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Man against machine: Do fungal fruitbodies and eDNA give similar biodiversity assessments across broad environmental gradients?

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ABSTRACT

The majority of Earth's biodiversity is unknown. This is particularly true for the vast part of soil biodiversity, which rarely can be observed directly. Metabarcoding of DNA extracted from the environment (eDNA) has become state-of-the-art in assessing soil biodiversity. For assessing fungal communities, eDNA metabarcoding is seen as an attractive alternative to classical surveying based on fruitbodies. However, it is unknown whether eDNA metabarcoding provides a representative sample of fungal diversity and census of threatened species. Therefore conservation planning and assessment are still based on fruitbody inventories. Based on a dataset of large ecological width, representing both soil eDNA metabarcoding and expert inventories of fungal fruitbodies in Denmark, we document for the first time the validity of eDNA sampling and metabarcoding as a practical inventory method and a measure of conservation value for fungi. Fruitbody data identified fewer species in total and per site, and had larger variance in site richness. Focusing on macrofungi – the class Agaricomycetes, and in turn the order Agaricales – metrics of total richness and compositional similarity converged between the methods. eDNA was suboptimal for recording the non-soil dwelling fungi such as lichens and polypores. β-Diversity was similar between methods, but more variation in community composition could be explained by environmental predictors in the eDNA data. The fruitbody survey was slightly better at finding red listed species. We find a better correspondence between biodiversity indices derived from fungal fruitbodies and DNA-based approaches than indicated in earlier studies. We argue that (historical) fungal community data based on fruitbody forays – with careful selection of taxonomic groups – may be interpreted together with modern DNA-based approaches to obtain a richer picture of the full mycobiota of the site, and for addressing historical changes. We estimated the costs of the two inventory approaches to be approximately similar for practical applications.

1. Introduction

1.1. Methods for inventorying fungi

For decades, inventory and identification of fungal fruitbodies were – together with isolation and culturing – the only ways to assess fungal communities (Hueck, 1953; Lange, 1948; Kjøller and Struwe, 1980; Rayner and Todd, 1980; Tyler, 1985; Schmit and Lodge, 2005). Since the 1990s, these methods have been supplemented with DNA-based methods, e.g. sequencing root samples to identify mycorrhizal fungi (Gardes and Bruns, 1996; Horton and Bruns, 2001; Helgason et al., 1998), sequencing of cloned PCR products from soil/litter samples (Schadt et al., 2003; O'Brien et al., 2005; Taylor et al., 2014), and more species specific PCR based assays to target selected fungi of conservation interest (e.g. Van der Linde et al., 2012; Gordon and Van Norman, 2014) – methods that allow for a more targeted study of some compartments or species, but are difficult to apply to ecosystem-wide inventories of large sampling sites. More recently, massive parallel sequencing of environmental DNA (eDNA) – now known as eDNA metabarcoding (Taberlet et al., 2012) – has gained ground in studies of fungal communities (e.g. Schmidt et al., 2013; Pellissier et al., 2014; Tedersoo et al., 2014; Barnes et al., 2016), and allows for such wide inventories. In this study we compare a thorough fruitbody inventory with eDNA metabarcoding for ecosystem-wide inventorying of the

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fungal community in Denmark. Fruitbody surveys and eDNA-based methods both have their strengths and limitations and may be seen as complementary, rather than competing approaches (Truong et al., 2017).

1.2. Advantages and limitations of fruitbody inventorying

Fruitbody surveys are low tech but laborious, requiring life-long expert taxonomic skills if thorough and reproducible data are to be achieved (Newton et al., 2003). However, many fungi do not produce fruitbodies and are systematically omitted. Other taxa are likely to be under-sampled, as they are rarely fruiting, or produce very small, inconspicuous, short-lived or below-ground fruitbodies (Taylor and Finlay, 2003; Lõhmus, 2009; Van der Linde et al., 2012). Fruitbody formation and duration are highly influenced by local variations in season and weather conditions, which may hamper comparisons of sites, unless sampling is repeated over several years (Newton et al., 2003; O'Dell et al., 2004). On the other hand, fruitbody surveys can systematically target small and locally rare substrates, and is suitable for fruitbodies on both the soil surface and other substrates, and for targeting species of conservation interest. Also, the constant flux of fungal taxonomy and nomenclature makes interpretation of historical data and even data collected by colleagues difficult.

1.3. Advantages and limitations of eDNA metabarcoding

Although eDNA metabarcoding requires high tech lab facilities and advanced post sequencing bioinformatics, methods are low tech when it comes to field sampling, which can be done according to a standardized protocol with relatively little training. It provides a broader taxonomic sample of the fungal community of not only the sexually reproducing, fruitbody forming fungi, but of virtually all fungi at the site. Sampling of soil eDNA is less dependent on seasonality and climatic variation. Although DNA from epiphytic lichens, polypores and other non-soil fungi may be expected to be present in the soil either as spores/propagules, cryptic stages or as decomposing tissue, it has to our knowledge not been tested whether soil eDNA can be used to make a representative sample of the fungi not having their active growing life-stages within the soil, and to what extent. Also, the majority of fungal biodiversity has yet to be described (Hibbett et al., 2011) and a large proportion of available barcode references lack proper annotation (Hibbett et al., 2011; Hibbett et al., 2016; Nilsson et al., 2016; Yahr et al., 2016). This limits ecological interpretation of detected community differences in relation to guild structure, trait space or taxonomic composition. Furthermore, when sampling for eDNA only a tiny fraction of a particular site surface area can be sampled, even with an intensive design. Hence, the sample representativity depends on the heterogeneity of species distributions within habitats and the size of mycelia (Lilleskov et al., 2004) – factors not easily assessed. This is a potential caveat, especially for detection of rare species – e.g. red listed taxa – important for nature conservation (Van der Linde et al., 2012). Although eDNA metabarcoding has been shown to successfully identify red listed species (Geml et al., 2014; Van der Linde et al., 2012), these may be more easily detected as fruitbodies, which may be targeted by trained experts over large study areas in relatively short time, particularly for species with long-lived fruitbodies (e.g. perennial polypores; Runnel et al., 2015).

1.4. Fruitbodies versus eDNA metabarcoding

Several studies detect a limited overlap in communities between fruitbody surveys and DNA-based approaches on a habitat scale (Gardes and Bruns, 1996; Dahlberg et al., 1997; Hawksworth and Luecking, 2017; Jonsson et al., 1999; Porter et al., 2008; Geml et al., 2009; Fischer et al., 2012; Baptista et al., 2015). However, it remains an open question whether key community metrics nonetheless correlate along environmental gradients, so that results from either method can be used as proxy for the other. In the context of nature conservation and monitoring, it would be attractive if eDNA metabarcoding can be proven to detect target species (e.g. red listed species) – for which e.g. historical data of decline is known, or where monitoring programs are already running – independent of optimal fruiting seasons and availability of taxonomic expertise. Finally, it would be valuable if historical data based on fruitbody surveys hold valid information on fungal communities, and may be compared or combined with modern eDNA metabarcoding for inferrence of temporal change.

1.5. Approach and expectations

In this study, we compare richness, community composition and community-environment relationships in a large ecological space using two parallel data sets, a thorough fruitbody inventory and data obtained by eDNA metabarcoding of soil. All data were gathered from the same 130 40 m × 40 m sample plots in Denmark and taken over the same 2–3 year period.

Overall, we expected eDNA metabarcoding to detect more OTUs (operational taxonomic units) than species in the fruitbody sampling. The conversion between these molecular taxonomic units and morphological species is not trivial, and as many bioinformatic approaches vastly overestimate species richness, we employed analytical approaches that are assumed to approach species level delimitation of molecular entities (Frøslev et al., 2017). However, we expected the fruitbody survey to detect more red listed species, due to the targeted survey across the whole study area and the fact that most red listed species produce conspicuous fruitbodies, that are relatively easily located if present. We expected eDNA metabarcoding to provide stronger correlation with environmental gradients, due to the expected better coverage of taxonomic diversity. We furthermore expected comparability between the two approaches to be highest for community composition, and lowest for red listed species detection, as previous studies have indicated stochastic variation in noisy data to affect richness estimates more than community composition (Abrego et al., 2016; Lekberg et al., 2014).

We expected that soil eDNA would be suboptimal for detecting non-soil fungi, and thus would show less concordance with the fruitbody community collected at non-soil substrates. Thus, we also expected higher correspondence between fruitbody and eDNA metabarcoding data, when the former were restricted to species recorded at soil level. Also, we expected a higher correspondence when both datasets were restricted to Agaricomycetes and Agaricales, as these taxa contain mainly fruitbody producing species and also constitute a major share of all conspicuous fruitbody forming fungi, and thus can be assumed to be good proxies for the mycobiota possible to survey as fruitbodies.

2. Materials and methods

2.1. Study sites

130 sites of 40 m × 40 m spread out across Denmark were studied (Fig. 1). The study sites covered an ecospace spanning the major environmental gradients of terrestrial ecosystems, i.e. soil moisture, soil fertility and successional stage (Brunbjerg et al., 2017b). The 130 sites were selected by stratified random sampling to represent 24 environmental strata (habitat types). Six habitat types were cultivated: three types of fields (rotational, grass leys, set aside) and three types of forest plantations (beech, oak, spruce). The remaining 18 strata were natural habitat types, constituting all factorial combinations of: fertile and infertile; dry, moist and wet; open, tall herb/scrub and forest. We replicated these 24 strata in each of five geographical regions across Denmark. We further included a subset of 10 perceived biodiversity hotspots, two within each region. This study was part of the Danish biodiversity study, Biowide, and an elaborate description of design and

Fig. 1. Map of Denmark showing the location of the 130 sites.

data collection is available in Brunbjerg et al. (2017a).

2.2. Fruitbody survey

Each site was visited twice during the main fungal fruiting season in 2014 (August–early September and October–early November) and once during the main fruiting season in 2015 (late August–October), focussing on all groups of Basidiomycota and Ascomycota, but excluding non-stromatic Pyrenomycetes and Discomycetes with fruitbodies regularly smaller than 1 mm and lichenized fungi. Most woody debris was turned over to locate e.g. corticioid fungi, but no structured attempts to find hypogeous fungi were conducted, although a few were found by chance. In sites with tall and dense herbaceous vegetation, regular inspections were carried out in a kneeling position. A site visit lasted approximately 1 h, in very open monotonous sites sometimes less, e.g. in newly ploughed arable sites. All visits were led by expert field mycologist Thomas Læssøe typically accompanied by one helper. Numerous samples were taken back to a mobile lab for immediate microscopic investigation, and more interesting or critical material was dried as voucher material and in part deposited at the fungarium (C) of the National History Museum of Denmark. Some specimens difficult to identify were forwarded to external experts.

2.3. Environmental variables

A complete inventory of vascular plants was done for each site. Ellenberg Indicator Values, EIV (Ellenberg et al., 1991) reflect plant species' abiotic optima and have often been used in vegetation studies to describe local conditions (Diekmann, 2003). Mean Ellenberg Indicator Values were calculated based on the plant lists for each site for the light conditions (EIV.L), soil nutrient status (EIV.N) and soil moisture (EIV.F). Ellenberg values together with measured variables (see Supplementary methods) for precipitation, soil pH, soil organic matter content, soil carbon content, soil phosphorous and light were used in the models to explain community structure.

2.4. Sequence data

Soil was collected from all sites followed by DNA-extraction, PCR-amplification and sequencing (more details given in Supplementary material). For each site, 81 soil samples approximately 5 cm diam. and 15 cm depth were collected in a virtual grid with samples 4 m apart using a simple gardening tool. For each site, a large bulk soil sample was constructed by thorough mixing of 81 single soil samples, and subjected to DNA extraction of a 4 g subsample with the MoBio PowerMax kit using a slightly modified protocol. Extraction blanks (4 in total) were included in each extraction batch. The fungal ITS2 region was amplified using tagged versions of the primers gITS7 (Ihrmark et al., 2012) and ITS4 (White et al., 1990). Tags were 8 to 9 nucleotides and differed in at least three positions from each other. Each sample was amplified three times with different tag combinations. No forward or reverse tag was used more than once in any library, and no combination of tags was reused in the study. Libraries were built with the Illumia TruSeq PCR free kit, and MiSeq sequenced (Illumina Inc., San Diego, CA, USA), at the Danish National Sequencing Centre using two 250 bp PE runs. Extraction blanks (4 in total) were amplified (in triplicates) and sequenced along with the samples, as well as 1–2 PCR negative controls per sequencing library (9 in total).

2.5. Bioinformatics and statistical analyses

OTU tables (species-site table) were constructed, aiming for a definition of OTUs (operational taxonomic units, Blaxter et al. (2005)) that approximates species level delimitation adequate for addressing biodiversity questions (see Frøslev et al., 2017). This was achieved by an initial processing with DADA2 (v 1.8) (Callahan et al., 2016) to identify exact amplicon sequence variants (ESVs, see Callahan et al., 2017) including removal of chimeras, followed by ITS extraction with ITSx (v 1.0.11) (Bengtsson-Palme et al., 2013) and clustering with VSEARCH (v 2.3.2) (Rognes et al., 2016) at 98.5% – the consensus clustering level used to delimit species hypotheses (SHs) in the UNITE database (Kõljalg et al., 2014), and subsequent post-clustering curation using LULU (Frøslev et al., 2017) to eliminate remaining redundant sequences. Taxonomic assignment of the OTUs was done using the v8.0 UNITE general FASTA release (UNITE Community, 2019) (see Supplementary material for more details).

The number, identity and abundance of OTUs in the negative controls and extraction blanks were assessed together with their frequency in other samples.

Statistical analyses (and parts of the sequence bioinformatics) were done in R (v. 3.4.3) (R Core Team, 2018). For the ecological analyses we used the vegan package (v. 2.5-2) (Oksanen et al., 2018), and for plotting we used ggplot2 (Wickham, 2016). Files documenting the analyses are deposited in GitHub (https://github.com/tobiasgf/man_vs_machine).

For the more descriptive analyses, we used full fruitbody data. For some more direct comparisons, we restricted the fruitbody data to species collected at the soil surface for a more qualified comparison, as it was evident from initial analyses that only a small proportion of these non-soil fungi, found as fruitbodies (e.g. many polypores and corticioid fungi), were registered by the soil-based eDNA metabarcoding. Furthermore both datasets were filtered to obtain two increasingly taxonomically focussed subsets – Agaricomycetes and Agaricales. Thus, in total we analyzed 6 datasets: full data, Agacomycetes data and Agaricales data for eDNA and fruitbodies respectively. Species composition was the focus of the study, and as biological abundance is difficult to assess with either method, presence/absence data was used for all analyses.

2.6. Overlap between methods

The frequency of each species/OTU (from here on: species = fruitbody ID, OTU = metabarcode ID) across the 130 sites was assessed for the full datasets, and the proportion of species/OTUs recorded with both methods or only as fruitbody or OTU was assessed. As incomplete and insufficiently annotated DNA reference data exacerbate the discrepancies between fruitbody and metabarcoding data, some focussed analyses were performed on only the species recorded with both methods ('coinciding species').

2.7. Richness and sampling effort

Species accumulation was assessed for all datasets, and the variation of the recorded richness per site was assessed by calculation of the relative standard deviation of richness for each dataset. Pearson correlation was used to test for correspondence between estimates of species richness and OTU richness across the 130 sites.

For the eDNA data we assessed the effect of sequencing depth by correlating the OTU count of sites based on the full data and on data resampled to 10,000 reads per sample.

2.8. Red listed species

We assessed both the total number of red listed species identified with either method, as well as the correspondence of site-wise counts of red listed species. Red list status was assigned by matching fruitbody id or the taxonomic assignment of OTUs to the red listed fungal species in the IUCN categories from near threatened to critically endangered on the official Danish red list (IUCN, 2012; Wind and Pihl, 2010). To account for changes and differences in taxonomic use, names from the UNITE database, the inventory and the red list were translated into current name use according to the Danish Fungal Atlas (Danish Mycological Society, 2018).

'Unknown' fungal lineages are repeatedly found in soil eDNA studies. Nilsson et al. (2016) implemented a function – 'top 50 most wanted fungi' – at the UNITE website to allow for sorting through these. These lineages could in some respects be thought of as the eDNA equivalents of red listed fruitbody species. We tested how many of our OTUs matched these 'unknown' lineages at different taxonomic levels (see Supplementary material).

2.9. Community composition

Community dissimilarity was estimated with the Sørensen dissimilarity metric using the vegdist function in vegan. Five out of the 130 sites had fewer than 4 observed fruitbody species and were removed prior to analyses of community dissimilarity. Correlations between community dissimilarity measures based on different datasets were tested with the Mantel test (method = "pearson", 999 permutations) and procrustes test (999 permutations) using the functions in vegan. Community turnover along gradients (assessed as dissimilarity) was tested for correlation with environmental distance using the *bioenv* function in vegan. Here Sørensen distance was used for community dissimilarity and Euclidean distance for environmental dissimilarity, and we allowed up to four explanatory variables to be selected.

3. Results

3.1. Sequence data

After bioinformatic processing, clustering and exclusion of non-fungal sequences, the full dataset contained 7,813,551 paired fungal reads and 10,490 OTUs. As the frequency and abundance fungal OTUs in the extraction blanks and negative controls was low (see Supplementary material), and as these potential contaminant fungi (or more likely ubiquitous fungi) would be of negligible impact on the analyses we did not exclude them. As both DADA2 and LULU do a good job in identifying low abundance errors, the dataset contained no > 44

singletons – OTUs with an abundance (read count) of one – and most sites contained no singleton OTUs and a site maximum of 3 singletons was observed. Thus we chose not to remove any OTUs or observations due to low frequency or abundance.

3796 OTUs (36.2%) matched a Species Hypothesis from UNITE with 98.5% or more and 5501 (52.4%) with 97% or more. 2262 (21.6%) OTUs received an exact species level annotation, and 9936 OTUs (94.7%) could be assigned to a phylum level taxon (see Supplementary material). 5, 15 and 12 OTUs were among the 'top 50 most wanted fungi' (Nilsson et al., 2016) at the phylum, class and order level respectively (see Supplementary material).

Very few OTUs (avg 0.7 OTU pr site) could be assigned to lichenized fungi (see Supplementary material for more information), and we did not exclude these OTUs from analyses although lichens were not assessed in the fruitbody dataset.

OTU richness was not greatly influenced by sequencing depth – Spearman rank r = 0.98 between OTU count based on rarefied data (10,000 reads per sample) and full data – and OTU richness measures were thus estimated from the full (not rarefied) data (Supplementary Fig. 5).

3.2. Overlap between methods

The fruitbody survey recorded fewer species than the eDNA metabarcoding approach (Fig. 2a, Supplementary Fig. 1). The fruitbody survey included 8793 observations (a record of species in a site), and recorded 1751 species (1359 Agaricomycetes of which 845 belonged to Agaricales). The eDNA metabarcoding included total 37,289 observations (an OTU in a site), and recorded 10,490 OTUs (2741 Agaricomycetes and 1480 Agaricales). 1204 (69%) of the fruitbody species were recorded as fruitbodies only, while 547 (31%) were found also as OTUs. 9796 (93%) of the OTUs were found with eDNA metabarcoding only, while 694 (7%) were recorded also as fruitbodies (i.e. had species name annotations corresponding to the 547 species mentioned above). For these 547 coinciding species there was a tendency towards pairwise correspondence of species and OTU frequency (Figs. 2a and 1c). Three coinciding species were common as fruitbodies, but rare as OTUs. Four of these (*Clitopilus hobsonii*, *Phloeomana speirea*, *Mollisia cinerea* and *Xylaria hypoxylon*) are normally observed on woody or herbaceous substrates, and the last (*Galerina vittiformis*) is associated with bryophytes. The top ten most frequent coinciding OTUs (Fig. 2b) were less frequent as fruitbodies – all were common soil fungi, except *Ganoderma applanatum*, a wood decomposer not generally perceived as a soil fungus. Site OTU richness and fruitbody species richness was highly

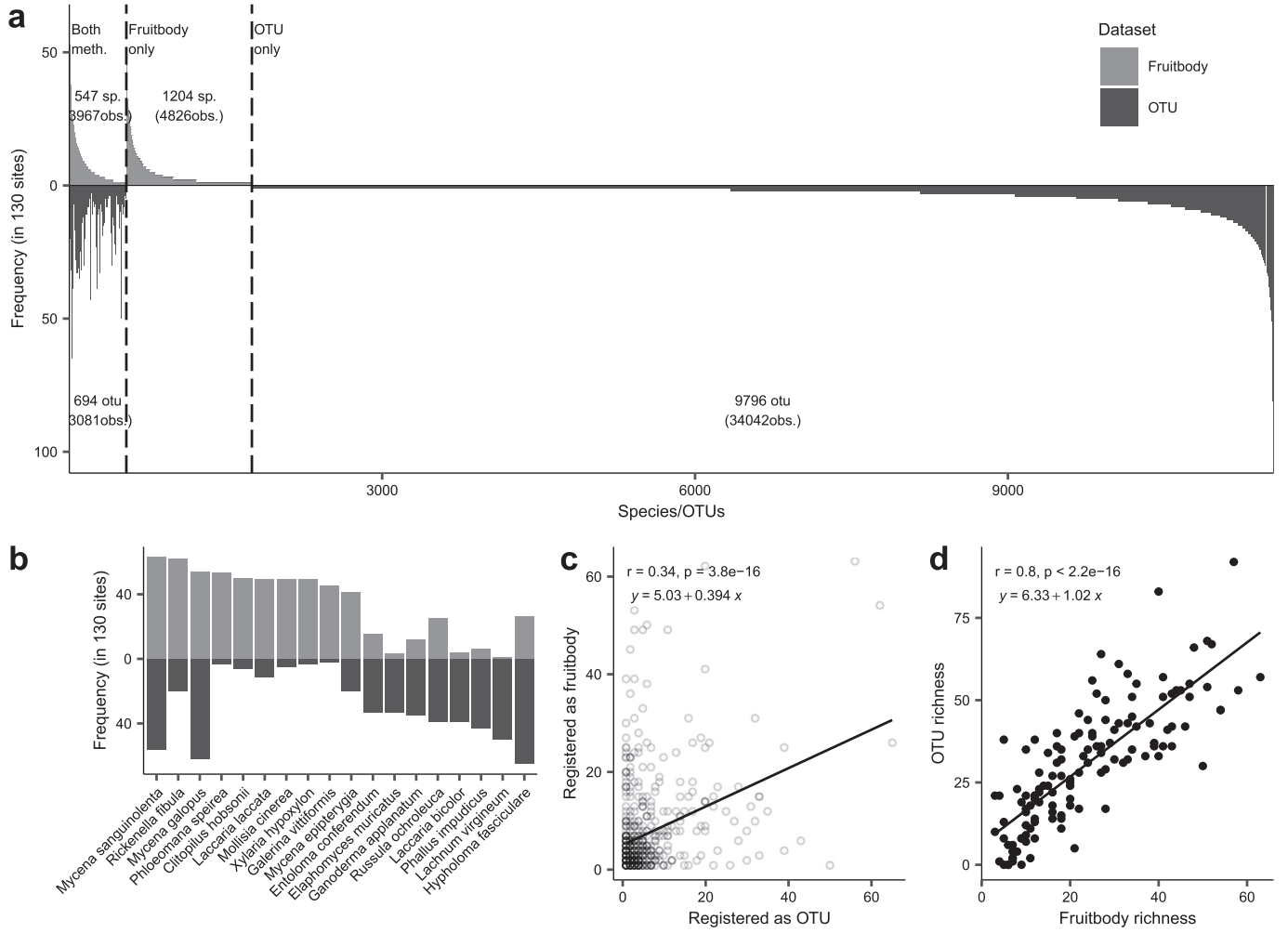

Fig. 2. Frequency of species and OTUs among the 130 sampling sites. a) Frequency of species sorted by decreasing frequency, and grouped by species recorded with both methods or only as OTU or as fruitbody, y-axis indicates the number of sampling sites (of 130) in which a species was recorded, number of species and number of observations (a species/OTU in a site) are indicated for each group. b) Top 10 most frequent species recorded with either method. c) Scatterplot of presences across sites of fruitbodies vs. DNA of the 463 species recorded by both methods. d) Species richness of the 130 sites as recorded with fruitbodies or OTUs for the 463 species recorded with both methods.

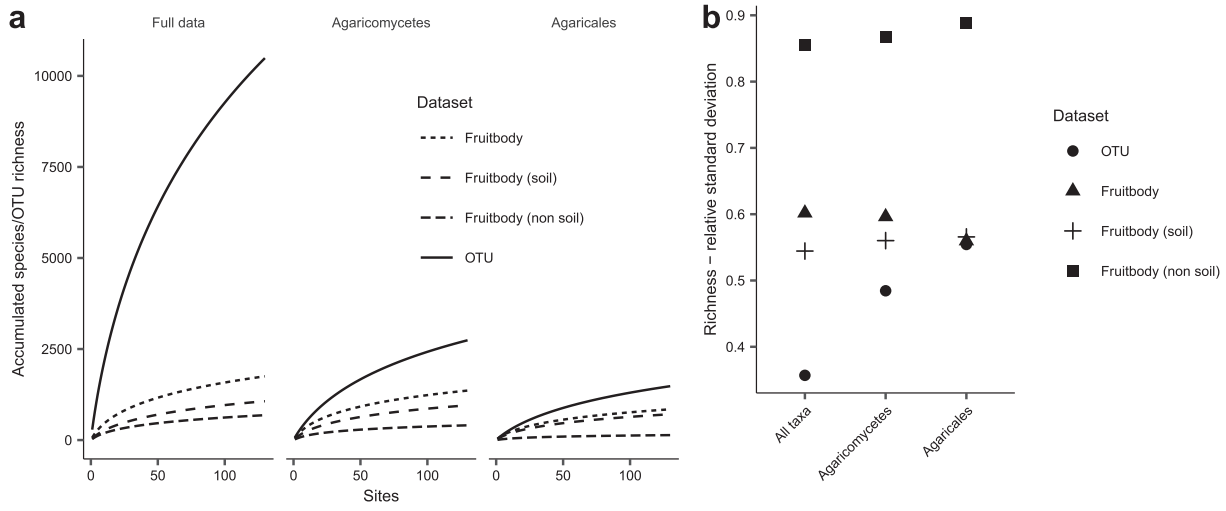

Fig. 3. Sampling effort and richness variation. a) Cumulative species richness when sampling the 130 sites for the full data, Agaricomycetes and Agaricales. b) Relative standard deviation (RSD) of site species/OTU richness across the 130 sampling sites for the full data, Agaricomycetes and Agaricales.

correlated when considering only the 547 coinciding species, r = 0.8 with a slope close to 1 (Fig. 2d).

3.3. Overall richness and sampling effort

Although the exact logging of expenses was not part of the project, we estimate that the costs of the two approaches would be approximately equal, if repeated with the focussed aim of monitoring. The fruitbody survey included 3 × 10,000 km of driving, and four months of salary (three months of collecting, and one month of identification) – excluding the aid from volunteers in the fruitbody survey, whereas the eDNA metabarcoding included 10,000 km of driving, approximately 6000 USD lab consumables, and three months of salary (one month collecting, and two months lab work and bioinformatics). Species accumulation curves did not reach an asymptote for any of the datasets after sampling of the 130 sites (Fig. 3a). This was most pronounced for the full eDNA metabarcoding dataset and least pronounced for the non-soil Agaricales fruitbody dataset. eDNA metabarcoding became increasingly similar to fruitbody data with narrowed taxonomic focus. The variation in site species/OTU richness across the 130 sites was lowest for eDNA metabarcoding, and highest for fruitbody data (markedly higher for non-soil fungi), but more similar with a narrowed taxonomic focus (Fig. 3b).

3.4. Richness correlation between methods

Of the 1751 total fruitbodies, 1067 were collected on soil. 443 (42%) of these 1067 soil fruitbody species were also registered as OTUs, whereas only 104 (15%) of the 684 non-soil fruitbody species were also registered as OTUs (Supplementary Fig. 6). Per site, an average of 4.7% of the soil fruitbody species were captured as OTUs, but only 0.43% of the non-soil fruitbody species.

As it was evident that the soil eDNA captured little of the non-soil mycobiota, we made comparison analyses of site richness and red list recording both using the full fruitbody data but also on fruitbody data that excluded the non-soil species.

Soil fruitbody species richness of the 130 study sites (Fig. 4) ranged from 0 to 102 (0–98 Agaricomycetes and 0–76 Agaricales), while OTU richness ranged from 81 to 636 (10–148 Agaricomycetes, 3–110 for Agaricales). Correlation between site species richness and OTU richness (Fig. 4) was moderate for the full datasets (r = 0.39), but strong when restricted to Agaricomycetes (r = 0.65) and Agaricales (r = 0.61). As expected, correlations became even stronger (r 0.67–0.8) when only considering the 547 'coinciding species' – species recorded with both methods (Fig. 2d, Supplementary Fig. 1d and h). OTU richness based on only Agaricomycetes or Agaricales were strongly correlated with OTU richness based on the full data (r = 0.75 and r = 0.8, respectively, Supplementary Fig. 3).

3.5. Overall taxonomic composition

Taxonomic composition of eDNA metabarcoding and fruitbody data became increasingly similar when going from full data to Agaricomycetes and Agaricales (Fig. 5, Supplementary Fig. 2, Supplementary Tables 2–5). Fruitbody data was heavily skewed towards Basidiomycota (90%), whereas the eDNA metabarcoding was composed of 47% Ascomycota, 33% Basidiomycota, and 20% species from other phyla (Fig. 5a, Supplementary Table 2). However, the relative proportions and absolute frequencies of taxa progressively converged when focussing on Agaricomycetes (Fig. 5b, Supplementary Fig. 2c) and Agaricales (Fig. 5c, Supplementary Fig. 2d). The non-soil fruitbody data was less dominated by Agaricomycetes and Agaricales than the soil-fungi data. All phyla and classes (except Dacrymycetes and Atractiellomycetes) were represented by more species/OTUs in the eDNA metabarcoding than in the fruitbody data (Fig. 5, Supplementary Fig. 2ab). A few Agaricomycetes orders (Russulales, Polyporales, Hymenochaetales, Auriculariales, Gomphales, and Amylocorticiales) were represented by more species in the fruitbody data than in the eDNA metabarcoding (Supplementary Fig. 2c). Almost all Agaricales genera were detected by both methods and with roughly similar species numbers.

3.6. Red listed species

502 of the 656 Danish red listed fungi were found in the UNITE database after synchronizing name usage, slightly more (511) red list names were found in GenBank, but as UNITE includes all fungal ITS GenBank data, it must be assumed that the GenBank sequences with these further names were genetically redundant and most likely with a wrong annotation in GenBank. The soil surface fruitbody survey recorded more red listed species than the eDNA metabarcoding (Fig. 6, Supplementary Table 6). 100 red listed species were recorded as fruitbodies on the soil surface (144 including the non-soil fungi), whereas 85 red listed species were found as OTUs. 39 red listed species were recorded with both methods, 46 red listed species was detected as OTUs only, and 105 as fruitbodies only. Only three red listed species from the non-soil part of the fruitbody data (*Buglossoporus quercinus*, *Jaapia ochroleuca* and *Nemania diffusa*) were also detected as OTUs. A

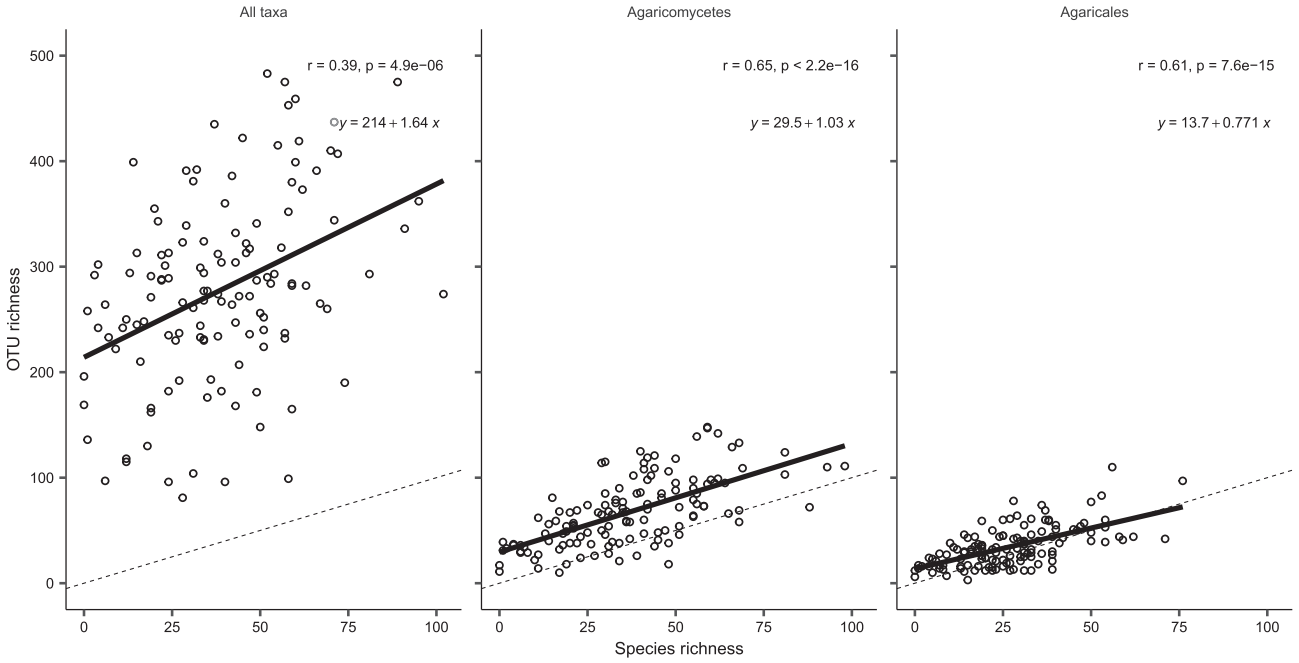

Fig. 4. Correlation between site fruitbody species richness and OTU richness. Solid lines represent the linear regression of OTU richness against species richness, while the dotted line shows the identity line (x = y). Correlations are shown for the taxonomic subsets (all taxa, Agaricomycetes, Agaricales). Fruitbody data is restricted to species registered at the soil surface.

substantial portion of currently red listed species are lacking from sequence databases (ISNDC, UNITE), but when restricting the comparison of red listed species to species present in the molecular reference database the figures for soil fruitbodies (74 species) was almost equal to the figures for OTUs (Fig. 6). When the 130 sites were grouped into categories with 0, 1–2 or 3 or more red listed species recorded as fruitbodies or as OTUs, there was a good correspondence between the two methods (Supplementary Fig. 4). The vast majority of the eDNA red list detections were in line with expectation from current knowledge of the relevant species and sampled habitats, with a few exceptions (see Supplementary material for more details).

3.7. Community – environment relation

Mantel tests showed very similar and strong correlations between community dissimilarity measures from fruitbody and eDNA metabarcoding data, mantel r = 0.67. In this case no improvements were achieved by increased taxonomic overlap (Mantel-r = 0.67 and 0.66 and 0.62 for Agaricomycetes and Agaricales respectively), or when restricted to soil-fungi (Mantel-r = 0.68, 0.69 and 0.64 for full data, Agaricomycetes and Agaricales respectively). These correlations were corroborated by procrustes analyses with correlation coefficients of 0.88, 0.87 and 0.83, and 0.87, 0.87, 0.83 for soil-fungi for the same comparisons (all p-values < 0.01). As could be expected, environmental variables explained more of the community dissimilarity for eDNA metabarcoding data than for the survey data, and the amount of explained variation was largest for the taxonomically more inclusive datasets (Fig. 7) with the maximum explained variation for the full eDNA metabarcoding dataset (0.68) and the minimum explained variation for the soil-fruitbody Agaricales (0.49). Adding a fourth explanatory variable did not increase the amount of explained variation for most datasets. Based on all subsets of the fruitbody data, the best three explanatory variables for community composition were mean Ellenberg soil nutrient status (EIV.N), mean Ellenberg light indicator value (EIV.L) and soil phosphorous, whereas mean Ellenberg soil nutrient status (EIV.N), mean Ellenberg soil moisture (EIV.F) and soil pH were the best for the DNA metabarcoding.

4. Discussion

More species (OTUs) were detected by eDNA metabarcoding than by the classic fruitbody survey. This could mainly be attributed to the detection of groups, which tend to go undetected in a fruitbody survey, e.g. diverse groups of moulds and yeasts. The fruitbody survey data was strongly dominated by fruitbody forming basidiomycetes. In general, there was a relatively poor correlation for richness measures and taxonomic composition between the two full datasets, but increased strength of correlation when narrowing the focus to Agaricomycetes and subsequently to just Agaricales. Similarly, excluding wood-inhabiting and other non-soil fungi improved the correspondence between the datasets, showing that these largely go undetected in soil-based eDNA sampling. The fruitbody survey identified more red listed species, but the difference was less pronounced than anticipated, and results were almost similar when delimited to soil-dwelling fungi only.

4.1. Taxonomic composition

The taxonomic composition was remarkably similar between eDNA metabarcoding and fruitbody data when focussing on the Agaricomycetes, and even more pronouncedly the Agaricales. Many of the major discrepancies align with expectations – i.e. taxonomically difficult groups like *Inocybe* (Larsson et al., 2009; Ryberg et al., 2008) and *Cortinarius* (Frøslev et al., 2007) were markedly more species rich as eDNA OTUs than as well-delimitated species identified from fruitbodies. Approximately half of the Agaricales species recorded as fruitbodies were also found as OTUs and vice versa – i.e. half of the species level annotations of OTUs were also found as fruitbodies (Supplementary Fig. 1e). Considering the very similar proportions of Agaricales genera between the methods, it could be assumed that a large part of non-overlapping species can be explained by incomplete DNA reference data and different taxonomic concepts in handbooks for species identification of fruitbodies compared to sequence databases. Part of the explanation could also be that the targeted visually guided registration of small individuals of fungi (fruitbodies) will capture species that go unseen with the more random soil sampling. Also, our eDNA data

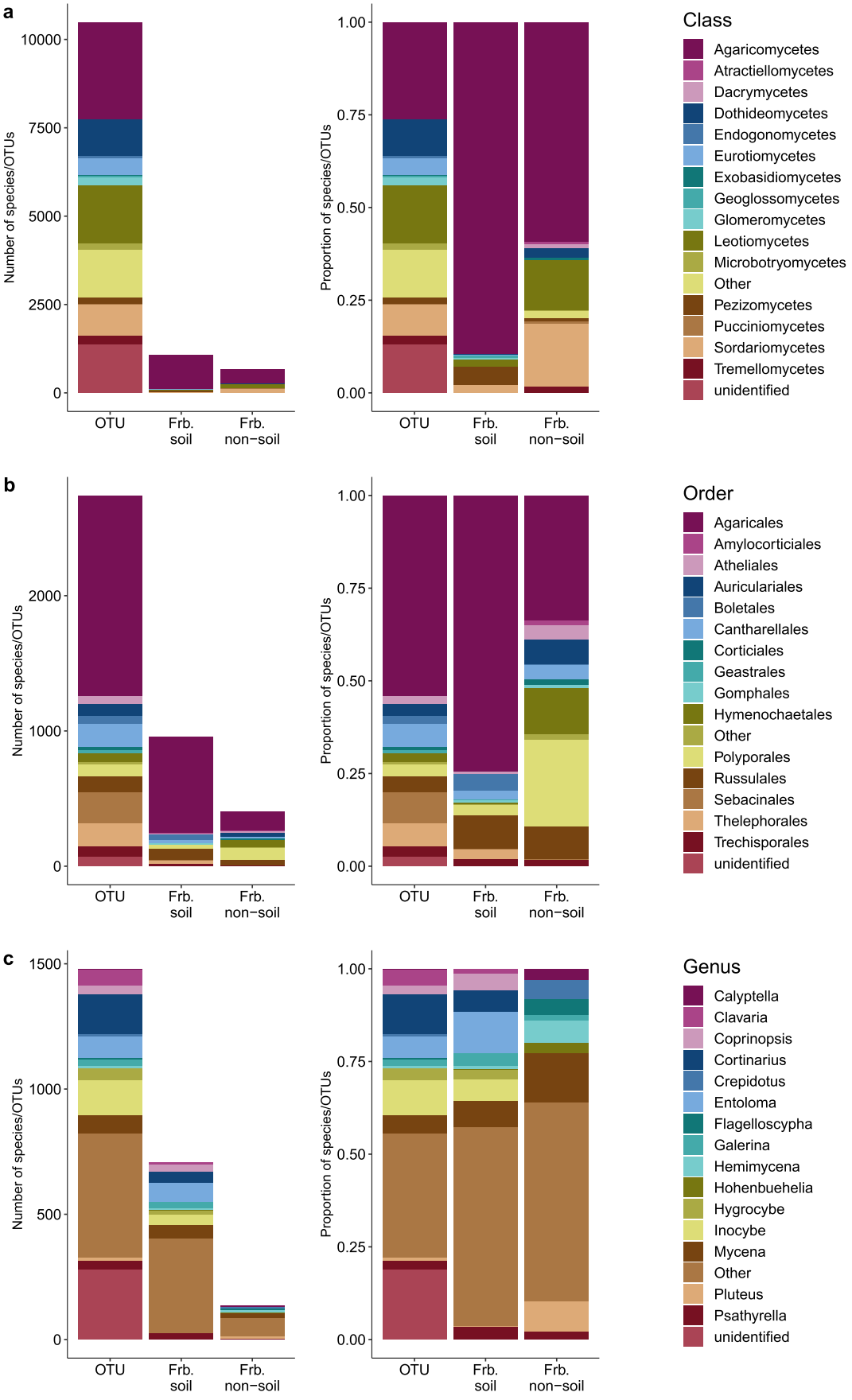

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Fig. 5. Taxonomic composition. Plots show the number of species and OTUs assigned to higher taxa. Composition is shown for OTUs, fruitbody (soil) and fruitbody (non-soil). Left plot in each panel shows the absolute richness (number of species) within different taxa, right plot shows the relative richness. a) Number of species in each class for full datasets. b) Number of species in each order in Agaricomycetes. c) Number of species in each genus of Agaricales. Most frequent taxa for each dataset is shown for all datasets, the rest are pooled in the category 'Other'.

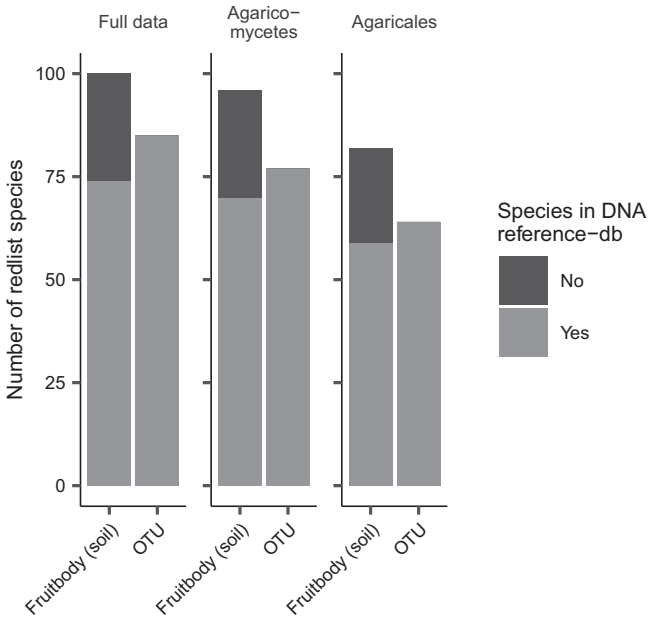

Fig. 6. Number of red listed species recorded. Total number of red listed species found as fruitbodies (restricted to soil fungi) and OTUs. Taxa present in the DNA reference database (and thus possible to identify also as OTU) are indicated in light grey, and taxa not present in the DNA reference database (and thus not possible to identify as OTU) are indicated in dark grey.

contained a large number of 'Agaricales spp.' and 'Clavariaceae spp.' that may in part be species that do not produce fruitbodies. Despite rigorous bioinformatics, some of the unassigned Agaricales OTUs may still be analytical errors, although the number and annotation of genetically well-examined genera – e.g. *Tricholoma* (Heilmann-Clausen et al., 2017) – came very close to current Danish knowledge. An effort to expand and curate DNA reference databases is hence essential to improve future DNA-based ecological studies as already suggested by other researchers (Hibbett et al., 2011; Hibbett et al., 2016; Nilsson et al., 2016; Wurzbacher et al., 2018; Yahr et al., 2016).

4.2. Soil DNA captures soil-fungi

In this study, we extracted DNA from soil samples. Although DNA from non-soil fungi may be expected to be present in the soil, it has not earlier been tested to what extend soil DNA can be used to register fungi not having their active growing life-stages within the soil, such as wood decomposing fungi. Several non-soil fungi were detected in this study, but they were observed in many fewer sites than soil-fungi when comparing to the corresponding fruitbody data. Likewise we detected only very few epiphytic lichenized fungi in the soil DNA. The few higher taxa that were more speciose in the fruitbody data, were primarily non-soil taxa like *Polyporales*, *Hymenochaetales*, *Crepidotus*, etc. It was, however, interesting to note that *Ganoderma applanatum* (a wood decaying polypore) was found as an OTU in 35 of 130 sites, including a few sites with no trees, suggesting that the species is frequently present in the spore bank, or that the species mycelium can be found outside wood. *G. applanatum* is known as a massive producer of spores (Ingold, 1971) and the first is more likely. Although soil sampling catches fungi associated with above-ground carbon sources, our results indicate that these are heavily undersampled by soil studies alone. Studies indicate that this compartment compose a major proportion of the total mycobiota (Unterseher et al., 2011; Arnold and Lutzoni, 2007; Arnold, 2007), so to get a more complete estimate of the total fungal community, DNA-based methods will need to include sampling of above-ground structures. The scarsity on non-soil fungi in the eDNA dataset indicate that this approach mainly sample the active fungal community in soils. However, we cannot rule out that part of the mycobiota detected with eDNA metabarcoding is composed of non-active (dead tissue, spores) stages of fungi. Hence it would be relevant to see if metatranscriptomics would give an even better (taxonomic) correlation with the observed fruitbody community.

4.3. Detection of red listed species

The fruitbody survey registered more red listed species in total and average per site. However, when adjusting for red listed species not present in the DNA reference database (502 of the 656 Danish red listed species were present with sequence data in UNITE), and omitting red listed species associated with dead wood and other non-soil resources, the eDNA metabarcoding approach performed almost as good. Interestingly, the methods partly recorded different red listed species, but the species recorded only as eDNA were in all cases except a few (see Supplementary material) evaluated to be likely inhabitants of the actual sites, based on expert knowledge. This indicate that fruitbody surveys and eDNA metabarcoding could be used complementarily to get a more reliable assessment of local conservation value, which to some degree conflicts with the findings of Runnel et al. (2015) that found fruitbody surveys to be superior to eDNA-based sampling of red listed wood-inhabiting polypores at stand scale. The detection of red listed species from environmental DNA samples must be expected to increase as sequence databases become more complete and well-annotated. Manual searches of the UNITE database revealed that several of the missing red list species have been added and/or annotated after the analyses carried out here, and now await formal incorporation in the next version of the UNITE general FASTA release. A large proportion of fungal species do not produce fruitbodies, and has so far not received much focus in conservation biology although many of these may in fact be rare or threatened. But without good reference databases and better knowledge of distribution and ecological requirements of these "invisible" taxa, they will be difficult to target in nature conservation. Thus it may be more appropriate to target habitats that contain many unique taxa (OTUs) using approaches that are independent of exact taxonomic annotation and random sampling, e.g. the uniquity metric (Ejrnæs et al., 2018), which is applicable to OTU data.

4.4. Species turnover is comparable

Despite marked differences in taxonomic composition (Fig. 5), our results showed that measures of community composition changes estimated from DNA-metabarcoding data correlated well with estimates based on fruitbody data (Mantel-tests and procrustes). This correlation did not change much after narrowing the taxonomic focus to Agaricomycetes/Agaricales, indicating that all approaches are suitable for describing community turnover along environmental gradients. However, eDNA metabarcoding outperformed fruitbody data when it came to correlation with environmental gradients expressed by independent environmental variables for all subsets of data. Further, it appears that the wider soil fungal community is more predictable than the fruitbody community. This could be caused by fruitbodies constituting a more stochastic subset of the total mycobiota, or alternatively that

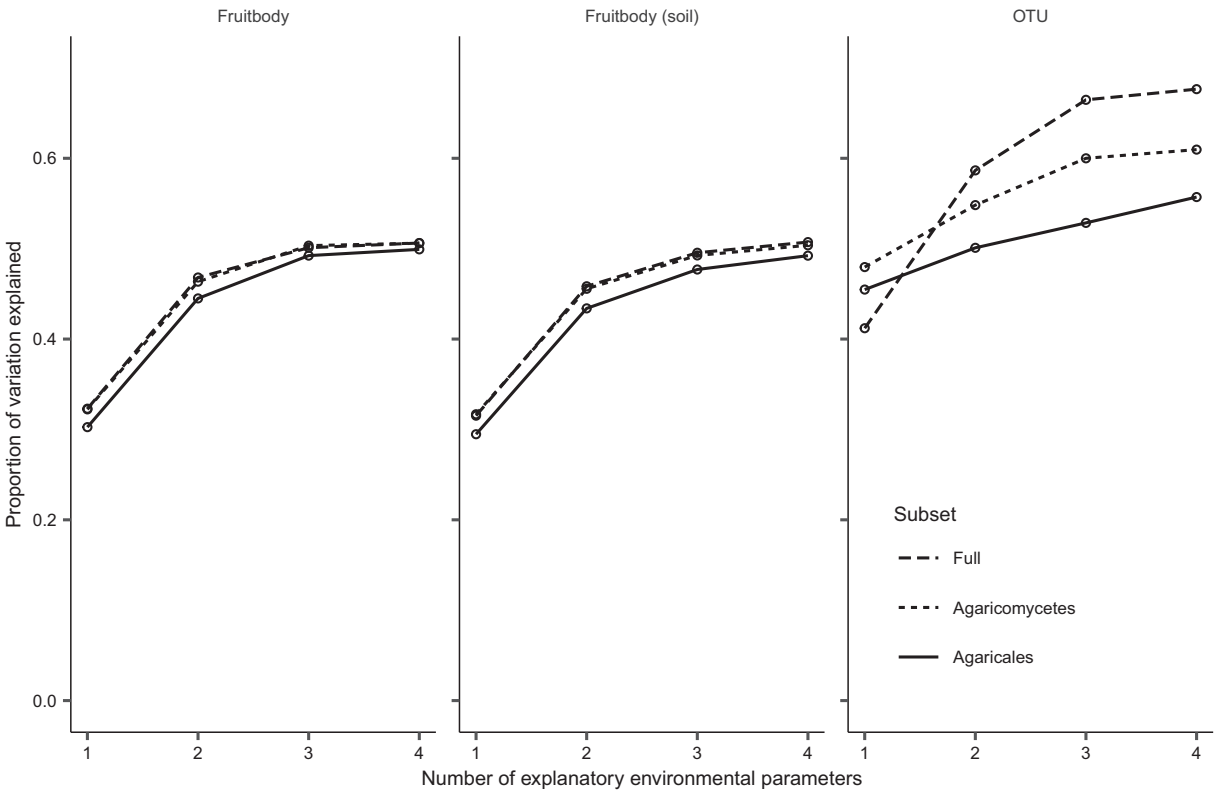

Fig. 7. Proportion variation of community dissimilarity. X-axis shows the number of explanatory environmental variables selected by the model, and y-axis shows the total amount of explained variation. Line type indicates the three taxonomic subsets (all taxa, Agaricomycetes and Agaricales). One panel per dataset – OTU, fruitbody (all data) and fruitbody (soil species).

Agaricomycetes and Agaricales depend on less easily measured properties of the environment. Most species of Agaricomycetes and Agaricales produce billions of spores that are effectively dispersed (Peay and Bruns, 2014) – which is not the case for some of the other main groups of fungi in this study (Money, 2016). Hence the detected community of these by eDNA may in part be a signal from the spore bank. The spore bank community has been shown to have relatively low correlation with the active community of the same taxa for pine-associated ectomycorrhizal fungi (Glassman et al., 2015), and thus, the lower correlation seen in our study may potentially be caused by a similar discrepancy. The lower performance of fruitbody survey data likely also indicates that fruitbody formation is more sensitive to e.g. unpredictable variation in weather conditions.

As seen from the taxonomic composition, eDNA metabarcoding has a much higher proportion of Ascomycota and other phyla of 'microfungi', but also a relatively lower proportion of non-soil Ascomycota and Basidiomycota. DNA-metabarcoding thus targets a community with a larger proportion of micro-fungi (possibly also due to PCR amplification biases), which must be assumed to be more dependent on soil composition and humidity, whereas the fruitbody data targets a community of macrofungi with a larger dependence on the vegetation and above ground conditions. This is reflected in light being among the best explanatory variables for the fruitbody data, and soil moisture and pH for the eDNA metabarcoding data.

4.5. Sampling efficiency/depth

The results obtained in this study reflect the exact sampling protocols for both fruitbody survey and eDNA sampling, as well as the bioinformatics processing of the sequence data. The fruitbody survey included three visits to each site, and it is obvious that more sampling visits will continue to add to the species list, and may be necessary to get a fully representative sample (Halme and Kotiaho, 2012; Newton et al., 2003; Straatsma et al., 2015) (but see Abrego et al., 2016). The (eDNA) soil sampling method included the mixing of 81 soil cores and thus several kilos of soil for each site, and was uniquely large compared to previous studies (e.g. Porter et al., 2008; Geml et al., 2010; Baptista et al., 2015; Pellissier et al., 2014; Geml et al., 2009; Schmidt et al., 2013). However, it still covered only 0.01% of the soil surface of the 40 m × 40 m sites, and of the approximately 5–20 kg soil sampled from each plot only 4 g of soil was used for DNA extraction. Also, we made no attempt to maximize coverage of visible variation at the sites but sampled completely systematically. Hence, both sampling approaches could be both up- and down-scaled for applications in practice. A study in Switzerland (Straatsma et al., 2015) recorded fruitbodies on a weekly basis over 21 years, and identified 101 species on average per year (408 species in total) in a forest study area close to ours in size (1500 vs. 1600 m^2). Although their total number of recorded species exceeds the site average of 68 fruitbody species (and 286 OTUs) in our study, their yearly average of 101 species is only slightly higher than the average (96.6) of our forest/plantation sites after three 1 h visits, and we predict that it would require much further effort to get a significantly larger average species number for the fruitbody data.

Presently, there is little knowledge on which parameters are the most important for obtaining a representative sample with the eDNA metabarcoding approach. We expect that extracting and sequencing the 81 soil cores separately or sequencing many sub-samples of the bulk sample would increase the number of detected OTUs, but this would also pose a marked increase of lab consumables and processing time. As many fungal mycelia must be assumed to be restricted in size and/or time, and as our bulk sample only covers 0.01% of the soil surface, we expect that additional bulk samples – done at the same time or at another time of the year – would be the most cost-efficient way of capturing a larger sample of the real fungal community.

4.6. Practical applications

Both approaches represent surveys that are realistic to perform within the limits of standard surveys and research studies, and expenses were roughly comparable. For the full data, eDNA metabarcoding resulted in more species (OTU) observations (37,289) than the fruitbody survey (8793), whereas the numbers were more similar for Agaricomycetes (8739 vs. 7233) and Agaricales (4474 vs. 4326). In our data, fruitbody richness was a relatively weak predictor of total fungal richness as assessed with DNA metabarcoding, but was a relatively good predictor of the richness of fruitbody forming fungi (Agaricomycetes and Agaricales). This indicates that species richness of these groups may be assessed interchangeably with eDNA metabarcoding or as fruitbodies.

For detection of red listed species, eDNA metabarcoding performed much better than expected, but we still would recommend a manual search for fruit-bodies in all cases where larger areas need to be surveyed e.g. for conservation value, as also suggested for wood-inhabiting fungi (Runnel et al., 2015). However, eDNA metabarcoding may supply valuable information in cases of poor fruiting conditions or in more targeted plot-based monitoring programs, and may be combined with lidar-derived variables (Peura et al., 2016; Thers et al., 2017). Fruitbody and eDNA metabarcoding data result in comparable measures of species turnover, and thus, our results indicate that data may be combined for example to evaluate long time series including historical fruitbody data and future DNA-based surveys.

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Conflicts of interest

All authors declare no conflicting interests.

Authors' contributions

All authors designed the study, TL collected the fruitbody data, TGF collected and analyzed the molecular data, TGF carried out the statistical analyses, TGF, JHC and RK wrote first draft, all authors contributed to revising the paper.

Data accessibility

Files documenting the analyses and all files necessary to reproduce the analyses – including links to raw sequence data and observational data – are available on GitHub (https://github/tobiasgf/man_vs_machine).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biocon.2019.02.038.

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