New Phytologist

Research

Plant nutrient-acquisition strategies drive topsoil microbiome structure and function

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Received: *5 February 2020*
Accepted: *31 March 2020*

New Phytologist (2020)
doi: 10.1111/nph.16598

Key words: biotic interactions, metagenomics, mycorrhizal type, plant–soil feedback, symbiosis.

Summary

- Plant nutrient-acquisition strategies drive soil processes and vegetation performance, but their effect on the soil microbiome remains poorly understood. This knowledge is important to predict the shifts in microbial diversity and functions due to increasing changes in vegetation traits under global change.
- Here we documented the topsoil microbiomes of 145 boreal and temperate terrestrial sites in the Baltic region that broadly differed in vegetation type and nutritional traits, such as mycorrhizal types and symbiotic nitrogen-fixation.
- We found that sites dominated by arbuscular mycorrhizal (AM) vegetation harbor relatively more AM fungi, bacteria, fungal saprotrophs, and pathogens in the topsoil compared with sites dominated by ectomycorrhizal (EM) plants. These differences in microbiome composition reflect the rapid nutrient cycling and negative plant–soil feedback in AM soils. Lower fungal diversity and bacteria : fungi ratios in EM-dominated habitats are driven by monodominance of woody vegetation as well as soil acidification by EM fungi, which are associated with greater diversity and relative abundance of carbohydrate-active enzymes.
- Our study suggests that shifts in vegetation related to global change and land use may strongly alter the topsoil microbiome structure and function.

Introduction

Climate change poses an increasing threat to biodiversity and carbon (C) stores in terrestrial ecosystems by shifting vegetation types, aboveground foliar traits and belowground nutrient acquisition strategies (Stocker *et al.*, 2016; Jo *et al.*, 2019). Human-induced shifts in land use and pollution affect soil nitrogen (N) availability and terrestrial C cycling (Stocker *et al.*, 2016; Douglas *et al.*, 2018). Although shifts in N cycling are more localized and related to sources of N pollution and fertilization, soil C losses induced by warming and elevated CO_2 are globally more uniform (Stocker *et al.*, 2016; Jo *et al.*, 2019). However, shifts in soil C cycling depend on the costs of acquiring N, and thus on nutrient acquisition strategies of plants such as mutualistic root associations with mycorrhizal fungi and N-fixing bacteria (Norby, 1987; Terrer *et al.*, 2018).

Nearly 90% of plant families have evolved root symbioses with mycorrhizal fungi that benefit their hosts through enhanced nutrient and water uptake, and protection against pathogens and environmental stress (Smith & Read, 2008; Brundrett & Tedersoo, 2018). Based on the anatomy and taxonomic identity of the phyto- and mycobionts, mycorrhizas are broadly classified into four types: arbuscular mycorrhiza (AM), ectomycorrhiza (EM), ericoid mycorrhiza and orchid mycorrhiza (Brundrett & Tedersoo, 2018). Arbuscular mycorrhiza and EM plants dominate in most natural and anthropogenic ecosystems (Soudzilovskaia *et al.*, 2019), and they differ in belowground C allocation, capacity of organic nutrient acquisition, and impact on soil C and nutrient cycling (Phillips *et al.*, 2013; Tedersoo & Bahram, 2019). For example, EM systems have evolved relatively higher N-acquisition efficiency from organic material to cope with slower decomposition processes and lower litter quality (Smith & Read, 2008; Terrer *et al.*, 2018; Tedersoo & Bahram, 2019). Thus, depending on limiting nutrients and plant nutrient acquisition strategies, global change may hamper or support C sequestration, with further implications on nutrient cycling and climate

change (Averill *et al.*, 2014; Jo *et al.*, 2019; Soudzilovskaia *et al.*, 2019). Together with contrasting nutrient dynamics, EM and AM systems have contrasting patterns of plant–soil feedbacks influencing plant community dynamics (Tedersoo *et al.*, 2020). In general neutral or positive plant–soil feedbacks prevail in EM systems, compared to the negative plant–soil feedbacks prevailing in AM systems, likely resulting from contrasting effects of mycorrhizal type on soil properties and the activity of various microbial functional groups, namely antagonists such as soilborne pathogens (Bennett *et al.*, 2017; Teste *et al.*, 2017; Kadowaki *et al.*, 2018). In addition to mycorrhizal symbionts, root-associated bacteria from Rhizobiaceae and Frankiaceae families fix atmospheric N_2 and sustain plant N nutrition. However, despite such general mycorrhizal type and N-fixing effects on soil processes and plant community dynamics, knowledge about direct and indirect effects of plant nutrient-acquisition strategies on the diversity and composition of free-living soil microbes and microbial functions relevant to C and N cycling is still lacking.

Free-living soil microorganisms both affect and respond to shifts in rhizosphere processes because of their integral roles in plant nutrition, cycling of organic material and regulation of plant communities (Bardgett & Wardle, 2010). Bacteria drive most soil N-cycling processes such as N-fixation, nitrification and denitrification (Philippot *et al.*, 2007; Reed *et al.*, 2011). Saprotrophic Basidiomycota have evolved efficient mechanisms for degrading lignin and soil organic complexes, whereas other microfungi and bacteria decompose less recalcitrant polymers such as cellulose, hemicellulose and chitin, but also by-products of the lignin degradation pathway. The relative importance of bacteria and fungi in decomposition processes depend on soil acidity and C : N ratio (Waring *et al.*, 2013; Bahram *et al.*, 2018), which are driven strongly by the dominant vegetation via litter input (Waring *et al.*, 2015) and potentially by mycorrhizal type (Lin *et al.*, 2017). Due to high plant C allocation to EM fungi and the use of similar substrates, EM fungi may outcompete saprotrophic fungi for soil organic N sources, which may result in hampered degradation activity, termed the Gadgil effect (Gadgil & Gadgil, 1975; Fernandez & Kennedy, 2016). Soilborne pathogens have a direct negative effect on plant performance and thus can drive patterns of plant diversity (Bardgett & Wardle, 2010). Ectomycorrhizal fungi compared to AM fungi offer greater physical protection against soilborne pathogens, but also may maintain unfavourable conditions for disease progression such as high C : N ratios and low pH (Tedersoo & Bahram, 2019).

Here we performed a regional-scale investigation of topsoil microbes and their potential gene functions in 145 sites with contrasting edaphic conditions (Supporting Information Fig. S1), dominated either by AM or EM vegetation and/or N-fixing plants, to estimate the effects of these nutrient acquisition strategies on the free-living topsoil microbiome and its potential gene functions. We used metabarcoding and shotgun metagenomics techniques for identification of taxa and gene functions, respectively. We tested a hypothesis that temperate/boreal EM, AM and N-fixing dominated systems have contrasting soil microbial community compositions and potential functions, driven by direct interactions and indirectly by altering the soil conditions of C : N ratio and pH. More specifically, we predicted that with EM vegetation dominance EM fungi become the dominant soil organism group, favouring fungi to bacteria driven processes, as reflected in lower bacteria : fungi ratios in EM compared to AM systems. We further hypothesized that EM fungi suppress soilborne pathogens and saprotrophs in EM systems, with negative consequences for plant community diversity and turnover (Mariotte *et al.*, 2018).

Materials and Methods

Sampling

We performed sampling and documented vegetation parameters (including plant identity, functional type and basal area) in 145 plots (5800 subsamples) representing various soil types and various plant nutrient acquisition strategies across the northern Baltic region in Estonia and Latvia (Table S1). The sites were selected to represent various vegetation types, contrasting soil conditions and relative abundance of individual ectomycorrizal (EM), arbuscular muycorrhizal (AM) and nitrogen (N)-fixing plant species but keeping climatic variation minimal (Fig. S1). These sites included 73, 16, 50 and six 2500-m^2 plots in natural forests dominated by EM deciduous woody plants (total 16 species), EM coniferous trees (Pinaceae; three species), AM deciduous woody plants (48 species), and AM coniferous trees (Cupressaceae; two species), respectively. Altogether 12 EM plots (*Alnus incana*, *A. glutinosa*) and four AM plots (*Caragana arborescens, Hippophae rhamnoides*) were dominated by N-fixing plants. Grasslands and fields comprising no woody plants included six and eight plots, respectively. To determine plant composition, we recorded the relative abundance of plant species based on basal area (for tree) or relative cover (for grasses and crops) in each plot. We used a broad classification of AM and EM types, because dual mycorrhizas as well as other mycorrhizal types such as ericoid mycorrhiza were under-represented in our study. Time since last fire, vegetation age and the proportion of each woody plant species (basal area basis) also were recorded. We downloaded climate data from the WorldClim database (www.worldclim.org). Samples were taken mostly during growing seasons from 2011 to 2016 (Table S1). Mean annual temperature and precipitation for all samples ranges from 4.7–7.0°C and 549–745 mm, respectively. We excluded climatic variables from statistical analyses, because of little climate variation across the 400 × 400 km study area and little climate effect on microbes in our study based on preliminary analyses. In each site, 40 soil subsamples (5 cm diameter × 5 cm depth – no distinction was made between organic and mineral layers) were collected, and air-dried within 12 h of collection and homogenization. Total carbon (C), ^{13}C, total N and ^{15}N content were measured using an elemental analyzer (Eurovector, Milan, Italy) and an isotope ratio mass spectrometer (MAT 253; Thermo Electron, Bremen, Germany), following Tedersoo *et al.* (2012). Total phosphorus (P) and potassium (K) concentration were measured in ammonium lactate (Tecator ASTN 9/84; AOAC, 1990). Concentrations of

calcium (Ca) and magnesium (Mg) were measured in 1 M ammonium acetate (Tecator ASTN 90/92; Page *et al.*, 1982).

Molecular analysis

In order to determine the taxonomic and functional gene composition of soil, we used an amplicon-based approach for soil prokaryotes and eukaryotes as well as a shotgun metagenomics approach for gene-encoded functions. Total DNA was extracted from 2.0 g sample material (soil and fine root powder from homogenized soil samples) using the PowerMax Soil DNA Isolation Mini kit (MoBio, Carlsbad, CA, USA) following the manufacturer's instructions (Tedersoo *et al.*, 2014). Total DNA concentration was used as a proxy for microbial biomass, although it also may represent microbial necromass (Torti *et al.*, 2015). The entire soil metagenome was sequenced from 5 μg extracted DNA that was sonicated to fragments of 300–400 bases and further ligated to adaptors using the TruSeq Nano DNA HT Library Prep Kit (Illumina Inc., San Diego, CA, USA). DNA libraries were sequenced on three runs in Illumina HiSeq 2500 platform (2 × 250 bp paired-end chemistry, rapid run mode). The DNA samples were subjected to metabarcoding of Bacteria and Archaea using primers 515FB and 926R to target the ribosomal rRNA 16S gene V4 region (Walters *et al.*, 2016). All eukaryotes including fungi were targeted based on partial 18S rRNA gene (V9 subregion) and full-length internal transcribed spacer (ITS) region using the primers ITS9MUNngs and ITS4ngsUni (Tedersoo & Lindahl, 2016). Samples were amplified using one of 115 primer pairs, in which both primers were tagged with a unique 10-base Golay index (at least four differences to each other; all starting with A; AT : GC ratio between 0.4 and 0.6). The 25-μl PCR mix consisted of 16 μl sterilized H_2O, 5 μl 5 × HOT FIREPol Blend MasterMix polymerase (Solis Biodyne, Tartu, Estonia), 0.5 μl each primer (200 nM) and 3 μl DNA extract. Thermal cycling conditions were the following: 95°C for 15 min, 30 cycles of 95°C for 30 s, 50°C 45 s and 72°C for 1 min, with a final extension step at 72°C for 10 min. PCR products of two technical replicates were pooled and their relative quantity was estimated by running 2 μl DNA on 1% agarose gel for 15 min. DNA samples producing no visible band or an overly strong band were re- amplified using 35 and 25 cycles, respectively. The amplicons were pooled and purified using FavorPrep™ Gel/PCR Purification Kit (Favorgen, Vienna, Austria) and shipped for library preparation in sequencing service providers' laboratories in the Estonian Biocenter, University of Tartu (Illumina MiSeq and HiSeq platforms) and Oslo University Sequencing Center (PacBio). For fungal identification, we used the PacBio platform that outperforms other platforms in terms of distinguishing species, taxonomic precision and (lower) proportion of artefacts (Tedersoo *et al.*, 2018). Negative and positive controls were used throughout the experiment including sequencing. All metagenomics and metabarcoding sequences have been deposited in the European Bioinformatics Institute Sequence Read Archive database: PRJEB24121 (ERP105926); 16S and ITS metabarcoding data of global soil samples, accession nos. PRJNA598043.

Bioinformatics analysis

Metagenomics Metagenomics analysis followed the procedure described in (Bahram *et al.*, 2018). Briefly, reads were quality-filtered by removing those with < 70% max length of run (i.e. 150 bp), an accumulated error > 2 or an estimated accumulated error > 2.5, with a probability of ≥ 0.01 and > 1 ambiguous position. Reads were trimmed if a quality window of 15 bases dropped < 20 at the 3′ end. This resulted in 980 810 505 reads. Quality-filtered reads were merged using FLASH (Magoč & Salzberg, 2011) and mapped against the reference databases eggnog (Huerta-Cepas *et al.*, 2015) and custom-modified CAZY (using DIAMOND in BLASTX mode; parameters $k = 5$, $e = e^{-4}$) to quantify the abundance of functional genes. The scores of two unmerged query reads that mapped to the same target were combined to avoid double counting reads. To combine DIAMOND hit scores on target proteins, we summed the hit score of the forward and reverse reads matching a given target, using custom Perl scripts. To calculate the corresponding e-values independently from sequence as well as database parameters, we selected the lower e-value of either forward and reverse reads. We note that this approach reflects the potential function of organisms, which are not necessarily expressed at the transcript and enzyme level. Compared to bacterial and fungal pathogens, forest soil fungi are under-represented in genome databases, which will underpower assignment of functions to specific fungal guilds.

Metabarcoding The LOTUS 1.462 pipeline was used for 16S amplicon sequence processing (Hildebrand *et al.*, 2014). Reads were demultiplexed with a modified quality-filtering procedure to trim reads to 170 bp and reject substandard reads: accumulated error ≤ 2; presence of unique reads > 7 times in one, > 3 times in two or > 2 times in three samples. In total 22 335 463 of 31 286 576 reads passed the quality control and these were clustered with UPARSE (Edgar, 2013) at 97% sequence similarity. Chimeric operational taxonomic units (OTUs) were detected and removed based on both reference-based and *de novo* chimera checking, using the RDP reference (http://drive5.com/uchime/rdp_gold.fa) in UCHIME (Edgar *et al.*, 2011). Because our intention was to focus on the most abundant taxa, low-abundance OTUs with < 4 sequences were removed by UPARSE, as implemented in LOTUS by default, to minimize artefactual taxa in our datasets. The bacterial OTU abundance matrix was filtered from sequences of eukaryotic and chloroplastic origin, and rarefied to the lowest number of shared OTUs to remove the effect of sequencing depth across samples. Bacterial OTUs were assigned into different functional groups using FAPROTAX (Louca *et al.*, 2016).

PacBio amplicon sequences were processed using PIPECRAFT (Anslan *et al.*, 2017), resulting in 394 067 quality-filtered reads. Clustering at 97% sequence similarity was used for calculating OTUs. Representative sequences of OTUs were BLASTN-queried against the UNITE 7.1 reference dataset. Taxonomic assignments were performed at 70%, 75%, 80%, 85% and 90% sequence similarity to roughly match phylum, class, order, family and genus level, respectively. Taxa with sequence similarity

< 70% to any taxon or match e-value > e^{-50} were considered unidentified at the kingdom level. Fungal taxa were functionally assigned to principal guilds (Nguyen *et al.*, 2016; Tedersoo & Smith, 2017).

Data analysis

The OTU abundance matrices were rarefied once to an equal number of reads (20 000 for bacteria and 500 for fungi) per sample to reduce the effect of variation in terms of sequenced reads using the function rrarefy in R/VEGAN (Oksanen *et al.*, 2007). Alternatively, residuals of models with square root of total read abundance were used for analyzing richness (Tedersoo *et al.*, 2014). The results were very similar between two approaches, and thus we only report the results of the latter approach (McMurdie & Holmes, 2014). To deal with compositionality of abundance matrices (Gloor *et al.*, 2017), we transformed the abundance-based compositional dataset by using centred log ratio transformation (CLR) in the MIXOMICS package (Rohart *et al.*, 2017). Five samples were removed from the analyses, because they were either from anoxic habitats and hence outliers in community analyses, or contained a few bacterial reads and were therefore excluded from both the fungal and bacterial dataset. For better visualization of the data in Fig. 1 and Fig. S6, the percentage of EM plants more and less than 50% were rescaled with $|EM\%\text{-}50|_4$ and $-|EM\%\text{-}50|_4$, respectively.

The B : F ratio was calculated based on the proportion of bacterial to fungal metagenomic rRNA genes, as shown previously to correlate strongly to phospholipid-derived fatty acids (PLFA)-based B : F ratio (Bahram *et al.*, 2018; Fig. 2a). To include plant community composition in our univariate analysis, we used the first two axes from a principal coordinates analysis (PCoA) as implemented in the APE package (Paradis *et al.*, 2004) (Fig. S2). For the PCoA analysis, a Bray–Curtis distance matrix for the plant community was generated based on Hellinger-transformed abundance data in VEGAN. Plant diversity was calculated based on Shannon diversity index in VEGAN.

For univariate analysis, the best predictors of microbial richness and relative abundances were identified and included in a final model selection procedure using a machine learning approach as implemented in the *randomForest* function of RANDOMFOREST package (Liaw & Wiener, 2002). This approach estimates variable importance while training the random forest (Breiman, 2001). For this analysis, we included 25 biotic and abiotic variables, including vegetation and soil parameters as well as latitude, longitude and altitude (Table S1). We tested spatial autocorrelation in our data in Random Forest analysis by including spatial distance (principal coordinates of neighbourhood matrix (PCNM) vectors; Borcard & Legendre, 2002), generated based on a matrix of geographical distances among samples in VEGAN. To further test direct and indirect effects of variables, we built structural equation modelling (SEM) models in the AMOS software (SPSS) by including predictors based on their importance in the Random Forest models. In a prior model, all indirect and direct links between variables were established based on their correlations. Then, we removed nonsignificant links and variables or created new links between error terms until a significant model fit was achieved. Differences between the relative abundance of the main taxonomic and functional groups across mycorrhizal types were tested using a nonparametric Wilcoxon rank sum test, with Benjamini–Hochberg multiple testing correction. To model these based on EM vegetation cover (Fig. 1), we used beta regression for proportions (Ferrari & Cribari-Neto, 2004) as implemented in R/BETAREG (Zeileis *et al.*, 2016).

Multivariate models were constructed in permutational multivariate analysis of variance (PERMANOVA) (Anderson, 2005) with the Adonis function of VEGAN (using 10^3 permutations), following variable selection in forward selection mode based on F_{pseudo}-values. PERMANOVA was performed to test discrimination of the relative abundance of different bacterial phyla and functional categories across mycorrhizal types. We further visualized taxonomic (OTU) and functional (orthologous gene; OG) composition of bacteria using global nonmetric multidimensional scaling (GNMDS) in the VEGAN package based on the following options: two dimensions, initial configurations = 100, maximum iterations = 200, and minimum stress improvement in each iteration = 10^{-7}. For constructing OG and OTU distance matrices, the Bray–Curtis dissimilarity was calculated between each pair of samples. Spatial autocorrelation as well as correlation in composition of different organism groups was calculated using the Mantel test. Furthermore, to determine the relative importance of soil, vegetation and spatial variables (PCNMs) in shaping the composition of microbial taxa and functions, variation partitioning analysis was used as implemented in *varpart* function of VEGAN. To infer direct and indirect effects of mycorrhizal type at the multivariate level, we used the two first axes from a PCoA analysis of plant communities as explanatory or response variables in SEM.

Results

Microbial richness and abundance

Our analyses revealed 10 325 fungal OTUs (304 248 reads) and 29 813 bacterial OTUs (7022 893 reads). Among abiotic variables, soil pH and $\delta^{15}N$ (an integrator of the N cycle) showed the strongest correlation with the EM : AM plant abundance ratio (based on basal area). Soil C : N ratio was strongly correlated with the proportion of coniferous EM trees (Fig. S3). Plant diversity correlated to coniferous EM abundance ratio ($r = 0.423$, $P < 0.001$) but not to the EM : AM plant abundance ratio ($P > 0.05$). Total microbial biomass was positively associated with tree species richness ($r = 0.471$, $P < 0.001$) and the proportion of deciduous EM trees ($r = 0.203$, $P = 0.023$), but negatively with the proportion of coniferous trees (EM: $r = -0.240$, $P = 0.007$; AM: $r = -0.341$, $P < 0.001$; Fig. S3). Microbial biomass ($P > 0.1$) was not different but the B : F ratio was relatively lower ($R^2_{adj} = 0.197$, $P < 0.001$) in EM ecosystems, especially in coniferous sites (Fig. 1). Random Forest analysis revealed that plant diversity, functional and mycorrhizal traits were the major determinants of absolute microbial biomass, whereas the B : F ratio was positively related to soil $\delta^{15}N$ and pH (Fig. 2).

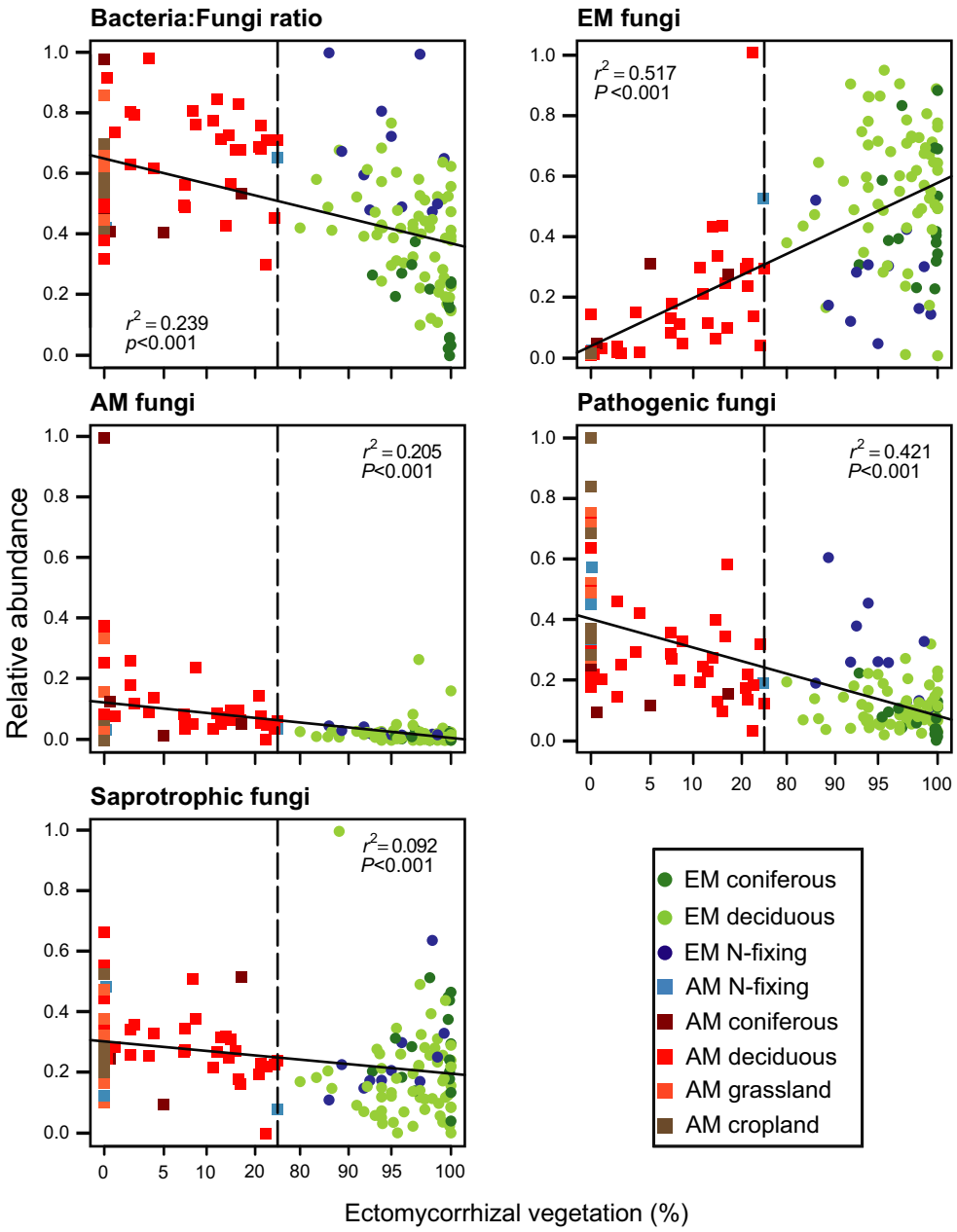

Fig. 1 Functional guild composition of soil bacteria (% relative to fungi) and fungal functional guild relative abundance in relation to ectomycorrhizal (EM) dominance. Data points show the relative abundance of bacteria and fungi (and fungal guilds) in each plot. EM dominance corresponds to the percentage of EM vegetation estimated on a basal-area basis. Values on the *x*-axis were rescaled and the relative abundances on the *y*-axes were scaled from 0 to 1 for better visualization.

Bacterial taxonomic richness was most strongly determined by soil pH (unimodal association; peak at pH 5–6; $R^2_{adj} = 0.455$, $P < 0.001$; Fig. 2), with the effect of vegetation traits remaining of secondary importance ($R^2_{adj} = 0.027$; Fig. 2). Nevertheless, bacterial richness was highest in habitats dominated by N-fixing plants independently of soil pH ($R^2_{adj} = 0.076$, $P = 0.0005$) and lowest in coniferous EM-dominated ecosystems ($R^2_{adj} = 0.218$, $P < 0.001$; Figs 2, S4). In a similar way to fungi, plant pathogenic bacteria were relatively more abundant in AM-dominated plots (0.358, $P < 0.001$; Fig. S5). Nevertheless, N-fixing bacteria were more abundant in EM habitats ($r = 0.527$, $P < 0.001$), where soil N is bound mostly in organic material. Nitrogen-fixing plants had no effect on relative abundance of N-fixing or denitrifier soil bacteria ($P > 0.1$). SEM models indicated that EM relative abundance (basal area basis) had a direct negative effect but additional indirect negative effects on fungal richness through enhancing soil C : N ratio (Fig. S4). Of fungal functional groups, the relative abundance and richness of all guilds (except EM fungi) declined with increasing EM dominance (Fig. 1). When excluding mycorrhizal fungi (both AM and EM) from the analysis, differences in these groups became weaker but remained significant (Fig. S6). As fungal pathogens in current databases (e.g. UNITE) mostly comprise agricultural plant pathogens, we excluded croplands from these analyses, but the strong negative correlation between pathogens and EM : AM ratio remained ($r = -0.626$, $P < 0.001$), indicating that the observed pattern for plant pathogens (Fig. 1) was not driven solely by this potential bias. Spatial vectors had little effect compared with other variables on microbial richness and relative abundance (Fig. S7).

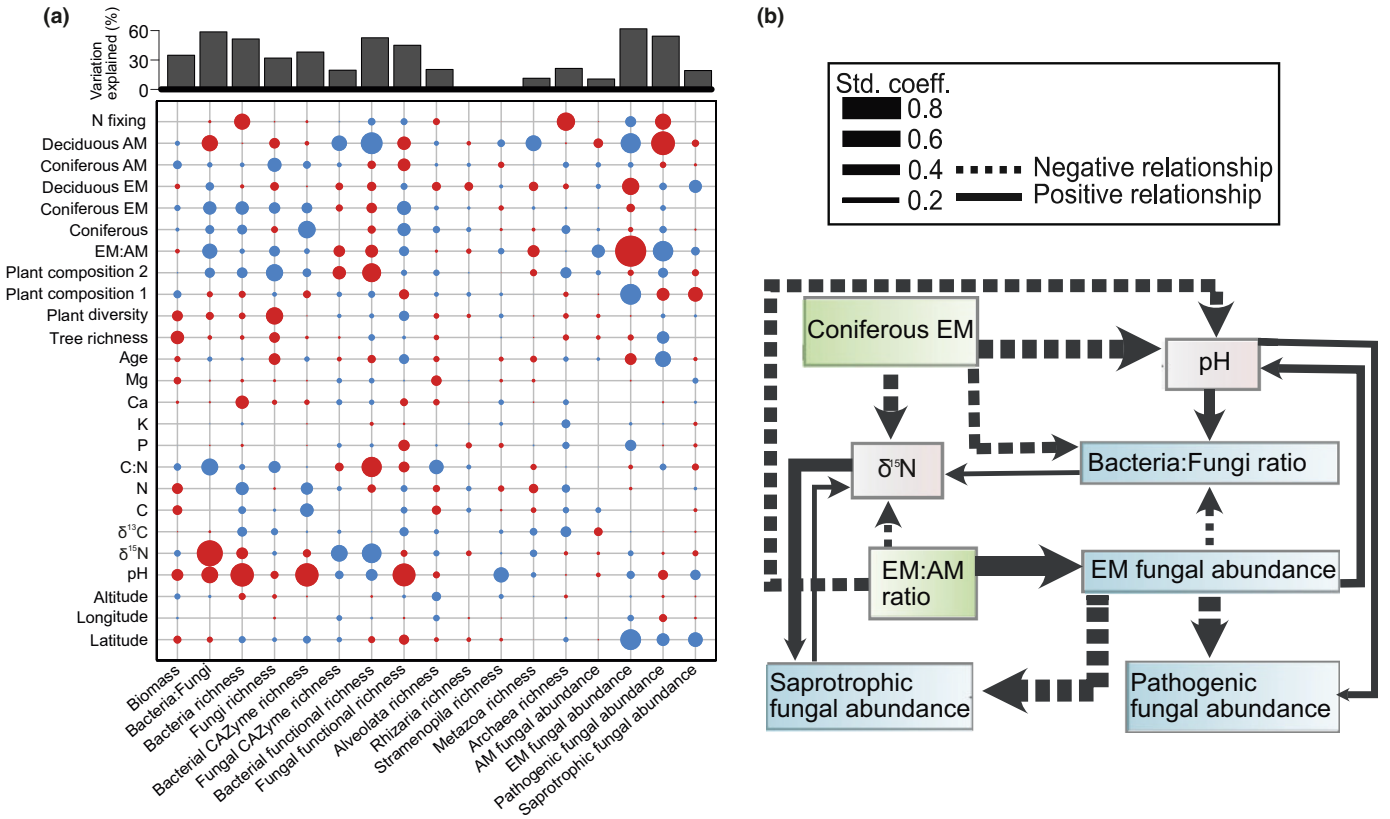

Fig. 2 Microbial diversity and composition associate with biotic and abiotic factors. (a) Random Forest heatmap indicates relationship of microbial taxa and functional groups to plant traits, edaphic and geographical variables. The size of circles corresponds to the variable importance (% of mean decrease accuracy estimated based on out-of-bag-CV); blue and red depict negative and positive Spearman correlations, respectively. Plant composition 1 & 2 are the first two princioal components analysis (PCA) axes representing changes in the composition of plants across the plots. The top barplot shows the out-of-bag variance explained for each model with the dependent variables on the *x*-axis. (b) Best-fitting structural equation model based on relationships retrieved in (a) for the relative abundance of bacteria and fungi. All relationships were significant ($P < 0.05$) and model fits were acceptable according to chi-square test ($P > 0.1$) and PCLOSE test ($P > 0.1$). See Supporting Information Table S2 for statistical details. We tested both directions for the relationships between $\delta^{15}N$ or pH and the relative abundance of functional groups or bacteria : fungi (B : F) ratio, and kept those that improved model fit (based on PCLOSE test).

Microbial composition

Bacterial community composition also was strongly driven by soil pH (PERMANOVA: $F_{1,143} = 61.8$, $R^2_{adj} = 0.302$, $P = 0.001$; Figs 2, S8) but not by mycorrhizal type or other environmental variables. By contrast, fungal composition was determined mainly by mycorrhizal type both directly (PERMANOVA: $F_{1,143} = 7.37$; $R^2_{adj} = 0.049$, $P = 0.001$; Fig. S4) and indirectly via reduced soil pH in EM habitats (Figs S3, S4). Except for Umbelopsidomycetes (Mucoromycota molds) and Agaricomycetes (Basidiomycota), relative abundance of most fungal higher-level taxa increased with AM tree dominance (Fig. 3). SEM analyses suggested that EM fungal relative abundance and richness drive those of bacteria and saprotrophs (Figs 2, S3). Mantel test and variation partitioning analysis revealed weak spatial autocorrelation in our data. (Fig. S9).

Microbial functions

Plant nutrient-acquisition strategies affected the relative abundance and composition of microbial functional genes. Bacterial and fungal carbohydrate-active enzyme (CAZyme) profiles showed remarkable differences among habitats with different mycorrhizal types for both bacteria ($F_{1,143} = 23.6$, $R^2_{adj} = 0.116$, $P = 0.001$) and fungi ($F_{1,143} = 16.2$, $R^2_{adj} = 0.090$, $P = 0.001$). These differences among mycorrhizal types were particularly pronounced in coniferous EM plots compared with any AM plots (Figs 3, S4). In spite of reduced taxonomic richness, the diversity of bacterial (Mantel $r = 0.445$, $P = 0.001$); and fungal ($r = 0.586$, $P < 0.001$) CAZyme genes increased significantly with increasing EM dominance (Figs 2a, S1). Bacterial functional gene composition was determined mainly by soil pH ($F_{1,143} = 61.81$, $R^2_{adj} = 0.302$, $P = 0.001$) and dominance of conifers ($F_{1,143} = 23.65$, $R^2_{adj} = 0.141$, $P = 0.001$), with lower importance of mycorrhizal type ($F_{1,143} = 13.77$, $R^2_{adj} = 0.088$, $P = 0.001$). Bacterial OGs related to *inorganic ion transport and metabolism* were relatively more abundant in EM-dominated plots ($R^2_{adj} = 0.276$, $P < 0.001$; Fig. 3). Unlike in bacterial OGs ($P > 0.1$), plant mycorrhizal type was the strongest determinant of fungal OG composition ($F_{1,143} = 20.2$, $R^2_{adj} = 0.124$, $P = 0.001$; Figs 2, S5), with $\delta^{15}N$ ($F_{1,143} = 27.3$, $R^2_{adj} = 0.165$, $P = 0.001$) as an important

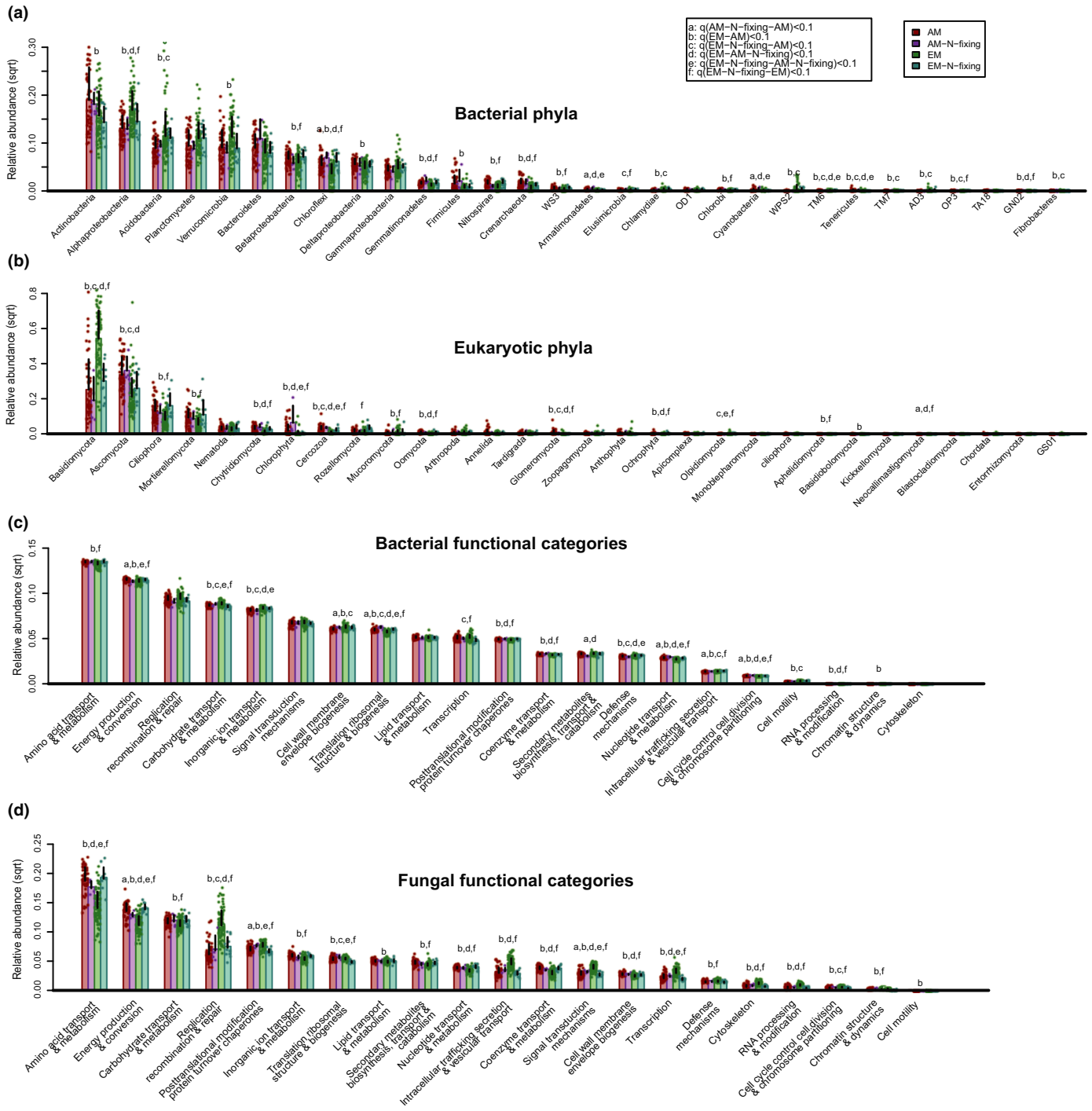

Fig. 3 The distribution of microbial taxa and functional genes differ across habitats with different plant nutrient-acquisition strategies and the dominant vegetation type. The figure shows the relative abundance of (a) major prokaryotic phyla (classes for Proteobacteria), (b) eukaryotic phyla and the functional gene categories of (c) bacteria and (d) fungi in arbuscular mycorrhizal (AM)- and ectomycorrhizal (EM)-dominant plots. Letters denote significant differences at the 0.05 probability level on the basis of Kruskal–Wallis tests corrected for multiple testing. Jittered points and bars represent individual relative abundances per sample, whereas bars represent the mean of relative abundances per category. Values on the *y*-axes are the square root of relative abundances. The error bars represent SEs.

edaphic predictor. Fungal OG richness was significantly higher in EM- than AM-dominated plots ($R^2_{adj} = 0.201$, $P < 0.001$). Of the main fungal OG categories, *inorganic ion transport and metabolism* significantly increased with AM dominance, whereas *replication and recombination* increased with EM dominance (Fig. 3).

Discussion

Microbiome diversity

Our study demonstrates that the composition of microbial taxa and functional genes consistently differs among ecosystems

dominated by plants with different nutrient-acquisition strategies, especially mycorrhizal type. Plant–soil feedbacks involving soil-inhabiting microorganisms contribute to the substantial differences in ecosystem processes such as soil carbon (C) and nutrient cycling among forest stands dominated by different tree species (Waring *et al.*, 2015) and mycorrhizal types (Phillips *et al.*, 2013; Tedersoo & Bahram, 2019). In particular, we found that nonfungal eukaryotes and saprotrophic fungi are more enriched in AM habitats. This is in line with higher decomposition rates in AM ecosystems (Tedersoo & Bahram, 2019) and implies strong competitive interactions of EM fungi with free-living saprotrophs (Bödeker *et al.*, 2016), bacteria and potentially other soil microbes. Of decomposer organisms, only saprotrophic Agaricomycetes were relatively more common in EM forests, particularly EM coniferous ecosystems, compared with AM-dominated habitats (Figs 1, S6), which reflects the production of low-quality litter by conifers (Cornelissen *et al.*, 2001).

Ectomycorrhizal plants generally accumulate recalcitrant litter with a high C : N ratio (Lin *et al.*, 2017; Sasse *et al.*, 2017), which has strong direct effects on the soil microbiome (Bahram *et al.*, 2018). Our model suggests that coniferous EM plants are the major drivers of an increase in soil C : N ratio and decrease in pH, which in turn reduce the richness of bacteria, archaea, several protist groups and the bacteria-to-fungi (B : F) ratio (Fig. S3). The strong effect of pH on soil bacterial richness corroborates previous reports and validates our analyses (Rousk *et al.*, 2010; Bahram *et al.*, 2018). Due to differences in physiology, capacity to withstand H^+ stress and nutrient stoichiometry (Rousk *et al.*, 2010), bacterial decomposition pathways dominate over those of fungi in soils with high pH and low C : N ratio (Waring *et al.*, 2013; Bahram *et al.*, 2018).

Although bacterial functional and taxonomic composition was relatively insensitive to factors other than pH, both functional and taxonomic diversity of fungi responded strongest to mycorrhizal type and plant diversity. Biotrophic fungal guilds such as plant pathogens, AM and EM mutualists are intimately associated with living plants and thus exhibited stronger and more specific plant interactions compared with most bacteria. Our analyses also indicate that the effect of mycorrhizal type on soil communities may depend strongly on other vegetation parameters, as reflected by large differences between deciduous and coniferous EM-dominated ecosystems.

Functional genes

The distribution of overall functional gene categories was similar in EM and AM ecosystems, except ligninolytic carbohydrate-active enzymes (CAZymes) that were more abundant and family-rich in EM habitats, which we attribute to the accumulation of recalcitrant litter and high abundance of saprotrophic Agaricomycetes with efficient ligninolytic weaponry (Nagy *et al.*, 2017). Although other OGs were generally represented at similar abundance, EM and AM ecosystems differed substantially in gene composition of bacterial vs fungal origin. Most gene families are fundamental to signalling, cellular growth and both metabolic and anabolic processes that are required equally for functioning by various functional guilds of the microbiome. Conversely, bacterial and fungal degradation and nutrient cycling pathways exhibit great differences in emission of gases (NH_4, NO_3, CH_4) and intracellular vs extracellular biopolymer degradation (Bugg *et al.*, 2011; Frey-Klett *et al.*, 2011).

The relatively greater B : F ratio in AM habitats explains the higher proportion of bacterial genes related to nutrient and C cycling, which is consistent with the more dynamic and leaky nutrient cycling in AM ecosystems (Tedersoo & Bahram, 2019). Although our metagenomics data cannot be used to infer production and efficiency of specific enzymes, we advocate that bacterial and fungal pathways of the seemingly redundant functions may differentially affect soil nutrient fluxes and C cycling (Caspi *et al.*, 2011). *In situ* measurements of soil processes integrated with proteome and transcriptome analyses will provide deeper insights into quantitative functional differences among plant nutrient acquisition strategies (Tedersoo & Bahram, 2019). Overall, the relative abundance and diversity of N-fixing plants and N-fixing bacteria showed weak association with the diversity and composition of bacterial and fungal gene functions, indicating the more prominent role of mycorrhizal types and other vegetation parameters in driving these patterns.

Pathogens and plant community dynamics

According to the Janzen–Connell hypothesis, density-dependent accumulation of plant species-specific antagonists regulates species abundance and promotes diversity (Bagchi *et al.*, 2014; Mariotte *et al.*, 2018). Yet, EM fungi may interfere with the general Janzen–Connell model (Dickie *et al.*, 2005; Chen *et al.*, 2019), as reflected in often conspecific monodominant EM-dominated systems compared to more species-rich mixed AM-dominated systems. Ectomycorrhizal associations could counteract negative density-dependent mechanisms through positive plant–soil feedbacks that favour the aggregation of conspecific individuals rather than a diverse plant community (Bever *et al.*, 2010; Peh *et al.*, 2011; Johnson *et al.*, 2018). In line with this, recent experimental studies indicate that AM trees experience greater antagonisms from their associated soil microbiota compared with EM trees (Bennett *et al.*, 2017; Teste *et al.*, 2017; Kadowaki *et al.*, 2018). Our metabarcoding results complement the idea of pathogen protection and suppression as a key mechanism driving positive plant–soil feedbacks in EM systems, demonstrating that putative plant pathogens are on average 2.6-fold more abundant in AM-dominated ecosystems, especially in AM deciduous forests (Fig. 1). However, we did not find support for the effect of this mechanism on plant community and diversity, as EM basal area showed rather weak, or even positive correlation in the case of coniferous EM, to plant diversity. Other mechanisms also may be important in promoting positive EM plant–soil feedbacks, such as extensive common EM mycelial networks redistributing nutrients and promoting EM seedlings (McGuire, 2007; Kadowaki *et al.*, 2018), and EM fungi trapping EM-dominated systems in a N-limitation feedback loop that reinforces the dominance and obligatory nature of the EM symbiosis (Franklin *et al.*, 2014). Our results support the importance of the latter to

some extent, specifically in coniferous EM-dominated systems that were related to higher C : N and lower B : F ratios, soil $\delta^{15}N$ and pH (Fig. S3), as well as the greater relative abundance of EM fungi representing the dominant fungal functional guild (Fig. S4). Although our results need greater empirical support, we suggest that the negative plant-soil feedback in AM systems compared to positive or neutral soil feedback in EM systems (Bennett *et al.*, 2017; Teste *et al.*, 2017; Kadowaki *et al.*, 2018) may be attributed to four nonexclusive mechanisms: species-specific damage by pathogens (Mariotte *et al.*, 2018), a relatively greater ability of EM fungi to physically protect their hosts from the soil environment (Kadowaki *et al.*, 2018), direct antagonistic effects of EM mycelium against antagonists and competitors, and the maintenance by EM fungi of unfavourable acidic and low-nutrient soil conditions for many microbial groups including pathogens (Figs 1, S3). In mixed EM–AM forests, these contrasting mechanisms driving a collection of positive, neutral and negative plant–soil feedbacks may generate complex microsites supplying regeneration niches for various species and promoting overall plant diversity (Mariotte *et al.*, 2018).

Global implications

We demonstrated that mycorrhizal type may be one of the strongest predictors of soil microbiome diversity and functioning across contrasting soil and vegetation types at the regional scale, specifically in temperate and boreal ecosystems. At large geographical scales, the effects of vegetation parameters on bacterial and fungal composition are less pronounced, perhaps due to the interplay of other predictors such as climatic variables and historical factors including dispersal limitation (Tedersoo *et al.*, 2014; Maestre *et al.*, 2015; Bahram *et al.*, 2018; Delgado-Baquerizo *et al.*, 2018). Modelling from regional to global scales demonstrates that climatic factors and land use play additional important roles in determining the distribution of mycorrhizal types and that EM vegetation may enhance soil C storage (Soudzilovskaia *et al.*, 2019). Alternatively, litter decomposition potential was proposed as a key driver of mycorrhizal type distribution globally (Steidinger *et al.*, 2019). However, for our studied temperate/boreal region, our SEM models suggest that mycorrhizal type affects both relative and absolute abundances of soil saprotrophs and bacteria as well as genes related to decomposition, not *vice versa*. The directionality is important, because both mycorrhizal fungi and pathogens determine plant establishment success at a landscape scale, which further shapes the habitat for particular saprotrophic groups. The local influential variables such as soil and vegetation parameters and as well as climatic variables on larger scales have strong implications for the potential effects of global change on vegetation type, soil microbial diversity and the processes governed by these (Soudzilovskaia *et al.*, 2019). Ectomycorrhizal vegetation, which is patchily distributed in tropical ecosystems and limited by rainfall, may suffer strongest from extended drought periods and atmospheric pollution (Terrer *et al.*, 2016; Tedersoo, 2017; Jo *et al.*, 2019).

To conclude, our results suggest that shifts in the balance between EM–AM vegetation may alter the soil microbiome structure and function in temperate and boreal ecosystems. However, it remains unclear to what extent these functional differences among plant nutrient-acquisition strategies can be extrapolated to arctic and tropical ecosystems, because differences among mycorrhizal types on soil chemistry and ecosystem processes are somewhat weaker at low latitudes (Keller & Phillips, 2019; Soudzilovskaia *et al.*, 2019). We certainly need controlled experiments to test the interactions of soil pH and temperature with mycorrhizal type effects on soil microbiome structure and functioning and to what extent these effects are bidirectional.

Acknowledgements

We thank Rasmus Puusepp and Emma Johanna Sova for help with molecular analysis and Franz Buegger for help with stable isotope analyses. We also thank Ian Dickie and four anonymous reviewers for providing constructive comments on an earlier version of the manuscript. This study was funded by grants from the Estonian Research Council (PUT1317) and Swedish Research Council (Vetenskapsrådet, 2017-05019).

Author contributions

MB, RD, KL and LT contributed data; MB, TN, FH, SA and LT analyzed the data; FH conducted bioinformatic analysis; KP contributed to Isotopic analysis; MB and LT wrote the manuscript with input from TN, FH, PB and other authors.

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Fig. S1 Distribution map of sampling plots.

Fig. S2 Principal coordinate analysis (PCoA) plot showing the variation in the composition of plant communities among samples (symbols).

Fig. S3 Pairwise Spearman correlations between environmental variables used in statistical analyses.

Fig. S4 Fungal communities drive bacterial and saprotrophic fungal communities and the abundance of pathogens.

Fig. S5 The main biotic and abiotic determinants of the relative abundance of nitrogen fixing and denitrifying bacteria.

Fig. S6 Functional guild composition of soil eukaryotes across plots dominated by different nutrient acquisition strategies.

Fig. S7 Random Forest heatmap indicates relationship of microbial taxa and functional groups to plant traits, edaphic, geographical and spatial variables.

Fig. S8 Functional and taxonomic composition of microbes differ between plant nutrient-acquisition strategies.

Fig. S9 Venn diagram based on the variation partitioning analysis of microbial taxonomic and functional composition.

Fig. S10 Diversity of CAZymes increases with increasing relative abundance of ectomycorrhizal plants.

Table S1 Samples used in this study.

Table S2 Results of structural equation modelling (SEM) shown in Fig. 2(b).

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