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Comparing eDNA metabarcoding with morphological analyses: Fungal species richness and community composition of differently managed stages along a forest conversion of Norway spruce towards European beech in Germany

Peggy Heine *, Jonas Hausen [1], Richard Ottermanns, Martina Roß-Nickoll

Institute for Environmental Research, ABBt Aachen Biology & Biotechnology, RWTH Aachen University, Worringer Weg 1, 52074 Aachen, Germany

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ABSTRACT

Analyzing fungal diversity and community composition through environmental DNA (eDNA) metabarcoding and high-throughput sequencing relies on sequence databases and their taxonomic coverage which are often doubted in regards of data accuracy. To assess the potential of eDNA metabarcoding to distinguish differently managed forest conversion stages, we compared an extant morphological dataset created through sporocarp surveys with a metabarcoding dataset from the same study sites. The study was conducted along a spruce forest conversion project of Norway spruce towards European beech in the Eifel National Park in Germany. Using the UNITE ITS reference database, a total of 198 fungal operational taxonomic units (OTUs) were assigned up to the species level. Comparing the morphological and metabarcoding dataset, a low species overlap was observed with 27 shared fungi. The metabarcoding dataset revealed that all investigated forest conversion management stages shared beech-associated fungi (even the spruce forests), while within the morphological dataset, only the beech-inhabiting forest conversion management stages showed beech-associated fungi. The metabarcoding dataset could not show the same fungal community response patterns on the spruce forest conversion, compared to the morphological dataset, but revealed the genetic refugium of the soil fungal community. We conclude that fungal eDNA metabarcoding should always be evaluated by taxonomic experts to identify potential sequence database errors. eDNA metabarcoding cannot be used interchangeably for morphological community analyses to identify response patterns of fungal communities on forest management strategies. However, both approaches performed well in combination and showed that beech-associated fungal communities with high functional redundancy can develop after a spruce forest conversion by restoring natural European beech forests with an appropriate close-to-nature management strategy.

1. Introduction

Most forest ecosystems in Germany are degraded or deforested by direct or indirect effects of natural or anthropogenic disturbance events (Sabatini et al., 2018). Norway spruce is, together with European beech, the dominant forest tree species in German forest ecosystems (BMEL, 2014). Warming climate and associated natural disturbance events are predicted to facilitate native European beech dominance over the non-native Norway spruce, since the latter is more vulnerable for droughts, bark beetle attacks and windstorm events, while European beech seems to increase its resistance (Bolte et al., 2010; Marini et al., 2017). More than ever, the question is, how forest ecosystems can become more stable or resistant against natural and anthropogenic disturbance events. Anthropogenic disturbance events, such as large-scale clear-cutting or other non-sustainable forest management, could also negatively affect the forest ecosystem functions such as regional climate regulation (Foley et al., 2005). Thus, sustainable forestry is needed for the effective conservation of forest ecosystems and all inhabiting organisms to restore resistant and stable terrestrial habitats. In the last decades, reconverting Norway spruce plantation to more natural European beech forests has become an important task (Ammer et al., 2008; Verstraeten et al., 2013), where close-to-nature forest management can support such spruce forest

* Corresponding author.
E-mail address: peggy.heine@rwth-aachen.de (P. Heine).
[1] Deceased.

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conversions (Heine et al., 2019; Vacek et al., 2019). Nevertheless, ecological studies on spruce forest conversion and its consequences for forest ecosystems and their inhabitants are still rare. The Eifel National Park in Germany offered an opportunity to study a climate-oriented, close-to-nature managed spruce forest conversion towards a forest dominated by European beech that is typical and suitable of this region.

In general, when assessing forest management, a bioindicator should be used that is essential to the forest ecosystem and interacts with many other soil organisms. Soil fungi are highly diverse and valuable for forest ecosystems, but also sensitive and threatened. Soil fungi support essential ecological functions, such as carbon sequestration, litter decomposition, and are in symbiotic association with trees (Courty et al., 2010; Tedersoo et al., 2014). Fungi provide high-priority ecosystem services such as influencing tree growth and survival, affecting soil aggregation, or enmeshing of soil particles (Miller and Jastrow, 1992). Thus, soil fungal communities can structure plant diversity and vegetation composition (van der Heijden et al., 2008; Tedersoo et al., 2020), but, vice versa, plant communities are shaping fungal soil structures by environmental condition or vegetation composition changes (Wubet et al., 2012; Nacke et al., 2016; Nakayama et al., 2019). In forest ecosystems, fungal diversity and community composition depend on many different biotic and abiotic factors, such as forest stand age, plant diversity, host tree species as well as organic matter quality (Nordén et al., 2004; Waldrop et al., 2006; Wallander et al., 2010; Rosinger et al., 2018). Furthermore, fungi serve as a food source and habitat for other soil- and litter-dwelling organism groups such as oribatid mites or saproxylic beetles (Crowther et al., 2013; Maraun et al., 2014). Thus, fungal communities are an important research topic of soil ecology (Egan et al., 2018) and are promising bioindicators to study forest ecosystems (Halme et al., 2017).

The high fungal diversity has proven difficult to study only with the traditional morphological identification approach, thus molecular approaches came in focus. Obtaining as many comprehensive DNA-based taxonomic profiles as possible for a forest ecosystem renders soil sampling, in combination with metabarcoding, an effective strategy for investigating species-rich fungal communities (Hibbett et al., 2011; Schmidt et al., 2013; Thomsen and Willerslev, 2015; Anslan et al., 2018). An increasing number of eDNA (environmental DNA) metabarcoding studies assessed the effects of environmental conditions on fungal communities at various biogeographic levels (Wubet et al., 2012; Yahr et al., 2016; Deiner et al., 2017; Matsuoka et al., 2019). Over the years, metabarcoding became less expensive and is now included in standard investigational frameworks because many fungi do not exhibit morphological structures and features for differentiation via traditional morphological identification. In contrast, morphological studies realize satisfactory taxonomic resolution up to the species level (Straatsma et al., 2001; Ji et al., 2013) which is an important information e.g., for red-listed fungal species (Frøslev et al., 2019) or functional studies (Raja et al., 2017). Most of the metabarcoding studies are focused on sequence-based OTU (operational taxonomic unit) abundance and diversity. However, metadata should be identified up to a taxonomic level with ecological information to gain insights into ecological functions (Raja et al., 2017).

The UNITE sequence database is one of the most commonly used ITS reference databases for environmental sequencing of fungal communities (Nilsson et al., 2019). Despite efforts to create systematic OTU identifiers such as the DOI-based species hypothesis (SH) of UNITE (Nilsson et al., 2019), scientific taxa names are the better possibility for cross-study comparison (Yahr et al., 2016), especially with a focus on functional group analysis of fungi (Geml et al., 2016).

Only a few studies (Porter et al., 2008; Runnel et al., 2015; Frøslev et al., 2019) have focused on the combined assessment of data from fungal eDNA metabarcoding and traditional morphological identification for the ecological evaluation of forest conversion management. Such comprehensive concepts probably have substantial potential to support conservation policies or to increase our understanding of fungal diversity, fungal distributions, and trends in changing communities (Yahr et al., 2016).

Therefore, one objective of this study was to investigate the fungal diversity, community composition, and functional structure with the facilitation of eDNA metabarcoding (i) to assess the genetic reservoir of the topsoil, (ii) to identify response patterns in fungal communities to forest conversion management, (iii) to determine the ecologically functional group affiliations, and (iv) to compare all these results with those of an extant morphological dataset at the same study sites.

The study sites were differently managed forest areas, classified as management stages along a forest conversion from Norway spruce towards European beech in the Eifel National Park in Germany. In addition to this forest conversion project with active management of beech introduction after selective cutting of spruce trees, the Eifel region has been influenced by devastating windstorm events before, therefore windthrow areas were also included in this study. An extant morphological dataset of 235 macrofungal species (Heine et al., 2018, 2019) which showed distinctive response patterns to the different forest conversion stages was used as reference dataset within this paper to answer the following questions:

1. Do the eDNA metabarcoding and traditional morphological identification approaches yield similar conclusions regarding the effects of the spruce forest conversion on fungal communities?
2. Can traditional morphological identification be outperformed or even replaced by the eDNA metabarcoding approach?

The morphological dataset from Heine et al. (2018, 2019) contains two phyla, namely, Basidiomycota and Ascomycota. Its 235 fungal species could be classified into 70 families and 25 orders, with the most species-richest order being Tricholomataceae followed by Strophariaceae. All visible macrofungi were examined while ignoring, e.g., lichens and moulds.

We hypothesized that it is possible to identify comparable community response patterns via eDNA metabarcoding to those of the morphological dataset, while the morphologically identified taxa cover a subset of the genetic pool in topsoil that is observed via eDNA metabarcoding.

2. Materials and methods

2.1. Study sites

The 15 study sites were located in the Eifel National Park (50°34′12.60″N 6°21′38.50″E) and in the neighbouring forest Monschauer Stadtwald (50°31′51.43″N 6°18′53.93″E) in Germany. We used the same study sites as described in Heine et al. (2019). The locations, site characteristics, and management history of each study site are detailed in Heine et al. (2018). Some of the forest sites were influenced by devastating windthrow events, while others were close-to-nature managed or were without any forest management for greater than 100 years. Briefly, we covered differently managed stages that represent a spruce forest conversion towards European beech forests (three replicated study sites each): (I) even-aged Norway spruce forest (*spruce*), (II) unmanaged Norway spruce windthrow (*wtplus*), (III) salvage-logged Norway spruce windthrow (*wtminus*), (IV) single Norway spruce tree selection cutting (close-to-nature managed) with European beech underplanting (*spb*), and (V) old-growth, uneven-aged European beech forest (*beech*).

2.2. Study site characteristics

Heine et al., 2018 summarises all parameter used in this study to classify the forest conversion stages. Briefly, topsoil C:N (TCN), litter C:N (LCN), %total C in litter (C_litter), %total N in litter (N_litter), mean surface temperature (Ts), mean surface humidity (Hs), EIV (Ellenberg

indicator value) for nutrient availability (EIVN), EIV for light availability (EIVL), EIV for soil reaction (EIVR), EIV for soil moisture (EIVM), EIV for temperature (EIVT), canopy closure (CC), plant richness of light-demanding plants (PRL), mean fungal species richness (FSR), topsoil pH (TpH), litter pH (LpH), %total C in topsoil (C_topsoil), and %total N in topsoil (N_topsoil) were detected or calculated.

2.3. Soil sampling and DNA extraction

The soil sampling was conducted in August/September of 2011 at the same study sites where the sporocarp surveys were performed over three years (2010–2012). For the soil sampling, at five locations (the centre and corners) in each study site (10 m × 10 m), topsoil (0–10 cm depth) was sampled after removing the aboveground litter by using a cylindrical soil core sampler (ø 5 cm). The 75 samples were separated into sterile plastic bags and were transported at 4 °C for further processing. After sieving (<2 mm), we maintained the soil at −20 °C until DNA extraction following Lindahl et al. (2013).

Environmental DNA (eDNA) was extracted from 0.5 g of each topsoil sample using the NucleoSpin® Soil DNA extraction kit (Macherey-Nagel, Germany) with a modified sample lysis procedure; bead beating was conducted for 3 × 15 s at speed 5 using a Fast Prep FP120 cell disrupter (Qbiogene, Heidelberg, Germany). Each eDNA probe was re-suspended in 50 µl elution buffer and stored at −20 °C until DNA amplification. Previously, the quantity and quality of each eDNA probe were assessed via gel electrophoresis using a 1.5–1.6% agarose gel and the MassRuler DNA Ladder Mix (SM0403, Thermo Fisher Scientific, USA).

2.4. DNA amplification

Following Schoch et al. (2012), we used the fungal internal transcribed spacer (ITS) region of the ribosomal DNA (rDNA) which is highly evolutionarily variable (Nilsson et al., 2015). After extensive primer checks, we used the primer pair ITS1FI2 (forward primer, 5́-GAACCWGCGGARGGATCA-3́, Schmidt et al., 2013) and ITS2 (reverse primer, 5́-GCTGCGTTCTTCATCGATGC-3́, White et al., 1990) which target the full fungal ITS1 region.

Both primer which were acquired from Invitrogen (Germany), were synthesized with a tag of three degenerated (random) nucleotide mixes (4 N, 2 N, and 0 N) for the adjustment of the Illumina microscope (Kircher et al., 2011). Each 12.5 µl PCR amplification mixture consisted of 6.25 µl Phusion® High-Fidelity PCR master mix (Finnzymes by Thermo Fisher Scientific, USA), 0.5 µl of each primer (0.4 µM), 5.25 µl Milli-Q water, and 2 µl purified eDNA probe. Each PCR mixture sample was denatured for 30 s at 98 °C before undergoing 35 cycles of 10 s at 98 °C, 30 s at 50 °C, and 30 s at 69 °C. Final elongation was conducted at 72 °C for 5 min. PCR reactions were conducted in duplicate with three positive and negative controls. Each amplicon was purified with the QIAquick® PCR purification kit (Qiagen, Germany) in accordance with the manufacturer's protocol to remove primer and short DNA fragments. The quantity and quality of all amplicons were judged via gel electrophoresis using a 1.6% agarose gel and the MassRuler DNA Ladder Mix (SM0403, Thermo Fisher Scientific, USA). The resulting PCR amplicons had a size of 200–400 bases and were subsequently stored at −20 °C for further processing.

2.5. Sequence library preparation

A sequence library was prepared by attaching unique sequence indices to individual adapters to enable sample multiplexing (measurement of multiple DNA sequences simultaneously). A customized standard desalted adapter primer was used (Invitrogen, Germany) for the labelling-PCR step (Indexing-PCR) of all purified PCR amplicons. For multiplexing, a single-index library design of 6 bases for an individual TruSeq index adapter (indices 1–17) was incorporated in a reverse TruSeq adapter which was unique for each of the 17 samples (14 study sites and three technical replicates of the study site b1).

Each 12.5 µl PCR amplification consisted of 6.25 µl Phusion® High-Fidelity PCR Master Mix (Finnzymes by Thermo Fisher Scientific, USA), 0.5 µl TruSeq universal adapter (10 µM), 0.5 µl individual reverse TruSeq Index adapter, 5.25 µl Milli-Q water, and 1 µl purified PCR amplicon of the first amplification step. Each sample was denatured at 98 °C for 30 s, followed by 35 cycles for 10 s at 98 °C, 30 s at 50 °C, 30 s at 72 °C, and a final extension at 72 °C for 30 s. The final elongation was conducted at 72 °C for 2 min. PCR reactions were conducted in duplicate and with three positive and negative controls. All amplicons were purified with the QIAquick® PCR purification kit (Qiagen, Germany) in accordance with the manufactureŕs protocol and eluted in 30 µl elution buffer. The library inputs with inserts between 200 and 400 bases have a specific adapter attached to the 5′ and 3′ ends. At the Usadel Lab (Institute for Biology I, RWTH Aachen University, Germany), all PCR amplicons were normalized after quantification with the Qubit dsDNA HS assay kit (Invitrogen, Germany) and a Qubit 2.0 fluorometer (Invitrogen, Germany). Subsequently, all samples were pooled and stored at −20 °C until high-throughput sequencing.

2.6. High-throughput sequencing

The pooled PCR amplicons were sequenced on a HiSeq 2000 Illumina platform (Illumina, USA) in the Usadel Lab using the 2 × 250 bp paired-end mode protocol. Additionally, technical replicates of sample *b1* (subsamples) were measured, an approach inspired by the study of Bálint et al. (2014). The quality of each paired-end read was assessed using FastQC version 0.11.5. In total, we observed ~ 24.8 million raw reads with a nearly even distribution among the 17 multiplexed samples. Only samples *b1.2* and *sp3* showed fewer raw reads.

2.7. Metabarcoding pipeline

We followed the recommendations of Bálint et al. (2014) by using a desktop computer to implement a customized metabarcoding pipeline for de-multiplexing, trimming, merging paired-end reads, sorting, quality filtering, sample pooling, de-replication, singleton removal, OTU picking, and alignment with the fungal ITS database UNITE (Abarenkov et al., 2010). The fungal metabarcoding pipeline was applied with UPARSE (Edgar, 2013) based on the USEARCH version 10.0.240 (Edgar, 2010), by running the Cygwin64 version 2.874 for Windows.

2.8. Demultiplexing & trimming

De-multiplexing and trimming (barcode removal) of the single reads of each sample were conducted using the command line tool Trimmomatic version 0.36 (Bolger et al., 2014) with the default parameters. Via this approach, 2,435,388 forward and reverse reads were obtained for the 17 samples.

2.9. Paired-end read assembly

We assembled 1,759,268 paired-end reads by merging the forward and reverse reads. During the paired-end assembly, 27.7% of the reads were discarded due to either differences during alignment (>5 bases) or failure to align. The mean merged length of the paired-end reads was 329 bases, and the average alignment length was ~ 170 bases.

2.10. Quality filtering

Quality filtering of paired-end reads was run by using the *-fastq_filter* USEARCH parameter based on a maximum expected error of 1 error/read, and the read tail was trimmed to 271 bases, thereby resulting in 1,660,781 filtered sequences for the 17 samples.

2.11. Sample pooling, de-replication and singleton removal

Sample pooling improves the estimation of read abundance and singleton detection in the subsequent de-replication step. De-replication (*–fastx_uniques* parameter) summarized 158,816 unique sequences (100% match), out of which 113,208 were unique sequences (singletons).We assumed that all singletons were potential artefactual sequences due to sequencing errors, as we obtained similar community results after OTU picking with or without singletons. Consequently, the singletons (71.3%) were removed from subsequent analyses to prevent the overestimation of species richness and the contamination of databases with artefactual data by using the *-minsize 2* parameter. All 45,608 unique sequences were sorted in accordance with the abundance (with the *–sortbysize* parameter), where the maximum abundance of the most common sequence was 142,883.

2.12. OTU picking

All sorted unique sequences (45,608) were clustered into OTUs based on 97% similarity identity using the UPARSE algorithm with the *-cluster_otu* parameter. The centroid sequence of each cluster (a representative sequence from the most common sequence type in each OTU) was used for the annotation of fungal reads. The centroid sequences were relabelled and provided as FASTA files. We used the USEARCH parameter *-usearch_global* to generate an OTU table for determining the abundance of each OTU per sample. OTU picking and chimera filtering were conducted as an integrated process. Overall, 288 chimeras were identified and discarded. At the 97% similarity cut-off level, all unique sequences were assigned to 884 OTUs (See Supplementary Appendix 1 [crosstable_884_OTU_tax]).

2.13. Taxonomic identification

For a better comparison of the metabarcoding dataset with the extant morphological dataset from Heine et al. (2019), all OTUs were aligned with a fungal ITS sequence database to obtain assignments with taxonomy up to the species level. The UNITE database has a valuable collection of fungal ITS sequences (Kõljalg et al., 2005; Abarenkov et al., 2010; Xu, 2016) which are obtained by considering SHs (species hypotheses) that are based on one or more fungal ITS sequences (Kõljalg et al., 2013). UNITE follows the Index Fungorium (www.indexfungorium.org) classification. We blasted all 884 OTUs against the *sh_general_release_dynamic* UNITE database version 7.2 (10/10/2017, 30,548 sequences, https://unite.ut.ee/repository.php). The alignment used a threshold of 97% sequence similarity which is still widely used in fungal high-throughput sequencing approaches (Baldrian et al., 2021). The output table was created with the USEARCH parameter *-usearch_local* and presented all hits in the default UNITE BLAST format which included all OTU labels, the taxonomic entry, alignment-specific parameters (percentage identity, alignment length, number of mismatches, number of gap opens, start position in the query, end position in the query, start position in the target, and end position in the target), the calculated E-value, and the bit score as quality indicators for the alignment.

Out of the 884 OTUs, 869 (98.3%) could be assigned to either fungal or unidentified entries through the UNITE reference database and further assigned to 361 alignment hits (at the genus level or lower), revealing 198 OTUs assigned up to species level within the phyla Basidiomycota and Ascomycota. Thereby, we used OTU-identified species that resulted from an alignment length up to 274 bases.

For the functional group analysis of fungi, those 198 OTUs with a species name were matched to guilds in the FUNGuild online tool and database (Nguyen et al., 2016). The FUNGuild database (http://www.stbates.org/guilds/app.php) consisted of almost 10,000 records at the time of this study, and the functional group affiliation was conducted predominantly at the species level due to the fact that the FUNGuild outcomes of the 361 alignment hits or the outcomes the 198 OTUs (with a species name), respectively, did not differ significantly from each other.

2.14. Statistical analysis

Statistical analyses were conducted using R software version 3.3.1 (R Core Team, 2016) unless stated otherwise.

To determine whether the management of each forest conversion stage influenced the mean OTU richness, a generalized linear model (glm) was utilized. The dependent variable was the mean valuewhile the independent variable represented the stage (*glm* function, *stats* package in R, R Core Team, 2016). Similar to the morphological dataset from Heine et al. (2019), the values were not significantly over-dispersed (*dispersiontest* function, *AER* package in R, Kleiber and Zeileis, 2008). Therefore, it was also modelled based on the Poisson distribution ($p < 0.05$) which was followed by a Tukey's honestly significantly different (TukeyHSD) *post hoc* test (*glht* function, *multcomp* package in R, Hothorn et al., 2008) for pairwise comparison.

The proportions of unique and shared OTUs of the metabarcoding dataset were calculated and visualized in a pairwise Venn diagram (*draw.pairwise.venn* function, *VennDiagram* package in R, Chen and Boutros, 2011) in comparison to the fungal species of the morphological dataset from Heine et al. (2019). In addition, the proportions of unique and shared OTUs among the five forest conversion stages were calculated and visualized in non-proportional Venn diagram (*venn* function, *venn* package in R, Dusa, 2017) and compared with the Venn diagram of the unique and shared fungal species of the morphological dataset.

Hierarchical clustering analyses of the metabarcoding dataset were conducted using the Jaccard measure and complete clustering (*vegdist* and *hclust* function, *vegan* package in R, Oksanen et al., 2018). The resulting clusters were coloured in accordance with the *a priori* classified forest conversion stages and set against the cluster analysis of the morphological dataset from Heine et al. (2019) to investigate the dissimilarity of the dendrograms within a tanglegram (*tanglegram* function, *dendextend* package in R, Galili, 2015).

The multiple response permutation procedure (MRPP, Mielke Jr., 1991) was conducted in PC-Ord (McCune and Mefford, 2011) to test the null hypothesis of similar species compositions for the forest conversion stages. It was conducted with non-transformed species data using Jaccard dissimilarities and 999 permutations at the p-value of $p < 0.1$, and it was followed by a TukeyHSD *post hoc* test (Hothorn et al., 2008) for pairwise comparison of the fungal species composition within each forest conversion stage.

Unconstrained NMDS (non-metric multidimensional scaling, Shepard, 1962; Kruskal, 1964) ordinations were employed (*metaMDS* function, *vegan* package in R, Oksanen et al., 2018) to visualize the spatial distribution of the community composition. Procrustes correlation analysis (*procrustes* function, *vegan* package in R, Oksanen et al., 2018 in accordance with Gower, 1971) and the Protest test (*Protest* function, *vegan* package in R, Oksanen et al., 2018 in accordance with Jackson, 1995) were used to determine whether the effects of the forest conversion stages were similar across both datasets.

3. Results

3.1. NGS data characteristics

We obtained 884 OTUs using the UNITE reference database (See Supplementary Appendix 1 [crosstable_884_OTU_tax]). Of these, 4 (identified as plantae spec.) OTUs and 11 (classified into the kingdoms Protista, Chromista and Cercozoas) OTUs were removed. Of the remaining 869 OTUs, 361 could be clearly assigned up to the fungal kingdom, while 508 OTUs could not be assigned. As it was not clear whether these unidentified OTUs were sequence artefacts or currently unknown fungal OTUs, all 869 OTUs were included in the community analyses. The metabarcoding revealed a mean OTU richness with

decreasing numbers of OTUs, namely, 345, 338, 331, 279, and 245 OTUs, for *wtminus*, *spb*, *wtplus*, *beech*, and *spruce*, respectively (Fig. 1). The 361 fungal alignment hits at various taxonomic ranks led to 198 OTUs that corresponded to species names of the phyla Basidiomycota and Ascomycota. However, this study assumed that the UNITE reference database represented an equal fungal distribution and not an accumulation of one phylum or one fungal genus of a single forest type which would generate an overrepresentation of fungal sequences in the database and, thus, in the metabarcoding dataset at the species level. This assumption is supported, as similar ratios of OTUs with species names compared to all identified OTUs among all 15 study sites could be recorded (not shown in this paper) and the same species response pattern across the five forest conversion stages could be recognized (Fig. 1). Thus, we assumed that the taxonomic assignment reflected a representative part of the entire OTU data matrix.

3.2. Fungal species richness

The highest mean fungal species richness (OTUs with a species names) of the metabarcoding dataset was identified in the spruce-beech mixed stage (*spb*, 62.7 ± 19.6) which was followed by the unmanaged windthrow stage (*wtplus*, 57.3 ± 3.8) and the salvage-logged windthrow stage (*wtminus*, 55.7 ± 7.1). The lowest mean fungal species richness was identified in the even-aged spruce stage (*spruce*, 35.7 ± 22.0), while the old-growth beech stage showed more (*beech*, 40.7 ± 7.2) (Fig. 1). The OTUs with a species name showed contrasting response patterns of the fungal species richness among the five forest conversion stages compared the results of the morphological dataset (Fig. 1). The managed stages (*spb*, *wtplus*, and *wtminus*) showed the highest values of mean species richness that were observed via metabarcoding, in contrast to the fungal species richness of the morphological dataset, where the managed stages showed lower species richness than the old-growth beech forests (Fig. 1).

At the same study sites, 235 macrofungal species were identified via traditional morphological identification, as presented in Heine et al. (2019). Jointly, the two approaches produced 433 species of fungi: 406 of these were unique to one of the approaches while 27 were recovered by both approaches (Fig. 1 and Supplementary Appendix 1 [shared species-both approaches]). Of those, 24 overlapping fungal species could be identified within the same study site, while 3 species (*Clitocybe nebularis*, *Hypholoma lateritium*, and *Mycena metata*) could not be identified at the very same study site. In addition, *Phaeoclavulina* cf. *abietina* was identified in both datasets but was not included in this context as the morphological identification result was uncertain.

The metabarcoding dataset showed the most shared OTUs among all five forest conversion stages (Fig. 2 and Supplementary Appendix 1 [shared OTUs via metabarcoding]), while within the morphological dataset, very few fungal species (6) were equally distributed among all five forest conversion stages (see Heine et al., 2018, 2019).

3.3. Community analysis

A cluster analysis of the metabarcoding dataset separated the five *a priori* classified forest conversion stages into three groups: the windthrow sites (*wtminus*1-3 and *wtplus*1-3), two of the close-to-nature managed spruce-beech mixed forest sites (*spb*1-2), and all three beech sites (*b*1-3) together with all spruce sites (*sp*1-3). Hierarchical clustering of the morphological dataset from Heine et al. (2019) yielded four distinct groups of sites. All three beech sites are separated from the remaining sites. All spruce sites and all spruce-beech mixed forest sites

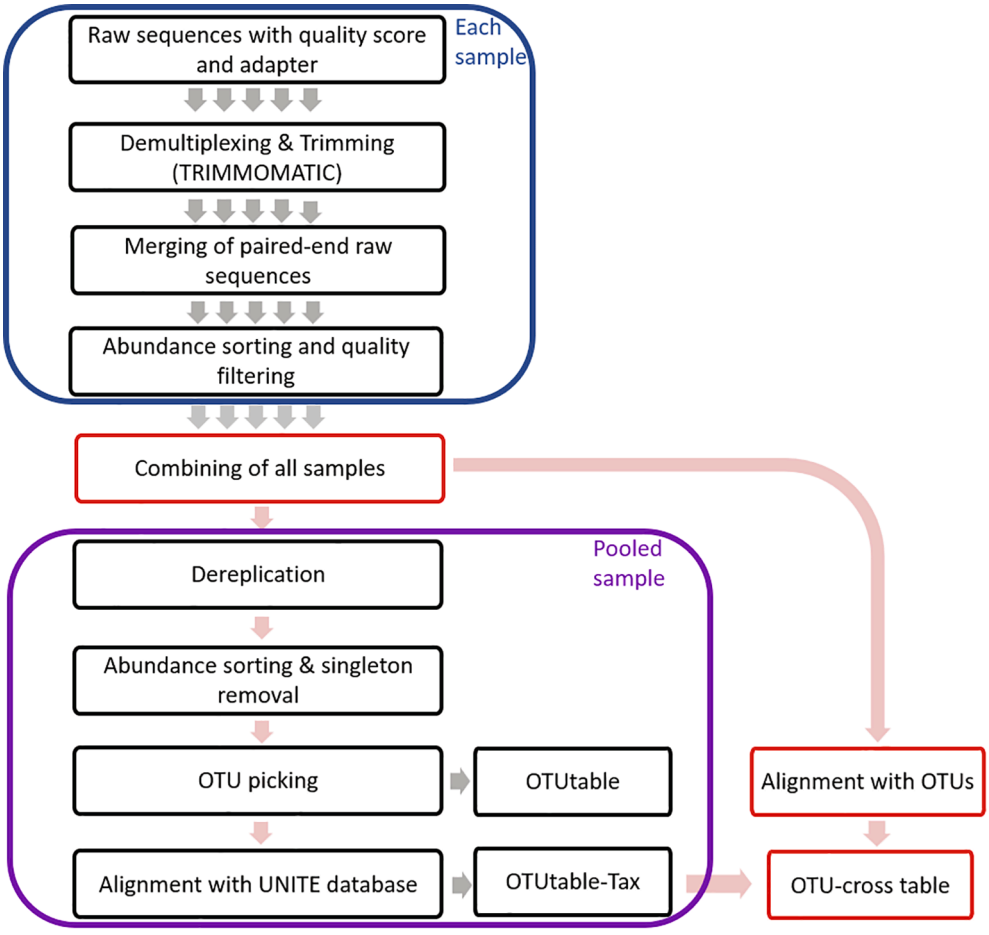

Fig. 1. first graph: The mean species richness of the morphological dataset, the mean OTU richness of the OTUs that could be assign up to the species level of the metabarcoding dataset, and the mean OTU richness of all OTUs of the metabarcoding dataset among the five forest conversion stages (three replicated study sites each): beech forest (beech), spruce forest (spruce), unmanaged windthrow (wtplus), salvage-logged windthrow (wtminus), and close-to-nature managed spruce-beech mixed forest (spb). Bars that share the same letter are not significantly different from each other ($p < 0.05$, Tukey HSD post hoc test). The standard deviation is specified as the above and below error. Second graph: The morphological dataset from Heine et al. (2019) contained 235 macrofungal species, while 198 OTUs of the metabarcoding dataset could be assigned with fungal species names using the UNITE database (version from 10/10/20217), revealing 27 shared fungal species; 361 OTUs were assigned up to the genus level; and 508 OTUs remained unassigned. List of all 27 shared fungal species can be found in the Supplementary Appendix 1 [shared species-both approaches]).

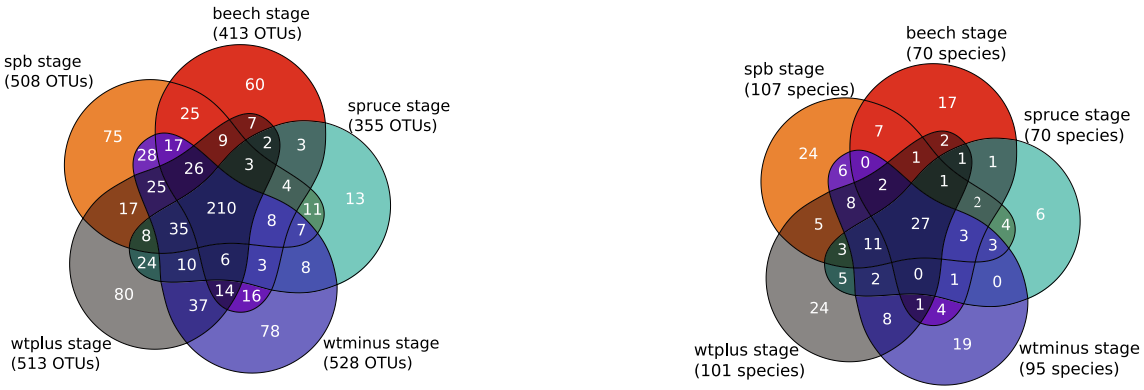

Fig. 2. Shared and unique (number on the outermost portion of each ellipse) OTUs (first graph) and OTUs that could be assigned up to the species level though the UNITE database (version 10/10/2017) (second graph) of the metabarcoding dataset among the five forest conversion stages: beech forest (beech), spruce forest (spruce), unmanaged windthrow (wtplus), salvage-logged windthrow (wtminus), and close-to-nature managed spruce-beech mixed forest (spb). The highest amount of shared OTUs (either of all OTUs or the OTUs with a species name) were detected within all five differently managed forest conversion stages. A list of the 27 OTUs which could be assigned up to the species level and that were the same among the five forest conversion stages can be found in the Supplementary Appendix 1 [shared OTUs via metabarcoding].

form separate sub-clusters of a larger cluster. The fourth group contained all sites of the windthrow sites: *wtplus* and *wtminus*. Comparison of the resulting dendrograms of both clustering revealed different combinations of clusters regarding the five *a priori* classified forest conversion stages (Fig. 3). In accordance with the morphological dataset, the beech sites are clearly separated, whereas within the metabarcoding dataset, these sites are grouped together with spruce sites and one spruce-beech mixed forest site (*spb3*). Sites *spb1* and *spb2* form a separate cluster within the metabarcoding dataset (orange) which is more similar to the windthrow sites. Overall, the tanglegram revealed an entanglement coefficient of 0.07 which indicates satisfactory alignment.

The smallest changes in study site constellation were observed in the largest cluster (*wtplus* and *wtminus*) which was followed by the red cluster (*beech*) and the orange cluster (*spb*) within both datasets. Both datasets showed high similarity and separation into two large clusters: the windthrow cluster (blue) and the beech cluster (red) (Fig. 3).

The MRPP results of the metabarcoding dataset (abundance data) demonstrated that close-to-nature managed spruce-beech mixed forest stage (*spb*) did not differ from the beech stage (*beech*) (Table 1). Using the binary data of the metabarcoding dataset, significant differences in community composition were identified, especially among *spb* and *beech*, while *spb* and *spruce* still did not show difference in the fungal

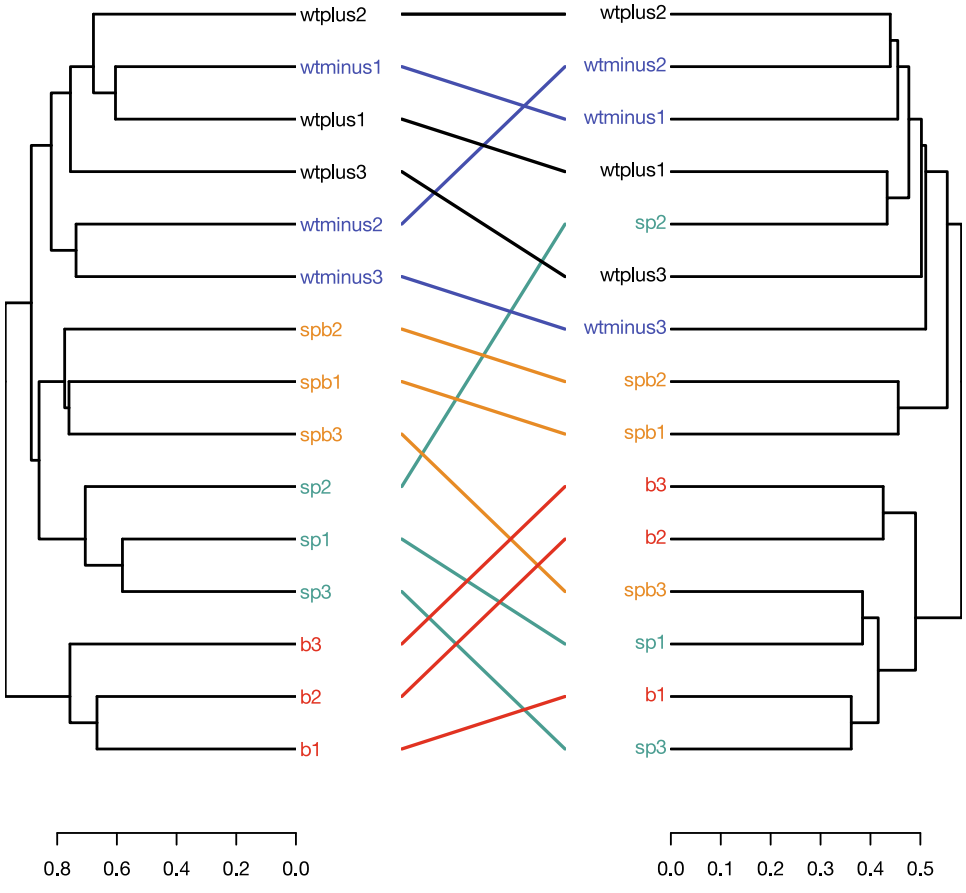

Fig. 3. Tanglegram of the macrofungal community that is based on the morphological (left, 235 species) dataset from Heine et al. (2019) and the metabarcoding dataset (right, 869 OTUs). The tanglegram was obtained using binary data for direct comparison of all fifteen study sites of the five forest conversion stages: beech forest (b1-b3), spruce forest (sp1-sp3), unmanaged windthrow (wtplus1-wtplus3), salvage-logged windthrow (wtminus1-wtminus3), and close-to-nature managed spruce-beech mixed forest (spb1-spb3). The entanglement coefficient is 0.07 which indicated satisfactory alignment. The Jaccard similarity index and the complete linkage method were used for both datasets.

Table 1
Results of pairwise comparison (PC-Ord) of all fungal species of the morphological dataset (235 species, binary data), all 869 OTUs of the metabarcoding dataset (abundance and binary data) and the 198 OTUs with a species name of the metabarcoding dataset (abundance and binary data) for the five forest conversion stages: beech forest (beech), spruce forest (spruce), unmanaged windthrow (wtplus), salvage-logged windthrow (wtminus), and close-to-nature managed spruce-beech mixed forest (spb). Significant p-values of < 0.1 are marked as *.

Correlated study sites	235 species (binary data)			869 OTUs (abundance data)			869 OTUs (binary data)			198 OTUs at species level (abundance data)			198 OTUs at species level (binary data)		
	A	p		A	p		A	p		A	p		A	p	
spruce - beech	0.172	0.022	*	0.079	0.023	*	0.055	0.026	*	0.02	0.039	*	0.05444083	0.039	*
spb - beech	0.092	0.023	*	0.019	0.225		0.058	0.030	*	0.00	0.524		0.09531336	0.027	*
wtminus - beech	0.157	0.022	*	0.135	0.026	*	0.077	0.023	*	0.03	0.027	*	0.10937865	0.027	*
wtplus - beech	0.151	0.022	*	0.129	0.024	*	0.093	0.022	*	0.03	0.043	*	0.14331879	0.023	*
spb - spruce	0.047	0.029	*	0.072	0.027	*	0.045	0.077	*	0.02	0.049	*	0.00707363	0.346	
wtminus - spruce	0.107	0.022	*	0.009	0.299		0.058	0.026	*	0.01	0.023	*	0.01857282	0.104	.
wtplus - spruce	0.111	0.022	*	0.026	0.181		0.037	0.038	*	0.01	0.152		0.03391331	0.039	*
wtminus - spb	0.073	0.022	*	0.129	0.028	*	0.025	0.066	*	0.02	0.037	*	0.03276253	0.052	*
wtplus - spb	0.073	0.022	*	0.130	0.025	*	0.056	0.024	*	0.02	0.097	*	0.06479675	0.025	*
wtplus - wtminus	0.017	0.177		−0.017	0.583		0.004	0.372		0.02	0.135		0.02523269	0.118	

community composition. Again, the windthrow stages did not differ from each other (Table 1), thereby supporting the results of the tanglegram (Fig. 3).

The NMDS ordination of the metabarcoding dataset (of all OTUs or of the 198 OTUs that could be assigned with taxonomy up to the species level) revealed mostly a similar group separation to the *a priori* forest conversion stages (Fig. 4) which likely demonstrates effects of the forest type and the forest management on the fungal species composition. Along the first axis, the sites are separated into windthrow sides (*wtplus*: black and *wtminus*: blue), spruce sites (*spruce*, cyan), spruce-beech mixed forest sites (*spb*, orange), and beech sites (*beech*, red). The second axis separated *wtplus* from *wtminus* and *spruce* from *spb* (Fig. 4).

The most congruent NMDS ordination in comparison to the morphological dataset was identified within the binary data of all 869 OTUs (Procrustes analysis, 61.25%; Protest analysis $p = 0.0033$), while the abundance data of all 869 OTUs showed a low congruence (Procrustes analysis 15.2%; Protest analysis $p = 0.8$). Thus, the comparison was further conducted with the binary data. When considering only the 198 OTUs with a species name, a slim version of the NMDS ordination of all 869 OTUs was identified. Comparing the NMDS of the 198 OTUs with the NMDS of the morphological dataset, a high congruence (Procrustes analysis 67.2%, Protest analysis $p = 0.0015$) was calculated. The NMDS of the morphological dataset from Heine et al. (2019) showed a more distinct separation of all five *a priori* classified forest conversion stages and a lower variability within each forest conversion stages: The beech sites (*b1-b3*) are more clearly separated from the remaining sites. The spruce sites (*spruce*) and spruce-beech mixed forest sites (*spb*) are distributed closer to each other (Fig. 4). The same is observed for the *wtplus* and *wtminus* sites, thereby supporting the results of the tanglegram (Fig. 3) and pairwise comparison (Table 1).

Regarding the community-influencing study site characteristics, both datasets were similarly affected by two main drivers: the nutrient availability and the light availability (Fig. 4). In detail, the morphological dataset correlated to 9 environmental variables, whereas the molecular dataset with all 869 OTUs was additionally related to litter C:N (LCN [$R^2 = 38\%$]) instead of EIV for soil reaction (EIVR) and EIV for nutrient availability (EIVN). The nutrient availability in topsoil and litter was characterised in accordance with the correlations of the topsoil C:N (TCN [$R^2 = 53\%$], the %total C in litter (C_litter [$R^2 = 40\%$]) and LCN (Fig. 4). The windthrow stages are highly distinct from the three forested stages and correlated to variables that represent high light availability with higher daily temperature fluctuation and light transmission (canopy closure, CC [$R^2 = 66\%$], mean surface temperature Ts [$R^2 = 41\%$], plant richness of light-demanding plants PRL [$R^2 = 74\%$], and EIV for light availability EIVL [$R^2 = 67\%$]). The fungal species richness (FSR [$R^2 = 56\%$]) was highly related to the fungal community distribution and showed the highest quantity within the beech-inhabiting stages (*beech* and *spb*). The mean surface humidity (Hs), carbon in topsoil (C_topsoil), pH in topsoil (TpH), pH in litter (LpH), EIVR, EIVN, EIVT (EIV for temperature) and EIVM (EIV for moisture) were not significantly correlated to the DNA-based fungal community compositions ($p < 0.1$).

Using only the 198 OTUs, additional explanatory variables were related to the fungal community, such as the pH in litter [LpH $R^2 = 41\%$], mean surface humidity [Hs $R^2 = 32\%$], and nitrogen in litter [N_litter $R^2 = 34\%$] (Fig. 4). In total, 6 correlating variables were similar within all three community compositions (morphological dataset, metabarcoding dataset at OTU level, and metabarcoding dataset at species level): canopy closure (CC), plant richness of light-demanding plants (PRL), mean surface temperature (Ts), C:N in topsoil (CN_topsoil), fungal species richness (FSR), and EIV for light availability (EIVL).

3.4. Functional structure

In total, 508 OTUs remained unassigned (Fig. 1) as the sequences were either not present in the UNITE database at the time of this study or the sequences were artefacts. This leads to a loss of information, especially in regard to the ecosystem functioning. The 198 OTUs that could be assigned up to the species level were classified by FUNGuild into the following dominant (defined as > 6%) functional groups: saprotroph (88 species, 44.4%), symbiotroph (55 species, 27.8%), pathotroph-saprotroph (22 species, 11.1%), pathotroph (15 species, 7.6%), saprotroph-symbiotroph (13 species, 6.6%), pathotroph-symbiotroph (1 species, 0.5%), and OTUs without functional group classification (4 OTUs, 2.0%). Similar to the morphological dataset from Heine et al. (2019), saprotrophs accounted for the highest proportion followed by those of symbiotrophs and of pathotrophs. Comparing the functional structures among the forest conversion stages, the DNA of the saprotrophs contributed the most, namely, 43%, within all five forest conversion stages, while the DNA of the pathotrophs and the pathotroph-saprotroph was found more rarely in the spruce forest and the beech forest. Between both datasets, the functional group affiliations differed (Fig. 5). The saprotrophs, pathotroph-saprotroph, and pathotrophs of the metabarcoding dataset showed similar response patterns with higher species richness in the forest conversion stages *spb*, *wtplus,* and *wtminus,* compared to the lower species richness within the forested stages *beech* and *spruce* (in contrast to the morphological dataset), whereas the symbiotrophs showed no significant differences among the forest conversion stages (Fig. 5) but a slight increase from *spruce* < *wtplus* < *wtminus* < *spb* < *beech*.

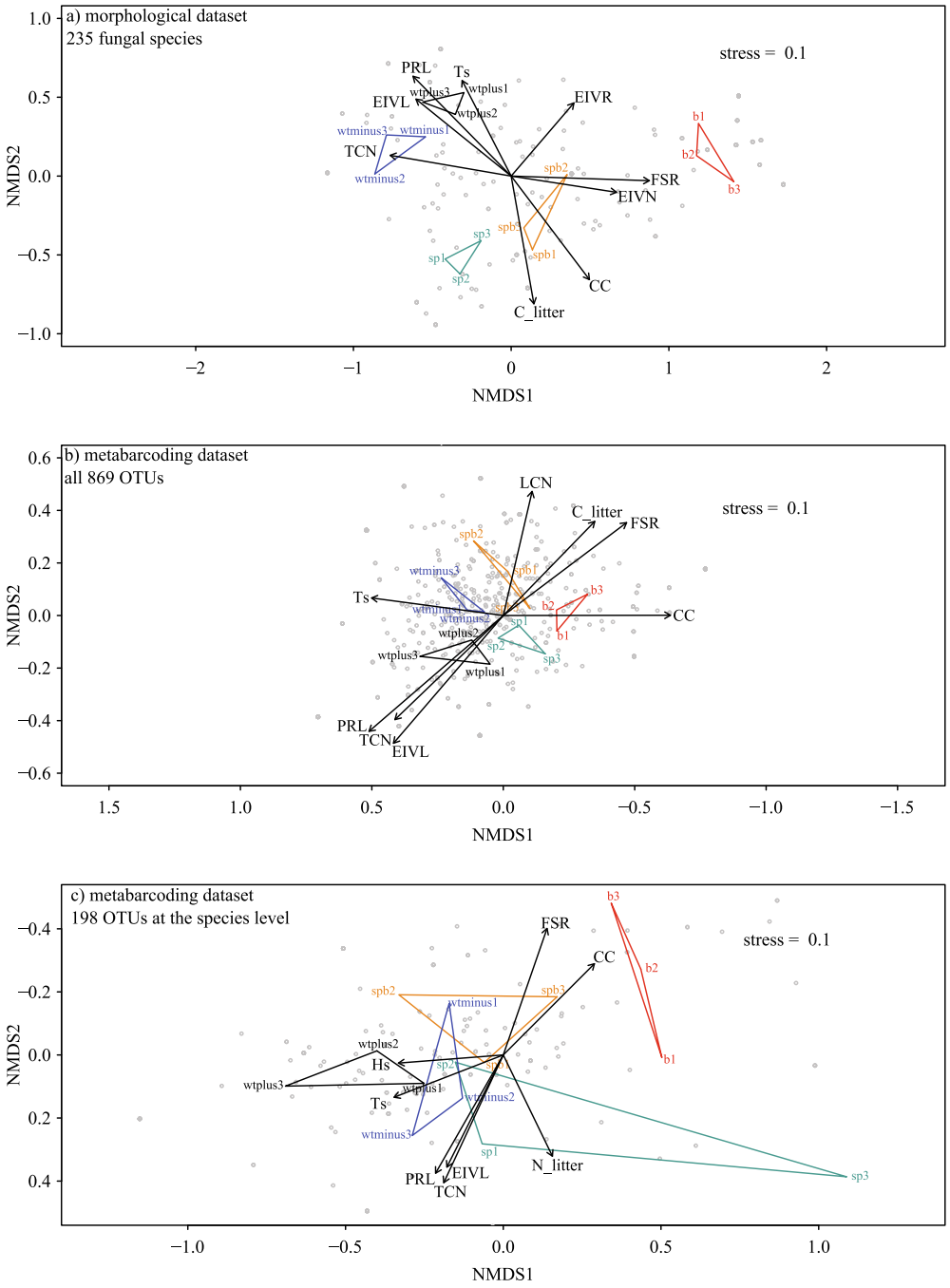

Fig. 4. Two-dimensional non-metric multi-dimensional scaling (NMDS) ordination of the morphological dataset from Heine et al. (2019) with 235 fungal species (binary data) (a), the metabarcoding dataset with 869 OTUs (binary data) (b) and the metabarcoding dataset with 198 OTUs with a species name (binary data) (c). The first two axes are shown in all graphs. The ordinations are based on the Jaccard distance. The ordinations are based on Jaccard distance, including 9 significantly correlated environmental variables as fitted vectors ($p < 0.1$): Topsoil C:N (TCN), litter C:N (LCN), % total C in litter (C_litter), %total N in litter (N_litter), mean surface temperature (Ts), mean surface humidity (Hs), EIV for nutrient availability (EIVN), EIV for light availability (EIVL), EIV for soil reaction (EIVR), canopy closure (CC), plant richness of light-demanding plants (PRL), and mean fungal species richness (FSR) (999 permutations). All other studied environmental variables (litter pH (LpH), topsoil pH (TpH), EIV for soil moisture (EIVM), EIV for temperature (EIVT), %total C in topsoil (C_topsoil) and % total N in topsoil (N_topsoil) did not correlate and were excluded in the graphs. The grey points correspond to fungal species and OTUs, respectively. Coloured points and envelopes represent the fifteen study sites among the five forest conversion stages: beech forest (b1-b3), spruce forest (sp1-sp3), unmanaged windthrow (wtplus1-wtplus3), salvage-logged windthrow (wtminus1-wtminus3), and close-to-nature managed spruce-beech mixed forest (spb1-spb3). The final stress values are presented in the graphs. of the morphological dataset (left graph, 235 fungal species, Heine et al., 2019) and the metabarcoding dataset (right graph, 198 OTUs that could be assigned up to the species level) of 4 functional groups (saprotroph, symbiotroph, pathotroph-saprotroph, and pathotroph) among the five forest conversion stages (with an average of three replicate study sites each): beech forest (beech), spruce forest (spruce), unmanaged windthrow (wtplus), salvage-logged windthrow (wtminus), and close-to-nature managed spruce-beech mixed forest (spb). GLM was modelled based on the Poisson distribution. Bars that share the same letter did not significantly differ from each other ($p < 0.05$, Tukey HSD post hoc test). The standard deviation is presented as the above and below error.

4. Discussion

4.1. Fungal species richness

The metabarcoding dataset of this study revealed that the highest amount of OTUs were equally distributed among all five forest conversion stages, including the spruce dominated stages (Fig. 2). From those 210 shared OTUs, 27 OTUs could be assigned up to the species level, including highly beech-associated species, e.g., *Mycena rosea*, a characteristic species in forests dominated by beech trees and oak trees in accordance to the study results of Karadelev and Rusevska (2004) or *Lactarius blennius* which is a characteristic fungus for beech forests. Also, broadleaved forest-associated fungi were included among all forest conversion stages, such as *Pluteus brunneidiscus*, while other shared fungal species, such as *Paxillus involutus,* were primarily related to mixed forested habitats with host trees of either broadleaved or coniferous species. The beech- or broadleaved-associated fungi within all study sites, even in the spruce forests, seem to be indicators for historically similar soil structures of beech habitats. Hence, the metabarcoding dataset revealed that beech was included in the previous vegetation form of the National Park area (before the spruce afforestation). As some plant species that are typical for beech forests occur in the spruce forests (Heine et al., 2018), there seems to be a potential for the development of native plant and fungal communities after spruce forest conversion. In

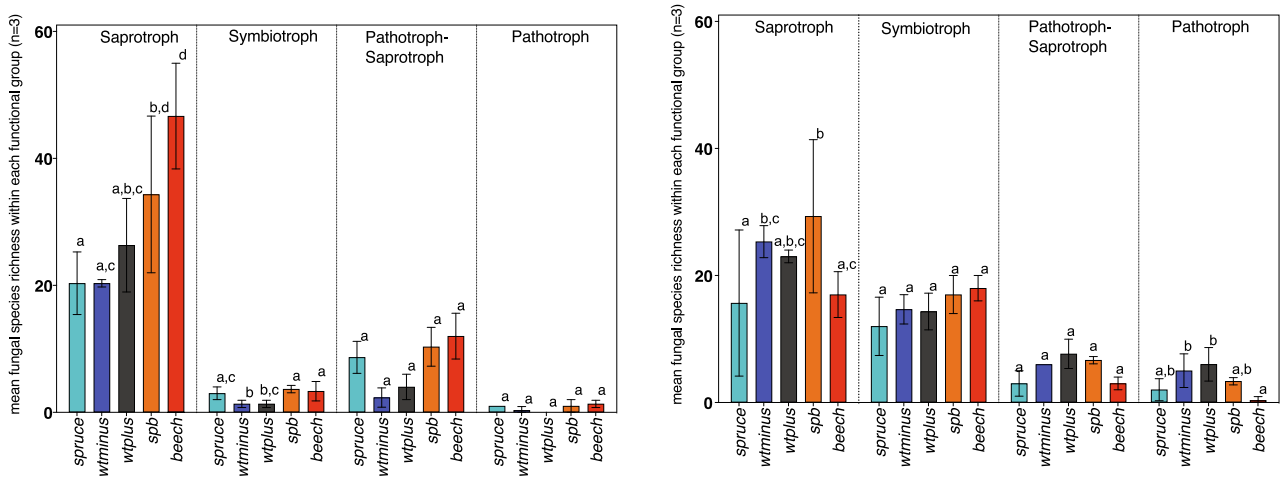

Fig. 5. Fungal species distribution patterns of the morphological dataset (left graph, 235 fungal species, Heine et al., 2019) and the metabarcoding dataset (right graph, 198 OTUs that could be assigned up to the species level) of 4 functional groups (saprotroph, symbiotroph, pathotroph-saprotroph, and pathotroph) among the five forest conversion stages (with an average of three replicate study sites each): beech forest (beech), spruce forest (spruce), unmanaged windthrow (wtplus), salvage-logged windthrow (wtminus), and close-to-nature managed spruce-beech mixed forest (spb). GLM was modelled based on the Poisson distribution. Bars that share the same letter did not significantly differ from each other ($p < 0.05$, Tukey HSD post hoc test). The standard deviation is presented as the above and below error.

contrast, the fungal communities observed with the morphological surveys did not contain shared beech-associated fungi among all five forest conversion stages (Heine et al., 2019) that indicate the different scopes and perspectives of both approaches.

The mean OTU richness differed substantially among the forest conversion stages. Both windthrow stages had significantly higher mean OTU richnesses than the dense beech forest stage or the spruce forest stage. For example, the fungus *R. medullata* was detected only through eDNA metabarcoding within the stage *wtminus*. This species is highly coupled to deciduous forests. It could not be observed through the traditional morphological analyses and was not identified in other forest conversion stages via eDNA metabarcoding. Some of these fungal species that could only be recorded via eDNA metabarcoding may have been introduced into the habitat via management or wind or organism dispersal. One explanation could be the open and partly managed habitat structure of the windthrow stages which can lead to a better introduction of new fungal spores by wind, as suggested by Buée et al. (2005). A similar scenario was identified for the managed spruce-beech mixed stage (*spb*), the forest management via selective single tree cutting and beech tree underplanting can probably increase the possible entrance of new fungal species.

Another explanation for higher OTU richness in the managed stages could be that some fungi build fungal residues in the soil from former habitat structures. This explanation is supported by results from previous studies that have demonstrated the survival of fungal DNA in soil after many decades (Straatsma et al., 2001; Egli et al., 2006), while not only active but also metabolically inactive fungi can persist beyond their active lifecycles (Egli et al., 2006; van der Linde et al., 2012) and can likely recover from environmental stress, such as tree mortality after windthrow events. Furthermore, the disparity in the species richnesses among the forest conversion stages could correspond to a slower adaption to the management by the fungal species pool in soil. As DNA metabarcoding can detect fungi from a variety of time periods and size classes, while the morphological surveys represent a single moment in time, it is possible that DNA from before the forest management change is still extant and remains quantifiable (van der Linde et al., 2012). However, the degradation of DNA in habitats is a complex process that is not yet fully understood (Bohmann et al., 2014). Managed forest or windthrow areas, compared to the unmanaged forests, seem to have a high reservoir of fungal residues, while fruiting fungi are at an extremely low level. Due to these different results and limitations of both approaches, studies seeking to clarify the fungal diversity of some given locality should always employ both approaches (Lawson Handley, 2015).

Regarding the 198 OTUs that could be assigned up to the species level, 171 OTUs (86%) were identified with eDNA metabarcoding only, while 27 (14%) were recorded also during the traditional morphological surveys (Fig. 1). The species overlap between both approaches would be much higher if the 235 fungal species that were recorded by traditional morphological surveys where compared against all 13,810 species entries of the used version of the UNITE reference database (10/10/2017). Theoretically, 141 would be the maximum number of shared species of the approaches, while we observed only 27 in this study, for example *Stropharia hornemannii* and *M. metata* which seem to be locally rare (found only once in < 3 replicated study sites). The remaining 25 shared fungal species between both datasets are common. This small overlapping quantity is likely the result of variations in the scopes of both approaches. Both approaches identify fungi with various levels of metabolic and functional activities. Some metabolically and functionally active macrofungi produce no visible sporocarps due to environmental conditions or asexual reproduction (Gardes and Bruns, 1996). These are not detectable during morphological surveys, while DNA metabarcoding is likely to measure such soil fungi, or even single spores of metabolically inactive fungi (Lindahl et al., 2013), as representatives from the past (Egli et al., 2002; Jones et al., 2003). The low blast success rate of 14% indicated missing records of conspicuous fungal species in the UNITE database at the time of this study, although it seems to be the most comprehensive ITS fungal database that is managed by curators. Previous DNA metabarcoding studies (e.g., Schön et al., 2018) reported also incompleteness of other ITS fungal databases, such as NCBI ITS RefSeq. In addition, missing records from the field surveys could also contribute to this low overlap. That was supported by an intensive dataset analysis which demonstrated that many OTUs are basidiomycete microfungi, such as *Fibulochlamys chilensis* (Madrid et al., 2010). Such tiny fungal species are difficult to discover via traditional morphological surveys as a media plate in the lab and a microscope would be needed to identify the conidiogenous cells. As accurate analysis of OTUs is difficult (Blaxter et al., 2005; Anslan et al., 2018), and further interpretation of taxonomic identities requires expertise (Ryberg et al., 2008), manual generation and interpretation of OTUs continues to be an important step in obtaining robust sequence-based datasets (Hofstetter et al., 2019).

In accordance with previous results (Frøslev et al., 2019), eDNA

metabarcoding resulted in more detected OTUs in comparison to the number of fungal species that were detected by traditional morphological surveys. However, our study showed that the number of OTUs that could be assigned up to the fungal species level was lower than the number of fungal species that were detected by traditional morphological surveys maybe due to incompleteness of the used ITS database. Another reason of the low species overall between both approaches is probably the used ITS region, as the ITS1 region is meanwhile known for their taxonomic bias towards some Basidiomycota (for example Boletales) by favouring various sequence lengths during PCR (Bellemain et al., 2010). However, the reverse primer was newly by the time of this study but used in pioneering studies about fungal metabarcoding of environmental sample (Schmidt et al., 2013; Bálint et al., 2014). Nowadays, it should be using the ITS2 region or the entire ITS region.

4.2. Fungal community composition and structure

Most previous barcoding and metabarcoding studies considered the fungal OTU abundance (e.g., Wubet et al., 2012), while this study concludes that presence/absence data (binary data) should be used for fungal community composition studies. Our results with the metabarcoding approach demonstrated that binary data responded similarly to the results of the morphological dataset from Heine et al. (2019) which served as a reference dataset for this paper. We showed that the abundance data of the metabarcoding dataset yielded contrasting results: the eDNA metabarcoding approach could not distinguish between the fungal community composition of the managed spruce-beech mixed stage (*spb*) and those of the even-aged single-species spruce forest. Thus, the morphological dataset from Heine et al. (2019) could show that the community compositions of the forested stages (*spb*, *beech*, and *spruce*) appeared to vary more than those of the eDNA metabarcoding dataset from this paper. In the morphological dataset, the community compositions of the three beech (b1, b2 and b3) sites are more distinct from the fungal community composition of the other forest conversion stages than those within the metabarcoding dataset. The stronger separation of the spore-based fungal community could probably enable by higher tree host specifications. Nevertheless, the community compositions of the morphological dataset and the metabarcoding dataset (binary data) showed similar community response patterns when evaluated against each other. The Procrustes correlation coefficient was significant, thereby supporting the hypothesis that both approaches are comparable in terms of the influence of the forest type and environmental parameter. The results accord with those of the studies of Ji et al. (2013) and Frøslev et al. (2019), in which ecological assessment using traditional morphological surveys and eDNA metabarcoding are compared. Ji et al. (2013) arrived at similar conclusions for conservation applications, such as restoration ecology or systematic conservation planning. Together with an increase of forest resistance (by higher tree species richness, higher spatial and tree age structure) against climate change and associated disturbance events (Vacek et al., 2019), the environmental conditions were improved. The study site characteristics demonstrated a higher soil fertility and high-quality litter within the beech-inhabiting stages (*beech* and *spb*) compared to those without beech. Indeed, introduction of beech in spruce plantations can increase the macronutrient turnover (Achilles et al., 2021). Such positive effects of beech introduction on spruce plantation should merits additional attention from foresters.

In accordance with the clustering results of the 869 OTUs (binary data), the effect of the disturbance event on the fungal community in topsoil seemed to be more important than the dominant tree species change or the management strategy. Within the metabarcoding dataset, it does not matter if the disturbance event occurs naturally through windstorms or anthropogenically through selective tree cutting, as both types of disturbance events substantially affected the sequence-based fungal community. Heine et al. (2019) showed that a strong effect on the fungal community occurred when the dominant tree species changed from spruce to beech. In comparison to these results of the morphological dataset, the clusters formed via metabarcoding are less distinct, and the separation of the *a priori* forest conversion stages and their vegetation structure is inferior. The separation of study site replicates into windthrow sites with or without post-windthrow management appeared to be difficult for both datasets; hence, the disturbance that was caused by the windthrow was an active driver of the fungal community composition change within both datasets, while further salvage-logging did not measurably affect either community. This well accords with the findings of Taeroe et al. (2019) regarding vegetation re-establishment after windthrow studies: neither windthrow events nor subsequent salvage logging stops the forest recovery but demonstrates the resilience of dominant forest types.

Nevertheless, even at the current level of knowledge regarding the high-throughput sequencing analysis of fungal communities, traditional morphological surveys identify qualified information as suitable shortcuts and form a simple approach for the identification of complex biodiversity in forest ecosystems and for forest management strategies (Halme et al., 2017; Heine et al., 2019). The present study suggests that the verification of the metabarcoding results with a reference dataset that was developed via traditional morphological surveys at the same study sites is extremely helpful for obtaining comprehensive results for forest management. eDNA metabarcoding did not show a significant difference when comparing the fungal community between the spruce forest stage and the spruce-beech underplanting stage. Instead, the eDNA metabarcoding dataset shows soil fungal inventory that can be used as prerequisites to plan the forest conversion. Thus, we agree with the result of Yoccoz (2012): DNA metabarcoding is a useful tool in combination of fungal morphological approaches.

4.3. Functional structure

In the present study, the FUNGuild database was used for ecological functional group affiliation to assign OTUs (at species level) to functional groups (trophic mode) according to definitions by Nguyen et al. (2016). This method is important to link metabarcoding data and fungal functions within ecosystemswhich is not common as most of the metabarcoding approaches are based on OTUs at genus level. Overall, the metabarcoding dataset and the morphological dataset contain several different functional groups while saprotrophs were the most present functional group. Among the five differently managed forest conversion stages, the managed spruce-beech mixed forest stage (*spb*) showed the highest richness of saprotrophs within the metabarcoding dataset. With the traditional morphological analyses, significant differences in the species richness among four functional groups, except for the pathotrophs, could be identified with an increasing tendency from *spruce* to *spb* to *beech*. Thus, Heine et al. (2019) concluded that, the beech introduction into spruce forests and its high-quality litter can support the redundancy of species richness within dominant functional groups. With the eDNA metabarcoding approach, only the symbiotrophs showed a trend from *spruce* to *spb* to *beech* (Fig. 5). All other functional groups showed the highest species richness with increasing natural or anthropogenic disturbance such as windthrow events or salvage-logging which was in contrast to the results of the symbiotrophs and to the findings of Vašutová et al. (2018). Although saprotrophs can inhabiting the soil in similar amounts as in the wood above the soil ground (Mäkipää et al., 2017), the metabarcoding dataset showed comparable small amounts of saprotrophs of the dense forest stages *spruce* and *beech*. In contrast, a high amount of saprotrophs was observed within the morphological dataset, especially within the beech forest stage which was likely due to the presence of sufficient fine wood debris (FWD) and coarse wood debris (CWD) (Komonen and Müller, 2018). One explanation may be that many saprotrophs and pathotrophs were not detected through the eDNA metabarcoding as the average amount of all OTUs was also low (Fig. 1). Another explanation could be that the saprothrophic and pathotrophic fungal community of dense forests are highly conserved,

may be due to the smaller amounts of external fungal species that are introduced via wind-dispersal or forest management. In this context, Komonen and Müller (2018) suggested that not only the dispersal but also colonization and establishment can be limiting factors for the presence of saprotrophs in forest ecosystems while forest-type specific deadwood would support fungal diversity. Accordingly, we conclude that more high-quality deadwood or retained trees in the converted forests would increase the number of saprotrophs in both investigated spheres. In generell, retention forestry would be an option to increase biodiversity (Fedrowitz et al., 2014).

5. Conclusions

This paper compared two fungal community datasets that were obtained through eDNA metabarcoding and through a three-year morphological sporocarp survey (published in Heine et al., 2018, 2019), respectively, at the same study sites during a spruce forest conversion project in the Eifel National Park in Germany. This comprehensive study should provide an example of the potential contributions of fungal eDNA metabarcoding of topsoil samples with high-throughput sequencing, in addition to traditional morphological identification, to advanced fungal community monitoring and interpretation of management strategies of forest ecosystems. The eDNA metabarcoding dataset showed that the soil fungal communities shared many fungal OTUs among all differently managed forest conversion stages. These shared species contained beech-associated and deciduous-associated fungi, even within the soil of the even-aged spruce forests. Whereas, within the morphological dataset, only a few fungal generalists could be identified among all differently managed forest conversion stages (see Heine et al., 2018, 2019). The fungal eDNA metabarcoding monitors the fungal biodiversity of the genetic species pool in soil. This perspective can be used to answer questions about historical soil and habitat structures which is an important information for forest conversion or restoration projects. We conclude that the soil offers a giant fungal reservoir, developed through many centuries, that can support and influence the prevailing or future aboveground vegetation. We showed that a traditional morphological survey at the same study sites should be conducted to calibrate the eDNA metabarcoding dataset because it was not able to distinguish between the managed spruce-beech mixed forest (*spb*) and the other forest conversion stages. However, we showed that the eDNA metabarcoding results with OTUs which could be assigned up to the species level by the ITS reference database, were useable to evaluate the forest conversion project while the full potential of the used UNITE ITS reference database (version 10/10/2017) has not yet be realized. In total, we observed only 27 shared fungal species among both datasets. Thus, we could not support our initial hypothesis that the metabarcoding dataset included most of the morphologically determined fungal taxa. Both approaches were useful tools but differed in terms of the scope and the number of potentially sampled fungal species. We suggest that fungal ITS database results should always be evaluated by taxonomic experts and that fungal eDNA metabarcoding should not be used interchangeably for morphological fungal community analyses to interpret forest management strategies. The results on the metabarcoding dataset and morphological dataset differed in terms of the species richness response pattern and functional structure while the community analyses of both datasets seem to be more comparable. However, the metabarcoding approach could not indicate differences between close-to-nature managed spruce-beech mixed stage (beech introduction under spruce) compared to the even-aged spruce stage. Integration of fungal eDNA metabarcoding in traditional morphological community analyses is suitable for the ecological interpretation of long-term forest conversion projects. We recommend using both approaches together to study fungal communities above and below the soil ground simultaneously as we could show that beech-associated fungal communities with high functional redundancy can develop after a spruce forest conversion by restoring natural European beech forests with an appropriate close-to-nature management strategy.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data accessibility

The high-throughput sequences of the metabarcoding dataset were deposited in the European Bioinformatics Institute (EBI) database under study number PRJEB29665 (https://www.ebi.ac.uk/ena/browser/view/PRJEB29665).

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foreco.2021.119429.

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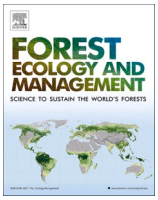

Corrigendum

Corrigendum to "Comparing eDNA metabarcoding with morphological analyses: Fungal species richness and community composition of differently managed stages along a forest conversion of Norway spruce towards European beech in Germany" [For. Ecol. Manage. 496 (2021) 119429]

Peggy Heine [*], Jonas Hausen [1], Richard Ottermanns, Martina Roß-Nickoll

Institute for Environmental Research, ABBt Aachen Biology & Biotechnology, RWTH Aachen University, Worringer Weg 1, 52074 Aachen, Germany

The authors regret a wrong image file for Fig. 1 in the original article. The correct Fig. 1 can be seen below.

In addition, we would like to provide the figure description of the current Fig. 1 within the original article (see Fig. 2). We would also like to provide the correct link (https://github.com/UMNFuN/FUNGuild) for the FUNGuild tool.

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* Corresponding author.
E-mail address: peggy.heine@rwth-aachen.de (P. Heine).
[1] Deceased.

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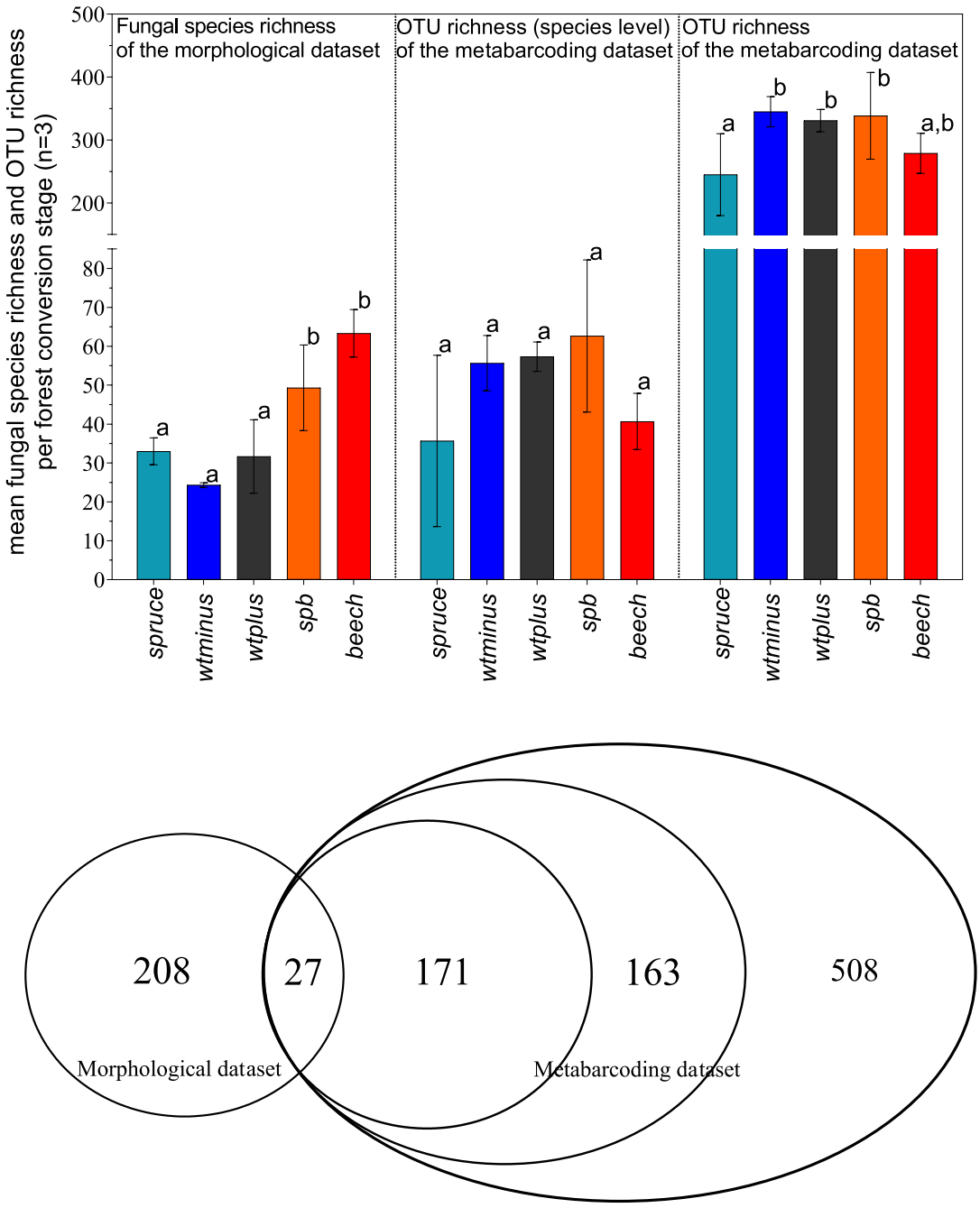

Fig. 1. First graph: The mean species richness of the morphological dataset, the mean OTU richness of the OTUs that could be assign up to the species level of the metabarcoding dataset, and the mean OTU richness of all OTUs of the metabarcoding dataset among the five forest conversion stages (three replicated study sites each): beech forest (beech), spruce forest (spruce), unmanaged windthrow (wtplus), salvage-logged windthrow (wtminus), and close-to-nature managed spruce-beech mixed forest (spb). Bars that share the same letter are not significantly different from each other ($p<0.05$, Tukey HSD post hoc test). The standard deviation is specified as the above and below error. Second graph: The morphological dataset contained 235 macrofungal species, while 198 OTUs of the metabarcoding dataset could be assigned with fungal species names using the UNITE database (version from 10/10/20217), revealing 27 shared fungal species; 361 OTUs were assigned up to the genus level; and 508 OTUs remained unassigned. List of all 27 shared fungal species can be found in the Supplementary Appendix 1 [shared species-both approaches] of the original article.

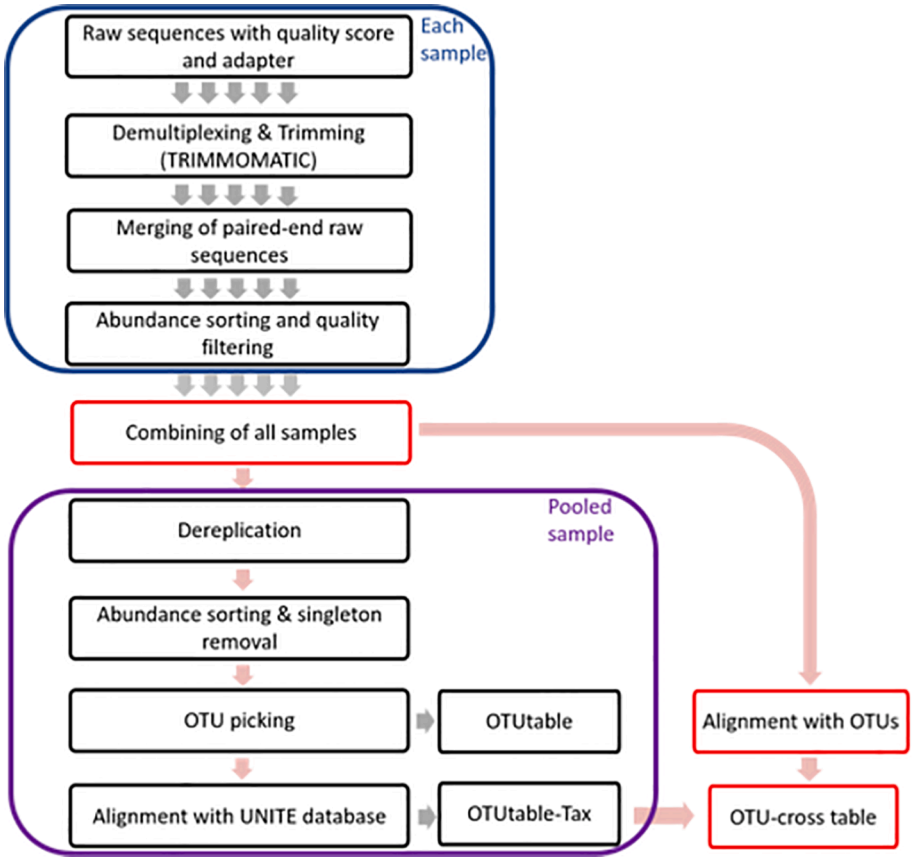

Fig. 2. Each working step of the customized metabarcoding pipeline for de-multiplexing, trimming, merging paired-end reads, sorting, quality filtering, sample pooling, de-replication, singleton removal, OTU picking, and alignment with the fungal ITS database UNITE.