Environmental drivers of ectomycorrhizal communities in Europe's temperate oak forests

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Abstract

Ectomycorrhizal fungi are major ecological players in temperate forests, but they are rarely used in measures of forest condition because large-scale, high-resolution, standardized and replicated belowground data are scarce. We carried out an analysis of ectomycorrhizas at 22 intensively monitored long-term oak plots, across nine European countries, covering complex natural and anthropogenic environmental gradients. We found that at large scales, mycorrhizal richness and evenness declined with decreasing soil pH and root density, and with increasing atmospheric nitrogen deposition. Shifts in mycorrhizas with different functional traits were detected; mycorrhizas with structures specialized for long-distance transport related differently to most environmental variables than those without. The dominant oak-specialist *Lactarius quietus*, with limited soil exploration abilities, responds positively to increasing nitrogen inputs and decreasing pH. In contrast, *Tricholoma*, *Cortinarius* and *Piloderma* species, with medium-distance soil exploration abilities, show a consistently negative response. We also determined nitrogen critical loads for moderate (9.5–13.5 kg N/ha/year) and drastic (17 kg N/ha/year) changes in belowground mycorrhizal root communities in temperate oak forests. Overall, we generated the first baseline data for ectomycorrhizal fungi in the oak forests sampled, identified nitrogen pollution as one of their major drivers at large scales and revealed fungi that individually and/or in combination with others can be used as belowground indicators of environmental characteristics.

Keywords: bioindicator, critical load, exploration type, ICP Forests, mycorrhizas, pollution, *Quercus*

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Introduction

Globally, mycorrhizal mutualisms underpin terrestrial ecosystems. Mycorrhizas are ancient, obligate and ubiquitous mutualisms between the vast majority of plants and members of several fungal phyla to exchange photosynthates for fungal-acquired soil nutrients. Ectomycorrhizal (ECM) fungi cover nearly all fine tree roots in boreo-temperate biomes, thus functioning as the interface between trees and soils, playing a dominant role in the water and nutrient acquisition of trees. In gaining 75% of their required nitrogen (N), plants pay 15% of their carbon (C); for the fungi, this represents all of their required C at a cost of 40% of their N (Hobbie & Hobbie 2006).

To date, the emphasis in mycorrhizal research has been on laboratory or local-scale studies to provide a mechanistic understanding of symbiotic physiology. However, the main projected impacts of environmental change on forest processes stem from global perturbations in the C and N cycles (Schulze *et al.* 2000), regional changes in soil organic matter and declines in soil biodiversity (Janssens *et al.* 2010). In addition to direct impacts at regional and landscape scales, the maintenance of ecosystem services provided by temperate forests of the Northern Hemisphere is critical for climate change mitigation (Bonan 2008). Several lines of evidence suggest that a wider mycorrhizal perspective is required to understand the impact of environmental change. Both a meta-analysis of ECM fungal species composition and root biomass (Cudlin *et al.* 2007) and long-term phenological data sets on fruiting of forest fungi in England and Norway indicate large-scale responses that differ from those of plants and animals (Gange *et al.* 2007; Kauserud *et al.* 2008). Functional traits of mycorrhizas drive C and N dynamics at global scales (Averill *et al.* 2014), and there is evidence that determinants of ECM diversity at local scales are not necessarily their primary drivers at larger spatial scales (Lilleskov & Parrent 2007). Consequently, ecologists have called repeatedly for unbiased, regional-to-continental-scale, DNA-sequence-based, ecosystem-level baseline data on mycorrhizal distributions (Lilleskov & Parrent 2007; Courty *et al.* 2010; Peay *et al.* 2010; Kjøller *et al.* 2012).

Due to their distinctive ecological niche, ECM fungi are at particular risk due to changes in either their soil environment or host C allocation (Pardo *et al.* 2011). Since the 1980s, some national ECM extinctions have been documented within European countries (Arnolds 2010), 31 countries maintain or are preparing Red Lists based on observations of fungal fruiting, and an IUCN Global Fungal Red List Initiative (http://iucn.ekoo.se) is now finally underway. Declines in oligotrophic forest floor plant cover, but not richness, have been related to

N critical loads in the European-wide long-term forest monitoring data set (Dirnböck *et al.* 2014). Similarly, declines in fruiting of many forest mycorrhizal fungi in northern and central European countries have been linked to N deposition (Arnolds 1991; Wallenda & Kottke 1998; Lilleskov *et al.* 2011), and in general, less species-rich communities dominated by N-tolerant fungi have been observed under increasing N deposition gradients in mycorrhizal studies at regional-to-subcontinental scales (e.g. Taylor *et al.* 2000; Cox *et al.* 2010 and references in Table S1, Supporting information). The mapping, monitoring and evaluation of fungi still need to reach beyond national boundaries to reflect potentially large-scale fungal distributions.

Oak forests are an extensive forest type across temperate regions, receiving some of the highest N deposition rates on Earth (Galloway & Cowling 2002), but so far, the vast majority of mycorrhizal studies have been undertaken in conifer forests. We carried out a survey of mycorrhizal fungi in one of the largest and most intensive long-term forest monitoring networks in the world (ICP Forests) to answer the following questions: (i) What are the patterns of mycorrhizal diversity and community structure and composition in Europe's oak forests? (ii) What are the main environmental factors that control mycorrhizal distributions in temperate oak forests at large scales? (iii) Are there fungi that can act as belowground indicators of particular environmental conditions? and (iv) Do fungi with different soil exploration strategies (Agerer 2001) respond differently to environmental gradients? Moreover, we aimed to generate baseline mycorrhizal diversity data against future change at a continental scale.

Materials and methods

Field sampling and mycorrhizal assessment

Standardized mycorrhizal sampling was carried out in twenty-two 0.25 ha plots across nine European countries (Table 1; Fig. 1). All plots were Level II plots of the International Cooperative Programme on Assessment and Monitoring of Air Pollution Effects on Forests, or ICP Forests (www.icp-forests.org). *Quercus robur* and/or *Q. petraea* plots were selected based on the environmental data available to cover a wide gradient of environmental conditions. We adapted the sampling methodology applied by Cox *et al.* (2010) in pine forests. In each plot, 24 numbered oaks were randomly selected, and a transect was traced from each chosen tree to the nearest neighbouring oak. Four soil cores were removed at evenly-spaced distances using a 2 cm diameter and 25 cm length soil corer. Transect distance and depth of each soil core were recorded for root den-

Table 1 Main characteristics of the intensively monitored oak plots included in this study

PLOT	ICP Forests code	Country	Oak species	Age* (years)	Altitude (m)	Total precipitation (mm/year)	Mean temperature (°C)	Soil type[†]	Throughfall N deposition[‡] (kg/ha/year)	Location (Lat, Long)
SP01	33Qpe	Spain	*Quercus petraea*	41–60	1150	904.8	8.9	Eutric cambisol	5.1 ± 1.8	42.52, −4.33
FR01	CHS41	France	*Quercus petraea*	108	127	943.4	11.3	Stagnic luvisol	5.6 ± 1.5	47.57, 1.26
FR02	CPS77	France	*Q. petraea + Q. robur*	129	80	678.6	13.1	Cambic podzol	7.2 ± 1.7	48.45, 2.72
AU01	02	Austria	*Quercus petraea*	87	290	777.4	9.0	Haplic cambisol	8.8 ± 2.3	48.06, 16.04
HU01	M16	Hungary	*Quercus petraea*	83	260	876.3	10.1	Haplic luvisol	9.8 ± 2.3	46.49, 16.24
EN01	512	England	*Quercus robur*	76	80	816.1	10.1	Eutric vertisol	9.8 ± 2.8	51.16, −0.86
FR03	CHS35	France	*Quercus petraea*	117	80	972.4	11.2	Stagnic luvisol	10.3 ± 2.1	48.18, −1.53
HU02	M03	Hungary	*Quercus petraea*	73	660	812	8.7	Mollic leptosol	11.8 ± 2.6	47.51, 19.58
FR04	CHP59	France	*Quercus robur*	86	149	990.8	9.7	Stagnic luvisol	12.2 ± 3.5	50.17, 3.75
EN02	516	England	*Quercus robur*	61	107	818.4	10.2	Eutric vertisol	12.7 ± 4.1	51.60, −1.92
GE01	706	Germany	*Quercus robur*	112	129	764.8	10.6	Mollic gleysol	13.1 ± 2.0	49.02, 8.13
GE02	913	Germany	*Quercus petraea*	133	475	711.4	7.9	Stagnic cambisol	13.4 ± 2.4	48.55, 11.45
EN03	517	England	*Quercus petraea*	91	120	1865	9.4	Cambic podzol	14.1 ± 3.9	54.33, −2.95
GE03	921	Germany	*Quercus petraea*	117	330	637.7	9.1	Stagnic cambisol	14.4 ± 5.6	49.43, 9.53
GE04	914	Germany	*Quercus petraea*	114	470	1008.1	7.0	Stagnic cambisol	14.9 ± 3.5	49.58, 9.27
RO01	13	Romania	*Quercus petraea*	62	573	672.3	11.1	Eutric cambisol	15.6 ± 3.5	45.01, 24.59
GE05	705	Germany	*Quercus petraea*	205	550	983.7	9.1	Haplic cambisol	16.3 ± 2.5	49.25, 7.73
RO02	05	Romania	*Quercus robur*	61	90	603.7	11.3	Mollic preluvisol	18.3 ± 3.9	44.30, 26.10
GE06	1001	Germany	*Quercus petraea*	136	320	954.4	10.6	Stagnic cambisol	21.3 ± 6.4	49.32, 7.02
BE01	16	Belgium	*Quercus robur*	76	26	876.3	10.8	Dystric podzoluvisol	22.7 ± 5.6	50.97, 3.80
GE07	502	Germany	*Q. petraea + Q. robur*	134	28	862.8	10.1	Stagnic cambisol	26.3 ± 4.5	51.81, 6.13
NE01	1040	Netherlands	*Quercus robur*	82	50	852.5	10.4	Fimic anthrosol	35.5 ± 5.8	52.11, 5.23

*Tree age in the year of sampling.
[†]Soil types are named according to World Reference Base for Soil Resources (WBR) classifications.
[‡]Standard deviations are given (±) after the mean for N deposition values.

sity calculations. A total of 96 soil cores were collected per plot and stored in sealed plastic bags at 4°C for up to 7 days until processed. Soil samples were rinsed on a 0.5 mm sieve, and roots were collected over a five minute period using a dissecting microscope. To minimize observer bias and maximize sample independence, the three longest roots were selected from each soil sample. One live mycorrhiza (assessed by its turgor and appearance after breaking it apart from the root) was sampled from one end of each root. A total of 288 mycorrhizas were analysed per plot (24 transects × 4 soil cores × 3 mycorrhizas per soil core). Brief descriptions of each mycorrhiza sampled, including the presence of rhizomorphs, were recorded helped by 10 years of experience in morphotyping oak mycorrhizas by the first author. Representative morphotypes were photographed. In addition, we recorded the presence of resistant *Cenococcum* propagules (sclerotia). Roots collected in each soil core were freeze-dried, and their dry weight recorded for root density calculations.

Environmental data

Geographical coordinates, forest age, altitude of the plots and long-term averaged environmental data for a minimum of 3 years for each plot on atmospheric N throughfall deposition, soil (including soil type, organic C, total N and C:N ratio in organic and mineral horizons) and foliar chemistry (N, P, Mg^{2+}, Ca^{2+}, K^+ and S), soil solution (nitrate and ammonium collected at up to 30 cm depth in soil) and meteorology (mean temperature and total precipitation) were obtained through the national contact point of ICP Forests in each country or through the Programme Coordinating Centre (see data accessibility). These *in situ* data reflect plot conditions better than modelled estimates (Dirnböck *et al.* 2014). Additionally, three randomly selected samples from both the organic and the mineral soil horizons were obtained in each plot, and their pH measured in water with a soil pH-meter (Hannah Instruments, Rhode Island).

Mycorrhizal assessment

Genomic DNA from individual mycorrhizas was obtained using Extract-N-Amp (Sigma), and the internal transcribed spacer (ITS) region of the nuclear rDNA was amplified using ITS1F (Gardes & Bruns 1993) and ITS4 (White *et al.* 1990) primers. Amplicons were purified using ExoSAP-IT (USB, Cleveland, OH, USA) and
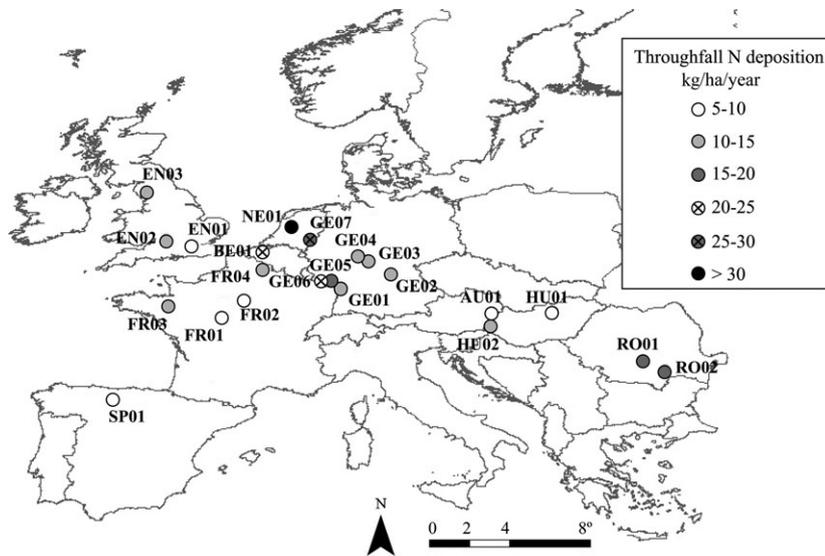
sequenced bidirectionally using BigDye3.1 with an ABI 3730 (Applied Biosystems, Foster City, CA, USA). Sequences were edited with Sequencher v. 4.2 (Gene Codes Corp., Ann Arbor, MI, USA), and a first identification through BLAST searches in GenBank (Altschul et al. 1990) was carried out assigning them to genus or family to facilitate subsequent alignment. Chimera Checker (Nilsson et al. 2010a) was used to detect potential chimeric sequences. The full ITS region including 5.8S was isolated when possible using the Fungal ITS Extractor (Nilsson et al. 2010b). All ITS sequences with less than 2% ambiguities were aligned by family with SATé v. 2.0.3 (Liu et al. 2009) and clustered in MO-THUR v. 1.16.0 (Schloss et al. 2009) at a 97% cut-off. A representative DNA sequence of each operational taxonomic unit (OTU) was compared against the GenBank and UNITE (Kõljalg et al. 2005) databases for their taxonomic placement. Phylogenetic trees generated by SATé were used to check taxon assignments. Sequences presenting over 2% ambiguities, originally discarded from the clustering analyses, but that had similarity to identified taxa in the above databases and for which we were able to match their morphotypes with already identified mycorrhizas, were included in further analyses. A representative sequence of each OTU was submitted to GenBank (accession nos KM576293–KM576684; Table S2, Supporting information). Once mycorrhizas were assigned to ECM fungal taxa, when possible they were classified into fruiting body types for further analyses. Mycorrhizal exploration types were assigned to each taxon (Table S3, Supporting information) following Agerer (2001, 2006), based on our descriptions, our image database of morphotypes and DEEMY (Information System for Characterization and Determination of Ectomycorrhizae; www.deemy.de).

They were further classified as low biomass (contact, short- and medium-distance smooth exploration types) and high biomass (medium-distance fringe, medium-distance mat and long-distance exploration) based on Hobbie & Agerer (2010) and by the presence/absence of rhizomorphs.

Statistical analyses

We used R v. 2.15.0 for statistical analyses (R Foundation for Statistical Computing, 2012). Observed and estimated richness (Abundance-based Coverage Estimator, ACE; Chao & Lee 1992) were calculated using EstimateS (v 8.2; Colwell 2009). Fungal OTU (proxy for species) accumulation curves were constructed in R by sampling soil cores randomly without replacement using 1000 permutations. Taxon evenness was calculated using Pielou's (1966). Relative abundance was calculated by dividing the number of mycorrhizas of each taxon by the total number of mycorrhizas in each plot. Relative abundance was also calculated in a similar way for each exploration type.

Relationships between throughfall N deposition, foliar N, soil solution nitrate and ammonium, total N in the organic and mineral soil horizons, root density and forest age were explored by Pearson's or Spearman rank correlations. Linear regressions were carried out to test for the influence of all N-related variables (N deposition, foliar N, soil solution nitrate and ammonium, C:N ratio and total N in organic and mineral soil horizons) on ECM community richness (ACE) and evenness (Pielou's index). Multiple regressions were carried out to explore the combined effect of N deposition and pH on community richness and evenness. We tested for normality of the variables using Shapiro & Wilk (1965).

Nitrogen deposition, soil solution nitrate and ammonium, N in the mineral horizon, root density and stand age were log-transformed, while the logit transformation was applied to community evenness. Relative abundances of fungal taxa were log-transformed or square-root transformed.

Bray–Curtis and Euclidean dissimilarity matrices were generated for community and environmental data, respectively. The Bray–Curtis matrix was based on the relative abundance of each fungus in each plot to account for differences in DNA sequencing success. Fungal taxa occurring only once in the data set were removed from the analyses to reduce the effect of rare species. To identify the main factors affecting community composition, community dissimilarity among plots was visualized by nonmetric multidimensional scaling (NMDS) and 20 environmental variables (latitude, longitude, forest age, mean annual temperature, total annual precipitation, altitude, throughfall N deposition, foliar Ca^{2+}, Mg^{2+}, K^+ and P, pH and total N in organic and mineral horizons, organic C, C:N ratio in the organic horizon and root density) were fitted to the ordination plots using the 'envfit' function with the vegan package. Tree species and soil type were categorical factors. The NMDS was also carried out for exploration types and taxa found in at least half of the 22 plots, defined as ECM dominant fungi. Due to missing values, soil solution data and foliar N were not included. To address the relative importance of geographical distance, sampling month and the subset of variables found to be significant in NMDS analyses of the fungal communities, we carried out a multivariate ANOVA using the Adonis routine of the vegan package of R (Oksanen *et al.* 2012). The effect of geographical distance was addressed by reducing the Euclidean distances among plot coordinates to spatial Principal Coordinates of Neighbourhood Matrix (PCNM) vectors (Borcard & Legendre 2002). Significant PCNM vectors were further selected ($\alpha = 0.05$). Soil type was reduced to seven categories in Adonis analysis: eutric, stagnic, cambic, haplic, mollic, dystric and fimic.

The function tree in the package Tree in R was used to estimate the N critical loads for mycorrhizal communities. We estimated two critical loads based on the partition of the variance of the mycorrhizal richness and evenness, the highest one above which a more drastic effect in the ECM communities was observed. Mantel and partial Mantel tests, based on the above dissimilarity matrices, were performed using the Ecodist package to independently assess the correlation of N-related variables, geography, soil characteristics or tree species on community composition, removing the correlation of the other matrices of variables. Linear regressions were

carried out to test the response of individual fungal taxa to different environmental conditions using the relative abundance of each taxon and excluding absence data. Similar analyses were carried out for exploration and fruiting body types. Species indicator analyses were performed by combining *a priori* groups of plots to detect species and/or combinations of species significantly associated with plots presenting similar environmental characteristics that could act as belowground indicators of environmental variables using the Indicspecies package (Dufrêne & Legendre 1997; De Cáceres *et al.* 2010, 2012). To determine the number of groups of plots for each environmental variable, a plot of the within-groups sum of squares by the number of groups extracted by partitioning was used. Only fungal taxa present in five or more plots were tested. We selected as candidate species those whose frequency was larger than 50% in each group of target plots. We considered as valid indicators from single and/or combinations up to five species, reducing the number of combinations by restricting both A (specificity) and B (fidelity) components of the indicator value (IndVal) to 0.8 and 0.6, respectively.

Results

Mycorrhizal diversity, community composition and distribution patterns

We sampled roots from 2112 soil cores and morphologically examined over 6300 mycorrhizas, from which we recovered 5513 DNA sequences belonging to ECM fungi. Approximately 79% of the ECM fungi were Basidiomycota and 21% Ascomycota. Across plots we recovered on average 70% of the estimated diversity, based on the ACE-richness estimator, from 57.6% in plot FR04 (France) to 83.7% in plot BE01 (Belgium). Overall, we detected 392 ECM fungal taxa of which 87% were identified at least to genus and 41% to species (Table S2, Supporting information). The average richness was 55, ranging from 24 fungal taxa in plot NE01 (the Netherlands) to 83 in plot RO01 (Romania) (Fig. S1, Supporting information).

Rank abundance curves indicated that communities had two or three dominants and many rare fungal taxa (data not shown) although the higher relative abundance of dominants in plots under higher N deposition led to a decrease in community evenness in these plots (Fig. 2). Nearly 45% of mycorrhizas belonged to the family Russulaceae with the genera *Lactarius* and *Russula* almost equally represented (Table S2, Supporting information), followed by Gloniaceae (represented exclusively by *Cenococcum geophilum* with *c.* 10% of roots), Thelephoraceae (7%, mainly *Tomentella* spp.) and

Cortinariaceae *s.l.* (4.8%, mainly *Cortinarius* spp.). Over-all, *Lactarius quietus, Cenococcum geophilum* and *Russula ochroleuca* were the most abundant. *Lactarius quietus* colonized *c.* 11.5% of all roots (from 5 to 33%) was dominant in seven plots and among the three most abundant in 14 plots. *Cenococcum geophilum*, representing 50% of the ascomycetes, was dominant in four plots and among the three most abundant in eight plots. This species complex can be represented by abundant large resistant propagules (sclerotia) in forest soils, but we did not find a relationship between the number of cores with sclerotia of *C. geophilum* and its abundance as mycorrhizas (data not shown). However, we found that in plots with lower pH, the number of cores with these structures was higher ($P \leq 0.05$, data not shown). Twenty-one fungal taxa formed mycorrhizas in at least half of the plots (dominant fungi). We avoided transects near other ECM trees, so we identified only seven rare fungal taxa generally not considered oak associates (indicated with asterisks in Table S2, Supporting information).

Environmental variables and ECM communities at large scales

Soil solution nitrate ($r = 0.55$, $P = 0.02$, $n = 17$) and ammonium ($r = 0.58$, $P = 0.013$, $n = 17$) were positively correlated with N deposition (Table S4, Supporting information). There was no significant correlation between N deposition and N concentration in the leaves or in the two soil horizons. Root density was negatively correlated to N deposition ($r = -0.50$, $P = 0.018$, $n = 22$), soil solution nitrate ($r = -0.67$, $P = 0.003$, $n = 17$) and foliar N ($r = -0.47$, $P = 0.049$, $n = 18$). The pH in the organic horizon was negatively related to its total N concentration ($r = -0.47$, $P = 0.03$, $n = 22$). None of the previous variables were related to the age of the forest.

Estimated richness in the plots (ACE) was negatively correlated with N deposition and positively correlated to pH in both horizons (Fig. 2). The partial correlation test between N deposition and ACE, excluding the effect of forest age, was significant ($P \leq 0.05$). Similarly, Pielou's evenness index was negatively correlated to N deposition and pH in both horizons (Fig. 2). The interaction among N deposition and soil pH did not significantly affect richness across plots, and when removed from the regression model, only pH had a significant effect. Similarly, in the case of community evenness, the interaction between both parameters was not significant and only N deposition had a significant effect.

Of the 20 environmental variables fitted in NMDS, the latitude, longitude, N deposition, root density, C:N ratio, total N in the organic soil horizon, total precipitation, oak species, soil type and pH in both horizons were significantly related to community composition among plots (Fig. 3). When soil type was reduced to seven categories, it became nonsignificant ($r = 0.40$, $P = 0.13$). Partial Mantel tests indicated that only N-related variables (N deposition, foliar N, total N of organic and mineral horizons, soil solution nitrate and ammonium), and geography (latitude, longitude), were significantly related to community composition when removing the effect of other environmental variables (Table S5, Supporting information). When using the significant spatial PCNM vectors to account for geographical distance, results were similar (data not shown). In the case of the 21 dominant fungal taxa in these forests, NMDS ordination showed that geography (latitude and longitude), pH, total N in the organic horizon, soil type and mean temperature were significantly related to dissimilarities in their abundance among plots (Fig. S3, Supporting information). The ECM fungal taxa forming resupinate (crust) fruiting bodies were less abundant in the roots of high N sites (data not shown).

The pH of the organic soil horizon, N deposition and root density alone explained *c.* 27% of the variation in the community data (Table 2). The effect of the dominant oak species, soil type, sampling month and the PCNM geographical vectors was not significant. The estimated N critical load for mycorrhizal diversity and evenness ranges between 9.5 and 13.5 kg N/ha/year. There is a second N deposition threshold at approximately 17 kg N/ha/year with a steeper community change (Fig. 2).

Indicator species analyses and fungal responses to environmental variables

Forty-nine ECM fungal taxa were selected as significant single species indicators and/or in combination with others (Table 3). Individual fungal taxa whose relative abundance varied significantly under particular environmental conditions are listed in Table S6 (Supporting information). Species such as *Russula parazurea* and *Scleroderma citrinum* were significantly associated with plots with N deposition over 18 kg/ha/year and soil pH below 4.0, while *Lactarius chrysorrheus* and *Boletus reticulatus* were associated with plots with N deposition below 10 kg/ha/year and C:N ratios over 40. *Lactarius quietus* consistently dominated plots above *c.* 13 kg/ha/year and associated with low soil pH and high total N in the organic horizon. Other common species, such as *Elaphomyces muricatus* and *Cenoccocum geophilum*, only appeared as indicators combined with others. Different *Cortinarius* species, individually or combined with other species, were
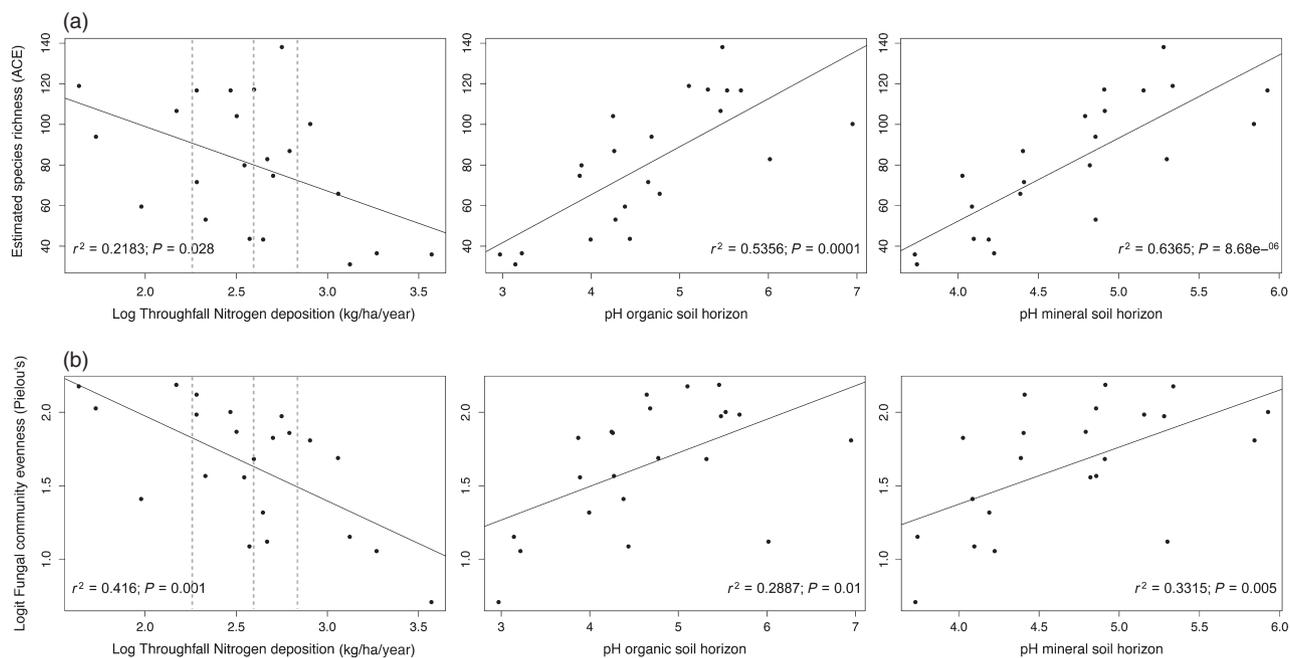
Fig. 2 Regressions between (a) estimated species richness (ACE) and (b) community evenness (Pielou's index) recorded in the plots and their N deposition and soil pH values. From left to right, the vertical dashed lines represent 9.5, 13.5 and 17 kg N/ha/year, respectively.
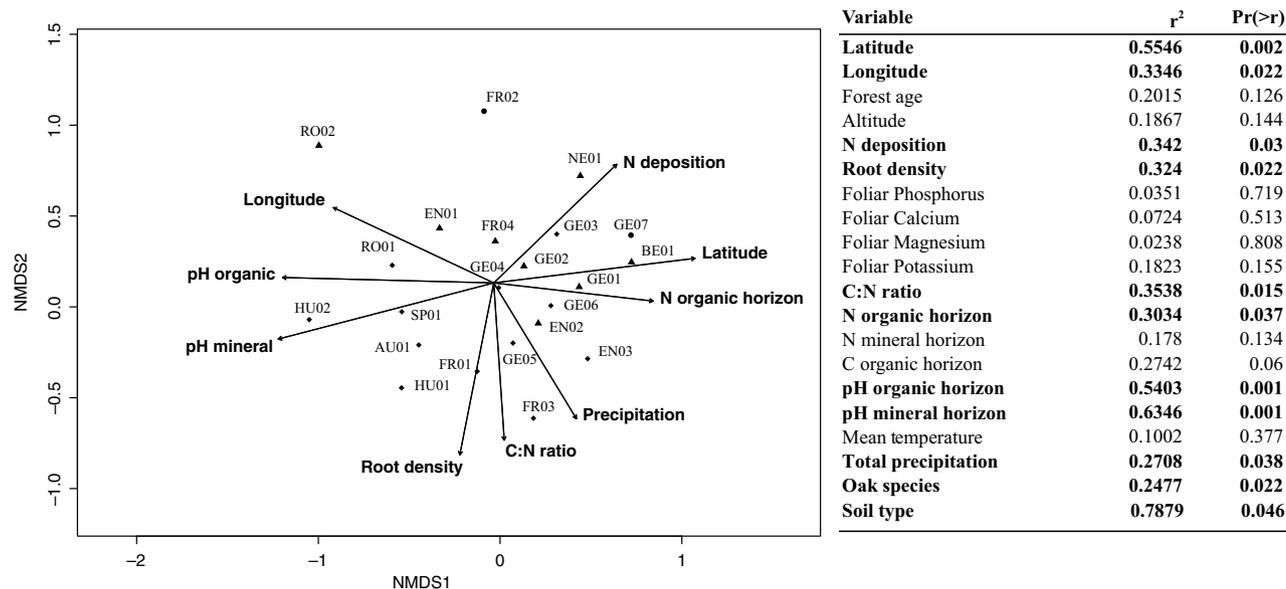

Variable	r^2	Pr(>r)
Latitude	**0.5546**	**0.002**
Longitude	**0.3346**	**0.022**
Forest age	0.2015	0.126
Altitude	0.1867	0.144
N deposition	**0.342**	**0.03**
Root density	**0.324**	**0.022**
Foliar Phosphorus	0.0351	0.719
Foliar Calcium	0.0724	0.513
Foliar Magnesium	0.0238	0.808
Foliar Potassium	0.1823	0.155
C:N ratio	**0.3538**	**0.015**
N organic horizon	**0.3034**	**0.037**
N mineral horizon	0.178	0.134
C organic horizon	0.2742	0.06
pH organic horizon	**0.5403**	**0.001**
pH mineral horizon	**0.6346**	**0.001**
Mean temperature	0.1002	0.377
Total precipitation	**0.2708**	**0.038**
Oak species	**0.2477**	**0.022**
Soil type	**0.7879**	**0.046**

Fig. 3 Fungal community compositions of the 22 plots displayed using NMDS. Stress of the ordination is 0.19. Significant variables ($P < 0.05$) are shown by arrows whose length is proportional to the strength of the correlation, and they are shown in bold in the table. Oak species are identified by symbols as follows: diamonds for *Quercus petraea*; triangles for *Q. robur;* and circles for both oak species.

associated with and indicate low and medium levels of N-related variables and medium and high soil pH, and the genus correlated negatively with soil solution nitrate and positively with C:N ratio (Table S6, Supporting information).

Exploration types: linking function to environmental conditions

Of the 20 environmental variables fitted in the NMDS ordination, the latitude, altitude, N deposition, oak spe-

cies, soil type and soil pH were significantly related to exploration type composition (Fig. S2, Supporting information). Partial Mantel tests showed that N-related variables and geography are related to exploration type composition when removing the effect of other variables (data not shown). Responses to the environmental variables tested when grouping fungi by exploration type and low vs. high biomass are in Table 4. Contact mycorrhizas responded positively to N deposition and total N in the organic soil horizon, and negatively to altitude and pH (Table 4). Medium-distance fringe exploration types show a negative response to increasing soil solution nitrate concentration and a positive response to altitude (Table 4); belonging to this soil exploration type, *Tricholoma*, *Cortinarius* and *Piloderma* were not present in the three plots with highest N deposition (over 21 kg N/ha/year). Four *Tricholoma* were only present in six plots below 16 kg N/ha/year. The six *Piloderma* detected responded negatively to N deposition and positively to pH in both horizons. But, only *Piloderma* sp.3 combined with *Humaria* sp.1 or with *Lactarius quietus* emerged as an indicator of pH or C:N ratio, respectively (Table 3). We only found medium-distance mat exploration type mycorrhizas (*Hysterangium* and *Ramaria*) in three plots below 12 kg N/ha/year. Variation in sensitivity to N deposition within low biomass and long-distance exploration types was also reflected in our data set: both *Russula parazurea* (contact or medium-distance smooth) and *Lactarius quietus* (contact) increased with N deposition, while *Russula amoenipes* (contact or medium-distance smooth) and *Lactarius chrysorrheus* (medium-distance smooth) were only in plots with low N availability. Similarly, long-distance exploration types such as *Boletus* were mainly at low or medium N, while *Scleroderma* increased in abundance with increasing N.

Mycorrhizas with high biomass showed a positive relationship with organic C concentration and C:N ratios in the organic soil horizon (Table 4). Overall, rhizomorph-forming fungi (medium and long distance) were differently related than fungi without them (contact and short distance) to all variables tested except root density.

Discussion

We carried out a continental-scale belowground study covering a wide range of environmental conditions recorded *in situ* in each plot and across years. We captured on average 70% of the estimated diversity in our plots and identified most of the fungal taxa detected at least to genus level. Because the data are DNA-sequence-based, we have generated a baseline that allows for direct and accurate comparisons with studies of mycorrhizas, sporocarps, bulk roots and soil. We also examined the exploration type of every mycorrhiza sampled, allowing us to link biodiversity with functional traits.

ECM communities: patterns of diversity and distribution

Russulaceae, *Cortinariaceae* and *Thelephoraceae* were dominant in oaks, as previously reported (Avis *et al.* 2003, 2008; Courty *et al.* 2008). The most abundant fungus was the oak milkcap, *Lactarius quietus*, an oak specialist, followed by the *Cenococcum geophilum* species complex, the most widely distributed ECM fungi with over 200 angiosperm and gymnosperm hosts (Trappe 1964; LoBuglio 1999). By sampling roots, we detected dominants that would have been overlooked if assessing fruiting aboveground; from the 21 fungal taxa present in at least half of the plots, *C. geophilum* does not produce sexual structures – and its resistant propagules are not related to its ectomycorrhizal abundance – *Elaphomyces muricatus*, *Hydnotrya tulasnei* and *Genea hispidula* form truffles, and *Tomentella sublilacina* forms inconspicuous crusts. Hardly any of these dominant fungal taxa have been the focus of physiology or -omics studies. Interestingly, *E. muricatus* and *H. tulasnei* are being considered for red listing by IUCN based on fruiting body records.

Environmental variables and large-scale mycorrhizal distributions

With our sampling we covered the historical gradient of N deposition across Europe, with higher values in central industrialized areas reaching maximum levels in the Netherlands, Belgium and parts of Germany, and lower in Alpine regions and northern Europe with Scandinavia showing the lowest values (Lorenz *et al.* 2008). We did not sample plots at the edge of oak's distribution in southern Sweden, but low N deposition there resembles other plots included in this study. The

Table 2 Relative importance of pH in the organic soil horizon, throughfall N deposition and oak root density on the ectomycorrhizal communities across the 22 oak plots, as revealed from the Adonis function

	d.f.	F value	R^2	P value
Soil pH	1	3.19	0.126	0.001
N deposition	1	1.95	0.077	0.006
Root density	1	1.74	0.069	0.030
Residuals	16	0.63		
Total	21			

Table 3 Results of indicator species analyses in the 22 oak forests sampled

Single species	Cov.	A	B	IndVal	Species combinations	A	B	sqrtIV	Cov.
Throughfall N deposition (kg/ha/year)									
5.10–10.3	100				5.10–10.3				100
Lactarius chrysorrheus		0.88	0.86	0.87**	*Lactarius chrysorrheus*	0.83	0.86	0.85**	
Boletus reticulatus		0.94	0.57	0.73*	*Elaphomyces muricatus + Russula amoenipes*	1.00	0.71	0.85**	
Russula amoenipes		0.75	0.71	0.73*					
Pseudocraterellus undulatus		0.93	0.57	0.73*					
Boletus subtomentosus		0.92	0.57	0.72*					
11.8–16.3	100				11.8–16.3				100
Boletus pruinatus		0.79	0.80	0.79*	*Boletus pruinatus*	0.85	0.80	0.83**	
Genea hispidula		0.78	0.70	0.74*	*Amanita rubescens + Russula rosea*	0.82	0.60	0.70*	
Clavulina coralloides		0.84	0.60	0.71*					
Cortinarius sp.12		0.81	0.60	0.70*					
18.3–35.5	60				18.3–35.5				80
Scleroderma citrinum		0.96	0.60	0.76*	*Amanita rubescens + Russula cyanoxantha + Tomentella sublilacina*	0.88	0.80	0.84**	
Russula parazurea		0.96	0.60	0.76**					
pH organic soil horizon									
6.90–5.10	87.5				6.90–5.10				87.5
Humaria sp.1		1.00	0.75	0.87**	*Russula rosea + Russula vesca*	0.84	0.75	0.79*	
Russula rosea		0.83	0.75	0.79*	*Elaphomyces muricatus + Russula atropurpurea + Russula vesca*	0.84	0.63	0.73	
Thelephoraceae sp.8		0.88	0.63	0.74*					
4.80–3.90	100				4.80–3.90				100
Laccaria amethystina		0.76	1.00	0.87***	*Laccaria amethystina*	0.83	1.00	0.91***	
Russula densifolia		1.00	0.73	0.85*					
Lactarius camphoratus		0.96	0.73	0.84*					
Tomentella botryoides		0.93	0.64	0.77*					
Cortinarius casimiri		0.71	0.82	0.76*					
Russula brunneoviolacea		1.00	0.55	0.74*					
3.20–3.00	100				3.20–3.00				100
Scleroderma citrinum		0.98	1.00	0.99***	*Hydnotrya tulasnei + Russula cyanoxantha + Tomentella sublilacina*	0.90	1.00	0.95**	
Russula parazurea		0.98	1.00	0.99***					
*Laccaria laccata*1		0.67	1.00	0.82*					
Hydnotrya tulasnei		0.64	1.00	0.80*					
Lactarius quietus		0.59	1.00	0.77*					
Amanita rubescens		0.57	1.00	0.76*					
pH mineral soil horizon									
5.90–5.10	100				5.90–5.10				83.3
Cortinarius sp.2		0.79	0.67	0.73*	*Humaria* sp.1 + *Piloderma* sp.3	0.90	0.67	0.77**	
Russula rosea		0.63	0.83	0.72*	*Cortinarius* sp.2 + *Russula vesca*	0.82	0.67	0.74*	
Cortinarius sp.18		0.74	0.67	0.70*					

Table 3 *Continued*

Single species	Cov.	A	B	IndVal	Species combinations	A	B	sqrtIV	Cov.
4.80–4.90					4.80–4.90				
Russula nigricans	100	0.56	1.00	0.75*	*Lactarius chrysorrheus* + *Lactarius quietus*	0.87	0.67	0.76*	100
Cortinarius anomalus		0.79	0.67	0.73*	*Boletus pruinatus* + *Cortinarius casiniri* + *Lactarius subumbonatus*	0.80	0.67	0.73*	
4.40–3.70					4.40–3.70				
Amanita rubescens	100	0.69	0.90	0.79**	*Amanita rubescens* + *Cenococcum geophilum2*	0.81	0.90	0.85**	90
Scleroderma citrinum		0.99	0.60	0.77*					
Tomentella sublilacina		0.73	0.80	0.77*					
Lactarius quietus		0.57	1.00	0.75*					
Russula parazurea		1.00	0.50	0.71*					
Total N organic soil horizon (g/kg)									
8.70–10.0					8.70–10.0				
Cortinarius sp.18	100	0.66	1.00	0.81*	*Cortinarius diasemospermus* + *Cortinarius* sp.8 + *Elaphomyces muricatus*	1.00	1.00	1.00**	100
12.6–13.9					12.6–13.9				
None	—	—	—	—	None	—	—	—	—
14.7–15.6					14.7—15.6				
None	—	—	—	—	None	—	—	—	
16.3–20.3					16.3–20.3				
Lactarius quietus	100	0.61	1.00	0.78***	*Amanita rubescens* + *Lactarius tabidus*	0.84	0.63	0.72*	87.5
					A. rubescens + *E. muricatus* + *Laccaria laccata1* + *Lactarius quietus*	0.82	0.63	0.72*	
Total N mineral soil horizon (g/kg)									
1.00–2.30					1.00–2.30				
Humaria sp3	100	0.81	0.67	0.73*	*Boletus pruinatus* + *Pezizales* sp.2	0.89	0.67	0.77*	100
Meliniomyces bicolor		0.80	0.67	0.73*	*Amanita rubescens* + *Meliniomyces bicolor*	0.85	0.67	0.75*	
2.60–00					2.60–4.00				
None	—	—	—	—	None	—	—	—	—
4.8–5.7					4.8–5.7				
Tomentella terrestris2	100	0.83	0.83	0.83**	*Cortinarius diasemospermus* + *Cortinarius* sp.12	0.82	0.67	0.74*	100
Russula amoenipes		0.81	0.67	0.73*	*Russula amoenipes*	0.81	0.67	0.73*	
Cortinarius sp.12		0.65	0.67	0.66*					
7.20–11.1					7.20–11.1				
Russula ochroleuca	100	0.58	1.00	0.76*	*C. geophilum1* + *R. cyanoxantha* + *R. nigricans* + *Tomentella sublilacina*	0.84	0.67	0.75*	100
					Ascomycota sp.1 + *C. diasemospermus* + *Lactarius chrysorrheus*	0.81	0.67	0.73*	
Soil NO$_3$ (mg/l)									
0.01–0.95					0.01–0.95				
Laccaria amethystina	100	0.53	1.00	0.73*	*Cenococcum geophilum2* + *Russula ochroleuca*	0.83	0.86	0.85*	100
					C. geophilum1 + *C. geophilum2* + *Laccaria amethystina* + *R. atropurpurea*	0.84	0.71	0.77*	
1.80–3.10					1.80–3.10				
Boletus pruinatus	100	0.70	1.00	0.84**	*Boletus pruinatus* + *Russula cyanoxantha*	0.82	1.00	0.91**	100
6.00–7.10					6.00–7.10				
None	—	—	—	—	None	—	—	—	—

Table 3

Table 3 Continued

Single species	Cov.	A	B	IndVal	Species combinations	A	B	sqrtIV	Cov.
8.80–14.6					8.80–14.6				
None	—	—	—	—	Laccaria sp.1	0.83	1.00	0.91*	100
Foliar N (mg/g)									
22.8–24.5					22.8–24.5				
None	—	—	—	—	Cenococcum geophilum2 + Lactarius camphoratus + Russula fragilis	0.88	0.60	0.73*	100
					Cenococcum geophilum1 + Hydnotrya tulasnei + Tomentella subtilacina	0.81	0.60	0.70*	100
24.7–25.4					24.7–25.4				
Thelephoraceae sp.7	100	1	0.9	0.93**	Thelephoraceae sp.7	1.00	0.86	0.93**	100
Tomentella botryoides		0.70	0.9	0.77*	Cortinarius diasomospermus + Tomentella botryoides	0.83	0.86	0.85*	100
Laccaria amethystina		0.48	1.0	0.69*					
26.2–26.5					26.2–26.5				
None					Boletus pruinatus + Russula nobilis	0.86	1.00	0.92*	100
27.0–28.7					27.0–28.7				
Lactarius subumbonatus	100	0.6	1.0	0.76*	Laccaria laccata1 + Russula vesca	0.90	0.83	0.87*	100
Boletus pruinatus		0.6	1.0	0.75*	Hydnotrya tulasnei + Laccaria laccata1	0.80	0.83	0.82*	
Laccaria laccata1		1.0	0.74	0.05*					
C:N organic soil horizon									
38.1–33.9					38.1–33.9				
Cortinarius casimiri	100	0.68	1.00	0.82**	Cortinarius casimiri + Thelephoraceae sp.7	0.804	1	0.90**	100
Lactarius chrysorrheus		0.67	1.00	0.82*					
Tomentella botryoides		0.62	1.00	0.79*					
Lactarius subumbonatus		0.57	1.00	0.76*					
Boletus reticulatus		0.83	0.67	0.74*					
28.9–24.9					28.9–24.9				
Pezizales sp.1	83.3	0.76	0.80	0.80*	Hydnotrya tulasnei + Pezizales sp.1	0.931	0.667	0.79*	66.7
22.9–20.5					22.9–20.5				
None	—	—	—	—	Lactarius quietus + Piloderma sp.3	0.84	0.60	0.71*	90
					C. geophilum1 + C. geophilum2 + H. tulasnei + Laccaria laccata1	0.80	0.60	0.69*	
19.3–16.9					19.3–16.9				
None	—	—	—	—	Cenococcum geophilum2 + Clavulinaceae sp.3 + Pezizales sp.2	1	0.667	0.8165*	100
					Elaphomyces muricatus + Laccaria sp.1 + Tomentella castanea	1	0.667	0.8165*	

Significance codes: (†0.1 > P > 0.05; *0.05 > P > 0.01; **0.01 > P > 0.001; ***0.001 > P). Only fungi present in five or more plots are included in these analyses. Abbreviations are as follows: cov.: pooled coverage (%); A: specificity; B: fidelity; IndVal: indicator value, sqrtIV: square root of the indicator value.

Table 4 Response of mycorrhizas with different exploration types to the environmental variables tested.

Soil exploration type	Throughfall N deposition (kg/ha/year)	N organic horizon (g/kg)	N mineral horizon (g/kg)	Soil solution nitrate (mg/l)	pH organic horizon	pH mineral horizon	C organic horizon (mg/g)	C:N ratio	Altitude (m)	Root density (mg/cm^3)
Contact	+(*)	+(*)	+	+	−	−(*)	+	−(†)	−(*)	
Short distance	−	−	−	−	+	+	−(*)	−	+	+
Medium-distance smooth	−	−(†)	−	−	+(*)	+(**)	−	+(†)	+	+
Medium-distance fringe	−	+	+	−(*)	+	+	+	+	+(*)	+(†)
Long distance	+	+	−(*)	+	−(*)	−(†)	+	+	−	−(***)
Low biomass	+	+	−	+	−(*)	−	−	−	−	−
High biomass	−	+	−(*)	−	−	−	+(*)	+(*)	+	−
Without rhizomorphs	+(†)	+	+	+(†)	−	+(†)	−	−(**)	−(*)	−
With rhizomorphs	−	−	−(†)	−	+	−(*)	+	+(*)	+(†)	−

Low (contact, short- and medium-distance smooth exploration types) and high (medium-distance fringe, medium-fringe mat and long-distance exploration types) biomass classification is based on Hobbie & Agerer (2010). Only variables affecting significantly the relative abundance of at least one exploration type are shown.
Significance codes: (†0.1 > P > 0.05; *0.05 > P > 0.01; **0.01 > P > 0.001; ***0.001 > P).

impact of N deposition passing through oak forest canopies was reflected in root density, soil N availability, foliage N and possibly soil pH. Root density was negatively correlated to N deposition, soil solution nitrate and foliar N, suggesting (i) direct N deposition effects on forest belowground production and (ii) that at greater N availability, lower fine root density supplies canopy N (Helmisaari et al. 2009). Root responses to N have been reported as reductions in number of roots colonized by ECM fungi (Newton & Pigott 1991) but not consistently (Taylor et al. 2000; Avis et al. 2008).

One of the main effects of N deposition is soil acidification; increased N can decrease soil pH and alter nutrient availability (Avis et al. 2008). Even though soil pH variation is mainly driven by soil characteristics (e.g. bedrock composition), in our study the pH of both horizons was negatively related to N deposition, and organic horizon pH was negatively related to N concentration (Table S4, Supporting information). While richness seems to be mainly affected by soil pH, N deposition is the main factor affecting evenness. In fact, elevated available soil N and acidity have been associated with severely declining eucalypt forests (Horton et al. 2013) and with a decrease in ECM mycelial production in Norway spruce forests (Bahr et al. 2013).

Nitrogen deposition sufficient to elevate soil inorganic N, especially nitrate availability, affects mycorrhizal fungi (Pardo et al. 2011). In the forests sampled, N deposition, through changes in N availability for plants and fungi, decreased the richness and evenness of ECM communities and shifted them towards nitrophilic and acidophilous fungi (e.g. *Scleroderma citrinum*, *Russula parazurea* and *Amanita rubescens*) resulting in a decrease or even loss of N-sensitive fungi (e.g. *Cortinarius*, *Piloderma* and *Tricholoma* species). These continental-scale results agree with some studies at smaller scales on both mycorrhizas and fruiting bodies (Avis et al. 2003; Lilleskov 2005; Cox et al. 2010).

The N critical load for mycorrhizal diversity and evenness in the oak forests sampled is 9.5–13 kg N/ha/year, assuming our least polluted plots (c. 5 kg N/ha/year) are undisturbed. The distribution of fungi considered N-intolerant (*Tricholoma*, *Piloderma* spp.) supports this assumption. Our estimate fits that for acidophilous, *Quercus*-dominated woodlands in Europe (10–15 kg/ha/year, Bobbink & Hettelingh 2011), lies slightly below the 10–20 kg/ha/year estimated based on mycelial growth in oak forests of southern Sweden (Nilsson et al. 2007), and it is higher than in North America (Pardo et al. 2011) and Scots pine stands in Scotland (Jarvis et al. 2013). In a recent study of Norway spruce in Sweden, Bahr et al. (2013) reported that ECM mycelial biomass is reduced even at moderate levels of N deposition (<10 kg/ha/year). Based on our data, it is above 17 kg N/ha/year that communities change drastically, but oak mycorrhizas are affected at much lower values as well.

Aboveground fruiting body data suggest host specialist fungi are more susceptible to decline at high N inputs than generalists (Arnolds 1991). Accordingly,

mycorrhizas of the oak-specialist *Lactarius chrysorrheus* were only dominant in plots up to 10 kg N/ha/year. However, the specialist *Lactarius quietus*, which increased at higher N loads, and the usually oak-associated *Russula vesca* and *R. atropurpurea* were among the three dominants in plots up to 20 kg N/ha/year. This indicates that a decline in their mycorrhizas may only occur above those higher N inputs. Nevertheless, abundant ectomycorrhizas do not necessarily lead to abundant fruiting (Gardes & Bruns 1996). Combined data from mycorrhizas, fruiting bodies, mycelia and spore banks as well as experimental physiological studies are needed to determine precisely how these communities are threatened.

Ectomycorrhizal community composition was correlated to eleven environmental variables (Fig. 3), highlighting the complexity of disentangling the factors that structure diverse communities over complex gradients where physical barriers and dispersal limitations likely also play a role (Peay *et al.* 2010; Tedersoo *et al.* 2012a). The strong effect of N-related variables might swamp the effect of the geographical location of the plots; when accounting for spatial autocorrelation, N deposition, soil pH and root density explained over a quarter of the variation in the community data. Adding mycorrhizal data from more plots would allow testing the effects of more environmental variables to gain insight into the still unexplained variability. Root density is a major factor related to community composition; plots with higher N pollution had lower root density and less even communities, leading to an increase in ECM competition for roots. This, together with the sensitivity of some ECM fungi to high N availability and its side effects (i.e. acidification and litter accumulation), could lead to less even ECM fungal communities in polluted plots. *Quercus robur* usually occurs at lower altitudes and higher water availability than *Q. petraea*, but they overlap extensively in their distributions. Taxonomically close host species tend to harbour similar ECM communities (Ishida *et al.* 2007); nonetheless, *Q. douglasii* (deciduous) and *Q. wislizeni* (evergreen) communities overlap only by *c.* 30% (Morris *et al.* 2008). In our study, both deciduous oaks share *c.* 64% of the nonsingleton species and we did not find any fungal taxon, present in at least five plots, that was associated with only one oak species. Mean precipitation was found to have a significant effect on the ECM communities in the NMDS ordination, similarly to Jarvis *et al.* (2013) who found rainfall to have a strong influence on pine ECM in Scotland. But in our study, when removing the effect of other variables, precipitation became nonsignificant. This is probably due to the narrower range of precipitation in our plots (Table 1) compared to the range in their study.

Mycorrhizas as belowground indicators of forest condition

Based on a meta-analysis, Cudlin *et al.* (2007) proposed mycorrhizas as potential indicators of environmental conditions to infer forest status. Our large set of both environmental and fungal data allowed us to detect a suite of belowground indicators of different environmental conditions in temperate oak forests. These include oak specialists such as *Lactarius quietus*, *L. subumbonatus* and *L. chrysorrheus* and various generalists. *Lactarius chrysorrheus* is an excellent indicator of low N deposition levels (Table 3) and did not appear in plots above 13 kg N/ha/year. Among the generalists, *Scleroderma citrinum* and *Russula parazurea* associate with high N pollution and low soil pH – the former is known to thrive with N fertilization (Newton & Pigott 1991). Single indicator species associated with groups of plots with similar environmental conditions give us insight into the species' niche preferences or tolerance. If used as a prediction tool, the higher the number of single indicator species found in a new plot, the higher the confidence on the forest condition assignment. Following De Cáceres *et al.* (2012), we also identified sets of indicators at more restricted conditions; in this case, all species involved must occur to be used as indicators, which does not necessarily mean that those species are associated. To our knowledge, this is the first time this analysis is carried out for fungi. Similarly extensive and intensive environmental and fungal data would be needed for applying these analyses to ECM communities outside Europe.

Diversity and function: linking exploration types and environment

The functional traits that define each exploration type can confer different capabilities with regard to storing C, taking up and translocating nutrients (Courty *et al.* 2010; Hobbie & Agerer 2010). Thus, a decrease in exploration type diversity can make communities less resilient to environmental change. We found that N pollution and geography are the main factors structuring soil exploration type abundance. In contrast, temperature can affect exploration type distribution at smaller scales (Jarvis *et al.* 2013). Usually, mycorrhizas with contact, short- and medium-distance smooth exploration types seem to use labile N, mainly inorganic, and they are thought to be C-cost effective in inorganic N-rich environments. For instance, some *Russula* and *Lactarius* tend to increase with increasing N loads (Lilleskov *et al.* 2002; Cox *et al.* 2010); it has been proposed that some members of the Russulaceae are able to provide the host with nitrate by diffusion, being

less C costly for the host and avoiding the toxicity of high nitrate concentrations (Nygren *et al.* 2008). Moreover, some Russulaceae belonging to the short exploration type present cystidia, which might confer them a unique role among this functional group (Avis 2012). In oak forests, the positive response of contact mycorrhizas to N-related variables, and negative to pH, is probably driven by the contact dominant *Lactarius quietus*, whose mycorrhizas characteristically appear tightly sandwiched between decomposing leaves. At a Danish site, Kjøller *et al.* (2012) found that contact exploration types, including *L. quietus*, responded similarly to N. Medium-distance fringe and mat, and long-distance exploration types are believed to use other organic N sources; they have long-distance hydrophobic rhizomorphs and hydrolytic exoenzymes including proteases. Based on their similarity with medium-distance fringe exploration types, they have been hypothesized to use organic N (Lilleskov *et al.* 2011). These morphotypes rich in extra-radical mycelium, generally display the strongest potential activities of degradation enzymes, except for laccase (Tedersoo *et al.* 2012b). At high N availability, the exploratory investment of these so called protein fungi may be too costly, leading to their decline (Hobbie & Agerer 2010). Our results agree with these hypotheses: medium-distance fringe type species show consistently negative responses to high N, and medium-distance mat mycorrhizas appeared only below 12 kg N/ha/year.

Apart from N, root density and soil organic C concentration may also be related to differences in the relative abundances of exploration types. For instance, long-distance exploration types were negatively related to oak root density, while medium-distance fringe types were positively related (Table 4). In a study along a pine root density gradient, long-distance exploration types were also negatively related to root density, highlighting the importance of rhizomorphs in colonizing new roots (Peay *et al.* 2011). Medium-distance fringe mycorrhizas have rhizomorphs too, but they were not abundant in low root density oak plots, probably because these protein fungi do not thrive at high inorganic N levels. Oak mycorrhizas with high biomass showed a positive relationship with organic C and C:N ratios in soil. This may show (i) a high C allocation rate from the roots to those fungi, since at high C availability, investment in thick mantles and rhizomorphs is worthwhile (Ekblad *et al.* 2013) and (ii) the contribution of these fungi to C sequestration in soil in the form of mycelium and rhizomorphs (Clemmensen *et al.* 2013). In addition, we found that at higher C:N ratios, mycorrhizas with rhizomorphs are more abundant. Most potential enzymatic activities of ECM depend on exploration type, particularly the presence or absence of rhizomorphs, rather than on fun-

gal lineage or host species (Tedersoo *et al.* 2012b) and fungi forming rhizomorphs can produce *c.* 15 times more biomass than short-distance exploration types (Weigt *et al.* 2012). The plasticity of these functional traits needs further investigation. Soil C:N ratio has been hypothesized to affect preferred N source by soil microorganisms (Geisseler *et al.* 2010), and it is reduced by N deposition in forest soil (Aber *et al.* 2003). At high C:N ratios, ECM fungi may take whatever source of N is available, initially depleting inorganic sources (Avolio *et al.* 2012). Moreover, rhizomorphs allow fungi to gather nutrients when they are patchily distributed in the soil (Koide *et al.* 2014); therefore, fungi with rhizomorphs using organic N will be more competitive in low N environments. However, rhizomorph-forming fungi may directly increase those C:N ratios by rapidly mobilizing N and transferring it to the host tree in low N soils (Lindahl *et al.* 2007). Thus, these high-biomass and rhizomorph-forming fungi might be driving soil characteristics, such as C storage and C:N ratios, instead of the other way around.

Conclusions

Our intensive sampling generated robust data on diversity and distribution of ectomycorrhizas for European oak forests showing the structure and function of a major functional guild in these ecosystems belowground. This reveals dominant organisms and the ranges of biotic and abiotic factors in which they thrive or decline that can be used to complement data obtained from fruiting bodies for preparing global red lists for fungi. Across Europe, N deposition and soil pH drive ECM community richness, evenness and functional type composition. There is still much to learn on the relationship between the extent of colonization of roots and soil by a fungus, its production of fruiting bodies and formation of spore banks, and their links to population size changes and geographic distribution. Here we provide a reference list of fungal taxa to predict the condition of temperate oak forests and to provide evidence for the impacts of environmental change. Because mycorrhizal communities hold predictive value for forest status, and viceversa, long-term assessment of mycorrhizas merits incorporation into long-term monitoring.

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Data accessibility

Details on data collection, harmonization and quality assessment of the environmental variables are available in http://icp-forests.net/page/icp-forests-manual.

The DNA sequences of each OTU are available at GenBank with the accession nos KM576293 to KM576684.

Data mentioned in the text and not directly available are uploaded as supplementary material. The environmental variables not included in Table 1, the R scripts for an example of the NMDS and Species Indicator Analyses and the alignments used for OTU assignments are uploaded as supplementary material.

Supporting information

Additional supporting information may be found in the online version of this article.

Fig. S1 Species accumulation curves for each plot.

Fig. S2 Exploration type composition of the 22 plots displayed using NMDS.

Fig. S3 Ectomycorrhizal fungal taxa present in at least 11 plots (dominant ECM taxa) displayed using NMDS.

Table S1 Studies reporting effects of increasing nitrogen on below-ground ectomycorrhizal communities (from Cox *et al.* 2010, extended).

Table S2 Relative abundances (%) of the ectomycorrhizal fungi detected.

Table S3 Exploration types assigned to each mycorrhizal fungus.

Table S4 Correlation matrix (Pearson) between variables.

Table S5 Results of the partial Mantel and Mantel tests.

Table S6 Individual fungi and genera responding significantly to any of the environmental variables tested.

Appendix S1 Environmental variables for the 22 plots.

Appendix S2 R scripts.

Appendix S3 Sequence alignments.