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Review

The biogeography and conservation of Earth's 'dark' ectomycorrhizal fungi

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SUMMARY

Breakthroughs in DNA sequencing have upended our understanding of fungal diversity. Only ~155,000 of the 2–3 million fungal species on the planet have been formally described and named, and 'dark taxa' — species known only from sequences — represent the vast majority of species within the fungal kingdom. The International Code of Nomenclature requires physical type specimens to officially recognize new fungal species, making it difficult to name dark taxa. This is a significant problem for conservation because, without names, species cannot be recognized for environmental and legal protection. Symbiotic ectomycorrhizal (EcM) fungi play a particularly important role in forest carbon drawdown, but at present we have little understanding of how many EcM fungal species exist, or where to prioritize research activities to survey and describe EcM fungal lineages. In this review, we use global soil metabarcoding databases (GlobalFungi and the Global Soil Mycobiome consortium) to evaluate current estimates of the total number of EcM fungal species on Earth, outline the current state of undescribed EcM dark taxa, and identify priority regions for future dark taxa exploration. The metabarcoding databases include up to 219,730 EcM fungal operational taxonomic units (OTUs) detected from almost 39,500 samples. Using Chao richness estimates corrected for extrapolating species numbers from metabarcoding datasets, we predict that the global diversity of EcM fungi could be ~25,500–55,500 species. Dark taxa — those that do not match species-level identities — account for 79–83% of OTUs. Oceania contains the highest percentage of dark taxa (87%), and Europe the lowest (78%). Priority 'darkspots' for future research occur predominantly in tropical regions, but also in selected temperate forests at both southern and northern latitudes. We propose concrete steps to reduce the prevalence of EcM darkspots, including performing targeted field surveys, barcoding fungaria voucher specimens, and developing new ways to describe and conserve fungal taxa from DNA alone.

Introduction

Ectomycorrhizal (EcM) fungi form symbiotic associations with plants, providing access to essential nutrients and other resources in exchange for carbohydrates photosynthesized by the plant[1]. Roughly 6,000 tree species representing ~25% of all global vegetation cover are known to form EcM associations[2,3]. These fungi are critical to the functioning of Earth's forests by driving major biogeochemical cycles and enhancing plant stress tolerance[4,5]. Recent estimates indicate that trees allocate ~2.5 Gt of carbon annually to EcM hyphal networks[6]. Consequently, protecting and restoring EcM forests and fungi is an important part of global climate-change mitigation strategies[7–9].

Environmental DNA (eDNA) metabarcoding is increasingly used to document fungal diversity[10–12]. However, the vast majority of sequences detected as eDNA cannot be matched to named species and are therefore termed 'dark taxa'[13]. Functional roles, organismal interactions, and ecosystem services remain mostly unknown for dark taxa. Additionally, conserving unnamed species is challenging because they cannot be included in initiatives such as the IUCN Red List of Threatened Species[14]. The high prevalence of dark taxa limits the usefulness of global eDNA

Table 1. Estimates of ectomycorrhizal (EcM) fungal diversity.

Source	Number of EcM taxa[a]	Number of named EcM taxa[b]	Details
Published EcM richness estimates			
Molina *et al.*[90]	5,000–6,000	–	Total EcM species estimate based on expert opinion of EcM fungal genera known at the time
Rinaldi *et al.*[18]	20,000–25,000	–	Total EcM species estimate based on a literature search of EcM fungal species that was extrapolated using a previously calculated known:unknown ratio for all macromycetes
He *et al.*[35]	36,500	–	Predicted number of described species by 2030 within Basidiomycota orders that contain EcM functional groups, based on publication rates of new species from 2009–2020 (note: this estimate also includes non-EcM species)
Database EcM taxa records			
DEEMY[30]	420	N/A	Species included in the DEEMY database of descriptive EcM fungal data
FUNGuild reference database[91]	491	58 (11.8%)	Number of taxa (primarily genus-level) with EcM listed as one of the guild descriptions
FungalTraits reference database[17]	11,296	4,374[c] (38.7%)	Number of EcM Species Hypotheses present in the FungalTraits database (v.1.1)
Catalogue of Life[92]	13,961	N/A	Number of species listed in the Catalogue of Life that belong to the 327 fungal genera classified as EcM in the FungalTraits genus-level database (subspecies, duplicates, and synonym records were removed before totalling)
GlobalFungi sequence database[12]	95,114	16,141[c] (17.0%)	EcM fungal OTUs (based on ITS1 or ITS2 sequences clustered at 97% similarity) in the GlobalFungi database v.5.
Global Soil Mycobiome consortium sequence database[11]	124,616	25,489[c] (20.5%)	EcM fungal OTUs (based on full-length ITS and sometimes also part of 18S-V9 regions; clustered at 98% similarity).

[a]Here, 'taxa' refers to either genera, species, Species Hypotheses, or operational taxonomic units (OTUs) depending on the database (see Details column). Species Hypotheses are species-level clusters of sequences in reference databases that are linked to a DOI[17]. Note that the numbers of OTUs in the GlobalFungi and Global Soil Mycobiome consortium databases are not comparable due to the different sequencing and OTU clustering methods used. [b]Number of taxa in databases that are assigned species-level names. The percentage of total taxa that are named is shown in parentheses. N/A = not applicable, because those databases only include named taxa. See Details column and Supplemental information for details of the criteria used to name taxa in different databases. [c]Named OTUs in the FungalTraits database matched 2,858 unique EcM fungal species names, those in the GlobalFungi database matched 3,200 unique names, and those in the Global Soil Mycobiome consortium database matched 2,504 unique names. ITS = internal transcribed spacer; 18S-V9 = V9 region of the 18S rRNA gene.

databases — such as GlobalFungi[12] and the Global Soil Mycobiome consortium (GSMc)[11] — which are becoming a critical resource for mapping and conserving fungal species. For eDNA studies to reach their full potential, work to identify dark fungal taxa is urgently needed. At present, the prevalence and geographical distribution of dark EcM fungal taxa remains unquantified at the global level, making it difficult to target research towards reducing the dark taxa problem.

To address these gaps, here we provide an updated global estimate of total EcM fungal diversity from eDNA databases, quantify the current state of undescribed EcM fungal dark taxa, identify priority regions for future research, and outline concrete steps to reduce the dark taxa problem and improve fungal species identification from eDNA. Identifying and understanding dark taxa is crucial if fungal diversity and function are to be adequately conserved and protected in the future.

Global estimates of ectomycorrhizal fungal diversity

EcM fungi are among the most diverse groups of mycorrhizal symbionts[3] and have potentially evolved independently over 80 times within the phyla Basidiomycota, Ascomycota and, to a lesser extent, Mucoromycota[15,16]. Over 300 fungal genera are known to contain EcM species[17], although true numbers may be considerably higher. The most recent published estimate of EcM fungal species diversity suggests that there could be 25,000 species[18] (Table 1). This estimate is based on

Box 1. Estimating total EcM species diversity from environmental DNA.

We provide an updated estimate of Earth's total EcM fungal species diversity from the largest soil metabarcoding databases to date: the GlobalFungi database (v.5)[12] and the Global Soil Mycobiome consortium (GSMc)[11]. The GSMc database contains data from 3,200 plots, with samples sequenced in central laboratories using standardized long-read sequencing methods of the full-length ITS (internal transcribed spacer) and part-length 18S-V9 (V9 region of the 18S rRNA gene) regions[11]. While this dataset likely has more reliable fungal taxonomic assignments than operational taxonomic units (OTUs) generated from short-read amplicons, it has far lower sequencing depth per sample and could be missing rarer members of soil fungal communities. The GlobalFungi database includes short-read ITS1 and ITS2 sequences from more varied metabarcoding protocols, but from a greater number of samples that cover a larger geographical area (39,495 samples contain EcM OTUs; this dataset includes samples from the GSMc, using only the ITS2 region).

For the GlobalFungi dataset, we used OTUs clustered at 97% similarity that have been passed through rigorous quality control measures and used previously to estimate and describe fungal diversity patterns[22,93]. For the GSMc dataset, OTUs were clustered at 98% similarity by Tedersoo *et al.*[16]. Further details of differences between the databases are provided in the Supplemental information. The GlobalFungi dataset contained 95,114 EcM fungal OTUs from the 39,495 soil samples (Table 1 and Figure 1), and the GSMc dataset contained 124,616 EcM OTUs across the 3,200 plots.

For each dataset, we used Chao richness extrapolation to estimate total EcM fungal diversity[93,94]. This resulted in global estimates of 96,705 (± 204 SE) and 477,303 (± 4,455 SE) EcM fungal OTUs on Earth from the GlobalFungi and GSMc datasets, respectively. However, these two numbers are not directly comparable due to the different clustering thresholds used by the two databases (97% for GlobalFungi versus 98% for GSMc). Additionally, there are limitations to extrapolating species richness from metabarcoding studies that use OTU clustering to distinguish taxa[71]. For instance, using a single similarity threshold when clustering OTUs is common practice in fungal ecology analyses but may insufficiently capture true species-level divergence between fungal lineages. One recent analysis comparing OTUs to more accurate 'phylogenetic entities' created using sequence alignment methods suggests that a corrective OTU:species ratio of 3.8:1 should be used when extrapolating species estimates from 97% OTUs, and 8.6:1 for 98% OTUs[71]. After applying these corrective factors, we conservatively estimate that the total number of EcM fungi on Earth is between 25,449 (± 54) and 55,500 (± 518) species. The lower estimate (from the GlobalFungi dataset), is remarkably similar to past estimates[18], but the higher estimate from the GSMc dataset is more than double.

The difference in estimates from the two databases could reflect differences in sequencing platform, OTU clustering thresholds, or methods of taxonomic assignment for long- and short-read data. The corrective ratios identified by Niskanen *et al.*[71] were specifically calculated using subsets of the GlobalFungi and GSMc databases and should equalize any differences in total species estimates due to different methods. However, these corrective ratios contain considerable uncertainty because they were calculated using only a small number of samples and have not been calibrated for EcM fungi in particular (see Supplemental information). Additionally, many true EcM sequences are likely missing from both the GlobalFungi and GSMc databases due to their absence from reference databases used to assign taxonomy and guilds (discussed later in this review). Therefore, we suggest considering these species estimates as a conservative starting range until future, more uniform analyses can be conducted that are able to quantify the differences in richness estimates resulting from distinct sequence processing methodologies in more detail.

extrapolating the number of species from published literature using a known:unknown ratio previously calculated for all macromycete fungal species[18]. However, the increased accessibility of eDNA sequencing technologies has led to a rapid rise in the detection of EcM fungal sequences from environmental soil samples at global scale[12,19], with many thousands more EcM fungal operational taxonomic units (OTUs) detected (Table 1).

We examined EcM fungal OTUs present in the largest fungal eDNA metabarcoding databases currently available — GlobalFungi[12] and the GSMc[11] — to provide an updated global estimate of total EcM fungal diversity (Box 1). Although considerable uncertainty remains (Box 1 and Supplemental information), this analysis suggests that EcM fungal species numbers could be up to ~55,500.

Global predictions of the geographical distribution of EcM fungal diversity have repeatedly demonstrated that EcM fungi exhibit an opposite latitudinal diversity gradient to most other taxa, with diversity primarily peaking in high northern and southern latitudes[20–22]. However, the majority of EcM research attention has focused on northern temperate and boreal regions where EcM associations are dominant[23]. EcM fungal communities of central and southern latitudes remain poorly characterized, despite their critical ecological impacts in many tropical and southern temperate ecosystems[24–28]. This geographical research bias is problematic for properly understanding and conserving the threatened EcM species in those regions. Furthermore, these undersampled regions may cause global EcM diversity to be underestimated if unique species in these regions have never been sampled. Future work is required to improve the diversity estimates we provide and quantify any potential effect of bias in eDNA databases on calculations.

Naming EcM fungi and the problem of dark taxa

Traditionally, EcM fungal species have been delimited based on the morphology of their sporing bodies (e.g., fruiting bodies like mushrooms) and the spores they produce. However, methods relying on fungaria specimens can limit our understanding of EcM biodiversity due to the rarity of some taxa that have restricted environmental niches and geographical distributions[16]. Variation in sporing body production can also bias diversity estimates, given that some species may only produce such bodies sporadically or may produce inconspicuous sporing

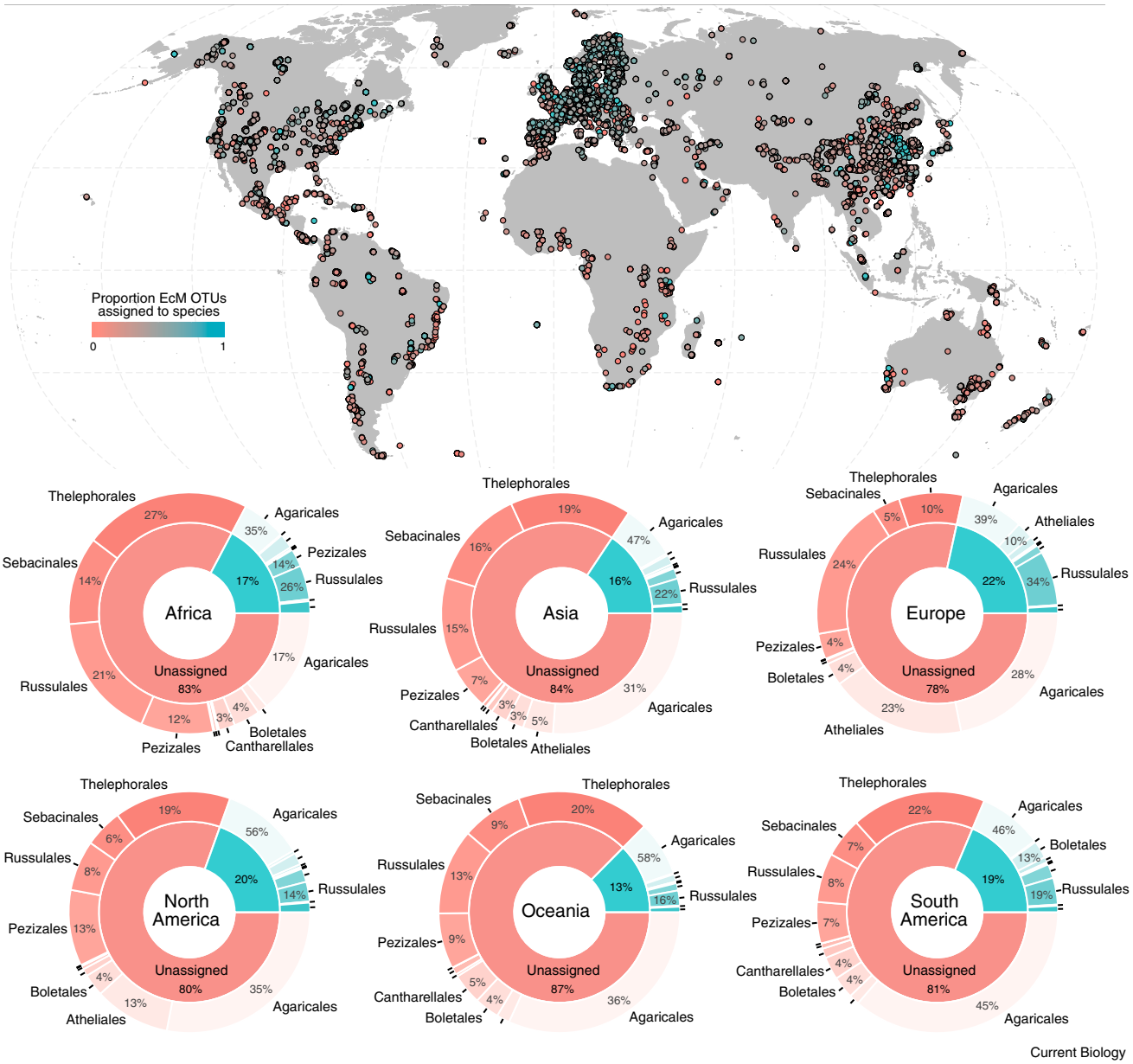

Figure 1. Overview of EcM fungal OTUs in the GlobalFungi dataset (v.5)[12] assigned species-level taxonomy in soil samples from different continents.

The top map shows locations of samples with EcM fungal taxa (39,495 samples) with points colored by the proportion of EcM OTUs assigned to species level. The lower plots show the percentage of EcM species assigned by continent at the order level, which are calculated for assigned and unassigned categories independently (low percentages not labelled). Figure created using data from[12] (CC BY 4.0).

bodies (e.g., resupinates and truffles) that are less likely to be collected[19,24]. Other methods based on morphotyping or chemical staining of EcM root tips[29,30] remain impractical for species delimitation because the morphology of EcM root tips varies with host species. Additionally, culture-based methods routinely used to detect and identify fungal pathogens are not suitable for many EcM fungi that are difficult or impossible to isolate into axenic culture[31].

With the rise in DNA-based methods for studying fungi, fungal systematics have increasingly relied on molecular data, using phylogenetic, coalescence-based and, more recently, genomic approaches to delimit EcM species[32,33]. However, the International Code of Nomenclature for algae, fungi, and plants requires a physical type specimen to formally name fungal species[34]. Whilst it is now common practice to include DNA sequences along with type specimens when describing new fungal species, it is not an absolute requirement, and formal species description based on DNA alone is not currently accepted. Therefore, DNA sequence repositories that hold the data from high-throughput sequencing approaches (e.g., soil metabarcoding) are primarily composed of undescribed dark taxa. Because they are nameless, dark taxa are not easily

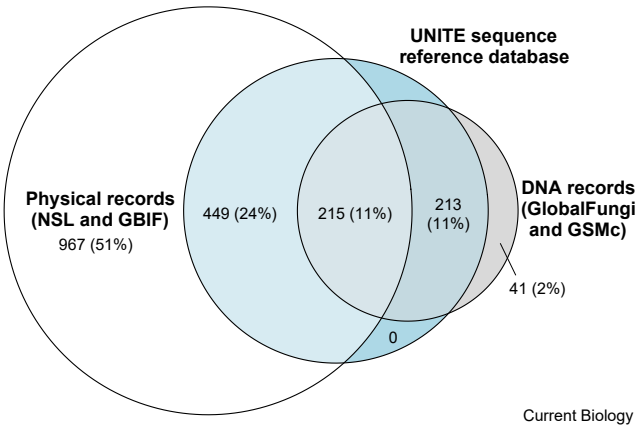

Figure 2. Euler diagram illustrating the number and percentages of unique EcM fungal species names that have been detected in Australia.

Physical records include those from the National Species List (NSL) of literature records[39] and fungaria specimens and/or observations in the Global Biodiversity Information Facility (GBIF)[40]. DNA records are unique names from 460 Australian soil samples in GlobalFungi v.5 and 73 Australian plots in the Global Soil Mycobiome consortium (GSMc)[11,12]. The number and percentage of these species names linked to a reference sequence in UNITE v.10[37], which is the most commonly used database to assign fungal taxonomy to metabarcoding datasets, are indicated within the blue circle. Note that Euler diagrams are approximations, and so 21 named species occurring within only the physical record and DNA record lists (and not UNITE) are not pictured. A more detailed breakdown showing overlap separately for the NSL, GBIF, GlobalFungi and GSMc lists is provided in Figure S1 in the Supplemental information.

incorporated into biodiversity surveys and are often excluded from nature conservation decision-making[13]. Therefore, there is an urgent need to classify, record and communicate the distribution, identity and ecological importance of fungal dark taxa[13,33,35,36].

To quantify the dark taxa problem for EcM fungi, we examined the level of taxonomy assigned to the EcM fungal OTUs present in the GlobalFungi v.5 database described in Box 1. Taxonomy was assigned using the UNITE database v.10[37], and OTUs that matched named reference sequences with at least 97% similarity were given species names. Of the 95,114 EcM fungal OTUs present, only 16,141 (17%) were assigned species-level names (with 3,200 unique names represented). Therefore, 83% (78,973 OTUs) can be considered dark taxa. The percentage of dark taxa varies slightly across continents but is relatively high in all regions (Figure 1). Oceania, Asia and Africa contain the highest percentages of dark taxa (87%, 84% and 83%, respectively), and Europe the lowest (78%). At higher taxonomic levels, the level of unidentified OTUs also differs between continents. For instance, compared to South America, European samples have half the proportion of unassigned Thelephorales OTUs and triple the proportion of unassigned Russulales OTUs (Figure 1).

Most dark taxa likely represent undescribed species, but some may represent described species that lack a representative barcode sequence in reference databases[38]. If a fungal species is not represented in reference databases (e.g., UNITE and FungalTraits used to assign taxonomy and filter EcM species), then sequences from that species in metabarcoding datasets may be wrongly discarded as non-EcM or match only higher taxonomic ranks and be classified as EcM dark taxa. We extracted EcM fungal species names from the Catalogue of Life (which encompasses Species Fungorum Plus) for each of the 327 EcM-defined genera in the FungalTraits database[17] (see Supplemental information). This showed that roughly 14,000 putative EcM fungal species with names exist (Table 1). However, only 2,858 unique EcM fungal species names are listed in the FungalTraits database, indicating that many EcM fungal species are not included in FungalTraits and therefore not identified as EcM in metabarcoding datasets. Using Australia as a case study highlights this problem further. Out of the 1,906 named EcM fungal species that have been recorded in Australia to date (from the literature[39], the Global Biodiversity Information Facility[40], and named OTUs from the Australian samples in the GlobalFungi and GSMc databases), only 877 (46%) have sequences with matching species names in the most recent UNITE reference sequence database (v.10; Figure 2). This highlights the paucity of reference sequences currently available in UNITE for this area. It is likely that sequencing available voucher material (i.e., permanently preserved specimens) would reduce this gap by allowing more sequences from metabarcoding databases to be matched to named EcM species.

Mapping 'darkspots' of EcM fungal diversity: priority regions for conservation and future research

Although metabarcoding databases provide a useful overview of the abundance of dark EcM fungal taxa in sampled regions, the global distribution of dark taxa, and locations of 'darkspots' (diversity hotspots of dark taxa), are largely unknown. To address this gap, we created a global geospatial model of dark EcM fungal richness by recreating models based on the GlobalFungi database v.4 developed by Van Nuland *et al.*[22] to map the geographical distribution of total EcM fungal richness (Figure 3A). We developed models using identical methods but trained only on the dark taxa EcM fungal OTUs unassigned to species level (see Supplemental information for details). We mapped: (i) the raw dark taxa OTU richness predictions (Figure 3B); (ii) the percentage of total OTU-predicted richness belonging to dark taxa (dark taxa richness map divided by the total taxa richness map from Van Nuland *et al.*[22] multiplied by 100; Figure 3C); and (iii) a metric of dark taxa research priority areas, calculated by multiplying normalized versions of (i) and (ii) and scaling values to range between 0 and 1 (Figure 3D). This research priority metric aims to identify regions that contain both a high percentage of dark taxa in the EcM fungal communities as well as high overall dark taxa richness. These areas are likely to be of most interest for targeting future EcM fungal research and taxonomic work.

Dark EcM fungal richness was strongly correlated with total EcM richness (Pearson's R^2 = 0.89), but the total EcM fungal richness hotspots of northern temperate and boreal zones (Figure 3A) were somewhat diminished for EcM dark taxa (Figure 3B). Interestingly, the regions predicted to contain the highest percentage of dark taxa (Figure 3C) primarily occurred in tropical forests and grasslands/shrublands across all continents. This is unsurprising considering that EcM fungal communities are vastly understudied in tropical systems compared to temperate regions[41], and tropical systems contain high levels

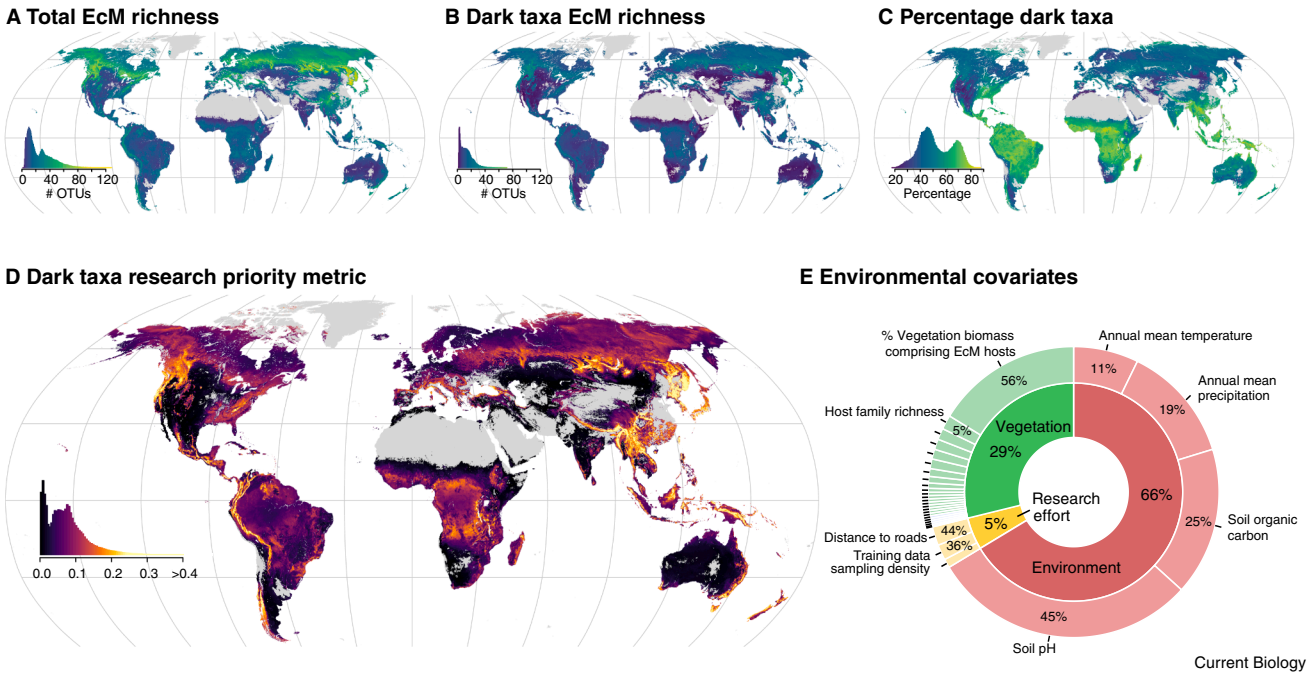

Figure 3. The predicted distribution of EcM fungal 'dark diversity' (fungal OTUs unassigned to named species).
The total EcM richness predictions from Van Nuland *et al.*[22] (A) and the predictions of EcM dark taxa richness from this study (B) were used to estimate the percentage of total EcM OTUs that represent dark taxa (C), calculated as (B) divided by (A) multiplied by 100. Priority regions for targeting future research efforts are shown in (D), which is the product of (B) and (C) normalized to a range between 0 and 1. (B) and (C) were also normalized prior to multiplying to ensure they contributed equally. Grey regions were masked due to high uncertainty in richness predictions. Uncertainty maps are provided in Figure S2. (E) The contribution of mean absolute SHapley Additive exPlanations (SHAP) values from the model describing the relationship between the dark taxa research priority scores and vegetation, research effort, and environmental covariates (see Supplemental information). Percentages in the outer ring are calculated separately within each of the three categories. See Figure S3 for values for all variables and relationship directions, and Table S1 for a description of the covariates. Figure created using data from Van Nuland *et al.*[22].

of fungal endemicity[42]. Regions likely to be of highest priority for directing future research efforts occurred predominantly in tropical coniferous and broadleaf forests in southeast Asia and Central and South America, tropical forests and grasslands/savannas/shrublands in central Africa, temperate broadleaf forests of China and southeast Australia, Sayan montane conifer forests above Mongolia, and western US temperate conifer forests (Figure 3D).

We explored whether EcM fungal priority 'darkspots' are related to vegetation characteristics (e.g., host plant dominance, diversity, and identity), potential past 'research effort' (e.g., regions with high rates of soil sampling or that are easily accessible), and/or environmental factors (e.g., climate and soil properties), using a SHapley Additive exPlanations (SHAP) model. The model was run with dark taxa research priority metric values (Figure 3D) from 1,000 randomly selected grid cells across the globe (see Supplemental information for details). Despite environmental variables exhibiting the strongest influence on the location of darkspots, the dominance and diversity of host plant families also had substantial effects (Figure 3E). Dark EcM fungal taxa were not strongly influenced by proxy variables of relative research effort (e.g., density of soil samples used to train the richness models, distance to roads, and human population density). However, it seems likely that dark taxa patterns are influenced by geographical research biases towards certain climatic biomes and host plant families[23,41]. Variables describing other research activities, such as fungal sporing body field survey effort, were not included here and may also influence dark taxa distributions.

Conservation implications and future directions

It is clear that discovery and description of EcM fungal taxa must be accelerated, particularly in priority darkspot regions (Figure 3). Much of the darkspot research priority land area (top 95th percentile of research priority metric values) falls in the tropical moist forest biome, particularly in Asia and South America (Figure 4A). Looking at mean research priority scores across entire biomes shows that, on average, tropical conifer forests are also of high research priority (Figure 5A). Many of these regions are historically undersampled due to research inequities and low numbers of academically trained mycological experts, and the outdated idea that EcM systems are rare in the tropics[24,43]. This follows the so-called biodiversity paradox, which describes the mismatch between the concentration of biodiversity in the tropics and the availability of human, institutional and financial resources primarily located at higher latitudes[44]. It is now recognized that a considerable number of plant species associate with EcM fungi in tropical forests[24] and these likely host a high number of undescribed species (Figure 3). For example, Central American tropical coniferous forests represent the southernmost distribution of native *Pinus* dominated forest and potentially represent relict populations isolated in tropical

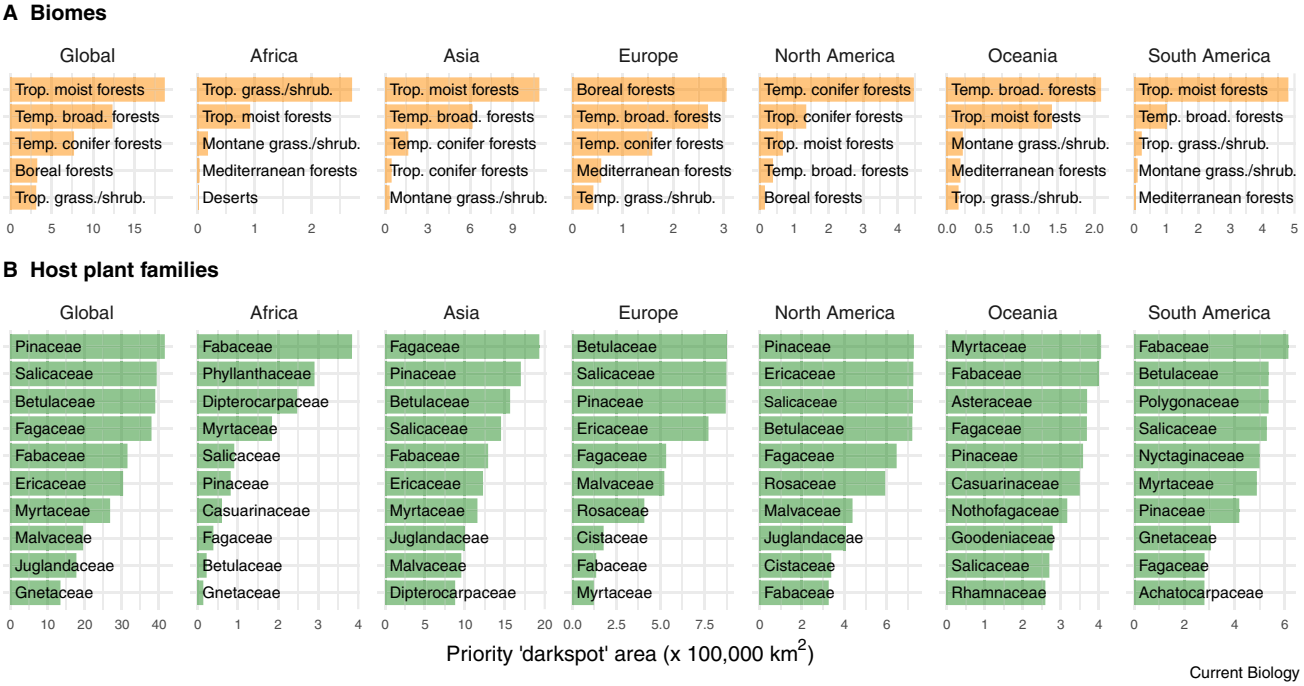

Figure 4. The distribution of land area that contains the upper 95th percentile of research priority scores (priority 'darkspot' regions, calculated from Figure 3D).

The darkspot area (km^2) that falls within each biome (A) and potential range of each host plant family (B) are shown at both the global level and within each continent. The top five biomes and top 10 host families are shown for each continent. Only plant genera classified as EcM host plants were included when calculating host plant family distributions, but both native and introduced ranges are included (see Supplemental information). Three biomes (tropical dry forests, flooded grasslands, and mangroves) were excluded due to them being inadequately represented (10 samples or less) in the training data used to build the dark taxa richness models. A map of darkspot regions is provided in Figure S4, and maps of host plant ranges in Figure S5. Trop. = tropical; temp. = temperate; broad. = broadleaf; grass. = grasslands; shrub. = shrublands.

mountainous areas. Temperate broadleaf forests are also of high research priority in some continents (Figures 4A and 5A), particularly southern-hemisphere regions in southeastern Australia, New Zealand, and Patagonia. A large proportion of these forests are dominated by Nothofagaceae and Myrtaceae host plants that comprise many ecologically, economically, and culturally important EcM tree genera[26,45–48]. These plants are also the focus of restoration efforts in these regions[49,50]. EcM fungi play an important role in restoration[8,51,52], and much more work is needed to fully characterize the fungal communities of these forests[28,53–57].

Much of the research priority darkspot area overlaps with the distribution of large well-known EcM host plant families, such as Pinaceae, Salicaceae, Betulaceae, and Fagaceae (Figure 4B). Many EcM-forming plant genera in the family Fabaceae also occupy darkspot zones, particularly in Africa, Oceania, and South America (Figure 4B). However, many of these families also dominate well-studied low-priority regions. Examining range of priority scores across the entire distribution of each plant family shows that plant families with the highest mean priority research metric scores are tropical families, such as Ticodendraceae (Central America), Dipterocarpaceae (predominantly Africa and southeast Asia) and Gnetaceae (South America, Africa and southeast Asia) (Figure 5B). Numerous endangered and critically endangered EcM fungal species are associated with the family Dipterocarpaceae in Asia and tropical lowland forests in the Amazon (e.g., *Austroboletus amazonicus*[58])[41]. Moreover, detailed studies on EcM associations from many Dipterocarpaceae genera and species, some of which have been classified as threatened themselves[59], are lacking[24,41]. Similarly, several *Gnetum* (Gnetaceae) plant species are also threatened, and studies investigating their EcM fungal symbionts are limited[60,61]. The associations that form between EcM fungi and Ticodendraceae hosts are even less well understood[24]. Although host plants from families like Ticodendraceae and Gnetaceae have small ranges and may not support as many EcM fungal species as other hosts[61], documenting their fungal communities, and any unique fungal species they host, is still of conservation importance. However, it is possible that these regions are emerging as research priority darkspots due to other host plants in the area. Regardless, these regions require further investigation and research. The EcM status of many plant lineages in families not typically known to form EcM associations, such as members of Rhamnaceae or Casuarinaceae in Oceania[62,63], also needs further investigation. This is especially important for endemic plant groups that are likely to host unique ECM fungal species[41].

Reducing the prevalence of darkspots will likely require a combination of strategies, involving targeted field surveys to identify and describe new fungal species, improving sequence reference databases to encompass a wider range of taxa (including yet-to-be-sequenced fungaria voucher specimens), and developing new ways to name and describe taxa from DNA alone without the need for physical material. We strongly advocate for further investment in field surveys in parallel with soil metabarcoding,

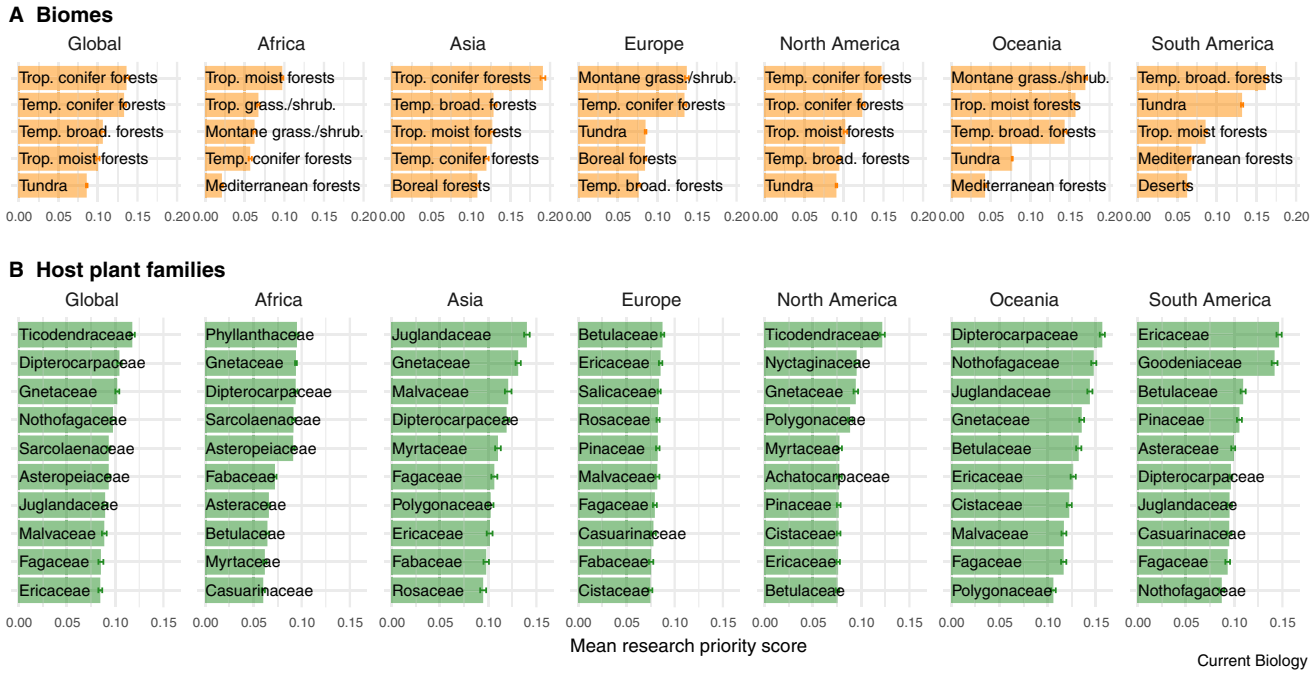

Figure 5. Mean dark taxa research priority metric scores within biomes and within the ranges of potential host plant families.
Bars show the mean (± one standard error) priority score (Figure 3D) from 1,000 randomly selected grid cells within each biome (A) or host family presence location (B). Only plant genera classified as EcM host plants were included when calculating host plant family distributions, but both native and introduced ranges are included (see Supplemental information). Within each continent, only biomes and host plant families that cover at least 150,000 km^2 of land area are shown. Three biomes (tropical dry forests, flooded grasslands, and mangroves) were additionally excluded due to them being inadequately represented (10 samples or less) in the training data used to build the dark taxa richness models. Maps of host plant ranges are provided in Figure S5. Trop. = tropical; temp. = temperate; broad. = broadleaf; grass. = grasslands; shrub. = shrublands.

to aid the compilation of more comprehensive reference sequence databases for EcM fungi[64,65]. As discussed earlier, metabarcoding datasets must be passed through the 'filter' of fungal reference databases to assign taxonomy and fungal guilds. Geographical research gaps create biases in reference databases that are currently skewed towards northern-hemisphere taxa[66,67]. For example, Corrales *et al.*[68] found that, when detecting EcM fungi via metabarcoding in Panama, adding sequences from locally collected voucher sporing bodies to the reference database that was used to assign taxonomy more than doubled the accuracy of taxonomic assignment (i.e., more than 50% of the environmental sequences had local specimens as their closest match). In particular, more focus on EcM fungal groups with inconspicuous sporing-body morphologies (i.e., truffloid, resupinate or cup-forming) is needed because most named taxa are those with typical 'mushroom' sporing-body types (Figure 1). Due to our reliance on taxonomic assignment (largely at genus level) to designate OTUs as EcM, our analyses of dark EcM taxa only represent 'known' dark taxa at generic level. Consequently, we have no information on 'unknown' dark EcM taxa that cannot be assigned taxonomy at the genus level, or those that have not yet been described in trait databases as EcM. Further traditional-style field surveys to collect, sequence, and confirm the EcM status of fungi present in understudied regions are therefore critical for uncovering unknown taxa and reducing reference database biases[65].

In addition to generating data through field surveys, furthering efforts to sequence named specimens from fungaria collections could substantially increase the percentage of fungal sequences that can be assigned to a species name and classified as EcM, as exemplified by our Australian case study (Figure 2)[65,69–71]. DNA extraction and sequencing of old specimens can be challenging due to DNA degradation resulting from time and preservation conditions, but high-throughput sequencing methods can help overcome issues[70]. Many herbaria are now developing large-scale specimen barcoding projects[72]. However, wide disparities remain between regions that house specimens and contain resources for sequencing, and regions where the specimens were collected[73]. Many of the sequencing projects include little to no involvement of the researchers and institutions from where the specimens originate, and these researchers can later struggle to gain access to the specimens through institutional loan exchange. The colonial legacy embedded within herbaria and fungaria collections is beginning to receive some attention[73]. Future work to collect, curate and sequence specimens should consider how to improve equitability and protect and benefit from indigenous cultural knowledge.

Describing species based on DNA alone has been highly debated[33,36,44,74], but it is becoming evident that the abundance of dark taxa can only be reduced by developing new approaches for scenarios where no physical specimens are available[33]. The hesitation toward DNA-based species description is partially due to the abundance of low-quality uncurated sequences in public databases[75]. However, advancements, such as robust, reproducible guidelines for data analysis and the reduced price of long-read metabarcoding that allows for more accurate phylogenetic

reconstruction and the production of high-resolution fungal taxonomic databases[76–78], allow for a vision of the future where some dark taxa are described solely through robust molecular analyses.

Naming and describing species has important implications for fungal conservation and the evaluation of extinction risk[64]. The IUCN Red List of Threatened Species[79] is an important tool used by many conservation decision-makers at local and global scales[14], but current criteria only allow named species to be assessed[80]. In addition, these assessments require information about population size, population trends, and geographic distribution based on specimen-only information, a carry-over from initial criteria established for macroscopic animals and plants. As of 2022, only 276 EcM fungal species have undergone an IUCN Red List assessment, of which 26% were determined to be globally threatened[14]. Consequently, we currently lack information on the threat status of potentially over 99% of EcM fungal species. A recent case study in Estonia used information from environmental DNA (eDNA) samples to improve the range and population parameters of Red Listed EcM fungal species[81], and such methods could be used more widely to aid the classification of species that are difficult to physically detect. Many challenges remain regarding the inclusion of fungal species that are detected only from eDNA in Red List assessments. Methods for defining and tracking eDNA-detected taxa so that they can be unambiguously referenced, such as the Species Hypotheses in UNITE[37,82], are promising approaches that could be explored to evaluate the threat status of such taxa in the future. However, calculating the number of mature individuals and estimating population sizes and ranges based purely on eDNA remains a challenge[80,81].

Conclusions

The dark taxa problem is a major conservation concern for EcM fungi. The fact that 79–83% of EcM fungal OTUs in global fungal sequencing databases do not match species-level assignments highlights the vast diversity of EcM fungi yet to be uncovered and formally described. Naming these species is critical for conducting species-threat evaluations and including species in the IUCN Red List and legislations for their conservation[14,80]. Without overcoming this barrier, many EcM species may be lost before we understand and document their value for ecosystems and global biodiversity. In particular, the high deforestation rates affecting many EcM systems that contain research priority regions identified in this study are of serious concern[41,83–85]. Protecting intact ecosystems, and the biodiversity and species interactions they contain, is among the best, most cost-effective of the natural climate solutions[7]. As one of the major drivers of forest carbon dynamics[6,86], EcM fungi form a critical part of these solutions.

The dark taxa research priority regions identified by our review (Figures 3–5) provide an indication of where to focus future research to improve knowledge gaps. Uncovering and naming dark taxa in these regions will involve a combination of collecting and sequencing field specimens[64,65], conducting large-scale DNA barcoding of identified but unsequenced species in fungaria collections[69], and developing standardized methods to delimit and describe fungal taxa from sequence data without physical specimens[36]. The prevalence of EcM priority darkspots in tropical regions supports previous calls for the expansion of EcM fungal research activities in tropical systems[24,41]. However, many temperate regions are also of high priority, especially in host plant groups endemic to specific regions, and dark EcM fungal taxa dominate communities across all parts of the world (Figure 1). The abundance of dark taxa is of global concern not only for EcM fungi — guidance for directing research efforts to uncover undescribed species is needed for many organisms, even plants[87]. However, despite the influence of fungi on ecosystem structure and function[6,88], and their importance for many other aspects of planetary health[89], they remain particularly underdescribed compared to other eukaryotic taxa[71]. Closing this research gap for EcM and other fungal groups is critical for understanding and protecting present and future biodiversity and ecosystem health.

DATA AVAILABILITY

Data used to conduct analyses and create figures are available at https://doi.org/10.6084/m9.figshare.28830371. These include:1) data from GlobalFungi v.5 used to create Figure 1; 2) the methods and list of fungal species extracted from the Catalogue of Life; 3) the R code used to generate the list of Australian species and create Figure 2; 4) geospatial layers of dark taxa metrics, R code used to create Figures 3–5, and R code used to conduct the SHAP analysis; and 5) the names and Global Biodiversity Information Facility (GBIF) download DOIs of EcM host plant genera and R code used to create the host plant family distribution maps.

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DECLARATION OF INTERESTS

C.T. is a member of the UNITE management board. P.K., P.D., and T.V. are creators of GlobalFungi, which is a service of the ELIXIR-CZ infrastructure. E.T.K. is a member of *Current Biology*'s advisory board.

SUPPLEMENTAL INFORMATION

Supplemental information including five figures, two tables and methodological details can be found with this article online at https://doi.org/10.1016/j.cub.2025.03.079.

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