Archives of Microbiology (2023) 205:269
https://doi.org/10.1007/s00203-023-03606-9

ORIGINAL PAPER

Comparison of survey methods for fungi using metabarcoding and fruit body inventories in an altitudinal gradient

Markus Blaschke[1] · **Angela Siemonsmeier**[1,3] · **Janno Harjes**[2] · **Daniel O. Okach**[2] · **Gerhard Rambold**[2]

Received: 17 October 2022 / Revised: 7 June 2023 / Accepted: 8 June 2023

Abstract

Metabarcoding of environmental samples is nowadays an established method in biodiversity research. When it comes to studying fungal populations in various ecotypes, fruit body inventories are the traditional method to assess the diversity of fungal communities. In this study, both methods—metabarcoding of soil samples and a traditional fruit body inventory—were conducted on 144 sample plots in an altitudinal gradient in the Bavarian Forest (Germany) and the results were compared. Metabarcoding detected significantly more species than the traditional fruit body inventory. The majority of taxa recorded in the fruit body inventory belonged to the *Basidiomycota*, whereas in the metabarcoding data, the distribution of species between *Basidiomycota* and *Ascomycota* was approximately balanced. Species of several orders forming inconspicuous or hypogeous fruit bodies were detected only by metabarcoding, while several wood decomposers were recorded only in the fruit body inventory. The proportion of detected wood-colonising species with melanized spores was considerably higher with metabarcoding than with the fruit body inventory, where more than 70% of recorded wood-colonisers had hyaline spores. Based on the metabarcoding data, a decline of species richness with increasing altitude was evident, but this was not visible in the fruit body inventory data. Detrended correspondence analyses yielded similar results for relative species community similarities with both survey methods.

Keywords Bavarian Forest · Biodiversity research · DNA community barcoding · Fungal community · Melanized spores

Introduction

Biodiversity research has been in a state of transition for many years. New techniques for assessing biodiversity via genetic material (DNA) in environmental samples by means of metabarcoding using next generation sequencing techniques have enabled high throughput of samples to be studied (Porter et al. 2008; Begerow et al. 2010; Ovaskainen et al. 2013; Purahong et al. 2018; Frøslev et al. 2019; Flessa et al. 2021). In metabarcoding, short DNA sections, i.e., sections of 'barcoding genes', are amplified and sequenced. A subsequent comparison of the sequence data obtained with reference sequences in pertinent databases such as UNITE (Nilsson et al. 2019) finally allows identification of species as operational taxonomic units (OTUs) at the species level or at a higher taxonomic level. The choice of barcode genes or gene segments to be used depends on the group of organisms to be studied. In fungi, the 'Internal Transcribed Spacer region' (ITS nrDNA region) is used as a quasi-standard (Schoch et al. 2012).Consequently, an immense number of fungal species can be identified in a soil sample in a manageable amount of time.

In research, DNA community barcoding has been established as an important supplement to traditional recording and identification of species. For fungi, in particular, the results generated in this way, besides the monitoring and identification of taxa based on macrophenotypic traits of fruit bodies in the field, play an important role for decision-making in the applied field (Schmidt-Stohn and Oertel

Communicated by Yusuf Akhter.

✉ Markus Blaschke
markus.blaschke@lwf.bayern.de

✉ Gerhard Rambold
gerhard.rambold@uni-bayreuth.de

[1] Bavarian State Institute of Forestry, Hans-Carl-von-Carlowitz-Platz 1, 85354 Freising, Germany

[2] Department of Mycology, University of Bayreuth, Universitätsstraße 30, 95447 Bayreuth, Germany

[3] University of Applied Forest Science Rottenburg, Schadenweilerhof, 72108 Rottenburg am Neckar, Germany

Published online: 24 June 2023

2015) and are highly suitable to contribute to biodiversity monitoring in a conservation context (Geml et al. 2014a; Thomsen and Willerslev 2015). However, the new methods also represent an important tool for biodiversity research to record fungal diversity (Begerow et al. 2010). This applies, in particular, to regions that have hardly been studied mycologically (Geml et al. 2014b), but also enables studies in well-investigated areas on a larger scale due to the extended possibilities of high-throughput methods. Direct comparisons between metabarcoding and traditional field study methods with fruit body inventories have rarely been published (Porter et al. 2008; Ovaskainen et al. 2013; Frøslev et al. 2019).

As part of a research project in the Bavarian Forest on the effects of climate change and forest management on biodiversity (Siemonsmeier et al. 2020), both a community barcoding-based method on soil samples (Harjes et al. 2023) and a traditional fruit body inventory on 144 circular plots along an altitudinal gradient were conducted in parallel (Blaschke and Siemonsmeier 2021). In the present article, the differences between the two methods are examined using the example of the altitudinal gradient project and possible reasons for divergent results are highlighted.

Material and methods

For the research project 'Höhengradient', 144 forest sample plots in the Bavarian Forest were investigated in 2018 and 2019, from the lowest sites near the city of Passau to the highest summit of the Bavarian Forest, the Großer Arber. Of these plots, 48 are located in eight strict forest reserves and 96 in selected commercial forest areas. They cover an altitudinal gradient from approximately 320 m to 1400 m above sea level.

Seven species groups were studied in more detail on the plots. These included vegetation, lichens, birds, snails, ground beetles, xylobiont beetles, and fungi (Blaschke and Siemonsmeier 2021). The fungi were analysed using a community barcoding approach from different soil depths as well as traditionally mapped in a fruit body inventory on a plot of 1000 m^2.

The soil samples were taken during the 2018 growing season. Three soil cores per study plot were collected using a soil auger and their positions on the plots were randomly selected. The material, separated into litter layer, organic layer and mineral layer, was transferred to sterile containers, dried, and stored in a freezer. For details on processing the samples in the laboratory, see Gkoutselis et al. (2021). After extraction of total DNA, amplification of the ITS region was performed in a two-step PCR. The first reaction used primer pairs ITS1F and ITS4, which carry an additional, unique TAG sequence, and the second amplification used Illumina sequencing primers, carrying a unique index sequence at the 5' ends (Gardes and Bruns 1993; Guerreiro et al. 2018; White et al. 1990). Sequencing was performed on an Illumina MiSeq™ 3000 platform at the Department of Genetics at the LMU Munich Biocenter, using the MiSeq™ Reagent Kit v3 (MS-102-3003). Sequence data was processed using QIIME1 pipeline implemented demultiplexing tools, extract_barcodes.py and split_libraries_fastqc.py (Caporaso et al. 2010). The output of the sorted sequence reads was imported into the QIIME2 pipeline using the CASAVA 1.8 format to be trimmed using the 'cutadapt' plugin, and for subsequent denoising and dereplication, DADA2 was used as a QIIME2 plugin (Callahan et al. 2016; Nearing et al. 2018). The obtained feature tables of amplicon sequence variants (ASVs) and the corresponding representative sequence files for each sequencing library were merged using the QIIME2 pipeline function 'feature-table merge', resulting in one ASV feature table for all separate sequencing runs. Taxonomic classification was performed with a 70% confidence threshold, applying the Naïve Bayes classifier implemented in QIIME2, which was previously trained on the UNITE v. 8.0 dynamic dataset with a chunk size of 20,000. The ASVs of the merged feature tables were collapsed into taxa based on their assigned taxonomies, using the QIIME2 function 'taxa collapse', forming operational taxonomic units (OTUs) (Bolyen et al. 2019).

Mapping of the fruit bodies of macrofungi was carried out in two campaigns in 2019. During the first campaign in August and September, warm and dry weather conditions prevailed after an extremely hot and dry summer. In the second campaign, damp and cool weather prevailed after intensive rainfall in October, as is typical of the season. The mapping was carried out in a time-normalised manner. Each plot was investigated for a maximum of 30 min and all fruit bodies identifiable in the field at species level were noted (Blaschke and Siemonsmeier 2021).

For the metabarcoding results presented here, all species detected by sequencing (OTUs) and all OTUs with at least ten reads per sample plot (number of detections of the respective base sequences during sequencing) were considered, as misidentifications or contaminations could not be entirely excluded in the case of a small number of reads.

For formal functional classification of detected fungi, the guilds of the species from the metabarcoding for the three most important altitudinal zones were compiled using the database FUNGuild. The spore colors were compiled for all wood-colonising species that were recorded either in metabarcoding or in fruiting body mapping.

Statistical analyses were performed using base R (R Core Team 2019) and the R package vegan (Oksanen 2011). The function "decorana" was used to perform Detrended correspondence analyses (DCA) of species communities based on presence-absence-data of both survey methods. The

influence of environmental factors, such as crown cover of tree species and skidder trails, on fungal communities was analysed with the function "adonis2".

Results

Species and OTU numbers

Based on metabarcoding, the number of OTUs detected per plot was on average five times higher than the number of species in the traditional fruit body inventory (Fig. 1). Even when all OTUs that yielded less than ten reads by metabarcoding were excluded, the number of species was still four times higher than in fruit body mapping. A comparison of the species numbers per plot determined with the two methods did not show a high correlation (Pearson's correlation coefficient – 0.26).

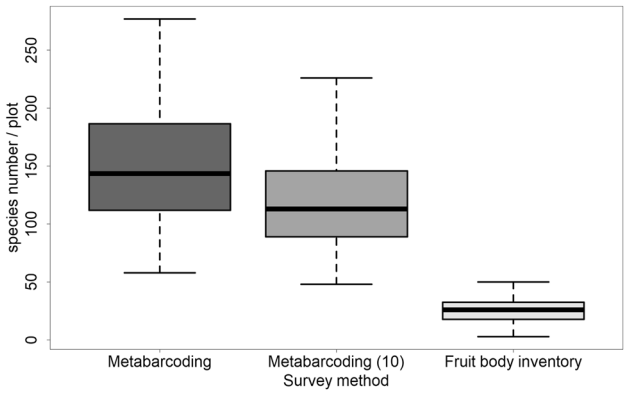

Fig. 1 Number of species per sample plot from metabarcoding (for all species and for secured species with more than ten reads) and from the fruit body inventory

Traditional mapping based on the fruit bodies essentially revealed fungal species belonging to the *Basidiomycota* (Fig. 2). These include in particular species from the class *Agaricomycetes*, such as the *Boletales* and *Russulales*. Two further classes of Ascomycetes that were regularly detected are the Leotiomycetes, which include plant-pathogenic taxa such as the Erysiphales and the Rhytismatales, and the Sordariomycetes, which include in particular species of the Xylariales. In total, about 86% of recorded species belonged to the *Basidiomycota* and only 12% to the *Ascomycota*.

However, when recording the species by metabarcoding, 53% of the detected species belonged to the *Basidiomycota* and 43% to the *Ascomycota*. The species were distributed much more evenly over a higher number of classes since many more (inconspicuous) taxa were identified via metabarcoding while the fruit body survey included only species identified in the field. Thus, the *Dothideomycetes* (*Ascomycota*), to which *Herpotrichia parasitica* belongs, the Eurotiomycetes (*Ascomycota*), with the genera *Penicillium* and *Aspergillus*, and the *Tremellomycetes* (*Basidiomycota*) occupy a larger space here (Fig. 2).

In almost all classes, the number of species detected was significantly higher than in the fruit body inventory. Only the number of *Dacrymycetes* species was higher in the traditional approach with five species, whereas the metabarcoding of soil samples, only one species was detected. Rust fungi (*Pucciniomycotina*, *Basidiomycota*) were documented with two representatives in the metabarcoding, while three species were detected in the fruit body inventory.

Differences in some orders

Among the *Basidiomycota*, some differences were found between the recording methods at the order level of the Agaricomycetes (Fig. 3). For example, the proportion of the

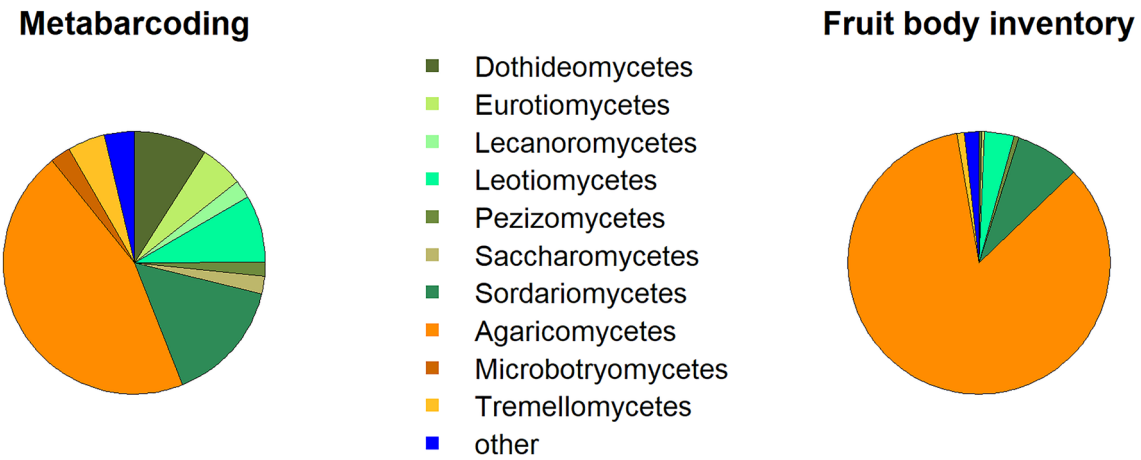

Fig. 2 Distribution of detected species within the classes of higher fungi in both detection methods. Greenish colours–*Ascomycota*; orange–*Basidiomycota*; blue–other (colour figure online)

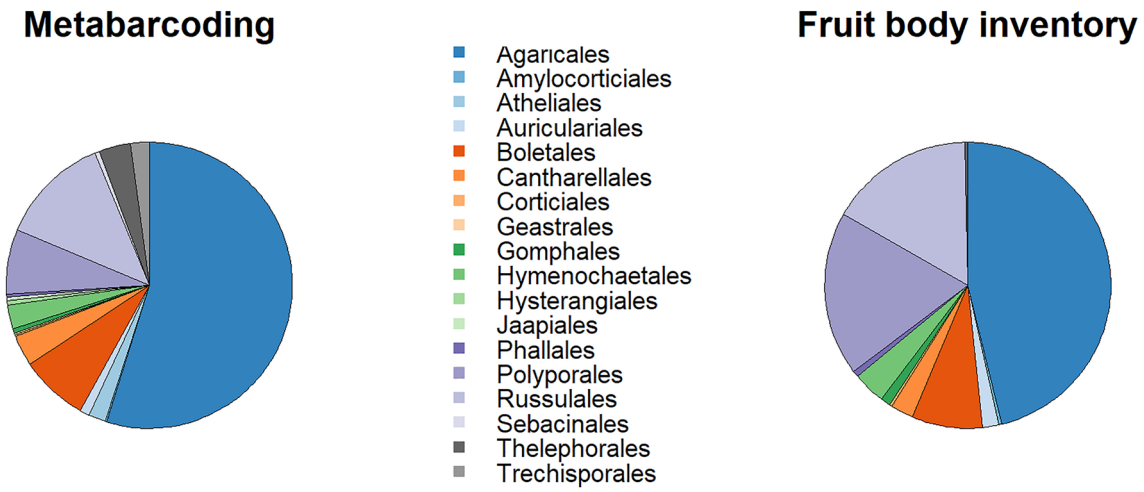

Fig. 3 Distribution of detected species within the orders of Agaricomycetes in both detection methods

order Agaricales was slightly larger in metabarcoding than in traditional mapping. In contrast, the Polyporales, which are predominantly wood decomposers, were represented with a significantly larger proportion in the traditional fruit body inventory. The Russulales, which include the genus *Russula* and the genus *Hericium*, were also represented with a larger proportion in the fruit body inventory than in the metabarcoding. Several orders, namely species of the Atheliales, Geastrales, Hysterangiales, Jaapiales, Sebacinales and Trechisporales, were only recorded by metabarcoding.

Dominant species of the ecological guilds

Among the mycorrhizal fungi, five species—*Craterellus tubaeformis, Imleria badia*, *Lactarius subdulcis, Russula nigricans*, and *Russula ochroleuca* were among the ten most common species—both in the metabarcoding and in the traditional inventory (Table 1). *Xerocomellus pruinatus*, which was subsumed under *Xeromellus chrysenteron* s.l. during mapping, must also be listed here. In addition, metabarcoding brought two mycorrhizal crust fungi, *Tomentella sublilacina* and *T. badia*, into focus as dominant species, while the fruit body inventory included *Laccaria amethystina* and *L. laccata* s.l. among the ten most common species.

With *Ganoderma applanatum*, a wood decomposer was the only fungal species that was detected by metabarcoding on all 144 study plots (Table 2). In the traditional mapping, however, this species did not appear among the ten dominant wood decomposers. Within the genus *Hypholoma*, only *Hypholoma fasciculare* was dominant in both inventories, whereas in the metabarcoding, also *H. capnoides* and *H. lateritium* were among the dominant wood decomposers.

Contrary, other wood decomposers were only detected in the fruit body survey. These include in particular very widespread species such as *Calocera viscosa, Dacrymyces stillatus*, *Diatrype decorticata*, *Fomes fomentarius* and *Fomitopsis pinicola*.

Among the soil-dwelling litter decomposers, three species, *Clavulina coralloides*, *Clitocybe nebularis* and *Rhodocollybia butyracea*, dominated in both survey methods (Table 3). Of the conspicuous species, *Phallus impudicus* was found on half of the plots in metabarcoding, while

Table 1 Steadiness of the ten most abundant mycorrhizal fungi (*Agaricomycotina*, *Basidiomycota*), each in comparison of metabarcoding and traditional fruit body inventory

Metabarcoding	Sample plots	Fruit body inventory	Sample plots
Russula ochroleuca	138	*Xerocomellus chrysenteron* s.l	68
Xerocomellus pruinatus	129	*Russula ochroleuca*	56
Imleria badia	121	*Laccaria amethystina*	49
Tomentella sublilacina	103	*Lactarius subdulcis*	43
Russula cyanoxantha	100	*Russula nigricans*	32
Lactarius subdulcis	100	*Craterellus tubaeformis*	31
Russula nigricans	76	*Imleria badia*	30
Amanita muscaria	75	*Paxillus involutus*	29
Tomentella badia	71	*Laccaria laccata*	27
Craterellus tubaeformis	68	*Amanita citrina*	22

Table 2 Steadiness of the ten most abundant wood decomposers (*Agaricomycotina*, *Basidiomycota*), each in comparison of metabarcoding and traditional fruit body inventory

Metabarcoding	Sample plots	Fruit body inventory	Sample plots
Ganoderma applanatum	144	*Phragmotrichum chailletii*	93
Hypholoma fasciculare	125	*Diatrype decorticata*	76
Coprinellus micaceus	113	*Dacrymyces stillatus*	74
Hypholoma capnoides	97	*Fomitopsis pinicola*	73
Kretzschmaria deusta	90	*Calocera viscosa*	72
Hypholoma lateritium	86	*Fomes fomentarius*	63
Hyphodontia pallidula	81	*Bisporella citrina*	62
Gymnopilus penetrans	79	*Hypoxylon fragiforme*	60
Kuehneromyces mutabilis	77	*Jackorogersella cohaerens*	59
Heterobasidion annosum s.l	73	*Hypholoma fasciculare*	59

Table 3 Steadiness of the ten most abundant litter decomposers (*Agaricomycotina*, *Basidiomycota*), each in comparison of metabarcoding and traditional fruit body inventory

Metabarcoding	Sample plots	Fruit body inventory	Sample plots
Clavulina coralloides	88	*Rhodocollybia butyracea*	65
Phallus impudicus	72	*Hygrophoropsis aurantiaca*	44
Paralepista flaccida	70	*Mycena zephirus*	35
Mycena sanguinolenta	68	*Clavulina coralloides*	32
Galerina calyptrata	59	*Mycena galopus*	30
Entoloma conferendum	59	*Mycena pura*	26
Rhodocollybia butyracea	58	*Mycena rosea*	24
Clitocybe nebularis	55	*Psathyrella piluliformis*	19
Entoloma cetratum	54	*Clitocybe nebularis*	17
Lycoperdon nigrescens	47	*Gymnopus androsaceus*	17

Hygrophoropsis aurantiaca and several *Mycena* species (*M. galopus*, *M. pura, M. rosea*, and *M. zephirus*) were regularly observed in the traditional fruit body inventory.

Species numbers and functional guilds along the altitudinal gradient

Within the altitudinal gradient, the metabarcoding approach showed a clear negative correlation between the number of species and sea level (Fig. 4). This applies to all species or OTUs as well as to the confirmed species with more than ten reads. This correlation was not pronounced in the traditional fruit body inventory. Here, species richness was relatively constant up to altitudes of 1250 m above sea level. Only in the higher altitude study areas, significantly fewer species were recorded.

A comparison of the axis values for the respective first axis of each of the separately calculated ordinations with data from metabarcoding and from the fruit body inventory (Fig. 5) showed that the two methods yielded comparable results with regard to the relative similarity of the fungal species communities on the study plots (Fig. 6).

Here, the influence of altitude on the formation of species communities was evident in both survey methods and was confirmed to be highly significant when environmental factors were fitted to the DCA results (Table 4).

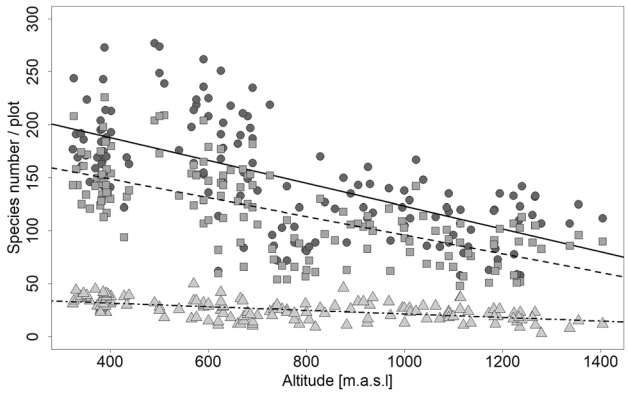

Fig. 4 Number of species per sample plot in dependence on altitude above sea level from metabarcoding (for all species (circles) and for confirmed species with more than ten reads (squares)) and from the fruit body inventory (triangles)

For the functional guilds derived from the metabarcoding data, there was an increase in reads and percent of total reads of the ectomycorrhizal fungi from around 45% at the low altitude sites (up to 420 m) to around 55% in the higher altitude levels (Table 5). On the other hand, there was a

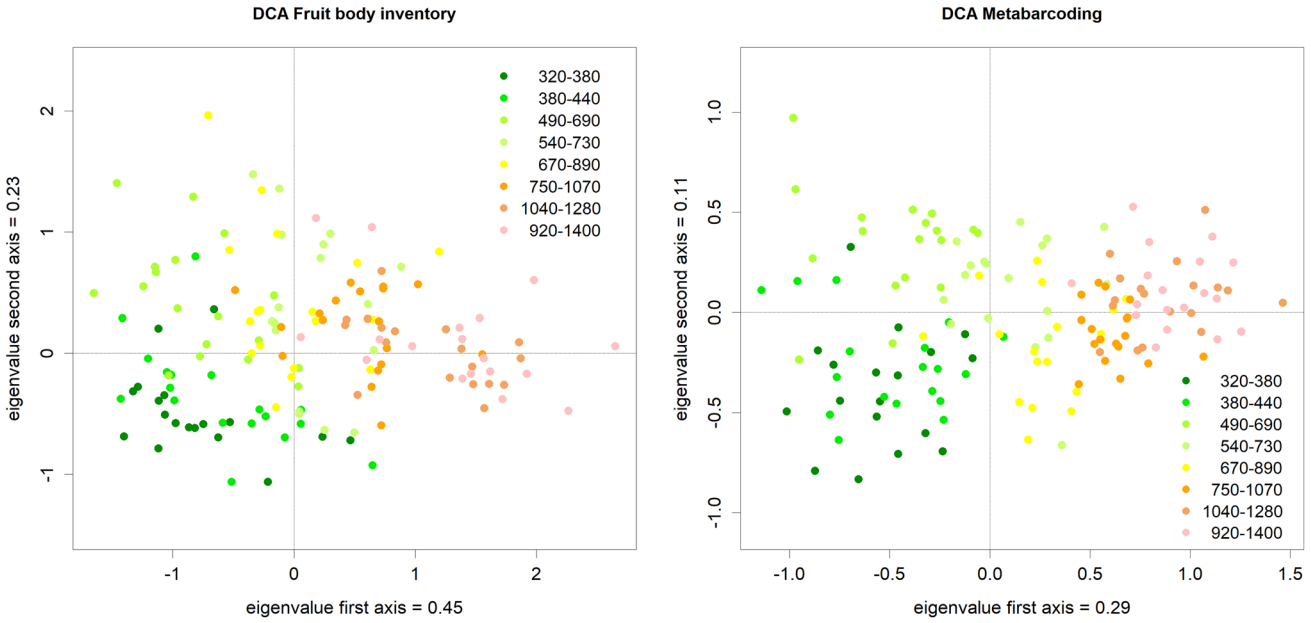

Fig. 5 DCA-Ordinations of the fungal species communities on the study plots, separated by survey method based on presence-absence data. Sea level heights for the eight area groups with colour code from dark green and green in lower altitude areas over yellow for medium altitude areas to brown and pink at high altitude sites (colour figure online)

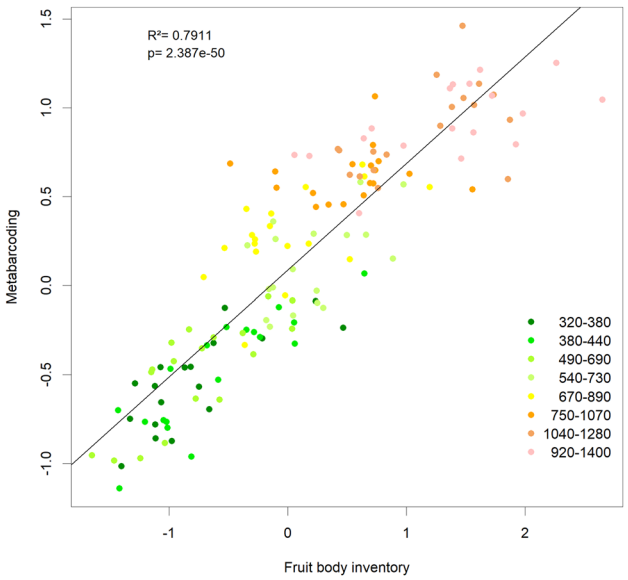

Fig. 6 Values of the respective first axis from the ordinations calculated with metabarcoding data and fruit body inventory data. Sea level heights for the eight area groups with colour code from dark green and green in lower altitude areas over yellow for medium altitude areas to brown and pink at high altitude sites (colour figure online)

decrease in the share of saprotrophic species from more than 15% in the low altitudinal belt to 12.3% and 11.2% in the higher areas, with the number of reads strongly decreasing at altitudes above 775 m a.s.l. Based on the number of OTUs, the highest gamma diversity over ectomycorrhizal, saprotroph and all guilds was found in areas at medium altitudes between 460 and 820 m a.s.l.

Detection of wood-colonizing fungi depending on spore colour

The proportions of wood-colonising species in terms of spore colour differed relatively clearly between metabarcoding and fruit body inventory (Fig. 7). In the former, a much higher proportion of species with melanised spores was found. In the latter, 76.1% of the wood-colonising species had hyaline spores. In contrast, this value was only 55% in the metabarcoding data. The remaining proportions were accounted for by black-spored species with 26%, brown-spored with 14.2% and pink-spored with 4.8%. In the fruit body inventory, in particular the proportion of brown-spored species was significantly lower at 3.9%.

Discussion

The two methods for assessing fungal diversity yielded very different species numbers. When metabarcoding soil samples, the number of recorded species was four to five times higher than with the traditional fruit body inventory. Frøslev et al. (2019), who investigated 130 plots distributed throughout Denmark in 24 different habitats using traditional fruitbody mapping and metabarcoding of soil samples,

Table 4 R^2 and F values of environmental factors with significant influence ($p < 0.05$) on the composition of the fungal species communities on the study plots for at least one recording method

Environmental factors	Metabarcoding			Fruit body inventory		
	R^2	F	p	R^2	F	p
Altitude above sea level	0.166	30.7169	0.001***	0.118	20.7619	0.001***
Crown cover: % spruce	0.024	4.4977	0.001***	0.036	6.48	0.001***
Crown cover: % silver fir	0.023	4.3136	0.001***	0.023	4.1035	0.001***
Rejuvenation: % spruce	0.007	1.2780	0.146	0.008	1.4815	0.092
Rejuvenation: % beech	0.009	1.7184	0.041*	0.010	1.7546	0.026*
Rejuvenation: mean height	0.171	1.0857	0.101	0.169	1.0315	0.286
Skidder trails: % area	0.006	1.1211	0.217	0.011	1.9948	0.011*
Rocks: % area	0.008	1.4115	0.093	0.014	2.5040	0.002**
Slope exposure	0.008	1.5537	0.078	0.008	1.5208	0.066

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Table 5 OTU, read counts and proportion of total reads from the metabarcoding analyses for the most important ecological guilds classified via the FUNGuild database

	Low 310–420 [m]			Mid 460–820 [m]			High 775–1260 [m]		
	OTU	Reads	%	OTU	Reads	%	OTU	Reads	%
Ectomycorrhizal	178	2,450,020	45.5	215	3,854,993	55.1	176	3,012,851	54.9
Endophyte	42	216,882	4.0	47	265,877	3.8	40	211,223	3.8
Ericoid Mycorrhizal	6	65,577	1.2	3	38,629	0.6	4	34,463	0.6
Lichenized	19	4501	0.1	26	5845	0.1	21	3453	0.1
Other	154	273,690	5.1	162	285,341	4.1	135	180,513	3.3
Saprotroph	322	843,357	15.7	384	858,378	12.3	319	613,326	11.2
Unassigned	233	1,524,760	28.3	265	1,681,021	24.0	225	1,434,142	26.1
Sum	954	5,378,787	100.0	1,102	6,990,084	100.0	920	5,489,971	100.0

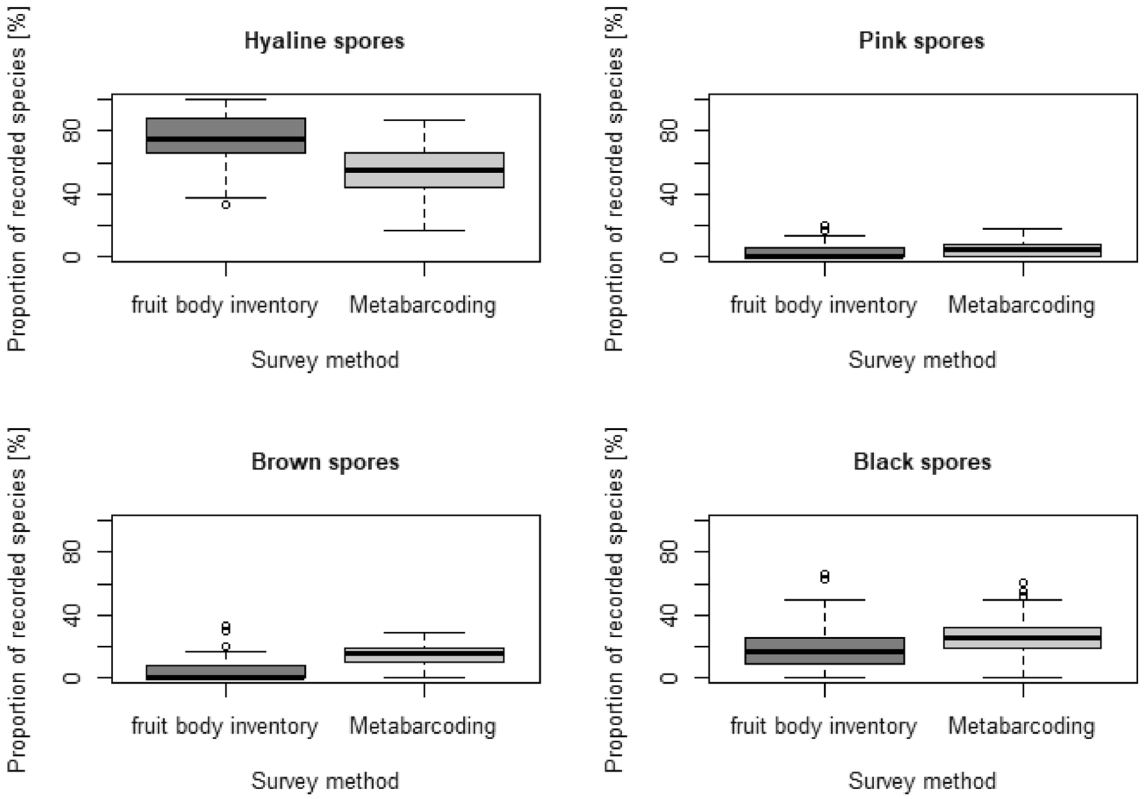

Fig. 7 Comparison of the proportion of wood-colonising species with different spore colours between fruit body inventory and metabarcoding

obtained similar results, with species numbers being 4.6 times higher with the metabarcoding approach than with the fruit body inventory. Similar to the present study, Agaricomycotina accounted for about 31% of OTUs in the study in Denmark. Of these, slightly more than half were assigned to the Agaricales. Dahl et al. (2018) analysed populations of soil-dwelling myxomycetes along an elevational transect in the northern German Alps using metabarcoding of soil extracts and sequencing of fruit bodies collected during a quantitative fruit body survey. Here again, overall species numbers were about 4.6 times higher in the eDNA data from soil extracts than in the fruit body-derived sequence dataset.

In the present study, the distribution of species detected by metabarcoding was approximately balanced between *Basidiomycota* (53%) and *Ascomycota* (43%), which is in agreement with Dahl et al. (2019). In a metabarcoding study of soil samples from an altitudinal gradient in the northern Alps, the authors reported that 48.9% of OTUs belonged to the Ascomycetes and 41.9% to the Basidiomycetes (Dahl et al. 2019). A different picture emerges from metabarcoding analyses in an altitudinal gradient in the Andes (Geml et al. 2014b). Here, *Ascomycota* accounted for about half of the OTUs, while *Basidiomycota* accounted for only about 25%. However, within the *Basidiomycota*, species were distributed among orders in similar proportions as in the present study. Thus, by far the largest number of species belonged to the Agaricales. Other orders rich in species were the Cantharellales, the Hymenochaetales, the Polyporales and the Tremellales. Representatives of the Atheliales, Trechisporales and Sebacinales, as well as species of the Hysterangiales, which predominantly form hypogeous (subterranean) fruit bodies, were recorded using metabarcoding of soil samples but remained undetected in the traditional fruit body inventory, a result that was similarly reported by Porter et al. (2008) from a study site in a hemlock-dominated forest in Ontario (USA). As in our study, Dahl et al. (2019) mentioned Sordariomycetes, Dothideomycetes and Leotiomycetes among the most dominant groups of *Ascomycota* in the metabarcoding dataset.

The species most frequently recorded by metabarcoding in the present study, with detections in all 144 study plots, was *Ganoderma applanatum*, a widespread wood decomposer. This fungus was also among the ten species most frequently detected by metabarcoding in the study from Denmark (Frøslev et al. 2019). Frøslev et al. (2019) assume that many detections are due to spores, which are relatively large and melanised in this species. The fungus was also frequently detected on plots where no wood was present as a substrate for the white-rot agent. This was also the case in the Bavarian Forest study, where the fungus was found on sample plots in the high altitudes where typical host tree species such as oaks and beeches were absent. It can, therefore, be assumed that the spores of *Ganoderma applanatum* are blown over greater distances and can withstand the degrading effects of weather and sunlight better than smaller, colourless spores of other species. The influence of the melanised spores in particular was also confirmed in the present study by the fact that eight species with dark spores were among the ten most abundant wood decomposers found in the metabarcoding data. In contrast, the seven most abundant wood decomposers detected by fruit body mapping all had hyaline spores and were not represented among the most common species in the metabarcoding data. The white-spored wood-decomposer *Fomitopsis pinicola*, although its fruit bodies were found in 73 plots, was detectable in soil samples by metabarcoding in only 15 study plots. In contrast, a species of the genus *Heterobasidion*, which also has white spores but occurs with its hyphae in the rhizosphere of trees, was present in the soil samples of more than half of the plots. In metabarcoding studies of wood samples from Norway spruce in Finland, *F. pinicola* and *Heterobasidion* sp. were found to be the dominant species among wood-colonising fungi (Ovaskainen et al. 2013). Analysis of the proportions of wood-colonising species with different spore colours in the two survey methods applied in the Bavarian Forest showed that species with melanised spores were obviously easier to detect in soil samples than species with hyaline (white) spores. This may be due to the fact that the melanised spores can survive longer in the soil than non-melanised spores and explains why *Ganoderma applanatum* with its thick-walled brown spores was detected in all sample plots by metabarcoding. This might also be a reason why representatives of the Dacrymycetes can be frequently observed in traditional field studies but remain virtually undetected by metabarcoding of soil samples, a finding which was also reported by Frøslev et al. (2019) for Dacrymycetes and Porter et al. (2008) for Dacrymycetales. In addition, PCR amplification of the ITS region in at least two genera of the Dacrymycetes using the ITS4 primer has been reported to show only limited success due to multiple mismatches in the annealing sites (Zamora and Ekman 2020). As a result, the detection of the species belonging to these genera by PCR-based sequencing strategies can only be limited. Nevertheless, this study allows a comparison between the two methodological approaches, as even with the limited PCR success rates, yeast species have been detected and tentatively taxonomically identified using metabarcoding.

Within the altitudinal gradient in the Bavarian Forest, the metabarcoding data showed a strong negative correlation between altitude and species number (Fig. 4). In contrast, this trend was not evident in the data from the fruit body inventory. The background for the high species diversity at lower altitudes and highest gamma diversity of ectomycorrhizal and saprotroph fungi at medium altitudes (Table 5) may be the higher diversity of tree species, which also leads to an increased number of corresponding

specific mycorrhizal fungi and saprotrophic fungal species. This observation has led to the consideration that altitude itself is an indirect factor, primarily shaping the vegetational cover which alters nutritional input via different litter types (Collins et al. 2018; Siles and Margesin 2016). Zinger et al. (2011) showed that plant species composition and soil organic matter had a significant effect on fungal communities in the soil of study plots in the French Alps. Similarly, the strong influence of plant species composition, indicated by crown cover proportions of the main tree species shaping the forest ecosystems, on fungal communities was evident also in our study. Collins et al. (2018) reported that the observed decline of fungal diversity in the metabarcoding data was consistent with change in litter type and increasing abiotic stress in higher altitudes. Dahl et al. (2019) performed metabarcoding analyses of soil samples from a transect in the German Alps and found vegetation type to be the most important environmental parameter to shape the communities of fungi, bacteria and myxomycetes. The authors reported that differences in fungal communities were mainly due to plant-symbiotic taxa and hence concluded that fungal-plant-interactions rather than litter quality were the dominating driver to shape fungal communities on the examined study sites. In the Bavarian Forest, we generally saw more ectomycorrhizae in the conifer-dominated plots at higher altitudes, which formed fruit bodies above the soil surface, this observation being in line with the conclusion of Dahl et al. (2019). Moisture conditions are also generally more favourable for fruit body formation at higher altitudes, so these effects cancelled each other out in the fruit body inventory across the altitudinal gradient resulting in roughly similar species numbers at the different altitudes. Unlike our results from the Bavarian Forest, Dahl et al. (2019) did not find a decrease of fungal diversity towards higher altitudes but a strong species turnover, which was also observed in the Bavarian Forest.

Analysis of the fungal species communities on the study plots using ordination models showed that comparable relative similarities between the local species communities were detected with both methods. The key environmental parameters that shaped the species community structure also showed high similarities, although the explanation power of the environmental parameters with significant influence, especially the parameter altitude, was higher in the analysis of the metabarcoding data than in that of the fruit body inventory data. Similar results were also obtained by Frøslev et al. (2019). The authors explained this discrepancy by the fact that fruit bodies are a rather random occurrence within the totality of mycota present on a plot and are, therefore, more difficult to calculate or predict than the assumed stable occurrence in the soil. The dependence of fruit body formation on short-term weather conditions, especially for ephemeral fruit bodies of cap fungi, makes representative mapping difficult when only a limited time frame is available. All the more astonishing is the good agreement of the relative similarities of the fungal species communities along the altitudinal gradient studied here, showing that the traditional fruit body inventory is still useful as a complementary measure in scientific studies. The results hence underline the relevance of fruit body monitoring in addition to high-throughput molecular methods, as shown by Kranabetter et al. (2009) in a study on important ecological aspects in forest ecosystems, i.e., soil fertility and nitrogen cycling.

Conclusions

Metabarcoding of fungi is a relatively new method for improving our knowledge of biodiversity in nature. Sequencing of environmental DNA offers the possibility of discovering species that cannot be detected in traditional fruit body inventories. Especially species forming hypogeous or very small, inconspicuous fruit bodies are often overlooked in fruit body inventories. The presented work shows that both survey methods yielded comparable results regarding relative species community similarities in the studied area although considerably higher species numbers were recorded in the metabarcoding approach than in the fruit body inventory. However, the metabarcoding approach also has limitations. For instance, sequencing has been restricted to certain barcoding genes, being ITS 1 and 2 nrDNA, which does not necessarily provide a fully reliable delimitation of species. Furthermore, good knowledge of the native mycota is necessary to verify species assignments of OTUs from metabarcoding data for plausibility.

One question that arose in the context of this project is to what extent the results from the metabarcoding analyses are due to established mycelia and/or to spore deposits since the dark-spored wood decomposer *Ganoderma applanatum* was detected by metabarcoding on many plots where its required substrate was absent. Furthermore, to what extent species with melanised, i.e., dark-pigmented spores consequently are more frequently detected. Future studies should critically analyse species lists obtained from metabarcoding surveys for species habitat requirements matching the conditions at the sampled locations.

Metabarcoding of fungal species is a very suitable approach to improve our knowledge of fungal ecology. However, such studies must also include a critical review of the results when applied to the traditional species concept. With no doubt, metabarcoding of environmental samples plays and will play a major role in biodiversity research now and in future, not least due to the fact that the number of field specialists is constantly decreasing for most taxa. Knowing the benefits and limitations of both survey methods is vital

for the interpretation of obtained results. This requires more studies comparing traditional inventories and metabarcoding of environmental samples to provide a comprehensive view of their respective power.

Acknowledgements The authors thank Finn Wahl (Bavarian State Institute of Forestry, Freising, Germany) for assistance with trait mapping, Johannes Matt (Nature Park Bavarian Forest) for assistance in the field work, Theresa M. L. Janssen and Gerasimos Gkoutselis (University of Bayreuth, Dept. of Mycology) for assistance in the laboratory. Furthermore, we want to thank the two anonymous reviewers for their valuable comments on the manuscript.

Author contributions This project was designed by AS, MB and GR. The fruit body monitoring campaigns were designed by MB and the sampling campaigns and lab workflow for the metabarcoding part by GR and JH. Metabarcoding samples were collected by DO and processed by JH who also analysed the sequence data. The combined analysis of both datasets was conducted by MB and AS. The manuscript was drafted by MB and AS with additions of JH and further elaborated by all co-authors. All authors read and approved the final manuscript.

Funding This study was conducted within the research project "Höhengradient" funded by the Forest Climate Fund, sponsored by the Federal Ministry of Food and Agriculture and the Federal Ministry for the Environment, Nature Conservation and Nuclear Safety, Germany (References: 22WC412201, 22WC412203).

Data availability Survey data of metabarcoding, including coordinates and collection dates are archived in NCBI BioSamples under Submission ID SUB11928789 and SUB9332799.The datasets generated by the fruit body inventory or analysed during the current study available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors have no relevant financial or non-financial interests to disclose.

References

Begerow D, Nilsson H, Unterseher M, Maier W (2010) Current state and perspectives of fungal DNA barcoding and rapid identification procedures. Appl Microbiol Biotechnol 87:99–108. https://doi.org/10.1007/s00253-010-2585-4

Blaschke M, Siemonsmeier A (2021) Pilzartengemeinschaften im Höhengradienten. Z Mykol 87(2):363–385

Bolyen E, Rideout JR, Dillon MR et al (2019) Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat Biotechnol 37:852–857. https://doi.org/10.1038/s41587-019-0209-9

Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP (2016) DADA2: high-resolution sample inference from Illumina amplicon data. Nat Methods 13:581–583. https://doi.org/10.1038/nmeth.3869

Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK et al (2010) QIIME allows analysis of high-throughput community sequencing data. Nat Methods 7:335–336. https://doi.org/10.1038/nmeth.f.303

Collins CG, Stajich JE, Weber SE, Pombubpa N, Diez JM (2018) Shrub range expansion alters diversity and distribution of soil fungal communities across an alpine elevation gradient. Mol Ecol 22:2461–2476. https://doi.org/10.1111/mec.14694

Dahl MB, Shchepin O, Schunk C, Menzel A, Novozhilov YK, Schnittler M (2018) A four year survey reveals a coherent pattern between occurrence of fruit bodies and soil amoebae populations for nivicolous myxomycetes. Sci Rep 8:11662. https://doi.org/10.1038/s41598-018-30131-3

Dahl MB, Brejnrod AD, Russel J, Sørensen SJ, Schnittler M (2019) Different degrees of niche differentiation for bacteria, fungi, and myxomycetes within an elevational transect in the German Alps. Microb Ecol 78:764–780. https://doi.org/10.1007/s00248-019-01347-1

Flessa F, Harjes J, Cáceres ME, Rambold G (2021) Comparative analyses of sooty mould communities from Brazil and Central Europe. Mycol Prog 20(7):869–887. https://doi.org/10.1007/s11557-021-01700-0

Frøslev TG, Kjøller R, Bruun HH, Ejrnæs R, Hansen AJ, Læssøe T, Heilmann-Clausen J (2019) Man against machine: do fungal fruitbodies and eDNA give similar biodiversity assessments across broad environmental gradients? Biol Conserv 233:201–212. https://doi.org/10.1016/j.biocon.2019.02.038

Gardes M, Bruns TD (1993) ITS primers with enhanced specificity for basidiomycetes–application to the identification of mycorrhizae and rusts. Mol Ecol 2:113–118. https://doi.org/10.1111/j.1365-294X.1993.tb00005.x

Geml J, Gravendeel B, van der Gaag KJ, Neilen M, Lammers Y, Raes N, Semenova TA, de Knijff P, Noordeloos ME (2014a) The contribution of DNA metabarcoding to fungal conservation: diversity assessment, habitat partitioning and mapping red-listed fungi in protected coastal Salix repens communities in the Netherlands. PLoS ONE 9(6):e99852. https://doi.org/10.1371/journal.pone.0099852

Geml J, Pastor N, Fernandez L, Pacheco S, Semenova TA, Becerra AG, Wicaksono CY, Nouhra ER (2014b) Large-scale fungal diversity assessment in the Andean Yungas forests reveals strong community turnover among forest types along an altitudinal gradient. Mol Ecol 23(10):2452–2472. https://doi.org/10.1111/mec.12765

Gkoutselis GM, Rohrbach S, Harjes J, Obst M, Brachmann A, Horn M, Rambold G (2021) Microplastics accumulate fungal pathogens in terrestrial ecosystems. Sci Rep 11:13214. https://doi.org/10.1038/s41598-021-92405-7

Guerreiro MA, Brachmann A, Begerow D, Peršoh D (2018) Transient leaf endophytes are the most active fungi in 1-year-old beech leaf litter. Fungal Divers 8:237–251. https://doi.org/10.1007/s13225-017-0390-4

Harjes J, Janssen TML, Gkoutselis G, Okach DO, Siemonsmeier A, Kinge RT, Blaschke M, Rambold G (submitted) Relations of betweenness centrality and topological coefficient of ectomycorrhizome and soil fungal saprobiome networks may be indicative for total C sequestration trends in soil. Fungal Diversity.

Kranabetter JM, Friesen J, Gamiet S, Kroeger P (2009) Epigeous fruiting bodies of ectomycorrhizal fungi as indicators of soil fertility and associated nitrogen status of boreal forests. Mycorrhiza 19:535–548. https://doi.org/10.1007/s00572-009-0255-0

Nearing JT, Douglas GM, Comeau AM, Langille MGI (2018) Denoising the denoisers: an independent evaluation of microbiome sequence error-correction approaches. PeerJ 6:e5364. https://doi.org/10.7717/peerj.5364

Nilsson RH, Larsson KH, Taylor AFS, Bengtsson-Palme J, Jeppesen TS, Schigel D, Kennedy P, Picard K, Glöckner FO, Tedersoo L, Saar I, Koljalg U, Abarenkov K (2019) The UNITE database for molecular identification of fungi: handling dark taxa and parallel taxonomic classifications. Nucleic Acids Res 47(D1):D259–D264. https://doi.org/10.1093/nar/gky1022

Oksanen J (2011) Multivariate analysis of ecological communities in R: vegan tutorial. R Package Version 1:1–43

Ovaskainen O, Schigel D, Ali-Kovero H, Auvinen P, Paulin L, Nordén B, Nordén J (2013) Combining high-throughput sequencing with fruit body surveys reveals contrasting life-history strategies in fungi. ISME J 7(9):1696–1709. https://doi.org/10.1038/ismej.2013.61

Porter TM, Skillman JE, Moncalvo JM (2008) Fruiting body and soil rDNA sampling detects complementary assemblage of *Agaricomycotina* (*Basidiomycota*, Fungi) in a hemlock-dominated forest plot in southern Ontario. Mol Ecol 17(13):3037–3050. https://doi.org/10.1111/j.1365-294X.2008.03813.x

Purahong W, Wubet T, Lentendu G, Hoppe B, Jariyavidyanont K, Arnstadt T, Baber K, Otto P, Kellner H, Hofrichter M, Bauhus J, Weisser WW, Krüger D, Schulze E-D, Kahl T, Buscot F (2018) Determinants of deadwood-inhabiting fungal communities in temperate forests: Molecular evidence from a large scale deadwood decomposition experiment. Front Microbiol 9:2120. https://doi.org/10.3389/fmicb.2018.02120

R Core Team (2019) R: A language and environment for statistical computing. – R foundation for statistical computing, Wien. http://www.R-project.org/ [Accessed 12 Mar 2020].

Schmidt-Stohn G, Oertel B (2015) Warum DNA-Sequenzierungen an Pilzen auch für Amateur-Mykologen wichtig sind. J JEC 17:75–88

Schoch CL, Seifert KA, Huhndorf S, Robert V, Spouge JL, Levesque CA, Chen W, Fungal Barcoding Consortium (2012) Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. Proc Natl Acad Sci USA 109(16):6241–6246. https://doi.org/10.1073/pnas.1117018109

Siemonsmeier A, Förster B, Blaschke M (2020) Forest structures and carbon storage in managed and unmanaged forests along an altitudinal gradient in a central European low mountain range. Waldökologie, Landschaftsforschung und Naturschutz – Forest Ecology. Landsc Res Nat Conserv 19:71–88

Siles JA, Margesin R (2016) Abundance and diversity of bacterial, archaeal, and fungal communities along an altitudinal gradient in alpine forest soils: what are the driving factors? Microb Ecol 72:207–220. https://doi.org/10.1007/s00248-016-0748-2

Thomsen PF, Willerslev E (2015) Environmental DNA–an emerging tool in conservation for monitoring past and present biodiversity. Biol Conserv 183:4–18. https://doi.org/10.1016/j.biocon.2014.11.019

White TJ, Bruns TD, Lee SB, Taylor JW (1990) Amplification and direct sequencing of fungal ribosomal RNA Genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds) PCR protocols: a guide to methods and applications. Academic Press Inc, New York, pp 315–322

Zamora JC, Ekman S (2020) Phylogeny and character evolution in the Dacrymycetes, and systematics of Unilacrymaceae and Dacyonaemataceae fam. nov. Persoonia 44:161–205. https://doi.org/10.3767/persoonia.2020.44.07

Zinger L, Lejon DPH, Baptist F, Bouasria A, Aubert S, Geremia RA, Choler P (2011) Contrasting diversity patterns of crenarchaeal, bacterial and fungal soil communities in an alpine landscape. PLoS ONE. https://doi.org/10.1371/journal.pone.0019950