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

Meike Widdig[1*], Anna Heintz-Buschart[2,3], Per-Marten Schleuss[1], Alexander Guhr[1], Elizabeth T. Borer[4], Eric W. Seabloom[4], Marie Spohn[1]

[1]Department of Soil Ecology and Soil Biogeochemistry, University of Bayreuth, Bayreuth, Germany

[2]Department of Soil Ecology, UFZ-Helmholtz Centre for Environmental Research, Halle (Saale), Germany

[3]Bioinformatics Unit, German Centre for Integrative Biodiversity Research (iDiv), Halle-Jena-Leipzig, Germany

[4]Department of Ecology, Evolution, and Behavior, University of Minnesota, St. Paul, USA

*Corresponding author: Meike Widdig

E-Mail: meike.widdig@uni-bayreuth.de

Phone: ++49- (0)921-55-5731

Paper type: Research Article (Regular Paper)

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 N_2 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 g N m^{-2} yr^{-1}, and it has been predicted that by 2050 some regions may receive up to 5 g N m^{-2} yr^{-1} (Galloway et al., 2004). 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; Ramirez et al., 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 x 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 ($(NH_2)_2CO$). Further, 10 g P m^{-2} yr^{-1} was added as triple-super phosphate ($Ca(H_2PO_4)_2$) without N and in combination with 10 g N m^{-2} yr^{-1}.

2.2 Soil sampling

Soils were sampled from 0-15 cm depth (called topsoil hereafter) and in a second depth increment from 15-30 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 Elementar, Hanau, Germany). TP was determined by ICP-OES (Vista-Pro radial, Varian) after pressure digestion in aqua regia (HNO_3 + 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 one hour. 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 K_2SO_4 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 CO_2 was trapped in 0.6 M KOH and changes in electrical conductivity were measured by a respirometer (Respicond V, Nordgen Innovations). Cumulative CO_2 was measured continuously (every 2 hours) and respiration rates were calculated based on the linear increase in accumulated C-CO_2 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 one hour 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-NH_4^+) and nitrate (N-NO_3^-) via flow injection analysis (FIA-Lab, MLE Dresden) and ICP-OES, respectively. Net N

mineralization rates were calculated based on the linear increase of $N\text{-}NH_4^+$ and $N\text{-}NO_3^-$ (dissolved inorganic N, DIN) over time.

2.7 Non-symbiotic N_2 fixation

Non-symbiotic fixation of atmospheric N_2 was measured based on a ^{15}N 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 $^{15}N_2$ (99.8 atom% $^{15}N_2$, Sigma Aldrich, batch number: MBBB5815V) and 0.8 ml O_2 and incubated in the dark at 15 °C for 72 h in the ^{15}N enriched artificial atmosphere. To control the artificial ^{15}N-enriched atmosphere, pressure changes were noted before and after adding $^{15}N_2$ and O_2. The average atmospheric composition consisted of 72.5 % $^{15}N_2$, 8.2 % O_2, and 19.2 % Ar in v/v%. The samples being exposed to $^{15}N\text{-}N_2$ as well as the samples that were not exposed to $^{15}N\text{-}N_2$ (natural abundance) were dried at 50°C, milled and analyzed for ^{15}N (Delta plus, Conflo III, Thermo Electron Cooperation, Bremen, Germany). The ^{15}N atom% was calculated using the isotope ratio of each sample (R_{sample}= $^{15}N/^{14}N$). The $^{15}N_2$ fixation was calculated using an isotope mixing model (Zechmeister-Boltenstern, 1996):

$$^{15}N_2 \text{ fixation rate (ng N g soil}^{-1}\text{ h}^{-1}) = \text{TN (mg N g soil}^{-1}) \times \frac{(\,^{15}N_{labeled}\,(\text{at\%}) - \,^{15}N_{NA}\,(\text{at\%}))}{100 \ast t\,(h)} \times 10^6$$

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

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 x 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 x 1 m strips within each 5 x 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 m^2 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 adonis2 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 ~ 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.

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

289 increment, nutrient addition did not change soil pH. Mean topsoil TOC concentration across all
290 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
291 TP concentrations to 0.5 ± 0.1 g P kg soil^{-1} (Table 1). Total element concentrations were not
292 significantly affected by nine years of element addition (Table 1).

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

304

305 *3.2 Microbial biomass carbon and element cycling processes*

306 Nutrient addition did not significantly change microbial biomass C or microbial respiration in
307 either depth increment compared to the control (Table 2). Further, microbial respiration per unit
308 microbial biomass C (metabolic quotient; qCO_2) was about twice as high in the control treatment
309 than in the lowest N addition treatment, but differences were not statistically different due to the
310 large variation among the plots (Table 2). Topsoil net N mineralization rates were 2 - 4 times
311 higher under any level of N and combined NP addition compared to the control (Table 2). Non-

312 symbiotic N_2 fixation rates in the second soil depth increment were 1.7 times higher under the
313 lowest level of N addition and 1.8 times higher under P addition compared to the control (Table
314 2).

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

323

324 *3.3 Plant diversity, biomass, and community composition*

325 Plant diversity was not significantly different in any of the nutrient addition treatments compared
326 to the control. However, diversity tended to decline with increasing N addition and was lowest in
327 the NP treatment (Figure S1a). Plant biomass amounted to 283.4 ± 8.8 g m^{-2} in the control plots
328 and was significantly higher under combined NP addition (975.7 ± 144.4 g m^{-2}, Figure S1b).
329 Single N or P addition did not significantly affect plant biomass compared to the control (Figure
330 S1b). There were no strong changes/trends in the plant community composition due to nutrient
331 addition in the three sampled blocks (Figure S2).

332

3.4 Prokaryotic community composition

Combined NP addition significantly shifted the prokaryotic community composition across both depth increments compared to the community in the control (Table S1). Both N10 and NP addition significantly altered the prokaryotic community composition as compared to P addition 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 (Figure 2).

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 archaean 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 (Figure 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 % (Figure 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 (Figure 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 % (Figure 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 (Figure 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 N_2 fixation that play a key role in soil C and N cycling. We 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 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 (Ramirez 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 *Nitrocosmicu*s 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 (Ramirez 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; Keiblinger 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 saprothropic Basidiomycota genera *Hygrocybe* and *Clavaria* (Tedersoo et al., 2014) under N addition can be explained by their sensitivity to elevated

493 nutrient inputs (Griffith et al., 2002). For instance, the number of *Hygrocybe* strongly decreased
494 through intensification of management in European grasslands (Griffith et al., 2002; Griffith and
495 Roderick, 2008). Decreasing Basidiomycota abundances due to N addition have been found in
496 other studies as well (Leff et al., 2015; Nemergut et al., 2008; Klaubauf et al., 2010; Morrison et
497 al., 2016).

498 In contrast to the prokaryotic community, the fungal community composition was not as strongly
499 controlled by soil pH, indicated by the correlation analysis. Previous studies also confirmed that
500 fungi were less sensitive to soil pH changes than bacteria (Rousk et al., 2010a; Rousk et al.,
501 2010b; Schleuss et al., 2019). However, plant and fungal community composition were highly
502 correlated, as found in other grasslands (Chen et al., 2017; Prober et al., 2015), whereas a more
503 detailed analysis revealed that nutrient availabilities were more important drivers of fungal
504 communities than plant biomass or plant community structure. A global study confirmed that
505 climatic and edaphic factors, not plant diversity, predicted fungal richness at a global scale
506 (Tedersoo et al., 2014).

507

508 *4.4 Conclusion*

509 Nine years of N addition altered prokaryotic and fungal community composition. The response of
510 the prokaryotic and fungal community composition to nutrient addition was more tightly coupled
511 to soil properties such as pH, TOC concentration, and DOC:DN ratio than to changes in the plant
512 community. The changes in microbial communities did not affect microbial biomass and
513 respiration rates indicating functional redundancy of these variables. In general, element-cycling
514 rates were mainly mediated by soil factors as opposed to plant and microbial community shifts.
515 Yet, over several decades, the observed changes in 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.

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). We thank Renate Krauss, Uwe Hell, and Karin Söllner for technical assistance, the chemical analytics (CAN) of the Bayreuth Center 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.

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., Rasplus, J.-Y., Rodriguez, L.J., Cruaud, A., 2017. High-throughput sequencing of multiple amplicons for barcoding and integrative taxonomy. Scientific Reports 7, 41948.

598 Cui, J., Wang, J., Xu, J., Xu, C., Xu, X., 2017. Changes in soil bacterial communities in an
599 evergreen broad-leaved forest in east China following 4 years of nitrogen addition. Journal of
600 Soils and Sediments 17, 2156–2164.
601 de Boer, W., Folman, L.B., Summerbell, R.C., Boddy, L., 2005. Living in a fungal world: impact
602 of fungi on soil bacterial niche development. FEMS Microbiology Reviews 29, 795–811.
603 De Deyn, G.B., Cornelissen, J.H.C., Bardgett, R.D., 2008. Plant functional traits and soil carbon
604 sequestration in contrasting biomes. Ecology Letters 11, 516–531.
605 Delgado-Baquerizo, M., Giaramida, L., Reich, P.B., Khachane, A.N., Hamonts, K., Edwards, C.,
606 Lawton, L.A., Singh, B.K., Brophy, C., 2016. Lack of functional redundancy in the
607 relationship between microbial diversity and ecosystem functioning. Journal of Ecology 104,
608 936–946.
609 Dixon, R., Kahn, D., 2004. Genetic regulation of biological nitrogen fixation. Nature Reviews.
610 Microbiology 2, 621–631.
611 Dray, S., Dufour, A.-B., 2007. The ade4 Package: Implementing the Duality Diagram for
612 Ecologists. Journal of Statistical Software 22, 1–20.
613 Falkowski, P.G., Fenchel, T., Delong, E.F., 2008. The Microbial Engines That Drive Earth's
614 Biogeochemical Cycles. Science 320, 1034–1039.
615 Fierer, N., Jackson, R.B., 2006. The diversity and biogeography of soil bacterial communities.
616 Proceedings of the National Academy of Sciences of the United States of America 103, 626–
617 631.
618 Fierer, N., Lauber, C.L., Ramirez, K.S., Zaneveld, J., Bradford, M.A., Knight, R., 2012.
619 Comparative metagenomic, phylogenetic and physiological analyses of soil microbial
620 communities across nitrogen gradients. The ISME Journal 6, 1007–1017.
621 Fierer, N., Strickland, M.S., Liptzin, D., Bradford, M.A., Cleveland, C.C., 2009. Global patterns
622 in belowground communities. Ecology Letters 12, 1238–1249.
623 Freedman, Z.B., Romanowicz, K.J., Upchurch, R.A., Zak, D.R., 2015. Differential responses of
624 total and active soil microbial communities to long-term experimental N deposition. Soil
625 Biology and Biochemistry 90, 275–282.
626 Galloway, J.N., Dentener, F.J., Capone, D.G., Boyer, E.W., Howarth, R.W., Seitzinger, S.P.,
627 Asner, G.P., Cleveland, C.C., Green, P.A., Holland, E.A., 2004. Nitrogen cycles: past,
628 present, and future. Biogeochemistry 70, 153–226.

Galloway, J.N., Townsend, A.R., Erisman, 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 (*Hygrocybe* 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, 1st 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., 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., Gorfer, M., 2010. Molecular diversity of fungal communities in agricultural soils from Lower Austria. Fungal Diversity 44, 65–75.

Kõljalg, 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., Martín, M.P., Matheny, P.B., Nguyen, N.H., Niskanen, T., Oja, J., Peay, K.G., Peintner, U., Peterson, M., Põldmaa, 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.C., 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., Tscherko, 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 (New York, N.Y.) 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., Szoecs, 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 (Oxford, England) 35, 526–528.

Peñuelas, J., Poulter, B., Sardans, J., Ciais, P., van der Velde, M., Bopp, L., Boucher, O., Godderis, Y., Hinsinger, P., Llusia, 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., DeLorenze, 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. URL http://www.R-project.org/, Vienna, Austria.

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

786 phosphorus addition in a mesic grassland in South Africa. Soil Biology and Biochemistry
787 135, 294–303.
788 Schloss, P.D., Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M., Hollister, E.B., Lesniewski,
789 R.A., Oakley, B.B., Parks, D.H., Robinson, C.J., Sahl, J.W., Stres, B., Thallinger, G.G., van
790 Horn, D.J., Weber, C.F., 2009. Introducing mothur: Open-source, platform-independent,
791 community-supported software for describing and comparing microbial communities.
792 Applied and Environmental Microbiology 75, 7537–7541.
793 Semenov, M.V., Manucharova, N.A., Stepanov, A.L., 2016. Distribution of Metabolically Active
794 Prokaryotes (Archaea and Bacteria) throughout the Profiles of Chernozem and Brown
795 Semidesert Soil. Eurasian Soil Science 49, 217–225.
796 Six, J., Frey, S.D., Thiet, R.K., Batten, K.M., 2006. Bacterial and Fungal Contributions to Carbon
797 Sequestration in Agroecosystems. Soil Science Society of America Journal 70, 555–569.
798 Smith, V.H., 1992. Effects of nitrogen: phosphorus supply ratios on nitrogen fixation in
799 agricultural and pastoral ecosystems. Biogechemistry, 19–35.
800 Spohn, M., 2015. Microbial respiration per unit microbial biomass depends on litter layer carbon-
801 to-nitrogen ratio. Biogeosciences 12, 817–823.
802 Spohn, M., Chodak, M., 2015. Microbial respiration per unit biomass increases with carbon-to-
803 nutrient ratios in forest soils. Soil Biology and Biochemistry 81, 128–133.
804 Sterner, R.W., Elser, J.J., 2002. Ecological stoichiometry: The biology of elements from
805 molecules to the biosphere. Princeton University Press.
806 Stevens, C.J., Lind, E.M., Hautier, Y., Harpole, W.S., Borer, E.T., Hobbie, S., Seabloom, E.W.,
807 Ladwig, L., Bakker, J.D., Chu, C., Collins, S., Davies, K.F., Firn, J., Hillebrand, H., La Pierre,
808 K.J., MacDougall, A., Melbourne, B., McCulley, R.L., Morgan, J., Orrock, J.L., Prober, S.M.,
809 Risch, A.C., Schuetz, M., Wragg, P.D., 2015. Anthropogenic nitrogen deposition predicts
810 local grassland primary production worldwide. Ecology 96, 1459–1465.
811 Strickland, M.S., Lauber, C.L., Fierer, N., Bradford, M.A., 2009. Testing the functional
812 significance of microbial community composition. Ecology 90, 441–451.
813 Strickland, M.S., Rousk, J., 2010. Considering fungal:bacterial dominance in soils - Methods,
814 controls, and ecosystem implications. Soil Biology and Biochemistry 42, 1385–1395.
815 Sul, W.J., Asuming-Brempong, S., Wang, Q., Tourlousse, D.M., Penton, C.R., Deng, Y.,
816 Rodrigues, J.L.M., Adiku, S.G.K., Jones, J.W., Zhou, J., Cole, J.R., Tiedje, J.M., 2013.

817 Tropical agricultural land management influences on soil microbial communities through its
818 effect on soil organic carbon. Soil Biology and Biochemistry 65, 33–38.
819 Tedersoo, L., Bahram, M., Põlme, S., Kõljalg, U., Yorou, N.S., Wijesundera, R., Villarreal Ruiz,
820 L., Vasco-Palacios, A.M., Thu, P.Q., Suija, A., Smith, M.E., Sharp, C., Saluveer, E., Saitta,
821 A., Rosas, M., Riit, T., Ratkowsky, D., Pritsch, K., Põldmaa, K., Piepenbring, M., Phosri, C.,
822 Peterson, M., Parts, K., Pärtel, K., Otsing, E., Nouhra, E., Njouonkou, A.L., Nilsson, R.H.,
823 Morgado, L.N., Mayor, J., May, T.W., Majuakim, L., Lodge, D.J., Lee, S.S., Larsson, K.-H.,
824 Kohout, P., Hosaka, K., Hiiesalu, I., Henkel, T.W., Harend, H., Guo, L.-d., Greslebin, A.,
825 Grelet, G., Geml, J., Gates, G., Dunstan, W., Dunk, C., Drenkhan, R., Dearnaley, J., Kesel, A.
826 de, Dang, T., Chen, X., Buegger, F., Brearley, F.Q., Bonito, G., Anslan, S., Abell, S.,
827 Abarenkov, K., 2014. Global diversity and geography of soil fungi. Science (New York,
828 N.Y.) 346, 1256688.
829 van den Heuvel, R.N., van der Biezen, E., Jetten, M.S.M., Hefting, M.M., Kartal, B., 2010.
830 Denitrification at pH 4 by a soil-derived Rhodanobacter-dominated community.
831 Environmental Microbiology 12, 3264–3271.
832 Vance, E.D., Brookes, P.C., Jenkinson, D.S., 1987. An extraction method for measuring soil
833 microbial biomass C. Soil Biology and Biochemistry 19, 703–707.
834 Vestgarden, L.S., Selle, L.T., Stuanes, A.O., 2003. In situ soil nitrogen mineralisation in a Scots
835 pine (Pinus sylvestris L.) stand: effects of increased nitrogen input. Forest Ecology and
836 Management 176, 205–216.
837 Vourlitis, G.L., Zorba, G., Pasquini, S.C., Mustard, R., 2007. Chronic Nitrogen Deposition
838 Enhances Nitrogen Mineralization Potential of Semiarid Shrubland Soils. Soil Science
839 Society of America Journal 71, 836.
840 Wang, R., Balkanski, Y., Boucher, O., Ciais, P., Peñuelas, J., Tao, S., 2015. Significant
841 contribution of combustion-related emissions to the atmospheric phosphorus budget. Nature
842 Geoscience 8, 48–54.
843 Wardle, D.A., Gundale, M.J., Jäderlund, A., Nilsson, M.-C., 2013. Decoupled long-term effects
844 of nutrient enrichment on aboveground and belowground properties in subalpine tundra.
845 Ecology 94, 904–919.
846 Weand, M.P., Arthur, M.A., Lovett, G.M., McCulley, R.L., Weathers, K.C., 2010. Effects of tree
847 species and N additions on forest floor microbial communities and extracellular enzyme
848 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., Labilitrichaceae 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.

Figure captions

Figure 1: Proportion of microbial respiration (a), net nitrogen (N) mineralization (b), and non-symbiotic N_2 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.

Figure 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.

Figure 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.

Figure 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 ~ N * P. Differentially abundant genera are indicated with an asterisk in the legend and displayed in Table S4.

Figure 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 ~ N * P. Differentially abundant genera are indicated with an asterisk in the legend and displayed in Table S4.

Tables

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

[1] Data were log10 transformed for statistical tests, [2]reciprocally transformed (1/x) for statistical tests

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 ± 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 ± 51.18	8.30 ± 3.03	51.59 ± 16.09	0.09 ± 0.05^{a}	0.69 ± 0.09
	1	0	155.81 ± 35.26	3.41 ± 0.27	22.73 ± 3.83	0.20 ± 0.03^{bc}	1.13 ± 0.20
	5	0	163.70 ± 47.23	3.80 ± 1.29	23.11 ± 4.43	0.32 ± 0.07^{d}	1.28 ± 0.13
	10	0	235.22 ± 36.29	5.75 ± 1.87	25.97 ± 12.03	0.25 ± 0.02^{bcd}	1.02 ± 0.41
	0	10	192.71 ± 7.12	6.69 ± 3.97	34.73 ± 20.79	0.16 ± 0.03^{ab}	1.08 ± 0.17
	10	10	168.81 ± 80.65	4.01 ± 1.80	29.32 ± 13.13	0.28 ± 0.05^{cd}	0.79 ± 0.21
15-30	0	0	118.62 ± 83.95	2.32 ± 0.85*	27.29 ± 17.16	0.06 ± 0.01	1.05 ± 0.21^{a}
	1	0	113.07 ± 66.81	2.03 ± 0.97	39.34 ± 26.35	0.09 ± 0.03*	1.78 ± 0.27^{b*}
	5	0	95.29 ± 37.12	1.62 ± 0.48	17.85 ± 2.47	0.12 ± 0.01*	1.50 ± 0.29^{ab}
	10	0	100.30 ± 38.56*	1.48 ± 0.33*	16.74 ± 5.44	0.11 ± 0.01*	1.74 ± 0.19^{ab*}
	0	10	45.87 ± 21.92*	1.97 ± 0.77*	39.49 ± 17.56	0.07 ± 0.02*	1.87 ± 0.28^{b*}
	10	10	133.81 ± 87.67	2.19 ± 0.47	27.75 ± 14.93	0.13 ± 0.01*	1.20 ± 0.03^{ab}

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			
Net N mineralization	(Intercept)	-0.1944	0.335		0.77	0.60	0.014
	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			
Non-symbiotic N_2 fixation	(Intercept)	-0.9078	0.254		0.59	0.42	0.038
	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

Figure 2

Click here to download high resolution image

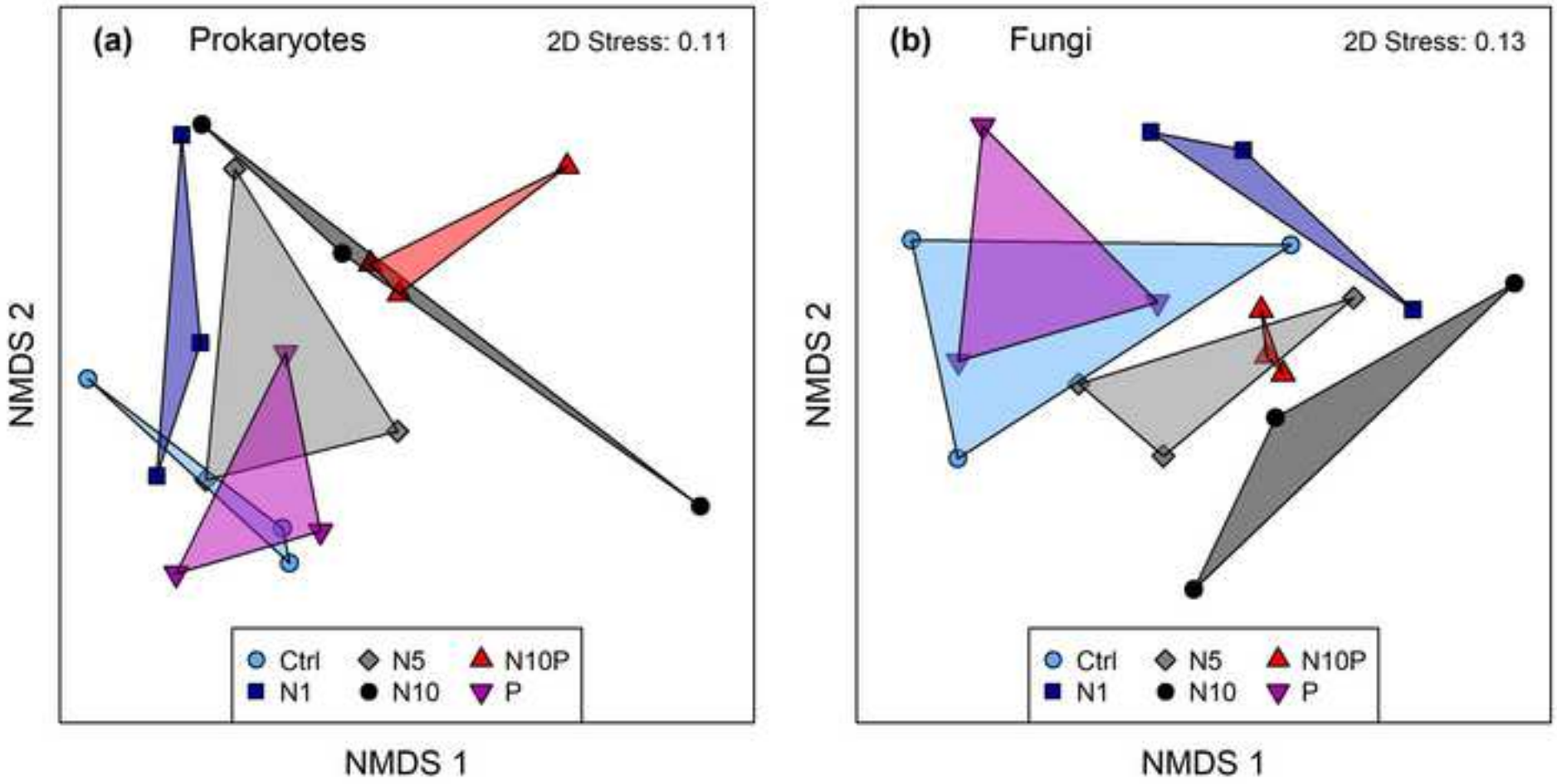

Figure 4

Click here to download high resolution image

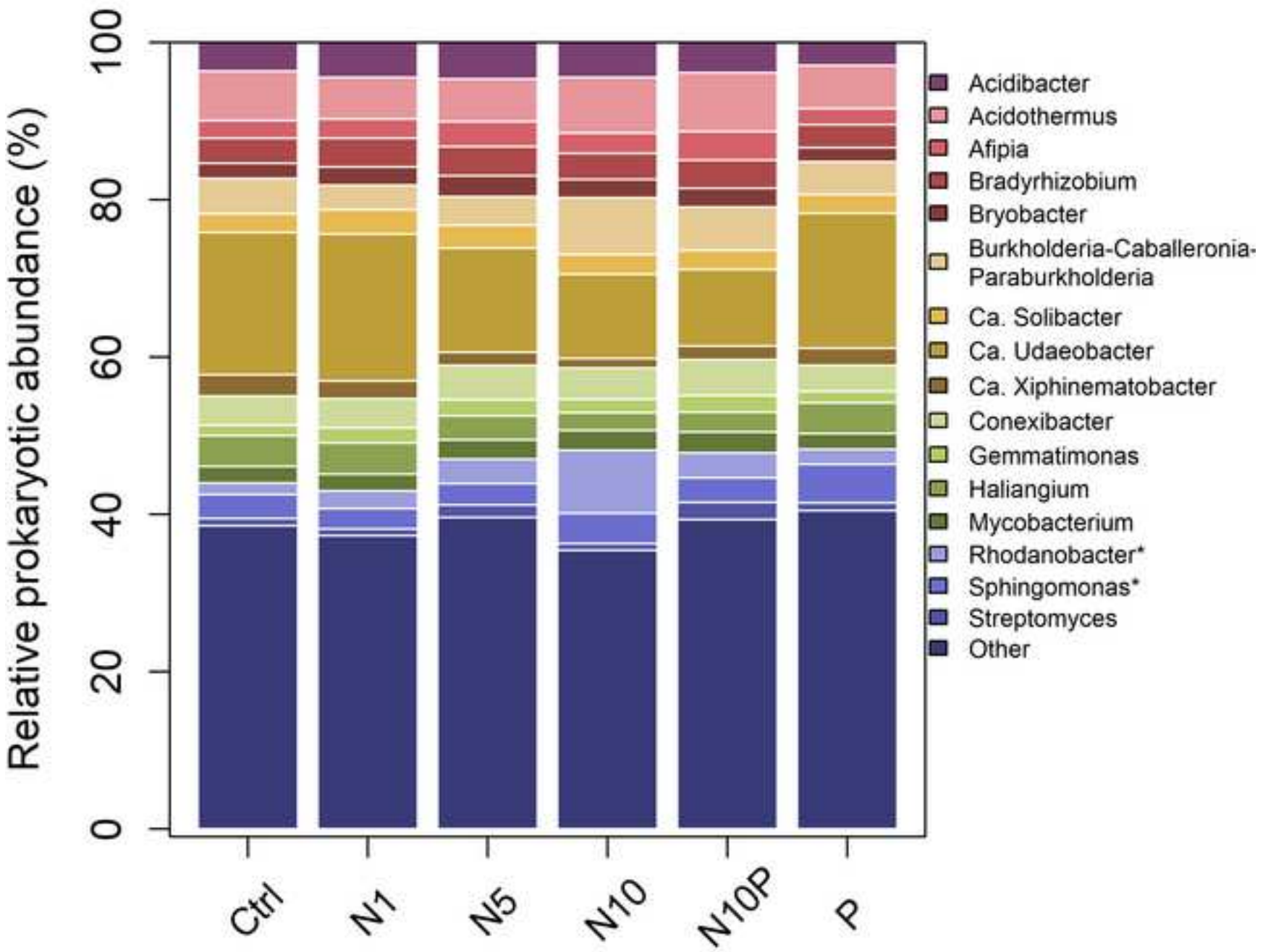

Figure 5

Click here to download high resolution image