Received: 28 January 2025 | Revised: 29 July 2025 | Accepted: 11 September 2025

DOI: 10.1002/ecs2.70466

ECOSPHERE
AN ESA OPEN ACCESS JOURNAL

ARTICLE
Microbial Ecology

Microbial functional guilds and genes are key to explaining soil nutrient cycling alongside soil and plant variables

Corinne R. Vietorisz[1] | **Nahuel Policelli**[1,2] | **Abigail Li**[1] | **Lindsey A. Adams**[1] | **Kathryn F. Atherton**[1,3] | **Jennifer M. Bhatnagar**[1]

[1]Department of Biology, Boston University, Boston, Massachusetts, USA

[2]Instituto Patagónico para el Estudio de los Ecosistemas Continentales IPEEC, Consejo Nacional de Investigaciones Científicas y Técnicas CONICET, Puerto Madryn, Chubut, Argentina

[3]Bioinformatics Graduate Program, Boston University, Boston, Massachusetts, USA

Correspondence
Corinne R. Vietorisz
Email: cviet@bu.edu

Funding information
Biological and Environmental Research, Grant/Award Numbers: DE-SC0012704, DE-SC0020403; National Science Foundation Graduate Research Fellowship Program

Handling Editor: Weile Chen

Abstract

Microbes play central roles in soil nutrient cycling; yet, a limited range of microbial community characteristics have been used to explain ecosystem nutrient cycling rates, and their importance relative to plant and abiotic factors remains unclear. In this study, we assessed which of 126 commonly measured soil fungal and bacterial community characteristics best explained net soil ammonium, nitrate, and phosphate mineralization rates in temperate forests in the Northeastern United States, as well as the relative contributions of microbial, plant, and abiotic factors. Using boosted regression tree modeling, we identified the microbial variables with the highest contributions to models explaining nutrient cycling rates: the relative abundances of ectomycorrhizal fungi and nitrogen (N)-decomposition genes from oligotrophic bacteria were the most important for net ammonification, the relative abundances of indicator taxa in bacterial networks, nitrifying bacteria, and copiotrophic bacteria were the most important for net nitrification, and the relative abundance of fungal phosphorus (P)-cycling oxidoreductase genes was the most important for net soil phosphate change. Microbial variables explained more variation than plant and abiotic variables in multivariate linear models of net nitrification and net phosphate release rates, but not net ammonification rates, which were largely explained by soil edaphic factors. Leaf litter traits were also important in explaining variation in net nitrification rates, and soil temperature was important in explaining rates of net phosphate release in soil. Collectively, our findings suggest that the N-cycling capacity of microbial functional guilds and P-cycling capacity of fungi should be incorporated into ecosystem biogeochemical models to improve our predictions and understanding of nutrient cycling and related ecological processes.

KEYWORDS
ammonification, bacteria, ectomycorrhizae, fungi, microbial communities, nitrification, nitrogen, nutrient cycling, phosphorus

INTRODUCTION

Soil microbes, including fungi and bacteria, are the driving force behind nutrient cycling within forest soils, secreting enzymes that release key elements like nitrogen (N) and phosphorus (P) from dead organic matter, immobilizing them in microbial biomass, and transferring these elements to plant hosts (Schneider et al., 2012; Smith & Read, 2008). Despite their central functions in nutrient cycling, we still do not understand which aspects of microbial communities best explain variation in soil nutrient cycling rates. Meta-analyses show that, on average, abiotic drivers alone such as climate, pH, and soil organic matter (SOM) can explain roughly half of the variation in nutrient cycling (Graham et al., 2016), while plant factors such as leaf litter quality and fine root distribution can explain between one-third and three-quarters of variation on their own (Finzi et al., 2015; Hobbie, 2015; Scott & Binkley, 1997; Thomas & Prescott, 2000). Incorporating microbial factors sometimes improves models explaining biogeochemical cycling, though bacterial community structure and diversity are often the only microbial factors considered (Graham et al., 2016). A wide range of soil microbial community characteristics can now be measured efficiently and at low cost, making them potential candidates to improve biogeochemical model predictions and our understanding of nutrient cycling and related ecological processes—such as carbon (C) cycling and primary productivity—across spatial and temporal scales.

Some microbial variables have been linked to nutrient cycling rates, such as microbial (usually bacterial) diversity, which can be positively correlated with N cycling, especially in species-poor communities (Bell et al., 2005; Philippot et al., 2013; Salonius, 1981; Setälä & McLean, 2004). Relative abundances of microbial functional guilds have also correlated with N cycling: soil nitrification rates can positively correlate with the relative abundance of ammonia-oxidizing prokaryotes (Isobe et al., 2015; Sorensen et al., 2019; Tatsumi et al., 2020; Zhang et al., 2019) and net N mineralization rates can positively correlate with the relative abundance of ectomycorrhizal fungi (EMF; Sorensen et al., 2019; Zhang et al., 2023). Additionally, abundances of functional marker genes encoding enzymes that perform nutrient transformations (primarily bacterial genes) have sometimes correlated with N cycling processes (Chen et al., 2020; Isobe et al., 2015; Petersen et al., 2012; Tang et al., 2019), but multiple studies have found no correlation (Graham et al., 2014; Philippot et al., 2013; Rocca et al., 2015; Saifuddin et al., 2021; Zhang et al., 2021). This discrepancy could be because community-level functional gene abundances are not linked to microbial taxonomy, marker genes may not be representative of a complete metabolic pathway (Albright et al., 2019), or gene copy abundances may not reflect gene or protein expression levels in the community.

Groups of genes involved in biochemical transformation pathways can be related to a microbe's functional guild (Auer et al., 2024; Romero-Olivares et al., 2021; Talbot et al., 2015), but it is unclear if these gene groups from individual microbial functional guilds can predict rates of nutrient cycling. For example, EMF can have higher expression of chitinases and other fungal cell wall-degrading enzymes than saprotrophic fungi (Auer et al., 2024; Maillard et al., 2023), so chitinase genes from EMF may explain rates of N degradation in soils. P decomposition rates in soils may also be explained by organic matter-degrading genes from mycorrhizal fungi, as they are hypothesized to perform decomposition to acquire nutrients instead of C (Lindahl & Tunlid, 2015; Talbot et al., 2015) and many EMF have diverse methods of P mobilization in soils (Plassard et al., 2011). Nitrification rates may be explained by nitrification gene abundances from narrow groups of nitrifying bacteria: in wastewater, nitrification correlates with nitrification gene expression in communities dominated by a single order of bacteria (*Nitrosomonodales*, Kapoor et al., 2016). Methods to estimate functional gene copies of entire microbial communities, such as PICRUSt2 (Douglas et al., 2020), have caveats, such as relying on reference phylogenies for amplicon sequence variants (ASVs) without taxonomic assignments and predicting gene abundances that may not be phylogenetically conserved. Nevertheless, there is an indication that taxonomy-based estimation of gene functional profiles could prove useful for explaining nutrient cycling rates in soil. For example, the relative abundances of functional gene copies derived from specific microbial guilds have been shown to correlate with tree growth (Anthony et al., 2022), but this approach remains untested for soil nutrient cycling.

Another untested microbial metric that may explain soil nutrient cycling rates includes associations between microbial groups, as interactions between species can drive community assembly and function (Guidi et al., 2016; Maynard et al., 2018; Ratzke et al., 2020). For example, competition for ammonium between EMF and ammonia-oxidizing prokaryotes may decrease nitrification rates (Tatsumi et al., 2020), so the correlation between these two groups could explain variation in net nitrification rates. Networks of commonly co-occurring taxa have also been linked to certain ecosystem processes, such as ocean C export, litter decomposition, and denitrification (Guidi et al., 2016; Wagg et al., 2019). However, it is unclear whether the collective relative abundances of co-occurring taxa central to microbial networks—which we refer to as indicator co-occurrence modules (ICMs)—can explain nutrient cycling rates. Despite the diversity of microbial community metrics that are relatively easy to measure, it is unknown which of these metrics best explain soil N and P cycling rates, or their importance relative to plant and soil abiotic factors.

In this study, we sought to determine which commonly measured soil microbial community characteristics best explain soil ammonium, nitrate, and phosphate mineralization rates and the relative contributions of microbial, plant, and abiotic factors in explaining these processes. We hypothesized that relative gene abundances from specific functional guilds will be the best microbial metric to explain rates of soil ammonium, nitrate, and phosphate mineralization. We expected that net ammonification and net phosphate change would be best explained by EMF N- and P-decomposition gene abundances, respectively, while net nitrification rates would be best explained by nitrifying bacterial nitrification gene abundances. We also hypothesized that microbial metrics will explain more of the variation in models explaining soil ammonium, nitrate, and phosphate mineralization rates than plant or edaphic factors. To test our hypotheses, we sampled soils across six mixed deciduous mid-successional temperate forest sites in Massachusetts, USA, that maximized variation in soil microbial communities, vegetation, and soil edaphic factors. We measured soil ammonium, nitrate, and phosphate mineralization rates, fungal and bacterial community characteristics, plant community characteristics, and soil abiotic attributes. We assessed the relative contributions of microbial, plant, and abiotic factors in explaining rates of soil ammonium, nitrate, and phosphate mineralization using a combination of boosted regression trees (BRTs) and multivariate linear models. We considered the "best" variables explaining nutrient cycling rates to be those that explained the most variation in nutrient cycling rates within the models.

METHODS

Site description and sampling design

We sampled a set of temperate forest sites in eastern and central Massachusetts, USA, that maximized variation in soil microbial community composition, plant community characteristics, and soil nutrient cycling rates, but minimized confounding correlations between microbial and plant factors. We selected six field sites (Appendix S1: Section S1: Methods), where each site contains four forest stand types, all within 2.5 km of each other: mature white pine (*Pinus strobus*)-dominated stands, mature hardwood-dominated stands (mostly *Quercus* spp.), hardwood-dominated stands with white pine saplings encroaching into the understory, and mixed mature white pine and hardwood stands (Appendix S1: Figure S1). The stands with pine encroachment contained no mature pine trees, but had scattered white pine saplings throughout the understory. Young pines associate with unique pine-specific EMF (i.e., EMF that form host-specific associations with pines, Policelli et al., 2020) but contribute very little pine litter to the forest floor (Appendix S1: Figure S2), allowing us to isolate effects of pine-associated soil microbial communities from litter inputs on soil nutrient cycling. Within each forest type at each site, one transect was laid from the forest edge to the interior, perpendicular to the forest edge (6 sites × 4 forest types = 24 total transects). We included a forest edge to interior transect because microbial communities and soil characteristics vary with distance from the forest edge in these forests, even when plant communities do not (Tatsumi et al., 2023), further isolating the effects of plant and microbial communities on soil nutrient cycling. Two sampling points were established within 5 m from the transect at 0, 15, 30, 60, and 90 m from the forest edge. This resulted in a total of 204 sampling points (24 transects × 4 or 5 distances per transect × 2 sampling points per distance).

Soil sampling, nutrient cycling, and edaphic factors

Organic horizon soil was sampled for microbial metrics, nitrogen cycling rates, and edaphic measurements in July 2021, and for phosphorus cycling rates in July 2022 as described in Appendix S1: Section S1: Methods. During the bulk soil collections, fresh sieved soil was saved at 4°C for inorganic nutrient extractions, pH, and moisture measurements; an aliquot of sieved soil was frozen at −80°C for subsequent DNA extraction; and an aliquot of sieved soil was air-dried for total elemental analysis. During soil sampling in July 2021 and 2022, soil temperature (Rapitest digital soil thermometer, Luster Leaf, Woodstock, IL) and depth of organic horizon were measured directly along the transect, equidistant between each A and B sampling point at all distances from the edge. In the same 204 soil samples from July 2021 that were used for inorganic N extractions, %SOM, gravimetric soil moisture, and pH were measured following the protocols described in Caron et al. (2023).

To measure soil net ammonification and nitrification, we used the "buried bag" method, where an initial soil sample is taken to measure ammonium and nitrate, then another soil sample is buried beneath the litter layer in the same location within a polyethylene bag and incubated in the field for a month (Caron et al., 2023; Durán et al., 2012;

Eno, 1960; Hanselman et al., 2004; Westermann & Crothers, 1980). Initial soil sampling was conducted in mid-July 2021, and the buried bags were collected 4 weeks later in mid-August 2021. Soil samples were collected, extracted in 2 M potassium chloride, and analyzed colorimetrically for inorganic nitrogen (ammonium and nitrate) content as described in Appendix S1: Section S1: Methods. To measure net soil phosphate change, a second year of soil sampling was completed in early July 2022–early August 2022. We followed the same buried bag and soil sampling protocol in the same sampling locations as for the net ammonification and net nitrification assays, except that we measured Olsen inorganic P (a measure of plant-available P, Olsen, 1954). We performed extractions in 0.5 M sodium bicarbonate and analyzed the extracts for phosphate concentrations colorimetrically as described in Appendix S1: Section S1: Methods (Frank et al., 1998; Olsen, 1954). On the 144 soils from the 0, 15, and 60 m distances collected in 2021, an aliquot of sieved soil was air dried and sent to the Ohio State Service Testing and Research Laboratory for total phosphorus analysis via the EPA 3051A acid digestion (U.S. Environmental Protection Agency, 2007). The same 144 soils were also ground to a fine powder in a ball-mill, dried at 65°C, and combusted on an elemental analyzer (Vario EL cube, Elementar) for total C and N analysis.

Vegetation factors

In September 2021, forest floor leaf litter depth and composition, and root density were measured at each distance from the forest edge along the transect as described in Appendix S1: Section S1: Methods. In September 2021–September 2022, litterfall weight and chemistry were measured once per transect as described in Appendix S1: Section S1: Methods. To measure aboveground vegetation community composition, in June 2022, a 10 m × 10 m square plot at each transect was created at each distance from the forest edge centered around the sampling points. Within the plot, we measured the total basal area of canopy trees by species and understory plant cover by growth form using the Braun–Blanquet method (Braun-Blanquet, 1932; Matteucci & Colma, 1982) as described in Appendix S1: Section S1: Methods.

Microbial DNA extraction, amplicon sequencing, and bioinformatics

Total soil DNA was extracted from approximately 0.25 g of each soil sample (204 samples) using the DNeasy PowerSoil Kit (QIAGEN, Hilden, Germany). To amplify microbial DNA, we used modified versions of the primer set fITS7 and ITS4 for fungi (amplifying the ITS2 region of rDNA, Anthony et al., 2017) and modified versions of the primer set 515f and 806r for bacteria (amplifying the v4 16S region of rDNA, Caporaso et al., 2011) that contained both the Illumina adapter and individual sample indexes. DNA amplification and sequencing were performed following the procedure outlined by Tatsumi et al. (2023) and in Appendix S1: Section S1: Methods. Bioinformatics were performed in R (v 4.2.1), where the R package "dada2" (v 1.26.0) was used for sequence quality control, paired-end assembly, identification of ASVs, and taxonomy assignment (Callahan et al., 2016; Caporaso et al., 2011), with any deviations from default noted in Appendix S1: Section S1: Methods. To identify and remove outliers based on community composition and sequencing depth, we ran a nonmetric multidimensional scaling analysis using an Aitchison distance matrix (Gloor et al., 2017; Appendix S1: Section S1: Methods). In all, 13 samples with under 8000 reads were removed for ITS sequences, and 3 samples determined as outliers due to sequencing errors or contamination were removed for 16S sequences.

To estimate total fungal and bacterial abundances, total ITS2 and 16S v4 gene abundances were quantified on all DNA extracts via quantitative polymerase chain reaction (qPCR) using the same primer sets used for amplicon sequencing (without the Illumina adapter and indexes; Caporaso et al., 2011; White et al., 1990), following the protocol outlined in Tatsumi et al. (2023).

Microbial community metrics

We chose to calculate microbial community characteristics based on amplicon sequence data because it is the most common way to characterize microbial communities and currently the most easily accessible to microbial ecologists. Thousands of amplicon sequencing datasets are publicly available globally, such that using existing amplicon sequencing data to predict nutrient cycling rates is an actionable method that could improve models of soil-level nutrient cycling processes across ecosystems.

Based on DNA amplicon sequence analysis, we quantified 126 microbial community characteristics across seven different categories of microbial community traits (Table 1). To obtain the relative abundances of fungal and bacterial functional guilds, the ITS and 16S ASV tables were converted to relative abundances by dividing each ASV count in each sample by the total number of reads in that sample (Weiss et al., 2017). Fungal genera were assigned functional guilds and EMF were assigned exploration types using the FungalTraits database (Põlme et al., 2020). Bacterial taxa were assigned as copiotrophs

TABLE 1 Definitions of the microbial community characteristics used to explain nutrient cycling rates.

Microbial community characteristic type	Definition	Example characteristic
Functional guild relative abundances	The summed relative abundance of ASVs belonging to a microbial functional group within a sample	Relative abundance of ectomycorrhizal fungi
Functional gene relative abundances	The estimated relative abundance of functional gene copies in the bacterial or fungal communities in a sample, calculated via PICRUSt2 (for bacteria) or using fungal genome annotations (for fungi) in a sample	Relative abundance of fungal chitinase genes
Functional gene relative abundances within functional guilds	The estimated relative abundance of functional gene copies, summed for a functional guild in a sample	Relative abundance of chitinase genes from ectomycorrhizal fungi
Indicator co-occurrence modules	The summed relative abundance of all fungal or bacterial ASVs in an indicator co-occurrence module (i.e., group of co-occurring ASVs identified via network analysis) in a sample	Relative abundance of the fungal "high ammonification" ICM
Diversity metrics	Metrics of fungal or bacterial alpha diversity	Fungal Shannon diversity
16S and ITS gene abundances	The total number of 16S v4 or ITS2 gene copies in a sample (per microliter DNA extract) as determined by qPCR, a proxy for total bacterial and fungal abundance, respectively	Fungal ITS gene copies
Functional guild correlations	The strength of the Spearman correlation between the proportion of a bacterial and fungal functional guild, calculated at the transect-level using all samples within a transect	Correlation between ectomycorrhizal fungi and nitrifying bacteria

Abbreviations: ASVs, amplicon sequence variants; ICM, indicator co-occurrence module; ITS, internal transcribed spacer; qPCR, quantitative polymerase chain reaction.

or oligotrophs, and/or nitrifying bacteria, using an in-house database compiled from literature reviews and genomic pathway presence where we assigned guilds at the genus through phylum level (Albright et al., 2019; Averill et al., 2021; Ho et al., 2017; Naylor et al., 2020), which allowed us to include many bacterial ASVs that did not have taxonomy assignments at lower taxonomic levels. The relative abundance of functional guilds in each sample was calculated by summing the relative abundances of ASVs belonging to each functional guild. Fungal and bacterial alpha diversity values were calculated on rarefied ASV tables (Weiss et al., 2017) using Shannon's diversity index. The 16S samples were rarefied to a depth of 14,906 reads, and ITS samples to a depth of 7,345 reads. Fungal evenness and bacterial evenness were calculated by dividing Shannon's diversity index by the natural log of the richness (Pielou, 1966).

To estimate the relative abundances of functional genes, Enzyme Commission (E.C.) numbers per ASV per sample were estimated via PICRUSt2 for bacteria (Douglas et al., 2020), and via the method described in Anthony et al. (2022) with modifications by Atherton et al. (Forthcoming) for fungi. All groups of E.C. numbers had strong phylogenetic signals (Appendix S1: Figure S3a–c) and in a test dataset, E.C. number estimations per sample (using the methods above) matched well to E.C. number counts obtained through metagenome sequencing of the same soil samples used for ITS and 16S amplicon sequencing (Appendix S1: Section S1: Methods, Figure S3d). To calculate the relative abundances of genes encoding enzymes involved in N and P cycling in each sample, we summed the relative abundances of all E.C. numbers encoding the enzymes listed in Appendix S1: Table S1. We focused only on N- and P-cycling genes directly involved in ammonification, nitrification, and phosphate release from organic matter, which are well annotated in environmental samples relative to other gene groups (Albright et al., 2019; Zeng et al., 2022). To calculate the relative abundances of genes derived from specific functional guilds in each sample, we summed the relative abundances of all E.C. numbers encoding N and P cycling enzymes that are derived from each functional guild.

In addition, we created ICMs of fungal or bacterial taxa that are central to microbial networks associated with each nutrient cycling rate. We first ran a weighted gene correlation network analysis (WGCNA) using the "WGCNA" (v 1.72.1; Langfelder & Horvath, 2008) and "flashClust" (v 1.1.2; Langfelder & Horvath, 2012) R packages on our 16S and ITS ASV tables as described in Appendix S1: Section S1: Methods. This analysis identifies microbial community network structures that are positively or negatively associated with nutrient cycling rates. We then identified the taxa that were most central to each network module (using a statistical metric of module membership, kME, Langfelder & Horvath, 2008) and created a new metric of ICM relative abundance by summing their relative abundances. Taxa included in each module are shown in Appendix S1: Figures S4 and S5.

The strength of correlations between fungal and bacterial guilds was also calculated for all soil samples within each of the 24 transects using Spearman correlations with the "rcorr" function in the "Hmisc" package (v 5.2.1; Harrell & Dupont, 2024). Guild correlations were calculated between all combinations of a fungal guild (e.g., EMF or saprotrophic fungi) and a bacterial guild (copiotrophic, oligotrophic, or nitrifying bacteria).

We chose not to use gene abundances measured via qPCR to explain nutrient cycling rates because they are not strongly linked to nutrient cycling rates in our study system. Saifuddin et al. (2021) found no relationship between *amoA*, *nosZ*, or *nifH* gene abundances and N cycling rates (including net nitrification) in soils at half of our study sites. In addition, we found no relationship between net nitrification rates and *amoA* gene abundances (measured via qPCR, Appendix S1: Section S1: Methods) in our dataset (Appendix S1: Figure S6). For these reasons, we chose not to measure marker genes via qPCR or use them in further analyses in this study.

Statistical methods

To test our hypotheses, we selected a unique set of potential explanatory variables for modeling each nutrient cycling process. A microbial variable was included in a model only if it directly related to a nutrient cycling mechanism (e.g., phosphate-cycling gene relative abundances were not included for N-cycling processes, and vice versa). All plant and soil abiotic variables were included as potential explanatory variables for all processes. This yielded 111 total variables for net ammonification (82 microbial, 20 plant, 9 soil), 68 variables for net nitrification (39 microbial, 20 plant, 9 soil), and 86 variables for net phosphate change (57 microbial, 20 plant, 9 soil). Because many soil abiotic variables co-correlated with each other, a principal components analysis (PCA) was run on the soil abiotic variables (Appendix S1: Figure S7), and a new variable "edaphic PC1" was created by extracting the first principal component. To examine the strength of correlation between explanatory variables and nutrient cycling processes, we ran Pearson correlations between all potential explanatory variables and the respective nutrient cycling rate. To reduce the total number of variables input to the multivariate model selection processes, for net ammonification and net nitrification models, we only input variables that had a significant correlation ($p < 0.05$) with the nutrient cycling rates. For net phosphate change, which had significant correlations with few variables, we input variables that had at least a marginally significant correlation ($p < 0.1$). See Appendix S1: Section S1: Methods for further detail on input variable selection for each nutrient cycling rate. This process yielded 17 input variables for net ammonification, 17 for net nitrification, and 6 for net phosphate change.

To compare the relative contributions of all microbial, plant, and soil abiotic variables explaining variation in net ammonification, net nitrification, and net phosphate change, we ran BRTs, which determine the relative contributions of many potential variables to explaining nutrient cycling rates and can fit complex, nonlinear relationships, do not require data transformation, and can handle interaction effects and correlations (Appendix S1: Figure S8) between predictors (Allen et al., 2017; De'ath, 2007; Elith et al., 2008). All BRTs were fit using the R package "dismo" (v 1.3.14; Elith et al., 2008) and parameters were optimized following the procedure described in Appendix S1: Section S1: Methods. Because there is slight variation in the predictor variable contributions in each run, we ran 1000 replicate model runs for each BRT.

To examine univariate linear relationships between nutrient cycling rates and explanatory variables identified as most important by the BRTs, we ran univariate linear mixed-effects models using the "lme4" package in R (v 1.1.35.5; Bates et al., 2015), where the sampling site was included as a random effect. If necessary, response and predictor variables were square-root transformed to meet linear model assumptions. For net nitrification, which could not be transformed to meet linear model assumptions, generalized linear mixed models were run using the "glm" command in the "stats" package (v 4.4.2; R Core Team, 2024) with the family set to "gamma" and the link set to "log," and a pseudo count of 1 was added to net nitrification values.

To determine which linear combinations of variables best explain variation in each nutrient cycling process, we constructed multivariate linear models for each nutrient cycling process and selected the best-fit model that explained the greatest amount of variation in nutrient cycling rates using the fewest possible independent variables. For net ammonification and net phosphate change, best-fit linear models were chosen by selecting the models with the lowest Akaike information criterion (AIC) where all explanatory variables are significant using the "leaps" package (v 3.2; Miller, 2024) testing for the Mallows' C-p score of each candidate model, which maximizes variation explained in the data, and minimizes correlation between predictors and the number of independent variables (Mallows, 1973). Site was included as a random effect unless it explained zero variance in the model. To construct multivariate models explaining net nitrification rates, we performed stepwise model selection manually on generalized linear mixed models using the "glm" command (R Core Team, 2024) as described in Appendix S1: Section S1: Methods and shown in Figure S9.

To test for the relationship between soil nutrient cycling rates and litterfall variables and microbial guild correlations, transect-level multivariate linear models were constructed. Litterfall variables and microbial guild correlations were measured once per transect, and all other variables were averaged by transect to create one observation per transect (24 total). Model selection was performed as described above using the "leaps" package. Net nitrification was log transformed (plus a pseudocount of 0.01) prior to input. All multivariate and univariate linear model assumptions were checked by examining plots of the residuals versus fitted values, normal quantile–quantile plot, and covariance between predictors. Generalized linear models were checked for over-dispersion using the "DHARMa" package (v 0.4.7; Hartig, 2022). R^2 values were estimated for all generalized linear models and models with random effects using the "r.squaredGLMM" function in the "MuMIn" package (v 1.48.4; Bartoń, 2024).

RESULTS

In contrast to our first hypothesis, functional guild abundances were the microbial factor most strongly tied to soil net ammonification and nitrification rates. For net ammonification, the microbial variable with the highest contribution to the BRTs was the relative abundance of EMF (Figure 1). The relative abundance of N-decomposition genes from oligotrophic bacteria had the next highest contribution, followed by the fungal high ammonification ICM, which all had positive relationships with net ammonification rates. Of all N-decomposition gene groups, chitinases and N-cycling glycosidases (hydrolyzing O- and S-glycosyl compounds) correlated most strongly with net ammonification. In the fungal high ammonification ICM, 4 of the genera in the module are EMF (Appendix S1: Figure S5) that comprise 34% of all reads in our dataset. Net ammonification rates also negatively correlated with fungal and bacterial diversity metrics, most strongly with bacterial Shannon diversity and fungal richness (Appendix S1: Figure S10).

The most important microbial variables contributing to the BRTs explaining net nitrification rates were the relative abundances of the bacteria in the high nitrification ICM, nitrifying bacteria, and copiotrophic bacteria (Figure 2). In contrast to our prediction, nitrifying genes from nitrifying bacteria were not found in our dataset. Instead, net nitrification rates correlated negatively with the relative abundance of bacterial nitrate reductase genes, the main enzyme involved in denitrification (Appendix S1: Figure S11). Factors related to EMF, especially EMF Shannon diversity and the relative abundance of medium-distance exploration EMF, also negatively correlated with net nitrification. For both net ammonification and nitrification rates, the highest-ranked microbial variables in the BRTs did not always have the strongest linear correlations with the process rate (Figures 1 and 2; Appendix S1: Figures S10 and S11), indicating that these are nonlinear relationships.

The most important variable contributing to the models explaining net phosphate change was the relative abundance of all fungal P-cycling oxidoreductase genes, not EMF P-decomposition genes as we originally predicted. Fungal P-cycling oxidoreductase genes had the highest contribution to BRTs and were positively related to net phosphate change (Figure 3). Few factors overall correlated with net phosphate change (Appendix S1: Figure S12), but most were relative abundances of functional genes, including the relative abundance of hydrolase genes (acting on P-bonds) from oligotrophic bacteria and the relative abundance of bacterial hydrolase genes (acting on C–P bonds), which correlated positively with rates of net phosphate change.

In contrast to our second hypothesis, microbial metrics did not explain more variation in net ammonification rates than abiotic factors, but they were still included in the best multivariate linear models explaining net ammonification (Table 2; Appendix S1: Table S2). These models included a soil edaphic variable (PC axis) and either the relative abundance of EMF or the fungal high ammonification ICM. When removing microbial variables from their respective models, the total variance explained by the model did not decrease, but the model fit declined (AIC change of +64). This finding is corroborated by the BRTs, where the soil edaphic PC axis was by far the most important variable explaining variation in net ammonification rates (Figure 1a).

By contrast, microbial variables explained more variation than plant and soil edaphic factors in models of net nitrification and phosphate change rates. The best model for net nitrification included the relative abundances of copiotrophic bacteria and medium-distance exploration EMF, bacterial Shannon diversity, fern abundance, and litter depth as explanatory variables (Table 2; Appendix S1: Table S3). Microbial variables explained the most variation in the linear model for net nitrification:

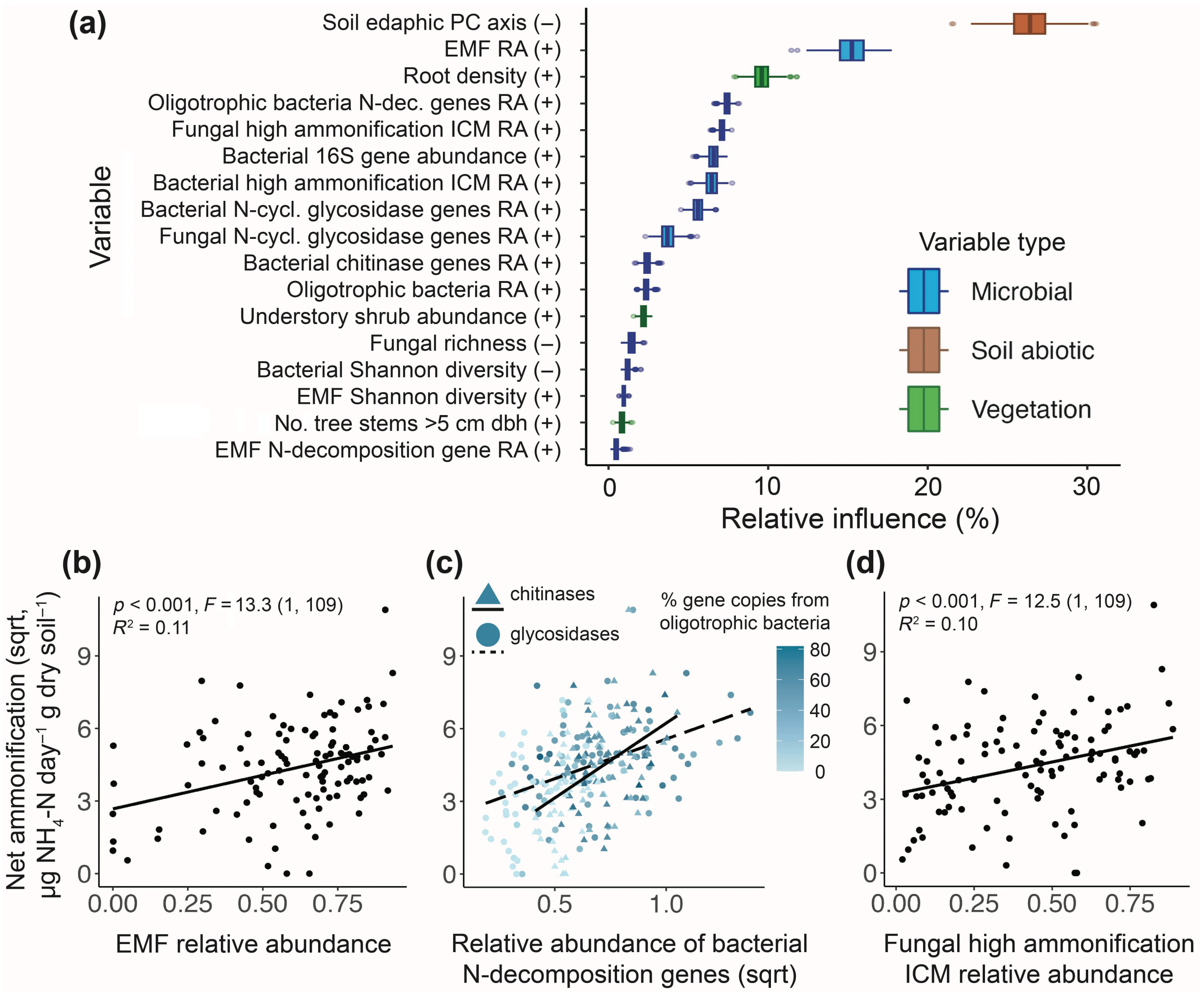

FIGURE 1 Relationships between net ammonification rates and explanatory variables. (a) Box plots show the relative contributions of each variable to explaining net ammonification rates across 1000 replicate model runs of boosted regression tree modeling. The direction of association with net ammonification is shown in parentheses after the variable name. Boxes represent the interquartile range of the data, midpoint lines represent the median, whiskers represent data within 1.5 times the interquartile range, and points represent outliers. Linear mixed-effects models show that net ammonification is correlated positively with the relative abundances (RA) of (b) ectomycorrhizal fungi (EMF), (c) the square roots of all bacterial chitinase ($R^2 = 0.24$, $p < 0.001$, $F_{1,43} = 33.9$) and nitrogen-cycling (N-cycl.) glycosidase genes (hydrolyzing O- and S-glycosyl compounds, $R^2 = 0.23$, $p < 0.001$, $F_{1,35} = 31.4$), and oligotrophic bacterial chitinase ($R^2 = 0.13$, $p < 0.001$, $F_{1,118} = 17.7$) and N-cycling glycosidase genes ($R^2 = 0.11$, $p < 0.001$, $F_{1,115} = 14.7$), and (d) the fungal high ammonification indicator co-occurrence module (ICM). PC, principal component; N-dec., nitrogen decomposition.

when microbial variables were removed from the model, the R^2 decreased by 0.37, or 80% of the total variance explained by the best model. Vegetation factors were also important in both the BRTs and the multivariate linear model, especially AM tree basal area and litter depth (Figure 2a).

Net phosphate change was best explained by a combination of microbial, plant, and soil edaphic factors: the best multivariate linear model included the relative abundances of fungal P-cycling oxidoreductase genes and bacterial hydrolase (targeting C–P bonds) genes, soil temperature, and the number of pine saplings (Table 2; Appendix S1: Table S4). When microbial variables are removed from the model, the R^2 decreases by 0.11, or 61% of the total variance explained by the best model, yet this best model explains relatively low variance in net phosphate change (18%). The results from the BRTs similarly showed that the relative abundance of fungal P-cycling oxidoreductase genes was more important than soil and vegetation variables, but soil temperature is still

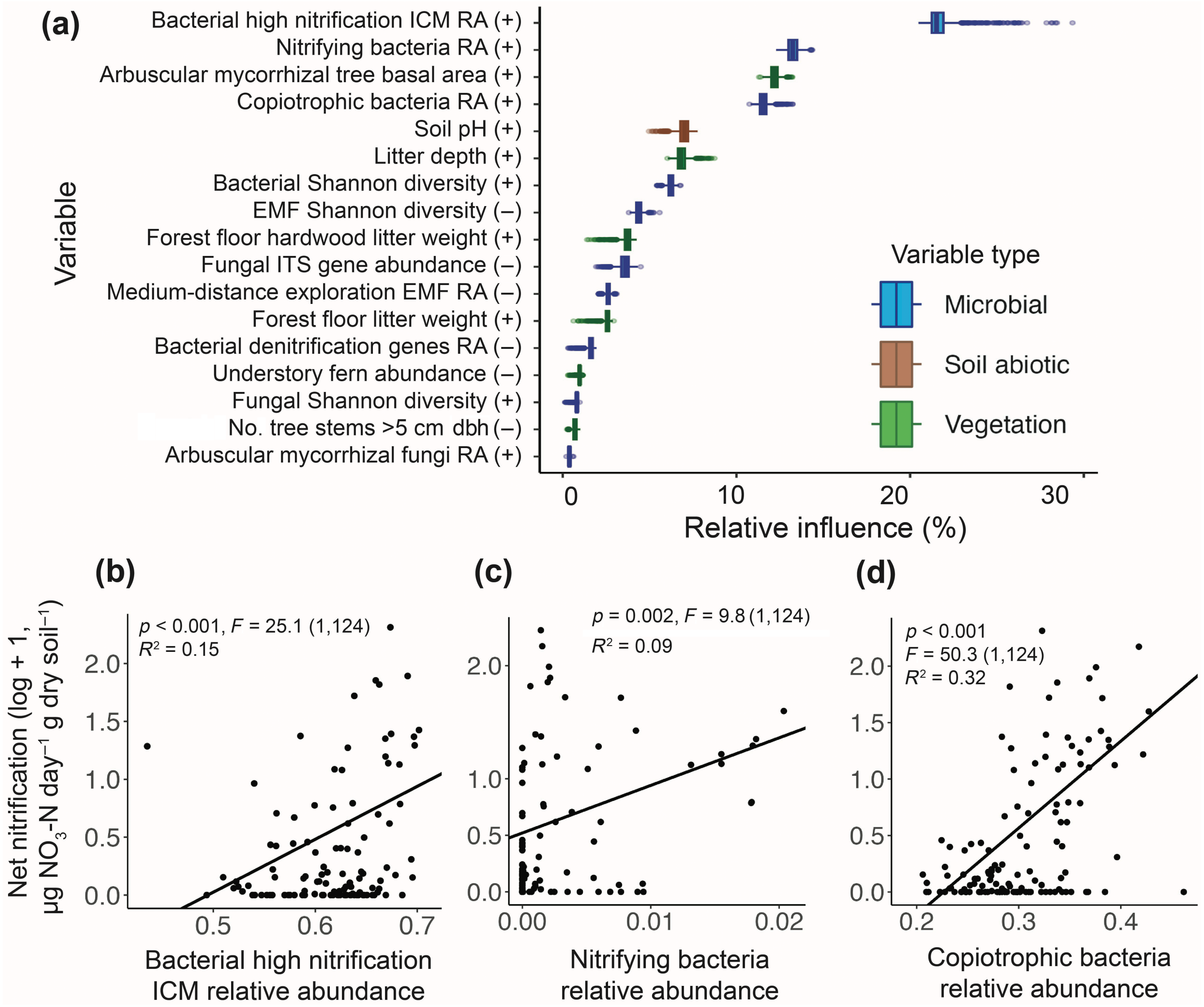

FIGURE 2 Relationships between net nitrification rates and explanatory variables. (a) Box plots show the relative contributions of each variable to explaining net nitrification rates across 1000 replicate model runs of boosted regression tree modeling. The direction of association with net nitrification is shown in parentheses after the variable name. Boxes represent the interquartile range of the data, midpoint lines represent the median, whiskers represent data within 1.5 times the interquartile range, and points represent outliers. Generalized linear mixed models show that net nitrification is correlated positively with the relative abundances (RA) of (b) the bacterial high nitrification indicator co-occurrence module (ICM), (c) nitrifying bacteria, and (d) copiotrophic bacteria. EMF, ectomycorrhizal fungi; ITS, internal transcribed spacer.

important as the second-highest contributing variable (Figure 3a). Additionally, litterfall chemistry variables (magnesium and molybdenum concentrations) were important in the transect-level models explaining net phosphate change (Appendix S1: Table S5).

DISCUSSION

Despite decades of soil microbial ecology research, key aspects of microbial communities that are useful for explaining soil nutrient cycling have remained elusive. Our study addresses this outstanding question, finding that functional guild relative abundances and ICMs were the most important microbial variables explaining ammonium and nitrate mineralization, while fungal P-cycling gene abundances were most important for soil phosphate mineralization within deciduous and pine forests of the Northeast United States. Microbial variables explained more variance in net nitrification and phosphate change rates than plant and soil abiotic variables, suggesting that microbial activity is a key regulator of these processes. These results suggest a

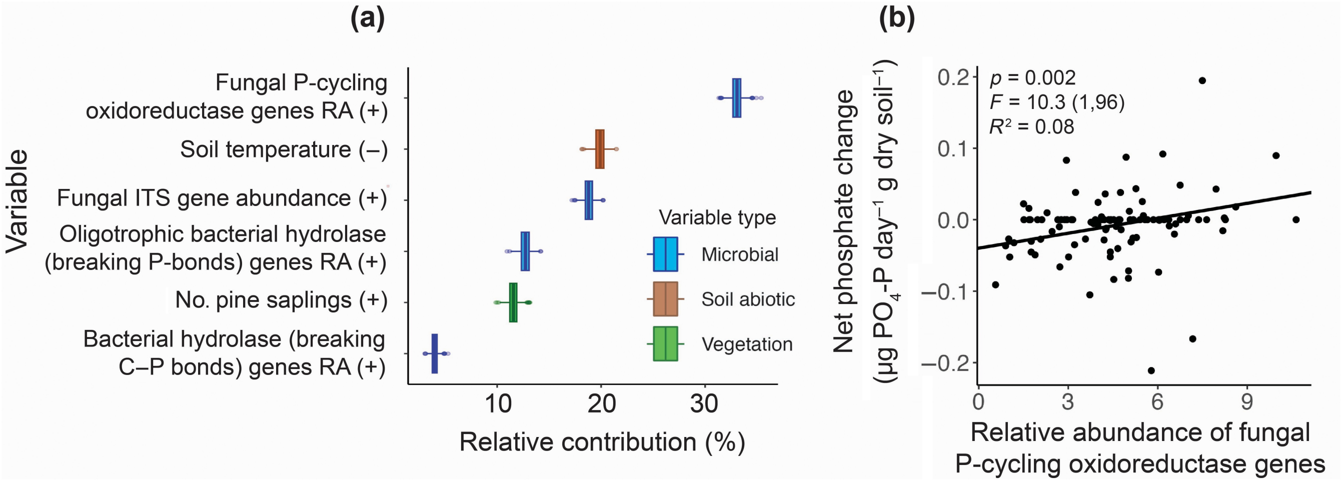

FIGURE 3 Relationships between net phosphate change rates and explanatory variables. (a) Box plots show the relative contributions of each variable to explaining net phosphate change rates across 1000 replicate model runs of boosted regression tree modeling. The direction of association with net phosphate change is shown in parentheses after the variable name. Boxes represent the interquartile range of the data, midpoint lines represent the median, whiskers represent data within 1.5 times the interquartile range, and points represent outliers. (b) Linear mixed-effects model shows that net phosphate change is correlated positively with the relative abundance (RA) of fungal phosphorus (P)-cycling oxidoreductase genes. ITS, internal transcribed spacer.

new framework for the roles of microbial, plant, and soil abiotic factors in inorganic nutrient cycling processes in these forests (Figure 4).

Microbial metrics explaining nutrient cycling rates

The microbial metrics explaining the most variation in net ammonification were consistently related to EMF and oligotrophic bacteria. The relative abundances of these functional groups and their N-decomposition genes were positively associated with net ammonification (Figure 1a–c; Appendix S1: Figure S10), suggesting that EMF and oligotrophic bacteria may drive ammonification via secretion of N-decomposing enzymes (Figure 4a). EMF and oligotrophic bacteria are characteristic of slow nutrient-cycling systems, where most nutrients are in organic forms (Fierer et al., 2007; Ho et al., 2017; Phillips et al., 2013), such as the nutrient-limited northern temperate forests in our study (Groffman et al., 2018). One hypothesis is that some EMF may decompose organic matter in search of N rather than C (Lindahl & Tunlid, 2015; Nicolás et al., 2019; Talbot et al., 2015), producing high levels of organic matter- and N-decomposing enzymes (Bödeker et al., 2014; Lindahl & Tunlid, 2015; Talbot et al., 2008; Zak et al., 2019) such as CAZymes, chitinases, ureases, and peroxidases (Argiroff et al., 2022; Auer et al., 2024; Maillard et al., 2023; Zhang et al., 2023). While little work has examined N-decomposition by oligotrophic bacteria, oligotrophic bacterial genomes tend to have abundant genes involved in amino acid transport and metabolism (Dragone et al., 2024) and marine oligotrophic bacteria may target N-rich molecules through extracellular leucine amino peptidase activity (Rath et al., 1993). Abundant oligotrophic bacterial phyla in our study sites, such as *Acidobacteria*, also have high genomic potential to degrade complex N-containing polysaccharides (Belova et al., 2018; Kalam et al., 2020; Zhou et al., 2019). Our findings support the idea that EMF and oligotrophic bacteria play a role in N-decomposition in temperate forest soils and provide novel evidence linking the N-cycling genomic potential of these microbial groups to net ammonification and nitrification rates.

Net nitrification rates were best explained by relative abundances of bacteria in the high nitrification ICM, nitrifying bacteria, and copiotrophic bacteria (Figures 2 and 4b), suggesting that these taxa may be primary drivers of nitrification in temperate forest soils. This aligns with findings that nitrification is largely performed by bacteria and archaea (Stein & Klotz, 2016). Known nitrifiers fall within the copiotrophic bacteria group and high nitrification ICM (Appendix S1: Figure S4), but more taxa in these groups may perform nitrification than we were able to annotate based on literature and genomic databases: multiple samples with positive net nitrification rates contained no nitrifying bacteria based on our annotations. This could be due to many bacterial ASVs (47%) lacking genus-level taxonomy needed to identify

TABLE 2 Best-fit multivariate linear models explaining soil nutrient cycling rates based on minimum Akaike information criterion (AIC) value.

Response variable	Independent variables	R^2	Coefficient	95% CI	p	F ratio (df)
Net ammonification (sqrt)	Soil edaphic PC axis	0.38	−0.57	−0.75, −0.37	<0.001	34.7 (1,85)
	EMF relative abundance		1.95	0.65, 3.26	0.005	8.5 (1,86)
Net ammonification (sqrt)	Soil edaphic PC axis	0.38	−0.59	−0.77, −0.40	<0.001	38.7 (1,86)
	Fungal high ammonification module abundance		1.37	0.71, 3.21	0.003	9.3 (1,85)
Net nitrification (log + 1)	Copiotrophic bacteria relative abundance	0.46	5.61	3.44, 7.82	<0.001	44.4 (1,109)
	Medium-distance exploration EMF relative abundance		−0.84	−1.32, −0.35	<0.001	33.8 (1,110)
	Bacterial Shannon diversity		0.56	0.06, 1.04	0.03	4.8 (1,108)
	Understory fern abundance		−0.12	−0.20, −0.03	0.02	5.7 (1,111)
	Litter depth		0.12	0.04, 0.20	<0.001	15.2 (1,112)
Net soil phosphate change	Fungal P-cycling oxidoreductase gene relative abundance	0.18	0.005	0.001, 0.009	0.03	4.9 (1,93)
	Soil temperature		−0.003	−0.006, −0.001	0.02	6.1 (1,93)
	No. pine saplings		0.001	<0.001, 0.002	0.009	7.2 (1,93)
	Bacterial hydrolase (targeting C–P bonds) gene relative abundance		1.0	0.20, 1.82	0.007	7.6 (1,93)

Abbreviations: EMF, ectomycorrhizal fungi; PC, principal component.

nitrifiers, or some bacterial lineages containing unidentified nitrifiers (Liao et al., 2024). Archaea or other microbes may also perform nitrification in these samples, especially because archaeal nitrifiers can dominate in acidic soils like the ones in this study (Isobe et al., 2015; Nicol et al., 2008). In addition, copiotrophic bacteria and taxa in the high nitrification ICM may grow and thrive in the environmental conditions created by nitrifiers (i.e., high inorganic nitrogen availability). The high nitrification ICM, which is calculated via correlation networks between taxa (Langfelder & Horvath, 2008), may capture associations between nitrifying bacteria and taxa that may depend on nitrifying bacterial activity (McClure et al., 2022). Net nitrification was also negatively associated with EMF, especially medium-distance exploration types, potentially due to competition for inorganic N between mycorrhizae and nitrifying bacteria and archaea (Sun et al., 2023; Tatsumi et al., 2020). Medium-distance exploration EMF have longer hyphae and increased nutrient foraging distance compared to contact or short-distance exploration types (Agerer, 2001; Chen et al., 2018) and may have limited capacity to degrade organic N (Hobbie & Agerer, 2009), such that they may take up large quantities of inorganic N. Because of their longer foraging distances, it is possible that EMF outcompete nitrifiers for access to ammonium in soil, limiting nitrification metabolism and/or growth of nitrifying microbes.

Net phosphate change was linked most strongly to fungal and bacterial P-cycling oxidoreductase and hydrolase genes (Figure 3). While the role of microbial oxidoreductases in soil P cycling has been understudied, redox reactions are being recognized as increasingly important in the soil P cycle (Ewens et al., 2021; Kehler et al., 2021; White & Metcalf, 2007). These results indicate that the cumulative sum of the entire microbial (especially fungal) community's P-targeting enzyme activity may be the primary driver of phosphate release from SOM (Figure 4c).

Relative contributions of microbial, vegetation, and soil abiotic factors

Although soil edaphic variables are widely used to explain nutrient cycling rates (Elrys et al., 2021), they were only key to explaining two of the three rates we measured. The soil edaphic PC axis (that includes pH, % SOM, % soil moisture, soil temperature, and organic layer depth) explained by far the most variation in net

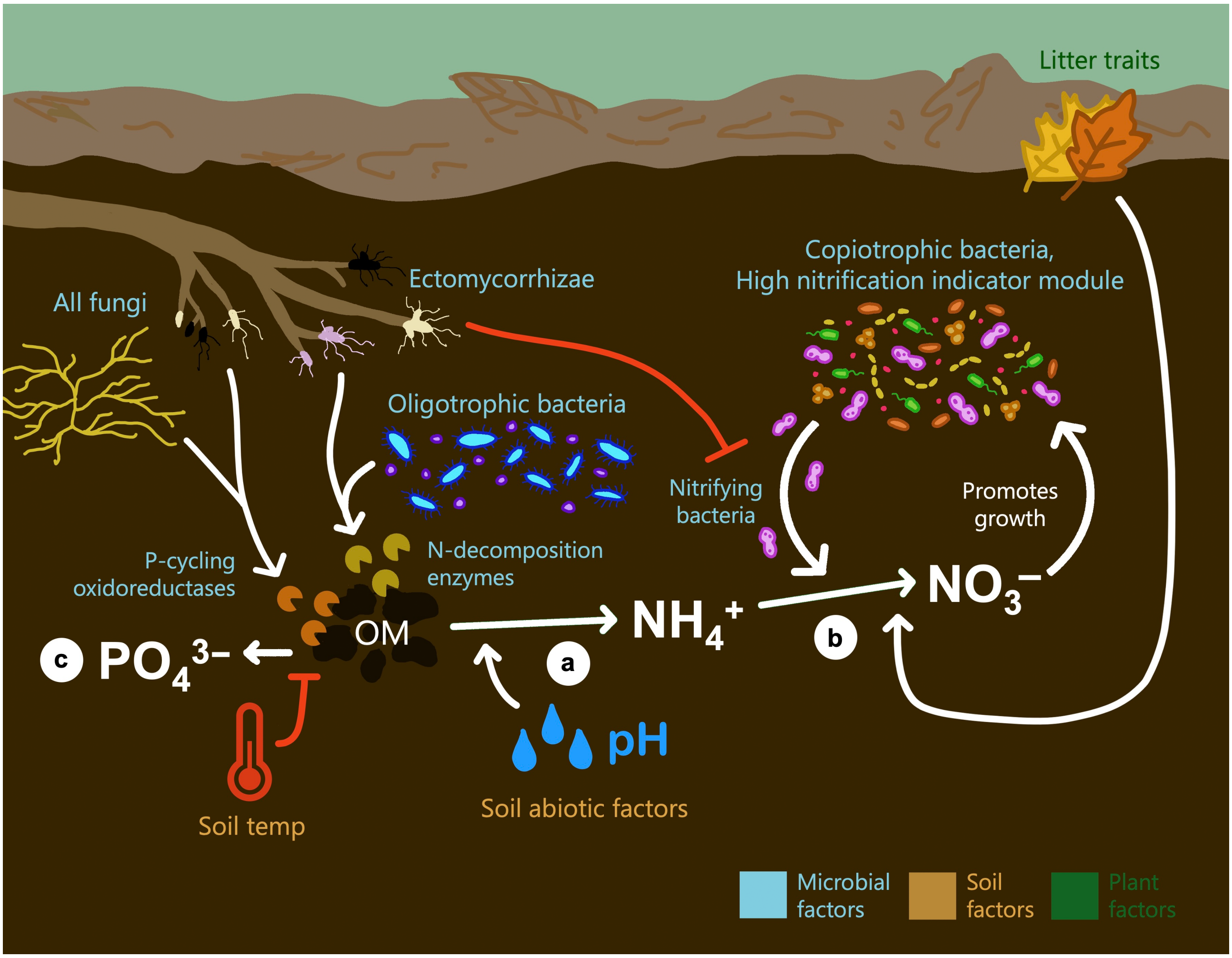

FIGURE 4 Hypothetical drivers and controls over soil nutrient cycling based on study results. Results from our field system analysis suggest that: (a) Net ammonification is driven by N-decomposition enzymes produced by ectomycorrhizal fungi and oligotrophic bacteria, which are regulated by edaphic factors like soil organic matter (OM) content, moisture, and pH. (b) Nitrification is driven by nitrifying bacteria within the guild of copiotrophic bacteria and the high nitrification indicator co-occurrence module, which in turn promotes the growth of other bacteria within these groups. Nitrification is also influenced positively by litter traits like litter depth, and negatively by ectomycorrhizal fungi, which inhibit the growth and activity of nitrifying bacteria, potentially through competition for ammonium, decreasing net nitrification rates. (c) Net phosphate change is driven by P-cycling oxidoreductases produced by a wide range of soil fungi and is inhibited by soil temperature. Illustration credit: Corinne Vietorisz.

ammonification rates of any variable (Figure 1a, Table 2), suggesting that these factors collectively control the release of ammonium from SOM (Figure 4a). In contrast, for net phosphate change, soil temperature was the only soil abiotic variable included in the BRTs or multivariate linear model (Figure 3a, Table 2). Soil P availability globally is negatively associated with temperature, potentially due to increased plant P uptake or reduced microbial P-decomposition activity under high temperatures (Hou et al., 2018; Hu et al., 2022; Unger et al., 2010). Microbial stress tolerance (e.g., heat tolerance) may trade off with nutrient acquisition activity (Malik et al., 2020), which could result in decreased P-decomposition activity under stressful temperatures. Surprisingly, for net nitrification, no soil abiotic variables were included in the multivariate linear model or were among the top variables contributing to the BRT (Figure 2a, Table 2). Only pH significantly correlated with net nitrification (Appendix S1: Figure S11), perhaps through affecting the abundances of pH-sensitive nitrifying microbes (Nicol et al., 2008; Stempfhuber et al., 2015). Because nitrification is a primarily microbe-mediated process (Stein & Klotz, 2016), microbial variables may overshadow soil variables that affect nitrification indirectly.

Multiple plant factors explained variance in nutrient cycling rates. Consistent with previous findings, arbuscular mycorrhizal (AM) tree basal area and litter depth (Figure 2a, Table 2) were especially important in explaining net nitrification rates and may affect nitrification indirectly through litter quality (Figure 4b). AM trees often have higher litter quality (e.g., low litter C:N; Laughlin, 2011) and increase soil inorganic N concentrations (Lin et al., 2017, 2018; Phillips et al., 2013), and nitrifier microbe abundances (Teutscherova et al., 2019). For net ammonification, root density was the third-most influential variable in the BRTs (Figure 1a), potentially through controlling the abundance and composition of EMF (Peay et al., 2011) or increasing the availability of C-rich root exudates which prime microbial decomposer activity (Dijkstra et al., 2013; Yin et al., 2018). For net phosphate change, pine sapling abundance was included in both the BRTs and final linear model as an important explanatory variable (Figure 3a, Table 2). Because pine saplings contributed negligible pine litter to the forest floor (Appendix S1: Figure S2), but can associate with a unique EMF community (Policelli et al., 2020), phosphate mineralization may be shaped by pine-associated EMF communities. Early-successional pine-associated EMF may promote P decomposition (Delucia et al., 1997), potentially by producing P-cycling enzymes and low-molecular-weight organic anions (Alvarez et al., 2012; Courty et al., 2006; Wang et al., 2021; Wang & Lambers, 2020) or by hosting bacteria with high phosphatase activity (Yuan et al., 2024).

Few factors explained net phosphate change and even the best multivariate linear model explained low variance (18%; Table 2). This may be because many of our samples had zero net phosphate change and low overall variance in rates. Northern temperate forests often have low concentrations of soil inorganic P, such that all P liberated from organic matter may be taken up by microbes and plants nearly immediately (Forstner et al., 2019; Sohrt et al., 2017), making it difficult to quantify gross rates of phosphate release from organic matter. Gross rates of P mineralization measured via isotopic tracing may be an especially valuable method to apply in these systems, as it can reveal microbial activity by quantifying P that is released from organic matter and rapidly immobilized. More studies examining the biotic drivers of P-cycling rates, especially gross rates of P mineralization or uptake via isotopic tracing, will improve our understanding of the mechanisms behind P cycling in temperate forests.

While our results apply directly to temperate, ectomycorrhizal-dominated forests, it remains unknown if similar factors are important predictors of nutrient cycling rates in other ecosystems. The patterns we found could be dependent on environmental conditions specific to northern temperate forests, such as acidic soils, intermediate soil moisture, and low rates of net nitrification and phosphate change. Indeed, other studies with similar findings, such as high EMF relative abundance corresponding with high rates of net N mineralization (Sorensen et al., 2019; Zhang et al., 2023) and low rates of net nitrification (Saifuddin et al., 2021; Tatsumi et al., 2020), are also from temperate forests. Nevertheless, our hypothesized framework of controls over soil nutrient cycling (Figure 4) could also apply to other types of ecosystems: N-limited boreal forests can have high abundances of oligotrophic bacteria and EMF, which can correspond with high organic matter turnover and/or N mineralization rates (Lindahl et al., 2021; Xiao et al., 2022). AM-dominated forests, especially tropical forests, typically are not N-limited and have high nitrification rates (Brookshire et al., 2012; Phillips et al., 2013), possibly due to a lack of competition between nitrifying bacteria and EMF for ammonium, faster litter decomposition (Lin et al., 2018), and potentially, abundant leaf litter inputs in high-productivity tropical forests that could select for copiotrophic bacteria.

Despite the limitations of DNA-based amplicon sequencing, our findings show that commonly measured microbial variables from amplicon sequencing are linked to soil nutrient cycling rates in northern temperate forests. Community-level genetic capacity for specific nutrient transformations can be measured via qPCR of particular genes (e.g., *amoA*), though the abundances of single marker genes rarely correlate with nutrient cycling processes (only 38% of the time, Rocca et al., 2015), and the taxonomic composition of marker genes is unknown. Estimating community- and taxon-level genomic capacity for nutrient transformations—as we did in this study—may correlate better with process rates if this method captures more complete microbial metabolic pathways than measuring single marker genes. This may be especially true for processes performed by diverse groups of enzymes, like the release of ammonium or phosphate from organic matter (Albright et al., 2019; Jarosch et al., 2019). However, many microbial nutrient cycling pathways remain unannotated (Albright et al., 2019) and functional gene abundances may not always reflect gene expression and enzyme activity. PICRUSt2 or similar methods for fungi (Anthony et al., 2022) likely underestimate certain gene groups, including nitrification genes that were not found in our dataset, a phenomenon that has been observed in other studies (Tatsumi et al., 2023). For fungi, the relatively small number of available fungal genomes means we must assign most gene counts at the genus level, which ignores species-level variation in gene content (Bödeker et al., 2014; Lofgren et al., 2025). When calculating community-level abundances of certain genes, such as fungal P-cycling oxidoreductases, these estimates are biased toward fungal lineages that have more sequenced genomes. Gene expression data (e.g., meta-transcriptomic data) may better reflect functional activity, but gene counts can reflect accumulated functional potential over evolutionary timescales. Our analyses show that even conservative estimates of functional gene abundances can significantly correlate with nutrient cycling rates and may be useful for predicting flux rates in areas where only soil microbial community composition data are available.

CONCLUSIONS

Through the first comprehensive test comparing a broad range of microbial community traits, we found that the relative abundances of functional guilds and ICMs explain soil net ammonification and nitrification rates, while the estimated relative abundance of fungal P-cycling genes explains soil net phosphate change rates at our sites. These results suggest a new conceptual model for these biogeochemical processes (Figure 4), where net ammonification is driven by soil abiotic factors, EMF, and oligotrophic bacteria; net nitrification is coupled to copiotrophic bacteria, the bacterial high nitrification ICM, and litter traits; and net phosphate release is driven by fungal oxidoreductases and soil temperature. This analysis is a key step toward distilling the complexity of microbial communities into quantifiable terms explaining and predicting ecosystem function that could improve predictions of nutrient movement through ecosystems.

AUTHOR CONTRIBUTIONS

Corinne R. Vietorisz, Nahuel Policelli, and Jennifer M. Bhatnagar established sampling sites and design. Corinne R. Vietorisz, Nahuel Policelli, Lindsey A. Adams, and Jennifer M. Bhatnagar conducted field sampling. Corinne R. Vietorisz, Nahuel Policelli, Abigail Li, and Lindsey A. Adams performed laboratory analysis on samples. Corinne R. Vietorisz, Jennifer M. Bhatnagar, and Kathryn F. Atherton conducted data analysis. Corinne R. Vietorisz and Jennifer M. Bhatnagar wrote the first draft of the manuscript, and all authors edited the manuscript.

ACKNOWLEDGMENTS

We would like to thank members of the Bhatnagar lab for their assistance with field work, manuscript edits, and laboratory analysis, especially Chikae Tatsumi, Michael Silverstein, Zoey Werbin, Victoria Moscato, Katherine Sotiropoulou, Long Hong, Li Lin, and Anthony Avila. We also thank those who gave us permission to sample at the field sites, including the Harvard Forest, the Newton Massachusetts Conservation Commission, the Burlington Massachusetts Select Board, the Lexington Massachusetts Conservation Commission, and the Maronite Monks of Adoration in Petersham, Massachusetts. We thank Drs. Peter Kennedy and Richard Primack for their helpful feedback on drafts of this manuscript. This research was funded by the Department of Energy Biological and Environmental Research awards DE-SC0020403 and DE-SC0012704 to Jennifer M. Bhatnagar, an National Science Foundation Graduate Research Fellowship Program award (10.13039/100023581, solicitation 19-590) to Corinne R. Vietorisz, and Research Awards from Boston University's Graduate Student Organization and Biogeoscience program to Corinne R. Vietorisz.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data (Bhatnagar et al., 2025) are available from the Environmental Data Initiative (EDI) Data Portal: https://doi.org/10.6073/pasta/a387b147236481983365b6c01a47c2b1. Processed data frames used for statistical analysis (Vietorisz, 2025) are available from Zenodo: https://doi.org/10.5281/zenodo.17364155. Raw DNA sequence files for the ITS2 and 16S datasets are available from the National Center for Biotechnology Information (NCBI): BioProject accession PRJNA1345857.

ORCID

Corinne R. Vietorisz https://orcid.org/0000-0002-5577-4922

Jennifer M. Bhatnagar https://orcid.org/0000-0001-6424-4133

REFERENCES

Agerer, R. 2001. "Exploration Types of Ectomycorrhizae." *Mycorrhiza* 11(2): 107–114.

Albright, M. B. N., B. Timalsina, J. B. H. Martiny, and J. Dunbar. 2019. "Comparative Genomics of Nitrogen Cycling Pathways in Bacteria and Archaea." *Microbial Ecology* 77(3): 597–606.

Allen, T., K. A. Murray, C. Zambrana-Torrelio, S. S. Morse, C. Rondinini, M. Di Marco, N. Breit, K. J. Olival, and P. Daszak. 2017. "Global Hotspots and Correlates of Emerging Zoonotic Diseases." *Nature Communications* 8(1): 1124.

Alvarez, M., D. Huygens, L. M. Díaz, C. A. Villanueva, W. Heyser, and P. Boeckx. 2012. "The Spatial Distribution of Acid Phosphatase Activity in Ectomycorrhizal Tissues Depends on Soil Fertility and Morphotype, and Relates to Host Plant Phosphorus Uptake." *Plant, Cell & Environment* 35(1): 126–135.

Anthony, M. A., T. W. Crowther, S. van der Linde, L. M. Suz, M. I. Bidartondo, F. Cox, M. Schaub, et al. 2022. "Forest Tree Growth Is Linked to Mycorrhizal Fungal Composition and Function across Europe." *The ISME Journal* 16(5): 1327–36.

Anthony, M. A., S. D. Frey, and K. A. Stinson. 2017. "Fungal Community Homogenization, Shift in Dominant Trophic Guild, and Appearance of Novel Taxa with Biotic Invasion." *Ecosphere* 8(9): e01951.

Argiroff, W. A., D. R. Zak, P. T. Pellitier, R. A. Upchurch, and J. P. Belke. 2022. “Decay by Ectomycorrhizal Fungi Couples Soil Organic Matter to Nitrogen Availability.” *Ecology Letters* 25(2): 391–404.

Atherton, K. F., Z. R. Werbin, D. Segrè, and J. M. Bhatnagar. Forthcoming. “MycoMetaGenome Predictor: A Pipeline for Predicting Fungal Metagenomes from Taxonomic Composition.” https://github.com/k-atherton/picrust-for-fungi (in preparation).

Auer, L., M. Buée, L. Fauchery, V. Lombard, K. W. Barry, A. Clum, A. Copeland, et al. 2024. “Metatranscriptomics Sheds Light on the Links between the Functional Traits of Fungal Guilds and Ecological Processes in Forest Soil Ecosystems.” *New Phytologist* 242(4): 1676–90.

Averill, C., Z. R. Werbin, K. F. Atherton, J. M. Bhatnagar, and M. C. Dietze. 2021. “Soil Microbiome Predictability Increases with Spatial and Taxonomic Scale.” *Nature Ecology & Evolution* 5(6): 747–756.

Bartoń, K. 2024. “MuMIn: Multi-Model Inference.” https://cran.r-project.org/web/packages/MuMIn/index.html.

Bates, D., M. Mächler, B. Bolker, and S. Walker. 2015. “Fitting Linear Mixed-Effects Models Using Lme4.” *Journal of Statistical Software* 67(October): 1–48.

Bell, T., J. A. Newman, B. W. Silverman, S. L. Turner, and A. K. Lilley. 2005. “The Contribution of Species Richness and Composition to Bacterial Services.” *Nature* 436(7054): 1157–60.

Belova, S. E., N. V. Ravin, T. A. Pankratov, A. L. Rakitin, A. A. Ivanova, A. V. Beletsky, A. V. Mardanov, J. S. Sinninghe Damsté, and S. N. Dedysh. 2018. “Hydrolytic Capabilities as a Key to Environmental Success: Chitinolytic and Cellulolytic Acidobacteria from Acidic Sub-Arctic Soils and Boreal Peatlands.” *Frontiers in Microbiology* 9(November): 2775.

Bhatnagar, J., C. Vietorisz, and N. Policelli. 2025. “Microbial, Plant, and Soil Impacts on Soil Nutrient Cycling in Harvard Forest and Greater Boston 2021–2022.” Harvard Forest Data Archive: HF456 (v.1). Environmental Data Initiative. https://doi.org/10.6073/pasta/a387b147236481983365b6c01a47c2b1.

Bödeker, I. T. M., K. E. Clemmensen, W. de Boer, F. Martin, Å. Olson, and B. D. Lindahl. 2014. “Ectomycorrhizal Cortinarius Species Participate in Enzymatic Oxidation of Humus in Northern Forest Ecosystems.” *New Phytologist* 203(1): 245–256.

Braun-Blanquet, J. 1932. *Plant Sociology: The Study of Plant Communities*, 1st ed. New York and London: McGraw-Hill book Company, Inc.

Brookshire, E. N. J., S. Gerber, D. N. L. Menge, and L. O. Hedin. 2012. “Large Losses of Inorganic Nitrogen from Tropical Rainforests Suggest a Lack of Nitrogen Limitation.” *Ecology Letters* 15(1): 9–16.

Callahan, B. J., P. J. McMurdie, M. J. Rosen, A. W. Han, A. J. A. Johnson, and S. P. Holmes. 2016. “DADA2: High-Resolution Sample Inference from Illumina Amplicon Data.” *Nature Methods* 13(7): 581–83.

Caporaso, J. G., C. L. Lauber, W. A. Walters, D. Berg-Lyons, C. A. Lozupone, P. J. Turnbaugh, N. Fierer, and R. Knight. 2011. “Global Patterns of 16S rRNA Diversity at a Depth of Millions of Sequences per Sample.” *Proceedings of the National Academy of Sciences of the United States of America* 108(Suppl 1): 4516–22.

Caron, S., S. Garvey, J. Gewirtzman, K. Schultz, J. Bhatnagar, C. Driscoll, L. Hutyra, and P. Templer. 2023. “Urbanization and Fragmentation Have Opposing Effects on Soil Nitrogen Availability in Temperate Forest Ecosystems.” *Global Change Biology* 29(January): 2156–71.

Chen, Q.-L., J. Ding, C.-Y. Li, Z.-Z. Yan, J.-Z. He, and H.-W. Hu. 2020. “Microbial Functional Attributes, Rather than Taxonomic Attributes, Drive Top Soil Respiration, Nitrification and Denitrification Processes.” *Science of the Total Environment* 734(September): 139479.

Chen, W., R. T. Koide, and D. M. Eissenstat. 2018. “Nutrient Foraging by Mycorrhizas: From Species Functional Traits to Ecosystem Processes.” *Functional Ecology* 32(4): 858–869.

Courty, P.-E., R. Pouysegur, M. Buée, and J. Garbaye. 2006. “Laccase and Phosphatase Activities of the Dominant Ectomycorrhizal Types in a Lowland Oak Forest.” *Soil Biology and Biochemistry* 38(6): 1219–22.

De’ath, G. 2007. “Boosted Trees for Ecological Modeling and Prediction.” *Ecology* 88(1): 243–251.

Delucia, E. H., R. M. Callaway, E. M. Thomas, and W. H. Schlesinger. 1997. “Mechanisms of Phosphorus Acquisition for Ponderosa Pine Seedlings under High CO_2 and Temperature.” *Annals of Botany* 79(2): 111–120.

Dijkstra, F. A., Y. Carrillo, E. Pendall, and J. A. Morgan. 2013. “Rhizosphere Priming: A Nutrient Perspective.” *Frontiers in Microbiology* 4(July): 216.

Douglas, G. M., V. J. Maffei, J. R. Zaneveld, S. N. Yurgel, J. R. Brown, C. M. Taylor, C. Huttenhower, and M. G. I. Langille. 2020. “PICRUSt2 for Prediction of Metagenome Functions.” *Nature Biotechnology* 38(6): 685–88.

Dragone, N. B., M. Hoffert, M. S. Strickland, and N. Fierer. 2024. “Taxonomic and Genomic Attributes of Oligotrophic Soil Bacteria.” *ISME Communications* 4(1): ycae081.

Durán, J., J. L. Morse, and P. M. Groffman. 2012. “Comparison of *In Situ* Methods to Measure N Mineralization Rates in Forest Soils.” *Soil Biology and Biochemistry* 46(March): 145–47.

Elith, J., J. R. Leathwick, and T. Hastie. 2008. “A Working Guide to Boosted Regression Trees.” *Journal of Animal Ecology* 77(4): 802–813.

Elrys, A. S., A. Ali, H. Zhang, Y. Cheng, J. Zhang, Z.-C. Cai, C. Müller, and S. X. Chang. 2021. “Patterns and Drivers of Global Gross Nitrogen Mineralization in Soils.” *Global Change Biology* 27(22): 5950–62.

Eno, C. F. 1960. “Nitrate Production in the Field by Incubating the Soil in Polyethylene Bags.” *Soil Science Society of America Journal* 24(4): 277–79.

Ewens, S. D., A. F. S. Gomberg, T. P. Barnum, M. A. Borton, H. K. Carlson, K. C. Wrighton, and J. D. Coates. 2021. “The Diversity and Evolution of Microbial Dissimilatory Phosphite Oxidation.” *Proceedings of the National Academy of Sciences of the United States of America* 118(11): e2020024118.

Fierer, N., M. A. Bradford, and R. B. Jackson. 2007. “Toward an Ecological Classification of Soil Bacteria.” *Ecology* 88(6): 1354–64.

Finzi, A. C., R. Z. Abramoff, K. S. Spiller, E. R. Brzostek, B. A. Darby, M. A. Kramer, and R. P. Phillips. 2015. “Rhizosphere Processes Are Quantitatively Important Components of Terrestrial Carbon and Nutrient Cycles.” *Global Change Biology* 21(5): 2082–94.

Forstner, S. J., V. Wechselberger, S. Stecher, S. Müller, K. M. Keiblinger, W. Wanek, P. Schleppi, et al. 2019. "Resistant Soil Microbial Communities Show Signs of Increasing Phosphorus Limitation in Two Temperate Forests after Long-Term Nitrogen Addition." *Frontiers in Forests and Global Change* 2 (December): 73.

Frank, K., D. Beegle, and J. Denning. 1998. "Phosphorus." In *Recommended Chemical Soil Test Procedures for the North Central Region No. 221 (Revised)*, edited by J. R. Brown, 21–30. Columbia, MO: North Central Regional Research Publication.

Gloor, G., J. Macklaim, V. Pawlowsky-Glahn, and J. J. Egozcue. 2017. "Microbiome Datasets Are Compositional: And this Is Not Optional." *Frontiers in Microbiology* 8(November): 2224.

Graham, E. B., J. E. Knelman, A. Schindlbacher, S. Siciliano, M. Breulmann, A. Yannarell, J. M. Beman, et al. 2016. "Microbes as Engines of Ecosystem Function: When Does Community Structure Enhance Predictions of Ecosystem Processes?" *Frontiers in Microbiology* 7: 214.

Graham, E. B., W. R. Wieder, J. W. Leff, S. R. Weintraub, A. R. Townsend, C. C. Cleveland, L. Philippot, and D. R. Nemergut. 2014. "Do We Need to Understand Microbial Communities to Predict Ecosystem Function? A Comparison of Statistical Models of Nitrogen Cycling Processes." *Soil Biology and Biochemistry* 68(January): 279–282.

Groffman, P. M., C. T. Driscoll, J. Durán, J. L. Campbell, L. M. Christenson, T. J. Fahey, M. C. Fisk, et al. 2018. "Nitrogen Oligotrophication in Northern Hardwood Forests." *Biogeochemistry* 141(3): 523–539.

Guidi, L., S. Chaffron, L. Bittner, D. Eveillard, A. Larhlimi, S. Roux, Y. Darzi, et al. 2016. "Plankton Networks Driving Carbon Export in the Oligotrophic Ocean." *Nature* 532(7600): 465–470.

Hanselman, T. A., D. A. Graetz, and T. A. Obreza. 2004. "A Comparison of In Situ Methods for Measuring Net Nitrogen Mineralization Rates of Organic Soil Amendments." *Journal of Environmental Quality* 33(3): 1098–1105.

Harrell, F. E., Jr., and C. Dupont. 2024. "Hmisc: Harrell Miscellaneous." https://cran.r-project.org/web/packages/Hmisc/index.html.

Hartig, F. 2022. "DHARMa: Residual Diagnostics for Hierarchical (Multi-Level/Mixed) Regression Models." https://cran.r-project.org/web/packages/DHARMa/index.html.

Ho, A., D. P. Di Lonardo, and P. L. E. Bodelier. 2017. "Revisiting Life Strategy Concepts in Environmental Microbial Ecology." *FEMS Microbiology Ecology* 93(3): fix006.

Hobbie, E., and R. Agerer. 2009. "Nitrogen Isotopes in Ectomycorrhizal Sporocarps Correspond to Belowground Exploration Types." *Plant and Soil* 327(June): 71–83.

Hobbie, S. E. 2015. "Plant Species Effects on Nutrient Cycling: Revisiting Litter Feedbacks." *Trends in Ecology & Evolution* 30 (6): 357–363.

Hou, E., C. Chen, Y. Luo, G. Zhou, Y. Kuang, Y. Zhang, M. Heenan, X. Lu, and D. Wen. 2018. "Effects of Climate on Soil Phosphorus Cycle and Availability in Natural Terrestrial Ecosystems." *Global Change Biology* 24(8): 3344–56.

Hu, W., J. Tan, X. Shi, T. R. Lock, R. L. Kallenbach, and Z. Yuan. 2022. "Nutrient Addition and Warming Alter the Soil Phosphorus Cycle in Grasslands: A Global Meta-Analysis." *Journal of Soils and Sediments* 22(10): 2608–19.

Isobe, K., N. Ohte, T. Oda, S. Murabayashi, W. Wei, K. Senoo, N. Tokuchi, and R. Tateno. 2015. "Microbial Regulation of Nitrogen Dynamics along the Hillslope of a Natural Forest." *Frontiers in Environmental Science* 2: 63.

Jarosch, K. A., E. Kandeler, E. Frossard, and E. K. Bünemann. 2019. "Is the Enzymatic Hydrolysis of Soil Organic Phosphorus Compounds Limited by Enzyme or Substrate Availability?" *Soil Biology and Biochemistry* 139(December): 107628.

Kalam, S., A. Basu, I. Ahmad, R. Z. Sayyed, H. A. El-Enshasy, D. J. Dailin, and N. L. Suriani. 2020. "Recent Understanding of Soil Acidobacteria and their Ecological Significance: A Critical Review." *Frontiers in Microbiology* 11(October): 580024.

Kapoor, V., X. Li, K. Chandran, C. A. Impellitteri, and J. W. Santo Domingo. 2016. "Use of Functional Gene Expression and Respirometry to Study Wastewater Nitrification Activity after Exposure to Low Doses of Copper." *Environmental Science and Pollution Research* 23(7): 6443–50.

Kehler, A., P. Haygarth, F. Tamburini, and M. Blackwell. 2021. "Cycling of Reduced Phosphorus Compounds in Soil and Potential Impacts of Climate Change." *European Journal of Soil Science* 72(6): 2517–37.

Langfelder, P., and S. Horvath. 2008. "WGCNA: An R Package for Weighted Correlation Network Analysis." *BMC Bioinformatics* 9(1): 559.

Langfelder, P., and S. Horvath. 2012. "Fast R Functions for Robust Correlations and Hierarchical Clustering." *Journal of Statistical Software* 46(March): 1–17.

Laughlin, D. C. 2011. "Nitrification Is Linked to Dominant Leaf Traits Rather than Functional Diversity." *Journal of Ecology* 99 (5): 1091–99.

Liao, T., S. Wang, H. Zhang, E. E. Stüeken, and H. Luo. 2024. "Dating Ammonia-Oxidizing Bacteria with Abundant Eukaryotic Fossils." *Molecular Biology and Evolution* 41(5): msae096.

Lin, G., D. Guo, L. Li, C. Ma, and D.-H. Zeng. 2018. "Contrasting Effects of Ectomycorrhizal and Arbuscular Mycorrhizal Tropical Tree Species on Soil Nitrogen Cycling: The Potential Mechanisms and Corresponding Adaptive Strategies." *Oikos* 127(4): 518–530.

Lin, G., M. L. McCormack, C. Ma, and D. Guo. 2017. "Similar Below-Ground Carbon Cycling Dynamics but Contrasting Modes of Nitrogen Cycling between Arbuscular Mycorrhizal and Ectomycorrhizal Forests." *New Phytologist* 213(3): 1440–51.

Lindahl, B. D., J. Kyaschenko, K. Varenius, K. E. Clemmensen, A. Dahlberg, E. Karltun, and J. Stendahl. 2021. "A Group of Ectomycorrhizal Fungi Restricts Organic Matter Accumulation in Boreal Forest." *Ecology Letters* 24(7): 1341–51.

Lindahl, B. D., and A. Tunlid. 2015. "Ectomycorrhizal Fungi – Potential Organic Matter Decomposers, Yet Not Saprotrophs." *New Phytologist* 205(4): 1443–47.

Lofgren, L., F. Maillard, T. Michaud, A. Gredeby, A. Tunlid, and P. G. Kennedy. 2025. "Diverse Nitrogen Acquisition Strategies of Conifer-Associated Ectomycorrhizal Fungi Shape Unique Responses to Changing Nitrogen Regimes." *Frontiers in Plant Science* 16(September): 1666003.

Maillard, F., A. Kohler, E. Morin, C. Hossann, S. Miyauchi, I. Ziegler-Devin, D. Gérant, et al. 2023. "Functional Genomics Gives New Insights into the Ectomycorrhizal Degradation of Chitin." *New Phytologist* 238(2): 845–858.

Malik, A. A., J. B. H. Martiny, E. L. Brodie, A. C. Martiny, K. K. Treseder, and S. D. Allison. 2020. "Defining Trait-Based Microbial Strategies with Consequences for Soil Carbon Cycling under Climate Change." *The ISME Journal* 14(1): 1–9.

Mallows, C. L. 1973. "Some Comments on CP." *Technometrics* 15 (4): 661–675.

Matteucci, S., and A. Colma. 1982. *Metodología Para El Estudio de La Vegetación*. Washington, D.C.: SERBIULA (Sistema Librum 2.0).

Maynard, D. S., K. R. Covey, T. W. Crowther, N. W. Sokol, E. W. Morrison, S. D. Frey, L. T. A. van Diepen, and M. A. Bradford. 2018. "Species Associations Overwhelm Abiotic Conditions to Dictate the Structure and Function of Wood-Decay Fungal Communities." *Ecology* 99(4): 801–811.

McClure, R., Y. Farris, R. Danczak, W. Nelson, H.-S. Song, A. Kessell, J.-Y. Lee, et al. 2022. "Interaction Networks Are Driven by Community-Responsive Phenotypes in a Chitin-Degrading Consortium of Soil Microbes." *mSystems* 7 (5): e00372-22.

Miller, T. L. 2024. "Leaps: Regression Subset Selection." https://cran.r-project.org/web/packages/leaps/index.html.

Naylor, D., N. Sadler, A. Bhattacharjee, E. B. Graham, C. R. Anderton, R. McClure, M. Lipton, K. S. Hofmockel, and J. K. Jansson. 2020. "Soil Microbiomes under Climate Change and Implications for Carbon Cycling." *Annual Review of Environment and Resources* 45: 29–59.

Nicol, G. W., S. Leininger, C. Schleper, and J. I. Prosser. 2008. "The Influence of Soil pH on the Diversity, Abundance and Transcriptional Activity of Ammonia Oxidizing Archaea and Bacteria." *Environmental Microbiology* 10(11): 2966–78.

Nicolás, C., T. Martin-Bertelsen, D. Floudas, J. Bentzer, M. Smits, T. Johansson, C. Troein, P. Persson, and A. Tunlid. 2019. "The Soil Organic Matter Decomposition Mechanisms in Ectomycorrhizal Fungi Are Tuned for Liberating Soil Organic Nitrogen." *The ISME Journal* 13(4): 977–988.

Olsen, S. R. 1954. *Estimation of Available Phosphorus in Soils by Extraction with Sodium Bicarbonate*. Washington, D.C.: U.S. Department of Agriculture.

Peay, K. G., P. G. Kennedy, and T. D. Bruns. 2011. "Rethinking Ectomycorrhizal Succession: Are Root Density and Hyphal Exploration Types Drivers of Spatial and Temporal Zonation?" *Fungal Ecology* 4(3): 233–240.

Petersen, D. G., S. J. Blazewicz, M. Firestone, D. J. Herman, M. Turetsky, and M. Waldrop. 2012. "Abundance of Microbial Genes Associated with Nitrogen Cycling as Indices of Biogeochemical Process Rates across a Vegetation Gradient in Alaska." *Environmental Microbiology* 14(4): 993–1008.

Philippot, L., A. Spor, C. Hénault, D. Bru, F. Bizouard, C. M. Jones, A. Sarr, and P.-A. Maron. 2013. "Loss in Microbial Diversity Affects Nitrogen Cycling in Soil." *The ISME Journal* 7(8): 1609–19.

Phillips, R. P., E. Brzostek, and M. G. Midgley. 2013. "The Mycorrhizal-Associated Nutrient Economy: A New Framework for Predicting Carbon–Nutrient Couplings in Temperate Forests." *New Phytologist* 199(1): 41–51.

Pielou, E. C. 1966. "The Measurement of Diversity in Different Types of Biological Collections." *Journal of Theoretical Biology* 13: 131–144.

Plassard, C., J. Louche, M. A. Ali, M. Duchemin, E. Legname, and B. Cloutier-Hurteau. 2011. "Diversity in Phosphorus Mobilisation and Uptake in Ectomycorrhizal Fungi." *Annals of Forest Science* 68(1): 33–43.

Policelli, N., T. R. Horton, R. A. García, M. Naour, A. Pauchard, and M. A. Nuñez. 2020. "Native and Non-Native Trees Can Find Compatible Mycorrhizal Partners in each Other's Dominated Areas." *Plant and Soil* 454(1–2): 285–297.

Põlme, S., K. Abarenkov, R. H. Nilsson, B. D. Lindahl, K. E. Clemmensen, H. Kauserud, N. Nguyen, et al. 2020. "FungalTraits: A User-Friendly Traits Database of Fungi and Fungus-Like Stramenopiles." *Fungal Diversity* 105(1): 1–16.

R Core Team. 2024. *R: A Language and Environment for Statistical Computing*. Vienna: R Foundation for Statistical Computing.

Rath, J., C. Schiller, and G. Herndl. 1993. "Ectoenzymatic Activity and Bacterial Dynamics along a Trophic Gradient in the Caribbean Sea." *Marine Ecology Progress Series* 95(December): 89–96.

Ratzke, C., J. Barrere, and J. Gore. 2020. "Strength of Species Interactions Determines Biodiversity and Stability in Microbial Communities." *Nature Ecology & Evolution* 4(3): 376–383.

Rocca, J. D., E. K. Hall, J. T. Lennon, S. E. Evans, M. P. Waldrop, J. B. Cotner, D. R. Nemergut, E. B. Graham, and M. D. Wallenstein. 2015. "Relationships between Protein-Encoding Gene Abundance and Corresponding Process Are Commonly Assumed Yet Rarely Observed." *The ISME Journal* 9(8): 1693–99.

Romero-Olivares, A. L., E. W. Morrison, A. Pringle, and S. D. Frey. 2021. "Linking Genes to Traits in Fungi." *Microbial Ecology* 82 (1): 145–155.

Saifuddin, M., J. M. Bhatnagar, R. P. Phillips, and A. C. Finzi. 2021. "Ectomycorrhizal Fungi Are Associated with Reduced Nitrogen Cycling Rates in Temperate Forest Soils without Corresponding Trends in Bacterial Functional Groups." *Oecologia* 196(3): 863–875.

Salonius, P. O. 1981. "Metabolic Capabilities of Forest Soil Microbial Populations with Reduced Species Diversity." *Soil Biology and Biochemistry* 13(1): 1–10.

Schneider, T., K. M. Keiblinger, E. Schmid, K. Sterflinger-Gleixner, G. Ellersdorfer, B. Roschitzki, A. Richter, L. Eberl, S. Zechmeister-Boltenstern, and K. Riedel. 2012. "Who Is Who in Litter Decomposition? Metaproteomics Reveals Major Microbial Players and their Biogeochemical Functions." *The ISME Journal* 6(9): 1749–62.

Scott, N. A., and D. Binkley. 1997. "Foliage Litter Quality and Annual Net N Mineralization: Comparison across North American Forest Sites." *Oecologia* 111(2): 151–59.

Setälä, H., and M. A. McLean. 2004. "Decomposition Rate of Organic Substrates in Relation to the Species Diversity of Soil Saprophytic Fungi." *Oecologia* 139(1): 98–107.

Smith, S. E., and D. J. Read. 2008. *Mycorrhizal Symbiosis*. London: Academic Press.

Sohrt, J., F. Lang, and M. Weiler. 2017. "Quantifying Components of the Phosphorus Cycle in Temperate Forests." *WIREs Water* 4(6): e1243.

Sorensen, P. O., J. M. Bhatnagar, L. Christenson, J. Duran, T. Fahey, M. C. Fisk, A. C. Finzi, P. M. Groffman, J. L. Morse, and P. H. Templer. 2019. “Roots Mediate the Effects of Snowpack Decline on Soil Bacteria, Fungi, and Nitrogen Cycling in a Northern Hardwood Forest.” *Frontiers in Microbiology* 10: 926.

Stein, L. Y., and M. G. Klotz. 2016. “The Nitrogen Cycle.” *Current Biology* 26(3): R94–R98.

Stempfhuber, B., M. Engel, D. Fischer, G. Neskovic-Prit, T. Wubet, I. Schöning, C. Gubry-Rangin, et al. 2015. “pH as a Driver for Ammonia-Oxidizing Archaea in Forest Soils.” *Microbial Ecology* 69(4): 879–883.

Sun, D., M. Kotianová, M. Rozmoš, H. Hršelová, P. Bukovská, and J. Jansa. 2023. “Arbuscular Mycorrhizal Hyphae Selectively Suppress Soil Ammonia Oxidizers – But Probably Not by Production of Biological Nitrification Inhibitors.” *Plant and Soil* 491(1): 627–643.

Talbot, J. M., S. D. Allison, and K. K. Treseder. 2008. “Decomposers in Disguise: Mycorrhizal Fungi as Regulators of Soil C Dynamics in Ecosystems under Global Change.” *Functional Ecology* 22(6): 955–963.

Talbot, J. M., F. Martin, A. Kohler, B. Henrissat, and K. G. Peay. 2015. “Functional Guild Classification Predicts the Enzymatic Role of Fungi in Litter and Soil Biogeochemistry.” *Soil Biology and Biochemistry* 88(September): 441–456.

Tang, Y., G. Yu, X. Zhang, Q. Wang, D. Tian, J. Tian, S. Niu, and J. Ge. 2019. “Environmental Variables Better Explain Changes in Potential Nitrification and Denitrification Activities than Microbial Properties in Fertilized Forest Soils.” *Science of the Total Environment* 647(January): 653–662.

Tatsumi, C., K. F. Atherton, S. M. Garvey, E. Conrad-Rooney, L. L. Morreale, L. R. Hutyra, P. H. Templer, and J. M. Bhatnagar. 2023. “Urbanization and Edge Effects Interact to Drive Mutualism Breakdown and the Rise of Unstable Pathogenic Communities in Forest Soil.” *Proceedings of the National Academy of Sciences of the United States of America* 120(36): e2307519120.

Tatsumi, C., T. Taniguchi, S. Du, N. Yamanaka, and R. Tateno. 2020. “Soil Nitrogen Cycling Is Determined by the Competition between Mycorrhiza and Ammonia-Oxidizing Prokaryotes.” *Ecology* 101(3): e02963.

Teutscherova, N., E. Vazquez, J. Arango, A. Arevalo, M. Benito, and M. Pulleman. 2019. “Native Arbuscular Mycorrhizal Fungi Increase the Abundance of Ammonia-Oxidizing Bacteria, but Suppress Nitrous Oxide Emissions Shortly after Urea Application.” *Geoderma* 338(March): 493–501.

Thomas, K. D., and C. E. Prescott. 2000. “Nitrogen Availability in Forest Floors of Three Tree Species on the Same Site: The Role of Litter Quality.” *Canadian Journal of Forest Research* 30(11): 1698–1706.

U.S. Environmental Protection Agency. 2007. “Method 3051A (SW-846): Microwave Assisted Acid Digestion of Sediments, Sludges, and Oils, Revision 1. Washington, DC.” https://www.epa.gov/esam/us-epa-method-3051a-microwave-assisted-acid-digestion-sediments-sludges-and-oils

Unger, M., C. Leuschner, and J. Homeier. 2010. “Variability of Indices of Macronutrient Availability in Soils at Different Spatial Scales along an Elevation Transect in Tropical Moist Forests (NE Ecuador).” *Plant and Soil* 336(1): 443–458.

Vietorisz, C. 2025. “crvietorisz/MA_forest_NPcycling: MA_forest_NPcycling_Publication (v1.0).” Zenodo. https://doi.org/10.5281/zenodo.17364156.

Wagg, C., K. Schlaeppi, S. Banerjee, E. E. Kuramae, and M. G. A. van der Heijden. 2019. “Fungal-Bacterial Diversity and Microbiome Complexity Predict Ecosystem Functioning.” *Nature Communications* 10(1): 4841.

Wang, Y., and H. Lambers. 2020. “Root-Released Organic Anions in Response to Low Phosphorus Availability: Recent Progress, Challenges and Future Perspectives.” *Plant and Soil* 447(1): 135–156.

Wang, Y., R. Wang, B. Lu, A. Guerin-Laguette, X. He, and F. Yu. 2021. “Mycorrhization of Quercus Mongolica Seedlings by Tuber Melanosporum Alters Root Carbon Exudation and Rhizosphere Bacterial Communities.” *Plant and Soil* 467(1): 391–403.

Weiss, S., Z. Z. Xu, S. Peddada, A. Amir, K. Bittinger, A. Gonzalez, C. Lozupone, et al. 2017. “Normalization and Microbial Differential Abundance Strategies Depend upon Data Characteristics.” *Microbiome* 5(1): 27.

Westermann, D. T., and S. E. Crothers. 1980. “Measuring Soil Nitrogen Mineralization under Field Conditions.” *Agronomy Journal* 72(6): 1009–12.

White, A. K., and W. W. Metcalf. 2007. “Microbial Metabolism of Reduced Phosphorus Compounds.” *Annual Review of Microbiology* 61: 379–400.

White, T., T. Bruns, S. Lee, J. Taylor, M. Innis, D. Gelfand, and J. Sninsky. 1990. “Amplification and Direct Sequencing of Fungal Ribosomal RNA Genes for Phylogenetics.” In *PCR Protocols: A Guide to Methods and Applications*, edited by Michael A. Innis, David H. Gelfand, John J. Sninsky, and Thomas J. White, Vol. 31, 315–322. New York, NY: Academic Press.

Xiao, R., X. Man, B. Duan, T. Cai, Z. Ge, X. Li, and T. Vesala. 2022. “Changes in Soil Bacterial Communities and Nitrogen Mineralization with Understory Vegetation in Boreal Larch Forests.” *Soil Biology and Biochemistry* 166(March): 108572.

Yin, L., F. A. Dijkstra, P. Wang, B. Zhu, and W. Cheng. 2018. “Rhizosphere Priming Effects on Soil Carbon and Nitrogen Dynamics among Tree Species with and without Intraspecific Competition.” *The New Phytologist* 218(3): 1036–48.

Yuan, J., R. Yan, X. Zhang, K. Su, H. Liu, X. Wei, R. Wang, et al. 2024. “Soil Organic Phosphorus Is Mainly Hydrolyzed Via Phosphatases from Ectomycorrhiza-Associated Bacteria Rather than Ectomycorrhizal Fungi.” *Plant and Soil* 504 (April): 659–678.

Zak, D. R., P. T. Pellitier, W. A. Argiroff, B. Castillo, T. Y. James, L. E. Nave, C. Averill, et al. 2019. “Exploring the Role of Ectomycorrhizal Fungi in Soil Carbon Dynamics.” *New Phytologist* 223(1): 33–39.

Zeng, J., Q. Tu, X. Yu, L. Qian, C. Wang, L. Shu, F. Liu, et al. 2022. “PCycDB: A Comprehensive and Accurate Database for Fast Analysis of Phosphorus Cycling Genes.” *Microbiome* 10(1): 101.

Zhang, G., J. Huang, M. Jia, F. Liu, Y. Yang, Z. Wang, and G. Han. 2019. “Ammonia-Oxidizing Bacteria and Archaea: Response to Simulated Climate Warming and Nitrogen Supplementation.” *Soil Science Society of America Journal* 83(6): 1683–95.

Zhang, M., P. J. O’Connor, J. Zhang, and X. Ye. 2021. “Linking Soil Nutrient Cycling and Microbial Community with Vegetation Cover in Riparian Zone.” *Geoderma* 384(February): 114801.

Zhang, M., S. Liu, X. Cao, M. Chen, J. Chen, G. Xu, and Z. Shi. 2023. “The Effects of Ectomycorrhizal and Saprotropic Fungi on Soil Nitrogen Mineralization Differ from those of Arbuscular and Ericoid Mycorrhizal Fungi on the Eastern

Qinghai-Tibetan Plateau." *Frontiers in Plant Science* 13 (January): 1069730.

Zhou, Z., T. Yan, Q. Zhu, X. Bu, B. Chen, J. Xue, and Y. Wu. 2019. "Bacterial Community Structure Shifts Induced by Biochar Amendment to Karst Calcareous Soil in Southwestern Areas of China." *Journal of Soils and Sediments* 19(1): 356–365.

SUPPORTING INFORMATION

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

How to cite this article: Vietorisz, Corinne R., Nahuel Policelli, Abigail Li, Lindsey A. Adams, Kathryn F. Atherton, and Jennifer M. Bhatnagar. 2025. "Microbial Functional Guilds and Genes are Key to Explaining Soil Nutrient Cycling Alongside Soil and Plant Variables." *Ecosphere* 16(11): e70466. https://doi.org/10.1002/ecs2.70466