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Molecular microbial diversity of deadwood and leaf litter in forests of different forest management intensity

Witoon Purahong

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PREFACE

This thesis is written as a cumulative doctoral thesis. Chapters 1 to 8 are independent papers that were accepted or submitted for publication in different international peer-reviewed journals. A list of publications and my contribution to each is provided at the end of the thesis. The thesis also includes a summary that indicates the importance of each manuscript (chapter), an introduction part with general introductory and methodology sections, an overall discussion and outlook and conclusion part with an integrating discussion that overarches all publication chapters. The format of each chapter was according to the required format of the journals where the manuscript has been accepted or submitted.

SUMMARY

Changes within a single land use category that include forest management and conversion of forest types potentially affect most characteristics, organismic compositions and structures of the entire forest ecosystem. However the effects of such changes on microbial diversity and microbial mediated ecological functions and services are still unclear to date. Here, I investigated the effect of changes within a single land use category "forest" on microbial diversity and community structure in deadwood and leaf litter using the experimental forest plots distributed across Germany as part of the German Biodiversity Exploratories. I used molecular methods, either Automated Ribosomal Intergenic Spacer Analysis, ARISA or 454 pyrosequencing to assess bacterial and fungal operational taxonomic units (OTUs). I reviewed methods for characterizing total and active fungal communities in decomposing substrates (chapter 1). Methodology establishment and optimization of bacterial ARISA was shown in chapter 2.

In chapter 3, fungal diversity and community structure in different forest management regimes were compared. Overall, 20 – 24% and 25 – 27% of native fungal OTUs from forest reserves and semi-natural forests became undetectable or were lost in managed and converted forests, respectively. Fungal richness was significantly reduced in age-class beech forests with a high level of logs and wood extraction, while fungal community structures were not significantly affected. Conversion of forests from native, deciduous to coniferous species caused significant changes in the fungal community structure and could reduce fungal richness which may depend on which coniferous species was introduced.

In chapter 4, I investigated whether management close to natural forest functioning (selection cutting forest) is a promising silvicultural practice that could maintain biodiversity in forest. I compared wood-inhabiting fungal richness and community composition between selection cutting and two other management types of Central European beech forests (age-class and unmanaged) using fungal-ARISA. I found the promising results that fungal OTU richness in selection cutting and unmanaged forests was not significantly different but it was significantly higher, in both cases, than that in the age-class forest. Fungal community composition was not significantly different among the three forest types. However, strong shifts of fungal OTU

abundances were found in the intensive forest management (age-class forest) compared to selection cutting forest.

In chapter 5, I disentangled the factors significantly correlated with wood-inhabiting fungal community structure and tried to link the fungal OTU richness and abundances as two key elements of the "fungal community structure" to potential ecological functions and services. The results clearly show that both anthropogenic (forest management) and wood physicochemical factors significantly correlate with wood-inhabiting fungal community structure. Abundances of specific fungal families and OTUs rather than total OTU richness were found to link with the potential ecological function (lignin decomposition) and service (wood decomposition rates).

In chapter 6, the effects of forest system management practices (FMPs) on leaf litter decomposition rates, nutrient dynamics (C, N, Mg, K, Ca, P) and the activity of ligninolytic enzymes were studied in a 473 day long litter bag experiment. I found that age-class beech and spruce forests (high forest management intensity) displayed significantly higher decomposition rates and nutrient release (most nutrients) than unmanaged deciduous forest reserves. Sites with close-to-nature forest management (low forest management intensity) exhibited no significant differences in litter decomposition rate, C release, lignin decomposition, and C/N, lignin/N and ligninolytic enzyme activity patterns compared to the unmanaged deciduous forest reserves, but most nutrient dynamics examined in this study were significantly faster under such close-to-nature forest management practices. Different FMPs affect litter decomposition by changing microbial enzyme activities, (both laccase and manganese peroxidase). The results also suggest that lignin decomposition is the rate limiting step in leaf litter decomposition and that MnP is one of the key oxidative enzymes for litter degradation. In conclusion, FMPs can significantly affect important ecological processes and services such as decomposition and nutrient cycling.

In chapter 7, I investigated the patterns of and the connections between microbial community structure and potential enzyme activities (microbial-mediated ecological function) across different FMPs and temporal change in leaf litter across temperate forest ecosystems. The results clearly show a distinct spatial and temporal pattern in microbial community structure while potential extra-cellular enzyme activities exhibit neither clear spatial nor temporal pattern. Uncoupling of microbial community and potential enzyme activities in our experiment demonstrates a paradigm

shift in ecology that structure determines function. I demonstrate that the disconnection of microbial community structure and potential enzyme activities can be due to (i) some degree of the functional redundancy of the microbial communities occurs in leaf litter of these complex forest ecosystems and (ii) different drivers of microbial growth versus potential enzyme activities.

In chapter 8, the effects of FMPs on bacterial and fungal communities in leaf litter overtime were studied. Furthermore, I tested whether alteration of microbial community development is the mechanism for non-additive decomposition rates occurred in mixed leaf litter in different FMPs. The results showed that both bacterial and fungal communities in leaf litter were significantly influenced by FMPs as well as days after incubation (DAI). The results from NMDS ordination revealed distinct patterns of bacterial and fungal community development overtime in leaf litter of different FMPs. Many factors including FMPs, DAI, litter quality, macro- and micronutrients and pH significantly influenced microbial community development.

In total, the performed works represent a contribution to unravel the relationship between forest composition and management and the structure and function of fungal and/or bacterial communities involved in decomposition of wood and litter, a key aspect of element cycling in forest ecosystems. Understanding these functional aspects provides rationale to evaluate ecosystem services provided by microbial communities and the impact of forest management on such services.

ZUSAMMENFASSUNG

Veränderungen innerhalb einer einzelnen Landnutzungskategorie, die die Fortwirtschaft und die Veränderung von Waldtypen betreffen, beeinflussen potentiell die meisten Eigenschaften, die organismische Zusammensetzung sowie die Strukturen des gesamten Waldökosystems. Allerdings sind die Auswirkungen solcher Veränderungen auf die mikrobielle Vielfalt und die ökologischen Funktionen und Leistungen der Mikroben derzeit noch unklar. Hier habe ich die Auswirkungen von Veränderungen innerhalb der Landnutzungskategorie "Wald" auf die mikrobielle Diversität und die Gemeinschaftsstruktur im Totholz und in der Laubstreu untersucht. Hierbei nutzte ich experimentellen Waldflächen, die Teil der "Deutschen Biodiversitätsexploratorien" sind. Ich verwendete molekulare Methoden, entweder Automated Ribosomal Intergenic Spacer Analysis (ARISA) oder 454 Pyrosequencing, um die "Operational Taxonomic Units" (OTU) der Bakterien und Pilze zu bewerten. Ich vergleiche die Methoden zur Charakterisierung der gesamten und der aktiven Pilzgemeinschaften in zersetzenden Substraten (Kapitel 1). Die Etablierung und Optimierung der Methode der bakteriellen ARISA werden in Kapitel 2 aufgezeigt.

In Kapitel 3 werden Pilzvielfalt und Gemeinschaftsstrukturen unter unterschiedlichen Waldbewirtschaftungsmaßnahmen verglichen. Insgesamt sind 20 - 24% und 25 - 27% der OTU heimischer Pilze aus Waldreservaten und naturnahen Wäldern nicht nachweisbar oder durch Bewirtschaftung und Umwandlung der Waldflächen verloren gegangen. Die Pilzdiversität ist in den Altersklassenbuchenwäldern durch einen hohen Grad an Abholzung gesunken, während die Gemeinschaftsstrukturen der Pilze nicht signifikant beeinflusst wurden. Die Umwandlung der heimischen Laubwälder in Nadelwälder führte zu signifikanten Veränderungen innerhalb der Pilzgemeinschaftsstrukturen. Dies kann, abhängig davon, welche Nadelbaumart eingeführt wurde, zur Verringerung der Pilzvielfalt führen.

In Kapitel 4 untersuche ich, ob eine naturnahe Ausrichtung der Forstwirtschaft (selektive Einschlagwirtschaft) eine vielversprechende Bewirtschaftung ist, bei der die biologische Vielfalt beibehalten wird. Ich vergleiche die holzbewohnende Pilzvielfalt und die Zusammensetzung der Gemeinschaften in Plenterwäldern und bei zwei anderen Bewirtschaftungsmaßnahmen in mitteleuropäischen Buchenwäldern (Altersklassenwälder und unbewirtschaftete Wälder). Dazu verwendete ich Fungal-

ARISA. Ich erhielt das vielversprechende Ergebnis, dass die Diversität der OTU der Pilze in Plenterwäldern und unbehandelten Wäldern sich nicht signifikant unterscheidet, jedoch im Vergleich zu einem Altersklassenwald erheblich höher ist. Die Zusammensetzung der Pilzgemeinschaften innerhalb der 3 Bewirtschaftungstypen unterscheidet sich kaum. Jedoch wurden starke Verschiebungen der Pilz-OTU-Häufigkeiten in intensiv bewirtschafteten Wäldern (Altersklassenwälder) im Vergleich zu Plenterwäldern festgestellt.

In Kapitel 5 zeige ich die Faktoren, die mit der holzbewohnenden Pilzgemeinschaftsstrukturen korrelieren, auf und versuche die Pilz-OTU-Häufigkeit und Diversität als die zwei Schlüsselelemente der "Pilzgemeinschaftsstruktur" mit den potentiellen ökologischen Funktionen und Leistungen zu verknüpfen. Die Ergebnisse zeigen deutlich, dass die anthropogenen (Bewirtschaftung) und die physikalisch-chemischen Faktoren (des Holzes) mit der holzbewohnenden Pilzgemeinschaftsstruktur stark korrelieren. Die Abundanzen spezifischer Pilzfamilien und OTUs eher als die Gesamtzahl der OTUs wurden als verbunden mit den potentiellen ökologischen Funktionen (Ligninzersetzung) und Leistungen (Holzabbauraten) vorgefunden.

In Kapitel 6 wird auf die Auswirkungen der Waldbewirtschaftungsmethoden (FMP) auf die Laubzersetzungsrate, Nährstoffdynamik (C, N, Mg, K, Ca, P) und die Aktivität von ligninolytischen Enzymen eingegangen. Dies wurde in einem 473 Tage andauernden Streubeutelexperiment untersucht. Es stellte sich heraus, dass die Altersklassenbuchenwälder und Fichtenwälder (intensive Forstwirtschaft) deutlich höhere Zersetzungsraten und Nährstofffreisetzungen (meiste Nährstoffe) als unbewirtschaftete Laubwaldreservate aufzeigten. Standorte mit naturnaher Forstwirtschaft zeigten keine signifikanten Unterschiede in der Streuzersetzungsrate, Kohlenstofffreisetzung, Ligninzersetzung und C/N-, Lignin/N und ligninolytischen Enzymaktivitätsmustern im Vergleich zu unbewirtschafteten Laubwaldreservaten. Jedoch wurde in dieser Studie eine deutlich höhere Nährstoffdynamik bei solch naturnaher Forstwirtschaft festgestellt. Unterschiedliche Bewirtschaftungsmethoden beeinflussen die Streuzersetzung durch veränderte mikrobielle Enzymaktivitäten (Laccase- und Manganperoxidasen). Die Ergebnisse lassen vermuten, dass die Ligninzersetzung der limitierende Schritt der Laubstreuzersetzung ist. Die Manganperoxidase ist hierbei eine der oxidativen Schlüsselenzyme für die

Streuzersetzung. Zusammenfassend kann man sagen, dass die Forstwirtschaftspraktiken einen hohen Einfluss auf die ökologischen Prozesse und Leistungen wie Zersetzung und den Nährstoffkreislauf haben.

In Kapitel 7 untersuche ich die Muster und Zusammenhänge zwischen den Strukturen der Mikrobengemeinschaften und potentiellen Enzymaktivitäten (durch Mikroben vermittelte ökologische Funktionen) in der Laubstreu in den Waldökosystemen der gemäßigten Klimazone bei verschiedenen Waldbewirtschaftsmethoden über die Zeit hinweg. Die Resultate zeigen ausgeprägte räumliche und zeitliche Muster in der Struktur der Mikrobengemeinschaften, während die potentiellen extrazellulären Enzymaktivitäten weder räumliche noch zeitliche Muster aufweisen. Die Entkopplung mikrobieller Gemeinschaften und potentieller Enzymaktivitäten stellt in unserem Experiment einen Paradigmenwechsel in der Ökologie dar, dass die Struktur die Funktionen bestimmt. Ich zeige auf, dass die Trennung von Mikrobengemeinschaftsstruktur und potentieller Enzymaktivitäten bis zu einem bestimmten Maß aufgrund (i) funktioneller Redundanzen der Mikrobengemeinschaften in der Laubstreu dieser komplexen Waldökosysteme und (ii) verschiedener Treiber von mikrobiellem Wachstum im Vergleich zu potentiellen Enzymaktivitäten bestehen könnte.

In Kapitel 8 werden die Auswirkungen der Waldbewirtschaftsmaßnahmen auf Bakterien- und Pilzgemeinschaften in Laubstreu während des Experiments erläutert. Darüber hinaus wurde von mir getestet, ob die Änderung der Entwicklung der Mikrobengemeinschaften ausschlaggebend für die nicht-additive Abbaurate ist, welche sich in der gemischten Laubstreu bei verschiedenen Forstwirtschaftsverfahren ist. Die Ergebnisse zeigen, dass sowohl die Bakterien- als auch die Pilzgemeinschaften in Laubstreu signifikant von den Bewirtschaftungsmaßnahmen sowie der Inkubationszeit (DAI) beeinflusst werden. Die Ergebnisse der NMDS-Ordination zeigten im Verlauf des Experiments eindeutige Entwicklungsmuster der Bakterien- und Pilzgemeinschaften in der Laubstreu der unterschiedlich bewirtschafteten Wälder. Viele Faktoren, einschließlich FMP's, DAI, Streuqualität, Makro- und Mikronährstoffe und pH-Wert, beeinflussen die Entwicklung der Mikrobengemeinschaften signifikant.

Insgesamt sind die geleisteten Arbeiten ein Beitrag zur Erforschung der Beziehungen zwischen Waldzusammensetzung und Bewirtschaftung sowie der Strukturen und Funktionen der Pilz- und/oder Bakteriengesellschaften, die in der

Zersetzung von Holz und Streu involviert sind. Die Zersetzung stellt einen Schlüsselaspekt des Stoffkreislaufes in Waldökosystemen dar. Das Verstehen der funktionellen Aspekte liefert die Begründung, Ökosystemdienstleistungen der mikrobiellen Gemeinschaften und den Einfluss der Waldbewirtschaftung auf diese zu evaluieren.

A. INTRODUCTION

Although forest management has been implemented for many centuries, its impacts on biodiversity are still unclear to date.

"It appears that the negative effect of forest management on biodiversity has become an axiom. Whether the negative effect, however, is a fact based on solid empirical evidence is not self-evident."

(Halme et al., 2010)

1. GENERAL INTRODUCTION

1.1 Current situation of forest management

Almost one-third of the world's total forest area (ca. 1.2 billion ha) is managed primarily for wood biomass production (FAO, 2010). This proportion is even higher in European forests (excluding those in the Russian Federation), where 57% of the total cover is managed for wood production (FAO, 2010). Forest system management practices include the forest management itself, but also conversion from one forest type to another (Luyssaert et al., 2011; Salazar et al., 2011). Forest management can shift tree species composition, stand density, and/or age structure (Chazdon et al., 2008). In addition, a number of studies have reported that deadwood, an important energy source and habitat for organisms in forest ecosystems, is significantly scarcer in intensively managed than in unmanaged forests (Müller et al., 2007; Lonsdale et al., 2008). Large areas of forests are also facing conversion from natural or semi-natural systems to non-native monoculture forest plantations of conifers, eucalyptus, or other economically valuable timber tree species (Chazdon et al., 2008). Forest conversion may constitute a switch to a different dominant tree species or be the trigger for changes in tree species richness and composition. Forest conversion directly affects the quality of deadwood with respect to the decomposer community because the wood of different tree species has different characteristics and chemical compositions (Kögel-Knabner, 2002). Thus, changes in forest management or conversion to different forest types may result in large effects on the entire forest ecosystem, whichmay directly or indirectly affect biodiversity and community structure within forests. Forest management and conversion result in a decline "naturalness" and an increase in the "artificialness" of forest ecosystems (Brumelis et al., 2011) e.g. from natural to semi-natural (natural regeneration) and to artificial regeneration systems (planted forest).

Forest management practices and harvesting methods vary and are not consistent around the globe (Foley et al., 2005). In Central Europe, clear cutting is normally not allowed or restricted to small areas of few hectares. However, intensive thinning operations are commonplace (Hessenmöller et al., 2011). To prevent soil damage, manual harvesting methods and/or light harvesting equipment are preferable

in this area. Selection cutting (in Germany known as Plenterwald) is encouraged as eco-friendly forest management system in hardwood forests (Hessenmöller et al., 2011). In North America generally as well as in boreal forests of the Northern hemisphere, clear cutting is very common, especially in softwood stands and this harvesting is usually done with heavy machinery (FAO, 1999). In the Southern hemisphere, in countries such as Chile and New Zealand, natural forests were converted to plantation of *Pinus radiatus* and various Eucalypti, as also happens on the Iberian Peninsula in Europe (FAO, 1999). The British isles, both UK and Ireland, saw large scale afforestation utilizing exotic trees such as, Sitka spruce; Norway spruce; Lodgepole pine; and Douglas fir (FAO, 1999). With climate change allowing forest cover to regain their hold on Nordic countries such as Greenland and Iceland, managed forestry is expected to become even more economically important, as well as of concern because forests are carbon sinks and climate modulators (Roach, 2013), thus of high societal importance.

Despite the well-established concept of sustainability in forestry (expressed by Hans Carl von Carlowitz in his seminal *Sylvicultura oeconomica, oder haußwirthliche Nachricht und Naturmäßige Anweisung zur wilden Baum-Zucht* in 1713) that coincides with the advent of modern forestry that originated in Germany, such activities also strongly affect the composition of tree species in German forests. Based on data on a potentially natural vegetation form before the introduction of managed forestry, German forests would be highly dominated by European beech (*Fagus sylvatica*) (74%) and Oak (*Quercus* spp) (18%) (BMELV, 2011). However, due to the forest management strategies, they have been replaced on a large scale by the non-native conifers Norway spruce (*Picea abies*) and Scots pine (*Pinus sylvestris*). Nowadays European beech and oak account for only 15% and 9.6% of the total forest cover compared with 52% for the introduced coniferous species combined (Purahong et al., 2014a).

1.2 Impacts of forest management on biodiversity and ecosystem functioning and services

Although forest system management practices have the potential to affect most characteristics, compositions and structures of the entire forest ecosystem, effects on ecological processes in soil are still unclear (IPCC, 2003; Torras and Saura, 2008,

Luyssaert et al., 2011). For example thinning and pruning operations, which are common in intensively managed forests, have been found to affect microclimatic conditions such as light penetration, air movement and temperature (Ma et al., 2010) and therefore should also influence abiotic and biotic soil properties. Even more pronounced changes can be expected if, as a result of conversion of forest type, dominant tree species are replaced; this will influence the diversity and community structure of both above- and below-ground biota, which interact with the plants (Torras and Saura, 2008; Zak et al., 2003).

Most previous studies on the effects of changes in management on forest biodiversity have focused on plant and invertebrate communities (e. g. Werner and Raffa, 2000; du Bus de Warnaffe and Lebrun, 2004; Torras and Saura, 2008; Lange et al., 2011). These studies have shown that a change in forest management can affect plant and invertebrate diversity. However, despite the importance of the microbial community for the functioning of ecosystems, very few studies have investigated the effects of forest management and forest conversion on microbial diversity (Nacke et al., 2011). In forests, fungi play an important role in plant performance, especially as mycorrhizal symbionts or plant pathogens and also as litter and deadwood decomposers that maintain nutrient cycles (Watkinson et al., 2006; Purahong et al., 2010; Orwin et al., 2011; Purahong and Hyde, 2011; Fukasawa et al., 2012; Kahl et al., 2012).

Effects of forest management on ecosystem functioning and services have been extensively studied in some ecosystems (Waldrop et al., 2003). Potential activities of enzymes important for biogeochemical cycle of macronutrient such as C, N and P have been used as the indicators for potential ecosystem function (Frossard et al., 2012). Decomposition of plant debris is an important ecological process mainly mediated by microbes and has been used as the indicator for ecosystem services. Different FMPs were found to affect ecosystem functioning by altering microbial communities, soil enzyme activities and also the correlations under these soil enzyme activity profiles (Salazar et al., 2011). Intensive forest managements, especially those that include burning of the forest stand were reported to highly negatively affect potential activities of many enzymes important for biogeochemical cycling such as β-N-acetyl-

glucosaminidase, phenol oxidase and phosphatase and also decomposition rate of leaf litter (Waldrop et al., 2003). Nevertheless, the impacts of different forest management in Central Europe where European beech forests have been intensively managed or converted to conifer forests are still unclear to date.

1.3 Biodiversity *vs.* functional diversity: how defined and how related to each other?

Biodiversity generally is defined as "the variety of life, distributed heterogeneously across the Earth" (Gaston, 2000). Practically it can be conceived as the diversity of genes, species and ecosystems, three hierarchical levels of biological organization (Convention on Biological Diversity, 1992). Biodiversity as applied to taxonomic entities can be measured as alpha, beta, and gamma diversity over spatial scales according to Whittaker (1960, 1972). Alpha diversity (α) is defined as "the diversity or number of species within a particular area or ecosystem", beta diversity (β) as "comparison of diversity between ecosystems", and gamma diversity (γ) as numerical descriptions of "the total diversity within a large region". The relationship among these 3 diversity measures can be shown with the following formulas:

$$\gamma = \alpha \times \beta \text{ (Whittaker 1960)}$$

Biodiversity only seen as species richness (or in the modern molecular world OTU richness) has been initially used to investigate effects of biodiversity on ecosystem functioning. Whoever and whatever carries out ecosystem processes matters, as biological entities such as species have different characteristics, and even different, plastic characteristics under different circumstances. However, in some experiments, species richness did not provide a clear answer or explain effects of biodiversity on ecosystem functioning (Hooper et al., 2002). This could be related to the nature of ecosystem processes. Generally, ecosystem processes are influenced by the functional characteristics of organisms involved rather than their taxonomic identity (Hooper et al., 2002). Hence, to understand the effects of biodiversity on ecosystem functioning (including the mechanisms), the functional attributes of organisms should be taken into account (e.g. diversity of functional groups) (Hooper et al., 2002). Although the

functional diversity is considered as an important component of biodiversity, methods for quantifying the functional diversity are not well developed as compare to species richness (species diversity). Petchey et al. (2001) demonstrate that the relationships between species richness and functional diversity in 5 natural communities (2 plant and 3 animal communities) are all relatively linear. From this point of view, we may assume that ecosystem functioning is stable at high species richness, thus also high functional diversity levels.

1.4 Role of microbes in biogeochemical cycles in forest soils

Microscopic life plays important roles in biogeochemical cycle by transforming and recycling both organic and inorganic substances in the total environment including, atmosphere, hydrosphere, biosphere, lithosphere, and anthroposphere (Fortuna, 2012). In deadwood and leaf litter, the fungi and bacteria are the main decomposers that use secreted or membrane-bound digestive enzymes (both oxidative and hydrolytic enzymes) to degrade polymeric substances. These microbes rely on the access the small molecules of degradation products (i.e., glucose, amino acids, phosphate) that are important for their growth and metabolism (German et al., 2011). Different groups of microbes have different life strategies and this affecting how microbes access the substrates. Roughly, microbes live as unicellular (most bacteria and yeast) and filamentous (most fungi and some bacteria). In soil and litter (both deadwood and leaf litter), some of the most important enzymes for carbon, nitrogen and phosphorous acquisition that can be produced by microbes are listed (German et al., 2011).

Deadwood and leaf litter are the main sources of organic plant matter input into forest soils (Swift et al., 1973; Müller et al., 2007; Purahong et al., 2014c). In forest, the amount of deadwood and leaf litter combined (represent as above-ground litter) were estimated to contribute at least 52% of the total annual litter input and can be considered as an intermediate phase between living plant and soil (Freschet et al., 2013). Both deadwood and leaf litter from higher plants are comprised of both simple molecules and complex substances such as sugars, amino acids, proteins, nucleic acids, waxes, celluloses, hemi-celluloses and especially quite recalcitrant lignins (Kögel-Knabner, 2002; Fukasawa et al., 2012). The latter makes a marked difference between

plant litter and for example certain animal excrements, carcasses or fern and moss litter also present in forests. Microorganisms play an important role in the decomposition process of deadwood and leaf litter finally resulting in a release of nutrients as free ions into the forest soil (Fukasawa et al., 2012; Purahong et al., 2014a; Purahong et al., 2014b; Purahong et al., 2014c). These free nutrient ions increase soil fertility and can be up taken by plants (Dighton, 2003; Purahong et al., 2010). However, in forest soils, there is no complete mineralization but also humification, and humus (a complex of organic macromolecules resulting from re-polymerization of litter and lignin compounds), also participate to fertility, as they have a notable cation exchange and water holding capacity.

For deadwood, fungi are widely assumed to be the main decomposers because they as a group are known to secrete wide ranges of hydrolytic and oxidative exoenzymes in woody substrates causing high mass loss (Fukasawa et al., 2012). Woody matter in terrestrial environments is constantly degraded by biological means (activities of both microbes as well as larger organisms such as insects and vertebrates), and to usually lesser degree by abiotic physical and chemical means such as photodegradation (George et al., 2005; Austin and Ballare 2010). Due to the ability to produce potent enzymes and the characteristics of the wood after being colonized by fungi, there are three main types of biological decay recognized: white rot, brown rot and soft rot (Schwarze, 2007). White rot fungi are found in all Dikarya fungi (basidiomycetes and certain ascomycetes) and can efficiently degrade lignin, cellulose and hemicelluloses, rendering the wood a lightweight, whitish stringy mass. The relative rate of lignin and cellulose degradation may vary greatly depending on fungal species and condition of the wood (Schwarze, 2007). Brown rot fungi are able to degrade cellulose and hemicelluloses but have limited ability for lignin decomposition (Schwarze, 2007) the result of their activity is characteristically brown and consists of brittle cubicles. Brown rot seems more dominant in conifers. Finally, soft rot fungi create a spongy texture at the wood surface (including ligninolytic basidiomycetes and some of the large ascomycetes). They are able to decompose cellulose and hemicellulose efficiently but they can only slightly modify lignin. The developmental pattern involving hyphae tunneling inside the lignified cell walls in soft rot fungi enable us to distinguish them from brown and white rot fungi (Schwarze, 2007). Soft rot are more common in

wet places. Special kinds of wood decay are termed so due to other characteristics such as red rot "Rotfäule", which biochemically is a white rot that appears reddish in coloration, and butt- and root rots for their location on the tree (Schwarze, 2007). For leaf litter, fungi directly partake in the decomposition process probably even before (when they are epiphytes and endophytes) but definitely after leaf shedding (then called saprobes) (Purahong and Hyde, 2011). When leaf litter is attacked by fungi and micro- and mesofauna, its surface area increases and bacteria can efficiently decompose leaf litter (Word, 1997; Plowman, 2012). Due to the importance of microbes in decomposition processes, changes in microbial diversity and community composition could therefore affect decomposition rates, nutrient cycling and fertility of forest soils (Purahong et al., 2014c).

Bacteria play a role in wood decomposition as well, as they can also produce hydrolytic and some oxidative enzymes such as laccase (benzenediol oxygen oxidoreductases, EC 1.10.3.2) (Sharma et al., 2007) and they may support fungal colonization and fructification on deadwood by providing generally scarce nitrogen through fixation (Cowling and Merrill, 1966; Hoppe et al., 2014). Generally, fungi and bacteria are often associated with each other in commensal to symbiotic ways, and this should not be different when wood and litter are their substrates.

1.5 Study areas and main forest system management practices: the German Biodiversity Exploratories

The study was conducted in experimental forest plots distributed across Germany as part of the German Biodiversity Exploratories (http://www.biodiversity-exploratories.de). The forests are located in North-Eastern Germany within the Schorfheide-Chorin region, Schorfheide-Chorin Exploratorium Wald, SEW (ca. 1300 km^2; 53°01'N 13°77'E), in the Hainich-Dün region in Central Germany including the Hainich National Park and its surroundings, Hainich-Dün Exploratorium Wald, HEW; (ca. 1300 km^2; 51°16'N 10°47'E) and in the Schwäbische Alb region of South-Western Germany, Schwäbische Alb Exploratorium Wald, AEW (ca. 422 km^2; 48°44'N 9°39'E) (Fischer et al., 2010; Hessenmöller et al., 2011). The experimental forest plots are representative of the forest management types as well as the dominant tree species in

each region. All information pertaining to these forest plots has been described in detail by Fischer et al. (2010) and Hessenmöller et al. (2011). The plot selection criteria for each experiment were based on the objectives; generally it considered forest history and management regimes, dominant tree species and deadwood status (Fischer et al., 2010; Hessenmöller et al., 2011; Luyssaert et al., 2011). All selected forest plots had, apparently, been subjected neither to clearing nor to a period of agricultural use in the past (Luyssaert et al., 2011), something that is overall rare in long term cultural landscapes such as in Germany.

Figure 1. The map of 3 biodiversity exploratories.

1.6 Main forest system management practices

Four types of forest management practices were included in this study. The forest management intensity is lowest in unmanaged deciduous forest reserves dominated by European beech (beech unmanaged forest, BU, uneven-age forest structure), low in European beech selection cutting forest (beech selection cutting forest, BS, near-to-nature forest management with natural regeneration, uneven-age forest structure), high in European beech age-class forest (beech age-class forest, BA, semi-natural forest with natural regeneration, even-age forest structure) and highest in conifer age-class forest

(spruce age-class forest, SA and pine age-class forest, PA, planted Norway spruce and Scots pine forests converted from European beech forest, even-age forest structure). The intensity of forest management in this study was measure according to Land-Use and Disturbance Intensity (LUDI) index) (Luyssaert et al., 2011) and a Silvicultural Management Intensity indicator (SMI) (Schall and Ammer, 2013), which also reflect the level of naturalness (Brumelis et al., 2011) (Figure 2).

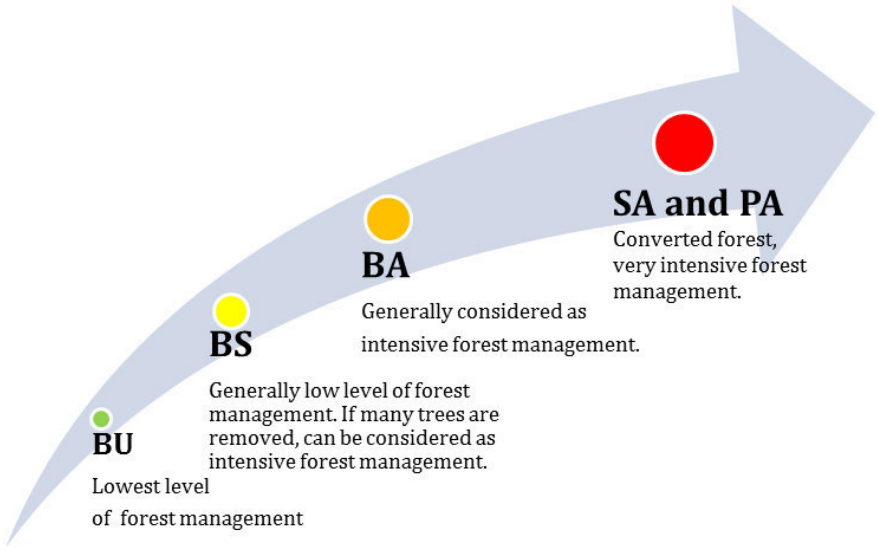

Figure 2. Different level of forest management in this study (BU = unmanaged deciduous forest reserves dominated by European beech, BS = European beech Selection cutting forest, BA = European beech Age-class forest, SA = Norway Spruce Age-class forest and PA = Scots Pine Age-class forest).

1.6.1 ***Unmanaged deciduous forests*** are mainly located in National Parks or forest reserves (Figure 3). The composition of tree species in this forest type depends on the forest development stage, however in all study forest plots, European beech (*Fagus sylvatica*) is the most dominant tree species (Purahong et al., 2014a, b, c). In some forest plots, they are also contained some fraction of admixed tree species of *Acer* spp., *Fraxinus* spp., *Quercus* spp., *Prunus* spp. and others, but in some plots, European beech covers the entire forest area (Purahong et al., 2014a, b, c). No wood extraction has been performed in unmanaged forests for at least approximately 60 years (Hessenmöller et al., 2011). This forest type normally contains large amounts of coarse woody debris (CWD).

Figure 3. Unmanaged deciduous forest reserves dominated by European beech located in Hainich National Park.

1.6.2 ***European beech selection cutting forests*** are considered as close-to-nature forest management (Figure 4) as this forest type is results from natural regeneration and has uneven-age forest structure (Hessenmöller et al., 2011; Purahong et al., 2014b; Purahong et al., 2014c). This forest type is located at HEW (Hessenmöller et al., 2011; Purahong et al., 2014b; Purahong et al., 2014c). Dominant tree species in all plots is European beech (Purahong et al., 2014b; Purahong et al., 2014c). Although the forest structure is more similar to unmanaged forest compared to age-class forest, this forest type can be considered as intensive forest management when most woody biomass has been harvested (Purahong et al., 2014b). Due to an efficient wood harvesting, CWD amount may be reduced in this forest types, especially when compared to unmanaged forest (Purahong et al., 2014b).

Figure 4. European beech Selection cutting forest located in the Hainich-Dün region.

1.6.3 ***European beech age-class forests*** are characterized by the even-age forest structure (Hessenmöller et al., 2011) (Figure 5). In this study, all plots of this management type originated from natural regeneration. The development stages of this forest type are as followed (i) thicket stage (< 7 cm diameter at breast height of 1.3 m: DBH for the stem of average basal area), (ii) pole stage (7 to 15 cm DBH), (iii) young timber stage (15 to 30 cm DBH), and (iv) old timber stage (> 30 cm DBH) (Hessenmöller et al., 2011). This forest type is considered as an intensive forest management because the thinning operation takes place at the thicket stage and continues until the young timber stage (Hessenmöller et al., 2011). The thinning operation is intensively done to improve the timber quality of the remaining trees at the harvest. With the thinning operation, approximately 50% of forest growth biomass is removed before harvest (Hessenmöller et al., 2011). At the harvest, most mature large trees are clear cut and removed from the forest, thus the amount of CWD in this forest type is normally low. However, the amount of fine woody debris may be increased in this forest type if the slash or thinned wood biomass is not removed from the forest (Purahong et al., 2014a). Nevertheless, the intensity level of this forest type may be considered as not stable over

time, as at the old timber stage there is no thinning operation or harvest anymore, so that the management is less intensive at that stage (Purahong et al., 2014a).

Figure 5. European beech age-class forest located in the Hainich-Dün region.

1.6.4 ***Conifer age-class forests*** are characterized by an even-age forest structure (Hessenmöller et al., 2011; Purahong et al., 2014a) (Figure 6). In this study all conifer plots originated from planting (Hessenmöller et al., 2011). Two different conifer species: Norway spruce (*Picea abies*) and Scots pine (*Pinus sylvestris*) were grown at AEW and SEW, respectively. The development stages and thinning operation are similar to European beech age-class forest. Coniferous age-class forest is considered as the most intensively managed forest in this study because it requires intensive thinning operations and the forest is considered as non-native forest (Schall and Ammer, 2013; Purahong et al., 2014a). Based on the level of naturalness, it is also considered as the least natural forest compared to European beech age-class, selection cutting and unmanaged forests (Brumelis et al., 2011).

Figure 6. Norway spruce age-class forest located in the Hainich-Dün region.

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2. DEFINITION OF A MOLECULAR TOOLBOX

2.1 Molecular methods for microbial community analyses

In this thesis, I addressed different levels of the microbial taxonomic units ranging from the biomass of broad microbial groups to Operational Taxonomic Units (OTUs). Definitions of OTUs coming from different molecular methods vary. At community level, different groups of microbial biomass (microbial biomarkers) or detected OTUs were combined and analyzed together as whole and represent the microbial community. Where the information for the abundances of microbial biomarkers and OTUs were used for analyzing the microbial community, this refers to microbial community structure. If only presence/absence data were used, this only refers to microbial community composition. I briefly explain the molecular methods for microbial community analyses used in this thesis below.

2.1.1 *Phospholipid-derived fatty acid (PLFA) analysis*

Different subsets of the microbial community have different PLFA patterns and specific individual PLFAs were used as indicators for key groups of microbes: i15:0 for Gram-positive bacteria; 16:1ω7c for Gram-negative bacteria; 17:0 cyclo and 19:0 cyclo for anaerobic bacteria; 10Me16:0 for Actinobacteria; 18:2ω6,9c for general fungi and 16:1 ω5c for arbuscular mycorrhizal fungi (AMF) (Frostegård et al., 1993, Wu et al., 2012). Thus, changes in overall microbial community structure can be accessed by analyzing the PLFA composition (Frostegård et al., 1993). PLFA are the most abundant class of amphipathic lipids in the lipid bilayer plasma membranes. Only viable organisms have an intact membranes containing PLFA and after microbial death, their polar phosphate group is hydrolyzed by enzymes within minutes to hours leaving the lipid core as diglyceride (White et al., 1993). Biomass of key groups of microbes detected with PLFA analysis thus is assumed to be a quantitative measure of the viable biomass (White et al., 1993). Limitations of the PFLA analysis were published for example by Frostegård et al., (2011). The most important limits are related to the interpretation of PLFAs, the specificity of PLFA markers and the use of number of PLFAs as the estimation of microbial richness (Frostegård et al., 2011). It is clear that with PLFA analysis, we can

only infer about the changes of microbial community structure (without inferring richness or diversity) and in specific cases, the individual PLFAs can be used as indicators for key groups of microbial biomass. For example, in my study, I did not analyze PLFAs in leaf litter at time point 0 but started from leaf litter at 89 days after incubation. This is to avoid the plant derived PLFA (18:1ω9, 18:2ω6,9 and 18:3ω3,6,9). At 89 DOI, a residual effect from PLFAs from litter would be minimal, especially because the litter was dried and processed before plot establishment. In addition, any residue would be by then far surpassed by new microbial growth.

2.1.2 *Automated ribosomal intergenic spacer analysis (ARISA)*

ARISA (automated robosomal intergenic spacer analysis) in the form used today as Bacterial (B)-and Fungal (F)-ARISA (on the Internal Transcribed Spacer (ITS) barcode region) stems from RISA (ribosomal intergenic spacer analysis) that targeted bacteria and was originally run on electrophoretic gels (Ranjard et al., 2000). As such it relates to other targeted amplicon gel fingerprinting methods such as Denaturing Gradient Gel Electrophoresis (DGGE)/ Temperature Gradient Gel Electrophoresis (TGGE)/ Constant Denaturant Gel Electrophoresis (CDGE). It is rather based on length heterogeneity rather than base composition, hence it is a form of amplicon length heterogeneity (LH)-PCR and also a simple cousin of Terminal Restriction Fragment Length Polymorphism (T-RFLP), lacking the restriction cutting step of the latter. A number of other fingerprinting methods can be applied to microbial communities today, such as Single-Strand Conformation Polymorphism (SSCP), Amplified Fragment Length Polymorphism (AFLP), and the non-targeted Random Amplified Polymorphic DNA (RAPD)-PCR.

Run on capillary electrophoresis sequencers with fluorescent tags and size markers it lacks the ability to excise, clone and sequence the individual bands, yet it is relatively fast and inexpensive, and it can be multiplexed. With ARISA, it is possible to obtain OTUs that can be used as proxies for microbial richness. The relative fluorescence intensity of each OTU can be normalized within the sample and can be used for the relative abundance of particular OTU (Ramette 2009). OTUs were defined as described previously by Jones et al. (2007) and Ramette (2009) for the ARISA approach and may differ from those OTU definitions relying on sequences. They could,

alternatively, be called ribotypes (Gleeson et al. 2005). OTUs derived from ARISA may not be equivalent to species, however, they do provide a basis for richness estimates, and allow highly consistent measurement of community structure through space and time (Jones et al. 2007). In some cases, it is also possible to identify the species that is behind the OTU. This is much dependent on the database used. In my dissertation research, I linked some F-ARISA OTUs obtained from deadwood logs to fungal taxonomy (genus or species level) by comparing the length of the fragments with a clone sequence database. Clone libraries were constructed from pooled DNA samples of deadwood logs from the same study site and host tree species used in the experiment. F-ARISA was carried out separately on all selected clones to confirm the lengths of the ITS region (a.k.a ITS1-5.8S-ITS2; Purahong et al., 2014). Only the clones that gave similar length from both cloning sequencing and F-ARISA were considered valid.

2.1.3 *Cloning/sequencing*

One of the most commonly used methods to analyze microbial community in environmental sample is amplicon cloning/sequencing. DNA is extracted from environmental samples, amplified with PCR with specific primers for different groups of microbes or with universal primers. PCR products amplified are cloned and sequenced (DeSantis et al. 2007). The generated sequences are compared to known sequences in databases. This method is laborious, expensive and time consuming. For fungal communities, it has been estimated that at least 100 sequences per sample are needed to get reliable data on fungal community composition (Avis et al., 2010). Despite these disadvantages, the cloning/sequencing approach can provide a great advantage for studying microbial community structure compared to fingerprinting and also the next generation sequencing (NGS) platforms. Cloning/sequencing can provide long sequences, thus makes good taxonomic assignment possible (Krüger et al. 2012; Jumpponen and Brown, 2014). For fungi, this method can also include partial sequencing of the more conserved Large Subunit (LSU) together with the full-range of more variable, ITS barcoding region. The longer sequences allow for better OTU assignment, whereas shorter sequences derived from NGS platforms may inflate richness estimators (Jumpponen and Brown, 2014).

2.1.4 *Targeted 454 Pyrosequencing*

A number of different NGS platforms and technologies exist today that are a big step beyond the capillary and gel electrophoresis sequencing varieties based on the Sanger dye- ddNTP, chain interrupting sequencing method, that along with the Maxam-Gilbert method started nucleic acid sequencing. There are several platforms of the NGS such as Ion Torrent PGM, Roche-454 Titanium (454 pyrosequencing) and Illumina MiSeq. The 454 pyrosequencing was used in my thesis because it can generate quite long read sequences (400-500bp) and there has been already a standard protocol in the lab at Department of Soil Ecology, UFZ-Helmholtz Centre for Environmental Research. The 454 pyrosequencing system is based on sequencing by synthesis that has been described by Balzer et al. (2010). The procedures are consists of the repeatedly flowing of nucleotide reagents over a PicoTiterPlate™ (Balzer et al., 2010). The plate contains ca. one million wells, and each well consists of at most one bead with a unique single-stranded template (DNA fragment to be sequenced). The nucleotides are flowed over the beads in the presence of polymerase enzyme and using the template on the beads. There are many cycles of the flow of the nucleotides and every time the plate is cleansed of nucleotides. During each cycle if that nucleotide is complementary with the template a photon of light is given off and can be recorded by camera. If the nucleotide is not complementary, there is no light signal. At the end, bioinformatics software uses this information to construct the sequence and go through several levels of trimming and quality control. Good quality sequences of targeted DNA obtained by 454 pyrosequencing can be used for taxonomical assignment (e.g. OTUs). The community composition or community structure can be constructed with presence/absence and abundance data, respectively.

2.2 Methodological selection: Why PLFA? Why ARISA? Why 454 pyrosequencing?

In this thesis, I used different methods to access the microbial community structure and/or diversity. The most important factors for method selection are the objectives and the budget available to best meet these. For biomass estimation, especially as no other method to quantify the viability and activity of present microorganisms was

available (for example, no RNA sampled, rDNA copy number heterogeneity, no stable isotopes used), PLFA was chosen (chapters 7), also over ergosterol estimation that would have to be done separately and would only gain information on some fungi (Weete et al., 2010). While fingerprinting methods (PLFA and ARISA) are less sensitive compared to NGS (i.e. 454 pyrosequencing), their cost is much lower (more than 10 times). Therefore, in chapters 2 to 4, 7 and 8, I decided to use fingerprinting methods to rapidly get an overview of the effects of forest management on fungal community in deadwood and leaf litter. Emphasis is more on the fungal community structure (especially, chapter 7 and 8), thus the fingerprinting methods are sensitive enough and get along with the objectives. The results from ARISA fingerprints do not directly provide any taxonomic information, thus amplicon cloning followed by sequencing was used to link the ARISA peaks to species of known systematic position (Chapter 3). Overall, the results from fingerprinting methods revealed that forest management and forest type conversion have significant effect on microbial community and/or diversity. More sensitive methods (454 pyrosequencing) are also used to more deeply investigate the effects of forest management and forest type conversion on microbial community and/or diversity (Chapter 5). In particular, the effect of conversion of forest type from beech to spruce dominated forests on fungal community structure and richness obtained by F-ARISA and 454 pyrosequencing are similar. In chapter 3 (F-ARISA), I found that this conversion caused significantly reduced of fungal richness ($P < 0.05$) and alter wood-inhabiting fungal community structure ($R_{ANOSIM} = 0.65$, $P < 0.001$). In chapter 5 (454 pyrosequencing), this kind of conversion also caused significantly reduced fungal richness ($P < 0.05$) and alter wood-inhabiting fungal community structure ($R_{ANOSIM} = 0.60$, $P < 0.001$). This is consistent with a study that compares B-ARISA and 454 pyrosequencing that also found similar results from these two methods (Gobet et al., 2013). I summarize the usages, advantages and disadvantages of the microbial community analysis methods that are used in all of the experiments in table 1.

Table 1 Usages, advantages and disadvantages of the microbial community analysis methods used in this study.

Methods	Usages	Advantages	Disadvantages
Phospholipid-derived fatty acids (PLFA) (Frostegård et al., 1993)	- Chemotaxonomic markers of microorganisms - Microbial community fingerprint - Biomass of functional microbial group estimation	- Inexpensive - Fast and easily done - High-throughput - Data analysis is straight forward and not complex - PLFAs degrade rapidly after the mortality, so the microbial biomass estimation leans toward the viable microbes. -Stress or physiological status of microbes may be estimated.	- Temperature sensitive (necessitates careful handling and transport) - Very low taxonomic resolution - The functional microbial group markers are not specific. Especially, the marker for general fungi is also found in plants. - Composition of fatty acids may differ at different life stages of organisms.
Automated ribosomal intergenic spacer analysis (ARISA) (Fisher and Triplett, 1999; Ranjard et al., 2001)	- Microbial community fingerprint - Microbial richness estimator	- Inexpensive - Fast and easily done - High-throughput - Data analysis is straight forward and not complex - Provides very useful information on microbial community structure - Highly reproducible - More automated than other fingerprint methods	- Bias toward primer selection - Quality of DNA is important - Under or over estimations are possible which are difficult to precise - Less sensitive compared with next generation sequencing
Cloning/sequencing (DeSantis et al., 2007)	- Microbial community analysis - Microbial richness estimator	- Taxonomic resolution can be high and possibly entails information on phylogenetic distance (depending on primers used and hence alignability and phylogenetic informativeness -Statistical analysis is more complex compared with the fingerprinting method but it is still less complex compare with next generation sequencing	- Expensive (especially when comparing the cost per number of sequences) - Laborious - Bias toward primer selection - When only low number of sequences are taken from each sample, the information is strongly biased and not reliable - Cloning adds an additional layer of bias to the PCR biases

Methods	Usages	Advantages	Disadvantages
Targeted* 454 Pyrosequencing (and other methods of next generation sequencing) (Buée et al., 2009)	- Microbial community analysis - Microbial richness estimator	- Taxonomic information is possible (DNA barcoding, established pipelines for analyses) - Sensitive method to infer microbial richness and community changes - High-throughput	- Bias toward primer selection - Complicated bioinformatics and statistical analyses - Expensive and more laborious than fingerprinting methods - Depending on methods, amplicons may be too short for reliable direct phylogenetic estimates of the relatedness of the sequences diversity - Highly dependent on automated pipelines for data cleaning and assembly, little ability to check errors - * PCR bias (unless shotgun sequencing)

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3. AIMS, QUESTIONS AND HYPOTHESES

In chapter 1, I reviewed how precursor rRNA containing ITS regions could potentially be used for determining fungal activity and characterizing the active fungal community in decomposing substrates. I estimated the life span of this marker and suggested how to deal with sample preparation. Active and total fungal community derived from RNA and DNA based approaches were discussed. This chapter is to establish the concept for the following chapters. Based on this chapter, the DNA based approach was selected for characterizing the total fungal community.

In chapter 2, I established bacterial-ribosomal intergenic spacer analysis (B-ARISA) in the laboratory for investigating bacterial diversity and community composition in plant samples. To date, the most commonly used primer sets for B-ARISA have not yet been tested for the specificity to bacterial taxa. Specifically, I evaluate, for the first time, the specificity of three commonly used B-ARISA primer sets. I tested whether the three primer sets are able to amplify (*in silico*) mitochondrial and when relevant chloroplast, from fungal, invertebrate and plant DNA. The final goal was to identify the most useful and informative primer set to use for characterizing the bacterial diversity and community structure in plant soil interphase.

Chapters 3 and 4 aimed to investigate the influence of changes in management regimes or conversion to a different forest type on the wood-inhabiting mycobiota. Here we assessed following hypotheses.

Hypotheses: (1) Forest management regimes with a high level of wood extraction strongly reduce fungal richness and alter community structure and composition of wood-inhabiting fungi; and (2) forest conversion from beech to conifers reduces fungal richness and alters community structure and composition.

Chapter 5 aimed to study the wood-inhabiting fungal communities in beech and spruce deadwood across different forest management types and, to link the fungal taxonomy data (both fungal OTUs and abundances) to potential ecosystem functions and services.

Hypotheses: (3) Different deadwood species (beech vs. spruce) could cause significant differences in fungal community structure, (4) anthropogenic (such as forest

management) as well as wood physicochemical factors significantly correlate with fungal community structure within and between *Fagus* and *Picea* wood and (5) positive correlations between the ecological function (ligninolytic enzyme activities) and service (wood decomposition rates) and total fungal richness.

In chapter 6: Effects of forest system management practices on some important ecosystem functioning and services such as leaf litter decomposition rates and nutrient dynamics were studied.
Hypotheses: (6) Decomposition rate of deciduous litter is lower in spruce forest than in other deciduous beech dominated forests (Ayres et al., 2009) and (7) in deciduous beech dominated forests, I expected variations according to the level of naturalness and disturbance: that leaf litter decomposition rate would be highest in unmanaged natural forest, next highest in near-to-nature forest and lowest in high management intensity age-class forest (Waldrop et al., 2003; Brumelis et al., 2011).

The results from chapter 5 indicate that forest system management practices significantly influence important ecosystem functioning and services such as leaf litter decomposition rates and nutrient dynamics. Non-additive decomposition rates were found in leaf litter of different forest system management practices. **Chapters 7 and 8** aimed to identify the mechanism explaining the differences of leaf litter decomposition rates and nutrient dynamics in different forest system management practices.
Hypothesis: (8) Microbial community and potential enzymes activity in leaf litter significantly different among different forest system management practices.

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B. PUBLICATIONS AND MANUSCRIPTS

CHAPTERS 1-2: Method development and optimization

CHAPTERS 3-5: Molecular fungal diversity in deadwood

CHAPTERS 6-8: Molecular microbial diversity in leaf litter, decomposition rates and nutrient cycling.

CHAPTER 1

A better understanding of functional roles of fungi in the decomposition process: using precursor rRNA containing ITS regions as a marker for the active fungal community

Witoon Purahong[1, 2], Dirk Krüger[1]

[1]UFZ-Helmholtz Centre for Environmental Research, Department of Soil Ecology, Theodor-Lieser-Str. 4, D-06120 Halle (Saale), Germany

[2]Technical University of Munich, Chair for Soil Science, Ingolstädter Landstr. 1, D-85758 Oberschleissheim, Germany

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LETTER TO THE EDITOR

A better understanding of functional roles of fungi in the decomposition process: using precursor rRNA containing ITS regions as a marker for the active fungal community

Witoon Purahong · Dirk Krüger

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1 Introduction

Decomposition and biogeochemical cycling are important ecosystem services provided by fungi (Kulhánková et al. 2006; Gange et al. 2007; Trap et al. 2011). Hitherto, different approaches have been used to examine the fungal community and its functional role in the decomposition process. Culture-dependent approaches have limitations because most fungi in environmental samples are likely to be uncultivable or difficult to isolate and such approaches are biased towards fast-growing fungi and affected by media selection (Anderson and Cairney 2004). On the other hand, culture-independent molecular approaches are used routinely for fungal diversity and community assessment. The main targets of these approaches are the rRNA genes and spacer regions of the RNA operon (rDNA), thus basing diversity and community composition on DNA content. However, some studies show that DNA may have a long life span in the environment (Nielsen et al. 2004; Levy-Booth et al. 2007; Corinaldesi et al. 2008; Pietramellara et al. 2009). DNA can stem from fungal resting structures and spores or even senescent mycelium, all of which would be metabolically inactive at a given sampling time and even permanently. Consequently, fungal community details from DNA carry some memories about the previous community composition, and may poorly reflect the fungi that are active in decomposition processes at the time of sample collection. Additionally, it is possible that dispersed propagules leave their DNA signatures when they are carried there by wind or animals (Stenlid and Gustafsson 2001). The operational taxonomic unit (OTU) richness of decomposer fungal communities revealed by DNA-based community assessment is usually higher than that based on RNA because of the inclusion of dead or inactive fungal DNA (Anderson and Parkin 2007; Rajala et al. 2011). However, there were few cases reported vice versa or no significant differences between OTU richness of the fungal decomposers derived either by DNA- or RNA-based approaches (Bastias et al. 2007; Baldrian et al. 2012). These are because some fungal OTUs are only detected using RNA and they compensate the inactive fungi detected by DNA. Nevertheless, we suggest caution that the presence of background communities of dormant or senescent fungi in environmental samples as an artifact is a potentially major hindrance to unbiased interpretation of real functional roles and correlations with enzyme production and environmental parameters. A DNA-based study (Kulhánková et al. 2006; Kubartová et al. 2009) has revealed that a large proportion of ascomycetes produce few oxidative enzymes and so are restricted in their ability to digest complex substances in the late stages of decomposition. Inactive or dead Ascomycota may be remnants of earlier stages of decomposition (Purahong and Hyde 2011; Rajala et al. 2011), which strongly supports the suggestion that there is an inert background fungal community, and that

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Dirk Krüger: writing the paper and supervising the work

W. Purahong (✉) · D. Krüger
Department of Soil Ecology, UFZ—Helmholtz Centre for Environmental Research,
Theodor-Lieser-Strasse 4,
06120 Halle (Saale), Germany
e-mail: witoon.purahong@ufz.de

W. Purahong
Chair for Soil Ecology, Technical University of Munich,
Ingolstädter Landstrasse 1,
85758 Oberschleissheim, Germany

DNA-based approaches may not be suitable for studies of functional ecology. When the objective of a study is to characterize active fungi, the inert background community is not relevant.

2 Conservation vs. understanding functionality

DNA-based approaches generally provide information on the successional stages that the fungal community has passed through, over time, because DNA accumulates within the environmental pool. Although DNA is a phosphorus-rich molecule which plants and microbes may compete for in the soil, it has been reported to persist in the top layer (0–15 cm) for few days up to several years (Nielsen et al. 2004; Pietramellara et al. 2009). Levy-Booth et al. (2007) reviewed that the persistence of recombinant DNA in field soil ranged from 77 days to at least 2 years. This is because DNA can be protected against nuclease degradation by its adsorption on surface-reactive particles in soil, i.e. clay, sand, silt and humic substance (Pietramellara et al. 2009). Thus, both active and inactive fungal taxa can be identified using DNA-based approaches in environmental samples (Anderson and Parkin 2007; Bastias et al. 2007). This can deliver results particularly relevant to conservation or natural resource management research. In an environment where DNA is retained, sampling at a late stage of decomposition can provide much information about the fungal taxa associated with the entire decomposition process. However, the drawback is that it is impossible to assign correct functional roles within successional stages of decay as some fungal taxa may have been actively growing at different successional stages. This situation is more severe in an environment where DNA is not retained. The uneven persistence of DNA from different fungal species and/or substrate quality (i.e. soil types, humic substances, Levy-Booth et al. 2007; Pietramellara et al. 2009) may bias both the existence and relative abundance of each fungal taxon within the community. This is the major obstacle for the understanding of the functional roles of fungi in the decomposition process and of their relationships with environmental factors. According to the definition of an ecological community given by Palmer and White (1994) as "the living organisms present within a space–time unit of any magnitude", dead fungi identified from DNA-based analysis should be therefore excluded from the community.

3 What is a suitable target for assessing fungal activity?

Metabolically active cells transcribe more rRNA for ribosome synthesis than inactive cells. In the case of bacteria, it has been proved that rRNA content is highly correlated with metabolic activity and therefore commonly used for quantification of their activity (Wagner 1994). However, this correlation has not yet been validated for fungi and some concerns to the use of rRNA content as a fungal activity marker have been proposed (Rajala et al. 2011). Some specific types of RNA have been used as markers for fungal activity, i.e. 18S rRNA, 28S rRNA, mRNA of protein-coding genes and the ITS region still contained in precursor RNA (Anderson and Parkin 2007; Bastias et al. 2007; McGrath et al. 2008; Rajala et al. 2011; Baldrian et al. 2012). However, to date, the best possible marker has not been identified. A suitable marker for fungal activity should have short life span but allow direct detection from environmental samples, be specific, easy to detect and provide high resolution for fungal identification (Anderson and Parkin 2007; Rajala et al. 2011). Life spans of mRNA (3 min to more than 90 min; Wang et al. 2002) and precursor RNA containing ITS regions (a few minutes; Kos and Tollervey 2010) are shorter than that of 18S rRNA (Rajala et al. 2011). Secondary structure and the presence of structural proteins can increase the life span of 18S rRNA because of slower degradation, thus the fungal activity indicated by mRNA and precursor rRNA can be considered to be more recent and represent the active community during the last few minutes or hours before sampling (Rajala et al. 2011). Both 18S rRNA and mRNA obtained by direct extraction suffer from PCR amplification bias (Rajala et al. 2011), and restricted nucleotide sequence databases limit the potential for determining taxonomic affiliation (Anderson and Parkin 2007). Precursor RNA containing ITS regions has been proposed as a suitable marker (Rajala et al. 2011). It is amplifiable by RT-PCR directly from environmental samples (Anderson and Parkin 2007; Bastias et al. 2007; Rajala et al. 2011; Baldrian et al. 2012), and it also provides good resolution for fungal taxonomy via BLAST searches at the genera or species level (Anderson and Parkin 2007). However, some cases of intraspecific variation have been reported (Müller et al. 2007) and for fungal taxa, it is too variable to allow reliable multiple sequence alignment and subsequent phylogenetic placement and OTU delimitation. Other than rRNA, some specific types of biochemical markers have been used for quantification of fungal biomass and/or fungal activity, for example certain types of fatty acids in the neutral lipid and phospholipid fatty acid fractions as well as ergosterol content. The reliability of these markers, alone or coupled with stable isotope probing, has been questioned because different fungal species may vary greatly with respect to the amount of these biochemical markers; indeed, various studies have produced conflicting results (Malosso et al. 2004; Högberg 2006). Ergosterol has been shown to persist in the environment after fungal death, and it may be detected from inactive fungi, thus it may

not reflect the active fungal community (Mille-Lindblom et al. 2004).

4 Detection of precursor rRNA containing ITS regions in environmental samples

In fungi, the main rRNA operon comprises 18S rRNA gene, ITS1, 5.8S rRNA gene, ITS2 and 25/28 rRNA gene. rDNA is transcribed by RNA polymerase I to generate large single precursor molecules. Although, ITS regions are removed from the main precursor rRNA molecule in post-transcriptional processing, leaving the mature 5.8S rRNA, 18S rRNA and 25/28S rRNA for ribosome formation (Kos and Tollervey 2010), metabolically active fungi are continuously transcribing rRNA precursor molecules and their ITS regions can be detected in the precursor rRNA pool (Anderson and Parkin 2007; Bastias et al. 2007; Rajala et al. 2011; Baldrian et al. 2012). Nevertheless, due to the fast turnover rate of this precursor rRNA, appropriate sampling and processing methods are needed, since any manipulations after sampling can induce transcription of certain species. Fixing of RNA extracts after in situ extraction of the samples with liquid nitrogen is a promising method to deal with this problem. Alternatively, immediate freezing of the samples using liquid nitrogen also can be used (Rajala et al. 2011; Baldrian et al. 2012). Chemical stabilization of nucleic acids such with commercial preservation solutions could be a viable option too. To date, ITS rRNA sequences have been successfully detected in various types of environmental samples, including soil (Anderson and Parkin 2007; Baldrian et al. 2012), litter (Baldrian et al. 2012) and wood (Rajala et al. 2011). RT-PCR is capable of detecting precursor rRNA ITS regions (Anderson and Parkin 2007; Bastias et al. 2007; Rajala et al. 2011; Baldrian et al. 2012). Pure RNA can be reverse transcribed into complementary DNA (cDNA) using Moloney murine leukaemia virus reverse transcriptase and fungal ITS primers (Anderson and Parkin 2007; Rajala et al. 2011) or random hexamer primers (Baldrian et al. 2012). Amplified cDNA can then be used for further analysis of the active fungal community.

5 Comparisons of fungal communities obtained by rDNA and precursor rRNA containing ITS regions

It has been reported that, in soil and wood samples, fungal communities characterized on the basis of rDNA and precursor rRNA molecules are clearly different in their structure, composition and richness (Anderson and Parkin 2007; Bastias et al. 2007; Rajala et al. 2011). Baldrian et al. (2012) recently found no differences in fungal diversity using rDNA and precursor rRNA from litter and organic layers of spruce forest, but significant differences in the composition of fungal taxa and, for example, several highly active fungi were of low abundance or even disappeared from the DNA pool. In addition, Rajala et al. (2011) found that the richness of fungal species determined using precursor rRNA analysis increased with decay classes but the same trend was not observed using rDNA analysis. This work also demonstrated the clear functional role of different active fungi in the successional community of decomposing wood based on precursor rRNA analysis, whilst DNA analysis was unable to show this. Rajala et al. (2011) also compared relationships between fungal community structures obtained either by rDNA- or precursor rRNA-based techniques and 13 wood quality factors, 5 among them length, surface area, volume, carbon content and C/N ratio, yielding different results. The rDNA-based technique revealed a significant relationship between fungal community structure and these five factors, while the precursor rRNA-based technique indicated that there were no significant relationships. This is the first recorded occurrence of such a discrepancy (Rajala et al. 2011) and poses a number of questions for the ecologist: (1) how do we deal with and interpret the relationships between fungal community structure and substrate or environmental factors derived from rDNA? And (2) do such relationships tell us anything? The results from the comparisons of rDNA- and precursor rRNA-based techniques demonstrate that the background revealed by rDNA analysis is real but can be excluded from the results by using precursor rRNA-based techniques. Once this background is no longer clouding the picture, the active fungi in the community, which do have functional roles in the decomposition process, can be identified (Rajala et al. 2011).

6 Applications

Precursor rRNA containing ITS regions can be used in conjunction with fingerprinting and non-fingerprinting techniques to characterize the active fungal community. To date, denaturing gradient gel electrophoresis and terminal restriction fragment length polymorphism are the only fingerprinting techniques which have been combined with the analysis of precursor rRNA molecules, although there is great potential to use precursor rRNA molecules in conjunction with other fingerprinting techniques. Baldrian et al. (2012) successfully isolated the fungal precursor rRNA containing ITS1, 5.8S rRNA and ITS2 from an environmental rRNA pool, and this is the key to success for other fingerprinting techniques, such as automated ribosomal intergenic spacer analysis and single-strand conformation polymorphism, which target this region. Although fingerprinting techniques are commonly used for investigating the differences between microbial communities, it has been suggested that direct comparison of different community fingerprints

obtained by rDNA and precursor rRNA molecules is inappropriate because of their dissimilar motilities (Rajala et al. 2011). Comparisons using richness and/or diversity indices are more appropriate. For non-fingerprinting techniques, precursor rRNA molecules have been successfully combined with 454 pyrosequencing to characterize the active fungal community (Baldrian et al. 2012). However, there appears to be no published research describing the use of precursor rRNA molecules with cloning techniques to investigate fungal communities.

7 Conclusions

Precursor rRNA containing ITS regions is an emerging and interesting molecular marker for determining fungal activity and characterizing the active fungal community in decomposing substrates. Although a small number of studies using this marker have been reported, they do have high impact on molecular fungal ecology, and increase our understanding of the functional roles of fungi in the decomposition process. Further research on the application of precursor rRNA-based techniques to characterize the active fungal community in different ecosystems and/or geographical areas is needed, especially on investigating relationships between the active community structures and enzyme production and/or environmental factors. These relationships are still unclear, and results may differ from those obtained by rDNA-based techniques.

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CHAPTER 2

Influence of commonly used primer systems on automated ribosomal intergenic spacer analysis of bacterial communities in environmental samples

Witoon Purahong[1,2], Barbara Stempfhuber[3], Guillaume Lentendu[1], Davide Francioli [1], Thomas Reitz[1], François Buscot[1, 4, 5], Michael Schloter[2,3], Dirk Krüger[1]

[1]UFZ-Helmholtz Centre for Environmental Research, Department of Soil Ecology, Theodor-Lieser-Str. 4, D-06120 Halle (Saale), Germany

[2]Technical University of Munich, Chair for Soil Science, Ingolstädter Landstr. 1, D-85758 Oberschleissheim, Germany

[3]Helmholtz Zentrum München, Research Unit for Environmental Genomics, Ingolstädter Landstr. 1, D-85758 Oberschleissheim, Germany

[4]University of Leipzig, Institute of Biology, Johannisallee 21-23, D-04103 Leipzig, Germany

[5]German Centre for Integrative Biodiversity Research (iDiv), Halle-Jena-Leipzig, Deutscher Platz 5e, D-04103 Leipzig, Germany

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Title: Influence of Commonly Used Primer Systems on Automated Ribosomal Intergenic Spacer Analysis of Bacterial Communities in Environmental Samples

Authors : Witoon Purahong[1,2]¶*, Barbara Stempfhuber[3]¶, Guillaume Lentendu[1,4]¶, Davide Francioli[1], Thomas Reitz[1], François Buscot[1, 4, 5], Michael Schloter[2, 3]*, Dirk Krüger[1]

Affiliations:

[1]UFZ-Helmholtz Centre for Environmental Research, Department of Soil Ecology, Halle (Saale), Germany, [2]Technical University of Munich, Chair for Soil Science, Oberschleissheim, Germany, [3]Helmholtz Zentrum München, Research Unit for Environmental Genomics, Oberschleissheim, Germany, [4]University of Leipzig, Institute of Biology, Leipzig, Germany, [5]German Centre for Integrative Biodiversity Research (iDiv), Leipzig, Germany.

***Corresponding authors**:

1)Tel: +49 345-5585-207, Fax: +49 345-5585-449, E-mail: witoon.purahong@ufz.de

2)Tel: +49 893-1873-543, Fax: +49 893-1872-136, E-mail: schloter@helmholtz-muenchen.de

¶These authors contributed equally

Abstract

Due to the high diversity of bacteria in many ecosystems, their slow generation times, specific but mostly unknown nutrient requirements and syntrophic interactions, isolation based approaches in microbial ecology mostly fail to describe microbial community structure. Thus cultivation independent techniques, which rely on directly extracted nucleic acids from the environment, are a well-used alternative. For example, bacterial automated ribosomal intergenic spacer analysis (B-ARISA) is one of the widely used methods for fingerprinting bacterial communities after PCR-based amplification of selected regions of the operon coding for rRNA genes using community DNA. However, B-ARISA alone does not provide any taxonomic information and the results may be severely biased in relation to the primer set selection. Furthermore, amplified DNA stemming from mitochondrial or chloroplast templates might strongly bias the obtained fingerprints. In this study, we determined the applicability of three different B-ARISA primer sets to the study of bacterial communities. The results from *in silico* analysis harnessing publicly available sequence databases showed that all three primer sets tested are specific to bacteria but only two primers sets assure high bacterial taxa coverage (1406f/23Sr and ITSF/ITSReub). Considering the study of bacteria in a plant interface, the primer set ITSF/ITSReub was found to amplify (*in silico*) sequences of some important crop species such as *Sorghum bicolor* and *Zea mays*. Bacterial genera and plant species potentially amplified by different primer sets are given. These data were confirmed when DNA extracted from soil and plant samples were analyzed. The presented information could be useful when interpreting existing B-ARISA results and planning B-ARISA experiments, especially when plant DNA can be expected.

Keywords: Biodiversity, Bacterial diversity, Bacterial community, Culture-independent method, B-ARISA.

Introduction

Bacterial automated ribosomal intergenic spacer analysis (B-ARISA) is a widely used, culture-independent, molecular technique for analyzing bacterial diversity and community structure in various types of habitats, including both terrestrial and aquatic ecosystems [1]–[5]. B-ARISA is a PCR-based method that estimates the number of bacterial operational taxonomic units (OTUs) based on the length heterogeneity of the 16S-23S ribosomal intergenic spacer region (IGS) [1], [2]. This method is highly sensitive, reliable and reproducible [3], [4]. Considering the length of the bacterial IGS region (100 – 1500 bps), B-ARISA can potentially discriminate at least 700 bacterial OTUs (using a 2 bp window for binning), so this method may be suitable to use for a large number of samples collected over a range of locations and at different times [4]. However, B-ARISA alone does not provide any taxonomic information and the results may be severely biased in relation to the primer set selection [2].

Thus, the aim of this study was to compare the coverage and specificity of three primer sets *in silico* and *in vitro*, mainly to investigate their applicability for studies of bacterial communities at the plant–soil interface: 1406f/23Sr [1], ITSF/ITSReub [2] and S-D-Bact-1522-b-S-20/L-D-Bact-132-a-A-18 [6]. We used the updated databases from December 2012 to March 2014 and improved B-ARISA PCR conditions [7], [8]. In addition, we evaluated the primer sets in a more meaningful way by examining both forward and reverse primers together (with 1 to 3 mismatches) instead of evaluating each primer separately. Furthermore, we evaluated, for the first time the specificity of these three B-ARISA primer sets to bacteria. To this end, we also tested whether the primer sets would amplify (*in silico*) chloroplast, mitochondrial, fungal, plant and invertebrate sequences.

Materials and methods

Ethics Statement

Field work permits were issued by the responsible environmental offices of the state of Baden-Württemberg, Germany (according to § 72 BbgNatSchG).

In silico testing

To determine the most valuable primer set for the B-ARISA technique, ecoPCR software (http://www.grenoble.prabi.fr/trac/ecoPCR) [9], [10] was used for theoretical sequence amplification by virtual PCR using data from four sets of databases (Supporting Information, S1 Databases, Table S1–S4). The two primer sets (1406f/23Sr and ITSF/ITSReub) that produced the best results from ecoPCR were evaluated further for their coverage and specificity to bacteria using the FastM and ModelInspector tool, implemented in the Genomatix software suite (http://www.genomatix.de/solutions/genomatix-software-suite.html). Some archaeal sequences were also contained in some databases.

In vitro testing

Ten soil samples were obtained from a long-term soil fertilization experiment that has been running for 110 years in Bad Lauchstädt, Germany [11], where different levels of fertilizer application have been compared. Furthermore, wood samples were taken from 10 different logs of two tree species in the Schwäbische Alb Biodiversity Exploratory (five samples from European beech, *Fagus sylvatica* and five samples from Norway spruce, *Picea abies*) [12]. DNA extracts from all samples were processed with B-ARISA as described by Cardinale et al. [2] for primer set ITSF/ITSReub, and as described by Borneman and Triplett [1] modified

according to Yannarell et al. [7] and Frossard et al. [8] for primer set 1406f/23Sr (for more details about the material, methods and statistical analysis, see Supporting Information, S1 Methods).

Results

Coverage and specificity of primer sets revealed by the ecoPCR software

Proportions of bacterial taxa virtually amplified by different primer sets for different levels of bacterial taxonomic classification (from phylum to species) in the prokaryote Whole Genome Sequences database (wgs-embl-pro) retrieved from EMBL are presented in Table 1. Primer set 1406f/23Sr achieved a much higher proportion of bacterial taxa virtually amplified than the other two primer sets at all levels of taxonomic classification when either zero or one mismatch was allowed. For Genome Sequence Scan, High Throughput Genome Sequencing and Standard sequence classes of prokaryotes retrieved from the EMBL (embl-pro) and constrained bacterial 16S-23S spacer (ncbi-bact-spacer) databases, the 1406f/23Sr and ITSF/ITSReub primer sets amplified similar percentages of the bacterial species (zero and one mismatch), with proportions much higher than achieved using the S-D-Bact-1522-b-S-20/L-D-Bact-132-a-A-18 primer set (Table S1, S2). Fig. 1 shows the total number of bacterial taxa virtually amplified by each primer set allowing zero to three mismatches on both forward and reverse primers from the wgs-embl-pro database. The total numbers of bacterial species or sequences with a positive virtual amplification (separated by phylum) for each primer set from the embl-pro, ncbi-bact-spacer and wgs-embl-pro databases are shown in Tables S1–S3. Overall, the 1406f/23Sr and ITSF/ITSReub primer sets perform better than the S-D-Bact-1522-b-S-20/L-D-Bact-132-a-A-18 primer set by amplifying more bacterial species and sequences in all databases especially when zero and one mismatches was allowed.

Table 1 Proportion of bacterial taxa with positive virtual amplification in the wgs-embl-pro database revealed by ecoPCR

Taxonomic rank	No. of taxa	Primer set 1406F/23Sr		Primer set ITSF/ITSReub		Primer set S-D-Bact-1522-b-S-20/L-D-Bact-132-a-A-18	
		0 mismatch	1 mismatch	0 mismatch	1 mismatch	0 mismatch	1 mismatch
phylum	19	84.2	89.5	36.8	68.4	0	52.6
class	32	78.1	87.5	43.8	59.4	0	46.9
order	72	83.3	91.7	44.4	63.9	0	31.9
family	163	79.8	87.1	49.7	64.4	0	34.4
genus	483	65.4	72.7	33.3	48.9	0	16.8
species	1389	48.2	56.7	28.9	40.3	0	12.0

	No. of species	Primer set 1406F/23Sr				Primer set ITSF/ITSReub				Primer set S-D-Bact-1522-b-S-20/L-D-Bact-132-a-A-18			
		0 mismatch	1 mismatch	2 mismatches	3 mismatches	0 mismatch	1 mismatch	2 mismatches	3 mismatches	0 mismatch	1 mismatch	2 mismatches	3 mismatches
Bacterial phyla:													
Actinobacteria	209	99	133	139	189	103	128	134	134	0	94	121	131
Aquificae	2	2	2	2	2	0	1	1	1	0	1	2	2
Bacteroidetes	126	45	80	84	95	7	15	16	16	0	1	34	64
Chlamydiae	5	4	4	4	4	0	0	0	0	0	0	4	4
Chlorobi	1	1	1	1	1	1	1	1	1	0	0	0	1
Chloroflexi	2	1	1	1	2	0	1	1	1	0	0	0	1
Cyanobacteria	26	23	23	24	24	0	1	21	21	0	0	8	21
Deinococcus-Thermus	2	0	0	0	1	0	0	0	1	0	0	0	0
Firmicutes	378	147	155	172	204	84	117	129	133	0	42	82	145
Fusobacteria	9	0	0	2	5	0	0	0	0	0	0	0	1
Lentisphaerae	1	1	1	1	1	0	0	0	0	0	0	1	1
Planctomycetes	6	0	0	0	6	0	0	0	0	0	0	0	0
Proteobacteria	582	336	357	359	450	204	290	294	294	0	21	194	289
Spirochaetes	28	1	2	2	5	0	0	0	1	0	2	2	2
Synergistetes	7	3	6	6	6	0	1	1	1	0	1	1	3
Tenericutes	18	2	14	16	17	0	0	2	2	0	0	1	8
Thermotogae	3	0	2	2	2	2	2	2	2	0	2	2	2
Verrucomicrobia	6	2	4	4	5	1	1	1	1	0	1	4	4
Archaeal phyla:													
Crenarchaeota	3	0	0	0	0	0	0	3	3	0	0	0	0
Euryarchaeota	19	0	0	0	8	0	0	3	8	0	0	0	0
Thaumarchaeota	1	0	0	0	0	0	0	0	1	0	0	0	0

Fig. 1. Number of potentially amplified species for different prokaryotic phyla in the wgs-embl-pro database revealed by ecoPCR by increasing mismatches allowed on both forward and reverse primers. The overlaid heatmap (white = 0, darkest = maximum number of sequences) illustrates rising anticipated amplification success with increasing mismatches and was applied per row (i.e. heatmap is proportional to the sequence number available per phylum). The in-cell bar illustrates the relative contribution of the phyla in the used database.

We tested whether the three primer sets are able to amplify *in silico* the non-target sequences of chloroplast (ncbi-chloro), mitochondria (ncbi-mito), fungi (embl-fun), plant (embl-pln) and invertebrates (embl-inv) (Table S4). The S-D-Bact-1522-b-S-20/L-D-Bact-132-a-A-18 primer set was most specific for bacterial sequences, only amplifying the IGS region from 4 out of 4945 and 5 out of 115186 species represented in the embl-pln database, respectively for Chlorophyta and Streptophyta. This primer set was unlikely to amplify any chloroplast, mitochondrial, fungal or invertebrate sequences when zero to three mismatches were allowed, except for chloroplast sequences where only one species of Chlorophyta was virtually amplified when 3 mismatches were allowed. The 1406f/23Sr and ITSF/ITSReub primer sets were also specific to bacterial sequences with zero or one mismatch. Primer set 1406f/23Sr virtually amplified 5 out of 27 Chlorophyta (ncbi-chloro), 1 out of 2 Chromerida (ncbi-chloro), 2 out of 5 Euglenida (ncbi-chloro), 16 out of 4945 Chlorophyta (embl-pln) and 2 out of 115186 Streptophyta (embl-pln) species. Primer set ITSF/ITSReub amplified only 8 out of 115186 Streptophyta (embl-pln) and 1 out of 1339 Echinodermata (embl-inv) species. The plant sequences amplified *in silico* by the primer sets 1406f/23Sr and ITSF/ITSReub with zero and one mismatch are presented in the supporting information (S1 Sequences) and most of them match with bacterial sequences when blasted against GenBank. When three mismatches were allowed, the 1406f/23Sr and ITSF/ITSReub primer sets amplified *in silico* more chloroplast, fungal and plant species; in addition, ITSF/ITSReub also amplified more invertebrate species. Nevertheless, the proportions of non-bacterial species amplifiable by these two primer sets were very low even when three mismatches were allowed.

Coverage and specificity of primer sets revealed by the Genomatix software suite

The total number of virtually amplified sequences using each primer set and the number of different genera to which these sequences belong was analyzed. Genera have been grouped into the corresponding bacterial phyla. Representatives of the phyla Chloroflexi, Deinococcus-Thermus, Gemmatimonades and Planctomycetes were only covered by primer set 1406f/23Sr (Table S5). Primer set ITSF/ITSReub, however, seems not to amplify sequences belonging to any representative of these phyla. The number of sequences and of genera within each phylum varied between the primer sets tested. A table containing a detailed list of the genera included in the analysis can be found in the supporting information (Tables S6, S7). Twenty one amplifiable sequences were found with primer set1406f/23Sr and 12 sequences with ITSF/ITSReub in the Genomatix database for plant-assigned sequences (Table S5). Primer set 1406f/23Sr resulted in sequence hits for green algae (Chlorophyta) to a large extent, whilst red algae (Rhodophyta) and sequences of genus *Zea* were only found for the set ITSF/ITSReub. *Paulinella* , belonging to Rhizaria, could be amplified by both primer sets. In both cases, most sequences were identified as plastid / chloroplast sequences. Besides a few sequences without known genus and not reported in the result tables, the ITS1F/ITSReub primer pair could amplify *Methanocella* of the domain Archaea.

In vitro testing

The figures for bacterial richness and community structure obtained using the 1406f/23Sr and ITSF/ITSReub primer sets were similar (Table S8; Fig. 2). The correlation between the two primer sets with respect to OTU richness and Shannon diversity was significant for plant samples (OTU richness: $r = 0.67$, $P = 0.03$; Shannon diversity $r = 0.89$, $P = 0.0007$) but not for soil samples (OTU richness: $r = -0.10$, $P = 0.43$; Shannon diversity $r = 0.24$, $P = 0.46$). When we examined the results for each primer set in order to determine the effects of fertilization (unfertilized *vs*. fertilized soil) and tree species (Norway spruce *vs*. European beech) on bacterial richness and community structure, similar results were obtained regardless of the primer set used.

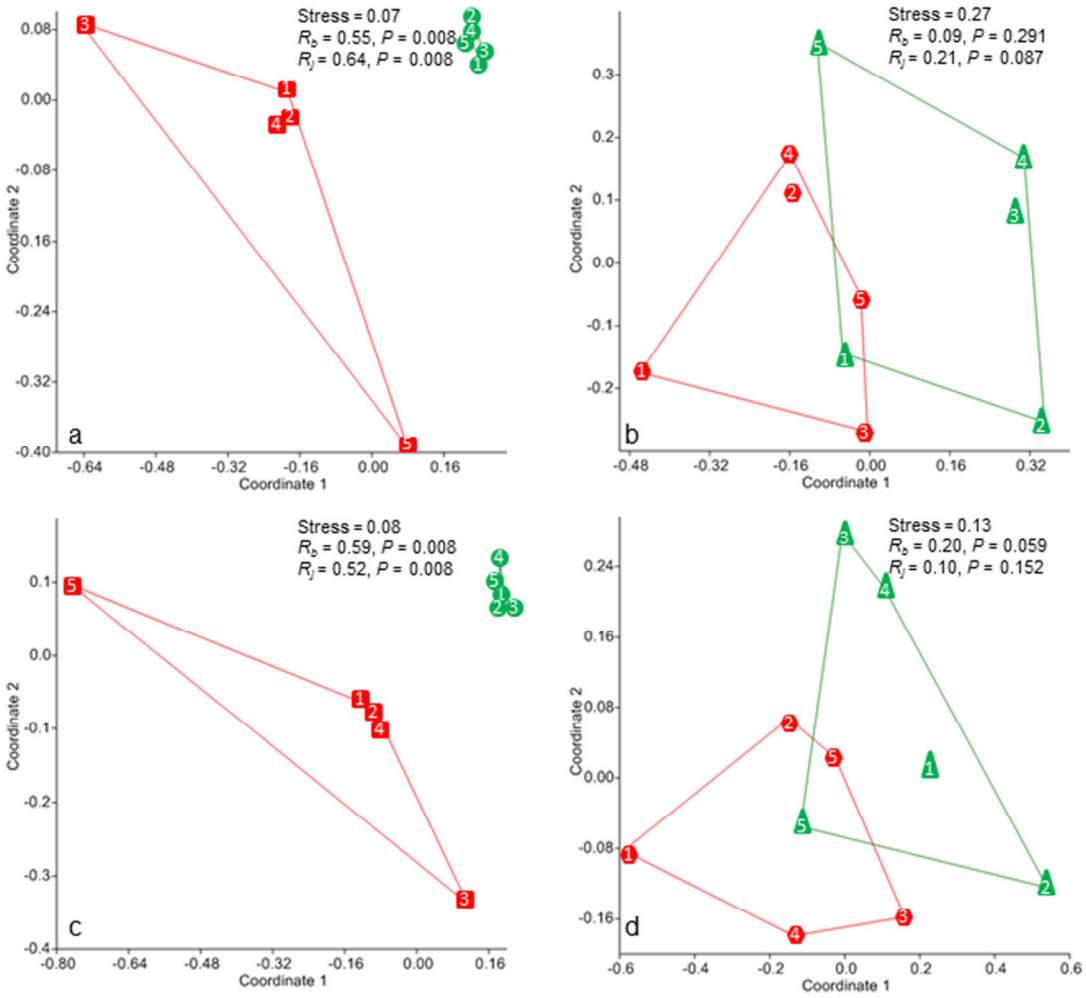

Fig. 2. NMDS ordination plots of bacterial community structure in soil (a, c) and wood (b, d) samples using different primer sets: 1406f/23Sr (a, b) and ITSF/ITSReub (c, d). Stress values from the NMDS ordinations and R_{ANOSIM} based on Bray-Curtis (R_b) and Jaccard (R_j) distance measures are shown on the right. Square = fertilized soil, circle = unfertilized soil, hexagon = *Picea abies*, triangle = *Fagus sylvatica*. Each number (1 to 5) represents one individual replicate.

Discussion and conclusions

Under changing conditions attributed to the rapid database expansions and new software tools for analyzing the specificity of primer systems, in our study we could show that the 1406f/23Sr and ITSF/ITSReub can be considered as the most promising primer sets for B-ARISA. However those results have to be interpreted in the light of the paucity of the publicly available sequence databases. The wgs-embl-pro was used as the most accurate database to estimate the amplification potential of the IGS region as all tested species were supposed to have the same chance to be virtually amplified by the different primer sets. This is equally true for the non-target ncbi-chloro and ncbi-mito databases as they contain full genomes of chloroplast and mitochondria, respectively. For the other databases however, most of the sequences do not cover the IGS region (embl-pro, -pln, -fun, -inv) or do not contain the region targeted by the primers (ncbi-bact-spacer) resulting in low virtual amplification rates, even for the targeted prokaryotic phyla (Table S1, S2).

For the primer set ITSF/ITSReub, we could confirm its high coverage and specificity for bacteria when 0 to 1 mismatch was allowed, as obtained by Cardinale et al. [2]. However, for the primer set 1406F/23Sr, current bioinformatics as well as advances in analytical methodology reveal contrasting results compared to a similar study carried out on a datasets almost a decade older [2]. An improvement in the soil DNA extraction method and/or different PCR conditions we used for our B-ARISA for the 1406f/23Sr primer set could also have increased the quality of the B-ARISA fingerprints obtained. Cardinale et al. [2] reported that with the 1406f/23Sr primer set no B-ARISA peak from soil samples (including natural and polluted soil) could be obtained. However, in our study we found that the 1406f/23Sr primer set was quite able to amplify bacterial DNA templates in natural and fertilized soil and the numbers of B-ARISA peaks (OTUs) obtained by 1406f/23Sr and ITSF/ITSReub were not

significantly different. When we examined the results for each primer set in order to determine the effects of fertilization (unfertilized vs fertilized soil) and tree species (Norway spruce *vs.* European beech) on bacterial richness and community structure, similar results were obtained regardless of the primer set used. We could also show that, if primers used have comparable properties based on *in silico* analysis, the data obtained for diversity and richness of bacterial communities based on ARISA were highly similar, independent of the studied habitat, which has also been postulated by others [4]. However, we suggest that the bias of each primer set should be taken into consideration when selecting a suitable primer set for each particular experiment. We list bacterial genera and plant species potentially amplified by primer sets 1406F-23Sr and ITSF/ITSFReub; this information could be useful when interpreting existing B-ARISA results and planning B-ARISA experiments involving samples containing plant material.

In conclusion, we consider that B-ARISA is still a powerful tool for analyzing bacterial communities, especially for simple communities originating from a restricted area or a controlled system with known bacterial community composition and biases. Using B-ARISA to investigate complex bacterial communities may still be valuable as it can provide a quick snapshot of bacterial richness and community composition before applying more sensitive approaches such as amplicon sequencing. The usefulness of B-ARISA patterns can also be seen in the study of Gobet et al. [13] where they were ecologically coherent with the data obtained from 454 pyrosequencing.

Supporting Information

Table S1.

Number of species with positive virtual amplification for different prokaryotic phyla in the embl-pro database (41 phyla, 1200281 sequences) revealed by ecoPCR.

Table S2.

Number of species with positive virtual amplification for different bacterial phyla in ncbi-bac-spacer database (19 phyla, 37134 sequences) revealed by ecoPCR.

Table S3.

Number of amplified sequences for different prokaryotic phyla in wgs-embl-pro database (21 phyla, 178462 sequences) revealed by ecoPCR.

Table S4.

Non-target species amplifiable by the different primer sets. Table shows the potential ability of primer sets to amplify mitochondrial, chloroplast, fungal, plant and invertebrate sequences.

Table S5.

Number of amplifiable sequences from different bacterial, archaeal, plant and invertebrate phyla and genera revealed by Genomatix software suite.

Table S6.

List of bacterial genera included for Genomatix analysis.

Table S7.

Bacterial genera potentially amplified by primer sets 1406f/23Sr (a) and ITSF/ITSFReub (b).

Table S8.

B-ARISA analyses in soil and wood samples for 1406f/23Sr and ITSF/ITSReub primer sets.

S1 Databases

S1 Methods

S1 Sequences

Acknowledgments

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Supporting Information

Influence of commonly used primer systems on automated ribosomal intergenic spacer analysis of bacterial communities in environmental samples

Witoon Purahong, Barbara Stempfhuber, Guillaume Lentendu, Davide Francioli, Thomas Reitz, François Buscot, Michael Schloter, Dirk Krüger

S1 Databases

ecoPCR analyses were performed on four sets retrieved from databases to test coverage and specificity of different B-ARISA primer sets. Though the plant databases actually include other groups of organisms such as algae and protozoa, we simply used the term "plant" to refer to the original name of each database.

First, all Genome Sequence Scan, High Throughput Genome Sequencing and Standard sequence classes of fungi, invertebrates, plants and prokaryotes were retrieved from the EMBL release 118 of December 2013 (embl-fun, embl-inv, embl-pln, embl-pro).

Second, Whole Genome Sequences of prokaryotes were retrieved from the EMBL release 114 of December 2012, and only one entry per species name was conserved for further analysis (wgs-embl-pro).

Third, a more constrained database was retrieved from the NCBI GenBank nucleotide database using the key search "((16S-23S spacer) AND "bacteria"[porgn:__txid2]) AND 200:1000000[Sequence Length]" on March 2013 (Release 194, ncbi-bact-spacer) in order to restrict the database to the potential target sequences (i.e. bacterial intergenic spacer) of the analysed primer pairs.

Finally, the available full chloroplast and mitochondrial genomes were retrieved from the NCBI Organelle Genome Ressources on March 2014 (ncbi-chloro and ncbi-mito).

S1 Methods

Material and methods

Soil and plant samples

Ten soil samples were obtained from the Static Fertilization Experiment in Bad Lauchstädt (Northeastern Germany), a long-term soil fertilization experiment running for over 110 years, (Merbach and Schulz 2012). Five soil samples belong to control (unfertilized soil) and the rest belongs to samples that were treated with manure and chemical fertilized soil (20 t/ha). In addition, ten wood samples were taken from ten different logs of two different tree species (5 samples from European beech, *Fagus sylvatica* and another 5 samples from Norway spruce, *Picea abies*), all from the Schwäbische Alb Biodiversity Exploratory (Fischer et al., 2010). The logs from these two tree species were comparable in length, diameter and decay class. The samples taken from the same log were combined, homogenized, ground into a fine powder with the aid of liquid nitrogen using a swing mill (Retsch, Haan, Germany) and stored at -80 ºC.

DNA extraction and B-ARISA

DNA was extracted from 250 mg homogenized soil or 100 mg homogenized wood samples, using the ZR Soil Microbe DNA MiniPrep kit (Zymo Research, Irvine, CA, USA), according to the manufacturer's instructions. The presence and quantity of genomic DNA was checked using a NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Dreieich, Germany) and the extracts were then stored at -20ºC. B-ARISA was performed in duplicate reactions using 1µl DNA template solution (20 ng template as determined by NanoDrop) under the conditions described by Cardinale et al. (2004) (primer set ITSF/ITSReub) and Borneman and Triplett (1997) modified according to Frossardet al. (2012) (primer set 1406f/23Sr). For the primer set 1406f/23Sr, briefly, the PCR mixture (20 µl) contained 1 µl DNA template (~20 ng DNA template as determined by NanoDrop); 10 µM of primer 1406f (5′-TGYACACACCGCCCGT-3′) labeled with FAM at 5′-end and an unlabeled 23Sr primer (5′-GGGTTBCCCCATTCRG-3′); 4 µl FIREPol 5x Master Mix (Solis BioDyne, Tartu, Estonia); and water to 20 µl. PCR was carried out with an initial denaturation at 94 °C for 5 min, followed by 30 cycles of 94 °C for 35 s, 55 °C for 45 s and 72 °C for 2 min, with a final

extension at 72 °C for 5 min. The PCR products were purified using a PCRExtract Mini Kit (5PRIME, Hamburg, Germany). A standardized quantity of DNA (40 ng of DNA, as determined by NanoDrop) was mixed with 14 µl of deionized Hi-Di formamide (Applied Biosystems, Foster City, CA, USA) and 0.1 µl of internal size standard Map Marker 1500 ROX (50-1500 bp) (BioVentures, Inc, Murfreesboro, TN, USA). The mixture was denatured for 5 min at 95^{o}C and chilled on ice for at least 10 min before being further processed on a capillary sequencer (ABI PRISM 3730xl Genetic Analyzer, Applied Biosystems). The two independent PCR replicates were highly correlated ($\rho = 0.84$, $P < 0.0001$; data not shown). All peaks of the fragments between 200 and 1500 bp that appeared in two technical PCR replicates were used for further analyses (Frossardet al., 2012). Operational taxonomic unit (OTU) binning was carried out using an interactive custom binning script (Ramette, 2009) in R version 2.14.1 (The R Foundation for Statistical Computing, 2011-2012) (binning size = 2 bp, cut off = 0.09%). Double DNA normalization steps before the initial PCR and the separation of DNA fragments via capillary electrophoresis make this standard F-ARISA robust for inferring changes in community structure (Ramette, 2009).

Primer pairs targeting the bacterial 16S-23S intergenic spacer

	Forward	Reverse	Reference
1406f/23Sr	TGYACACACCGCCCGT	GGGTTBCCCCATTCRG	Borneman and Triplett, 1997
ITSF/ITSReub	GTCGTAACAAGGTAGCCGTA	GCCAAGGCATCCACC	Cardinale et al., 2004
S-D-Bact-1522-b-S-20/L-D-Bact-132-a-A-18	TGCGGCTGGATCCCCTCCTT	CCGGGTTTCCCCATTCGG	Ranjard et al., 2000

Statistical analysis

B-ARISA fingerprint data were analyzed using the PAST program (Hammer et al., 2001). OTU richness of different treatments in soil and wood samples were analyzed using the paired t-test incorporating the Jarque-Bera JB test for normality and the *F* test for the equality of group variances, while the bacterial community structures were assessed using one-way ANOSIM based on Bray-Curtis and Jaccard distance measures. Non-metric multidimensional scaling (NMDS) based on Bray-Curtis distance measure was performed to visualize the bacterial community structure of different treatments.

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S1 Sequences

Fragment of the plant sequences *in silico* amplified in the embl-pln databases at 0 and 1 mismatch allowed. Sequences are annotated with their original sequence identifier, their species name, their phylum (if available or "None") and the primer pair which virtually amplified it.

>FJ789639 *Brassica rapa* Streptophyta 1406f/23Sr
cacaccatgggagttggattcactcgaaggcgttgagctaaccgtaaggaggcaggcgac
cacagtgggtttagcgactggggtgaagtcgtaacaaggtagccgtaggggaacctgcgg
ctggatcacctcctttctaaggatcgtgacgaaagcgtcagtgctagacactgaaagagc
ttcgtcatttccaaagaacatagccgccgtcctcatgtcccttcatcactagagattagc
gcagttcgctgcgctgatagctgagcaggctcaagcgcctctggctgctaacgcagcctg
atttggcagctgggccggtagctcaggtggttagagcgcacgcctgataagcgtgaggtc
ggaggttcaactcctccccggcccaccagcatttggtgaggggctttagctcagctggga
gagcggttgctttgcaagcatcaggtcatcggttcgatcccgataagctccaccatttgc
tgcgcaaatggtcttttgatttgggtccgctgcataagcagcgacggccgttcggccttg
cgaaccttcggttcgttggagcgccaatcaagcgatctgccagatactctagttgatgaa
gatagcggtttaccagccaaggctggtgatatggctcgcacaagcgagcctctttgacat
tgtgaatgggtttttttaatcgatgccgtggcgacatggttcggtttttggtgtttccgca
agggagcatccggaaagcgggcgatgttgtacacacaagattatctggctgagtttaata
accacaccgatacagctttcgacaaatgctacccagtattgtcgttggtggtgtggactc
tcaagcgtgaggtaagggcatctggtgaatgccttggcatgtacaggcgatgaaggacgt
ggcacgctgcgataagcgtgggggagccgtgagcaggctttgatcccgcgattt

>GU799579 *Carica papaya* Streptophyta 1406f/23Sr
cacaccatgggagtgggttgcaaaagaagtaggtagcttaaccttcgggagggcgcttac
cactttgtgattcatgactggggtgaagtcgtaacaaggtaaccgtaggggaacctgcgg
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>HE610157 *Oltmannsiellopsis sp. CCMP1240* Chlorophyta 1406f/23Sr
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tacggtgtaactagtaactatggtgaagtcgtaacaaggtatccgtactggaaggtgtgg
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tagctcagttggttagagcgcgcccctgataagggcgaggtcgctggttcaaatccagca
tagcccactcgaacaaatacgcagacbcggcgcaatcttgcgccgagtcattgcggtcaa
atgacaataggcttatggtgaatacctaggcacccaaagttgatgaagggcgcggaaacc
ggcgaaacgcttcggggagttggaaacacgctttgatccgaagatac

>HE610167 *Pedinomonas sp. UTEX 'LB 1027'* Chlorophyta 1406f/23Sr
cacaccatgggagctggctatgcccaaagtcgttaccccaaccgtttggagggggatgcc
taaggcagagctagtgactggggtgaagtcgtaacaaggtagccgtactggaaggtgcgg
ctggatcacctccttttaaggtccattcaaaattttcaagaaacaacttgatgaagtca
cgggctattagctcaggtggttagagcgcacccctgataagggtgaggtcgctggttcga
gtccagcatagcccatgactccgcaatgttgaagttgaaggacactgttcagcaagcata
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ctgcctttgcaaggcagatgtcagcggttcgagtccgcttacctccaccaaatcgaaaaa
cttttggatcaagcatttttttgttaagttaaaacttagcaataaacatatttcttttaa
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cgggaagctggaaacaagctatgatccggagattc

>HE610168 *Pedinomonas tuberculata* Chlorophyta 1406f/23Sr
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aagatagaaaaacaaatacggtcaaaggaaatacagattacggtggatacctaggcatct
agagtcgaagaagggcgtcgaaaccaacgaaaagcttcggggaagctgtaaacaagcaaa
gctggaaacaagctatgatccggagattc

>HE610169 *Pedinomonas sp. M2079/1* Chlorophyta 1406f/23Sr
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gggtgaggtcgctggttcgagtccagcatagcccatgacttcaaatcaagacgcgattct
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tggatcagccgaaactcgcaagaggatcgcaagattcctcgcaagagaatctttttaact
tttactcgaaagaggtcaaaggaattacggattacggtggatacctaggcatctagagtc
gaagaagggcgtcgaaaccaacgaaaagcttcgggaagctggaaacaagctatgatccgg
agatac

>HQ700713 *Schizomeris leibleinii* Chlorophyta 1406f/23Sr
caaaggtcggaaggagatagggtcggaagtggtttcgcttaacgatgaaaatcgagggca
gctacaaattctctgtttccaactaatcttaagtcgtaacaaggtagccctactggaagg
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tgtgtgttgttacaaggtttatactaaaaagaacctctaaaggtcaaatgaagtaaggcg
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>ED495993 *Sorghum bicolor* Streptophyta ITSF/ITSFReub
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cagattgtctgatgaaaatgagcagtaaaacctctacaggcttgtagctcaggtggttag
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ctctcaaattttcgcaacacgatgatggatcgcaagaaacatcttcgggttgtgaggtta
agcgactaagcgtacac

>EI077474 *Oryza nivara* Streptophyta ITSF/ITSFReub
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gcgtgataagcaattttcgtgtccccttcgtctagaggcccaggacaccgccctttcacg
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aattttcgcaacacgatgatgaatcgaaagaaacatcttcgggttgtgaggttaagcgac
taagcgtacac

>FJ789639 *Brassica rapa* Streptophyta ITSF/ITSFReub
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gctgagtttaataaccacaccgatacagctttcgacaaatgctacccagtattgtcgttg
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>GU799579 *Carica papaya* Streptophyta ITSF/ITSFReub
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cagattgtctgatagaaagtgaaaagcaaggcgtttacgcgttgggagtgaggctgaaga
gaataaggccgttcgctttctattaatgaaagctcaccctacacgaaaatatcacgcaac
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acctgccttaatatctcaaaactcatcttcgggtgatgtttgagatatttgctctttaaa
aatctggatcaagctgaaaattgaaacactgaacaacgaaagttgttcgtgagtctctca
aattttcgcaacacgatgatgaatcgaaagaaacatcttcgggttgtgaggttaagcgac
taagcgtacac

>AM084273 *Paulinella chromatophora* None ITSF/ITSFReub
ccggaaggtgcggctggatcacctcctaacagggagacaaaatgtgttactaatgtttgg
ctacttatattgccattctggtatcctgtcaccttaggtcgatcggtatcttatttcgta
agtctggattttgtaattcagctatcatttcagttcctaaactttgtctaagtcatgccc
cggcaagggtctcctgggccattagctcaggtggttagagcgcacccctgataagggtga
ggtccctggttcaagtccaggatggcccattcgtgtttgggggtttagctcagttggtag
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ttaatctggttaagctacaaagagctcac

>CP000815 *Paulinella chromatophora* None ITSF/ITSFReub
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agtctggattttgtaattcagctatcatttcagttcctaaactttgtctaagtcatgccc
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>CP000815 *Paulinella chromatophora* None ITSF/ITSFReub
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>DQ369902 *Zea mays* Streptophyta ITSF/ITSFReub
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acacgaattgcttgattcattgaagaagacgataaagaagcagcccgaaattgggtctgt
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>DQ369903 *Zea mays* Streptophyta ITSF/ITSFReub
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gtacac

>DQ369905 *Zea perennis* Streptophyta ITSF/ITSFReub
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aataccaaaatgctagatcaaggtatgaagggcgtac

>DQ369907 *Zea diploperennis* Streptophyta ITSF/ITSFReub
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cagattgtctgataaaaagtaatgagcaagacggctgcgaagtcgtgacactacccgtgt
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agcgtacac

>DQ369909 *Zea mays* Streptophyta ITSF/ITSFReub
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aagcgtacac

>DQ369911 *Zea mays* Streptophyta ITSF/ITSFReub
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gtgtctcacgagacatcttcgggttgtgaggttaagcgactaagcgtacac

>DQ369913 *Zea mays* Streptophyta ITSF/ITSFReub
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aagatagactgaacgttactttcactggtaacggatcaggctaaggtaaaatttgtaagt
aattgcgaattttcggcgaatgtcgtcttcacagtataaccagattgcttggggttatat
ggtcaagtgaagaagcgcatac

>HF562234 *Chondrus crispus* None ITSF/ITSFReub
ctggaaggtgcggctggatcacctccttaattagggaaaatagttatacaacaattctat
taaccttagatcgtatattaaataatttttaagggctattagctcagatggttagagcgc
acccctgataagggtgaagtccctggttcaattccaggatagcccaaatgaaatagtagg
ggggtatagctcagctggtagagcgctgcctttgcaaggcagatgtcagcggttcgagtc
cgcttatctccacaaagttaagttgtacttctcaaataaaattttattatttgataataa
aaaaaagtaaagtaagtaagagcttac

>JF810595 *Nitella hyalina* Streptophyta ITSF/ITSFReub
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cttcgagcctctgtgaatgtcgatcgtctcatcagactagtatcaatcatacaaaggcac
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gcaaccacgccctgacgggccttaggctctaagagtcgggatataaacaaaaagcaagtt
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>KC894740 *Grateloupia taiwanensis* None ITSF/ITSFReub
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tacatcccagatcgaaaatataagagctcttaatttaagtgttctaagaaatttgagtaa
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aagtaaatttaaggcttac

>X54299 *Antithamnion sp.* None ITSF/ITSFReub
ctggaaggtgcggctggatcacctccttatttagggatatactagattaatttataataa
tcttatcctaggtcgtaattttcgggctattagctcagttggttagagcgcacccctgat
aagggtgaggtccctggttcaaatccaggatggcccaagcaaaaggggtatagctcagt
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attttatttatagttttatgtataataaaatatcaagtcattaagggcttac

>Z29521 *Chondrus crispus* None ITSF/ITSFReub
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taaccttagatcgtatattaaataatttttaagggctattagctcagatggttagagcgc
acccctgataagggtgaagtccctggttcaattccaggatagcccaaatgaaatagtagg
ggggtatagctcagctggtagagcgctgcctttgcaaggcagatgtcagcggttcgagtc
cgcttatctccacaaagttaagttgtacttctcaaataaaattttattatttgataataa
aaaaaagtaaagtaagtaagagctta

Table S1 Number of species with positive virtual amplification for different prokaryotic phyla in the embl-pro database (41 phyla, 1200281 sequences) revealed by ecoPCR.

	No. of species	1406f/23Sr				ITSF/ITSReub				S-D-Bact-1522-b-S-20/L-D-Bact-132-a-A-18			
		0 mismatch	1 mismatches	2 mismatches	3 mismatches	0 mismatch	1 mismatches	2 mismatches	3 mismatches	0 mismatch	1 mismatches	2 mismatches	3 mismatches
Bacterial phyla:													
Acidobacteria	452	7	8	8	8	6	7	7	7	0	0	7	7
Actinobacteria	42746	165	202	207	216	219	259	264	265	7	169	210	223
Aquificae	244	11	13	13	13	7	11	11	11	0	6	13	13
Armatimonadetes	4	1	1	1	1	0	1	1	1	0	1	1	1
Bacteroidetes	10499	46	80	85	86	18	26	27	28	0	0	21	57
Caldiserica	1	1	1	1	1	0	0	1	1	0	0	1	1
Calescamantes	1	0	0	0	0	0	0	0	0	0	0	0	0
Chlamydiae	121	14	16	17	17	6	6	6	6	0	0	13	16
Chlorobi	176	9	9	10	10	10	10	10	10	0	0	1	9
Chloroflexi	158	10	11	11	13	0	1	3	3	0	1	10	11
Chrysiogenetes	4	1	1	1	1	1	1	1	1	0	0	0	1
Cloacimonetes	1	0	1	1	1	0	0	1	1	0	0	1	1
Cyanobacteria	5852	268	270	273	273	0	57	282	309	0	4	18	69
Deferribacteres	31	1	4	4	4	0	1	1	1	0	0	3	4
Deinococcus-Thermus	540	6	6	6	7	0	1	6	8	0	1	6	6
Dictyoglomi	8	2	2	2	2	0	2	2	2	0	0	2	2
Elusimicrobia	3	0	0	1	1	0	0	1	1	0	0	0	0
Fibrobacteres	4	1	1	1	1	0	0	0	0	0	0	1	1
Firmicutes	39314	266	286	299	302	214	345	375	378	1	77	190	303
Fusobacteria	178	0	1	3	6	0	0	1	1	0	0	0	2
Gemmatimonadetes	29	1	1	1	1	0	0	0	0	0	1	1	1
Gracilibacteria	7	0	0	0	0	0	0	0	0	0	0	0	0
Ignavibacteriae	2	1	2	2	2	1	1	2	2	0	0	1	2
Lentisphaerae	11	0	0	0	0	0	0	0	0	0	0	0	0
Marinimicrobia	15	0	0	0	0	0	0	0	0	0	0	0	0
Nitrospinae	23	0	0	0	0	0	0	0	0	0	0	0	0
Nitrospirae	61	4	5	5	5	1	3	4	4	0	1	3	5
None*	24184	35	51	55	57	32	35	45	46	0	23	27	31
Omnitrophica	1	0	0	0	0	0	0	0	0	0	0	0	0
Planctomycetes	411	0	6	7	13	0	1	6	7	0	0	3	6
Proteobacteria	109836	624	691	703	711	476	730	763	766	1	85	496	634
Spirochaetes	1349	1	17	17	19	2	3	11	11	0	7	17	18
Synergistetes	84	2	6	6	7	0	3	3	3	0	1	2	4
Tenericutes	1989	50	122	132	136	0	0	80	86	0	0	67	176
Thermodesulfobacteria	21	2	2	2	2	0	0	0	0	0	0	0	2
Thermotogae	123	4	20	20	20	12	12	13	13	0	9	17	20
Verrucomicrobia	400	2	3	5	7	1	1	2	2	0	2	4	5
Archaeal phyla:													
Crenarchaeota	315	0	0	2	29	0	1	37	37	0	0	1	1
Euryarchaeota	2706	0	0	0	60	0	2	84	107	0	0	0	0
Korarchaeota	2	0	0	0	0	0	0	1	1	0	0	0	0
Nanoarchaeota	1	0	0	0	0	0	0	0	0	0	0	0	0
Thaumarchaeota	11	0	0	0	0	0	0	0	3	0	0	0	0
Total (% of total species)	241918	0.63	0.76	0.79	0.84	0.42	0.63	0.85	0.88	0.00	0.16	0.47	0.67

* species without phylum annotation

The overlaid heatmap (white = 0, darkest = maximum number of sequences) illustrates rising anticipated amplification success with increasing mismatches and was applied per row (i.e. heatmap is proportional to the sequence number available per phylum). The in-cell bar illustrates the relative contribution of the phyla in the used database.

Table S2 Number of species with positive virtual amplification for different bacterial phyla in ncbi-bac-spacer database (19 phyla, 37134 sequences) revealed by ecoPCR.

	No. of species	1406f/23Sr				ITSF/ITSReub				b-S-20/L-D-Bact-132-a-A-18			
		0 mismatch	1 mismatches	2 mismatches	3 mismatches	0 mismatch	1 mismatches	2 mismatches	3 mismatches	0 mismatch	1 mismatches	2 mismatches	3 mismatches
Acidobacteria	5	4	4	4	4	3	4	4	4	1	4	4	4
Actinobacteria	587	49	53	53	53	116	131	134	135	2	54	62	66
Aquificae	35	0	0	0	0	0	0	0	0	0	0	0	0
Armatimonadetes	1	1	1	1	1	1	1	1	1		1	1	1
Bacteroidetes	85	9	13	14	14	2	3	3	3	0	0	1	8
Chlamydiae	16	12	13	13	13	3	3	3	3	0	0	12	14
Chloroflexi	5	2	2	2	2	1	1	2	2	0	1	2	2
Cyanobacteria	810	114	114	114	115	0	36	156	163	0	0	7	21
Fibrobacteres	1	1	1	1	1	0	1	1	1	0	0	0	0
Firmicutes	591	58	66	71	71	125	199	221	221	1	29	65	91
Fusobacteria	16	0	0	0	3	0	0	1	1	0	0	0	1
Gemmatimonadetes	3	2	2	2	2	2	2	2	2	1	3	3	3
Nitrospirae	8	2	2	2	2	1	3	3	3	0	2	2	2
None*	135	38	44	45	46	31	42	50	51	1	18	34	43
Planctomycetes	78	2	6	8	8	1	3	7	8	0	0	4	6
Proteobacteria	2060	179	198	201	204	245	351	357	358	4	51	186	228
Spirochaetes	54	1	2	2	2	1	1	2	2	0	2	3	3
Synergistetes	1	1	1	1	1	0	0	0	0	0	0	0	0
Tenericutes	795	38	91	98	102	0	0	75	79	0	0	65	165
Verrucomicrobia	3	1	2	2	2	0	1	1	1	0	1	1	2
Total (% of total species)	5289	9.72	11.63	11.99	12.21	10.06	14.79	19.34	19.63	0.19	3.14	8.55	12.48

* species without phylum annotation

The overlaid heatmap (white = 0, darkest = maximum number of sequences) illustrates rising anticipated amplification success with increasing mismatches and was applied per row (i.e. heatmap is proportional to the sequence number available per phylum). The in-cell bar illustrates the relative contribution of the phyla in the used database.

Table S3 Number of amplified sequences for different prokaryotic phyla in wgs-embl-pro database (21 phyla, 178462 sequences) revealed by ecoPCR.

	No. of sequences	1406f/23Sr				ITSF/ITSReub				S-D-Bact-1522-b-S-20/L-D-Bact-132-a-A-18			
		0 mismatch	1 mismatches	2 mismatches	3 mismatches	0 mismatch	1 mismatches	2 mismatches	3 mismatches	0 mismatch	1 mismatches	2 mismatches	3 mismatches
Bacterial phyla:													
Acidobacteria	71	2	2	2	2	0	3	3	3	0	0	3	3
Actinobacteria	39430	134	191	201	458	154	195	204	204	0	141	182	196
Aquificae	779	7	7	7	8	0	2	2	2	0	1	7	7
Bacteroidetes	7448	79	166	175	224	18	29	30	30	0	1	67	122
Chlamydiae	104	4	4	4	4	0	0	0	0	0	0	4	4
Chlorobi	47	1	1	1	1	1	1	1	1	0	0	0	1
Chloroflexi	157	4	4	4	5	0	4	4	4	0	0	0	4
Cyanobacteria	2960	35	35	37	47	0	1	34	34	0	0	13	33
Deinococcus-Thermus	39	0	0	0	1	0	0	0	1	0	0	0	0
Firmicutes	39769	329	351	406	471	192	309	332	336	0	96	201	327
Fusobacteria	652	0	0	3	6	0	0	0	0	0	0	0	2
Lentisphaerae	81	4	4	4	4	0	0	0	0	0	0	4	4
Planctomycetes	1924	0	0	0	16	0	0	0	0	0	0	0	0
Proteobacteria	80470	634	667	673	1039	283	463	467	468	0	30	336	530
Spirochaetes	2162	1	2	2	5	0	0	0	1	0	2	2	2
Synergistetes	421	3	9	9	11	0	1	1	1	0	1	1	4
Tenericutes	269	2	15	17	18	0	0	3	3	0	0	1	9
Thermotogae	69	0	2	2	2	2	2	2	2	0	2	2	2
Verrucomicrobia	147	2	4	4	8	1	1	1	1	0	1	4	4
Archaeal phyla:													
Crenarchaeota	631	0	0	0	0	0	0	3	3	0	0	0	0
Euryarchaeota	831	0	0	0	10	0	0	3	8	0	0	0	0
Thaumarchaeota	1	0	0	0	0	0	0	0	1	0	0	0	0
Total (% of total sequences)	178462	0.70	0.82	0.87	1.31	0.36	0.57	0.61	0.62	0.00	0.15	0.46	0.70

The overlaid heatmap (white = 0, darkest = maximum number of sequences) illustrates rising anticipated amplification success with increasing mismatches and was applied per row (i.e. heatmap is proportional to the sequence number available per phylum). The in-cell bar illustrates the relative contribution of the phyla in the used database.

Table S4 Non-target species amplifiable by the different primer sets. Table shows the potential ability of primer sets to amplify mitochondrial, chloroplast, fungal, plant and invertebrate sequences. The in-cell bar illustrates the relative contribution of the taxonomic groups in the used database.

	No. of species	1406f/23Sr				ITSF/ITSReub				S-D-Bact-1522-b-S-20/L-D-Bact-132-a-A-18			
		0 mismatch	1 mismatches	2 mismatches	3 mismatches	0 mismatch	1 mismatches	2 mismatches	3 mismatches	0 mismatch	1 mismatches	2 mismatches	3 mismatches
ncbi-mito (mitochondrial database from Metazoa), 3943 sequences													
Acanthocephala	6	0	0	0	0	0	0	0	0	0	0	0	0
Annelida	15	0	0	0	0	0	0	0	0	0	0	0	0
Arthropoda	695	0	0	0	0	0	0	0	0	0	0	0	0
Brachiopoda	4	0	0	0	0	0	0	0	0	0	0	0	0
Bryozoa	7	0	0	0	0	0	0	0	0	0	0	0	0
Chaetognatha	5	0	0	0	0	0	0	0	0	0	0	0	0
Chordata	2611	0	0	**3**	**13**	0	0	0	0	0	0	0	0
Cnidaria	83	0	0	0	0	0	0	0	0	0	0	0	0
Ctenophora	2	0	0	0	0	0	0	0	0	0	0	0	0
Echinodermata	32	0	0	0	0	0	0	0	0	0	0	0	0
Entoprocta	2	0	0	0	0	0	0	0	0	0	0	0	0
Hemichordata	4	0	0	0	0	0	0	0	0	0	0	0	0
Mollusca	169	0	0	0	0	0	0	0	0	0	0	0	0
Nematoda	80	0	0	0	0	0	0	0	0	0	0	0	0
Nemertea	9	0	0	0	0	0	0	0	0	0	0	0	0
Onychophora	4	0	0	0	0	0	0	0	0	0	0	0	0
Placozoa	5	0	0	0	0	0	0	0	0	0	0	0	0
Platyhelminthes	57	0	0	0	0	0	0	0	0	0	0	0	0
Porifera	49	0	0	0	0	0	0	0	0	0	0	0	0
Priapulida	2	0	0	0	0	0	0	0	0	0	0	0	0
Rotifera	3	0	0	0	0	0	0	0	0	0	0	0	0
Tardigrada	2	0	0	0	0	0	0	0	0	0	0	0	0
Xenacoelomorpha	1	0	0	0	0	0	0	0	0	0	0	0	0
Xenoturbellida	1	0	0	0	0	0	0	0	0	0	0	0	0
Total (% of total species)	3848	0.00	0.00	0.08	0.34	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ncbi-chloro (chloroplast database), 498 sequences													
Apicomplexa	7	0	0	0	0	0	0	0	0	0	0	0	0
Bacillariophyta	6	0	0	**1**	**5**	0	0	0	0	0	0	0	0
Chlorophyta	27	**2**	**5**	**19**	**19**	0	0	**6**	**10**	0	0	0	**1**
Chromerida	2	**1**	**1**	**1**	**1**	0	0	**1**	**1**	0	0	0	0
Euglenida	5	0	**2**	**3**	**4**	0	0	0	0	0	0	0	0
Eustigmatophyceae	6	0	0	**6**	**6**	0	0	0	0	0	0	0	0
None*	25	**1**	**1**	**15**	**18**	0	**3**	**9**	**11**	0	0	0	0
Phaeophyceae	3	0	0	0	**3**	0	0	0	0	0	0	0	0
Streptophyta	406	0	0	**11**	**12**	0	0	**14**	**15**	0	0	0	0
Xanthophyceae	1	0	0	**1**	**1**	0	0	0	0	0	0	0	0
Total (% of total species)	488	0.82	1.84	11.68	14.14	0.00	0.61	6.15	7.58	0.00	0.00	0.00	0.20

Table S4 Non-target species amplifiable by the different primer sets. Table shows the potential ability of primer sets to amplify mitochondrial, chloroplast, fungal, plant and invertebrate sequences. (continue).

	No. of species	1406f/23Sr				ITSF/ITSReub				S-D-Bact-1522-b-S-20/L-D-Bact-132-a-A-18			
		0 mismatch	1 mismatches	2 mismatches	3 mismatches	0 mismatch	1 mismatches	2 mismatches	3 mismatches	0 mismatch	1 mismatches	2 mismatches	3 mismatches
embl-fun (fungal database), 1287591 sequences													
Ascomycota	50855	0	0	**1**	**18**	0	0	**1**	**7**	0	0	0	0
Basidiomycota	23108	0	0	0	**2**	0	0	0	**11**	0	0	0	0
Blastocladiomycota	43	0	0	0	0	0	0	0	0	0	0	0	0
Chytridiomycota	409	0	0	0	0	0	0	0	0	0	0	0	0
Cryptomycota	3	0	0	0	0	0	0	0	0	0	0	0	0
Entomophthoromycota	102	0	0	0	0	0	0	0	0	0	0	0	0
Glomeromycota	1100	0	0	0	0	0	0	0	0	0	0	0	0
Microsporidia	818	0	0	**1**	**3**	0	0	0	0	0	0	0	0
Monoblepharidomycota	30	0	0	0	0	0	0	0	0	0	0	0	0
Neocallimastigomycota	305	0	0	0	0	0	0	0	0	0	0	0	0
None*	9442	0	0	0	0	0	0	0	**9**	0	0	0	0
Total (% of total species)	86215	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.00
embl-pln (plant database), 18132367 sequences													
Aurearenophyceae	2	0	0	0	0	0	0	0	0	0	0	0	0
Bacillariophyta	1334	0	0	**1**	**5**	0	0	**2**	**24**	0	0	0	0
Bolidophyceae	7	0	0	0	0	0	0	0	0	0	0	0	0
Chlorophyta	4945	**7**	**16**	**52**	**62**	0	0	**21**	**37**	0	0	0	**4**
Eustigmatophyceae	135	0	0	**6**	**6**	0	0	0	0	0	0	0	0
None*	10511	**1**	**1**	**27**	**41**	0	**4**	**90**	**116**	0	0	0	0
Phaeophyceae	1076	0	0	0	**4**	0	0	0	**12**	0	0	0	0
Pinguiophyceae	7	0	0	0	0	0	0	0	0	0	0	0	0
Streptophyta	115186	**1**	**2**	**13**	**55**	**3**	**8**	**53**	**172**	0	0	**4**	**5**
Xanthophyceae	148	0	0	**1**	**1**	0	0	**1**	**1**	0	0	0	0
Total (% of total species)	133351	0.01	0.01	0.07	0.13	0.00	0.01	0.13	0.27	0.00	0.00	0.00	0.01

Table S4 Non-target species amplifiable by the different primer sets. Table shows the potential ability of primer sets to amplify mitochondrial, chloroplast, fungal, plant and invertebrate sequences. (continue).

	No. of species	1406f/23Sr				ITSF/ITSReub				S-D-Bact-1522-b-S-20/L-D-Bact-132-a-A-18			
		0 mismatch	1 mismatches	2 mismatches	3 mismatches	0 mismatch	1 mismatches	2 mismatches	3 mismatches	0 mismatch	1 mismatches	2 mismatches	3 mismatches
embl-inv (invertebrate database), 3913563 sequences													
Acanthocephala	130	0	0	0	0	0	0	0	0	0	0	0	0
Annelida	3675	0	0	0	0	0	0	**3**	**69**	0	0	0	0
Apicomplexa	4536	0	0	0	**2**	0	0	**14**	**16**	0	0	0	0
Arthropoda	132792	0	0	0	**12**	0	0	**289**	**690**	0	0	0	0
Brachiopoda	213	0	0	0	0	0	0	0	**1**	0	0	0	0
Bryozoa	351	0	0	0	**1**	0	0	0	**13**	0	0	0	0
Chaetognatha	52	0	0	0	0	0	0	**1**	**1**	0	0	0	0
Chordata	350	0	0	0	0	0	0	0	**8**	0	0	0	0
Chromerida	2	**1**	**1**	**1**	**1**	0	0	**1**	**1**	0	0	0	0
Cnidaria	4670	0	0	0	0	0	0	**354**	**390**	0	0	0	0
Ctenophora	53	0	0	0	0	0	0	**25**	**25**	0	0	0	0
Cycliophora	7	0	0	0	0	0	0	0	0	0	0	0	0
Echinodermata	1339	0	0	**1**	**1**	0	**1**	**1**	**4**	0	0	0	0
Entoprocta	24	0	0	0	0	0	0	0	0	0	0	0	0
Euglenida	265	0	**2**	**3**	**6**	0	0	0	0	0	0	0	0
Gastrotricha	175	0	0	0	0	0	0	0	0	0	0	0	0
Gnathostomulida	26	0	0	0	0	0	0	0	0	0	0	0	0
Haplosporidia	49	0	0	0	0	0	0	0	0	0	0	0	0
Hemichordata	48	0	0	0	0	0	0	0	0	0	0	0	0
Kinorhyncha	49	0	0	0	0	0	0	0	0	0	0	0	0
Loricifera	2	0	0	0	0	0	0	0	0	0	0	0	0
Mollusca	13006	0	0	0	0	0	0	**7**	**209**	0	0	0	0
Myzostomida	46	0	0	0	0	0	0	0	0	0	0	0	0
Nematoda	5337	0	0	0	**5**	0	0	**33**	**258**	0	0	0	0
Nematomorpha	42	0	0	0	0	0	0	0	0	0	0	0	0
Nemertea	333	0	0	0	0	0	0	0	0	0	0	0	0
None*	6874	0	0	**2**	**12**	0	**1**	**15**	**38**	0	0	0	0
Onychophora	128	0	0	0	0	0	0	0	0	0	0	0	0
Orthonectida	1	0	0	0	0	0	0	0	0	0	0	0	0
Picozoa	5	0	0	0	0	0	0	0	0	0	0	0	0
Placozoa	95	0	0	0	0	0	0	0	0	0	0	0	0
Platyhelminthes	4313	0	0	0	**1**	0	0	**4**	**275**	0	0	0	0
Porifera	1943	0	0	0	0	0	0	**31**	**153**	0	0	0	0
Priapulida	8	0	0	0	0	0	0	0	0	0	0	0	0
Rhombozoa	10	0	0	0	0	0	0	0	0	0	0	0	0
Rotifera	350	0	0	0	0	0	0	**11**	**12**	0	0	0	0
Tardigrada	419	0	0	0	0	0	0	0	0	0	0	0	0
Xenacoelomorpha	185	0	0	0	0	0	0	0	0	0	0	0	0
Xenoturbellida	2	0	0	0	0	0	0	0	0	0	0	0	0
Total (% of total species)	181905	0.00	0.00	0.00	0.02	0.00	0.00	0.43	1.19	0.00	0.00	0.00	0.00

* species without higher taxon annotation

Table S5 Number of amplifiable sequences from different bacterial, archaeal, plant and invertebrate phyla and genera revealed by Genomatix software suite.

	1406f/23Sr		ITSF/ITSReub	
	No. of sequences	No. of genera	No. of sequences	No. of genera
Bacteria:				
Acidobacteria	3	2	2	1
Actinobacteria	150	20	161	22
Aquificae	1	1	1	1
Bacteroidetes	41	7	30	3
Chlamydiae	7	4	7	4
Chlorobi	7	1	6	1
Chloroflexi	4	2	-	-
Cyanobacteria	62	10	22	3
Deferribacteres	2	1	2	1
Deinococcus-Thermus	1	1	-	-
Firmicutes	590	28	648	29
Gemmatimonades	1	1	-	-
Planctomycetes	1	1	-	-
Proteobacteria*:				
α-Proteobacteria	203	36	207	32
β-Proteobacteria	139	20	145	21
γ-Proteobacteria	692	47	692	41
δ-Proteobacteria	41	9	34	7
ε-Proteobacteria	13	4	20	3
Spirochaetes	1	1	2	1
Tenericutes	5	1	3	1
Thermotogae	6	2	2	1
Plants/Algae:				
Chlorophyta	18	12	-	-
Cercozoa (Rhizaria)	3	1	3	1
Streptophyta	-	-	7	3
Rhodophyta	-	-	2	2
Others:				
Annelida (Metazoa)	-	-	-	-
Euryarchaeota (Archaea)	-	-	2	1

* Due to the large numbers of genera within the Proteobacteria, this phylum was subdivided into its respective classes.

Table S6 List of bacterial genera included for Genomatix analysis.

Bacteria phyla	1406f/ 23Sr	Bacterial genera/species	ITSF/ ITSReub	Bacteria phyla	1406f/ 23Sr	Bacterial genera/species	ITSF/ ITSReub
Acidobacteria	1	*Candidatus Koribacter*	2	Aquificae	1	*Hydrogenobacter*	1
	2	*Candidatus Solibacter*	-	Bacteroidetes	13	*Bacteroides*	15
Actinobacteria	1	*Acidothermus*	1		3	*Capnocytophaga*	-
	11	*Arthrobacter*	11		3	*Cytophaga*	-
	21	*Bifidobacterium*	17		6	*Flavobacterium*	-
	30	*Corynebacterium*	31		7	*Parabacteroides*	7
	-	*Curtobacterium*	8		8	*Porphyromonas*	8
	2	*Frankia*	2		1	*Salinibacter*	-
	4	*Kineococcus*	-	Chlamydiae	2	*Candidatus Fritschea*	2
	9	*Kitasatospora*	9		3	*Chlamydia*	2
	3	*Kocuria*	2		1	*Chlamydophila*	2
	2	*Microbacterium*	2		1	*Waddlia*	1
	-	*Microlunatus*	1	Chlorobi	7	*Chlorobium*	6
	1	*Micromonospora*	1				
	25	*Mycobacterium*	27	Chloroflexi	2	*Anaerolinea*	-
	2	*Nocardia*	2		2	*Roseiflexus*	-
	2	*Nocardioides*	2				
	-	*Rathayibacter*	1	Cyanobacteria	4	*Anabaena*	-
	13	*Rhodococcus*	14		1	*Aphanizomenon*	-
	3	*Rothia*	3		1	*Gloeobacter*	-
	1	*Rubrobacter*	1		4	*Microcystis*	-
	3	*Salinispora*	3		4	*Nostoc*	-
	12	*Streptomyces*	18		11	*Prochlorococcus*	4
	4	*Thermobifida*	4		32	*Synechococcus*	16
	1	*Tropheryma*	1		2	*Synechocystis*	2
					1	*Thermosynechococcus*	-
					2	*Trichodesmium*	-

Table S6 List of bacterial genera included for Genomatix analysis (continue).

Bacteria phyla	1406f/ 23Sr	Bacterial genera/species	ITSF/ ITSReub	Bacteria phyla	1406f/ 23Sr	Bacterial genera/species	ITSF/ ITSReub
Deferribacteres	2	*Deferribacter*	2	Firmicutes	83	*Staphylococcus*	67
Deinococcus-Therm	1	*Deinococcus*	-		119	*Streptococcus*	120
Firmicutes	9	*Alkaliphilus*	10		3	*Syntrophomonas*	3
	99	*Bacillus*	95		-	*Tuberibacillus*	3
	15	*Brevibacillus*	15		-	*Virgibacillus*	27
	3	*Caldicellulosiruptor*	3		24	*Weissella*	24
	4	*Carboxydothermus*	4	Gemmatimonades	1	*Gemmatimonas*	-
	28	*Clostridium*	68	Planctomycetes	1	*Candidatus Brocadia*	-
	6	*Desulfitobacterium*	6	Proteobacteria			
	8	*Desulfotomaculum*	8	α-Proteobacteria	39	*Acetobacter*	43
	11	*Erysipelothrix*	-		4	*Acidiphilium*	4
	8	*Finegoldia*	8		51	*Agrobacterium*	33
	18	*Geobacillus*	19		3	*Azorhizobium*	3
	-	*Halobacillus*	42		9	*Azospirillum*	9
	82	*Lactobacillus*	73		6	*Bartonella*	7
	7	*Lactococcus*	7		3	*Bradyrhizobium*	11
	4	*Leuconostoc*	4		3	*Brucella*	3
	4	*Macrococcus*	4		1	*Candidatus Pelagibacter*	-
	4	*Melissococcus*	4		2	*Chelativorans*	2
	1	*Moorella*	1		1	*Erythrobacter*	1
	7	*Oceanobacillus*	9		-	*Gluconacetobacter*	1
	2	*Oenococcus*	2		3	*Gluconobacter*	6
	17	*Paenibacillus*	-		3	*Granulibacter*	3
	3	*Pasteuria*	-		1	*Hyphomonas*	-
	7	*Pediococcus*	2		1	*Jannaschia*	1
	2	*Pelotomaculum*	2		3	*Magnetococcus*	3
	12	*Solibacillus*	12		2	*Magnetospirillum*	2
	-	*Sporolactobacillus*	6		2	*Maricaulis*	2

Table S6 List of bacterial genera included for Genomatix analysis (continue).

Bacteria phyla	1406f/ 23Sr	Bacterial genera/species	ITSF/ ITSReub	Bacteria phyla	1406f/ 23Sr	Bacterial genera/species	ITSF/ ITSReub
α-Proteobacteria	2	*Mesorhizobium*	2	β-Proteobacteria	1	*Methylibium*	1
	1	*Methylobacterium*	1		2	*Methylobacillus*	2
	2	*Nitrobacter*	2		4	*Neisseria*	4
	3	*Novosphingobium*	3		1	*Nitrosomonas*	1
	4	*Ochrobactrum*	-		1	*Nitrosospira*	1
	4	*Paracoccus*	4		3	*Polaromonas*	3
	1	*Phenylobacterium*	-		1	*Polynucleobacter*	1
	3	*Rhizobium*	20		7	*Ralstonia*	7
	11	*Rhodobacter*	11		1	*Ramlibacter*	1
	9	*Rhodopseudomonas*	7		2	*Rhodoferax*	2
	8	*Rhodospirillum*	8		-	*Taylorella*	6
	1	*Roseobacter*	1		2	*Thiobacillus*	2
	3	*Ruegeria*	3		3	*Verminephrobacter*	3
	5	*Silicibacter*	5	γ-Proteobacteria	5	*Acinetobacter*	5
	3	*Sinorhizobium*	-		14	*Actinobacillus*	12
	3	*Sphingobium*	3		19	*Aeromonas*	-
	2	*Sphingomonas*	2		4	*Alcanivorax*	4
	1	*Sphingopyxis*	1		2	*Alkalilimnicola*	2
β-Proteobacteria	8	*Acidovorax*	8		16	*Arsenophonus*	13
	9	*Bordetella*	9		2	*Candidatus Blochmannia*	2
	77	*Burkholderia*	77		1	*Candidatus Hamiltonella*	-
	6	*Candidatus Accumulibacter*	6		1	*Candidatus Portiera*	23
	1	*Collimonas*	1		5	*Candidatus Regiella*	-
	4	*Cupriavidus*	4		1	*Candidatus Serratia*	-
	4	*Dechloromonas*	4		5	*Chromohalobacter*	5
	2	*Janthinobacterium*	2		2	*Cobetia*	2

Table S6 List of bacterial genera included for Genomatix analysis (continue).

Bacteria phyla	1406f/ 23Sr	Bacterial genera/species	ITSF/ ITSReub	Bacteria phyla	1406f/ 23Sr	Bacterial genera/species	ITSF/ ITSReub
γ-Proteobacteria	9	*Colwellia*	-	γ-Proteobacteria	-	*Serratia*	1
	1	*Coxiella*	-		117	*Shewanella*	108
	4	*Dichelobacter*	3		28	*Shigella*	28
	-	*Edwardsiella*	25		7	*Sodalis*	7
	7	*Enterobacter*	24		3	*Thiomicrospira*	3
	7	*Erwinia*	7		41	*Vibrio*	12
	83	*Escherichia*	84		2	*Wigglesworthia*	-
	9	*Francisella*	9		7	*Xanthomonas*	22
	23	*Haemophilus*	23		35	*Yersinia*	35
	1	*Haererehalobacter*	2	δ-Proteobacteria	2	*Anaeromyxobacter*	2
	5	*Hahella*	5		2	*Bdellovibrio*	-
	96	*Halomonas*	111		12	*Desulfovibrio*	12
	2	*Halorhodospira*	2		4	*Geobacter*	4
	8	*Klebsiella*	8		4	*Myxococcus*	-
	4	*Legionella*	4		6	*Pelobacter*	6
	2	*Marinobacter*	3		8	*Polyangium*	7
	8	*Marinomonas*	-		2	*Syntrophobacter*	2
	3	*Nitrosococcus*	3		1	*Syntrophus*	1
	7	*Pantoea*	7	ε-Proteobacteria	5	*Arcobacter*	-
	7	*Photorhabdus*	7		11	*Campylobacter*	13
	-	*Piscirickettsia*	1		3	*Nitratiruptor*	-
	1	*Proteus*	1		4	*Sulfurimonas*	4
	5	*Pseudoalteromonas*	-		3	*Sulfurovum*	-
	45	*Pseudomonas*	41		3	*Wolinella*	3
	13	*Psychrobacter*	13	Spirochaetes	1	*Spirochaeta*	2
	10	*Psychromonas*	10	Tenericutes	-	*Acholeplasma*	3
	1	*Saccharophagus*	1		5	*Mycoplasma*	-
	14	*Salmonella*	14	Thermotogae	4	*Thermosipho*	-
					2	*Thermotoga*	2

Table S7 Bacterial genera potentially amplified by primer sets 1406f-23Sr (a) and ITSF/ITSFReub (b).

Bacteria phyla													
Acidobacteria		Actinobacteria		Aquificae		Bacteroidetes		Chlamydiae		Chlorobi		Chloroflexi	
Candidatus Koribacter	ab	*Acidothermus*	ab	*Hydrogenobacter*	ab	*Bacteroides*	ab	*Candidatus Fritschea*	ab	*Chlorobium*	ab	*Anaerolinea*	a
Candidatus Solibacter	a	*Arthrobacter*	ab			*Capnocytophaga*	a	*Chlamydia*	ab			*Roseiflexus*	a
		Bifidobacterium	ab			*Cytophaga*	a	*Chlamydophila*	ab				
		Corynebacterium	ab			*Flavobacterium*	a	*Waddlia*	ab				
		Curtobacterium	b			*Parabacteroides*	ab						
		Frankia	ab			*Porphyromonas*	ab						
		Kineococcus	a			*Salinibacter*	a						
		Kitasatospora	ab										
		Kocuria	ab										
		Microbacterium	ab										
		Microlunatus	b										
		Micromonospora	ab										
		Mycobacterium	ab										
		Nocardia	ab										
		Nocardioides	ab										
		Rathayibacter	b										
		Rhodococcus	ab										
		Rothia	ab										
		Rubrobacter	ab										
		Salinispora	ab										
		Streptomyces	ab										
		Thermobifida	ab										
		Tropheryma	ab										

Table S7 Bacterial genera potentially amplified by primer sets 1406f-23Sr (a) and ITSF/ITSFReub (b) (continue).

Bacteria phyla											
Cyanobacteria		Deferribacteres		Deinococcus-Thermus		Firmicutes		Gemmatimonades		Planctomycetes	
Anabaena	a	*Deferribacter*	ab	*Deinococcus*	a	*Alkaliphilus*	ab	*Gemmatimonas*	a	*Candidatus Brocadia*	a
Aphanizomenon	a					*Bacillus*	ab				
Gloeobacter	a					*Brevibacillus*	ab				
Microcystis	a					*Caldicellulosiruptor*	ab				
Nostoc	a					*Carboxydothermus*	ab				
Prochlorococcus	ab					*Clostridium*	ab				
Synechococcus	ab					*Desulfitobacterium*	ab				
Synechocystis	ab					*Desulfotomaculum*	ab				
Thermosynechococcu	a					*Erysipelothrix*	a				
Trichodesmium	a					*Finegoldia*	ab				
						Geobacillus	ab				
						Halobacillus	b				
						Lactobacillus	ab				
						Lactococcus	ab				
						Leuconostoc	ab				
						Macrococcus	ab				
						Melissococcus	ab				
						Moorella	ab				
						Oceanobacillus	ab				
						Oenococcus	ab				
						Paenibacillus	a				
						Pasteuria	a				
						Pediococcus	ab				
						Pelotomaculum	ab				
						Solibacillus	ab				
						Sporolactobacillus	b				
						Staphylococcus	ab				
						Streptococcus	ab				
						Syntrophomonas	ab				
						Tuberibacillus	b				
						Virgibacillus	b				
						Weissella	ab				

Table S7 Bacterial genera potentially amplified by primer sets 1406f-23Sr (a) and ITSF/ITSFReub (b) (continue).

Bacteria phyla															
Proteobacteria															
α-Proteobacteria		β-Proteobacteria		γ-Proteobacteria		δ-Proteobacteria		ε-Proteobacteria		Spirochaetes		Tenericutes		Thermotogae	
Acetobacter	ab	*Acidovorax*	ab	*Acinetobacter*	ab	*Anaeromyxobacte*	ab	*Arcobacter*	a	*Spirochaeta*	ab	*Acholeplasma*	b	*Thermosipho*	a
Acidiphilium	ab	*Bordetella*	ab	*Actinobacillus*	ab	*Bdellovibrio*	a	*Campylobacter*	ab			*Mycoplasma*	a	*Thermotoga*	ab
Agrobacterium	ab	*Burkholderia*	ab	*Aeromonas*	a	*Desulfovibrio*	ab	*Nitratiruptor*	a						
Chelativorans	ab	*Candidatus Accumulibacter*	ab	*Alcanivorax*	ab	*Geobacter*	ab	*Sulfurimonas*	ab						
Azorhizobium	ab	*Collimonas*	ab	*Alkalilimnicola*	ab	*Myxococcus*	a	*Sulfurovum*	a						
Azospirillum	ab	*Cupriavidus*	ab	*Arsenophonus*	ab	*Pelobacter*	ab	*Wolinella*	ab						
Bartonella	ab	*Dechloromonas*	ab	*Candidatus Blochmannia*	ab	*Polyangium*	ab								
Bradyrhizobium	ab	*Janthinobacterium*	ab	*Candidatus Hamiltonella*	a	*Syntrophobacter*	ab								
Brucella	ab	*Methylibium*	ab	*Candidatus Portiera*	ab	*Syntrophus*	ab								
Candidatus Pelagibacte	a	*Methylobacillus*	ab	*Candidatus Regiella*	a										
Erythrobacter	ab	*Neisseria*	ab	*Candidatus Serratia*	a										
Gluconacetobacter	b	*Nitrosomonas*	ab	*Chromohalobacter*	ab										
Gluconobacter	ab	*Nitrosospira*	ab	*Cobetia*	ab										
Granulibacter	ab	*Polaromonas*	ab	*Colwellia*	a										
Hyphomonas	a	*Polynucleobacter*	ab	*Coxiella*	a										
Jannaschia	ab	*Ralstonia*	ab	*Dichelobacter*	ab										
Magnetococcus	ab	*Ramlibacter*	ab	*Edwardsiella*	b										
Magnetospirillum	ab	*Rhodoferax*	ab	*Enterobacter*	ab										
Maricaulis	ab	*Taylorella*	b	*Erwinia*	ab										
Mesorhizobium	ab	*Thiobacillus*	ab	*Escherichia*	ab										
Methylobacterium	ab	*Verminephrobacter*	ab	*Francisella*	ab										
Nitrobacter	ab			*Haemophilus*	ab										
Novosphingobium	ab			*Haererehalobacter*	ab										
Ochrobactrum	a			*Hahella*	ab										
Paracoccus	ab			*Halomonas*	ab										
Phenylobacterium	a			*Halorhodospira*	ab										

Table S7 Bacterial genera potentially amplified by primer sets 1406f-23Sr (a) and ITSF/ITSFReub (b) (continue).

Bacteria phyla			
Proteobacteria			
α-Proteobacteria		γ-Proteobacteria	
Rhizobium	ab	*Klebsiella*	ab
Rhodobacter	ab	*Legionella*	ab
Rhodopseudomonas	ab	*Marinobacter*	ab
Rhodospirillum	ab	*Marinomonas*	a
Roseobacter	ab	*Nitrosococcus*	ab
Ruegeria	ab	*Pantoea*	ab
Silicibacter	ab	*Photorhabdus*	ab
Sinorhizobium	a	*Piscirickettsia*	b
Sphingobium	ab	*Proteus*	ab
Sphingomonas	ab	*Pseudoalteromonas*	a
Sphingopyxis	ab	*Pseudomonas*	ab
		Psychrobacter	ab
		Psychromonas	ab
		Saccharophagus	ab
		Salmonella	ab
		Serratia	b
		Shewanella	ab
		Shigella	ab
		Sodalis	ab
		Thiomicrospira	ab
		Vibrio	ab
		Wigglesworthia	a
		Xanthomonas	ab
		Yersinia	ab

Table S8 B-ARISA analyses in soil and wood samples for 1406f/23Sr and ITSF/ITSReub primer sets.

Environmental sample	1406f/23Sr			ITSF/ITSReub		
	No. of OTUs ± SD	Range of OTU size (bp)	Shannon diversity (mean± SD)	No. of OTUs ± SD	Range of OTU size (bp)	Shannon diversity (mean± SD)
Natural soil (unfertilized soil)	41.00±9.06 a	385 - 1093	3.47±0.23 a	70.20±17.09 a	229 - 1297	3.90±0.23 a
Fertilized soil	39.80±11.28 a	383 - 1053	3.40±0.28 a	59.80±22.64 a	221 - 1301	3.70±0.51 a
Norway Spruce wood	48.20±15.34 a	478 - 1236	3.55±0.36 a	65.40±29.62 a	270 - 1300	3.65±0.64 a
European Beech wood	64.40±19.44 a	446 - 1346	3.58±0.47 a	69.80±29.84 a	234 - 1298	3.42±0.76 a
***Different letters represent statistically significant differences ($P < 0.05$) after the paired t-test . Jarque-Bera JB test was used to test for normality in all datasets.						

CHAPTER 3

Changes within a single land-use category alter microbial diversity and community structure: molecular evidence from wood-inhabiting fungi in forest ecosystems

Witoon Purahong[1, 2], Björn Hoppe[1, 3], Tiemo Kahl[3], Michael Schloter[4], Ernst-Detlef Schulze[5], Jürgen Bauhus[3], François Buscot[1, 6,7], Dirk Krüger[1]

[1]UFZ-Helmholtz Centre for Environmental Research, Department of Soil Ecology, Theodor-Lieser-Str. 4, D-06120 Halle (Saale), Germany

[2]Technical University of Munich, Chair for Soil Science, Ingolstädter Landstr. 1, D-85758 Oberschleissheim, Germany

[3]University of Freiburg, Faculty of Environment and Natural Resources, Chair of Silviculture, Tennenbacherstr. 4, D-79085 Freiburg i. Brsg., Germany

[4]Helmholtz Zentrum München, Research Unit for Environmental Genomics, Ingolstädter Landstr. 1, D-85758 Oberschleissheim, Germany

[5]Max Planck Institute for Biogeochemistry, Hans-Knöll-Str. 10, D-07745 Jena, Germany

[6]University of Leipzig, Institute of Biology, Johannisallee 21-23, D-04103 Leipzig, Germany

[7]German Centre for Integrative Biodiversity Research (iDiv), Halle-Jena-Leipzig, Deutscher Platz 5e, D-04103 Leipzig, Germany

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Changes within a single land-use category alter microbial diversity and community structure: Molecular evidence from wood-inhabiting fungi in forest ecosystems

Witoon Purahong [a,b,*], Björn Hoppe [a,c], Tiemo Kahl [c], Michael Schloter [d], Ernst-Detlef Schulze [e], Jürgen Bauhus [c], François Buscot [a,f,g], Dirk Krüger [a,**]

[a] UFZ-Helmholtz Centre for Environmental Research, Department of Soil Ecology, Theodor-Lieser-Str. 4, D-06120 Halle (Saale), Germany
[b] Technical University of Munich, Chair for Soil Science, Ingolstädter Landstr. 1, D-85758 Oberschleissheim, Germany
[c] University of Freiburg, Faculty of Environment and Natural Resources, Chair of Silviculture, Tennenbacherstr. 4, D-79085 Freiburg i. Brsg., Germany
[d] Helmholtz Zentrum München, Research Unit for Environmental Genomics, Ingolstädter Landstr. 1, D-85758 Oberschleissheim, Germany
[e] Max Planck Institute for Biogeochemistry, Hans-Knöll-Str. 10, D-07745 Jena, Germany
[f] University of Leipzig, Institute of Biology, Johannisallee 21-23, D-04103 Leipzig, Germany
[g] German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, Deutscher Platz 5e, D-04103 Leipzig, Germany

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ABSTRACT

The impact of changes within a single land-use category or land-use intensity on microbial communities is poorly understood, especially with respect to fungi. Here we assessed how forest management regimes and a change in forest type affect the richness and community structure of wood-inhabiting fungi across Germany. We used molecular methods based on the length polymorphism of the internal transcribed spacers and the 5.8S rRNA gene to assess fungal operational taxonomic units (OTUs). A cloning/sequencing approach was used to identify taxonomic affinities of the fungal OTUs. Overall, 20–24% and 25–27% of native fungal OTUs from forest reserves and semi-natural forests became undetectable or were lost in managed and converted forests, respectively. Fungal richness was significantly reduced during a regeneration phase in age-class beech forests with a high level of wood extraction ($P = 0.017$), whereas fungal community structures were not significantly affected. Conversion of forests from native, deciduous to coniferous species caused significant changes in the fungal community structure ($R = 0.64–0.66$, $P = 0.0001$) and could reduce fungal richness ($P < 0.05$) which may depend on which coniferous species was introduced. Our results showed that *Ascocoryne cylichnium*, *Armillaria* sp., *Exophiala moniliae*, *Hyphodontia subalutacea* and *Fomes fomentarius*, all known for wood-decaying abilities were strongly reduced in their abundances when forests were converted from beech to coniferous. We conclude that changes within a single land-use category can be regarded as a major threat to fungal diversity in temperate forest ecosystems.

1. Introduction

There are many reasons for changes in biodiversity, including various switches or drivers that may be abiotic or biotic, man-made or natural; the emerging properties that result may give rise to new ecosystem functions (Wardle et al., 2011). Many studies have identified land-use change as one of the most important drivers affecting biodiversity in terrestrial ecosystems (Chapin et al., 2000; Sala et al., 2000; Zebisch et al., 2004). In 2003, the Intergovernmental Panel on Climate Change (IPCC) defined land-use change as changes between broad land-use categories (e.g. forest land, cropland, grassland, wetlands, settlements, and other land). Although the IPCC also proposed that subcategory changes exist within land-use types e.g. as a result of management (IPCC, 2003); such changes have received far less attention (Luyssaert et al., 2011; Nacke et al., 2011) and their effects on biodiversity are still poorly understood (Halme et al., 2010).

* Corresponding author. UFZ-Helmholtz Centre for Environmental Research, Department of Soil Ecology, Theodor-Lieser-Str. 4, D-06120 Halle (Saale), Germany. Tel.: +49 345 5585 219; fax: +49 345 5585 449.
** Corresponding author. UFZ-Helmholtz Centre for Environmental Research, Department of Soil Ecology, Theodor-Lieser-Str. 4, D-06120 Halle (Saale), Germany. Tel.: +49 345 5585 425; fax: +49 345 5585 449.
E-mail addresses: witoon.purahong@ufz.de, witoon.purahong@gmail.com (W. Purahong), dirk.krueger@ufz.de (D. Krüger).

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A major portion of the biological diversity in forests is associated or dependent on woody materials (Stokland et al., 2012). Forest management activities and conversion from one forest type to another are commonplace anthropogenic activities that can be regarded as moderate changes within the land-use category "forest", even though they have the potential to largely impact on the wood associated organisms (Stokland et al., 2012) and to change most characteristics and components of the entire forest ecosystem (IPCC, 2003; Torras and Saura, 2008). Thirty percent of the world's total forest area (ca. 1.2 billion ha) is subject to forest management regimes designed for wood production, and this is especially true in Europe, where 57% of the total forest cover (excluding Russian Federation) is managed for woody biomass (FAO, 2010). Forest management can shift tree species composition, stand density, and/or age structure either subtly over a number of decades, or suddenly, in a way comparable to catastrophic disturbances – e.g. because of complete logging or burning of old growth stands (Chazdon, 2008). In addition, a number of studies have reported that deadwood, an important energy source and habitat for organisms in forest ecosystems, is significantly scarcer in intensively managed than in unmanaged forests (Müller et al., 2007; Lonsdale et al., 2008). Large areas of forests are also facing conversion from natural or semi-natural systems to non-native monoculture forest plantations of conifers, eucalyptus, or other economically valuable timber tree species (Chazdon, 2008). This has already happened in Germany a century ago, where forests dominated by European beech (*Fagus sylvatica*) once prevailed but have been replaced on a large scale by the non-native conifers Norway spruce (*Picea abies*) and Scots pine (*Pinus sylvestris*). Nowadays European beech accounts for only 15% of the total forest cover compared with 52% for the introduced coniferous species combined (BMELV, 2011). Forest conversion may constitute a switch to a different dominant tree species or be the trigger for changes in tree species richness and composition. Forest conversion directly affects the quality of deadwood with respect to the decomposer community because the wood of different tree species has different characteristics and chemical compositions (Kögel-Knabner, 2002). Thus, changes in forest management or conversion to different forest types may result in large effects on the entire forest ecosystem that may directly or indirectly affect biodiversity and community structure within forests.

Most previous studies on the effects of changes in management on forest biodiversity have focused on plant and invertebrate communities (e.g. Werner and Raffa, 2000; du Bus de Warnaffe and Lebrun, 2004; Torras and Saura, 2008; Lange et al., 2011). These studies have shown that a change in forest management can affect plant and invertebrate diversity across different biomes. However, despite the importance of the microbial community for the functioning of ecosystems, very few studies have investigated the effects of forest management and forest conversion on microbial diversity (Nacke et al., 2011). In forests, fungi play an important role in plant performance, especially as mycorrhizal symbionts or plant pathogens and also as litter and deadwood decomposers that maintain nutrient cycles (Watkinson et al., 2006; Purahong et al., 2010; Orwin et al., 2011; Purahong and Hyde, 2011; Fukasawa et al., 2012; Kahl et al., 2012). In this study, we investigated the influence of changes in management regimes or conversion to a different forest type on wood-inhabiting fungi across Germany. Specifically, we tested the following hypotheses: (i) forest management regimes with a high level of wood extraction strongly reduce fungal richness and alter community structure and the composition of wood-inhabiting fungi; and (ii) forest conversion from beech to conifers reduces fungal richness and alters community structure and composition.

2. Materials and methods

2.1. Study areas

The study was conducted in 72 of the 150 experimental forest plots (1 ha each) distributed across Germany as part of the German Biodiversity Exploratories (Fischer et al., 2010). The forests are located in North-Eastern Germany within the Schorfheide-Chorin region (ca. 1300 km^2; 53°01′N 13°77′E), in the Hainich-Dün region in Central Germany (including the Hainich National Park and its surroundings; (ca. 1300 km^2; 51°16′N 10°47′E) and in the Schwäbische Alb region of South-Western Germany (ca. 422 km^2; 48°44′N 9°39′E) (Fischer et al., 2010; Hessenmöller et al., 2011). The experimental forest plots represent the forest management types as well as the dominant tree species in each region. All information pertaining to these forest plots has been described in detail by Fischer et al. (2010) and Hessenmöller et al. (2011). The plot selection criteria were based on forest history and management regimes, dominant tree species and deadwood status (Fischer et al., 2010; Hessenmöller et al., 2011; Luyssaert et al., 2011). All selected forest plots had, apparently, been subjected neither to clearing nor to a period of agricultural use in the past (Luyssaert et al., 2011). To investigate the first hypothesis regarding the effects of forest management regimes on fungal diversity and community structure and composition, we examined wood-inhabiting fungal diversity in 24 comparable forest plots located in the Hainich-Dün region (experiment 1) (Fischer et al., 2010). All forests plots in experiment 1 (24 forest plots/1 ha each) were located at least many hundred meters away from each other and in different forest stands, in some cases up to a maximum of 40 km distance. These 24 plots represent three treatments (with eight replicates each) based on forest management regimes and relative intensities of wood extraction: (i) European beech forest reserves with a fraction of admixed tree species *Acer* spp., *Fraxinus* spp., *Quercus* spp., *Prunus* spp. and others (no wood extraction for 60 years; uneven-age forest structure, beech unmanaged forest; BU), (ii) old age-class beech forests with a low level of wood extraction (even-age forest structure, BAL) and (iii) age-class beech forests following a high level of wood extraction (a stage of upgrowing young trees after final harvest; even-age forest structure, BAH) (Hessenmöller et al., 2011; Luyssaert et al., 2011). The data on wood extraction intensities were derived from the relative amount of woody biomass extracted per unit of land compared with reference unmanaged/pristine forests (land-use and disturbance intensity index = LUDI; Luyssaert et al., 2011). LUDI was calculated as the sum of $LUDI_p$ and $LUDI_o$ (Luyssaert et al., 2011):

$$LUDI_p = d_{AB}/d_{max} \times 100$$

$$LUDI_o = (1 - dbh_{obs}/f_1\,(N_{obs})) \times 100$$

where $LUDI_p$ = planning intensity, $LUDI_o$ = operational intensity, d_{AB} = distance on the self-thinning line between any two points A and B with density N_A and N_B, d_{max} = the length of the self-thinning curve between N_{min} and N_{max}, N = density, dbh_{obs} = observed stand diameter, N_{obs} = observed density. High level of woody biomass extraction creates large gaps in the forest canopy which enhances tree regeneration within these gaps. This early stage of regeneration is a thicket with high stand density of young trees and this results in a high value of the LUDI index, irrespective of the fact, that this stage may contain a large store of deadwood from slash of the preceding final harvest (Luyssaert et al., 2011; Schliemann and Bockheim, 2011). In this study, the LUDI of BAH forests (82.60 ± 15.30) was significantly higher than that of BU (37.26 ± 10.87) and BAL

(38.12 ± 10.78) forests ($F = 34.47$, $P < 0.01$) (Luyssaert et al., 2011). To address the second hypothesis, we compared fungal diversity and community structure in semi-natural beech forests (no conversion) with that of conifer forests (conversion from *Fagus sylvatica* to *Picea abies* or *Pinus sylvestris*; experiment 2). For this, a total of 48 comparable forest plots in two regions were investigated. In the Schwäbische Alb region, twelve plots each of European beech and Norway spruce forest and in the Schorfheide-Chorin region, twelve plots each of European beech and Scots pine forest were sampled (Fischer et al., 2010). There was no significant difference in the relative amount of woody biomass extraction and total deadwood volume per hectare (Table S1), either between beech and Norway spruce forests or beech and Scots pine forests ($P > 0.05$) and all 48 forests were under age-class management (data not shown). Thus, we expected that differences in fungal diversity or community structure and composition between beech and conifer forests were mainly attributable to the forest type.

2.2. Sampling methodologies and wood sample preparations

Fallen deadwood logs in all the forest plots were surveyed and their characteristics and locations were recorded. Only large logs with a diameter >7 cm and species specific (*Fagus sylvatica*, *Picea abies*, *Pinus sylvestris*) to each treatment (all decay stages: fresh, medium and wood in advanced decay are included) were sampled between October and November 2011, using a cordless drill (Makita BDF 451) equipped with a wood auger (diameter: 20 mm, length 450 mm), which was dipped into alcohol, flamed and wiped with ethanol between drillings to avoid cross-contamination. The drill was operated slowly and introduced at an angle of ~45° in relation to a vertical line perpendicular to the stem axis; in order to avoid overheating the sample, the operation was paused periodically. The positions and numbers of wood samples from each deadwood log were estimated according to length of the log, i.e. three samples were taken up to 5 m (one 0.5 m from each end and one in the middle), with another sample taken from each additional 5 m length of deadwood. This sampling strategy yielded a high correlation between total and sampling volume of deadwood logs ($\rho = 0.88$, $P < 0.01$; data not shown). Three composite samples from each plot were used for DNA isolation and fungal automated ribosomal intergenic spacer analysis (F-ARISA, originally developed for the bacterial intergenic spacer and since extended to fungal internal transcribed spacers). Wood samples were subsampled, frozen, transported on wet ice (ca. 0 °C) to the laboratory within 3–6 h and stored at −80 °C. Each composite wood sample was homogenized and ground into a fine powder with the aid of liquid nitrogen using a swing mill (Retsch, Haan, Germany).

2.3. DNA isolation and fungal community analysis by F-ARISA

DNA was extracted from 100 mg of each homogenized wood sample using the ZR Soil Microbe DNA MiniPrep kit (Zymo Research, Irvine, CA, USA), according to the manufacturer's instructions. The presence and quantity of genomic DNA was checked using a NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Dreieich, Germany) and the extracts were then stored at −20 °C. F-ARISA polymerase chain reaction (PCR) amplification was performed in duplicate under the conditions described by Gleeson et al. (2005) but with the following modifications: the PCR mixture (20 μl) contained 1 μl DNA template (~20 ng DNA template as determined by NanoDrop); 10 μM of fungal-specific plant-excluding primer ITS1-F (5′- CTTGGTCATTTAGAGGAAGTAA-3′, Gardes and Bruns, 1993) with a 5′ FAM-labeled modification and an unlabeled ITS4 primer (5′-TCCTCCGCTTATTGATATGC-3′, White et al., 1990); and 4 μl FIREPol 5× Master Mix (Solis BioDyne, Tartu, Estonia). PCR was carried out with an initial denaturation at 95 °C for 5 min, followed by 35 cycles of 95 °C for 60 s, 55 °C for 60 s and 72 °C for 75 s, with a final extension at 72 °C for 7 min. The PCR products were purified using a PCRExtract Mini Kit (5PRIME, Hamburg, Germany). A standardized quantity of DNA (20 ng of DNA, as determined by NanoDrop) was mixed with 14 μl of deionized Hi-Di formamide (Applied Biosystems, Foster City, CA, USA) and 0.1 μl of internal size standard Map Maker 1000 ROX (50-1000 bp) (BioVentures, Inc, Murfreesboro, TN, USA). The mixture was denatured for 5 min at 95 °C and chilled on ice for at least 10 min before being further processed using a sequencer (ABI PRISM 3730xl Genetic Analyzer, Applied Biosystems). The amplified PCR fragments were discriminated by capillary electrophoresis. The raw ARISA profiles were analyzed using the Gene Mapper Software 4.0 (Applied Biosystems) with a threshold of 100 fluorescent units. All peaks of the fragments between 390 and 1000 bp that appeared in two technical PCR replicates were used for further analyses (Ranjard et al., 2001). The two independent PCR replicates were highly correlated ($\rho = 0.93$, $P = 0.0001$; Fig. S1). Operational taxonomic unit (OTU) binning was carried out using an interactive custom binning script (Ramette, 2009) in R version 2.14.1 (The R Foundation for Statistical Computing, 2011–2012). The total peak area per sample was normalized to one and relative fluorescent intensity (RFI) was calculated. All peaks with RFI values lower than 0.09% were excluded as background noise. A strategy involving a binning size of 2 bp was applied to the F-ARISA data and the binning frame that gave the highest pairwise similarity among samples was used for further statistical analyses. Double DNA normalization steps before the initial PCR and the separation of DNA fragments via capillary electrophoresis make this standard F-ARISA robust for inferring change in community structure (Ramette, 2009).

2.4. Linking F-ARISA fingerprints with fungal taxonomy

The F-ARISA fingerprints were linked to the fungal taxonomy by comparing the length of the fragments with a clone sequence database (880 sequences). Clone libraries were constructed from pooled DNA samples of deadwood logs from the same study site and host tree species used in this experiment. Briefly, the internal transcribed spacers and the 5.8S rRNA gene (in short, ITS barcode region) of the fungal ribosomal DNA were amplified from pooled environmental DNA samples using the same primer set as in F-ARISA. PCR products were cloned into the pGEM-T Vector System (Promega GmbH, Mannheim, Germany) and *Escherichia coli* JM109 according to the manufacturer's instructions. In total, 880 clones were selected and screened by reamplification of the insert with primers M13F and M13R with the following PCR-conditions: 94 °C for 10 min, 32 cycles of 94 °C for 40 s, 54 °C for 30 s and 72 °C for 40 s and a final elongation step of 72 °C for 4 min. Amplicons were checked on 1.5% agarose gels under UV light. PCR products of clones with insert were purified with ExoSAP-IT (Affymetrix, Cleveland, OH, USA) and then used in cycle sequencing with the M13 primers as sequencing primers using Big Dye Terminator Cycle Sequencing Reaction Kit v.3.1 (Applied Biosystems). After an ethanol precipitation sequencing was done on an ABI PRISM 3730xl Genetic Analyzer (Applied Biosystems). F-ARISA (as described previously) was carried out separately on all selected clones to confirm the lengths of the ITS region. Only the clones that gave similar length from both cloning sequencing and F-ARISA were considered valid and reported in this study.

2.5. Statistical analysis

F-ARISA fingerprint data were analyzed using the PAST program (Hammer et al., 2001). Fungal OTUs and their normalized RFI values

were used as the proxy for fungal taxa and their relative abundances, respectively (Ramette, 2009). Fungal OTU richness and Shannon diversity were calculated using the PAST function "diversity indices". The differences in fungal OTU richness and diversity among different forest managements regimes at the Hainich-Dün region were analyzed for differences among means ($P < 0.05$) by performing one-way analysis of variance (ANOVA) incorporating the Shapiro–Wilk W test for normality, the Levene statistic was used to test for the equality of group variances, and Student-Newman-Keuls *post hoc* test was also applied (IBM SPSS Statistics 19, New York, NY, USA). One-way analysis of similarities (ANOSIM) based on three commonly used distance measures using abundance data (Bray–Curtis; Manhattan; Horn) and presence/absence data (Bray–Curtis; Jaccard; Kulczynski) was conducted to test for significant differences in fungal community structures and compositions among different forest management regimes, respectively. Three different distance measures were used to ensure that results were consistent across such measures. Statistical significances were based on 9999 permutations. Bonferroni-corrected *P* values were applied because more than two groups were compared. ANOSIM produces a sample statistic (R), which represents the degree of separation between test groups ranging from −1 to 1 ($R = 0$, not different; $R = 1$, completely different) (Rees et al., 2004; Ramette, 2007). Effects of forest conversion on fungal diversity and community structures and compositions were analyzed along with the effects of region and the interaction between region and forest conversion, since the experiment was conducted in two regions. Two-way ANOVA and two-way NPMANOVA (elsewhere called PERMANOVA; with the same distance measures and number of permutations used in the one-way ANOSIM) were used to investigate the fungal OTU richness and Shannon diversity, and fungal community structures and compositions, respectively. In addition, two-way ANOSIM (with the same distance measures as used for the one-way ANOSIM) were carried out to test the effects of regions and conversion of forest types on fungal community structure and composition. To test for the effects of different coniferous tree species on fungal OTU richness and diversity, we used an independent two-sample *t*-test incorporating the Shapiro–Wilk W test for normality and the *F* test for the equality of group variances, while the effects on fungal community structure and composition were assessed using one-way ANOSIM. To investigate the magnitude of the OTU abundance shift in the treatments (forests with different management regimes or conversion of forest types) compared with the control (unmanaged or semi-natural beech forests), the abundance of all shared OTUs (accounting for 88.40–94.60% of total abundances) was investigated with respect to percent abundance shift and share (see supporting information). In addition, the average abundance of the ten most abundant OTUs in each treatment and the same OTUs in the control and *vice versa* (pairwise comparisons) were visualized using the Voronoi algorithm with curved tessellations implemented in Treemap v. 2.7.2. (Macrofocus, Zurich, Switzerland). Voronoi treemaps have been used to visualize biodiversity data and gene expression previously (Balzer and Deussen, 2005; Horn et al., 2009; Santamaria and Pierre, 2012) but have never before been used to show microbial fingerprint data. This software allows us to visualize clearly the shifts in OTU abundance in each treatment. One-way ANOVA or *t*-test was used to test for the differences among or between means of total deadwood volume per hectare and LUDI in experiment 1 and 2 (comparisons were done only in forests located at the same regions). To investigate the correlation between total volume or sampling volume of deadwood in all study plots, and ARISA peak areas between two independent PCR replicates, non-parametric correlations (Spearman's rho test) were performed using the SPSS program (IBM SPSS Statistics 19).

3. Results

3.1. General data on wood-inhabiting fungal OTUs obtained by F-ARISA

In the Hainich-Dün region (experiment 1), 168 fungal OTUs were detected from 24 beech forests. The number of fungal OTUs detected in BAH was lower (110) than in BU (122) and BAL (124) forests (Table 1). There were 215 fungal OTUs detected in 48 forests located in Schwäbische Alb and Schorfheide-Chorin (experiment 2). Semi-natural beech forests at these two regions had a higher number of fungal OTUs (158 in Schwäbische Alb and 147 in Schorfheide-Chorin) than Norway spruce and Scots pine forests (132 and 136, respectively) (Table 1). The percentage of OTUs that were present only in beech forests and not in converted conifer forests (spruce and pine) was high (25–27%). Surprisingly, the percentage of newly detected OTUs, when compared to the original beech forest community, was low in Norway spruce and Scots pine forests (13–19%). The percentage of shared OTUs was high and stable across different management regimes and conversion of forest types; the figures ranged from 56 to 61% (Table 1). Newly detected OTUs from managed beech forests (73–82%) and conifer forests (68–78%) were mainly common at the landscape level.

Table 1
Analysis of similarity (ANOSIM) based on Bray–Curtis and Manhattan distance measures (identical results in all cases) using abundance data comparing different wood-inhabiting fungal community structures in forests subjected to different forest management regimes or forest type conversions. Percent newly detected and undetected and shared OTUs are shown on the right.

Changes within land-use category	Region	*N*	*R*	*P*	OTUs detected (total OTUs)	Fungal OTU (%)		
						Newly detected	Undetected	Shared
Forest management regime								
Unmanaged beech forest vs. low-intensity wood extraction age-class beech forest	HEW	16	−0.09	1.0000	122 vs.124 (155)	33 (21.29)	31 (20.00)	91 (58.71)
Unmanaged beech forest vs. high-intensity wood extraction age-class beech forest	HEW	16	−0.04	1.0000	122 vs.110 (144)	22 (15.28)	34 (23.61)	88 (61.11)
Low- vs. high-intensity wood extraction age-class beech forest	HEW	16	−0.03	1.0000	124 vs.110 (149)	25 (16.78)	39 (26.17)	85 (57.05)
Forest type conversion								
No conversion (beech forest) vs. converted Norway spruce forest	AEW	24	0.66	**0.0001**	158 vs.132 (181)	23 (12.71)	49 (27.07)	109 (60.22)
No conversion (beech forest) vs. converted Scots pine forest	SEW	24	0.64	**0.0001**	147 vs.136 (181)	34 (18.78)	45 (24.86)	102 (56.35)

Bonferroni-corrected *P* values were applied in all cases when more than two groups were compared with ANOSIM. (R = degree of separation between test groups ranging from −1 to 1; $R = 0$, not different; $R = 1$, completely different) (*P* values were based on 9999 permutations (significant values ($P < 0.05$), are given in bold); N = population size; HEW = forests in the Hainich-Dün region; AEW = forests in the Schwäbische Alb region; SEW = forests in the Schorfheide-Chorin region).

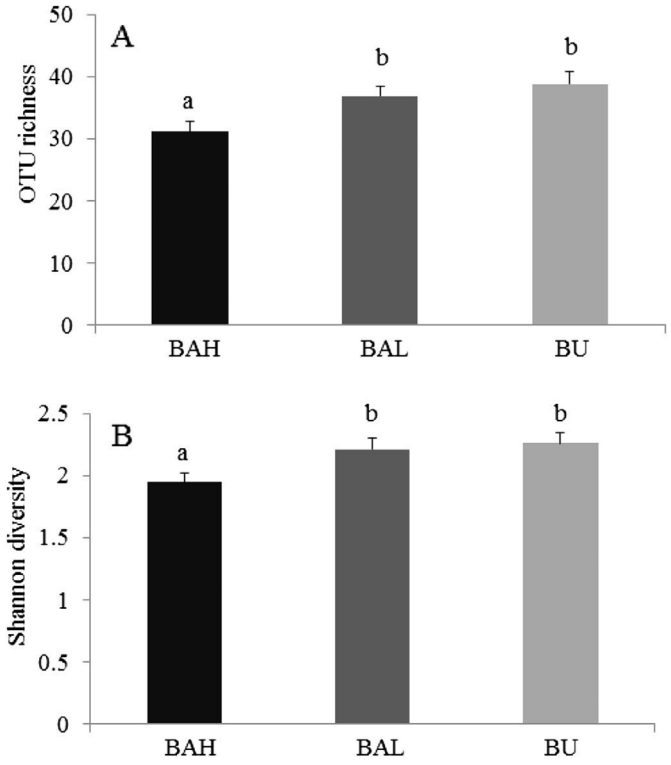

Fig. 1. Two diversity parameters for wood-inhabiting fungi across different forest management regimes in the Hainich-Dün region (BAH = age-class beech forest with a high level of wood extraction; BAL = age-class beech forest with a low level of wood extraction; BU = unmanaged beech forest): (A) Mean OTU richness and (B) Mean Shannon diversity (mean + S.E., $n = 8$). Different letters above each bar represent statistically significant differences ($P < 0.05$) after an analysis of variance and a Student-Newman-Keuls *post hoc* test.

3.2. Fungal community structure in different forest management regimes (experiment 1)

The OTU richness and Shannon diversity of wood-inhabiting fungi were significantly greater in BU and BAL than in BAH forests ($F = 5.01$, $P = 0.017$ and $F = 4.14$, $P = 0.031$, respectively; Fig. 1A and B). OTU richness and Shannon diversity were not significantly different between BU and BAL forests ($P > 0.05$). Interestingly, our results from one-way ANOSIM pair-wise comparisons of fungal community structures showed no significant differences among these three treatments (Bonferroni-corrected $P = 1.000$ in all treatments), and the R values from ANOSIM were close to zero in all treatments (Tables 1 and S2). The abundance data were transformed to presence/absence data and analyzed using one-way ANOSIM in order to investigate fungal community composition. The results were similar to those relating to community structure (Tables 1 and 2). This agreement confirmed that there was no significant difference in either fungal community structure or composition between forest management regimes either with low- or high- intensity woody biomass extraction (Tables 1 and 2).

3.3. Fungal diversity and communities in semi-natural beech forests vs. converted forests at different regions (experiment 2)

The effects of region and forest conversion on fungal OTU richness and diversity were pronounced in this study, whereas the effects of interaction of these two variables were not significant (Table 3). Specifically, fungal OTU richness and diversity (per plot) in forests at the Schwäbische Alb region were significantly lower than at Schorfheide-Chorin ($F = 4.96$, $P = 0.031$; $F = 4.73$, $P = 0.035$). Semi-natural beech forests had significantly higher fungal OTU richness and diversity than forests converted to conifers ($F = 10.70$, $P = 0.002$; $F = 4.81$, $P = 0.034$). We also investigated the responses of fungi to different conifer species in each region. In Schwäbische Alb, the forest was converted from beech to Norway spruce, resulting in a significant reduction of both fungal OTU richness and Shannon diversity ($t = -2.99$, $P = 0.007$ and $t = -2.20$, $P = 0.038$, respectively; Fig. 2A and B) compared to semi-natural beech forests. On the other hand, in Schorfheide-Chorin, where the beech forests were converted to Scots pine, there were no significant differences in either fungal OTU richness and diversity between these two forest types ($t = -1.46$, $P = 0.16$ and $t = -0.58$, $P = 0.57$, respectively; Fig. 2C and D). Changes in community structure ($F = 8.14$, $P = 0.0001$; $R = 0.65$, $P = 0.0001$) and composition ($F = 5.96$ and 3.87, $P = 0.0001$; $R = 0.57$, $P = 0.0001$) are obvious responses to the conversion of forest types; however they are also influenced by other factors such as region (community structure: $F = 3.40$, $P = 0.0002$; $R = 0.27$, $P = 0.0001$; community composition: $F = 3.57$ and 2.46, $P = 0.0001$; $R = 0.30$, $P = 0.0001$) and the interaction between region and change in forest type (community structure: $F = 2.15$, $P = 0.0098$ and 0.0079; community composition: $F = 2.23$ and 1.74, $P = 0.0016$) (Tables 3 and S4). Comparisons of the fungal community structure and composition between conifer forests and semi-natural beech forests in each region confirm that both community structure and composition were strongly modified in both Norway spruce (community structure: $R = 0.66$, $P = 0.0001$; community composition: $R = 0.59$, $P = 0.0001$) and Scots pine (community structure: $R = 0.64$, $P = 0.0001$; community composition: $R = 0.55$, $P = 0.0001$) forests (Tables 1, 2 and S2).

Table 2
One-way analysis of similarity (ANOSIM) based on three distance measures using presence-absence data for different wood- inhabiting fungal community compositions in forests subjected to different forest management regimes or conversion to a different forest type.

Changes within land-use category	Region	*N*	Bray–Curtis		Jaccard		Kulczynski	
			R	*P*	*R*	*P*	*R*	*P*
Forest management regime								
Unmanaged beech forest vs. low-intensity wood extraction age-class beech forest	HEW	16	0.02	1.0000	0.02	1.0000	0.02	1.0000
Unmanaged beech forest vs. high-intensity wood extraction age-class beech forest	HEW	16	0.12	0.2739	0.12	0.2823	0.09	0.4467
Low- vs. high-intensity wood extraction age-class beech forest	HEW	16	0.04	0.8892	0.04	0.9036	0.02	1.0000
Forest type conversion								
No conversion (beech forest) vs. converted Norway spruce forest	AEW	24	0.59	**0.0001**	0.59	**0.0001**	0.60	**0.0001**
No conversion (beech forest) vs. converted Scots pine forest	SEW	24	0.55	**0.0001**	0.55	**0.0001**	0.55	**0.0001**

Bonferroni-corrected *P* values were applied in all cases when more than two groups were compared with ANOSIM. (R = degree of separation between test groups ranging from −1 to 1; $R = 0$, not different; $R = 1$, completely different); P values were based on 9999 permutations (significant values ($P < 0.05$), are given in bold); N = population size; HEW = forests in the Hainich-Dün region; AEW = forests in the Schwäbische Alb region; SEW = forests in the Schorfheide-Chorin region.

Table 3
Effects of region and forest type conversion on fungal OTU richness, Shannon diversity (H') and community structure and composition. Two-way NPMANOVA and Two-way ANOSIM were carried out based on different distance measures depending on the data type (BC = Bray–Curtis; M = Manhattan; J = Jaccard; two other distance measures are shown in the supporting information). Significant values ($P < 0.05$), are given in bold. Two-way ANOSIM does not examine the interaction between region and forest type conversion. (Population size (N) = 48; AEW = forests in the Schwäbische Alb region; SEW = forests in the Schorfheide-Chorin region.).

	Statistical analysis	Region (AEW vs. SEW)		Forest type conversion		Region × forest type conversion	
		Statistics	*P*	Statistics	*P*	Statistics	*P*
OTU richness	Two-way ANOVA	$F = 4.96$	**0.0312**	$F = 10.70$	**0.0021**	$F = 2.34$	0.1333
H'	Two-way ANOVA	$F = 4.73$	**0.0351**	$F = 4.813$	**0.0336**	$F = 2.58$	0.1153
Community structure	Two-way NPMANOVA (BC)	$F = 3.40$	**0.0002**	$F = 8.14$	**0.0001**	$F = 2.15$	**0.0098**
	Two-way NPMANOVA (M)	$F = 3.40$	**0.0002**	$F = 8.14$	**0.0001**	$F = 2.15$	**0.0079**
	Two-way ANOSIM (BC)	$R = 0.27$	**0.0001**	$R = 0.65$	**0.0001**	–	–
	Two-way ANOSIM (M)	$R = 0.27$	**0.0001**	$R = 0.65$	**0.0001**	–	–
Community composition	Two-way NPMANOVA (BC)	$F = 3.57$	**0.0001**	$F = 5.96$	**0.0001**	$F = 2.23$	**0.0016**
	Two-way NPMANOVA (J)	$F = 2.46$	**0.0001**	$F = 3.87$	**0.0001**	$F = 1.74$	**0.0016**
	Two-way ANOSIM (BC)	$R = 0.30$	**0.0001**	$R = 0.57$	**0.0001**	–	–
	Two-way ANOSIM (J)	$R = 0.30$	**0.0001**	$R = 0.57$	**0.0001**	–	–

3.4. Abundance shift of fungal OTUs in forests with different forest management regimes or forest types

Overall, the abundance shift (change in abundance) caused by different management regimes (33–39%, experiment 1) was much lower than that caused by conversion from semi-natural beech to conifer forests (65–70%, experiment 2) (Table S3). Comparisons of the average OTU abundance (ten most abundant shared OTUs; converted *vs.* semi-natural beech forests), revealed that fungal OTU abundance in secondary spruce and pine forests had significantly shifted from that of semi-natural beech forests. In beech forests with different wood extraction intensities the abundance shifts were relatively small (Fig. 3). A list of some of the most abundant OTUs shared between forest types is shown as Table 4. In particular the average RFI (= abundance) of the top 10 shared ARISA OTUs, displayed by tessellation area, stayed relatively similar (Fig. 3A), while the top 10 average RFI decreased in the compared forest types (Fig. 3B). There were only few cases of OTUs among the top 10 of one forest type which were more abundant in the compared forest types. For example, (Top 10 Spruce vs. Beech), the OTUs 914 (*Armillaria* sp.), 674 (*Exophiala moniliae*), and 664 (*Hyphodontia subalutacea*) each individually were more abundant in beech than in spruce, where they are among the top 10 abundant shared OTUs (Fig. 3B). All three also were among the top 10 in beech (Fig. 3B Beech vs. Spruce). The OTU 914 (*Armillaria* sp.) was commonly found in the beech stands undergoing different wood removal intensities, and was also frequently encountered in Spruce. Indeed *Armillaria* are known to be common on both coniferous and deciduous wood (Banik et al., 1995).

4. Discussion

In this work, a commonly used molecular fungal community fingerprinting technique (F-ARISA) was successfully applied to investigate fungal richness and community structure in deadwood samples. OTUs derived from ARISA may not be equivalent to species, however, they do provide a basis for richness estimates, and allow highly consistent measurement of community structure through space and time (Green et al., 2004; Jones et al., 2007; Weig et al., 2013). This technique has a relatively high resolution and is a reproducible method for investigating differences between complex fungal communities (Ranjard et al., 2001; Green et al., 2004).

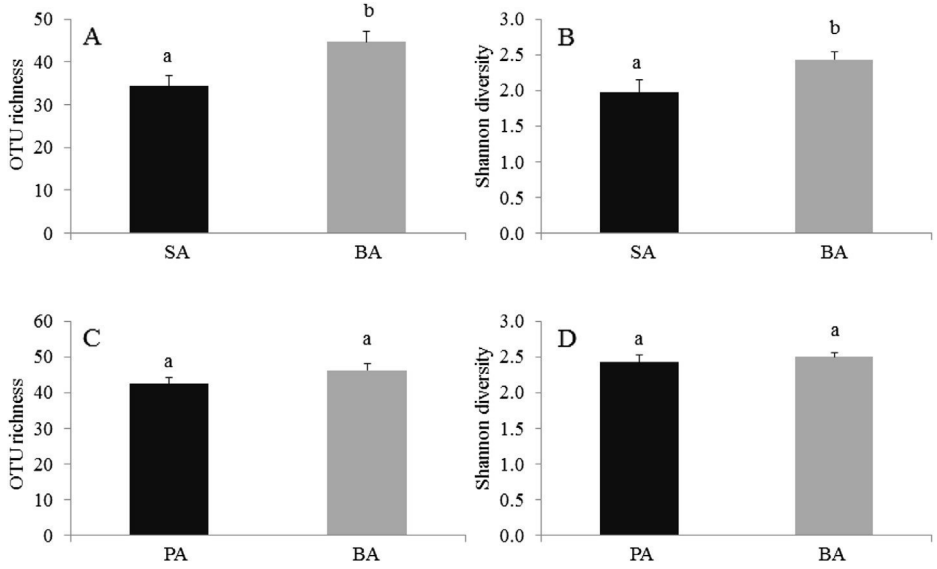

Fig. 2. Two diversity parameters for wood-inhabiting fungi experiencing conversion between forest types in the Schwäbische Alb region (A, B) and the Schorfheide-Chorin region (C, D) (SA = age-class Norway spruce forests; BA = age-class beech semi-natural forests; PA = age-class Scots pine forests): (A, C) Mean OTU richness and (B, D) Mean Shannon diversity (mean + S.E., $n = 12$). Different letters above each bar represent statistically significant differences ($P < 0.05$) after independent two-sample *t*-test.

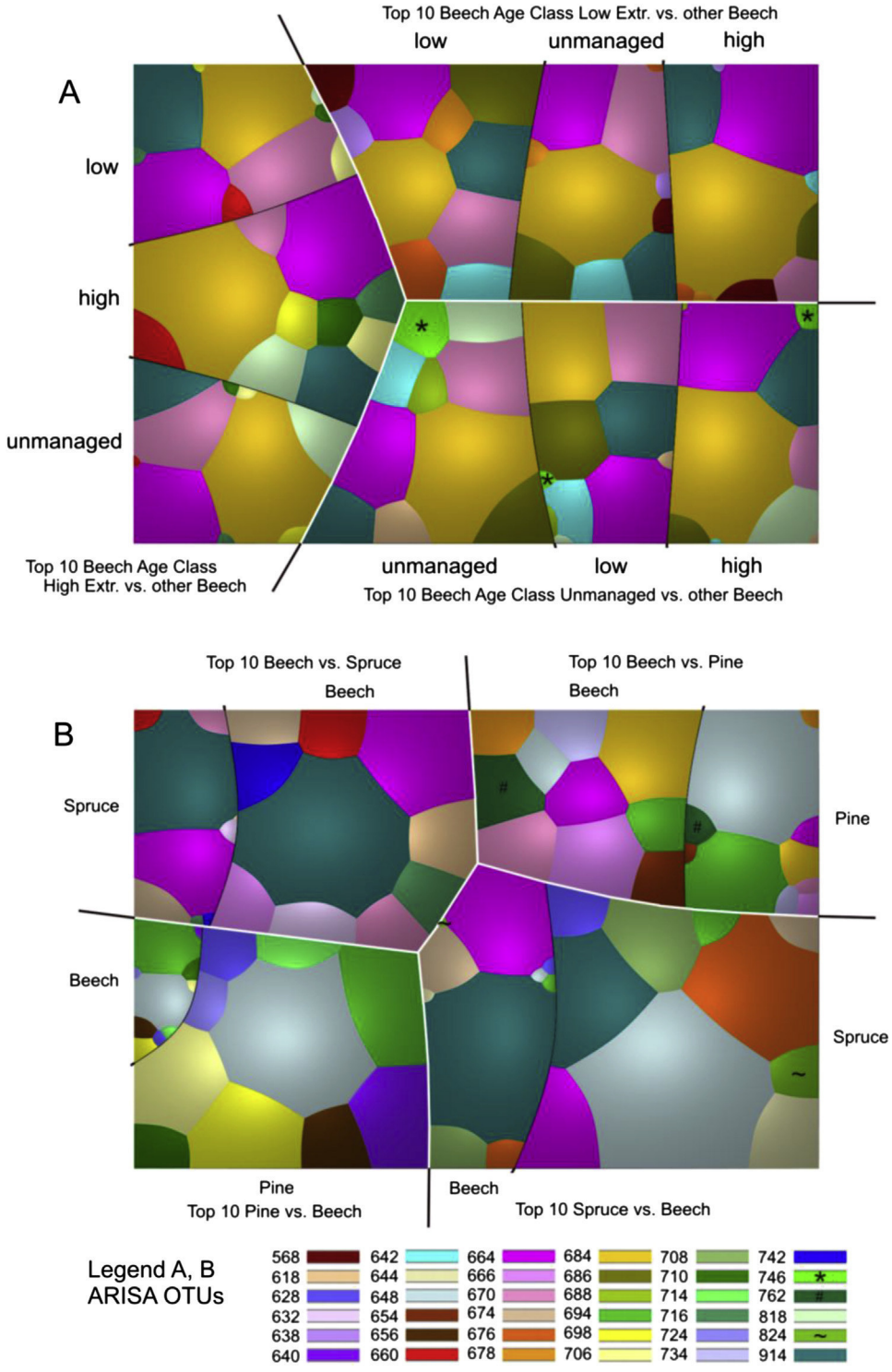

Fig. 3. Comparisons of community structure by Voronoi treemaps. The ten most abundant OTUs (= tessellation cells) shared between forest types being compared (top 10 by abundance in one type) are plotted next to the same set of OTUs in the other type. (A) Beech forests with different management regimes (unmanaged = unmanaged beech forest, high = age-class beech forest with a high level of wood extraction, low = age-class beech forest with a low level of wood extraction) compared for all possible combinations, (B) No conversion (beech forest) *vs.* converted Norway spruce forest and no conversion (beech forest) *vs.* converted Scots pine forest. Sizes are based on average RFI values, normalized within Fig. 3A and B separately. Colors indicate the same OTU and are consistent between the two subfigures. OTUs are listed according to their binning fragment size.

However, because of the normal F-ARISA procedure for excluding background noise (hence only keeping the peaks above a threshold of 50 or 100 fluorescence units and with an RFI value of 0.09% or greater), the sensitivity of the method may have been reduced. Thus, the richness estimated from ARISA accounts for community evenness rather than the total richness because some extremely rare taxa may not be included. We can assume that undetected OTUs were probably lost, i.e. that they disappeared due to the

Table 4
Linking F-ARISA fingerprints to fungal taxonomy for some of the most abundant OTUs shared between forest types.

Fungal OTU	Identified fungal species	Ecological role	Reference
628	*Ascocoryne sarcoides* (Ascomycota, Helotiales) (GQ411510)	Saprobe (deadwood, pine litter)	Farr and Rossman (2013)
632	*Ascocoryne cylichnium* (Ascomycota, Helotiales) (AY789395)	Saprobe (deadwood, stump)	Farr and Rossman (2013)
648	Fungal sp. TRN447 (AY843145)	nd	Ruibal et al. unpublished results
660	*Fomes fomentarius* (Basidiomycota, Polyporales) (EF155498)	Saprobe (white rot), tree pathogen (living and deadwood)	Farr and Rossman (2013)
664	*Hyphodontia subalutacea* (Basidiomycota, Hymenochaetales) (DQ340341)	Saprobe (white rot) (deadwood)	Farr and Rossman (2013)
674	*Exophiala moniliae* (Ascomycota, Chaetothyriales) (GU225948)	Saprobe (soil, plants, water, deadwood)	Chee and Kim (2002)
676	*Peniophora aurantiaca* (Basidiomycota, Russulales) (HQ604854)	Saprobe (white rot) (deadwood)	Farr and Rossman (2013)
716	*Exophiala* spp. (Ascomycota, Chaetothyriales) (EU035422)	Saprobe (soil, plants, water, deadwood)	Chee and Kim (2002)
914	*Armillaria* spp. (Basidiomycota, Agaricales) (AJ250053)	Saprobe (white rot), tree pathogen (root, deadwood, stump)	Banik et al. (1995)

Complete list of fungal sequences matched with F-ARISA OTUs have been submitted to GenBank under accession numbers KF823586–KF823629 (Table S5).

experimental settings. However, it is also feasible that some simply remained undetected because they fell below the detection threshold. At the same time we can only assume that newly detected OTUs are primarily the result of experimental factors. To confirm whether an OTU really was gained, lost, or was too rare to be recorded, more sensitive approaches like OTU-specific real-time PCR (after sequencing of fragments) or pyrosequencing-based barcoding would be helpful.

Our results indicate that increasing wood extraction and land-use and disturbance intensity causes significant declines in fungal OTU richness and diversity despite large amounts of deadwood remaining in these stands. Overall, in BAH, 24% of wood-inhabiting fungal OTUs became undetected compared with beech forest reserves (Table 1). This could be related to the effects of canopy structure, disturbance, and stand age. Large areas of thicket stage or young forest caused by a high disturbance after wood extraction may reduce fungal OTU richness and diversity as some works demonstrate that, in soil fungal richness and diversity increase with the forest age (Ágreda et al., 2006; Twieg et al., 2007). However, this response is tree species specific and the evidence in beech forests from the literature is still lacking (Twieg et al., 2007). Our results clearly show that fungal richness and diversity of age-class managed beech forests increase to similar level to that of unmanaged forest when the forest stands become older and changing the canopy structure (from BAH to BAL forest). In other words, the negative effect of forest management on fungal diversity is a temporary one and dependent on stand age. It is generally understood that a managed forest has lower deadwood amounts than unmanaged forests, and it was assumed that this determines fungal species richness. However, from our study we emphasize that managed forests (even with high levels of wood biomass extraction) can contain the same amount of deadwood as unmanaged forests, depending on the stage of forest maturation/management and the time passed since the forests became unmanaged (Table S1). In our case, although BAH forests have comparable deadwood to unmanaged forests and significantly higher than BAL forests (Table S1), all forests considered have a lower amount of deadwood than the generally recommended (60 m^3/ha) to maintain high wood-inhabiting fungal diversity (Müller et al., 2007). Thus, instead of the amount of deadwood, we suggest that stand age, canopy structure and disturbance may also play an important role to regulate fungal OTU richness and diversity in these forests. Although the negative effects of wood extraction on wood-inhabiting fungal diversity caused by intensive forest management (Junninen et al., 2006; Müller et al., 2007) or even low-intensity timber harvest (Josefsson et al., 2010) have been hypothesized before our study, this effect has only been shown to be of marginal or no significance. The discrepancies between the results of our study and previous studies may largely be due to the method used to determine wood-inhabiting fungal diversity. Whilst most previous studies relied on classical fungal sporocarp surveys and morphological species identification (Junninen et al., 2006; Müller et al., 2007; Josefsson et al., 2010), we applied F-ARISA, a molecular fingerprinting technique that targets DNA. Fungal diversity based on classical morphology is focused on currently active fungi that are producing sporocarps on deadwood at the time of sampling and normally only basidiomycetes and some ascomycetes are included in the investigation (Junninen et al., 2006; Müller et al., 2007; Josefsson et al., 2010). On the other hand, fungal diversity determined by F-ARISA with the conditions used in this study provides information on the total fungal community and most taxonomic groups of fungi (both from Dikarya and more basal fungal lineages) are presumably included (Manter and Vivanco, 2007; Baldrian et al., 2012; Purahong and Krüger, 2012; Toju et al., 2012). In addition, a molecular wood-inhabiting fungal diversity study based on 454 pyrosequencing has also revealed discrepant results compared to classical fungal sporocarp surveys and suggested that currently fruiting species may respond differently to environmental factors compared with the complete fungal community (Kubartová et al., 2012; Ovaskainen et al., 2013). F-ARISA and other culture-independent molecular methods are indeed useful but not always perfect. Their limitations have been discussed elsewhere (Forney et al., 2004). Nevertheless, such molecular methods have greatly advanced studies of microbial ecology (Jones et al., 2007). In BAL forests fungal OTU richness and diversity were not reduced when compared with BU forests, even though the latter were also exposed to wood extraction. This is because the level of wood extraction and disturbance intensity were much lower than in BAH forests (Luyssaert et al., 2011). In fact, 20% of fungal OTUs in BAL forest also became undetectable but there was an additional 21% of fungal OTUs that were newly detected (Table 1). The net number of newly detected OTUs outweighed undetected OTUs, thus the richness was maintained. We hypothesized that age-class management, even with low-intensity timber extraction, can change forest structure, tree species composition, the dynamics of deadwood and microclimatic conditions substantially (Josefsson et al., 2010; Luyssaert et al., 2011), resulting in the loss of some native fungal OTUs and in the gain of some fungal OTUs that had not been detected in the unmanaged forests. Surprisingly, all three forest management regimes had similar fungal community structures and compositions. This could be related to the fact that the habitat quality (deadwood) provided by these three forest management regimes is similar as there is no change in dominant tree species. We found that major parts of the fungal communities (57–61% of fungal OTUs) in different forest management regimes were shared between forest management types and that most of the OTUs were highly abundant and frequently detected in most of the samples (Table 1). In addition, we found

little or no abundance shift or rearrangement of these shared OTUs (Fig. 3, Table S3). This situation is likely to result in maintenance of fungal community structure and composition among different forest management regimes.

In our study, we found great changes in community structure and composition of wood-inhabiting fungi and significantly reduced fungal OTU richness and diversity in forests that had been converted from semi-natural European beech to secondary conifer species. These results were consistent with a classical fungal sporocarp survey carried out in the same regions (Blaser et al., 2013). Fungal richness and diversity were also dependent on the new dominant tree species, with fungal richness and diversity in Norway spruce forests being significantly reduced but no decrease in Scots pine forests compared to beech forests. However, because of our experimental design, the effect of the different conifer species could not be separated from the effect of region, since Scots pine and Norway spruce forests each occurred only in one region. In our study, fungal OTU richness in spruce forests is lower than for beech forests, thus it is reasonable that in forests converted from spruce to beech there might be increased fungal richness. Nevertheless, we did not design our experiment to investigate this aspect and the conversion from semi-natural spruce forest to beech forest is not an issue in these studied areas (BMELV, 2011). Our results demonstrate that the responses of the wood-inhabiting fungal community to conversion of forest types are also similar to the responses of soil bacteria to land-use change (from forest to grassland or cropland) for both tropical (Jesus et al., 2009) and temperate forests (Nacke et al., 2011). These responses showed that changes in community structure and composition are obvious consequences but OTU richness depends greatly on the dominant plant species present. We conclude that forest type conversion may have effects on microbial diversity and community structure similar to those of other land-use changes. Changes in species richness, diversity or community structure and composition may affect ecosystem processes, services and functions (Dangles and Malmqvist, 2004; Fukami et al., 2010). Thus, the changes resulting from either conversion to a different forest type or management regimes should receive more attention and they may be regarded as having a major impact on the diversity of microorganisms in terrestrial habitats. The fungi inhabiting and decomposing wood – the focus of this study – have an important role for element cycling in ecosystems and hence deserve our attention. We investigated the ecological roles of wood-inhabiting fungal majorities (Table 4) that were influenced by forest management and/or conversion of forest type in our study. We found that they occupy different ecological niches including plant pathogen, endophyte and saprobe, thus they may play different ecological roles in wood decomposition. *Fomes fomentarius* and *Armillaria* spp. are important plant pathogens causing root rot (Banik et al., 1995; Farr and Rossman, 2013) and they are also able to change their life strategy to a saprobic one (Valmaseda et al., 1990). They have been reported as effective decomposers on plant residues causing high mass and lignin losses (Valmaseda et al., 1990). *Ascocoryne sarcoides* is an endophytic fungus that may be capable to switch its life style to saprobic as it is often found on deadwood (Gianoulis et al., 2012). It has been reported to decompose cellulose and potentially produce an extraordinary diversity of metabolites (Gianoulis et al., 2012). The saprobes *Hyphodontia subalutacea* and *Peniophora aurantiaca* cause white rot on deadwood (Farr and Rossman, 2013). *Peniophora* sp. has been reported to efficiently produce ligninolytic enzymes, particularly laccase (Jordaan, 2005). The question how changes in these fungal abundances affect ecological functioning such as wood decomposition is interesting but it goes beyond the scope of the study. Although ecological functions may be compensated by species redundancy, our results on the wood-inhabiting fungal majorities do show that different fungi have quite unique functions. Furthermore, changes in abundances or losses of particular fungal species correspond to an altered community assembly and interactions among fungi in deadwood, which in turn could significantly affect ecosystem functioning (Boddy, 2000; Fukami et al., 2010). Fukami et al. (2010) and Dickie et al. (2012) reported that subtle differences in fungal species assembly history can significantly affect *Nothofagus* wood decomposition rate and carbon and nitrogen dynamics under laboratory and also natural conditions. Nevertheless, based on our results, it is still very difficult to draw the conclusion whether changes in fungal abundances affect ecological functioning. The definition of fungal species in our study is only based on DNA homology and the functional redundancy of fungi in deadwood is still unknown.

In this study, we provide empirical evidence to answer questions about what could happen to fungal communities when forest management regimes are changed or forests are transformed to different types. Species gain and loss and abundance shifts are the consequences of human disturbance, which often homogenizes species composition (Wardle et al., 2011). In our work, we demonstrate that these three events all occur: abundance shifts, in particular, were clearly observed. We reported on numbers of undetected or lost OTUs which could also mean how many native fungal OTUs in beech forest reserves or semi-natural forests are threatened by different forest management regimes or changes in forest type. These numbers are an important outcome often neglected in biodiversity studies. Most studies emphasize the increase or decrease in species richness and diversity without examining shifts in community structure and composition (Barlow et al., 2007). Details of all the native OTUs or species that become undetectable or lost have seldomly been reported or discussed in metagenomic studies, thus we do not actually know how many native taxa are under threat. The importance of reporting the number of undetected OTUs is demonstrated clearly in our work: we show that richness, diversity and community structure and composition are not significantly changed in BAL compared to BU forests, however one-fifth of the total native fungal OTUs became undetectable or were lost (Table 1). Thus, information on species richness, diversity and community structure and composition may underrepresent biodiversity effects of land-use. Our experiment also demonstrates how fungal communities may respond to dramatic changes to their habitat. In our case, beech forests were converted to two types of coniferous forest in recent, historical time, so the quality of deadwood as the habitat of wood-inhabiting fungi changed with respect to chemical composition (Kögel-Knabner, 2002). We found that around one-fourth of native fungal OTUs were undetectable and less than 19% were newly detected in converted forests. Surprisingly, we found that the main part of the community (ca. 56–60%) from semi-natural beech forest still existed, however the abundances of the fungal OTUs were shifted and many OTUs were only detected from a few samples within the same treatments. Specifically, many highly abundant semi-natural beech forest OTUs were less frequently detected or undetected in conifer forests, and *vice versa*, highly abundant conifer forest OTUs dwindled in these beech forests. This may be explained by differences in wood chemistry (substrate quality) alter the colonization ability of different wood-inhabiting fungi as many of them prefer certain tree species (Stokland et al., 2012). We also postulate that changes of wood chemistry could alter interactions among different fungi, e.g. a good competitor in one tree species is a weak competitor in another. Wood decayers *Ascocoryne cylichnium*, *Armillaria* spp., *Exophiala moniliae*, *Hyphodontia subalutacea* and *Fomes fomentarius* were strongly reduced in their abundances when forests were converted from beech to coniferous.

5. Conclusion

Intensive forest management caused a significant reduction of wood-inhabiting fungal diversity at young forest stage while conversion of forest type caused significant changes in the fungal community structure and could reduce fungal diversity which may depend on which coniferous species was introduced. Based on the results from our study and the previous works (Werner and Raffa, 2000; du Bus de Warnaffe and Lebrun, 2004; Lange et al., 2011), we conclude that changes within a single land-use category can be regarded as a major threat to biodiversity of saproxylic (deadwood-dependent) organisms in terrestrial ecosystems.

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Appendix A. Supplementary material

Supplementary material related to this article can be found at http://dx.doi.org/10.1016/j.jenvman.2014.02.031.

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Supporting Information

Changes within a single land-use category alter microbial diversity and community structure: molecular evidence from wood-inhabiting fungi in forest ecosystems

Witoon Purahong, Björn Hoppe, Tiemo Kahl, Michael Schloter, Ernst Detlef Schulze, Jürgen Bauhus, François Buscot and Dirk Krüger

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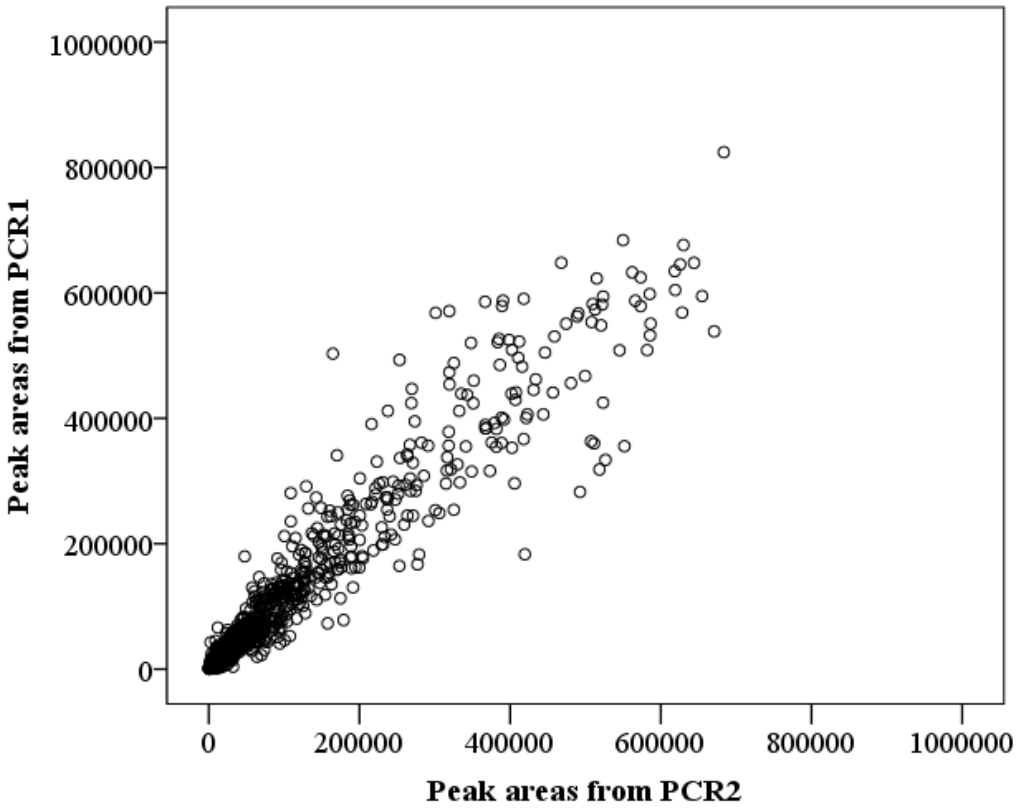

Figure S1: Non-parametric correlation between raw data of ARISA peak areas (fluorescent units) from two independent PCR replicates ($\rho = 0.93$; $n = 3935$; $P = 0.0001$).

Table S1 Total deadwood volume (m^3/ha) of forests experiencing different management regimes (mean±SD, n = 8) and conversion between forest types (mean±SD, n = 12, except in Scots pine forest (n = 11) where total deadwood data of 1 plot is missing) at different regions (HEW = forest in the Hainich-Dün region; AEW = forest in the Schwäbische Alb region; SEW = forest in the Schorfheide-Chorin region).

Changes within land-use category	Region	*n*	Total deadwood volume
Forest management regime			
Beech age-class with high-intensity wood extraction	HEW	8	39.82±15.51[a]
Beech age-class with low-intensity wood extraction	HEW	8	16.87±5.14[b]
Beech forest reserves	HEW	8	26.82±14.24[ab]
Forest type conversion			
Norway spruce forest	AEW	12	33.82±13.83[a]
Semi-natural beech forest	AEW	12	33.01±21.42[a]
Scots pine forest	SEW	11	18.37±4.59[a]
Semi-natural beech forest	SEW	12	18.37±9.52[a]

Different letters above each number of total deadwood volume per hectare represent statistically significant differences (P<0.05) after an analysis of variance and a Student-Newman-Keuls *post hoc* test (forest management regime) or independent two-sample t-test (forest type conversion).

Table S2: One-way analysis of similarity (ANOSIM) based on three distance measures using abundance data for different wood- inhabiting fungal community structures in forests subjected to different forest management regimes or conversion to a different forest type. Bonferroni-corrected *P* values were applied in all cases when more than two groups were compared with ANOSIM. (*R* = degree of separation between test groups ranging from -1 to 1; *R* = 0, not different; *R* = 1, completely different); *P* values were based on 9999 permutations; N = population size; HEW = forests in the Hainich-Dün region; AEW = forests in the Schwäbische Alb region; SEW = forests in the Schorfheide-Chorin region.

Changes within land-use category	**Region**	**N**	Bray-Curtis		Manhattan		Horn	
			R	***P***	***R***	***P***	***R***	***P***
Forest management regime								
Unmanaged beech forest vs. low-intensity wood extraction age-class beech forest	HEW	16	-0.09	1.0000	-0.09	1.0000	-0.09	1.0000
Unmanaged beech forest vs. high-intensity wood extraction age-class beech forest	HEW	16	-0.04	1.0000	-0.04	1.0000	-0.01	1.0000
Low- vs. high-intensity wood extraction age-class beech forest	HEW	16	-0.03	1.0000	-0.03	1.0000	-0.03	1.0000
Forest type conversion								
No conversion (beech forest) vs. converted Norway spruce forest	AEW	24	0.66	**0.0001**	0.66	**0.0001**	0.70	**0.0001**
No conversion (beech forest) vs. converted Scots pine forest	SEW	24	0.64	**0.0001**	0.64	**0.0001**	0.67	**0.0001**

Table S3: Total, shifted and overlapping abundances of all OTUs common to the control (unmanaged beech forest or unconverted beech forest) and treatments (low- or high- intensity wood extraction from age-class beech forest or converted Norway spruce and Scots pine forest). Abundance shift is calculated as the sum of differences (absolute values) between relative OTU abundances in each control and treatment pair; the overlapping abundance is the sum of shared relative abundances in each control and treatment pair. (N = population size; Regions are as in table S1). The shared OTU abundances account for 88.40 – 94.60% of total abundances of all fungal OTUs.

Changes within land-use category	N	Shared OTU abundance		
		Total	Shift (%)	Share (%)
Forest management regime				
Unmanaged beech forest vs. low-intensity wood extraction age-class beech forest	16	1513.62	501.72 (33.15)	1011.90 (66.85)
Unmanaged beech forest vs. high-intensity wood extraction age-class beech forest	16	1497.58	580.32 (38.75)	917.26 (61.25)
Forest type conversion				
No conversion (beech forest) vs. converted Norway spruce forest	24	2214.47	1431.89 (64.66)	782.58 (35.34)
No conversion (beech forest) vs. converted Scots pine forest	24	2121.52	1475.80 (69.56)	645.72 (30.44)

Table S4: Effects of region and conversion to a different forest type on fungal community structures and compositions. Two-way NPMANOVA and Two-way ANOSIM were carried out based on three different distance measures depending on data types (abundance data: BC = Bray-Curtis, M = Manhattan, H = Horn; presence/absence data: BC = Bray-Curtis, J = Jaccard, K = Kulczynski). Significant values (P<0.05) were based on 9999 permutations and are given in bold. Two-way ANOSIM does not examine the interaction between region and forest type conversion. (Population size (N) = 48; AEW = forests in the Schwäbische Alb region; SEW = forests in the Schorfheide-Chorin region.).

	Statistical Analysis	**Region (AEW vs. SEW)**		**Forest type conversion**		**Region × forest type conversion**	
		Statistics	***P***	**Statistics**	***P***	**Statistics**	***P***
Community structure	Two-way NPMANOVA (BC)	F = 3.40	**0.0002**	F = 8.14	**0.0001**	F = 2.15	**0.0098**
	Two-way NPMANOVA (M)	F = 3.40	**0.0002**	F = 8.14	**0.0001**	F = 2.15	**0.0079**
	Two-way NPMANOVA (H)	F = 7.07	**0.0001**	F = 16.76	**0.0001**	F = 3.47	**0.0037**
	Two-way ANOSIM (BC)	R = 0.27	**0.0001**	R = 0.65	**0.0001**	-	-
	Two-way ANOSIM (M)	R = 0.27	**0.0001**	R = 0.65	**0.0001**	-	-
	Two-way ANOSIM (H)	R = 0.31	**0.0001**	R = 0.68	**0.0001**	-	-
Community composition	Two-way NPMANOVA (BC)	F = 3.57	**0.0001**	F = 5.96	**0.0001**	F = 2.23	**0.0016**
	Two-way NPMANOVA (J)	F = 2.46	**0.0001**	F = 3.87	**0.0001**	F = 1.74	**0.0016**
	Two-way NPMANOVA (K)	F = 3.55	**0.0001**	F = 5.97	**0.0001**	F = 2.27	**0.0016**
	Two-way ANOSIM (BC)	R = 0.30	**0.0001**	R = 0.57	**0.0001**	-	-
	Two-way ANOSIM (J)	R = 0.30	**0.0001**	R = 0.57	**0.0001**	-	-
	Two-way ANOSIM (K)	R = 0.30	**0.0001**	R = 0.57	**0.0001**	-	-

Table S5: Complete list of fungal sequences matched with F-ARISA OTUs (A = ascomycetes, B = basidiomycetes, C= Chytridiomycetes, n.d. = not determined)

Fungal OTU	Identified fungal species*	Class	Fungal OTU	Identified fungal species*	Class
525	*Ascocoryne sarcoides*	A	635	*Cladosporium musae*	A
565	*Passalora graminis*	A	636	*Scoliciosporum umbrinum*	A
571	*Degelia gayana*	A	648	Fungal sp. TRN447	n.d.
575	*Fibulobasidium murrhardtense*	B	664	*Hyphodontia subalutacea*	B
576	*Pseudocercosporella* sp.	A	665	*Hyphodontia subalutacea*	B
577	*Catenulostroma germanicum*	A	674	*Exophiala moniliae*	A
588	*Paecilomyces* sp.	A	676	*Peniophora aurantiaca*	B
589	*Vertexicola confusa*	A	680	*Ascocoryne sarcoides*	A
589	*Lecythophora* sp.	A	705	*Ceratobasidium* sp.	B
590	*Paecilomyces* sp.	A	716	*Exophiala* sp.	A
590	*Leptodontidium elatius*	A	717	*Fusarium merismoides*	A
592	*Leptodontidium elatius*	A	727	*Hyphodontia floccosa*	B
594	*Neonectria punicea*	A	742	*Powellomyces* sp.	C
594	*Neonectria ramulariae*	A	745	*Mycena purpureofusca*	B
599	*Paecilomyces inflatus*	A	747	*Mycena* sp.	B
599	*Pseudaegerita corticalis*	A	749	*Mycena cinerella*	B
602	*Myrmecridium* sp.	A	764	*Megacollybia platyphylla*	B
605	*Rhodoveronaea varioseptata*	A	764	*Megacollybia platyphylla*	B
608	*Cercophora sulphurella*	A	764	*Megacollybia platyphylla*	B
626	Pleosporales sp.	A	841	Ascomycota sp.	A
628	*Ascocoryne sarcoides*	A	896	*Marasmius alliaceus*	B
632	*Ascocoryne cylichnium*	A	914	*Armillaria* sp	B

All fungal sequences matched with F-ARISA OTUs reported in this table have been submitted to GenBank under accession numbers KF823586-KF823629.

Statement of authorship: JB, FB, TK, DK, EDS, WP designed the experiment. WP and TK carried out the fieldwork. BH and WP established and optimized F-ARISA. WP carried out the laboratory work. WP and DK analyzed the results. EDS contributed forest management expertise. WP and DK wrote the manuscript. WP, DK, MS, FB, JB, EDS and TK revised the manuscript.

CHAPTER 4

Comparing fungal richness and community composition in coarse woody debris in Central European beech forests under three types of management

Witoon Purahong [1, 2], Tiemo Kahl[3], Michael Schloter[4], Jürgen Bauhus[3], François Buscot[1, 5, 6], Dirk Krüger[1]

[1] UFZ-Helmholtz Centre for Environmental Research, Department of Soil Ecology, Theodor-Lieser-Str. 4, D-06120 Halle (Saale), Germany

[2]Technical University of Munich, Chair for Soil Science, IngolstädterLandstr. 1, D-85758 Oberschleissheim, Germany

[3]University of Freiburg, Faculty of Environment and Natural Resources, Chair of Silviculture, Tennenbacherstr. 4, D-79085 Freiburg i. Brsg., Germany

[4]Helmholtz Zentrum München, Research Unit for Environmental Genomics, Ingolstädter Landstr. 1, D-85758 Oberschleissheim, Germany

[5]University of Leipzig, Institute of Biology, Johannisallee 21-23, D-04103 Leipzig, Germany

[6]German Centre for Integrative Biodiversity Research (iDiv), Halle-Jena-Leipzig, Deutscher Platz 5e, D-04103 Leipzig, Germany

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SHORT COMMUNICATION

Comparing fungal richness and community composition in coarse woody debris in Central European beech forests under three types of management

Witoon Purahong · Tiemo Kahl · Michael Schloter · Jürgen Bauhus · François Buscot · Dirk Krüger

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Abstract Managing forests by selection cutting is a promising silvicultural technique for maintaining forest biodiversity. Despite the importance of fungi in decomposition and nutrient cycling in forest ecosystems, no study to date has investigated the effects of selection cutting on fungal communities, especially using a culture-independent molecular technique to assess more than just the species that are fruiting at the time of sampling. Based on operational taxonomic units (OTUs) found in coarse woody debris, we compared the richness and community composition of wood-inhabiting fungi from selection cutting, age-class, and unmanaged European beech-dominated forests. We found that fungal OTU richness in selection cutting and unmanaged forests was not significantly different ($P>0.05$), but it was higher, in both cases, than that in the age-class forest ($P=0.0002$). Fungal community composition was not significantly different among the three forest types ($P>0.05$). Abundances of common, wood-inhabiting fungal OTUs in different forest types were significantly correlated: the highest and lowest correlations were found between unmanaged forests and selection cutting ($\rho=0.52$, $P<0.0001$, $n=94$), and between unmanaged and age-class forests ($\rho=0.30$, $P=0.0080$, $n=79$), respectively.

Keywords Fungal diversity · Silviculture · Forest management · F-ARISA · Culture-independent molecular method

W. Purahong (✉) · F. Buscot · D. Krüger
Department of Soil Ecology, UFZ-Helmholtz Centre for Environmental Research, Theodor-Lieser-Str. 4, 06120 Halle (Saale), Germany
e-mail: witoon.purahong@ufz.de

W. Purahong
e-mail: witoon.purahong@gmail.com

W. Purahong
Technical University of Munich, Chair for Soil Science, Ingolstädter Landstr. 1, 85758 Oberschleissheim, Germany

T. Kahl · J. Bauhus
Faculty of Environment and Natural Resources, Chair of Silviculture, University of Freiburg, Tennenbacherstr. 4, 79085 Freiburg i. Brsg., Germany

M. Schloter
Research Unit for Environmental Genomics, Helmholtz Zentrum München, Ingolstädter Landstr. 1, 85758 Oberschleissheim, Germany

F. Buscot
Institute of Biology, University of Leipzig, Johannisallee 21-23, 04103 Leipzig, Germany

F. Buscot
German Centre for Integrative Biodiversity Research (iDiv), Halle-Jena-Leipzig, Deutscher Platz 5e, 04103 Leipzig, Germany

Introduction

Although a difficult substrate to attack and decay, coarse woody debris (CWD) is one of the most important nutrient sources in forest ecosystems (Küffer et al. 2008). Wood-inhabiting fungi are capable of degrading complex substances such as cellulose, hemi-cellulose, and lignin (Fukasawa et al. 2012; Osono et al. 2013), and they play a crucial role in wood decomposition and nutrient cycling (Watkinson et al. 2006; Küffer et al. 2008; Purahong and Hyde 2011; Kahl et al. 2012). Intensive forest management, such as age-class clear cutting, could reduce species richness and alter community composition of wood decaying fungi because, under this management, most CWD (which represents both habitat and the source of nutrients and energy for wood-inhabiting fungi) is removed or harvested (Müller et al. 2007; Brunet et al. 2010).

Selection cutting is considered to constitute a near-to-nature forest management because it is based on natural regeneration and creates unevenly-aged forest structure as found in unmanaged or pristine forests (Gossner et al. 2013).

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However, since in selection cutting most large trees are harvested (Ren-hui et al. 2006), this management approach can also be considered to be intensive. Most previous studies that compared forest biodiversity in selection cutting and unmanaged forests have focused on plant and animal communities (Torras and Saura 2008; Légaré et al. 2011). These studies have shown that selection cutting forests have similar or even higher diversity than unmanaged forests. However, despite the importance of wood-inhabiting fungi for the functioning of forest ecosystems, no study to date has compared their richness and community composition, especially using a culture-independent molecular technique that assesses not only those fungi that are fruiting at the time of sampling, but also those present in the wood as vegetative mycelium.

The objective of our study was to compare wood-inhabiting fungal richness and community composition between selection cutting and two other types of Central European beech forests (age-class and unmanaged European beech (*Fagus sylvatica*) dominated forests). The molecular approach we used was fungal automated ribosomal intergenic spacer analysis (F-ARISA, originally developed for the bacterial intergenic spacer and extended to fungal internal transcribed spacers), a technique that provides a basis for richness estimates and allows highly consistent measurement of community composition through space and time (Green et al. 2004; Jones et al. 2007; Weig et al. 2013).

Materials and methods

The study was conducted in the Hainich-Dün region in Central Germany (including the Hainich National Park and its surroundings; ca. 1,300 km^2; 51°16'N 10°47'E) as part of the German Biodiversity Exploratories (Fischer et al. 2010). The main soil types in the study area are luvisol and stagnosol (Fischer et al. 2010). The soil pH is weakly acidic (5.1±1.1; mean±SD) (Naether et al. 2012). The annual mean temperature and precipitation ranges are 6.5–8 °C and 500–800 mm, respectively (Fischer et al. 2010; Hessenmöller et al. 2011). In total, 21 forest plots (1 ha for each plot) that represent three management types (with seven replicates each) were selected. The plot selection criteria were based on three forest management regimes: (i) age-class beech forests following a high level of wood extraction; (ii) selection cutting; and (iii) unmanaged forests. In all forest types the dominant tree species was the European beech (*Fagus sylvatica*) (Fischer et al. 2010; Hessenmöller et al. 2011). All selected forest plots had apparently been subjected neither to clearing nor to a period of agricultural use in the past (Luyssaert et al. 2011). All 21 studied plots were located less than 40 km apart, thus minimizing the influence of geography and climate.

Fallen CWD logs in all the forest plots were surveyed and their characteristics and locations were recorded. Only the three largest European beech CWD logs (diameter>7 cm) in each plot were sampled in November 2011. The decay class of each CWD log was determined as described by Müller-Using and Bartsch (2009). Wood samples were taken using a cordless drill (Makita BDF 451) equipped with a wood auger (diameter: 20 mm, length 450 mm), which was flamed and wiped with ethanol between drillings to avoid cross-contamination between samples. The positions and numbers of wood samples taken from each CWD log were estimated according to the length of the log, i.e., three samples were taken from 5-m-long logs (one 0.5-m sample from each end and one from the middle), and additional samples were taken from each additional 5-m length of any CWD log. This sampling strategy yielded a high correlation between total and sampling volume of CWD logs (ρ=0.87, P<0.0001; data not shown). Wood samples from each CWD log were pooled, resulting in three composite samples from each plot; each composite sample was used for DNA extraction and F-ARISA. Immediately after collection, the subsamples were frozen, transported on wet ice (ca. 0 °C) to the laboratory within 3 h, and stored at -80 °C. Each composite wood sample was separately homogenized and ground into a fine powder with the aid of liquid nitrogen, using a swing mill (Retsch, Haan, Germany).

DNA was extracted from 100 mg of each homogenized composite wood sample using a ZR Soil Microbe DNA MiniPrep kit (Zymo Research, Irvine, CA, USA), according to the manufacturer's instructions. The presence and quantity of genomic DNA was checked using a NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Dreieich, Germany) and the extracts were then stored at -20 °C. F-ARISA polymerase chain reaction (PCR) amplification was performed in duplicate under the conditions described by Gleeson et al. (2005) with the following modifications: the PCR mixture (20 μl) contained 1 μl DNA template (~20 ng DNA template as determined by NanoDrop); 10 μM of fungal-specific, plant-excluding primer ITS1F (5′- CTTGGT CATTTAGAGGAAGTAA-3′, Gardes and Bruns 1993) with a 5′ FAM-labeled modification and an unlabeled ITS4 primer (5′-TCCTCCGCTTATTGATATGC-3′, White et al. 1990); and 4 μl FIREPol 5x Master Mix (Solis BioDyne, Tartu, Estonia). PCR was carried out with an initial denaturation at 95 °C for 5 min, followed by 35 cycles of 95 °C for 60 s, 55 °C for 60 s and 72 °C for 75 s, with a final extension at 72 °C for 7 min. The PCR products were purified using a PCRExtract Mini Kit (5PRIME, Hamburg, Germany). A standardized quantity of 20 ng DNA, as determined by NanoDrop, was mixed with 14 μl of deionized Hi-Di formamide (Applied Biosystems, Foster City, CA, USA) and 0.1 μl of internal-size standard Map Maker 1000 ROX (50-1000 bp) (BioVentures, Inc, Murfreesboro, TN, USA). The mixture was denatured for 5 min at 95 °C and chilled on ice for at least 10 min before being further processed using a sequencer (ABI PRISM 3730xl Genetic Analyzer, Applied Biosystems).

Table 1 Characteristics of the studied plots and sampled logs (mean ± S.E., n=21) in age-class, selection cutting, and unmanaged forests

Parameter	Age-class forest	Selection cutting forest	Unmanaged forest
Dominant tree species	*Fagus sylvatica*	*Fagus sylvatica*	*Fagus sylvatica*
Forest structure	Even age	Uneven age	Uneven age
Mean length of logs (m)	8.14 ± 1.35^{a}	5.23 ± 0.58^{a}	12.94 ± 1.35^{b}
Mean diameter (cm)	34.90 ± 3.34^{ab}	27.10 ± 2.93^{a}	43.81 ± 3.62^{b}
Mean wood volume, three CWD logs (m^3)	1.12 ± 0.34^{b}	0.25 ± 0.07^{a}	1.36 ± 0.23^{b}
Mean decay class	2.76 ± 0.14^{a}	3.10 ± 0.12^{a}	2.95 ± 0.15^{a}
Mean entire wood volume* (m^3)	43.00 ± 5.15^{b}	20.81 ± 3.28^{a}	27.59 ± 5.75^{ab}

*Mean entire wood volume per plot (mean ± S.E., n=7). Different letters represent statistically significant differences (P<0.05) based on analysis of variance and Tukey's post hoc test

The raw ARISA profiles were analyzed using the Gene Mapper Software 4.0 (Applied Biosystems) with a threshold of 100 fluorescence units. All peaks of the fragments between 390 and 1000 bp that appeared in the two technical PCR replicates were considered for further analyses (Ranjard et al. 2001). The pairs of independent PCR replicates were highly correlated (ρ=0.91, P=0.0001; data not shown). Operational taxonomic unit (OTU) binning was conducted using an interactive custom binning script (Ramette 2009) in R version 2.14.1 (The R Foundation for Statistical Computing, 2011-2012). The total peak area per sample was normalized to 1 and the relative fluorescent intensity (RFI) was calculated. All peaks with RFI values lower than 0.09 % were excluded as background noise. A strategy involving a binning size of 2 bp was applied to the F-ARISA data and the binning frame that gave the highest pairwise similarity among samples was used for further statistical analyses. F-ARISA fingerprint data were analyzed using PAST version 2.10 (Hammer et al. 2001). Fungal OTUs and their normalized RFI values were used as proxies for fungal taxa and their relative abundance, respectively (Ramette 2009). OTUs were defined as described previously by Green et al. (2004), Jones et al. (2007) and Ramette (2009) for the F-ARISA approach and may differ from those OTU definitions relying on sequences. They could, alternatively, be called ribotypes (Gleeson et al. 2005; Thakuira et al. 2010). The differences in fungal OTU richness and in wood parameters (Table 1) between the three forest types were analyzed (P<0.05) by performing one-way analysis of variance (ANOVA), incorporating the Jarque-Bera JB test for normality. The Levene statistic was used to test for the equality of group variances, and Tukey's post hoc test was also applied. In some cases, data were square root or logarithmically transformed to meet the requirements for parametric testing. To assess the effect of different forest management types on wood-inhabiting fungal community composition, abundance data were transformed to presence/absence and analyzed using one-way analysis of similarities (ANOSIM) and non-parametric multivariate analysis of variance (NPMANOVA) based on Bray-Curtis and Jaccard distance measures. Correlations between i) total wood volumes (m^3) and sample wood volume (cm^3) ii) raw data of ARISA peak areas (fluorescence units) from two independent PCR replicates and iii) fungal OTU abundances among three forest types (using both all and common fungal OTUs) were assessed using Spearman's rho non-parametric correlation (ρ).

Results and discussion

Characteristics of sampled CWD logs in different forest types

The characteristics of sampled CWD logs are shown in Table 1. We found that those from unmanaged forests were significantly longer (P<0.0001) and thicker (P=0.0030) than those from

Table 2 Number of fungal OTUs detected in different forest types and community composition analysis by means of analysis of similarity (ANOSIM) and non-parametric multivariate analysis of variance (NPMANOVA) (N=14 in all cases), based on Bray-Curtis distances (presence-absence data), comparing wood-inhabiting fungal community composition in forests subjected to different forest management types

Forest management types	Total OTUs	Shared OTUs	R	P	F	P
Unmanaged beech forest vs. age-class beech forest	137 (116 vs. 100)	79	0.18	0.164	1.574	0.086
Unmanaged beech forest vs. selection cutting beech forest	158 (116 vs. 136)	94	-0.12	1.000	0.745	1.000
Selection cutting beech forest vs. age-class beech forest	153 (136 vs. 100)	83	0.13	0.410	1.307	0.483

Bonferroni-corrected P values were applied. Tests statistics, columns left to right: R = degree of separation between test groups based on ANOSIM ranging from -1 to 1; R = 0, not different; R = 1, completely different; F = F values based on NPMANOVA (Bray-Curtis distances); P values were based on 9,999 permutations

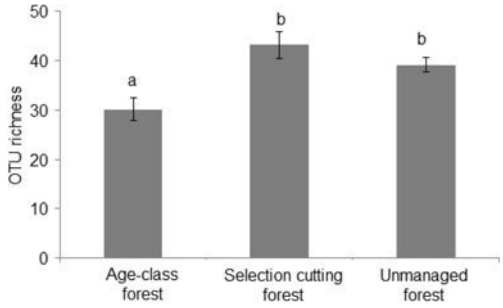

Fig. 1 Mean fungal OTU richness in different forest types (mean ± S.E., n= 7). Different letters above each bar represent statistically significant differences (P<0.05) based on analysis of variance and Tukey's post hoc test

selection cutting forests, so they also had significantly larger volumes (P=0.0001) (Table 1). CWD logs from unmanaged forests were also significantly longer (Table 1) than those from age-class forests, but the differences in their diameters and volumes were not quite significant (P=0.06 - 0.07). Decay status of the sampled CWD logs was similar among the three forest types (P>0.05), with most CWD logs in decay class 3 (moderate decay) (Table 1).

Wood-inhabiting fungal richness and community structure in different forest types

In total, we detected 168 wood-inhabiting fungal OTUs in the three forest types investigated. One hundred thirty-seven fungal OTUs were detected in unmanaged and in age-class forests; of these, 37 were exclusive to unmanaged and 21 to age-class forests (Table 2). In the unmanaged and selection cutting forests there was a total of 158 fungal OTUs, with 94 common to both, 22 exclusive to unmanaged forests, and 42 to selection cutting forests (Table 2). For the selection cutting and age-class forests, a total of 153 fungal OTUs was detected, with 53 only found in selection cutting and 17 in age-class forests (Table 2). The mean fungal OTU richness per plot (3 CWD logs) did not differ significantly between selection cutting (43.14) and unmanaged (39.14) forests (P>0.05), but both values were significantly higher than that for the age-class forest (30.14) (P=0.0002) (Fig. 1). One-way ANOSIM (Bray-Curtis distance measure) showed that the communities of wood-inhabiting fungi were not significantly different between the three forest types (Table 2). A one-way NPMANOVA (Bray-Curtis distance measure) confirmed the results from the one-way ANOSIM, that different forest management did not significantly affect wood-inhabiting fungal community composition (Table 2). Using Jaccard distances instead of Bray-Curtis distances did not lead to different results for one-way ANOSIM or NPMANOVA (data not shown). Abundances of wood-inhabiting fungal OTUs among different forest types were significantly correlated, either when all fungal OTUs or only the common OTUs were considered. However, the correlations between fungal OTUs in unmanaged and selection cutting forests were much higher than those for unmanaged and age-class forests (Fig. 2).

In contrast to our results, some fungal diversity studies based on sporocarp surveys have demonstrated a positive relationship between the size of CWD logs and fungal richness (Heilmann-Clausen and Christensen 2003, 2004). Interestingly, although we found CWD logs in selection cutting forests to be significantly smaller than in unmanaged forests, just as they are in managed forests according to Müller et al. (2007), there was no significant difference in fungal richness determined by F-ARISA. In addition, although the volumes of CWD logs in age-class forests were significantly higher than in selection cutting forests, but not significantly different from unmanaged forests, there was significantly lower fungal OTU richness in age-class forests. The discrepancy between fungal sporocarp surveys and direct molecular detection in the logs may be due to the methodology: classical sporocarp surveys focus on basidiomycetes and some ascomycetes, whilst direct molecular detection, as used here, covers most fungi (Manter and Vivanco 2007; Baldrian et al. 2012; Purahong and Krüger 2012). Although the volume of the three sampled CWD logs in unmanaged forests was higher than in selection cutting forests, the total CWD volume (entire plot) did not significantly differ between these two management types (Table 1). This implies that there are more, but smaller, CWD logs in

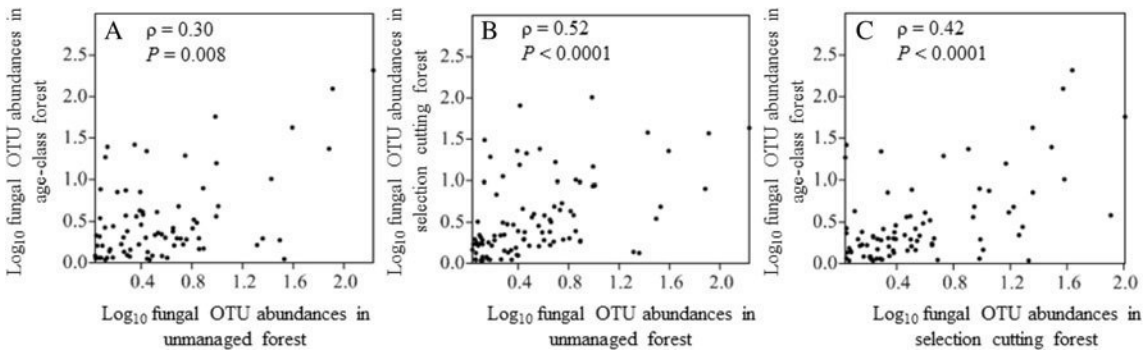

Fig. 2 Non-parametric correlation between common fungal OTU abundances in different forest types ($\log_{10}$ transformed). **a** unmanaged and age-class forests (n=79). **b** unmanaged and selection cutting forests (n=94). **c** selection cutting and age-class forests (n=83)

selection cutting forests than in unmanaged forests. These smaller logs may play an important role in maintaining wood-inhabiting fungal diversity in selection cutting forests. In our experiment, there was no significant difference in CWD volume over the entire plot in age-class forests compared to unmanaged forests, but the former contained a significantly higher CWD volume compared to selection cutting forests (Table 1). This indicates that forest management may affect fungal OTU richness in CWD logs in a complex manner by influencing a range of factors, including forest structure, plant diversity, level of disturbance, microclimatic conditions and deadwood dynamics (Torras and Saura 2008; Ma et al. 2010; Luyssaert et al. 2011; Hessenmöller et al. 2011; Purahong et al. 2013). In this study, the results show that changes in forest management that maintain the same dominant tree species did not significantly alter wood-inhabiting fungal community composition compared with unmanaged forests. This could be partly explained by the fact that the CWD quality is similar among the different forest types (all sampled wood was beech CWD) and the two forest types (age-class and selection forests) in our experiment still contain similar amounts of CWD compared to unmanaged forests. This indicates that maintaining the amount and quality of dead wood is important for sustaining the wood-inhabiting fungal community in forest ecosystems (Müller et al. 2007; Purahong et al. 2013).

Effect of selection cutting forest management on different groups of organisms

The results from our study and other recent studies comparing biodiversity and community composition in selection cutting and old-growth, natural or unmanaged forests are consistent and reveal that there are no significantly negative effects of selection cutting on biodiversity. Torras and Saura (2008) reported that selection cutting forests have significantly higher shrub species richness, tree species richness, and tree species diversity than unmanaged forests. Légaré et al. (2011) also found that there was no change in abundance, diversity or community composition of beetle species (saproxylic flying beetles, saproxylic epigaeic beetles and non-saproxylic epigaeic beetles) when comparing old growth and selection cutting forests, but they reported significant differences when comparing clear cut and old growth forests. Ren-hui et al. (2006) reported that selection cutting with low and moderate woody biomass extraction intensities only causes small variations in forest structure and in tree species evenness, whereas selection cutting with high and ultra-high intensities significantly changes forest structure, tree species evenness, and the dominant tree species. Changes to these three characteristics of forests can negatively affect some organisms (including wood-inhabiting fungi) that are dependent on forest structure (Pardini et al. 2005); adversely affecting them can cause a decline in species richness and abundances (Echeverría et al. 2007).

Outlook

Forest management may affect fungal OTU richness in CWD logs in complex ways by influencing a range of factor,s as we have discussed. The amount of CWD logs is important for maintaining wood-inhabiting fungal richness, however, the amount of CWD alone may not reflect the richness of wood-inhabiting fungi in different forest types. Although we did not find an indication of shifts in OTU richness related to selection cutting in our study, which focused on short-term effects, there is a need to consider long-term effects on biodiversity and community structure if such forest management is applied over longer periods. Josefsson et al. (2010) provided evidence that minor forest logging carried out a century ago may continually affect dead wood dynamics and the wood-inhabiting fungal community, in particular the abundance of red-listed species.

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CHAPTER 5

Fungal presence and activity in dead *Fagus* and *Picea* wood tackled by nrITS metagenomics and lignin-modifying enzyme analysis

Björn Hoppe[1, 2*†], Witoon Purahong[1, 3†], Tesfaye Wubet[1, 4†], Tiemo Kahl[2], Jürgen Bauhus[2], Tobias Arnstadt[5], Martin Hofrichter[5], François Buscot[1,4], Dirk Krüger[1]

[1]UFZ-Helmholtz Centre for Environmental Research, Department of Soil Ecology, Theodor-Lieser-Str. 4, D-06120 Halle (Saale), Germany

[2]University of Freiburg, Faculty of Environment and Natural Resources, Chair of Silviculture, Tennenbacherstr. 4, D-79085 Freiburg i. Brsg., Germany

[3]Technical University of Munich, Chair for Soil Science, Ingolstädter Landstr. 1, D-85758 Oberschleissheim, Germany

[4]German Centre for Integrative Biodiversity Research (iDiv), Halle-Jena-Leipzig, Deutscher Platz 5e, D-04103 Leipzig, Germany

[5] Technical University of Dresden, International Institute (IHI) Zittau, Markt 23, D-02763 Zittau, Germany

*These authors contributed equally to this work.

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Title: Fungal presence and activity in *Fagus* and *Picea* deadwood tackled by nrITS metagenomics and lignin-modifying enzyme analysis

Authors (e-mail addresses):

Björn Hoppe[1, 2*†], Witoon Purahong[1, 3†], Tesfaye Wubet[1, 4†], Tiemo Kahl[2], Jürgen Bauhus[2], Tobias Arnstadt[5], Martin Hofrichter[5], François Buscot[1,4], Dirk Krüger[1]

Affiliations:

[1]UFZ-Helmholtz Centre for Environmental Research, Department of Soil Ecology, Theodor-Lieser-Str. 4, D-06120 Halle (Saale), Germany, [2]University of Freiburg, Faculty of Environment and Natural Resources, Chair of Silviculture, Tennenbacherstr. 4, D-79085 Freiburg i. Brsg., Germany, [3]Technical University of Munich, Chair for Soil Science, Ingolstädter Landstr. 1, D-85758 Oberschleissheim, Germany, [4]German Centre for Integrative Biodiversity Research (iDiv), Halle-Jena-Leipzig, Deutscher Platz 5e, D-04103 Leipzig, Germany, [5] Technical University of Dresden, International Institute (IHI) Zittau, Markt 23, D-02763 Zittau, Germany

***Corresponding authors**:

1)Tel: +49 345-5585-219, Fax: +49 345 5585225, E-mail: bjoern.hoppe@ufz.de

† These authors contributed equally to this work.

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Abstract

Fungi play vital roles in the decomposition of deadwood due to their secretion of various enzymes that break down plant cell-wall complexes. However, the ecological mechanisms that underpin these processes are not well understood. The compositions of wood-inhabiting fungal (WIF) communities change over the course of the decomposition process as the remaining mass of wood decreases. It is currently unclear which substrate-related and anthropogenic factors govern these changes in WIF communities and whether such changes influence the deadwood decomposition rate. Here we report a study on fungal diversity and community structure in deadwood of Norway spruce and European beech in temperate forest ecosystems under variable management regimes using 454 pyrosequencing. Our aims were to disentangle the factors that correspond to WIF community composition and to investigate the links between richness, taxonomically-resolved fungal identity, and microbial-mediated ecosystem functions and processes by analyzing physico-chemical wood properties, lignin-modifying enzyme activities and wood decomposition rates. Unlike the fungal richness, we found significant differences in community structure between the two deadwood species. The composition of WIF communities was related to both the physico-chemical properties of the deadwood substrates and the applied forest management regime. Decomposition rates and the activities of lignin-modifying enzymes were controlled by the succession of the fungal communities and competition scenarios rather than fungal OTU richness. Our results clarify the links between fungal community structure and microbial-mediated ecosystem functions and processes.

Introduction

Between 400,000 and 1,000,000 species are associated with or depend on woody substrates for either energy, nutrition, or shelter (Stokland *et al.* 2012). Coarse woody debris (CWD) is one of the most important organic carbon pools in forest ecosystems (Floudas *et al.* 2012). Due to its lignin content of 15-40 %, CWD is resistant to decomposition and is therefore an important temporal store of carbon and macronutrients (Kopra & Fyles 2005; Krankina *et al.* 1999; Sarkanen 1971). Microorganisms, mainly fungi, play crucial roles in forest ecosystems due to their ability to disintegrate and mineralize lignin. Filamentous fungi of the phyla Basidiomycota and (to a lesser extent) Ascomycota are particularly important in this respect. White-rot fungi secrete a set of extracellular oxidative enzymes such as laccase (Lac, EC 1.10.3.2), manganese peroxidase (MnP, EC 1.11.1.13) and/or manganese independent peroxidases (MiP) to oxidize the recalcitrant lignin polymer (Hofrichter *et al.* 2010). In contrast, brown-rot fungi do not secrete these enzymes. Instead, they oxidize lignin via a mechanism relying on hydroxyl radicals, which are generated via the Fenton reaction (Hatakka & Hammel 2011; Jellison *et al.* 1997; Martinez *et al.* 2005). The decomposition processes driven by decomposer organisms are complex and influenced by the host tree species and environmental factors (Boddy 2001). Different fungal species have different capacities for wood decomposition (Valmaseda *et al.* 1990). Moreover, the identity of the host tree determines several key abiotic substrate factors that influence the interactions between the resident microbiota along with their growth, reproduction, and metabolism (Gadd 2010; Kögel-Knabner 2002). These factors include the chemical properties and cell structure of the wood (Schwarze *et al.* 2000) as well as its pH and water content. A recent study showed that different host tree species (European beech vs. Norway spruce) were associated with different fungal occupancy patterns: a strong competitor in one tree species could be outcompeted in another (Purahong *et al.* 2014a).

The existing ecological data on wood-inhabiting fungi (WIF), including information on their diversity patterns, resource use and the determinants of their community structure, was primarily obtained via sporocarp surveys (Blaser *et al.* 2013; Heilmann-Clausen & Christensen 2003; Müller *et al.* 2007), which only record the composition of the actively reproducing fungal community at a specific point in time. However, the macroscopically observable fungal flora may not be fully representative of the fungal community that is present, especially if sampling is only conducted once or over a short period of time (Halme & Kotiaho 2012). In contrast, high-throughput sequencing allows detailed analyses of fungal community dynamics in CWD. This approach has been used to study WIF communities within and among Norway spruce (*Picea abies*) logs (Kubartova *et al.* 2012) and to test the link between fungal life history and population dynamics (Ovaskainen *et al.* 2013). As expected, these studies revealed much higher fungal species richness than previously published sporocarp surveys and suggested that highly abundant fruiting species may be only weakly representing the fungal community as a whole.

All three of the European high-throughput sequencing studies on WIF conducted to date focused on the CWD of a single gymnosperm tree species, *Picea abies*, in a single biome: the boreal forest zone of Northern Europe (Kubartova *et al.* 2012; Ovaskainen *et al.* 2010; Ovaskainen *et al.* 2013). Therefore, our information on the ecological dynamics of WIF in CWD of different tree species in different locations is limited. Links between fungal diversity and richness, taxonomically resolved community composition, and ecosystem processes are rarely studied, especially under natural conditions. In this study, we aimed to narrow these knowledge gaps by: (i) using 454 pyrosequencing to compare the richness and community structure of WIF in deadwood of two silviculturally important tree species found in Central European temperate forests - the coniferous *Picea abies* and the deciduous *Fagus sylvatica*, (ii) disentangling the ecological and environmental factors (including forest management

regimes) that correlate with WIF community structure, and (iii) using the resulting data to link WIF richness and taxonomically-resolved identity to microbial-mediated ecosystem functions and processes. We hypothesized that (1) the different wood physico-chemical properties of the two model tree species could give rise to significant differences in fungal diversity and community structure (Purahong *et al.* 2014a). We further hypothesized that (2) anthropogenic changes in the quantity and quality of substrate material due to changes in forest management regimes would have profound effects on fungal community structure in *Fagus* and *Picea* deadwood (Purahong *et al.* 2014a; Rajala *et al.* 2012). Microbial-mediated ecosystem functions and processes were determined by measuring the activities of lignin-modifying enzymes and wood decomposition rates, respectively. Since the loss of microbial biodiversity could alter ecosystem functioning and stability, we (3) expected positive correlations between fungal diversity and ecosystem functions and processes.

Material and methods

Experimental design, deadwood selection and sampling

The study was conducted on forest plots of the German Biodiversity Exploratories (Fischer *et al.* 2010) located in the UNESCO Biosphere Reserve "Schwäbische Alb" in southwestern Germany. Our survey took place on deadwood logs in intensively investigated 1 ha plots, representing the following three forest management types: (i) extensively managed beech forests, (ii) managed beech forests dominated by *Fagus sylvatica* and (iii) managed spruce forests dominated by *Picea abies*. In April 2009, a set of 48 logs, equally representing the two tree species (*P. abies* and *F. sylvatica*) located on the forest floor were randomly selected and their properties (length, diameter, tree species, *e.t.c*) were characterized. Our selection assured that *Fagus* logs were present in *Picea*-dominated plots and *vice versa*. In June 2009, wood chips from the logs were sampled using a cordless Makita BDF451 drill (Makita, Anja,

Japan) equipped with a 2 x 42 cm wood auger as described in Hoppe *et al.* (2014). Sporocarp data were available (Hoppe *et al.* 2014) and used as corroborative evidence for the presence of particular fungi that were detected as OTUs in the sequencing analysis.

Wood physico-chemical properties and lignin-modifying enzyme assays

The concentrations of C and N in wood samples were determined by total combustion using a Truspec elemental analyzer (Leco, St. Joseph, MI, USA). Klason lignin content was determined gravimetrically as the dry mass of solids remaining after sequential hydrolysis with sulfuric acid (72% w/w); in a second step, acid soluble lignin was measured by UV-photometry in 4% H_2SO_4 (Effland 1977; Liers *et al.* 2011). Total lignin was obtained by summing acid insoluble Klason lignin and acid soluble lignin (Raiskila *et al.* 2007). The wood samples' pH values and contents of nutrient ions and lignin-modifying enzymes were measured in aqueous extracts. The extractions were performed using 10 ml distilled water per 1g dry mass of wood for 120 min on a rotary shaker (120 rpm). Macronutrients (Mg, K, Ca, Fe) and micronutrients (Cu, Mn, Zn, Ni) were determined using inductively coupled plasma (ICP) optical emission spectrometry (ICP-OES) and mass spectrometry (ICP-MS), according to the instrument manufacturers' specifications. Three oxidative extracellular oxidoreductases important for lignin degradation (laccase - Lac, manganese peroxidase – MnP, manganese-independent peroxidases - MiP) (Hatakka & Hammel 2011) were measured as described by Hahn *et al.* (2013). Nutrient ion and lignin-modifying enzyme analyses were conducted in triplicate and in duplicate, respectively, on the same subsamples.

Deadwood logs were assigned to four decay classes based on remaining mass (%) data by k-means cluster analysis as described in Hoppe *et al.* (2014) and Kahl *et al.* (2012). Decay rates were calculated based on a single exponential model (Harmon *et al.* 1986) using information

on mass loss (density and volume loss) and time since death obtained by dendrochronological dating of the deadwood.

DNA isolation, PCR and pyrosequencing

Total community DNA was isolated from 1 g of each homogenized wood sample using a modified CTAB-protocol (Doyle & Doyle 1987) as described in Hoppe *et al.* (2014). All DNA extracts from the wood samples of each log were pooled into a composite extract prior to PCR. Primers ITS1-F (Gardes & Bruns 1996) and ITS4 (White *et al.* 1990) were used to amplify the fungal nuclear ribosomal internal transcribed spacer (nrITS) rDNA. We used a set of 10nt MID-barcodes provided by Roche Applied Science (Mannheim, Germany). Each composite DNA extract for the amplicon libraries was amplified separately by PCR in triplicate 50 µl reaction mixtures containing 25 µl 2x GoTaq Green Mastermix (Promega, Madison, WI, USA), 25 µM of each primer and approximately 20 ng template DNA. Amplification was performed using a touchdown PCR program with denaturation at 95°C for 5 min followed by 10 cycles of denaturation at 94°C for 30 sec, annealing at 60–50°C for 45 sec (-1°C per cycle), and extension at 72°C for 2 min, followed by 30 cycles of 94°C for 30 sec, 50°C for 45 sec and 72°C for 2 min, with a final 10 min extension step at 72°C. The PCR products were separated on a 1.5% agarose gel and equimolar volumes of the amplified products of the expected size from the three positive replicate amplicons per sample were homogenized. The pooled products were gel purified using a Qiagen Gel Extraction Kit (Qiagen, Hilden, Germany). The purified DNA was quantified using a fluorescence spectrophotometer (Cary Eclipse, Agilent Technologies, Waldbronn, Germany). An equimolar mixture of each library was subjected to unidirectional pyrosequencing from the ITS4 end of the amplicons, using a 454 Titanium amplicon sequencing kit and a Genome Sequencer FLX 454 System (454 Life Sciences/ Roche Applied Science) at the UFZ Department of Soil Ecology.

Bioinformatic analysis

We performed multiple levels of sequence quality filtering. The fungal ITS sequences were extracted based on 100% barcode similarity. Sequences were clipped of barcodes and trimmed to a minimum length of 300nt to best cover the ITS2 part of the nrITS using MOTHUR (Schloss *et al.* 2009). Sequence reads with an average quality score of <20 bases, and homo-polymers of >8 bases were removed. Unique good quality sequences from the dataset were filtered and checked for chimeras using the uchime algorithm (Edgar *et al.* 2011) as implemented in MOTHUR. The sequence dataset was then clustered and assigned to OTUs using CD-HIT-454 (Li & Godzik 2006) at a 97% threshold of pairwise sequence similarity. We used MOTHUR to taxonomically assign representative sequences of the OTUs against the UNITE reference database (as downloaded in May 2013) (Abarenkov *et al.* 2010).

Statistical analysis

To link WIF richness and taxonomic identity to microbial-mediated enzyme activity and decomposition rates, we defined cumulative OTUs (cOTUs) as species synonyms by aggregating OTUs that were unequivocally given the same name by BLAST reanalysis against GenBank yielding at least 95% (species level) and 90% (genus level) maximum identity scores (Ovaskainen *et al.* 2013) for the same database species.

The Chao1 and ACE (abundance- based coverage estimator) diversity indices were calculated for all OTUs (including 1-3tons) using the *estimate* function in the R package "vegan" (Oksanen *et al.* 2013).

Analysis of similarities (ANOSIM) and nonmetric multidimensional scaling (NMDS) based on Bray-Curtis distances were conducted using PAST (Hammer *et al.* 2001) and the "vegan" package in R, respectively, to compare the fungal community structure of *Fagus* and *Picea*.

The influence of selected wood physico-chemical parameters and environmental factors (e.g. fungal family abundances and forest management regimes) on fungal community structure was investigated by fitting data on each factor to the NMDS ordinations of the fungal communities. The wood physico-chemical parameters considered in these analyses were decay class, concentrations of macronutrients (C, N, K, Ca, Mg, Fe) and micronutrients (Cu, Mn, Ni and Zn), relative wood moisture, wood density, remaining mass and pH. Goodness-of-fit statistics (R^2) for environmental variables fitted to the NMDS ordinations of fungal communities were calculated using the *envfit* function of "vegan", with *P* values being based on 999 permutations (Oksanen *et al.* 2013). The *P* values were Bonferroni-corrected in all cases. We calculated non- parametric Kendall-Tau correlations (τ) (pairwise comparisons) to link the fungal taxonomic groups to lignin-modifying enzyme activities and decomposition rates using PAST. The differences in OTU richness (observed OTU and cOTU richness and estimated Chao1 and ACE richness) and wood physico-chemical properties among different decay classes were analyzed for differences among means ($P < 0.05$) by performing one-way analysis of variance (ANOVA) incorporating Shapiro-Wilk's W test for normality and Levene's test to check for the equality of group variances. Fisher's Least Significant Difference (LSD) *post hoc* test was also performed.

3. Results

Wood physico-chemical properties in different decay classes

The C/N ratios of the deadwood increased as it decayed, and were significantly higher in *Picea* logs ($P < 0.0001$, ranging from 630 ± 48.4 to 423 ± 52.4) than in *Fagus* logs (365 ± 14.9 to 194 ± 15.6) (Fig. 1 and Table S1). This difference can be attributed to the significantly higher C concentrations in *Picea* deadwood compared to *Fagus* ($P < 0.0001$),

which ranged from 49.3 % ± 0.31 in decay class 1 to 51.4 % ± 0.94 in decay class 4, and also to the significantly higher N concentrations in *Fagus* logs ($P < 0.0001$) compared to *Picea*. Nitrogen concentrations also increased ($P < 0.0001$ for *Fagus* logs, $P < 0.05$ for *Picea* logs) as wood decay progressed (Fig. 1). The total lignin concentrations in *Fagus* deadwood were lower than in that of *Picea* (28.9 % and 36.0 %, $P < 0.0008$) and the relative proportion of lignin in the wood increased significantly as it decayed. There were no differences in the mean relative wood moisture, mass loss and decay rate between the two tree species, but these parameters differed significantly between different decay classes within each tree species (Fig. 1). For example, wood moisture (mass percent dry wood) increased in parallel with decay, from 49.8 % ± 5.5 for decay class 1 to 152.2 % ± 9.1 for decay class 4 in *Fagus* ($P < 0.0001$) and from 48.7 % ± 11.6 to 163.1 % ± 24.6 in *Picea* ($P < 0.0001$). The pH of *Fagus* deadwood was constant across decay classes and significantly higher than that of *Picea* deadwood, which declined from 4.6 ± 0.1 for decay class 1 to 4.3 ± 0.2 for decay class 4. The allocation of micronutrients and the activities of the lignin-modifying enzymes laccases (Lac), manganese peroxidase (MnP) and manganese independent peroxidases (MiP) are provided in supplementary Table S2.

Pyrosequencing and community sampling statistics

In total, 139,352 reads were obtained from 454 pyrosequencing of 48 deadwood samples. Sequences were initially quality checked, trimmed, normalized per sample and screened for potential chimeras. CD-HIT-454 clustering of the remaining 86,935 sequences yielded 2,386 OTUs at a 97% cutoff, of which 1,090 appeared as singletons and 305 as doubletons. We performed a Mantel test on Bray-Curtis dissimilarities to assess the correlations between the whole matrix and a matrix excluding the rare taxa (singletons, doubletons and tripletons). This indicated that the removal of rare taxa from the community composition had no effect (*R*

= 0.99, P = 0.0001). In total, 81,803 sequences clustering into 779 OTUs were retained for further statistical analysis after removing sequences that could not be taxonomically assigned to fungi. By applying a species-level sequence divergence threshold of 3%, we were able to taxonomically assign 93.1% of the filtered OTUs at the phylum level. Basidiomycota accounted for 338 of the filtered OTUs, Ascomycota for 375, and zygomycetes for 9. Chytridiomycota, Entomophthoromycota, and Glomeromycota were represented by 1 OTU each. Further, 614 (78.8%), 552 (70.9%) and 434 (55.7%) of the filtered OTUs were classified at the order, family and genus levels, respectively. The remaining 6.9% (54 OTUs) were grouped as unknown fungal OTUs.

Wood-inhabiting fungal richness and community structure

The total observed fungal OTU richness (excluding rare taxa) per sample ranged from 17 to 102 (25-159 including rare taxa) in logs of *Fagus sylvatica* and from 28 to 102 (38-151 including rare taxa) in *Picea abies* logs (Fig. S1). We did not observe significant variation in mean OTU richness between the two tree species (P = 0.32) (Table S3). All four measures of fungal OTU richness (total observed, cumulative, Chao1, and ACE) correlated significantly and positively with decay class, whether it was quantified in terms of declining wood density ($P < 0.05$ *Fagus sylvatica*, $P < 0.001$ on *Picea abies*) or remaining wood mass (Fig. S2).

While the distribution of OTUs belonging to the Basidiomycota or Ascomycota was comparatively balanced in terms of their presence or absence, their relative abundances differed significantly. For example, the 201 Basidiomycota OTUs identified in *Fagus* samples contained 25,053 sequences (63.2% of all sequences) whereas the 265 Ascomycota OTUs (Table S4) only contained 14,091 (35.5%). There was an even more pronounced pattern in *Picea,* where the 217 identified Ascomycota OTUs contained only 5,614 (13.3%)

sequences whereas the Basidiomycota accounted for almost 83% (34,985) of all sequences clustered into 242 OTUs. More specifically, 2,487 (6.35%) sequences were assigned to *Annulohypoxylon cohaerens*, a common European beech saprotroph of the family *Xylariaceae*, which also represented the dominant family in the *Fagus* logs, accounting for 5,377 sequences in total (Figs. 2 and S3). Other important families in the *Fagus* fungal communities were the *Meruliaceae*, *Polyporaceae*, *Mycenaceae*, *Physalacriaceae* and *Marasmiaceae,* that together accounted for 42% of all identified fungi (Fig. 2). OTUs assigned to the white-rot causing fungus *Resinicium bicolor*, which is listed as Agaricomycetes I.S. (*incertae sedis*) in the Index Fungorum (www.index-fungorum.org), were dominant in *Picea* deadwood, accounting for 25.3% of all sequences. This fungus was detected in 83.3% of all *Picea* logs (Fig. S3). *Bondarzewiaceae*, represented by *Heterobasidion sp*., *Stereaceae*, *Fomitopsidaceae* and *Mycenaceae* also occurred frequently and accounted for 32.64% of the identified sequences/ OTUs. Unidentified species of the *Helotiaceae* and an OTU identified as the potential soft-rot agent *Phialophora sp.* of the family *Herpotrichiellaceae* were the most abundant Ascoymcota in *Picea* but only accounted for 2% and 1.9% respectively of all identified sequences.

We also examined the sharedness of WIF at species (cOTU) and genus level (Fig. S4ab). Among the 160 cOTUs with the same genus and species epithets, 74 (22 Ascomycota, 50 Basidiomycota, and 2 zygomycetes) were found in both, *Picea* and *Fagus* logs (Fig. S4a). Genus-level comparisons revealed that 35 genera were unique to *Fagus* and, 34 to *Picea*, while 58 genera were shared (Fig. S4b).

WIF dynamics on the two deadwood species

Different WIF dynamics were clearly observed in the *Fagus* and *Picea* logs (Figs. S5, S6). WIF communities in *Fagus* logs were highly dynamic with respect to wood decay, with no

cOTU being dominant in all decay classes. The dominant fungal families and cOTUs in *Fagus* logs of decay classes 1-4 were *Xylariaceae* (mainly *Annulohypoxylon cohaerens*) and *Polyporaceae* (*Trametes versicolor*) in decay class 1, *Polyporaceae* (*Fomes fomentarius*) and *Meruliaceae* (*Phlebia livida*) in decay class 2, *Meruliaceae* (*Ceriporiopsis gilvescens*), *Polyporaceae* (*Fomes fomentarius*) and *Marasmiaceae* (*Megacollybia platyphylla*) in decay class 3, and *Mycenaceae* (*Mycena alnetorum*), *Marasmiaceae* (*Megacollybia platyphylla, Marasmius alliaceus*) and *Polyporaceae* (*Trametes versicolor*) in decay class 4. In contrast, all of the *Picea* decay classes were dominated by a single cOTU assigned to Agaricomycetes I.S. (*Resinicium bicolor*); the mean abundances of this cOTU in decay classes 1, 2, 3, and 4 were 26.1%, 36.6%, 27.6%, 16.5%, respectively. Some fungal families and cOTUs were also co-dominant in different decay classes: *Stereaceae* (mainly *Amylostereum areolatum*) in decay class 1, *Fomitopsidaceae* (*Fomitopsis pinicola*) in decay class 3, and *Bondarzewiaceae* (*Heterobasidion* sp.) in decay classes 3 and 4. The WIF dynamics of both deadwood species are described at greater detail in the supporting information.

Factors correlating to fungal community structure in the two deadwood species

NMDS analysis clearly separated the WIF communities of the two tree species (Fig. 3ab; Table 1). Both anthropogenic factors and wood physico-chemical parameters correlated significantly with the fungal community structure (Fig. 3a). Factors that contributed significantly to the variation in the WIF community structure in both tree species were the applied forest management regime, decay class, relative water content, pH, remaining mass, wood volume, wood density, C/N ratio and the concentrations of total lignin, C, N, Mg, Al and Zn (P = 0.021-0.0001). At the individual tree species level, WIF community structure correlated significantly with forest management type, decay class, relative water content, remaining mass, wood density, C/N ratio, total lignin and C (Table 1). In addition, wood

volume and N concentration were important in *Fagus* deadwood, while the concentrations of Klason lignin and Ca were contributing significantly in shaping the fungal community structure in *Picea* deadwood. Fungal families that correlated significantly with WIF community structure in *Fagus* and *Picea* logs are listed in Fig. 3a.

Relationships between wood-inhabiting fungal richness, taxonomy and lignin-modifying enzyme activities

Correlations between WIF richness (in terms of total observed, cumulative, or estimated OTUs), WIF abundance (at both the family and cOTU levels), and the activities of lignin-modifying enzymes are presented in Table S5. There were no positive correlations between any actual richness of OTU/cOTU as well as the estimated richness of total OTU and the activities of lignin-modifying enzyme activities. Interestingly, however, there were some significant positive correlations between the abundances of certain fungal families and the measured activity of lignin-modifying enzymes. In *Fagus* logs, the abundance of the *Schizoporaceae* and *Xylariaceae* correlated positively with Lac and MiP activity, respectively ($P < 0.05$). In *Picea* logs, the abundance of *Bondarzewiaceae* correlated positively with MiP and MnP activity ($P = 0.002$ - 0.009). We hence found many significant correlations between fungal cOTU abundance and the activity of potential lignin-modifying enzymes (Table S5). In *Fagus* deadwood, the strongest significant positive correlations were found between *Mycena alnetorum* and Lac ($\tau = 0.43$, $P = 0.003$), *Hypoxylon rubiginosum* and MiP ($\tau = 0.42$, $P = 0.004$), and *Marasmius alliaceus* and MnP ($\tau = 0.31$, $P = 0.036$). In *Picea* deadwood, the strongest significant positive correlations were found between *Armillaria gallica* and Lac ($\tau = 0.32$, $P = 0.028$), *Heterobasidion sp*. and MiP ($\tau = 0.37$, $P = 0.010$), and *Heterobasidion sp*. and MnP ($\tau = 0.45$, $P = 0.002$). In addition, the abundances of certain families and cOTUs exhibited significant negative correlations with lignin-modifying enzyme activity (Table S5).

Relationships between wood-inhabiting fungal richness, taxonomic identity and wood decomposition rates

There were no significant correlations between total OTU richness (in terms of total observed, cumulative or estimated OTU richness) and wood decomposition rates (Table S6). However, the abundances of specific fungal taxonomic groups and cOTUs correlated significantly with wood decomposition rates. In *Fagus* deadwood, the abundance of the *Xylariaceae* family correlated significantly and negatively with decomposition rates (τ = -0.44, P = 0.003). The abundances of three individual species also correlated negatively with decomposition rates: *Hypoxylon fragiforme* and *Xylaria hypoxylon* (both *Xylariaceae*), and *Neobulgaria pura* (*Leotiaceae*). On the other hand, *Mycena purpureofusca, Phialocephala dimorphospora, Trametes versicolor* correlated positively with decomposition rates (τ = 0.31 – 0.41, P = 0.034 – 0.005; Table S6). In *Picea* deadwood, the abundances of the *Schizoporaceae* were positively correlated with decomposition rates (τ = 0.43, P = 0.005). Individual species whose abundances correlated positively with decomposition rates were *Botryobasidium botryosum, Hyphodontia alutacea, Hyphodontia alutaria* and *Mycena alnetorum* (τ = 0.32 – 0.45, P = 0.036 – 0.003; Table S6).

4. Discussion

This work builds on earlier studies that used high-throughput sequencing to study fungal community structures in deadwood (Kubartova *et al.* 2012; Ovaskainen *et al.* 2013) by providing the first comparison of two morphologically different tree species often occurring in close proximity in temperate European forests. By assessing a very comprehensive set of dataset on physico-chemical wood properties, we were able to identify key factors that correlate with fungal community structure. Moreover, by linking fungal diversity and

community composition to enzyme activities and decomposition rates, we demonstrated that ecosystem processes are controlled by complex mechanisms such as assembly histories and competition scenarios.

Fungal diversity and community composition

We found no significant differences between the two deadwood species in terms of total or mean OTU richness. The mean fungal OTU richness tended to increase with increasing decay class. This is inconsistent with the findings of fructification pattern studies, which indicated that the abundance of fruiting bodies was highest at intermediate stages of decay (Heilmann-Clausen 2001; Hoppe *et al.* 2014). However, the fact that fungal OTU richness increased with losses of mass and density (i.e. as the decay class increased) is in agreement with the results of previous studies on spruce deadwood that used molecular techniques (Kubartova *et al.* 2012; Rajala *et al.* 2012). The discrepancy between these findings may be due to the fact that many fungi tend to reside as vegetative mycelia in deadwood and therefore do not develop fruiting bodies (Kubartova *et al.* 2012).

WIF richness is affected by various anthropogenic factors and activities, such as forest management and structure, the level of forest disturbance, and the amount and quality of available deadwood (Josefsson *et al.* 2010; Junninen *et al.* 2006; Müller *et al.* 2007; Purahong *et al.* 2014a; Purahong *et al.* 2014b). Interestingly, in a specific subset of forest types (*Picea* deadwood in an age-class spruce forests and *Fagus* deadwood in an age-class beech forests), the estimated fungal OTU richness based on Chao1 and ACE for *Picea* deadwood was significantly lower than that for *Fagus* deadwood (P = 0.01-0.02, Fig. S7). This is consistent with a study conducted in the same area that compared the fungal OTU richness in *Picea* deadwood in converted forests (age-class spruce forest converted from

beech forest) to that of *Fagus* deadwood in a semi-natural age-class beech forest (Purahong *et al.* 2014a). This study demonstrated the negative effect of forest conversions on WIF diversity, e.g. if an autochthonous tree species is replaced by an introduced one.

WIF dynamics during decomposition processes and driving factors

Fungal community structure differed significantly between the two tree species as indicated by the NMDS analysis. The community structure was also more dependent on the species origin of deadwood than on the surrounding forest type. More specifically, fungal communities in *Fagus* logs in beech stands were more similar to fungal communities in *Fagus* logs in spruce forests than to fungal communities in *Picea* deadwood in beech forests. The same was true for *Picea* logs in spruce and beech stands. This further demonstrates that substrate type has a greater impact on WIF community structure than forest types. Our results indicate that both an anthropogenic factor (the applied forest management regime) and the physico-chemical properties of the wood (decay class, relative water content, mass remaining, wood density, C/N ratio, total lignin, and C concentration) correspond significantly to community structure in both tree species, which is consistent with the report of Rajala *et al.* (2012).

The two deadwood species also differed substantially with respect to the way in which their WIF communities changed as the wood decayed. In *Picea* logs, *Resinicium bicolor* was dominant in all decay classes; other fungi such as *Amylostereum areolatum*, *Heterobasidion sp*. and *Fomitopsis pinicola* were co-dominant in specific decay classes but much less abundant. Conversely, in *Fagus* logs, no single fungus was dominant in all decay classes. The dominant species changed from *Annulohypoxylon cohaerens* and *Trametes versicolor* in decay class 1 to *Fomes fomentarius* in decay classes 2 and 3 and then *Mycena alnetorum* and

Trametes versicolor in decay class 4. It may be surprising that *Polyporaceae* were still dominant in the decay class 4 since species of this family require substrates with high energy contents to produce fruiting bodies and are therefore consequently rarely found in wood at later stages of the decomposition when using methods that focus on fruiting bodies (Bader *et al.* 1995; Lindblad 1998). This may be due to the presence of vegetative mycelia and/ or DNA residues in the wood (Kubartova *et al.* (2012), both of which would be captured by our methodology. However, the change in the dominant polypore species from *Fomes fomentarius* in decay class 3 to *Trametes versicolor* in decay class 4 indicates that this phenomenon may not be entirely related to DNA residues.

The fungal community dynamics in *Picea* deadwood observed in this study were distinctly different compared to those reported for boreal forest ecosystems in Fennoscandia (Kubartova *et al.* 2012; Ovaskainen *et al.* 2013; Rajala *et al.* 2011; Rajala *et al.* 2012) . First, the ascomycetes that were reported to be most dominant during the early stages of decay in boreal forests (Rajala *et al.* 2012) were largely absent in the Central European forests, where basidiomycetes were most abundant across all stages of decay. Secondly, different fungi were dominant at different stages of decay in the boreal environment (Kubartova *et al.* 2012; Ovaskainen *et al.* 2013; Rajala *et al.* 2012), whereas in our study *Resinicium bicolor* was most abundant in all decay classes. Third, species such as *Hyphodontia alutaria*, *Ascocoryne cylichnium*, *Heterobasidion parviporum* and *Fomitopsis pinicola* were classified as early colonizers in boreal forests (Kubartova *et al.* 2012) but were common in all decay classes in our study and even dominant in *Picea* logs of decay class 4. Finally, ectomycorrhizal (ECM) fungi that were already detected during the early stages of decay in previous studies and became strongly dominant during the final stages of decomposition in boreal forests (Rajala *et al.* 2012) were largely absent in our study. We did not observe any increase in the abundance of ECM species in the more mineralized wood of decay class 4. Only 4 ECM

cOTUs were detected in a single *Picea* log in the later stages of decay: *Lactarius* sp. (42 sequences), *Laccaria amethystina* (22 sequences) *Russula fellea* (9 sequences) and *Xerocomus pruinatus* (4 sequences). Together, these species accounted for only 0.18% of all sequences detected in *Picea abies*. The relatively low abundance of ECM in *Picea* logs from temperate forests as compared to boreal forests could be due to differences in N-availability in the two forest ecosystems (Nasholm *et al.* 1998). We assume that ECM fungi in temperate forests preferentially acquire N from the soil, whereas in the N-limited boreal environment it is worthwhile for ECM fungi to acquire nitrogen by attacking deadwood.

The role of WIF in ecosystem functions and processes

Both the activities of lignin-modifying enzymes and the decomposition rate were related to the abundances of particular fungal families and cumulative OTUs. Laboratory scale studies demonstrated that members of different fungal taxa that were detected in this study (such as *Armillaria* sp., *Fomes fomentarius*, *Trametes versicolor*) can efficiently produce similar amounts of lignin-modifying enzymes (Baldrian 2006) and cause similarly high levels of mass loss (Valmaseda *et al.* 1990). The co-occurrence of these fungi in deadwood suggests that there is some functional redundancy within the studied WIF communities. Several authors have reported increased Lac and MnP activities due to two-species-interactions (Baldrian 2004; Freitag & Morrell 1992; Snajdr *et al.* 2011; White & Boddy 1992). Chi *et al.* (2007) found that some combinations of two fungi can accelerate the decay of wood due to increases in MnP production relative to that observed in equivalent cases featuring only a single fungal species. Whether enzymes are actually secreted due to fungus-fungus interactions or for other reasons may depend on the community structure and the state of degradation of the colonized wood. However, recent studies (Dickie *et al.* 2012; Dowson *et al.* 1988a, b; Fukami *et al.* 2010; Fukasawa *et al.* 2009) have demonstrated a high degree of

interaction among co-existing fungal species, suggesting that WIF may invest more energy into competing with one-another than on producing wood-degrading enzymes under natural field conditions. Coatesand Rayner (1985) also found that interaction reduced the rate of wood decay. Therefore, as demonstrated in this work, the high species richness of deadwood-resident fungal communities need not be associated with any increase in the production of degradative enzymes or wood decomposition rate. We speculate that there may be a threshold for the co-occurrence of WIF below which they will efficiently produce enzymes and accelerate the decomposition of the deadwood. We identified some fungal OTUs that are known to be active producers of lignin-modifying enzymes and strong wood decomposers that cause a white-rot with high mass loss (Table S7). *Trametes versicolor* is among the most important decomposers, occurring in all decay classes of *Fagus* deadwood. However, it only caused high mass losses in deadwood samples where it accounted for at least 11% of the total sequence abundance.

We further show that different wood types (deciduous vs. coniferous) directly relate to decomposer (fungal) community structure and dynamics. The decomposition rates on the two deadwood species were not significantly different. This may be due to functional redundancy in their WIF communities and demonstrates that the fungi in each community are adapted to their host tree species. Fungi in coniferous wood have to deal with larger amounts of extractives as well as more recalcitrant and condensed lignin than is encountered in *Fagus* wood (Blanchette 1991; Higuchi 2006). In respect to anatomy, coniferous woods have simpler structures than deciduous woods (Fengel & Wegener 1983). Such factors will affect fungal substrate preferences. Brown-rot fungi prefer coniferous wood while most white-rot fungi prefer deciduous wood (Hibbett & Donoghue 2001). The preference of brown-rot fungi for coniferous wood was apparent in our data: the three most abundant brown-rot fungi *Fomitopsis pinicola, Dacrymyces stillatus* and *Antrodia sinuosa* were found to be much more

abundant in *Picea* than in *Fagus*. Surprisingly, we found more sequences for white-rot fungi in *Picea* wood than we did for brown-rot fungi. The mean enzyme activities of MiP and MnP in *Picea* were only marginally lower than those observed for *Fagus*, further demonstrating the presence of white-rot species in the studied *Picea* deadwood. These observations are consistent with those of Olsson *et al.* (2011) and Rajala *et al.* (2012), who also found white-rot fungi to be more abundant than brown-rot fungi on *Picea abies*.

The relationships between fungal cOTU richness, family abundances and the activities of lignin-modifying enzymes and decomposition rates were very different in *Fagus* and *Picea* logs. The fungal communities in both deadwood species were composed of different cOTUs that were positively correlated and/ or expected to play roles with all lignin-modifying enzyme activities and wood decomposition rates. Interestingly, there were some fungi whose abundances did not correlate positively with lignin-modifying enzyme activity and/ or wood decomposition rates in this field study even though they secreted high titers of lignin-modifying enzymes and caused high mass losses under laboratory conditions. This could be due to the succession of the studied communities (i.e. priority effects) and the interspecific interactions among different fungal species, as discussed above.

Our results also revealed significant negative correlations between the abundances of *Xylariaceae* species and decomposition rates on *Fagus* logs. Different members of the *Xylariaceae* were abundant in many *Fagus* logs of early to intermediate decay classes, and logs harboring these species often exhibited low rates of decomposition. This could be related to the ability of fungi of this family (which cannot produce MnP) to impede deadwood colonization by secondary saprotrophic basidiomycetes (Fukasawa *et al.* 2009). *Xylariaceae* were shown to act very defensively against saprotrophic basidiomycetes on 2% malt agar and were not displaced by saprotrophic basidiomycetes in twigs over an incubation period of 6

months (Fukasawa *et al.* 2009). *Xylariaceae* can form pseudosclerotial plates (PSPs) to delineate decay columns (demarcation lines) within the wood and protect them from attacks by competing saprotrophs. These decay columns can persist even after several years of decomposition (Fukasawa *et al.* 2009; Purahong & Hyde 2011). We observed similar recalcitrant dense matrices of melanized hyphae (PSPs) in *Fagus* logs that were highly dominated by *Xylariaceae* (Fig. S8). *Trametes versicolor* was also highly abundant in the *Fagus* deadwood at various stages of decay, and its abundance correlated negatively with that of *Xylariaceae* members. Interestingly, some logs in the early stages of decay that had been colonized by *Trametes versicolor* rather than *Xylariaceae* exhibited very high rates of wood decomposition. This demonstrates the importance of priority effects and interspecific interactions among different fungal species. *Trametes versicolor* has been shown to secrete large quantities of different lignin-modifying enzymes and yields high wood decomposition rates under laboratory condition (Valmaseda *et al.* 1990). We assume that the properties and species origin of deadwood affects both the dynamics of the fungal community and the interactions among different fungal species. Many fungal OTUs were present in both *Fagus* and *Picea* deadwood but the dominance patterns and temporal dynamics of the communities in each case differed substantially (Figs. S3, S4).

Conclusion

Fungal community structure was significantly different between deadwood of *Fagus sylvatica* and *Picea abies* occurring in close proximity in temperate German forests of Germany. Wood physico-chemical properties and anthropogenic activities are the main drivers corresponding to the fungal communities in these deadwood species. Under the studied natural conditions, microbial-mediated ecosystem functions (i.e. the activities of lignin-modifying enzymes) and processes (wood decomposition rate) were controlled by

successional assembly history, interspecific interactions and competition scenarios rather than total OTU/ -species richness (Dickie *et al.* 2012; Fukami *et al.* 2010).

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Data accessibility: The raw sequence data are available from the NCBI Sequence Read Archive (http://www.ncbi.nlm.nih.gov/Traces/study/) under experiment SRX589508. Corresponding MIDs and metadata are provided in Table S8.

Figure legends

Figure 1. Decay rates, mass losses, and wood physico-chemical properties of *Fagus* and *Picea* deadwood logs of different decay classes. The figure shows the relative deviation from the sample mean in each case. Mean values of all parameters for deadwood logs of both species are shown in the middle. The differences between the two deadwood species and also among different decay classes for individual deadwood species were analyzed by t-tests and one-way analysis of variance, incorporated in the form of Fisher's Least Significant Difference (ns = not significant, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$). The four FASY (*Fagus sylvatica*) and PIAB (*Picea abies*) decay classes are shown in different color shades.

Figure 2. Patterns of cOTUs on *Fagus* and *Picea* deadwood displayed as sunburst charts (beatexcel.com). The outer rings show the cumulative relative abundances of different fungal OTUs (cOTUs) at the species and genus levels with at least 95% and 90% probability of correct identification, respectively, based on secondary BLAST re-analysis. The middle rings display the cumulative relative abundances at the family level. The inner rings display the cumulative relative abundances of different concordant fungal phyla. Only cOTUs that accounted for > 1% of the total abundances and identified at least to genus are labeled.

Figure 3. 3D-Nonmetric multidimensional scaling (NMDS) ordination of fungal community structure in *Fagus* (green) and *Picea* (red) deadwood using the *plot3d* and *ordigl* functions in R. The NMDS ordination (stress = 0.16) was fitted to wood physico-chemical and anthropogenic factors (a) and also abundances of different fungal families (b) by using the *envfit* command in "vegan". ANOSIM revealed significant separation of fungal community structure according to tree species ($R = 0.60$, $P < 0.001$, 999 permutations). To better show the 3-dimensionality of the ordination, two movies in .flv Flash format (BB Flashback

Express Recorder, Blueberry Software, Birmingham, UK) are available in Suppl. files S1a and S1b.

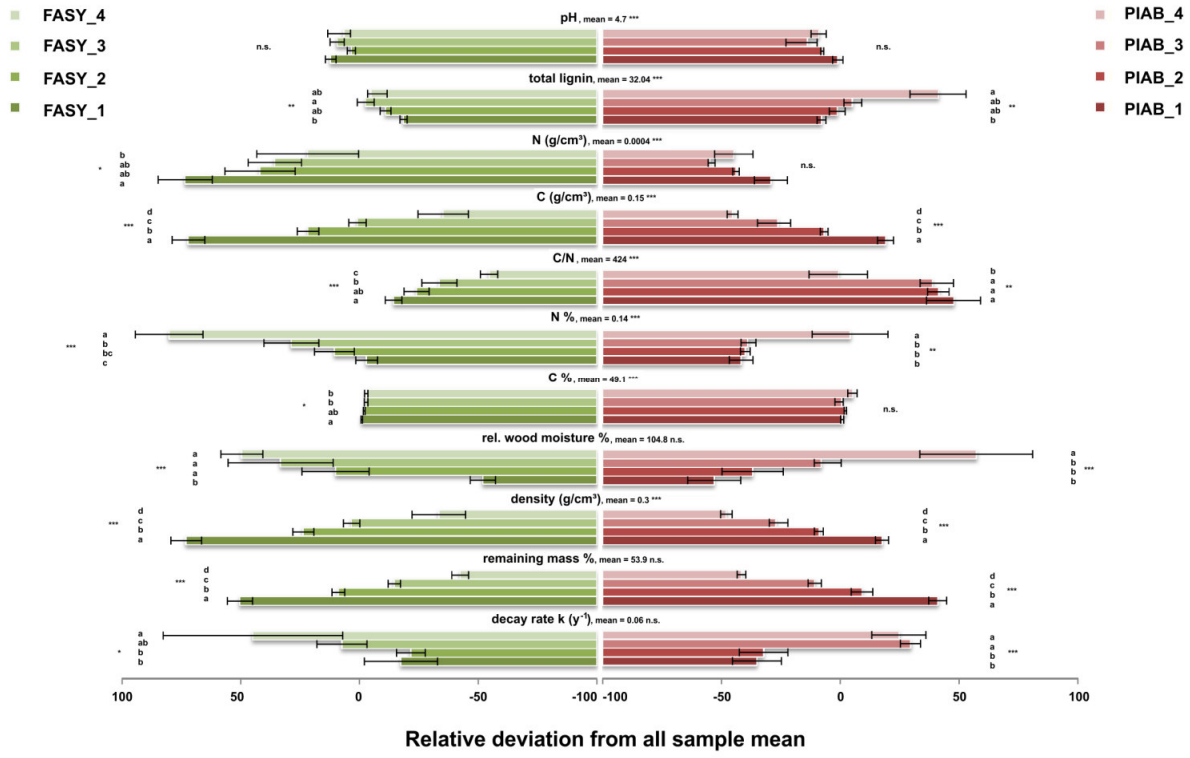
FASY_4
FASY_3
FASY_2
FASY_1
PIAB_4
PIAB_3
PIAB_2
PIAB_1
pH, mean = 4.7 ***
total lignin, mean = 32.04 ***
N (g/cm³), mean = 0.0004 ***
C (g/cm³), mean = 0.15 ***
C/N, mean = 424 ***
N %, mean = 0.14 ***
C %, mean = 49.1 ***
rel. wood moisture %, mean = 104.8 n.s.
density (g/cm³), mean = 0.3 ***
remaining mass %, mean = 53.9 n.s.
decay rate k (y⁻¹), mean = 0.06 n.s.
100
50
0
-50
-100
Relative deviation from all sample mean

Figure 1.

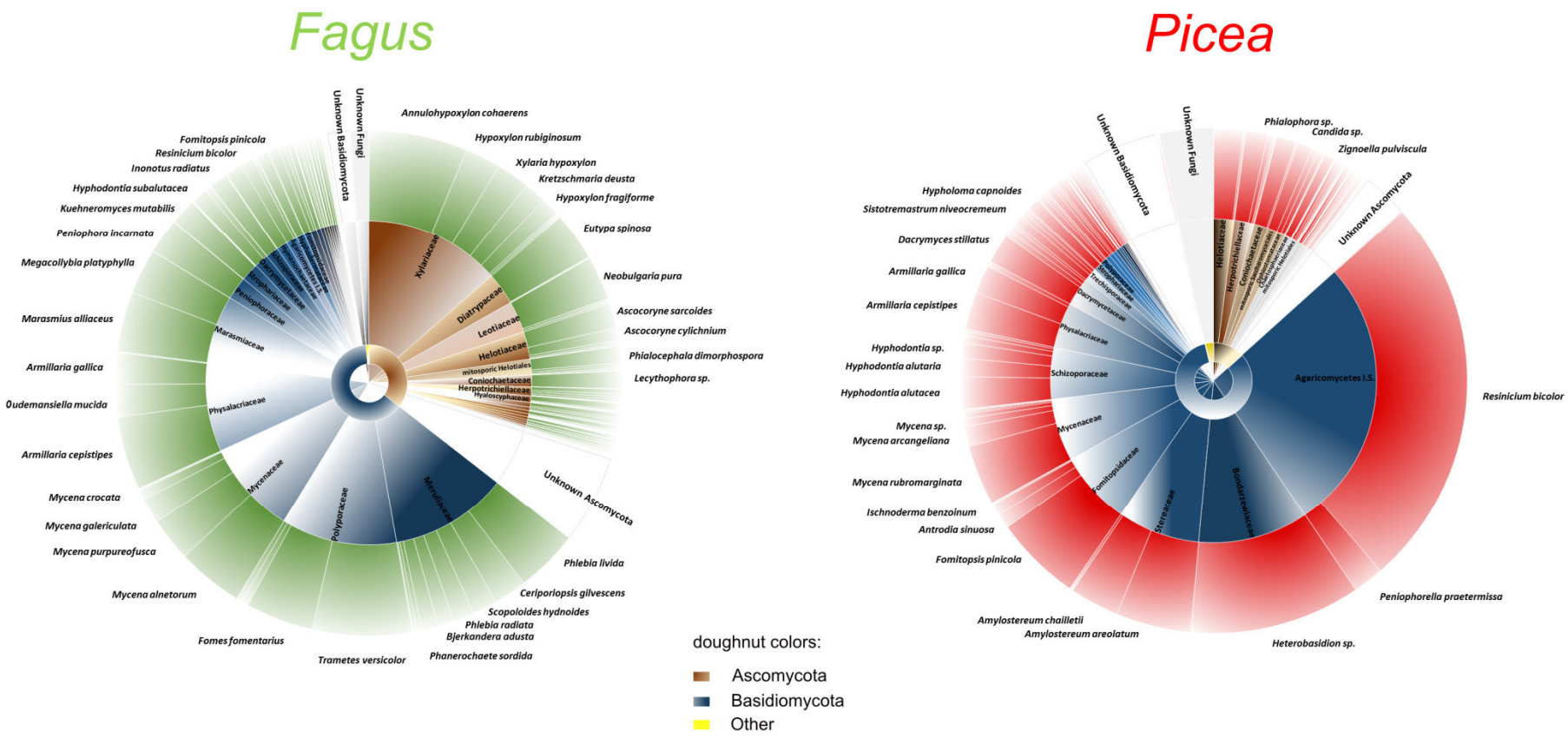
Fagus
Picea
Annulohypoxylon cohaerens
Hypoxylon rubiginosum
Xylaria hypoxylon
Kretzschmaria deusta
Hypoxylon fragiforme
Eutypa spinosa
Neobulgaria pura
Ascocoryne sarcoides
Ascocoryne cylichnium
Phialocephala dimorphospora
Lecythophora sp.
Unknown Ascomycota
Phlebia livida
Ceriporiopsis gilvescens
Scopoloides hydnoides
Phlebia radiata
Bjerkandera adusta
Phanerochaete sordida
Trametes versicolor
Fomes fomentarius
Mycena alnetorum
Mycena purpureofusca
Mycena galericulata
Mycena crocata
Armillaria cepistipes
Oudemansiella mucida
Armillaria gallica
Marasmius alliaceus
Megacollybia platyphylla
Peniophora incarnata
Kuehneromyces mutabilis
Hyphodontia subalutacea
Inonotus radiatus
Resinicium bicolor
Fomitopsis pinicola
Unknown Basidiomycota
Unknown Fungi
Xylariaceae
Diatrypaceae
Leotiaceae
Helotiaceae
mitosporic Helotiales
Coniochaetaceae
Herpotrichiellaceae
Hyaloscyphaceae
Meruliaceae
Polyporaceae
Mycenaceae
Physalacriaceae
Marasmiaceae
Peniophoraceae
Phialophora sp.
Candida sp.
Zignoella pulviscula
Unknown Ascomycota
Resinicium bicolor
Peniophorella praetermissa
Heterobasidion sp.
Amylostereum areolatum
Amylostereum chailletii
Fomitopsis pinicola
Antrodia sinuosa
Ischnoderma benzoinum
Mycena rubromarginata
Mycena arcangeliana
Mycena sp.
Hyphodontia alutacea
Hyphodontia sp.
Armillaria cepistipes
Armillaria gallica
Dacrymyces stillatus
Sistotremastrum niveocremeum
Hypholoma capnoides
Unknown Basidiomycota
Unknown Fungi
Helotiaceae
Herpotrichiellaceae
Coniochaetaceae
Agaricomycetes I.S.
Schizoporaceae
Physalacriaceae
Mycenaceae
Fomitopsidaceae
Stereaceae
Bondarzewiaceae
doughnut colors:
Ascomycota
Basidiomycota
Other

Figure 2.

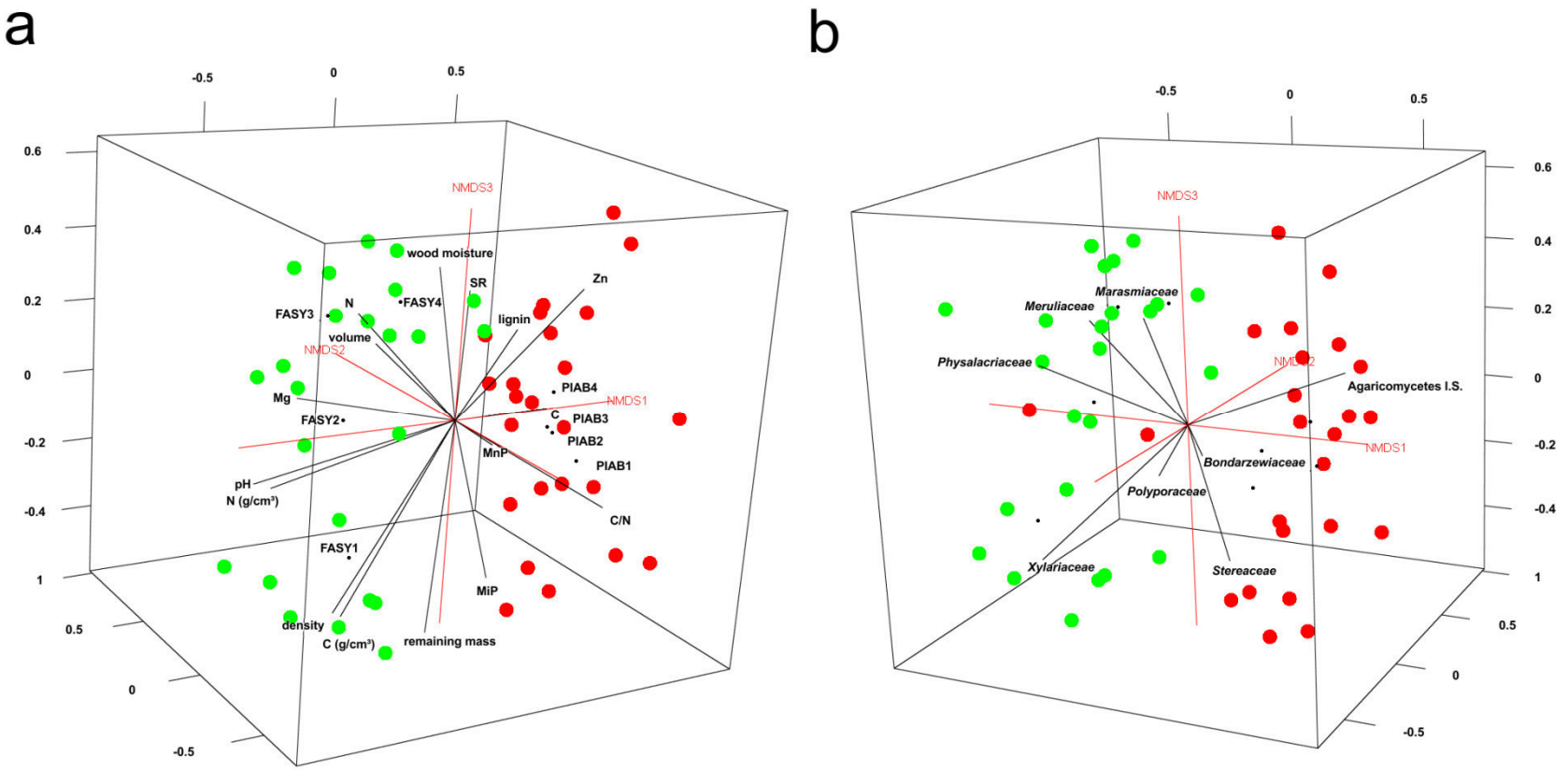
a
b
NMDS1
NMDS2
NMDS3
wood moisture
SR
Zn
FASY3
N
FASY4
lignin
volume
Mg
PIAB4
C
PIAB3
FASY2
PIAB2
MnP
PIAB1
pH
N (g/cm³)
C/N
FASY1
MiP
density
C (g/cm³)
remaining mass
Marasmiaceae
Meruliaceae
Physalacriaceae
Agaricomycetes I.S.
Bondarzewiaceae
Polyporaceae
Xylariaceae
Stereaceae

Figure 3.

Table 1. Goodness-of-fit statistics (R^2) for parameters fitted to the nonmetric multidimensional scaling (NMDS) ordination of fungal community structure. The significance estimates were based on 999 permutations. Significant factors (Bonferroni corrected $P < 0.05$) are indicated in bold. Marginally significant variables (Bonferroni corrected $P < 0.10$) are indicated in italics.

Parameter	*Fagus vs Picea*		*Fagus*		*Picea*	
	R^2	*P*	R^2	*P*	R^2	*P*
Forest management	0.1876	**0.001**	0.2683	**0.001**	0.3034	**0.001**
Fungal OTU richness	0.1846	**0.024**	0.6127	**0.001**	0.8205	**0.001**
Decay class	0.3827	**0.001**	0.7099	**0.001**	0.4691	**0.004**
Remaining mass	0.4511	**0.001**	0.7984	**0.001**	0.4675	**0.004**
Volume	0.2161	**0.009**	0.3983	**0.013**	0.0939	0.615
Density	0.6223	**0.001**	0.7749	**0.001**	0.476	**0.003**
Rel. wood moisture	0.2351	**0.004**	0.6527	**0.002**	0.6009	**0.001**
Total lignin	0.3732	**0.001**	0.3194	0.053	0.6028	**0.001**
pH	0.5679	**0.001**	0.2327	0.142	0.1547	0.341
Decay rate	0.052	0.526	0.2711	*0.086*	0.3016	*0.085*
Laccase	0.0486	0.524	0.0542	0.781	0.0857	0.648
Manganese independent peroxidase	0.2407	**0.009**	0.3941	**0.016**	0.3046	**0.048**
Manganese peroxidase	0.1747	**0.032**	0.2092	0.156	0.3874	**0.017**
C/N	0.4268	**0.001**	0.342	**0.035**	0.3605	**0.02**
C	0.3401	**0.001**	0.2217	0.182	0.4594	**0.004**
N	0.2159	**0.009**	0.3247	**0.048**	0.276	*0.07*
C (g/cm³)	0.5956	**0.001**	0.7744	**0.001**	0.4373	**0.006**
N (g/cm³)	0.6797	**0.001**	0.5371	**0.003**	0.0808	0.634
Mn	0.1451	*0.077*	0.0377	0.902	0.2107	0.18
Mg	0.2928	**0.001**	0.1175	0.475	0.1079	0.531
Ca	0.0161	0.882	0.0494	0.801	0.346	**0.034**
K	0.0941	0.224	0.2829	0.07	0.1161	0.503
Fe	0.1181	0.149	0.1398	0.354	0.0519	0.801
Cu	0.0565	0.477	0.1521	0.353	0.08	0.664
Zn	0.3158	**0.002**	0.2494	0.14	0.2027	0.187
Ni	0.0904	0.222	0.1827	0.19	0.2113	0.173

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Supporting Information

Title: Fungal presence and activity in *Fagus* and *Picea* deadwood tackled by nrITS metagenomics and lignin-modifying enzyme analysis

Björn Hoppe, Witoon Purahong, Tesfaye Wubet, Tiemo Kahl, Jürgen Bauhus, Tobias Arnstadt, François Buscot, Dirk Krüger

Detailed WIF dynamics and sharedness of cOTUs and families: *Fagus vs. Picea* logs

(A) Detailed WIF dynamics in Fagus and Picea logs

In *Fagus* deadwood (Fig. S5), the decay class 1 was highly dominated by *Xylariaceae* (total abundance 26%; mainly comprising *Annulohypoxylon cohaerens* 17.4%, *Hypoxylon rubiginosum* 5.45%), followed by *Polyporaceae* (total abundance 14.6%; mainly *Trametes versicolor* 12.3%), *Physalacriaceae* (total abundance 10.4%; mainly *Armillaria cepistipes* 6.39%, *Armillaria gallica* 3.78%) and *Leotiaceae* (total abundance 8.18%; mainly *Neobulgaria pura* 7.43%). In decay class 2, *Polyporaceae* (mean abundance 17.8%; mainly *Fomes fomentarius* 13.3% and *Meruliaceae* (mean abundance 17%; mainly *Phlebia livida* 8.37% were dominant fungal families followed by *Xylariaceae* that dropped in their abundances to 10% (dominated by *Xylaria hypoxylon* 7.65%) when compared to decay class 1. With advancing decay (decay class 3), *Xylariaceae* further dropped to 5.15% (dominated by *Kretzschmaria deusta* 4.54%), whereas other families that dominated the deadwood decay class 2 remained dominant. In decay class 4, *Mycenaceae* (mean abundance 23%; dominated by *Mycena alnetorum* 16.1%) became the most dominant family followed by *Marasmiaceae* (mean abundance 15.5%; mainly *Megacollybia platyphylla* 7.99%) and *Polyporaceae* (mean abundance 10.1%; dominated by *Trametes versicolor* 10.1%). *Xylariaceae* (mean abundance 0.74%) almost disappeared from this most advanced decay class.

In *Picea* deadwood (Fig. S6), all decay classes were highly dominated by Agaricomycetes I.S. (mean abundance 26.4%, 42.7%, 29.6%, 17% in decay classes 1-4, respectively; almost fully corresponding to *Resinicium bicolor*). *Stereaceae* were frequently detected in decay class 1 (mean abundance 21.7%; dominated by *Amylostereum areolatum* 19.9%), however, they declined in abundance in decay class 2 and 3 (mean abundances 1.18% and 2.69%, respectively) and increased in their abundance in decay class 4 again (mean abundance 7.31%; dominated by *Amylostereum chailletii* 7.29%). *Physalacriaceae* were frequently detected in decay class 1 (mean abundance 14.2%; dominated by *Armillaria cepistipes* 6.85% and *Armillaria gallica* 6.11%) but were of low abundance in other decay classes. *Bondarzewiaceae* (represented by *Heterobasidion sp.*) were frequently detected in all decay classes (mean abundances 10.3 – 18.9%) except in decay class 2 (1.87%). *Mycenaceae* were frequently found in decay classes 1 and 2 (mean abundances 7.88% and 12.5% both dominated by *Mycena rubromarginata*), whereas in decay class 3 and 4 they were much less frequent (mean abundances 2.77% and 3.71%). *Fomitopsidaceae* were frequently detected from decay class 2, 3 and 4 (mean abundances 7.44%, 17.1% and 5.81% dominated by *Fomitopsis pinicola*). *Schizoporaceae* were only highly frequently detected in decay class 4 (mean abundance 11.9%; dominated by *Hyphodontia alutacea* 5.73%, and *Hyphodontia alutaria* 3.08%).

(B) Sharedness of cOTUs and families between Fagus and Picea

We examined the sharedness of WIF cOTUs and genera between the tree species (Fig. S4). At the level of species (Fig. S4A), this was only possible for species that received unequivocally the same epitheth. Among the 160 where this was the case, 74 species (22 Ascomycota, 50 Basidiomycota, and 2 zygomycetes) were found on both *Picea* and *Fagus* logs. Some of them are not normally reported on this particular substrate, e.g. *Xylariaceae* species *Annulohypoxylon cohaerens*, *Hypoxylon fragiforme*, *Hypoxylon rubiginosum*,

Kretzschmaria deusta and *Nemania serpens* are known from literature to exclusively occur on *Fagus* logs and few other deciduous tree species, but in our case could also be detected with marginal abundances on *Picea* logs. At the level of genus, to assume sharedness identification to sp. of same genus minimally suffices, and with this, 35 genera are unique to *Fagus*, 34 to *Picea*, and 58 genera are shared (Fig. S4B).

Table S1. Decay rate, remaining mass and wood physico-chemical parameters of *Fagus* (FASY) and *Picea* (PIAB) deadwood logs in different decay classes (DC 1 through 4).

DC:	FASY_1	FASY_2	FASY_3	FASY_4	PIAB_1	PIAB_2	PIAB_3	PIAB_4
Decay rate k (y^{-1})	0.05 ± 0.01	0.05 ± 0	0.07 ± 0.01	0.09 ± 0.02	0.04 ± 0.01	0.04 ± 0.01	0.08 ± 0	0.08 ± 0.01
Remaining mass (%)	80.28 ± 2.82	58.16 ± 1.46	45.57 ± 1.4	30.75 ± 1.9	75.25 ± 2.01	58.26 ± 2.4	47.64 ± 1.5	31.19 ± 0.97
Density ($g*cm^{-3}$)	0.51 ± 0.02	0.37 ± 0.01	0.31 ± 0.01	0.2 ± 0.03	0.35 ± 0.01	0.27 ± 0.01	0.22 ± 0.01	0.15 ± 0.01
Rel. wood moisture (% dry mass)	49.83 ± 5.5	114.21 ± 14.66	138.22 ± 22.94	155.15 ± 9.14	48.72 ± 11.59	65.45 ± 13.38	95.27 ± 8.91	163.06 ± 24.62
C %	48.41 ± 0.16	47.92 ± 0.18	47.52 ± 0.37	47.51 ± 0.33	49.29 ± 0.31	49.92 ± 0.22	49.16 ± 0.35	51.39 ± 0.94
N %	0.13 ± 0.01	0.15 ± 0.01	0.18 ± 0.02	0.25 ± 0.02	0.08 ± 0.01	0.08 ± 0	0.08 ± 0	0.14 ± 0.02
C/N	365.22 ± 14.87	324.02 ± 22.45	282.87 ± 31.53	193.7 ± 15.62	629.69 ± 48.44	602.56 ± 19.24	591.22 ± 38.75	422.87 ± 52.44
C ($g*cm^{-3}$)	0.248 ± 0.01	0.175 ± 0.007	0.145 ± 0.005	0.093 ± 0.015	0.172 ± 0.005	0.134 ± 0.002	0.106 ± 0.008	0.079 ± 0.003
N ($g*cm^{-3}$)	0.0007 ± 0	0.0006 ± 0.0001	0.0005 ± 0	0.0005 ± 0.0001	0.0003 ± 0	0.0002 ± 0	0.0002 ± 0	0.0002 ± 0
Total Lignin (%)	26.09 ± 0.48	28.52 ± 0.73	31.21 ± 1.14	30.49 ± 2.18	29.5 ± 0.59	31.63 ± 1.06	33.63 ± 2.31	45.23 ± 3.78
pH	5.25 ± 0.1	4.84 ± 0.08	5.12 ± 0.14	4.99 ± 0.13	4.63 ± 0.1	4.33 ± 0.03	4.04 ± 0.16	4.26 ± 0.15

Table S2. Mean ligninolytic enzyme activities and macro/ micro nutrients of *Fagus* and *Picea* deadwood logs.

Parameters	***Fagus sylvatica***	***Picea abies***
Lac (mU/g)	51.5 ± 11.2	26.6 ± 10.3
MiP (mU/g)	15 ± 4.9	14.4 ± 5.8
MnP (mU/g)	18 ± 9	13.8 ± 6.1
Mn (µg/g)	12.2 ± 3	24.2 ± 3.2
Mg (mg/g)	0.2 ± 0	0.1 ± 0
Ca (mg/g)	0.4 ± 0	0.4 ± 0
K (mg/g)	0.6 ± 0.1	0.4 ± 0.1
Fe (µg/g)	3.8 ± 1.2	5.1 ± 1
Al (µg/g)	0.8 ± 0.2	1.5 ± 0.3
Cu (µg/g)	0.4 ± 0	0.4 ± 0
Zn (µg/g)	30.2 ± 3.3	45.2 ± 2.8
Ni (µg/g)	0.5 ± 0.3	0.1 ± 0

Table S3. Total observed OTU richness and estimated OTU richness (Chao1 and ACE) of *Fagus* and *Picea* deadwood logs in different decay classes (DC).

Deadwood species	Parameters	DC 1	DC 2	DC 3	DC 4	Total
Fagus sylvatica	OTU richness	44.8 ± 6.7	62 ± 10.2	68.9 ± 9	62.7 ± 14.8	58.3 ± 5.1
	Chao1	173.3 ± 24.3	163.1 ± 20.6	161.4 ± 22.1	182.6 ± 38.8	168.4 ± 12.6
	ACE	162 ± 22.2	171.1 ± 15.6	160.9 ± 20.7	176.2 ± 31.9	165.7 ± 11.1
	cOTU richness	36.25 ± 5.38	50.83 ± 8.96	60.57 ± 8	55.67 ± 9.92	49.42 ± 4.57
Picea abies	OTU richness	41.8 ± 5.4	69.6 ± 6.1	68 ± 5.5	74.8 ± 8.6	65.4 ± 4.6
	Chao1	125.4 ± 15.3	162.5 ± 19.4	162.1 ± 12.7	170.7 ± 19.6	157.7 ± 10
	ACE	139.1 ± 19.5	166.8 ± 16.2	173.4 ± 16.8	186 ± 22.5	169.6 ± 11.1
	cOTU richness	31.6 ± 8.24	55.2 ± 6.04	53.6 ± 5.17	66.89 ± 8.76	54.33 ± 4.71

Table S4. Summary of fungal sequences, OTU statistics and fungal phyla distribution from MOTHUR analysis.

Parameter	***Fagus***	***Picea***
Total sequences	39,640	42,163
Average sequences per sample	1,651.63	1,756.79
Total OTUs in all samples	506	504
Average OTUs per sample	58.29	65.41
Number of OTUs per phylum:		
Ascomycota	265	217
Basidiomycota	201	242
zygomycetes	6	7
Entomophthoromycota	0	1
Glomeromycota	0	1
Chytridiomycota	1	1
Unknown fungal OTUs	33	35

Table S5. Relationships between fungal richness, abundances of fungal families and cOTUs and activities of ligninolytic enzymes.

Parameter	Enzyme activities in *Fagus* (mU/g)			Enzyme activities in *Picea* (mU/g)		
	Laccase	**MiP**	**MnP**	**Laccase**	**MiP**	**MnP**
Fungal OTU richness (observed)	-0.09	-0.23	-0.04	**-0.34**	-0.14	-0.24
Fungal cOTU richness	-0.12	-0.25	-0.10	-0.16	-0.16	-0.26
Fungal OTU richness (Chao1)	0.01	-0.13	0.07	*-0.26*	-0.04	-0.15
Fungal OTU richness (ACE)	0.02	-0.22	0.09	-0.24	-0.07	-0.18
Family abundances						
Agaricomycetes I.S.	*0.27*	0.00	0.11	0.22	0.01	0.06
Bondarzewiaceae	NA	NA	NA	0.23	**0.37**	**0.45**
Chaetosphaeriaceae	-0.16	**-0.30**	*-0.25*	-0.12	-0.07	-0.08
Coniochaetaceae	-0.18	-0.06	-0.20	**-0.34**	-0.23	*-0.27*
Dacrymycetaceae	0.02	-0.13	**-0.32**	**-0.43**	**-0.38**	**-0.44**
Diatrypaceae	-0.18	-0.01	0.07	NA	NA	NA
Fomitopsidaceae	-0.21	-0.18	0.07	-0.24	-0.21	*-0.24*
Helotiaceae	-0.06	-0.01	-0.11	-0.09	-0.02	-0.14
Helotialaceae	NA	NA	NA	-0.27	-0.14	*-0.25*
Herpotrichiellaceae	-0.11	0.07	-0.15	**-0.29**	-0.17	**-0.36**
Hyaloscyphaceae	-0.12	**-0.37**	-0.19	-0.01	-0.02	-0.02
Leotiaceae	0.02	0.23	-0.08	NA	NA	NA
Marasmiaceae	0.05	0.04	0.22	-0.05	-0.02	-0.12
Meruliaceae	0.23	0.06	0.23	-0.23	-0.10	-0.17
mitosporic Helotiales	-0.04	**-0.42**	-0.09	0.09	0.04	0.12
Mycenaceae	0.19	0.10	-0.20	**-0.30**	-0.14	**-0.33**
Peniophoraceae	0.10	0.22	0.04	NA	NA	NA
Physalacriaceae	0.11	0.01	0.07	0.22	0.06	0.18
Polyporaceae	0.09	-0.08	0.23	-0.07	-0.12	-0.14
Schizoporaceae	**0.30**	0.01	0.10	-0.22	0	-0.06
Stereaceae	NA	NA	NA	0.08	0	0.03
Strophariaceae	-0.21	0.24	0.25	-0.15	-0.11	0.14
Trechisporaceae	NA	NA	NA	0.02	0.22	0.19
Xylariaceae	-0.02	**0.32**	0.17	-0.08	0.01	-0.11
cOTU abundances						
Amylostereum areolatum	NA	NA	NA	-0.11	-0.20	-0.16
Amylostereum chailletii	NA	NA	NA	0.16	0.13	0.09
Annulohypoxylon cohaerens	-0.07	**0.37**	-0.19	NA	NA	NA
Armillaria cepistipes	0.02	-0.04	-0.02	0.14	0.00	0.14
Armillaria gallica	-0.20	-0.22	-0.03	**0.32**	0.17	**0.30**
Ascocoryne cylichnium	0.14	**-0.30**	-0.12	-0.11	-0.02	-0.09
Ascocoryne sarcoides	-0.12	0.08	-0.18	NA	NA	NA
Bjerkandera adusta	0.10	**0.30**	0.19	NA	NA	NA
Botryobasidium botryosum	NA	NA	NA	-0.03	0.10	0.01
Calocera cornea	-0.01	-0.22	-0.22	NA	NA	NA
Ceriporiopsis gilvescens	0.13	-0.01	0.18	NA	NA	NA
Dacrymyces stillatus	NA	NA	NA	**-0.30**	**-0.30**	**-0.35**
Eutypa spinosa	-0.16	-0.03	0.18	NA	NA	NA
Fomes fomentarius	0.12	0.12	*0.26*	NA	NA	NA
Fomitopsis pinicola	-0.21	-0.18	0.07	-0.19	-0.22	-0.20
Heterobasidion sp.	NA	NA	NA	0.23	**0.37**	**0.45**
Hyphodontia alutacea	NA	NA	NA	*-0.25*	-0.11	-0.19
Hyphodontia alutaria	NA	NA	NA	0.05	0.23	0.16
Hypoxylon fragiforme	-0.09	**0.32**	-0.06	NA	NA	NA
Hypoxylon rubiginosum	**0.33**	**0.42**	0.19	NA	NA	NA
Marasmius alliaceus	0.09	0.22	**0.31**	NA	NA	NA

Parameter	Enzyme activities in *Fagus* (mU/g)			Enzyme activities in *Picea* (mU/g)		
	Laccase	**MiP**	**MnP**	**Laccase**	**MiP**	**MnP**
Megacollybia platyphylla	-0.05	-0.23	0.09	NA	NA	NA
Mycena alnetorum	**0.43**	0.12	0.13	-0.05	0.08	-0.06
Mycena purpureofusca	-0.08	-0.24	**-0.42**	NA	NA	NA
Mycena rubromarginata	NA	NA	NA	**-0.41**	*-0.25*	**-0.41**
Neobulgaria pura	0.08	**0.35**	0.02	NA	NA	NA
Oudemansiella mucida	0.14	0.21	0.20	NA	NA	NA
Peniophora incarnata	0.10	0.22	0.04	NA	NA	NA
Peniophorella praetermissa	*0.26*	-0.03	0.01	0.03	-0.05	-0.10
Phialocephala dimorphospora	-0.12	**-0.37**	-0.18	NA	NA	NA
Phlebia livida	0.05	0.06	0.20	-0.12	0.05	-0.19
Resinicium bicolor	0.21	0.09	0.02	**0.29**	0.10	0.12
Scopoloides hydnoides	0.02	**-0.29**	-0.18	NA	NA	NA
Sistotrema brinkmanii	0.01	0.13	0.09	-0.09	-0.13	-0.11
Trametes versicolor	0.00	-0.15	0.09	NA	NA	NA
Xylaria hypoxylon	-0.13	0.16	0.00	NA	NA	NA

Table S6 Relationships between fungal richness, abundances of fungal families and cOTUs and wood decomposition rates.

Parameter	*Fagus*		*Picea*	
	τ	***P***	***τ***	***P***
Fungal OTU richness (observed)	0.06	0.672	0.17	0.258
Fungal cOTU richness	0.07	0.636	0.20	0.20
Fungal OTU richness (Chao1)	0.01	0.921	0.13	0.382
Fungal OTU richness (ACE)	0.02	0.882	0.13	0.382
Family abundances				
Agaricomycetes I.S.	-0.12	0.421	*-0.29*	0.058
Bondarzewiaceae	NA	NA	0.08	0.596
Chaetosphaeriaceae	0.23	0.123	-0.19	0.181
Coniochaetaceae	0.10	0.501	-0.07	0.655
Dacrymycetaceae	*0.26*	0.070	-0.12	0.357
Diatrypaceae	0.10	0.495	NA	NA
Fomitopsidaceae	0.10	0.480	-0.23	0.185
Helotiaceae	0.08	0.588	0.13	0.312
Helotialaceae	NA	NA	0.21	0.224
Herpotrichiellaceae	-0.11	0.471	-0.06	0.793
Hyaloscyphaceae	0.17	0.233	-0.14	0.432
Leotiaceae	*-0.27*	0.065	NA	NA
Marasmiaceae	-0.01	0.955	-0.13	0.474
Meruliaceae	-0.12	0.394	0.09	0.705
mitosporic Helotiales	*0.26*	0.079	0.13	0.434
Mycenaceae	0.15	0.305	0.15	0.450
Peniophoraceae	-0.22	0.131	NA	NA
Physalacriaceae	-0.06	0.688	0.10	0.577
Polyporaceae	0.16	0.280	0.25	0.161
Schizoporaceae	0.23	0.110	**0.43**	0.005
Stereaceae	NA	NA	-0.08	0.668
Strophariaceae	-0.22	0.125	0.21	0.204
Trechisporaceae	NA	NA	0.24	0.150
Xylariaceae	**-0.44**	0.003	-0.07	0.605
cOTU abundances				
Amylostereum areolatum	NA	NA	-0.18	0.252
Amylostereum chailletii	NA	NA	-0.03	0.846
Annulohypoxylon cohaerens	-0.12	0.428	NA	NA
Armillaria cepistipes	-0.09	0.524	0.02	0.879
Armillaria gallica	0.00	1.000	*0.29*	0.059
Ascocoryne cylichnium	0.19	0.193	-0.03	0.868
Ascocoryne sarcoides	0.06	0.678	NA	NA
Bjerkandera adusta	-0.07	0.629	NA	NA
Botryobasidium botryosum	NA	NA	**0.43**	0.005
Calocera cornea	*0.25*	0.091	NA	NA
Ceriporiopsis gilvescens	0.01	0.919	NA	NA
Dacrymyces stillatus	NA	NA	-0.14	0.351
Eutypa spinosa	0.05	0.729	NA	NA
Fomes fomentarius	-0.01	0.929	NA	NA
Fomitopsis pinicola	0.10	0.480	-0.15	0.321
Heterobasidion sp.	NA	NA	0.08	0.617
Hyphodontia alutacea	NA	NA	**0.32**	0.036
Hyphodontia alutaria	NA	NA	**0.32**	0.036
Hypoxylon fragiforme	*-0.25*	0.083	NA	NA
Hypoxylon rubiginosum	-0.23	0.122	NA	NA
Marasmius alliaceus	-0.07	0.626	NA	NA

Parameter	*Fagus*		*Picea*	
	τ	***P***	***τ***	***P***
Megacollybia platyphylla	0.18	0.210	NA	NA
Mycena alnetorum	0.04	0.790	**0.45**	0.003
Mycena purpureofusca	**0.31**	0.034	NA	NA
Mycena rubromarginata	NA	NA	0.01	0.973
Neobulgaria pura	**-0.29**	0.049	NA	NA
Oudemansiella mucida	0.07	0.629	NA	NA
Peniophora incarnata	-0.22	0.131	NA	NA
Peniophorella praetermissa	0.14	0.353	-0.21	0.177
Phialocephala dimorphospora	**0.41**	0.005	NA	NA
Phlebia livida	-0.13	0.374	NA	NA
Resinicium bicolor	-0.15	0.319	-0.19	0.219
Scopoloides hydnoides	0.12	0.431	NA	NA
Sistotrema brinkmanii	-0.11	0.449	-0.15	0.315
Trametes versicolor	**0.32**	0.031	NA	NA
Xylaria hypoxylon	**-0.31**	0.033	NA	NA

Table S7. Fungal cOTUs and their potential roles in deadwood decomposition**.** FASY= *Fagus sylvatica*, PIAB = *Picea abies*.

Sample	Fungal cOTUs (Abundance in %)	Potential roles in deadwood decomposition	Published ecological role	Reference
8280 (FASY)	*Fomes fomentarius* (75.22)	laccase producer	important producer of lignocellulose-degrading enzymes endo-1,4-β-glucanase, 1,4-β-glucosidase, cellobiohydrolase, endo-1,4-β-xylanase, 1,4-β-xylosidase, MNP, and laccase	Větrovský *et al.* (2013)
8282 (FASY)	*Annulohypoxylon cohaerens* (46.18)	MiP producer	*Annulohypoxylon spp*. are excellent producers of hydrolytic enzymes	Robl *et al.* (2013)
8283 (FASY)	*Mycena alnetorum* (48.22)	laccase producer	laccase and peroxidase producer	Eduardo *et al*. (1997)
8298 (FASY)	*Trametes versicolor* (30.43)	important decomposer (causing high mass loss)	important decomposer (causing high mass and lignin loss)	Valmaseda *et al*. (1990)
8810 (FASY)	*Armillaria gallica* (18.92)	MnP producer	*Armillaria spp*. caused high mass and lignin loss	Valmaseda *et al*. (1990)
8818 (FASY)	*Kuehneromyces mutabilis* (36.62)	laccase producer	laccase producer	Cho *et al.* (1999)
8820 (PIAB)	*Resinicium bicolor* (66.50)	MiP and MnP producer	white-rot fungus able to produce ligninolytic enzymes, especially laccase	Shah *et al.* (2014)
8823 (PIAB)	*Amylostereum chailletii* (65.61)	important decomposer (causing high mass loss)	saprotrophic decay fungus occurring on fallen conifer logs	Vasiliauskas *et al*. (1998)
8825 (PIAB)	*Resinicium bicolor* (62.97)	laccase producer	white-rot fungus able to produce ligninolytic enzymes, especially laccase	Shah *et al.* (2014)
8828 (FASY)	*Trametes versicolor* (87.51)	important decomposer (causing high mass loss)	important decomposer (causing high mass and lignin loss)	Valmaseda v (1990)
8832 (FASY)	*Phlebia livida* (43.49)	laccase producer	*Phlebia spp*. known as laccase producers	Arora & Rampal (2002)

Figure S1. Number of sequence reads and number of detected OTUs (rarefaction curves calculated as individual rarefaction in PAST) of all samples used in the study. Four decay classes of (a) *Fagus* (FASY) and (b) *Picea* (PIAB) differentiated by line colors.

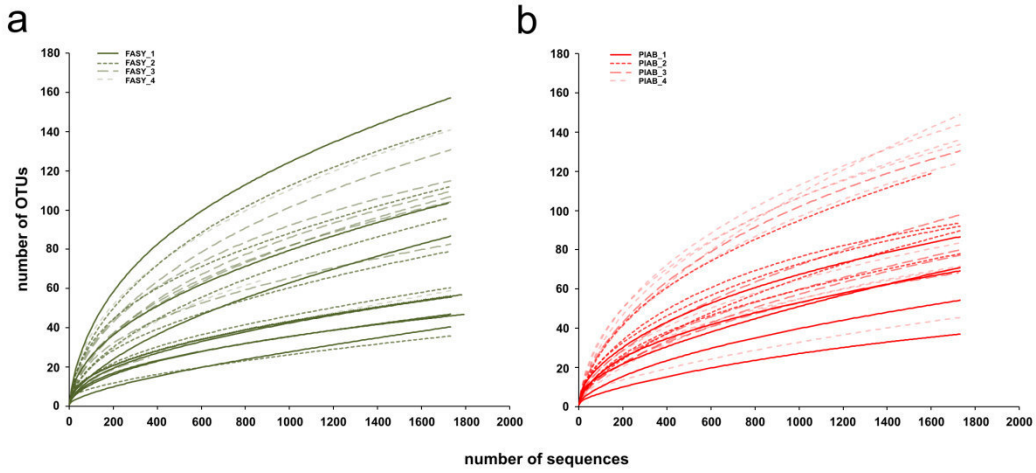

Figure S2. Relationships between fungal OTUs richness and decay classes (as determined by density (a) and remaining mass (b)), green = *Fagus sylvatica*, red = *Picea abies*.

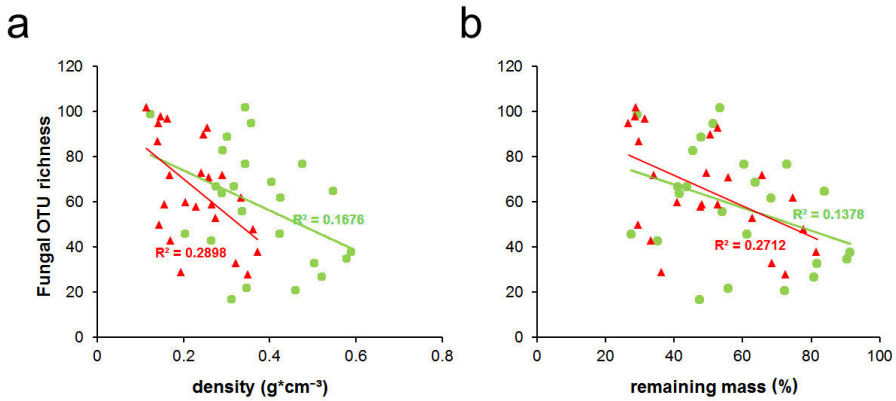

Figure S3. Heatmap displaying cOTU abundances and presences per sample. This figure is a companion to Fig. 2 of the main text and contains only the cOTUs that appear labeled in that figure. The rows are sorted by the number of sequences on the family level, which also only includes the cOTUs appearing in this table.

		Fagus sylvatica		*Picea abies*		total		sum on family level*	
cOTU = species	family#	n seqs	n samples	n seqs	n samples	n seqs	n samples	n seqs	n samples
Resinicium bicolor	Agaricomycetes I.S. (B)	319	11	10646	20	10965	31	10965	31
Mycena alnetorum	*Mycenaceae* (B)	1667	4	136	6	1803	10	5982	42
Mycena rubromarginata		0	0	1625	12	1625	12		
Mycena purpureofusca		1114	6	0	0	1114	6		
Mycena galericulata		635	4	11	2	646	6		
Mycena arcangeliana		0	0	535	5	535	5		
Mycena crocata		258	2	1	1	259	3		
Annulohypoxylon cohaerens	*Xylariaceae* (A)	2487	8	3	2	2490	10	5253	42
Hypoxylon rubiginosum		897	10	2	2	899	12		
Xylaria hypoxylon		837	3	0	0	837	3		
Kretzschmaria deusta		563	5	4	2	567	7		
Hypoxylon fragiforme		458	9	2	1	460	10		
Armillaria cepistipes	*Physalacriaceae* (B)	1858	13	999	8	2857	21	5243	45
Armillaria gallica		792	12	765	6	1557	18		
Oudemansiella mucida		829	6	0	0	829	6		
Heterobasidion sp.	*Bondarzewiaceae* (B)	6	2	4606	11	4612	13	4612	13
Trametes versicolor	*Polyporaceae* (B)	2513	7	1	1	2514	8	4409	17
Fomes fomentarius		1712	4	6	2	1718	6		
Datronia mollis		177	3	0	0	177	3		
Phlebia livida	*Meruliaceae* (B)	1588	4	4	3	1592	7	4405	33
Ceriporiopsis gilvescens		831	3	4	1	835	4		
Scopoloides hydnoides		629	9	0	0	629	9		
Phlebia radiata		523	2	0	0	523	2		
Bjerkandera adusta		413	6	1	1	414	7		
Phanerochaete sordida		412	4	0	0	412	4		
Amylostereum areolatum	*Stereaceae* (B)	4	2	2018	8	2022	10	3352	16
Amylostereum chailletii		1	1	1329	5	1330	6		
Fomitopsis pinicola	*Fomitopsidaceae* (B)	267	6	2326	8	2593	14	3300	21
Antrodia sinuosa		1	1	402	3	403	4		
Ischnoderma benzoinum		0	0	304	3	304	3		
Marasmius alliaceus	*Marasmiaceae* (B)	1520	7	5	4	1525	11	2678	21
Megacollybia platyphylla		1150	7	3	3	1153	10		
Hyphodontia alutacea	*Schizoporaceae* (B)	5	2	880	3	885	5	2043	23
Hyphodontia alutaria		1	1	493	11	494	12		
Hyphodontia subalutacea		472	2	1	1	473	3		
Hyphodontia nespori		0	0	191	3	191	3		
Eutypa spinosa	*Diatrypaceae* (A)	1239	9	0	0	1239	9	1239	9
Neobulgaria pura	*Leotiaceae* (A)	1108	5	0	0	1108	5	1108	5
Dacrymyces stillatus	*Dacrymycetaceae* (B)	10	4	808	16	818	20	1069	36
Calocera cornea		161	13	90	3	251	16		
Ascocoryne sarcoides	*Helotiaceae* (A)	622	13	27	5	649	18	1010	42
Ascocoryne cylichnium		313	13	48	11	361	24		
Kuehneromyces mutabilis	*Strophariaceae* (B)	662	2	0	0	662	2	983	8
Hypholoma capnoides		3	1	318	5	321	6		
Peniophorella praetermissa	Agaricomycetes I.S. (B)	100	6	831	11	931	17	931	17
Peniophora incarnata	*Peniophoraceae* (B)	894	4	0	0	894	4	894	4
Phialocephala dimorphospora	mitosporic Helotiales (A)	424	14	18	5	442	19	442	19
Inonotus radiata	*Hymenochaetaceae* (B)	437	2	2	2	439	4	439	4
Sistotrema brinkmanii	*Hydnaceae* (B)	144	10	158	11	302	21	302	21
Zignoella pulviscula	*Chaetosphaeriaceae* (A)	3	3	273	2	276	5	276	5
Sistotremastrum niveocremeum	*Trechisporaceae* (B)	16	2	245	4	261	6	261	6
Botryobasidium botryosum	*Botryobasidiaceae* (B)	67	3	109	8	176	11	176	11
Phialocephala fusca	*Ophiostomataceae* (A)	2	1	162	2	164	3	164	3

(A) = Ascomycota
(B) = Basidiomycota

#for Agaricomycetes I.S. the actual family level is not known
heatmap from white to dark within each column

Figure S4. Comparison of sharedness and species (= cOTU) distribution. Green colors indicate *Fagus*, red colors *Picea*. (a) Table of 74 species that are shared between *Fagus* and *Picea* made as MS Excel in-cell dot chart. The left and right columns are filled by a heatmap corresponding to overall cOTU size in number of sequences while the bars are relative to cOTU size within the tree species. Up to 150 sequences this dot chart is size-correct. (b) Area-correct Venn diagram for sharedness of genera (Pan-Omics Research, Pacific Northwest National Laboratory).

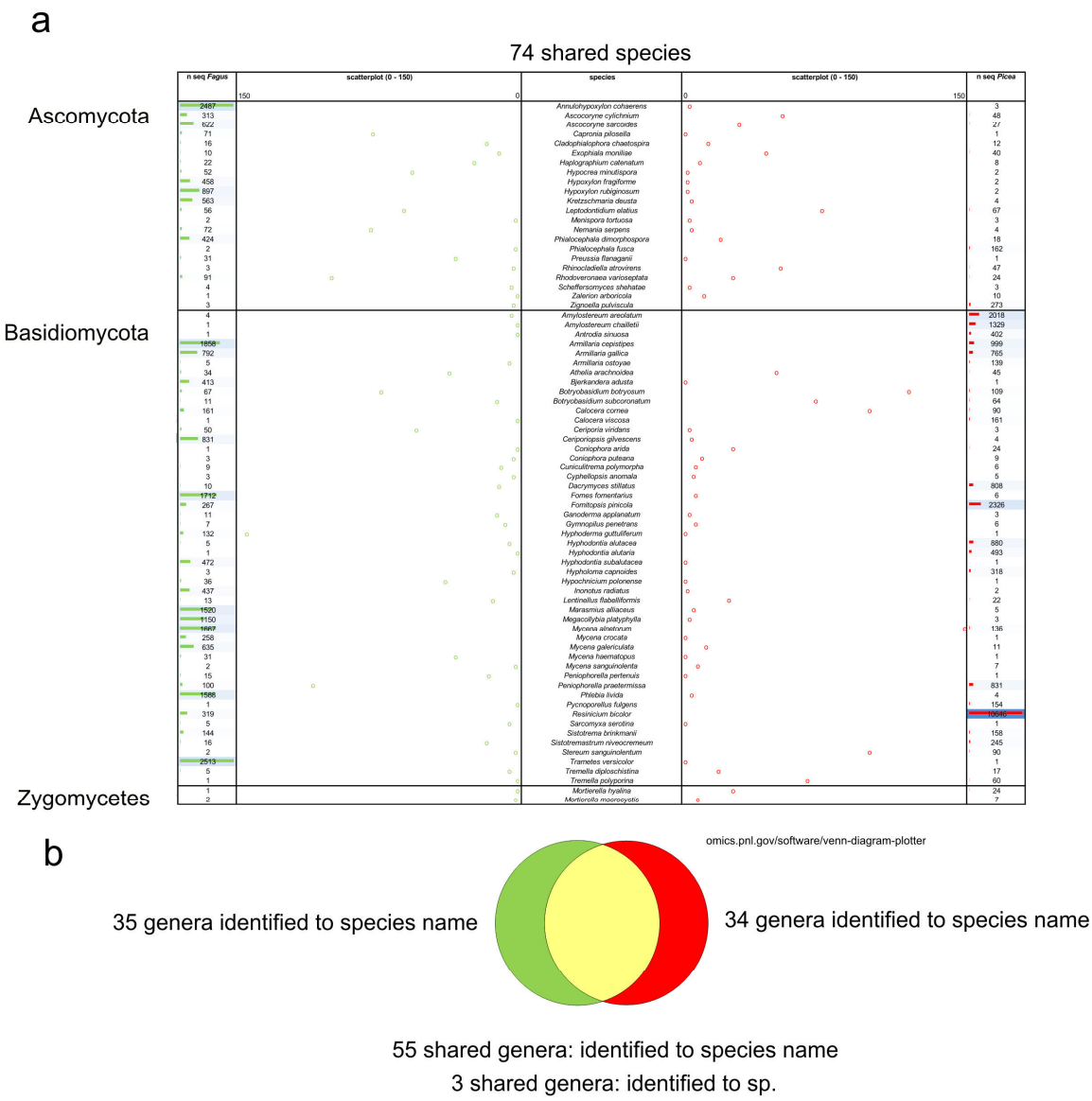

Figure S5. Heatmap (green-white) of mean abundances of highly detected fungal families and cOTUs in different decay classes (DC, labeled FASY_1 through FASY_4) in *Fagus sylvatica* deadwood logs.

Fagus sylvatica	FASY_1	FASY_2	FASY_3	FASY_4
Family				
Xylariaceae	26	10	5.15	0.74
Polyporaceae	14.6	17.8	11.4	10.1
Physalacriaceae	10.4	9.81	8.24	1.43
Leotiaceae	8.18	0.75	0.01	0.02
Meruliaceae	7.6	17	12	8.07
Marasmiaceae	0.29	8.4	11.1	15.5
Mycenaceae	4.95	5.95	9.92	23
cOTU				
Annulohypoxylon cohaerens	17.4	0.11	0	0
Trametes versicolor	12.3	4.44	0.03	10.1
Neobulgaria pura	7.43	0.5	0	0
Armillaria cepistipes	6.39	7.68	1.18	1.24
Peniophora incarnata	6.16	0.11	0	0
Hypoxylon rubiginosum	5.45	1.25	0	0
Mycena alnetorum	4.72	1.29	0	16.1
Armillaria gallica	3.78	0.9	1.43	0.19
Fomes fomentarius	0	13.3	11.3	0
Phlebia livida	0	8.37	4.04	4.93
Marasmius alliaceus	0.29	7.87	3.61	7.47
Xylaria hypoxylon	0.08	7.65	0	0
Eutypa spinosa	0.31	4.45	6.22	0
Inonotus radiata	0.04	4.18	0	0
Scopoloides hydnoides	0.32	4	0.26	3.09
Mycena purpureofusca	0.1	1.86	4.61	6.83
Megacollybia platyphylla	0	0.48	7.47	7.99
Ceriporiopsis gilvescens	0	0.6	6.26	0
Oudemansiella mucida	0.01	1.22	5.64	0
Mycena galericulata	0.02	0.02	5.15	0
Kretzschmaria deusta	0	0.19	4.54	0.74
Hyphodontia subalutacea	0	0	0	8.91
Bjerkandera adusta	0.66	3.19	0.01	0

Figure S6. Heatmap (red-white) of mean abundances of highly detected fungal families and cOTUs in different decay classes (DC, labeled PIAB_1 through PIAB_4) in *Picea abies* deadwood logs.

Picea abies	PIAB_1	PIAB_2	PIAB_3	PIAB_4
Family				
Agaricomycetes I.S.	26.4	42.7	29.6	17
Stereaceae	21.7	1.18	2.69	7.31
Physalacriaceae	14.2	0	5.94	0.79
Bondarzewiaceae	12.3	1.87	18.9	10.3
Mycenaceae	7.88	12.5	2.77	3.71
Fomitopsidaceae	0.29	7.44	17.1	5.81
cOTU				
Resinicium bicolor	26.1	36.6	27.6	16.5
Amylostereum areolatum	19.9	0.03	2.67	0.02
Heterobasidion sp.	12.3	1.87	18.9	10.3
Armillaria cepistipes	6.85	0	3.5	0.58
Armillaria gallica	6.11	0	2.43	0.03
Mycena rubromarginata	5.18	8.66	0.1	2.48
Dacrymyces stillatus	2.95	4.31	1.1	0.55
Mycena arcangeliana	2.2	2.67	0.74	0.27
Amylostereum chailletii	1.8	0.12	0.02	7.29
Peniophorella praetermissa	0.3	6.12	2.02	0.52
Fomitopsis pinicola	0.02	7.23	12.5	3.71
Antrodia sinuosa	0	0	4.49	0.03
Hyphodontia alutacea	0	0	0	5.73
Hyphodontia alutaria	0	0.01	0.09	3.08

Figure S7. Comparison of fungal ACE (P = 0.02) and Chao 1 (P = 0.01) richness of observed OTUs in *Picea* deadwood in converted forests (age-class spruce forest converted from beech forest) to that of *Fagus* deadwood in a semi-natural age-class beech forest.

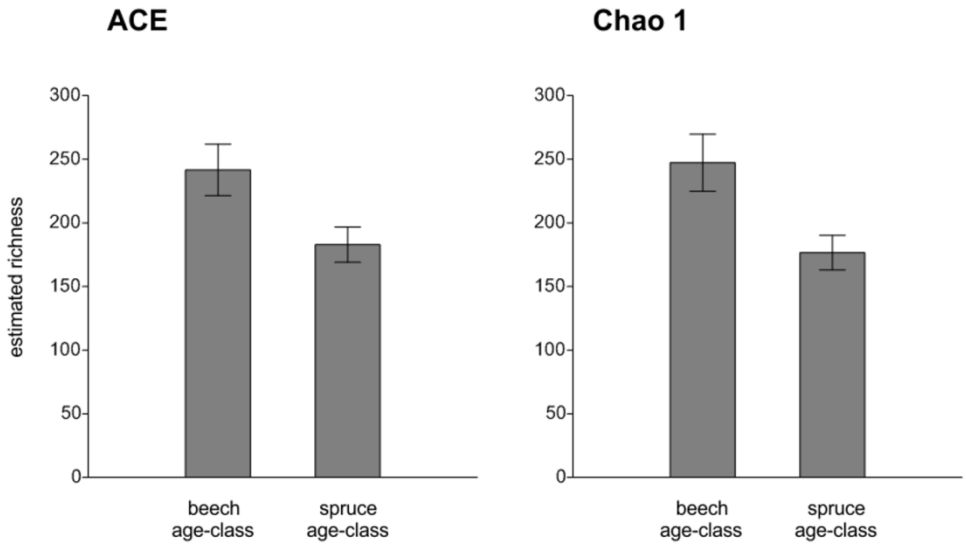

Figure S8. Some example *Fagus sylvatica* logs showing pseudosclerotial plates (PSPs) due to the colonization by *Xylariaceae*.

Table S8. List of MID tags for deadwood logs (samples) and additional characterization of sampling design. For further details see Hoppe *et al*. (2014).

CWD ID	MID tag used in pyrosequencing	Tree species	Forest manag.	Plot ID	Gauss-Krüger coordinates		Coordinates		Altitude	Soil type
					RW	HW	Latitude	Longitude		
8270	**ACGAGTGCGT**	*F. sylvatica*	spruce age-class	AEW1	3524799	5371201	48.478064 °N	9.334397 °E	763 m	Cambisol
8271	**ACGCTCGACA**	*P. abies*	spruce age-class	AEW1	3524799	5371201	48.478064 °N	9.334397 °E	763 m	Cambisol
8273	**AGACGCACTC**	*P. abies*	spruce age-class	AEW1	3524799	5371201	48.478064 °N	9.334397 °E	763 m	Cambisol
8276	**AGCACTGTAG**	*P. abies*	spruce age-class	AEW1	3524799	5371201	48.478064 °N	9.334397 °E	763 m	Cambisol
8277	**ATCAGACACG**	*F. sylvatica*	spruce age-class	AEW1	3524799	5371201	48.478064 °N	9.334397 °E	763 m	Cambisol
8820	**ATATCGCGAG**	*P. abies*	spruce age-class	AEW2	3526110	5360300	48.37998 °N	9.351452 °E	749 m	Leptosol
8821	**CGTGTCTCTA**	*F. sylvatica*	spruce age-class	AEW2	3526110	5360300	48.37998 °N	9.351452 °E	749 m	Leptosol
8822	**CTCGCGTGTC**	*P. abies*	spruce age-class	AEW2	3526110	5360300	48.37998 °N	9.351452 °E	749 m	Leptosol
8823	**TAGTATCAGC**	*P. abies*	spruce age-class	AEW2	3526110	5360300	48.37998 °N	9.351452 °E	749 m	Leptosol
8824	**TCTCTATGCG**	*P. abies*	spruce age-class	AEW2	3526110	5360300	48.37998 °N	9.351452 °E	749 m	Leptosol
8825	**TGATACGTCT**	*P. abies*	spruce age-class	AEW2	3526110	5360300	48.37998 °N	9.351452 °E	749 m	Leptosol
8279	**TACTGAGCTA**	*P. abies*	spruce age-class	AEW3	3526400	5363890	48.412253 °N	9.355592 °E	715 m	Cambisol
8280	**CATAGTAGTG**	*F. sylvatica*	spruce age-class	AEW3	3526400	5363890	48.412253 °N	9.355592 °E	715 m	Cambisol
8281	**CGAGAGATAC**	*P. abies*	spruce age-class	AEW3	3526400	5363890	48.412253 °N	9.355592 °E	715 m	Cambisol

CWD ID	MID tag used in pyrosequencing	Tree species	Forest manag.	Plot ID	Gauss-Krüger coordinates		Coordinates		Altitude	Soil type
					RW	HW	Latitude	Longitude		
8282	**ATACGACGTA**	*F. sylvatica*	spruce age-class	AEW3	3526400	5363890	48.412253 °N	9.355592 °E	715 m	Cambisol
8283	**TCACGTACTA**	*F. sylvatica*	spruce age-class	AEW3	3526400	5363890	48.412253 °N	9.355592 °E	715 m	Cambisol
8807	**CGTCTAGTAC**	*F. sylvatica*	spruce age-class	AEW3	3526400	5363890	48.412253 °N	9.355592 °E	715 m	Cambisol
8809	**TCTACGTAGC**	*F. sylvatica*	spruce age-class	AEW3	3526400	5363890	48.412253 °N	9.355592 °E	715 m	Cambisol
8842	**TGTACTACTC**	*F. sylvatica*	beech age-class	AEW4	3518205	5362394	48.399088 °N	9.244828 °E	783 m	Cambisol
8845	**ACGACTACAG**	*F. sylvatica*	beech age-class	AEW4	3518205	5362394	48.399088 °N	9.244828 °E	783 m	Cambisol
8846	**CGTAGACTAG**	*F. sylvatica*	beech age-class	AEW4	3518205	5362394	48.399088 °N	9.244828 °E	783 m	Cambisol
8826	**TACGAGTATG**	*P. abies*	beech age-class	AEW5	3530770	5364730	48.419609 °N	9.414683 °E	809 m	Cambisol
8827	**TACTCTCGTG**	*F. sylvatica*	beech age-class	AEW5	3530770	5364730	48.419609 °N	9.414683 °E	809 m	Cambisol
8828	**TAGAGACGAG**	*F. sylvatica*	beech age-class	AEW5	3530770	5364730	48.419609 °N	9.414683 °E	809 m	Cambisol
8810	**TCGTCGCTCG**	*F. sylvatica*	beech age-class	AEW6	3533100	5361900	48.394042 °N	9.445939 °E	767 m	Cambisol
8811	**ACATACGCGT**	*P. abies*	beech age-class	AEW6	3533100	5361900	48.394042 °N	9.445939 °E	767 m	Cambisol
8812	**ACGCGAGTAT**	*P. abies*	beech age-class	AEW6	3533100	5361900	48.394042 °N	9.445939 °E	767 m	Cambisol
8813	**ACTACTATGT**	*P. abies*	beech age-class	AEW6	3533100	5361900	48.394042 °N	9.445939 °E	767 m	Cambisol
8815	**ACTGTACAGT**	*F. sylvatica*	beech age-class	AEW6	3533100	5361900	48.394042 °N	9.445939 °E	767 m	Cambisol
8817	**AGACTATACT**	*P. abies*	beech	AEW6	3533100	5361900	48.394042	9.445939	767 m	Cambisol

CWD ID	MID tag used in pyrosequencing	Tree species	Forest manag.	Plot ID	Gauss-Krüger coordinates		Coordinates		Altitude	Soil type
					RW	HW	Latitude	Longitude		
			age-class				°N	°E		
8818	**AGCGTCGTCT**	*F. sylvatica*	beech age-class	AEW6	3533100	5361900	48.394042 °N	9.445939 °E	767 m	Cambisol
8819	**AGTACGCTAT**	*P. abies*	beech age-class	AEW6	3533100	5361900	48.394042 °N	9.445939 °E	767 m	Cambisol
8832	**ATAGAGTACT**	*F. sylvatica*	beech age-class	AEW7	3519430	5362080	48.396228 °N	9.261357 °E	773 m	Leptosol
8833	**CACGCTACGT**	*P. abies*	beech age-class	AEW7	3519430	5362080	48.396228 °N	9.261357 °E	773 m	Leptosol
8834	**CAGTAGACGT**	*P. abies*	beech age-class	AEW7	3519430	5362080	48.396228 °N	9.261357 °E	773 m	Leptosol
8835	**CGACGTGACT**	*F. sylvatica*	beech age-class	AEW7	3519430	5362080	48.396228 °N	9.261357 °E	773 m	Leptosol
8285	**TACACACACT**	*F. sylvatica*	beech natural forest	AEW8	3528400	5360600	48.38258 °N	9.382386 °E	779 m	Cambisol
8286	**TACACGTGAT**	*F. sylvatica*	beech natural forest	AEW8	3528400	5360600	48.38258 °N	9.382386 °E	779 m	Cambisol
8288	**TACAGATCGT**	*P. abies*	beech natural forest	AEW8	3528400	5360600	48.38258 °N	9.382386 °E	779 m	Cambisol
8289	**TACGCTGTCT**	*P. abies*	beech natural forest	AEW8	3528400	5360600	48.38258 °N	9.382386 °E	779 m	Cambisol
8290	**TAGTGTAGAT**	*P. abies*	beech natural forest	AEW8	3528400	5360600	48.38258 °N	9.382386 °E	779 m	Cambisol
8294	**TCGATCACGT**	*P. abies*	beech natural forest	AEW8	3528400	5360600	48.38258 °N	9.382386 °E	779 m	Cambisol

CWD ID	MID tag used in pyrosequencing	Tree species	Forest manag.	Plot ID	Gauss-Krüger coordinates		Coordinates		Altitude	Soil type
					RW	HW	Latitude	Longitude		
8295	**TCGCACTAGT**	*F. sylvatica*	beech natural forest	AEW8	3528400	5360600	48.38258 °N	9.382386 °E	779 m	Cambisol
8296	**TCTAGCGACT**	*F. sylvatica*	beech natural forest	AEW9	3530840	5359140	48.369336 °N	9.41522 °E	753 m	Leptosol
8297	**TCTATACTAT**	*F. sylvatica*	beech natural forest	AEW9	3530840	5359140	48.369336 °N	9.41522 °E	753 m	Leptosol
8298	**TGACGTATGT**	*F. sylvatica*	beech natural forest	AEW9	3530840	5359140	48.369336 °N	9.41522 °E	753 m	Leptosol
8801	**TGTGAGTAGT**	*P. abies*	beech natural forest	AEW9	3530840	5359140	48.369336 °N	9.41522 °E	753 m	Leptosol
8804	**ACAGTATATA**	*P. abies*	beech natural forest	AEW9	3530840	5359140	48.369336 °N	9.41522 °E	753 m	Leptosol

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CHAPTER 6

Influence of different forest system management practices on leaf litter decomposition rates, nutrient dynamics and the activity of ligninolytic enzymes: a case study from Central European forests

Witoon Purahong[1, 2], Danuta Kapturska[1, 3], Marek J. Pecyna[3], Elke Schulz[1], Michael Schloter[4], François Buscot[1, 5, 6], Martin Hofrichter[3], Dirk Krüger[1]

[1]UFZ-Helmholtz Centre for Environmental Research, Department of Soil Ecology,Theodor-Lieser-Str. 4, D-06120 Halle (Saale), Germany

[2]Technical University of Munich, Chair for Soil Science, Ingolstädter Landstr. 1, D-85758 Oberschleissheim, Germany

[3]Technical University of Dresden, International Graduate School (IHI) Zittau, Markt 23, D-02763 Zittau, Germany

[4]Helmholtz Zentrum München, Research Unit for Environmental Genomics, Ingolstädter Landstr. 1, D-85758 Oberschleissheim, Germany

[5]University of Leipzig, Institute of Biology, Johannisallee 21-23, D-04103 Leipzig, Germany

[6]German Centre for Integrative Biodiversity Research (iDiv), Halle-Jena-Leipzig, Deutscher Platz 5e, D-04103 Leipzig, Germany.

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Influence of Different Forest System Management Practices on Leaf Litter Decomposition Rates, Nutrient Dynamics and the Activity of Ligninolytic Enzymes: A Case Study from Central European Forests

Witoon Purahong[1,2*☯], Danuta Kaptursk[1,3☯], Marek J. Pecyna[3☯], Elke Schulz[1], Michael Schloter[4], François Buscot[1,5], Martin Hofrichter[3], Dirk Krüger[1]

1 UFZ-Helmholtz Centre for Environmental Research, Department of Soil Ecology, Halle (Saale), Germany, **2** Chair for Soil Science, Technical University of Munich, Oberschleissheim, Germany, **3** Department of Bio- and Environmental Sciences, International Institute Zittau, Technische Universität Dresden, Zittau, Germany, **4** Research Unit for Environmental Genomics, Helmholtz Zentrum München, Oberschleissheim, Germany, **5** German Centre for Integrative Biodiversity Research (iDiv) Halle - Jena - Leipzig, Leipzig, Germany

Abstract

Leaf litter decomposition is the key ecological process that determines the sustainability of managed forest ecosystems, however very few studies hitherto have investigated this process with respect to silvicultural management practices. The aims of the present study were to investigate the effects of forest management practices on leaf litter decomposition rates, nutrient dynamics (C, N, Mg, K, Ca, P) and the activity of ligninolytic enzymes. We approached these questions using a 473 day long litterbag experiment. We found that age-class beech and spruce forests (high forest management intensity) had significantly higher decomposition rates and nutrient release (most nutrients) than unmanaged deciduous forest reserves ($P<0.05$). The site with near-to-nature forest management (low forest management intensity) exhibited no significant differences in litter decomposition rate, C release, lignin decomposition, and C/N, lignin/N and ligninolytic enzyme patterns compared to the unmanaged deciduous forest reserves, but most nutrient dynamics examined in this study were significantly faster under such near-to-nature forest management practices. Analyzing the activities of ligninolytic enzymes provided evidence that different forest system management practices affect litter decomposition by changing microbial enzyme activities, at least over the investigated time frame of 473 days (laccase, $P<0.0001$; manganese peroxidase (MnP), $P=0.0260$). Our results also indicate that lignin decomposition is the rate limiting step in leaf litter decomposition and that MnP is one of the key oxidative enzymes of litter degradation. We demonstrate here that forest system management practices can significantly affect important ecological processes and services such as decomposition and nutrient cycling.

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* E-mail: witoon.purahong@ufz.de

☯ These authors contributed equally to this work.

Introduction

Almost one-third of the world's total forest area (ca. 1.2 billion ha) is managed primarily for wood biomass production [1]. This figure is even higher in European forests (excluding those in the Russian Federation), where 57% of the total cover is managed for wood production [1]. Forest system management practices include the forest management itself, but also conversion from one forest type to another [2,3]. Conversion of forests includes the maintenance of non-autochthonous forests in Central Europe, for example, where European beech dominated forests were converted to age-class Norway spruce or Scots pine forests [4]. Forest management and conversion result in a decline of "naturalness" and an increase in the "artificialness" of forest ecosystems [5] e.g. from natural to semi-natural (natural regeneration) and to artificial regeneration systems (planted forest). Therefore, shifts in forest system management practices can change tree species composition and richness, stand density and age structure, either slowly over a number of decades, or perhaps suddenly because of clear-felling or burning of old growth stands [6,7].

Although forest system management practices have the potential to affect most characteristics, compositions and structures of the entire forest ecosystem, effects on ecological processes in soils are still unclear [2,7,8]. For example thinning and pruning operations, which are common in intensively managed forests, have been found to affect microclimatic conditions such as light penetration, air movement and temperature [9] and therefore

should also influence abiotic and biotic soil properties. Even more pronounced changes can be expected if, as a result of conversion of forest type, dominant tree species are replaced; this will influence the diversity and community structure of both above- and below-ground biota, which interact with the plants [7,10].

Decomposition of plant litter is a complex ecological process that is regulated by three main drivers: climate, litter quality and the decomposer communities [11–13]. Litter quality (as determined by initial N, C/N ratio, lignin/N ratio, etc.) not only influences litter decomposition rate, but also the dynamics of nutrient mineralization and immobilization [13]. The nutrient dynamics directly affects the concentrations of nutrient elements in litter that in turn, could affect microbial decomposer and detritivore communities [14]. Microbial decomposers directly involve with litter decomposition by secreting extracellular enzymes that degrades both readily available substrates such as sugars, starch and amino acids, and larger complex substrates such as cellulose, hemi-cellulose and lignin [15]. Some studies show that lignin decomposition is the rate limiting step in litter decomposition [16,17], thus activities of ligninolytic enzymes may greatly influence the litter decomposition rates.

In an intensively managed forest, large fractions of woody biomass are normally removed or harvested, thus leaf litter fall remains the main source of organic plant matter input into forest soil [18–20]. For this reason, leaf litter decomposition is the key ecological process that determines the sustainability of managed forest ecosystems. However, despite the importance of leaf litter decomposition for semi-natural and artificially regenerated forests, very few studies have investigated this process with respect to the management practices [15,21], which is especially pronounced in Central Europe. In addition, leaf litter decomposition is strongly influenced by seasonal climatic variations, thus all short term experiments with a duration of less than 1 year are likely to suffer from biases as a result of seasonal variability and the true effects of forest system management practices on decomposition and nutrient dynamics could not be reasonably determined [22]. Nevertheless, in the South European Mediterranean climatic zone, some unique experiments investigated the decomposition rates and nutrient cycling of two different forests (natural beech vs. planted Scots pine forests) over 2 years [12,13]. These experiments showed that different forest management practices, with different dominant tree species, could alter decomposition rates and the annual amount of N returning to the soil [12,13].

In this study, we aimed to examine mixed deciduous leaf litter decomposition rates and the dynamics of macronutrients (N, Mg, K, Ca, P) under different forest system management practices, including natural, near-to-nature, age-class European beech dominated deciduous forests and converted forests (from European beech to monoculture Norway spruce) by means of a litterbag experiment lasting 473 days. We also investigated the activity of microbial ligninolytic enzymes and examined whether they can help to explain the leaf litter decomposition rates and nutrient dynamics under different forest system management practices. We hypothesized that the decomposition rate of deciduous litter is lower in spruce forest than in other deciduous beech dominated forests [23]. In deciduous beech dominated forests, we expected variations according to management intensity: that leaf litter decomposition rate would be highest in unmanaged natural forest, next highest in near-to-nature forest and lowest in high management intensity age-class forest [5,15]. Intensive forest management practices were found to negatively affect lignocellulytic enzyme activities which have been reported to be a good predictor of the litter decomposition rate [15]. The results obtained from converted forests do not directly demonstrate the sustainability of such systems, but rather increase our understanding of the efficiency of the artificial system decomposer communities to break down material derived from more natural systems in the study area.

Materials and Methods

Ethics Statement

Field work permits were issued by the responsible environmental offices of the Free State of Thuringia (according to § 72 BbgNatSchG).

Study Area and Treatments

The study was carried out at four forest sites across the Hainich-Dün region in Central Germany, including the Hainich National Park and its surroundings; (about 1,300 km^2; 51°16'N 10°47'E), part of the German Biodiversity Exploratories [24]. The forests in this region grow on soils over limestone bedrock [4]. The main soil types in the study area are Luvisol and Stagnosol [24]. The soil pH is weakly acidic (5.1±1.1; mean±SD; [25]). Thin litter layers (in the range 2–5 cm) were recorded at all four forest sites. The annual mean temperature and precipitation ranged, respectively, from 6.5–8°C and 500–800 mm [4,24]. Deciduous forests (mainly European beech, *Fagus sylvatica* dominated) are dominant in this area, covering about 83% of the total forest area. These forests can be classified, according to management types, as unmanaged forest reserves, farmers' forests, selectively cut forests, and age-class forests [4]. The age-class management type covers the largest forest area (around 57%). The remaining forests in this region (17%) are coniferous forests dominated by Norway spruce (*Picea abies*) [4]. All information pertaining to these forests has been described in detail by [4,24]. In this study, four forest sites (100 m × 100 m) were selected, based on their forest system management; each site represents a treatment (the main characteristics of the selected sites are presented in table 1). All sites were located less than 30 km apart, to reduce the influence of variations in geography and climate. Within each forest site, we assigned three plots (2 m × 8 m) located on flat land; these represent three replicates. The four treatments (forest management practice) in this study were: (i) Norway spruce age-class forest (SA, planted Norway spruce forest converted from European beech forest, even-age forest structure); (ii) European beech age-class forest (BA, semi-natural forest with natural regeneration, even-age forest structure); (iii) European beech selection cutting forest (BS, near-to-nature forest management with natural regeneration, uneven-age forest structure); and (iv) unmanaged deciduous forest reserves dominated by European beech (BU, uneven-age forest structure) [4,24]. We classified the sites on the basis of a silvicultural management intensity indicator (SMI; 0–1; 0 = lowest forest management intensity, 1 = highest forest management intensity) [26]. The SMI was low in the BU (0.09) and BS (0.14) forests but high in the SA (0.40) and BA (0.46) forests [26]. SMI was calculated as the average of a risk component (SMI_r, the risk of stand loss, function of tree species and stand age) and a density component (SMI_d, stand density, a function of the silvicultural regime, stand age and tree species.) [26].

Leaf Litter Collection and Litterbag Method

At each forest site, freshly fallen leaves of deciduous species were collected from the forest floor in October, 2009. Deciduous litter collected from SA was derived from deciduous trees naturally regenerated within this site. The leaves from each site were separated according to species and air dried to constant weight at room temperature. Ten grams of local mixed leaves (representa-

Table 1. Main characteristics of the selected forest sites in this study.

Site characteristic	Spruce age-class forest (SA)	Beech age-class forest (BA)	Beech selection cutting forest (BS)	Beech unmanaged forest (BU)
Location	Mühlhausen	Revier Sollstedt	Revier Keula	Hainich National Park
Mean annual T(°C)	6.5–8	6.5–8	6.5–8	6.5–8
Annual rainfall (mm)	500–800	500–800	500–800	500–800
Tree species richness (higher than 5 m)	5	3	2	1
Dominant tree species	*Picea abies* (>80%)	*Fagus sylvatica* (>80%)	*Fagus sylvatica* (>90%)	*Fagus sylvatica* (~100%)
Tree species cover ~5–10%	*Fagus sylvatica Acer* sp., *Quercus* sp.	*Acer* sp., *Fraxinus* sp.	*Acer* sp.	–
Forest system	Planted forest	Semi-natural	Semi-natural	Natural forest reserves
Forest history	Converted from beech forest	Natural regeneration	Natural regeneration	Unmanaged forest, no wood extraction > 60 yrs.
Forest age structure	Even-age (~80 yrs.)	Even-age (~30 yrs.)	Uneven-age	Uneven-age
SMI	High (0.40)	High (0.46)	Low (0.14)	Natural, very low (0.09)
Soil type	Stagnosol (Luvisol-Stagnosol)	Luvisols (Luvisol-Stagnosol)	Luvisols (Luvisol-Stagnosol)	Luvisols (Luvisol-Stagnosol)

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tive of the litter composition of the deciduous tree species at each respective site, Table 1, 2) were placed in nylon litterbags (25 cm × 25 cm, 2 mm mesh size). It has been reported that this mesh size is sufficiently small to minimize losses of leaf litter due to breakage, but to permit the activity of aerobic microbes and other decomposers such as micro-fauna [27–29]. A total of 216 litterbags were used for the experiment. At the end of the litter fall period (13 November 2009), 180 litterbags were placed in a horizontal position in the upper litter horizon at the study plots (45 litterbags per management type resulting in 15 bags per plot) while 36 litterbags (9 per treatment) were retained to determine the initial dry mass (oven-dried at 105°C ≥24 hr. until constant weight), nutrient element concentrations and lignin content of the litter. Litterbags were retrieved on five sampling dates: in 2010 on 10 February (89 days after incubation commenced, DAI), 12 May (180 DAI), 24 August (284 DAI), 10 November (362 DAI) and in 2011 on 1 March (473 DAI). On each sampling occasion, a random sample of nine litterbags per treatment (three litterbags per plot) were carefully removed, each put in a separate clean plastic bag to reduce the loss of small fragments and transported on ice (0°C) to the laboratory within 4 hrs. In the laboratory, litterbags were processed immediately. The extraneous organic materials adhering to the outside of the bags were removed and the leaf litter from the three litterbags retrieved from the same plot and treatment was pooled and its wet weight determined. Thus, three composite samples from each site were obtained for each treatment and DAI and each composite sample was homogenized and subsampled for determination of dry mass, nutrient concentrations, lignin content and ligninolytic enzyme activities.

Physicochemical Analyses

The dry mass of leaf litter samples was determined after oven-drying at 105°C to constant weight, i.e. for ≥24 hr. Initial and subsequent leaf litter samples from different incubation periods were analyzed for C, N, and lignin. Total C and N were determined by dry combustion at 1000°C with an Elementar Vario EL III (Hanau, Germany) elemental analyzer (DIN/ISO 10694 (Aug. 1996)). Nutrient ions were determined using inductively coupled plasma (ICP) optical emission spectrometry (ICP-OES) and mass spectrometry (ICP-MS) according to manufacturers' specifications. The ICP-OES device "Optima 3000" (PerkinElmer Inc., Waltham, MA, USA) was calibrated using "ICP multi-element standard solution IV" (Mg, K, Ca, Mn, Fe, Cu) as well as "phosphorus ICP standard" (both from Merck, Darmstadt, Germany) in 1 mg/L and 10 mg/L concentrations. The ICP-MS "Elan DRC-e" (PerkinElmer) device was calibrated using "ICP multi-element standard solution VI" (V, Mn, Fe, Co, Cu; Merck) in 2 μg/L, 15 μg/L and 50 μg/L concentrations. Samples were derived from filtered (0.7 μm Whatman GF/F filter, GE Healthcare, Buckinghamshire, UK), aqueous extracts of 10 g coarsely ground (on dry ice) litter material from one replicate. The mean values for the three biological replicates from each sampling point were calculated and presented as μg per gram of litter dry mass. Extractions and ion determinations were undertaken once for each biological replicate, dry mass determination was performed three times.

Total lignin was obtained by summing Klason lignin (acid insoluble lignin) and acid soluble lignin [30]. Klason lignin content was determined gravimetrically as the dry mass of solids after sequential hydrolysis with sulfuric acid (72% w/w); in a second step, acid soluble lignin was measured UV-photometrically in 4% H_2SO_4 [31,32]. All physico-chemical analyses were conducted in triplicate on the same subsample.

Ligninolytic Enzyme Activities

Aliquots of all samples from 89 to 473 DAI were used to determine ligninolytic enzyme activities. Three important enzymes were chosen: laccase (EC 1.10.3.2), peroxidase (EC 1.11.1.7) and manganese peroxidase (MnP; EC 1.11.1.13) [33,34].

Laccase and peroxidase activities were spectrophotometrically determined in 1 mL cuvettes by following the oxidation of ABTS (2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) at 420 nm ($\varepsilon_{420} = 36\ mM^{-1}cm^{-1}$) in a sequential assay in 50 mM sodium citrate buffer at pH 4.5 (final concentrations: 50 mM sodium citrate buffer, pH 4.5; 0,3 mM ABTS; 100 nM H_2O_2; 50 μL sample in 1 mL reaction). Measurement started with the addition of ABTS, the ABTS oxidation without H_2O_2 (laccase activity) was

Table 2. Information on the litter composition for individual forest sites.

Forest management practice	Litter composition (%)			
	Fagus sylvatica	*Acer* sp.	*Quercus* sp.	*Fraxinus* sp.
Spruce age-class forest (SA)	70	25	5	0
Beech age-class forest (BA)	85	10	0	5
Beech selection cutting forest (BS)	90	10	0	0
Beech unmanaged forest (BU)	100	0	0	0

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recorded for some time, before H_2O_2 was added; the peroxidase activities were corrected by those of laccase.

Manganese peroxidase activities were specifically assayed as described previously by monitoring the formation of Mn^{3+}–malonate complexes at 270 nm ($\varepsilon_{270} = 11{,}59\ mM^{-1}cm^{-1}$, [35]) (final concentrations: 50 mM Na-malonate buffer, pH 4.5; 0.5 mM $MnCl_2$, 100 nM H_2O_2; 50 µL sample in 1 mL reaction). Measurement started when H_2O_2 was added.

Samples were derived from 10-fold concentrated (''Vivaspin6'' centrifugal concentrator devices with 10 kDa MWCO; Sartorius Stedim Biotech GmbH, Göttingen, Germany) aqueous extracts of 10 g coarsely ground (on dry ice) litter material from one replicate. In all cases, the mean values of triplicate determinations were calculated and expressed in international units (U) defined as the amount of enzyme that forms 1 mmol product min^{-1} under the assay conditions used; enzyme activities are given per gram of dry mass of the particular litter sample. For each extract, enzyme activity and dry mass were determined three times. All chemicals used were purchased from Sigma-Aldrich (Steinheim, Germany) and Merck (Darmstadt, Germany).

Statistical Analysis

Total C and N were expressed as a percentage of the initial value, based on ash-free dried mass. Nutrient ions (Mg, K, Ca, P) were expressed as percentage of initial values based on dry litter mass. The expression of the N, Mg, K, Ca and P contents in the leaf litter as a percentage of the initial content allowed us to determine the net N, Mg, K, Ca and P dynamics [36]. Mass loss was expressed as the percentage of remaining dried mass compared with initial dried mass. Decomposition rates (k) for each forest type (treatment) were determined by fitting a single negative exponential decay equation [37] to remaining leaf litter dried mass over 1.30 years (473 days) in the form:

$$X/X_0 = e^{-kt}$$

where X = dried mass remaining at time t, X_0 = original mass, ''e'' = the base of natural logarithms, k = decomposition rate, and t = time (year). All data were analyzed using a one-way analysis of variance (ANOVA) incorporating the Fisher's Least Significant Difference (LSD) implemented in SPSS software (IBM SPSS Statistics 19, New York, NY, USA) to determine the differences ($P<0.05$) between different treatments at each DAI. All datasets were tested for normality using Shapiro-Wilk and Jarque-Bera tests and the equality of group variances was examined using Levene's test. ANOVA residuals were also plotted to determine the normal probability using Shapiro-Wilk test. In addition, ligninolytic enzyme activities were also analyzed using two-way (forest system management practice and DAI) ANOVA with $P<0.05$ considered significant, implemented in the PAST program [38].

Results

Effect of Forest Types on Litter Decomposition Rates

The remaining mass of leaf litter varied significantly with forest system management practice (treatments). Surprisingly, we did not find a negative effect of high forest management intensity and/or conversion of forest types on the leaf litter decomposition rate for any of the DAI sampling times. In general, leaf litter in unmanaged beech forest (BU) decomposed relatively slower than in other forests, and the results were significant at 362 and 473 DAI (Fig. 1). One-way ANOVA demonstrated that at 362 DAI, spruce age-class (SA), beech age-class (BA) and beech selecting cutting (BS) forests had significantly lower mass remaining (higher decomposition rates) than BU ($F = 10.37$, $P = 0.0039$). At the end of the experiment, SA and BA had significantly lower mass remaining (higher decomposition rates) than BU ($F = 4.58$, $P = 0.0378$) (Fig. 1; Table 3).

Nutrient Concentrations and Effect of Forest Types on Nutrient Dynamics

Leaf litter from BU had lower amounts of N and higher C/N and total lignin/N ratios compared with other forest management practices (Table 4). Initial metal ion concentrations (V, Fe, Co, Cu) under different forest management practices were relatively similar (Table 4). All initial nutrient concentrations and other quality parameters of leaf litter under different forest management practices are listed in table 4.

In general, the amounts of nutrients in leaf litter relative to the initial values (nutrient dynamics) were significantly influenced by forest system management practice. The changes in the amounts of different nutrients relative to their initial values, C/N and total lignin/N ratios over 473 days of incubation are shown in Figures 1, 2 and 3. For most nutrients (except C), litter from BU showed significantly slower nutrient release compared with other forest system management practices. For N and P dynamics, nutrient immobilization was clearly observed in the BU forest, where the percentage to initial values of N and P increased from the beginning of the experiment onwards, reaching peaks at 284 DAI (N = 166.49%) or 89–284 DAI (P = 277.70–247.80%) and decreasing thereafter. Similar patterns of N and P immobilizations were also observed in litter from BS, but with much lower magnitudes compared to the BU litter (N and P reached a peak at 284 DAI, with 115.27, 121.74%, respectively). Litter from SA only exhibited P immobilization and, at the first sampling date, N was released (89 DAI). In the BA forest, there were no clear patterns observed for either N or P immobilization, and N and P were

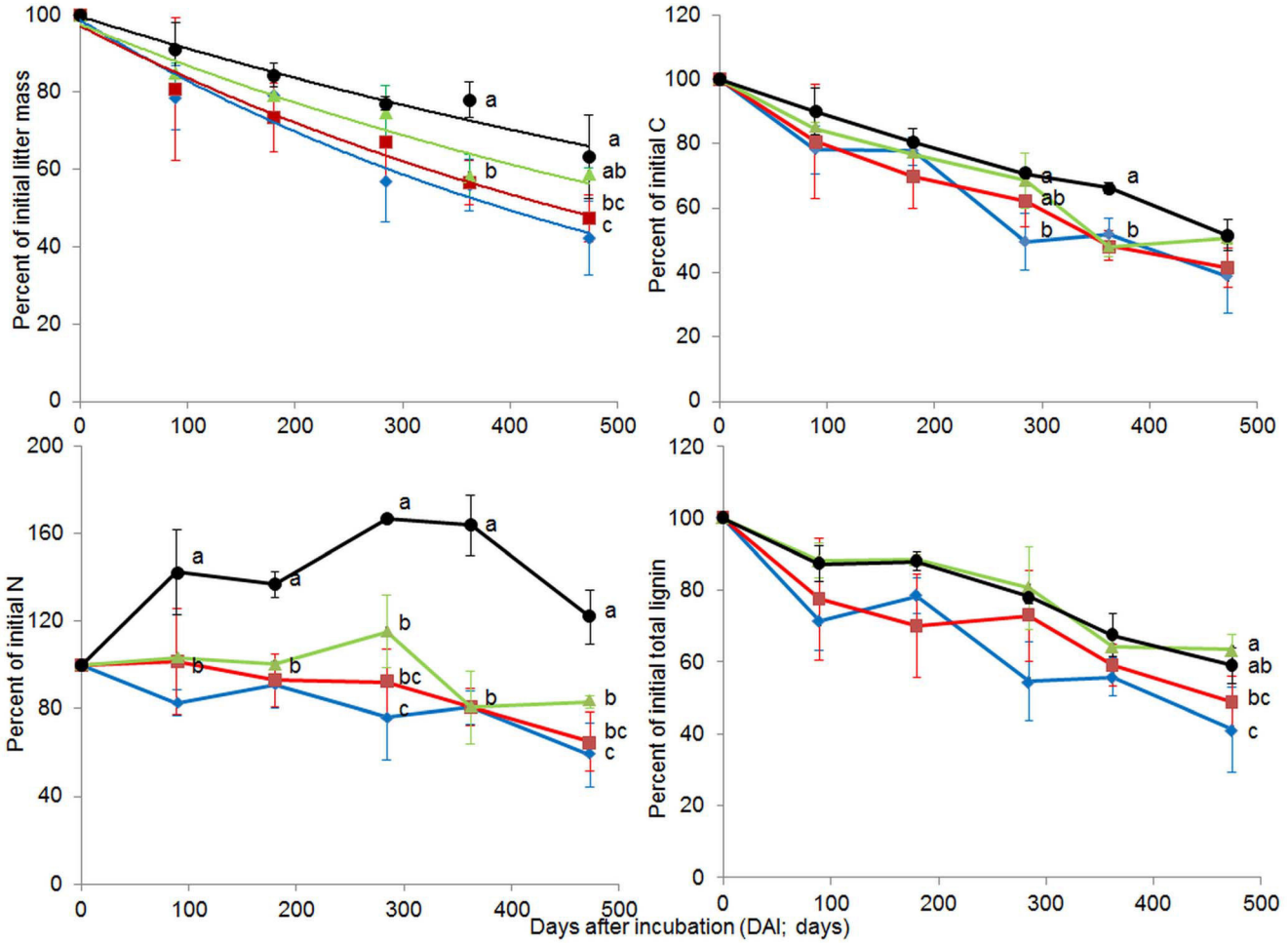

Figure 1. Remaining amount of leaf litter dry mass, carbon, nitrogen and total lignin during decomposition under different forest system management practices. Norway spruce age-class forest (blue, SA), European beech age-class forest (red, BA), European beech selective cut forest (green, BS) and unmanaged deciduous forest reserves dominated by European beech (black, BU) (mean ± SD, n = 3). Different letters indicate significant differences according to one-way ANOVA incorporating Fisher's Least Significant Difference (from 89 DAI to 473 DAI).
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released at 180 DAI. For C and K dynamics, all forests exhibited an exponential decrease from 89 DAI, however the reduced amounts of C and K varied significantly with forest system management practice (Figs. 1 and 3). Exponential nutrient release was also found for Mg and Ca under SA, BA and BS forest management practices but not under BU, where Mg and Ca had decreased at 89 DAI, increased at 180 DAI, reached a peak at 284 DAI (Mg = 113.88%, Ca = 239.21%) and decreased thereafter. The C/N ratio of leaf litter in different forests showed a similar trend: the C/N ratio decreased until 362 DAI then remained constant or slightly increased at 473 DAI (Fig. 2). At 89 DAI, the C/N ratios of all forest types were similar ($F = 1.25$, $P = 0.3545$). The BA forest had a significantly higher C/N ratio in leaf litter compared with other forests from 284 DAI until the end of the experiment (Fig. 2).

Table 3. Mean decay rate constants (k), coefficients of determination (r^2) describing the fit of the decay model ($P<0.05$), and mean percentage of leaf remaining mass at the end of the study (473 days after incubation) under different forest system management practices.

Forest system management practice	K (year^{-1})	r^2 (P value)	Remaining mass (%)
Age-class spruce forest (SA)	0.64±0.12 ^{c}	0.96 (**$P = 0.001$**)	42.17±9.61^{c}
Age-class beech forest (BA)	0.55±0.08 ^{bc}	0.98 (**$P<0.001$**)	47.35±6.18 ^{bc}
Selection cutting beech forest (BS)	0.42±0.02 ^{ab}	0.92 (**$P = 0.002$**)	58.76±1.60 ^{ab}
Unmanaged beech forest (BU)	0.32±0.09 ^{a}	0.94 (**$P = 0.001$**)	63.29±10.89 ^{a}

Different lower-case letters indicate significant differences according to one-way ANOVA incorporating Fisher's Least Significant Difference.
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Table 4. Initial chemical composition of dried leaf litter under different forest management practices (Mean±SD, n = 3).

Nutrient	Age-class spruce forest (SA)	Age-class beech forest (BA)	Selection cutting beech forest (BS)	Unmanaged beech forest (BU)
Total C (%)	49.05±0.19 ^{b}	47.61±0.17 ^{a}	47.34±0.16 ^{a}	48.81±0.22 ^{b}
Total N (%)	1.30±0.03 ^{d}	0.97±0.01 ^{b}	1.04±0.00 ^{c}	0.84±0.03 ^{a}
C/N	37.65±0.80 ^{a}	48.92±0.20 ^{c}	45.52±0.15 ^{b}	58.38±2.18 ^{d}
Total lignin/N	35.80±0.87 ^{a}	43.13±0.25 ^{c}	39.74±0.00 ^{b}	56.27±1.90 ^{d}
Initial Mg (μg/g dry mass)	619.33±257.02 ^{b}	204.00±50.27 ^{a}	294.33±197.28 ^{a}	157.93±54.19 ^{a}
Initial K (μg/g dry mass)	2120.00±1108.51 ^{a}	3076.67±545.01 ^{a}	4596.67±2500.11 ^{a}	2443.33±120.97 ^{a}
Initial Ca (μg/g dry mass)	1090.33±489.43 ^{b}	593.33±142.55 ^{ab}	964.33±576.11 ^{ab}	344.33±70.61 ^{a}
Initial P (μg/g dry mass)	103.87±10.74 ^{a}	187.67±41.50 ^{b}	152.67±27.43 ^{ab}	157.33±20.84 ^{a}
Initial Mn (μg/g dry mass)	69.90±33.40 ^{b}	32.87±9.64 ^{a}	26.47±11.65 ^{a}	20.53±5.56 ^{a}
Initial Fe (μg/g dry mass)	7.27±3.91 ^{a}	5.60±2.52 ^{a}	4.67±2.80 ^{a}	7.37±9.14 ^{a}
Initial Cu (μg/g dry mass)	1.27±0.22 ^{bc}	0.91±0.37 ^{b}	0.62±0.07 ^{ab}	0.40±0.04 ^{a}
Initial Co (μg/g dry mass)	0.01±0.01 ^{a}	0.01±0.00 ^{a}	0.02±0.01 ^{a}	0.01±0.00 ^{a}
Initial V (μg/g dry mass)	0.02±0.01 ^{a}	0.02±0.00 ^{a}	0.02±0.00 ^{a}	0.02±0.02 ^{a}

Different lower-case letters indicate significant differences according to one-way ANOVA incorporating Fisher's Least Significant Difference.
doi:10.1371/journal.pone.0093700.t004

Effect of Forest System Management Practices on Lignin Decomposition

Lignin amounts (both Klason and total lignin) in leaf litter relative to the initial values were significantly affected by forest system management practices. Patterns of lignin decomposition (both Klason and total lignin) between BU and BS (low forest management intensity) forests, and between BA and SA (high management intensity) forests were similar across the DAI sampling times (Fig. 1). Interestingly, across all DAIs, we found that the lignin decomposition rate (based on both Klason and total lignin) in the BU forest was never higher than in the BA and SA forests (Klason and total lignin were high in BU forest across DAI). The amount of lignin, relative to the initial values, between high management intensity (BA or SA) forests and low management intensity (BU and BS) forests was marginally significant at 180 DAI and 284 DAI (Klason lignin, $P = 0.0500$ and 0.0518) and at 180, 284 and 362 DAI (total lignin, $P = 0.0558$, 0.0541 and 0.0923, respectively; data not shown). Large fractions of lignin in the SA and BA forests were decomposed earlier than in the BU and BS forests; for example, at 89 and 180 DAI, SA and BA forest had lost 29–30% of Klason and total lignin while BU and BS forests reached this level around 362 DAI (Fig. 1). The Klason/N and total lignin/N ratios of leaf litter in different forests exhibited different patterns across the DAI (Fig. 2).

Ligninolytic Enzyme Activities under Different Forest System Management Practices

Different forest system management practices significantly affected laccase ($F = 14.00$; $P < 0.0001$) and MnP ($F = 3.43$; $P = 0.0260$) activities, while the effects of DAI and the interaction between forest system management practice and DAI were not significant ($P > 0.05$; Table 5). In contrast to these two enzymes, general peroxidase activity was significantly influenced only by time ($F = 2.66$, $P = 0.0466$), whilst the effects of forest system management practices and the interaction between forest types and time were not significant ($P > 0.05$; Table 5). Forest system management practices with high and low management intensities had distinct patterns of ligninolytic enzyme activities over the incubation period (Fig. 4). SA and BA forests (high management intensity) maintained high activities of MnP at all sampling times; specifically, the MnP activities ranged from 206–830 and 251–2,694 mU/g litter dry mass in the SA and BA forests, respectively. These two forests had relatively lower laccase activities compared to the BU forest at all sampling times. On the other hand, the BU and BS forests (low management intensity) showed relatively high laccase activities but low MnP activities compared with SA and BA forests at most sampling times. Increasing MnP activity in the BU and BS forests was observed after 284 and 362 days, respectively. Considerable decreases in the remaining lignin in leaf litter of the BU and BS forests were observed after the increase in MnP activity (Figs. 1 and 4).

Discussion

Effect of Forest Types on Litter Decomposition Rates

The decomposition rate and the patterns of nutrient dynamics of leaf litter in the BU forest were similar to other published litterbag experiments carried out in this area [39,40]. In our experiments we used leaf litter taken from each site individually (within each forest management practice site) to avoid deviations from the normal process of leaf litter decomposition at each particular site. It is known that senescent leaves cover microbial communities (endophytes and epiphytes) that initiate the decomposition process [41,42]. Thus, mixing leaf litter from different sites may affect the microbial communities and their interactions; this could create results that do not represent what normally happens at each site. Therefore our study provides clear answers about how and to what extent the forest system management practices influence decomposition rates and thereby nutrient dynamics in Central European forest ecosystems. Interestingly, our results revealed that there were no significantly negative effects of intensive forest management or conversion of forest type on leaf litter decomposition and nutrient release to the forest soil. In contrast to what has been generally expected [15,23], we found that leaf litter decomposition rates and nutrient release (most nutrients) in the converted and/or intensively managed forests (SA and BA) were significantly higher than in the unmanaged forest reserve (BU). In addition, despite having the same forest age structure (uneven-age), the decomposition rate was relatively

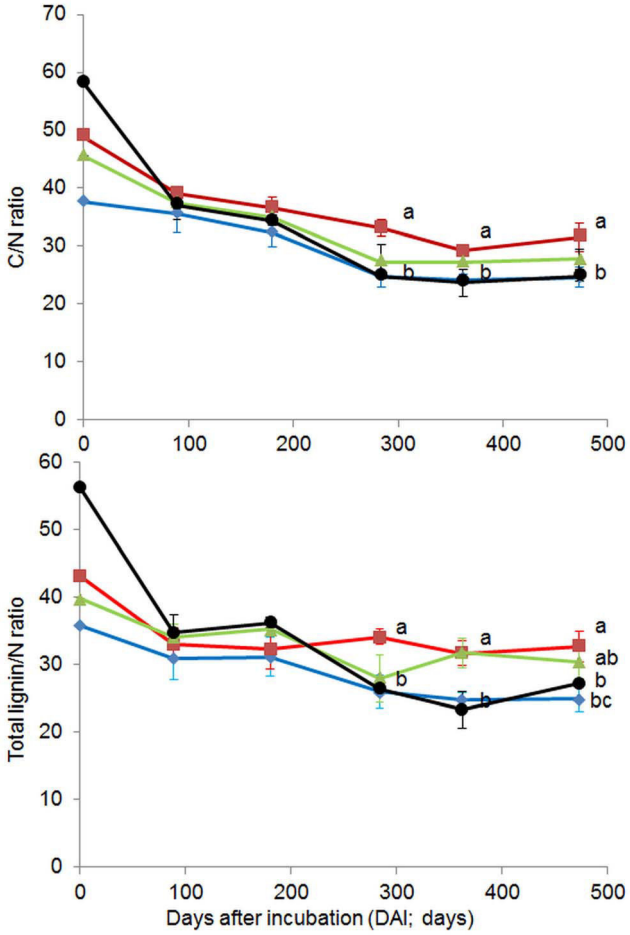

Figure 2. C/N and total lignin/N ratios in leaf litter during 473 days of decomposition under different forest system management practices. Norway spruce age-class forest (blue, SA), European beech age-class forest (red, BA), European beech selective cut forest (green, BS) and unmanaged deciduous forest reserves dominated by European beech (black, BU) (mean ± SD, n = 3). Different letters indicate significant differences according to one-way ANOVA incorporating Fisher's Least Significant Difference (from 89 DAI to 473 DAI).
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higher and N, Ca, K and P mineralization significantly faster in BS (near-to-nature forest management) than BU forests. This may be because forest management practices alter leaf litter composition and quality. We found that leaf litter in SA, BA and BS forests was of better quality (lower C/N or lignin/N ratios) than that in BU forest (Table 4); this was due to the mix of leaf litter from other tree species (better litter quality than beech) from natural regeneration at each site (Table 1). The SA forest originated from large scale disturbance of beech dominated deciduous forest (so there has been no change in land-use, for example an agricultural period or a time as grassland) [2], thus deciduous propagules still remain in the soil and are available to regenerate naturally within the SA forest (Table 1). In beech dominated forest, the woody biomass is harvested either by whole stand harvesting (BA) or individual tree harvesting (BS) [4]. These harvests could generate the forest gaps that are important for tree regeneration and the maintenance of plant diversity [4,43]. Such gaps tend to be nutrient rich and have relatively high light levels. In them, propagules of different plants (especially shade intolerant species) can germinate, grow quickly and maintain in the forest [43]. The unmanaged beech (BU) forest studied here is located in a National Park which, for at least 60 years, has not undergone wood harvesting or other serious disturbances that would have resulted in the loss of large numbers of trees; thus, European beech dominates the entire forest area [4]. This could explain the higher tree species richness in the BA and BS forests compared with the BU forest in our study. Due to the positive effects of tree diversity on leaf litter decomposition rate and nutrient release in managed forests, tree diversity should be maintained by allowing the natural regeneration of a range of native deciduous species, especially at the thinning stage. In this experiment, litter from the SA forest showed an interesting pattern of N and P dynamics (litter accumulates P but releases N). This may be again related to the initial leaf litter stoichiometry: initial C/N and C/P ratios. Manzoni et al. [44] reported that stoichiometry regulates carbon, nitrogen, and phosphorus dynamics in decomposing litter. In SA forest, the initial C/N ratio was low and possibly below critical ratio for N mineralization in this ecosystem while the initial C/P ratio was considerably high and does not reach the critical ratio for P mineralization [44].

Nevertheless, changes in leaf litter stoichiometry due to the higher tree richness in BA and BS forests do not explain all the effects of forest management practices on litter decomposition rates. It is unlikely that small fractions (5–10%) of leaf litter from an additional one or two tree species in BA and BS forest compared with BU forest could dramatically change the decomposition rate (31–72%). There is some evidence that forest management practices not only change tree species richness but also the microclimate and the microbial decomposer community [9,45].

The Role of Microbes

The higher mass loss and net nutrient release (for most of nutrients) in the BA forest as compared with the BU forest may be due to changes in the soil microbial community structure and activity induced by different forest system management practices, as reported in a recent soil fungal diversity study carried out at the same study sites [45]. Although these changes were not statistically significant for fungal communities, there was a substantial shift of 23% (121–123 fungal OTUs were unique to each forest type) when compared to fungal communities in BU and BA forests. It is reasonable to conclude that particular taxa may have substantial impact on litter degradation dynamics so that these community changes may not be so subtle in their effect.

When comparing SA and beech dominated forests, we found no significant home-field advantage. This is because the SA forest in this experiment is accustomed to periodic deciduous litter deposits. In our study, we were able to sample deciduous litter on this site simply because some deciduous trees (~20%) have established here as a result of natural regeneration. We also postulate that because decomposer communities in SA forest can cope with more recalcitrant substances, such as resins, polyphenols and guaiacyl lignin found in spruce needles, they may also have the capacity to decompose the main deciduous leaf litter (European beech), which is also considered to be a low quality litter, quite rapidly [20].

Lignin Degradation and Carbon Dynamic

In this study, we demonstrated that the decomposition of complex substances such as lignin was not negatively affected by forest system management practices and/or the naturalness of the forest ecosystems. We found that semi-natural and artificial systems (BA and SA forests) are more efficient at decomposing lignin than near natural (BS forest) and natural systems (BU forest). In addition, the percentage of remaining C in the leaf litter in BU forests was high (indicating slow decomposition) compared with

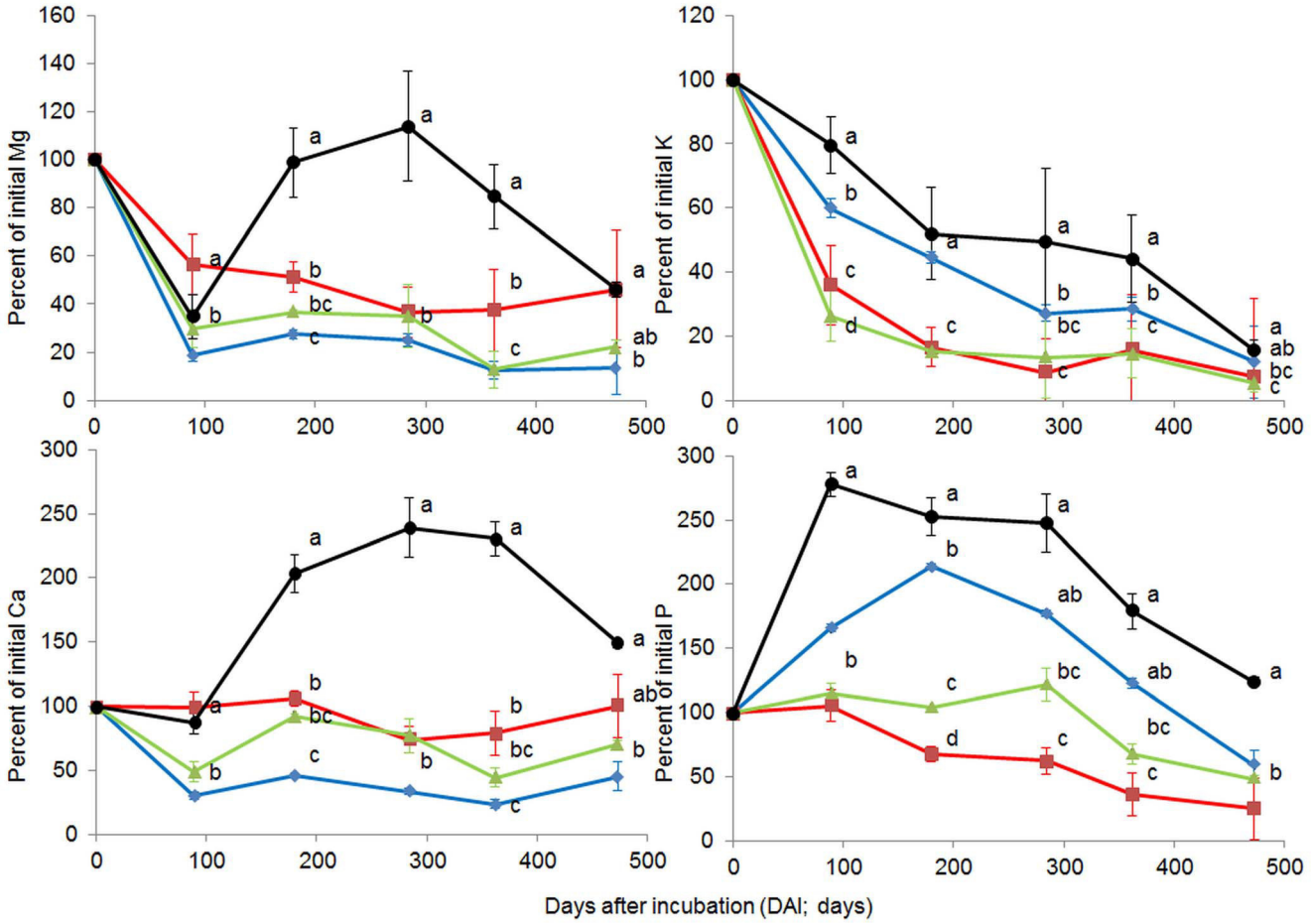

Figure 3. Percent of initial magnesium (Mg), potassium (K), calcium (Ca) and phosphorous (P) during decomposition under different forest system management practices. Norway spruce age-class forest (blue, SA), European beech age-class forest (red, BA), European beech selective cut forest (green, BS) and unmanaged deciduous forest reserves dominated by European beech (black, BU) (mean ± SD, n = 3). Different letters indicate significant differences according to one-way ANOVA incorporating Fisher's Least Significant Difference (from 89 DAI to 473 DAI).
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other forest system management practices at all sampling times, and at the end of the experiment there was no significant difference in remaining C in different forest management types. Since no significantly negative effects of forest system management practices were found for either lignin or C decomposition, we can conclude that C cycling was not negatively influenced by intensive forest management, although we could not quantify the exact amounts of leaf litter C release to the forest soil, since litter C can be released both to the soil and to the atmosphere [11]. Some recent studies carried out at the same study sites have also found that forest management had no detectable effect on turnover of mineral associated soil organic matter [46] or on current soil organic carbon (SOC) stocks [47].

The ligninolytic enzyme activities recorded indicate that forest system management practices control the litter decomposition rates via changes in microbial, in the first place fungal, activities. Although both laccase (high activity in BU and BS forests) and MnP (high activity in BA and SA) can catalyze the oxidation of phenolic lignin moieties, laccase has a generally lower oxidative strength and redox potential compared to peroxidases [48–51]. This may explain why BS and/or BU forests had lower lignin decomposition rates compared with BA and SA forests despite the high laccase activity. Interestingly, in BU and BS forests, the sharp decrease in the remaining lignin in leaf litter was only observed after the MnP activities started to increase. This strongly indicates that MnP is a key enzyme in lignin decomposition and supports the results of other studies demonstrating the ability of MnP to catalyze the disintegration and partial mineralization of lignin [52,53]. It can be assumed that there is a shift from microbial communities producing more laccase in BU and BS forests (low forest management intensity) to more MnP producing communities (dominated by basidiomycetes) in BA and SA forests (high forest management intensity). In BA and SA forests, where the litter decomposition rates were high, high MnP activities were also observed at all sampling times. In addition, the lignin contents (as compared to the initial amounts) decreased considerably from the first sampling date on (89 DAI). This confirms that lignin decomposition is the rate limiting step in litter decomposition [16,17] and that MnP is one of the oxidative key enzymes of litter degradation [33]. Mechanisms on how MnP is involved in the decomposition process of lignin and other recalcitrant substances have been reviewed [33]. Based on this knowledge, we propose that, also in leaf litter, MnP oxidizes Mn^{2+} (that is present in sufficient amounts in samples of all forest sites, table 4) into highly reactive Mn^{3+} that is stabilized by secreted fungal chelators such as oxalic acid. Chelated Mn^{3+} in turn performs as a small diffusible

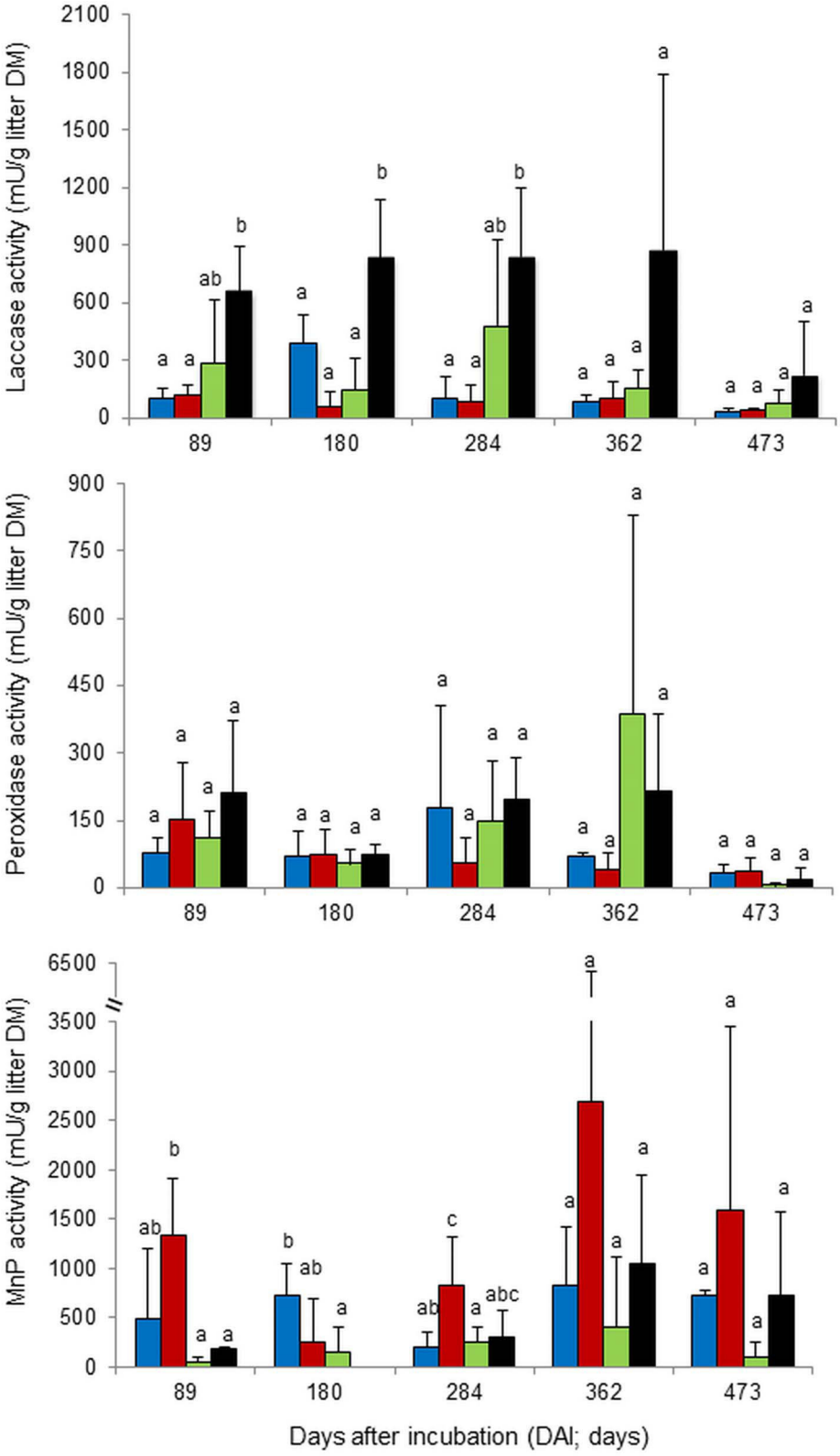

Figure 4. Mean ligninolytic enzyme activities in leaf litter under different forest system management practices. Norway spruce age-class forest (blue, SA), European beech age-class forest (red, BA), European beech selective cut forest (green, BS) and unmanaged deciduous forest reserves dominated by European beech (black, BU) (mean+SD, n = 3). Different letters indicate significant differences according to one-way ANOVA incorporating Fisher's Least Significant Difference (from 89 DAI to 473 DAI). *At 180 DAI, MnP activity of unmanaged deciduous forest reserves dominated by European beech = 0.
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Table 5. Effects of forest system management practice, days after incubation commenced and their interactions on ligninolytic enzyme activity ($P<0.05$).

Factor	Laccase activity	Peroxidase activity	Manganese peroxidase activity
Forest system management practice	$F=14.00$, $\mathbf{P<0.0001}$	$F=1.13$, $P=0.3483$	$F=3.43$, $\mathbf{P=0.0260}$
Days after incubation (DAI)	$F=1.89$, $P=0.1308$	$F=2.66$, $\mathbf{P=0.0466}$	$F=1.66$, $P=0.1780$
Forest system management practice × DAI	$F=0.87$, $P=0.5787$	$F=1.09$, $P=0.3967$	$F=0.48$, $P=0.9152$

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oxidant (redox mediator) attacking preferentially phenolic lignin structures which leads to the formation of instable free radicals that tend to disintegrate spontaneously [33].

Outlook

In our experiment, we found that the differences in management may affect litter decomposition by altering the composition of the vegetation. To improve our mechanistical understanding of this point, a common garden experiment where all litters are incubated in one place may be helpful. However, litter decomposition is not only depending on the litter material used but is also strongly impacted by the microclimatic and other environmental conditions (nutrients, water availability, etc.) [11]. Thus, such a common garden experiment will only help to figure out what is the role of litter quality but not the role of the environmental conditions present at the site.

Conclusion

We conclude that forest system management practices can significantly influence leaf litter decomposition rates and nutrient dynamics in Central European forests. The effects of forest system management practices on litter decomposition rate and nutrient dynamics in beech dominated forests (BA, BS and BU) are complex and are controlled by a range of factors, including tree diversity, microclimatic conditions and the microbial decomposer community. In managed forest ecosystems where a large fraction of woody biomass is harvested, as is the case in BA and BS forests, the system can balance the nutrient status by increasing leaf litter decomposition rates and nutrient dynamics.

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Author Contributions

Conceived and designed the experiments: D. Krüger FB MH D. Kapturska MP WP. Performed the experiments: MP D. Kapturska WP. Analyzed the data: WP. Contributed reagents/materials/analysis tools: D. Krüger FB MH ES. Wrote the paper: WP MP MS D. Krüger MH.

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CHAPTER 7

Uncoupling of microbial community structure and function in decomposing litter across beech forest ecosystems in Central Europe.

Witoon Purahong[1, 2], Michael Schloter[3], Danuta Kapturska[1,4], Veronika Däumlich[1], Marek J. Pecyna[4], Sanchit Mital[1], François Buscot[1, 5, 6], Martin Hofrichter[4], Jessica LM Gutknecht[1,7], Dirk Krüger[1]

[1]UFZ-Helmholtz Centre for Environmental Research, Department of Soil Ecology,Theodor-Lieser-Str. 4, D-06120 Halle (Saale), Germany.

[2]Technical University of Munich, Chair for Soil Science, Ingolstädter Landstr. 1, D-85758 Oberschleissheim, Germany

[3]Helmholtz Zentrum München, Research Unit for Environmental Genomics, Ingolstädter Landstr. 1, D-85758 Oberschleissheim, Germany

[4]Technical University of Dresden, International Graduate School (IHI) Zittau, Markt 23, D-02763 Zittau, Germany

[5]University of Leipzig, Institute of Biology, Johannisallee 21-23, D-04103 Leipzig, Germany

[6]German Centre for Integrative Biodiversity Research (iDiv), Halle-Jena-Leipzig, Deutscher Platz 5e, D-04103 Leipzig, Germany

[7]Present address: Department of Soil, Water, and Climate, University of Minnesota, 439 Borlaug Hall, 1991 Upper Buford Circle, St. Paul, MN 55108, USA.

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Correspondence and requests for materials should be addressed to J.L.M.G. (jgut@umn.edu) or D.Krüger (nasowasnun@googlemail.com)

* These authors contributed equally to this work.

† Current address: Department of Soil, Water, and Climate, University of Minnesota, 439 Borlaug Hall, 1991 Upper Buford Circle, St. Paul, MN 55108, USA.

Uncoupling of microbial community structure and function in decomposing litter across beech forest ecosystems in Central Europe

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[1]UFZ-Helmholtz Centre for Environmental Research, Department of Soil Ecology, Theodor-Lieser-Str. 4, D-06120 Halle (Saale), Germany, [2]Technical University of Munich, Chair for Soil Science, Ingolstädter Landstr. 1, D-85758 Oberschleissheim, Germany, [3]Helmholtz Zentrum München, Research Unit for Environmental Genomics, Ingolstädter Landstr. 1, D-85758 Oberschleissheim, Germany, [4]Dresden University of Technology (TU Dresden) - International Institute (IHI) Zittau, Markt 23, D-02763 Zittau, Germany, [5]University of Leipzig, Institute of Biology, Johannisallee 21-23, D-04103 Leipzig, Germany, [6]German Centre for Integrative Biodiversity Research (iDiv), Halle-Jena-Leipzig, Deutscher Platz 5e, D-04103 Leipzig, Germany.

The widespread paradigm in ecology that community structure determines function has recently been challenged by the high complexity of microbial communities. Here, we investigate the patterns of and connections between microbial community structure and microbially-mediated ecological function across different forest management practices and temporal changes in leaf litter across beech forest ecosystems in Central Europe. Our results clearly indicate distinct pattern of microbial community structure in response to forest management and time. However, those patterns were not reflected when potential enzymatic activities of microbes were measured. We postulate that in our forest ecosystems, a disconnect between microbial community structure and function may be present due to differences between the drivers of microbial growth and those of microbial function.

A major portion of Europe's forest cover (approx. 57% if the Russian Federation is excluded) is managed for woody biomass production[1]. Forest system management practices (FMPs) can significantly alter soil physical, biological and chemical properties, as well as microclimatic conditions. These in turn can have profound effects for many ecosystem functions including productivity, carbon storage and biodiversity conservation[2–4]. However despite their importance as drivers for most ecosystem functions, the influence of different forest management practices on microbial communities in different soil compartments and their functional traits is still not well understood. It is generally believed that the structure of microbial communities determines their function[5–9]. However, they also propose that there may be, in some instances, a disconnection between microbial community structure and its functions. For instance, findings from several ecosystems have demonstrated different responses between microbial community structure and functions[9,10]. Thus it can be difficult to predict how changes in environmental factors will affect microbial community structure and microbially-mediated ecosystem functions in different ecosystems because there may be differences in the resistance to change at either the the community and functional levels, and because of functional redundancy within the microbial community[11]. The aims of this study were to investigate the patterns of and connections between microbial community structure and microbially-mediated functions across different FMPs as a function of time.

Leaf litter degradation is a very interesting case study for examining microbial structure-function relationships. It provides an important basis for the nutrient supply of trees in most forest ecosystems and thus leaf litter degradation and recycling, which is mainly catalyzed by microbes, determines productivity to a large degree[12]. Forest system management practices can potentially change the chemical composition of leaf litter by changing the dominant tree species as well as tree species richness, abundance and performance[2,12]. In addition, microbial communities colonizing leaf litter undergo major temporal changes, both seasonally and over the course of litter decomposition[13,14]. In temperate forests, the bulk of the litter input (for both leaf and fine root litter) occurs in the

autumn and the concentrations of easily degradable substrates from fresh leaf-litter including carbohydrates cellulose, proteins, starch and tannins are the highest in soil at this time. This presence of labile substrates may in turn stimulate microbial biomass in winter[15–18]. Plant and soil microbial seasonal cycles can also significantly influence nutrient availability in forest soil, also potentially affecting microbial community composition and feedbacks on microbial decomposition of leaf litter[5]. In this study we investigated microbial communities colonizing the litter material as well as selected functional traits over time in leaf litter from unmanaged stands and stands subject to different forest management regimes (different FMPs). The tested regimes involved age-class and selection cutting, both of which leave rather many forest gaps that enable the establishment of diverse tree species[12,19]. The unmanaged stands had fewer gaps and were only dominated by European beech (*Fagus sylvatica*), which produces low-quality leaf litter with a high C:N ratio[12]. Microbial community structure was determined broadly using phospholipid fatty acids. The functional traits of microbial communities were evaluated by measuring the potential activities of specific microbial enzymes involved in the biogeochemical cycling of important elements such as carbon, nitrogen and phosphorus[10]. We hypothesized that FMP and time of litter incubation in soil (days after incubation, DAI) both have significant effects on microbial community structure and potential enzyme activities. However the extent to which microbial community structure and function will be influenced will differ leading to a decoupling of structure–function relationships in this soil compartment. FMP and DAI may strongly affect microbial community structure, however, different microbial communities from different FMPs and/or DAI may perform similar enzyme activities (functional redundancy)[10]. Furthermore, differences between the drivers of microbial growth and enzyme activities may also be responsible for the disconnection of microbial community structure and function[20]. We used multivariate analysis to identify leaf litter properties with significant effects on the overall microbial community and enzyme activity patterns. Factors considered in this analysis included leaf litter quality parameters (C:N and lignin:N ratios) and other abiotic properties of the litter such as its pH, water content, and concentrations of microbial macronutrients and micronutrients (trace elements).

Results

Effects of FMP and DAI on microbial community structure and potential enzyme activities. Abundance of phospholipid fatty acid (PLFA) indicators were significantly influenced by FMP and temporal (DAI) changes, and also by the interaction between FMP and DAI ($P < 0.001$ in all cases, Table S1, Figure 1). Overall in the leaf litter of managed forests (beech age-class, BA and beech selection cutting, BS) the highest amounts of PLFA (both total and key groups) were detected in winter (89 and 473 DAIs) while the lowest PLFA amounts were measured in spring and summer (180, 284 DAIs) (Figure 1). Similar results were obtained for litter material from the unmanaged beech forest (BU). However, during the second winter the microbial biomass in the BU forest did not increase to the same extent as in the BA and BS forests. The fungal: bacterial ratio decreased over time under all FMPs (Figure 1). At 89 and 180 DAI, the leaf litter from BU forest had a significantly higher fungi: bacteria ratio than that observed in the BA and BS forests (89 DAI: BU = 5.76, BS = 2.82, BA = 2.72; 180 DAI: BU = 3.43, BS = 2.42, BA = 2.78). PLFA indicators for all bacterial groups except Gram negative bacteria varied significantly with FMP in spring. In summer, no significant differences among different PLFA indicators were ascertained for bacterial groups, except for Gram positive bacteria whose indicators were highest in BS forest and lowest in BA forest. NMDS analysis of PLFA patterns revealed four distinct clusters representing samples taken at different DAI ($R_{DAI} = 0.97$, $P < 0.001$, Figure 2a). The different FMPs also affected microbial community structure, as shown by the existence of one cluster corresponding to samples from managed forests (BA and BS) and another corresponding to the BU forest at specific DAI values ($R_{FMP} = 0.69$, $P < 0.001$, Figure 2a).

The potential activities of the enzymes investigated in this study did not follow the same trends as lipid biomass or lipid indicators (Tables S1 and S2, Figures 2b and 3). Mantel tests revealed an insignificant correlation between the PLFA and enzyme activity matrices (Mantel R (Euclidean distance matrix) = 0.05, $P > 0.05$, Mantel R (Bray-Curtis distance matrix) = 0.09, $P > 0.05$). Enzyme activities did not vary according to the microbial biomass (Table S2). Enzyme activities in leaf litter under different forest management practices are presented in detail in Figure 3. Hydrolytic enzymes, cellobiohydrolase and β-glucosidase were significantly influenced by DAI and the interaction between FMP and DAI ($P < 0.05$, Table S1) but were not significantly influenced by FMPs. Xylosidase activity was significantly influenced by FMP and DAI ($P < 0.01$, Table S1). Xylosidase activity was generally high in BU (low forest management intensity) compared with BA (high forest management intensity). N-acetylglucosaminidase and acid phosphatase activities were significantly influenced by FMP and the interaction between FMP and DAI ($P < 0.05$, Table S1). The activities of the studied oxidative enzymes also behaved differently compared to microbial biomass: laccase activity was significantly affected by FMP and DAI ($P < 0.05$, Table S1), peroxidase by DAI ($P < 0.01$, Table S1), and manganese peroxidase by FMP ($P < 0.01$, Table S1). However, the interaction between FMP and DAI did not significantly affect the activities of any studied oxidative enzyme ($P > 0.05$, Table S1). Overall the observed NMDS patterns for microbial community structure were not reproduced in the NMDS analysis of enzyme activities where there was no recognizable clustering of samples representing either different FMPs or DAI values (Figure 2b). Although the different FMPs and DAI significantly influenced potential enzyme activity patterns, the degree of influence indicated by ANOSIM R values were much lower than for the microbial lipid abundances ($R_{FMP} = 0.36$, $R_{DAI} = 0.44$, $P < 0.001$ in both cases). In some cases, microbial communities collected from forests subject to different FMPs or collected at different times exhibited similar enzyme activities (Figure 2b).

The effects of the physicochemical properties of leaf litter on microbial community structure and function. Our results indicated that microbial community structure and potential enzymatic activities are regulated and controlled by different physicochemical properties of the leaf litter. The structure of the microbial communities was significantly influenced by all leaf litter parameters (i.e. C:N and lignin:N ratios), the levels of most microbial macronutrients other than P (C, N, Mg, K, Ca, Fe), the levels of one microbial micronutrient (Mn), and the pH (Table 1). In contrast, only C, K, P, Mn, and pH significantly influenced enzyme activities. Leaf litter quality parameters (C:N and lignin:N ratios) that had very significant effects on the structure of the microbial community ($P < 0.01$) did not significantly affect the pattern of enzymatic activities ($P > 0.05$) (Table 1). Changes in leaf litter chemistry over time are presented in Figure S1.

Discussion

In this experiment, we examined the patterns of and connections between microbial community structure and function across different forest management practices and as a function of time using leaf litter as an example. PLFA analysis was used for characterizing the microbial community structure. This method provides a broad estimation of the viable biomass of different microbial functional groups[21]. Some phospholipids (such as the fungal indicators 18:1ω9, 18:2ω6,9 and 18:3ω3,6,9) or potential enzymatic activities could be derived from plants[22]. While this consideration was taken into account for our interpretations, we assume that residual con-

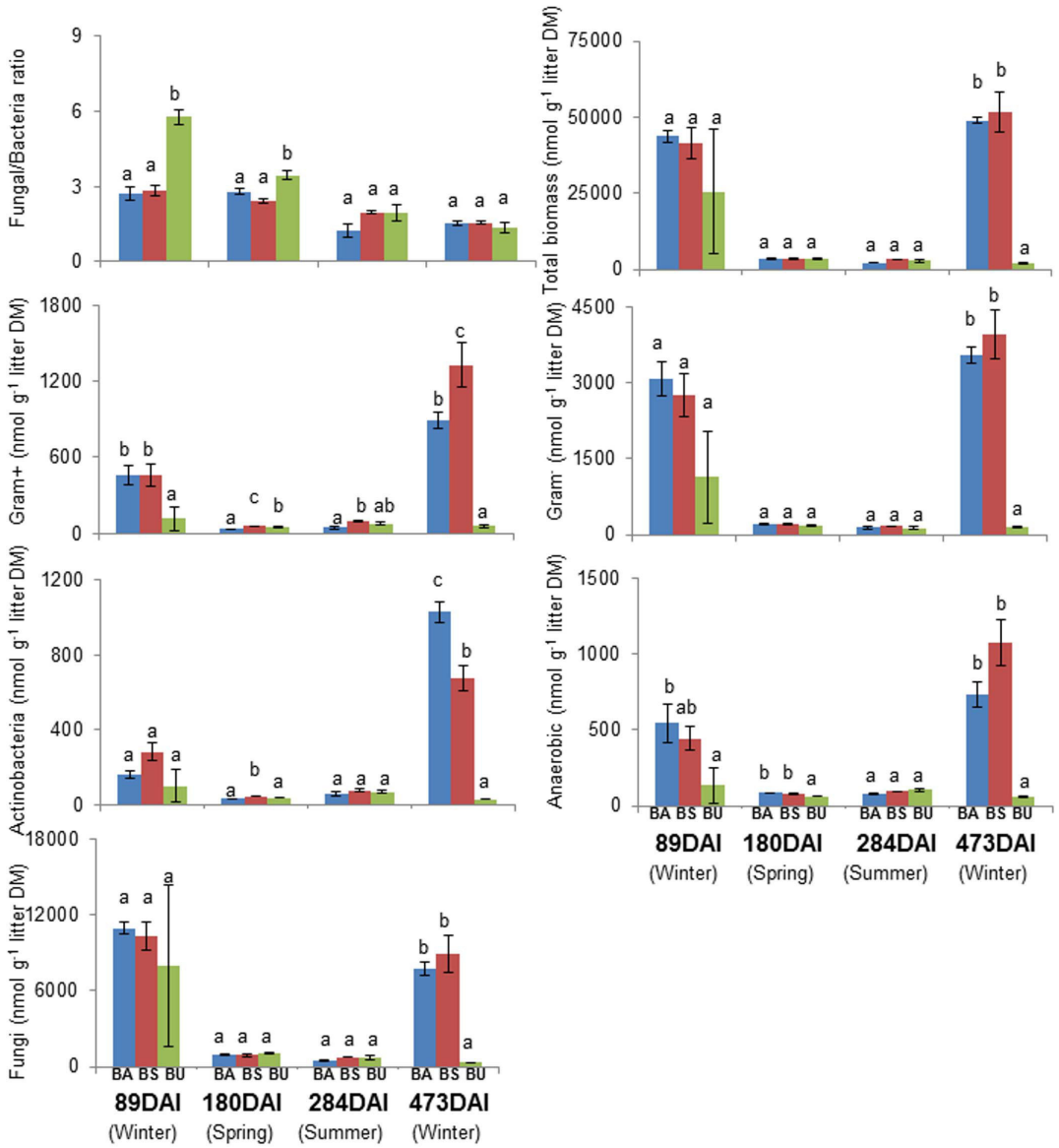

Figure 1 | Fungal: bacterial ratio and microbial PLFA abundances (both total and key groups) (mean ± SE) in leaf litter samples collected at four sampling dates (days after incubation commenced, DAI). European beech age-class forest (BA, blue), European beech selection cutting forest (BS, red) and unmanaged deciduous forest reserves dominated by European beech (BU, green). Litter DM = litter dry mass. Different letters indicate significant differences ($P < 0.05$) between different treatments at each DAI according to one-way ANOVA and Fisher's Least Significant Difference (from 89 DAI to 473 DAI).

tributions from plants would be far surpassed by new microbial growth. This assumption is based on sample timing at 89 DAI to 473 DAI. By 89 DAI, a residual effect from PLFA or enzymes from the residual litter would be minimal, especially because the litter was dried and processed before plot establishment[23]. Based on the results of leaf litter decomposition rates of the same experiment, we showed that forest management practices significantly influence the decomposition kinetics (BA forest has higher decomposition rate than BU forest)[12]. Thus, a caveat to this dataset is that at the same DAIs, leaf litter from different FMPs (BA and BU forests) may not be at the same decomposition stages. This may also affect the patterns of enzyme activities and PLFA in leaf litter at the same DAI (different FMPs).

As expected, high microbial biomass was observed for all FMPs in winter (89 and/or 473 DAI)[15–18]. Our biomass analyses indicated that fungi were the dominant leaf litter-decomposing organisms under all of the studied FMPs and DAI. On the other hand, bacteria increased in abundance as decomposition progressed. Fungi are generally considered to be the primary decomposers of leaf litter in temperate forest ecosystems[13] and they rapidly colonize leaf litter during the early stages of decomposition[24]. Biological fragmentation caused by the action of fungi and invertebrates and physical fragmentation results in an increased leaf litter surface area and the release of nutrients during the later stages of leaf litter decomposition[25], which may enhance the activity of bacteria during litter decomposition[26].

We identified clear differences between the microbial communities from leaf litter produced under different FMPs (i.e. from managed vs. unmanaged forests) and between microbial communities collected at different time points within the 473 day sampling period. This strongly suggests that the structure of microbial communities in the leaf litter of the studied forest systems is sensitive to forest management practices, the stage of litter decomposition, and seasonal factors. Similar conclusions have been drawn in a number of previous studies examining various types of disturbance: in general, the

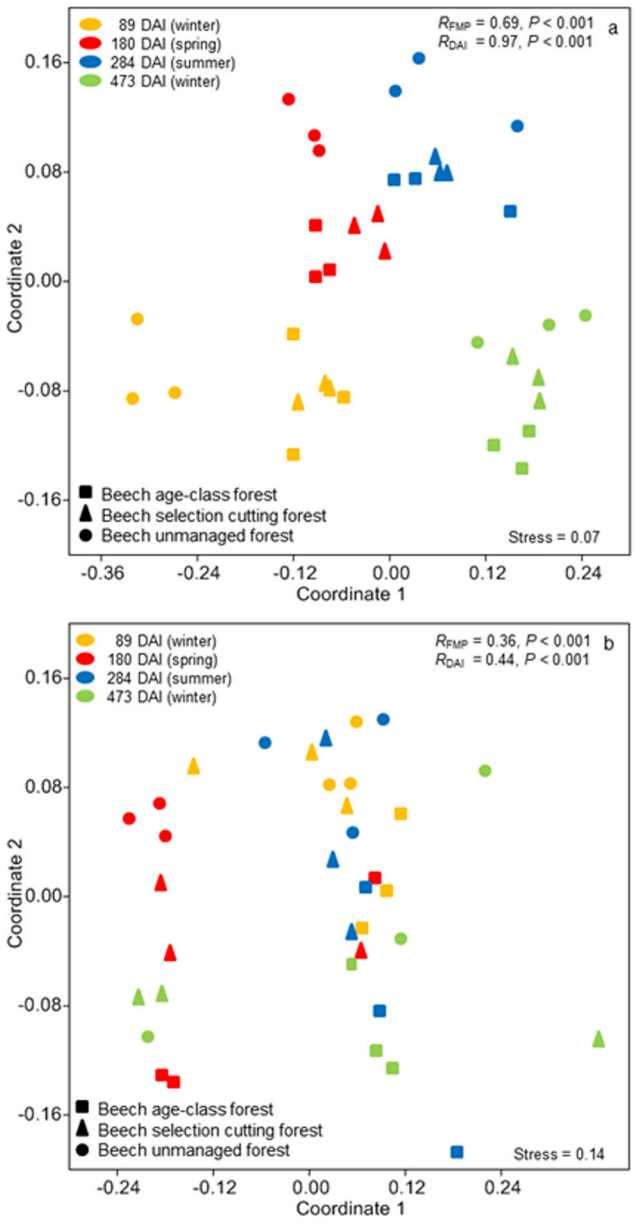

Figure 2 | Nonmetric multidimensional scaling (NMDS) ordination of (a) microbial community structure inferred from PLFA analysis and (b) activities of 8 different enzymes. FMP = forest system management practice; DAI = days after incubation commenced; R = degree of separation between test groups ranging from −1 to 1; $R = 0$, not different; $R = 1$, completely different; P values were based on 999 permutations.

structures of microbial communities are sensitive to many factors and not immediately resilient to disturbance[11]. In our studied sites, FMPs affect tree species composition that, in turn, significantly alters leaf litter quality and nutrients in BA and BS forests as compared to BU forest[12]. The results from multivariate analysis (Table 1) demonstrate that leaf litter quality and most macronutrients are significantly correlated with changes in microbial community composition. BU forest is located in a forest reserve area, which, for at least 60 years, has not undergone thinning, wood harvesting, or other serious disturbances that would have resulted in the loss of large numbers of trees. Thus the BU had fewer gaps and were only dominated by European beech (*Fagus sylvatica*), which produces low-quality leaf litter[12]. On the other hand, thinning operation and woody biomass harvesting in BA (whole stand harvesting) and BS (individual tree harvesting) could generate the forest gaps that are important for natural tree regeneration and the maintenance of plant diversity. Thus, *Acer* sp. successfully established by natural regeneration in both BA and BS forests (Table S3). In addition, a small fraction of *Fraxinus* sp. was also found in BA forest (Table S3). *Acer* sp. and *Fraxinus* sp. leaf litter are known to have better quality and nutrients compared with *Fagus sylvatica* leaf litter[12].

In contrast, we did not identify any clear changes in the patterns of potential enzyme activities in leaf litter samples due to FMPs or temporal factors. The responses of enzymatic activities to DAI and FMPs varied and were not always significant. Our results therefore indicate a decoupling of microbial community structure and the metabolic functions of potential microbial enzymatic activities across FMPs and temporal changes in temperate forest ecosystems. This might be explained, at least in part, by the widespread occurrence of hydrolytic enzymes (including polymer-disintegrating cellulases and xylanases) among different groups of microbes (fungi, bacteria, protists) so that they can replace each other in the course of community changes (functional redundancy)[27]. For example, the cellulases of fungi may disappear along with their producers during leaf litter succession, but they can be replaced by similarly active enzymes of actinobacteria or other Gram-positive bacteria[28]. In the case of oxidative enzymes, the situation is somewhat different. Oxidative enzymes were reported to be more organism-specific. Both laccase and generic peroxidases are primarily secreted by fungi (both ascomycetes and basidiomycetes)[29,30]. This is especially true for Mn-peroxidase that, in soil environments, is exclusively produced by specific basidiomycetous fungi causing a kind of white-rot in leaf litter[31,32]. Thus, the activities of oxidative enzymes may not be as widely spread as those of hydrolytic enzymes. This means, if appropriate fungi are present in a litter bag, there will be a high level of oxidative enzymes but if not, this activity will be lacking.

Insofar as our results deviate from the generally notion that structure determines function, these findings together with those of Frossard et al. can be regarded as supporting a paradigm shift[10]. Frossard and colleagues demonstrated a decoupling of bacterial community structure and function (represented by the activities of 10 separate enzymes) across temporal changes in nascent stream corridors. The disconnection between microbial community structure and function in these two cases may be due to functional redundancy within the studied microbial communities[10]. Our results show that different microbial communities (from either different FMPs or different points in time) can produce similar enzyme activities, thereby we speculate that at least some level of functional redundancy may occur. In addition to microbial functional redundancy, we demonstrated that differences in the drivers of microbial growth versus function can be used to explain the uncoupling between microbial community structure and the metabolic activity of microbial enzymes[20]. While all of the studied litter quality parameters and the levels of microbial macronutrients (except P) significantly influenced microbial community structure, only a few of these factors were found to significantly affect the overall pattern of potential enzyme activities. *Vice versa* P concentration did not significantly influence the microbial community structure but did have significant effects on the overall patterns of enzyme activity.

Based on our results, we suggest that there is some degree of functional redundancy in the microbial community. However, we also detected differences in litter decomposition rates and nutrient dynamics between forests with different FMPs (especially between unmanaged and age-class forests)[12]. This is due to the time lag in functional similarity. Our results show that microbial communities from different FMPs perform similar enzyme activities at different points in time. We have previously shown that the rates of leaf litter decomposition are highest in systems that decompose lignin most rapidly (i.e. those with high levels of manganese peroxidase, an efficient ligninolytic enzyme)[12]. Thus, leaf litter produced under FMPs

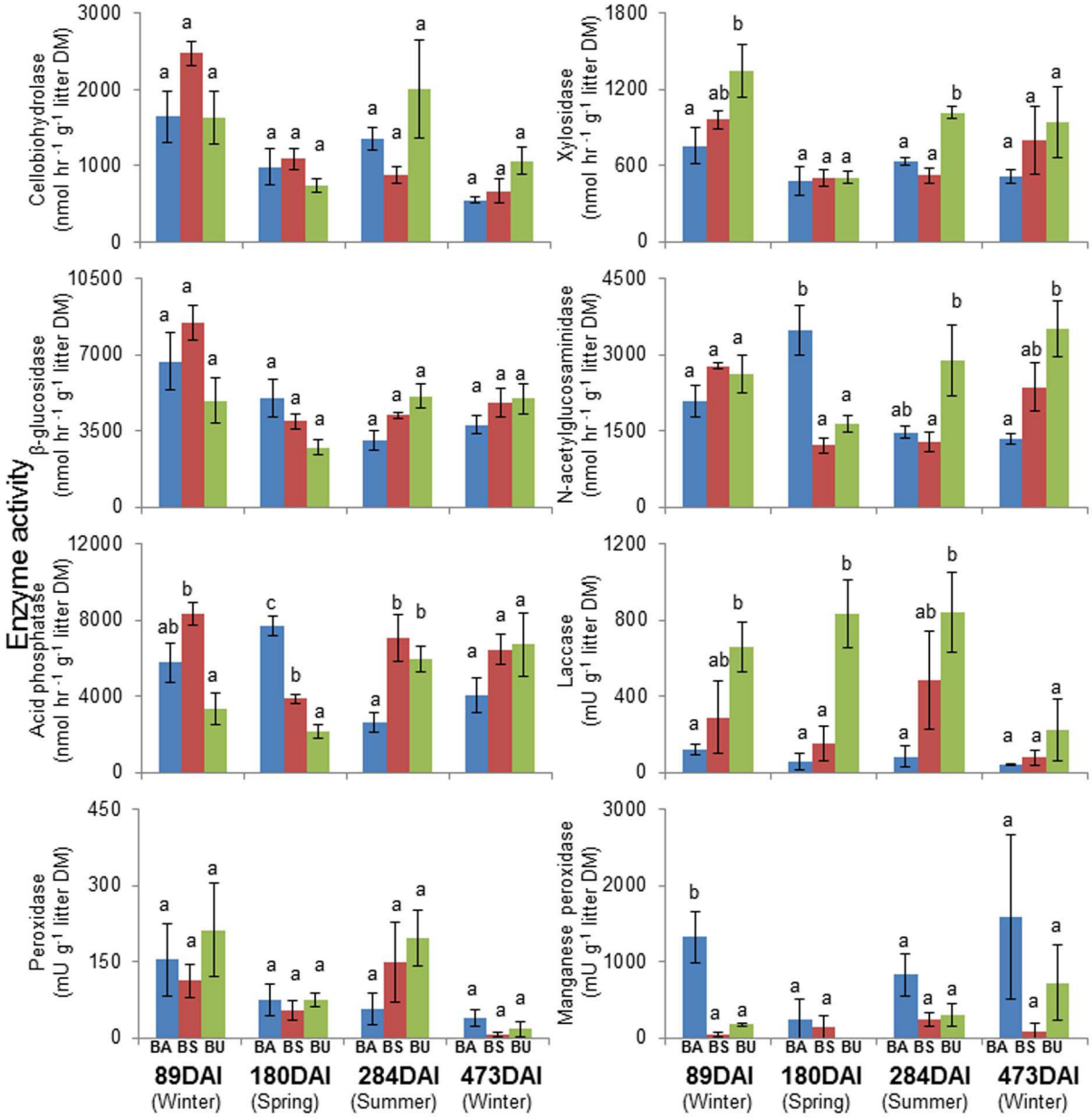

Figure 3 | Enzyme activities (mean ± SE) in leaf litter samples collected at four sampling dates (days after incubation commenced, DAI). European beech age-class forest (BA, blue), European beech selection cutting forest (BS, red) and unmanaged deciduous forest reserves dominated by European beech (BU, green). Litter DM = litter dry mass. Data on oxidative enzyme activities are derived from Purahong et al.[12]. Different letters indicate significant differences ($P < 0.05$) between different treatments at each DAI according to one-way ANOVA and Fisher's Least Significant Difference (from 89 DAI to 473 DAI).

that are more amenable to rapid colonization by microbial communities producing efficient ligninolytic enzymes may decompose relatively quickly.

In summary, the structure of microbial communities and microbial ecological functions of metabolic enzymes are dependent on the complex interactions between numerous environmental factors. In temperate forest ecosystems, forest management is one of the most important factors affecting both microbial community structure and its ecological function.

Methods

Study site. The study was conducted at three forest sites in the Hainich-Dün region of Central Germany (about 1,300 km^2; 51°16′N 10°47′E), which is part of the German Biodiversity Exploratories[33]. The forests in this region grow on soils over limestone bedrock[33]. The main soil type in the study area according to the German soil classification system and the World Reference Base of Soil Resources is a Stagnosol[33]. The soil pH is weakly acidic (5.1 ± 1.1; mean ± SD)[34]. Thin litter (in the range 2–5 cm) were recorded at all three forest sites[12]. The annual mean temperature and precipitation ranged, respectively, from 6.5–8°C and 500–800 mm[33]. All information pertaining to these forests has been described in detail elsewhere[12,33].

Three forest sites (10,000 m^2 each) were selected based on their forest system management practices with each site representing one treatment. All sites were dominated by European beech (*Fagus sylvatica*) and located less than 30 km apart, to reduce the influence of variations in geography and climate. Within each forest site, we assigned three plots (2 × 8 m^2) located on flat land to represent three experimental replicates of each forest management practice. The three FMPs considered in this study were: (i) European beech age-class forest (BA, semi-natural forest with natural regeneration, even-age forest structure); (ii) European beech selection cutting forest (BS, near-to-nature forest management with natural regeneration, uneven-age forest structure); and (iii) unmanaged deciduous forest reserves dominated by European beech (BU, uneven-age forest structure)[12]. These three forest sites differ in terms of the silvicultural management intensity indicator (SMI)[35]. The SMI was low for the BU and BS forests but high in the BA forest[35]. The litter composition for individual forest sites and the initial chemical composition of the litter material are given in Tables S3 and S4.

Table 1 | Goodness-of-fit statistics (R^2) of environmental factors fitted to the nonmetric multidimensional scaling (NMDS) ordination of microbial community structure and enzyme activities. The significance was based on 999 permutations. Significant factors ($P < 0.05$) are indicated in bold: *$P < 0.05$, ** $P < 0.01$, ***$P < 0.001$

	Microbial biomass		Enzyme activities	
Varibles	R^2	P	R^2	P
Total C	**0.701**	**0.001*****	**0.266**	**0.007****
Total N	**0.424**	**0.001*****	0.073	0.309
C:N ratio	**0.717**	**0.001*****	0.032	0.566
Lignin:N ratio	**0.280**	**0.006****	0.090	0.209
pH ($CaCl_2$)	**0.460**	**0.001*****	**0.315**	**0.002****
pH (H_2O)	0.086	0.258	0.025	0.634
Mg	**0.235**	**0.009****	0.010	0.872
K	**0.396**	**0.001*****	**0.608**	**0.001*****
Ca	**0.288**	**0.006****	0.063	0.354
P	0.052	0.414	**0.352**	**0.002****
Mn	**0.271**	**0.010****	**0.218**	**0.013***
Fe	**0.383**	**0.001*****	0.000	0.999
Cu	0.006	0.910	0.026	0.650
Co	0.073	0.265	0.009	0.919
V	0.001	0.988	0.119	0.149
Water content	0.103	0.166	0.085	0.216
Total lignin	0.094	0.211	0.086	0.218

Experimental design and sampling. At each forest site, freshly fallen leaves of deciduous species were collected from the forest floor in October, 2009. The leaves from each plot were separated according to species and air dried to constant weight at room temperature. Ten grams of local mixed leaves, representative of the litter composition of the deciduous tree species at each respective site were then placed in litterbags (25 × 25 cm, 2 mm nylon mesh size) for deployment[12]. At the end of the litter fall period (13 November 2009), 12 replicate litter bags were placed in each plot, for a total of 36 litterbags per management type and 108 litterbags in total. 27 additional litterbags (9 per treatment) were retained to determine the initial dry mass (oven-dried at 105°C ≥ 24 h until constant weight), nutrient element concentrations, and lignin content of the litter mixtures (Data supplement, Table S4). To preserve the situation in the temperate forest, we allowed the fresh leaf canopy litter to fall above the litter bags. Litterbags were retrieved on four sampling dates: in 2010 on 10 February (89 days after incubation commenced, DAI), 12 May (180 DAI), 24 August (284 DAI), and in 2011 on 1 March (473 DAI). On each sampling occasion, 3 replicate litter bags per plot (nine litterbags per treatment) were randomly removed, placed into a separate clean plastic bag to reduce the loss of small fragments, and transported on ice (0°C) to the laboratory within 4 h. Once in the laboratory, litter bags were processed immediately. First, the three replicate litterbags retrieved from the same plot and treatment were pooled and their wet weight was determined. Thus, three composite samples from each site were obtained for each treatment and DAI. Each composite sample was then homogenized and subsampled for determination of dry mass, nutrient element concentrations, lignin content, phospholipid fatty acids analysis (PLFA) and enzyme activities.

Details of phospholipid fatty acid analysis (PLFA). Microbial community structure was assessed using the PLFA method described by Wu et al.[36]. Briefly, phospholipids were first extracted three times from leaf litter using a methanol, chloroform, citric acid solution. Phospholipids were then separated using silica columns and methylated by alkaline methanolysis. A total of 42 lipids with 20 or fewer carbon atoms each were used to calculate the total microbial lipid abundance (nmol lipid g litter DM^{-1}). Several individual PLFAs were used as indicators for key groups of microbes: 15:0 iso for Gram-positive bacteria; 16:1ω7c for Gram-negative bacteria; 17:0 cyclo and 19:0 cyclo for anaerobic bacteria; 10Me16:0 for Actinobacteria and 18:2ω6,9c for general fungi. In our study, 3–5 individual PLFA markers that can be used to quantify the abundances of Gram-positive bacteria (15:0 iso, 15:0 ante, 16:0 iso, 17:0 iso and 17:0 ante), Gram-negative bacteria (16:1ω5c, 16:1ω7c and 16:1ωw9c) and general fungi (18:1ω9, 18:2ω6,9c and 18:3ω3,6,9c) were highly correlated (R = 0.93–1.00, $P < 0.001$; Table S5)[5,37–39]. Thus, we found that results were similar whether we used one *versus* more indicators for each microbial group. For our final analysis, we only used one indicator (except for anaerobic bacteria).

Determination of potential enzymatic activities. A total of 8 enzyme activities were measured. Specifically, five hydrolytic enzymes which are important for carbon (β-glucosidase (EC 3.2.1.21), cellobiohydrolase (EC 3.2.1.91), and xylosidase (EC 3.2.1.37), nitrogen (N-acetylglucosaminidase, EC 3.1.6.1), and phosphorus (acid phosphatase, EC 3.1.3.2) acquisition were assessed based on methods described by Sinsabaugh et al. and German et al.[40,41]. Three oxidative enzymes important for lignin degradation (laccase (EC 1.10.3.2), general peroxidase (EC 1.11.1.7), and manganese peroxidase (EC 1.11.1.13), MnP) were measured as described by Purahong et al.[12]. Manganese peroxidase activities were not captured by the assay for general peroxidases because, without sufficient amounts of Mn(II) in the assay solution, manganese peroxidase cannot work. Thus in this experiment we found no correlation between the activities of these two enzymes (R = 0.09, P = 0.582) (Table S2).

Measurements of physicochemical parameters of the leaf litter. The water content and pH (in H_2O and 0.01 M $CaCl_2$) of all leaf litter samples were also measured. The pH measurement in 0.01 M $CaCl_2$ was found to provide more consistent and reproducible results than pH measured in H_2O. Total C and N were determined by dry combustion at 1,000°C with an Elementar Vario EL III elemental analyzer (Elementar Analysensysteme GmbH, Hanau, Germany) (DIN/ISO 10694 (Aug. 1996)). Nutrient ions (Mg, K, P, Ca, Fe, Cu, V, Mn, Co) were determined using inductively coupled plasma (ICP) optical emission spectrometry (ICP-OES) and mass spectrometry (ICP-MS) according to manufacturers' specifications. Total lignin was calculated by summing Klason lignin (acid insoluble lignin) and acid soluble lignin[42]. Klason lignin content was determined gravimetrically as the dry mass of solids after sequential hydrolysis with sulfuric acid (72% w/w); in a second step, acid soluble lignin was measured UV-photometrically in 4% H_2SO_4[43,44]. All physicochemical analyses were conducted in triplicate on the same subsample.

Statistical analysis. Total microbial biomass, the abundance of microbial indicators, and enzyme activities were analyzed using two-way (FMP and DAI) analysis of variance (ANOVA) with $P < 0.05$ considered significant, implemented in the PAST program[12,45]. All data were also analyzed using a one-way ANOVA to determine the differences ($P < 0.05$) among different treatments at each DAI[12]. When the differences were significant, they were further analyzed using a post-hoc test (Fisher's Least Significant Difference, $P < 0.05$). All datasets were tested for normality using Shapiro-Wilk and Jarque-Bera tests and the equality of group variances was examined using Levene's test. ANOVA residuals were also plotted to determine the normal probability using the Shapiro-Wilk W test. A Log_{10} transformation ($\log_{10}(x + 1)$) was applied to all data sets that did not meet the parametric assumptions. Non-metric multidimensional scaling (NMDS) based on a Bray-Curtis similarity measures (100 random restarts) were performed on a matrix of PLFAs information (expressed as mole percentages) and a matrix including information on all enzyme activities (Log_{10} transformation) using PAST program[45] and vegan package of R version 2.13.1[46]. Two-way (FMP and DAI) Analysis of Similarity (ANOSIM) based on Bray-Curtis measure was conducted to test for differences among clusters[45]. Correlation between microbial community structure (PLFA matrix) and function (enzyme activity matrix) were analyzed using Mantel tests in PAST program[45]. To investigate the influence of leaf litter physicochemical factors on patterns of microbial community and enzyme activities, we fitted the physicochemical factors onto NMDS ordinations and calculated goodness-of-fit statistics (R^2) using the envfit function in the vegan package[46]. P values were based on 999 permutations[46].

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Author contributions

D. Krüger, F.B., M.H., J.G., D. Kapturska, M.P. and W.P. conceived and designed the experiments. M.P., D. Kapturska, W.P., V.D. and S.M. performed the experiments. W.P. analyzed the data. D.Krüger, F.B., M.H., J.G. and W.P. contributed reagents/materials/analysis tools. W.P., M.S., M.P., D. Krüger, M.H., J.G. wrote the paper.

Additional information

Supplementary information accompanies this paper at http://www.nature.com/scientificreports

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Supplementary information

Uncoupling of microbial community structure and function in decomposing litter across beech forest ecosystems in Central Europe

Witoon Purahong, Michael Schloter, Marek J Pecyna, Danuta Kapturska, Veronika Däumlich, Sanchit Mital, François Buscot, Martin Hofrichter, Jessica LM Gutknecht, Dirk Krüger

Table S1. Statistical values from 2-way ANOVA of all parameters used in this study. Data on oxidative enzyme activities are derived from Purahong et al.[1].

Parameter	2 way ANOVA		
	FMPs	DAI	FMPs $_x$ DAI
Fungal : Bacteria ratio	***F* = 28.47, *P* < 0.001**	***F* = 77.83, *P* < 0.001**	***F* = 16.87, *P* < 0.001**
Total microbial biomass	***F* = 8.791, *P* = 0.001**	***F* = 25.46, *P* < 0.001**	***F* = 4.21, *P* = 0.005**
i15:0, Gram-positive bacteria biomass	***F* = 37.04, *P* < 0.001**	***F* = 68.10, *P* < 0.001**	***F* = 19.43, *P* < 0.001**
16:1ω7c, Gram negative bacteria biomass	***F* = 21.50, *P* < 0.001**	***F* = 44.49, *P* < 0.001**	***F* = 8.63, *P* < 0.001**
10Me16:0, actinobacteria biomass	***F* = 51.13, *P* < 0.001**	***F* = 120.10, *P* < 0.001**	***F* = 40.69, *P* < 0.001**
17:0 cyclo and 19:0 cyclo, anaerobic bacteria biomass	***F* = 23.70, *P* < 0.001**	***F* = 37.05, *P* < 0.001**	***F* = 11.50, *P* < 0.001**
18:2ω6,9c, general fungal biomass	***F* = 19.99, *P* < 0.001**	***F* = 48.38, *P* < 0.001**	***F* = 10.91, *P* < 0.001**
Cellobiohydrolase	*F* = 0.75, *P* = 0.483	***F* = 11.33, *P* < 0.001**	***F* = 2.84, *P* = 0.031**
Xylosidase	***F* = 6.65, *P* = 0.005**	***F* = 6.87, *P* = 0.002**	*F* = 1.12, *P* = 0.379
β-glucosidase	*F* = 1.92, *P* = 0.169	***F* = 9.75, *P* < 0.001**	***F* = 3.35, *P* = 0.015**
N-acetylglucosaminidase	***F* = 4.61, *P* = 0.020**	*F* = 1.76, *P* = 0.183	***F* = 7.11, *P* < 0.001**
Acid phosphatase	***F* = 5.01, *P* = 0.015**	*F* = 1.31, *P* = 0.295	***F* = 7.82, *P* < 0.001**
Laccase	***F* = 16.82, *P* < 0.001**	***F* = 3.40, *P* = 0.034**	*F* = 1.29, *P* = 0.301
Peroxidase	*F* = 1.19, *P* = 0.320	***F* = 5.39, *P* = 0.006**	*F* = 0.80, *P* = 0.582
Manganese peroxidase	***F* = 5.90, *P* = 0.008**	*F* = 1.58, *P* = 0.220	*F* = 0.78, *P* = 0.593

Table S2. Correlations among total PLFA, PLFA indicators and enzyme activities (significant values ($P < 0.05$), are given in bold).

	total_bior	Actinomy	anaerobic	Gram+	Gram-	fungi	MNP	Per	L	P	X	B	N
total_biomass													
Actinomycetes													
anaerobic_bacteria		**0.954**											
Gram+		**0.956**	**0.967**										
Gram-		**0.861**	**0.908**	**0.930**									
fungi		**0.768**	**0.823**	**0.841**	**0.960**								
MNP	0.154	0.170	0.159	0.179	0.158	0.105							
Per	-0.085	-0.243	-0.225	-0.245	-0.158	0.011	0.095						
L	-0.216	**-0.336**	**-0.362**	*-0.279*	*-0.281*	-0.145	-0.119	**0.480**					
P	0.121	0.112	0.217	0.204	0.138	0.073	-0.009	-0.024	-0.022				
X	0.116	-0.093	-0.045	0.028	0.089	0.135	*0.297*	0.146	0.102	0.086			
B	*0.316*	0.115	0.247	0.262	**0.329**	*0.309*	0.086	0.209	0.109	**0.791**	**0.369**		
N	-0.046	-0.241	-0.099	-0.121	-0.036	-0.045	0.037	-0.026	0.025	**0.429**	**0.600**	**0.497**	
C	-0.068	-0.266	-0.168	-0.172	-0.089	-0.013	*0.289*	**0.443**	0.221	0.220	**0.688**	**0.533**	**0.530**

Abbreviations: MNP = Mn-peroxidase, Per = general peroxidase, L = laccase, P = acid phosphatase, X = Xylosidase, B = β-glucosidase, N = N-acetylglucosaminidase

Table S3. Information on the litter composition for individual forest sites[1].

Forest management practice	Litter composition (%)		
	Fagus sylvatica	*Acer* sp.	*Fraxinus* sp.
Beech age-class forest (BA)	85	10	5
Beech selection cutting forest (BS)	90	10	0
Beech unmanaged forest (BU)	100	0	0

Table S4. Initial chemical composition of dried leaf litter under different forest management practices (Mean±SD, n = 3) [1].

Nutrient	Age-class beech forest (BA)	Selection cutting beech forest (BS)	Unmanaged beech forest (BU)
Total C (%)	47.61±0.17	47.34±0.16	48.81±0.22
Total N (%)	0.97±0.01	1.04±0.00	0.84±0.03
C/N	48.92±0.20	45.52±0.15	58.38±2.18
Total lignin/N	43.13±0.25	39.74±0.00	56.27±1.90
Initial Mg (µg/g dry mass)	204.00±50.27	294.33±197.28	157.93±54.19
Initial K (µg/g dry mass)	3076.67±545.01	4596.67±2500.11	2443.33±120.97
Initial Ca (µg/g dry mass)	593.33±142.55	964.33±576.11	344.33±70.61
Initial P (µg/g dry mass)	187.67±41.50	152.67±27.43	157.33±20.84
Initial Mn (µg/g dry mass)	32.87±9.64	26.47±11.65	20.53±5.56
Initial Fe (µg/g dry mass)	5.60±2.52	4.67±2.80	7.37±9.14
Initial Cu (µg/g dry mass)	0.91±0.37	0.62±0.07	0.40±0.04
Initial Co (µg/g dry mass)	0.01±0.00	0.02±0.01	0.01±0.00
Initial V (µg/g dry mass)	0.02±0.00	0.02±0.00	0.02±0.02

Table S5. Correlation among different PLFA markers for (A) Gram-positive bacteria (15:0 iso, 15:0 ante, 16:0 iso, 17:0 iso and 17:0 ante), (B) Gram-negative bacteria (16:1ω5c, 16:1ω7c and 16:1ωw9c) and (C) general fungi (18:1ω9, 18:2ω6,9c and 18:3ω3,6,9c). All correlations were significant ($P < 0.001$).

(A) Gram-positive bacteria

	a15:0	i15:0	i16:0	a17:0
a15:0				
i15:0	**0.97**			
i16:0	**0.98**	**0.99**		
a17:0	**0.98**	**0.98**	**1.00**	
i17:0	**0.93**	**0.99**	**0.98**	**0.97**

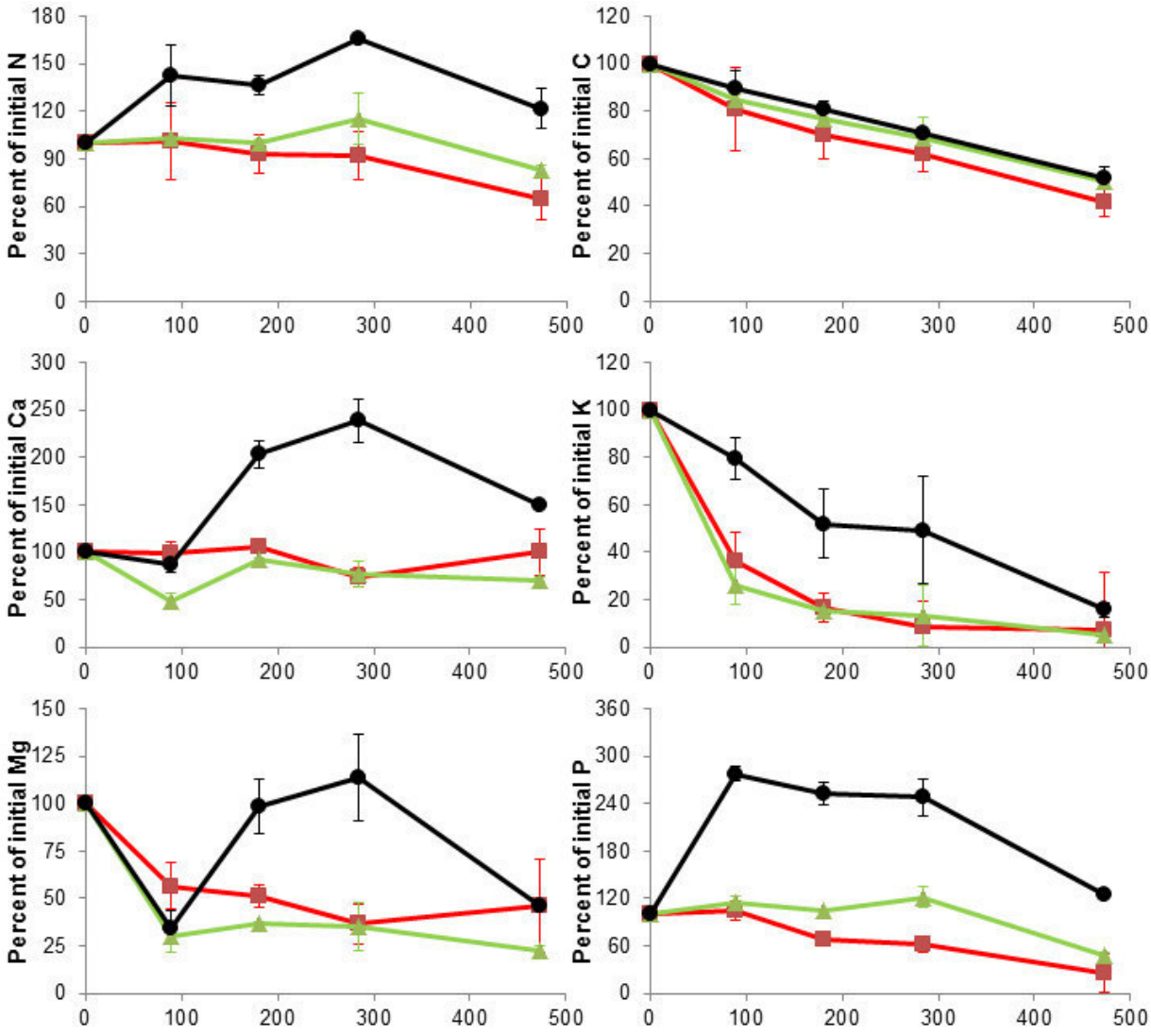
(B) Gram-negative bacteria

	16:1ω5c	16:1ω7c
16:1ω5c		
16:1ω7c	**0.94**	
16:1ωw9c	**0.98**	**0.97**

(C) General fungi

	18:1ω9	18:2ω6,9c
18:1ω9		
18:2ω6,9c	**0.97**	
18:3ω3,6,9c	**0.95**	**0.94**

Figure S1. Percent of initial nitrogen (N), carbon (C), calcium (Ca), potassium (K), magnesium (Mg), and phosphorous (P) during decomposition under different forest system management practices[1]. European beech age-class forest (red, BA), European beech selection cutting forest (green, BS) and unmanaged deciduous forest reserves dominated by European beech (black, BU) (mean ± SD, n = 3).



Reference

1. Purahong, W. et al. Influence of different forest system management practices on leaf litter decomposition rates, nutrient dynamics and the activity of ligninolytic enzymes: a case study from Central European forests. *PLoS ONE* 9: e93700 (2014).

CHAPTER 8

Effects of forest management practices in temperate beech forests on bacterial and fungal communities involved in leaf litter degradation

Witoon Purahong[1, 2], Danuta Kapturska[1, 3], Marek J. Pecyna[3], Katalee Jariyavidyanont[1], Jennifer Kaunzner[1], Kantida Juncheed[1], Tanaporn Uengwetwanit[1], Renate Rudloff[1], Elke Schulz[1], Martin Hofrichter[3], François Buscot[1, 4, 5], Michael Schloter[6], Dirk Krüger[1]

[1]UFZ-Helmholtz Centre for Environmental Research, Department of Soil Ecology,Theodor-Lieser-Str. 4, D-06120 Halle (Saale), Germany

[2]Technical University of Munich, Chair for Soil Science, Ingolstädter Landstr. 1, D-85758 Oberschleissheim, Germany

[3]Technical University of Dresden, International Graduate School (IHI) Zittau, Markt 23, D-02763 Zittau, Germany

[4]University of Leipzig, Institute of Biology, Johannisallee 21-23, D-04103 Leipzig, Germany

[5]German Centre for Integrative Biodiversity Research (iDiv), Halle-Jena-Leipzig, Deutscher Platz 5e, D-04103 Leipzig, Germany.

[6]Helmholtz Zentrum München, Research Unit for Environmental Genomics, Ingolstädter Landstr. 1, D-85758 Oberschleissheim, Germany

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SHORT COMMUNICATION

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Authors:

Witoon Purahong[1, 2*†], Danuta Kapturska[1, 3†], Marek J. Pecyna[3†], Katalee Jariyavidyanont[1], Jennifer Kaunzner[1], Kantida Juncheed[1], Tanaporn Uengwetwanit[1], Renate Rudloff[1], Elke Schulz[1], Martin Hofrichter[3], Michael Schloter[4], Dirk Krüger[1†], François Buscot[1, 5*]

Affiliations:

[1]UFZ-Helmholtz Centre for Environmental Research, Department of Soil Ecology, Theodor-Lieser-Str. 4, D-06120 Halle (Saale), Germany, [2]Technical University of Munich, Chair for Soil Science, Ingolstädter Landstr. 1, D-85758 Oberschleissheim, Germany, [3]Technische Universität Dresden, International Institute (IHI) Zittau, Markt 23, D-02763 Zittau, Germany, [4]Helmholtz Zentrum München, Research Unit for Environmental Genomics, Ingolstädter Landstr. 1, D-85758 Oberschleissheim, Germany, [5]German Centre for Integrative Biodiversity Research (iDiv), Halle-Jena-Leipzig, Deutscher Platz 5e, D-04103 Leipzig, Germany.

***Corresponding authors**:

Tel: +49 345-5585-219, Fax: +49 345-5585-449, E-mail: witoon.purahong@ufz.de

Tel: +49 345-5585-221, Fax: +49 345-5585-449, E-mail: francois.buscot@ufz.de

† These authors contributed equally to this work.

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Abstract

Forest management practices (FMPs) significantly influence important ecological processes and services in Central European forests, such as leaf litter decomposition and nutrient cycling. Changes in leaf litter diversity and thus its quality as well as microbial community structure and function induced by different FMPs were hypothesized to be the main drivers causing shifts in decomposition rates and nutrient release in managed forests. In a litterbag experiment lasting 473 days, we aimed to investigate the effects of FMPs (even-aged timber management, selective logging and unmanaged) on bacterial and fungal communities involved in leaf litter degradation over time. Our results showed that microbial communities in leaf litter were strongly influenced by both FMPs and sampling date. The results from NMDS ordination revealed distinct patterns of bacterial and fungal successions over time in leaf litter. We demonstrated that FMPs and sampling dates can influence a range of factors, including leaf litter quality, microbial macronutrients and pH, which significantly correlate with microbial community successions.

Keywords: Forest management; fungal diversity; bacterial diversity; microbial community; lignocellulose decomposition; decomposition rates; nutrient release.

Introduction

Forest management practices (FMPs) alter the composition and performance of most forest biota including plants, animals and microorganisms [1–5]. Intensive forest management can change the quantity and quality of leaf litter by modifying the diversity and community structure of higher plants in the tree, shrub and herb strata [2, 6, 7]. Changes in physical and chemical properties of leaf litter are known to affect leaf litter decomposition rates and consequently impact nutrient cycling and carbon sequestration rates in forest ecosystems [8–10]. It has been proposed that a managed forest (such as even-aged timber management) has higher decomposition and nutrient release rates compared to unmanaged forests as a result of highly heterogeneous litter quality [7]. Several studies have indicated that an increase in plant diversity and, thus, a larger spectrum of leaf litter qualities causes non-additive decomposition rates, meaning that litter mixtures decompose at a different rate compared to that predicted from decomposition rates of single litter types [11, 12]. Alternatively, Chapman et al. (2013) suggested that the alteration of microbial succession could be the mechanism for non-additive effects in litter decomposition [12].

In Central Europe as well as elsewhere in the Northern hemisphere, even-aged forests are widespread. In North America, and elsewhere in the palearctic boreal forest belt, even-aged timber management includes clear cutting by heavy machinery, especially in softwood stands [13]. However, in Central Europe, clear cutting without intended abandonment of land-use as forest is normally not allowed or restricted to small areas of few hectares. Nevertheless, even-aged forests in different areas are all subjected to intensive thinning operations, wood harvesting and lead to an even age of timber trees [14]. Thinning operations and forest structure significantly affect the microclimate such as light penetration and temperature. Wood harvesting and transport can significantly impact soil physical characteristics, e.g.

through compaction, that in turn could significantly influence soil microbial communities. Selective logging (in German known as 'Plenterwald') is encouraged as eco-friendly forest management system in hardwood forests [14]. Although selectively logged forests have uneven-aged forest structures, they can nevertheless differ strongly from unmanaged forest. If many trees are harvested, this forest management practice can be considered as intensive. Selectively logged forests have been suggested to be similarly efficient in maintaining diversity of different groups of saproxylic organisms including both fungi and invertebrates [4, 5] as unmanaged forests; however, to the best of our knowledge the effect of selective logging on microbial communities in leaf litter is still unknown. Due to the wide distribution of even-aged forests and the increasing interest for eco-friendly forest management practices, even-aged timber management and selective logging were chosen for our study.

In managed forest ecosystems, large amounts of wood are often harvested. Thus, leaf litter may be considered as the major source of nutrients that return to the soil [7]. Effects on nutrient dynamics and decomposition rates can significantly affect the nutrient status, productivity and sustainability of such managed forests. Microbial communities, both bacterial and fungal, play important roles in decomposition processes in leaf litter as well as other substrates [4, 5, 7]. Thus, investigating microbial community successions in leaf litter under different FMPs may provide crucial information to explain the differences in leaf litter decomposition rates in forests with different FMPs [12]. In our study, we hypothesized that non-additive effects in litter decomposition rates in even-aged and selectively logged forests can be partly explained by differences of microbial community successions as compared to unmanaged forest [12].

To test this hypothesis, this study aimed at (i) investigating the effects of different FMPs on bacterial and fungal communities in leaf litter over time and (ii) testing whether alteration of

microbial community development could be the mechanism for non-additive decomposition rates occurring in mixed leaf litter under different FMPs. Therefore, we carried out a 15-month litterbag experiment and investigated microbial diversity patterns at four time points using automated ribosomal intergenic spacer analysis (ARISA) of the bacterial intergenic spacer (IGS) and the fungal nuclear ribosomal internal transcribed spacer (nrITS) regions [4, 5, 15]. ARISA is a widely used cost-effective molecular culture-independent technique for analyzing microbial diversity and community structure in various types of habitats encompassing both terrestrial and aquatic ecosystems [4, 5, 15–20]. We carried out multivariate analysis to investigate how different factors under different FMPs, including leaf litter quality parameters (carbon: nitrogen and lignin: N ratios) and other abiotic factors in leaf litter (pH, water content, concentrations of microbial macronutrients and micronutrients) shaped the bacterial and fungal communities and their succession.

Materials and methods

Study area and FMPs

Leaf litter samples used in this study were obtained from a 473 day litterbag experiment carried out in the Hainich-Dün region in Central Germany (about 1,300 km^2; 51°16'N 10°47'E), part of the German Biodiversity Exploratories (http://www.biodiversity-exploratories.de), as described by Purahong et al. [7]. The three FMPs used in this study were: (i) European beech even-aged forest (BA, semi-natural forest with natural regeneration, even-age forest structure); (ii) European beech selectively logged forest (BS, close-to-nature forest management with natural regeneration, uneven-age forest structure) and (iii) unmanaged deciduous forest reserves dominated by European beech (BU, uneven-age forest structure). All sites were dominated by European beech (*Fagus sylvatica*) but, depending on

the management, had varying degrees of admixed additional tree species (Table S1). The BA was dominated by three tree species (85% *Fagus sylvatica*, 10% *Acer* sp. and *Fraxinus* sp. 5%), whereas the BS was dominated by two tree species (90% *Fagus sylvatica* and 10% *Acer* sp.). and the BU by *Fagus sylvatica* alone. All study sites are located within a 30 km radius and thus varied little in geographic and climatic conditions. The soil of all sites has been characterized as Stagnosol [21], with a pH of 5.1 ± 1.1 [22] and a thin litter layer (2-5 cm depth).

Experimental design and litterbag experiment

Within each FMP area (10,000 m^2 each), we assigned three plots (2 m × 8 m) located on available flat land; these represented three replicates. The distances between the plots were at least 2.5 m. See Purahong et al. [7] for detailed information on the experimental design.

A total of 135 litterbags were used for the experiment. Each litterbag (25-cm × 25-cm, 2-mm nylon mesh size) contained 10 g of mixed local leaves, representative of the litter composition of the deciduous tree species at each site (Table S1) [7]. Freshly fallen leaves were collected from the forest floor during the peak litter fall period. At the end of the litter fall period (13 November 2009), 108 litterbags were placed horizontally among fallen leaves in the upper litter horizon of the study plots (12 bags per plot resulting in 36 litterbags per management type); 27 litter bags (9 per treatment) were retained to determine the initial dry mass (oven-dried at 105 ºC ≥ 24 h until constant weight). Three litterbags per plot were retrieved on four sampling dates: in 2010 on 10 February (89 days, winter), 12 May (180 days, spring), 24 August (284 days, summer) and in 2011 on 1 March (473 days, winter). Leaf litter from the three litterbags retrieved from the same plot and treatment were pooled. Thus, three composite samples from each site were obtained for each treatment and sampling

date. Each composite sample was homogenized and divided into subsamples for DNA extraction and determination of physical properties, nutrient concentrations (microbial macronutrients: C, N, P, K, Ca, Mg, Fe; microbial micronutrients: Mn, Co, Cu, V) and lignin content. Samples were stored at -20 °C until analysis. Further details on the experimental procedure can be found in supporting information.

Physicochemical analyses of leaf litter

The water content was analyzed by oven-drying the leaf litter samples at 105 ºC ≥ 24 h or until constant weight. Leaf litter pH was determined in H_2O and $CaCl_2$ [23] using 0.5 g of ground litter sample and 10 ml demineralized water after overnight incubation (see more detailed information in supporting information). Total C and N were determined by dry combustion at 1,000 °C with an Elementar Vario EL III elemental analyzer (Elementar Analysensysteme GmbH, Hanau, Germany). Nutrient ions (Mg, K, P, Ca, Fe, Cu, V, Mn, Co) were determined using inductively coupled plasma (ICP) optical emission spectrometry (ICP-OES) and mass spectrometry (ICP-MS) according to manufacturers' specifications (PerkinElmer Inc., Waltham, MA, USA). Klason lignin content was determined gravimetrically as the dry mass of solids after sequential hydrolysis with sulfuric acid (72% w/w); in a second step, acid soluble lignin was measured by UV-spectrophotometry in 4% H_2SO_4 [24, 25]. Total lignin was obtained by summing acid insoluble (Klason) lignin and acid soluble lignin [26]. All physicochemical analyses were conducted in triplicate on the same subsample.

DNA extraction and determination of bacterial and fungal ARISA patterns

DNA was extracted from 100 mg of each of the homogenized freeze-dried leaf litter samples using a ZR Soil Microbe DNA MiniPrep kit according to the manufacturer's instructions (Zymo Research, Irvine, CA, USA) [4, 5]. The presence and quantity of genomic DNA were determined using a NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Dreieich, Germany) and the extracts were then stored at -20ºC.

B-ARISA (bacterial) and F-ARISA (fungal) were conducted under the conditions described by Frossard et al. [15] and Purahong et al. [4], respectively. Operational taxonomic units (OTUs) derived from ARISA were defined as described previously by Green et al. [27] and Jones et al. [16] and may differ from those OTU definitions relying on sequences. Briefly, B-ARISA polymerase chain reaction was performed in duplicate reactions under the conditions described by Borneman and Triplett [28] and modified according to Frossard et al. [15] and Purahong et al. [29]: the PCR mixture (20 µl) contained 1 µl DNA template (~20 ng DNA template as determined by NanoDrop); 10 µM of primer 1406f (5′-TGYACACACCGCCCGT-3′) labeled with FAM at 5′-end and an unlabeled 23Sr primer (5′-GGGTTBCCCCATTCRG-3′); 4 µl FIREPol 5x Master Mix (Solis BioDyne, Tartu, Estonia); and water to 20 µl. PCR was carried out with an initial denaturation at 94 °C for 5 min, followed by 30 cycles of 94 °C for 35 s, 55 °C for 45 s and 72 °C for 2 min, with a final extension at 72 °C for 5 min. F-ARISA amplification was done in duplicate under the conditions described by Purahong et al. [4]: the PCR mixture (20 µl) contained 1 µl DNA template (~20 ng DNA template as determined by NanoDrop); 10 µM of fungal-specific, plant-excluding primer ITS1F (5′- CTTGGTCATTTAGAGGAAGTAA-3′, Gardes and Bruns [30]) labeled with FAM at 5′-end and an unlabeled ITS4 primer (5′-TCCTCCGCTTATTGATATGC-3′, White et al. [31]); 4 µl FIREPol 5x Master Mix (Solis

BioDyne, Tartu, Estonia); and water to 20 µl. PCR was carried out with an initial denaturation at 95 °C for 5 min, followed by 35 cycles of 95 °C for 60 s, 55 °C for 60 s and 72 °C for 75 s, with a final extension at 72 °C for 7 min. The PCR products were purified using a PCRExtract Mini Kit (5PRIME, Hamburg, Germany). A standardized quantity of 40 ng (B-ARISA) or 20 ng (F-ARISA) DNA, as determined by NanoDrop, was mixed with 14 µl of deionized Hi-Di formamide (Applied Biosystems, Foster City, CA, USA) and 0.1 µl of internal-size standard Map Maker 1500 ROX (50-1500 bp) (BioVentures, Inc, Murfreesboro, TN, USA). The mixture was denatured for 5 min at 95 °C and chilled on ice for at least 10 min before being further processed using a capillary sequencer (ABI PRISM 3730xl Genetic Analyzer, Applied Biosystems). DNA normalization was done twice in ARISA (before the initial PCR and before the separation of DNA fragments using capillary electrophoresis) to make this standard ARISA robust for inferring changes in community structure [4, 5, 32].

All peaks of the fragments between 200 and 1,500 bp that were present in two analytical PCR replicates were used for further analyses [15]. The two independent PCR replicates were highly correlated. OTU binning was carried out using an interactive custom binning script in R version 2.14.1[32]. The total peak area per sample was normalized to 1 and the relative fluorescent intensity (RFI) was calculated. All peaks with RFI values lower than 0.09% were discarded as background noise. A strategy involving a binning size of 2 bp was applied to the bacterial and fungal ARISA data and the binning frame that gave the highest pairwise similarity among samples was used for further statistical analyses.

Statistical analyses

Decomposition rates for each forest type were determined by fitting a single negative exponential decay equation [33]. Our experimental design may contain potential sources of dependencies among samples as each plot within one replicate site represents only one stand per FMP. We analyzed the data as suggested by Yannarell et al. [34] by treating the experiment as a split-plot design, with FMP as a whole-plot factor replicated 3 times, the forest plots as the plot factor, and time as the within-plot factor. B-ARISA and F-ARISA fingerprint data were analyzed according to this split-plot design with permutational multivariate analysis of variance (PERMANOVA) using the 'adonis' function of the 'vegan' package in R [35]. Our null distribution was generated based on 1,999 permutations of the rows of the sample-by-OTU data tables according to our restricted permutation scheme. Non-metric multidimensional scaling (NMDS), based on Bray-Curtis distance measurements (including plotting correlations with environmental variables), was carried out using the software PAST to visualize the patterns of bacterial and fungal community succession over time and to investigate the factors related to bacterial and fungal community assemblages [36]. Goodness-of-fit statistics (R^2) of environmental variables fitted to the NMDS ordination of bacterial and fungal communities were calculated using the 'envfit' function in the vegan package, with P values based on 999 permutations [35]. All significant environmental variables ($P < 0.05$) were plotted in the NMDS ordination using PAST. Ordinations of detailed bacterial and fungal communities in leaf litter under different forest management practices at each sampling date were analyzed using principle component analysis (PCA) with the biplots option of PAST.

Results

Effect of FMPs on bacterial and fungal community structure

Microbial communities were strongly influenced by FMPs (bacterial community: Pseudo-F = 4.65, P = 0.001; fungal community: Pseudo-F = 6.76, P = 0.001) and sampling dates (bacterial community: Pseudo-F = 10.23, P = 0.001; fungal community: Pseudo-F = 13.69, P = 0.001; Table 1, Figures 1A, 1B). Bacterial communities from the BA forest formed a distinct cluster in the NMDS biplot (Figure 1A). Bacterial communities in unmanaged beech (BU) and BS forests were more similar to each other; however, bacterial communities from both forests at the same sampling date showed no overlap in NMDS ordination space (Figure 1A). Fungal communities in the BA forest also showed distinct community succession patterns compared to those of BU and BS forests (Figure 1B). Communities changed more slowly and/or were resistant to temporal changes. Fungal communities from BU and BS forests developed over time, overlapping on two sampling dates (180 and 284 days, Figure 1B). Ordination plots of bacterial and fungal communities in leaf litter under different FMPs at each sampling date are given in the supporting information (Figures S1, S2, respectively).

Overall, 267 bacterial and 220 fungal OTUs were detected in the three forest types. Approximately 54 – 58% of the bacterial and 44 – 55% of the fungal OTUs were shared between all forest types (Table 2). Patterns of bacterial OTU richness in leaf litter from managed forests (BA and BS) were similar and increased over time but differed from that of the BU forest (Figure 2A). Bacterial OTU richness in the BU forest was higher compared to BA and BS forests at two sampling dates (89 and 180 days, Figure 2A). Distinct patterns of fungal OTU richness in leaf litter were observed in different FMPs (Figure 2B).

Factors corresponding with bacterial and fungal community successions

We explored the relationships between environmental variables and bacterial and fungal community succession patterns by fitting leaf litter physicochemical properties to the NMDS ordination of bacterial and fungal communities [34, 35]. For bacterial communities, we found that litter quality parameters (C: N and lignin: N ratios), macronutrients in leaf litter (total C, total N, K, Ca) and pH were strongly related to bacterial community succession ($P < 0.01$, Table 3. Litter Fe was marginally related to bacterial community composition ($P = 0.081$). In the case of fungi, similar factors that corresponded with bacterial communities (C: N and lignin: N ratios, total C, total N, K, Ca, pH) also significantly correlated with the fungal communities (Table3). In addition, total lignin ($P = 0.006$) and litter Fe ($P = 0.003$) concentrations were significantly related to fungal community succession.

Discussion

FMPs influence tree species richness that in turn significantly affected the leaf litter quality in forests with different FMPs. The initial chemical composition of dried leaf litter under different forest management practices are shown in Table S2. Decomposition rates of leaf litter in even-aged (BA) and selectively logged (BS) beech forests were strongly increased (31 – 72%) when small fractions (5 – 10%) of leaf litter from *Acer* sp. and/or *Fraxinus* sp. were mixed with *Fagus sylvatica* leaf litter (Tables S1, S3) [7]. Overall, our results support the idea that different microbial community succession could be a mechanism explaining the non-additive decomposition rates of mixed leaf litter in forests under different FMPs [12]. Some studies have provided experimental evidence of the effects of substrate quality on decomposers [37–41]. Interestingly, both synergistic and antagonistic interactions have been observed in litter mixtures [42–44]. Obviously in our study, synergistic interactions played an

important role and may be explained, for example, by a positive feedback of soil invertebrates and decomposers due to diverse habitat and energy sources and the nutrient transfer (leaching) from nutrient-rich to nutrient-poor (e.g. *Fagus* sp.) litter species [42, 43].

Our study showed that bacterial and fungal communities were strongly influenced by FMPs and incubation time of leaf litter material (sampling dates). The succession patterns of both bacterial and fungal communities differed significantly under different FMPs. This may be explained by the fact that forest management can greatly alter tree species richness, quality and quantity of leaf litter input, forest structure and microclimate [4–6, 45] that, in turn, could significantly affect microbial communities [4, 46]. In addition, we found that FMPs also affect the initial bacterial and fungal community structure (Figure S3). Thus, it is conceivable that any differences in litter among FMPs are related to the assembly or activity of these initial microbial communities. The combination of our results on microbial community structure (especially fungi) and previous work on oxidative enzyme activities from the same samples [7] could provide some indication of which aspects of community succession influence decomposition rates. The fungal communities from BA forest at the early decomposition stage (sampling dates: 89 and 180 days) differed from those of BU and BS forests (Figures 1B, S2), which was reflected in differences in enzyme activities in the same samples. BA forest samples had high activities of manganese peroxidase, an exclusively fungal, extracellular enzyme pivotal for enzymatic lignin decomposition [7]. On the contrary, BU and BS litter samples showed low or no manganese peroxidase activity but high activities of the weaker oxidative enzyme laccase, an extracellular enzyme of bacterial or fungal origin which only plays an inferior role in enzymatic lignin degradation [7]. It is known that lignin decomposition is the rate-limiting step in litter decomposition, thus samples of leaf litter in which lignin is decomposed faster will likely have higher decomposition rates [7]. Nevertheless, using microbial community structure to predict microbially-mediated

ecological functions such as patterns of enzyme activities may not give a definitive answer. A recent study reported a disconnection between microbial community structure and function in forest ecosystems due to functional redundancy within the microbial community and the differences between the drivers of microbial growth and those of microbial function [47]. To further investigate the functional redundancy, studies using sequence-based approaches and microbial ecosystem function analysis are needed.

It is noteworthy from our study that litter quality, microbial macronutrients, and pH are important factors shaping the microbial community in leaf litter under different FMPs. Nutrient elements are able to affect microbial growth, activity and survival [48 49]. Specifically, C and N are important elements in all macromolecules including carbohydrates, proteins, lipids, and nucleic acids [49]. K and Ca are required for the activity of enzymes as cofactors [49]. Highly concentrated transition metals may be toxic to microorganisms; however, this was not the case in our study because all transition metal concentrations we measured were below the toxic levels reported for microbes [50]. Of all the transition metals, iron (Fe) had the highest impact on both bacterial and fungal community development in leaf litter. Fe is required in large amounts as it is involved in many important metabolic processes [49]. Ligninolytic peroxidases (i.e. aforementioned manganese peroxidase, lignin peroxidase and versatile peroxidase) are secreted, exclusively fungal heme proteins that indeed depend on Fe^{3+} as the central ion in the catalytically active porphyrin ring. This also applies to most monoxygenases and dioxygenases (e.g. P450 and Fe-S cluster enzymes) that are the responsible biocatalysts in the intracellular metabolism of aromatics [51].

Interestingly, the total lignin content only significantly correlates with fungal communities. This may be related to the ability of fungi (in particular of certain basidiomycetes) to degrade and chemically modify the recalcitrant lignin polymer to a substantial extent [7, 52]. Such

fungi were reported to secrete a range of ligninolytic and lignin-modifying enzymes: high-redox potential ligninolytic peroxidases as mentioned as well as laccase (copper-containing phenol oxidases) [52]. In contrast, few bacteria are able to produce extracellular laccase and they are generally lacking manganese-oxidizing peroxidases that initiate lignin oxidation [52, 53]. All these enzymes can catalyze the oxidation of phenolic lignin moieties, however, the more recalcitrant non-phenolic lignin accounting for 90% of the aromatic rings in the lignin polymer [54], can only be attacked by the high-redox potential peroxidases, while laccase has a generally lower oxidative strength [55, 56] and is therefore only considered as a lignin-modifying rather than a ligninolytic enzyme [57]. Overall our results support the hypothesis that different FMPs could alter bacterial and fungal community succession patterns, which are involved in leaf litter degradation and explain the different ligninolytic enzyme activities and leaf litter decomposition rates, which were found under different FMPs [7].

Conclusion and outlook

We conclude that FMPs significantly affect bacterial and fungal community succession and richness patterns. FMPs influence bacterial and fungal community succession in leaf litter over time via a number of factors, including leaf litter quality, microbial macronutrients and pH. Alteration of microbial community succession is one of the mechanisms, which could explain non-additive decomposition rates occurring in mixed leaf litter under different FMPs. Further studies using sequence-based approaches may be needed in the future to unravel the major responders to management and time factors.

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Table 1. Permutational multivariate analysis of variance for bacterial and fungal community structure.

Microbial community	Source	Pseudo-*F*	R^2	*P* value
Bacterial community	Overall sampling date	10.23	0.21	0.001***
	Sampling date (only BA)	4.12	0.29	0.001***
	Sampling date (only BS)	6.00	0.38	0.001***
	Sampling date (only BU)	8.01	0.45	0.001***
	Overall FMP	4.65	0.09	0.001***
	FMP (only 1st sampling date)	1.90	0.21	0.033*
	FMP (only 2nd sampling date)	1.94	0.22	0.017*
	FMP (only 3rd sampling date)	3.74	0.35	0.001***
	FMP (only 4th sampling date)	3.88	0.36	0.001***
	Sampling date x FMP	2.71	0.05	0.007**
Fungal community	Overall sampling date	13.69	0.25	0.001***
	Sampling date (only BA)	7.39	0.42	0.002**
	Sampling date (only BS)	7.30	0.42	0.001***
	Sampling date (only BU)	4.76	0.32	0.001***
	Overall FMP	6.76	0.12	0.001***
	FMP (only 1st sampling date)	6.86	0.50	0.004**
	FMP (only 2nd sampling date)	8.54	0.55	0.003**
	FMP (only 3rd sampling date)	2.53	0.27	0.013*
	FMP (only 4th sampling date)	2.73	0.28	0.005**
	Sampling date x FMP	3.14	0.06	0.011*

P values were based on 1,999 restricted permutation of samples; *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. FMP = forest management practice, BA = beech even-aged forest, BS = beech selectively logged forest and BU =beech unmanaged forest.

Table 2. Pairwise comparison of bacterial and fungal OTUs from different forest management practices.

Microbial group	Pairwise comparison	Number of OTUs		
		Only in 1st forest	Only in 2nd forest	Shared
Bacteria	Beech unmanaged *vs.* Beech even-aged	47	52	139
	Beech unmanaged *vs.* Beech selectively logged	58	50	128
	Beech selectively logged *vs.* Beech even-aged	47	60	131
Fungi	Beech unmanaged *vs.* Beech even-aged	65	44	87
	Beech unmanaged *vs.* Beech selectively logged	47	39	105
	Beech selectively logged *vs.* Beech even-aged	55	42	89

Table 3. Goodness-of-fit statistics (R^2) of environmental variables fitted to the non-metric multidimensional scaling (NMDS) ordination of bacterial and fungal communities.

Variables	Bacterial community		Fungal community	
	R^2	P	R^2	P
Total C	**0.754**	**0.001*** **	**0.410**	**0.001 *** **
Total N	**0.435**	**0.001*** **	**0.506**	**0.001 *** **
C:N ratio	**0.741**	**0.001*** **	**0.699**	**0.001 *** **
Lignin:N ratio	**0.408**	**0.001*** **	**0.240**	**0.011***
pH ($CaCl_2$)	**0.298**	**0.004****	**0.232**	**0.011***
Mg	0.092	0.219	0.104	0.155
K	**0.464**	**0.002****	**0.204**	**0.027 ***
Ca	**0.301**	**0.004 ****	**0.193**	**0.026 ***
P	0.090	0.214	0.098	0.156
Mn	0.073	0.292	0.001	0.984
Fe	*0.1301*	*0.081*	**0.257**	**0.003****
Cu	*0.160*	*0.051*	0.003	0.955
Co	0.020	0.730	0.060	0.372
V	0.041	0.498	0.087	0.233
Water content	0.068	0.285	0.352	0.533
Total lignin	0.021	0.723	**0.240**	**0.006****

Figure Legends

Figure 1. NMDS ordination biplots showing microbial community succession in leaf litter in forests with different forest management practices (FMPs): bacterial communities (A) and fungal communities (B). Permutational multivariate analysis of variance was conducted to test the effects of sampling date (SD) and FMP on bacterial and fungal community structure (*P* values were based on 1,999 permutations). Environmental variables were fitted to the non-metric multidimensional scaling (NMDS) ordination of bacterial and fungal communities. The significance was based on 999 permutations and only significant variables ($P < 0.05$) are shown.

Figure 2. Patterns of bacterial (A) and fungal (B) OTU richness in leaf litter across different forest management practices over time.

Supporting information

Litterbag experiment procedure

Leaf litter pH measurement

Table S1. Information on the litter composition for individual forest sites.

Table S2. Initial chemical composition of dried leaf litter under different forest management practices.

Table S3. Mean decay rate constants (k) of leaf litter, coefficients of determination (r^2) describing the fit of the decay model ($P < 0.05$) under different forest management practices.

Figure S1. PCA biplot showing bacterial communities in leaf litter under different forest management practices at each sampling date.

Figure S2. PCA biplot showing fungal communities in leaf litter under different forest management practices at each sampling date.

Figure S3. NMDS ordinations showing bacterial and fungal communities in freshly fallen leaf litter under different forest management practices.

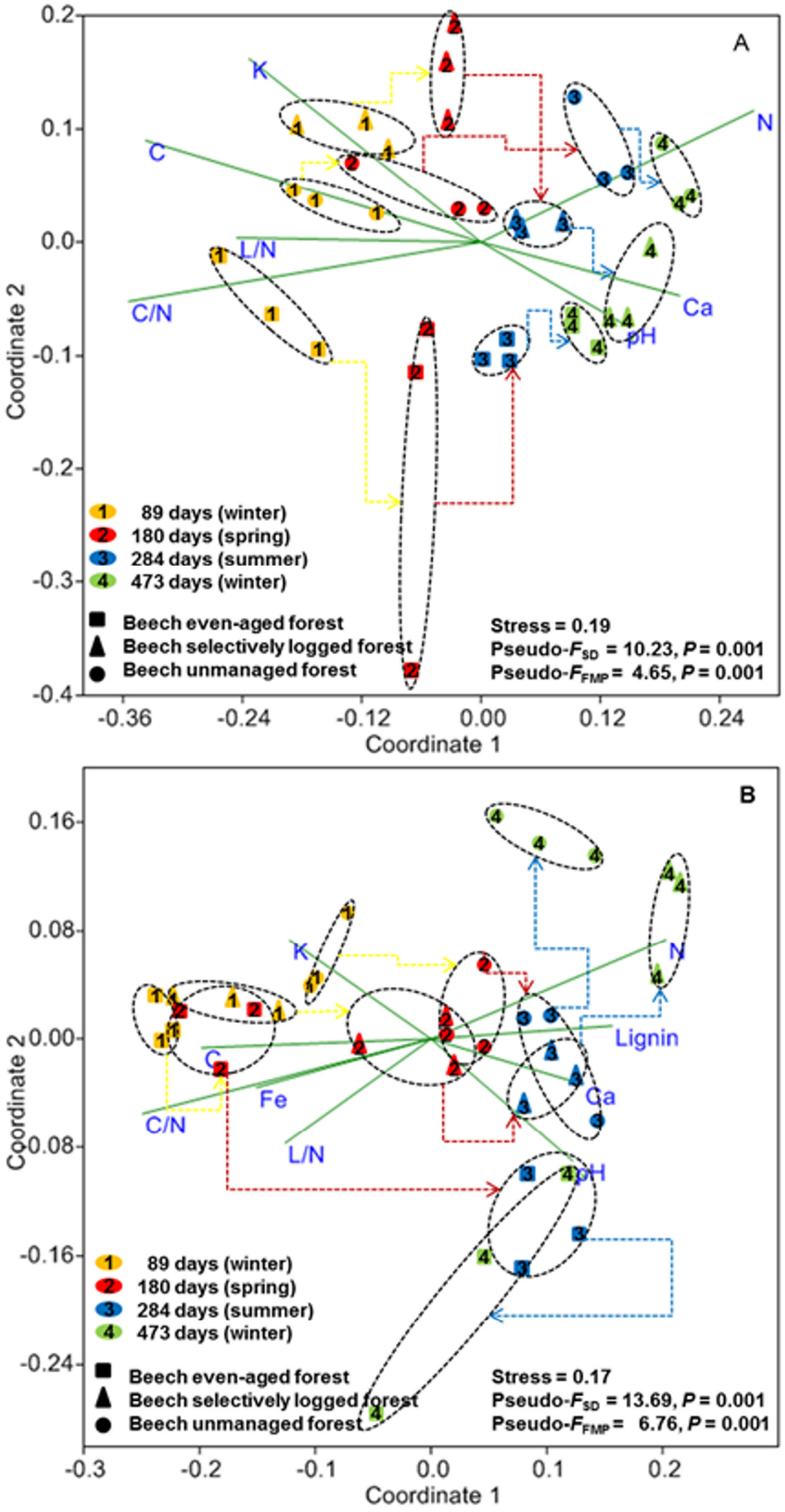

A
K
N
C
L/N
C/N
Ca
pH
1 89 days (winter)
2 180 days (spring)
3 284 days (summer)
4 473 days (winter)
Beech even-aged forest
Beech selectively logged forest
Beech unmanaged forest
Stress = 0.19
Pseudo-F_{SD} = 10.23, P = 0.001
Pseudo-F_{FMP} = 4.65, P = 0.001
Coordinate 1
Coordinate 2
B
K
N
Lignin
C
Fe
Ca
C/N
L/N
pH
1 89 days (winter)
2 180 days (spring)
3 284 days (summer)
4 473 days (winter)
Beech even-aged forest
Beech selectively logged forest
Beech unmanaged forest
Stress = 0.17
Pseudo-F_{SD} = 13.69, P = 0.001
Pseudo-F_{FMP} = 6.76, P = 0.001
Coordinate 1
Coordinate 2

Figure 1.

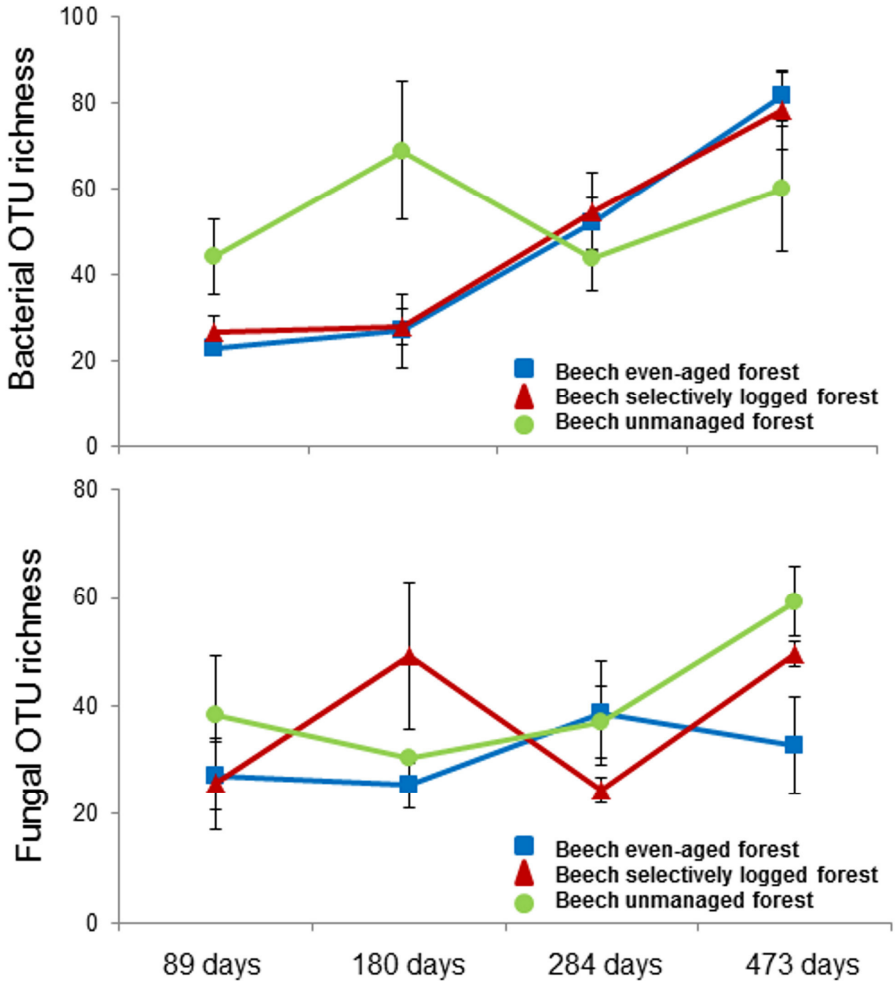

Bacterial OTU richness
100
80
60
40
20
0
Beech even-aged forest
Beech selectively logged forest
Beech unmanaged forest
Fungal OTU richness
80
60
40
20
0
Beech even-aged forest
Beech selectively logged forest
Beech unmanaged forest
89 days
180 days
284 days
473 days

Figure 2.

Supporting information

Effects of forest management practices in temperate beech forests on bacterial and fungal communities involved in leaf litter degradation

Witoon Purahong, Danuta Kapturska, Marek J. Pecyna, Katalee Jariyavidyanont, Jennifer Kaunzner, Kantida Juncheed, Tanaporn Uengwetwanit, Renate Rudloff, Elke Schulz, Martin Hofrichter, Michael Schloter, Dirk Krüger, François Buscot.

Litterbag experiment procedure

At each forest site, freshly fallen leaves of deciduous species were collected from the forest floor in October, 2009. The leaves from each plot were separated according to species and air dried to constant weight at room temperature. Ten grams of local mixed leaves (representative of the litter composition of the deciduous tree species at each respective site, Table S1) were placed in litterbags (25 cm × 25 cm, 2 mm nylon mesh size). A total of 135 litterbags were used for the experiment. At the end of the litter fall period (13 November 2009), 108 litterbags were placed in a horizontal position in the upper litter horizon of the study plots (36 litter bags per management type resulting in 12 bags per plot) while 27 litterbags (9 per treatment) were retained to determine the initial dry mass (oven-dried at 105 ºC ≥24 h until constant weight). Litterbags were retrieved on four sampling dates: in 2010 on 10 February (89 days), 12 May (180 days), 24 August (284 days), and in 2011 on 1 March (473 days). On each sampling occasion, a random sample of nine litterbags per treatment (three litterbags per plot) was carefully removed, each put in a separate clean plastic bag to reduce the loss of small fragments and transported on ice (0ºC) to the laboratory within 4 h In the laboratory, litterbags were processed immediately. The extraneous organic materials adhering to the outside of the bags were removed and the leaf litter from the three litterbags retrieved from the same plot and treatment was pooled. Thus, three composite samples from

each site were obtained for each treatment and sampling date and each composite sample was homogenized and kept frozen at -20ºC until DNA extraction.

Leaf litter pH measurement

Leaf litter pH was determined in H_2O and $CaCl_2$ [23] using 0.5 g of ground litter sample and 10 ml demineralized water after overnight incubation (see more detailed information in supporting information). The samples were vortexed for 2 min and incubated over night at room temperature. The following day, the samples were briefly vortexed, centrifuged at 4,000 rpm for 1 min and the pH in the supernatant liquid (H_2O) was measured. After the addition of 100 µl 1 M $CaCl_2$ (final concentration of 0.01 M $CaCl_2$ in 10-ml suspension), the samples were vortexed for 2 min and incubated for 2 h at room temperature. Following another brief vortexing step, the suspension was centrifuged at 4,000 rpm for 1 min and pH in the supernatant liquid (pH in $CaCl_2$) was determined.

Table S1. Information on the litter composition for individual forest sites (reproduced from Purahong et al., 2014).

Forest management practice	Litter composition		
	Fagus sylvatica	***Acer* sp.**	***Fraxinus* sp.**
Beech even-aged forest	85	10	5
Beech selectively logged forest	90	10	0
Beech unmanaged forest	100	0	0

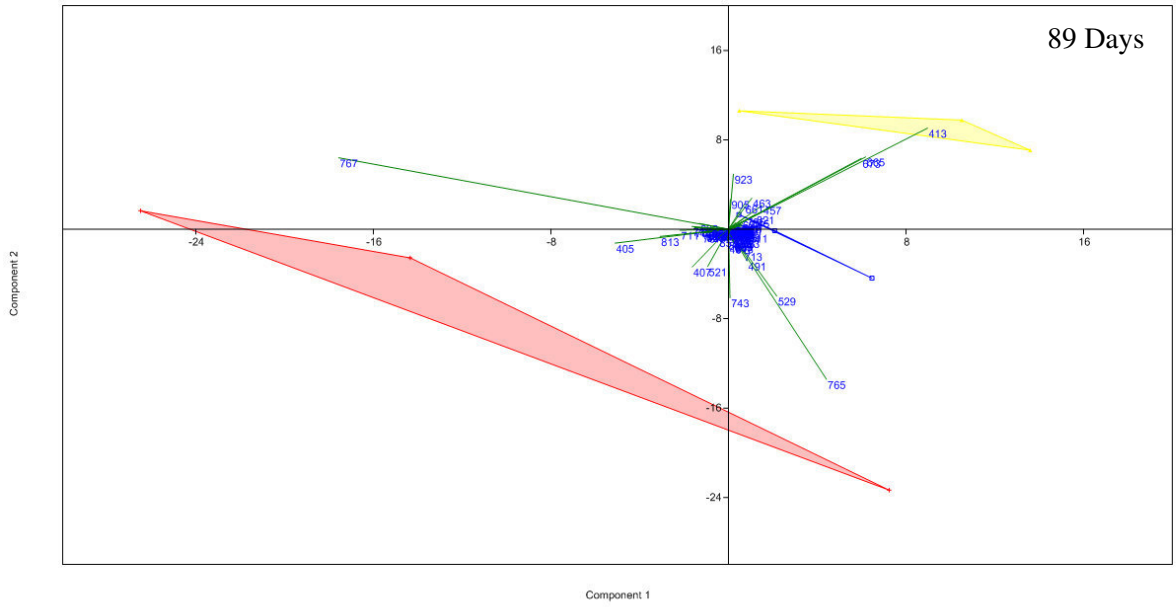
Table S2. Initial chemical composition of dried leaf litter under different forest management practices (heatmapped [yellow to green, per row], Mean±SD, n = 3) (reproduced from Purahong et al., 2014).

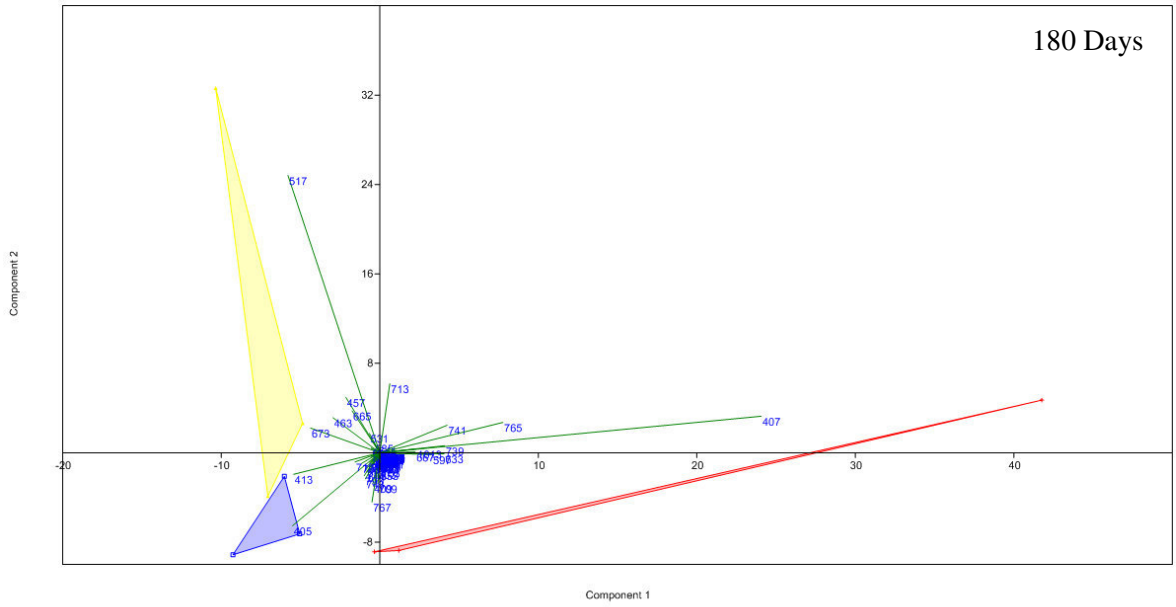
Nutrient	Age-class beech forest (BA)		Selection cutting beech forest (BS)		Unmanaged beech forest (BU)	
Total C (%)	47.61	± 0.17	47.34	± 0.16	48.81	± 0.22
Total N (%)	0.97	± 0.01	1.04	± 0	0.84	± 0.03
C/N	48.92	± 0.2	45.52	± 0.15	58.38	± 2.18
Total lignin/N	43.13	± 0.25	39.74	± 0	56.27	± 1.9
Initial Mg (µg/g dry mass)	204	± 50.27	294.33	± 197.28	157.93	± 54.19
Initial K (µg/g dry mass)	3076.67	± 545.01	4596.67	± 2500.11	2443.33	± 120.97
Initial Ca (µg/g dry mass)	593.33	± 142.55	964.33	± 576.11	344.33	± 70.61
Initial P (µg/g dry mass)	187.67	± 41.5	152.67	± 27.43	157.33	± 20.84
Initial Mn (µg/g dry mass)	32.87	± 9.64	26.47	± 11.65	20.53	± 5.56
Initial Fe (µg/g dry mass)	5.6	± 2.52	4.67	± 2.8	7.37	± 9.14
Initial Cu (µg/g dry mass)	0.91	± 0.37	0.62	± 0.07	0.4	± 0.04
Initial Co (µg/g dry mass)	0.01	± 0	0.02	± 0.01	0.01	± 0
Initial V (µg/g dry mass)	0.02	± 0	0.02	± 0	0.02	± 0.02

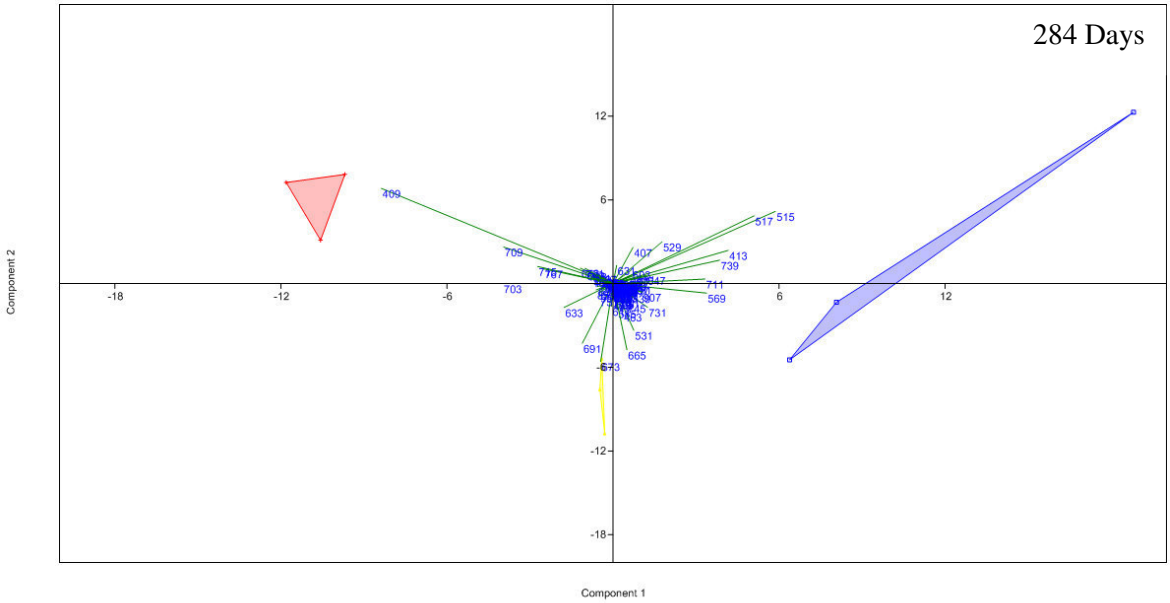
Table S3. Mean decay rate constants (k) of leaf litter, coefficients of determination (r^2) describing the fit of the decay model ($P < 0.05$) under different forest management practices (reproduced from Purahong et al., 2014).

Forest management practice	***K*** (year^{-1})	r^2 (***P*** value)
Even-aged beech forest (BA)	0.55 ± 0.08	0.98 (***P* < 0.001**)
Selectively logged beech forest (BS)	0.42 ± 0.02	0.92 (***P* = 0.002**)
Unmanaged beech forest (BU)	0.32 ± 0.09	0.94 (***P* = 0.001**)

Figure S1. PCA biplot showing bacterial communities in leaf litter under different forest management practices (blue = beech unmanaged forest, yellow = beech selectively logged forest and red = beech even-aged forest) at each sampling date.






284 Days
Component 2
Component 1
12
6
-12
-18
-18
-12
-6
6
12
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711
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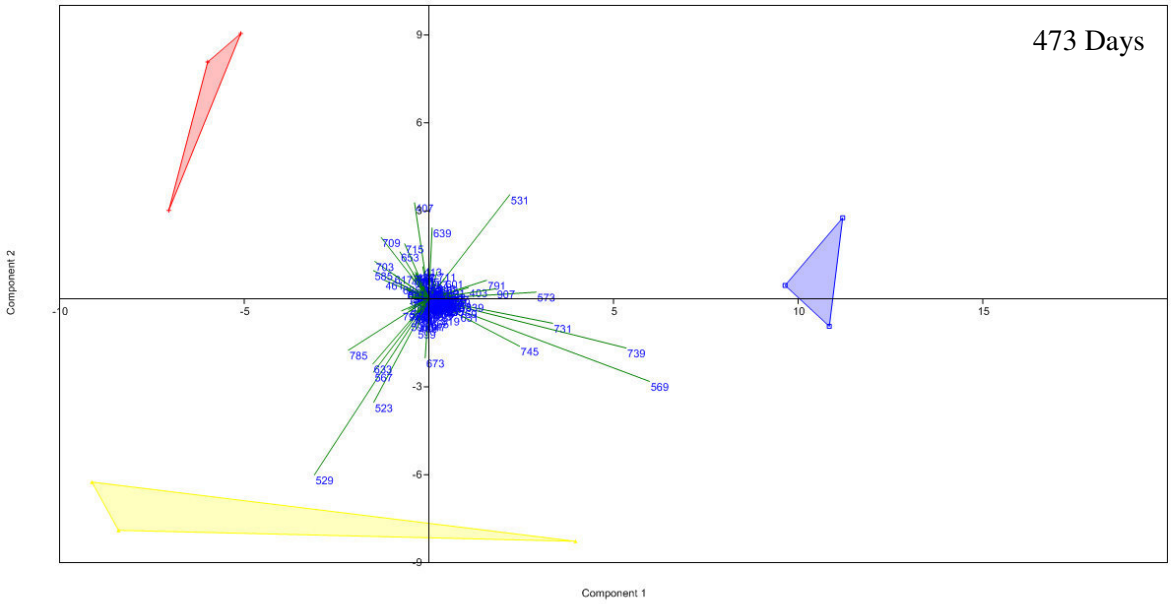
731
531
691
665
573


473 Days
Component 2
Component 1
9
6
-3
-6
-9
-10
-5
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10
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531
639
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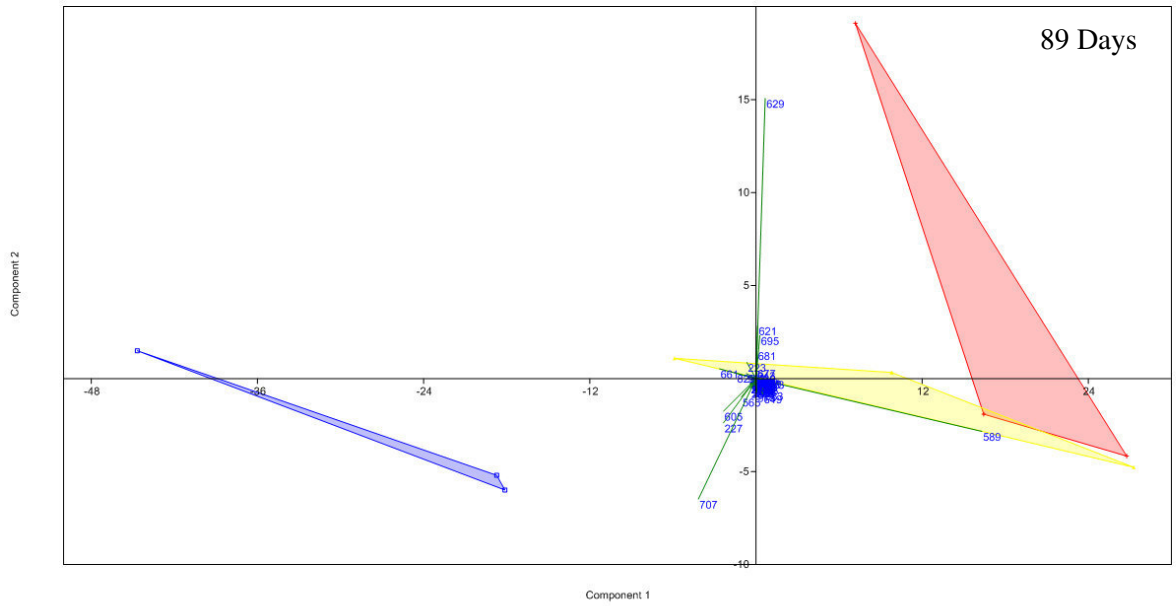
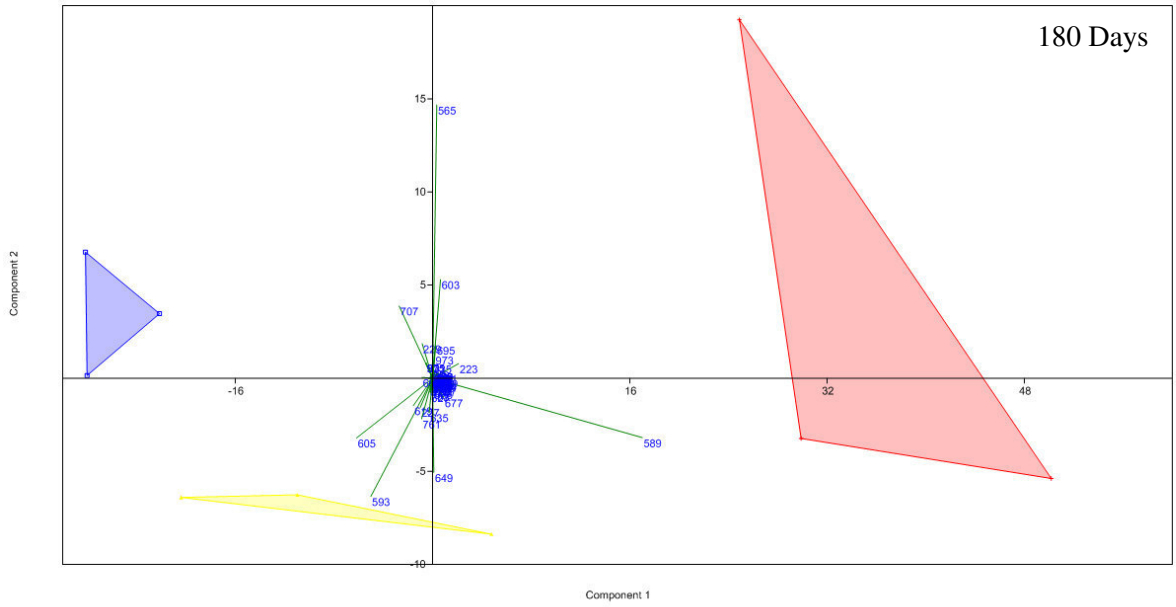
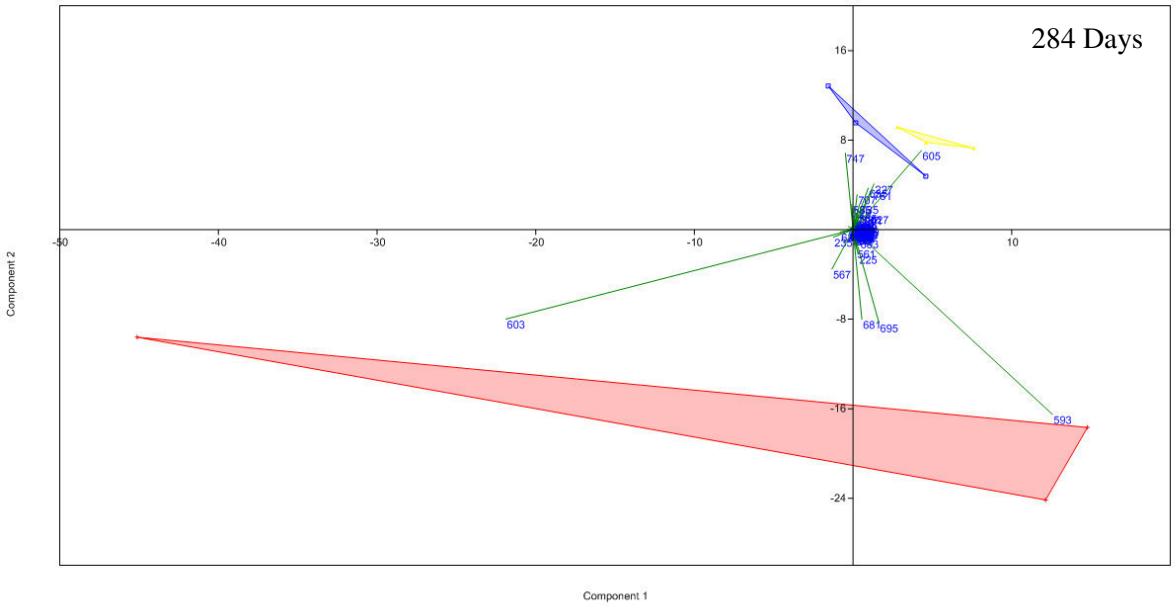
633
703
791
907
573
731
745
739
569
785
673
523
529

Figure S2. PCA biplot showing fungal communities in leaf litter under different forest management practices (blue = beech unmanaged forest, yellow = beech selectively logged forest and red = beech even-aged forest) at each sampling date.






284 Days
Component 2
Component 1
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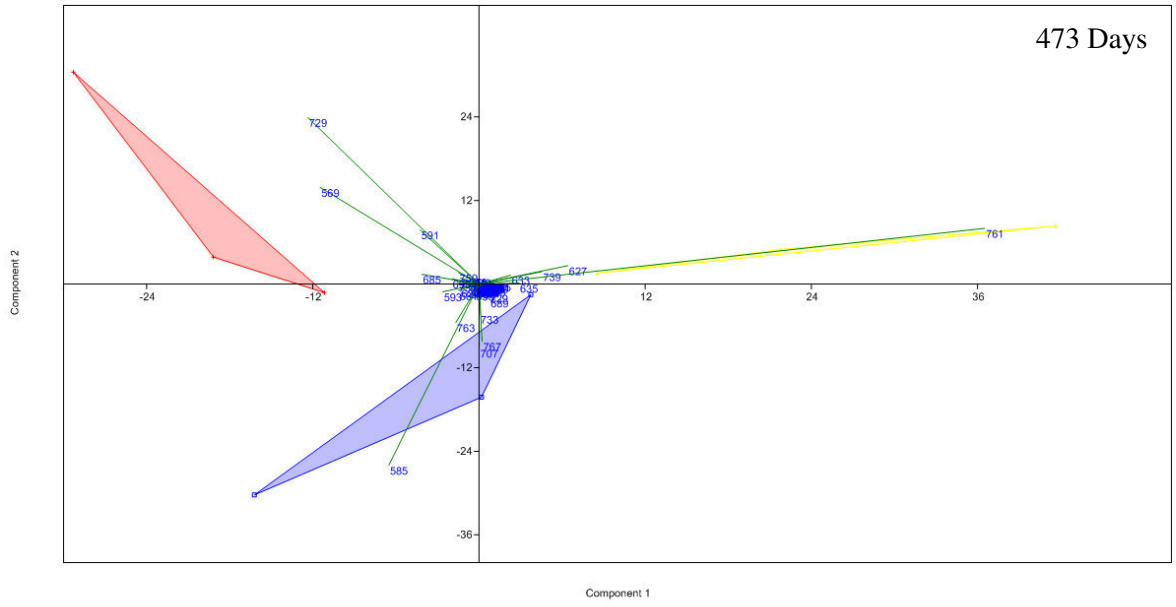
695
593


473 Days
Component 2
Component 1
24
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-12
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729
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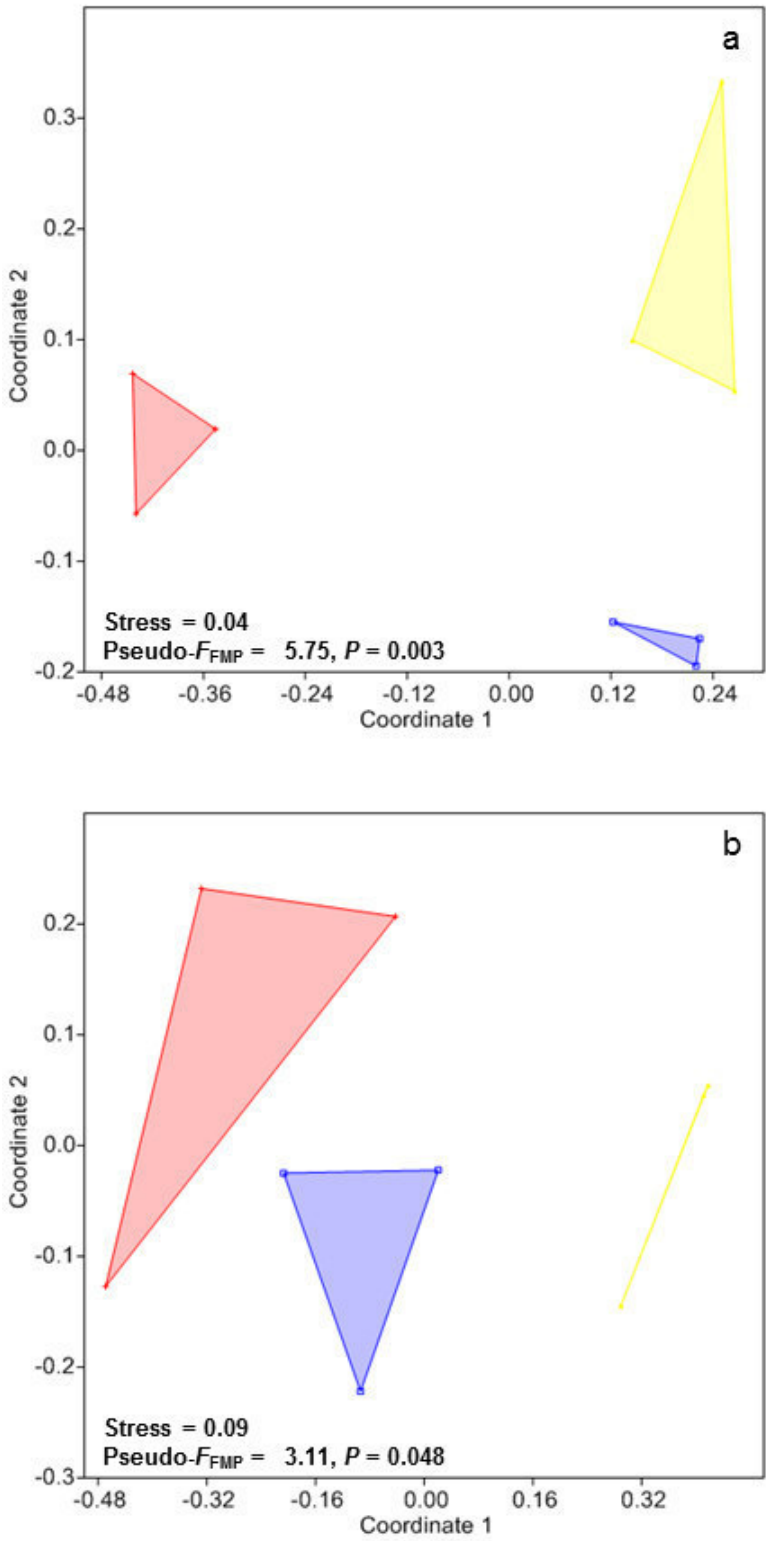
591
761
627
739
763
733
767
707
585

Figure S3. NMDS ordinations showing bacterial (a) and fungal (b) communities in freshly fallen leaf litter under different forest management practices (blue = beech unmanaged forest, yellow = beech selectively logged forest and red = beech even-aged forest). Permutational multivariate analysis of variance was conducted to test the effect of FMP on bacterial and fungal community structure (*P* values were based on 1,999 permutations).



Reference

Purahong W, Kapturska D, Pecyna MJ, Schloter M, Buscot F, Hofrichter M, Krüger D (2014) Influence of different forest system management practices on leaf litter decomposition rates, nutrient dynamics and the activity of ligninolytic enzymes: a case study from Central European forests. PLoS ONE 9(4): e93700. doi:10.1371/journal.pone.0093700

C. Overall Discussion

Definition of biodiversity in this study

In this thesis, I take biodiversity as the richness of the Operational Taxonomic Units (OTUs) equaling the number of discernable peaks from the ARISA fingerprint method (chapter 2, 3, 4 and 8) or by the number of clearly different sequence reads from 454 pyrosequencing (after quality checking and removal of singletons to tripletons, chapter 5). Microbial community is defined as the community members of different microbial populations. Thus, I assume that microbial structure and composition can be derived from the information of OTUs and their relative abundances (community structure) and OTUs with presence/absence data (community composition) (Barlow et al., 2007). For microbial ecology these molecular culture independent techniques are important as most of microbes are too small to be distinguished by naked human eyes and also very small portion of the microbial community can be isolated on artificial media in laboratory (Poole et al., 2012). This makes microbial diversity different from the diversity of butterflies, birds or flowers that fascinates naturalists and is described in the literature for a long time (Poole et al., 2012). The question remains what to do with the peaks or sequences in nature? And what does it really mean when the number of peaks or sequences from a particularly assessed and amplified target marker increases or decreases, when there is no definite correlation between them and "species" (another human concept with fuzzy boundaries) or them and "functionally active individual"? The answer for now is still unclear, since up to date, no method was reported that can examine the microbial community without any bias (Forney et al., 2004; Poole et al., 2012). Thus, while employing current state of the art it is now still difficult to judge whether the number of OTUs as shown by either different peaks or sequences represents the microbial diversity in the environmental samples.

Hypothesis 1: Forest management regimes with a high level of wood extraction strongly reduce fungal richness and alter community structure and the composition of wood-inhabiting fungi

This hypothesis is partly verified. Managing as age-class beech forest with high wood extraction does significantly reduce fungal richness compared with age-class beech forest with low wood extraction, with selection cutting beech forest and with unmanaged beech forest (Purahong et al., 2014a, b). This could be related to the effects of canopy structure, disturbance, and stand age (Ágreda et al., 2006; Twieg et al., 2007; Ma et al., 2010; Luyssaert et al., 2011). Large areas of thicket stage or young forest caused by a high disturbance after wood extraction may reduce fungal OTU richness and diversity (Purahong et al., 2014a). However, there is no significant difference in fungal community structure among different forest management regimes. This could be related to the fact that the habitat quality (deadwood) provided by these three forest management regimes is similar as there is no change in dominant tree species (Kögel-Knabner, 2002; Purahong et al., 2014a, b).

Hypothesis 2: Forest conversion from beech to conifers reduces fungal richness and alters community structure and composition.

This hypothesis is partly verified. The overall analysis revealed that conversion from age-class beech forest to age-class conifer forest significantly reduces fungal richness and alters community structure and composition (Purahong et al., 2014c). However, when we consider the effect of forest type conversion within in each region, inconsistent results were found. At AEW (where age-class beech forests were converted to age-class spruce forests) the hypothesis was fully verified. In contrast, at SEW (where age-class beech forests were converted to age-class Scots pine forests) only community structure and composition were altered, but the richness was not significant different (Purahong et al., 2014a). This divergence could be related to the different conifer species introduced at the two sites, which in turn, generate differences concerning both wood quality and microclimatic condition in the forest plots (Chen et al., 1999; Kögel-Knabner, 2002).

Hypothesis 3: Different deadwood species (beech vs. spruce) could cause significant differences in fungal diversity and community structure.

This hypothesis was partially confirmed. Wood-inhabiting fungal community structure in dead wood under beech significantly differed from the one under spruce. In the next hypothesis (hypothesis 4), I show that the difference in fungal communities derived from these two deadwood species is due to the effect of wood physicochemical properties and to the forest management regimes (Rajala et al., 2012; Purahong et al., 2014a). When comparing richness of wood-inhabiting fungi from beech age-class and spruce age-class forest (similar to the previous hypothesis in chapter 2), the richness is significantly reduced, thus a consistent conclusion was obtained using different molecular culture independent methods (ARISA and pyrosequencing) (Gobet et al., 2013). Nevertheless, when considering the full design that includes beech deadwood in spruce forest and spruce deadwood in beech forest, there is no significant difference in fungal richness derived from beech and spruce. This could be related to the effect of forest management regimes itself and also the interaction of forest management regimes and deadwood species (Purahong et al., 2014a). I hypothesize that forest management may affect wood-inhabiting fungal OTU richness in a complex manner by influencing a range of factors, including forest structure, plant diversity, level of disturbance, microclimatic conditions and deadwood dynamics (Purahong et al., 2014a).

Hypothesis 4: Anthropogenic (such as forest management) as well as wood physicochemical factors significantly correlate with fungal community structure within and between *Fagus* and *Picea* wood.

This hypothesis is fully verified. Anthropogenic (forest management regimes) as well as wood physicochemical significantly correlate with fungal community structure both within and between *Fagus* and *Picia* deadwood (Rajala et al., 2012). The effect of forest management types (including forest conversion from beech to spruce) on wood-inhabiting fungal community structure has been reported by Purahong et al., 2014a. The correlation of wood physicochemical factors on wood-inhabiting fungal community

structure is consistent to many similar studies that investigate this effect in *Picia* deadwood (Rajala et al., 2012).

Hypothesis 5: Positive correlations between the ecological function (ligninolytic enzyme activities) and service (wood decomposition rates) and total fungal richness.

This hypothesis is not verified. There is no positive correlation found between the potential ecological functions and wood decomposition rates, and total fungal richness. The positive correlations were rather found between richness and abundances of particular fungal families and OTUs and potential ecological functions and wood decomposition rates. This could be explained by the fact that the decomposition rates and ligninolytic enzyme activities do not relate to the total OTU richness, but are controlled by interspecific differences and concurrence scenarios (Boddy, 2001; Fukami et al., 2010; Dickie et al., 2012).

Hypothesis 6: The decomposition rate of deciduous litter is lower in spruce forest than in other deciduous beech dominated forests.

This hypothesis based on the home field advantage is not verified (Ayres et al., 2009). I found that decomposition rates of leaf litter (mixed deciduous leaf litter, mainly beech) is significantly higher in spruce forest compared to beech dominated forests. This could be explained by the fact that decomposers in spruce forest are also accustomed to periodic deciduous litter deposits (due to the presence of deciduous trees in this spruce forest) (Purahong et al., 2014c). In addition, I hypothesize that decomposer communities in spruce forest can cope with more recalcitrant substances, such as resins, polyphenols and guaiacyl lignin found in spruce needles. Therefore, they may also have the capacity to decompose the main deciduous leaf litter (European beech), which is also considered to be a low quality litter (Purahong et al., 2014c).

Hypothesis 7: In deciduous beech dominated forests, leaf litter decomposition rate would be highest in unmanaged natural forest, next highest in near-to-nature forest and lowest in high management intensity age-class forest

This hypothesis is not verified. Leaf litter decomposition rates are low in unmanaged natural beech forest, low to intermediate in near-to-nature forest and high in age-class beech forest (Purahong et al., 2014c). This could be related to the positive effect of tree diversity in selection cutting and age-class forests (Purahong et al., 2014c). In these forests, the woody biomass is harvested either by whole stand harvesting (age-class forest) or individual tree harvesting (selection cutting forest). These harvests could generate forest gaps that are important for tree regeneration and the maintenance of plant diversity (Hessenmöller et al., 2011; Schliemann and Bockheim, 2011). Such gaps tend release ressources by accelerated mineralization and have relatively high light levels. In such gaps, propagules of different plants (especially shade intolerant species) can germinate, grow quickly and maintain in the forest (Schliemann and Bockheim, 2011). In contrast, the unmanaged beech (BU) forest in this study is located in a National Park which, for at least 60 years, has not undergone wood harvesting or other serious disturbances that would have resulted in the loss of large numbers of trees; thus, European beech dominates the entire forest area (Purahong et al., 2014c). This could explain the higher tree species richness in the BA and BS forests compared with the BU forest in our study. Despite small proportions (5-10%) of leaf litter of different tree species (other than beech) in BA and BS forests, this mixture do significantly change leaf litter quality, enzyme activities patterns (especially ligninolytic enzymes) and development of microbial communities (Purahong et al., 2014c). I demonstrate that the increasing litter decomposition rates in BA and BS forests can be caused by non-additive effect of leaf litter decomposition rates. In the future, it is interesting to know what will happen if the managed forests containing low tree diversity. However, due to the positive effects of tree diversity on leaf litter decomposition rate and nutrient release in managed forests, tree diversity should be maintained by allowing the natural regeneration of a range of native deciduous species, especially at the thinning stage (Purahong et al., 2014c).

Hypothesis 8: Microbial community and potential enzymes activity in leaf litter significantly different among different forest system management practices.

This hypothesis is partly verified. Microbial community and potential enzyme activities significantly influence by both FMPs and DAI. However, only clear separation of microbial communities according to FMP (managed vs unmanaged forests) and DAI were observed. On the other hand, the potential enzyme activity patterns are overlapping among forests with different FMPs and DAI. This suggests the uncoupling of microbial community structure and potential ecosystem functions in forest ecosystem. The disconnection between microbial community and potential ecological functions has been reported before in aquatic ecosystem (Frossard et al., 2012). Along with this study, this could be related to functional redundancy of microbial community in leaf litter. Furthermore, I demonstrate that this disconnection can be due to the different drivers of microbial growth and ecosystem functions (potential enzyme activities).

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D. CONCLUSIONS and OUTLOOK

Forest management significantly affects microbial community structure and/or diversity revealed in deadwood and leaf litter by molecular approaches. Forest management is definitely not as a single factor but it results from a combination of many factors. Changes in forest management type (including forest type conversion) potentially affect most characteristics of the entire forest ecosystem. Chemical, physical and biological characteristics of soil can be significantly altered by forest management. For deadwood, intensive forest management (age-class with high wood extraction) causes changes in fungal OTU abundances and significantly reduces wood-inhabiting fungal OTU richness. Conversion of forest types from beech to spruce forest significantly reduce wood-inhabiting fungal OTU richness and strongly changes fungal community structure. Wood-inhabiting fungal OTUs abundances in the near to nature forest management (selection cutting) were more similar to that of in unmanaged forest than in age-class forest. The wood-inhabiting fungal OTU richness in selection cutting and unmanaged forests was not significantly different, but it was higher, in both cases, than that in the age-class forest. Overall, wood-inhabiting fungal community structure is correlating with both wood physicochemical parameters as well as anthropogenic factors such as forest management types. Importantly, wood decomposition rate and enzyme activity does not rely on total fungal OTU richness, but is controlled by assembly history, interspecific interactions and concurrence scenarios. In leaf litter, the results demonstrate that forest management significantly affects microbial community structure, potential enzyme activities, nutrient dynamics and decomposition rates. Intensive forest management that allows natural regeneration and maintenance of tree diversity could positively affect leaf litter decomposition rates and nutrient dynamics. Positively non-additive effect of leaf litter decomposition rate due to the changes of microbial community structure is identified as one of the mechanism explaining how leaf litter decomposition rates are different across forest system management practices. Similar to deadwood, microbial community structure in leaf litter is also correlating with both litter physicochemical and anthropogenic factors.

Although I did not find an indication of shifts in wood-inhabiting fungal OTU richness related to selection cutting in this study, which focused on short-term effects, there is a need to consider long-term effects on biodiversity and community structure if such forest management is applied over longer periods. For fungal community structure, although the wood-inhabiting fungal community structure is not different among different forest management regime (not including conversion of forest type), the shift of OTUs abundances are found. Importantly, many native fungal OTUs in unmanaged forest were lost or undetected in managed forests. A study provided evidence that minor forest logging carried out a century ago may continually affect deadwood dynamics and the wood inhabiting fungal community, in particular the abundance of red-listed species (Josefsson et al., 2010). To maintain the wood-inhabiting fungal community, it is important to incorporate the native tree species in forest management plan.

In the litter bag experiment, I found the differences in management to affect litter decomposition by altering the composition of the vegetation. To improve our mechanistic understanding of this point, a common garden experiment where all litters are incubated in one place may be helpful. However, litter decomposition is not only depending on the litter material used, but is also strongly impacted by the microclimatic and other environmental conditions (nutrients, water availability, etc.). Thus, such a common garden experiment will only help to figure out what is the role of litter quality but not the role of the environmental conditions present at the site. The effects of forest management on microbial communities, potential ecological functions and services are also complex and can be influenced by a range of factors including, tree species, tree diversity, geographical region, nutrient available, etc.

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Contributions to individual manuscripts/publications

Manuscript	Contributions
1. A better understanding of functional roles of fungi in the decomposition process: using precursor rRNA containing ITS regions as a marker for the active fungal community. **Witoon Purahong**, Dirk Krüger.	Conceived and designed the experiments: **-** Performed the experiments: **-** Analyzed the data: **Yes** Contributed reagents/materials/analysis tools: **Yes** Wrote the paper: **Yes**
2. Influence of commonly used primer systems on automated ribosomal intergenic spacer analysis of bacterial communities in environmental samples**.** **Witoon Purahong**, Barbara Stempfhuber, Guillaume Lentendu, Davide Francioli, Thomas Reitz, François Buscot, Michael Schloter, Dirk Krüger.	Conceived and designed the experiments: **Yes** Performed the experiments: **Yes** Analyzed the data: **Yes** Contributed reagents/materials/analysis tools: **Yes** Wrote the paper: **Yes**
3. Changes within a single land-use category alter microbial diversity and community structure: molecular evidence from wood-inhabiting fungi in forest ecosystems. **Witoon Purahong**, Björn Hoppe, Tiemo Kahl, Michael Schloter, Ernst-Detlef Schulze, Jürgen Bauhus, François Buscot, Dirk Krüger.	Conceived and designed the experiments: **Yes** Performed the experiments: **Yes** Analyzed the data: **Yes** Contributed reagents/materials/analysis tools: **Yes** Wrote the paper: **Yes**
4. Comparing fungal richness and community composition in coarse woody debris in Central European beech forests under three types of management. **Witoon Purahong** , Tiemo Kahl, Michael Schloter, Jürgen Bauhus, François Buscot, Dirk Krüger	Conceived and designed the experiments: **Yes** Performed the experiments: **Yes** Analyzed the data: **Yes** Contributed reagents/materials/analysis tools: **Yes** Wrote the paper: **Yes**
5. Fungal presence and activity in dead Fagus and Picea wood tackled by nrITS metagenomics and lignin modifying enzyme analysis. Björn Hoppe*, **Witoon Purahong***, Tesfaye Wubet*, Tiemo Kahl, Tobias Arnstat, Jürgen Bauhus, François Buscot, Dirk Krüger. *These authors contribute equally	Conceived and designed the experiments: **No** Performed the experiments: **No** Analyzed the data: **Yes** Contributed reagents/materials/analysis tools: **Yes** Wrote the paper: **Yes**
6. Influence of different forest system management practices on leaf litter decomposition rates, nutrient dynamics and the activity of ligninolytic enzymes: a case study from Central European forests. **Witoon Purahong**, Danuta Kapturska, Marek J. Pecyna, Michael Schloter, François Buscot, Martin Hofrichter, Dirk Krüger.	Conceived and designed the experiments: **Yes** Performed the experiments: **Yes** Analyzed the data: **Yes** Contributed reagents/materials/analysis tools: **No** Wrote the paper: **Yes**
7. Uncoupling of microbial community structure and function in decomposing litter across beech forest ecosystems in Central Europe. **Witoon Purahong**, Michael Schloter, Danuta Kapturska, Veronika Däumlich, Marek J. Pecyna, Sanchit Mital, François Buscot, Martin Hofrichter, Jessica LM Gutknecht, Dirk Krüger.	Conceived and designed the experiments: **Yes** Performed the experiments: **Yes** Analyzed the data: **Yes** Contributed reagents/materials/analysis tools: **Yes** Wrote the paper: **Yes**
8. Effects of forest management practices in temperate beech forests on bacterial and fungal communities involved in leaf litter degradation. **Witoon Purahong**, Danuta Kapturska, Marek J. Pecyna, Katalee Jariyavidyanont, Jennifer Kaunzner, Kantida Juncheed, Tanaporn Uengwetwanit, Renate Rudloff, Elke Schulz, Martin Hofrichter, Michael Schloter, Dirk Krüger, François Buscot.	Conceived and designed the experiments: **Yes** Performed the experiments: **Yes** Analyzed the data: **Yes** Contributed reagents/materials/analysis tools: **Yes** Wrote the paper: **Yes**

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Curriculum Vitae

Name: Mr. WITOON, PURAHONG

BIRTH DATE: April 7, 1985

BIRTH PLACE: Ayutthaya, Thailand.

EDUCATION:

2007 B.Sc (Biology) (1st Class Honors), the highest GPA in the Faculty of Science, 3.95/4.00
Srinakharinwirot University (SWU), Bangkok Thailand.

2008 Graduate Diploma in Teaching Profession (Master of Professional in Teaching Profession)(Teaching Biology), SWU.

2010 Erasmus Mundus Joint Master Degrees:

- M.Sc (passed with high distinction), Technische Universität München, Germany.
- Laurea specialistica Ortofrutticoltura Internazionale (110/110 Cum Laude), Università di Bologna, Italy.
- Diplom-Ingenieur (with distinction), Universität für Bodenkultur Wien, Vienna, Austria.

2014 (expected) Dr. rer. nat., Technische Universität München, Germany.

INTERNATIONAL PUBLICATIONS (Listed in ISI):

Purahong W., Lerstaveesin P., Ampornpan L. (2010) Succession of fungi associated with decomposition of leaf litter in tropical ever green forest (Northeastern Thailand), Polish Journal of Ecology 58(3): 515–522.

Purahong W., Hyde K.D. (2011) Effects of fungal endophytes on grass and non-grass litter decomposition rates. Fungal Diversity 47:1–7 (IF=5.074; 1/30; Mycology).

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separated by the Apennines in Northern-Central Italy. Plant Pathology Journal 27(4): 354-359.

Purahong W., Alkadri D., Nipoti P., Pisi A., Lemmens M., Prodi A. (2012) Validation of a modified Petri-dish test to quantify aggressive levels of *Fusarium graminearum* in durum wheat. European Journal of Plant Pathology 132:381–391.

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Purahong W., Kapturska D., Pecyna M. J., Schloter M., Buscot F., Hofrichter M., Krüger D. (2014) Influence of different forest system management practices on leaf litter decomposition rates, nutrient dynamics and the activity of ligninolytic enzymes: a case study from Central European forests. PLoS ONE, doi: 10.1371/journal.pone.0093700.

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structure and function in decomposing litter across beech forest ecosystems in Central Europe. Scientific Reports, doi: 10.1038/srep07014.

Purahong W., Stempfhuber B., Lentendu G., Francioli D., Reitz T., Buscot F., Schloter M., Krüger D. (2015) Influence of commonly used primer systems on automated ribosomal intergenic spacer analysis of bacterial communities in environmental soil and plant samples. PLoS ONE (Accepted), doi: 10.1371/journal.pone.0118967.

Purahong W., Kapturska D., Pecyna M J., Buscot F., Jariyavidyanont K., Kaunzner J., Juncheed K., Uengwetwanit T., Rudloff R., Schulz E., Hofrichter M., Schloter M.,Krüger D. (2015) Effects of forest management practices on bacterial and fungal communities in leaf litter: a case study from Central European beech forest ecosystems Microbial Ecology (Accepted).

WORK EXPERIENCES:

2011-present: Researcher (PhD position) at Department of Soil Ecology, Helmholtz Centre for Environmental Research – UFZ, Germany.
2009: Research assistance in plant pathology laboratory, Faculty of Agriculture, University of Bologna, Italy.
2004-2007: Teaching assistant on botanical courses in Department of Biology, Faculty of Science, SWU, Thailand.
2006: (Summer) Student trainee in Mycology Laboratory, BIOTEC (National Center for Genetic Engineering and Biotechnology), Thailand.
2005: (Summer) Field assistant for the thesis entitled "Tree species diversity and regeneration of seasonally dry forest in northeast Thailand" of a PhD- level student from Norwegian University of Life Sciences.
2004: (Summer) Field assistant for the thesis entitled "Economics importance and ecology of two locally consumed and marketed forest species" of a MSc- level student from Norwegian University of Life Sciences.
2003-2004: Research assistant in the project entitled "Maintenance of Biodiversity: Conservation and Cultural Practice" Funded by the ASEAN Regional Centre for Biodiversity Conservation and the European Commission.

ENGLISH LANGUAGE COMPETENCY:

IELTS overall band score 6.5 (Valid until 30 / May / 2011).

HONOURS AND AWARDS:

2011: Best graduate student (highest GPA) for the 2010 academic year, Weihenstephan, Technische Universität München.
2010: Best oral presentation award, the international symposium on fungal diversity and resources, Mae Fah Luang University, Chiang Rai, Thailand.
2008 - 2010: Erasmus Mundus Scholarship (International Master of Horticultural Science), European Union.
2007: Scholarship for a training course in insect taxonomy and collection management from the Belgian Focal Point to the Global Taxonomy Initiative at the Royal Belgian Institute of Natural Sciences, Brussels, Belgium.
2003-2007: Scholarship from the Project for the Promotion of Science and Mathematics Talented Teachers (PSMT) (4 years in science and 1 year in education)
2003-2006: Certificate on the "The highest GPA in the Department of Biology", Faculty of Science, SWU.
2004: Certificate and Token of Honour of Professor Tab Nillaniti Foundation on the "The highest GPA in the Faculty of Science"