Global Fungal Diversity Estimated from High-Throughput Sequencing

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Abstract

Fungi are key players in vital ecosystem services, spanning carbon cycling, decomposition, and varied plant symbioses. Due to their cryptic lifestyle, it was difficult to assess their diversity until the advent of methods of high-throughput sequencing. Based on the papers utilizing high-throughput sequencing approaches to study fungi in natural habitats using the nuclear ribosomal internal transcribed spacer 2 (ITS2) contained in the public open database GlobalFungi (https://globalfungi.com), the current estimate of global fungal diversity is 6.3 million species, considering 97% sequence similarity as a species-level threshold. Of the observed fungi, most belong to Ascomycota and Basidiomycota: 57% and 37% of taxa, respectively. Soil and litter represent the habitats with the highest alpha diversity of fungi followed by air, plant shoots, plant roots, and deadwood. Based on the high-throughput sequencing data, the highest proportion of unknown fungal species is associated with samples of lichen and plant tissues. Climate was identified as the key driver of fungal biogeography. In contrast to plants and most other taxa, fungal diversity in tropics appears to be lower than at high latitudes. Despite limitations, the use of high-throughput sequencing is an important tool for the assessment of diversity, biogeography, and ecology of fungi.

Keywords

Fungal diversity · High-throughput sequencing · Metabarcoding · Biogeography · Fungal ecology

10.1 Introduction

Fungi represent one of the most diverse groups of organisms in the world (Purvis and Hector 2000; Hawksworth 2001). However, the cryptic lifestyle of most fungal species makes estimates of global biodiversity challenging. Given their critical roles in many ecosystem processes (Peay et al. 2016), understanding the extent of global fungal biodiversity and the factors that determine it can provide crucial information about the stability and functioning of ecosystems. In the past, mycology has mostly relied on morphological characteristics, but early studies of amplified molecular markers derived from high-throughput sequencing (HTS) (Buéé et al. 2009; Amend et al. 2010; Baldrian et al. 2012) have suggested that traditional approaches greatly underestimate the diversity of fungi. The ability to recognize and

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classify fungi based on appropriate molecular markers obtained by DNA sequencing has opened up the opportunity to explore the composition and diversity of fungal communities (Koljalg et al. 2013), and HTS-based metabarcoding has demonstrated its scientific predictive power for studying global fungal diversity and biogeography (Tedersoo et al. 2014; Davison et al. 2015) as well as for elucidating regional drivers of fungal community diversity and composition (Talbot et al. 2014; Tedersoo et al. 2020b; Odriozola et al. 2021). Moreover, mycological efforts supported by HTS are able to address a wide range of questions regarding ecological and functional aspects of fungal communities (Baldrian et al. 2012; Peay et al. 2016; Nilsson et al. 2019a). Metabarcoding of fungal communities has become a well-established method readily available to the broader scientific community with a solid methodological backing (Lindahl et al. 2013; Větrovský and Baldrian 2013; Anslan et al. 2018; Nilsson et al. 2019a). As a result, several hundred papers have been published to date using high-throughput sequencing to analyse fungal community composition (Větrovský et al. 2020).

With the exception of a few large-scale attempts (Tedersoo et al. 2014; Davison et al. 2015), the power of HTS to determine the global diversity of fungi by assembling data from multiple studies is only now beginning to be exploited. This cumulative approach allows for more precise estimates of global fungal diversity (Větrovský et al. 2019; Větrovský et al. 2020; Baldrian et al. 2022).

10.2 Global Database of Fungal Metabarcoding Data

An appreciation of the number of studies that use metabarcoding to characterize fungal communities in terrestrial and aquatic communities has led to the creation of a community resource: a database of results from all available studies that use fungal internal transcribed spacer (ITS) as a metabarcoding marker—the GlobalFungi database (Větrovský et al. 2020). This curated database (https://globalfungi.com) is a public repository of metabarcoding data that continuously catalogues the fungal records generated by high-throughput sequencing studies (Fig. 10.1). To achieve the goal of making published data findable, accessible, interoperable, and reusable (FAIR), the web interface allows users to search the entire database or within a selected set of samples for individual sequences, fungal species hypotheses—groups of sequences that share similarity approximately corresponding to a species level created using the data of the UNITE database (Koljalg et al. 2013), species, or genera; obtain a visual representation of their distribution in the environment; and access and download sequence data and metadata (Fig. 10.1). In addition, the user interface allows authors to submit data from studies not yet included, thereby contributing to building a resource for the community of researchers in fungal systematics, biogeography, and ecology (Větrovský et al. 2020).

The GlobalFungi database includes high-throughput studies of fungal community composition published since 2009. All studies that fulfilled the following criteria are included: (1) the samples represent natural habitats that have not been experimentally treated in a laboratory or greenhouse, (2) the exact geographic location of each sample is available, (3) the primers used for amplicon sequencing were designed to target the entire fungal community (rather than subsets of it), (4) internal transcribed spacer regions (ITS1, ITS2, or both) were the subject of amplification, and (5) the sequencing data were publicly available. In its third release, the database covered up to 36,684 samples of fungal communities from 367 studies that contained over 1.1 billion sequence records from more than 200 million unique variants (Fig. 10.2).

10.3 Global Fungal Diversity Estimate

It was long in the past when scientists realized that fungi are extremely diverse. Since then, there have been repeated to estimate the total number of

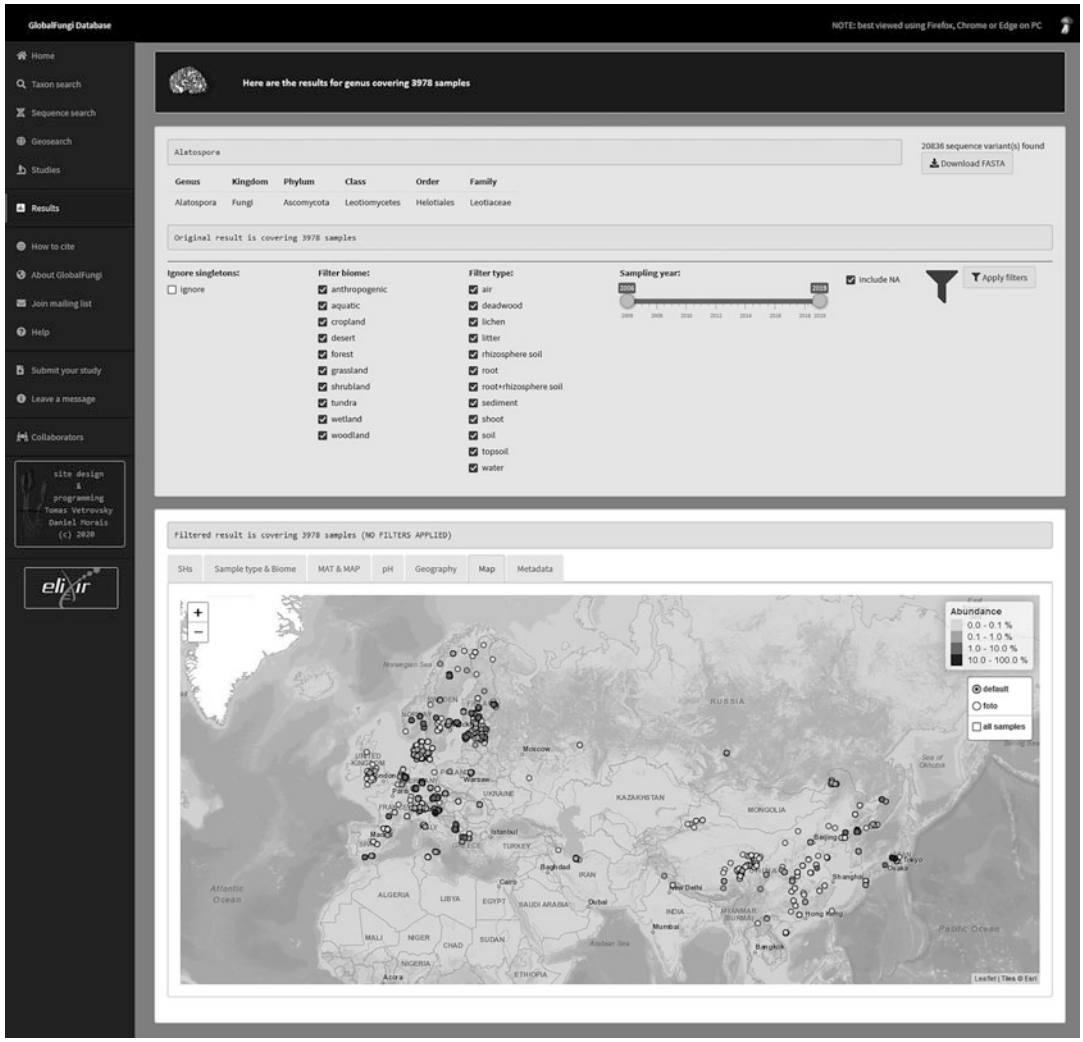

Fig. 10.1 User interface of the GlobalFungi database (https://globalfungi.com) provides a quick yet complex access to the data in the GlobalFungi Database. It is possible to search for individual fungal taxa—genera, species, or Species Hypotheses (Taxon search) or to search for sequences and taxa by sequence similarity (Sequence search). The list of studies can be accessed through the Studies button and regional mycoflora can be retrieved using Geosearch. There is also the option to submit new data using the Submit your study option

fungal species on Earth. The estimates were originally obtained by quantifying of host-specific fungi and multiplying them with putative number of fungal specialists per host (i.e. plant or insect) species (Hawksworth and Lücking 2017; Hyde et al. 2020). The high-throughput sequencing opened in the recent years another opportunity to assess the global fungal richness, and with increasing numbers of studies, the opportunities offered by this approach increase.

As GlobalFungi is currently the largest collection of fungal ITS marker data, it can serve as a data source for estimating global fungal diversity. Based on 97% sequence similarity clustering of full-length fungal ITS2 sequences, we recently identified more than a million fungal nonsingleton

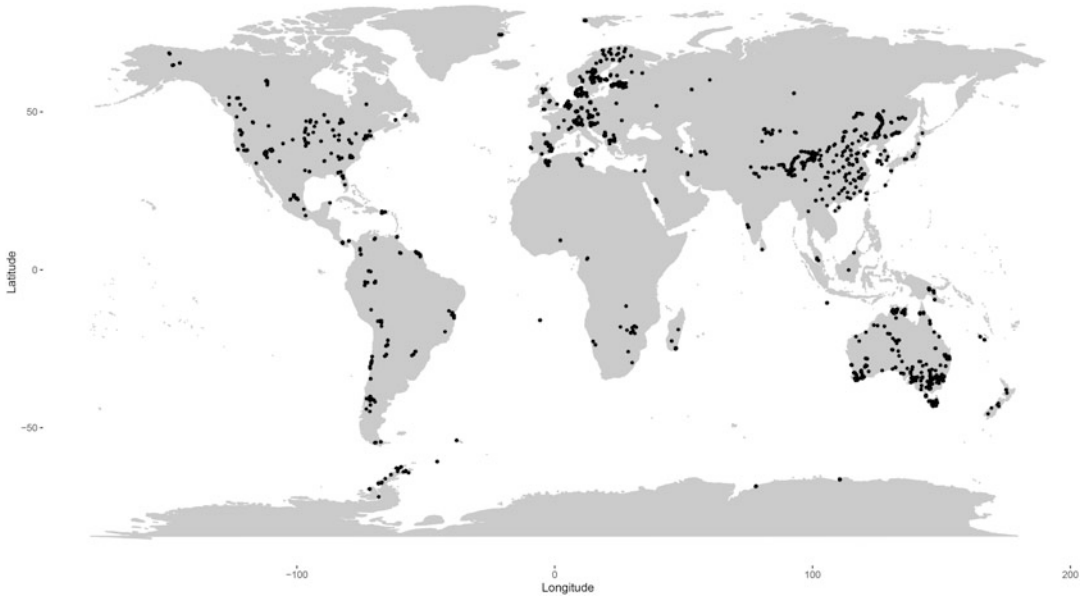

Fig. 10.2 Location of 36,684 samples covered in GlobalFungi database, release 3 (https://globalfungi.com)

Operational Transcription Units (OTUs) that were already reported and are contained in the database. The total global diversity of fungi was estimated to be approximately 6.3 million OTUs based on the Chao1 estimate calculated from the entire dataset (Baldrian et al. 2022).

Although this estimate is significantly higher than some previously proposed estimates of global fungal diversity, e.g., 2.2 or 3.8 million species (Hawksworth and Lücking 2017; Hyde et al. 2020), there are several reasons why this might be a rather conservative value. First, the threshold for judging a sequence as fungal was set at a conservative e-value of e-50 after using BLAST. Second, although some of the global doubletons and OTUs with higher sequence counts are likely to be undetected chimaeras or otherwise compromised data, many of the global singletons that were excluded from the analysis as potential technical sequencing errors or chimeric sequences are likely to represent true fungal species. Third, although some fungal species harbour several different copies of ITS2 with less than 97% similarity (Stockinger et al. 2010; Lindner et al. 2013; Větrovský et al. 2016), and some OTUs may represent rRNA pseudogenes (Glass et al. 2013), identical or very similar ITS sequences shared between different species are known from many species-rich genera of Pezizomycotina, including *Cladosporium*, *Penicillium*, *Fusarium,* and *Aspergillus* (Schoch et al. 2012). It should also be noted that there are several geographical gaps in knowledge where our information about fungal communities is limited. The high-throughput sequencing work published to date shows considerable geographic variation with one sample per 4000 km2 reported from Europe and only one sample per 200,000 km^2 in Africa. Habitat sampling has thus far been largely dominated by studies focusing on soils, particularly forest soils (Větrovský et al. 2020). It is very likely that more representative sampling covering a wider range of environmental conditions would lead to higher estimates of overall diversity. In addition, there is for sure additional fungal diversity associated with other habitats: marine environments, animal-associated microbiomes, or engineered systems. As has been shown for bacteria, these habitats contain a high proportion of specific microbial taxa (Thompson et al. 2017), and the same pattern should be true in fungi. It should be noted that metabarcoding studies on fungal communities in marine habitats are even fewer than studies on bacteria. Additionally,

surveys of animal-host-associated mycobiomes are much less frequent than studies on host-associated bacteria (Peay et al. 2016; Nilsson et al. 2019a; Seibold et al. 2019). Finally, many fungal taxa, such as the phylum Glomeromycota, show limited recovery when general fungal primers are used (Kohout et al. 2014).

Metabarcoding has previously been used to demonstrate the existence of so-far undescribed but widespread groups of fungi and provide their putative taxonomic placement (Nilsson et al. 2016; Tedersoo et al. 2020b). The present dataset also lends itself to such an endeavour. The majority of OTUs in our data belonged to Ascomycota (613,755 OTUs; 57%), followed by Basidiomycota (395,877; 37%), Glomeromycota (12,780; 1%), Mucoromycota (8263; 0.8%), Mortierellomycota (4621; 0.4%), Rozellomycota (3027; 0.3%), and fungal organisms with an unclear phylum-level classification (39,246 OTUs; 3.6%, Fig. 10.3). The most common families are listed in Table 10.1 (Baldrian et al. 2022).

The largest proportion of the terrestrial habitats was represented by soil samples—approximately 60% of all samples. Together with other samples from the rhizosphere or litter, these topsoil samples accounted for almost 70% of the total. Aboveground plant biomass (shoots) and plant roots were also frequently targeted by HTS (14% and 6%, respectively) as was deadwood (9% of samples). When examining the diversity across all samples, the Chao1 estimate increased with sampling depth across the dataset, but Chao1 estimates at fixed sampling depths (5000 sequences) were higher for soil than for deadwood, roots, and shoots (Baldrian et al. 2022) (Fig. 10.4).

Accordingly, when comparing samples of different habitat types after random subsampling to 5000 sequences, the highest OTU richness was found in soil (mean ± SE: 366 ± 4), followed by litter, with deadwood showing the lowest value (82 ± 2). At this sampling depth, Chao1 estimates were highest for soil and lowest for shoots, roots, and deadwood (Table 10.2) (Baldrian et al. 2022). Although no significant correlation was found between the volume of material sampled and alpha diversity (Větrovský et al. 2019), we can assume that larger samples or samples that are composite and represent larger volume of material capture higher diversity of potential fungal habitats and thus more fungal species (Smith and Peay 2014).

The comparison of clustered sequences used in a study with the UNITE reference corpus (Baldrian et al. 2022) allowed us to estimate the relative proportion of taxa and sequences that belong to previously undescribed fungal species. Across all samples, 78% of sequences (representing 52% of OTUs) mapped to previously characterized taxa, showing a similarity of greater than 97% to any UNITE 8.2 species hypothesis. The relative proportion of sequences of known species ranged from 75% in soil samples to almost 97% in air samples. The lichen habitat appears to be the most promising for finding new fungal species, as 69% of recorded OTUs were not mapped to any characterized taxon; this share was significantly higher than in any other habitat. This observation is consistent with previous reports documenting a surprisingly high number of unrecognized species among lichenized fungi (Lücking et al. 2014). In the litter, the proportion of new taxa averaged a moderate 35%, perhaps reflecting the fact that this has been investigated by mycologists for several centuries. It should be noted that fungi associated with animal hosts were not included in the analysis due to limited data availability. These and other undersampled habitats may still harbour an unknown proportion of total fungal diversity (Wu et al. 2019) and definitely worth further exploration.

10.4 Global Patterns and Determinants of Fungal Diversity

In the context of ongoing global change, it is critical to determine how climate and other environmental factors affect the diversity and distribution of fungal communities (Hillebrand 2004). Fungal symbionts may benefit plants by mitigating abiotic stressors associated with climate change such as heat and drought

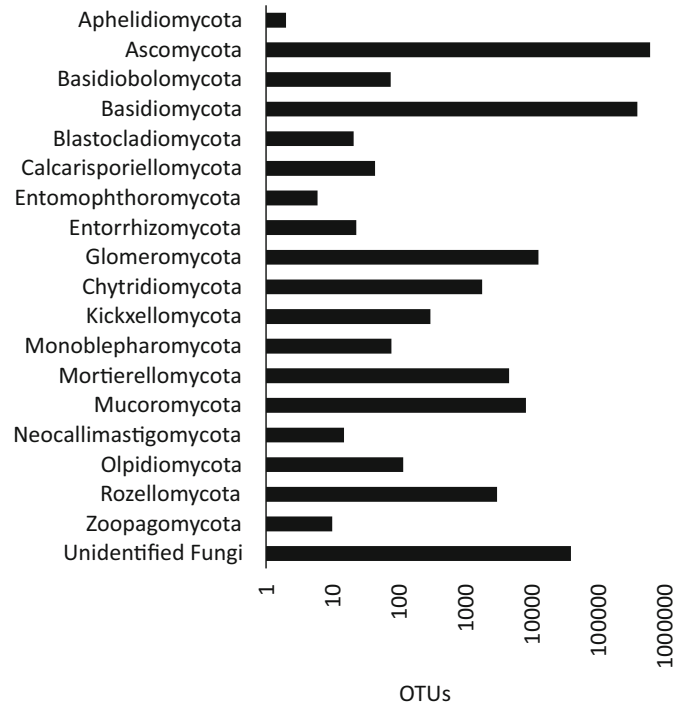

Fig. 10.3 Taxonomic breakdown of the best hits of nonsingleton OTUs present in the GlobalFungi database (Baldrian et al. 2022). The taxonomy follows (Tedersoo et al. 2018)

(Kivlin et al. 2013), but the distribution of these symbionts may be controlled by mechanisms other than climate (Kreft and Jetz 2007; Bardgett and van der Putten 2014). Because the geographic distribution and environmental preferences of almost all fungi remain unknown (Tedersoo et al. 2014), it is difficult to assess their current status and future threats to their existence (Jetz et al. 2012; Joppa et al. 2016). Given the importance of plant–fungal interactions, the ability to predict shifts in fungal distribution could help to understand or predict ecosystem-level changes. Recently, fungal community composition has been found to be influenced by climatic

Table 10.1 Families with the most predicted fungal species-level taxa (OTUs) based on the GlobalFungi database (https://globalfungi.com), release 3. The numbers indicate predicted OTU counts in each family and the numbers of sequences of fungi in each family in the database

Family	OTUs	Sequences (thousands)
Cortinariaceae (Basidiomycota)	67,940	4265
Inocybaceae (Basidiomycota)	65,952	5358
Hydnangiaceae (Basidiomycota)	37,583	1166
Aspergillaceae (Ascomycota)	36,708	3717
Nectriaceae (Ascomycota)	34,350	5480
Herpotrichiellaceae (Ascomycota)	31,699	5733
Bolbitiaceae (Basidiomycota)	27,700	933
Tricholomataceae (Basidiomycota)	26,127	2665
Pezizaceae (Ascomycota)	24,886	1545
Agaricaceae (Basidiomycota)	24,004	1000
Chaetomiaceae (Ascomycota)	23,959	4705
Thelephoraceae (Basidiomycota)	16,774	1468
Helotiaceae (Ascomycota)	15,615	5265
Pleosporaceae (Ascomycota)	15,358	4603

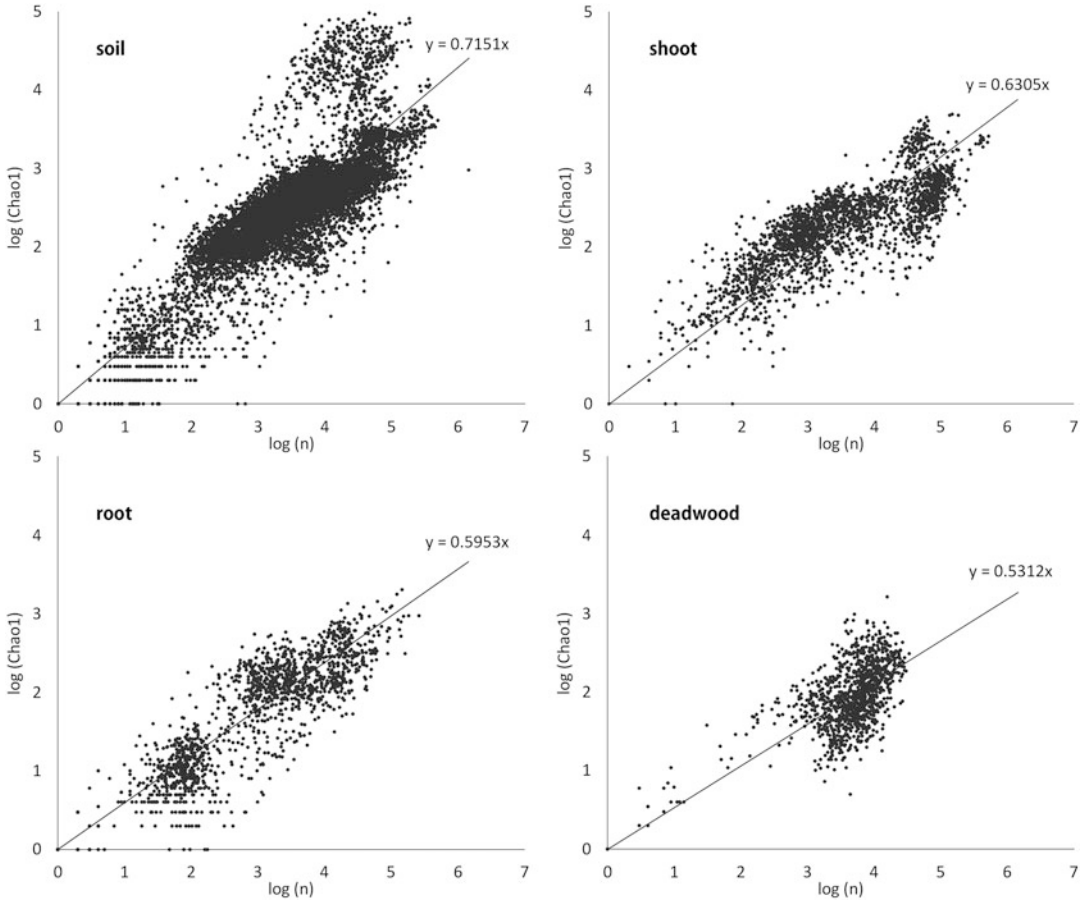

Fig. 10.4 Alpha diversity of fungal communities based on ITS2 sequencing. Correlation of the Chao1 diversity estimate and sequencing depth across soil (n = 9743), shoot (n = 2298), root (n = 1581), and deadwood (n = 1126) samples in the GlobalFungi database (Baldrian et al. 2022). The lines represent linear correlation fits

(Wollan et al. 2008; Maestre et al. 2015; Newsham et al. 2016) and edaphic variables (Tedersoo et al. 2020b; Odriozola et al. 2021) as well as vegetation elements (Crowther et al. 2016).

It is less clear whether global patterns of fungal biodiversity correspond to the higher diversity at low latitudes previously demonstrated for terrestrial macroorganisms and bacteria (Amundson

Table 10.2 Mean fungal species-level taxon (OTU) richness and Chao1 estimates in samples from various habitats. The values were calculated after subsampling each sample to 5000 sequences, and the data are based on the GlobalFungi database (https://globalfungi.com), release 3

Habitat	n	Chao1	OTU Richness
Soil	4113	1219 ± 48	366 ± 4
Litter	178	569 ± 18	332 ± 9
Air	36	392 ± 24	202 ± 10
Lichen	84	276 ± 16	134 ± 9
Shoot	933	228 ± 6	107 ± 3
Root	416	215 ± 6	135 ± 4
Deadwood	630	140 ± 5	82 ± 2

et al. 2015; Thompson et al. 2017). The distribution of plant and animal species also exhibits strong biogeographic partitioning (Kreft and Jetz 2010). Although some biogeographic patterns have been observed for bacteria, other data indicate that dominant microbes are widely distributed and thus can occur in all regions of suitable environments (Hanson et al. 2012; Delgado-Baquerizo et al. 2018).

In a previous study (Větrovský et al. 2019), data from 3084 soil samples in 36 independent studies covering a wide range of climatic conditions were evaluated in terms of the diversity of these communities. Both species richness and the Chao1 index across the different sets consistently revealed relatively low fungal diversity in the tropics. In contrast, areas of higher latitude showed considerable variation in fungal diversity with the most diverse areas concentrated near the high latitudes (Fig. 10.5). Based on these results, there is no convincing support for high fungal diversity in the tropics, which contrasts dramatically with well-known patterns for plants, arthropods, vertebrates (Amundson et al. 2015), and some bacteria (Thompson et al. 2017). Moreover, fungal diversity showed a moderate inverse relationship with temperature, confirming higher diversity at higher latitudes with colder and more seasonal climates (Větrovský et al. 2019). Although these results will need to be further refined and re-evaluated as more data become available, particularly from currently understudied areas, the present comparisons strongly suggest surprisingly low fungal diversity in the tropics.

Fungi can be categorized into ecological guilds reflecting their nutrition or other features (Põlme et al. 2020). When comparing the distribution of fungal species-level taxa belonging to different ecological guilds, ectomycorrhizal fungi have a lower upper temperature limit than that of fungi from other guilds. The temperature valence, defined as a range of temperatures where 90% of all observations of a particular taxon were observed, averaged 4.2 °C in ectomycorrhizal fungi compared with those in other fungal guilds (6.7–9.6 °C). The precipitation valence (330 mm) of this group was also narrower than those of all other guilds (430 to 860 mm), except for endophytes and wood-associated saprotrophs, which was due to the relatively low abundance of ectomycorrhizal fungi in samples with low precipitation. The narrower climatic niche of ectomycorrhizal fungi suggests that these mutualistic plant symbionts may need to respond to climate change-related problems by altering their phenology, range of distribution, or physiology. If they fail to do so, they are likely to become extinct under future climatic conditions. Plant pathogens, in contrast, appear to have much broader climatic niches (Fig. 10.6) (Větrovský et al. 2019).

10.5 Limitations of Molecular Barcoding of Fungi

Although it is tempting to consider HTS as the best solution to explore fungal diversity and biogeography, molecular barcoding and metabarcoding approaches have several limitations that prevent their use as the sole tool for diversity studies. The first important problem is the definition of species. Species are biological entities whose existence is subject to assessment by experimental tools, although these tools may be difficult to apply to all species. Metabarcoding relies on the mathematical construction of taxa. These constructions are an approximation of reality and typically build on an average barcoding gap (sequence divergence) that delineates DNA sequences into molecular taxa or OTUs. The barcoding gap varies significantly among fungi, such that some biological species appear to be multiple OTUs, whereas others may be clustered into a single OTU (Schoch et al. 2012; Liu et al. 2015; Větrovský et al. 2016). Another important issue is the scale of observations. The distribution of fungi can be extremely heterogeneous in space, and samples collected over a small area may share only a few dominant taxa (Šťursova et al. 2016). Metabarcoding is also prone to errors from technical sample processing as well as platform-specific sequencing issues, which adds more sources of bias (Nilsson et al. 2019a). One such potential bias is nucleic acid extraction, and

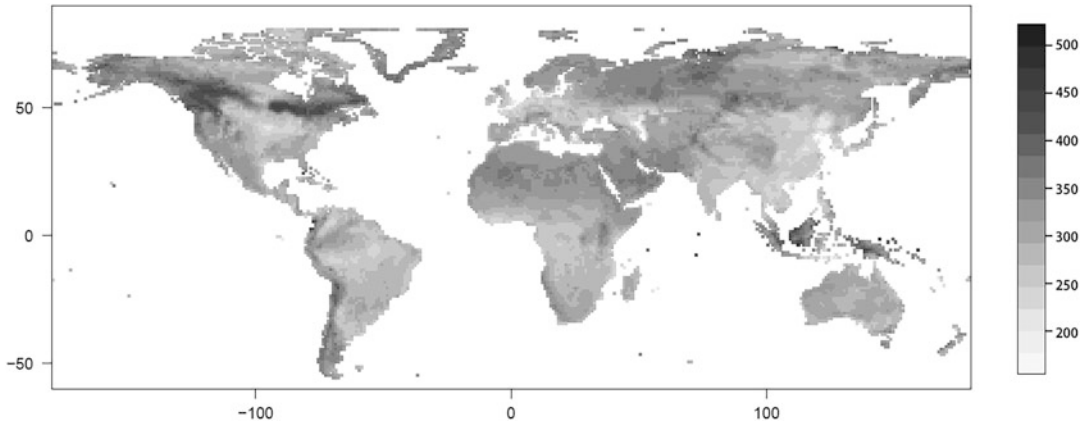

Fig. 10.5 Inferred patterns of fungal species diversity (OTU richness) predicted by the best-subset GLM (Větrovský et al. 2019)

another is the formation of chimeric sequences during marker amplification. The limited ability of bioinformatics tools to recognize chimaeras results in an unknown number of OTUs representing artificial taxa, whereas an unknown number of authentic OTUs are incorrectly removed as potential chimaeras (Schloss et al. 2011). For alpha diversity studies, cross-contamination of samples is a major and largely undescribed problem. There are also many important aspects related to the choice of molecular marker (Schoch et al. 2012). The ITS2 region has the advantage of high taxonomic resolution and limited length variability; moreover, a battery of well-tested PCR primers with varying degrees of specificity is available (Ihrmark et al. 2012; Schoch et al. 2012; Tedersoo et al. 2015). Despite the reasonable coverage of some ITS2 primers, all fungus-specific primer pairs used thus far in HTS studies, which were designed to be generally applicable to all fungi, miss at least a few of the 467 most abundant fungal taxa in GlobalFungi (Větrovský et al. 2019). It should be noted that existing ITS primers have been designed based on databases of available sequences, and the extent to which they work well for previously undiscovered and possibly genetically divergent fungal groups remains largely unknown.

Due to the fact that the number of copies of rDNA (and thus ITS) per genome varies among fungi (Lofgren et al. 2019), single-copy markers were considered as an alternative metabarcoding marker. While some of them were proven successful for analysis of closely taxonomically related taxa and appeared to possibly better estimate species-level diversity, they typically miss a large proportion of taxa across the fungal tree of life and show highly variable barcoding gap (Větrovský et al. 2016).

10.6 Conclusions

Using a large number of metabarcoding datasets from the GlobalFungi database, the conservative estimate of global fungal species richness was 6.3 million species (Baldrian et al. 2022). The global distribution of fungal diversity is shaped by multiple environmental factors, but climate appears to be the major factor affecting the most common fungal species. This finding underscores how profound the impacts of ongoing climate change can be on ecosystem functioning and food security. For example, beneficial ectomycorrhizal fungi exhibit narrow climate niches compared with those of plant pathogens. Thus, the impact of climate on fungal distribution could seriously impair plant productivity. These results open the way for further such research and identify climate as a major determinant of biogeographic patterns

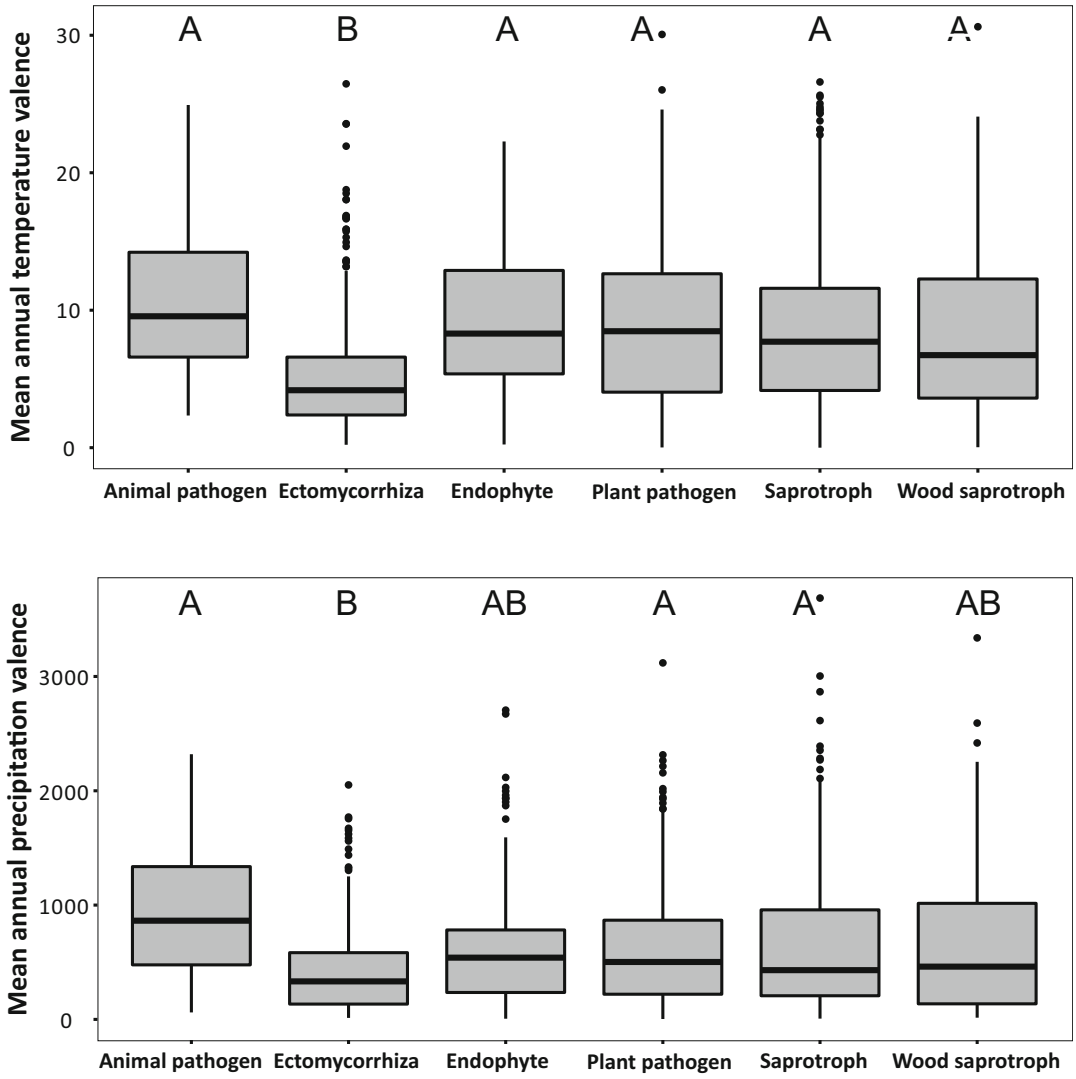

Fig. 10.6 Climatic determinants of ecological guilds of fungi. (**a**) The first and ninth deciles of sample mean annual temperature and (**b**) annual precipitation for SHs belonging to selected ecological guilds with occurrence in >10 samples (Větrovský et al. 2019)

in fungi, which appears to differ dramatically from those known for other eukaryotes and bacteria. It is clear that the estimation of diversity from HTS data needs to be viewed in light of the many potential sources of bias mentioned above. However, attempts to estimate extant fungal diversity without considering the HTS perspective appear unrealistic. Mycology as a whole stands to gain from the successful implementation of HTS, and classical mycology is entering the age of big data; there is considerable benefit for the future of fungal ecology and biogeography arising from this paradigm change.

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