

An assessment of biodiversity data shortfalls for ectomycorrhizal fungi in Canada

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Abstract

Ectomycorrhizal (EcM) fungi play critical roles in global carbon and nutrient cycling. In Canada, vast forest ecosystems dominated by EcM trees are experiencing rapid environmental and anthropogenic change that threaten the symbiosis. Here we quantify and discuss seven biodiversity data shortfalls that constrain our knowledge of EcM fungi in Canada, and impede the development of effective conservation plans. We based our assessments on publicly accessible sequence data from GlobalFungi and GenBank, filtered to samples within Canada. We found **2,805** unique EcM fungal species hypotheses, of which only **58%** could be assigned a species name. The **1,405** unique named species comprised **124** genera, roughly **38%** of the genera known globally. An overriding shortfall is that samples are lacking from large areas of the country, and with respect to both abiotic and biotic niche space. For example,

host information is missing or unresolved for **73%** of the named species, and among **1,002** species georeferenced in GlobalFungi, 50% have **5** or fewer occurrence records nationally. Although our assessment highlights large biodiversity data shortfalls for EcM fungi in Canada, it also provides the foundation for a coordinated, pan-Canadian sampling effort that can be optimized to address multiple shortfalls simultaneously.

Keywords

ectomycorrhizal fungi, species hypotheses, biodiversity shortfalls, fungal conservation, DNA metabarcoding, boreal forest

Introduction

Large scale biodiversity data are often incomplete and unrepresentative, owing to several shortfalls that limit ecological and evolutionary knowledge and affect land stewardship (conservation, management, restoration) ([Hortal et al. 2015](#)). These shortfalls include gaps in knowledge about formal species descriptions (Linnean), species' geographic distributions (Wallacean), abundance and population dynamics (Prestonian), evolution of species lineages (Darwinian), species traits and functions (Raunkiaeran), abiotic tolerance limits (Hutchinsonian), and species interactions (Eltonian).

Significant progress has been made in addressing these shortfalls for animals and plants, yielding extensive new data to inform conservation and management strategies. For example, billions of new species observations made over the past decade have enabled efforts to estimate

the geographic ranges of nearly all vertebrates, many plant species, and even an increasing number of arthropods (www.inaturalist.org; [Lumbierres et al. 2022](#); [Daru 2024](#); [Daru 2025](#); [Moulatlet et al. 2025](#)). The IUCN Red List, which curates a variety of biodiversity data to perform extinction risk assessments, has now assessed over 90% of extant vertebrates and provides a wealth of standardized information on species geographic distributions, habitat preferences, elevational ranges, threats, and demographic trends ([IUCN 2025](#)). In addition, global efforts to collate data on species functional traits, genomes, and biotic interactions have produced or expanded large open access databases ([Poelen et al. 2014](#); [Kattge et al. 2020](#); [Schmidt et al. 2020](#); [Karachaliou et al. 2025](#); [Matias Rodrigues et al. 2026](#)), enabling researchers to predict vertebrate food webs ([Hao et al. 2025](#)) and trait-environment relationships ([Li and Prentice 2024](#)) at global scales, resolve megaphylogenies ([Smith and Brown 2018](#); [Upham et al. 2019](#); [Kawahara et al. 2023](#)), and track widespread genetic response to ongoing global change ([Shaw et al. 2025](#)). Even some of the most challenging shortfalls such as the Prestonian (i.e., population abundance and dynamics) are being filled. Updated global abundance tracking datasets like BioTIME ([Dornelas et al. 2025](#)), PREDICTS ([Hudson et al. 2017](#)), and the Living Planet Index ([Ledger et al. 2023](#)) are routinely relied on to detect and attribute biodiversity change, and taxa-specific efforts have identified widespread declines in what are historically data deficient groups like butterflies ([Edwards et al. 2025](#)) and other insects ([Van Klink et al. 2024](#)). These efforts to address major biodiversity data shortfalls, aside from increasing our biodiversity knowledge writ large, have directly benefited both applied and predictive research to inform the conservation of Earth’s biodiversity amid accelerating global change ([Steinke et al. 2025](#)).

Far less progress has been made, however, in addressing these shortfalls for fungi (Mueller et al. 2022), including ectomycorrhizal (EcM) fungi. EcM fungi form obligate associations with the majority of tree stems on earth (Steidinger et al. 2019) as well as many shrub and herbaceous species that are widespread in alpine and tundra ecosystems globally (e.g. *Salix*, *Betula*, *Bistorta*, *Dryas*; Timling et al. (2012)). EcM fungi mediate soil nutrient and carbon cycling (Fry et al. 2019; Hawkins et al. 2023), are associated with forest productivity (Anthony et al. 2022), and promote expansion of plant ranges (Nuñez et al. 2009; Nuñez and Dickie 2014; Policelli et al. 2023). EcM fungi also are sensitive to shifts in forest management (Tomao et al. 2020; Lindahl et al. 2026) and climate change (van Nuland et al. 2024). Despite their importance and sensitivity to the drivers of global change, fungi have only recently been considered in conservation assessments or plans (Bazzicalupo et al. 2022; Niskanen et al. 2023; van Galen et al. 2025; van Nuland et al. 2025; Qin et al. 2026). While biodiversity assessments have become increasingly common and our ability to accurately predict global distributions of fungi is improving (Tedersoo et al. 2010; Tedersoo et al. 2012; Tedersoo et al. 2014; Větrovský et al. 2019; Větrovský et al. 2020; Tedersoo et al. 2021; Tedersoo et al. 2022; Qin et al. 2023; Abrego et al. 2024; Delhaye et al. 2024; Ovaskainen et al. 2024; van Galen et al. 2025; van Nuland et al. 2025; Qin et al. 2026), there remains a considerable gap in ecologically and evolutionarily relevant data required to inform broadscale conservation (Cameron et al. 2018; García-Roselló et al. 2023; Li et al. 2023; Mikryukov et al. 2023; Qin et al. 2023; Copot et al. 2024).

With the aim of inspiring and directing progress to gather the biodiversity data required to inform conservation and management, here we assess each of seven key shortfalls for

EcM fungi in Canada. Canada hosts around 12% of the Earth’s forests (spanning 40% of Canadian land, an area equivalent to six times the size of France; ([Natural Resources Canada 2022](#))), 24% of Earth’s remaining intact ecosystems ([Allan et al. 2017](#)), and 20% of global soil carbon stocks in the top 1m ([Sothe et al. 2022](#)). With the majority of trees in Canada requiring symbiosis with EcM fungi (cf. [Taylor et al. 2000](#); [Steidinger et al. 2019](#)), and large expanses of arctic tundra hosting EcM plants ([Timling et al. 2012](#); [Parisy et al. 2025](#)), the conservation of EcM fungi across Canada carries planetary consequences for biodiversity and critical ecosystem services like carbon cycling.

For each of the seven shortfalls, we discuss past progress in gathering data on EcM fungi, and also consider data requirements that distinguish fungi from other groups like animals and plants, when appropriate. Finally, we estimate the breadth of current data gaps, laying a foundation for future assessments to track progress on addressing the shortfalls.

Data and Methods

Detailed methods are provided in the Supplemental Materials SM1. Briefly, our assessments draw on publicly accessible fungal sequence records from GlobalFungi v5 [<https://global-fungi.com/>; Větrovský et al. ([2020](#))] and NCBI GenBank (accessed 29 June 2026), via targeting internal transcribed spacer (ITS) sequence of the small-subunit ribosomal RNA. GlobalFungi is a curated database of community-survey amplicon data, consistently processed but aggregated from studies with varied protocols and mostly derived from soil samples; GenBank is a general-purpose repository spanning diverse studies and sampling efforts, including targeted root sampling. For GlobalFungi we retained ITS2 and ITS1+ITS2 ampli-

con records and used the pre-assigned fungal species hypothesis (SH) codes and taxonomy that GlobalFungi provides from UNITE v10 [<https://dx.doi.org/10.15156/BIO/2959332>; 4 April 2024; Nilsson et al. (2019)]. In their pipeline, representative sequences meet a 98.5% similarity threshold for each SH. As noted by van Galen et al. (2025), GlobalFungi includes samples from the Global Soil Mycobiome Consortium (Tedersoo et al. 2021), 12 of which are located in Canada. For processing GenBank records, we aimed to match the GlobalFungi pipeline as closely as possible. We retrieved all fungal ITS records for Canada and used ITSx (Bengtsson-Palme et al. 2013) to detect and extract the ITS2 sub-region, discarding records with no detectable ITS2 or with an ITS2 fragment shorter than 100 bp. This retains only records carrying ITS2, matching the ITS2/ITSboth barcoding_region restriction applied to GlobalFungi. We assigned SH codes and taxonomy to the extracted ITS2 fragments using the same UNITE release (above) and vsearch v2.30.4 (Rognes et al. 2016) at 98.5% global-alignment identity. Because GenBank’s content and assigned taxonomy change continuously, all GenBank-derived counts reported here reflect the state of the database on the access date; the retrieved record set is archived with the code and data (YYY) to ensure reproducibility. We combined the GlobalFungi and GenBank-based SH and taxonomy datasets, then assigned the sequences to EcM guilds based on genus-level guild assignments in the FungalTraits database (Pölme et al. 2020). For any analyses that required georeferenced samples, we binned sample coordinates to three decimal places of latitude and longitude (~100 m at temperate latitudes), and these are henceforth referred to as sample “sites”. Methodological details for each shortfall analysis are provided in the Supplemental Materials SM1. Code for our entire workflow is publicly available (XXX) and aims to be computationally

reproducible. All input and output data are also archived and publicly available at [YYY](#).

Table 1 summarizes the key properties of our Canada-specific GlobalFungi dataset, alongside comparator figures derived from all GlobalFungi samples worldwide. Table 2 summarizes relevant information about the GenBank data. Figure 1 shows the locations of both GlobalFungi and GenBank samples in Canada (**486** GlobalFungi and **1,137** GenBank unique locations).

Linnean shortfall

The Linnean shortfall is operationalized as the discrepancy between the number of species that have been formally described and the number of species that exist or have ever existed ([Lomolino 2004](#)). The magnitude of this shortfall is difficult to quantify for EcM fungi, since it depends on the total number of species (which is generally unknown) as well as our ability to delineate and describe new species ([Hortal et al. 2015](#)). While EcM fungi have been traditionally delineated based on morphological characteristics using approaches like microscopy, the advent of genetic sequencing and high-throughput technologies completely transformed fungal systematics ([Miralles et al. 2020](#)) and greatly increased species description rates ([Cunningham et al. 2024](#)). These molecular techniques and the corresponding species concepts (referred to as species hypotheses) have been widely adopted; in 2018 over 94% of studies describing new fungal taxa relied on molecular techniques ([Miralles et al. 2020](#)). [Cunningham et al. \(2024\)](#) predict that Kingdom Fungi could see an additional 64,000–74,000 newly described species by the end of the century, thanks to high-throughput workflows. A recent, conservative estimate of the global diversity of EcM fungi is 25,499 (+/- 54) – 55,500 (+/- 518) species based on sequence datasets from GlobalFungi and the Global Soil

167 Mycobiome consortium, respectively ([van Galen et al. 2025](#)). However, these estimates
168 represent lower-bound estimates and are highly uncertain for reasons discussed below.

169 Despite this progress, the dependency on molecular species concepts means that almost all
170 newly identified species remain undescribed, or “dark”, taxa. Many identified species have
171 only been detected a handful of times, often solely by molecular barcoding, and often only by
172 single sequences ([Cazabonne et al. 2024](#)). In both GlobalFungi ([Větrovský et al. 2020](#)) and
173 the Global Soil Mycobiome ([Tedersoo et al. 2021](#)) databases, a minority of taxa (< 25%)
174 are assigned species-level names. As a result, when it comes to assessing existing knowledge
175 for fungi, global assessments like the IUCN Red List have evaluated only a tiny fraction of
176 described species ([Mueller et al. \(2022\)](#) estimated 0.4%, a percent that is now larger but still
177 much less than 5%). The requirement that species be described before qualifying for Red List
178 assessment represents a serious bottleneck, and contributes to why fungi are the most poorly
179 assessed group across all Eukaryotes excluding protists ([Niskanen et al. 2023](#); [IUCN 2025](#)).

180 Quantifying and addressing the Linnean shortfall for EcM fungi has also been hindered by
181 the lack of standardized and integrated global taxonomic repositories. Unlike for vertebrates
182 and plants, comprehensive and harmonized taxonomies for EcM fungi rely on databases that
183 combine more traditional species concepts (e.g., morphological) with modern approaches
184 (e.g., molecular). Fortunately, progress has been made on this front. Originally released in
185 2003, the publicly accessible UNITE database, which offers standardized fungal taxonomies
186 based on molecular records, has grown rapidly over the past decade ([Abarenkov et al. 2024](#)).
187 For example, since 2014, the number of fungal species (species hypotheses) represented in
188 UNITE has more than tripled. Importantly, as of 2019, UNITE has started linking molecular

189 sequences (e.g., metabarcodes) to standardized species hypotheses harmonized against the
190 Global Biodiversity Information Facility (GBIF) backbone taxonomy, thereby facilitating
191 their use in informing policy. These advances have been instrumental in standardizing species
192 concepts across EcM fungal research, increasing reproducibility and reusability of data, and
193 facilitating crosstalk with other global databases that leverage UNITE.

194 Our assessment of the Linnean shortfall for EcM fungi in Canada starts with a basic accounting
195 of EcM fungal taxonomic data. We emphasize at the outset that the sampling footprint
196 in Canada is geographically biased and clustered (Figure 1), especially with respect to
197 the potential abiotic and biotic niche space of EcM fungi (see Hutchinsonian and Eltonian
198 shortfalls sections), so all results should be interpreted with this in mind. Within the combined
199 GlobalFungi and GenBank dataset there were **2,805** unique UNITE SH codes, and the **2,062**
200 codes from the Canada-specific GlobalFungi dataset represents **16%** of the **12,970** EcM
201 fungal SH codes within the entire GlobalFungi dataset.

202 Among the **2,805** unique EcM fungal SH codes, **1,621** (**58%**) were assigned to a UNITE
203 named species, and **1,405** of these were unique (some SH codes are assigned to the same
204 species epithets). Of the named SH codes, **681** were shared between GlobalFungi and
205 GenBank, **442** were unique to GlobalFungi, and **498** were unique to GenBank.

206 The **1,405** named species comprise **124** unique genera, which is around **38%** of the estimated
207 **327** EcM fungal genera known globally.

208 The difference between the **2,805** unique SH codes and the **1,621** with names attached can be
209 considered a form of “dark diversity”, specific to our dataset: about **42%** (**1,184**) of codes in
210 the combined GlobalFungi + GenBank dataset have no match above the UNITE species-level

211 assignment threshold, and thus cannot be assigned a species name at this time. This figure is
212 not directly comparable to the OTU-level dark diversity estimates recently reported by van
213 Galen et al. (2025), as they used a custom bioinformatics pipeline that, for example, used a
214 97% similarity threshold for taxonomic assignment rather than the 98.5% used by GlobalFungi
215 (and by us). Nevertheless, for reference, we (i) provide a Canada-specific map of dark EcM
216 fungal richness, adapted from the geospatial model of van Galen et al. (2025) (Supplemental
217 Materials Figure S1), and (ii) accessed their sample-specific estimates of the proportion of
218 OTUs unassigned to species (available at: <https://doi.org/10.6084/m9.figshare.28830371>),
219 which is analogous to our measure of dark diversity used above, and calculated an average of
220 57% among their 1,368 Canadian forest and tundra samples (the most comparable subset to
221 ours).

222 The Linnean shortfall for EcM fungi in Canada is clearly substantial, but also challenging to
223 estimate accurately because the available sample data are (i) so few relative to the target land
224 area, (ii) geographically clustered, and (iii) restricted to a small portion of the potential EcM
225 fungal niche space (Figure 1; see also Hutchinsonian, Eltonian, and Wallacean shortfalls).
226 The same limitations apply to the full GlobalFungi dataset (see global sampling density map,
227 Figure S2), upon which the estimate of 25,500–55,500 EcM fungal species globally was based
228 (van Galen et al. 2025).

229 Moreover, and the reason we do not provide estimates of sample coverage ($\hat{C}$) or EcM fungal
230 taxon richness in Canada, the available estimation methods make assumptions that cannot
231 be met by GlobalFungi or comparable community eDNA data. Specifically, Chao1, Chao2,
232 and $\hat{C}$ (Chao 1987; Chiu and Chao 2016) all derive from the same underlying quantities: the

number of rare taxa observed exactly once (f_1) or twice (f_2) in a sample (Chao1), or across sites (Q_1 and Q_2 , the incidence-based analogue, for Chao2). Coverage is, in its simplest form $\hat{C} = 1 - f_1/n$, where “ n ” represents the total sample reads. All of these methods assume that f_1 and f_2 (or Q_1 and Q_2) faithfully reflect how much rare diversity remains undetected in the true underlying community. This assumption cannot be met with GlobalFungi data because, when estimating richness of specific taxonomic guilds (like EcM fungi), the guild-specific read count of every GlobalFungi sample is the outcome of an upstream taxonomic (EcM fungal genus) filtering step applied to each sample’s whole-community sequencing output. To illustrate, across the **1,388** soil-only Canadian GlobalFungi samples with at least one EcM fungal read, a median of **94%** (IQR **79–99%**) of total sample sequencing depth is discarded by the EcM filtering step before any richness estimator would see the data (**Figure S3**). It is therefore problematic to assume that the f_1 and f_2 (or Q_1 and Q_2) values that come from the remaining EcM fungal reads represent what the methods assume they represent. Notably, this problem is separate from the widely-recognized challenges that PCR / sequencing methodologies pose for diversity estimation ([Boshuizen and Te Beest 2023](#); [van Galen et al. 2025](#)), and from other problems highlighted in a recent study ([Elena Schmitz and Rahmann 2025](#)).

Wallacean shortfall

The Wallacean shortfall encompasses our lack of knowledge of where species occur (i.e., species geographic distributions). For fungi, the Wallacean shortfall was once considered negligible, as longstanding hypotheses predicted that microbial species were ubiquitous across

space (reviewed in [Green et al. 2008](#)). The advent of molecular methods enabling whole-community sequencing of fungal diversity, however, has shown the opposite. Field surveys detecting putatively endemic fungi are common ([Talbot et al. 2014](#); [Kivlin and Hawkes 2020](#); [Bazzicalupo et al. 2022](#)), including in Canada or western North America ([Kranabetter et al. 2018](#)), and studies have demonstrated geographically constrained dispersal for most EcM fungal spores ([Peay et al. 2012](#); [Abrego et al. 2024](#)). Thus, although some EcM fungal taxa may be distributed over large areas (e.g. circumpolar; [Geml et al. \(2015\)](#); [Noffsinger et al. \(2026\)](#)), many more taxa likely exhibit more constrained distributions, mirroring what is observed among other taxonomic groups ([Brown et al. 1996](#)).

In recent years, species distribution models (SDMs), which relate species occurrences to abiotic and sometimes also biotic predictors to estimate species geographic distributions, have increasingly been used to predict the potential distributions of EcM fungi ([Qin et al. 2023](#); [van Nuland et al. 2025](#); [Qin et al. 2026](#)). For example, in the most comprehensive modeling of EcM fungal species distributions thus far, [Qin et al. \(2026\)](#) used Hierarchical Modelling of Species Communities (HMSC) ([Tikhonov et al. 2020](#)), informed by worldwide GlobalFungi soil samples binned into 30 arc-second grid cells ($\sim 1 \text{ km} \times 1 \text{ km}$), to model the distributions of 2,669 EcM fungal species hypotheses (based on a tailored pipeline that used OTUs composed of sequences clustered to 97% similarity). This equates to approximately 10% of the estimated minimum number of EcM fungal species globally (see above). [Qin et al.](#) used a minimum occurrence cutoff of 31 grid cells, which is consistent with common recommendations (e.g. [van Proosdij et al. 2016](#)).

For comparison, and serving as our first, straightforward measures of the Wallacean shortfall,

we highlight that among the **2,062** unique SH codes that were georeferenced in Canada (from GlobalFungi samples exclusively), **969 (47%)** have fewer than 30 occurrence records globally, while **1,855 (90%)** have fewer than 30 occurrence records within Canada (Table 3).

Our second approach to quantifying the Wallacean shortfall focuses on the frequencies of occurrence (or detections) of EcM fungal taxa more generally. Figure 2 shows histograms of those frequencies, enumerated within Canada (panels a and b) and globally (c, d) for both named species (a,c) and SH codes (b, d), again limiting analyses to the GlobalFungi dataset and sites equating with 30 arc-second grid cells. The histograms show that most taxa have been detected in few sites whereas a few have been detected in many sites. However, unlike for macro-organisms like mammals and birds, sampling is currently insufficient to estimate the true frequency distributions of occurrence, at any spatial scale, for EcM fungi native to Canada, even among taxa that are putatively endemic to Canada or western North America (Kranabetter et al. 2018). Together with Figure 2, the numbers presented in Table 3 provide a straightforward picture of the data limitations that underlie the substantial Wallacean shortfall for EcM fungal taxa in Canada.

Prestonian shortfall

Of the seven shortfalls, the Prestonian, which represents our lack of knowledge about the abundance of species, is often most pronounced (Hochkirch et al. 2021). This is especially true for fungi, since abundance trends and population dynamics over space and time remain mostly unknown (Koch et al. 2018; Martinović et al. 2021). Evidence from vertebrate communities suggests that most populations require relatively continuous monitoring over

decadal timespans to provide robust estimates of abundance change (White 2019). In contrast, fungi often exhibit fast generation times and rapid community turnover (Ning et al. 2021); thus, resolving population dynamics likely requires fungal-specific sampling approaches (Tóth and Barta 2010). Short-term population dynamics (resolved via sequencing host root tissue) can incorporate stable-isotope probing via isotopically-labeled carbon substrates or water to track actively growing EcM fungal taxa (Koch et al. 2018) on the order of days to weeks. Fungal turnover in community composition during intermediate time scales can be addressed via repeated sampling (Kivlin and Hawkes 2020; Martinović et al. 2021) and may be important for understanding succession or response to disturbances. Long-term population dynamics meant to indicate factors like extinction risk are likely more complicated and could involve the monitoring of spore deposition (Kranabetter et al. 2026) or spore banks which can sustain viable propagules for many years (Nguyen et al. 2012; Pither and Pickles 2017), identifying genetic structures of fungal populations through whole genome sequencing (Vincenot and Selosse 2017), and/or incorporating phylogenetic methods to detect radiations versus extirpations (Beaulieu and O'Meara 2016). Unfortunately, studies with repeated sampling designs over both short and long time scales are uncommon for EcM fungi. Correspondingly, estimates of fungal persistence within a single location, much less across a species range, are exceedingly rare. Even when samples are collected repeatedly over time (Averill et al. 2019), the destructive nature of chemical assays, microscopic assays, and DNA sequencing of soils and/or roots means that temporal estimates of fungal persistence must account for a spatial component as well (Carini et al. 2020).

Unlike other taxa, approaches taken to measure the abundance of EcM fungi vary considerably,

which makes aggregating data across regions, species, and studies challenging. For example, fungal abundance can be represented as estimates of total biomass detected from chemical signatures (e.g., phospholipid fatty acids in (Olsson 1999); ergosterol in (Ekblad et al. 1998); biomass inferred from carbon use efficiencies in (Jørgensen et al. 2025)) or microscopic counts of EcM fungal root tip colonization (Soudzilovskaia et al. 2015). Minirhizotron tube measurements that track mycorrhizal fungal growth over time are rare but those estimates suggest that EcM fungal hyphae turn over on the order of weeks to months (Treseder et al. 2010; Allen and Kitajima 2013; Defrenne et al. 2021). Estimates of fungal abundance based on DNA sequences (e.g., qPCR and next generation sequencing) are often biased by unequal target gene copy number (Taylor et al. 2016), PCR bias against certain EcM fungal clades (Janowski and Leski 2023), and the fact that the DNA concentration of fungal biomass varies across growthforms (e.g., aseptate arbuscular mycorrhizal fungi). Relative abundance estimates of EcM fungi compared to total read counts suffer from the fact that the former are a simplex (sum to 1) and often are not corrected for total unequal sequencing depth among samples (see above), sequencing technologies, and/or gene regions (Weiss et al. 2017; Quinn et al. 2019). In the future, estimates of fungal abundance with deeper next generation sequencing may more closely approximate biomass, and targeting sequencing of whole genomes may allow us to estimate the number of individuals in a sample more reliably. Because it is difficult to estimate the number of fungal individuals in a sample, it is nearly impossible to model population dynamics of most EcM fungal lineages. Demographic models require tracking individuals through survival, growth, and reproduction over time. But, even when sampling of EcM fungi is repeated at monthly time intervals, the same individuals may

not be detected. For the purpose of conservation monitoring, Dahlberg and Mueller (2011) propose fruiting body counts as an operational definition for population size; however, to be reliable, they suggest that EcM fungal population trends should be assessed over at least 50 years. Instead, a focus on genetic diversity over time and among populations across an EcM fungal species' range may be the most promising avenue forward to understand persistence of fungal populations (Douhan and Rizzo 2005; Abe et al. 2024). Using this approach, Golan and colleagues demonstrated that genets of the EcM fungus *Amanita phalloides* can persist for more than three decades across multiple locations (Golan et al. 2024). As longitudinal data accumulate for EcM fungi, future studies can begin to incorporate changes in abundance into population demographic models, at least as stage-based estimates of population growth and longevity.

To quantify the Prestonian shortfall for EcM fungi in Canada, we used the BioTIME database, which represents the largest and most comprehensive effort to compile abundance data (Dornelas et al. 2025). Yet, despite decent coverage for other kingdoms, BioTIME includes only a single study that tracks fungal abundance over time based on data collected from Bavarian Forest National Park in south-eastern Germany (Thorn et al. 2016) over a period of 5 years. Of the **110** fungal species included in that study, two have been recorded in Canada. Here, we operationalize the Prestonian shortfall as the percentage of the **1,405** named EcM fungal species present in Canada that lack some form of abundance time series data recorded anywhere on Earth. We estimate this shortfall to be **1,403** out of **1,405**, or **99.9%**.

Darwinian shortfall

The Darwinian shortfall describes the lack of knowledge about how species and their traits have evolved (Diniz-Filho et al. 2013). Traditional species concepts have been especially problematic in microbes as criteria for delineating species differ among groups of fungi (Cai et al. 2011; Kwon-Chung et al. 2017; Xu 2020; Janowski and Leski 2023); and previously agreed-upon phylogenies can rapidly and drastically shift (Tedersoo et al. 2010; Tedersoo et al. 2014; Niskanen et al. 2023).

The hyperdiversity and short generation times of microorganisms (including fungi) can make resolving phylogenetic relationships more challenging than in other organisms that lack abundant horizontal gene transfer, multiple genomes, high phenotypic plasticity, or increased potential rates of mutation accumulation (James et al. 2020; Xu 2020; Li et al. 2021; Janowski and Leski 2023). Although the discussion of the evolution of the EcM fungal habit has been ongoing since at least the 1980s (Malloch et al. 1980; Read 1991), the topic has continuously been revisited and revised (Hibbett et al. 2000; Matheny et al. 2009; Sato 2024). The timing and geographic origins of the original emergence of EcM fungi are another area that has been limited by the lack of available taxonomic data and has been much discussed and summarized (Ryberg and Matheny 2011; Looney et al. 2016; Niskanen et al. 2023; Sato 2024). Despite this ongoing research, there is still a lack of agreement on how many times the EcM symbiosis has independently evolved - from as few as 27 (Sánchez-García et al. 2020; Cai et al. 2024; Sato 2024) to upwards of 80 times (Tedersoo et al. 2010; Tedersoo and Smith 2013; Sánchez-García et al. 2014; Tedersoo et al. 2014; Tedersoo and Smith 2017; Strullu-Derrien et al. 2018) - and which fungal taxa truly belong in the EcM lineage

([Tedersoo and Smith 2013](#); [Tedersoo and Smith 2017](#)), and even which genes should be taken into account when building phylogenies ([Sato et al. 2017](#); [Xu 2020](#); [Li et al. 2021](#); [Cai et al. 2024](#)).

The limited taxonomic data on EcM fungi necessitates a large amount of extrapolation and reliance on closely related taxa to infer relationships ([Tedersoo et al. 2010](#); [Tedersoo and Smith 2013](#)). Many attempts to delineate phylogenies have been focused on single lineages ([Lebreton et al. 2022](#); [Kobayashi et al. 2023](#); [Cai et al. 2024](#); [Noffsinger et al. 2026](#)), and there have been limited attempts to synthesize across studies ([Tedersoo et al. 2010](#); [Tedersoo and Smith 2013](#); [Tedersoo and Smith 2017](#)), impeding a broader understanding of the overall evolution. Additionally, the majority of attempts have been biased towards the Agaricomycetes class ([Sánchez-García et al. 2014](#); [Tedersoo et al. 2014](#); [Cai et al. 2024](#); [Sato 2024](#)).

Given their symbiotic lifestyle, EcM fungal diversification could be linked to host diversification and host switching ([Sato et al. 2017](#); [Wilson et al. 2017](#); [Strullu-Derrien et al. 2018](#); [van der Linde et al. 2018](#); [Sato and Toju 2019](#); [Cai et al. 2024](#); [Sato 2024](#)). Time since major disturbance, such as the Cretaceous–Paleogene (K–Pg) boundary or global cooling events in the Late Paleogene–Neogene ([Strullu-Derrien et al. 2018](#)), and functional traits, such as fruiting body forms ([Varga et al. 2019](#); [Sánchez-García et al. 2020](#)) or the formation of spores ([Sato 2024](#)), are also potential drivers of EcM fungal diversification. Due to a lack of available taxonomic data and the difficulties in resolving fungal phylogenies, function or trait-based approaches might be a substitute for evolutionary relationships ([Aguilar-Trigueros et al. 2015](#); [Zanne et al. 2020](#)). However, context-dependence and the lack of agreed upon

life-history strategies and functional groups for EcM fungi, combined with the fact that there is not a standardized set of morphological or functional data collected in EcM studies (see Raunkiaeran shortfall section), has made this approach challenging. For an approach to be viable, studies must move beyond metabarcoding to investigate the functional roles of EcM fungi, and a standardized framework for studying and recording trait data must be implemented.

More recently, there has also been growing interest in the rate of modern EcM fungal evolutionary processes (Dauphin and Peter 2024). While there is evidence of fungal evolution on ecologically-relevant timescales, such as adaptation to heavy metals (Bazzicalupo et al. 2020) as well as temperature and precipitation (Sønstebo et al. 2022), these traits are still relatively poorly characterized, inhibiting effective conservation strategies (Dauphin and Peter 2023; Dauphin and Peter 2024). This is another area that will require studies to move away from metabarcoding and towards whole genome sequencing to better investigate speciation, as well as changes in functional genes that barcoding cannot capture (Shelton et al. 2026). For example, *Suillus* species are emerging as model organisms due to the availability of a high quality genome and detailed knowledge of life cycles (Branco et al. 2015; Bazzicalupo et al. 2020; Dauphin and Peter 2024; Lofgren et al. 2024). Overall, fungi lag behind in whole genome studies and, while there has been an increasing number of whole fungal genome sequencing initiatives, the number for EcM fungi in particular is still low with an estimated 0.5% of the 50,000 known EcM fungi genomes sequenced (Dauphin and Peter 2024).

An increase in whole genome sequencing, as well as improved trait characterization, are necessary to move beyond a single model organism and fully capture the large genetic and

functional diversity of EcM fungi. However, whole genome sequencing may be prohibitively expensive and computationally intensive for some studies. In this case, sequencing for conserved functional genes of interest, such as those relating to melanin production or heavy metal tolerance, in addition to traditional ITS sequencing could help to elucidate the potential niche and functionality of EcM fungal communities at a lower cost (Suenaga 2015; Dauphin and Peter 2023). Strategies in landscape genomics that have been developed for other organisms can also be well-applied to EcM fungi, as noted in Dauphin and Peter (2024), such as targeting gaps in spatial and temporal resolution and employing stratified sampling to increase statistical power (Dauphin and Peter 2023).

Quantifying the Darwinian shortfall is challenging, since approaches to gather and analyze evolutionary data change over time. Here, we decided to focus on the representation of Canadian EcM fungal species in terms of available genomic data. While many fungi, including EcM, are often delineated based on reference barcodes of commonly sequenced regions like ITS (nuclear ribosomal) and CO1 (mitochondrial), these highly conserved regions of the genome are poorly suited to understanding the evolutionary relationships among species (Dentinger et al. 2011), which is best done using whole genome or long-read approaches (Varga et al. 2019). For fungi, the MycoCosm genomic database (Grigoriev et al. 2014) is the primary source of compiled genomic data. Using this database, we quantified the Darwinian shortfall as the percentage of the **1,405** named EcM fungal species in Canada absent from the database. This amounted to **1,338** species, which represents **95.2%**, indicating this shortfall remains severe.

Raunkiaeran shortfall

The Raunkiaeran shortfall is the lack of data on species traits and functions. Characterizing species by functional traits instead of phylogenetic relatedness is important for understanding how species contribute to ecosystem functions and interactions (Ackerly and Cornwell 2007; Aguilar-Trigueros et al. 2015; Kardol et al. 2016; Fry et al. 2019). Importantly, and more so in fungi compared to other kingdoms, phylogenetic relatedness does not always correlate well with the ecological or functional roles that species perform (Martiny et al. 2013). For example, while fungi in the genus *Cenococcum* (Ascomycota) have many functional, ecological, and genomic traits in common with EcM fungi in phylum Basidiomycota (Peter et al. 2016), fungal species within the same genus can exhibit considerable functional diversity (Carrillo et al. 2025). This disconnect between phylogeny and function highlights the need to collect functional trait data to connect fungal species and sequences with ecosystem functioning to better understand the vital role of EcM fungi in ecosystems across Canada.

The large diversity of functions across fungi complicates efforts to standardize trait data collection. Even among mycorrhizal fungal researchers, there are various approaches to collecting and reporting trait data. While researchers of arbuscular mycorrhizal (AM) fungi have adopted Grime's C-S-R framework (Chagnon et al. 2013) to organize and standardize measures of functional traits like life history strategy across multiple dimensions (hosts, soil, fungal ecology; Antunes et al. (2025)), no such framework exists for EcM fungi. Assigning exploration types to EcM fungi holds promise, especially as these traits appear to covary with capacities for decomposition and nutrient cycling (Mansfield et al. 2026). However, traits focused on specific genes, enzymes, and/or resource flows can be context-dependent for

471 both the environmental conditions and the specific mycorrhizal fungus being investigated.
 472 Thus, while unified trait databases are a valuable tool for summarizing broad patterns and
 473 generating testable hypotheses, producing these for EcM fungi remains a challenge.

474 Currently, FungalTraits is the most comprehensive functional traits database for fungi, with
 475 trait descriptions for over 160,000 fungal genera (Pöhlme et al. 2020), including 327 EcM
 476 fungal genera. Decades of field and laboratory observations form the basis of these trait
 477 descriptions that broadly describe fungal genera in terms of morphology (e.g., growth form,
 478 and/or mycelial exploration type) and ecology/interactions, which collectively point toward
 479 broad ecological roles for fungi.

480 We quantified the Raunkiæran shortfall as the coverage of trait information for EcM fungi
 481 in Canada, using six coarse ecological/morphological traits recorded in the FungalTraits
 482 database that are relevant to EcM fungi. A seventh trait, “growth form”, is exclusively
 483 “filamentous mycelium” for all 327 EcM fungal genera, so is not considered further. It should
 484 be noted that the FungalTraits database does not include traits on enzymatic function or
 485 carbon acquisition, features critical to EcM fungal function.

486 As shown in Table 4, trait knowledge is concentrated in morphological characters derivable
 487 from taxonomy (almost 100% coverage) and is near-absent for ecological-interaction traits.

488 We acknowledge that the host-specificity trait is relevant to the Eltonian shortfall (below),
 489 but we discuss it here due to its inclusion in the FungalTraits dataset. The 6 genera
 490 with a documented host-specificity entry (all Pinaceae) are *Chroogomphus*, *Gomphidius*,
 491 *Pseudoboletus*, *Fuscoboletinus*, *Rhizopogon*, and *Suillus*. However, the first two of these genera
 492 appear to be primarily epiparasites of the latter two genera (Olsson et al. 2000), and based

on morphology and multigene phylogenies, *Fuscoboletinus* has recently been reclassified as a subgenus of *Suillus* (Shi et al. 2025). *Pseudoboletus* is a monospecific genus whose single species (*P. parasiticus*) is a parasite of the earthball *Scleroderma citrinum* (though it has been shown to form ectomycorrhiza with *Pinus resinosa* roots in culture; Richter and Bruhn (1989)). The two ecological/interaction traits with near-zero coverage are documented for the same two genera, both members of the Endogonales (Mucoromycota): *Densospora* and *Jimgerdemannia* (Chang et al. 2019). *Densospora* is the only genus assigned a secondary lifestyle of “soil saprotroph” and an endophytic interaction capability of “root endophyte”, while *Jimgerdemannia* carries the value “root-associated” for both fields. No other Canadian EcM genus has an entry for either trait, so these two characters provide essentially no resolving information across the assemblage. The Raunkiaeran shortfall for Canadian EcM fungi is therefore primarily a shortfall in ecological-interaction knowledge specifically.

Hutchinsonian shortfall

The Hutchinsonian shortfall refers to the lack of knowledge about species tolerances to abiotic environmental conditions (Hortal et al. 2015). Addressing the Hutchinsonian shortfall will not only improve our understanding of the factors underlying spatio-temporal variation in the abundance and distributions of EcM fungal species, but is also particularly relevant for predicting fungal responses to climate change (Steidinger et al. 2020).

For a taxonomic group as diverse as the EcM fungi, a species-level focus has not been the norm in primary research; instead, most studies assess community-level features such as species richness, composition, community aggregated traits, or total biomass in relation to

environmental conditions. For example, soil properties can influence diversity and composition at fine spatial scales (Taylor et al. 2014), whereas climate typically emerges as the primary driver at broad spatial scales (Tedersoo et al. 2014; Větrovský et al. 2019; Mikryukov et al. 2023). A community-level focus has also been the classical approach in plant macroecology, which culminated in the creation of plant functional types and the development of dynamic global vegetation models in contrast to individual species response curves (Box 1996). While informative, these assemblage-level patterns provide limited insights into the abiotic niches of individual taxa. However, like the trajectory of plant biogeography, which has shifted from a community to species-level focus, EcM fungal biogeography has begun to shift research focus from describing community-level responses to characterizing individual species-level response curves via ecological niche models and SDMs (Davison et al. 2021; Kivlin et al. 2021; Qin et al. 2023; van Nuland et al. 2024; Qin et al. 2026). Given that the diversity and symbiotrophy of EcM fungi make it infeasible to estimate species' tolerances at scale via controlled physiological experiments, niche modeling will remain the primary means for estimating EcM fungal species' responses to abiotic conditions (but see Lofgren et al. (2024)). To achieve best accuracy, niche models should be informed by sampling that captures all the environmental conditions in which the target species occurs (Moudrý et al. 2024). This is especially important for presence-only occurrence data, for which niche modeling additionally requires that environmental conditions are sampled in proportion to their actual availability (Hastie and Fithian 2013). Thus, for our first assessment of the Hutchinsonian shortfall, we evaluate the degree to which available samples cover Canada's climate space (Figure 3a,d), defined here in simplified terms by mean annual temperature and mean annual precipitation.

Figure 3 makes clear that this aspect of the Hutchinsonian shortfall is enormous for EcM fungi in Canada, particularly for the systematic samples from GlobalFungi (Figure 3b,e). For example, the most common climate conditions in Canada - those covering northern boreal, sub-arctic and arctic regions (Fig. 3a) - are among the most poorly represented among GlobalFungi (Figure 3e) and GenBank samples (Fig. 3f). GenBank samples appear biased towards areas with high mean annual temperature (Figure 3f), which are comparatively rare. The global sampling density map (Figure S2), which shows few samples across vast expanses of north-eastern Europe and northern Russia, implies similar climate sampling gaps at the global scale, with implications for EcM fungi SDMs based on global data (Qin et al. 2026). The preceding assessment considered only climate. Evaluating sampling in relation to additional abiotic variables, such as soil and physiographic conditions, would undoubtedly reveal additional gaps, thus broadening the Hutchinsonian shortfall further. As a preliminary assessment of this, Figure S4 shows a map of GlobalFungi and GenBank samples overlaid on Canada's 15 ecozones, and Table S1 shows the sample numbers and sampling density (number of samples per 10,000 km²) for each ecozone. Taken together with the climate space sampling results (Figure 3), these ecozone findings show that the Hutchinsonian shortfall is extensive for EcM fungal taxa in Canada, and a priority will be designing sampling strategies that appropriately represent the potential abiotic niche space of species.

Eltonian shortfall

The Eltonian shortfall describes the lack of knowledge of biotic interactions. This is especially relevant for EcM fungi as they are primarily obligate symbionts, meaning that most species

require a compatible plant host, or “phytobiont”, to reproduce. Here we first consider the Eltonian shortfall from the perspective of the EcM fungi (mycobiont), and specifically what data exist about potential hosts for the **1,405** named EcM fungal species in Canada. Given that the majority of Canada’s forests and much of the tundra are dominated by EcM tree or shrub species, the arena within which functional EcM interactions could be realized is enormous. However, not all EcM plant species are suitable hosts for all EcM fungal species; EcM fungi exhibit a range of host breadths, from the comparatively narrow host-breadth of *Suillus*, which predominantly associates with members of the Pinaceae family, to the broad host-breadth of *Cenococcum geophilum*, which associates with a wide array of EcM tree species including gymnosperms and angiosperms (Molina and Horton 2015). While the mechanisms thought to influence host breadth are varied, and implicate processes at both the molecular and genomic level and landscape and regional scales (Molina and Horton 2015; Martin et al. 2016), much remains to be learned about why some EcM fungal taxa can colonize a wide range of hosts, while others are more restricted (Martin and Van Der Heijden 2024; Voller et al. 2024).

Demonstrating the existence of an EcM symbiosis is itself logistically demanding. The gold standard for evidence is demonstrating the presence of a Hartig net, and, once confirmed, using DNA sampled from the ectomycorrhiza and host roots to identify the mycobiont and phytobiont partners (Brundrett and Tedersoo 2020). Such evidence is rare in practice. Most common are samples of environmental DNA of fungi (e.g. from soil samples) for whom the potential host species is inferred as the nearest plant, which can be problematic when there are multiple potential host species in the vicinity. Thus, for our first assessment of the

Eltonian shortfall, we report that of the **1,469** GlobalFungi samples in Canada that included at least one EcM fungal SH code, **684** (**47%**) lacked information about potential hosts, i.e. the “dominant plant species” metadata field was empty. Of the **785** samples that did include potential host information, **704** (**89.7%**) were collected from soil, and thus potential host information must be inferred. Only **44** samples were collected directly from roots (**5.6%**), and would therefore provide more confidence about the potential host identity. Among the **9,149** GenBank sequence records, **7,366** (**80.5%**) were missing host information (in the “host taxon” field). Among the **1,783** records that did include host information, **339** (**19%**) had no information about the source of the DNA (the “isolation_src” field was empty), and an additional **369** (**21%**) were likely derived from samples other than ectomycorrhiza or host roots (based on the “isolation_src” field).

Next, we summarize the gap in what is known about potential EcM fungi – host interactions, again using information derived from the GlobalFungi and GenBank samples. On the mycobiont side, among the **1,405** named EcM fungal species, **1,020** (**73%**) lacked any associated host information. Among the **385** named EcM fungal species that were associated with at least one host species, the maximum number of unique host species associated with an EcM fungal species was **7**. If we expand the scope to include metadata from all global records from GlobalFungi and GenBank, then **521** (**37%**) of the **1,405** named EcM fungal species in Canada lacked any associated host information anywhere, and among the **884** species that were associated with at least one host species, the maximum number of unique host species associated with an EcM fungal species was **16**.

On the phytobiont side, there were **50** unique host plant species documented in the metadata.

For comparison, we cross-referenced data from the FungalRoot dataset (Soudzilovskaia et al. 2020, GBIF source: doi:10.15468/a7ujmj) with the BIEN database and its native species resolver (Maitner et al. 2018; Boyle et al. 2024; Enquist et al. 2026) to assemble a list of vascular plant species native to Canada that are EcM (see detailed methods in Supplemental Materials SM1). This yielded **272** species; **120** tree species, **119** shrub species, and **33** herbaceous species. Our estimate of **272** is conservative: species of *Acer*, *Fraxinus*, and *Juglans*, for example, are excluded from our list despite published evidence of genuine EcM associations for these genera elsewhere in the literature (Teste et al. 2020). FungalRoot’s genus-level recommendation does not close this gap, since it independently classifies all three genera as AM. We therefore emphasize that our remaining Eltonian shortfall estimates, which rely on data about potential host species, are conservative estimates. Among the list of **50** unique host plant species names documented in the metadata, **28** matched names in our list of native EcM host plant species in Canada (Table S2). The remaining **22** names that did not overlap included ornamental species (which we did not consider), mis-spelled names, and other problematic entries (Table S3).

The preceding data about host information can be summarized as an Eltonian shortfall: **244** (**90%**) of the **272** potential EcM host species native to Canada lack any records of associated EcM fungal partners in either GlobalFungi or GenBank.

Considering the full potential interaction matrix for Canada (**272** host species $\times$ **1,405** named EcM fungal species = **382,160** cells), **99.8%** lack data. Of the **739** filled cells, **389** (**52.6%**) are supported by a single observation. Coarsening to genus, the matrix collapses to **7,440** cells, of which **96.5%** lack data; of the **260** filled cells, **73** (**28.1%**) rest on a single

observation.

Lastly, Figure 4 provides a geographic perspective of the Eltonian shortfall, simultaneously mapping the estimated richness (within 0.5° grid cells) of EcM host species across Canada and the proportion of species in each cell with at least one associated fungal DNA sequence record in Canada.

The way forward

Across the tree of life, our knowledge and understanding of biodiversity is biased and incomplete due to limitations in the extent of available biodiversity data. Since 2015, summarizing these data gaps under the shortfall framework proposed by Hortal et al. (2015) has proved effective, and we have made substantial progress in overcoming these shortfalls for animals (mostly vertebrates) and plants. For groups like EcM fungi, progress has been slower. In countries like Canada, with vast ecosystems dominated by EcM trees and shrubs, these shortfalls hinder the application of eco-evolutionary knowledge in conservation and land management. Our assessment reveals that current knowledge in Canada is severely limited, characterized by large gaps in available data for EcM fungi and exacerbated by shifting methodological practices, multiple species concepts, and changing technologies. As such, our ability to integrate EcM fungi into conservation strategies to protect biodiversity is lacking. These findings suggest that there is an urgent and broadscale need for additional data and knowledge on the diversity, distribution, and functioning of EcM fungi across Canadian ecosystems.

From a conservation point of view, the most critical shortfalls to address are the Linnean (taxonomic diversity), Wallacean (geographic distributions), Hutchinsonian (environmental niches), and Eltonian (biotic interactions), especially when it comes to systematically prioritizing land for actions like area-based protection amid ongoing climate change (Eckert et al. 2023; O'Connor et al. 2026; Qin et al. 2026). For example, SDMs are almost certainly going to play an increasingly central role in closing the Wallacean shortfall, and the efforts of Qin et al. (2026) and van Nuland et al. (2024) provide valuable foundations upon which to build. Key to this will be improving model discriminatory power and calibration, which at present is weak for EcM fungal taxa (Qin et al. 2026), presumably because (i) most taxa are represented by comparatively few occurrence records; and / or (ii) the occurrence records that are available likely capture only a small portion of the true realized niches (for symbionts like EcM fungi, this includes the abiotic and biotic niche) (see Hutchinsonian and Eltonian shortfalls).

Fortunately, future data collection to overcome these shortfalls for EcM fungi has the potential to benefit from promising synergies. For example, high-throughput molecular methods to identify EcM fungi from spatially-referenced root samples from known host species can simultaneously contribute taxonomic, geographic, environmental, and species interaction data (Figure 5). Moreover, optimizing sampling programs across specific host species, geographies, and environments could rapidly accrue gains in data coverage (Figure 5). Importantly, generating these data can also advance research priorities (e.g. Qin et al. (2026)) and immediately benefit conservation planning to reach rapidly approaching targets like Canada's commitment to protecting 30% of land by 2030 (United Nations Convention on Biological

Diversity 2022), both of which provide researchers with powerful incentives to expand sampling using more optimized designs.

While we fundamentally need more data on EcM fungi in Canada, solving data limitations alone will not be sufficient to overcome all of the seven shortfalls. This is because, unlike other clades, research on EcM fungi lags behind other fields in producing the open access, standardized, and unified databases that have facilitated broadscale synthesis. For example, while plants and other fungi (like AM fungi) benefit from standardized trait databases (Kattge et al. 2020; Antunes et al. 2025) and integrated, openly licensed data infrastructures (Enquist et al. 2026), general trait frameworks are more difficult to develop for EcM fungi owing to their high functional diversity, though there may be promise in using exploration types (Mansfield et al. 2026). By improving consistency among existing trait data, we can promote broadscale spatial or taxonomic investigations of variation in functional diversity. That said, the existing databases (e.g. GlobalFungi, Global Soil Mycobiome, GenBank) have led to rapid progress in studying EcM fungal diversity at scale, and the numerous, recent contributions from the Society for the Protection of Underground Networks (SPUN; <https://www.spun.earth/>) and collaborators exemplify these efforts (e.g. Větrovský et al. 2019; Tedersoo et al. 2021; Mikryukov et al. 2023; van Nuland et al. 2024; van Galen et al. 2025; van Nuland et al. 2025; Qin et al. 2026).

Finally, for some shortfalls like the Prestonian (abundance patterns) and Darwinian (evolutionary relationships), Canada is essentially starting from scratch. While daunting, this also opens the door for optimizing how we collect data from the beginning, learning from efforts made in other regions, and targeting the most pressing aspects or questions pertaining to

687 the different data shortfalls. For example, while studies have explored shifts in EcM fungal
 688 abundance following specific disturbances like nitrogen addition (Högberg et al. 2021) or
 689 warming (Clemmensen et al. 2006), understanding how EcM fungi respond to fire is also
 690 critical in a region like Canada, where single fire seasons can produce burns greater in area
 691 than ~40% of European nations (in 2023, over 165 thousand km² of forest burned in Canada).
 692 Post-fire fungal community surveys often show a reduction in detectable EcM fungi, especially
 693 following high severity fire that kills EcM host trees (Dove and Hart 2017; Pulido-Chavez
 694 et al. 2021; Packard et al. 2023). But EcM fungi have been shown to persist, dormant
 695 in the soil, until new host seedlings begin to establish (Baar et al. 1999; Yamaoka et al.
 696 2025). Better understanding of EcM fungal community distribution, dynamics, dispersal, and
 697 persistence is therefore critical for supporting ecosystem recovery and management efforts.

698 Finally, while we are confident about the data and methods we used to evaluate the seven
 699 shortfalls, our study has some important limitations that warrant discussion. First, we based
 700 our evaluations exclusively on publicly available and discoverable DNA sequence data from
 701 GlobalFungi and GenBank, and did not consider other kinds of data including physical
 702 specimen occurrence records available through the Global Biodiversity Information Facility
 703 (GBIF). While taxonomic inconsistencies and cryptic diversity render such data less reliable
 704 for the present goals, they can nonetheless complement future, molecular-based sampling
 705 efforts. For example, Figure S5 shows a map of physical specimen occurrence records, pulled
 706 from GBIF, for **142** EcM fungal genera, with records distinguished according to whether the
 707 genus is represented within our Canada-specific DNA sequence datasets. Table S4 shows the
 708 **32** genera that currently lack associated DNA sequence data in Canada. This list could be

709 used to target future sampling efforts. Second, our shortfall assessments were not informed
710 in any systematic way by data available solely in the primary research literature; rather,
711 we relied exclusively on curated, discoverable, and publicly accessible data repositories like
712 Mycocosm, BioTime, and FungalRoot. Some shortfalls may therefore be less severe in reality
713 than our limited data suggest. Third, like all studies of this kind, the data acquisition,
714 processing, and analysis pipeline (including bioinformatics pipelines implemented by original
715 sources) involves many decisions, some of which are less well-rationalized than others. This
716 yields a “multiverse” of unique analysis pipelines whose outcomes could vary materially
717 (Steege et al. 2016). Ideally, one would conduct a “multiverse analysis” that reports
718 the results from all unique pipelines (Short et al. 2025). We are currently exploring that
719 option, but in the interim, for full transparency we provide a thorough “Decisions Log” in the
720 Supplemental Information S2 that documents all decisions and their rationales. Lastly, the
721 data presented in this study represent the shortfalls realized at a given point in time. We are
722 in the process of developing a web-based dashboard that will update our shortfall analyses
723 (plus complementary analyses) quarterly - a form of “digital twin” (Visser et al. 2026).

724 Conclusion

725 While we are making progress in gathering and synthesizing the data we need to advance
726 biodiversity knowledge and conservation, we still have a long way to go when it comes to
727 understanding the diversity and distribution of EcM fungi in Canada. That said, rapid
728 progress is possible if we optimize future sampling and take advantage of the lessons learned
729 from other taxa and regions. To that end, our assessment marks an important first step

in improving our knowledge and understanding of EcM fungi in Canada and provides the foundation for a coordinated, pan-Canadian sampling effort that can be optimized to address multiple shortfalls simultaneously.

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Data and code availability

Code for the entire workflow is publicly available (XXX). All input and output data are archived and publicly available at (YYY). Supplemental Materials SM1 and SM2 are also archived at the same location.

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Tables

Table 1: Summary of data derived from v5 of the GlobalFungi dataset. Summary data are provided for the Canada-specific samples, and for the full GlobalFungi dataset for comparison.

Table 2: Properties of the Canadian GenBank EcM sequence dataset, after ITS2/ITS-both filtering and UNITE SH assignment ($\geq 98.5\%$ identity). Taxon counts are over all Canadian GenBank records.

Table 3: Occupancy of georeferenced Canadian EcM fungal taxa (GlobalFungi only; sites defined as 30 arc-second grid cells). For each taxonomic level: number of taxa; the first quartile (Q1), median, third quartile (Q3), and maximum number of occupied cells within Canada; and the number (percentage) of taxa recorded in fewer than 30 cells within Canada and across the worldwide GlobalFungi v5 dataset.

Table 4: Trait coverage for the 124 EcM fungal genera detected in Canada, across the six traits within the FungalTraits database applicable to EcM fungi. Coverage is the percentage of genera with a non-missing value; explicit “unknown” entries are counted as missing.

Category	Metric	Canada	GlobalFungi-wide	Canada as % of GlobalFungi-wide
Sampling effort	Quality-filtered samples, all fungal taxa	1,567	47,606	3.3%
Sampling effort	Quality-filtered samples with ≥ 1 EcM detection	1,469	34,928	4.2%
Geographic coverage	Unique EcM sampling locations	486	11,651	4.2%
Detection volume	EcM detection records (sample $\times$ SH-code, abundance > 0)	39,867	829,687	4.8%
Detection volume	EcM total read abundance (Σ reads)	8,641,968	154,022,074	5.6%
Taxonomic richness	EcM named species detected	1,002	3,759	26.7%
Taxonomic richness	EcM genera detected	103	225	45.8%
Taxonomic richness	EcM SH codes (UNITE v10) detected	2,062	12,970	15.9%

Property	Value
EcM fungal sequence records	9,149
Records with coordinates	3,163
EcM fungal SH codes	1,616
SH codes with a UNITE species-level name	1,179
EcM fungal genera	109
EcM fungal named species	1,056
Unique georeferenced sampling locations	1,137

Taxonomic level	Taxa	Q1 cells (Canada)	Median cells (Canada)	Q3 cells (Canada)	Max cells (Canada)	< 30 cells in Canada	< 30 cells worldwide
Named species	1,002	2	5	17	280	846 (84%)	351 (35%)
SH codes	2,062	1	3	10	306	1,855 (90%)	969 (47%)

1314 **Table 4**

Trait	Trait class	Genera documented	Coverage (%)
Host specificity	Ecological/interaction	6 / 124	4.8
Secondary lifestyle	Ecological/interaction	3 / 124	2.4
Endophytic interaction capability	Ecological/interaction	2 / 124	1.6
Fruitbody type	Morphological/structural	124 / 124	100.0
Hymenium type	Morphological/structural	124 / 124	100.0
Mycorrhiza exploration type	Morphological/structural	119 / 124	96.0

Figures

Figure 1: Sampling locations from which EcM fungal records were publicly available from either GlobalFungi v5 (n = 486 unique locations) or GenBank (n = 1,137 unique locations), for a combined total of 1,623 unique locations. Many locations overlap so are not clearly visible.

Figure 2: Frequency distributions of the number of 30 arc-second grid cells (~1 km²) occupied by EcM fungal taxa, GlobalFungi only. Top row: occupancy within Canada for (a) named species (n = 1,002 species) and (b) SH codes (n = 2,062 SH codes). Bottom row: occupancy across the full global GlobalFungi v5 dataset for the same (c) named species and (d) SH codes.

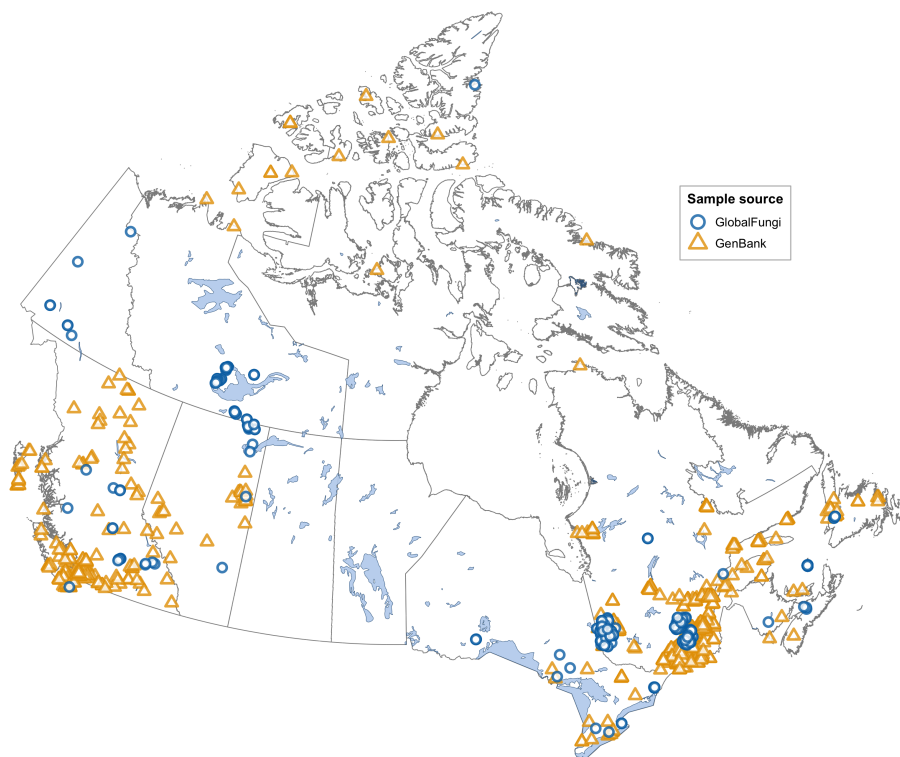
Figure 3: Available climate space and Canadian EcM fungal sampling coverage, in geographic and climate space (WorldClim v2.1 BIO1 × BIO12, 50 × 50 grid of climate zones). Top row (a–c): geographic maps. Bottom row (d–f): the same in climate space. Left (a, d): available climate frequency; in (d), grid cells outlined in white indicate climates never sampled by GlobalFungi + GenBank combined, i.e. the grey zones in (f). Middle (b, e): GlobalFungi coverage; grey areas denote climates not sampled by any of the 486 GlobalFungi sampling locations. Right (c, f): combined GlobalFungi + GenBank coverage; grey areas denote climates not sampled by any of the 1,623 combined sampling locations. Figure style adapted from Hébert et al. (2026).

Figure 4: Bivariate maps of EcM host species richness (BIEN2 modelled ranges) versus proportion of host species with EcM fungal molecular detection data in Canada, shown

1336 separately for non-tree (shrub and herbaceous; n = 139 species; top) and tree (n = 88
1337 species; bottom) EcM host species. Counts are the host species with a BIEN2 modelled range
1338 overlapping Canada, which is fewer than the full native host list (see Supplemental Materials
1339 SM1). Sampling unit: 0.5° grid cell; only cells with predicted EcM host habitat are shown.

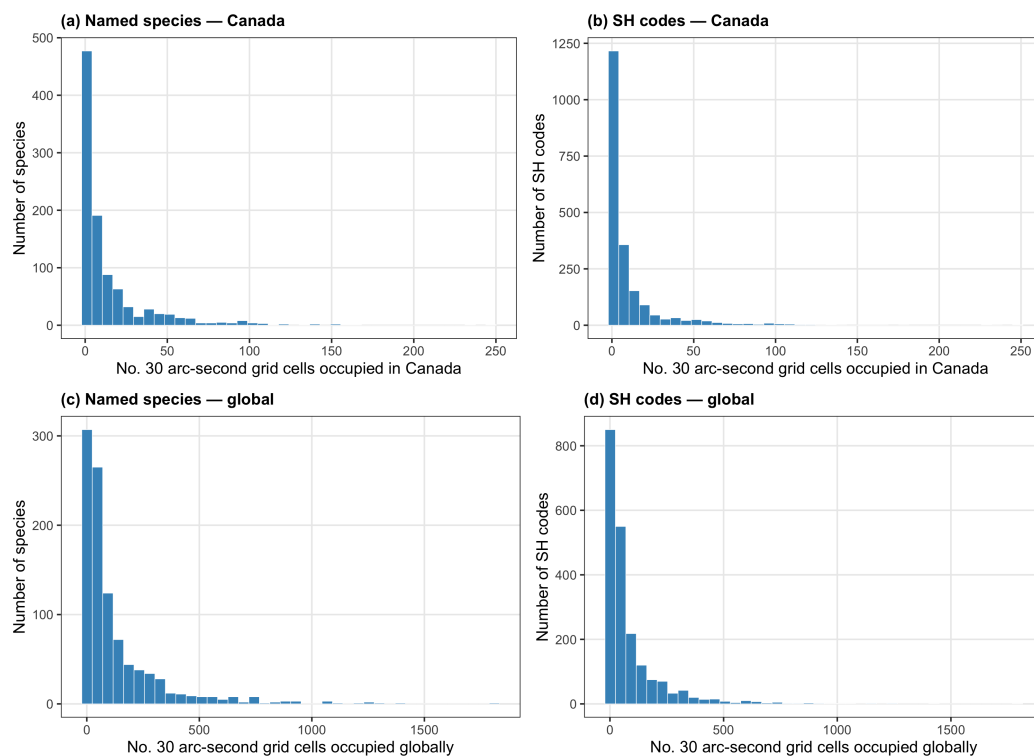
1340 **Figure 5:** Efforts to address current data shortfalls for EcM fungi in Canada can benefit
1341 from informed and optimized sampling programs that synergize progress to rapidly improve
1342 our taxonomic, geographic, and abiotic knowledge of EcM fungi across many host plant
1343 species. Ellipses show how sampling targeting a particular region (Wallacean) or abiotic suite
1344 of conditions (Hutchinsonian) can simultaneously address other data shortfalls, including
1345 Eltonian and Linnean. For example, the blue ellipse shows how sampling the under-represented
1346 climate conditions of the arctic (top-right quadrant) can simultaneously address the Linnean
1347 shortfall (top left quadrant). Likewise, the green ellipse shows how filling the large geographic
1348 sampling gap across the provinces of Manitoba and Saskatchewan (bottom left quadrant) could
1349 simultaneously address both the Hutchinsonian and Eltonian shortfalls (right quadrants).

1350 Figure 1



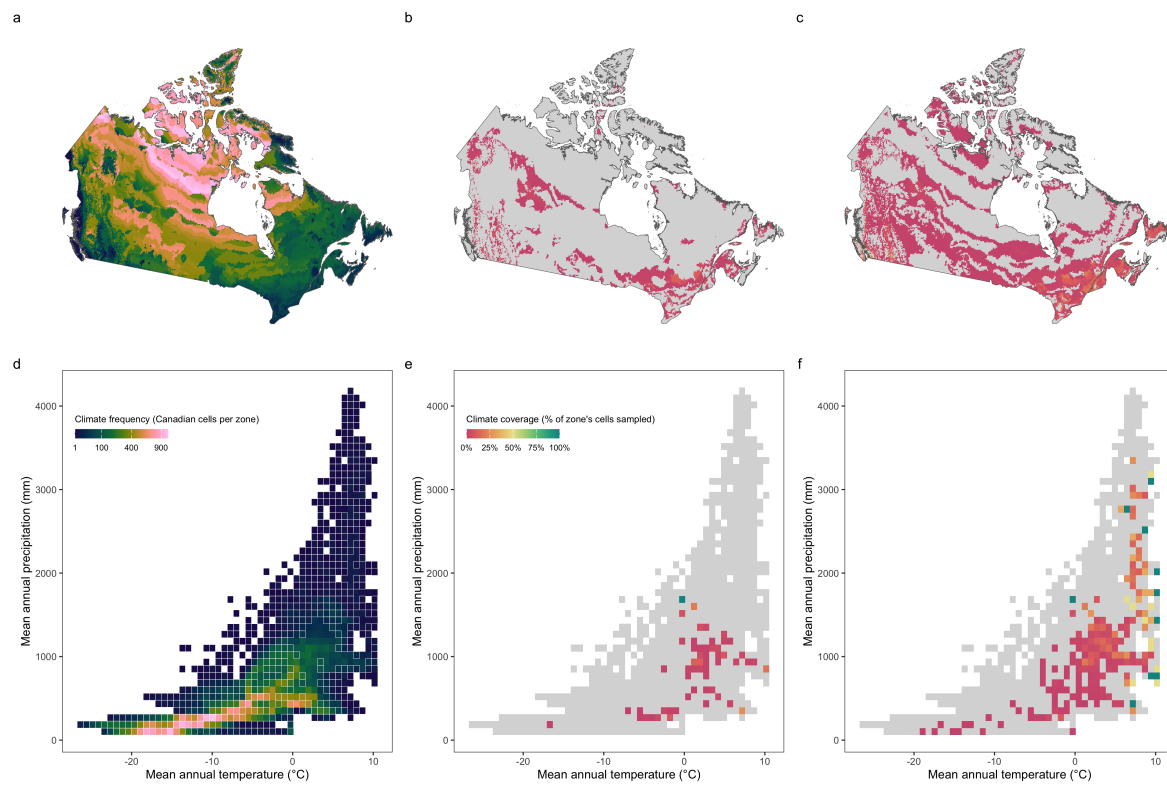
1351

1352 Figure 2

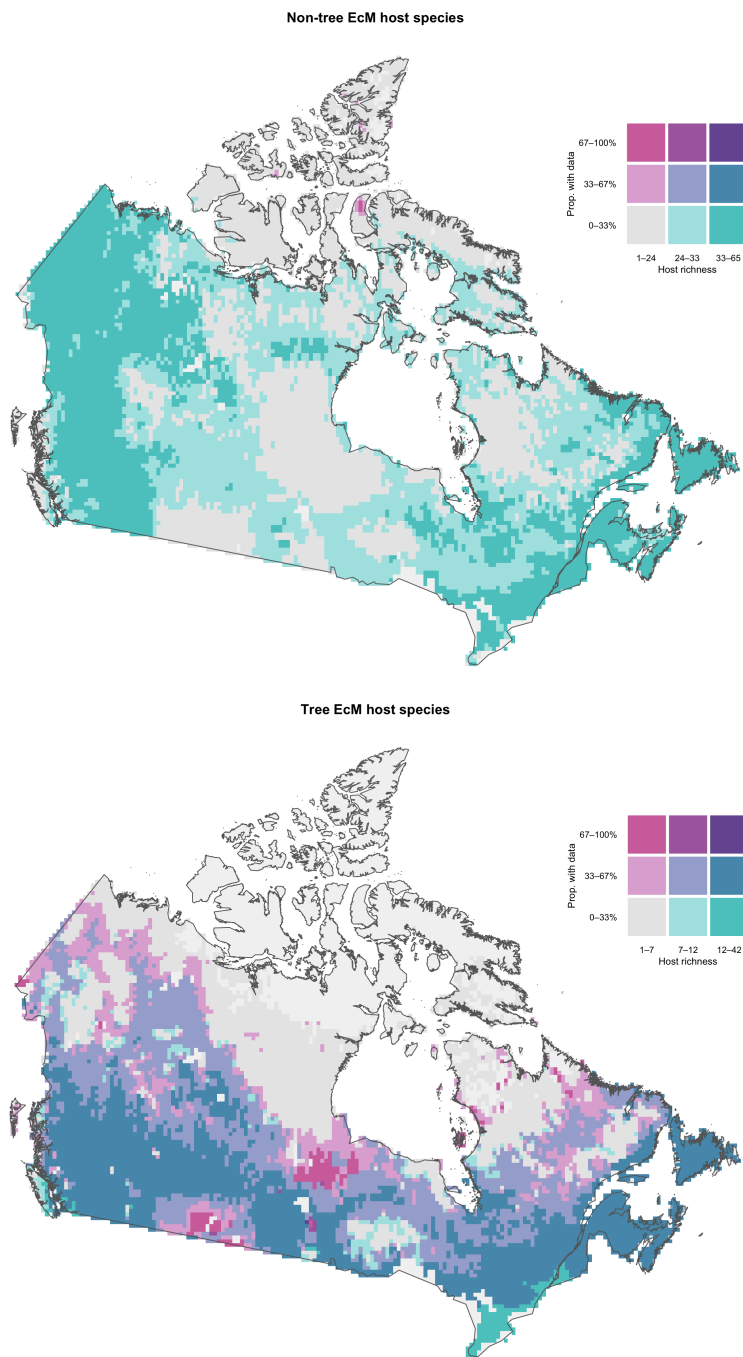


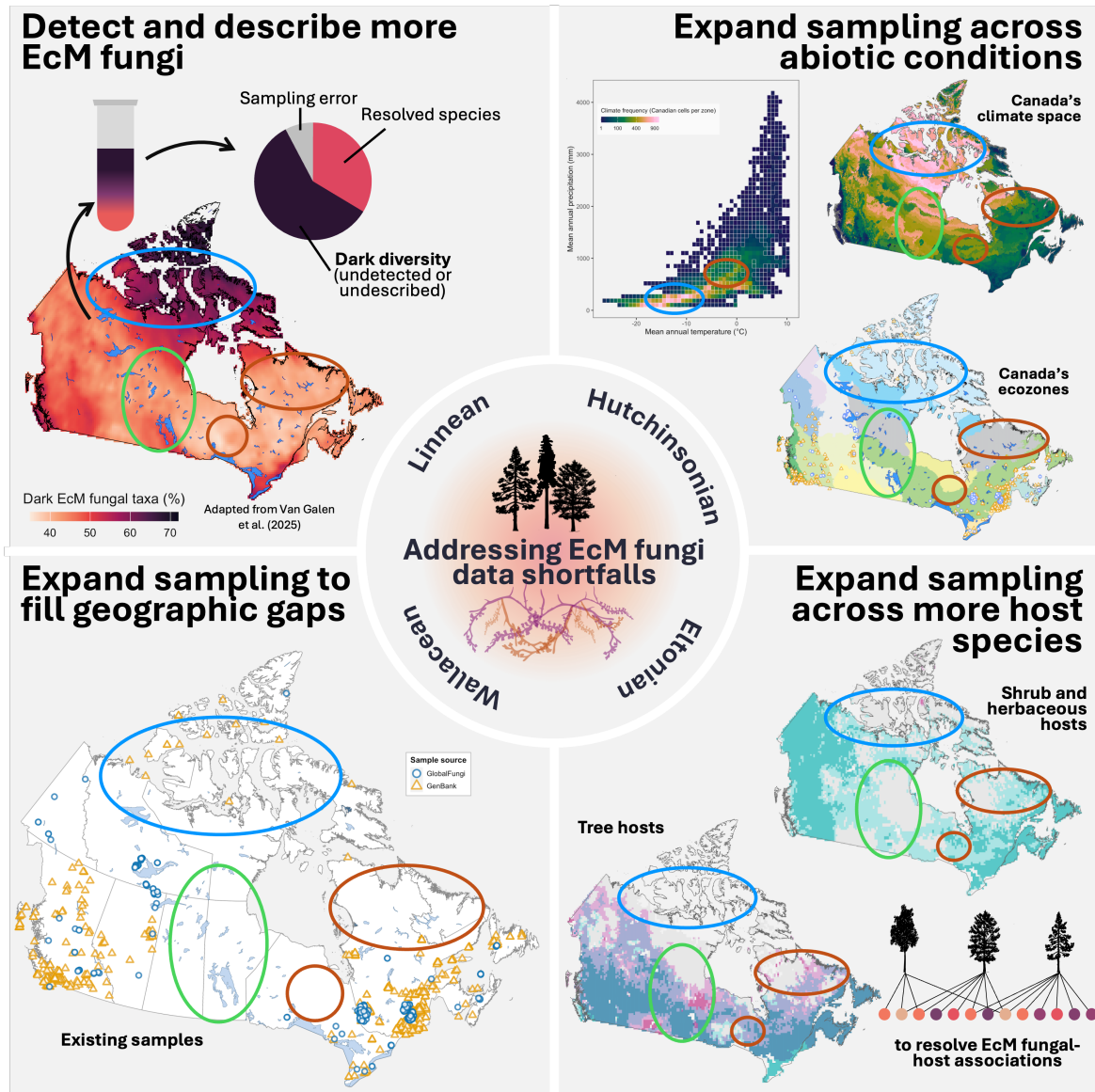
1353

1354 Figure 3



1355





Supplemental materials (SM1) for: An assessment of biodiversity data shortfalls for ectomycorrhizal fungi in Canada

Jason Pither, with assistance from Claude (Sonnet 5 and Opus 4.8)

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1 Detailed methods

1.1 Software

We conducted analyses in R (v4.5.2; R Core Team (2026)). We used the following R packages: `sf` (Pebesma (2018)) for vector spatial operations; `terra` (Hijmans (2025)) for rasters; `ggplot2` (Wickham (2016)) and `patchwork` (Pedersen (2024)) for figures; `rentrez` (Winter (2017)) for GenBank retrieval; `rgbif` (Chamberlain et al. (2024)) for GBIF access; `BIEN` (Maitner et al. (2018)) for plant range and native-status data; `geodata` (Hijmans (2026)) and `rnaturalearth` (Massicotte and South (2026)) for spatial base layers; `httr` (Wickham (2026)) for programmatic download of BIEN2 range shapefiles; `NSR` (Maitner and Boyle (2024)) for native-status resolution; `GIFT` (Denelle and Weigelt (2024)) for the plant growth-form traits used to fill gaps in the BIEN trait coverage; `readxl` for reading FungalRoot’s supplementary workbook; and `data.table` for column-selective reads of the multi-gigabyte GlobalFungi matrices. We used ITSx v1.1.3 (Bengtsson-Palme et al. (2013)), with its `HMMER` 3.x backend, to extract ITS sub-regions from GenBank sequences; `vsearch` v2.30.4 (Rognes et al. (2016)) for sequence-similarity searching; and `awk` for extraction from the GlobalFungi abundance matrix.

1.2 Assembly of the Canadian EcM fungal dataset

1.2.1 Data sources

We compiled EcM fungal records for Canada from two publicly accessible sequence sources: version 5 of the GlobalFungi database (Větrovský et al. (2020)) and NCBI GenBank (accessed 29 June 2026). GlobalFungi is a curated, purpose-built database of community-survey amplicon data, consistently processed but aggregated from studies using varied sequencing platforms and protocols, and primarily derived from soil samples. GenBank is a general-purpose sequence repository comprising records deposited from diverse studies and sampling efforts.

1.2.2 UNITE reference database and SH assignment

We assigned fungal species hypothesis (SH) codes and taxonomy using the UNITE general FASTA release (build 4 April 2024, UNITE v10.0, DOI 10.15156/BIO/2959332; dynamic clustering, full-length ITS; Nilsson et al. (2019)). This release provides one representative sequence per SH code, each with a single unambiguous taxonomy string in its header. We used

the same UNITE reference database for both the GlobalFungi taxonomy and the GenBank `vsearch` assignment described below, so both sources reference an identical SH definition.

1.2.3 GlobalFungi records

We downloaded the GlobalFungi v5 sample metadata file and the combined ITS1/ITS2 SH abundance matrix. The GlobalFungi dataset comes pre-populated with SH codes that are based on the same UNITE release described above, and a pipeline described at globalfungi.com. We retained Canadian samples by filtering for `country = Canada`; barcoding region in `{ITS2, ITSboth}`; non-manipulated conditions (`manipulated == "NO"`); and exclusion of substrate types irrelevant to EcM fungi (shoot, air, water, sediment). We pivoted the resulting wide abundance matrix to long format and removed zero-abundance records, then joined UNITE taxonomy by SH code.

1.2.4 GenBank records

We searched GenBank for fungal ITS sequences from Canada using the query `"Canada"[Country] AND "Fungi"[Organism] AND ("internal transcribed spacer"[All Fields] OR "ITS"[All Fields])`, with no date cutoff and no lifestyle keyword, so the search returns all matching fungal ITS records regardless of guild. We retrieved FASTA sequences and record metadata (coordinates, collection date, host, isolation source) in batches through the NCBI Entrez API (`rentrez`; Winter (2017)).

To make the GenBank component comparable to the GlobalFungi component (which we restricted to samples with a `barcoding_region` of ITS2 or ITSboth) we used ITSx v1.1.3 (Bengtsson-Palme et al. (2013); HMMER backend, Fungi profile set) to detect and extract the ITS1 and ITS2 sub-regions from each retrieved sequence. We excluded records with no detectable ITS2 or with an extracted ITS2 fragment shorter than 100 bp before SH assignment. We recorded whether each record also carried a genuine standalone ITS1 detection in an `its_region` column (ITS2 or ITSboth), directly analogous to GlobalFungi's `barcoding_region`.

We matched every eligible record against UNITE on its extracted ITS2 fragment alone. The `its_region` flag is for provenance only and does not influence assignment. This differs from the GlobalFungi pipeline, which independently classified each ITS region. Our pipeline preserves a one-row-per-accession schema, so we do not achieve exact parity with GlobalFungi at the level of individual sub-region observations. To gauge whether discarding ITS1 measurably costs resolution, our pipeline cross-tabulates each record's assignment outcome (below) against whether it carried a standalone ITS1 detection (`genbank_its1_resolution_crosstab.csv`).

We used `vsearch` v2.30.4 (Rognes et al. (2016); `usearch_global`) to compare sequences against the UNITE reference at 98.5% identity, matching GlobalFungi's documented SH-assignment criterion. We searched both strands exhaustively (`--strand both --maxaccepts 0 --maxrejects 0`) and retained all top-scoring hits per query (`--top_hits_only`) so that

we could resolve ties. Where a query’s top hits spanned more than one UNITE reference, we resolved the tie by taxonomic agreement. We compared named-species hits by species name, and “dark” hits (UNITE placeholders ending `_sp`) by SH code, following UNITE’s own dark-taxon convention (Nilsson et al. (2019); Ryberg and Nilsson (2018)). When all tied hits agreed at that level, we assigned the record that SH (`taxonomic_resolution = "sh"`). When they disagreed but shared a genus, we retained the record at genus-only resolution (`taxonomic_resolution = "genus"`, with `sh_code` and `species` set to NA and the genus preserved), following the principle of climbing to the lowest unambiguous rank (Heeger et al. (2018)). We treated only records whose tied hits disagreed at genus level as genuinely ambiguous and excluded them (logged to `genbank_ambiguous_species_excluded.csv`). Downstream SH-richness counts therefore treat genus-resolved records as carrying no SH.

We obtained the taxonomy for SH-resolved records directly from the matched SH representative in the UNITE reference. We obtained the host taxon from the structured GenBank `host` qualifier where available, and used a fallback regex extraction of binomial names from the `isolation_source` field. We preserve the raw extracted string as `host_taxon_raw` (see below). We also assigned each GenBank record a `canada_basis` provenance category, recording the evidence for its inclusion (structured country field, coordinates, both, or search-only).

We addressed inconsistencies in raw host-plant names across the source datasets (the GenBank `host` qualifier and `isolation_source` field, and GlobalFungi’s `dominant_plant_species` and `other_plant_species` fields) using a single shared normalization function, `canonicalize_host()` (`00_setup.R`), applied identically wherever we compare a raw host string against a species list (GenBank assembly here, and the Eltonian and Hutchinsonian analyses below). It reduces each raw string to a standardized “Genus species” (or bare genus) form, or to a missing value where the string cannot be resolved to a plausible Latin name. This step is necessary because host names in both sources are free text rather than a controlled vocabulary, so the same species may appear as a full binomial, a misspelling, an abbreviation, a common name, or with inconsistent capitalization or stray punctuation.

1.2.5 Combining sources and spatial validation

We combined the GlobalFungi and GenBank datasets by row-binding. For all records with parsed coordinates we applied a spatial-containment check against the GADM administrative boundary for Canada (see below), buffered by 2 km (applied in the Canada Albers Equal Area Conic projection, so the buffer distance is a true 2,000 m regardless of latitude), recording the result in a logical `coord_in_canada` column (TRUE = validated inside the buffered boundary; FALSE = coordinates present but outside; NA = no coordinates); original coordinates are always preserved. The buffer absorbs GADM coastline-simplification and topology artefacts, which would otherwise exclude a handful of coastal and small-island records (e.g. Gulf of St. Lawrence) carrying `country == "Canada"` metadata. All spatial analyses filter on `coord_in_canada == TRUE`, while non-spatial enumerations (taxon richness, record counts) include all records,

treating a GlobalFungi `country == Canada` label as authoritative even where coordinates fall marginally outside the buffered Canada polygon.

1.2.6 Assigning EcM guild status

We determined fungal EcM status at the genus level using FungalTraits (Pölme et al. (2020)): we retained records whose UNITE-assigned genus carries `primary_lifestyle = ectomycorrhizal` in FungalTraits (genus names lower-cased before matching), and carried two additional FungalTraits fields, EcM lineage and exploration type, into the final dataset. The resulting primary analysis dataset (`data_derived/emf_canada_em_only.csv`) has one row per (sample $\times$ SH code) detection.

1.2.7 Definition of a “site”

For analyses requiring georeferenced sampling units, we binned sample coordinates to three decimal places of latitude and longitude (~ 100 m at temperate latitudes), and refer to these bins as sampling “sites”. This collapses potential GPS-precision noise across repeat readings at one general location. We used site-binning for the ecozone sampling-coverage counts; the Wallacean occurrence counts (below) are intentionally left unbinned so that each distinct coordinate contributes an occurrence.

1.3 Reference data preparation

1.3.1 Spatial layers

We obtained Canadian national, provincial, and territorial boundaries from GADM via `geodata`, a higher-resolution Canada boundary and major-lake polygons from Natural Earth via `rnaturalearth`, and Canadian ecoregions (National Ecological Framework for Canada) with their ecozone-name lookup. We reprojected layers to WGS84 for coordinate operations and to the Canada Albers Equal Area Conic projection for mapping, simplified them with `terra` (`simplifyGeom`) where appropriate, and wrote them to `data_derived/spatial/`.

1.3.2 FungalRoot host-determination rule

We determined EcM host status for plant species and genera from FungalRoot (Soudzilovskaia et al. (2020)) using two independent routes.

For the species-level route, we used Darwin Core Archive occurrence records from GBIF (DOI 10.15468/a7ujmj), joining the occurrence file to the “measurements” extension on the mycorrhiza-type field. We classified a species as EcM if FungalRoot carries at least one

occurrence with an unambiguous EcM-positive label (“EcM”, “EcM, no AM colonization”, or “ErM, EcM”). Two further labels - “EcM, AM undetermined” and “EcM, AM” - do not on their own qualify a species as EcM-demonstrated, because they are too ambiguous to serve as positive evidence. However, records carrying these labels are not treated as evidence against EcM status either: a species with such a record is still classified as EcM-demonstrated if at least one other, unambiguous record for that species exists elsewhere in FungalRoot. We excluded EcM, AM undetermined because, in at least one case we identified (the fern *Dryopteris filix-mas*), it was contradicted by separate AM and non-mycorrhizal records for the same species, consistent with this label denoting an EcM report for which AM status was simply not surveyed, rather than a confirmed AM-negative result. We excluded “EcM, AM” (a dual-positive call, indicating both EcM and AM colonization were reported) as qualifying evidence at the species level because it was frequently the only EcM signal available for a species, with no unambiguous EcM record found anywhere else in that species’ genus. Of an initial 698 candidate species that qualified under a broader rule, 118 species (spanning 20 genera, including *Acer*, *Allium*, *Fraxinus*, and *Juglans*) relied solely on this dual-positive label. Given the lack of any corroborating unambiguous evidence, we judged this label too weak on its own to support an EcM-demonstrated classification (but see below).

For the genus-level route, we used FungalRoot’s own published genus-level recommendation, Supplementary Table S2 (Soudzilovskaia et al. (2020)), which assigns each genus a mycorrhizal-type call based on at least 67% consistency of species-level diagnosis. A genus qualifies as an EcM host genus if Table S2 assigns it “EcM” or “EcM-AM”; the latter captures genera with genuine, well-supported dual mycorrhizal associations (e.g., *Salix*, *Populus*, *Eucalyptus*) that the binary species-level occurrence rule cannot express. A handful of Table S2 rows carry non-standard free-text or apparent data-entry-error values (e.g., “species-specific: EcM or NM-AM”); these do not match either qualifying label, so we exclude them by default. The two routes are independent: a species/genus can qualify via its own direct occurrence record, via Table S2, or both, and a species need not have an occurrence record of its own to qualify if its genus is included via Table S2.

We acknowledge the species-level criterion is conservative. Some genera excluded under it, including *Acer*, *Fraxinus*, and *Juglans*, have published evidence of genuine EcM associations elsewhere in the literature (e.g., *Fraxinus* is listed among confirmed dual-mycorrhizal genera by Teste et al. (2020)), evidence that is not captured by any unambiguous occurrence record in the FungalRoot GBIF extract used here. The source Table S2 does not resolve this gap: it independently classifies *Acer*, *Fraxinus*, and *Juglans* as AM, so the genus-level route adds no evidence for these three genera either. We chose not to supplement FungalRoot with case-by-case literature additions, to keep host determination reproducible from a single, clearly defined source; the cost of this choice is that our EcM host list under-counts true EcM hosts for a small number of genera whose FungalRoot evidence (occurrence- and genus-level alike) is exclusively ambiguous or absent.

1.3.3 Native Canadian EcM host species

We identified native EcM host plant species occurring in Canada by querying BIEN (Enquist et al. (2026); Maitner et al. (2018)) for the plant species with Canadian distributions, filtering to natives via the Native Species Resolver (NSR; Boyle et al. (2024)), one of BIEN’s modular data services (Enquist et al. (2026)), which determines whether a species is native or introduced to the political division of the observation by comparing it against curated regional checklists. For Canadian determinations NSR draws on the Database of Vascular Plants of Canada (VASCAN; Brouillet (2025)), which NSR last refreshed on 16 September 2024, and the World Checklist of Vascular Plants, delivered via Plants of the World Online (WCVF v13; Govaerts (2024)), refreshed 21 May 2024. We queried NSR through its R package (Maitner and Boyle (2024)), and intersected the resulting native flora against the EcM host determination described above. We retain a native species if it qualifies under **either** route: its own species-level occurrence record satisfies the species rule, **or** its genus qualifies under FungalRoot’s Table S2 genus rule. We combine the two routes with OR, as implemented in `06_host_species.R` (Step 4). The resulting list (`data_derived/ecm_native_canada_host_species.csv`) records which route(s) applied for each species (`evidence_source`: “occurrence”, “table_s2”, or “both”) and is used in the Hutchinsonian and Eltonian analyses.

Combining the two routes with OR also mitigates a taxonomic-resolver limitation. Because BIEN’s occurrence-name scrubbing, the Native Status Resolver, and the GBIF backbone underlying FungalRoot are independent taxonomic resolvers, a genuinely native, EcM-demonstrated species can fail the species-level intersection when no single name string clears both the native filter and the species-level demonstrated filter simultaneously. The green/mountain alder complex (*Alnus alnobetula* / *A. crispa* / *A. viridis*) is a case in point: the native filter returns “Native” only under *Alnus alnobetula* while FungalRoot’s unambiguous species-level EcM evidence is filed under *Alnus crispa*, so the species-level route alone would not match. Here the genus route rescues the species — genus *Alnus* is called “EcM” in Table S2, so *Alnus alnobetula* is retained (via `evidence_source == "table_s2"`). A residual limitation remains only for species whose species-level name fails to match across resolvers **and** whose genus is absent from Table S2; we flag this as a known limitation of unresolved cross-database synonymy rather than applying a general synonym-harmonization step (which could shift counts for many species).

We assigned each host species a growth form (tree, shrub, or herbaceous), which we use for the tree / non-tree split reported in the main text and in Figure 4. Growth form comes from a four-stage chain, applied in order and recorded per species in the `growth_form_source` column of `data_derived/ecm_native_canada_host_species.csv`: (i) the modal value in the BIEN trait database (Enquist et al. (2026); Maitner et al. (2018)); (ii) for species BIEN does not cover, GIFT trait 1.2.1 (Denelle and Weigelt (2024), queried at `agreement = 0.66`); (iii) for species neither database covers, the modal growth form of that genus’s other species in this host list; and (iv) a curated manual override for any species still unresolved. Of the **272** host species, **215** growth forms come from BIEN, **15** from GIFT, **42** from the congener-modal step, and **0** from the manual override. We flag the congener-inferred group explicitly because those

values are inferred from congeners rather than taken from a trait record for the species itself.

1.3.4 BIEN2 host ranges and richness rasters

We obtained MaxEnt-based modelled range polygons (BIEN2; Moulatlet et al. (2025)) for the native Canadian EcM host species from biendata.org, downloaded programmatically through the REST API, reprojected to WGS84, and clipped to the Canada boundary. We rasterized these polygons onto a 0.5° geographic grid to produce three layers: host species richness (count of host species with a range polygon per cell); host data richness (count of host species with at least one Canadian EcM molecular-detection record per cell); and host data proportion (the ratio of the two).

Not every host species contributes a range. Of the **272** native Canadian EcM host species, **227** have a BIEN2 modelled range that overlaps Canada and therefore appear in the richness rasters; the remaining **45** do not, either because BIEN2 has no modelled range for the species or because the modelled range does not intersect the Canada boundary once clipped. The host-richness and host-proportion layers, and Figure 4, are therefore based on the **227** species with usable ranges rather than the full host list. This makes the mapped host coverage conservative: a cell may hold EcM host species whose ranges BIEN2 does not model.

1.4 Shortfall analyses

1.4.1 Linnean shortfall

We quantified the Canadian EcM fungal inventory at three taxonomic levels: unique UNITE SH codes, genera, and named species (excluding unresolved UNITE entries ending in `_sp`). SH codes are the primary richness unit because they provide a consistent operational taxonomic unit applicable to both sources, and because many EcM taxa are not yet formally named. We compared observed Canadian EcM genera to the global EcM genus inventory in Fungal-Traits, and quantified the overlap between the GlobalFungi and GenBank components at the named-species SH level using set operations (shared, GlobalFungi-only, GenBank-only). We characterized the dataset “dark fraction” as the proportion of detected SH codes lacking a species-level UNITE name, computed for the combined dataset and for each source separately. This SH-level dark fraction is not directly comparable to the OTU-level dark-diversity estimates of van Galen et al. (2025), whose denominators include OTUs matched only to genus level.

We also computed the average per-sample proportion of species-unassigned (“dark”) OTUs among van Galen et al.’s Canadian forest and tundra samples, using their published sample-specific estimates (Figshare DOI 10.6084/m9.figshare.28830371) — a table giving, for each GlobalFungi v5 sample in their global compilation, the proportion of EcM OTUs assigned and unassigned to a named species, together with sample-level metadata including country and biome. We filtered this table to samples with country = Canada and biome in {forest, tundra}

(the subset of their global sampling most comparable to our own) and took the unweighted mean across those samples. We report this in the main text alongside our own SH-level dark-fraction estimate as a rough, non-equivalent point of reference rather than a direct comparison: the two measures use different taxonomic units (OTU versus SH code) and different underlying clustering/assignment pipelines.

1.4.2 Wallacean shortfall

We characterized geographic sampling breadth for each EcM taxon at three levels (SH code, genus, named species). For each taxon we counted unique sampling occurrences as distinct 30 arc-second grid cells ($\sim 1 \text{ km}^2$), and produced frequency distributions of occurrences per taxon within Canada and globally. We report the proportion of taxa known from a single occurrence, and from fewer than 30 occurrences, both within Canada and globally, as straightforward measures of the shortfall. The within-Canada and global occurrence counts are drawn from the GlobalFungi dataset only, because it provides the structured sampling frame required for meaningful occupancy comparison; the global counts map each named Canadian species (or SH code) to all globally-known SH codes carrying that identity and match them against the full global GlobalFungi v5 SH abundance matrix under the same quality filters used for the Canadian data. As a complementary view relevant to species distribution modelling, we tabulated how many taxa meet common minimum-occurrence thresholds for modelling (30 occurrence records).

1.4.3 Prestonian shortfall

We obtained BioTIME (Dornelas et al. (2018)) directly from the BioTIME data portal (biotime.st-andrews.ac.uk) on 5 March 2026, downloading both the raw biodiversity time-series data (as an RDS file) and the accompanying study metadata (as a CSV) via the portal’s direct-download endpoints. We searched this database for time-series records of EcM fungal communities in Canada, filtering to studies with at least one Canadian location, at least two sampling time points, and an organism type consistent with fungi, and cross-referencing matching studies against the EcM dataset by taxonomy. We operationalize the Prestonian shortfall as the percentage of named EcM fungal species present in Canada that lack any abundance time-series data recorded anywhere on Earth.

1.4.4 Darwinian shortfall

We assessed genome-sequence availability for Canadian EcM genera by cross-referencing detected genera against the JGI MycoCosm fungal-genomics portal. We obtained MycoCosm’s organism list on 5 March 2026 from its public “All Fungi” listing page (myco-cosm.jgi.doe.gov/fungi/fungi.info.html, no login required), which lists every fungal genome in the portal; at the time of data collection the portal’s automated bulk-export feature required a

JGI login and was not functioning, so we retrieved the listing table by selecting and copying its contents directly from the page. Because the source page lists fungal genomes exclusively, we applied no additional kingdom- or phylum-level filtering. We operationalize the Darwinian shortfall as the percentage of named Canadian EcM fungal species absent from the database.

1.4.5 Raunkiaeran shortfall

We quantified trait-data availability using FungalTraits (a genus-level compilation) for all EcM genera detected in Canada. We assessed the six FungalTraits columns carrying information meaningful for EcM fungi, in two classes: three morphological/structural traits derivable from taxonomic description (fruitbody type, hymenium type, mycorrhizal exploration type) and three ecological/interaction traits (secondary lifestyle, endophytic-interaction capability, host specificity). We excluded the remaining FungalTraits columns as inapplicable to EcM fungi by lifestyle — those describing decay and saprotrophic modes, lichen photobionts, plant pathogenicity, animal biotrophy, and aquatic habitat. The seventh potentially relevant column, growth form, is “filamentous mycelium” for all EcM genera, and we excluded it as uninformative. Coverage is the proportion of genus $\times$ trait cells with a documented value, where “documented” means non-missing and not the literal entry “unknown”. Blanks in secondary lifestyle and endophytic-interaction capability are ambiguous between “not assessed” and “trait absent”, so coverage for those two traits is a lower bound.

1.4.6 Hutchinsonian shortfall

We addressed environmental coverage at two levels.

For **climate-space coverage**, we characterized how completely Canada’s climate space has been sampled. We defined climate space using WorldClim 2.1 bioclimatic variables BIO1 (mean annual temperature) and BIO12 (mean annual precipitation), aggregating the 30 arc-second rasters to ~ 10 arc-minutes and masking to the Canada boundary, then binned the two-dimensional temperature $\times$ precipitation space into a 50×50 grid of equal-width climate zones. For each zone we computed coverage as the proportion of its Canadian grid cells containing at least one EcM sample (each cell counted once), with a coverage of zero denoting a climate present in Canada but never sampled. We mapped coverage in both geographic and climate space, separately for GlobalFungi samples and for the combined GlobalFungi + GenBank dataset, against the available-climate frequency (the number of Canadian cells per zone). We adapted the code producing this figure from Hébert et al. (2026).

For **ecozone coverage**, we determined which of Canada’s 15 terrestrial ecozones (National Ecological Framework for Canada; ecozones.ca) contain at least one EcM sampling location, and report the number and proportion of ecozones sampled at a range of site thresholds.

1.4.7 Eltonian shortfall

We first assessed the **availability of host information** in the raw sample metadata. For GlobalFungi, among Canadian samples carrying at least one EcM SH code we tallied how many had an entry in the `dominant_plant_species` field, and cross-tabulated the samples with host information by `sample_type` (soil vs. root) (`18_eltonian.R`, Step A2b). For GenBank, we binned each Canadian EcM record’s `isolation_source` string into tissue categories (root/ectomycorrhiza, soil/rhizosphere, other, or not recorded) using keyword matching (`18_eltonian.R`, Step A2c). Note that this availability diagnostic keys on the single `dominant_plant_species` field for GlobalFungi, whereas the fungus–host association analysis below draws host identities from **both** `dominant_plant_species` and `other_plant_species`; the two therefore measure subtly different quantities (presence of a primary host label vs. any usable host identity), so their sample counts need not coincide.

We then focused on EcM fungus–host plant associations, in two scopes. For the **Canadian scope**, we extracted host information from the GenBank `host` qualifier (with fallback from `isolation_source`) and from GlobalFungi’s `dominant_plant_species` and `other_plant_species` fields, restricted to root samples. We canonicalized all host strings with the shared helper and matched them against the native Canadian EcM host species list, which serves as the denominator of potential interactions. We built fungus $\times$ host interaction matrices at the SH-code, named-species, and genus levels, and computed the proportion of Canadian EcM host species with at least one documented fungal association, the fill rate of the full host $\times$ named-fungal-species matrix, and the proportion of observed pairs supported by only a single record. For the **global scope**, we queried the full GlobalFungi global metadata for root samples matching Canadian host species, and queried GenBank globally for each Canadian EcM genus (retrieving all matching records), to establish which Canadian hosts and fungi have any documented association anywhere. To contextualize the sampling gap relative to host diversity, we produced a bivariate map classifying each 0.5° grid cell by EcM host species richness versus the proportion of those host species with EcM molecular-detection data in Canada, shown separately for tree and non-tree hosts (this bivariate map is produced in `18_eltonian.R`).

1.4.8 Extra analyses

We compiled physical EcM specimen records from GBIF as a vouchered complement to the sequence data: an occurrence download for Kingdom Fungi in Canada (basis of record `PRESERVED_SPECIMEN` or `LIVING_SPECIMEN`; records with geospatial issues excluded), filtered to species-rank records in EcM genera. For the primary map (Figure S5), the “with-sequence” genera are those observed in our own Canada-specific dataset (itself restricted to FungalTraits EcM genera), while Table S4’s “without-sequence” genera are drawn directly from the FungalTraits EcM genus list (`09_linnean.R`). The download (retrieved 6 March 2026; 673,532 occurrence records across 294 datasets before filtering) is archived at <https://doi.org/10.15468/dl.92rns5>

(download key 0017649-260226173443078; predicates country = CA, HAS_GEOSPATIAL_ISSUE = FALSE, taxonKey = 5, BASIS_OF_RECORD in {PRESERVED_SPECIMEN, LIVING_SPECIMEN}, format SIMPLE_CSV; licence CC BY-NC 4.0).

1.5 Figure preparation

Every figure in the manuscript and in these supplemental materials is generated directly by the analysis scripts, with one exception described below. Each analysis script writes its figure in two versions: a white-background version, which is the version reproduced in the manuscript and supplements, and an otherwise identical version on a light grey background (#F2F2F2).

We used the grey-background versions to assemble Figure 5, which is a designed conceptual composite rather than a computed figure. We laid out its five source panels manually in Microsoft PowerPoint and added the annotations that appear in it — the quadrant labels and the ellipses that link sampling strategies to the shortfalls they would jointly address. Figure 5 is therefore the only figure that cannot be regenerated by running the pipeline. Its source panels can be, and are listed below.

Source panels used to assemble Figure 5, and the analysis script that produces each.

Source panel (grey-background version)	Produced by
Figure-01_sampling_map_grey.png	19_sampling_maps.R
Figure-03_climate_gap_grey.png	17_hutchinsonian.R
Figure-04_host_bivariate_map_grey.png	18_eltonian.R
Figure-S1_dark_diversity_grey.png	10_dark_diversity.R
Figure-S4_ecozone_sampling_map_grey.png	17_hutchinsonian.R

19_sampling_maps.R also produces a grey-background version of Figure S5 (Figure-S5_gbif_specimens_grey.png), following the same convention as the panels above, but it is not used in Figure 5.

2 Supplemental figures and tables

Table S1. Unique EcM fungal sampling locations per Canadian ecozone (National Ecological Framework), for the combined GlobalFungi + GenBank dataset (n = 1,623 locations across 15 ecozones) and for GlobalFungi only (n = 486 locations), with ecozone area and combined sampling density. Points are assigned to ecozones by a within-polygon join against the detailed (unclipped) ecoregion layer, with points falling just offshore of the ecoregion coastline snapped to the nearest ecozone within 5 km; ecozone areas use the same polygon basis, so counts and areas are consistent. Ordered by decreasing combined density.

Ecozone	GlobalFungi (loc.)	Total (loc.)	Area (km ²)	Density (loc. / 10,000 km ²)
Mixedwood Plains	58	387	169258	22.86
Pacific Maritime	2	209	251087	8.32
Atlantic Maritime	104	156	282969	5.51
Boreal Shield	222	667	2079895	3.21
Montane Cordillera	17	60	489977	1.22
Taiga Plains	46	47	657947	0.71
Boreal Plains	20	38	740741	0.51
Boreal Cordillera	6	16	470758	0.34
Taiga Shield	9	24	1453302	0.17
Arctic Cordillera	1	2	340729	0.06
Northern Arctic	0	13	2838182	0.05
Prairies	1	2	466981	0.04
Southern Arctic	0	2	1683284	0.01
Hudson Plains	0	0	448432	0.00
Taiga Cordillera	0	0	266972	0.00

Table S2. The 28 FungalRoot/BIEN native Canadian EcM host species with at least one recorded, species-level host taxon in Canadian GlobalFungi or GenBank EcM records (the intersection between the native host list and the distinct species-level host taxa recorded across both sources).

Host species
<i>Abies balsamea</i>
<i>Alnus alnobetula</i>
<i>Alnus incana</i>
<i>Betula nana</i>
<i>Betula papyrifera</i>
<i>Dryas integrifolia</i>
<i>Gaultheria shallon</i>
<i>Monotropa uniflora</i>
<i>Picea engelmannii</i>
<i>Picea glauca</i>

Host species
<i>Picea mariana</i>
<i>Picea rubens</i>
<i>Pinus albicaulis</i>
<i>Pinus banksiana</i>
<i>Pinus contorta</i>
<i>Pinus ponderosa</i>
<i>Pinus rigida</i>
<i>Pinus strobus</i>
<i>Populus balsamifera</i>
<i>Populus tremuloides</i>
<i>Populus trichocarpa</i>
<i>Pseudotsuga menziesii</i>
<i>Pterospora andromedea</i>
<i>Quercus garryana</i>
<i>Quercus rubra</i>
<i>Salix arctica</i>
<i>Salix reticulata</i>
<i>Tsuga heterophylla</i>

Table S3. The 22 species-level host names recorded in the Canadian GlobalFungi or GenBank metadata that did not match the FungalRoot/BIEN native EcM host species list (Table S2). These include ornamental or non-native species, misspellings, and other problematic or truncated entries, and are excluded from the host-interaction analyses.

Host name (metadata)
<i>Abies balsamifera</i>
<i>Alnus crispa</i>
<i>Ascophyllum nodosum</i>
<i>Betula in</i>
<i>Betula payrifera</i>
<i>Corylus avellana</i>
<i>Deschampsia cespitosa</i>
<i>Douglas fir</i>
<i>Fagus or</i>
<i>Fagus sylvatica</i>
<i>Kalmia angustifolia</i>
<i>Not available</i>
<i>Oak beech</i>
<i>Picea abies</i>
<i>Pinus mugo</i>

Host name (metadata)
<i>Pinus radiata</i>
<i>Populus alba</i>
<i>Populus and</i>
<i>Populus in</i>
<i>Quercus robur</i>
<i>Ulva intestinalis</i>
<i>Zostera marina</i>

Table S4. The 32 EcM fungal genera (FungalTraits) represented in the Canadian GBIF physical-specimen records (Figure S5) that have no sequence data in Canada (not detected in the combined GlobalFungi + GenBank dataset), and so could be targeted for future molecular sampling. 'GBIF records' and 'GBIF species' are the count of physical-specimen occurrence records and distinct species names per genus in the GBIF download.

Genus	GBIF records	GBIF species
Boletinus	276	5
Turbinellus	190	2
Imleria	125	2
Gomphus	115	1
Albatrellopsis	109	3
Xanthoconium	84	2
Wynnella	41	2
Hortiboletus	33	2
Boletellus	27	1
Laeticutis	21	1
Gyrodon	19	1
Aleurina	18	3
Baorangia	14	1
Tremellodendron	14	3
Phallologaster	12	1
Cyanoboletus	11	3
Neoboletus	11	3
Underwoodia	10	1
Gastroboletus	6	2
Paragyrodon	6	1
Bankera	5	2
Boletochaete	5	1
Pseudocraterellus	5	2
Riessia	4	1
Porpoloma	3	1

Genus	GBIF records	GBIF species
Hydnangium	2	1
Plicariella	2	2
Zelleromyces	2	2
Brauniellula	1	1
Marcelleina	1	1
Mycolevis	1	1
Protoglossum	1	1

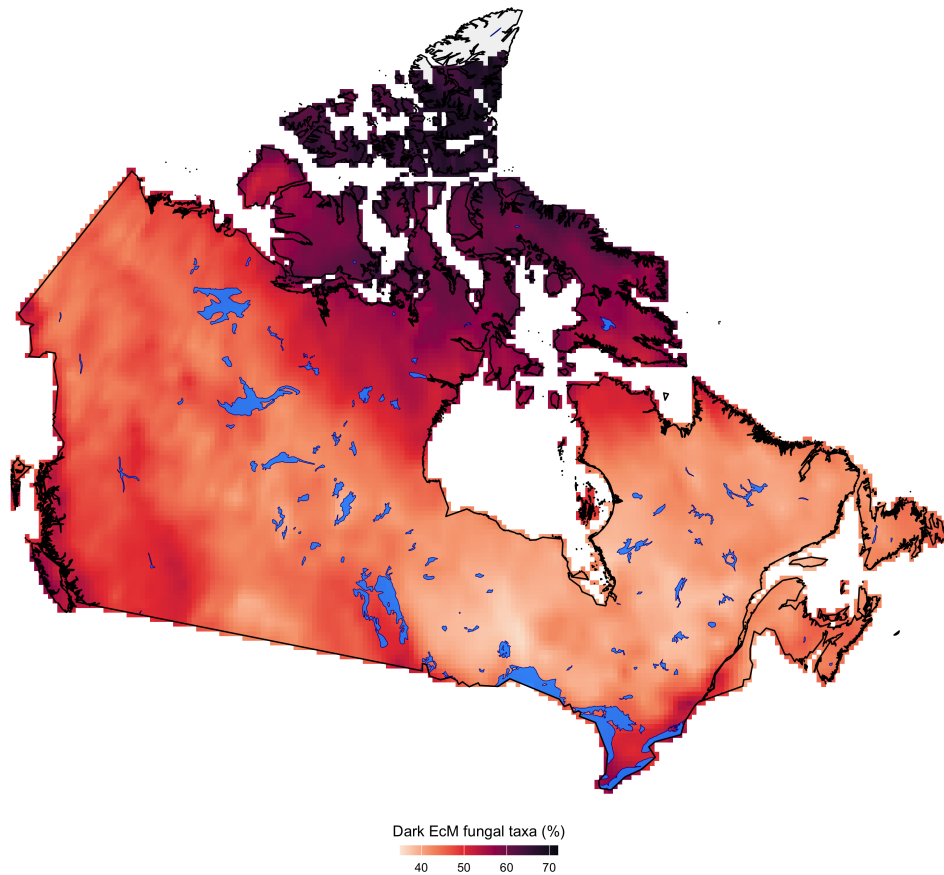


Figure S1. Modelled dark (“undescribed”) EcM fungal diversity across Canada (percentage of EcM OTUs undescribed), clipped from the global geospatial dataset of van Galen et al. (2025).

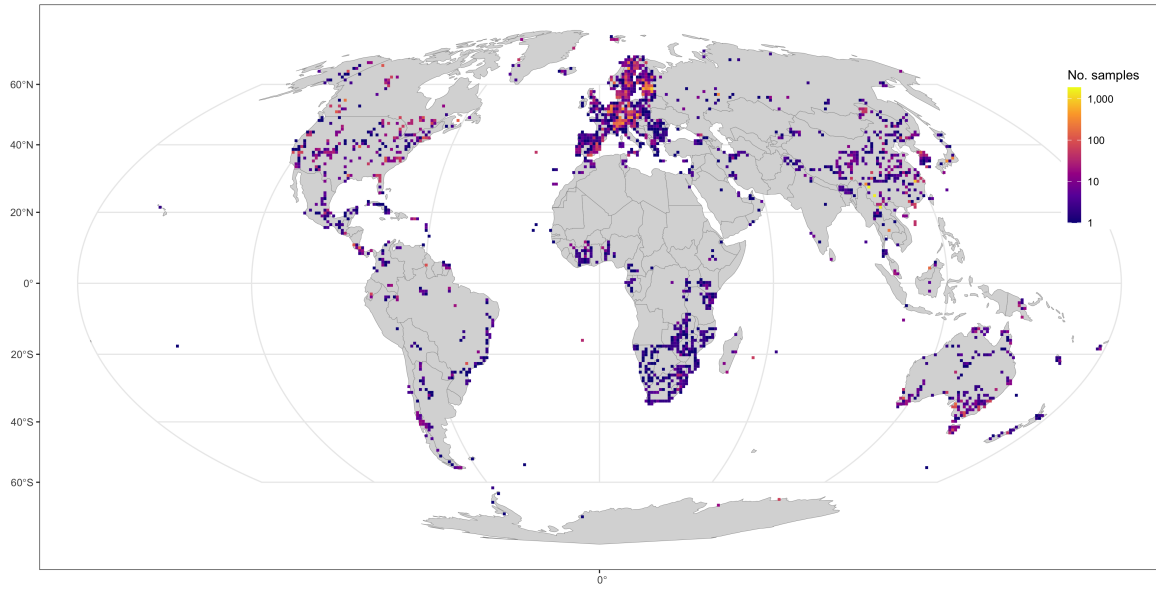


Figure S2. Sampling density of GlobalFungi samples in which at least one EcM fungal taxon was detected ($n = 34,925$; in Canada, $n = 1,469$ samples). Each coloured pixel represents a single 100×100 km region.

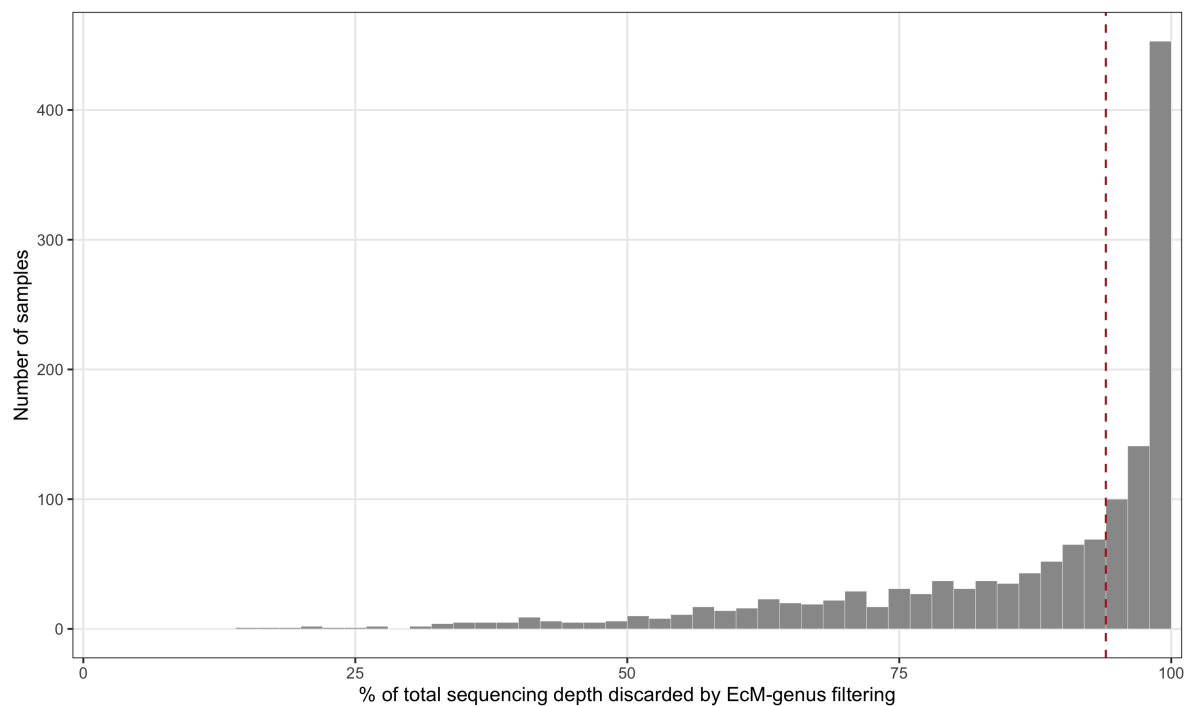


Figure S3. Distribution of the percentage of each Canadian GlobalFungi soil sample's total sequencing depth discarded by EcM-genus filtering, for every sample with at least one EcM-classified read ($n = 1,388$ samples). Q1–median–Q3: 79.2–94.0–99.0%. The vertical dashed line marks the median (94%).

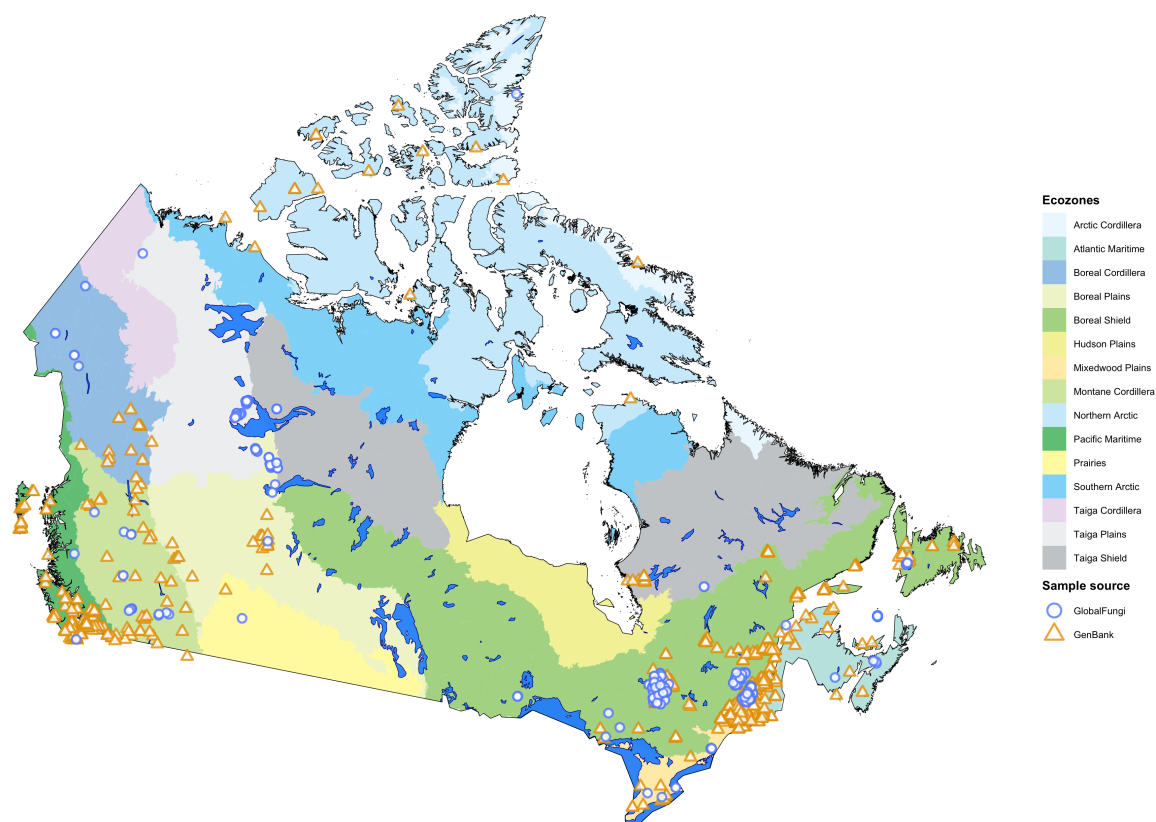


Figure S4. EcM fungal sampling locations (GlobalFungi and GenBank) projected in Canada Albers Equal Area and overlaid on Canadian ecozone polygons (National Ecological Framework for Canada). Sampling unit: one distinct raw lat/lon coordinate per record (GlobalFungi: $n = 486$; GenBank: $n = 1,137$; combined: $n = 1,623$ unique locations). Blue circles: GlobalFungi; orange triangles: GenBank.

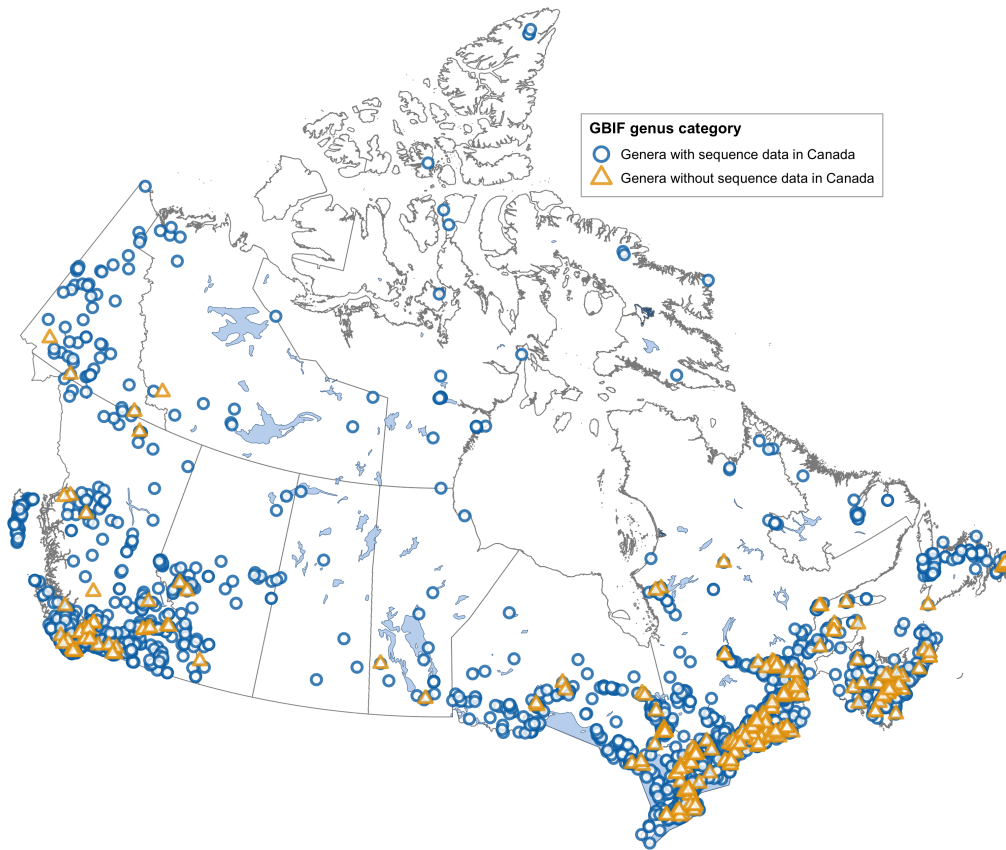


Figure S5. Occurrence records associated with physical EcM fungal specimen records in Canada, retrieved from GBIF on 6 March 2026 (PRESERVED_SPECIMEN and LIVING_SPECIMEN; unique occurrence records). Blue circles: occurrence records of EcM fungal genera (FungalTraits) with sequence data in Canada (combined GlobalFungi + GenBank; $n = 4,131$ plotted records). Orange triangles: occurrence records of EcM fungal genera without any sequence data in Canada ($n = 299$ plotted records). Plotted records are the georeferenced, distinct-coordinate occurrences within the Canada boundary, far fewer than the total specimen records because most GBIF records lack coordinates or share a coordinate. Source: GBIF Occurrence Download <https://doi.org/10.15468/dl.92rms5>.

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Supplemental materials (SM2) for: An assessment of biodiversity data shortfalls for ectomycorrhizal fungi in Canada

Jason Pither, with assistance from Claude (Sonnet 5 and Opus 4.8)

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1 Decisions log

Purpose

This log inventories the decisions **we** made in assembling and analyzing the dataset (Tables A1–A6), and, separately, the upstream decisions **outside our control** that are baked into the external datasets we rely on (Tables B1 - B9).

How to read the tables

Each row is one decision. The *Choice* column records what we did; the *Rationale* column records the stated reason

where one exists in the code or Supplemental Materials. Parameter values in `font` are taken directly from the pipeline scripts; the parenthetical script reference (e.g. `02_globalfungi.R`) points to the source.

1.1 PART A — Decisions made by us

1.1.1 Table A1. GlobalFungi record assembly (`02_globalfungi.R`)

#	Decision	Choice	Rationale
A1	Barcoding-region filter	Retain <code>barcoding_region %in% {ITS2, ITSboth}</code>	Restricts to samples carrying an ITS2 read, the region used for SH assignment; sets up parity with the GenBank ITS2 restriction (Detailed methods SM1).
A2	Manipulation filter	Retain <code>manipulated == "NO"</code>	Exclude experimentally manipulated samples so records reflect natural communities (Detailed methods SM1).
A3	Substrate exclusion	Drop substrate in <code>{shoot, air, water, sediment}</code>	These substrates are irrelevant to (soil/root-associated) EcM fungi (Detailed methods SM1).

#	Decision	Choice	Rationale
A4	Species-level GlobalFungi matrix	Not used ; work from the SH-level matrix only, decoded through the pinned UNITE build	Keeps a single, self-consistent SH definition across the project (<code>data_raw/DATA-DICTIONARY.md</code>).

1.1.2 Table A2. GenBank record assembly (`03_genbank.R`)

#	Decision	Choice	Rationale
B1	Search query	"Canada"[Country] AND "Fungi"[Organism] AND ("internal transcribed spacer"[All Fields] OR "ITS"[All Fields]); no date cutoff, no lifestyle keyword	Returns all matching fungal ITS records regardless of guild; we apply EcM filtering later and consistently (Detailed methods SM1).
B2	Sub-region extraction	ITSx (HMMER backend, Fungi profile set) to detect/extract ITS1 and ITS2	GenBank-side analogue of GlobalFungi's ITS2/ITSboth restriction, for cross-source comparability (Detailed methods SM1).
B3	ITS2 minimum length	Exclude ITS2 fragment < 100 bp	Floor to exclude spurious very short calls that trivially tie with many unrelated UNITE references (<code>03_genbank.R</code> , Detailed methods SM1).

#	Decision	Choice	Rationale
B4	SH matching input	Match on the extracted ITS2 fragment only ; ITS1 not independently classified	Deliberate simplification preserving a one-row-per-accession schema; we disclose the consequence (loss of exact sub-region parity with GlobalFungi) and diagnose it via <code>genbank_its1_resolution_crosstab.csv</code> (Detailed methods SM1).
B5	vsearch identity threshold	usearch_global at 98.5% identity	Matches GlobalFungi’s documented SH-assignment criterion (Detailed methods SM1).
B6	Tie resolution — named vs dark	Named-species hits compared by species name; dark hits (<code>_sp</code> placeholders) compared by SH code	Follows UNITE’s own dark-taxon convention (Nilsson et al. (2019); Ryberg and Nilsson (2018)) (Detailed methods SM1).
B7	Tie resolution — fallback rank	Agree at SH → assign SH; disagree but share genus → keep at genus-only (<code>sh_code/species = NA</code>); disagree at genus → exclude as ambiguous	“Climb to the lowest unambiguous rank” principle (Heeger et al. (2018)); excluded records logged to <code>genbank_ambiguous_species_excluded.csv</code> (Detailed methods SM1).

B8	Host taxon source	Structured <code>host</code> qualifier, with fallback regex extraction of binomials from <code>isolation_source</code>	<code>host</code> is the most reliable field; <code>isolation_source</code> recovers additional hosts (Detailed methods SM1).
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1.1.3 Table A3. Host-name normalization — shared across sources (`canonicalize_host()`, `00_setup.R`)

#	Decision	Choice	Rationale
C1	Single shared normalizer	One <code>canonicalize_host()</code> applied identically wherever we match a raw host string to a species list (GenBank host field; Eltonian; Hutchinsonian)	Raw host names are free text (binomials, misspellings, abbreviations, common names, inconsistent case/punctuation); one shared function avoids ad hoc per-analysis cleaning and silent match failures (Detailed methods SM1).
C2	Normalization steps	Strip surrounding punctuation → collapse whitespace → keep first two tokens → strip per-token punctuation → apply curated typo lookup → case-normalize (Titlecase genus, lowercase epithet) → reject non-Latin-name patterns → NA	Reduce each raw string to a standardized “Genus species”/“Genus” form, or NA if unresolvable (<code>00_setup.R</code>).

#	Decision	Choice	Rationale
C3	Typo-correction lookup	Curated typo_fixes (currently Abies balamifera → Abies balsamea), extended as new misspellings surface	Manual, auditable corrections rather than fuzzy auto-correction (00_setup.R).

1.1.4 Table A4. Source combination, spatial validation, EcM guild, and site/occurrence grain conventions
(**04_combine_ecm_dataset.R**, **00_setup.R**)

#	Decision	Choice	Rationale
D1	Coordinate validation	Spatial-containment check vs GADM Canada boundary, buffered by 2 km (in the Canada Albers Equal Area Conic projection) → logical coord_in_canada (TRUE / FALSE / NA); original coordinates always preserved	Distinguishes validated-inside from present-but-outside vs no-coordinates, without discarding data. The 2 km buffer absorbs GADM coastline-simplification and topology artefacts that would otherwise exclude a handful of genuinely Canadian coastal and island GlobalFungi records (Detailed methods SM1).

#	Decision	Choice	Rationale
D2	Spatial vs non-spatial use	Spatial analyses filter <code>coord_in_canada == TRUE</code> ; non-spatial enumerations (richness, counts) include all records	For counts, we treat a GlobalFungi <code>country == Canada</code> label as authoritative even where coordinates fall marginally outside the buffered polygon (Detailed methods SM1).
D3	EcM guild assignment	Genus-level: retain records whose UNITE-assigned genus is <code>primary_lifestyle == "ectomycorrhizal"</code> in FungalTraits (genus lower-cased before matching)	Genus-level EcM status from a single authoritative trait source (Detailed methods SM1).
D4	FungalTraits version	Pinned to v1.2 (FungalTraits_1-2.csv); one file used by all scripts via <code>paths\$fungaltraits</code>	Held fixed rather than tracked to a live source, so every script shares an identical EcM genus definition; provenance and SHA256 recorded in <code>fungaltraits_version.txt</code> (mirrors the UNITE build-pinning rationale given in Supplemental Materials SM1).

#	Decision	Choice	Rationale
D5	“Site” definition	Bin lat/lon to 3 decimal places (~100 m); <code>add_site_id()</code>	Collapses GPS-precision noise across repeat readings at one location without merging distinct sites (<code>00_setup.R</code> , Detailed methods SM1).
D6	Occurrence grain (Wallacean)	Distinct 30 arc-second (~1 km ²) grid cells; <code>snap_30arcsec()</code>	Aggregates occurrences to ~1 km ² cells matching common SDM practice (<code>00_setup.R</code> , Detailed methods SM1).
D7	Where we apply 3-decimal site-binning	We use the <code>add_site_id()</code> 3-decimal site-binning (D5) for the ecozone sampling-coverage counts, but do not apply it to the Wallacean per-taxon location table, whose within-Canada occurrence counts use raw distinct coordinates; we use the separate 30 arc-second occupancy grid (D6) for the worldwide occupancy comparison and Figure 2	Distinct raw coordinates preserve per-record occurrences for the Canada location counts, while the coarser 30 arc-second grid gives a like-for-like occupancy comparison against the worldwide GlobalFungi dataset (<code>11_wallacean.R</code> , Detailed methods SM1).

1.1.5 Table A5. Reference / host-data preparation (`05_prepare_fungalroot.R`, `06_host_species.R`, `07_bien2_ranges.R`, `08_host_rasters.R`)

#	Decision	Choice	Rationale
E1	Host determination — two routes	Species-level (FungalRoot occurrence records) OR genus-level (FungalRoot Table S2); combined with OR	Two independent routes; a species/genus can qualify via either or both (Detailed methods SM1).
E2	Qualifying species-level labels	Count as EcM-demonstrated only unambiguous EcM-positive labels: EcM, EcM, no AM colonization, ErM, EcM	Restrict to unambiguous positive evidence (Detailed methods SM1).
E3	Excluded label — EcM, AM undetermined	Excluded as qualifying evidence (but not disqualifying)	Denotes an EcM report with AM status unsurveyed, not a confirmed AM-negative; contradicted in a checked case (<i>Dryopteris filix-mas</i>) (Detailed methods SM1).
E4	Excluded label — EcM, AM (dual-positive)	Excluded as qualifying evidence	On inspection it was the sole EcM evidence for 118 of 698 candidate species across 20 genera (e.g. <i>Acer</i> , <i>Allium</i> , <i>Fraxinus</i> , <i>Juglans</i>) with no unambiguous record elsewhere (Detailed methods SM1).

#	Decision	Choice	Rationale
E5	Genus-level qualifying labels	Table S2 assignment of EcM or EcM-AM qualifies	EcM-AM captures genuine dual-mycorrhizal genera (<i>Salix</i> , <i>Populus</i> , <i>Eucalyptus</i>) the binary species rule cannot express; at least 67% species-consistency basis is FungalRoot’s own (Detailed methods SM1).
E6	Non-standard Table S2 rows	Excluded by default (e.g. “species-specific: EcM or NM-AM”)	Do not match either qualifying label (Detailed methods SM1).
E7	No literature supplementation	Do not add case-by-case literature EcM records (e.g. <i>Acer</i> , <i>Fraxinus</i> , <i>Juglans</i>)	Keep host determination reproducible from a single defined source; disclosed cost is under-counting a few genera (Detailed methods SM1).
E8	Native host list construction	BIEN species with Canadian distributions → filter to native via Native Status Resolver → retain species qualifying under either route in E1 (species-level occurrence list OR Table S2 genus list), combined with OR	Applies the two-route host determination (E1) to the native flora; no growth-form restriction. evidence_source records which route(s) applied (06_host_species.R , Step 4; Detailed methods SM1).

#	Decision	Choice	Rationale
E9	Growth-form assignment	Four-stage chain, applied in order: BIEN trait database → GIFT trait 1.2.1 (agreement = 0.66) → modal growth form of congeners already in the host list → curated manual override. The stage that supplied each value is recorded in growth_form_source	Growth form drives the tree / non-tree split reported in the main text and Figure 4, so it must be resolved for every host species; BIEN alone covers 215 of 272. Recording the source keeps the 42 congener-inferred values distinguishable from values taken from a trait record for the species itself (06_host_species.R , Steps 5–7c).

#	Decision	Choice	Rationale
E10	Unresolved synonymy	Accept drop-outs from independent taxonomic resolvers rather than harmonizing synonyms	A general synonym-harmonization step could shift counts for many species; we flag this as a known limitation instead. The genus route (E1) already rescues most such cases — e.g. we retain <i>Alnus alnobetula</i> via genus <i>Alnus</i> in Table S2 even though its species-level EcM evidence is filed under <i>A. crispa</i> — so a residual drop-out requires the genus to be absent from Table S2 as well (Detailed methods SM1).
E11	Host-range rasters	BIEN2 MaxEnt range polygons → reproject WGS84 → clip to Canada → rasterize to 0.5° grid ; three layers (host richness, host-data richness, host-data proportion)	Standardized grid for host richness and data-coverage comparison (Detailed methods SM1).

1.1.6 Table A6. Shortfall-analysis decisions (09–21)

#	Shortfall	Decision	Choice	Rationale
F1	Linnean	Richness units	Report unique UNITE SH codes, genera, and named species (excluding _sp)	SH codes are the primary unit: consistent OTU applicable to both sources, and many EcM taxa are unnamed (Detailed methods SM1).
F2	Linnean	Dark fraction	Proportion of detected SH codes lacking a species-level UNITE name (combined and per source)	Operational definition of the “dark fraction” (Detailed methods SM1).
F3	Linnean	van Galen comparison	Filter van Galen per-sample table to <code>Country == Canada</code> and <code>biome</code> <code>{forest, tundra}</code> ; report unweighted mean of per-sample unassigned proportion, as a non-equivalent reference only	Subset most comparable to our sampling; explicitly flagged as non-equivalent (different taxonomic units: OTU vs SH) (Detailed methods SM1).

#	Shortfall	Decision	Choice	Rationale
F4	Linnean	GBIF vouchered complement	GBIF Kingdom Fungi, Canada, basis PRESERVED_SPECIMEN/LIVING_SPECIMEN, geospatial issues excluded, filtered to species-rank records in EcM genera (FungalTraits)	Vouchered specimen complement to archived download key for reproducibility (retrieved 6 Mar 2026) (Detailed methods SM1).
F5	Wallacean	Occurrence levels	Count occurrences per taxon at SH, genus, and named-species levels	Multiple taxonomic resolutions of the shortfall (Detailed methods SM1).
F6	Wallacean	Reporting thresholds	Report proportion of taxa known from a single occurrence and from < 30 occurrences (Canada and global); tabulate common minimum-occurrence SDM thresholds	Straightforward shortfall measures; 30 is a common SDM minimum-sample rule of thumb (Detailed methods SM1).
F7	Wallacean	Data source	Occurrence/occupancy counts drawn from GlobalFungi only	GlobalFungi provides the structured sampling frame required for meaningful occupancy comparison (Detailed methods SM1).

#	Shortfall	Decision	Choice	Rationale
F8	Wallacean	Global comparator	Map each Canadian species/SH to all globally-known SH codes of that identity, matched against the full global GF v5 SH matrix under the same quality filters	Consistent global denominator (Detailed methods SM1).
F9	Prestonian	Source & filters	BioTIME (downloaded 5 Mar 2026): studies with at least 1 Canadian location, at least 2 time points, fungi-consistent organism type	Minimal criteria for a fungal time series in Canada (Detailed methods SM1).
F10	Prestonian	Shortfall metric	% of named Canadian EcM species lacking any abundance time series anywhere on Earth	Operational definition (Detailed methods SM1).
F11	Darwinian	Source	JGI MycoCosm “All Fungi” listing (retrieved 5 Mar 2026 by copying the page; bulk export required login and was non-functional)	Only complete accessible listing at the time; fungi-only page needs no extra filtering (Detailed methods SM1).

#	Shortfall	Decision	Choice	Rationale
F12	Darwinian	Matching	Exact set-membership match after normalization: genus = first token of MycoCosm taxon_name , species = first two tokens, both lower-cased/trimmed; matched against lower-cased emf genera and named species (placeholder epithets sp/spp/cf/aff excluded)	We use no fuzzy/edit-distance matching or tolerance (15_darwinian.R). Limitation: exact matching misses cross-database synonyms and spelling variants, which slightly <i>over</i> -states the shortfall.
F13	Darwinian	Shortfall metric	% of named Canadian EcM species absent from MycoCosm	Operational definition (Detailed methods SM1).

#	Shortfall	Decision	Choice	Rationale
F14	Raunkiaeran	Traits assessed	Six Fungal Traits columns: 3 morphological (fruitbody type, hymenium type, exploration type) + 3 ecological (secondary lifestyle, endophytic-interaction capability, host specificity)	Columns carrying information meaningful for EcM fungi (Detailed methods SM1).
F15	Raunkiaeran	Excluded trait	Growth form excluded	“Filamentous mycelium” for all EcM genera — uninformative (Detailed methods SM1).
F16	Raunkiaeran	Coverage definition	Proportion of genus $\times$ trait cells non-missing and not literal “unknown”; secondary lifestyle & endophytic capability treated as a lower bound	Blanks in those two traits are ambiguous between “not assessed” and “trait absent” (Detailed methods SM1).

#	Shortfall	Decision	Choice	Rationale
F17	Hutchinsonian	Ecozone unit	Canada's 15 terrestrial ecozones (National Ecological Framework); report number/proportion sampled across a range of site thresholds	Standard national ecozone framework; no ecozones excluded (all contain predicted host habitat) (Detailed methods SM1).
F18	Hutchinsonian	Offshore points	Points just offshore of the ecoregion coastline snapped to nearest ecozone within 5 km	Recover coastal samples misplaced by coastline generalization; ecozone areas use the same polygon basis (Detailed methods SM1, Table S1).
F19	Hutchinsonian	Climate variables	WorldClim 2.1 BIO1 (MAT) and BIO12 (MAP)	Two axes defining Canadian climate space (Detailed methods SM1).
F20	Hutchinsonian	Climate grid	Aggregate WorldClim 30 to ~10 (AGG_FACTOR = 20)	Match the spatial grain of the source workflow (Hébert et al. (2026)) (17_hutchinsonian.R).

#	Shortfall	Decision	Choice	Rationale
F21	Hutchinsonian	Climate binning	50 × 50 equal-width grid of temperature × precipitation; cell “sampled” if at least 1 EcM sample; coverage = sampled cells / Canada cells per zone	Coverage definition adapted from Hébert et al. (2026) (Detailed methods SM1, 17_hutchinsonian.R).
F22	Eltonian	Sample restriction (Canadian scope)	Host associations restricted to root samples (GenBank host/isolation_source; GlobalFungi dominant/other plant species)	Direct root extractions give strongest evidence of mycorrhizal association; bulk soil indicates only spatial proximity (Detailed methods SM1).
F23	Eltonian	Denominator	Native Canadian EcM host species list (Table 7) as denominator of potential interactions	Fixed, defensible universe of possible hosts (Detailed methods SM1).
F24	Eltonian	Matrices & metrics	Interaction matrices at SH, named-species, genus levels; report % hosts with at least 1 association, matrix fill rate, and % pairs from a single record	Multiple resolutions and a single-record fragility check (Detailed methods SM1).

#	Shortfall	Decision	Choice	Rationale
F25	Eltonian	Global scope retrieval	GenBank global query retrieves all matching records per genus (no cap), paginated via the NCBI history server	No truncation of heavily sequenced genera; a sensitivity check confirmed the earlier 2,000-record cap materially affected only a few macrofungal genera, so we removed the cap (<code>18_eltonian.R</code>).
F26	Sampling maps	Sampling unit (Fig S4)	One distinct raw lat/lon per record	Location-level depiction of sampling (Detailed methods SM1, Fig S4).
F27	Depth discard	Metric (Fig S3)	Per Canadian GF soil sample, % of total sequencing depth discarded by EcM-genus filtering, among samples with at least 1 EcM read	Quantifies the data cost of restricting to EcM genera (Detailed methods SM1, <code>20_depth_discard.R</code>).

1.2 PART B — Decisions outside our control (upstream datasets)

These decisions are embedded in the external datasets and tools we use. We report them as fully as the sources document them; where a source’s internal choices are not public in detail, that limitation is itself noted.

1.2.1 Table B1. GlobalFungi v5 (Větrovský et al. (2020))

#	Upstream decision	What we know	Note
U1	Sample inclusion & curation	GlobalFungi aggregates published community-survey amplicon studies, re-processed through its own consistent pipeline (documented at globalfungi.com)	Studies use varied sequencing platforms/protocols; predominantly soil samples (Detailed methods SM1).
U2	SH pre-assignment	SH codes pre-assigned against a specific UNITE release (the build we pin to; see Supplemental Materials SM1)	We inherit GlobalFungi's SH assignment for its records rather than re-calling them.
U3	SH-assignment identity criterion	Documented BLASTn criterion at ~98.5% identity	We match this on the GenBank side (B5).
U4	Read processing / quality filtering	Internal to the GlobalFungi pipeline (denoising, chimera removal, abundance tabulation)	Details not re-implemented or independently audited by us.

1.2.2 Table B2. UNITE (Nilsson et al. (2019))

#	Upstream decision	What we know	Note
U5	SH clustering	Dynamic clustering thresholds define SH codes; identifiers renumbered between builds	Motivates our build-pinning (Supplemental Materials SM1).
U6	Representative sequence & taxonomy	One representative sequence and one taxonomy string per SH	We read taxonomy directly from these (Supplemental Materials SM1).
U7	Dark-taxon naming	Placeholder names ending <code>_sp</code> for unnamed lineages	We follow UNITE’s convention in tie resolution (B6) and dark-fraction definition (F2).

1.2.3 Table B3. FungalTraits (Pölme et al. (2020))

#	Upstream decision	What we know	Note
U8	Trait scoring grain	Genus-level trait compilation	Constrains our EcM assignment and trait coverage to genus resolution (D3, F14).
U9	Primary-lifestyle categories	Includes <code>ectomycorrhizal</code> as a primary lifestyle	Basis of our EcM guild filter (D3).
U10	Blank vs “unknown” semantics	Blanks in some trait columns are ambiguous between “not assessed” and “trait absent”	Forces our lower-bound coverage treatment for two traits (F16).

1.2.4 Table B4. FungalRoot (Soudzilovskaia et al. (2020))

#	Upstream decision	What we know	Note
U11	Mycorrhiza-type labels	FungalRoot’s occurrence-level label vocabulary (incl. ambiguous EcM, AM undetermined, EcM, AM)	Drives our qualifying/excluded-label choices (E2–E4).
U12	Genus-level recommendation (Table S2)	Genus calls based on at least 67% species-level consistency	Basis of our genus-level host route (E5).
U13	Underlying GBIF taxonomy	Occurrence records resolved against the GBIF backbone	Independent from BIEN resolvers → unresolved synonymy drop-outs (E9).

1.2.5 Table B5. BIEN / BIEN2 (Maitner et al. (2018); Moulatlet et al. (2025))

#	Upstream decision	What we know	Note
U14	Occurrence name-scrubbing	BIEN applies its own name resolution	Independent resolver → synonymy drop-outs (E9).
U15	Native Status Resolver	BIEN’s algorithm classifies native vs introduced	We accept its native calls (E8).
U16	BIEN2 range models	MaxEnt-based modelled range polygons with fixed modelling settings (model name encodes settings)	We take ranges as given, reproject, and clip; we do not re-fit SDMs (E11).

1.2.6 Table B6. van Galen et al. (2025) dark-taxa data

#	Upstream decision	What we know	Note
U17	Dark-OTU definition	Proportion of EcM OTUs unassigned to species level, per GF v5 sample	Different taxonomic unit (OTU) from our SH-level dark fraction — comparison is non-equivalent (F3).
U18	Raster modelling	<code>percentage_dark_taxa</code> band from their modelled global layers	Used as-is for the Figure S1 map (<code>10_dark_diversity.R</code>).

1.2.7 Table B7. BioTIME (Dornelas et al. (2018))

#	Upstream decision	What we know	Note
U19	Study inclusion & structure	BioTIME's own criteria for what constitutes an included biodiversity time series	We filter within it (at least 1 Canadian location, at least 2 time points, fungi) but inherit its study boundaries (F9).

1.2.8 Table B8. JGI MycoCosm

#	Upstream decision	What we know	Note
U20	Genome inclusion	Which fungal genomes JGI has sequenced/portalized	Defines the Darwinian denominator; reflects JGI project priorities, not random sampling (F11).
U21	Listing completeness at retrieval	Page-copy snapshot on 5 Mar 2026 (bulk export non-functional)	Snapshot may lag the live portal; retrieval method documented (F11).

1.2.9 Table B9. WorldClim 2.1, GADM, Natural Earth, ecoregions, GBIF

#	Upstream decision	What we know	Note
U22	WorldClim interpolation	Climate surfaces interpolated from station data at 30 (Fick and Hijmans (2017))	We aggregate to ~10 (F20); interpolation error inherited.
U23	Administrative boundaries	GADM / Natural Earth boundary generalization	Affects coordinate validation and offshore snapping (D1, F18).
U24	Ecozone delineation	National Ecological Framework for Canada polygon definitions	Fixed unit for ecozone coverage (F17).
U25	GBIF specimen records	Basis-of-record labelling, georeferencing, and dataset coverage vary by publisher	We exclude geospatial-issue records but inherit publisher labelling (F4).

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