

Effects of nitrogen and phosphorus addition on microbial community composition and element cycling in a grassland soil

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ARTICLE INFO

Keywords:

Nutrient cycling
Bacterial community composition
Nitrogen and phosphorus fertilization
Functional redundancy
Carbon mineralization
Nutrient network (NutNet)

ABSTRACT

Microorganisms mediate nutrient cycling in soils, and thus it is assumed that they largely control responses of terrestrial ecosystems to anthropogenic nutrient inputs. Therefore, it is important to understand how increased nitrogen (N) and phosphorus (P) availabilities, first, affect soil prokaryotic and fungal community composition and second, if and how changes in the community composition affect soil element cycling. We measured soil microbial communities and soil element cycling processes along a nine-year old experimental N-addition gradient partially crossed with a P-addition treatment in a temperate grassland. Nitrogen addition affected microbial community composition, and prokaryotic communities were less sensitive to N addition than fungal communities. P addition only marginally affected microbial community composition, indicating that P is less selective than N for microbial taxa in this grassland. Soil pH and total organic carbon (C) concentration were the main factors associated with prokaryotic community composition, while the dissolved organic C-to-dissolved N ratio was the predominant driver of fungal community composition. Against our expectation, plant biomass and plant community structure only explained a small proportion of the microbial community composition. Although microbial community composition changed with nutrient addition, microbial biomass concentrations and respiration rates did not change, indicating functional redundancy of the microbial community. Microbial respiration, net N mineralization, and non-symbiotic N₂ fixation were more strongly controlled by abiotic factors than by plant biomass, plant community structure or microbial community, showing that community shifts under increasing nutrient inputs may not necessarily be reflected in element cycling rates. This study suggests that atmospheric N deposition may impact the composition of fungi more than of prokaryotes and that nutrient inputs act directly on element-cycling rates as opposed to being mediated through shifts in plant or microbial community composition.

1. Introduction

Microorganisms govern soil nutrient cycling, and hence might regulate ecosystem responses to the human induced increases in nitrogen (N) and phosphorus (P) availabilities (Galloway et al., 2008; Wang et al., 2015; Peñuelas et al., 2013). Microorganisms not only mediate the fate of nutrients in soils, they also respond to nutrient inputs by shifts in biomass and community structure. To predict effects of nutrient inputs on ecosystems, it is critical to understand how nutrient additions affect soil microbial community structure, and how these shifts are linked to

important processes of element cycling (Zhou et al., 2017). The relationships between changes in prokaryotic and fungal community composition and element cycling rates in response to nutrient addition are currently poorly understood (Zeng et al., 2016), and the drivers of these changes have rarely been analyzed.

The emission of biologically available N has more than doubled through anthropogenic activities since 1970 (Galloway et al., 2008; Gruber and Galloway, 2008). Large regions of the world receive N at deposition rates $>1 \text{ g N m}^{-2} \text{ yr}^{-1}$, and it has been predicted that by 2050 some regions may receive up to $5 \text{ g N m}^{-2} \text{ yr}^{-1}$ (Galloway et al., 2004).

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<https://doi.org/10.1016/j.soilbio.2020.108041>

Received 28 May 2020; Received in revised form 4 September 2020; Accepted 4 October 2020

Available online 11 October 2020

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Increases in N inputs are often not paralleled by increased P inputs (Peñuelas et al., 2012), changing ratios of bioavailable N to P (Peñuelas et al., 2013), and highlighting the need for studies on the independent and interactive effects of N and P addition.

Increased supplies of biologically limiting nutrients can alter microbial community composition (Ramirez et al., 2010, 2012; Fierer et al., 2012; Morrison et al., 2016; Leff et al., 2015). However, drivers of microbial community change are difficult to disentangle (Wardle et al., 2013; Manning et al., 2006), because the changes can arise for instance through changes in soil chemistry (e.g., soil pH, the bioavailability of carbon (C) and N) and biology (e.g. food webs, viruses), and they can be mediated through changes in above- and belowground plant biomass or plant community structure (Högberg et al., 2007; Ramirez et al., 2010; Chen et al., 2015; Leff et al., 2015; Prober et al., 2015; Zeng et al., 2016). Of these factors, soil pH can have particularly strong effects on the soil bacterial community composition (Lauber et al., 2009; Fierer et al., 2009; Fierer and Jackson, 2006) because low soil pH imposes stress on bacterial cells, exerting selection pressure on certain bacterial taxa (Lauber et al., 2008; Rousk et al., 2010a). In particular, low soil pH impedes the metabolism of bacteria (Rousk et al., 2010b), reducing the ability of bacteria to successfully compete with fungi (Rousk et al., 2010b).

Increased nutrient supplies have been shown to change plant biomass and plant community composition (Stevens et al., 2015; Harpole et al., 2016), which impacted bacterial (Leff et al., 2015) and fungal community structure (Heinemeyer and Fitter, 2004; Lauber et al., 2008). For example, nutrients can change root architecture offering different microhabitats and environmental conditions, and can change the food spectrum in the form of root exudates and litter inputs (Lange et al., 2015; Berg and Smalla, 2009).

Altered microbial community structure can influence ecosystem functions including soil element cycling (Philippot et al., 2013; Delgado-Baquerizo et al., 2016; Strickland et al., 2009), although functional redundancy of microbial communities also occurs (Louca et al., 2018; Nannipieri et al., 2003). Functional redundancy means that loss of species may not impact ecosystem functioning because each metabolic function can be performed by several coexisting, taxonomically distinct species (Allison and Martiny, 2008; Philippot et al., 2013; Louca et al., 2018).

Predicting the effects of nutrient supply on ecosystems requires an understanding of microbial responses to nutrient addition and the linkages between soil microbial communities and nutrient cycling. However, our mechanistic understanding of microbial communities and coupled physicochemical processes is in its infancy (Louca et al., 2018), and few studies have concurrently explored responses of bacterial and fungal communities (e.g. Allison et al., 2007; Ramirez et al., 2010; Fierer et al., 2012; Zeng et al., 2016). In particular, few sequence-based attempts have been made to simultaneously explore drivers of changes in prokaryotic and fungal community composition under nutrient addition (Leff et al., 2015; Schleuss et al., 2019) and link it to element cycling processes.

Here, we investigated how N and P addition (a) altered prokaryotic and fungal community composition and (b) changed processes of soil element cycling. Further, we explored the drivers of microbial community change and of element cycling processes to understand the links between nutrient addition and microbial communities and between microbial community change and element cycling. We hypothesized that N and P additions alter prokaryotic and fungal community composition (i). We expected that these alterations in prokaryotic and fungal community composition are mainly caused by altered soil pH and by altered plant biomass and plant community structure under N and P addition (ii). In addition, we hypothesized that changes in soil element cycling processes (microbial respiration, N mineralization, and non-symbiotic N_2 fixation) are mediated by changes in microbial community composition (iii). To close the critical knowledge gaps about microbial and ecosystem responses to nutrient inputs, we studied soil

microbial community structure and element cycling in a grassland N and P addition experiment in the USA after nine years of annual nutrient addition.

2. Material and methods

2.1. Site description and experimental design

We studied a grassland site in the Central Plains, USA, that belongs to a worldwide research cooperation called Nutrient Network (Borer et al., 2014). The site is located within the Cedar Creek Ecosystem Science Reserve, Minnesota, USA (45.43 N, -93.21 E). The mean annual temperature is 6 °C and mean annual precipitation is 800 mm yr⁻¹. The site is situated 270 m above sea level on the Anoka Sand Plain, an outwash plain of the Wisconsin Glacial Episode giving the soil a sandy texture (88.7% sand, 9.7% silt and 1.5% clay). The soil is an Arenosol according to WRB classification and the site is currently vegetated by tallgrass prairie.

Nutrients have been added annually to 5 × 5 m plots at the beginning of the growing season since 2008. Six different nutrient addition treatments were studied here that consist of different levels of N addition (control, N1, N5, and N10), a combined N and P addition treatment (N10P10, later referred to as N10P), and a P addition treatment (P10, later referred to as P). Nutrient addition treatments were each replicated in three blocks. The three different levels of N (1, 5, and 10 g N m⁻² yr⁻¹) were added as time-release urea ((NH₂)₂CO). Further, 10 g P m⁻² yr⁻¹ was added as triple-super phosphate (Ca(H₂PO₄)₂) without N and in combination with 10 g N m⁻² yr⁻¹.

2.2. Soil sampling

Soils were sampled from 0 to 15 cm depth (called topsoil hereafter) and in a second depth increment from 15 to 30 cm depth (called subsoil hereafter). Both depth increments covered the A horizon. Six soil samples were taken per plot and depth from three replicate blocks (blocks 1–3) using a soil corer with a diameter of 3.5 cm and combined into one mixed sample. Sampling was carried out in September 2017 at peak biomass and samples were shipped to University of Bayreuth, Germany, directly after sampling. Subsequently, soils were sieved (<2 mm), and stones and roots were removed. An aliquot of each soil sample was dried at 60 °C for soil chemical analyses, another aliquot was frozen for microbial analyses, and a third aliquot was adjusted to 60% water holding capacity and pre-incubated for 1 week at 15 °C before incubation experiments were started. Soil water content and water-holding capacity (WHC) were analyzed gravimetrically. To determine WHC, we oversaturated fresh samples with water, drained them for 24 h on a sand bath, determined the mass gravimetrically, and then dried them at 105 °C before determining the dry weight.

2.3. Soil chemical parameters

Soil pH was measured in deionized water in a soil:water ratio of 1:2.5 (m:v) using air-dried soil. Soil samples were milled prior to the determination of total organic C (TOC), total N (TN), and total P (TP). TOC and TN were measured using an element analyzer (Vario Max Elemental, Hanau, Germany). TP was determined by ICP-OES (Vista-Pro radial, Varian) after pressure digestion in aqua regia (HNO₃ + HCl). Dissolved organic C (DOC), dissolved total N (DN), and dissolved inorganic P (DIP) were extracted in deionized water in a ratio of 1:4 (soil: water) by shaking for 1 h. Water extracts were filtrated through 0.45 µm filters using an under-pressure device. Concentrations of DOC and DN were quantified by a TOC/TN Analyzer (multi N/C 2100, Analytik Jena, Germany), and DIP concentrations were quantified by a spectrophotometer (UV, 1800; Shimadzu) using the molybdenum blue method (Murphy and Riley, 1962).

2.4. Microbial biomass carbon

Microbial biomass C concentrations were determined using the chloroform fumigation-extraction method (Brookes et al., 1982; Vance et al., 1987). Each soil sample was split into two aliquots of which one was fumigated with chloroform for 24 h and the other not. Fumigated and non-fumigated samples were extracted in 0.5 M K₂SO₄ in a ratio of 1:5 (soil:extractant). Samples were diluted in a ratio of 1:20 before measuring dissolved C using a TOC/TN analyzer (multi N/C 2100, Analytik Jena, Jena, Germany). Microbial biomass C was calculated by subtracting the concentrations of the non-fumigated samples from the fumigated samples, and by multiplying the difference with a conversion factor of 2.22 (Jenkinson et al., 2004).

2.5. Microbial respiration

Soil samples of 40 g dry-weight-equivalent were incubated for 35 days at 15 °C in the dark. Respired CO₂ was trapped in 0.6 M KOH and changes in electrical conductivity were measured by a respirometer (Respicond V, Nordgen Innovations). Cumulative CO₂ was measured continuously (every 2 h) and respiration rates were calculated based on the linear increase in accumulated C–CO₂ over time (Heuck and Spohn, 2016).

2.6. Net N mineralization

Sub-samples of 20 g soil dry-mass equivalent were extracted in 80 ml distilled water on an overhead shaker for 1 h and filtrated through 0.45 µm filters using an under-pressure device. The extraction-filtration procedure was repeated after 0, 14, 28, and 42 days of soil incubation at 15 °C. Water extracts were measured for ammonium (N–NH₄⁺) and nitrate (N–NO₃⁻) via flow injection analysis (FIA-Lab, MLE Dresden) and ICP-OES, respectively. Net N mineralization rates were calculated based on the linear increase of N–NH₄⁺ and N–NO₃⁻ (dissolved inorganic N, DIN) over time.

2.7. Non-symbiotic N₂ fixation

Non-symbiotic fixation of atmospheric N₂ was measured based on a ¹⁵N stable isotope approach (Zechmeister-Boltenstern, 1996). A dry-mass equivalent of 4 g fresh soil (60% WHC) was filled into 12 ml exetainers (Labco). All exetainers were closed, flushed with argon (Ar), carefully evacuated, and finally filled with 7.2 ml ¹⁵N₂ (99.8 atom% ¹⁵N₂, Sigma Aldrich, batch number: MB5815V) and 0.8 ml O₂ and incubated in the dark at 15 °C for 72 h in the ¹⁵N enriched artificial atmosphere. To control the artificial ¹⁵N-enriched atmosphere, pressure changes were noted before and after adding ¹⁵N₂ and O₂. The average atmospheric composition consisted of 72.5% ¹⁵N₂, 8.2% O₂, and 19.2% Ar in v/v%. The samples being exposed to ¹⁵N–N₂ as well as the samples that were not exposed to ¹⁵N–N₂ (natural abundance) were dried at 50 °C, milled and analyzed for ¹⁵N (Delta plus, Conflo III, Thermo Electron Cooperation, Bremen, Germany). The ¹⁵N atom% was calculated using the isotope ratio of each sample ($R_{\text{sample}} = {}^{15}\text{N}/{}^{14}\text{N}$). The ¹⁵N₂ fixation was calculated using an isotope mixing model (Zechmeister-Boltenstern, 1996):

where TN is the total soil N (in mg N per g soil), ¹⁵N_{labeled} is the content of ¹⁵N atoms in the labeled sample, ¹⁵N_{NA} is the content of ¹⁵N atoms in the control samples, and *t* is the incubation time (in h).

$${}^{15}\text{N}_2 \text{ fixation rate (ng N g soil}^{-1} \text{ h}^{-1}) = \text{TN (mg N g soil}^{-1}) \times \frac{({}^{15}\text{N}_{\text{labeled}} (\text{at}\%) - {}^{15}\text{N}_{\text{NA}} (\text{at}\%))}{100 * t (\text{h})} \times 10^6$$

2.8. Sequencing

Before pre-incubation of soil for the incubation experiments, samples for amplicon sequencing were taken and frozen. The DNeasy PowerSoil Kit (Qiagen) was used to extract DNA of 400 mg soil of each sample. The V4 region of the prokaryotic 16S rRNA gene and the fungal ITS2 region were amplified (primers F-515- GTGCCAGCMGCCGCGGTAA, R-806- GGACTACHVGGGTWTCTAAT for prokaryotes (Caporaso et al., 2011); and F-ITS4-TCCTCCGCTTATTGATATGC (White et al., 1990), R-ITS7-GTGARTCATCGAATCTTTG for fungi (Ihrmark et al., 2012); modified with heterogeneity spacers according to Cruaud et al. (2017)) and sequenced using the Nextera XT kit (Illumina) on an Illumina MiSeq with 2 × 300 bp. Primer sequences were removed from reads using *cutadapt* v1.8 (allowing 4 mismatches). Both 16S and ITS amplicon reads were processed separately using DADA2 (Callahan et al., 2016: maximum estimated error: 0.7, truncation quality score: 2, length of first/second read: 230/200), to estimate error models for the whole run to yield counts at sequence variant level. Chimeras were removed. For the ITS data set, non-fungal reads were removed according to ITSx annotation (Bengtsson-Palme et al., 2013). Sequences were taxonomically classified using *mothur's classify.seqs* (Schloss et al., 2009) against the UNITE v8 database for ITS (Kõljalg et al., 2013), and the SILVA v132 database for 16S sequences (Quast et al., 2013). Amplicon sequence variants (ASVs) not classified as the target taxa ('Fungi' for the ITS data set, 'Bacteria' or 'Archaea' for the 16S data set) were removed. Chloroplast and mitochondria sequences were manually removed from the 16S data set.

2.9. Plant sampling

At peak biomass in 2017, all aboveground plant biomass was clipped in two 0.1 × 1 m strips within each 5 × 5 m plot, and the current year's growth was sorted to species level, dried to a constant mass at 60 °C, and weighted to the nearest 0.01 g. Areal cover and identity of all species was estimated visually in a 1 m² quadrat in each plot.

2.10. Accession numbers

The raw sequencing data from 16S rRNA genes and ITS regions were deposited in NCBI's sequence read archive under the accession number PRJNA596166.

2.11. Statistics

To test significant differences in soil properties and element cycling rates between treatments and depth increments, two-way ANOVA was conducted followed by Tukey-Test for multiple comparisons (*p* < 0.05). Previously, data were checked for normal distribution (Shapiro-Wilks test) and homogeneity of variance (Levene's test) and log- or square root-transformed if data were not normally distributed and variances were not homogenous.

Prokaryotic and fungal beta-diversity was calculated as Jensen-Shannon divergences (JSD) of sum-normalized community profiles at ASV level (using *vegan* and *phyloseq* packages (Oksanen et al., 2019; McMurdie and Holmes, 2013)). To test for pairwise differences in community profiles of all treatment levels, analyses of similarity (ANOSIM) were performed on JSDs, stratifying for sampling depth (using *vegan's* function *anosim* (Oksanen et al., 2019)). Non-metric multi-dimensional scaling (NMDS) plots were calculated based on the

JSD after sum-normalization at ASV level using the phyloseq implementation metaMDS (McMurdie and Holmes, 2013).

N and P addition were assessed as independent variables in a multivariate analysis of beta-diversity by permutational multivariate analysis of variance (PERMANOVA) of JSDs (using adonis 2 of the vegan package (Oksanen et al., 2019)). The same analyses were conducted at the taxonomic ranks of genus, family, order, class, phylum, and using weighted UniFrac distances for prokaryotic data and at the taxonomic ranks of genus, family, order, class, and division for fungal data (using vegan and phyloseq packages (Oksanen et al., 2019; McMurdie and Holmes, 2013)). Differentially abundant genera were detected from a data matrix containing the samples from the Ctrl, N10, N10P and P treatments with reads summed up at genus level using DESeq2 (Love et al., 2014) with the model $Y \sim N * P$. To test if plant beta-diversities correlated with beta-diversities of prokaryotes and fungi, Mantel tests were performed using the mantel. rtest function from the R-package ade4 (Dray and Dufour, 2007).

To identify the main controls of prokaryotic and fungal community composition, PERMANOVA was conducted as well. Only soil data from the first depth increment was used for these analyses to match the plant data. Plant communities were represented by plant Shannon diversity and the first three axes of a principal coordinate analysis based on plant community JSD (using the phyloseq and ape packages (McMurdie and Holmes, 2013; Paradis and Schliep, 2019)). The soil and plant variables with significant explanatory values in single-factor PERMANOVAs were determined and added to a combined model after removal of collinearity. As PERMANOVA is sensitive to factor order, variables were given by decreasing importance in single-factor models.

For identification of the main controls on microbial respiration, net N mineralization, and non-symbiotic N_2 fixation, multiple backward stepwise regression analysis was applied using the stepAIC function in R. The initial linear model contained soil pH, TOC, TN, DOC and DN concentrations, DOC:DN ratio, prokaryotic and fungal community composition (based on first axis of principal coordinates analysis), plant diversity and plant biomass (same variables as used to assess drivers of microbial community composition + prokaryotic and fungal community composition as potential drivers). Level of significance was chosen at $p < 0.05$. The first model with a p-value below 0.05 and the highest number of remaining variables was selected to show the influence of several variables. Variance inflation factors were used to check for multicollinearity and highly collinear variables were dropped. The order of variables in further analyses was based on AIC from stepwise regression analysis, except that DN was placed as first independent variable as we considered it to be the main factor related to the N treatment.

Table 1

Soil pH, total organic carbon (TOC), total nitrogen (TN), and total phosphorus (TP) contents, dissolved organic carbon (DOC), dissolved nitrogen (DN), and dissolved inorganic phosphorus (DIP) concentrations under N and P addition in 0–15 cm and 15–30 cm soil depth. Numbers depict means $\pm$ standard deviations ($n = 3$). Two-way ANOVA was conducted followed by Tukey test for multiple comparisons. Lower-case letters indicate significant differences between treatments tested separately for each depth increment. If no lower-case letters are shown, treatments did not differ significantly. Asterisks indicate significant differences between depth increments tested individually for each treatment.

Depth (cm)	N addition (g m ⁻² yr ⁻¹)	P addition (g m ⁻² yr ⁻¹)	pH _{H2O}	TOC ¹	TN ¹ (g kg ⁻¹)	TP ²	DOC (mg kg ⁻¹)	DN ¹	DIP ¹	DOC:DN ratio ²
0–15	0	0	5.27 $\pm$ 0.08 ^a	9.44 $\pm$ 0.87	0.71 $\pm$ 0.09	0.31 $\pm$ 0.03	14.59 $\pm$ 0.30 ^a	3.20 $\pm$ 3.61 ^a	0.16 $\pm$ 0.10 ^a	18.08 $\pm$ 11.31 ^a
	1	0	5.03 $\pm$ 0.16 ^{ab}	16.18 $\pm$ 3.84	1.14 $\pm$ 0.31	0.48 $\pm$ 0.21	17.37 $\pm$ 1.75 ^a	6.77 $\pm$ 6.04 ^{abc}	0.18 $\pm$ 0.07 ^a	6.09 $\pm$ 4.02 ^a
	5	0	4.98 $\pm$ 0.18 ^{ab}	11.79 $\pm$ 5.71	0.89 $\pm$ 0.43	0.36 $\pm$ 0.17	20.14 $\pm$ 4.15 ^{ab}	21.68 $\pm$ 8.65 ^{bc}	0.13 $\pm$ 0.08 ^a	1.19 $\pm$ 0.28 ^{bc}
	10	0	4.70 $\pm$ 0.14 ^b	15.68 $\pm$ 7.16	1.13 $\pm$ 0.53	0.46 $\pm$ 0.20	20.24 $\pm$ 3.91 ^{ab}	32.88 $\pm$ 12.71 ^c	0.13 $\pm$ 0.05 ^a	0.77 $\pm$ 0.15 ^c
	0	10	5.27 $\pm$ 0.08 ^a	8.99 $\pm$ 0.25	0.61 $\pm$ 0.04	0.58 $\pm$ 0.08	19.75 $\pm$ 0.87 ^{ab}	2.73 $\pm$ 1.68 ^{ab}	13.37 $\pm$ 2.25 ^b	12.04 $\pm$ 6.14 ^a
	10	10	4.84 $\pm$ 0.08 ^{ab}	11.01 $\pm$ 2.80	0.82 $\pm$ 0.21	0.56 $\pm$ 0.09	24.28 $\pm$ 2.15 ^b	13.42 $\pm$ 2.36 ^{abc}	12.29 $\pm$ 2.89 ^b	2.14 $\pm$ 0.17 ^{ab}
15–30	0	0	5.36 $\pm$ 0.08	5.17 $\pm$ 0.88	0.35 $\pm$ 0.07	0.24 $\pm$ 0.03	10.90 $\pm$ 0.50	1.32 $\pm$ 0.51 ^a	0.06 $\pm$ 0.02 ^a	10.89 $\pm$ 3.27 ^a
	1	0	5.23 $\pm$ 0.26	11.32 $\pm$ 6.33	0.77 $\pm$ 0.44	0.43 $\pm$ 0.25	12.59 $\pm$ 1.97 ^a	5.65 $\pm$ 3.84 ^{ab}	0.05 $\pm$ 0.03 ^a	3.59 $\pm$ 1.53 ^{ab}
	5	0	5.22 $\pm$ 0.29	8.64 $\pm$ 5.19	0.60 $\pm$ 0.34	0.36 $\pm$ 0.14	12.76 $\pm$ 2.05 ^a	12.77 $\pm$ 0.94 ^c	0.04 $\pm$ 0.02 ^a	1.18 $\pm$ 0.24 ^c
	10	0	5.17 $\pm$ 0.15 ^a	10.39 $\pm$ 6.40	0.67 $\pm$ 0.40	0.40 $\pm$ 0.16	12.48 $\pm$ 1.65 ^a	10.01 $\pm$ 0.85 ^{bc}	0.04 $\pm$ 0.01 ^a	1.45 $\pm$ 0.08 ^{bc}
	0	10	5.45 $\pm$ 0.19	4.44 $\pm$ 0.41	0.33 $\pm$ 0.05	0.36 $\pm$ 0.03	12.42 $\pm$ 0.23 ^a	2.06 $\pm$ 1.20 ^{ab}	4.19 $\pm$ 0.45 ^b	9.60 $\pm$ 4.69 ^a
	10	10	4.96 $\pm$ 0.18	5.76 $\pm$ 1.73	0.40 $\pm$ 0.08	0.38 $\pm$ 0.05	14.91 $\pm$ 1.03 ^a	6.96 $\pm$ 1.69 ^{ab}	5.01 $\pm$ 0.73 ^b	2.65 $\pm$ 0.64 ^{abc}

¹ Data were log 10 transformed for statistical tests, ²reciprocally transformed (1/x) for statistical tests.

Plant diversity was calculated using Shannon's Diversity ($H' = -\sum (p_i \ln(p_i))$), where p_i is the frequency of occurrence of each species). To test if nutrient additions significantly affected plant diversity and biomass, one-way-ANOVA was conducted followed by Tukey-Test for multiple comparisons. To assess the plant community composition, we first calculated Bray-Curtis distance matrices in PRIMER 7 (Clarke and Gorley, 2015) with 999 permutations before NMDS was applied to display the community composition. After the calculation of Bray-Curtis matrices, one-way-ANOSIM with 999 permutations was used to test significant effects of nutrient addition. Statistical analyses were done using R (R Core Team, 2018).

3. Results

3.1. Soil physical and chemical parameters

After nine years of N addition, topsoil pH was significantly lower in the highest N level compared to the control and the P addition treatments (Table 1), whereas in the second depth increment, nutrient addition did not change soil pH. Mean topsoil TOC concentration across all treatments amounted to 12.2 ± 5.1 g C kg soil⁻¹, TN concentrations to 0.9 ± 0.2 g N kg soil⁻¹, and TP concentrations to 0.5 ± 0.1 g P kg soil⁻¹ (Table 1). Total element concentrations were not significantly affected by nine years of element addition (Table 1).

The mean topsoil DOC concentration across all treatments amounted to 19.4 ± 3.9 mg C kg soil⁻¹. Under NP addition, topsoil DOC concentrations were significantly higher compared to the control and the lowest N addition level. In contrast, nutrient addition did not significantly change the DOC concentration in the second depth increment (Table 1). N addition rates of 1, 5, and 10 g N m⁻² yr⁻¹ gradually increased the topsoil DN concentration 2, 7, and 10 times, respectively, compared to the control, whereas P addition did not significantly change topsoil DOC and DN concentrations compared to the control. The molar topsoil DOC:DN ratio was highest in the control (18.1 ± 11.3) and under P addition (12.0 ± 6.1), and compared to control, decreased 3, 15, and 24 times exposed to 1, 5, and 10 g of N m⁻² yr⁻¹, respectively. Further, mean DIP concentrations were significantly higher under P addition compared to the control and to all levels of N addition in both depth increments (Table 1).

3.2. Microbial biomass carbon and element cycling processes

Nutrient addition did not significantly change microbial biomass C or microbial respiration in either depth increment compared to the control (Table 2). Further, microbial respiration per unit microbial biomass C

Table 2

Microbial biomass carbon (MBC), microbial respiration, microbial respiration per unit MBC (qCO_2), net nitrogen (N) mineralization, and non-symbiotic nitrogen (N_2) fixation under N and P addition in 0–15 cm and 15–30 cm soil depth. Numbers depict means $\pm$ standard deviations ($n = 3$). Two-way ANOVA was conducted followed by Tukey test for multiple comparisons. Lower-case letters indicate significant differences between treatments tested separately for each depth increment. If no lower-case letters are shown, treatments did not differ significantly. Asterisks indicate significant differences between depth increments tested individually for each treatment.

Depth (cm)	N addition (g m ⁻² yr ⁻¹)	P addition (g m ⁻² yr ⁻¹)	MBC (mg C kg soil ⁻¹)	Respiration (mg C kg soil ⁻¹ d ⁻¹)	qCO_2 (mg C (g MBC) ⁻¹ d ⁻¹)	N mineralization (mg N kg soil ⁻¹ d ⁻¹)	Non-symb. N_2 fixation (ng N g soil ⁻¹ d ⁻¹)
0–15	0	0	162.68 $\pm$ 51.18	8.30 $\pm$ 3.03	51.59 $\pm$ 16.09	0.09 $\pm$ 0.05 ^a	0.69 $\pm$ 0.09
	1	0	155.81 $\pm$ 35.26	3.41 $\pm$ 0.27	22.73 $\pm$ 3.83	0.20 $\pm$ 0.03 ^{bc}	1.13 $\pm$ 0.20
	5	0	163.70 $\pm$ 47.23	3.80 $\pm$ 1.29	23.11 $\pm$ 4.43	0.32 $\pm$ 0.07 ^d	1.28 $\pm$ 0.13
	10	0	235.22 $\pm$ 36.29	5.75 $\pm$ 1.87	25.97 $\pm$ 12.03	0.25 $\pm$ 0.02 ^{bcd}	1.02 $\pm$ 0.41
	0	10	192.71 $\pm$ 7.12	6.69 $\pm$ 3.97	34.73 $\pm$ 20.79	0.16 $\pm$ 0.03 ^{ab}	1.08 $\pm$ 0.17
	10	10	168.81 $\pm$ 80.65	4.01 $\pm$ 1.80	29.32 $\pm$ 13.13	0.28 $\pm$ 0.05 ^{cd}	0.79 $\pm$ 0.21
15–30	0	0	118.62 $\pm$ 83.95	2.32 $\pm$ 0.85*	27.29 $\pm$ 17.16	0.06 $\pm$ 0.01	1.05 $\pm$ 0.21 ^a
	1	0	113.07 $\pm$ 66.81	2.03 $\pm$ 0.97	39.34 $\pm$ 26.35	0.09 $\pm$ 0.03*	1.78 $\pm$ 0.27 ^{b*}
	5	0	95.29 $\pm$ 37.12	1.62 $\pm$ 0.48	17.85 $\pm$ 2.47	0.12 $\pm$ 0.01*	1.50 $\pm$ 0.29 ^{ab}
	10	0	100.30 $\pm$ 38.56*	1.48 $\pm$ 0.33*	16.74 $\pm$ 5.44	0.11 $\pm$ 0.01*	1.74 $\pm$ 0.19 ^{ab*}
	0	10	45.87 $\pm$ 21.92*	1.97 $\pm$ 0.77*	39.49 $\pm$ 17.56	0.07 $\pm$ 0.02*	1.87 $\pm$ 0.28 ^{b*}
	10	10	133.81 $\pm$ 87.67	2.19 $\pm$ 0.47	27.75 $\pm$ 14.93	0.13 $\pm$ 0.01*	1.20 $\pm$ 0.03 ^{ab}

(metabolic quotient; qCO_2) was about twice as high in the control treatment than in the lowest N addition treatment, but differences were not statistically different due to the large variation among the plots (Table 2). Topsoil net N mineralization rates were 2–4 times higher under any level of N and combined NP addition compared to the control (Table 2). Non-symbiotic N_2 fixation rates in the second soil depth increment were 1.7 times higher under the lowest level of N addition and 1.8 times higher under P addition compared to the control (Table 2).

Stepwise multiple regression analysis revealed that DOC:DN ratio, plant diversity, and DN concentrations could account for 15.7%, 21.4%, and 5.8% of microbial respiration, respectively (Table 3, Fig. 1, plant data is described in section 3.3). Further, DN (42.9%), DOC (18.8%), and fungal community composition (9.0%) were the best explanatory variables for net N mineralization rates (Table 3, Fig. 1, fungal data is described in section 3.5). For non-symbiotic N_2 fixation, TOC (24.2%), DOC:DN ratio (17.3%), plant biomass (9.3%), and prokaryotic community composition (7.5%) were the variables explaining the largest proportion of variation (Table 3, Fig. 1, prokaryotic data is described in section 3.4).

Table 3

Multiple regression analysis after backward stepwise selection for identification of environmental controls on microbial respiration, net nitrogen (N) mineralization, and non-symbiotic N_2 fixation in 0–15 cm depth. The initial model contained soil pH, total organic carbon (TOC), total N (TN) contents, dissolved organic carbon (DOC), and dissolved N (DN) concentrations, DOC:DN ratio, prokaryotic and fungal community composition (based on first axis of principal coordinates analysis), and plant biomass and diversity. Displayed is the first model with a p-value below 0.05 and the highest number of remaining variables to show the influence of several variables. Variance inflation factors were used to check for multicollinearity and highly collinear variables were dropped.

	Variable	Coefficient	p-value	% explained	Multiple R ² (model)	Adj. R ² (model)	p-value (model)
Respiration	(Intercept)	14.4002	0.010		0.43	0.31	0.044
	DOC:DN ratio	0.3424	0.008	15.74			
	Plant diversity	-6.1142	0.031	21.41			
	DN	0.0700	0.255	5.75			
	(Intercept)	-0.1944	0.335		0.77	0.60	0.014
Net N mineralization	DN	0.0030	0.114	42.93			
	Fungal comm.	0.0859	0.095	8.95			
	DOC	0.0110	0.117	18.77			
	Plant diversity	0.0697	0.344	0.62			
	Plant biomass	0.0001	0.392	3.09			
	TN	-0.0528	0.412	2.26			
	Prok. comm.	0.0014	0.969	0.00			
	(Intercept)	-0.9078	0.254		0.59	0.42	0.038
Non-symbiotic N_2 fixation	Plant diversity	0.6933	0.037	0.42			
	DOC:DN ratio	-0.0115	0.219	17.31			
	TOC	0.0227	0.128	24.16			
	Plant biomass	0.0006	0.089	9.34			
	Prok. comm.	-0.2140	0.166	7.47			

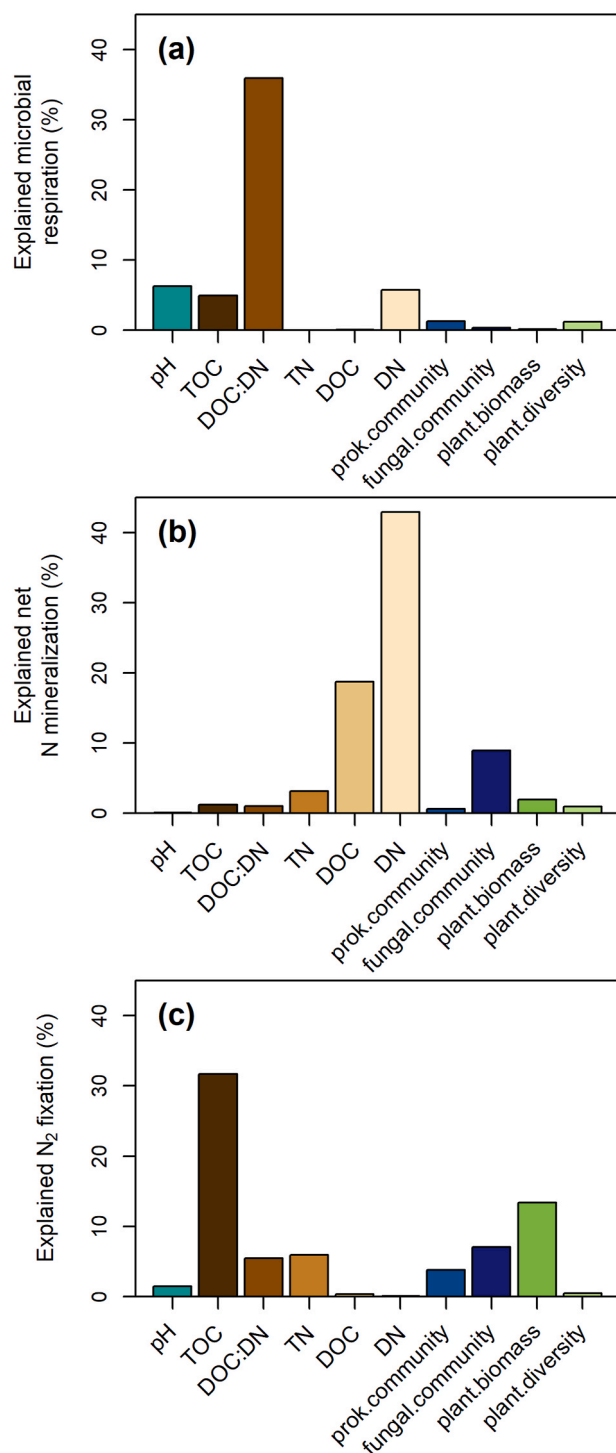


Fig. 1. Proportion of microbial respiration (a), net nitrogen (N) mineralization (b), and non-symbiotic N₂ fixation rates (c) in 0–15 cm soil depth explained by the displayed soil, microbial, and plant factors. Soil factors include soil pH, total organic carbon (TOC), dissolved organic carbon-to-dissolved nitrogen ratio (DOC:DN), total nitrogen (TN), dissolved organic carbon (DOC), and dissolved nitrogen (DN). Microbial factors include prokaryotic and fungal community composition at ASV level based on first axis of principal coordinates analysis. Plant factors include plant biomass, and plant diversity measured as Shannon diversity. All input variables are displayed, for a significant model, variables were removed stepwise as displayed in Table 3.

Addition of N (highest N addition treatment compared to control) accounted for between 10% and 28% of the variance in bacterial community composition across all taxonomic ranks (Table S2). Further, P addition accounted for a smaller proportion of bacterial community composition than N addition (between 5% and 8% of variance). Addition of N accounted for a significant proportion of variance of the archaeal community composition at ASV level (between 17% and 32%), whereas P addition had no explanatory power (Table S2). Together N and P addition accounted for between 18% and 35% of the variance in prokaryotic community composition in the highest N level compared to the control across all taxa (Table S2).

Variation in the prokaryotic community was highly correlated with the plant community (Mantel test $r = 0.48$, $p = 0.001$). However, plant community composition accounted for only 10% of variance in prokaryotic community composition in a multi-factor PERMANOVA (Table S3) considering all significant factors from single-factor PERMANOVA, whereas soil pH and TOC concentration accounted for a larger proportion of variance in community composition with 23% and 20%, respectively (Fig. 3a).

In total, bacterial reads were assigned to 41 different prokaryotic phyla with Proteobacteria, Acidobacteria, and Actinobacteria being the dominant phyla in both depth increments. Under N10 addition, the relative abundances of sequencing reads of nine prokaryotic genera increased, while the relative abundances of twelve genera decreased, compared to the control (Table S4, based on DESeq2 analysis). P addition decreased the relative abundances of two prokaryotic genera and two prokaryotic genera were affected by the interactive effects of N and P (Table S4). Of these genera, *Rhodanobacter* and *Sphingomonas* were above a relative abundance of 2% (Fig. 4).

3.5. Fungal community composition

The lowest and the highest level of N addition as well as combined NP addition significantly altered the fungal community composition compared to control and P addition calculated across both depth increments (Table S1). Combined NP addition significantly shifted the fungal community compared to the communities of all other treatments calculated across both depth increments (Table S1). Only considering 0–15 cm soil depth, community composition in all treatments was not significantly different due to statistical power issues (Fig. 2).

Considering only the highest N addition treatment compared to the control, N addition accounted for between 12% and 46% of variance of the fungal community composition across all taxonomic ranks (Table S2). In contrast, P addition only accounted for a significant proportion of variance (14%) at the division level but not at any other taxonomic rank (Table S2).

The variation in the fungal community was highly correlated with the plant community (Mantel test $r = 0.46$, $p = 0.001$). However, in multi-factor PERMANOVA (Table S3), considering all significant factors from single-factor PERMANOVA, plant community composition accounted for only 9% of variation in fungal community composition, whereas the DOC:DN ratio accounted for 21% (Fig. 3b).

Fungal reads were assigned to 13 different fungal divisions, of these Ascomycota, Basidiomycota, and Mortierellomycota showed the highest relative abundance across both depth increments. Under N10 addition, the relative abundances of sequencing reads of four fungal genera decreased, whereas one genus increased (Table S4, based on DESeq2 analysis). Of these genera, *Clavaria* and *Hygrocybe* (both Basidiomycota) were above 1% relative abundance (Fig. 5).

4. Discussion

By studying a unique nutrient-addition experiment, we investigated the link between changes in microbial communities and soil element-cycling processes such as microbial respiration, N mineralization, and non-symbiotic N₂ fixation that play a key role in soil C and N cycling. We

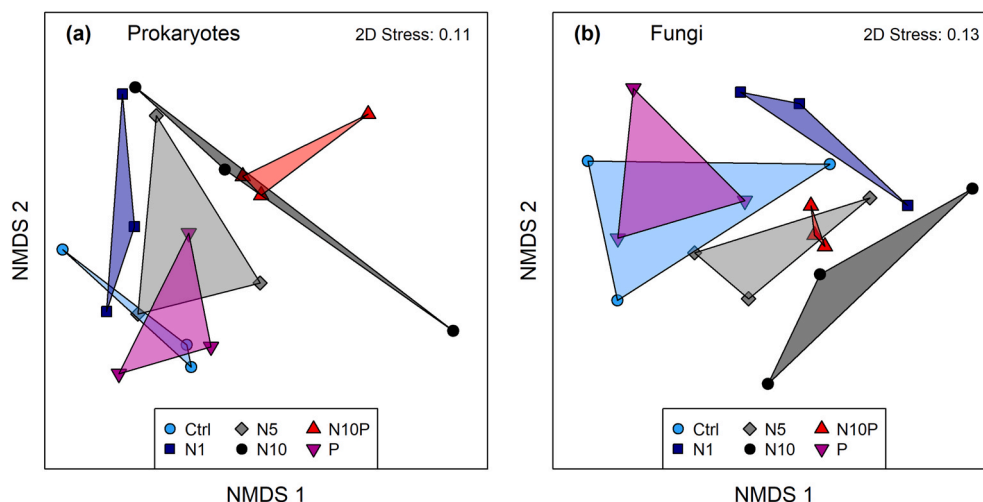


Fig. 2. Prokaryotic (a) and fungal (b) community composition at ASV level displayed via non-metric multidimensional scaling (NMDS) of Jensen-Shannon divergences for different treatments in 0–15 cm soil depth. To test for significant differences in community profiles of all treatments, analyses of similarity were performed on JSDs of both depth increments (Table S1) stratifying for sampling depth.

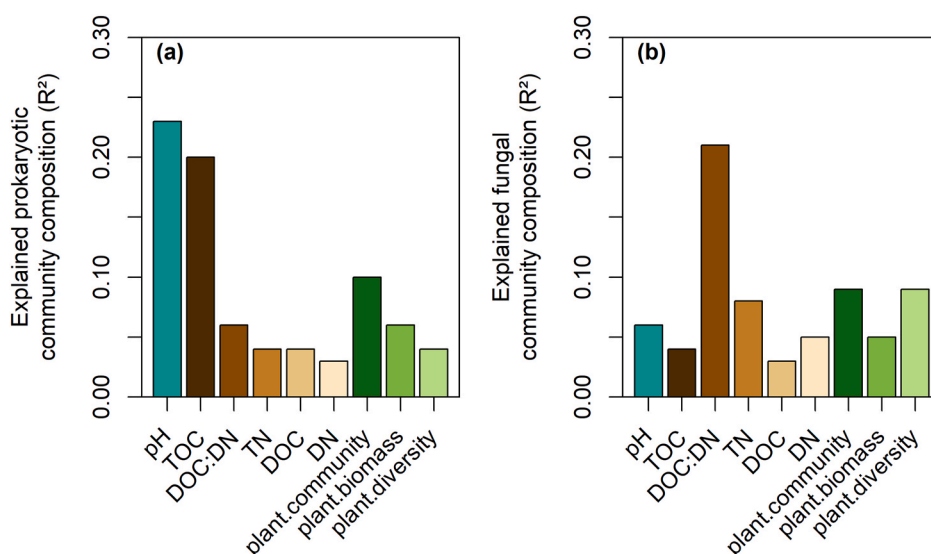


Fig. 3. Proportion of variation (R^2) of prokaryotic (a) and fungal (b) community composition explained by the displayed soil and plant factors in 0–15 cm soil depth. Results are based on permutational multivariate analyses of variance (PERMANOVA) using Jensen-Shannon divergence of microbial communities at ASV level (Table S3), included are all factors with significant explanatory value in single-factor PERMANOVA. Soil factors include soil pH, total organic carbon (TOC), dissolved organic carbon-to-dissolved nitrogen ratio (DOC:DN), total nitrogen (TN), dissolved organic carbon (DOC), and dissolved nitrogen (DN). Plant factors include plant community based on the first axis of principal coordinates analysis, plant biomass, and plant diversity measured as Shannon diversity.

found that nutrient addition significantly affected microbial community composition. We found that soil, not plant, properties affected microbial communities under nutrient addition and that changes in microbial communities were not reflected in most element cycling rates.

4.1. Changes in soil microbial element cycling under nutrient addition

Unchanged microbial respiration under nutrient addition, despite changed microbial community composition, indicates functional redundancy of the soil microbial community with respect to C mineralization as has been found in other studies (Banerjee et al., 2016; Rousk et al., 2009; Wertz et al., 2006). Thus, contrary to previous findings (Liu and Greaver, 2010), N inputs might not lower soil CO_2 emissions in this grassland as corroborated in a global meta-analysis (Yue et al., 2016). Correlational analyses indicated that abiotic factors, mainly the DOC:DN ratio that changed along our N gradient, accounted for a large proportion of microbial respiration demonstrating the importance of substrate stoichiometry for controlling microbial respiration (Spohn, 2015; Spohn and Chodak, 2015). Besides substrate stoichiometry, plant diversity explained a large proportion of variation in microbial respiration as

found in a global meta-analysis (Chen et al., 2019a). The maintenance of the microbial respiration rate and biomass production, despite changes in the microbial community, may have occurred because a limited set of metabolic pathways is associated with core processes, such as respiration, in soil microbial clades (Falkowski et al., 2008). Consequently, these functions shared by many microbial taxa, are less affected by changes in microbial community composition than more specific processes (Griffiths et al., 2000; Wertz et al., 2006; Louca et al., 2018; Schimel, 1995).

Further, N addition increased N mineralization rates as commonly observed (Vourlitis et al., 2007; Vestgarden et al., 2003; Ma et al., 2011) and this increase was associated with changes in DN and DOC concentrations. Whether N release or immobilization prevails also depends on the availability of C in relation to N (Manzoni et al., 2008). Microorganisms are most likely to release excess N when microbial C demands are not met, and N supplies exceed demands. In contrast, microorganisms likely retain N in their biomass when abundant C is available and N demands are not covered (Manzoni et al., 2008; Heuck and Spohn, 2016).

In contrast to processes, such as respiration, that are performed by

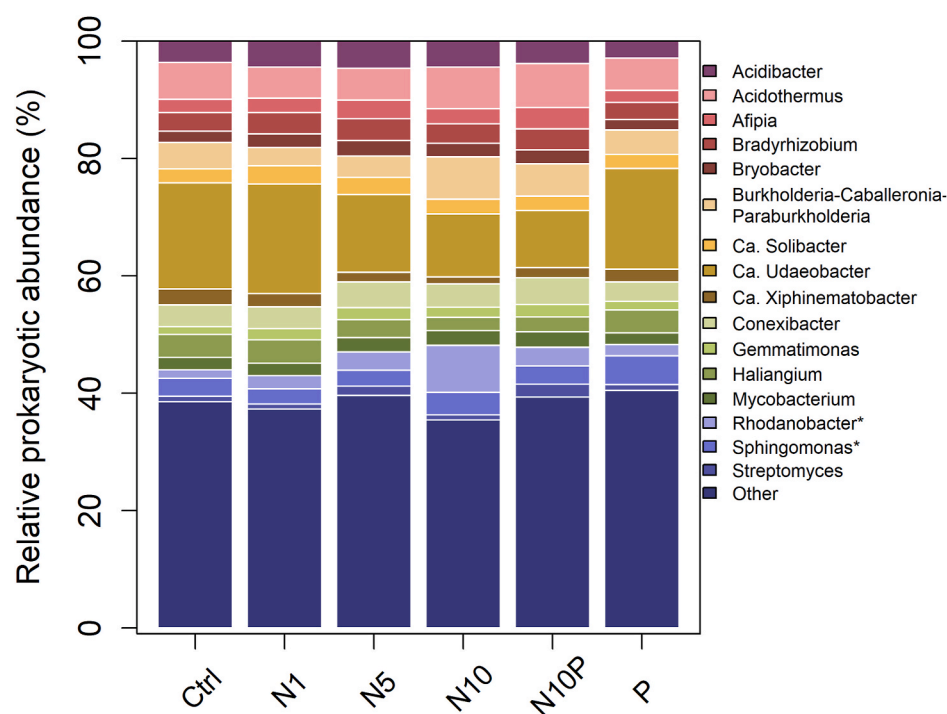


Fig. 4. Relative abundances of prokaryotic genera in 0–15 cm soil depth. Displayed are prokaryotic genera that made up > 2% of relative abundance. Prokaryotic genera < 2% relative abundance were grouped as “Other”. Differentially abundant genera were detected from a data matrix containing the samples from the Ctrl, N10, N10P and P treatments with reads summed up at genus level, using DESeq2 with the model $Y \sim N * P$. Differentially abundant genera are indicated with an asterisk in the legend and displayed in Table S4.

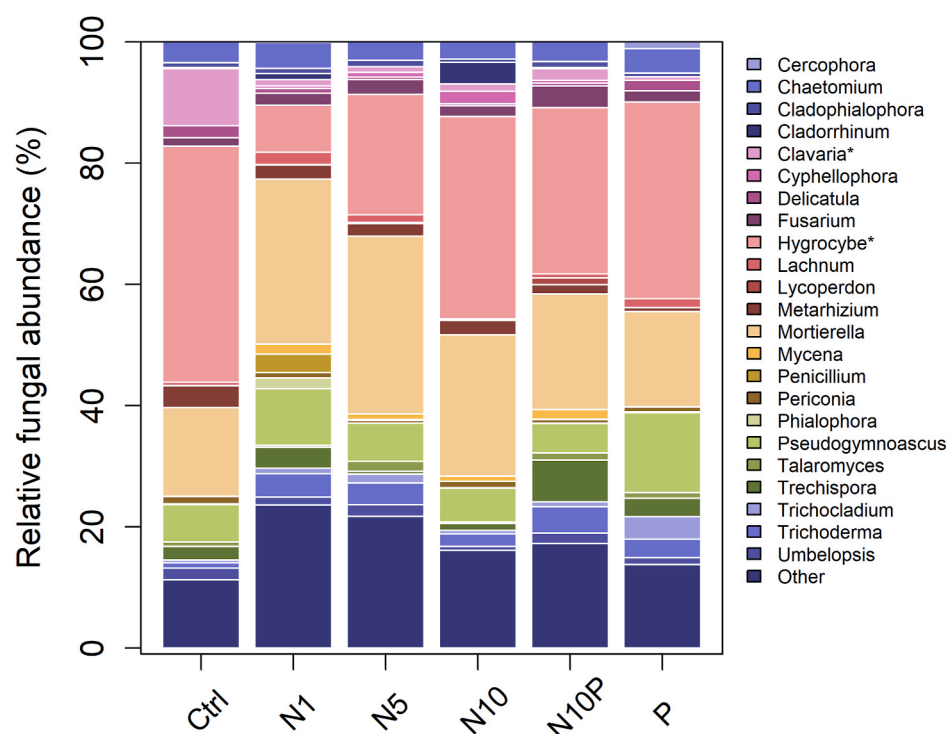


Fig. 5. Relative abundances of fungal genera in 0–15 cm soil depth. Displayed are genera with > 1% relative abundance. Unclassified fungi and fungal genera < 1% abundance were grouped as “Other”. Differentially abundant genera were detected from a data matrix containing the samples from the Ctrl, N10, N10P and P treatments with reads summed up at genus level, using DESeq2 with the model $Y \sim N * P$. Differentially abundant genera are indicated with an asterisk in the legend and displayed in Table S4.

many microorganisms, specialized functions that are restricted to a few groups, such as non-symbiotic N_2 fixation by free-living microorganisms (Dixon and Kahn, 2004), might be more affected by microbial community change (Schimel, 1995; Reed et al., 2010). However, correlational analysis indicated that the TOC concentration was the major driver of non-symbiotic N_2 fixation rates. This is likely because non-symbiotic N_2 fixation is one of the most energy-costly biological processes on earth (Hill, 1992; Smith, 1992) and enough energy- and C-sources need to be available to support non-symbiotic N_2 fixation (Reed et al., 2011).

Further, the experimental P addition increased non-symbiotic N_2 fixation likely because P is needed to produce sufficient ATP to fuel the energy-costly process of N_2 fixation (Reed et al., 2011). Surprisingly, the lowest level of N addition significantly enhanced N_2 fixation rates in the subsoil. An explanation could be that under N1 addition TOC concentrations were increased when calculated across both depth increments confirming the importance of C sources on non-symbiotic N_2 fixation.

Prokaryotic community composition accounted for around 8% of non-symbiotic N_2 fixation, indicating some importance of microbial

community composition on more specialized functions such as non-symbiotic N_2 fixation. Taken together, microbial community composition was largely unrelated to changes in element-cycling rates caused by N and P additions. Thus, community change under nutrient addition may not necessarily mean change in ecosystem functioning.

4.2. Drivers of prokaryotic community composition under nutrient addition

Prokaryotic community composition was only affected by the highest level of N addition, under which also soil pH decreased. Accordingly, correlation analyses also indicated that soil pH was one of the most important determinants of the prokaryotic community composition, as has been found in other studies (Ramírez et al., 2010; Lauber et al., 2009; Fierer and Jackson, 2006; Rousk et al., 2010a). Soil pH can directly induce physiological stress on soil prokaryotes impairing their growth or competitiveness (Fernández-Calviño and Bååth, 2010). We found decreased relative abundances under N10 addition of the myxobacterial genus *Labilithrix* (Yamamoto et al., 2014) that was also decreased in a South African grassland (Schleuss et al., 2019) and in a Chinese forest soil (Cui et al., 2017) due to N addition. The relative decrease in *Labilithrix* is likely associated with the decreased soil pH under N10 addition, since its growth range is pH 5–9 (Yamamoto et al., 2014). Decreased soil pH likely explained increased relative abundances of *Rhodanobacter* species that can be considered as acid-tolerant denitrifiers and dominated bacterial communities in acidic and nitrate-rich conditions (Green et al., 2012; van den Heuvel et al., 2010).

N addition enhanced abundances of ammonia-oxidizing bacteria such as of *Nitrosospira* and of ammonia-oxidizing archaea such as *Candidatus Nitrocosmicus* that oxidize ammonia to nitrate and thus perform the first step of nitrification, as previously observed (Carey et al., 2016; Yan et al., 2020). Further, under NP addition relative abundances of *Sphingomonas* increased, as previously observed for a Chinese agricultural (Chen et al., 2019b) and forest soil (Cui et al., 2017). *Sphingomonas* species are known as ubiquitously occurring generalists (Aschenbrenner et al., 2017) with high catabolic versatility (Asaf et al., 2020) and seem to be favored by increased N and P availabilities likely because nutrient addition makes the soil less oligotrophic.

Besides soil pH, TOC concentration accounted for a high proportion of prokaryotic community composition according to our correlation analysis, reflecting the importance of C as a limiting resource supporting and structuring microbial communities (Alden et al., 2001; Heuck et al., 2015; Sul et al., 2013). For example, C availability structured bacterial communities across N gradients in a grassland and an agricultural soil in the USA (Ramírez et al., 2010), TOC was the most important factor that accounted for differences in microbial community structure in an African savanna (Sul et al., 2013), and differences in tree species derived C inputs under N addition were the main factor driving microbial community composition in a hardwood forest (Weand et al., 2010). TOC concentration also has been shown to control the number of metabolically active cells in soil (Semenov et al., 2016). Although we sequenced total microbial communities and not active microbial communities based on RNA analysis, most of the community in the rhizosphere can be considered as active or potentially active rather than dormant (Blagodatskaya and Kuzyakov, 2013). The root density in the topsoil of the sampled grassland was very high suggesting that most of our soil volume was from the rhizosphere.

4.3. Drivers of fungal community composition under nutrient addition

Nutrient addition altered fungal more strongly than prokaryotic community. This is in line with previous studies indicating that fungi react more sensitively to nutrient addition than other microbial groups (Högberg et al., 2007; Freedman et al., 2015). In contrast to the main drivers of the prokaryotic community, DOC:DN ratio was the strongest predictor of the fungal community, according to correlation analysis. An

explanation for this finding could be that fungal and bacterial biomass show slightly different C:N ratios, with C:N ratios around 5 for bacteria and around 10 for fungi in soils (Strickland and Rousk, 2010; De Deyn et al., 2008). Generally speaking, fungi and bacteria prefer substrates with different C:N ratios (Sterner and Elser, 2002) with fungi preferring substrates with a higher C:N ratio in comparison to bacteria (Six et al., 2006; Keibinger et al., 2010; Grosso et al., 2016). Thus, smaller DOC:DN ratios under N addition could have affected especially fungal communities. However, certain fungi, mainly fast-growing fungi in the Ascomycota, such as molds (Lundell et al., 2014), also benefit from smaller DOC:DN ratios, whereas other fungi, mainly Basidiomycota, are specialists in decomposing complex C sources with high C:N ratios (de Boer et al., 2005).

Consequently, the relative decreases in the saprotrophic Basidiomycota genera *Hygrocybe* and *Clavaria* (Tedersoo et al., 2014) under N addition can be explained by their sensitivity to elevated nutrient inputs (Griffith et al., 2002). For instance, the number of *Hygrocybe* strongly decreased through intensification of management in European grasslands (Griffith et al., 2002; Griffith and Roderick, 2008). Decreasing Basidiomycota abundances due to N addition have been found in other studies as well (Leff et al., 2015; Nemergut et al., 2008; Klaubauf et al., 2010; Morrison et al., 2016).

In contrast to the prokaryotic community, the fungal community composition was not as strongly controlled by soil pH, as indicated by the correlation analysis. Previous studies also confirmed that fungi were less sensitive to soil pH changes than bacteria (Rousk et al., 2010a, 2010b; Schleuss et al., 2019). However, plant and fungal community composition were highly correlated, as found in other grasslands (Chen et al., 2017; Prober et al., 2015), whereas a more detailed analysis revealed that nutrient availabilities were more important drivers of fungal communities than plant biomass or plant community structure. A global study confirmed that climatic and edaphic factors, not plant diversity, predicted fungal richness at a global scale (Tedersoo et al., 2014).

5. Conclusion

Nine years of N addition altered prokaryotic and fungal community composition. The response of the prokaryotic and fungal community composition to nutrient addition was more tightly coupled to soil properties such as pH, TOC concentration, and DOC:DN ratio than to changes in the plant community. The changes in microbial communities did not affect microbial biomass and respiration rates indicating functional redundancy of these variables. In general, element-cycling rates were mainly mediated by soil factors as opposed to plant and microbial community shifts. Yet, over several decades, the observed changes in microbial community composition and element cycling will likely become stronger. Taken together, our results suggest that changes in the microbial community in response to increasing N inputs might not necessarily lead to a loss of microbial functioning that underlies soil element cycling in grassland ecosystems.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

M. Spohn thanks the German Research Foundation for funding this study through the Emmy Noether-program (grant SP1389/6-1). Coordination and data management of the Nutrient Network have been supported by funding to E. Borer and E. Seabloom from the National Science Foundation Research Coordination Network (NSF-DEB-1042132) and Long Term Ecological Research (DEB-1234162 and DEB-

1831944 to Cedar Creek LTER) programs, and the Institute on the Environment (DG-0001-13). A. Heintz-Buschart was funded by the German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig of the German Research Foundation (FZT 118, DFG 202548816). We thank Renate Krauss, Uwe Hell, and Karin Söllner for technical assistance, the chemical analytics (CAN) of the Bayreuth Centre of Ecological and Environmental Research (BayCEER) for performing parts of the chemical analyses, the Laboratory of Isotope Biogeochemistry at the University of Bayreuth for measuring ^{15}N isotopes, and Beatrix Schnabel of the Helmholtz-Centre for Environmental Research (UFZ) for sequencing. We thank Eduardo Vazquez for his helpful comments on a previous version of the manuscript. The community composition data have in part been computed at the High-Performance Computing (HPC) Cluster EVE, a joint effort of both the Helmholtz Centre for Environmental Research - UFZ and the German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.soilbio.2020.108041>.

References

- Alden, L., Demoling, F., Baath, E., 2001. Rapid method of determining factors limiting bacterial growth in soil. *Applied and Environmental Microbiology* 67, 1830–1838.
- Allison, S.D., Hanson, C.A., Treseder, K.K., 2007. Nitrogen fertilization reduces diversity and alters community structure of active fungi in boreal ecosystems. *Soil Biology and Biochemistry* 39, 1878–1887.
- Allison, S.D., Martiny, J.B.H., 2008. Resistance, resilience, and redundancy in microbial communities. *Proceedings of the National Academy of Sciences of the United States of America* 105, 11512–11519.
- Asaf, S., Numan, M., Khan, A.L., Al-Harrasi, A., 2020. Sphingomonas: from diversity and genomics to functional role in environmental remediation and plant growth. *Critical Reviews in Biotechnology* 40, 138–152.
- Aschenbrenner, I.A., Cernava, T., Erlacher, A., Berg, G., Grube, M., 2017. Differential sharing and distinct co-occurrence networks among spatially close bacterial microbiota of bark, mosses and lichens. *Molecular Ecology* 26, 2826–2838.
- Banerjee, S., Kirkby, C.A., Schmutter, D., Bissett, A., Kirkegaard, J.A., Richardson, A.E., 2016. Network analysis reveals functional redundancy and keystone taxa amongst bacterial and fungal communities during organic matter decomposition in an arable soil. *Soil Biology and Biochemistry* 97, 188–198.
- Bengtsson-Palme, J., Ryberg, M., Hartmann, M., Branco, S., Wang, Z., Godhe, A., Wit, P. de, Sánchez-García, M., Ebersberger, I., Sousa, F. de, Amend, A.S., Jumpponen, A., Unterseher, M., Kristiansson, E., Abarenkov, K., Bertrand, Y.J.K., Sanli, K., Eriksson, K.M., Vik, U., Veldre, V., Nilsson, R.H., Bunce, M., 2013. Improved software detection and extraction of ITS1 and ITS2 from ribosomal ITS sequences of fungi and other eukaryotes for analysis of environmental sequencing data. *Methods in Ecology and Evolution* 25, 914–919.
- Berg, G., Smalla, K., 2009. Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere. *FEMS Microbiology Ecology* 68, 1–13.
- Blagodatskaya, E., Kuzyakov, Y., 2013. Active microorganisms in soil: critical review of estimation criteria and approaches. *Soil Biology and Biochemistry* 67, 192–211.
- Borer, E.T., Harpole, W.S., Adler, P.B., Lind, E.M., Orrock, J.L., Seabloom, E.W., Smith, M.D., Freckleton, R., 2014. Finding generality in ecology: a model for globally distributed experiments. *Methods in Ecology and Evolution* 5, 65–73.
- Brookes, P.C., Powlson, D.S., Jenkinson, D.S., 1982. Measurement of microbial biomass phosphorus in soil. *Soil Biology and Biochemistry* 14, 319–329.
- Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A., Holmes, S.P., 2016. DADA2: high-resolution sample inference from Illumina amplicon data. *Nature Methods* 13, 581–583.
- Caporaso, J.G., Lauber, C.L., Walters, W.A., Berg-Lyons, D., Lozupone, C.A., Turnbaugh, P.J., Fierer, N., Knight, R., 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, 4516–4522.
- Carey, C.J., Dove, N.C., Beman, J.M., Hart, S.C., Aronson, E.L., 2016. Meta-analysis reveals ammonia-oxidizing bacteria respond more strongly to nitrogen addition than ammonia-oxidizing archaea. *Soil Biology and Biochemistry* 99, 158–166.
- Chen, C., Chen, H.Y.H., Chen, X., Huang, Z., 2019a. Meta-analysis shows positive effects of plant diversity on microbial biomass and respiration. *Nature Communications* 10, 1332.
- Chen, D., Lan, Z., Hu, S., Bai, Y., 2015. Effects of nitrogen enrichment on belowground communities in grassland. Relative role of soil nitrogen availability vs. soil acidification. *Soil Biology and Biochemistry* 89, 99–108.
- Chen, L., Redmile-Gordon, M., Li, J., Zhang, J., Xin, X., Zhang, C., Ma, D., Zhou, Y., 2019b. Linking cropland ecosystem services to microbiome taxonomic composition and functional composition in a sandy loam soil with 28-year organic and inorganic fertilizer regimes. *Applied Soil Ecology* 139, 1–9.
- Chen, Y.-L., Xu, T.-L., Veresoglou, S.D., Hu, H.-W., Hao, Z.-P., Hu, Y.-J., Liu, L., Deng, Y., Rillig, M.C., Chen, B.-D., 2017. Plant diversity represents the prevalent determinant of soil fungal community structure across temperate grasslands in northern China. *Soil Biology and Biochemistry* 110, 12–21.
- Clarke, K.R., Gorley, R.N., 2015. PRIMER V7: User Manual/Tutorial (Plymouth).
- Cruaud, P., Rasplis, J.-Y., Rodriguez, L.J., Cruaud, A., 2017. High-throughput sequencing of multiple amplicons for barcoding and integrative taxonomy. *Scientific Reports* 7, 41948.
- Cui, J., Wang, J., Xu, J., Xu, C., Xu, X., 2017. Changes in soil bacterial communities in an evergreen broad-leaved forest in east China following 4 years of nitrogen addition. *Journal of Soils and Sediments* 17, 2156–2164.
- de Boer, W., Folman, L.B., Summerbell, R.C., Boddy, L., 2005. Living in a fungal world: impact of fungi on soil bacterial niche development. *FEMS Microbiology Reviews* 29, 795–811.
- De Deyn, G.B., Cornelissen, J.H.C., Bardgett, R.D., 2008. Plant functional traits and soil carbon sequestration in contrasting biomes. *Ecology Letters* 11, 516–531.
- Delgado-Baquerizo, M., Giammar, L., Reich, P.B., Khachane, A.N., Hamonts, K., Edwards, C., Lawton, L.A., Singh, B.K., Brophy, C., 2016. Lack of functional redundancy in the relationship between microbial diversity and ecosystem functioning. *Journal of Ecology* 104, 936–946.
- Dixon, R., Kahn, D., 2004. Genetic regulation of biological nitrogen fixation. *Nature Reviews Microbiology* 2, 621–631.
- Dray, S., Dufour, A.-B., 2007. The ade4 package: implementing the duality diagram for ecologists. *Journal of Statistical Software* 22, 1–20.
- Falkowski, P.G., Fenchel, T., Delong, E.F., 2008. The microbial engines that drive earth's biogeochemical cycles. *Science* 320, 1034–1039.
- Fierer, N., Jackson, R.B., 2006. The diversity and biogeography of soil bacterial communities. *Proceedings of the National Academy of Sciences of the United States of America* 103, 626–631.
- Fierer, N., Lauber, C.L., Ramirez, K.S., Zaneveld, J., Bradford, M.A., Knight, R., 2012. Comparative metagenomic, phylogenetic and physiological analyses of soil microbial communities across nitrogen gradients. *The ISME Journal* 6, 1007–1017.
- Fierer, N., Strickland, M.S., Liptzin, D., Bradford, M.A., Cleveland, C.C., 2009. Global patterns in belowground communities. *Ecology Letters* 12, 1238–1249.
- Freedman, Z.B., Romanowicz, K.J., Upchurch, R.A., Zak, D.R., 2015. Differential responses of total and active soil microbial communities to long-term experimental N deposition. *Soil Biology and Biochemistry* 90, 275–282.
- Galloway, J.N., Dentener, F.J., Capone, D.G., Boyer, E.W., Howarth, R.W., Seitzinger, S. P., Asner, G.P., Cleveland, C.C., Green, P.A., Holland, E.A., 2004. Nitrogen cycles: past, present, and future. *Biogeochemistry* 70, 153–226.
- Galloway, J.N., Townsend, A.R., Erismann, J.W., Bekunda, M., Cai, Z., Freney, J.R., Martinelli, L.A., Seitzinger, S.P., Sutton, M.A., 2008. Transformation of the nitrogen cycle: recent trends, questions, and potential solutions. *Science* 889–892.
- Green, S.J., Prakash, O., Jasrotia, P., Overholt, W.A., Cardenas, E., Hubbard, D., Tiedje, J. M., Watson, D.B., Schadt, C.W., Brooks, S.C., Kostka, J.E., 2012. Denitrifying bacteria from the genus *Rhodanobacter* dominate bacterial communities in the highly contaminated subsurface of a nuclear legacy waste site. *Applied and Environmental Microbiology* 78, 1039–1047.
- Griffith, G.W., Easton, G.L., Jones, A.W., 2002. Ecology and diversity of waxcap (*Hygocybe* spp.) fungi. *Botanical Journal of Scotland* 54, 7–22.
- Griffith, G.W., Roderick, K., 2008. Saprotrophic basidiomycetes in grasslands: distribution and function. In: Boddy, L., Frankland, J.C., van West, P. (Eds.), *Ecology of Saprotrophic Basidiomycetes*, first ed. Elsevier, Amsterdam, Boston, pp. 277–299.
- Griffiths, B.S., Ritz, K., Bardgett, R.D., Cook, R., Christensen, S., Ekelund, F., Sørensen, S. J., Bååth, E., Bloem, J., de Ruiter, P.C., Dolfing, J., Nicolardot, B., 2000. Ecosystem response of pasture soil communities to fumigation-induced microbial diversity reductions: an examination of the biodiversity–ecosystem function relationship. *Oikos* 90, 279–294.
- Grosso, F., Bååth, E., Nicola, F. de, 2016. Bacterial and fungal growth on different plant litter in Mediterranean soils: effects of C/N ratio and soil pH. *Applied Soil Ecology* 108, 1–7.
- Gruber, N., Galloway, J.N., 2008. An Earth-system perspective of the global nitrogen cycle. *Nature* 451, 293–296.
- Harpole, W.S., Sullivan, L.L., Lind, E.M., Firn, J., Adler, P.B., Borer, E.T., Chase, J., Fay, P.A., Hautier, Y., Hillebrand, H., MacDougall, A.S., Seabloom, E.W., Williams, R.H., Bakker, J.D., Cadotte, M.W., Chaneton, E.J., Chu, C., Cleland, E.E., D'Antonio, C., Davies, K.F., Gruner, D.S., Hagenah, N., Kirkman, K., Knops, J.M.H., La Pierre, K.J., McCulley, R.L., Moore, J.L., Morgan, J.W., Prober, S.M., Risch, A.C., Schuetz, M., Stevens, C.J., Wragg, P.D., 2016. Addition of multiple limiting resources reduces grassland diversity. *Nature* 537, 93–96.
- Heinemeyer, A., Fitter, A.H., 2004. Impact of temperature on the arbuscular mycorrhizal (AM) symbiosis: growth responses of the host plant and its AM fungal partner. *Journal of Experimental Botany* 55, 525–534.
- Heuck, C., Spohn, M., 2016. Carbon, nitrogen and phosphorus net mineralization in organic horizons of temperate forests: stoichiometry and relations to organic matter quality. *Biogeochemistry* 131, 229–242.
- Heuck, C., Weig, A., Spohn, M., 2015. Soil microbial biomass C:N:P stoichiometry and microbial use of organic phosphorus. *Soil Biology and Biochemistry* 85, 119–129.
- Hill, S., 1992. Physiology of nitrogen fixation in free-living heterotrophs. In: Stacey, G., Burris, R.H., Evans, H.J. (Eds.), *Biological Nitrogen Fixation*. Chapman and Hall, New York.
- Högberg, M.N., Högberg, P., Myrold, D.D., 2007. Is microbial community composition in boreal forest soils determined by pH, C-to-N ratio, the trees, or all three? *Oecologia* 150, 590–601.
- Ihrmark, K., Bödeker, I.T.M., Cruz-Martinez, K., Friberg, H., Kubartova, A., Schenck, J., Strid, Y., Stenlid, J., Brandström-Durling, M., Clemmensen, K.E., Lindahl, B.D., 2012.

- New primers to amplify the fungal ITS2 region - evaluation by 454-sequencing of artificial and natural communities. *FEMS Microbiology Ecology* 82, 666–677.
- Jenkinson, D.S., Brookes, P.C., Powlson, D.S., 2004. Measuring soil microbial biomass. *Soil Biology and Biochemistry* 36, 5–7.
- Keiblinger, K.M., Hall, E.K., Wanek, W., Szukics, U., Hämmerle, I., Ellersdorfer, G., Böck, S., Strauss, J., Sterflinger, K., Richter, A., Zechmeister-Boltenstern, S., 2010. The effect of resource quantity and resource stoichiometry on microbial carbon-use efficiency. *FEMS Microbiology Ecology* 73, 430–440.
- Klaubauf, S., Inselsbacher, E., Zechmeister-Boltenstern, S., Wanek, W., Gottsberger, R., Strauss, J., Gorfner, M., 2010. Molecular diversity of fungal communities in agricultural soils from Lower Austria. *Fungal Diversity* 44, 65–75.
- Köljal, U., Nilsson, R.H., Abarenkov, K., Tedersoo, L., Taylor, A.F.S., Bahram, M., Bates, S.T., Bruns, T.D., Bengtsson-Palme, J., Callaghan, T.M., Douglas, B., Drenkhan, T., Eberhardt, U., Dueñas, M., Grebenc, T., Griffith, G.W., Hartmann, M., Kirk, P.M., Kohout, P., Larsson, E., Lindahl, B.D., Lücking, R., Martin, M.P., Matheny, P.B., Nguyen, N.H., Niskanen, T., Oja, J., Peay, K.G., Peintner, U., Peterson, M., Pöldmaa, K., Saag, L., Saar, I., Schüßler, A., Scott, J.A., Senés, C., Smith, M.E., Suija, A., Taylor, D.L., Telleria, M.T., Weiss, M., Larsson, K.-H., 2013. Towards a unified paradigm for sequence-based identification of fungi. *Molecular Ecology* 22, 5271–5277.
- Lange, M., Eisenhauer, N., Sierra, C.A., Bessler, H., Engels, C., Griffiths, R.I., Mellado-Vázquez, P.G., Malik, A.A., Roy, J., Scheu, S., Steinbeiss, S., Thomson, B., Trumbore, S.E., Gleixner, G., 2015. Plant diversity increases soil microbial activity and soil carbon storage. *Nature Communications* 6, 6707.
- Lauber, C.L., Hamady, M., Knight, R., Fierer, N., 2009. Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. *Applied and Environmental Microbiology* 75, 5111–5120.
- Lauber, C.L., Strickland, M.S., Bradford, M.A., Fierer, N., 2008. The influence of soil properties on the structure of bacterial and fungal communities across land-use types. *Soil Biology and Biochemistry* 40, 2407–2415.
- Leff, J.W., Jones, S.E., Prober, S.M., Barberán, A., Borer, E.T., Firn, J.L., Harpole, W.S., Hobbie, S.E., Hofmockel, K.S., Knops, J.M.H., McCulley, R.L., La Pierre, K., Risch, A.C., Seabloom, E.W., Schütz, M., Steenbock, C., Stevens, C.J., Fierer, N., 2015. Consistent responses of soil microbial communities to elevated nutrient inputs in grasslands across the globe. *Proceedings of the National Academy of Sciences of the United States of America* 112, 10967–10972.
- Liu, L., Greaver, T.L., 2010. A global perspective on belowground carbon dynamics under nitrogen enrichment. *Ecology Letters* 13, 819–828.
- Louca, S., Polz, M.F., Mazel, F., Albright, M.B.N., Huber, J.A., O'Connor, M.I., Ackermann, M., Hahn, A.S., Srivastava, D.S., Crowe, S.A., Doebeli, M., Parfrey, L.W., 2018. Function and functional redundancy in microbial systems. *Nature Ecology & Evolution* 2, 936–943.
- Love, M.I., Huber, W., Anders, S., 2014. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biology* 15, 550.
- Lundell, T.K., Mäkelä, M.R., Vries, R.P. de, Hildén, K.S., 2014. Genomics, lifestyles and future prospects of wood-decay and litter-decomposing Basidiomycota. In: Martin, F. M. (Ed.), *Advances in Botanical Research*. Elsevier Academic Press, pp. 329–370.
- Ma, L.-N., Lü, X.-T., Liu, Y., Guo, J.-X., Zhang, N.-Y., Yang, J.-Q., Wang, R.-Z., 2011. The effects of warming and nitrogen addition on soil nitrogen cycling in a temperate grassland, northeastern China. *PLoS One* 6, e27645.
- Manning, P., Newington, J.E., Robson, H.R., Saunders, M., Eggers, T., Bradford, M.A., Bardgett, R.D., Bonkowski, M., Ellis, R.J., Gange, A.C., Grayston, S.J., Kandeler, E., Marhan, S., Reid, E., Tschirko, D., Godfray, H.C.J., Rees, M., 2006. Decoupling the direct and indirect effects of nitrogen deposition on ecosystem function. *Ecology Letters* 9, 1015–1024.
- Manzoni, S., Jackson, R.B., Trofymow, J.A., Porporato, A., 2008. The global stoichiometry of litter nitrogen mineralization. *Science* 321, 684–686.
- McMurdie, P.J., Holmes, S., 2013. phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS One* 8, e61217.
- Morrison, E.W., Frey, S.D., Sadowsky, J.J., van Diepen, L.T.A., Thomas, W.K., Pringle, A., 2016. Chronic nitrogen additions fundamentally restructure the soil fungal community in a temperate forest. *Fungal Ecology* 23, 48–57.
- Murphy, J., Riley, J.P., 1962. A modified single solution method for the determination of phosphate in natural waters. *Analytica Chimica Acta* 27, 31–36.
- Nannipieri, P., Ascher, J., Ceccherini, M.T., Landi, L., Pietramellara, G., Renella, G., 2003. Microbial diversity and soil functions. *European Journal of Soil Science* 54, 655–670.
- Nemergut, D.R., Townsend, A.R., Sattin, S.R., Freeman, K.R., Fierer, N., Neff, J.C., Bowman, W.D., Schadt, C.W., Weintraub, M.N., Schmidt, S.K., 2008. The effects of chronic nitrogen fertilization on alpine tundra soil microbial communities: implications for carbon and nitrogen cycling. *Environmental Microbiology* 10, 3093–3105.
- Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., Szocs, E., Wagner, H., 2019. *vegan: community Ecology Package*. R package version 2.5-5. <https://CRAN.R-project.org/package=vegan>.
- Paradis, E., Schliep, K., 2019. Ape 5.0: an environment for modern phylogenetics and evolutionary analyses in R. *Bioinformatics* 35, 526–528.
- Peñuelas, J., Poulter, B., Sardans, J., Ciais, P., van der Velde, M., Bopp, L., Boucher, O., Goddard, Y., Hinsinger, P., Ilusija, J., Nardin, E., Vicca, S., Obersteiner, M., Janssens, I.A., 2013. Human-induced nitrogen-phosphorus imbalances alter natural and managed ecosystems across the globe. *Nature Communications* 4, 2934.
- Peñuelas, J., Sardans, J., Rivas-ubach, A., Janssens, I.A., 2012. The human-induced imbalance between C, N and P in Earth's life system. *Global Change Biology* 18, 3–6.
- Philippot, L., Spor, A., Hénault, C., Bru, D., Bizouard, F., Jones, C.M., Sarr, A., Maron, P.-A., 2013. Loss in microbial diversity affects nitrogen cycling in soil. *The ISME Journal* 7, 1609–1619.
- Prober, S.M., Leff, J.W., Bates, S.T., Borer, E.T., Firn, J., Harpole, W.S., Lind, E.M., Seabloom, E.W., Adler, P.B., Bakker, J.D., Cleland, E.E., DeCrappeo, N.M., DeLorenzo, E., Hagenah, N., Hautier, Y., Hofmockel, K.S., Kirkman, K.P., Knops, J.M.H., La Pierre, K.J., MacDougall, A.S., McCulley, R.L., Mitchell, C.E., Risch, A.C., Schuetz, M., Stevens, C.J., Williams, R.J., Fierer, N., 2015. Plant diversity predicts beta but not alpha diversity of soil microbes across grasslands worldwide. *Ecology Letters* 18, 85–95.
- Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., Glöckner, F.O., 2013. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Research* 41, 590–596.
- R Core Team, 2018. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria. URL: <http://www.R-project.org/>.
- Ramirez, K.S., Craine, J.M., Fierer, N., 2012. Consistent effects of nitrogen amendments on soil microbial communities and processes across biomes. *Global Change Biology* 18, 1918–1927.
- Ramirez, K.S., Lauber, C.L., Knight, R., Bradford, M.A., Fierer, N., 2010. Consistent effects of nitrogen fertilization on soil bacterial communities in contrasting systems. *Ecology* 91, 3463–3470.
- Reed, S.C., Cleveland, C.C., Townsend, A.R., 2011. Functional ecology of free-living nitrogen fixation: a contemporary perspective. *Annual Review of Ecology, Evolution and Systematics* 42, 489–512.
- Reed, S.C., Townsend, A.R., Cleveland, C.C., Nemergut, D.R., 2010. Microbial community shifts influence patterns in tropical forest nitrogen fixation. *Oecologia* 164, 521–531.
- Rousk, J., Bååth, E., Brookes, P.C., Lauber, C.L., Lozupone, C., Caporaso, J.G., Knight, R., Fierer, N., 2010a. Soil bacterial and fungal communities across a pH gradient in an arable soil. *The ISME Journal* 4, 1340–1351.
- Rousk, J., Brookes, P.C., Bååth, E., 2009. Contrasting soil pH effects on fungal and bacterial growth suggest functional redundancy in carbon mineralization. *Applied and Environmental Microbiology* 75, 1589–1596.
- Rousk, J., Brookes, P.C., Bååth, E., 2010b. Investigating the mechanisms for the opposing pH relationships of fungal and bacterial growth in soil. *Soil Biology and Biochemistry* 42, 926–934.
- Schimel, J.P., 1995. Ecosystem consequences of microbial diversity and community structure. In: Chapin, F.S., Körner, C. (Eds.), *Artic and Alpine Biodiversity: Patterns, Causes, and Ecosystem Consequences*. Springer, Berlin Heidelberg, pp. 239–254.
- Schleuss, P.-M., Widdig, M., Heintz-Buschart, A., Guhr, A., Martin, S., Kirkman, K., Spohn, M., 2019. Stoichiometric controls of soil carbon and nitrogen cycling after long-term nitrogen and phosphorus addition in a mesic grassland in South Africa. *Soil Biology and Biochemistry* 135, 294–303.
- Schloss, P.D., Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M., Hollister, E.B., Lesniewski, R.A., Oakley, B.B., Parks, D.H., Robinson, C.J., Sahl, J.W., Stres, B., Thallinger, G.G., van Horn, D.J., Weber, C.F., 2009. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Applied and Environmental Microbiology* 75, 7537–7541.
- Semenov, M.V., Manucharova, N.A., Stepanov, A.L., 2016. Distribution of metabolically active prokaryotes (archaea and bacteria) throughout the profiles of chernozem and Brown semidesert soil. *Eurasian Soil Science* 49, 217–225.
- Six, J., Frey, S.D., Thiet, R.K., Batten, K.M., 2006. Bacterial and fungal contributions to carbon sequestration in agroecosystems. *Soil Science Society of America Journal* 70, 555–569.
- Smith, V.H., 1992. Effects of nitrogen: phosphorus supply ratios on nitrogen fixation in agricultural and pastoral ecosystems. *Biogeochemistry* 19–35.
- Spohn, M., 2015. Microbial respiration per unit microbial biomass depends on litter layer carbon-to-nitrogen ratio. *Biogeosciences* 12, 817–823.
- Spohn, M., Chodak, M., 2015. Microbial respiration per unit biomass increases with carbon-to-nutrient ratios in forest soils. *Soil Biology and Biochemistry* 81, 128–133.
- Sterner, R.W., Elser, J.J., 2002. *Ecological Stoichiometry: the Biology of Elements from Molecules to the Biosphere*. Princeton University Press.
- Stevens, C.J., Lind, E.M., Hautier, Y., Harpole, W.S., Borer, E.T., Hobbie, S., Seabloom, E.W., Ladwig, L., Bakker, J.D., Chu, C., Collins, S., Davies, K.F., Firn, J., Hillebrand, H., La Pierre, K.J., MacDougall, A., Melbourne, B., McCulley, R.L., Morgan, J., Orrock, J.L., Prober, S.M., Risch, A.C., Schuetz, M., Wragg, P.D., 2015. Anthropogenic nitrogen deposition predicts local grassland primary production worldwide. *Ecology* 96, 1459–1465.
- Strickland, M.S., Lauber, C.L., Fierer, N., Bradford, M.A., 2009. Testing the functional significance of microbial community composition. *Ecology* 90, 441–451.
- Strickland, M.S., Rousk, J., 2010. Considering fungal:bacterial dominance in soils - methods, controls, and ecosystem implications. *Soil Biology and Biochemistry* 42, 1385–1395.
- Sul, W.J., Asuming-Brempong, S., Wang, Q., Tourlousse, D.M., Penton, C.R., Deng, Y., Rodrigues, J.L.M., Adiku, S.G.K., Jones, J.W., Zhou, J., Cole, J.R., Tiedje, J.M., 2013. Tropical agricultural land management influences on soil microbial communities through its effect on soil organic carbon. *Soil Biology and Biochemistry* 65, 33–38.
- Tedersoo, L., Bahram, M., Pölme, S., Köljal, U., Yorou, N.S., Wijesundera, R., Villarreal Ruiz, L., Vasco-Palacios, A.M., Thu, P.Q., Suija, A., Smith, M.E., Sharp, C., Saluveer, E., Saita, A., Rosas, M., Riit, T., Ratkowsky, D., Pritsch, K., Pöldmaa, K., Piepenbring, M., Phosri, C., Peterson, M., Parts, K., Pärtel, K., Östing, E., Nounra, E., Njounkou, A.L., Nilsson, R.H., Morgado, L.N., Mayor, J., May, T.W., Majuakim, L., Lodge, D.J., Lee, S.S., Larsson, K.-H., Kohout, P., Hosaka, K., Hiiesalu, I., Henkel, T.W., Harend, H., Guo, L.-d., Greslebin, A., Grelet, G., Geml, J., Gates, G., Dunstan, W.,

- Dunk, C., Drenkhan, R., Dearnaley, J., Kesel, A. de, Dang, T., Chen, X., Buegger, F., Brearley, F.Q., Bonito, G., Anslan, S., Abell, S., Abarenkov, K., 2014. Global diversity and geography of soil fungi. *Science* 346, 1256–1268.
- van den Heuvel, R.N., van der Biezen, E., Jetten, M.S.M., Hefting, M.M., Kartal, B., 2010. Denitrification at pH 4 by a soil-derived *Rhodanobacter*-dominated community. *Environmental Microbiology* 12, 3264–3271.
- Vance, E.D., Brookes, P.C., Jenkinson, D.S., 1987. An extraction method for measuring soil microbial biomass C. *Soil Biology and Biochemistry* 19, 703–707.
- Vestgarden, L.S., Selle, L.T., Stuanes, A.O., 2003. In situ soil nitrogen mineralisation in a Scots pine (*Pinus sylvestris* L.) stand: effects of increased nitrogen input. *Forest Ecology and Management* 176, 205–216.
- Vourlitis, G.L., Zorba, G., Pasquini, S.C., Mustard, R., 2007. Chronic nitrogen deposition enhances nitrogen mineralization potential of semiarid shrubland soils. *Soil Science Society of America Journal* 71, 836.
- Wang, R., Balkanski, Y., Boucher, O., Ciais, P., Peñuelas, J., Tao, S., 2015. Significant contribution of combustion-related emissions to the atmospheric phosphorus budget. *Nature Geoscience* 8, 48–54.
- Wardle, D.A., Gundale, M.J., Jäderlund, A., Nilsson, M.-C., 2013. Decoupled long-term effects of nutrient enrichment on aboveground and belowground properties in subalpine tundra. *Ecology* 94, 904–919.
- Weand, M.P., Arthur, M.A., Lovett, G.M., McCulley, R.L., Weathers, K.C., 2010. Effects of tree species and N additions on forest floor microbial communities and extracellular enzyme activities. *Soil Biology and Biochemistry* 42, 2161–2173.
- Wertz, S., Degrange, V., Prosser, J.I., Poly, F., Commeaux, C., Freitag, T., Guillaumaud, N., Le Roux, X., 2006. Maintenance of soil functioning following erosion of microbial diversity. *Environmental Microbiology* 8, 2162–2169.
- White, T.J., Bruns, T., Lee, S., Taylor, J., 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis, M.A., Gelfand, D.H., Sninsky, J.J., White, T.J. (Eds.), *PCR Protocols: A Guide to Methods and Applications*. Academic Press, San Diego, pp. 315–322.
- Yamamoto, E., Muramatsu, H., Nagai, K., 2014. *Vulgatibacter incomptus* gen. nov., sp. nov. and *Labilithrix luteola* gen. nov., sp. nov., two myxobacteria isolated from soil in Yakushima Island, and the description of *Vulgatibacteraceae* fam. nov., *Labilithrichaceae* fam. nov. and *Anaeromyxobacteraceae* fam. nov. *International Journal of Systematic and Evolutionary Microbiology* 64, 3360–3368.
- Yan, D., Gellie, N.J.C., Mills, J.G., Connell, G., Bissett, A., Lowe, A.J., Breed, M.F., 2020. A soil archaeal community responds to a decade of ecological restoration. *Restoration Ecology* 28, 63–72.
- Yue, K., Peng, Y., Peng, C., Yang, W., Peng, X., Wu, F., 2016. Stimulation of terrestrial ecosystem carbon storage by nitrogen addition: a meta-analysis. *Scientific Reports* 6, 19895.
- Zechmeister-Boltenstern, S., 1996. Non-symbiotic nitrogen fixation. In: Schinner, F., Öhlinger, R., Kandeler, E., Margesin, R. (Eds.), *Methods in Soil Biology*. Springer, Berlin, Heidelberg, pp. 122–134.
- Zeng, J., Liu, X., Song, L., Lin, X., Zhang, H., Shen, C., Chu, H., 2016. Nitrogen fertilization directly affects soil bacterial diversity and indirectly affects bacterial community composition. *Soil Biology and Biochemistry* 92, 41–49.
- Zhou, Z., Wang, C., Zheng, M., Jiang, L., Luo, Y., 2017. Patterns and mechanisms of responses by soil microbial communities to nitrogen addition. *Soil Biology and Biochemistry* 115, 433–441.