Received: 13 February 2023 | Accepted: 27 April 2023

DOI: 10.1111/1462-2920.16398

RESEARCH ARTICLE

ENVIRONMENTAL MICROBIOLOGY Applied Microbiology International

Mycena species can be opportunist-generalist plant root invaders

Christoffer Bugge Harder[1,2,3] | **Emily Hesling**[4] | **Synnøve S. Botnen**[1,5] | **Kelsey E. Lorberau**[1,6] | **Bálint Dima**[7,8] | **Tea von Bonsdorff-Salminen**[8] | **Tuula Niskanen**[8,9] | **Susan G. Jarvis**[10] | **Andrew Ouimette**[11] | **Alison Hester**[12] | **Erik A. Hobbie**[11] | **Andy F. S. Taylor**[4,12] | **Håvard Kauserud**[1]

[1]Department of Biosciences, University of Oslo, Oslo, Norway

[2]Department of Microbial Ecology, Lund University, Lund, Sweden

[3]Department of Biology, Section of Terrestrial Ecology, University of Copenhagen, Copenhagen, Denmark

[4]School of Biological Sciences, University of Aberdeen, Aberdeen, UK

[5]Oslo Metropolitan University, Oslo, Norway

[6]Department of Arctic and Marine Biology, UiT–The Arctic University of Norway, Tromsø, Norway

[7]Department of Plant Anatomy, Institute of Biology, Eötvös Loránd University, Budapest, Hungary

[8]Botany Unit, Finnish Museum of Natural History LUOMUS, University of Helsinki, Helsinki, Finland

[9]Jodrell Laboratory, Royal Botanic Gardens, Kew, Surrey, UK

[10]UK Centre for Ecology & Hydrology, Lancaster, UK

[11]Earth Systems Research Center, University of New Hampshire, Durham, New Hampshire, USA

[12]The James Hutton Institute, Aberdeen, UK

Correspondence
Christoffer Bugge Harder, Department of Biosciences, University of Oslo, Box 1066 Blindern, 0316 Oslo, Norway.
Email: cbharder@bio.ku.dk

Funding information
Carlsbergfondet, Grant/Award Number: CF18-0809; Danish Independent Research Fund, Grant/Award Number: 2032-00064B; European Commission, Grant/Award Number: 658849; Ministry of Environment, Finland, Grant/Award Number: YM23/5512/2013

Abstract

Traditional strict separation of fungi into ecological niches as mutualist, parasite or saprotroph is increasingly called into question. Sequences of assumed saprotrophs have been amplified from plant root interiors, and several saprotrophic genera can invade and interact with host plants in laboratory growth experiments. However, it is uncertain if root invasion by saprotrophic fungi is a widespread phenomenon and if laboratory interactions mirror field conditions. Here, we focused on the widespread and speciose saprotrophic genus *Mycena* and performed (1) a systematic survey of their occurrences (in ITS1/ITS2 datasets) in mycorrhizal roots of 10 plant species, and (2) an analysis of natural abundances of $^{13}C/^{15}N$ stable isotope signatures of *Mycena* basidiocarps from five field locations to examine their trophic status. We found that *Mycena* was the only saprotrophic genus consistently found in 9 out of 10 plant host roots, with no indication that the host roots were senescent or otherwise vulnerable. Furthermore, *Mycena* basidiocarps displayed isotopic signatures consistent with published $^{13}C/^{15}N$ profiles of both saprotrophic and mutualistic lifestyles, supporting earlier laboratory-based studies. We argue that *Mycena* are widespread latent invaders of healthy plant roots and that *Mycena* species may form a spectrum of interactions besides saprotrophy also in the field.

INTRODUCTION

Among ecologists, a consensus is emerging that the classical assignment of fungal species into single trophic groups, including mycorrhizal, saprotrophic or pathogenic lifestyles, may be too simplistic (Baldrian & Kohout, 2017; Selosse et al., 2018). Some otherwise free-living fungi can invade plant roots and exist as either asymptomatic endophytes (where no harm or benefit is apparent) and then switch from endophytic into becoming pathogenic (e.g., *Fusarium graminearum*, Löfgren et al., 2018), forming orchid mycorrhizas (*Piriformospora indica*, Weiß et al., 2016), or forming ericoid mycorrhizal associations (*Meliniomyces* spp., Martino et al., 2018). Numerous genera of saprotrophic fungi have been screened for their ability to colonize *Pinus sylvestris* and *Picea abies* seedling roots in vitro (Smith et al., 2017). Several genera, including *Mycena*, *Gymnopus*, *Phlebiopsis*, *Marasmius* or *Pleurotus*, invaded roots apparently without decomposing dead tissue in the process. However, beyond the invasion, the nature of the interactions with the plant host (if any) remains unknown.

For our target genus *Mycena*, several lines of direct and indirect evidence point to their ability to invade and interact with living plant roots, at least in vitro. *Mycena* is one the largest genera in Agaricales (over 500 species), widespread across habitats and climate zones (Kühner, 1938; Maas Geesteranus, 1992; Rexer, 1994; Robich, 2003; Aronsen & Læssøe, 2016). *Mycena* species have been identified as potential orchid mycorrhizal symbionts (Guo et al., 1997; Martos et al., 2009; Ogura-Tsujita et al., 2009; Selosse et al., 2010; Zhang et al., 2012) or endophytes in photosynthetic moss tissue (Davey et al., 2013) and non-mycorrhizal brassicaceous plants (Glynou et al., 2018). They have also formed mycorrhiza-like structures in the roots of *Vaccinium corymbosum* in growth studies (Grelet et al., 2017). Recently, Thoen et al. (2020) showed that multiple species and individual strains of *Mycena* could colonize roots of *Betula pendula* seedlings in vitro, and formed a gradient of interactions from harmful to neutral to beneficial, with some species or strains able to transfer nutrients to the plant host. This is significant, as prior to this, *Mycena* sensu stricto (Moncalvo et al., 2002) (henceforth simply '*Mycena*') was known primarily as quantitatively important litter and wood debris decomposers (Baldrian et al., 2012; Boberg et al., 2008; Kyaschenko et al., 2017). The spectrum of interactions seen by Thoen et al. (2020) is further noteworthy in light of the "waiting room hypothesis" (Selosse et al., 2009; van der Heijden et al., 2015) on mycorrhizal evolution, which suggests that the mycorrhizal habit evolves from saprotrophs gradually via neutral endophytic intermediate states. This hypothesis has remained controversial even though it is accepted that the mycorrhizal habit has evolved on numerous, independent occasions from saprotrophic ancestors (Kohler et al., 2015; Tedersoo & Smith, 2013). Thus, the genus *Mycena* may represent a promising research model for studying both ecological versatility in fungi and the possible ongoing evolution of fungi traditionally believed to be purely saprotrophic *en route* to developing mycorrhizal abilities.

Most evidence for trophic versatility in *Mycena* originates from in vitro studies, and it is uncertain to what extent this translates to the field. To investigate the trophic mode of fungi in natural environments, analysis of the isotope ratios of ^{13}C:^{12}C and ^{15}N:^{14}N (expressed as $\delta^{13}C$ and $\delta^{15}N$ values relative to known standards) can be applied directly to fungal carpophores and other field material. Ectomycorrhizal fungi are generally more enriched in ^{15}N and depleted in ^{13}C than saprotrophic fungi (Griffith, 2004; Hobbie et al., 1999; Hobbie et al., 2001; Kohzu et al., 1999; Mayor et al., 2009; Taylor et al., 1997). By comparing against fungi of known trophic status, the natural abundance of $\delta^{15}N$ and $\delta^{13}C$ can also give strong indications of the nutritional mode of fungi with unknown trophic status. Thus, Halbwachs et al. (2018) recently used this approach to strongly suggest that *Hygrocybe*, another genus traditionally believed to be saprotrophic, was most likely biotrophic with plants.

The occurrence and abundance of *Mycena* sequences retrieved from wild plant roots also suggest interactions with plant roots in situ. Such reports are particularly common from Arctic plants, including *Bistorta vivipara*, *Cassiope tetragona*, *Dryas octopetala* and *Salix polaris* (Blaalid et al., 2014; Botnen et al., 2014; Lorberau et al., 2017). In some cases, *Mycena* sequences comprised >30%–50% of the total reads, suggesting that they are not simply casual colonizers. The harsh and oligotrophic Arctic environments may stimulate otherwise free-living fungal genera (including *Mycena*) to explore new ecological niches (Botnen et al., 2014; Jumpponen & Trappe, 1998; Lorberau et al., 2017; Ryberg et al., 2009; Ryberg et al., 2011; Timling et al., 2012). Nevertheless, *Mycena* reads have also been recovered in high quantities from inside living *Picea abies* roots in temperate environments (Kohout et al., 2018). Overall, however, current information on the occurrence of *Mycena* and other saprotrophs in roots is unsystematic and too scattered to identify any clear patterns of their occurrence and abundance.

High throughput sequencing (HTS) studies of fungal communities in plant roots generally target mycorrhizal fungi (Bahram et al., 2011; Blaalid et al., 2014; Buee et al., 2009; Kaur et al., 2019; Tedersoo et al., 2010; Vasar et al., 2017). The workflow requires annotating hundreds or thousands of operational taxonomic units (OTUs)/clusters of fungi into ecological guilds. The genus level is traditionally considered the most relevant level for separating fungal taxa by nutritional mode (den Bakker et al., 2004; Fries & Mueller, 1984; Garnica et al., 2016; Molina & Trappe, 1994; Tedersoo &

Smith, 2013). Although it is widely accepted that evolution is a continuous process, most studies still favour those conservative taxonomic ecological generalizations, often for practical reasons. Thus, modern ecological annotation software for amplicon analyses (Nguyen et al., 2016, Pölme et al., 2020) remains largely based on this view. In studies of mycorrhizal fungi in roots, fungal genera identified as being saprotrophic are therefore often at best briefly mentioned, reported as one lumped ecological group (Menkis et al., 2012; Tedersoo & Smith, 2013), or simply dismissed as accidental contamination (Liao et al., 2014). Thus, large quantities of potentially informative data are often ignored, leading to a potentially oversimplified view of root ecology and interactions.

Here, we analyse the occurrence of *Mycena* in the roots of wild plants in a range of ecosystems and investigate the potential trophic versatility of *Mycena* in the field by presenting: (1) a systematic analysis of data from 10 plant species from Arctic and temperate regions from previously published and newly generated ITS1/ITS2 HTS data sets from living plant roots, and (2) a comparison of the natural abundance of ^{13}C and ^{15}N in carpophores of *Mycena* with other fungi, soils and mycorrhizal host plants from 253 fungal collections, host plants and soils from 5 field locations (see Figures S1, S2 for a map of sampling sites).

We investigated four main research questions:

(1) Are *Mycena* species (and other supposedly saprotrophic genera) systematically found associated with living plant roots in significant quantities?

(2) Do the data from 13C and 15N abundances support the view that Mycena species may form mutualist associations with plants?

(3) Are there indications of host preferences or specificity among invasive *Mycena* species?

(4) Are root invasions by *Mycena* or saprotrophic fungi more prevalent in arctic and alpine environments?

RESULTS

Analysis of ITS metabarcoding datasets from ectomycorrhizal plant roots

We data-mined our set of ectomycorrhizal plant host species systematically for *Mycena* presence in their roots. Our plant host sample consisted of 10 species in total, 3 amplified with ITS1 primers, and 7 with ITS2 primers from both previously published studies and new datasets (Table 1). We separated data into both OTUs (at the 3% threshold, retaining only those with ≥10 sequences) and amplicon sequence variants (ASVs) and investigated further OTUs/ASVs identified as *Mycena* (Table 2). As there were no major differences between the finer ASV-('haplotype') scale or at the coarser 3% OTU scale (Tables S8, S9), we focused on the OTU datasets in further analyses.

After quality sorting, a final dataset of 889,290 ITS1 sequences was clustered into 1193 OTUs for the ITS1 dataset and 992,890 sequences into 1032 OTUs in the ITS2 dataset (Table 1). For a detailed list of the sequence quality sorting steps on the ITS1 and ITS2 data and the respective counts for each host species, see Supplementary data and Tables S1–S6. Applying the 'coverage/completeness' method for assessing saturation (Chao & Jost, 2012), 111 samples failed to meet the 97% coverage cutoff value and were discarded. Although the iNEXT (Hsieh et al., 2016) extrapolations of observed species richness suggested that some slight undersampling remained in some samples (Figure S5), none of the 10 species showed correlations between *Mycena* infection levels (all p values >0.05, Table S7) with sampling depth.

For ITS1, 606 of 1193 OTUs (78.3% of the sequences) could be identified to genus level by SINTAX at the threshold of BPP >0.6; for ITS2, this number was 513 of 1032 ITS2 OTUs (84.5% of sequences).

The same SINTAX classification identified 13 *Mycena* OTUs (1.5% of all ITS1 sequences) in the ITS1 dataset and 14 *Mycena* OTUs in ITS2 (12.6% of all ITS2 sequences).In a second identification step, especially targeting *Mycena*, representative sequences of all OTUs were clustered together with our constructed *Mycena* ITS database of 576 sequences. This analysis revealed additional 7 ITS1 OTUs and 7 ITS2 OTUs not identified as *Mycena* by SINTAX at BPP >0.6. Thus, in total 20 ITS1 OTUs (2.1% of all sequences) and 21 ITS2 OTUs (15.8% of all sequences) could be identified as *Mycena* (s. str) with these two combined methods.

Of other (non-*Mycena*) taxa traditionally considered to be saprotrophic or endophytic, we found Sebacinales in the four Arctic host plants, the zygomycete *Mortierella* in *B. pubescens*, *A. uva-ursi* and *A. alpinus* (Scottish hosts), and *Phialocephala in B. vivipara* and *Clavulinopsis*/*Clavaria* in *C. tetragona* (Figure 2A–C). No other saprotrophic or endophytic genera made up more than 0.5% of the sequences in any of the host plants. The high and very variable infection levels and frequency patterns of *Mycena* were not found in other saprotrophic or endophytic taxa.

Mycena infection levels

Mycena infection levels reached up to 25%–80% of all reads in individual samples of 9 out of 10 examined plant host species (Figure 2A–C), with considerable intraspecific infection variation (Figure 1, Figure S3). *Pinus sylvestris* was the only plant host with consistently little or no infection by *Mycena*. For the ITS1 data, *Mycena* average read content for all 519 *B. vivipara* samples was significantly lower than for *S. polaris* ($n = 20$) and *D. octopetala* ($n = 22$; Figure 1A, B, Table S10). However, the *S. polaris* and *D. octopetala*

TABLE 1 Overview of all species and samples included in this study.

ITS1 studies	Plant hosts[a]	Locality	Sampling time
Blaalid et al. (2012)	*B. vivipara* ($n = 59$)	Finse (Norway)	July 2008
Yao et al. (2013)	*B. vivipara* ($n = 51$)	Finse (Norway)	July 2011
Blaalid et al. (2014)	*B. vivipara* ($n = 146$)	32 localities on Svalbard	August 2009–10
Botnen et al. (2014)	*B. vivipara* ($n = 19$), *D. octopetala* ($n = 22$), *S. polaris* ($n = 20$)	Blomsterdalen, Svalbard	Mid-July 2011
Mundra et al. (2015)	*B. vivipara* ($n = 84$)	Isdammen, Svalbard	July 2009
Davey et al. (2015)	*B. vivipara* ($n = 103$)	Svalbard, Finse (Norway)	July 2008
Botnen et al. (2019)	*B. vivipara* ($n = 57$)	Scotland, Austria, Iceland, Jan Mayen	July–August 2013–14
ITS1 summary	**Total plant host samples**	**No. of sequences/plant species (OTU analysis)**	**No. of sequences/plant species (ASV analysis)**
	B. vivipara ($n = 519$)	803,649	908,621
	D. octopetala ($n = 22$)	39,880	43,286
	S. polaris ($n = 20$)	30,069	33,141
ITS2 studies	**Plant hosts[a]**	**Locality**	**Sampling time**
Jarvis et al. (2015)	*P. sylvestris* ($n = 32$)	Scotland	July–September 2011
Lorberau et al. (2017)	*C. tetragona* ($n = 15$)	Endalen, Svalbard	Early August 2013
Biogeography project (new data)	*A. alpine* ($n = 10$), *A. uva-ursi* ($n = 8$) *B. nana* ($n = 8$) *S. herbacea* ($n = 7$)	Scotland	July–November 2011–12
Altitude project (new data)	*A. uva-ursi* ($n = 68$), *P. sylvestris* ($n = 9$)	Scotland	Late June-early July 2011
Birch (new data)	*B. pendula* ($n = 81$)	Scotland	August 2008
ITS2 summary	**Total plant host samples**	**No. of sequences/plant species (OTU analysis)**	**No. of sequences/plant species (ASV analysis)**
	A. alpine ($n = 10$)	59,829	61,703
	A. uva-ursi ($n = 76$)	241,418	247,091
	B. nana ($n = 8$)	49,927	51,182
	B. pendula ($n = 81$)	90,214	93,429
	P. sylvestris ($n = 41$)	212,577	219,124
	S. herbacea ($n = 7$)	25,298	25,497
	C. tetragona ($n = 15$)	313,627	385,763

Our plant samples consisted of the herbaceous ectomycorrhizal Arctic *Bistorta vivipara*, subshrub *Dryas octopetala* and the dwarf shrub *Salix polaris* (Blaalid et al., 2012; Blaalid et al., 2014; Botnen et al., 2014; Davey et al., 2015; Mundra et al., 2015; Yao et al., 2013); the Arctic ericaceous *Cassiope tetragona* (Lorberau et al., 2017) and the ectomycorrhizal conifer *Pinus sylvestris* (Jarvis et al., 2015) from Scotland; and new data also from Scottish (temperate) plants: the arbutoid mycorrhizal *Arctostaphylos alpine* and *Arctostaphylos uva-ursi*, ectomycorrhizal dwarf shrubs *Betula nana* and *Salix herbacea*, and the ectomycorrhizal trees *Betula pubescens* and additional *Pinus sylvestris*. The *Betula pubescens* samples all came from saplings of <1 m kept low by deer/sheep grazing; the other host plants collected were mature.

[a]Some samples with low sequence counts were omitted from our analyses. The numbers listed represent samples which were included in our final analyses.

data sets came from only one locality (Botnen et al., 2014), and when comparing them only with the *B. vivipara* dataset ($n = 19$) from the same locality, no significant differences were observed (one-way ANOVA, $df = 2$, $F = 1.36$, $p = 0.263$). *When it comes to the ITS2 sequences*, *the* host species could be roughly divided into three groups based on average *Mycena* infection level: (1) *P. sylvestris* with virtually no *Mycena*, (2) an intermediate group (median values about 5%–10%) with *S. herbacea*, *A. alpine*, *B. nana* and *A. uva-ursi*, and (3) *B. pubescens* and *C. tetragona* with median *Mycena* infection levels above 20% of all sequence reads. While all species (except *P. sylvestris*) harboured individual samples with few, if any, *Mycena* and some with >30%, there were still significant differences between these three rough categories. (Figure 1D, E, Table S11).

Environmental influences on *Mycena*

Disparities in sample size and study metadata only permitted limited testing of environmental influences on *Mycena* infection in three host species. In *C. tetragona*,

TABLE 2 Total sequence counts for operational taxonomic units (OTUs) and amplicon sequence variants (ASVs) for each host species.

ITS1	*B. vivipara* ($n = 519$)[a]		*D. octopetala* ($n = 22$)		S. polaris ($n = 20$)[a]	
OTUs ($n = 1193$)	803,649		39,880		30,069	
ASVs ($n = 2272$)	908,621		43,286		33,141	
ITS2	***A. alpine* ($n = 10$)**	***A. uva-ursi* ($n = 76$)[a]**	***B. nana* ($n = 8$)**	***B. pendula* ($n = 81$)[a]**	***P. sylvestris* ($n = 41$)**	***S. herbacea* ($n = 7$)**	***C. tetragona* ($n = 15$)**
OTUs ($n = 1032$)	59,829	241,418	49,927	90,214	212,577	25,298	313,627
ASVs ($n = 1559$)	61,703	247,091	51,182	93,429	219,124	25,497	385,763

[a]Some samples with low sequence counts were omitted from our analyses. The numbers listed represent samples which were included in our final analyses.

there were no differences in *Mycena* infection levels between samples derived from drought and control plots applied by Lorberau et al. (2017) (two-tailed *t*-test, unequal variances, $p = 0.57$). In *A. uva-ursi*, the level of *Mycena* infection decreased significantly with increasing altitude (65–805 m above sea level; $R^2 = 0.2$, $p < 0.0001$, data not shown), which does not agree with the assumption (question 4) that increasingly stressful environments should facilitate infection with *Mycena* or saprotrophs.

In *B. vivipara*, no correlations between *Mycena* infection level and annual mean temperature, latitude, or mean temperature of the wettest quartal were found (all adjusted $R^2 < 0.01$, all $p > 0.25$, see Table S12). A very weak correlation between decreasing *Mycena* species richness in roots and increasing the mean temperature of the wettest quartal ($R^2 = 0.01$, $p = 0.04$) disappeared when the Austrian outlier samples (which contained no *Mycena*) were excluded.

A chi-square test on the observed versus expected prevalence of *Mycena* in 222 *B. vivipara* host plants from 44 patches (a patch constituted multiple plants collected in close proximity) showed a significant, non-random association ($c^2 = 65.22$, $df = 43$, $p = 0.01$) of *Mycena* infections in host plants, suggesting that *Mycena*-infected *B. vivipara* were distributed in clumps.

Mycena phylogenetics of OTUs and species diversity

Among the 20 ITS1 and the 21 ITS2 OTUs that were identified as *Mycena*, we found no phylogenetic signal suggesting that root invasion might be linked to certain clades (Figure 3). Many of the same *Mycena* species were in both the ITS1 and ITS2 datasets; 12 ITS1-ITS2 pairs of OTUs clustered with >90% probability to the same branches. There were no indications of host specialization by *Mycena* species on particular host species, with large individual variations in all host plants (again except *P. sylvestris*) between which *Mycena* species that were found (Figure S3). Several species, such as *Mycena epipterygia* or *Mycena leptocephala*, occurred at infection levels of >10% in at least one individual of 6 of 10 host species or more (Figure S3). Indeed, the two OTUs were the only OTUs shared between *C. tetragona* from Svalbard and all Scottish host species (except *P. sylvestris*).

Stable isotope data

On average, carpophore values of $\delta^{15}N$ and $\delta^{13}C$ placed *Mycena* among the saprotrophs. They were higher in $^{TM}{}^{13}C$ and lower in $^{TM}{}^{15}N$ than the average of the remaining non-*Mycena* saprotrophs (Figure 4A–E). The $^{TM}{}^{13}C$ values of all saprotrophic species for all

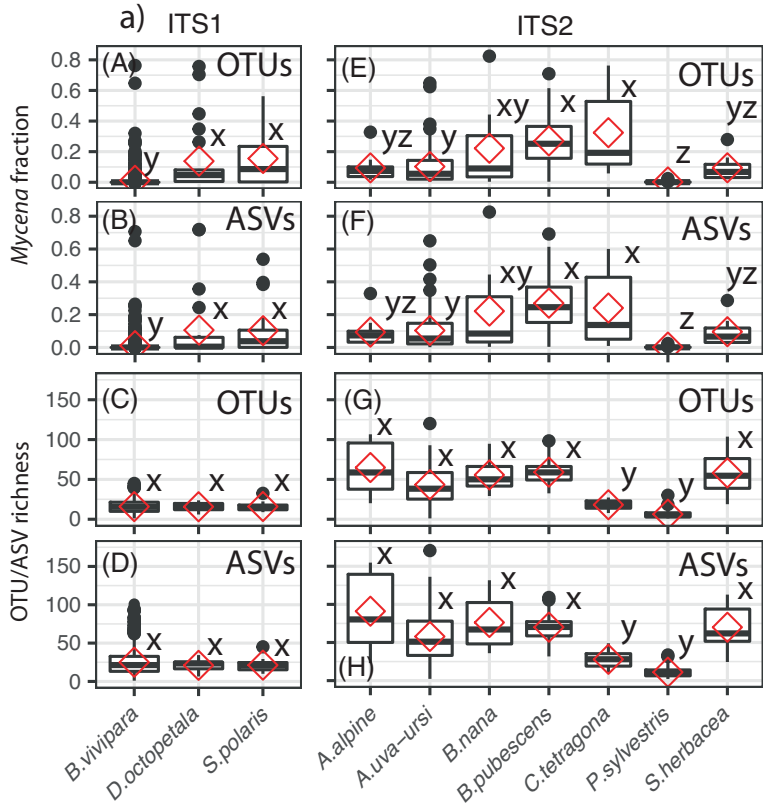

FIGURE 1 Mycena infection levels (fraction of read shares) and species richness at 97% coverage corrected for sampling depths (Chao & Jost, 2012) for the ITS1 (A-D) and ITS2 (E-H) datasets. Very little difference between the OTU and the ASV approaches were found. Letters x-y-z denote host species «significance groups» as found by ANOVA + Scheffes multiple comparisons test for a significance at the $p < 0.05$ level. Species sharing one identical letter (x, y or z) do not significantly differ from each other in mean Mycena infection level/overall species richness (at 97% coverage).

regions were between −26% and −22%, except for a *Phloeomana speirea* at Finse (Figure 4A) at −27.1%, and one *Mycena metata* collection from Svalbard (Figure 4C) at −26.9%. However, there were striking anomalies (and intraspecific variations) in the ^{15}N values for certain individual collections of *Mycena*, particularly *Mycena pura*, which varied between 1.7% for *Mycena pura* in Gribskov and 12.6% for *Mycena pura*1 at Finse. A *t*-test showed the *Mycena pura*1 and *Mycena pura*2 collections at Finse to be strongly and significantly higher in $\delta^{15}N$ than the average for the other *Mycena* at Finse ($p < 0.0001$), and slightly but still significantly higher in $\delta^{13}C$ ($p = 0.02$).

In the stepwise regression of $\delta^{15}N$, genera were separated by up to 12% into 5 groups (Table S13). Relative to the mean, *Mycena* grouped at −3% with the litter decay fungi *Calvatia*, *Lycoperdon* and *Rhodocollybia*. The overall adjusted r^2 of the regression model was 0.56 ($n = 253$), with site accounting for 8.1% of the variance and the remaining 48.0% accounted for by genus.

In the stepwise regression of $\delta^{13}C$, genera were separated by up to 5% into 8 groups (Table S14). Relative to the mean, *Mycena* grouped at +1% with both the ectomycorrhizal *Rhizopogon*, *Ramaria*, which appear to contain both saprotrophic and ectomycorrhizal members (Hobbie et al., 2002), and the litter decay fungi *Calvatia*, *Lepista* and *Rhodocollybia*. The overall adjusted r^2 of the regression model was 0.66 ($n = 252$), with site accounting for 11.7% of the variance, nitrogen concentration (%N) for 2.8% and the remaining 51.4% accounted for by genus.

The $\delta^{15}N$ values for host plants in all 5 regions were all below 0%, which is significantly lower than both carpophores and the soil (Figure 4). Soil $\delta^{15}N$ values were below 2%, with lower overall N content but higher $\delta^{15}N$ values for deeper soil depths. The $\delta^{15}N$ and $\delta^{13}C$ values differed significantly between the five regions, but the amount of variance for both measures explained by region in the mixed linear model was <20% for both isotopes and well below that explained by sample type and genus/sample type in combination (>75% for both). Overall, the other fungal genera had isotopic profiles that matched their expected nutritional mode.

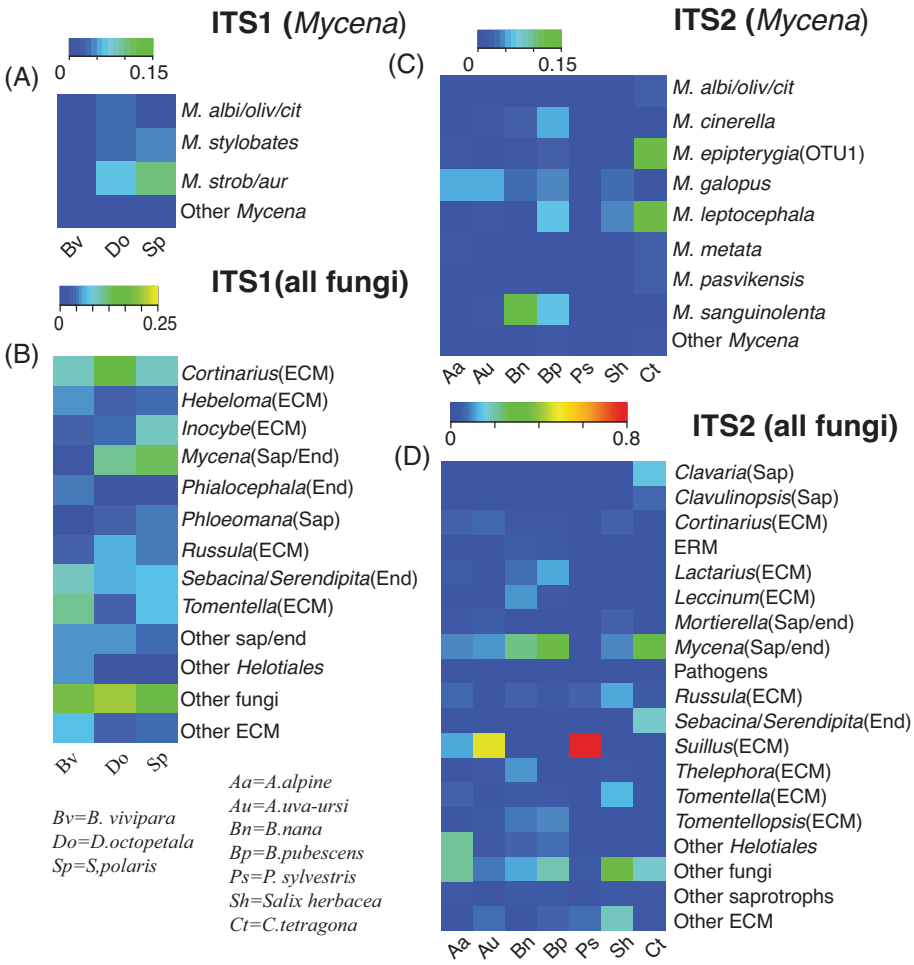

FIGURE 2 Heatmap of Mycena species occurrence (A-C) and overall genus occurrence (B-D). Note the slightly different colour bars in each plot. Only *Mycena* species that made up >1% of at least one sample were included as separate rows in A-C. In B-D, only genera that comprised >5% of reads as an average in at least one host species had its own separate row. Besides these criteria, we also included Helotiales that could not be identified to generic level, but might still conceivably harbour ericoid mycorrhizal fungi or dark septate endophytes (as Phialocephala). ERM, ericoid mycorrhiza; ECM, ectomycorrhiza; Sap, saprotroph; end, endophyte. Note the near-complete dominance of ectomycorrhiza (particularly Suillus) in *P. sylvestris* (Ps).

DISCUSSION

Mycena found in roots are not surface contaminants

This study constitutes a rare systematic analysis of the occurrence of a supposedly saprotrophic genus, *Mycena*, in wild plant roots. The results indicate that *Mycena* species are frequent root colonizers of a taxonomic range of mycorrhizal host plants, although infection levels are very variable. However, here in our analyses presented here, *Mycena* infection was widespread in Arctic and alpine hosts as well as in temperate hosts. The general lack of host-specificity in *Mycena* was also universal. The ability to colonize living roots appears to be a widely shared trait across the *Mycena* phylogeny, consistent with the findings of Thoen et al. (2020). We argue that the systematic findings of *Mycena* in multiple plant roots should settle concerns such as Vohník (2020), who suggested that the high numbers of *Mycena*/*Clavaria* sequences in *C. tetragona* (Lorberau et al., 2017) might be explained by a lack of root cleaning/washing. All samples (including *C. tetragona* (Lorberau et al., 2017)) were indeed collected following standard practice for studies aimed at mycorrhizal fungi, with healthy-looking roots selected and either serially washed or surface sterilized, and the work performed in different laboratories. It is thus unlikely that the systematic recovery of large numbers of *Mycena* reads from multiple plants is mainly due to mycelia living commensally on the root surface.

In 9 out of 10 host plants, *Mycena* infections were present in many samples and varying from little or no infection up to >40%–50% of the recovered reads. No other supposedly saprotrophic fungal genus was widely found across the different plant species. This is consistent with the findings of Kohout et al. (2018) in their study on root communities in a *Picea abies* stand

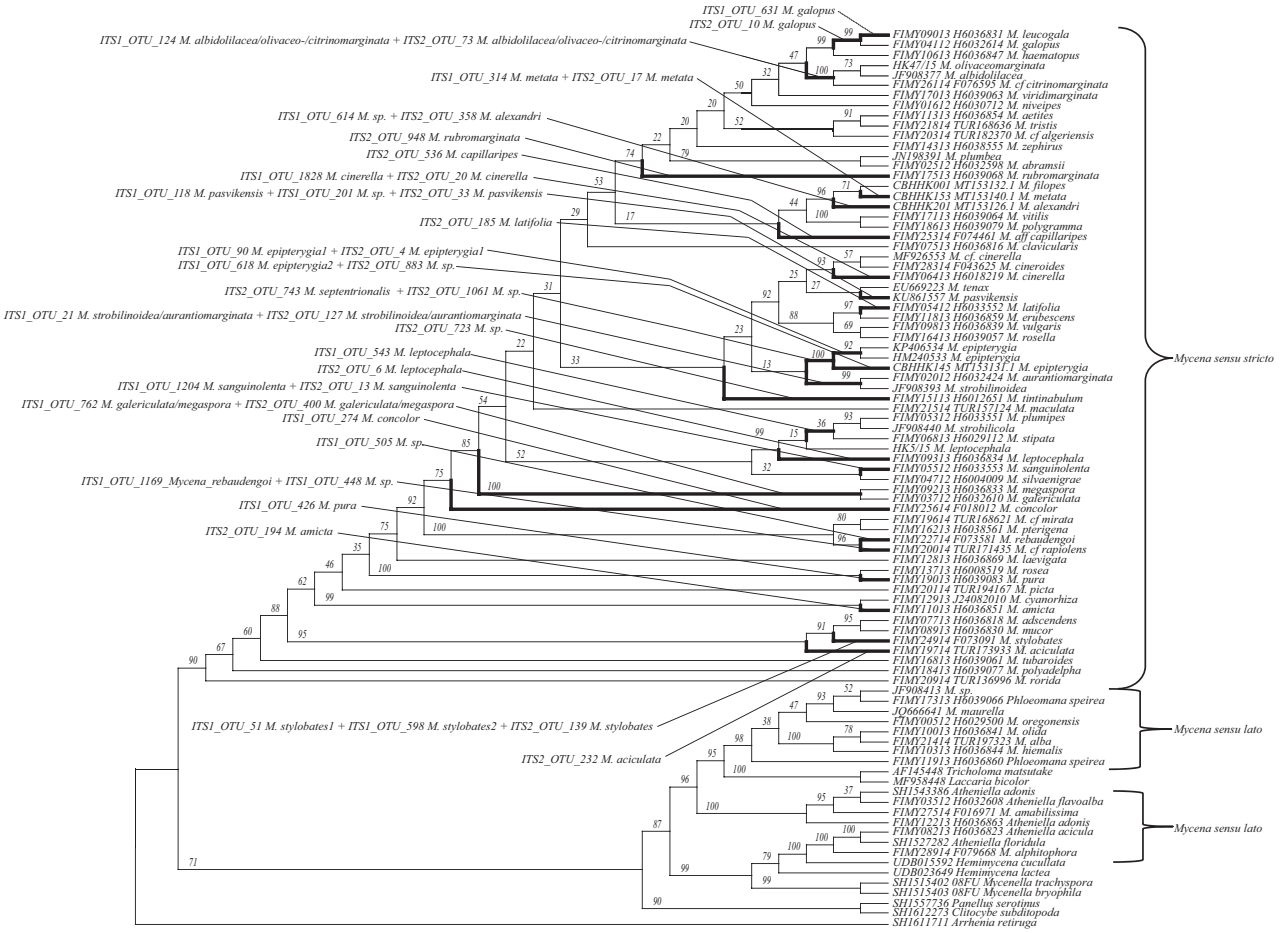

FIGURE 3 RaxML complete ITS phylogeny of 64 Mycena s.s. sequences, with 25 outgroups from 'Mycena s.l.' and other Agaricales. Bootstrap supports indicated about each branch. The 20 + 21 OTUs identified as Mycena from ITS1 and ITS2 are superimposed upon the branches (bolded) with their best fit.

before and after clearcutting. There, *Mycena* differed from several other saprotrophic fungi by being present in the tree roots already before the clearcutting took place (and going on to dominate the root communities after clearcutting), whereas the other saprotrophs invaded afterward. It is thus a robust conclusion that *Mycena* can systematically invade the living roots of multiple plant roots in the lab as well as in the field.

What characterizes root invasion patterns by *Mycena* species?

More different *Mycena* species possess the ability to invade living plant roots (Smith et al., 2017; Thoen et al., 2020) than those we found to regularly do so in the field in this study (Figures 2, 3). The most frequent root-invading species: *M. leptocephala*, *M. epipterygia. M. sanguinolenta*, *M. galopus* and *M. metata* are not phylogenetically related but are all broad generalists as litter decayers, and thus, their ecological versatility appears to translate into root invasion, too.

There are known challenges in identifying *Mycena* species molecularly with ITS (Harder et al., 2013), and some morphospecies could not be separated by our analysis here; however, the most abundant root-invading *Mycena* were fairly easily identified at the 3% OTU threshold. This is a relatively high value for species separation, but consistent with the average intra-specific ITS sequence variation of 3.6% (ITS1) and 2.7% (ITS2) in our 576 collection database (Figure S3) and with the range for true phylogenetically concordant *Mycena* species in known species complexes as *Mycena pura* (Harder et al., 2013). Lower thresholds of, for example, 1.5% (Blaalid et al., 2012) do not give a better species identification, and the ASV and the 3% OTU datasets give very similar conclusions. Thus, it is a robust conclusion that root invasion by *Mycena* is a frequent but opportunistic phenomenon found mostly in broad litter generalists.

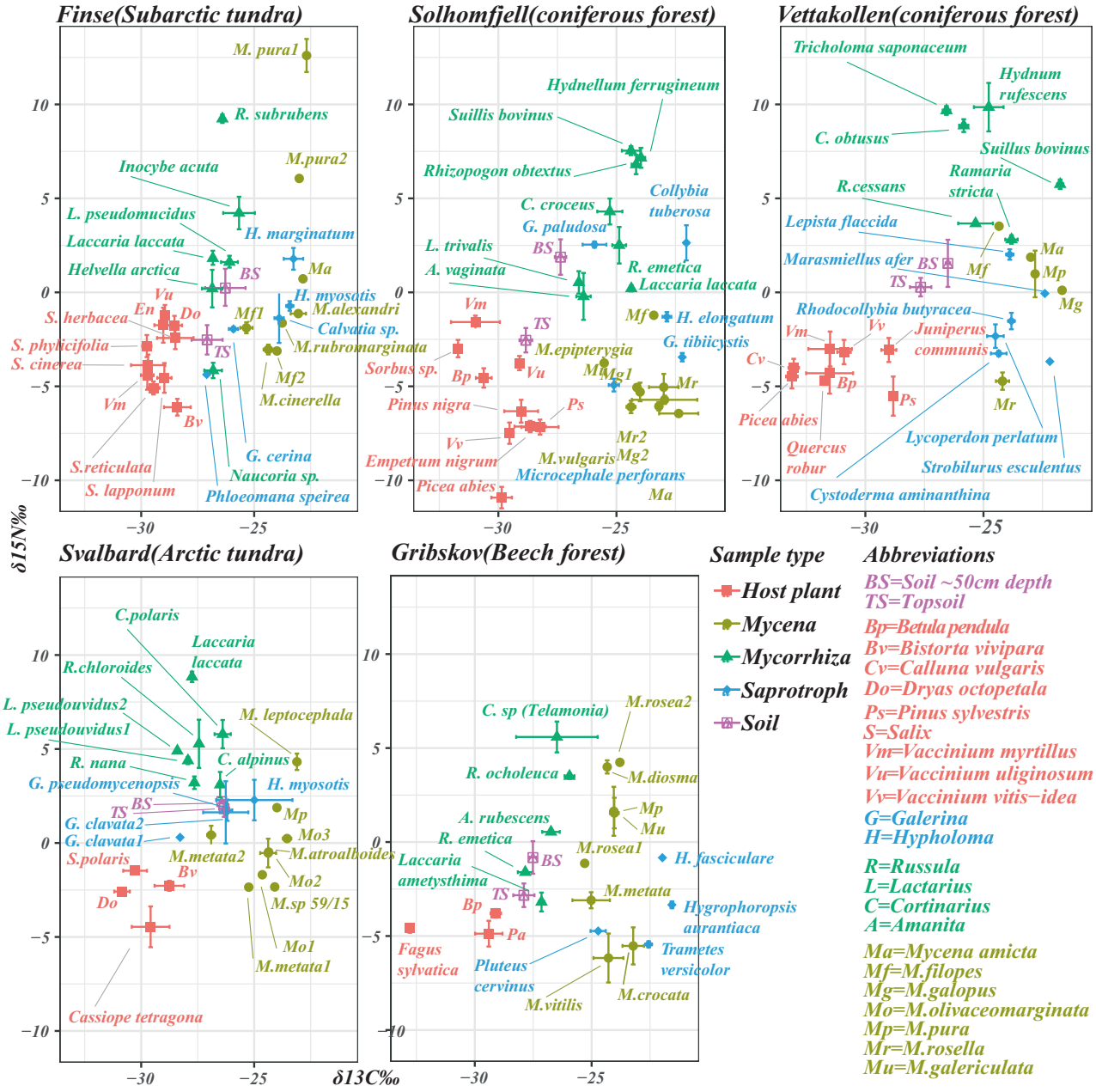

FIGURE 4 Biplots of stable isotopes ^{15}N and ^{13}C for soil, host plants and the three simplified categories *Mycena*, Mycorrhiza and saprotrophs. Overall, saprotrophs will be found predominantly bottom right, mycorrhizal fungi top left. Note the deviant particular *M. pura*s at Finse, upper left.

The question then is what constitutes an opportunity for *Mycena* invasion of a plant root. One answer could be that *Mycena* preferentially invade roots late in the season as many roots have become senescing and/or otherwise damaged. In twigs on trees, an effect termed 'late pruning' has been described, where wood-decay fungi invade still living but senescing or damaged branches towards the end of the growth season (Butin & Kowalski, 1986; Butin & Kowalski, 1990; Hendry et al., 1998). This is beneficial to the tree, as it causes dead branches to fall off more quickly, closes a possible entry point for pathogens, and allows the tree to spend the resources on new fresh branches. McKay (1968) and Keay and Brown (1990) showed a comparable effect in grass roots, where saprophytes such as *Psilocybe semilanceata* or *Melanoleuca* and *Conocybe* invaded dead cells and parts of senescing but otherwise healthy roots in growth experiments. A mechanism of 'late root pruning' of senescing roots by opportunistically early saprotrophic invaders could thus be invoked in an analogy to that in twigs. The small but significantly positive trend with increasing *Mycena* content and advancing season (June–October) in *Arctostaphylos uva-ursi* (Figure S6) suggests that this seasonal

senescence effect does exist; however, the r^2 of only 0.08 does not suggest that seasonality is the main reason for root invasion.

A disturbance may be another explanation. Defoliation by animal grazing is also known to affect plant root health with local dieoffs of fine roots, the abscissions increasing the vulnerability of otherwise healthy roots (Sanderson et al., 1997). The *B. pubescens* in this study were tree saplings of >1 m which were kept low by very high grazing pressure from sheep and deer. All other species with high *Mycena* infection levels were smaller dwarf shrubs of variable sizes or herbaceous plants, also known to be frequent subjects to grazing by deer or reindeer (Kolari et al., 2019). The clumped (local) distribution of high *Mycena* infection levels in *B. vivipara* could be interpreted as offering some support for this theory.

Finally, human management in forestry with the planting of dense monocultures of multiple even-aged seedlings in large cleared areas may favour saprotrophs present in the soil and create opportunities for them to invade multiple young and yet uninfected root systems, where they would otherwise tend to lose out in direct competition with ectomycorrhizal symbionts with dominant networks in more natural forest systems with uneven-aged trees and old trees left standing.

The complete absence of *Mycena* in our *P. sylvestris* (Figure 1) samples supports this hypothesis. As seen in the growth experiments by Smith et al. (2017), *Mycena* and several other saprotrophs are indeed able to invade roots in *P. sylvestris*; however, in vitro resynthesis experiments are necessarily done with tree seedlings, and such diaxenic in vitro resynthesis experiments are not realistic models of natural forests. Our *P. sylvestris* samples were mature trees from largely undisturbed, uneven-aged, and unmanaged stands within a national park, with strongly heterorhizic root systems where only the most distal feeder roots are neither suberised nor metacutinised, severely limiting colonization by fungi. For such mature trees, ectomycorrhizal colonization levels of the feeder roots can be close to 100% under field conditions, so the available surface for possible colonization by non-mycorrhizal fungi can be very limited. Indeed, the *P. sylvestris* in our study showed a near-complete dominance by one genus (*Suillus*, Figure 2D) and had an associated very low general root diversity (Figure 1G, H), consistent also with pre-HTP sequencing-era studies of undisturbed *P. sylvestris* roots (Jonsson et al., 1999).

In contrast, the roots of the fellow conifer (*Picea abies*) in Kohout et al. (2018) were heavily *Mycena*-infected (and had a high overall root fungal diversity), even though they were also sampled from fully mature trees (~80 year old). However, those *Picea abies* stands in that study were from a planted, largely even-aged and managed (and ultimately clear-cut) forest. Did the *Mycena* of Kohout et al. (2018) invade their *Picea abies* hosts already as seedlings, with the trees then 'carrying the seeds of their own destruction', as Parfitt et al. expressed it (2010), for the next eight decades? It has long been known that healthy sapwood tissue of trunks and twigs in trees and woody plants harbour propagules of wood-decaying poroid/corticioid basidiomycetes and xylarioid ascomycetes (Boddy & Griffith, 1989; Gilmartin et al., 2022; Parfitt et al., 2010). This has generally been interpreted as wood-decayers biding their time as seemingly commensal endophytes, waiting for the trunk and twigs, or parts thereof, to die off and turn into a suitable substrate (Parfitt et al., 2010), then having the advantage relative to later post-mortem-invaders. The findings of Kohout et al. (2018) appear to provide direct support for this interpretation of *Mycena*, which became dominant in the roots of the stumps left after the stand was clear-cut. If indeed the *Mycena* are latently present for decades, the fact that they invade the roots rather than the trunk or the twigs has further implications for their nutrition, as we discuss further in the subsection below.

Summing up, it is conceivable that *Mycena* root invasion should be seen as an opportunistic fungal response to plants that are young and susceptible, affected by animal or human impact, or otherwise vulnerable to new infections. This is also consistent with what is generally known to facilitate attacks from known fungal parasites (Walters, 2011). In forest management, there is increasing attention to how traditional planting and harvesting practices shape the associated fungal communities and the vulnerability of the planted forests to known pathogens (Hoeksema et al., 2020). Our results here suggest that besides classical pathogens, intensive forest management practices could also favour opportunistic novel root invasions by *Mycena* and other normally saprotrophic fungi.

What effect does *Mycena* have on the host upon invasion?

If we accept that *Mycena* regularly invades seemingly healthy plant roots, the important issue (question 2) then is what impact *Mycena* invasion in a root has on the host. As described above, the advantage of being latently present on a substrate before its death is obvious. However, the very long life of wood plant hosts means that the time from the first invasion to the significant weakening and actual death of the host could well last for decades or more. As seemingly asymptomatic endophytic fungi always have the potential to opportunistically turn symptomatic, fungi may easily start interacting more measurably with their host during this time in their 'waiting room' (Selosse et al., 2009). It is not hard to envision how the lifestyle of a supposedly patient commensal endophyte could quickly evolve into

parasitism or more active necrotrophy like in, for example, *Armillaria*, and, for *Mycena* or other saprotrophs that preferably invade roots rather than trunks and twigs, to armistice and mutualism.

Such a spectrum of responses reflects what was seen in the laboratory growth experiments by Thoen et al. (2020), where different *Mycena* species and conspecific strains displayed behaviours ranging from either little or no invasion into the *Betula* host plant root, over a primarily harmful or commensally endophytic effect, and ultimately with two strains of *M. pura* and *M. galopus* being able to transfer P *to their host*, with a similar plant growth effect on the host as the known ectomycorrhizal mushroom *Paxillus involutus*. In our field analyses here, while we cannot directly determine whether a *Mycena* carpophore sampled for analysis of stable isotopes is free-living or associated with a plant host, the variable isotopic patterns of particularly 15 N in *Mycena* here are broadly consistent with the patterns of variation observed in these growth experiments.

The average value of the isotopic signatures of *Mycena* do suggest a saprotrophic lifestyle as expected: The *Mycena pura* from Svalbard and Vettakollen (in mainland Norway) and a *Mycena rosea1* from Gribskov had such saprotrophic isotopic signatures close to the expected. All of our collections of *Mycena galopus* also displayed saprotrophic profiles with limited similar intraspecific variations and no signs of mycorrhizal-like isotopic profiles, as one might have expected based on Grelet et al. (2017) or Thoen et al. (2020).

However, some individual collections did have profiles that suggested alternative modes of nutrition. Most clearly, two collections of *Mycena pura* in Finse have isotopic profiles that resemble those of mycorrhizal fungi. In addition, the isotopic profiles of *Mycena pura* and its close relatives *Mycena rosea2* and *Mycena diosma* from Gribskov (in Denmark) are not far from those of the ectomycorrhizal *Russula* or *Cortinarius* (Figure 4). These species all belong to the *Mycena* section Calodontes which are notably hard to grow in culture, which has previously led to speculations about their nutrition (Boisselier-Dubayle et al., 1996; Harder et al., 2010; Harder et al., 2012; Perreau et al., 1992). On the other hand, *Mycena pura* was not commonly found in the root samples and only constituted a significant (>10%) fraction of the root community in one single *B. vivipara* individual (Figure S5). It is thus clear that *Mycena*-plant nutrient transfer is not a common phenomenon.

The $\delta^{13}C$ values in those mycorrhiza-like *Mycena* (Calodontes) collections in Figure 4 are on the high side for average mycorrhizal fungi if their main carbon source was derived from recent photosynthesis. However, some supposedly ectomycorrhizal fungi such as *Chalciporus piperatus* (Luptáková & Mihál, 2020), also have high $\delta^{13}C$ in carpophores. This has been used to argue that *Chalciporus piperatus* could be both saprotrophic (Högberg et al., 1999) or biotrophic on ectomycorrhizal fungi (Tedersoo et al., 2010). The ^{13}C enrichment of *Chalciporus* relative to autotrophic plants is comparable to our *Mycena* here.

The ^{15}N isotopic profiles of the same aforementioned mycorrhiza-like *Mycena* are more in line with that of mycorrhizal fungi, with the notable exception of the *Mycena pura1* sample from Finse. This is surprisingly high in $\delta^{15}N$, more than even known mycorrhizal fungi, almost appearing to suggest a naïve pro bono net N donation to the plant. There could be other explanations for a high $\delta^{15}N$ profile, mycoheterotrophic plants associated with ectomycorrhizal fungi are commonly also quite high in $\delta^{15}N$ (Trudell et al., 2003), which is likely due to them preferentially accessing the ^{15}N-enriched protein component of these fungi rather than the ^{15}N-depleted chitin. However, Hobbie et al. (2020) found that wood-decay *Mycena* were no more than 1% enriched in ^{15}N relative to their source nitrogen. It thus seems unlikely that our *Mycena* here accessed ^{15}N-enriched nitrogen from co-occurring ectomycorrhizal fungi. Thus, the high $\delta^{15}N$ values in *Mycena pura* from Finse do seem to reflect an unusually high source of $\delta^{15}N$ or high sequestration of ^{15}N-depleted N in mycelial chitin prior to carpophore formation. Furthermore, the *Mycena pura* culture in the study by Thoen et al. (2020), which seemingly disproportionally transferred ^{32}P to its plant host compared to the ^{14}C it received in return, was originally derived from the same *M. pura1* carpophore in Figure 4. This could be an unusual individual aberration or an indication of a recently evolved ability to transfer nutrients that is not yet well-adapted to a usual standard mutualistic relationship. Those results from the field and the laboratory are fairly consistent.

Overall, the bulk of the evidence suggests that the main ecological role of root-invasive *Mycena* is living commensally endophytically or as slight parasites within the root for some time. A similar role as largely asymptomatic (commensal) endophytes has been suggested for some Sebacinales (Blaalid et al., 2014; Botnen et al., 2014; Lorberau et al., 2017; Selosse et al., 2009) or as well as for dark septate endophytes (Newsham, 2011) in many Arctic plants. As this waiting process can be prolonged for years or decades in trees, and as some *Mycena* species have indeed evolved the ability to transfer nutrients in the meantime, this genus does provide an empirical example of saprotrophic fungi having progressed to the early intermediate stages from pure saprotrophy. This is in accordance with the predictions of the 'waiting room hypothesis' (Selosse et al., 2009; van der Heijden et al., 2015) of mycorrhizal evolution, which may lead to true reciprocal mycorrhizal *Mycena*-plant interactions one day.

Need for more directly targeted studies on saprotrophic root invasion

Until now, most data on the occurrence of saprotrophic fungi inside plant roots have arisen as a by-product of other research, and the sampling for these metabarcoding datasets here was not originally designed to investigate this question or to be analysed together. The differences (Figure 1) in average *Mycena* infection levels between 9 of the 10 host plants in our sample should be interpreted with the caution warranted by differences in sample sizes and site variations and in comparing two ITS regions (Harder et al., 2013). The reverse primer (ITS2_r) of the ITS1 primer set has a terminal mismatch with 99% of all *Mycena* species (Tedersoo & Lindahl, 2016), which suggests that *Mycena* content in the ITS1 dataset could be underestimated. If true, this would merely strengthen our overall conclusions about *Mycena* as an overlooked but significant root-invading genus; however, more studies directly targeting supposedly saprotrophic or endophytic (non-mycorrhizal) fungi in roots are certainly desirable.

To test our hypothesis that *Mycena* (or other saprotrophic) root infections result from opportunistic invasion under disturbance-related circumstances, future targeted metabarcoding root studies should directly analyse roots of multiple host species of different ages and disturbance levels in the field to identify particular factors that may affect root invasion. Besides disturbance, differences in nitrogen availability have also been shown to influence fungal nutrition facultatively (Peng et al., 2022), and nutrient variability in soil and its role in fungal nutritional mode shifts should certainly also be investigated further. Annotation databases should be continuously updated to reflect our best taxonomic knowledge, and further efforts should be undertaken to identify fungal OTUs or ASVs beyond the genus level, which may require more attention to detail than relying on SINTAX/RDP classification based on even the best possible databases.

Most importantly, more studies on direct ecological interactions between particular hosts and known fungal species are needed, specifically: (1) resynthesis host-fungus experiments with studies of nutrient and C transfer between the symbionts, and (2) stable isotope studies in the field that specifically target saprotrophic taxa. Direct visualization of root-invasive *Mycena* or other saprotrophic fungi with genus- or species-specific FISH hybridization probes (Schneider-Maunoury et al., 2020) would be particularly useful for conclusive demonstrations of the specific nature and degree of the root invasions in each case.

If fungal ecology can be versatile not only below the genus or even the species level, then this may lead to reconsideration of the high importance ascribed to nutrition as a decisive taxon-delimiting trait (as in *Serpulaceae* Skrede et al. (2011) or *Clavariaceae* Birkebak et al. (2013)). Redhead et al. (2016) proposed to split the monophyletic *Amanita* sensu lato into ectomycorrhizal *Amanita* sensu stricto and a new saprotrophic *Saproamanita*, precisely to make an ecological annotation in molecular studies easier, but this would be unnecessary with a greater appreciation of ecological versatility. This has important implications for the widely applied approach in HTP-sequencing/metabarcoding plant root studies where annotating ecology to a sequence with genus-level-based taxonomy could be misguided.

CONCLUSIONS

The investigation of the trophic status of the genus Mycena using sequence data from wild plant roots and $\delta^{15}N$ and $\delta^{13}C$ signatures yielded the following: (1) In 9 of 10 analysed herbaceous and ericaceous plants and tree mycorrhizal host plants from temperate, alpine and Arctic environments, *Mycena* was consistently present in living plant roots across species and in different environments, while other saprotrophic taxa were only occasionally present. (2) The stable isotopic data on carpophores suggested that, although the genus *Mycena* is indeed mostly saprotrophic, strains of certain *Mycena* species can display an ecological versatility in the field and exchange nutrients with plants, consistent with previous results from in vitro resynthesis experiments. (3) *Mycena* infections were not generally more prevalent in Arctic environments or at higher altitudes, but we hypothesize that infection may be more prevalent under conditions of disturbance. (4) The ability to invade living plant roots is a feature of multiple *Mycena* species that do not discriminate between plant hosts. The evidence that fungal trophic modes may be variable on the species level and that within a large genus such as *Mycena*, there may be several potential trophic options in addition to pure free-living saprotrophy, raises intriguing questions about the general understanding and study of fungal ecology and the evolution of plant-fungus interactions. More research directly targeting root-associated fungi with unclear or unknown ecologies is required to resolve these questions. This study highlights the importance of continued detailed studies on interactions among organisms at the species level. Such studies would enhance data usage from broad, environmental metabarcoding approaches to community characterization.

EXPERIMENTAL PROCEDURES

Sample site and sample descriptions

Betula pubescens roots were collected at the RSPB Nature Reserve at Corrimony in north-west Scotland in August 2008. The trees were regenerating saplings at

a maximum of 1 m in height, growing on moorland within heather-dominated vegetation on a site previously browsed by sheep and deer. Root samples (supporting 100–200 ECM tips) were taken from the trees by direct tracing fine roots from the main laterals. Roots from five trees from within a block were pooled to give one single sample. Roots from *Salix herbacea*, *Betula nana*, *Arctostaphylos alpina* and eight *A. uva-ursi* from an original biogeography study were collected from mountains across Scotland (Figure S1; Hesling & Taylor, 2013).

The remaining 68 samples of *A. uva-ursi* roots were from an altitudinal gradient study (and 9 additional *P. sylvestris* samples in addition to those from Jarvis et al. (2015) in this study), collected June–July 2011 in the Invereshie-Inshriach National Nature Reserve in the north-west of the Cairngorms National Park in Scotland (Figures S1, S2). Samples came from 9 elevation transects from 450 to 850 masl on a *Calluna-Arctostaphylos* subalpine heath with scattered Scots pine trees up until the tree limit at ~650 masl. This was in close proximity to the *P. sylvestris* forest studied by Jarvis et al. (2015).

The previously published datasets of *B. vivipara*, *S. polaris*, *D. octopetala* and *C. tetragona* were all collected in Arctic and alpine tundra above the treeline in Arctic Norway, Iceland and Austria, and from grassland below the treeline in Scotland. For more details on the previously published data, we refer to the original publications. A more detailed description of the plant species targeted and the sample sites for the new data can be found in the supplementary data.

Preparation of roots and old amplicon libraries for previously published ITS2 datasets

For the three new ITS2 datasets/454 runs representing 5 of 7 host species (see bioinformatics below), roots were sampled and cleaned under a dissection microscope to remove visible soil debris, woody and non-target species' roots, then lyophilized in 2 mL tubes and milled using a steel bead in a mixer mill (RETSCH, Düsseldorf, Germany). The dry weight for DNA extraction was adjusted for each sample so that extracted mass was proportional to the total sample dry weight: *extract weight* (mg) = (42.50 × *total sample dry weight* (mg)) + 47.98. DNA was extracted using 96 well, DNeasy Plant Minikits (QIAGEN, Hilden, Germany).

PCR amplification of the ITS2 region was conducted on a 2720 Thermal Cycler (Life Technologies, Carlsbad, CA, USA) in 10 μL reactions: 5 μL diluted template; 40 μM of each nucleotide; 0.55 mM $MgCl_2$; 40 nM ITS7A primer (Ihrmark et al., 2012); 40 nM ITS 4 primer with a 3′ 8 bp tag (unique by ≥2 bp between samples); and 0.005 U/μL polymerase (DreamTaq Green, Thermo Scientific, Waltham, MA, USA) in buffer. Cycling parameters were: 94°C for 5 min then 25, 30, or 35 cycles at 94°C for 30 s; 57°C for 30 s; 72°C for 30 s; with a final extension of 72°C for 10 min. PCR products were checked using gel electrophoresis (dilutions/cycles adjusted if products were out with the range 1–10 ng μL^{-1}), then purified using AMPure 96 (Beckman Coulter, Brea, USA). DNA concentrations were established using a Qubit 2.0 fluorometer (Invitrogen, Paisley, UK), samples combined in equal molar proportion, further purified using GeneJET PCR Purification (Thermo Scientific, Waltham, USA), and lyophilized. Adaptor ligation, 454-sequencing, and sequence adapter trimming were performed by the NERC genomics facility (Liverpool, UK) on one picotitre plate using the GL FLX Titanium system (Roche, Basel, Switzerland).

Bioinformatics

From the previously published studies of *B. vivipara*, a high-quality dataset of 119,054 sequences was compiled from Blaalid et al. (2012), 191,099 from Yao et al. (2013), 157,181 from Botnen et al. (2014), 244,523 from Blaalid et al. (2014) 272,595 sequences from Mundra et al. (2015), 249,888 from Davey et al. (2015), and 132,912 from Botnen et al. (2019), making a total of 1,095,997 ITS1 sequences for clustering into OTUs/ASVs.

For the ITS2 dataset, we first analysed the two previously published studies of Jarvis et al. (2015) and Lorberau et al. (2017), and obtained 175,829 and 1,952,314 sequences, For the three unpublished ITS2 454 runs, 327,480 raw reads were obtained on a run with 104 *Betula pubescens* samples; 49,187 raw reads on a run combining altitude and biogeography samples, and 232,125 for a run with 16 first year biogeography samples. After denoising, chimera check, length, primer/base pair match, and quality controls, 121,587, 326,380 and 154,121 high-quality reads remained, respectively. In total, this amounted to 2,730,231 high-quality ITS2 sequences of all fungal ecological groups. These were then used for clustering into OTUs/ASVs. The OTUs/ASVs were classified taxonomically with the non-Bayesian SINTAX classifier (Edgar, 2016) using the 8.2 utax eukaryote reference database (Abarenkov et al., 2020).

QIIME (Caporaso et al., 2010) 1.9.1 pipeline was employed for the 3 unpublished 454 runs through the same steps as in Jarvis et al. (2015) until the OTU clustering step. We retained those with a sequence length of 200–550 bp, only 100% match to in primer/tag sequences, passed chimera check in UCHIME (Edgar et al., 2011), a sliding window quality check of

50 bp applied to identify low-quality regions (average Phred score <25). The resulting fasta files from the individual ITS1 and ITS2 runs were combined, and clustered first into OTUs at 97% similarity using vsearch (Rognes et al., 2016) and its usearch_global command function, and then into ASVs using the standard settings in UNOISE (Edgar, 2016). The R decontam package (Davis et al., 2018) with the default settings to remove likely contaminants based on the negative controls on a per-sample basis for each of the six different datasets in the ITS1 part, and on the single negative control samples in the *Cassiope tetragona* ITS2 dataset (no negative controls were sequenced in Jarvis et al. (2015) nor in any of the new ITS2 datasets). Non-fungal sequences ($p < 0.95$), and OTUs and ASVs with respectively <10/<8 counts were removed. Finally, sampling saturation was assessed with the iNEXT package (Hsieh et al., 2016; see also Figure S5), and all samples not meeting coverage-based completeness (Chao & Jost, 2012) of 97% were discarded.

Mycena database

For the identification of *Mycena* sequence data to species level, we first generated 151 new sequences from herbarium specimens and personal collections, using the ITS1F/ITS4 primers and the PCR protocol of Gardes and Bruns (1993). All *Mycena* ITS full-length sequences from GenBank and from the UNITE database (1099 sq) were extracted. Sequences not identified to species level, which did not cover the regions amplified by the ITS1F-2/ITS3-4 primer target regions, and which were not inside the *Mycena* sensu stricto clade (Figure 3), or duplicates between both databases were discarded. Additionally, 14 complete *Mycena* sequences in GenBank were also discarded, as these were deemed to be misidentified (see Table S15), most of those from (Hofstetter et al., 2019). The final database comprised 576 high-quality sequences with 136 named *Mycena* species, 89 of which with ≥2 sequences. Sequences were aligned with the FFT-NS-i algorithm in MAFFT v 5 (Katoh & Standley, 2013). The complete (628 bp) and annotated *Mycena pura* EU517504 sequence was used to identify the ITS1 and ITS2 regions in the alignment. They clustered into 156 ITS1 and 139 ITS2 3% OTUs, respectively. Most species were correctly identified by a 3% separation threshold; however, for both regions, some OTUs contained two or more species (such as *Mycena galericulata* + *Mycena megaspora* and *Mycena olivaceomarginata*, *Mycena citrinomarginata* and *Mycena albidolilacea*), while other species were split into multiple OTUs (e.g., *Mycena pura* and *Mycena epipterygia*). Average intraspecific variation was 3.6% (ITS1) and 2.7% (ITS2; Figure S3).

Phylogenetics

The ITS phylogeny (Figure 3) was constructed by first aligning the selected high-quality subset of 89 ITS full-length sequences (those with ≥2 sequences for each species) with the Q-ins-i algorithm in MAFFT (Katoh & Standley, 2013) for a final alignment of 1502 positions (gaps included), and then running a maximum likelihood with 1000 bootstrap replications in RaxML (Stamatakis, 2014), saving branch lengths. Then, the 20 ITS1 and the 21 ITS2 OTUs from the ITS1 and ITS2 datasets that were identified as *Mycena* s.s. were added to the alignment, the Q-ins-i alignment redone, and the OTUs mapped to the branches using the EPA algorithm (Barbera et al., 2019). The tree was visualized in FIGTREE v. 1.4.3 (http://tree.bio.ed.ac.uk/software/figtree).

Collection of samples for isotope analysis

Fungal carpophores, host plants, and soil were collected from five different regions: Svalbard (Arctic Norway), Finse/Hardangervidda (alpine central Norway), Vettakollåsen (boreal forest) in southeastern Norway, Solhomfjell National Park (boreal forest) in South Norway, and Gribsø /Gribskov (North Zealand, Denmark), in a beech-dominated broadleaf forest patch (Figure S1) in 2015 and 2016. For more information and geographic coordinates of the field locations, see Figure S1 and legend. In Svalbard, the collection sites spanned several similar valleys on the southern banks of Isfjorden, with the sites separated by up to ~60 km (Figure S1); for the other four remaining collection sites, samples were taken from an area that extended over no more than 1 km^2.

Fungal carpophores, soil samples, and plants were dried with continuous airflow for 12–36 h at 70°C until dry. Plants and fungi were identified morphologically, and *Mycena* furthermore by ITS sequences. For Svalbard, some additional fungal samples were taken from dried mushroom specimens kept at the herbarium at Tøyen at the Natural History Museum in Oslo. It was assumed that individual carpophores collected within a distance of <50 cm between them originated from the same mycelium. Conspecific *Mycena* carpophores sampled from larger distances were treated as separate samples. Whenever possible, collections from a given site were triplicated or at least duplicated, using separate carpophores from the same collection. Fungi were divided into the three categories 'ectomycorrhizal', 'saprotrophic' or '*Mycena*'. For every 36 samples analysed, internal replicates of material from two samples from the same carpophore were used to verify consistent machine functioning. Soil samples were taken from top-soil (A horizon, 0 cm) and mineral soils in ~50 cm depths. We sampled soil from three different

locations on the different sites. Plant samples were all taken from leaves.

Stable isotope analysis

Dried samples (plants, fungi, soil) were ground by hand, weighed (see Table S4) into 5 x 9 mm tin capsules (Sercon), closed and compressed. Samples consisted of 5 mg of fungi/plant, 10 mg of topsoil, or 20 mg of 50 cm depth soil. Samples were analysed for $\delta^{13}C$, $\delta^{15}N$, % C and % N by continuous flow with a Costech ECS4010 elemental analyser (Costech Analytical Technologies Inc, Valencia, CA) coupled with a DELTAplus XP isotope ratio mass spectrometer (Thermo Scientific, Bremen, Germany) at the University of New Hampshire Stable Isotope Laboratory. All carbon and nitrogen isotope data are reported in delta notation according to this equation: $\delta X = [(R_{sample}/R_{standard}) - 1] \times 1000$ where X is ^{13}C or ^{15}N and R is the ratio $^{13}C/^{12}C$ or $^{15}N/^{14}N$. All $\delta^{13}C$ and $\delta^{15}N$ values were normalized on VPDB ($\delta^{13}C$) and AIR ($\delta^{15}N$) reference scales with the following internationally calibrated standards and values: IAEA CH6 (210.45%), CH7 (232.15%), N1 (0.4%) and N2 (20.3%). Laboratory working standards included NIST 1515 (apple leaves), NIST 1575a (pine needles), and tuna muscle, as well as a *Boletus* quality control.

Statistics/graphics

Stepwise multiple regression models of fungal $\delta^{15}N$ and $\delta^{13}C$ were analysed with genus, site and %N as the independent variables. Because of the declining $\delta^{13}C$ of atmospheric carbon dioxide, the year was also included as a continuous factor in the $\delta^{13}C$ regression. Genus and site were categorical variables and year and %N were continuous variables. These statistical analyses were carried out in JMP 13 Pro (SAS Institute, Middleton, Massachusetts, USA). Models that minimized the Bayesian Information Criterion (BIC) were selected. The variance inflation factor of each model factor was also calculated, which measures multicollinearity. This approach allowed a test of whether *Mycena* generally grouped with saprotrophic or ectomycorrhizal genera without a priori setting up specific contrasts among *Mycena*, saprotrophic genera and ectomycorrhizal genera.

All other statistics were done in R using the 'phyloseq' 1.19.1 R package (McMurdie & Holmes, 2013) for combining and rearranging OTU tables and taxonomy information, and the heatmap.2 function from the 'gplots' package (Warnes et al., 2016) for visualizing heatmaps. We applied a sequential ANOVA for Figure 1A–D at the 0.05 significance threshold with the Scheffe post-hoc test correction for multiple comparisons, using the 'agricolae' package (de Mendiburu, 2020).

AUTHOR CONTRIBUTIONS

Christoffer Bugge Harder: Conceptualization (lead); data curation (equal); formal analysis (lead); funding acquisition (equal); investigation (lead); methodology (lead); project administration (lead); software (lead); validation (lead); visualization (lead); writing – original draft (lead); writing – review and editing (lead). **Emily Hesling:** Data curation (supporting); formal analysis (supporting); methodology (supporting); validation (supporting); visualization (supporting); writing – original draft (supporting). **Synnøve Smebye Botnen:** Data curation (supporting); formal analysis (supporting); methodology (supporting); writing – original draft (supporting). **Kelsey Erin Lorberau:** Data curation (supporting); formal analysis (supporting); investigation (supporting); writing – original draft (supporting). **Balint Dima:** Data curation (supporting); writing – original draft (supporting). **Tuula Niskanen:** Data curation (supporting); writing – review and editing (supporting). **Tea von Bonsdorff-Salminen:** Data curation (supporting). **Susan Jarvis:** Data curation (supporting); formal analysis (supporting); methodology (supporting). **Andrew Ouimette:** Data curation (supporting); formal analysis (supporting); validation (supporting). **Alison Hester:** Data curation (supporting); validation (supporting). **Erik Hobbie:** Conceptualization (supporting); data curation (supporting); formal analysis (equal); funding acquisition (supporting); investigation (supporting); methodology (equal); project administration (supporting); resources (supporting); supervision (supporting); writing – original draft (supporting); writing – review and editing (supporting). **Andy Taylor:** Conceptualization (supporting); data curation (supporting); formal analysis (supporting); funding acquisition (supporting); investigation (supporting); methodology (supporting); project administration (supporting); supervision (supporting); validation (supporting); writing – original draft (equal). **Håvard Kauserud:** Conceptualization (supporting); data curation (supporting); funding acquisition (equal); investigation (supporting); methodology (supporting); project administration (supporting); resources (supporting); supervision (supporting); writing – original draft (supporting); writing – review and editing (supporting).

ACKNOWLEDGEMENTS

We thank Karl-Henrik Larsson and Arne Aronsen for provisions of specimens from the Natural History Museum of Oslo and help with the identification of field specimens from Svalbard. We further thank Cecilie Mathiesen and Mikayla Jacobs for technical assistance in the laboratory, Brendan J. Furneaux for valuable input to the R script, and the curators of H, TUR, and OULU. The Mycena ITS sequences originating from

the specimens deposited in H, TUR, and OULU were produced as part of the Finnish Barcode of Life Project (FinBOL) funded by the Ministry of Environment, Finland (YM23/5512/2013), Otto A Malm's Donationsfond, and the Kone Foundation. We thank the European Commission (grant no. 658849) and the Carlsberg Foundation (grant no. CF18-0809) for grants to C.B. Harder that made this research possible. C.B. Harder was financed by a grant from the Danish Independent Research Fund DFF/FNU 2032-00064B (SapMyc) at the time of writing.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

MiSeq/454 files are found at the respective sources listed in Table S16. Sanger sequences can be accessed through GenBank/UNITE, see Table S17 for accession numbers. R scripts and downstream analysis files can be obtained from C.B. Harder upon request.

ORCID

Christoffer Bugge Harder https://orcid.org/0000-0002-6973-5633

REFERENCES

Abarenkov, K., Zirk, A., Piirmann, T., Pöhönen, R., Ivanov, F., Nilsson, R.H. et al. (2020) *UNITE USEARCH/UTAX release for eukaryotes*. Version 04.02.2020. UNITE Community. https://doi.org/10.15156/BIO/786375

Aronsen, A. & Læssøe, T. (2016) The genus Mycena s.l. In: Frøslev, T.G., Læssøe, T., Petersen, J.H., & Elborne, S.A. (Eds.) *The fungi of northern Europe*. Copenhagen, Denmark: Danish Mycological Society, 373.

Bahram, M., Polme, S., Koljalg, U. & Tedersoo, L. (2011) A single European aspen (*Populus tremula*) tree individual may potentially harbour dozens of *Cenococcum geophilum* ITS genotypes and hundreds of species of ectomycorrhizal fungi. *FEMS Microbiology Ecology*, 75, 313–320.

Baldrian, P. & Kohout, P. (2017) Interactions of saprotrophic fungi with tree roots: can we observe the emergence of novel ectomycorrhizal fungi? *New Phytologist*, 215, 511–513.

Baldrian, P., Kolarik, M., Stursova, M., Kopecky, J., Valaskova, V., Vetrovsky, T. et al. (2012) Active and total microbial communities in forest soil are largely different and highly stratified during decomposition. *The ISME Journal*, 6, 248–258.

Barbera, P., Kozlov, A.M., Czech, L., Morel, B., Darriba, D., Flouri, T. et al. (2019) EPA-ng: massively parallel evolutionary placement of genetic sequences. *Systematic Biology*, 68, 365–369.

Birkebak, J.M., Mayor, J.R., Ryberg, K.M. & Matheny, P.B. (2013) A systematic, morphological and ecological overview of the Clavariaceae (Agaricales). *Mycologia*, 105, 896–911.

Blaalid, R., Carlsen, T., Kumar, S., Halvorsen, R., Ugland, K.I., Fontana, G. et al. (2012) Changes in the root-associated fungal communities along a primary succession gradient analysed by 454 pyrosequencing. *Molecular Ecology*, 21, 1897–1908.

Blaalid, R., Davey, M.L., Kauserud, H., Carlsen, T., Halvorsen, R., Hoiland, K. et al. (2014) Arctic root-associated fungal community composition reflects environmental filtering. *Molecular Ecology*, 23, 649–659.

Boberg, J., Finlay, R., Stenlid, J., Nasholm, T. & Lindahl, B. (2008) Glucose and ammonium additions affect needle decomposition and carbon allocation by the litter degrading fungus *Mycena epipterygia*. *Soil Biology and Biochemistry*, 40, 995–999.

Boddy, L. & Griffith, G.S. (1989) Role of endophytes and latent invasion in the development of decay communities in sapwood of angiospermous trees. *Sydowia*, 41(41), e73.

Boisselier-Dubayle, M.C., Perreau-Bertrand, J. & Lambourdiere, J. (1996) Genetic variability in wild populations of *Mycena rosea*. *Mycological Research*, 100, 753–758.

Botnen, S., Vik, U., Carlsen, T., Eidesen, P.B., Davey, M.L. & Kauserud, H. (2014) Low host specificity of root-associated fungi at an Arctic site. *Molecular Ecology*, 23, 975–985.

Botnen, S.S., Davey, M.L., Aas, A.B., Carlsen, T., Thoen, E., Heegaard, E. et al. (2019) Biogeography of plant root-associated fungal communities in the North Atlantic region mirrors climatic variability. *Journal of Biogeography*, *46*, 1532–1546.

Buee, M., Reich, M., Murat, C., Morin, E., Nilsson, R.H., Uroz, S. et al. (2009) 454 pyrosequencing analyses of forest soils reveal an unexpectedly high fungal diversity. *New Phytologist*, 184, 449–456.

Butin, H. & Kowalski, T. (1986) Die natürliche Astreinigung und ihre biologischen Voraussetzungen: III. Die Pilzflora von Ahorn, Erle, Birke, Hainbuche und Esche. *European Journal of Forest Pathology*, 16(3), 129–138.

Butin, V.H. & Kowalski, T. (1990) Die natürliche Astreinigung und ihre biologischen Voraussetzungen: V. *European Journal of Forest Pathology*, 20(1), 44–54 Die Pilzflor. von Fichte, Kiefer Und Lärche.

Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K. et al. (2010) QIIME allows analysis of high-throughput community sequencing data. *Nature Methods*, 7, 335–336.

Chao, A. & Jost, L. (2012) Coverage-based rarefaction and extrapolation: standardizing samples by completeness rather than size. *Ecology*, 93, 2533–2547.

Davey, M.L., Blaalid, R., Vik, U., Carlsen, T., Kauserud, H. & Eidesen, P.B. (2015) Primary succession of *Bistorta vivipara*(L.) Delabre (Polygonaceae) root-associated fungi mirrors plant succession in two glacial chronosequences. *Environmental Microbiology*, 17, 2777–2790.

Davey, M.L., Heimdal, R., Ohlson, M. & Kauserud, H. (2013) Host- and tissue-specificity of moss-associated Galerina and Mycena determined from amplicon pyrosequencing data. *Fungal Ecology*, 6, 179–186.

Davis, N.M., Proctor, D.M., Holmes, S.P., Relman, D.A. & Callahan, B.J. (2018) Simple statistical identification and removal of contaminant sequences in marker-gene and metagenomics data. *Microbiome*, 6, 226.

de Mendiburu, F. (2020) Package 'agricolae'. *R Package Version*, 1–2. https://cran.r-project.org/package=agricolae

den Bakker, H.C., Zuccarello, G.C., Kuyper, T.W. & Noordeloos, M.E. (2004) Evolution and host specificity in the ectomycorrhizal genus *Leccinum*. *New Phytologist*, 163, 201–215.

Edgar, R.C. (2016) UNOISE2: improved error-correction for Illumina 16S and ITS amplicon sequencing. *BioArxiv*. 2016, 081257.

Edgar, R.C., Haas, B.J., Clemente, J.C., Quince, C. & Knight, R. (2011) UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics*, 27, 2194–2200.

Fries, N. & Mueller, G.M. (1984) Incompatibility systems, cultural features and species circumscriptions in the ectomycorrhizal genus *Laccaria* (Agaricales). *Mycologia*, 76, 633–642.

Gardes, M. & Bruns, T.D. (1993) ITS primers with enhanced specificity for basidiomycetes–application to the identification of mycorrhizae and rusts. *Molecular Ecology*, 2, 113–118.

Garnica, S., Schon, M.E., Abarenkov, K., Riess, K., Liimatainen, K., Niskanen, T. et al. (2016) Determining threshold values for barcoding fungi: lessons from *Cortinarius* (Basidiomycota), a highly diverse and widespread ectomycorrhizal genus. *FEMS Microbiology Ecology*, 92, fiw045.

Gilmartin, E.C., Jusino, M.A., Pyne, E.J., Banik, M.T., Lindner, D.L. & Boddy, L. (2022) Fungal endophytes and origins of decay in beech (*Fagus sylvatica*) sapwood. *Fungal Ecology*, 59, 101161.

Glynou, K., Nam, B., Thines, M. & Macia-Vicente, J.G. (2018) Facultative root-colonizing fungi dominate endophytic assemblages in roots of nonmycorrhizal *Microthlaspi* species. *New Phytologist*, 217, 1190–1202.

Grelet, G.A., Ba, R., Goeke, D.F., Houliston, G.J., Taylor, A.F.S. & Durall, D.M. (2017) A plant growth-promoting symbiosis between *Mycena galopus* and *Vaccinium corymbosum* seedlings. *Mycorrhiza*, 27, 831–839.

Griffith, G.W. (2004) The use of stable isotopes in fungal ecology. *Mycologist*, 18, 177–183.

Guo, S.X., Fan, L., Cao, W.Q., Xu, J.T. & Xiao, P.G. (1997) *Mycena anoectochila* sp. nov. isolated from mycorrhizal roots of *Anoectochilus roxburghii* from Xishuangbanna, China. *Mycologia*, 89, 952–954.

Halbwachs, H., Easton, G.L., Bol, R., Hobbie, E.A., Garnett, M.H., Persoh, D. et al. (2018) Isotopic evidence of biotrophy and unusual nitrogen nutrition in soil-dwelling Hygrophoraceae. *Environmental Microbiology*, 20, 3573–3588.

Harder, C.B., Læssøe, T., Kjøller, R. & Frøslev, T.G. (2010) A comparison between ITS phylogenetic relationships and morphological species recognition within *Mycena* sect, Calodontes in Northern Europe. *Mycological Progress*, 9, 395–405.

Harder, C.B., Læssøe, T., Frøslev, T.G., Ekelund, F., Rosendahl, S. & Kjøller, R. (2013) A three-gene phylogeny of the Mycena pura complex reveals 11 phylogenetic species and shows ITS to be unreliable for species identification. *Fungal Biology*, 117, 764–775.

Harder, C.B., Lodge, D.J., Petersen, R.H., Hughes, K.W., Blanco, J. C., Frøslev, T.G. et al. (2012) Amyloidity is not diagnostic for species in the Mycena pearsoniana complex (Mycena sectio Calodontes). *Mycological Progress*, 11, 725–732.

Hendry, S.J., Lonsdale, D. & Boddy, L. (1998) Strip-cankering of beech (*Fagus sylvatica*): pathology and distribution of symptomatic trees. *The New Phytologist*, 140(3), 549–565.

Hesling, E. & Taylor, A.F.S. (2013) Ectomycorrhizal fungi associated with Arctostaphylos uva-ursi in Scotland: exploring the biogeography of undiscovered fungal communities. *Karstenia*, 53, 1–2.

Hobbie, E.A., Grandy, A.S. & Harmon, M.E. (2020) Isotopic and compositional evidence for carbon and nitrogen dynamics during wood decomposition by saprotrophic fungi. *Fungal Ecology*, 45, 100915.

Hobbie, E.A., Macko, S.A. & Shugart, H.H. (1999) Insights into nitrogen and carbon dynamics of ectomycorrhizal and saprotrophic fungi from isotopic evidence. *Oecologia*, 118, 353–360.

Hobbie, E.A., Weber, N.S. & Trappe, J.M. (2001) Mycorrhizal vs. saprotrophic status of fungi: the isotopic evidence. *New Phytologist*, 150, 601–610.

Hobbie, E.A., Weber, N.S., Trappe, J.M. & Van Klinken, G.J. (2002) Using radiocarbon to determine the mycorrhizal status of fungi. *New Phytologist*, 156, 129–136.

Hoeksema, J.D., Averill, C., Bhatnagar, J.M., Brzostek, E., Buscardo, E., Chen, K.H. et al. (2020) Ectomycorrhizal plant-fungal co-invasions as natural experiments for connecting plant and fungal traits to their ecosystem consequences. *Frontiers in Forests and Global Change*, 3, 84.

Hofstetter, V., Buyck, B., Eyssartier, G., Schnee, S. & Gindro, K. (2019) The unbearable lightness of sequenced-based identification. *Fungal Diversity*, 96(1), 243–284.

Högberg, P., Plamboeck, A.H., Taylor, A.F. & Fransson, P.M. (1999) Natural ^{13}C abundance reveals trophic status of fungi and host-origin of carbon in mycorrhizal fungi in mixed forests. *Proceedings of the National Academy of Sciences*, 96(15), 8534–8539.

Hsieh, T.C., Ma, K.H., Chao, A. & McInerny, G. (2016) iNEXT: an R package for rarefaction and extrapolation of species diversity (Hill numbers). *Methods in Ecology and Evolution*, 7, 1451–1456.

Ihrmark, K., Bödeker, I.T., Cruz-Martinez, K., Friberg, H., Kubartova, A., Schenck, J., et al. (2012) New primers to amplify the fungal ITS2 region–evaluation by 454-sequencing of artificial and natural communities. *FEMS Microbiology Ecology*, 82(3), 666–677.

Jarvis, S.G., Woodward, S. & Taylor, A.F. (2015) Strong altitudinal partitioning in the distributions of ectomycorrhizal fungi along a short (300 m) elevation gradient. *New Phytologist*, 206, 1145–1155.

Jonsson, L., Dahlberg, A., Nilsson, M.C., Kårén, O. & Zackrisson, O. (1999) Continuity of ectomycorrhizal fungi in self-regenerating boreal *Pinus sylvestris* forests studied by comparing mycobiont diversity on seedlings and mature trees. *New Phytologist*, 142, 151–162.

Jumpponen, A.R.I. & Trappe, J.M. (1998) Dark septate endophytes: a review of facultative biotrophic root-colonizing fungi. *New Phytologist*, 140, 295–310.

Katoh, K. & Standley, D.M. (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution*, 30, 772–780.

Kaur, J., Andrews, L. & Sharma, J. (2019) High specificity of a rare terrestrial orchid toward a rare fungus within the north American tallgrass prairie. *Fungal Biology*, 123, 895–904.

Keay, S.M. & Brown, A.E. (1990) Colonization by *Psilocybe semilanceata* of roots of grassland flora. *Mycological Research*, 94(1), 49–56.

Kohler, A., Kuo, A., Nagy, L.G., Morin, E., Barry, K.W., Buscot, F. et al. (2015) Convergent losses of decay mechanisms and rapid turnover of symbiosis genes in mycorrhizal mutualists. *Nature Genetics*, 47, 410–415.

Kohout, P., Charvatova, M., Stursova, M., Masinova, T., Tomsovsky, M. & Baldrian, P. (2018) Clearcutting alters decomposition processes and initiates complex restructuring of fungal communities in soil and tree roots. *The ISME Journal*, 12, 692–703.

Kohzu, A., Yoshioka, T., Ando, T., Takahashi, M., Koba, K. & Wada, E. (1999) Natural 13C and 15N abundance of field-collected fungi and their ecological implications. *New Phytologist*, 144, 323–330.

Kolari, T.H., Kumpula, T., Verdonen, M., Forbes, B.C. & Tahvanainen, T. (2019) Reindeer grazing controls willows but has only minor effects on plant communities in Fennoscandian oroarctic mires. *Arctic, Antarctic, and Alpine Research*, 51, 506–520.

Kühner, R. (1938) Le genre *Mycena:* étude cytologique et systèmatique des espèces d'Europe et d'Amérique du nord. *Encyclopédie Mycologique*, 10, 1–710.

Kyaschenko, J., Clemmensen, K.E., Hagenbo, A., Karltun, E. & Lindahl, B.D. (2017) Shift in fungal communities and associated enzyme activities along an age gradient of managed Pinus sylvestris stands. *The ISME Journal*, 11, 863–874.

Liao, H.L., Chen, Y., Bruns, T.D., Peay, K.G., Taylor, J.W., Branco, S. et al. (2014) Metatranscriptomic analysis of ectomycorrhizal roots reveals genes associated with *Piloderma-Pinus* symbiosis: improved methodologies for assessing gene expression in situ. *Environmental Microbiology*, 16, 3730–3742.

Löfgren, L.A., LeBlanc, N.R., Certano, A.K., Nachtigall, J., LaBine, K. M., Riddle, J. et al. (2018) Fusarium graminearum: pathogen or endophyte of north American grasses? *New Phytologist*, 217, 1203–1212.

Lorberau, K.E., Botnen, S.S., Mundra, S., Aas, A.B., Rozema, J., Eidesen, P.B. et al. (2017) Does warming by open-top chambers induce change in the root-associated fungal community of the arctic dwarf shrub Cassiope tetragona (Ericaceae)? *Mycorrhiza*, 27, 513–524.

Luptáková, E. & Mihál, I. (2020) Dynamics of ectomycorrhizal mycobiota (Basidiomycota) communities on a former agricultural land (West Carpathians). *Mycological Progress*, 19(9), 845–857.

Maas Geesteranus, R.A. (1992) *Mycenas of the northern hemisphere*, Vol. 2. Amsterdam: North-Holland.

Martino, E., Morin, E., Grelet, G.A., Kuo, A., Kohler, A., Daghino, S. et al. (2018) Comparative genomics and transcriptomics depict ericoid mycorrhizal fungi as versatile saprotrophs and plant mutualists. *New Phytologist*, 217, 1213–1229.

Martos, F., Dulormne, M., Pailler, T., Bonfante, P., Faccio, A., Fournel, J. et al. (2009) Independent recruitment of saprotrophic fungi as mycorrhizal partners by tropical achlorophyllous orchids. *New Phytologist*, 184, 668–681.

Mayor, J.R., Schuur, E.A.G. & Henkel, T.W. (2009) Elucidating the nutritional dynamics of fungi using stable isotopes. *Ecology Letters*, 12, 171–183.

McKay, R.I. (1968) *The association of higher fungi and sand-dune grasses*, PhD Thesis. St. Andrews: Department of Botany, University of St. Andrews.

McMurdie, P.J. & Holmes, S. (2013) Phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS One*, 8, e61217.

Menkis, A., Burokiene, D., Gaitnieks, T., Uotila, A., Johannesson, H., Rosling, A. et al. (2012) Occurrence and impact of the root-rot biocontrol agent Phlebiopsis gigantea on soil fungal communities in Picea abies forests of northern Europe. *FEMS Microbiology Ecology*, 81, 438–445.

Molina, R. & Trappe, J.M. (1994) Biology of the ectomycorrhizal genus, Rhizopogon: I. Host associations, host-specificity and pure culture syntheses. *New Phytologist*, 126, 653–675.

Moncalvo, J.-M., Vilgalys, R., Redhead, S.A., Johnson, J.E., James, T.Y., Aime, M.C. et al. (2002) One hundred and seventeen clades of euagarics. *Molecular Phylogenetics and Evolution*, 23, 357–400.

Mundra, S., Halvorsen, R., Kauserud, H., Muller, E., Vik, U. & Eidesen, P.B. (2015) Arctic fungal communities associated with roots of *Bistorta vivipara* do not respond to the same fine-scale edaphic gradients as the aboveground vegetation. *New Phytologist*, 205, 1587–1597.

Newsham, K.K. (2011) A meta-analysis of plant responses to dark septate root endophytes. *New Phytologist*, 190, 783–793.

Nguyen, N.H., Song, Z., Bates, S.T., Branco, S., Tedersoo, L., Menke, J. et al. (2016) FUNGuild: an open annotation tool for parsing fungal community datasets by ecological guild. *Fungal Ecology*, 20, 241–248.

Ogura-Tsujita, Y., Gebauer, G., Hashimoto, T., Umata, H. & Yukawa, T. (2009) Evidence for novel and specialized mycorrhizal parasitism: the orchid *Gastrodia confusa* gains carbon from saprotrophic *Mycena*. *Proceedings of the Royal Society–Biolocial Sciences*, 276, 761–767.

Parfitt, D., Hunt, J., Dockrell, D., Rogers, H.J. & Boddy, L. (2010) Do all trees carry the seeds of their own destruction? PCR reveals numerous wood decay fungi latently present in sapwood of a wide range of angiosperm trees. *Fungal Ecology*, 3(4), 338–346.

Peng, L., Zhang, Y., Druzhinina, I.S., Kubicek, C.P., Wang, Y., Zhu, Z. et al. (2022) A facultative ectomycorrhizal association is triggered by organic nitrogen. *Current Biology*, 32(24), 5235–5249.

Perreau, J., Lambourdière, J. & Boisselier, M.C. (1992) *Mycena rosea* et le complexe *Mycena pura*. *Cryptogamie Mycologie*, 13, 247–251.

Pölme, S., Abarenkov, K., Henrik Nilsson, R., Lindahl, B.D., Clemmensen, K.E., Kauserud, H. et al. (2020) FungalTraits: a user-friendly traits database of fungi and fungus-like stramenopiles. *Fungal Diversity*, 105(1), 1–16.

Redhead, S.A., Vizzini, A., Drehmel, D.C. & Contu, M. (2016) *Saproamanita*, a new name for both *Lepidella* E.-J. Gilbert and *Aspidella* E.-J. Gilbert (*Amaniteae*, *Amanitaceae*). *IMA Fungus*, 7, 119–129.

Rexer, K.H. (1994) *Die gattung Mycena s. l.–studien zu ihrer anatomie, morphologie und systematik*. Universität Tübingen. Dissertation.

Robich, G. (2003) *Mycena D'Europa*. A.M.B.: Fondazione Centro Studi Micologici. Trento, Vicenza.

Rognes, T., Flouri, T., Nichols, B., Quince, C. & Mahe, F. (2016) VSEARCH: a versatile open source tool for metagenomics. *PeerJ*, 4, e2584.

Ryberg, M., Andreasen, M. & Björk, R.G. (2011) Weak habitat specificity in ectomycorrhizal communities associated with *Salix herbacea* and *Salix polaris* in alpine tundra. *Mycorrhiza*, 21, 289–296.

Ryberg, M., Larsson, E. & Molau, U. (2009) Ectomycorrhizal diversity on *Dryas octopetala* and *Salix reticulata* in an Alpine cliff ecosystem. *Arctic, Antarctic, and Alpine Research*, 41, 506–514.

Sanderson, M.A., Stair, D.W. & Hussey, M.A. (1997) Of perennial forages to stress. *Advances in Agronomy*, 59, 171.

Schneider-Maunoury, L., Deveau, A., Moreno, M., Todesco, F., Belmondo, S., Murat, C. et al. (2020) Two ectomycorrhizal truffles, tuber melanosporum and T. aestivum, endophytically colonise roots of non-ectomycorrhizal plants in natural environments. *New Phytologist*, 225, 2542–2556.

Selosse, M.A., Dubois, M.P. & Alvarez, N. (2009) Do Sebacinales commonly associate with plant roots as endophytes? *Mycological Research*, 113(10), 1062–1069.

Selosse, M.A., Martos, F., Perry, B., Maj, P., Roy, M. & Pailler, T. (2010) Saprotrophic fungal symbionts in tropical achlorophyllous orchids: finding treasures among the 'molecular scraps'? *Plant Signaling & Behavior*, 5, 349–353.

Selosse, M.A., Schneider-Maunoury, L. & Martos, F. (2018) Time to re-think fungal ecology? Fungal ecological niches are often prejudged. *New Phytologist*, 217, 968–972.

Skrede, I., Engh, I.B., Binder, M., Carlsen, T., Kauserud, H. & Bendiksby, M. (2011) Evolutionary history of Serpulaceae (Basidiomycota): molecular phylogeny, historical biogeography and evidence for a single transition of nutritional mode. *BMC Evolutionary Biology*, 11, 230.

Smith, G.R., Finlay, R.D., Stenlid, J., Vasaitis, R. & Menkis, A. (2017) Growing evidence for facultative biotrophy in saprotrophic fungi: data from microcosm tests with 201 species of wood-decay basidiomycetes. *New Phytologist*, 215, 747–755.

Stamatakis, A. (2014) RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics*, 30(9), 1312–1313.

Taylor, A.F., Högbom, L., Högberg, M., Lyon, A.J., Näsholm, T. & Högberg, P. (1997) Natural 15N abundance in fruit bodies of ectomycorrhizal fungi from boreal forests. *New Phytologist*, 136, 713–720.

Tedersoo, L. & Lindahl, B. (2016) Fungal identification biases in microbiome projects. *Environmental Microbiology Reports*, 8, 774–779.

Tedersoo, L., May, T.W. & Smith, M.E. (2010) Ectomycorrhizal lifestyle in fungi: global diversity, distribution, and evolution of phylogenetic lineages. *Mycorrhiza*, 20(4), 217–263.

Tedersoo, L. & Smith, M.E. (2013) Lineages of ectomycorrhizal fungi revisited: foraging strategies and novel lineages revealed by sequences from belowground. *Fungal Biology Reviews*, 27, 83–99.

Thoen, E., Harder, C.B., Kauserud, H., Botnen, S.S., Vik, U., Taylor, A.F.S. et al. (2020) In vitro evidence of root colonization suggests ecological versatility in the genus Mycena. *New Phytologist*, 227, 601–612.

Timling, I., Dahlberg, A., Walker, D.A., Gardes, M., Charcosset, J.Y., Welker, J.M. et al. (2012) Distribution and drivers of ectomycorrhizal fungal communities across the north American Arctic. *Ecosphere*, 3, art111.

Trudell, S.A., Rygiewicz, P.T. & Edmonds, R.L. (2003) Nitrogen and carbon stable isotope abundances support the myco-heterotrophic nature and host-specificity of certain achlorophyllous plants. *New Phytologist*, 160(2), 391–401.

van der Heijden, M.G., Martin, F.M., Selosse, M.A. & Sanders, I.R. (2015) Mycorrhizal ecology and evolution: the past, the present, and the future. *New Phytologist*, 205, 1406–1423.

Vasar, M., Andreson, R., Davison, J., Jairus, T., Moora, M., Remm, M. et al. (2017) Increased sequencing depth does not increase captured diversity of arbuscular mycorrhizal fungi. *Mycorrhiza*, 27, 761–773.

Vohník, M. (2020) Ericoid mycorrhizal symbiosis: theoretical background and methods for its comprehensive investigation. *Mycorrhiza*, 30, 671–695.

Walters, D. (2011) *Plant defense: warding off attack by pathogens, herbivores and parasitic plants*. Hoboken, NJ: Wiley.

Warnes, M.G.R., Bolker, B., Bonebakker, L., Gentleman, R. & Huber, W. (2016) Package 'gplots'. Various R Programming Tools for Plotting Data.

Weiß, M., Waller, F., Zuccaro, A. & Selosse, M.A. (2016) Sebacinales–one thousand and one interactions with land plants. *New Phytologist*, 211, 20–40.

Yao, F., Vik, U., Brysting, A.K., Carlsen, T., Halvorsen, R. & Kauserud, H. (2013) Substantial compositional turnover of fungal communities in an alpine ridge-to-snowbed gradient. *Molecular Ecology*, 22, 5040–5052.

Zhang, L., Chen, J., Lv, Y., Gao, C. & Guo, S. (2012) *Mycena* sp., a mycorrhizal fungus of the orchid *Dendrobium officinale*. *Mycological Progress*, 11, 395–401.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Harder, C.B., Hesling, E., Botnen, S.S., Lorberau, K.E., Dima, B., von Bonsdorff-Salminen, T. et al. (2023) *Mycena* species can be opportunist-generalist plant root invaders. *Environmental Microbiology*, 1–19. Available from: https://doi.org/10.1111/1462-2920.16398