern study region. One module (containing bark-associated fungi) is clearly distinct from the other three modules. Another module (blue) contains ASVs that are found in samples from both habitats and tree species. We hypothesize that these fungi are able to connect the different habitats. One potential pathway for the connection of the aboveground- and belowground habitats is stemflow (Magyar et al., 2021). Rainwater, which runs down the branches and stems of trees carries a multitude of microorganisms, which are washed into the ground, and may become part of the soil microbiome (van Stan et al., 2020; Magyar et al., 2021; Teachey et al., 2022). Conversely, fungal particles, such as spores, could be transported by wind in the other direction, from soil to bark, and adhere to bark surfaces and bark biofilms. While the bark community forms a single module including fungi from all host tree species, the soil community differentiates into more than one module: one module contains soil fungi in general, another module contains predominantly soil fungi from beech stands. Boraks et al. (2021) describe a similar pattern for tropical fungi, and suggest that belowground fungal communities respond to tree species composition, while aboveground community turnover is more dependent on geographical distances.

So-called hub taxa are important "connectors" that ensure stability and functioning of the network. These taxa have the highest betweenness centrality, i.e., the highest number of shortest paths going through them. ASV 28 ('Phallus impudicus') and ASV_257 ('Cladosporium') are identified as hub taxa in both study regions. Phallus impudicus, the common stinkhorn, occurs in soil and - less abundantly - on bark surfaces. It is a widespread saprotrophic mushroom usually found in soil. Dispersal occurs via insects, which may explain a wide distribution of environmental DNA of this fungus also in aboveground habitats. The genus Cladosporium is cosmopolitan and occupies virtually all ecological niches (Bensch et al., 2012). It is regularly detected in aerobiological samples, also using eDNA methods (Tordoni et al., 2021). Thus, ASV_257 ('Cladosporium') appears to be a widespread member of this genus. One of the hub taxa identified in Schorfheide-Chorin is Phylctis argena, a common crustose lichen on bark, which we frequently encountered in all of the Biodiversity Exploratories. It forms thin thalli on bark, mostly on deciduous trees. Tiny dispersal propagules (soredia) cover individuals of this lichenized fungus and likely account for the wide aboveground and belowground distribution, as they can be transported to other trees by wind, and into the soil by stemflow. Neocucurbitaria quercina is one of the hub taxa identified in Swabian Alb. It belongs to a family of plant-associated or plant pathogenic fungi (Hyde et al., 2013; Wanasinghe et al., 2017). The other hub taxa are taxonomically not assignable at species level, underlining our incomplete knowledge of forest biodiversity also with regard to species with potential importance at ecosystem level.

5. Caveats and conclusion

Ecological inferences based on metabarcoding data are sensitive to the way the amplicons are treated, and it is an ongoing debate whether amplicon sequencing variants (ASVs), or operational taxonomic units (OTUs) better represent fungal communities. Here, we opted for ASVs, because they provide higher accuracy over 97% identity OTUs. Nonetheless, we are aware of limitations of the ASV approach, such as the risk of splitting species into separate ASVs, because of different

rRNA copies present in the same genome (Schloss, 2021). Recent comparative studies drew opposing conclusions as to which method to use, either favoring ASVs (e.g., Joos et al., 2020; Cholet et al., 2022), or OTUs (Tedersoo et al., 2022). We argue that to date, there is no consensus for the most appropriate method for amplicon treatment, and for the time being, both approaches are acceptable.

Overall, our study contributes knowledge to fungal diversity patterns in temperate forests, and the connections between aboveground and belowground fungal habitats. We show that rather few, but highly abundant fungal taxa overlap between habitats and tree species. It remains to be seen, if these taxa are random environmental fungi, or if they are part of a core "forest mycobiome." Our study also provides baseline data of fungi associated with common European tree species, which are potentially useful for biogeographic studies, or biodiversity monitoring. We show that lichenized fungi can be detected in eDNA swabbed from bark surfaces. These data could be assessed for their use in future forest lichen inventories. We also confirm gaps in our knowledge of fungal communities associated with bark surfaces. Future studies of this type, integrating over additional forest habitats, and more organismal groups, will potentially unravel not only diversity patterns and interactions, but also vulnerabilities of the forest ecosystem.

Data availability statement

The raw sequences are deposited in the NCBI SRA repository, accession number SRR23371988. All scripts and additional data necessary to replicate the analysis are available at https://github.com/LukDrey/fungal_habitat. The selection of the dominant tree species is based on a stand composition assessment available at https://www.bexis.uni-jena.de/ under Accession number 22907.

Author contributions

IS, FG, and LD conceived and planned the study. LD, IS, FG, and BH collected the samples. LD and JO generated the molecular data. BH and LD analyzed the data. BH, IS, and LD wrote the manuscript with contributions from all authors. All authors contributed to the article and approved the submitted version.

Funding

This work has been partly funded by the DFG Priority Program 1374 "Biodiversity-Exploratories" (SCHM 1711/8-1). Field work permits were issued by the responsible state environmental offices of Baden-Württemberg and Brandenburg.

Acknowledgments

We thank the managers of the two Exploratories Swabian Alb and Schorfheide-Chorin, Julia Bass and Franca Marian, as well as all former managers for their work in maintaining the plot and project infrastructure; Victoria Grießmeier for giving support through the central office, Andreas Ostrowski for managing the central data base, and Markus Fischer, Eduard Linsenmair, Dominik Hessenmöller, Daniel Prati, Ingo Schöning, François Buscot, Ernst-Detlef Schulze,

Wolfgang W. Weisser and the late Elisabeth Kalko for their role in setting up the Biodiversity Exploratories project. We also thank the administration of the UNESCO Biosphere Reserve Swabian Alb and the UNESCO Biosphere. Reserve Schorfheide-Chorin as well as all land owners for the excellent collaboration. We thank Anna Küchler, Dominik Merges and Henrique Valim for their great support during fieldwork.

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.

References

Abarenkov, K., Zirk, A., Piirmann, T., Pöhönen, R., Ivanov, F., Nilsson, R. H., et al. (2022). UNITE general FASTA release for eukaryotes Version 27.10.2022 UNITE Community.

Arrigoni, E., Antonielli, L., Pindo, M., Pertot, I., and Perazzolli, M. (2018). Tissue age and plant genotype affect the microbiota of apple and pear bark. *Microbiol. Res.* 211, 57–68. doi: 10.1016/j.micres.2018.04.002

Aschenbrenner, I. A., Cernava, T., Erlacher, A., Berg, G., and Grube, M. (2017). Differential sharing and distinct co-occurrence networks among spatially close bacterial microbiota of bark, mosses and lichens. *Mol. Ecol.* 26, 2826–2838. doi: 10.1111/mec.14070

Baldrian, P. (2017). Forest microbiome: diversity, complexity and dynamics. FEMS $Microbiol.\ Rev.\ 41,\ 109-130.\ doi: 10.1093/femsre/fuw040$

Baldrian, P., Kolařík, M., Štursová, M., Kopecký, J., Valášková, V., Větrovský, T., et al. (2012). Active and total microbial communities in forest soil are largely different and highly stratified during decomposition. *ISME J.* 6, 248–258. doi: 10.1038/ismej.2011.95

Baldrian, P., Větrovský, T., Lepinay, C., and Kohout, P. (2022). High-throughput sequencing view on the magnitude of global fungal diversity. *Fungal Div.* 114, 539–547. doi: 10.1007/s13225-021-00472-y

Bálint, M., Tiffin, P., Hallström, B., O'Hara, R. B., Olson, M. S., Fankhauser, J. D., et al. (2013). Host genotype shapes the foliar fungal microbiome of balsam poplar (*Populus balsamifera*). *PLoS One* 8:e53987. doi: 10.1371/journal.pone.0053987

Barbi, F., Prudent, E., Vallon, L., Buée, M., Dubost, A., Legout, A., et al. (2016). Tree species select diverse soil fungal communities expressing different sets of lignocellulolytic enzyme-encoding genes. *Soil Biol. Biochem.* 100, 149–159. doi: 10.1016/j.soilbio.2016.06.008

Barbosa, A. M., Real, R., Muñoz, A. R., and Brown, J. A. (2013). New measures for assessing model equilibrium and prediction mismatch in species distribution models. *Div. Distrib.* 19, 1333–1338, doi: 10.1111/ddi.12100

Bastian, M., Heymann, S., and Jacomy, M. (2009). Gephi: an open source software for exploring and manipulating networks. *ICWSM* 3, 361–362. doi: 10.1609/icwsm.v3i1.13937

Bensch, K., Braun, U., Groenewald, J. Z., and Crous, P. W. (2012). The genus cladosporium. Stud. Mycol. 72, 1–401. doi: 10.3114/sim0003

Bhadra, B., Rao, R. S., Singh, P. K., Sarkar, P. K., and Shivaji, S. (2008). Yeasts and yeast-like fungi associated with tree bark: diversity and identification of yeasts producing extracellular endoxylanases. $Curr.\ Microbiol.\ 56,\ 489-494.\ doi:\ 10.1007/s00284-008-9108-x$

Bien, S., and Damm, U. (2020). Prunus trees in Germany—a hideout of unknown fungi? Mycol. Progress 19, 667–690. doi: 10.1007/s11557-020-01586-4

Blondel, V. D., Guillaume, J.-L., Lambiotte, R., and Lefebvre, E. (2008). Fast unfolding of communities in large networks. *J. Stat. Mech.* 2008:P10008. doi: 10.1088/1742-5468/2008/10/p10008

Boch, S., Müller, J., Prati, D., Blaser, S., and Fischer, M. (2013). Up in the tree—the overlooked richness of bryophytes and lichens in tree crowns. *PLoS One* 8:e84913. doi: 10.1371/journal.pone.0084913

Bolger, A. M., Lohse, M., and Usadel, B. (2014). Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* 30, 2114–2120. doi: 10.1093/bioinformatics/

Boraks, A., Plunkett, G. M., Doro, T. M., Alo, F., Sam, C., Tuiwawa, M., et al. (2021). Scale-dependent influences of distance and vegetation on the composition of aboveground and belowground tropical fungal communities. *Microb. Ecol.* 81, 874–883. doi: 10.1007/s00248-020-01608-4

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2023.1067906/full#supplementary-material

Brandes, U. (2001). A faster algorithm for betweenness centrality*. *J. Math. Sociol.* 25, 163–177. doi: 10.1080/0022250X.2001.9990249

Buck, J. W., Lachance, M.-A., and Traquair, J. A. (1998). Mycoflora of peach bark: population dynamics and composition. Can. J. Bot. 76, 345–354. doi: 10.1139/b98-009

Buée, M., Reich, M., Murat, C., Morin, E., Nilsson, R. H., Uroz, S., et al. (2009). 454 pyrosequencing analyses of forest soils reveal an unexpectedly high fungal diversity. *New Phytol.* 184, 449–456. doi: 10.1111/j.1469-8137.2009.03003.x

Cale, J. A., Garrison-Johnston, M. T., Teale, S. A., and Castello, J. D. (2017). Beech bark disease in North America: over a century of research revisited. Forest Ecol. Manag. 394, 86–103. doi: 10.1016/j.foreco.2017.03.031

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., and Holmes, S. P. (2016). DADA2: high-resolution sample inference from Illumina amplicon data. *Nat. Methods* 13, 581–583. doi: 10.1038/nmeth.3869

Camacho-Sanchez, M., Burraco, P., Gomez-Mestre, I., and Leonard, J. A. (2013). Preservation of RNA and DNA from mammal samples under field conditions. *Mol. Ecol. Res.* 13, 663–673. doi: 10.1111/1755-0998.12108

Cholet, F., Lisik, A., Agogué, H., Ijaz, U. Z., Pineau, P., Lachaussée, N., et al. (2022). Ecological observations based on functional gene sequencing are sensitive to the amplicon processing method. *Msphere*. 7:e00324-22.

Csardi, G., and Nepusz, T. (2006): The igraph software package for complex network research. *Int. J. Comp. Sys.* 1695, 1–8.

Davis, N. M, Proctor, D. M, Holmes, S. P., et al (2018). Simple statistical identification and removal of contaminant sequences in marker-gene and metagenomics data. *Microbiome*, 6, 226. doi: 10.1186/s40168-018-0605-2

Deyn, B. D. G., and Van Der Putten Wim, H. (2005). Linking aboveground and belowground diversity. *Trends Ecol. Evol.* 20, 625–633. doi: 10.1016/j.tree.2005.08.009

Doty, S. L. (2011). "Growth-promoting endophytic fungi of Forest trees" in *Endophytes of Forest trees* (Dordrecht: Springer), 151–156. doi: $10.1007/978-94-007-1599-8_9$

Dreyling, L., Schmitt, I., and Grande, F. D. (2022). Tree size drives diversity and community structure of microbial communities on the bark of beech (Fagus sylvatica). bioRxiv 2022;6951. doi: 10.1101/2022.011.9476951

Durand, A., Maillard, F., Foulon, J., Gweon, H. S., Valot, B., and Chalot, M. (2017). Environmental metabarcoding reveals contrasting belowground and aboveground fungal communities from poplar at a hg phytomanagement site. *Microb. Ecol.* 74, 795–809. doi: 10.1007/s00248-017-0984-0

Fischer, M., Bossdorf, O., Gockel, S., Hänsel, F., Hemp, A., Hessenmöller, D., et al. (2010). Implementing large-scale and long-term functional biodiversity research: the biodiversity Exploratories. *Basic Appl. Ecol.* 11, 473–485. doi: 10.1016/j.base.2010.07.009

Freeman, L. C. (1977). A set of measures of centrality based on betweenness. Sociometry 40:3543. doi: 10.2307/3033543

Fritz, Ö., and Heilmann-Clausen, J. (2010). Rot holes create key microhabitats for epiphytic lichens and bryophytes on beech (*Fagus sylvatica*). *Biol. Conserv.* 143, 1008–1016. doi: 10.1016/j.biocon.2010.01.016

Frøsley, T. G., Kjøller, R., Bruun, H. H., Ejrnæs, R., Brunbjerg, A. K., Pietroni, C., et al. (2017). Algorithm for post-clustering curation of DNA amplicon data yields reliable biodiversity estimates. *Nat. Commun.* 8:1188. doi: 10.1038/s41467-017-01312-x

Fruchterman, T. M. J., and Reingold, E. M. (1991). Graph drawing by force-directed placement. Softw. Pract. Exper. 21, 1129–1164. doi: 10.1002/spe.4380211102

Gerdemann, J. W. (1968). Vesicular-arbuscular mycorrhiza and plant growth. *Annu. Rev. Phytopathol.* 6, 397–418. doi: 10.1146/annurev.py.06.090168.002145

Glassman, S. I., Wang, I. J., and Bruns, T. D. (2017). Environmental filtering by pH and soil nutrients drives community assembly in fungi at fine spatial scales. *Mol. Ecol.* 26, 6960–6973. doi: 10.1111/mec.14414

Gloor, G. B., Macklaim, J. M., Pawlowsky-Glahn, V., and Egozcue, J. J. (2017). Microbiome datasets are compositional: and this is not optional. *Front. Microbiol.* 8:2224. doi: 10.3389/fmicb.2017.02224

Goldmann, K., Schöning, I., Buscot, F., and Wubet, T. (2015). Forest management type influences diversity and community composition of soil fungi across temperate Forest ecosystems. Front. Microbiol. 6:1300. doi: 10.3389/fmicb.2015.01300

Goldmann, K., Schröter, K., Pena, R., Schöning, I., Schrumpf, M., Buscot, F., et al. (2016). Divergent habitat filtering of root and soil fungal communities in temperate beech forests. *Sci. Rep.* 6:31439. doi: 10.1038/srep31439

Hawksworth, D. L., and Lücking, R. (2017). Fungal diversity revisited: 2.2 to 3.8 million species. *Microbiol. Spect.* 5:2016. doi: 10.1128/microbiolspec.FUNK-0052-2016

Herre, E. A., Mejía, L. C., Kyllo, D. A., Rojas, E., Maynard, Z., Butler, A., et al. (2007). Ecological implications of anti-pathogen effects of tropical fungal endophytes and mycorrhizae. *Ecology* 88, 550–558. doi: 10.1890/05-1606

Hyde, K. D., Jones, E. B. G., Liu, J. K., Ariyawansa, H. A., Boehm, E., Boonmee, S., et al. (2013). Families of dothideomycetes. *Fungal Divers*. 63, 1–313. doi: 10.1007/s13225-013-0263-4

Ihrmark, K., Bödeker, I. T. M., Cruz-Martinez, K., Friberg, H., Kubartova, A., Schenck, J., et al. (2012). New primers to amplify the fungal ITS2 region--evaluation by 454-sequencing of artificial and natural communities. FEMS Microbiol. Ecol. 82, 666–677. doi: 10.1111/j.1574-6941.2012.01437.x

Kandlikar, G. S., Gold, Z. J., Cowen, M. C., Meyer, R. S., Freise, A. C., Kraft, N. J. B., et al. (2018). Ranacapa: an R package and shiny web app to explore environmental DNA data with exploratory statistics and interactive visualizations. *F1000Research* 7:1734. doi: 10.12688/f1000research.16680.1

Kassambara, A (2020): Ggpubr: 'ggplot2' based publication ready plots. R Package Version. Available online at: https://CRAN.R-project.org/package=ggpubr.

Kembel, S. W., and Mueller, R. C. (2014). Plant traits and taxonomy drive host associations in tropical phyllosphere fungal communities. *Botany* 92, 303–311. doi: 10.1139/cjb-2013-0194

Krah, F.-S., Seibold, S., Brandl, R., Baldrian, P., Müller, J., and Bässler, C. (2018). Independent effects of host and environment on the diversity of wood-inhabiting fungi. J. Ecol. 106, 1428–1442. doi: 10.1111/1365-2745.1293

Kurtz, Z. D., Müller, C. L., Miraldi, E. R., Littman, D. R., Blaser, M. J., and Bonneau, R. A. (2015). Sparse and compositionally robust inference of microbial ecological networks. PLOS Comput. Biol. 11:e1004226. doi: 10.1371/journal.pcbi.1004226

Joos, L., Bödeker, S., Haegeman, A., Debode, J., Vandecasteele, B., Baeyen, S., et al (2020). Daring to be differential: metabarcoding analysis of soil and plant-related microbial communities using amplicon sequence variants and operational taxonomical units. *BMC genomics.* 21, 1–17.

Lahti, Leo, and Shetty, Sudarshan (2017): Microbiome – tools for microbiome analysis in R. Available online at: http://microbiome.github.io

Lambais, M. R., Lucheta, A. R., and Crowley, D. E. (2014). Bacterial community assemblages associated with the phyllosphere, dermosphere, and rhizosphere of tree species of the Atlantic forest are host taxon dependent. *Microb. Ecol.* 68, 567–574. doi: 10.1007/s00248-014-0433-6.

Lang, C., Seven, J., and Polle, A. (2011). Host preferences and differential contributions of deciduous tree species shape mycorrhizal species richness in a mixed central European forest. Mycorrhiza 21, 297–308. doi: 10.1007/s00572-010-0338-y

Magyar, D. (2008): The tree bark: a natural spore trap. Asp. Appl. Biol. 89, 7–16.

Magyar, D., van Stan, J. T., and Sridhar, K. R. (2021). Hypothesis and theory: fungal spores in Stemflow and potential bark sources. *Front. For. Glob. Change* 4:623758. doi: 10.3389/ffgc.2021.623758

Martin, M. (2011). Cutadapt removes adapter sequences from high-throughput sequencing reads. $\it EMBnet J.~17:10.~doi:~10.14806/ej.17.1.200$

Martins, G., Lauga, B., Miot-Sertier, C., Mercier, A., Lonvaud, A., Soulas, M.-L., et al. (2013). Characterization of epiphytic bacterial communities from grapes, leaves, bark and soil of grapevine plants grown, and their relations. $PLoS\ One\ 8:e73013$. doi: 10.1371/journal.pone.0073013

McMurdie, P. J., and Holmes, S. (2013). Phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. $PLoS\ One\ 8:e61217.\ doi:\ 10.1371/journal.pone.0061217$

McMurdie, P. J., and Holmes, S. (2014). Waste not, want not: why rarefying microbiome data is inadmissible. *PLoS Comput. Biol.* 10:e1003531. doi: 10.1371/journal. pcbi.1003531

Meinshausen, N., and Bühlmann, P. (2006). High-dimensional graphs and variable selection with the lasso. *Ann. Statist.* 34:281. doi: 10.1214/009053606000000281

Menke, S., Gillingham, M. A. F., Wilhelm, K., and Sommer, S. (2017). Home-made cost effective preservation buffer is a better alternative to commercial preservation methods for microbiome research. *Front. Microbiol.* 8:102. doi: 10.3389/fmich.2017.00102

Müller, C. L., Bonneau, R., and Kurtz, Z. (2016). Generalized stability approach for regularized graphical models. doi: 10.48550/arXiv.1605.07072

Nacke, H., Goldmann, K., Schöning, I., Pfeiffer, B., Kaiser, K., Castillo-Villamizar, G. A., et al. (2016). Fine spatial scale variation of soil microbial communities under European beech and Norway spruce. Front. Microbiol. 7:2067. doi: 10.3389/fmicb.2016.02067

Newman, M. E. J. (2006). Modularity and community structure in networks. *Proc. Natl. Acad. Sci. U. S. A.* 103, 8577–8582. doi: 10.1073/pnas.0601602103

Oksanen, Jari F., Blanchet, Guillaume, Friendly, Michael, Kindt, Roeland, Legendre, Pierre, McGlinn, Dan, et al. (2020): Vegan: Community ecology package. Available online at: https://cran.-project.org/web/package/vegan/index.html.

Pellitier, P. T., Zak, D. R., and Salley, S. O. (2019). Environmental filtering structures fungal endophyte communities in tree bark. *Mol. Ecol.* 28, 5188–5198. doi: 10.1111/mec.15237

Prasetya, B., and Roffael, E. (1990). Zur Acidität der Rinde einiger Nadelbaumarten. Holz als Roh-und Werkstoff 48, 429–435. doi: 10.1007/BF02627626

Prescott, C. E., and Grayston, S. J. (2013). Tree species influence on microbial communities in litter and soil: current knowledge and research needs. *For. Ecol. Manag.* 309, 19–27. doi: 10.1016/j.foreco.2013.02.034

Purhonen, J., Ovaskainen, O., Halme, P., Komonen, A., Huhtinen, S., Kotiranta, H., et al. (2020). Morphological traits predict host-tree specialization in wood-inhabiting fungal communities. *Fungal Ecol.* 46:100863. doi: 10.1016/j.funeco.2019.08.007

R Core Team (2021): R: a language and environment for statistical computing. *Version* 4:10. Available online at: https://www.r-project.org/.

Rai, M., and Agarkar, G. (2016). Plant-fungal interactions: what triggers the fungi to switch among lifestyles? Crit. Rev. Microbiol. 42, 428–438. doi: 10.3109/1040841X.2014.958052

Rajala, T., Peltoniemi, M., Pennanen, T., and Mäkipää, R. (2010). Relationship between wood-inhabiting fungi determined by molecular analysis (denaturing gradient gel electrophoresis) and quality of decaying logs. *Can. J. For. Res.* 40, 2384–2397. doi: 10.1139/XIO-176

RStudio Team (2021). RStudio: Integrated Development Environment for R. Posit Software, PBC, Boston, MA. Available online at: http://www.posit.co/

Russel, Jakob (2021). MicEco: Various functions for microbial community data. Rehackage Version 0.9.19 doi: 10.5281/zenodo.4733747

Saitta, A., Anslan, S., Bahram, M., Brocca, L., and Tedersoo, L. (2018). Tree species identity and diversity drive fungal richness and community composition along an elevational gradient in a Mediterranean ecosystem. *Mycorrhiza* 28, 39–47. doi: 10.1007/s00572-017-0806-8

Schall, Peter, and Ammer, Christian (2018): Stand composition based on 2nd forest inventory (abundance, basal area, crown projection area and volume) on all forest EPs, 2014–2018. Version 2. Biodiversity exploratories information system dataset. Available online at: https://www.bevis.uni.eina.ei.gd/dat/data/s/bwydata/27901

Schnell, I. B., Bohmann, K., Gilbert, M., and Thomas, P. (2015). Tag jumps illuminated--reducing sequence-to-sample misidentifications in metabarcoding studies. *Mol. Ecol. Res.* 15, 1289–1303. doi: 10.1111/1755-0998.12402

Shannon, C. E. (1948). A mathematical theory of communication. *Bell Syst. Tech. J.* 27, 623–656. doi: 10.1002/j.1538-7305.1948.tb00917.x

Schloss, P. D. (2021). Amplicon sequence variants artificially split bacterial genomes into separate clusters. Msphere.6:e00191-21.

Stevens, Martin Henry H. (2019). Adonis: permutational multivariate analysis of variance using. Available online at: https://rdrr.io/rforge/vegan/man/adonis.html

Tănase, C., Coşarcă, S., Toma, F., Mare, A., Man, A., Miklos, A., et al. (2018). Antibacterial activities of beech bark (Fagus sylvatica L.) polyphenolic extract. *Environ. Eng. Man. J.* 17, 877–884. doi: 10.30638/eemj.2018.088

Teachey, M. E., Ottesen, E. A., Pound, P., and van Stan, J. T. (2022). Under the canopy: disentangling the role of stem flow in shaping spatial patterns of soil microbial community structure underneath trees. *Environ. Microbiol.* 24, 4001–4012. doi: 10.1111/1462-2920.15970

Tedersoo, L., Bahram, M., Zinger, L., Nilsson, R. H., Kennedy, P. G., Yang, T., et al. (2022). Best practices in metabarcoding of fungi: From experimental design to results. *Molecular ecology*. 31, 2769–2795.

Teunisse, Guus Martijn (2017): Fantaxtic - Fantaxtic plots for phyloseq objects!.

Tordoni, E., Ametrano, C. G., Banchi, E., Ongaro, S., Pallavicini, A., Bacaro, G., et al. (2021). Integrated eDNA metabarcoding and morphological analyses assess spatio-temporal patterns of airborne fungal spores. *Ecol. Indic.* 121:107032. doi: 10.1016/j.ecolind.2020.107032

Urbanová, M., Snajdr, J., and Baldrian, P. (2015). Composition of fungal and bacterial communities in forest litter and soil is largely determined by dominant trees. *Soil Biol. Biolems.* 84, 35–64. doi: 10.1016/j.soilbio.2015.02.011

van Stan, I. I., John, T., Morris, C. E., Aung, K., Kuzyakov, Y., Magyar, D., et al. (2020). "Precipitation partitioning—hydrologic highways between microbial communities of the plant microbiome?" in *Precipitation partitioning by vegetation* (Cham: Springer), 229–252. doi: 10.1007/978-3-030-29702-2_14

Appendix - Publications

10.3389/fmicb.2023.1067906 Hofmann et al.

Wanasinghe, D. N., Phookamsak, R., Jeewon, R., Wen, J. L., Hyde, K. D., Jones, E. B. G., et al. (2017). A family level rDNA based phylogeny of Cucurbitariaceae and Fenestellaceae with descriptions of new fenestella species and Neocucurbitaria gen. Nov. *Mycosphere* 8, 397–414. doi: 10.5943/mycosphere/8/4/2

White, T. J., Bruns, T., Lee, S., and Taylor, J. (1990). "Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics" in *PCR protocols*. (Elsevier), 315–322.

Wickham, Hadley. (2016). ggplot2: elegant graphics for data analysis. $\it Version.$ 3:5. doi: 10.1007/978-3-319-24277-4

Yang, S., Poorter, L., Kurmane, E. E., Sass-Klaassen, U., Leite, M. F. A., Costa, O. Y. A., et al. (2022b). Stem traits, compartments and tree species affect fungal communities on decaying wood. *Environ. Microbiol.* 24, 3625–3639. doi: 10.1111/1462-2920.15953

Yang, H., Yang, Z., Wang, Q.-C., Wang, Y.-L., Hu, H.-W., He, J.-Z., et al. (2022a). Compartment and plant identity shape tree Mycobiome in a subtropical Forest. *Microbiol. Spect.* 10:e0134722. doi: 10.1128/spectrum.01347-22

Supplementary Material to

Hofmann, B., Dreyling, L., Dal Grande, F., Otte, J. & Schmitt, I. (2023): Habitat and tree species identity shape aboveground and belowground fungal communities in central European forests. *Frontiers in Microbiology* 14:1067906. doi: 10.3389/fmicb.2023.1067906

Figure S1: Rarefaction curves of bark and soil samples for the two study regions.

Figure S2: Venn diagrams of the overlap between tree species for bark and soil microbiomes in each region (A + B Swabian Alb, C + D Schorfheide-Chorin), and overlap between soil and bark microbiome within tree species in each region (E + F Swabian Alb, G + H Schorfheide-Chorin).

Figure S1

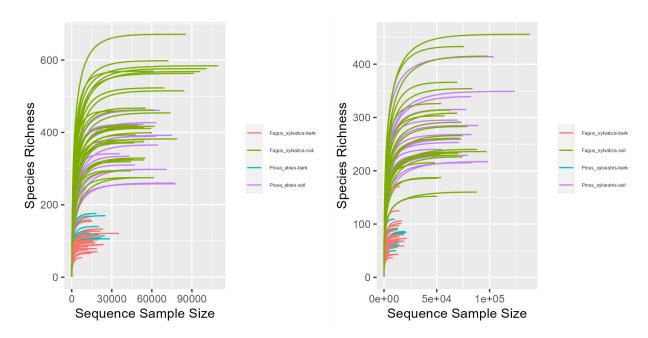
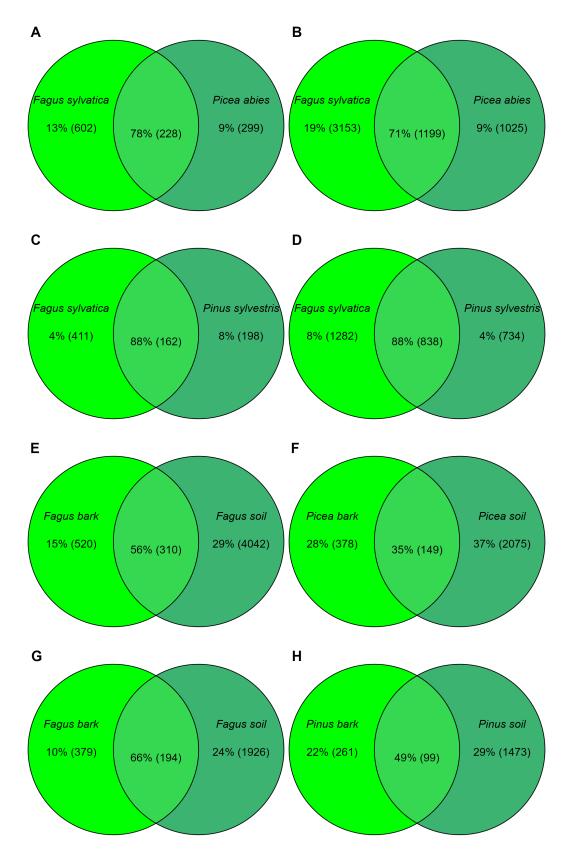


Figure S2



A.4. Surveying lichen biodiversity in forests: A comparison of expert mapping and simple eDNA metabarcoding

Declaration of author contributions to the manuscript:

Surveying lichen biodiversity in forests: A comparison of expert mapping and simple eDNA metabarcoding

Status: submitted

Contributing authors: Lukas Dreyling (LD), Steffen Boch (SB), H. Thorsten

Lumbsch (HTL), Imke Schmitt (IS)

What are the contributions of the doctoral candidate and his co-authors?

(1) Concept and design

Doctoral candidate (LD): 35% Co-author IS: 65%

(2) Conducting tests and experiments

Doctoral candidate (LD): 50% (sample collection from the field, PCR)
Co-author SB: 45% (sample collection from the field)
5% (sample collection from the field)

(3) Compilation of data sets and figures

Doctoral candidate (LD): 70% (processing of sequencing reads, ASV inference,

taxonomic assignment, preparation of figures and tables)

Co-author SB: 30% (data compilation of floristic lichen occurence)

(4) Analysis and interpretation of data

Doctoral candidate (LD): 40% (data curation, method comparison, intererpretation)

Co-author SB: 10% (data curation)

Co-author HTL: 20% (data curation and interpretation) Co-author IS: 30% (data curation data interpretation)

(5) Drafting of manuscript

Doctoral candidate (LD): 75% Co-author SB: 5% Co-author HTL: 5% Co-author IS: 15%

Dreyling et al. (2023), submitted

I hereby certify that the information above is correct.							
Date and place	Signature doctoral candidate						
Date and place	Signature supervisor						
Date and place	If required, signature of corresponding author						

Surveying lichen diversity in forests: A comparison of expert mapping and simple eDNA metabarcoding

Lukas Dreyling^{1,2}, Steffen Boch³, H. Thorsten Lumbsch⁴, Imke Schmitt^{1,2}

- 1: Senckenberg Biodiversity and Climate Research Centre (SBiK-F), Frankfurt am Main, Germany;
- 2: Goethe University Frankfurt, Institute of Ecology, Evolution and Diversity, Frankfurt am Main, Germany;
- 3: WSL Swiss Federal Research Institute, Birmensdorf, Switzerland
- 4: The Grainger Bioinformatics Center and Negaunee Integrative Research Center, Science & Education, The Field Museum, Chicago, IL 60605-2496, USA

Corresponding author: lukas.dreyling@senckenberg.de

Running title: Comparing eDNA to floristic surveys for lichen

Keywords: assessment, biodiversity, databases, floristic mapping, identification

Abstract

Lichens are important taxa in Central European forests, contributing to essential functions such as nutrient cycling. Because the identification of lichen species is usually difficult, it largely depends on the expertise of the collector. Thus, biodiversity assessments take a long time and often focus on small spatial scales. The application of eDNA methods promises to solve these challenges, but have not been evaluated for lichen surveys. Here we provide a comprehensive comparison of eDNA metabarcoding and an expert floristic survey from the same forest plots. We describe how the two methods differ in the identified species and their overlap. We find that eDNA identifies a higher diversity of inconspicuous species, that are difficult to distinguish. However, many prevalent taxa reported in the floristic survey could not be assigned due to gaps in the reference databases. Therefore, we conclude that currently eDNA has merit as a complementary tool to survey lichen biodiversity on large scales, but cannot be used on its own. We advocate for the further development of specialized and complete databases.

Introduction

Biodiversity assessments form the foundation of many ecological disciplines and inform a range of topics, from conservation ecology to land-use management (Ferrier et al. 2004). However, conventional biodiversity surveys are often costly, especially because of the high time expenditure (Yu et al. 2012). In addition, they are often focused on single taxon groups and thus require in-depth taxonomic expertise (Giordani et al. 2009). Often these limitations make it unfeasible to sample over extended spatial scales, and restrict biodiversity assessments to the local or regional level (Ferrier et al. 2004).

The application of environmental DNA (eDNA) promises to resolve many of these challenges, by allowing species level identification from samples of the complete DNA present in a simple environmental sample (Taberlet et al. 2012, Yoccoz 2012). With the emergence of methods such as eDNA metabarcoding, assessing biodiversity has become comparatively fast and cheap, and does not require extensive taxonomic expertise (Deiner et al. 2017). Environmental DNA metabarcoding, has several advantages over conventional assessment methods. Through the use of unspecific primers it is usually possible to identify a broad range of target taxa (Taberlet et al. 2012). Furthermore, collections of eDNA usually are non-invasive and do not require destruction of specimens, e.g., for voucher collection (Deiner et al. 2017). Despite some drawbacks, in particular due to incomplete databases and primer bias (Keck et al. 2022), eDNA has shown great potential for biodiversity assessments (Shirouzu et al. 2016, Frøslev et al. 2019). In comparisons to conventional methods the overlap with eDNA data was variable depending on the taxonomic group, but eDNA always identified taxa that were not picked up with other methods (Cordier et al. 2021). In a metaanalysis, eDNA was found to detect more species in general and significantly more rare species, exhibiting higher accuracy and efficiency, while being less costly than its conventional counterparts (Fediajevaite et al. 2021). Moreover, eDNA has high potential to detect higher rates of cryptic diversity (Deiner et al. 2017).

Lichens are one of the oldest known examples of symbiotic systems (Schwedener 1886). In modern lichenology, they are often described as the lichen holobiont, which includes many organisms in addition to the primary fungal-algal or fungal-cyanobacterial symbiosis (Muggia and Grube 2018, Grimm et al. 2021). In Central European forests, tree trunks are an essential habitat for many lichen species (Hofmeister et al. 2016), and consequently lichen and their symbionts are characteristic taxa of the bark surface community (Grube et al. 2015, Baldrian 2017, Dreyling et al. 2022, Hofmann et al. 2023). In fact, the bark of trees has been proposed as an important part of the forest microbiome, which sustains a high microbial biomass if lichens are present (Baldrian 2017). Furthermore, lichens and their associated microbiome are important for nutrient cycling, with a particularly high contribution to nitrogen cycles (Rousk et al. 2015). Additionally, they often serve as bioindicators for pollution and ecosystem health (Sancho et al. 2019, Grimm et al. 2021).

Unfortunately, assessments of lichen biodiversity can be challenging, even for taxonomic experts (Vondrák et al. 2016). Since species identification of lichens relies only on few morphological traits (Crespo and Lumbsch 2010) considerable expertise is necessary and often requires specimen collection for ex situ identification, e.g., through microscopy or chemical tests (Wright et al. 2019). As a result the outcomes of lichen surveys are highly dependent on the training of collectors (Giordani et al. 2009). Additionally, lichens are a group with high potential for cryptic

diversity that can not be distinguished in the field (Crespo and Lumbsch 2010, Altermann et al. 2014).

Since metabarcoding gained considerable popularity for the identification of fungi (Schmidt et al. 2013, Bálint et al. 2014, Tedersoo et al. 2022), it has also contributed to many advances in the field of lichenology (Dal Grande et al. 2018, Keepers et al. 2019, Hawksworth and Grube 2020). Applications of metabarcoding did not solely focus on the fungal part, but shed light on the diversity of algal photobionts (Dal Grande et al. 2018) and the lichen microbiome as a whole (Hawksworth and Grube 2020). However, studies have rarely investigated the biodiversity of lichens through the application of metabarcoding. A small number of studies have attempted to identify species and characterize the lichen community by metabarcoding bulk specimen collections (Wright et al. 2019, Henrie et al. 2022). They found that this method produces comparable results between minimally trained and expert collectors and thus potentially reduces the need for extensive training (Wright et al. 2019, Henrie et al. 2022). Furthermore, they conclude that metabarcoding surveys could enable a more efficient sampling over larger spatial extent (Wright et al. 2019). However, the metabarcoding of bulk samples is usually less challenging than eDNA methods (Taberlet et al. 2012).

In this study we apply eDNA metabarcoding to samples from the tree bark, the most important lichen habitat in Central European forests (Hofmeister et al. 2016). We use data from the full fungal diversity of the tree bark microbiome, and assign taxa to functional guilds to find species of lichenized fungi. We examine the diversity of lichens acquired by metabarcoding and compare the results to a previous floristic survey carried out in the exact same forests (Boch et al. 2013). We assess the following questions: I. Which species of lichenized fungi can be identified from environmental samples via eDNA metabarcoding? II. What are the differences to the diversity obtained through an expert survey? III. Is eDNA a reliable tool to survey lichen diversity in forests?

Material and Methods

Study sites

We surveyed communities of forest dwelling lichen species on 150 plots, spread across three regions, within the Biodiversity Exploratories framework (Fischer et al. 2010). The regions mark a south-west to north-east gradient across Germany and differ in their climate and topography as well as the human impact, e.g., through forest management (Fischer et al. 2010, Boch et al. 2021). As determined by using a forest inventory (Schall and Ammer 2018), beech (*Fagus sylvatica*) trees are dominant on most plots in all three regions with some plots in the SW and NE dominated by coniferous trees, spruce (*Picea abies*) and pine (*Pinus sylvestris*) respectively. In the Central region none of the sampled plots are dominated by conifers. Additionally other broad-leaved species (*Quercus sp.*, *Acer pseudoplatanus*, *Ulmus glabra*, *Carpinus betulus*) dominated on some plots. For both eDNA sampling and classical lichen mapping, we surveyed a 20 × 20 m subplot within the established 100 × 100 m experimental plots.

eDNA sampling & processing

We collected eDNA samples from the bark of six trees of the dominant species in May 2021 and pooled these samples into one composite sample per plot. Since we had previously shown large community differences between tree sizes (Dreyling et al. 2022) we included two trees of large (>

30 cm diameter at 150 cm height), medium (15-30 cm) and small (5-15 cm) size in each sample. If this was not possible we included more trees of the size class that best represented the forest around the sampling plot. To sample the bark eDNA we moisturised the bark surface with sterile water and then used a nylon-flocked medical swab (FLOQSwabsTM, Copan, Brescia, Italy) to collect the liberated material (Fig. 1). We swabbed around the full tree trunk at approximately 150 cm height from the forest floor, excluding large patches of bryophytes but explicitly including patches of lichen covered bark. The swabs where fixed with 5 ml nucleic acid preservation (NAP) buffer (Camacho-Sanchez et al. 2013) in 15 ml tubes directly after sampling and placed on ice in the field. Afterwards the samples where stored at 4 °C until DNA extraction in the following week.



Figure 1: Sampling procedure: We sprayed sterile water once around the tree trunk at breast height, and swabbed the bark surface in a zigzagging motion along a 10 cm wide band. The swabbing included smooth bark surfaces and crevices, as well as epiphytic organisms, if they were present.

A detailed description of the DNA extraction and bioinformatic processing of sequencing reads is given in (Dreyling et al. 2022). In brief: We extracted DNA, from samples as well as three extraction blanks, using an extraction kit (Quick-DNA Fecal/Soil Microbe Microprep, Zymo Research Europe GmbH, Freiburg, Germany) with an additional step ensuring liberation of material from the swab. Targeting the ITS2 region we subsequently amplified the fungal DNA in triplicates, using the universal primer pair fITS7 (GTGARTCATCGAATCTTTG) (Ihrmark et al. 2012) and ITS4 (TCCTCCGCTTATTGATATGC) (White et al. 1990). PCR reactions also included negative controls (without sample material) and multiplex controls (empty wells). We cleaned the amplicons via a magnetic bead protocol (MagSI-NGSPREP Plus, magtivio B.V., Geelen, Netherlands) and measured DNA concentration through fluorometry (Qubit dsDNA HS assay on a Qubit 3.0, Thermo Fisher Scientific, MA, United States), before equimolar pooling. The library preparation was carried out by Fasteris SA (Plan-les-Ouates, Switzerland) according to their MetaFast Protocol (avoiding additional PCR bias) as well as the sequencing on an Illumina MiSeq platform with 2 × 300 bp paired-end reads.

We used Cutadapt (v3.3, (Martin 2011)) to demultiplex the obtained sequencing reads and DADA2 (Callahan et al. 2016) to infer Amplicon Sequencing Variants (ASVs). Taxonomy was assigned against the UNITE database (Abarenkov et al. 2022, Version 9.0, incl. non-fungal eukaryotic DNA as outgroups). We used FUNGuild (Nguyen et al. 2016) to assign information on the functional guild to the fungal ASVs acquired after sequencing. Subsequently we filtered the dataset to only contain ASVs which were assigned as "Lichenized" fungi by FUNGuild. Additionally we conducted a BLAST (Altschul et al. 1990) run against the NCBI nucleotide database (Sayers et al. 2022), for all lichenized ASVs without any or with dubious species and genus level assignment. Taxa were included in the final dataset if the "percent identity" from BLAST was higher than 95%. All scripts on the bioinformatic processing, as well as the analysis, are available at Github at https://github.com/LukDrey/eDNA_lichen_survey.

Traditional floristic survey

In a floristic survey in 2007 and 2008, the occurrence of lichen species were recorded in over 600 plots of the Biodiversity Exploratories (Boch et al. 2013). The surveyed plots include the 150 plots in which the eDNA samples were collected. The survey covered a comparable area of $20 \text{ m} \times 20 \text{ m}$ and recorded all lichens occurring on bark (up to 2.5 m height), rocks, deadwood and soil. Most specimens were identified in situ, except when morphological identification was not directly possible. If identification was not possible in the field it was subsequently carried out in the laboratory, e.g., through chemical reactions.

Comparison of the two methods

Initially we reviewed the taxonomy used by both eDNA and floristic survey, and unified the classification by adopting the names currently listed in Index Fungorum (https://www.indexfungorum.org/). To facilitate comparisons between the two methods, we transformed the read counts from the eDNA metabarcoding to presence-absence data. In a conservative approach, we curated the obtained species list to include only species which could be assigned with a high confidence. Additionally, we filtered species from the floristic dataset that were not recorded on the bark of trees.

Using the two presence-absence datasets we compared the two methods and assess which and how many species were found with each method. Furthermore, we investigate the number of plots they were recorded in. In addition, we examine the number of species exclusively identified by each method and evaluate their overlap using Venn diagrams. Finally, we select five species to demonstrate geographical occurrence patterns and compare differences between the two methods in spatially proximate plots.

Results

In total we could identify 154 lichen species that were found on the tree bark in the Biodiversity Exploratories using both eDNA metabarcoding and floristic surveys. In general, we find only a small overlap in the set of taxa identified by the two methods. In fact, only 26%, or 40 species, could be identified in both eDNA and floristic mapping (Fig. 2). A list of species is given as supplementary material.

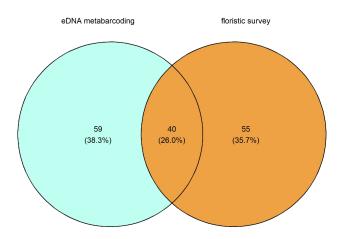


Figure 2: Number and proportion of lichenized fungi found either in eDNA metabarcoding or floristic survey, or in both methods.

We identified a total number of 99 species using our eDNA metabarcoding approach. The species recorded in the eDNA metabarcoding accounted for 25% of the total fungal reads on average. However, the proportions differed considerably between the three regions. The proportion of lichens was highest in the South-West region with approximately 36% of the total fungal reads, 26% in the North-East and lowest in the Central region with only 13%. Nevertheless, several lichen species, such as *Scoliciosporum umbrinum*, *Lepraria incana* and *Phlyctis argena*, were among the relatively most abundant taxa in the full eDNA dataset. Consequently, these three species were also among the most prevalent taxa in our study (Fig. 3). However, although *S. umbrinum* is a highly ubiquitous species and was recorded in 146/150 plots, it was not recorded in the floristic survey. Its sister species *Scoliciosporum chlorococcum* was also highly prevalent in the eDNA study (121 occurrences) but rarely found in the floristic dataset (11 occurrences).

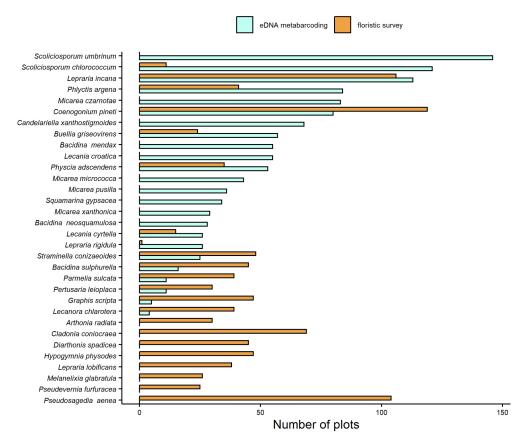


Figure 3: Most common species of lichenized fungi detected by either method (eDNA metabarcoding or floristic survey). We show taxa, which occurred in at least 25 plots across the three regions.

A sum of 95 epiphytic lichen species were recorded on bark in the floristic study. From those species, 86 were found in the South-West region, 37 in the Central and 36 in the North-East. The most prevalent species in the floristic survey were *Coenogonium pineti* and *Lepraria incana*, which were also found in the eDNA study (Fig. 3). *C. pineti* was found in 119/150 plots in the floristic study and in 80 plots in the eDNA dataset. The prevalence of *L. incana* was even more similar, being found in 106 plots with floristic and 113 plots with eDNA methods. Interestingly, *Pseudosagedia aenea*, a common species found in the floristic survey, was not found by the eDNA metabarcoding, albeit occurring in 104 plots. In addition, only very few species were found in a similar number of plots with both methods (Fig. 3).

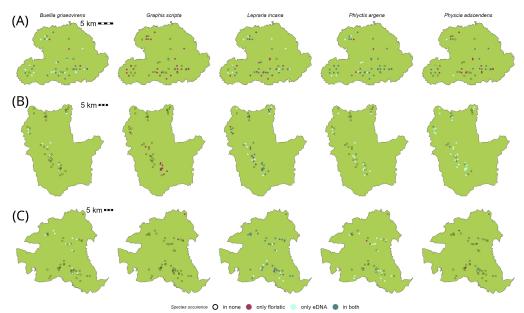


Figure 4: Distribution of five example species within the analyzed forest plots of the three regions. Shown are occurrence data based on the floristic survey and eDNA metabarcoding. Each map represents one region (Biodiversity Exploratory): A) South-West (Swabian Alb); B) Central (Hainich-Dün); C) North-East (Schorfheide-Chorin). Each circle depicts a 100 x 100 m forest plot.

The distribution maps of the five example species, Buellia griseovirens, Graphis scripta, Lepraria incana, Phlyctis argena and Physcia adscendens, highlight the clear differences between the three study regions. In general, the highest number of plots with lichen occurrences were in the South-West region (Fig. 4 A), while most plots of the North-East region (Fig. 4 C) were free of lichens. Furthermore there is no region in which the identification of these five lichen species seemed to work comparably well. Yet, the north-eastern region featured the highest number of plots in which species could be found with both the eDNA and floristic mapping. Interestingly, the occurrence does not follow a clearly distinguishable pattern of spatial clustering within the regions. Plots without any lichen record occur frequently close to other plots were lichens had been recorded with at least one method. Additionally, if a lichen species was frequently recorded by both methods, such as B. griseovirens, L. incana and P. argena in the South-West (Fig. 4 A), than it was also found by one of the methods in spatially close plots. In contrast, some species were only recorded by either method, such as G. scripta or P. adscendens in the Central region (Fig. 4 B). P. adscendens seems to be a special case. It was consistently picked up by both methods in the South-West (Fig. 4 A), but only by eDNA in the Central region (Fig. 4 B) and rarely only by floristic mapping in the North-East (Fig. 4 C).

Discussion

In our study we detected a comparable number of species with both eDNA metabarcoding and floristic mapping. The eDNA identified a slightly higher number of taxa which is congruent with lichen studies from other ecosystems (Wright et al. 2019). However, only 26% of the total taxa were shared between both methods. Previous studies, comparing eDNA to fruiting body collections of forest fungi, reported a similar overlap (Frøslev et al. 2019), and percentages of non-shared taxa (Shirouzu et al. 2016).

Many species identified by only one method were highly prevalent in the dataset. For example, *Scoliciosporum umbrinum* was almost ubiquitous in the three regions but was not identified in the floristic study. It is a taxon with a high morphologic variability and thus hard to identify and distinguish (Dymytrova 2011). Therefore, it is plausible that it has been overlooked or simply grouped with other taxa in the floristic survey. Other taxa commonly found in the eDNA metabarcoding dataset belong to the genus *Micarea* and *Candelariella*. At the time of the floristic survey, some species from these genera had not been formally described. For example, *C. xanthostigmoides* was reported from Europe for the first time in 2012 (Westberg and Clerc 2012) and *M. czarnotae* was only described as a distinct species in 2019 (Launis et al. 2019). However, *M. prasina* as the group that *M. czarnotae* had been grouped with, was also only found in eight plots in the floristic study.

In general, we recorded a high number of inconspicuous taxa from genera that are difficult to distinguish, such as *Micarea* (Launis et al. 2019), *Scoliciosporum* (Dymytrova 2011) and *Bacidina* (Czarnota and Guzow-Krzemińska 2018), only in the eDNA metabarcoding. Consistent with our findings, other studies have previously reported that eDNA was superior in revealing hidden diversity for fungi (Shirouzu et al. 2016) and lichens (Wright et al. 2019). An additional advantage of the eDNA approach might be the detection of taxa not directly occurring on the sampled substrate itself, for example from propagules (Wright et al. 2019, Henrie et al. 2022). In fact, we found *Squamarina gypsacea*, a species usually occurring on calcareous rocks (Timdal

1983), in over 30 plots in the South-West and Central region. Although the occurrence in these regions corresponds to the geology (Fischer et al. 2010), the occurrence on bark was surprising and likely due to dispersed propagules. Therefore, occurrence data from lichen eDNA need to be carefully interpreted when inferring distribution data.

The floristic dataset also includes numerous taxa which were not identified by the eDNA approach. One potential reason is that their habitat is outside of the sampled area, for example in the tree crowns or at the base of the tree. In fact, several species prevalent in the floristic dataset but not the eDNA, occur in these habitats, e.g., *Cladonia coniocraea* at the base of trees (Wirth et al. 2013), or *Pseudevernia furfuracea* on branches in the canopy (Kranner et al. 2003). Therefore, restricting the eDNA sampling, or any survey, to a single forest substrate is likely insufficient to describe the full lichen diversity (see also (Boch et al. 2013)).

Another apparent reason for the differences in eDNA and floristic surveys are related to the databases necessary for taxonomic assignment of the metabarcoding reads. Despite large efforts in recent years towards the development of reference databases for fungal taxonomy, like the UNITE database (Abarenkov et al. 2023) or the GlobalFungi project (Větrovský et al. 2020), many gaps remain. In our study, several species commonly found in the floristic study have no reference sequences in the UNITE database, including *Diarthonis spadicea*, *Lepraria lobificans* and all species from the genus *Arthonia*. In addition these species could also not be assigned against the NCBI nt database, indicating other issues. A potential reason might be a primer bias towards these taxa (Bellemain et al. 2010, Keck et al. 2022). Previous studies have proposed to close the gaps in the reference databases by large scale sequencing of lichen herbarium specimens (Gueidan and Li 2022) and recently a first database focussing on Central European lichens was published (Vondrák et al. 2023).

Between the eDNA sampling and the floristic survey was a considerable temporal gap of approximately 13 years. At the time of the floristic survey the influence of pollution, especially by sulphur dioxide (SO₂), was considerably higher than today and heavily polluted areas were considered to be "lichen deserts" (Sancho et al. 2019). Currently the SO₂ levels are decreasing and many species are expected to return to formerly uninhabitable ecosystems (Sancho et al. 2019). Other species that are tolerant to these conditions, especially acidofrequent species like *Straminella conizaeoides*, have been reported to decline in Central Europe (Farkas et al. 2022). In our study the number of plots in which *S. conizaeoides* was identified with eDNA in 2021, is only half of the floristic study in 2007/2008. Simultaneously to the decreasing SO₂ levels, temperate forests experience a higher deposition of nitrogen, e.g., through ammonia fertilizers (Carter et al. 2017). Therefore, nitrophytic species numbers might have increased in the recent past (Carter et al. 2017). However, *Xanthoria parietina*, arguably the most common nitrophytic lichen in Central Europe, was infrequently found in the floristic study and even less in the eDNA. Based on these developments an additioo

Conclusions

In its current form eDNA metabarcoding can not be used as a stand-alone tool to survey epiphytic lichen diversity. However it can serve as a valuable complementary tool, similarly to studies from many other taxonomic groups (see (Beng and Corlett 2020, Fediajevaite et al. 2021). Currently the largest limitation is the gap in the available databases together with the still fluidly developing taxonomy based on phylogenetic data. Both hamper the taxonomic assignment and comparison with

earlier studies. Looking forward the recent development of a lichen specific database might solve some of these issues. If eDNA biodiversity assessments are taken beyond the description of diversity, recently developed methods circumvent this issue altogether and are able to use unclassified taxa in the prediction of ecological states (Keck et al. 2023). Future studies of lichen biodiversity could employ these methods and expand the use of lichens as modern biomonitoring agents. Lastly we recommend to extend the sampling of environmental DNA to other substrates like rocks, branches and soil, to obtain a more complete picture of the lichen diversity in forest ecosystems.

Acknowledgments

We thank the managers of the three Exploratories, Max Müller, Julia Bass, Robert Künast, Anna K. Franke, Franca Marian and all former managers for their work in maintaining the plot and project infrastructure; Victoria Grießmeier for giving support through the central office, Andreas Ostrowski for managing the central data base, and Markus Fischer, Eduard Linsenmair, Dominik Hessenmöller, Daniel Prati, Ingo Schöning, François Buscot, Ernst-Detlef Schulze, Wolfgang W. Weisser and the late Elisabeth Kalko for their role in setting up the Biodiversity Exploratories project. We thank the administration of the Hainich national park, the UNESCO Biosphere Reserve Swabian Alb and the UNESCO Biosphere Reserve Schorfheide-Chorin as well as all land owners for the excellent collaboration. The work has been (partly) funded by the DFG Priority Program 1374 "Biodiversity- Exploratories" (SCHM 1711/8-1). Field work permits were issued by the responsible state environmental offices of Baden-Württemberg, Thüringen, and Brandenburg.

References

- Abarenkov K, Zirk A, Piirmann T, Pöhönen R, Ivanov F, Nilsson RH, Kõljalg U (2022) UNITE general FASTA release for eukaryotes. Version 27.10.2022. UNITE Community. https://doi.org/10.15156/BIO/2483913
- Abarenkov K, Nilsson RH, Larsson K-H, Taylor AFS, May TW, Frøslev TG, Pawlowska J, Lindahl B, Põldmaa K, Truong C, Vu D, Hosoya T, Niskanen T, Piirmann T, Ivanov F, Zirk A, Peterson M, Cheeke TE, Ishigami Y, Jansson AT, Jeppesen TS, Kristiansson E, Mikryukov V, Miller JT, Oono R, Ossandon FJ, Paupério J, Saar I, Schigel D, Suija A, Tedersoo L, Kõljalg U (2023) The UNITE database for molecular identification and taxonomic communication of fungi and other eukaryotes: sequences, taxa and classifications reconsidered. Nucleic Acids Research: gkad1039. https://doi.org/10.1093/nar/gkad1039
- Altermann S, Leavitt SD, Goward T, Nelsen MP, Lumbsch HT (2014) How Do You Solve a Problem like Letharia? A New Look at Cryptic Species in Lichen-Forming Fungi Using Bayesian Clustering and SNPs from Multilocus Sequence Data. PLOS ONE 9: e97556. https://doi.org/10.1371/journal.pone.0097556
- Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. Journal of Molecular Biology 215: 403–410. https://doi.org/10.1016/S0022-2836(05)80360-2
- Baldrian P (2017) Forest microbiome: diversity, complexity and dynamics. FEMS Microbiology Reviews 41: 109–130. https://doi.org/10.1093/femsre/fuw040
- Bálint M, Schmidt P-A, Sharma R, Thines M, Schmitt I (2014) An Illumina metabarcoding pipeline for fungi. Ecology and Evolution 4: 2642–2653. https://doi.org/10.1002/ece3.1107
- Bellemain E, Carlsen T, Brochmann C, Coissac E, Taberlet P, Kauserud H (2010) ITS as an environmental DNA barcode for fungi: an in silico approach reveals potential PCR biases. BMC Microbiology 10: 189. https://doi.org/10.1186/1471-2180-10-189
- Beng KC, Corlett RT (2020) Applications of environmental DNA (eDNA) in ecology and conservation: opportunities, challenges and prospects. Biodiversity and Conservation 29: 2089–2121. https://doi.org/10.1007/s10531-020-01980-0
- Boch S, Prati D, Hessenmöller D, Schulze E-D, Fischer M (2013) Richness of Lichen Species, Especially of Threatened Ones, Is Promoted by Management Methods Furthering Stand Continuity. PLOS ONE 8: e55461. https://doi.org/10.1371/journal.pone.0055461
- Boch S, Saiz H, Allan E, Schall P, Prati D, Schulze E-D, Hessenmöller D, Sparrius LB, Fischer M (2021) Direct and Indirect Effects of Management Intensity and Environmental Factors on the Functional Diversity of Lichens in Central European Forests. Microorganisms 9: 463–463. https://doi.org/10.3390/microorganisms9020463
- Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP (2016) DADA2: High-resolution sample inference from Illumina amplicon data. Nature Methods 13: 581–583. https://doi.org/10.1038/nmeth.3869
- Camacho-Sanchez M, Burraco P, Gomez-Mestre I, Leonard JA (2013) Preservation of RNA and DNA from mammal samples under field conditions. Molecular Ecology Resources 13: 663–673. https://doi.org/10.1111/1755-0998.12108

- Carter TS, Clark CM, Fenn ME, Jovan S, Perakis SS, Riddell J, Schaberg PG, Greaver TL, Hastings MG (2017) Mechanisms of nitrogen deposition effects on temperate forest lichens and trees. Ecosphere 8: e01717. https://doi.org/10.1002/ecs2.1717
- Cordier T, Alonso-Sáez L, Apothéloz-Perret-Gentil L, Aylagas E, Bohan DA, Bouchez A, Chariton A, Creer S, Frühe L, Keck F, Keeley N, Laroche O, Leese F, Pochon X, Stoeck T, Pawlowski J, Lanzén A (2021) Ecosystems monitoring powered by environmental genomics: A review of current strategies with an implementation roadmap. Molecular Ecology 30: 2937–2958. https://doi.org/10.1111/mec.15472
- Crespo A, Lumbsch HT (2010) Cryptic species in lichen-forming fungi. IMA Fungus 1: 167–170. https://doi.org/10.5598/imafungus.2010.01.02.09
- Czarnota P, Guzow-Krzemińska B (2018) Bacidina mendax sp. nov., a new widespread species in Central Europe, together with a new combination within the genus Bacidina. The Lichenologist 50: 43–57. https://doi.org/10.1017/S0024282917000627
- Dal Grande F, Rolshausen G, Divakar PK, Crespo A, Otte J, Schleuning M, Schmitt I (2018) Environment and host identity structure communities of green algal symbionts in lichens. New Phytologist 217: 277–289. https://doi.org/10.1111/nph.14770
- Deiner K, Bik HM, Mächler E, Seymour M, Lacoursière-Roussel A, Altermatt F, Creer S, Bista I, Lodge DM, de Vere N, Pfrender ME, Bernatchez L (2017) Environmental DNA metabarcoding: Transforming how we survey animal and plant communities. Molecular Ecology 26: 5872–5895. https://doi.org/10.1111/mec.14350
- Dreyling L, Schmitt I, Dal Grande F (2022) Tree Size Drives Diversity and Community Structure of Microbial Communities on the Bark of Beech (Fagus sylvatica). Frontiers in Forests and Global Change 5. https://doi.org/10.3389/ffgc.2022.858382
- Dymytrova L (2011) Notes on the genus Scoliciosporum (Lecanorales, Ascomycota) in Ukraine. Polish Botanical Journal 56: 61–75.
- Farkas E, Varga N, Veres K, Matus G, Sinigla M, Lőkös L (2022) Distribution Types of Lichens in Hungary That Indicate Changing Environmental Conditions. Journal of Fungi 8: 600. https://doi.org/10.3390/jof8060600
- Fediajevaite J, Priestley V, Arnold R, Savolainen V (2021) Meta-analysis shows that environmental DNA outperforms traditional surveys, but warrants better reporting standards. Ecology and Evolution 11: 4803–4815. https://doi.org/10.1002/ece3.7382
- Ferrier S, Powell GVN, Richardson KS, Manion G, Overton JM, Allnutt TF, Cameron SE, Mantle K, Burgess ND, Faith DP, Lamoreux JF, Kier G, Hijmans RJ, Funk VA, Cassis GA, Fisher BL, Flemons P, Lees D, Lovett JC, Van Rompaey RSAR (2004) Mapping More of Terrestrial Biodiversity for Global Conservation Assessment. BioScience 54: 1101–1109. https://doi.org/10.1641/0006-3568(2004)054[1101:MMOTBF]2.0.CO;2
- Fischer M, Bossdorf O, Gockel S, Hänsel F, Hemp A, Hessenmöller D, Korte G, Nieschulze J, Pfeiffer S, Prati D, Renner S, Schöning I, Schumacher U, Wells K, Buscot F, Kalko EKV, Linsenmair KE, Schulze E-D, Weisser WW (2010) Implementing large-scale and long-term functional biodiversity research: The Biodiversity Exploratories. Basic and Applied Ecology 11: 473–485. https://doi.org/10.1016/j.baae.2010.07.009

- Frøslev TG, Kjøller R, Bruun HH, Ejrnæs R, Hansen AJ, Læssøe T, Heilmann-Clausen J (2019) Man against machine: Do fungal fruitbodies and eDNA give similar biodiversity assessments across broad environmental gradients? Biological Conservation 233: 201–212. https://doi.org/10.1016/j.biocon.2019.02.038
- Giordani P, Brunialti G, Benesperi R, Rizzi G, Frati L, Modenesi P (2009) Rapid biodiversity assessment in lichen diversity surveys: implications for quality assurance. Journal of Environmental Monitoring 11: 730–735. https://doi.org/10.1039/B818173J
- Grimm M, Grube M, Schiefelbein U, Zühlke D, Bernhardt J, Riedel K (2021) The Lichens' Microbiota, Still a Mystery? Frontiers in Microbiology 12. https://doi.org/10.3389/fmicb.2021.623839
- Grube M, Cernava T, Soh J, Fuchs S, Aschenbrenner I, Lassek C, Wegner U, Becher D, Riedel K, Sensen CW, Berg G (2015) Exploring functional contexts of symbiotic sustain within lichen-associated bacteria by comparative omics. The ISME Journal 9: 412–424. https://doi.org/10.1038/ismej.2014.138
- Gueidan C, Li L (2022) A long-read amplicon approach to scaling up the metabarcoding of lichen herbarium specimens. MycoKeys 86: 195–212. https://doi.org/10.3897/mycokeys.86.77431
- Hawksworth DL, Grube M (2020) Lichens redefined as complex ecosystems. New Phytologist 227: 1281–1283. https://doi.org/10.1111/nph.16630
- Henrie JR, Thomson BM, Yungfleisch AA, Kerr M, Leavitt SD (2022) Characterizing Crustose Lichen Communities—DNA Metabarcoding Reveals More than Meets the Eye. Diversity 14: 766. https://doi.org/10.3390/d14090766
- Hofmann B, Dreyling L, Dal Grande F, Otte J, Schmitt I (2023) Habitat and tree species identity shape aboveground and belowground fungal communities in central European forests. Frontiers in Microbiology 14. https://doi.org/10.3389/fmicb.2023.1067906
- Hofmeister J, Hošek J, Malíček J, Palice Z, Syrovátková L, Steinová J, Černajová I (2016) Large beech (Fagus sylvatica) trees as 'lifeboats' for lichen diversity in central European forests. Biodiversity and Conservation 25: 1073–1090. https://doi.org/10.1007/s10531-016-1106-x
- Ihrmark K, Bödeker ITM, Cruz-Martinez K, Friberg H, Kubartova A, Schenck J, Strid Y, Stenlid J, Brandström-Durling M, Clemmensen KE, Lindahl BD (2012) New primers to amplify the fungal ITS2 region evaluation by 454-sequencing of artificial and natural communities. FEMS Microbiology Ecology 82: 666–677. https://doi.org/10.1111/j.1574-6941.2012.01437.x
- Keck F, Brantschen J, Altermatt F (2023) A combination of machine-learning and eDNA reveals the genetic signature of environmental change at the landscape levels. Molecular Ecology 32: 4791–4800. https://doi.org/10.1111/mec.17073
- Keck F, Blackman RC, Bossart R, Brantschen J, Couton M, Hürlemann S, Kirschner D, Locher N, Zhang H, Altermatt F (2022) Meta-analysis shows both congruence and complementarity of DNA and eDNA metabarcoding to traditional methods for biological community assessment. Molecular Ecology 31: 1820–1835. https://doi.org/10.1111/mec.16364
- Keepers KG, Pogoda CS, White KH, Anderson Stewart CR, Hoffman JR, Ruiz AM, McCain CM, Lendemer JC, Kane NC, Tripp EA (2019) Whole Genome Shotgun Sequencing Detects Greater Lichen Fungal Diversity Than Amplicon-Based Methods in Environmental Samples.

- Frontiers in Ecology and Evolution 7. Available from: https://www.frontiersin.org/articles/10.3389/fevo.2019.00484 (December 17, 2023).
- Kranner I, Zorn M, Turk B, Wornik S, Beckett RP, Batič F (2003) Biochemical traits of lichens differing in relative desiccation tolerance. New Phytologist 160: 167–176. https://doi.org/10.1046/j.1469-8137.2003.00852.x
- Launis A, Pykälä J, Boom PVD, Sérusiaux E, Myllys L (2019) Four new epiphytic species in the Micarea prasina group from Europe. The Lichenologist 51: 7–25. https://doi.org/10.1017/S0024282918000555
- Martin M (2011) Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet, journal 17: 10–10. https://doi.org/10.14806/ej.17.1.200
- Muggia L, Grube M (2018) Fungal Diversity in Lichens: From Extremotolerance to Interactions with Algae. Life 8: 15. https://doi.org/10.3390/life8020015
- Nguyen NH, Song Z, Bates ST, Branco S, Tedersoo L, Menke J, Schilling JS, Kennedy PG (2016) FUNGuild: An open annotation tool for parsing fungal community datasets by ecological guild. Fungal Ecology 20: 241–248. https://doi.org/10.1016/j.funeco.2015.06.006
- Rousk K, Sorensen PL, Lett S, Michelsen A (2015) Across-Habitat Comparison of Diazotroph Activity in the Subarctic. Microbial Ecology 69: 778–787. https://doi.org/10.1007/s00248-014-0534-y
- Sancho LG, Pintado A, Green TGA (2019) Antarctic Studies Show Lichens to be Excellent Biomonitors of Climate Change. Diversity 11: 42. https://doi.org/10.3390/d11030042
- Sayers EW, Bolton EE, Brister JR, Canese K, Chan J, Comeau DC, Connor R, Funk K, Kelly C, Kim S, Madej T, Marchler-Bauer A, Lanczycki C, Lathrop S, Lu Z, Thibaud-Nissen F, Murphy T, Phan L, Skripchenko Y, Tse T, Wang J, Williams R, Trawick BW, Pruitt KD, Sherry ST (2022) Database resources of the national center for biotechnology information. Nucleic Acids Research 50: D20–D26. https://doi.org/10.1093/nar/gkab1112
- Schall P, Ammer C (2018) Stand composition based on 2nd forest inventory (abundance, basal area, crown projection area and volume) on all forest EPs, 2014 2018. Version 2. Biodiversity Exploratories Information System. Dataset. Available from: https://www.bexis.uni-jena.de/ddm/data/Showdata/22907.
- Schmidt P-A, Bálint M, Greshake B, Bandow C, Römbke J, Schmitt I (2013) Illumina metabarcoding of a soil fungal community. Soil Biology and Biochemistry 65: 128–132. https://doi.org/10.1016/j.soilbio.2013.05.014
- Schwedener S (1886) Ueber die Beziehungen zwischen Algen und Flechtengonidien. Botanische Zeitung (Berlin): 289–292.
- Shirouzu T, Uno K, Hosaka K, Hosoya T (2016) Early-diverging wood-decaying fungi detected using three complementary sampling methods. Molecular Phylogenetics and Evolution 98: 11–20. https://doi.org/10.1016/j.ympev.2016.01.015
- Taberlet P, Coissac E, Pompanon F, Brochmann C, Willerslev E (2012) Towards next-generation biodiversity assessment using DNA metabarcoding. Molecular Ecology 21: 2045–2050. https://doi.org/10.1111/j.1365-294X.2012.05470.x

- Tedersoo L, Bahram M, Zinger L, Nilsson RH, Kennedy PG, Yang T, Anslan S, Mikryukov V (2022) Best practices in metabarcoding of fungi: From experimental design to results. Molecular Ecology 31: 2769–2795. https://doi.org/10.1111/mec.16460
- Timdal E (1983) The Genus Squamarina in Scandinavia. The Lichenologist 15: 169–179. https://doi.org/10.1017/S0024282983000262
- Větrovský T, Morais D, Kohout P, Lepinay C, Algora C, Awokunle Hollá S, Bahnmann BD, Bílohnědá K, Brabcová V, D'Alò F, Human ZR, Jomura M, Kolařík M, Kvasničková J, Lladó S, López-Mondéjar R, Martinović T, Mašínová T, Meszárošová L, Michalčíková L, Michalová T, Mundra S, Navrátilová D, Odriozola I, Piché-Choquette S, Štursová M, Švec K, Tláskal V, Urbanová M, Vlk L, Voříšková J, Žifčáková L, Baldrian P (2020) GlobalFungi, a global database of fungal occurrences from high-throughput-sequencing metabarcoding studies. Scientific Data 7: 228. https://doi.org/10.1038/s41597-020-0567-7
- Vondrák J, Malíček J, Palice Z, Coppins B, Kukwa M, Czarnota P, Sanderson N, Acton A (2016) Methods for obtaining more complete species lists in surveys of lichen biodiversity. Nordic Journal of Botany 34: 619–626. https://doi.org/10.1111/njb.01053
- Vondrák J, Svoboda S, Košnar J, Malíček J, Šoun J, Frolov I, Svensson M, Novotný P, Palice Z (2023) Martin7: a reference database of DNA barcodes for European epiphytic lichens and its taxonomic implications. Preslia 95: 311–345. https://doi.org/10.23855/preslia.2023.311
- Westberg M, Clerc P (2012) Five species of Candelaria and Candelariella (Ascomycota, Candelariales) new to Switzerland. MycoKeys 3: 1–12. https://doi.org/10.3897/mycokeys.3.2864
- White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal rna genes for phylogenetics. In: Innis M, Gelfland DH, Sninsky JJ, White TJ (Eds), PCR Protocols. Academic Press, San Diego, CA, 315–322. https://doi.org/10.1016/b978-0-12-372180-8.50042-1
- Wirth V, Hauck M, Schultz M (2013) Die Flechten Deutschlands. Ulmer, Stuttgart, 2 pp.
- Wright B, L. StC Larry, D. L Steven (2019) Is targeted community DNA metabarcoding suitable for biodiversity inventories of lichen-forming fungi? Ecological Indicators 98: 812–820. https://doi.org/10.1016/j.ecolind.2018.11.061
- Yoccoz NG (2012) The future of environmental DNA in ecology. Molecular Ecology 21: 2031–2038. https://doi.org/10.1111/j.1365-294X.2012.05505.x
- Yu DW, Ji Y, Emerson BC, Wang X, Ye C, Yang C, Ding Z (2012) Biodiversity soup: metabarcoding of arthropods for rapid biodiversity assessment and biomonitoring. Methods in Ecology and Evolution 3: 613–623. https://doi.org/10.1111/j.2041-210X.2012.00198.x

Supplementary Material to

Dreyling, L., Boch, S., Lumbsch, T. & Schmitt, I.: Surveying lichen diversity in forests: A comparison of expert mapping and simple eDNA metabarcoding. (submitted)

Table S1: Species List of taxa found in our study and whether they occurred in eDNA or metabarcoding, as well as the number of plots they were found in.

Table S1

				species	_list	
ASV_ID	Genus	Species	in_eDNA	in floristic	num_of_plots_eDNA num_of_plots_floris	stic
NA	Agonimia	allobata	no	yes	0	6
ASV_2337	Amandinea	punctata	yes	ves	12	3
NA	Anisomeridium	polypori	no	yes	0	18
NA	Arthonia	vinosa	no	ves	0	14
NA	Arthonia	punctiformis	no	yes	o	2
NA	Arthonia	radiata	no		Ö	30
				yes	5	0
ASV_10497	Arthopyrenia	cinereopruinosa	yes	no		
ASV_18078	Athallia	cerinella	yes	no	2	0
NA	Athallia	cerinelloides	no	yes	0	1
NA	Athallia	holocarpa	no	yes	0	4
NA	Athallia	inconnexa	no	yes	0	1
NA	Bacidia	arceutina	no	yes	0	1
ASV_2168	Bacidia	rubella	yes	no	1	0
NA	Bacidina	delicata	no	yes	0	1
ASV_5044	Bacidina	chloroticula	yes	no	5	0
ASV_1911	Bacidina	mendax	yes	no	55	0
ASV_3513	Bacidina	neosquamulosa	yes	no	28	0
ASV_784	Bacidina	sulphurella	yes	yes	16	45
ASV_54	Buellia	griseovirens	yes	yes	57	24
ASV_10295	Calicium	glaucellum	yes	no	1	0
ASV 3655	Calicium	montanum	yes	no	2	0
ASV_16946	Caloplaca	obscurella	yes	yes	2	1
ASV 15401	Caloplaca	turkuensis	ves	no	1	ō
ASV_1971	Candelariella	efflorescens	yes	no	7	0
NA	Candelariella	reflexa	no	yes	0	6
	Candelariella	vitellina		no	4	0
ASV_11377			yes		17	
ASV_1917	Candelariella	xanthostigma	yes	yes		16
ASV_17360	Candelariella	xanthostigmoides	yes	no	68	0
ASV_6241	Catillaria	nigroclavata	yes	no	4	0
NA	Cetrelia	olivetorum	no	yes	0	1
ASV_12182		chrysocephala	yes	yes	2	6
ASV_11703	Chaenotheca	ferruginea	yes	yes	8	10
NA	Chaenotheca	stemonea	no	yes	0	3
NA	Chaenotheca	trichialis	no	yes	0	4
ASV_4435	Chaenotheca	xyloxena	yes	no	1	0
ASV_2738	Cladonia	chlorophaea	yes	yes	4	3
NA	Cladonia	coniocraea	no	yes	0	69
NA	Cladonia	digitata	no	yes	0	20
NA	Cladonia	fimbriata	no	yes	0	10
ASV 7990	Cladonia	macilenta	ves	yes	1	4
ASV 1460	Cladonia	ASV_1460	yes	no	5	0
ASV_2230	Cladonia	ASV 2230	ves	no	1	0
ASV 3817	Cladonia	ochrochlora	ves	no	5	0
NA	Cliostomum	griffithii	no	ves	0	1
ASV_117	Coenogonium	pineti	yes	yes	80	119
ASV 16786	Coppinsiella	ulcerosa	ves	no	1	0
			,			4
NA	Dendrographa	decolorans	no	yes	0	
NA	Diarthonis	spadicea	no	yes	0	45
ASV_6658	Evernia	prunastri	yes	yes	1	7
ASV_1671	Fellhanera	bouteillei	yes	yes	15	1
ASV_1558	Fellhanera	viridisorediata	yes	no	12	0
ASV_9873	Fuscidea	pusilla	yes	no	6	0
NA	Glaucomaria	carpinea	no	yes	0	14
ASV_2340	Glaucomaria	subcarpinea	yes	yes	8	10
ASV_9639	Graphis	scripta	yes	yes	5	47
ASV_9683	Gyrographa	gyrocarpa	yes	no	1	0
ASV_349	Hypocenomyce	scalaris	yes	yes	16	9
NA	Hypogymnia	physodes	no	yes	0	47
ASV_3065	Hypogymnia	tubulosa	yes	yes	13	23
ASV_848	Lecania	croatica	yes	no	55	0
ASV_730	Lecania	cvrtella	yes	yes	26	15
NA	Lecanora	albella	no	yes	0	2
ASV_3549	Lecanora	albellula	yes	no	3	0
ASV_394	Lecanora	argentata	yes	yes	21	4
ASV_4722	Lecanora	chlarotera	ves	yes	4	39
ACV 1006	Lecanora	expallens	,		18	15
ASV_1886 ASV_1068	Lecanora	flavoleprosa	yes	yes no	18	12
W2A_T008	Lecanora Lecanora	intumescens	yes		10	10
ASV_5101			yes	yes		
NA NA	Lecanora	pulicaris	no	yes	0	9
ASV_5236	Lecanora	sarcopidoides	yes	no	2	0
NA	Lecanora	subrugosa	no	yes	0	3
ASV_1141	Lecidea	hypopta	yes	no	8	0
ASV_4419	Lecidea	nylanderi	yes	no	4	0
ASV_928	Lecidella	albida	yes	no	7	0
NA	Lecidella	elaeochroma	no	yes	0	24
ASV_5861	Lecidella	leprothalla	yes	no	1	0
NA _	Lecidella	stigmatea	no	yes	0	1
NA	Lepra	albescens	no	yes	0	1
	•					

Page 1

				specie	es_list	
NA	Lepra	amara	no	yes	0	13
NA	Lepraria	lobificans	no	yes	0	38
ASV_709 ASV_172	Lepraria	elobata	yes	no	23 113	0 106
ASV_172 ASV 812	Lepraria Lepraria	incana jackii	yes yes	yes no	18	0
ASV_1615	Lepraria	rigidula	yes	yes	26	1
ASV_3490	Lepraria	vouauxii	yes	no	1	0
NA	Melanelixia	glabratula	no	yes	0	26
NA	Melanelixia	subaurifera	no	yes	0	3
NA ACV 2760	Melanohalea Melanohalea	exasperata	no	yes	0	2 7
ASV_2769 ASV_6033	Melanohalea Melanomma	exasperatula pulvis-pyrius	yes yes	yes no	11 1	0
ASV_9341	Micarea	botryoides	yes	no	2	0
ASV_2140	Micarea	byssacea	yes	no	15	0
ASV_488	Micarea	czarnotae	yes	no	83	0
ASV_13524	Micarea	denigrata	yes	yes	1	3
ASV_6246	Micarea	fallax	yes	no	4	0
ASV_5771	Micarea Micarea	melanobola microareolata	yes yes	no	9 12	0
ASV_6340 ASV_168	Micarea	micrococca	yes	no no	43	0
ASV_15273	Micarea	misella	yes	no	2	0
NA _	Micarea	prasina	no	yes	0	9
ASV_2298	Micarea	pusilla	yes	no	36	0
ASV_358	Micarea	xanthonica	yes	no	29	0
NA	Naetrocymbe	punctiformis	no	yes	0	1
ASV_15632 ASV 2332	Ochrolechia Ochrolechia	microstictoides subviridis	yes yes	no no	1 4	0
NA	Parmelia	ernstiae	no	yes	0	3
NA	Parmelia	saxatilis	no	yes	0	4
ASV_1591	Parmelia	sulcata	yes	yes	11	39
NA	Parmelina	pastillifera	no	yes	0	1
NA	Parmeliopsis	ambigua	no	yes	0	8
ASV_1500	Pertusaria	coccodes	yes	no	4	0
NA ASV 1011	Pertusaria Pertusaria	coronata leioplaca	no yes	yes yes	0 11	2 30
ASV_1011 ASV 11320	Pertusaria	pertusa	yes	yes	1	7
ASV_16367	Phaeophyscia	NA	yes	no	1	0
ASV_2003	Phaeophyscia	orbicularis	yes	yes	10	22
ASV_27	Phlyctis	argena	yes	yes	84	41
ASV_1095	Physcia	adscendens	yes	yes	53	35
ASV_1697 ASV_1807	Physcia Placynthiella	tenella icmalea	yes	yes yes	20 2	10 11
ASV_1807 ASV 9893	Placynthiella	dasaea	yes yes	no	3	0
NA	Platismatia	glauca	no	yes	0	4
NA	Physconia	grisea	no	yes	0	5
ASV_10904	Polycauliona	candelaria	yes	no	1	0
NA	Polycauliona	polycarpa	no	yes	0	17
NA	Polyozosia	hagenii	no	yes	0	7
NA NA	Protoparmelia Pseudevernia	oleagina furfuracea	no no	yes yes	0 0	2 25
NA	Pseudosagedia	aenea	no	yes	0	104
NA	Pseudosagedia	chlorotica	no	yes	0	7
ASV_3142	Pseudoschismatomma	rufescens	yes	yes	15	2
NA	Punctelia	subrudecta	no	yes	0	1
NA	Punctelia	ulophylla	no	yes	0	1
NA NA	Pyrenula Pyrrhospora	nitida quernea	no no	yes yes	0 0	10 8
NA	Ramalina	farinacea	no	yes	0	9
ASV 4599	Rinodina	efflorescens	yes	no	5	0
ASV_9890	Rinodina	pyrina	yes	no	3	0
ASV_1764	Ropalospora	viridis	yes	yes	5	18
ASV_3867	Sarea	difformis	yes	no	16	0
ASV_3012	Sarea	resinae	yes	yes	16	1
ASV_58 ASV_907	Scoliciosporum Scoliciosporum	chlorococcum schadeanum	yes yes	yes no	121 8	11 0
ASV_191	Scoliciosporum	umbrinum	yes	no	146	0
NA	Segestria	leptalea	no	yes	0	2
ASV_1806	Squamarina	gypsacea	yes	no	34	0
ASV_6250	Stictis	friabilis	yes	no	5	0
ASV_336	Straminella	conizaeoides	yes	yes	25	48
ASV_2236	Toniniopsis Translionsis	separabilis	yes	no	12	0
ASV_2399 NA	Trapeliopsis Trapeliopsis	flexuosa granulosa	yes no	yes yes	5 0	10 12
ASV_7473	Violella	fucata	yes	no	4	0
ASV_14687	Xanthoria	parietina	yes	yes	3	20
ASV_6290	Xylopsora	friesii	yes	no	8	0



