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Monitoring ectomycorrhizal fungi at large scales for science, forest management, fungal conservation and environmental policy

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Abstract

• Key message The ICP Forests network can be a platform for large-scale mycorrhizal studies. Mapping and monitoring of mycorrhizas have untapped potential to inform science, management, conservation and policy regarding distributions, diversity hotspots, dominance and rarity, and indicators of forest changes.

• Context A dearth of information about fungi at large scales has severely constrained scientific, forest management, fungal conservation and environmental policy efforts worldwide. Nonetheless, fungi fulfil critical functional roles in our changing environments and represent a considerable proportion of terrestrial biodiversity. Mycorrhizal fungi are increasingly viewed as a major functional guild across forest

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ecosystems, and our ability to study them is expanding rapidly.

- **Aims** This study aimed to discuss the potential for starting a mycorrhizal monitoring programme built upon the existing forest monitoring network, raise questions, propose hypotheses and stimulate further discussion.
- **Results** An overview of the state-of-the-art regarding forest ectomycorrhizal ecology raises questions and recommendations for scaling up mycorrhizal assessments aimed at informing a variety of stakeholders, with a new focus on conservation and policy.
- **Conclusion** Fungal research and conservation are areas that can be informed by ICP Forests and may lead to useful spin-offs; research linked to long-term forest monitoring plots will enhance the relevance of science and conservation.

Keywords Mycorrhizas · ICP Forests · Monitoring · Air pollution · Indicator · Red list

1 Introduction

Nearly all tree roots in boreal and temperate forests form ectomycorrhizas. These intimate fungus–plant mutualisms play crucial ecological roles by determining the nutrient acquisition and drought tolerance of trees (Smith and Read 2008). There is concern that we lack baseline data on mycorrhizal species distributions and abundances against which to assess the effects of global change (Courty et al. 2010; Kjølter et al. 2012; Lilleskov and Parrent 2007; Peay et al. 2010). Research on mycorrhizas has been focused at local scales (i.e. stand level), leaving a widely recognised gap in our understanding of patterns of diversity and their drivers at landscape, regional, continental and global scales (Bruns 1995; Peay et al. 2010). We still know very little about diversity and its drivers for any key soil organisms above local scales, even though various ecological processes are only apparent at much larger spatial scales (Levin 2000). For ectomycorrhizal (ECM) fungi, mapping, monitoring and evaluation need to reach beyond national boundaries to reflect large-scale fungal distributions and their drivers of change.

The vast European forest monitoring network (www.icp-forests.org) is an ideal study platform because its intensively monitored long-term forest plots have tremendous potential for developing and scaling up forest mycorrhizal research and biomonitoring (Cox 2010; Cox et al. 2010a, b; Suz et al. 2014). In April 2014, a multidisciplinary workshop focused on how the network can be used to investigate mycorrhizal diversity and ecology at large scales (www3.imperial.ac.uk/ecosystemsandenvironment/events/workshops/nercimpactworkshops/macroecologyofmicrobes). Presentations and discussions assessed the state-of-the-art,

findings from ECM communities in a pine study across England and Germany (Cox et al. 2010b), a study of oak plots across Europe (Suz et al. 2014) and from an ongoing Europe-wide analysis of ECM fungi in spruce, pine and beech plots carried out in plots belonging to this network and using its rich *in situ* environmental data (listed below). The complementary needs and resources of the monitoring, nature conservation, scientific and forest management communities were discussed. A new focus was generating data on ECM communities to inform fungal conservation in the context of the recent International Union for the Conservation of Nature Initiative on Global Fungal Red Listing (Dahlberg and Mueller 2011, iucn.ekoo.se). Participants included government and NGO forest managers, government and academic conservation experts and ecologists. The outcomes of discussions and the questions raised are summarised here. The overarching goals of this article are to stimulate debate, to encourage expanding the spatial and temporal scales across which studies are carried out and to explain the rationale behind the ongoing assessments of mycorrhizas across Europe's forest monitoring network.

Overall, five main questions covering current knowledge gaps were formulated: (1) What are the spatial patterns of mycorrhizal diversity and community structure? (2) What are the environmental and ecological factors that control mycorrhizal distributions at landscape and larger scales? (3) What are the likely responses of dominant mycorrhizal fungi to global change and how will they affect the long-term spatial cohesion of communities? (4) Will communities change (e.g. shifts in dominant species and/or extinction of certain fungi) under changing environmental conditions, and if so, will these changes affect forest growth and resilience to global change? (5) How can forests be managed more sensitively to protect and enhance the diversity and functions of fungi?

Progress in answering some of these questions is being made by combining molecular ecology with geographic information systems (e.g. Tse-Laurence and Bidartondo 2011) to generate new data on ECM communities through sampling forest plots belonging to the ICP Forests network. These approaches reduce previous analytical limitations in large-scale mycorrhizal studies. Engaging stakeholders, such as forest managers, conservationists and policy makers, is essential to raise awareness, enhance collaborations and join fundraising efforts to include mycorrhizal surveys in forest monitoring.

2 How to assess fungal distributions

The distinctive life cycles and growth forms of fungi (see Peay et al. 2008 for an excellent review) pose unique challenges and opportunities for mapping fungal species distributions at large spatial and temporal scales. At present, the limited, typically national, data available on fungal species distributions and

how they change originate from studying the reproductive structures visible to the naked eye that are formed by some macroscopic fungi (e.g. mushrooms, truffles, crusts) (Arnolds 1991; Kausserud et al. 2012). Most of the more apparent mushrooms (and to a lesser extent the more cryptic truffles and crusts) are ephemeral (days to weeks) and sporadically produced but have been recorded for over a century and are well researched. Some fruiting bodies can be assessed in the field via multi-year surveys. Reproductive structures are partially represented in collections at museums for the purposes of taxonomic research, some of which have been databased and can be accessed via the public Global Biodiversity Information Facility (www.gbif.org).

In addition to visible fruiting bodies, there are fungal structures such as filaments (mycelium), dispersal propagules (spores and sclerotia) and structures formed together with plant host roots—called mycorrhizas—that are all found in the soil. These structures may not be visible to the naked eye but can be much larger and/or longer-lived than reproductive structures—e.g. the average size of individuals of the ECM fungus *Suillus variegatus* has been reported to range from 10 to 20 m (Dahlberg 1997) and sclerotia of the ubiquitous ECM fungus *Cenococcum geophilum* might rest in soil for over 1,000 years (Watanabe et al. 2007). Less well recorded and researched than fruiting bodies are the soil filaments that are also ephemeral (days to weeks) and the spores that are potentially viable for years (Nguyen et al. 2012). Soil filaments can be assessed through in-growth mesh bags or cores, and resistant spores and sclerotia can be assessed via soil bioassays. Spores and sclerotia of some ECM fungi can be abundant in soil, forming spore banks analogous to the seed banks of plants. This variety of forms merits thorough consideration when designing field sampling and interpreting data.

Despite the relatively short life (weeks) of the individual ECM roots, the diversity of ECM fungi can remain stable at scales >10 cm (Cox 2010; Douhan et al. 2011; Izzo et al. 2005; Koide et al. 2007; Lian et al. 2006). Crucially, ectomycorrhizas are always present as nearly all roots are colonised by mycorrhizal fungi—even under high nutrient availability—as shown by a meta-analysis (Cudlin et al. 2007). Sampling roots is thus a convenient and efficient way to monitor active ECM populations likely to give a more complete characterisation of the mycorrhizal communities present compared to spore, mycelium and fruiting body surveys. Functional traits of particular interest in ectomycorrhizas are conferred by their soil exploration types which are characterised by short, medium or long filaments linking mycorrhizal roots to soil (Agerer 2001, 2006) and their ability to uptake organic nitrogen (N) (driven by the production and release into soil of a battery of fungal enzymes, some of which may degrade recalcitrant organic matter as a side effect, Bödeker et al. 2014). Moreover, these functional traits that define each exploration type can confer different capabilities with regard to storing carbon and taking up and

translocating nutrients (Courty et al. 2010; Hobbie and Agerer 2010). We have seen that mycorrhizal communities in temperate oak forests respond differently to environmental variables depending on their soil exploration type (Suz et al. 2014). Because the effects of changes in the presence and proportions of different ECM functional groups across Europe may affect the resilience of forests to environmental change, they need more investigation. However, trait databases for mycorrhizas are in their infancy (e.g. www.deemy.de) with only about 320 mycorrhizal types characterized, although information on soil exploration strategies of 143 ECM genera has been recently compiled (Tedersoo and Smith 2013).

As with other organisms, the main public information repository for fungi as DNA sequence data is the International Nucleotide Sequence Database (www.insdc.org), though some specialised public sub-databases are also available—e.g. UNITE for the fungal DNA barcode for identification (unite.ut.ee) and MarjaAM for arbuscular mycorrhizal fungi (maarjam.botany.ut.ee)—. These DNA databases effectively integrate data from all fungal structures and life history stages. Globally, it is estimated that most fungal species, particularly those outside the developed world, await taxonomic description and are not yet represented in collections or databases (Blackwell 2011). Of the fungi that have a taxonomic description, relatively few have been sequenced (Brock et al. 2009). Some of these ‘unknown’ fungi—undescribed and/or unsequenced—can be dominant belowground even in relatively well-studied European forests (e.g. Cox et al. 2010b).

3 Monitoring mycorrhizas for improved forest management

Since mycorrhizal communities are integral to most forest ecosystems and play essential roles—accessing different inorganic and organic nutrient pools, conferring drought tolerance or plant resistance to pathogens—there is a need to better understand how they vary among forest types, across environmental gradients and management regimes in order to practice more sensitive management (e.g. thinning, clearing, fire control, harvest regulation of edible mushrooms). There is evidence, for example, that tree species composition influences mycorrhizal communities (Ishida et al. 2007) and that there are generalist and host-specific fungi (e.g. Molina et al. 1992). Where ‘non-native’ mycorrhizas are imported on planted trees, especially on non-native tree species (Pennington et al. 2011), what are the implications on existing mycorrhizal communities, particularly in any neighbouring native tree species stands? There are examples of invasive ECM fungi—see Vellinga et al. (2009) for a review on ECM introductions—such as the deadly European *Amanita phalloides* in North America (Wolfe et al. 2010). Moreover, what are the consequences of a much altered mycorrhizal

community? What is the link between major changes in forest condition (e.g. dieback) and the structure of mycorrhizal communities? It is intriguing that strong correlations have recently been reported between eucalyptus forest decline, soil chemistry and ECM community structure (Horton et al. 2013).

There is also a gap in understanding how much the success of forest restoration and afforestation is linked to restoring and building mycorrhizal communities. For instance, is it necessary to restore the mycorrhizal community of a native broadleaf woodland when converting from conifer to broadleaf or vice versa? Baskin (1997) describes a number of cases where highly disturbed forest soils no longer support the mycorrhizal communities required for the successful establishment of saplings, resulting in many failed reforestation attempts. The protection, restoration and creation of woodland both near and far from existing woodlands as well as conifer-broadleaf transitions are of interest to many land managers—how do mycorrhizas drive or constrain these different practices? How redundant are roles played by different ECM fungi in a forest? Under which conditions those roles might become unique and susceptible to disappearing? Many woodlands are heavily fragmented, e.g. 74 % of English woodland is ca. 100 m from a woodland edge (Riutta et al. 2014). Edge effects on light, water and pollution are relatively well understood, but woodland edges typically represent strong mycorrhizal transitions as well and these have only received limited attention (e.g. Branco et al. 2013).

4 The present and future of global fungal conservation

The very limited existing fungal diversity and distribution data (e.g. Fig. 1) are a critical gap in nature conservation; many fungi are now known to have discrete distributions, similar to those of animals and plants, but distribution and population trend data for fungi lag far behind equivalent datasets available for plants and animals. For detailed reviews of different aspects of the importance of fungal conservation, see Pringle et al. (2011) and others in the same issue of *Fungal Ecology*. Historically, there has been some resistance to red listing from within the fungal research community, largely due to a lack of basic ecological information about species. Consequently, fungi have long been overlooked by and left out of the influential International Union for the Conservation of Nature (IUCN) process of listing rare and endangered species to enhance their protection (i.e. currently only one macrofungus and two lichenised fungi are among the over 21,000 red-listed organisms). This is particularly striking when we consider the important functional role of many fungi in ecosystems and also the vast scale at which wild ECM forest fungi are commercially harvested and exported (Sitta and Davoli 2012).

Undoubtedly, more diversity data are needed—especially at large spatial and temporal scales—but it is already possible to identify targets and risk factors and to quantify uncertainty, at least locally, nationally or regionally (Dahlberg and Mueller 2011; Lilleskov et al. 2011; Molina et al. 2011). A consistent

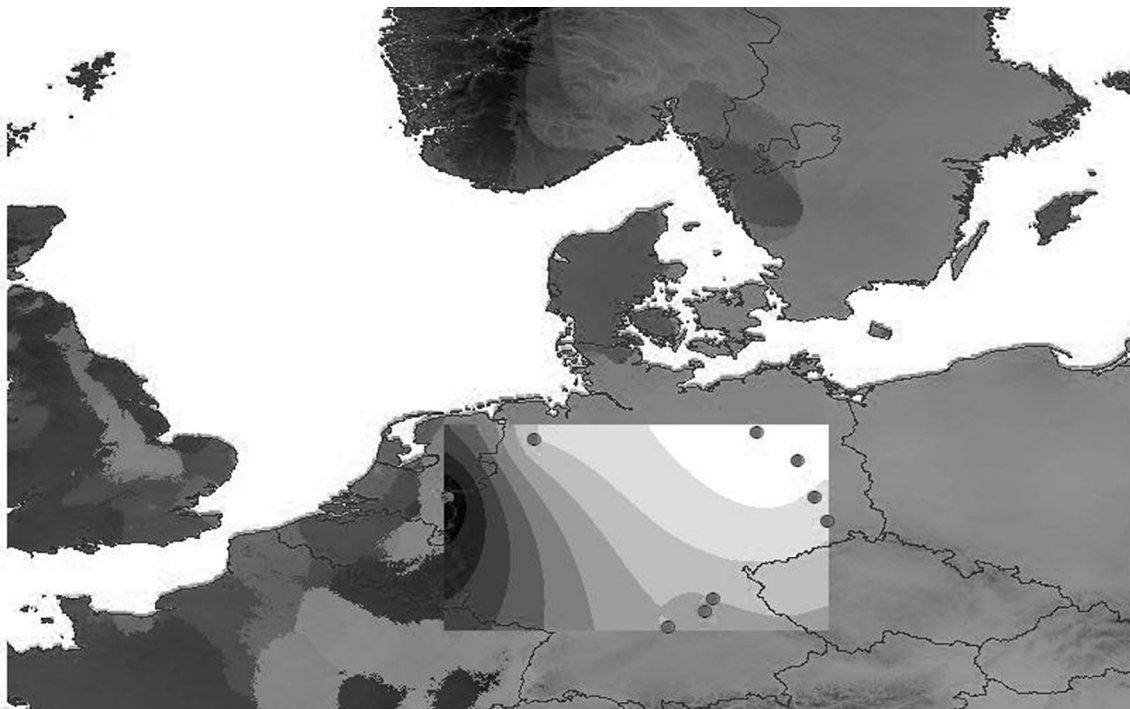


Fig. 1 A first geographic distribution map for the false truffle *Elaphomyces granulatus*—a dominant mycorrhizal fungus of pine forests overlooked in fruiting body surveys due to its underground fruiting that is currently a candidate for global red listing—generated

using inverse distance-weighted interpolation on DNA sequences from Level II pine sites and georeferenced GenBank accessions showing that large-scale mapping of dominant ectomycorrhizal fungi is feasible

problem for red listing of any particular species is how to scale up or extrapolate ecology and distribution information that justifies the need for conservation of fungi at risk beyond local, national or regional datasets, e.g. from relatively well-studied Nordic, Dutch or Swiss fungi, to nearby countries, pan-European scales and beyond. Generation time and persistence are key considerations when assessing changes in population size over time. Assessments following IUCN guidelines are based on three generations. A key question for fungal conservation remains developing biologically meaningful, defensible estimations of generation times for species of ECM fungi. The suggested generation times presented in Dahlberg and Mueller (2011) need additional validation from a diversity of species across disturbance gradients, soil differences and host associations. Belowground sampling coupled with population genetic studies of targeted taxa/habitats can provide these needed data. How long do fungi persist belowground under particular environmental pressures (e.g. pollution)? What is the host range of obligate symbiotic fungi? Thanks to research developments—largely driven by the molecular ecology revolution since the 1990s—many of these questions have been answered at local scales and that has now finally been sufficient to attract interest and support from IUCN, but extrapolation is risk-laden. The current first IUCN Red Listing Initiative for Fungi can ultimately lead to identifying endangered fungi, indicators of restoration success (e.g. UK waxcap grasslands) and fungal diversity hotspots or key fungal biodiversity areas. Better integration between the research and conservation communities is urgently needed. The biomonitoring community can act as a natural integrator with its platform for generating rigorous data on mycorrhizal diversity, distribution, turnover (spatially and temporally), host specificity, and responses to anthropogenic stressors and land management issues that are key needs of the fungal conservation community. These data are starting to be captured through the first mycorrhizal sampling of diverse ICP Forests plots. Ongoing communication between the conservation, research and biomonitoring communities and tentative statistical modelling approaches will help ensure sharing of data and ideas and also enable action on the ground in terms of potential funding and site availability for research.

5 Ectomycorrhizas and conservation policy

Ongoing barcoding of sporocarps initiatives, population genetic studies and regional collections database projects are providing the comparative data needed to integrate roots and other environmental samples to enable us to understand multiple aspects of biodiversity (taxonomic, ecological and genetic) across various scales in ways never before possible. Issues of scale and evidence quality are of paramount importance for informing policy makers on conservation issues, particularly

if costly interventions, such as industry regulation, are required in order to achieve conservation objectives. For instance, consideration of N deposition impacts needs to be based on both small-scale experimental manipulations and large-scale correlations between biodiversity change and deposition gradients (see Stevens et al. 2011; Emmett et al. 2011; ROTAP 2012). Once small-scale manipulations are combined with greater certainty that large-scale biodiversity change is being driven by N deposition, then it becomes possible to modify the critical value used in mapping and to start to infer pressures and threats on protected sites (Whitfield et al. 2012). Agencies such as the UK Joint Nature Conservation Committee support national fungal red listing (i.e. Boletaceae, Geastraceae), but they are also increasingly focused on acquiring information about fungal communities in the context of a range of ecosystem processes (for instance how N deposition will affect ecosystem function via impacts on fungal communities), rather than only on rare and/or declining species. Clear demonstrations of changes in mycorrhizal communities with increasing N deposition analogous to the changes that have been reported for other organisms (Stevens et al. 2011; Emmett et al. 2011) are emerging from analyses of ICP Forests mycorrhizal data (Cox et al. 2010b; Suz et al. 2014). There are also some hints as to which traits are likely to respond, e.g. soil exploration strategy. This leads to the question of how fungi recover through spore banks and/or via dispersal? And, when mycorrhizal community recovery is relatively slow, how does it limit vascular plant recovery?

6 Why monitor mycorrhizas in the ICP Forest Network Level II plots?

The ICP Forest Level II plots have been used successfully for national (e.g. Helmisaari et al. 2009) and increasingly for trans-national (e.g. Clarke et al. 2011; Cox et al. 2010b; Kristensen et al. 2004) research. European forests are uniquely suitable and tractable for the study of mycorrhizas because (1) there is relatively low forest biodiversity compared to North America and Asia, (2) an extensive and intensive forest monitoring network is available, (3) over 95 % of forest area is covered by ECM trees, (4) fungi are relatively well-characterised compared with other areas of the world and (5) there are strong natural and anthropogenic environmental gradients (e.g. pollution, Taylor et al. 2000). Thus, it is an excellent place to generate understanding and predictions on the behaviour of the natural environment and its resources by developing and testing bioindicators, contributing to assessments of carbon sequestration, macronutrient cycling, and identifying biotic and abiotic cause–effect relationships responsible for biome condition. There are about 800 intensively monitored (Level II) plots, 0.25 ha each, covering Europe’s major forest types. Monitoring in Level II plots includes (1)

continuous measurement of atmospheric deposition, soil solution chemistry and meteorology; (2) biannual measurement of foliar chemistry (i.e. C, Ca, Cd, Cu, Fe, K, Mg, Mn, N, P, Pb, S, Zn); (3) annual measurement of crown condition; (4) recording of tree growth and understory vegetation every 5 years; and (5) decadal measurements of solid phase parameters (most recently 2006–2007). Soil solution chemistry parameters include organic C, total N, carbonates, exchangeable acidity, exchangeable cations, Al, Ca, Cd, Cr, Cu, Fe, Hg, K, Mg, Mn, Na, P, Pb, S, Zn and total elements. Physical soil structure is characterised using organic layer weight, percentage of coarse fragments, bulk density, particle size distributions, clay, silt and sand content. At selected plots, additional information is gathered continuously on stand structure (including the amount of dead wood), lichen diversity, ambient air quality, phenology and/or litterfall. Growing season temperature and meteorological measurements are available from many plots. Land history is available from the national or regional agencies that are responsible for managing the plots. Monitoring in ICP Forests is discussed in detail by Ferretti and Fischer (2013).

Mycorrhizas are increasingly recognised as a major ‘missing’ biotic factor regulating development, biodiversity and productivity in terrestrial ecosystems (Peay et al. 2010). The ICP Forests network represents an unrivalled platform for research to fill this knowledge gap (Cox et al. 2010a). A first essential research step is to produce a large dataset for robust tests of key macroecological hypotheses and to provide the baseline high-quality mycorrhizal data to enable future monitoring of changes in community composition and diversity. Among them: (1) increased N availability impacts Europe’s mycorrhizal diversity; (2) soil pH, altitude, stand age and climatic conditions affect mycorrhizas; (3) there are dominant mycorrhizal fungi associated with Europe’s major trees; (4) there are geographic ‘hotspots’ of mycorrhizal biodiversity that correspond with those of vascular plants; (5) mycorrhizal richness follows latitudinal trends; and (6) there are mycorrhizal fungi that can act as indicators of environmental conditions.

A second step should involve multiple scale manipulative experiments across a range of sites in order to maximise predictive power. However, this second future step cannot be designed without the information arising from a baseline assessment of diversity and a macroecological understanding of that diversity across geographic gradients. Furthermore, the effects of experiments may differ from those resulting from long-term geographic gradients, so that both approaches are valuable and complementary.

7 Large-scale monitoring of mycorrhizas

So far, mycorrhizal assessments as part of the network (Fig. 2) have been carried out in 12 Scots pine (*Pinus sylvestris*) ICP

Forest Level II plots in Britain and Germany (Cox et al. 2010b) and 22 oak level II plots (*Quercus robur* and/or *Quercus petraea*) across nine countries (Suz et al. 2014). Over 3,000 soil cores have been examined and over 10,000 mycorrhizas analysed using DNA-based techniques that identified 112 mycorrhizal fungi in pine and ca. 400 in oak forests (see Fig. 3a for species accumulation curves). A further study of over 100 spruce, pine and beech plots is due for completion in 2016. The genetic screening approach used allows for the study of long-term associations, provides a least biased sample of the dominant active ECM fungi and preserves mycorrhizal root specimens for further study. Direct observation of fruiting bodies, inoculum potential bioassays to assess spore banks and detection of soil filaments may provide useful complementary information.

Overall, N pollution and soil pH are emerging as major factors driving ECM communities through impacts on tree roots, fungi and soil conditions (Fig. 3b). Both mycorrhizal richness and evenness decrease when soil pH decreases, leading to dominance by acidophilic fungi. Similarly, N deposition decreases the richness and evenness of mycorrhizal communities, and it shifts dominance towards nitrophilic fungi resulting in a decrease or even loss of N-sensitive fungi. There are species that show a consistently negative response to increasing N inputs and are not detected in plots receiving high N loads. There is a need to detect the critical N loads for mycorrhizal communities in European forests above which communities suffer changes that can affect forest ecosystem processes, as previously reported for forests in North America (Pardo et al. 2011) and oak forests in Europe (Bobbink and Hettelingh 2011; Suz et al. 2014). These thresholds may be similar to other taxa such as vascular plants that also demonstrate a sensitive response to N loading and changes in soil pH (Ikaunieca et al. 2013). Simultaneously, we need to identify species and sets of species that can indicate and predict ecosystem condition and vice versa (e.g. Suz et al. 2014).

So far, we have found that (1) nitrogen is a primary determinant of mycorrhizal distribution in Europe (Cox et al. 2010b; Suz et al. 2014; Fig. 3b); (2) rarely studied, poorly culturable and/or inconspicuously fruiting mycorrhizal fungi can dominate belowground both within and across plots (e.g. the truffles *Elaphomyces* and *Hydnotrya* and the crusts *Piloderma* and *Tomentella*), some of which (e.g. *Elaphomyces muricatus* and *Hydnotrya tulasnei*) are being considered for red listing by IUCN based on fruiting body records; (c) in common with other large perennial organisms, ECM communities can be spatially stable between seasons and years, even following seedling transplant between environmentally divergent plots (Cox 2010); (d) there can be abundant dormant propagules (i.e. sclerotia) in soil from fungi found rarely as mycorrhizas, questioning the assumption that bulk soil environmental DNA is from active fungi (e.g. Anderson et al. 2014; Talbot et al. 2014); and (e) dominant

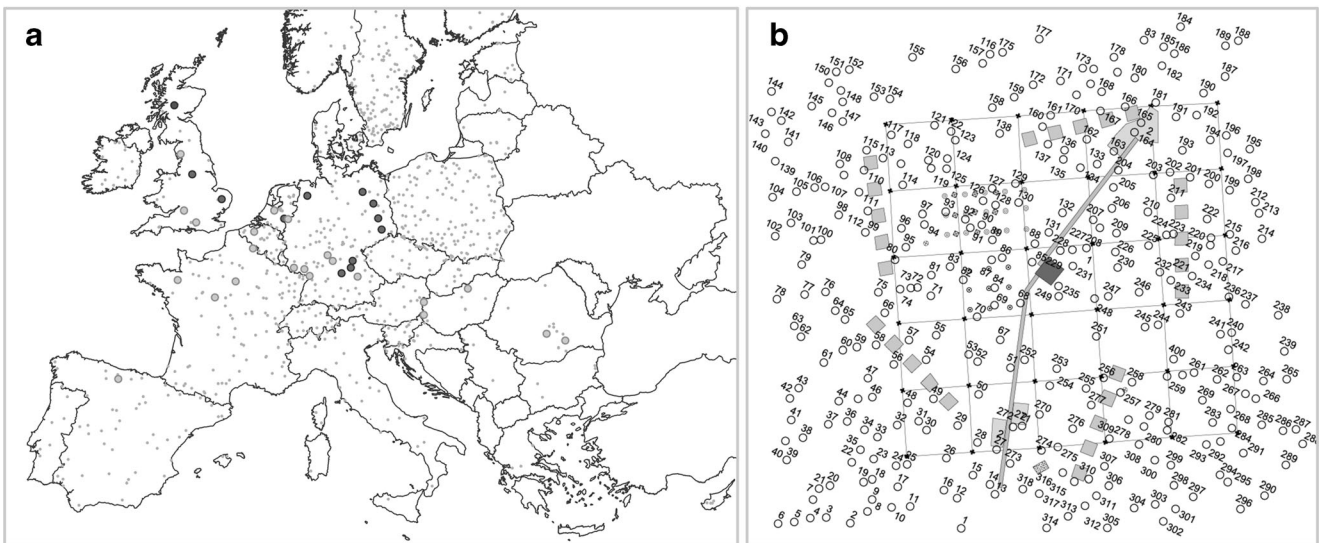


Fig. 2 a Thirty-four Level II plots belonging to the ICP Forests network have been used so far for mycorrhizal assessments. Dark grey circles are Scots pine plots sampled by Cox et al. (2010b), and light grey circles are oak plots sampled by Suz et al. (2014). b A schematic map of a typical

Level II plot shows the presence of numbered trees that facilitates randomisation of tree to tree transects for mycorrhizal root sampling within plots, as well as allowing the spatial location of samples to be recorded should within-site spatial analyses or re-sampling be required

but taxonomically unknown ECM fungi can be linked to previously informally described mycorrhizas (e.g. a Leotiomycete to ‘Piceirhiza sulfo-incrustata’ sensu Palfner et al. 2005).

8 Conclusion

The availability of long-term datasets is the strongest limiting factor in global change research (Willis and MacDonald 2011). Soil biology data will be of value

to forest monitoring efforts that can impact on policy by UNECE and the EU (e.g. Biodiversity Strategy). European trees and ground floor vegetation are being monitored and are leading to significant findings (e.g. Dirnboeck et al. 2014; Seidling et al. 2008; Van Dobben and de Vries 2010; Veresoglou et al. 2014), but fungi are not yet included in the current monitoring scheme. Science and conservation are areas that can be built into ICP Forests and/or may lead to useful spin-offs; research linked to these long-term plots has the potential to enhance the relevance of science and conservation.

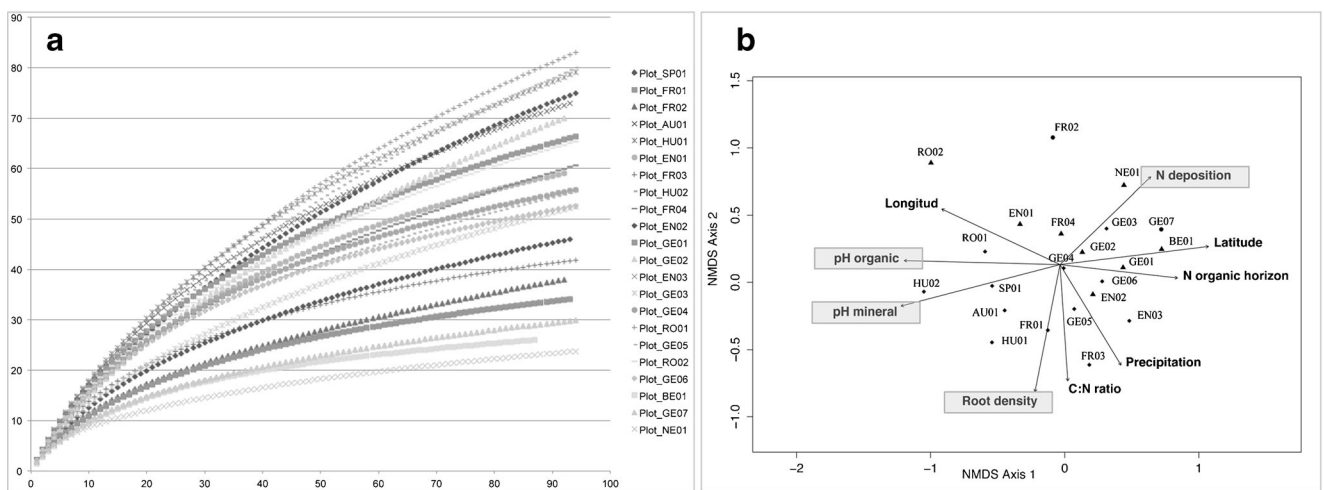


Fig. 3 A Species accumulation curves (Mao Tau) showing that the sampling design used recovered on average 70 % of the estimated richness across plots (x-axis: number of soil cores sampled for mycorrhizas; y-axis: number of fungal taxa detected). b Non-metric

multidimensional scaling (NMDS) analysis of mycorrhizal communities at oak Level II plots shows that among other variables, nitrogen availability, soil pH and root density are main drivers of mycorrhizal diversity and community composition (Suz et al. 2014)

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