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Environment and host as large-scale controls of ectomycorrhizal fungi

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Explaining the large-scale diversity of soil organisms that drive biogeochemical processes and their responses to environmental change is critical. However, identifying consistent drivers of below-ground diversity and abundance at large spatial scales remains problematic for some soil organisms. We investigated a major guild, the ectomycorrhizal fungi, at unprecedented scale and resolution across European forests to explore key biotic and abiotic predictors, and to identify dominant responses and thresholds across complex environmental gradients. Here we show the impact of 38 host, environment, climate and geographic variables on ectomycorrhizal diversity, and we define thresholds of community change for key variables. We quantify host specificity and reveal plasticity in functional traits involved in soil foraging across gradients. We conclude that environmental and host factors explain most variation in ectomycorrhizal diversity, the environmental thresholds used as major ecosystem assessment tools need strong adjustment, and the importance of specificity and plasticity below-ground has been underappreciated.

74 The main projected impacts of environmental change on forest processes stem from global
75 and regional perturbations in the carbon (C) and nitrogen (N) cycles[1,2] and declines in soil
76 biodiversity[3,4]. Globally, mycorrhizal mutualisms mediate soil processes in terrestrial
77 ecosystems[5] and are major drivers of ecosystem C and N dynamics[6]. Soil C sequestration[7,8],
78 tree population dynamics[9] and mitigation of CO_2 fertilization[10] have recently been linked to
79 ectomycorrhizal (EM) symbioses, ubiquitous drivers of photosynthetic C exchange for soil
80 nutrients across temperate and boreal forests[11].
81 How changes in ecosystem processes are underpinned by EM fungi is poorly understood, but
82 likely large-scale effects of those changes, e.g. deteriorating tree mineral nutrition and health,
83 are being observed[12,13]. Various ecological processes are only apparent at large spatial
84 scales[14], and there is concern about lacking baseline EM distribution data against which to
85 assess effects of global change[15,16]. Ectomycorrhizal research has emphasized laboratory or
86 local-scale studies, often reliant on few culturable fungi, to provide mechanistic
87 understanding of symbiotic physiology. However, determinants of EM diversity at local
88 scales are not necessarily their primary drivers at larger scales[17], and EM communities are
89 often dominated by hardly culturable and non- or inconspicuously-fruiting fungi[18].
90 Furthermore, EM community composition, richness, fine root biomass and morphology[19-21]
91 and fungal above-ground fruiting[22] indicate different large-scale patterns and responses from
92 plants and animals; and EM richness increases with sample area more than for microbes[17,23].
93 Consequently, there have been repeated calls for unbiased, large-scale, molecular, ecosystem-
94 level baseline data on EM fungi[15,18,20,24]. Elucidating large-scale EM diversity is crucial for
95 appropriate experimental design in ecosystem science and model organism selection for
96 experimental and comparative biology[25].
97 Unlike multiple local-scale studies where EM fungi are strongly determined by soil
98 environment[26,27], recent large-scale biogeographical studies report that, other than host

identity, soil, climate and atmospheric deposition explain remarkably limited variability[28-33] (Supplementary Information Table 1). Most EM fungi are thought to have broad host ranges, even though specialists can be widespread; but specificity is rarely quantified below-ground at large scales[34].

Current EM environmental thresholds rarely integrate occurrence, abundance and directionality of taxon responses, statistical analysis of large-scale standardized datasets, or studies of low pollution sites[16,35,36]. Critical loads are essential tools for international atmospheric emissions control[37,38], but for EM fungi they differ markedly between Europe and North America[36]. In addition, EM physiological and morphological plasticity are thought to enhance soil nutrient uptake of trees across environmental gradients[39]; however, foraging-related functional traits are assumed fixed at species- or genus-levels. Wide gradients with abundant observations are needed to link plasticity and environment.

We conducted a detailed mycorrhizal analysis using one of the world's largest and most intensive long-term monitoring networks of soil, atmospheric and vegetation parameters. We analysed 38 variables at 137 plots in 20 European countries across strong environmental gradients. We expected to (1) disentangle significant variability explained by co-varying climatic, soil and atmospheric deposition factors, (2) test the generality of host specificity, (3) detect precise thresholds of mycorrhizal change to inform environmental policy, and (4) infer trait plasticity linked to key environmental gradients.

Results

We examined 29,664 ectomycorrhizas from 9,888 soil cores from 103 plots of ca. 0.25 ha in 18 European countries. Including data from 34 plots from Cox et al.[18] and Suz et al.[16], resulted in 39,621 ectomycorrhizas from 137 plots in 20 countries across ca. 5.5 million km^2 (Fig. 1). After removing short low-quality (12,038), chimeric (231), non-mycorrhizal (848)

and unknown (1,308) ITS DNA sequences, we retained 25,196 resulting in 1,406 EM fungal operational taxonomic units (OTUs), 82% Basidiomycota and 18% Ascomycota (Fig 2); 914 were recorded more than once, and 90% were identified to genus or a higher taxonomic level, of which 47% were identified to species.

Composition and specificity

We explained 38% of variance in community composition with forward-selected variables according to the Akaike Information Criterion (AIC). Variables were divided in four partitions: host variables, soil+deposition, climate, and geographic distance (Supplementary Table 2). Nine host variables explained most overall community variance (23%), followed by soil+deposition (21%), geographic distance (14%) and climatic variables (12%). The partitions shared 20% of overall explained variance (Fig. 3).

We used global non-metric multidimensional scaling (NMDS) ordinations to visualize EM fungal community composition and we fitted environmental variables to the ordination to find the most influential variables (Extended Data Fig. 1, Extended Data Table 1). Thus, we identified five key variables for subsequent analyses: N throughfall deposition (N_{TFD}), forest floor pH, mean annual air temperature (MAT), K throughfall deposition (K_{TFD}) and foliar N:P ratio ($N:P_F$).

Almost two-thirds (62%) of ectomycorrhizas correspond to fungi that produce above-ground mushroom-like fruitbodies, the rest produce inconspicuous truffles, crusts or sclerotia. Based on abundance, 48% were generalists and 52% specialists to coniferous or broadleaf hosts. Only 7% of ectomycorrhizas were from specialists to one host tree species. Of the 88 OTUs forming 50 or more ectomycorrhizas, 41% were generalists and 60% coniferous or broadleaf specialists; eleven OTUs (12.5%) were specific to one host species.

Indicators, thresholds and plasticity

Threshold indicator species analyses identified decreasing (z-) and increasing indicator OTUs (z+) for all five key environmental variables (Fig. 4, Extended Data Fig. 2). We identified environmental thresholds of EM fungal community change by cumulating z- and z+ change points. For N_{TFD} we found a sum(z-) peak at 5.8 kg N ha^{-1} yr^{-1} and a sum(z+) peak at 15.5 kg N ha^{-1} yr^{-1}. For $N:P_F$ we detected peaks at 10.2 and 13.3 for sum(z-) and sum(z+), respectively. We found a sum(z-) peak at 6.9 kg K ha^{-1} yr^{-1} and an indistinct sum(z+) peak at 21.7 kg K ha^{-1} yr^{-1} for K_{TFD}. There was a distinct peak for forest floor pH for sum(z-) and sum(z+) at 3.8. Indicator OTUs showed a clear threshold of change for MAT, with a 7.4°C z-peak and a distinct 9.1°C z+ peak. Most z- for $N_{TFD,}$ $N:P_F$, K deposition, forest floor pH and MAT were conifer specialists while all z+ were generalists or broadleaf associates. Generally, threshold values based on accumulated change-points of individual taxa were less pronounced at genus than OTU level (Extended Data Fig. 3).

The observed frequencies of ectomycorrhizas with emanating hyphae and those with rhizomorphs differed significantly between tree species ($P < 0.0001$, df = 3) and soil types ($P < 0.0001$, df = 5; Extended Data Table 2ab); hyphal frequencies were higher than expected with beech and spruce and in Fe-Al soils, respectively. Thirty of the 88 most abundant OTUs (≥ 50 ectomycorrhizas) showed morphological plasticity and 26 of them were also indicators for a key environmental variable. The change in morphology of 17 of those EM taxa was significantly related with at least one environmental variable (Extended Data Tables 3a, 4a). Morphological plasticity related to at least one variable was found within 12 OTUs when a more stringent 99% sequence similarity was used (Extended Data Tables 3b, 4b).

Intraspecific plasticity of individual indicator EM fungi does not necessarily follow overall community morphological changes where logistic regressions showed that mean N_{TFD} was positively related with hyphal presence ($P < 0.0001$). There was negative correlation between

174 hyphal presence and forest floor pH, $N:P_F$ and K_{TFD}, but no correlation with MAT (Extended
175 Data Table 5). Community-wide, we found negative correlation between rhizomorph
176 presence and all tested environmental variables (Extended Data Table 5).
177
178 **Discussion**
179 This is the first large-scale high-resolution study of diversity and distribution of below-
180 ground tree symbionts covering all major European climatic regions for the most abundant
181 tree species. We explain considerable large-scale mycorrhizal diversity with an
182 unprecedented range and quality of environmental, host-related, climatic and geographic
183 variables. We identify large-scale environmental predictors, show the dominance of host
184 specificity, determine environmental indicators and new thresholds of change, and reveal
185 morphological plasticity along environmental gradients. These findings serve as a baseline to
186 assess future change and resilience.
187 Host-related, soil and atmospheric deposition variables were the most important predictors of
188 EM community structure across Europe. Four recent large-scale studies[29,31-33] found these
189 variables to be minor predictors, even though in local-scale studies soil environment shows
190 strong effects[26,27]. We distinguished five key environmental variables: N_{TFD}, $N:P_F$, forest
191 floor pH, K_{TFD} and MAT. Across previous large-scale studies, there is agreement that host
192 species and soil pH are important, but results about other variables disagree (Supplementary
193 Information Table 1). Inconsistent large-scale drivers of diversity and abundance have been
194 reported across different microbes[40], but host is also fundamental for prokaryotes at
195 macroecological scales[41]. Environmental effects on EM fungi in previous studies have
196 probably been confounded by: (i) environmental variables from modelled or extrapolated
197 regional sources*;* (ii) non-standardized sampling and spatial pseudo-replication; (iii) indirect
198 assignment of mycorrhizal status and traits using databases (e.g. UNITE, FunGuild,

199 DEEMY); (iv) semi-quantitative analysis of short DNA sequences; and (v) pooling DNA
200 samples from root hyphae, soil hyphae and dormant propagules even though EM spore banks
201 differ strongly from active communities on roots at local and large scales[42], and ephemeral
202 above-ground reproductive structures and soil hyphae correspond weakly with active
203 communities on roots[43,44]. As a result, up to 90% of variation in EM diversity at large scales
204 has remained unexplained by environmental models[33]. The approach used here is considered
205 more robust[45] and generates higher quality data[46], but had yet to be scaled up due to technical
206 challenges. The large unexplained part of community structure may be attributed to
207 unaccounted factors such as disturbance, management history, stochasticity, interactions
208 among variables masking individual effects, measurement and analytical errors, exclusion of
209 rare species, seasonality, using taxonomic instead of functional diversity, and/or not covering
210 complete gradients of each variable across whole geographic ranges of hosts and fungi. In our
211 study, conifers have larger distribution and thus cover larger environmental gradients that
212 likely explain the different number of environmental variables linked to community
213 dissimilarities among hosts.
214 Host-related variables strongly influence EM fungal communities, thus symbiosis plays a
215 major role in shaping EM distributions. Studies on host specificity of EM fungi at large scales
216 have been mainly based on fruitbody surveys and thus assess specificity on taxonomic rather
217 than abundance levels[47]. Host generalism is considered the rule[48], but intensive below-ground
218 analysis indicates EM fungal specificity to the most common European trees matches or
219 exceeds generalism on taxonomic and relative abundance levels, particularly for conifers. We
220 find more conifer specialists and they respond strongly to environmental gradients; the
221 implications of specificity and abundance merit investigation, as they can reflect,
222 respectively, more[34,49] and less[50] efficient nutritional mutualisms.

We use threshold indicator taxon analyses for the first time for fungi at a continental scale to identify distinct EM responses to key environmental variables and clear thresholds of change. Indicator species emerged for all key environmental variables, and several EM taxa were indicators for more than one. Different fungi within a family, and even a genus, can be both positive and negative indicators for a variable; for instance, *Thelephora terrestris* and *Tomentella castanea* are negative and positive indicators for $N:P_F$, respectively, and *Lactarius rufus* and *L. hepaticus* are negative and positive indicators for N_{TFD}, respectively. Nonetheless, genus-level analyses revealed most indicator species patterns hold true at higher taxonomic ranks (Extended Data Fig. 3). In some genera, the aggregate of species acts as indicator, although individual species do not (e.g. *Sistotrema*, *Clavulina* and *Boletus* for N_{TFD} and K_{TFD}). For several genera we find a different response to elevated N_{TFD} than previous studies, even those with consistent responses across studies[51] (i.e. *Tomentella*, *Tylospora*, *Cenococcum*, *Hebeloma*, *Amanita*). Furthermore, we confirm the response to elevated N_{TFD} of several genera only recorded in few studies[51] (i.e. *Clavulina*, *Elaphomyces*, *Boletus*, *Amphinema*).

With increasing N availability, metabolically costly ways of obtaining N from complex soil organic sources are less cost-effective; fungi that utilise those pathways (e.g. *Cortinarius*, *Piloderma*, *Tricholoma*) are at a disadvantage compared to fungi that utilise inorganic N (e.g. *Elaphomyces*, *Laccaria*)[51]. Indeed, organic N users tended to be negative indicators for N deposition, and inorganic N users tended to be positive.

Some indicator species for K_{TFD} are abundant and widespread in Europe (e.g. *Elaphomyces asperulus*, *Lactarius quietus*, *Piloderma sphaerosporum*); however, K_{TFD} has not been identified as a key variable in previous EM studies. A meta-analysis showed that in 69% of experiments tree growth responded positively to soil K increases[52], but K is highly diffusible in soil and easily accessible for plants. Some K_{TFD} may originate from canopy leaching; with

248 acidifying pollution, K leaches, and if depleted in foliage and litter, K availability in soil
249 organic matter could decrease. Moreover, K is taken up and translocated by EM fungi in a
250 specific manner (e.g. EM fungi with hydrophobins transfer less K)[11]. This agrees with our
251 results; most negative indicator genera were hydrophobic and most positive ones
252 hydrophilic[53].
253 Based on the large number of indicator species for MAT, climate should play an important
254 role in shaping EM communities, as suggested by fruiting phenology studies[54]. However, it is
255 difficult to distinguish MAT from climate and therefore to know whether a fungus occurs
256 somewhere because of prevalent temperatures. Nevertheless, current habitats may become
257 less favourable for many EM fungi as temperature increases.
258 Accumulated change-point values of all individual EM fungi indicate environmental
259 thresholds of change for most key environmental variables. There was a narrow range for
260 fungi negatively affected by N_{TFD} with a sharp threshold at 5.8 kg N ha^{-1} yr^{-1}. These mainly
261 conifer specialists thrive in poor soils and pre-industrial N levels (ca. < 2 kg N ha^{-1} yr^{-1}), but
262 cannot keep up with increased N_{TFD} from industrial, agricultural and transport emissions over
263 the last decades. They are likely out-competed by fungi that use the additional inorganic N or
264 avoid additional N uptake costs[55], particularly within the temperate distribution ranges of
265 beech and oak where N_{TFD} is greatest, and organic N users show some recovery in fruiting if
266 N pollution decreases[56]. Positively-affected fungi, mostly host generalists lacking proteolytic
267 abilities, initially do well with additional inorganic N, giving them a competitive advantage.
268 However, their much broader response range and less defined peak at 15.5 kg N ha^{-1} yr^{-1}
269 suggests adaptation by positively-affected fungi to increased N_{TFD} varies greatly. This might
270 be driven by geographically-divergent population-level evolutionary selection pressures on
271 fungi since the industrial revolution. Furthermore, naturally enriched microsites (e.g. animal

272 latrines, carcasses, disturbances) and macrosites (e.g. stands with N_2 fixers) could have pre-
273 adapted certain fungi.
274 We confirm and extend observations based on fruitbodies and roots at smaller scales[57] that
275 conifer specialists - most with abundant hyphae and rhizomorphs - are more negatively
276 affected by increasing N than broadleaf specialists and generalists. The strong differences
277 observed in host specificity between fungi negatively- and positively-affected by N_{TFD} may
278 be caused by differences in enzymatic capability to acquire N directly from complex soil
279 organic compounds, thus circumventing mineralization, and in resource exchange rate, e.g. if
280 specialists transfer more soil N per unit of tree C than generalists[34]. Comparative genetic,
281 physiological and ecological studies of the different sets of dominant indicators are now
282 needed to test alternative models of EM community optimisation versus parasitism under
283 changing C and N conditions[58] through species replacement, plasticity and/or evolution[59].
284 Large-scale below-ground analysis contributes important information on ecosystem
285 assessment tools for a uniquely important guild of forest organisms. Critical loads for
286 eutrophying N deposition were previously estimated for EM fungi, largely based on expert
287 opinion and above-ground data, at 5-10 kg N ha^{-1} yr^{-1} for North America[36] and 10-20 kg N ha^{-1} yr^{-1} for Europe[60]. Thresholds based on European EM data have focused on few sites across
289 smaller gradients or EM richness and evenness instead of community composition[16,35]. Our
290 large N deposition gradient leads to a much lower European threshold value for a substantial
291 EM shift at 5-6 kg N ha^{-1} yr^{-1}, based on both throughfall and open field deposition data,
292 approaching recent lower estimates for other forest organisms[61,62]. Caution is needed
293 inferring absolute values for critical loads, but based on our results critical loads for European
294 forests need strong adjustment towards those for North American forests, and EM and forest
295 change thresholds need aligning to explain alarming deterioration in European tree
296 nutrition[13]. Critical $N:P_F$ are considered plant specific[63] and $N:P_F$ has been linked to tree

297 health, with breakpoint values of 7.3 for conifers and 14.8 for broadleaf trees regarding
298 defoliation[12]. We show that lower (10.2) and upper (13.3) $N:P_F$ thresholds for EM
299 communities are linked to conifers and broadleaves, respectively. Community threshold
300 forest floor pH levels for negative and positive indicator species overlap. Although soil pH is
301 anthropogenically influenced (e.g. liming) and soil acidification affects parts of Europe[64], the
302 major soil pH differences across forests arise from soil parent material and climatic
303 differences over long timescales, and must have long influenced EM communities.
304 Nonetheless, individual species could be affected. For K_{TFD}, no threshold values for EM
305 composition have been published. We identify a 5-8 kg K ha^{-1} yr^{-1} threshold for declining
306 species; however, K_{TFD} results partly from K uptake and leaching by trees, which may be
307 influenced by EM fungi themselves. Therefore, research into K deposition and cycling is
308 needed for EM communities[11] and forests[52].
309 Physiological and morphological heterogeneity and plasticity of EM mycelium have been
310 considered responsible for enabling trees to rapidly take up soil nutrients[65,66], here we show
311 morphological plasticity within dominant EM taxa and changes over environmental
312 gradients. This has significant implications for functional diversity studies at large-scales
313 and/or across gradients. Indirect assignment of EM functional traits to taxonomic groups
314 merits caution and their temporal variation merits investigation.
315 We conclude that intensive and extensive organismal and environmental data collection, with
316 multiple biotic and abiotic co-varying factors, reveals soil, atmospheric deposition and
317 climate variables control large-scale patterns of species distributions in EM communities.
318 Such data allow linking species and community responses to environmental thresholds acting
319 across macroecological scales and deliver new insights into spatial variation in specificity and
320 functional trait plasticity below-ground.
321

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Author contributions M.B. conceived study. S.V., M.B., F.C., L.S., B.A. led most sampling design and fieldwork. S.V., B.A., L.S., F.C., Y.Z., M.B. processed and analysed samples. H.A., E.A., S.B., N.C., B.D., H.D., J.E., J.G., T.G., K.H., F.J., F.K., P.L., M.M., J.M., H.M., P.M., M.N., P.P., P.R., M.S., H.S., W.S., V.Š., A.T., I.T., H.T., E.V., A.V., L.V., P.W., S.W., D.Ž. assisted fieldwork and collected, collated and validated long-term environmental data. S.V., H.G., D.O. performed bioinformatics. S.V., D.O., L.S. performed data analysis. C.C. summarized literature. S.V. drafted manuscript, M.B. provided chief contributions, D.O., L.S. contributed extensively. All authors wrote and reviewed manuscript. S.V., L.S., D.O., M.B. led revision.

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ICPF permission. Authors declare no competing financial interests. Correspondence and material requests to S.V. (sietse.vanderlinde@forestry.gsi.gov.uk).

Figure legends

Figure 1: Map of Europe showing sampled UNECE ICP Forests Level II plots. Polygons depict outer boundaries of the sampled area for each host tree species.

Figure 2: Krona chart of taxonomic affiliation of ectomycorrhizas and their relative abundance. Inner circles represent higher taxonomic ranks, while more detailed taxonomic ranks (up to species level) are presented in outer circles. A full interactive version of this chart is available in the online version of this article (Supplementary Information Fig. 1).

Figure 3: Variation partitioning Venn diagram showing the percentages of individual contributions of host variables (host species, foliar chemistry and defoliation), soil+deposition variables, climatic variables and geographic distance. Percentage of variance explained by multiple partition models is shown where ellipses overlap. Values in brackets show the total percentage of variance explained by the four partitions. Residual variance represents the percentage unexplained by the four partition models.

Figure 4: Threshold indicator taxa analyses (TITAN) on individual OTU abundances in response to N_{TFD} (a). Black symbols show taxa declining with increasing N_{TFD} (z−), open symbols depict increasing taxa (z+). Symbol size is proportional to magnitude of response (z-score). Horizontal lines represent 5^{th} and 95^{th} quantiles of values resulting in the largest change in taxon z-scores among 1,000 bootstrap replicates. Tree shapes indicate host

generalist, conifer- or broadleaf-specific. Community-level output of accumulated z-scores per plot is shown in response to N_{TFD} (b).

Materials and methods

Sampling and processing

Since 1995, the International Co-operative Programme on Assessment and Monitoring of Air Pollution Effects on Forests[67,68] has been intensively monitoring ca. 800 plots (Level II) in major forest ecosystems across Europe[69]. Their extensive *in situ* data better reflect the local environmental conditions of plots than regional modelled or extrapolated data[70]. These Level II plots of at least 0.25 ha and located within homogenous forest stands are structurally diverse and cover a representative mixture of European managed forest types (ranging from plantations to natural regenerating forests)[71]. European forests are dominated by Scots pine, Norway spruce and European beech (60% of EU30 forest area), with the next three most common tree species together covering 10%. We selected all ICP Forests Level II plots where deposition, meteorology, foliar chemistry, soil and preferably soil solution data are measured simultaneously, and between September 2013 and September 2015 we sampled plots with European beech (*Fagus sylvatica* L.; n = 35), Norway spruce (*Picea abies* (L.) H. Karst; n = 36) or Scots pine (*Pinus sylvestris* L.; n = 32) as the dominant (>50% abundance) tree species. We combined these with additional data similarly collected from Scots pine Level II plots by Cox et al.[18] (n = 12) and pedunculate and sessile oak (*Quercus robur* L. and *Q. petraea* (Matt.) Liebl) by Suz et al.[16] (n = 22), to give a widespread coverage of European forest areas (Fig. 1).

We used Sanger DNA sequencing of the full internal transcribed spacer (ITS) amplicon from individual ectomycorrhizas to maximise resolution of identifications, obtain relative abundance data and link DNA sequences directly to morphology, following the standardized

563 sampling protocols of Cox et al.[18] and Suz et al.[16]. Briefly, on each plot (n = 137) 24 trees of
564 the investigated target tree species were randomly selected and from those trees a transect
565 was made to the nearest tree of the target species, then four soil samples (25 cm deep, 2 cm
566 diameter) were collected at equal distances on each transect. When plots contained multiple
567 tree species, areas with non-target tree species were avoided. Soil samples were stored at 4°C
568 up to ten days until processed. Roots from each soil core were rinsed on a 0.5 mm sieve, and
569 mycorrhizal roots were collected for five minutes using a dissecting microscope.
570 Subsequently, from each soil sample, an individual mycorrhiza was sampled from the three
571 longest roots, resulting in 288 mycorrhizas per plot. Morphological characteristics of each
572 mycorrhiza were recorded, including presence/absence of emanating hyphae and
573 rhizomorphs, and turgor to assess activity. Genomic DNA from individual mycorrhizas was
574 obtained using Extract-N-Amp (Sigma-Aldrich, St. Louis, MO, USA), and the ITS region of
575 the nuclear rDNA was amplified using ITS1F[72] and ITS4[73] primers. Amplicons were purified
576 using ExoSAP-IT (USB, Cleveland, OH, USA) and sequenced bidirectionally using
577 BigDye3.1 with an ABI 3730 DNA Analyzer (Applied Biosystems, Foster City, CA, USA).
578
579 *Environmental data*
580 On the Level II plots various environmental long-term measurements (average 14 years) were
581 carried out using national protocols based on a harmonized methodology[74] (see
582 Supplementary Information Table 2). Soil types were classified in ten types: Andosols,
583 Arenosols, Calcisols, Cambisols, Leptosols, Podzols, Regosols, Umbrisols, soil types
584 characterised by an Argic B horizon (i.e. Luvisols and Alisols), and soils with gleyic
585 properties (i.e. Gleysols and Stagnosols)[64,75]. Whilst maximizing the number of plots without
586 missing values (n = 108), we selected available data including forest age, level of
587 defoliation[76], geographical coordinates and elevation along with soil (eight variables) and

588 foliar (seven variables of investigated tree species)[77] data, atmospheric throughfall deposition
589 chemistry (wet and dry under forest canopy deposition, 11 variables)[78] and meteorology (six
590 variables)[79].
591
592 *Bioinformatics*
593 We used Phred[80] to obtain base quality scores (Q) for both forward and reverse DNA
594 sequences from all individual mycorrhizas, including DNA sequences from Cox et al.[18] and
595 Suz et al.[16]. The two sequences obtained from each mycorrhiza were assembled in Geneious
596 (version 8.1.8)[81], with the De Novo Assemble tool. We used Trimmomatic[82] to remove low
597 quality bases (Q < 20) at either end of the sequences and then discarded short reads (< 100
598 remaining bp). We then used the uchime_ref tool in vsearch[83] to match chimeric sequences
599 against the UNITE reference database (version 7.1, 22/08/2016).
600 We used the usearch_global tool in vsearch to identify remaining DNA sequences with a
601 percentage match ≥ 97% to UNITE 7.1 species hypotheses[84]. From the remaining unmatched
602 sequences, we first removed all sequences with ambiguous base pair codes and then used the
603 cluster_fast tool in vsearch, to identify *de novo* operational taxonomic unit (OTU) clusters.
604 The unmatched sequences were then matched to the centroids of these *de novo* clusters;
605 sequences were accepted with a percentage identity ≥ 97% and the remainder discarded.
606 We used three sources of information for each *de novo* centroid to confirm the identification
607 of the fungal sequences and to provide tentative classifications. First, we examined the ten
608 best alignments from BLAST searches[85] of the Genbank nucleotide database. Second, we
609 trained RDP Classifier[86] against the UNITE 7.1 database and then classified the *de novo*
610 centroids against the trained database. Third, we used vsearch to obtain the best match of
611 each centroid to the UNITE 7.1 species hypotheses.

612 Finally, we checked the EM status of all OTUs by comparing the taxonomic classification
613 based on UNITE with the literature[87,88]. When OTUs assigned in UNITE to species
614 hypothesis were identified to a taxonomic level that includes both EM and non-EM fungi
615 (e.g. Agaricomycetes sp.), we retrieved the taxonomic names associated with all UNITE
616 DNA sequences within that species hypothesis to assess the level of uncertainty in the
617 classification of the species hypothesis. We discarded *de novo* OTUs with less resolved
618 classification: (a) whose classification was distant from known EM fungi, (b) where the root
619 tip morphology suggested possibly dead plant or fungal tissue, and (c) which were based on
620 relatively short sequences (<150 bp). The set of identified EM fungal sequences was then
621 used to construct an abundance matrix of OTUs across sites. We used the Hellinger
622 transformation of proportion abundance[89] in subsequent analyses. Host specificity of
623 abundant OTUs (≥ 50 EM) was established by scoring occurrence at plots with the different
624 tree hosts. The OTUs occurring with one host tree species in a plot were considered strictly
625 specific and OTUs occurring with both coniferous and broadleaf or with more than two tree
626 species were considered generalists.
627
628 *Statistical analysis*
629 We used R (version 3.3.3) for statistical analyses and generating figures[90].
630 To quantify the importance of host variables, soil and deposition chemistry, climate and
631 geographic distance on EM fungal community composition, variances were partitioned
632 following Borcard et al.[91] and Legendre & Legendre[92]. Explanatory variables describing plot
633 and tree characteristics were grouped in the following partitions: (i) host (host species, foliar
634 chemistry and defoliation), (ii) soil and deposition chemistry (soil characteristics and
635 throughfall deposition), (iii) climate (climatic region, mean annual air temperature (MAT),
636 precipitation, growing season length, minimum and maximum annual temperatures,

637 elevation) and (iv) geographic distance (excluding elevation). The most relevant variables in
638 each partition were found through forward-selection model-building with the redundancy
639 analysis (RDA) method based on AIC and $P < 0.05$ using ordistep in the vegan package[93].
640 Geographic distances are the great circle distances, calculated using the mean Earth radius
641 between the minimum and maximum latitude of plots in this study (r = 6,365 km) with
642 rdist.earth in the fields package[94]. Great circle distances are commonly used in large scale
643 macroecological studies to approach real distances between sampling sites[95,96]. The
644 geographic distance matrix was transformed to rectangular data by extracting spatial vectors
645 with principal coordinates of neighbour matrices (PCNM) using pcnm (vegan). To build the
646 geographic distance model, PCNM vectors accounting for autocorrelation were extracted (P
647 < 0.05) using MoranI (lctools package)[97] and forward selected. Variation partitioning was
648 carried out for the 108 plots with the selected environmental data using varpart (vegan).
649 Global non-metric multi-dimensional scaling (NMDS) ordinations were used to explore and
650 visualise the main factors affecting EM fungal community composition with metaMDS
651 (vegan). Environmental variables (Supplementary Information Table 2) were fitted to the
652 ordination plots using envfit (vegan). Ordinations were performed for the 108 plots with the
653 selected environmental data. In order to limit co-linearity effects between variables, we
654 selected key environmental variables from the envfit results with $R^2 > 0.4$ and $P < 0.01$. In
655 case of correlations ($r \geq 0.7$) between those variables, the most commonly measured
656 environmental variable (Supplementary Information Table 1) was selected: N throughfall
657 deposition (N_{TFD}), forest floor pH, MAT, K throughfall deposition (K_{TFD}) and foliar N:P ratio
658 ($N:P_F$).
659 Indicator species for the key environmental variables were detected and their threshold values
660 were calculated using threshold indicator species analyses (TITAN2)[98]. The sums of the
661 indicator species scores of all OTUs were used to detect lower and upper EM community

thresholds for key environmental variables. In addition to N_{TFD} we also obtained EM community thresholds for N open field deposition since open field deposition measurements better reflect the data that is available in spatially mapped deposition datasets[99,100].

G-tests were performed to test if host species or soil type influence hyphal and rhizomorph presence or absence. We used logistic regression with each key environmental variable and the presence or absence of emanating hyphae and rhizomorphs within individual OTUs to test for environmental influences on their morphological plasticity. We considered OTUs where the indicator analysis suggested a response to a particular environmental variable and, for statistical power, we only tested OTUs with ≥ 15% presence and ≥ 15% absence of emanating hyphae or rhizomorphs (Extended Data Table 1). Target tree species and soil type was used as co-variate, to account for potential variation in hyphal and rhizomorph development in mycorrhizas belonging to the same OTU among different tree species and different soil types.

Code availability, R scripts for data analyses are available from the corresponding author upon reasonable request.

Data availability, Sequencing data generated during the current study are available through DRYAD under doi:10.5061/dryad.cr70qc8. Morphological characteristic and host specificity data generated during the current study are available from the corresponding author upon reasonable request. All environmental data (including deposition, foliar chemistry, soil and meteorological data) are available from UNECE ICP Forests but restrictions apply to the availability of these data, which were used under license for the current study. Data are available from the corresponding author upon reasonable request and with permission of UNECE ICP Forests.

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767
768 **Extended table titles and legends**
769 **Extended Data Table 1. Envfit results for the environmental variables used in the**
770 **NMDS ordination.** Significant variables are printed bold.
771
772 **Extended Data Table 2: Observed and expected frequencies of hyphae and rhizomorph**
773 **presence** for host tree species (a) and soil type (b). S1 = Fe Al soils, S2 = Clay soils, S3 =
774 Soils with little or no differentiation, S4 = Salt accumulation soils, S5 = Organic
775 accumulation soils, S6 = Limited root soil.
776
777 **Extended Data Table 3: Effects of key variables on hyphal plasticity** for 97% sequence
778 similarity OTUs (a) and 99% sequence similarity OTUs (b). *P* values < 0.05 are printed bold.
779 Logistic regressions were only calculated for OTUs where the indicator analysis suggested a
780 response to a particular environmental variable. With: $^{-}$ = declining indicator (z-), $^{+}$ =
781 increasing indicator (z+), ↓ = negative correlation, ↑ = positive correlation.
782
783 **Extended Data Table 4: Effects of key variables on rhizomorph plasticity** for 97%
784 sequence similarity OTUs (a) and 99% sequence similarity OTUs (b). *P* values < 0.05 are

printed bold. Logistic regressions were only calculated for OTUs where the indicator analysis suggested a response to a particular environmental variable. With: $^{-}$ = declining indicator (z-), $^{+}$ = increasing indicator (z+), ↓ = negative correlation, ↑ = positive correlation.

Extended Data Table 5: Effects of key variables on hyphal and rhizomorph presence on the total EM community. *P* values < 0.05 are printed bold. With: ↓ = negative correlation, ↑ = positive correlation.

Extended data figures

Extended Data Figure 1: Global non-metric multidimensional scaling ordination of community composition showing plots with host trees (brown squares: beech; blue circles: oak; green triangles: pine; yellow diamonds: spruce). Isoclines depict the forest floor pH and arrows show the direction and strength of correlation of the most influential environmental variables according to their R^2 values (> 0.4). A = MAT; B = mean minimum annual air temperature; C = growing season length; D = NH_4 throughfall deposition; E = N_{TFD}.

Extended Data Figure 2: Threshold indicator taxa analyses (TITAN) on individual OTU abundances in response to N:P_F (a), forest floor pH (c), K_{TFD} (e) and MAT (g). Black symbols correspond to taxa declining with the increasing variable (z−), open symbols depict increasing taxa (z+). Symbol size is proportional to magnitude of response (z-score). Horizontal lines represent 5th and 95th quantiles of values resulting in the largest change in taxon z-scores among 1,000 bootstrap replicates. Tree shapes indicate host generalist, conifer- or broadleaf-specific. Community-level output of accumulated z-scores per plot is shown in response to N:P_F (b), forest floor pH (d), K_{TFD} (f) and MAT (h).

Extended Data Figure 3: Threshold indicator taxa analysis at the genus level in response to N_{TFD} (a), $N:P_F$ (c), forest floor pH (e), K_{TFD} (g) and MAT (i). Black symbols correspond to taxa that declined with the increasing variable (z−), open symbols depict increasing taxa (z+). Symbol size is proportional to magnitude of response (z-score). Horizontal lines represent 5th and 95th quantiles of values resulting in the largest change in taxon z-scores among 1,000 bootstrap replicates. The community-level output of the accumulated z-scores per plot is shown in response to N_{TFD} (b), $N:P_F$ (d), forest floor pH (f), K_{TFD} (h) and MAT (j).