Soil Biology and Biochemistry 146 (2020) 107815

Contents lists available at ScienceDirect

Soil Biology and Biochemistry

journal homepage: http://www.elsevier.com/locate/soilbio

Microbial carbon use efficiency in grassland soils subjected to nitrogen and phosphorus additions

Meike Widdig [a], Per-M. Schleuss [a], Lori A. Biederman [b], Elizabeth T. Borer [c], Michael J. Crawley [d], Kevin P. Kirkman [e], Eric W. Seabloom [c], Peter D. Wragg [f], Marie Spohn [a,*]

[a] Department of Soil Biogeochemistry and Soil Ecology, University of Bayreuth, Germany
[b] Department of Ecology, Evolution and Organismal Biology, Iowa State University, USA
[c] Department of Ecology, Evolution, and Behavior, University of Minnesota, USA
[d] Department of Life Sciences, Imperial College London, England, UK
[e] School of Life Sciences, University of KwaZulu-Natal, South Africa
[f] Department of Forest Resources, University of Minnesota, USA

ARTICLE INFO

Keywords:
Carbon mineralization
Microbial respiration
Nutrient fertilization
Microbial growth efficiency
Nutrient network (NutNet)
Microbial growth

ABSTRACT

Soil microbial carbon use efficiency (CUE), defined as the ratio between carbon (C) allocated to growth and C taken up by microorganisms, is pivotal for the understanding of C cycling in terrestrial ecosystems. Soil microbial CUE is thought to increase under nitrogen (N) addition, thereby mediating the effects of atmospheric N deposition on C cycling in soils. We studied the effects of N, phosphorus (P), and combined N and P addition on soil microbial CUE from a total of six grassland soils from South Africa, USA, and UK. Microbial CUE varied between 25 and 57% with a mean value of 40% across all sites, depth increments, and treatments. Most of the site variability in microbial CUE was explained by sand content, mean annual precipitation and temperature, and the dissolved organic C:dissolved N ratio. Soil microbial CUE as well as microbial biomass turnover time were robust to changes in N, P, and NP supply. However, N addition significantly reduced microbial respiration and C uptake in the topsoil. Taken together, N, P, and NP addition did not influence microbial CUE and biomass turnover time in grassland soils on different continents, indicating that microbial CUE varies little despite large changes in element inputs. Consequently, increased N inputs to soil may have a smaller impact on microbial CUE and biomass turnover time, and therefore C cycling in grassland soils, than expected and models assuming increased CUE with increasing N inputs could overestimate future C storage.

1. Introduction

Soil microbial carbon use efficiency (CUE), which is defined as the ratio between the carbon (C) allocated to growth and C taken up by microorganisms (del Giorgio and Cole, 1998), and microbial biomass turnover time both shape soil C cycling in terrestrial ecosystems. Yet, it is poorly understood how changing environmental conditions, such as increasing availability of nitrogen (N) and phosphorus (P), affect soil microbial CUE and biomass turnover.

Humans have greatly increased the supply of nutrients to ecosystems through activities such as intensive agriculture and fossil fuel combustion (Galloway et al., 2004; Schlesinger, 2009; Wang et al., 2015). The increased supply of nutrients has affected plant growth (Fay et al., 2015; Stevens et al., 2015), plant diversity (Clark and Tilman, 2008; Harpole et al., 2016), and soil element cycling in grassland ecosystems (Janssens et al., 2010). Rising N and P supply has caused contradictory effects on soil C cycling and on C stocks as some studies report increasing soil C stocks due to N (Fornara and Tilman, 2012; Yue et al., 2016) or P addition (Bradford et al., 2008), while others report no change in grassland C stocks under N (Zeng et al., 2010; Lu et al., 2011; Crowther et al., 2019) or P addition (Fornara et al., 2013). Since grasslands contain up to 30% of the global soil C stocks (Scurlock and Hall, 1998), it is important to test how the addition of N and P impacts C cycling in grassland soils to improve future predictions of global C fluxes.

Soil C cycling is governed by microorganisms and changes in microbial CUE might critically influence the global C cycle (Li et al., 2018; Walker et al., 2018). Models predict an increase in CUE with increasing N availability (Ågren et al., 2001; Schimel and Weintraub, 2003;

* Corresponding author.
E-mail address: marie.spohn@uni-bayreuth.de (M. Spohn).

https://doi.org/10.1016/j.soilbio.2020.107815
Received 21 November 2019; Received in revised form 6 April 2020; Accepted 7 April 2020
Available online 11 April 2020

Eliasson and Ågren, 2011; Manzoni et al., 2017), and thus decreased C losses from soil to atmosphere. The reason for this prediction is that microorganisms might allocate more C to growth when N availability is high because of lower metabolic costs of N acquisition (Manzoni et al., 2012; Spohn et al., 2016b). However, empirical findings about how N inputs affect soil microbial CUE are contradictory and the effect of N on CUE was rarely studied across continents. Most studies found an increase in CUE under N addition: for example, long-term N addition in combination with P or K (Spohn et al., 2016b) and long-term NPK addition (Poeplau et al., 2019) increased CUE in temperate grasslands. In contrast, other studies have found a negative effect of N addition on soil microbial CUE in North American grasslands (Riggs and Hobbie, 2016), and no effect in croplands (Lee and Schmidt, 2014).

In comparison to the effects of N, less is known about the effects of P addition on microbial C processing. Addition of P could directly alleviate microbial nutrient limitation, and thus increase CUE. Further, addition of P could indirectly increase microbial CUE due to an increase in organic C supply through increased plant litter inputs (Elser et al., 2007) and desorption of organic compounds from the soil solid phase (Spohn and Schleuss, 2019). Yet, one study demonstrated that CUE was unchanged by long-term PK addition in a temperate grassland (Spohn et al., 2016b).

In addition to nutrient inputs, the C:N ratio of dissolved organic matter (DOC:DN ratio), which reflects the C:N ratio of substrate on which soil microorganisms feed, is an important factor influencing microbial CUE (Manzoni et al., 2012). The DOC:DN ratio usually exceeds the C:N ratio of the soil microbial biomass (Mooshammer et al., 2014) and in comparison to variations in the DOC:DN ratio, variations in microbial biomass C:N ratios are very small (Cleveland and Liptzin, 2007; Xu et al., 2013). It has been proposed that a large disparity between the stoichiometry (i.e. the element ratio) of the microbial biomass and its substrate decreases microbial CUE because microbes need to invest more C and energy into nutrient acquisition and since excess C might be metabolized by overflow respiration (Manzoni et al., 2012; Sinsabaugh et al., 2013). Confirming this concept, it has been found that soil microbial CUE declined with increasing DOC:DN ratio in soils without nutrient addition (Sinsabaugh et al., 2013, 2016; Manzoni et al., 2017). However, in fertilized soils, CUE increased moderately with increasing DOC:DN ratios (Manzoni et al., 2012) and the C:N ratio of rice straw had no effect on microbial substrate use efficiency (Devêvre and Horwáth, 2000). Thus, there are still open questions about the relationship between soil microbial CUE and soil stoichiometry.

Further, soil microbial biomass turnover time can directly influence the fate of C in soils, because it affects the amount of C that leaves the microbial biomass per unit time. The C that left the microbial biomass pool can either become mineralized by the soil microbial biomass or can contribute to the soil organic matter pool (Hagerty et al., 2014; Li et al., 2018). Microbial biomass turnover time is defined as the ratio of microbial biomass and microbial growth rate (Spohn et al., 2016a; Kouno et al., 2002). Recent studies have shown that an increase in temperature accelerates microbial biomass turnover (Hagerty et al., 2014; Li et al., 2018; Walker et al., 2018). However, the effect of nutrient addition on microbial biomass turnover time has rarely been studied. One study found that microbial biomass turnover time in a temperate grassland was unaffected by N or P addition (Spohn et al., 2016b). However, N addition could reduce microbial C uptake (Spohn et al., 2016a), which might lead to increased microbial biomass turnover time.

The contradictory effects of N addition on CUE and the lack of knowledge concerning the effects of nutrient addition on biomass turnover time show the need for a better understanding of how nutrient availability shapes microbial C cycling in soils. This is especially true since CUE is a critical factor in ecosystem C models (Allison et al., 2010; Six et al., 2006) that is expected to increase in response to N inputs to soil.

Here we studied the effects of N and P supply on soil microbial C processing (CUE and microbial biomass turnover) in a nutrient addition experiment replicated in a total of six grasslands soils in South Africa, the USA, and the UK. The sites represent a broad range of grasslands in terms of climate, soils, and biota (Borer et al., 2014). We used a recently developed method, which is based on the incorporation of soil water-derived ^{18}O into microbial DNA, to determine CUE and microbial biomass turnover times. We hypothesized that i) N and P addition will increase microbial CUE, that ii) CUE will be negatively related to the DOC:DN ratio, and that iii) N addition will increase microbial biomass turnover time.

2. Material and methods

2.1. Site description and experimental design

We chose to study six grasslands sites from South Africa, the USA, and the UK because they span a large, globally-relevant range of biotic and abiotic conditions (Tables 1, S1, S2) and represent some major grassland types worldwide, which enabled us to investigate C cycling under different environmental conditions. Two sites, Cedar Creek and Chichaqua Bottoms, are vegetated by tallgrass prairie and located in the Central Plains, USA (Table 1). The Cedar Creek site is situated on the Anoka Sand Plain, an outwash plain of the Wisconsin Glacial Episode. The Chichaqua Bottoms site is located on Pleistocene till and sand (Prior, 1991). The other two sites, Rookery and Heron's Brook, are mesic grasslands and are located in Silwood Park, UK (Table 1). The Rookery and Heron's Brook sites are both situated on sands of the Bagshot Formation (British Geological Survey, 1999). Two sites, Ukulinga and Summerveld, are mesic grasslands and located in KwaZulu-Natal, South Africa (Table 1). The Ukulinga site is located on top of a plateau formed by Ecca group shales (Fynn and O'Connor, 2005) and the Summerveld site is situated on a sandstone plateau (Wragg, 2017) and its soil is shallow with an average depth of 17 cm. All sites contribute to the Nutrient Network (Borer et al., 2014) and have been subject to a standardized nutrient addition treatment.

We sampled plots (5 × 5m) with and without N and P addition (Ctrl, N, P, and NP), which were replicated three times at each site. Nutrients had been added annually at the beginning of the growing season for at least seven years (Table 1) as 10 g m^{-2} yr^{-1} slow-release urea ($(NH_2)_2CO$) and 10 g m^{-2} yr^{-1} triple-super phosphate ($Ca(H_2PO_4)_2$).

2.2. Soil sampling and sample preparation

Soils were sampled in two depth increments, from 0 to 15 cm (termed "topsoil") and 15–30 cm depth (termed "subsoil"), both located in the A horizon of all soils. One mixed sample consisting of six individual samples from each plot was collected with a soil corer with a diameter of 3.5 cm. In Summerveld, only the first depth increment was sampled because of limited soil depth. At each site, the sampling coincided with the time of peak biomass (February 2017 in South Africa, September 2017 in the USA, and October 2017 in the UK). Samples were shipped to the University of Bayreuth within one week after collection. Soil samples were sieved (<2 mm) and stones and roots were removed. To determine soil water holding capacity and water content, samples were weighed, soaked with water, drained for 24 h in a sand bath and weighed again before and after drying at 105 °C. Samples were adjusted to 60% water holding capacity (except for the samples used for CUE analyses) and samples were pre-incubated for 1 week at 15 °C for subsequent measurements (i.e. soil water extracts, microbial respiration, and enzyme measurements) to allow the soil biota to recover from soil sieving and to allow soil respiration to reach basal rates.

2.3. Soil physical and chemical analyses

Samples were dried at 60 °C and milled to determine 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

Table 1
Site name, code, country, ecosystem, texture, duration of the nutrient addition treatment, elevation, latitude, longitude, mean annual precipitation (MAP), mean annual temperature (MAT), soil pH (in 0–15 cm depth), dominant plant species, grass, forb, and legume cover, and aboveground net primary productivity (ANPP) of the six grassland sites.

Site name	Cedar Creek	Chichaqua Bottoms	Rookery	Heron's Brook	Ukulinga	Summerveld
Site code	cdcr.us	cbgb.us	rook.uk	hero.uk	ukul.za	summ.za
Country	USA	USA	UK	UK	South Africa	South Africa
Ecosystem	Tallgrass prairie	Tallgrass prairie restored	Mesic grassland	Mesic grassland	Mesic grassland	Mesic grassland
Texture	Sand	Loamy sand	Sandy loam	Sandy loam	Silty clay	Loam
Nutrient addition (yr)	9	7	9	9	7	7
Elevation (m)	270	275	60	60	843	679
Latitude	45.43	41.79	51.41	51.41	−29.67	−29.81
Longitude	−93.21	−93.39	−0.64	−0.64	30.4	30.72
MAP (mm)	800	891	678	678	838	809
MAT (°C)	6	9	10	10	18	18
pH_{H2O}	5.27	5.73	3.76	5.12	5.89	5.20
Dominant plant species[a,b]	*Agrostis scabra, Andropogon gerardii, Carex sp., Conyza canadensis, Elymus repens, Pennisetum glaucum, Poa pratensis, Rumex acetosella, Schizachyrium scoparium, Solidago missouriensis*	*Ambrosia psilostachya, Andropogon gerardii, Bromus inermis, Chamaecrista fasciculata, Chenopodium album, Gaura biennis, Monarda fistulosa, Poa pratensis, Schizachyrium scoparium, Solidago canadensis, Solidago speciosa, Symphyotrichum pilosum*	*Agrostis capillaris, Festuca rubra, Galium saxatile, Holcus lanatus, Holcus mollis, Luzula campestris, Rumex acetosella, Senecio jacobaea*	*Agrostis capillaris, Anthoxanthum odoratum, Arrhenatherum elatius, Festuca rubra, Holcus lanatus, Holcus mollis, Lotus corniculatus, Ranunculus repens, Rumex acetosa, Trifolium repens, Veronica chamaedrys*	*Berkheya umbellata, Cymbopogon nardus, Eragrostis curvula, Hyparrhenia hirta, Scabiosa columbaria, Setaria nigrirostris, Tagetes minuta, Themeda triandra, Tristachya leucothrix*	*Aristida junciformis, Elionurus muticus, Helichrysum aureonitens, Monocymbium ceresiiforme, Panicum ecklonii, Sporobolus africanus, Tephrosia macropoda, Themeda triandra, Trachypogon spicatus*
Grass cover[b] (%)	94	74	45	104	116	120
Forb cover[b] (%)	21	42	61	26	44	56
Legume cover[b] (%)	1	6	0	19	12	10
ANPP[b] (g m^{-2} yr^{-1})	178	397	180	509	498	349

[a] Taxa are in alphabetic order.
[b] Data refer to the control (without any addition of N and P).

determined by ICP-OES (Vista-Pro radial, Varian) after pressure digestion in aqua regia (HNO_3 + HCl). For the determination of dissolved organic C (DOC), dissolved N (DN), and dissolved inorganic P (DIP), soils were extracted in deionized water in a ratio of 1:4 (soil:water) and shaken for 1 h. Subsequently, extracts were filtered through 0.45 μm cellulose acetate filters and quantified (DOC, DN: TOC:TN Analyzer, multi N/C 2100, Jena Analytics, Germany; DIP: UV 1800, Shimadzu). Labile P was extracted from soils with Bray-1 solution (0.03 M NH_4F, 0.025 M HCl) (Bray and Kurtz, 1945) in a ratio of 1:10 (soil:extractant) and determined by a multiplate reader (Infinite® 200 PRO, TECAN) using the molybdenum blue method (Murphy and Riley, 1962). To prevent interference with the color formation of the assay, fluoride ions were neutralized with 0.1 M boric acid (Kurtz, 1942). Soil pH was measured in deionized water and 1 M KCl in a soil:solution ratio of 1:2.5. Soil texture was analyzed according to Köhn (1928). Samples were pre-treated with H_2O_2 as oxidant to destroy organic substances. The sand fraction was separated through sieving. The samples were dispersed in 25 ml Na-Pyrophosphate and transferred into cylinders, where silt and clay content were assessed by sedimentation analysis (DIN ISO 11 277).

2.4. 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 h) and respiration rates were calculated based on the linear increase in accumulated C–CO_2 over time (Heuck and Spohn, 2016).

2.5. Carbon use efficiency and microbial biomass turnover time

Microbial CUE was determined based on the incorporation of ^{18}O from ^{18}O-labeled water into microbial DNA (Spohn et al., 2016a). A dilution of ^{18}O labeled water (97 at %) was prepared and added to one aliquot of each soil sample to reach 20 at% ^{18}O in the soil water and to adjust the soil water content to 60% of the soil's water holding capacity. Non-labeled Millipore water was added to another aliquot of the soil, serving as a natural isotope abundance sample. Both samples were incubated for 24 h at 15 °C. Subsequently, samples were frozen until DNA extraction. DNA was extracted using a DNA extraction kit (FastDNA™ SPIN Kit for Soil, MP Biomedicals) following the instruction of the manual except for some adjustments that were necessary to enhance purity and extraction quantity. First, samples were centrifuged for 15 min to enhance elimination of excessive debris and second, not just a part, but all DNA mixture was transferred to the filter. The weight of the DNA extract was determined gravimetrically. DNA concentration was measured with the picogreen assay (Sandaa et al., 1998) using a kit (Quant-iT™ PicoGreen® dsDNA Reagent, Life Technologies). An aliquot of 4 μl of each sample was diluted 250-fold and measured fluorimetrically using a microplate reader (Infinite® 200 PRO, TECAN). DNA extracts were dried in silver capsules at 60 °C, and the ^{18}O enrichment and the total amount of oxygen were measured using a TC/EA coupled to a Delta V Plus IRMS (Thermo Fisher). The microbial growth rate in terms of DNA produced per hour was calculated based on the incorporation of ^{18}O–H_2O into genomic DNA (Schwartz, 2007; Blazewicz and Schwartz, 2011; Spohn et al., 2016a) because new genomic DNA is only synthesized when cells are dividing. Based on a correlation between microbial DNA and microbial biomass C concentrations (see section 2.6), the growth rate in terms of biomass C produced per hour (C_{Growth}) was calculated. The correlation between microbial DNA and microbial biomass C concentrations across all samples analyzed here was used to calculate C_{Growth} following Spohn et al. (2016a). This prevents artificially created differences in soil microbial CUE caused by the measurement error. Further, several studies confirm a stable microbial biomass C:DNA ratio across soils from different locations and different soil depths (Anderson and Martens, 2013; Spohn et al., 2016a, 2016b; Spohn and Widdig, 2017). Finally, CUE was computed based on growth rate and respiration rate (Manzoni et al., 2012; Sinsabaugh et al., 2013):

$$CUE = \frac{C_{Growth}}{(C_{Growth} + C_{Respiration})}$$

To calculate the turnover time of microbial biomass, microbial biomass concentration was divided by microbial growth rate (Spohn et al., 2016a):

$$Turnover\ time = \frac{Microbial\ biomass\ C}{C_{Growth}}$$

2.6. Microbial biomass carbon and nitrogen

Microbial biomass C and N 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 was not fumigated. Both fumigated and non-fumigated samples were extracted in 0.5 M K_2SO_4 in a ratio of 1:5 and measured by a TOC/TN Analyzer. The concentration of the fumigated sample was subtracted from the concentration of the non-fumigated sample and the result was multiplied by a conversion factor of 2.22 for microbial biomass C (Jenkinson et al., 2004; Wu et al., 1990) and by a conversion factor of 1.85 for microbial biomass N (Brookes et al., 1985; Joergensen and Mueller, 1996).

2.7. Microbial community structure

DNA was extracted from 250 to 500 mg soil using the Nucleo-Spin Soil kit (No. 740780, Macherey-Nagel, Germany). Automated ribosomal intergenic spacer analysis (ARISA, Fisher and Triplett, 1999) for bacterial and fungal communities was performed as described in Heuck et al. (2015). Ribosomal intergenic spacers/internal transcribed sequences were PCR-amplified in two separate reactions using bacteria-specific primers (ITSF and ITSReub; Cardinale et al., 2004) and fungi-specific primers (ITS1F-Z and ITS2; Weig et al., 2013; White et al., 1990), respectively. Briefly, 5 ng metagenomic DNA was used in a 12.5 μl PCR volume as previously described (Weig et al., 2013). The following modifications were implemented: bacterial and fungal ARISA PCR products were separated independently on the fragment analyzer capillary electrophoresis instrument (Agilent, Waldbronn, Germany) equipped with a long capillary array (55 cm). Two microliter of ARISA PCR products were used for the double-stranded DNA kit DNF-910 (Agilent) and separated on the fragment analyzer. The electropherograms of each sample were manually inspected using the PROsize software (v3, Agilent) and a peak table including size of fragments and peak intensity (RFU) was exported. For statistical analyses of the ARISA data, only fragments between 200 and 1000 bp in size were selected and analyzed using Primer7 software (v 7.0.13, Primer-E Ltd.). PCR fragment profiles were compared between samples by the shape of cumulative frequency curves, separately for bacterial and fungal ITS amplification products. Finally, a resemblance matrix was calculated from the cumulative profile matrix using Manhattan distance as resemblance measure.

2.8. Enzyme activity

Activities of phosphatase (Pase), β-1,4-glucosidase (BG), β-1,4-N-acetyl-glucosaminidase (NAG), and L-leucine aminopeptidase (LAP) were determined using the fluorogenic substrates 4-methylumbelliferyl-phosphate, 4-methylumbelliferyl-β-D-glucoside, 4-methylumbelliferyl-N-acetyl-β-D-glucosaminide, and L-leucine-7-amino-4-methyl-coumarine following German et al. (2011) and Herold et al. (2014). A soil homogenate was prepared by mixing 1 g of moist soil and 50 ml of

sterile water. Four replicates of the soil homogenates were pipetted into black polystyrene 96-well microplates and distilled, sterile water was added instead of a buffer to remain close to natural soil pH conditions. Finally, 100 μl 1 mM fluorescent substrate solution were added to each sample well. The microplates were covered and pre-incubated in the dark at 15 °C for 30 min and measured fluorimetrically after 0, 60, 120, and 180 min with 360 nm excitation and 460 nm emission filters (Herold et al., 2014) using a microplate reader (Infinite® 200 PRO, TECAN). Enzyme activities were calculated using the slope of net fluorescence over time and were corrected for quenching of the soil, fluorescence of the substrate, and fluorescence of the homogenate.

2.9. Statistics

Data were checked for normal distribution (with Shapiro-Wilks test) and homogeneity of variance (with Levene test) and transformed, if data were not normally distributed and variances were not homogenous. After that, a two-way ANOVA followed by a Tukey post-hoc test for multiple comparisons was used to test differences between treatments and depth increments.

To assess the bacterial and fungal community composition, we first calculated Bray-Curtis distance matrices in PRIMER 7 (Clarke and Gorley, 2015) with 999 permutations before non-metric multi-dimensional scaling was applied to display the community composition. After the calculation of Bray-Curtis matrices, one-way-ANOSIM with 999 permutations was used to test for significant effects of either nutrient addition or site on bacterial and fungal community composition.

A linear mixed-effects model implemented in the R package nlme (Pinheiro et al., 2018) was used to test for the effects of nutrient addition on soil microbial CUE, microbial biomass turnover time as well as microbial respiration, growth, and C uptake across all sites. Treatment was set as fixed factor and random intercepts were included for sites. This approach was chosen because it quantifies and compares treatment effects across a set of sites controlling for between-site variation. Additionally, to test for the effects of nutrient addition on soil microbial CUE, linear mixed-effects models were calculated with treatments split into two main factors (N addition with levels 0 or 1 and P addition with levels 0 or 1) and their interaction.

A multi-model selection according to Grueber et al. (2011) was performed to assess the relative importance of topsoil TOC, TN, TP, DOC, DN, and labile P concentrations, TOC:TN, TOC:TP, and DOC:DN ratios, soil pH, sand content, mean annual temperature (MAT), and mean annual precipitation (MAP) on soil microbial CUE and to find the variables, which predict CUE in the different soils best. Further, we included aboveground net primary productivity (ANPP) into multi-model selection to test for significant effects of plant productivity on CUE. Silt and clay content were tested as explanatory variables in the model as well, but sand content obtained a greater model fit (R^2 and p-value) after multi-model selection. Random intercepts for the treatments at each site were included to compensate for among-site variation in intercept values. All input variables were standardized prior to analysis using "arm" R library (Gelman et al., 2018) to allow interpretation of the model estimates afterwards. To fit all possible models, we used the dredge function in MuMIn R library (Barton, 2018). Of all possible models, the best ones were selected using the AICc (AIC corrected for small sample size). Models within the top four AICc units of the model with the lowest AICc were selected and averaged using the MuMIn R library. Model variables having the highest relative importance (>0.90) were selected to fit a linear mixed-effects model, for which a conditional R^2 and p-value was calculated. The relative variable importance is the relativized sum of the AIC weights summed across all the models in which the parameter appears and ranges between 0 and 1. An importance of 1 represents variables with the highest explanatory weight. Model p-values were obtained by likelihood ratio test and R^2 was calculated as conditional R^2 (Nakagawa and Schielzeth, 2013). All statistical analyses were done using R version 3.3.1 (R Core Team,

Table 2
Total organic carbon (TOC), total nitrogen (TN), total phosphorus (TP), and soil pH in 0–15 and 15–30 cm soil depth in the control, N, P, and NP treatment at the six grassland sites. The soil at the site Summerveld (summ.za) was only sampled in 0–15 cm depth because of limited 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 site and depth increment. Asterisks indicate significant differences between depth increments tested individually for each site and treatment.

	Site	0–15 cm				15–30 cm			
		Ctrl	+N	+P	+NP	Ctrl	+N	+P	+NP
TOC (g C kg^{-1})	cdcr.us[a]	9.4 ± 1.1[a]	15.7 ± 8.8[a]	9.0 ± 0.3[a]	11.0 ± 3.4[a]	5.2 ± 1.1[a]	10.4 ± 7.8[a]	4.4 ± 0.5[a]*	5.8 ± 2.1[a]
	cbgb.us	7.2 ± 2.8[a]	8.2 ± 0.5[a]	6.9 ± 2.3[a]	7.4 ± 2.4[a]	4.1 ± 0.5[a]*	5.1 ± 0.2[a]*	4.0 ± 1.2[a]	4.1 ± 0.9[a]*
	rook.uk	24.3 ± 2.6[a]	28.7 ± 3.2[a]	26.9 ± 1.2[a]	24.9 ± 2.2[a]	10.5 ± 1.5[a]*	12.8 ± 1.3[a]*	11.6 ± 1.3[a]*	10.1 ± 0.7[a]*
	hero.uk	36.7 ± 6.8[a]	36.7 ± 6.1[a]	36.5 ± 1.8[a]	37.0 ± 7.7[a]	24.4 ± 3.5[a]*	24.5 ± 4.2[a]*	25.6 ± 4.7[a]*	23.9 ± 4.4[a]*
	ukul.za	42.0 ± 2.0[a]	42.5 ± 0.8[a]	44.4 ± 0.5[a]	45.7 ± 0.8[a]	37.5 ± 3.6[a]	3.2.0 ± 4.4[a]*	34.8 ± 5.3[a]*	36.4 ± 0.6[a]*
	summ.za[b]	49.1 ± 3.0[a]	51.1 ± 2.2[a]	51.7 ± 3.0[a]	51.7 ± 1.9[a]	NA	NA	NA	NA
TN (g N kg^{-1})	cdcr.us[a]	0.7 ± 0.1[a]	1.1 ± 0.6[a]	0.6 ± 0.1[a]	0.8 ± 0.3[a]	0.3 ± 0.1[a]*	0.7 ± 0.5[a]	0.3 ± 0.1[a]	0.4 ± 0.1[a]*
	cbgb.us	0.6 ± 0.2[a]	0.8 ± 0.1[a]	0.6 ± 0.2[a]	0.7 ± 0.2[a]	0.4 ± 0.1[a]*	0.5 ± 0.1[a]*	0.4 ± 0.1[a]*	0.4 ± 0.2[a]*
	rook.uk	2.1 ± 0.2[ab]	2.4 ± 0.3[b]	2.2 ± 0.1[ab]	2.0 ± 0.1[a]	1.0 ± 0.1[a]*	1.2 ± 0.1[a]**	1.0 ± 0.2[a]*	0.9 ± 0.1[a]*
	hero.uk	3.1 ± 0.7[a]	3.1 ± 0.5[a]	3.0 ± 0.2[a]	3.1 ± 0.8[a]	2.1 ± 0.3[a]*	2.1 ± 0.3[a]	2.1 ± 0.4[a]	2.0 ± 0.4[a]*
	ukul.za	2.9 ± 0.3[a]	3.1 ± 0.3[ab]	3.3 ± 0.1[ab]	3.4 ± 0.1[b]	2.6 ± 0.2[a]	2.4 ± 0.2[a]*	2.6 ± 0.3[a]*	2.7 ± 0.1[a]*
	summ.za[b]	2.8 ± 0.2[a]	3.0 ± 0.4[a]	2.9 ± 0.3[a]	3.0 ± 0.1[a]	NA	NA	NA	NA
TP (g P kg^{-1})	cdcr.us[a]	0.31 ± 0.03[a]	0.46 ± 0.24[a]	0.58 ± 0.09[a]	0.56 ± 0.11[a]	0.24 ± 0.03[a]	0.40 ± 0.19[a]	0.36 ± 0.04[a]	0.38 ± 0.06[a]
	cbgb.us	0.28 ± 0.02[a]	0.24 ± 0.05[a]	0.43 ± 0.04[b]	0.41 ± 0.06[b]	0.24 ± 0.03[a]	0.24 ± 0.02[a]	0.34 ± 0.03[b]*	0.35 ± 0.07[b]*
	rook.uk	0.38 ± 0.01[a]	0.38 ± 0.04[a]	0.60 ± 0.06[b]	0.61 ± 0.14[b]	0.27 ± 0.01[a]*	0.31 ± 0.04[a]	0.32 ± 0.03[a]*	0.31 ± 0.04[a]*
	hero.uk	0.62 ± 0.16[a]	0.57 ± 0.07[a]	0.93 ± 0.13[a]	0.96 ± 0.28[a]	0.54 ± 0.15[a]	0.48 ± 0.06[a]	0.55 ± 0.17[a]*	0.62 ± 0.25[a]*
	ukul.za[a]	0.45 ± 0.02[a]	0.46 ± 0.08[a]	1.33 ± 0.12[b]	1.20 ± 0.15[b]	0.39 ± 0.02[a]	0.38 ± 0.04[a]	0.74 ± 0.25[b]*	0.58 ± 0.01[b]*
	summ.za[b]	0.37 ± 0.01[a]	0.49 ± 0.18[a]	0.60 ± 0.33[a]	0.83 ± 0.7[a]	NA	NA	NA	NA
pH in H_2O	cdcr.us	5.27 ± 0.09[b]	4.70 ± 0.17[a]	5.27 ± 0.10[b]	4.84 ± 0.10[a]	5.36 ± 0.10[b]	5.17 ± 0.19[ab]*	5.45 ± 0.23[b]	4.96 ± 0.23[a]
	cbgb.us	5.73 ± 0.50[a]	5.68 ± 0.72[a]	5.86 ± 0.47[a]	5.72 ± 0.43[a]	5.40 ± 0.66[a]	5.56 ± 0.92[a]	5.58 ± 0.79[a]	5.34 ± 0.63[a]
	rook.uk	3.76 ± 0.04[a]	3.78 ± 0.02[a]	3.91 ± 0.02[a]	3.87 ± 0.03[a]	4.10 ± 0.13[a]*	4.08 ± 0.09[a]*	4.12 ± 0.10[a]*	4.06 ± 0.06[a]*
	hero.uk	5.12 ± 0.21[a]	5.18 ± 0.11[a]	5.08 ± 0.08[a]	5.09 ± 0.15[a]	5.24 ± 0.30[a]	5.30 ± 0.09[a]	5.20 ± 0.15[a]	5.22 ± 0.10[a]
	ukul.za	5.89 ± 0.08[a]	5.58 ± 0.42[a]	5.94 ± 0.09[a]	5.63 ± 0.11[a]	5.83 ± 0.10[a]	5.79 ± 0.33[a]	5.72 ± 0.16[a]	5.62 ± 0.16[a]
	summ.za[b]	5.20 ± 0.04[a]	5.03 ± 0.09[a]	5.01 ± 0.12[a]	4.97 ± 0.13[a]	NA	NA	NA	NA

[a] Data were LOG10 transformed.
[b] One-Way ANOVA and Tukey Test were performed.

Table 3
Dissolved organic carbon (DOC), dissolved nitrogen (DN), molar DOC:DN ratio, dissolved inorganic phosphorus (DIP), and microbial biomass carbon (MBC) in 0–15 and 15–30 cm depth in the sampled soils. The soil at the site Summerveld (summ.za) was only sampled in 0–15 cm 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 separately tested for each site and depth increment. Asterisks indicate significant differences between depth increments separately tested for each site and treatment.

	Site	0–15 cm				15–30 cm			
		Ctrl	+N	+P	+NP	Ctrl	+N	+P	+NP
DOC (mg C kg^{-1})	cdcr.us	15 ± 0.4^{a}	20 ± 4.8^{b}	20 ± 1.1^{b}	24 ± 2.6^{b}	11 ± 0.6^{a}	12 ± 2.0^{a*}	12 ± 0.3^{a*}	15 ± 1.3^{a*}
	cbgb.us	18 ± 2.7^{a}	22 ± 4.1^{a}	18 ± 2.1^{a}	22 ± 1.6^{a}	15 ± 1.6^{a}	22 ± 3.4^{a}	20 ± 5.6^{a}	20 ± 1.6^{a}
	rook.uk	23 ± 2.7^{a}	29 ± 7.5^{ab}	44 ± 8.3^{c}	36 ± 3.5^{bc}	15 ± 1.5^{a}	16 ± 3.1^{a*}	19 ± 1.0^{a*}	18 ± 1.0^{a*}
	hero.uk	29 ± 2.6^{a}	30 ± 3.4^{a}	36 ± 1.2^{ab}	40 ± 4.1^{b}	19 ± 1.2^{a*}	22 ± 4.2^{a*}	23 ± 3.0^{a*}	25 ± 3.9^{a*}
	ukul.za	115 ± 7^{a}	108 ± 15^{a}	127 ± 6^{a}	152 ± 34^{a}	127 ± 33^{a}	201 ± 52^{ab*}	238 ± 14^{b*}	217 ± 66^{b*}
	summ.za[b]	98 ± 3^{a}	98 ± 9^{a}	102 ± 10^{a}	100 ± 1^{a}	NA	NA	NA	NA
DN (mg N kg^{-1})	cdcr.us[a]	3.2 ± 4.4^{a}	32.9 ± 15.6^{b}	2.7 ± 2.1^{a}	13.4 ± 2.9^{b}	1.8 ± 1.5^{a}	10.0 ± 1.0^{b}	2.1 ± 1.5^{a}	7.0 ± 2.1^{ab}
	cbgb.us	4.1 ± 3.1^{a}	12.0 ± 1.4^{b}	4.7 ± 1.9^{a}	12.1 ± 3.9^{b}	2.6 ± 2.2^{a}	5.0 ± 2.8^{a*}	2.9 ± 1.1^{a}	4.8 ± 2.1^{a*}
	rook.uk	14.2 ± 2.7^{ab}	14.7 ± 3.2^{b}	10.9 ± 0.4^{ab}	10.3 ± 1.2^{a}	4.2 ± 2.4^{a*}	3.8 ± 1.0^{a*}	2.9 ± 0.8^{a*}	4.0 ± 0.8^{a*}
	hero.uk	12.9 ± 3.6^{a}	17.1 ± 3.9^{a}	11.1 ± 3.5^{a}	14.6 ± 8.7^{a}	9.7 ± 3.8^{a}	10.3 ± 0.8^{a}	11.9 ± 4.4^{a}	8.9 ± 3.2^{a}
	ukul.za	7.3 ± 0.3^{a}	43.2 ± 11.2^{b}	9.9 ± 5.0^{a}	34.4 ± 5.7^{b}	5.5 ± 1.1^{a}	17.3 ± 4.9^{ab*}	14.1 ± 3.3^{ab}	19.5 ± 5.3^{b*}
	summ.za[b]	5.0 ± 0.1^{a}	19.8 ± 5.0^{b}	5.1 ± 0.2^{a}	15.9 ± 3.1^{b}	NA	NA	NA	NA
DOC:DN ratio	cdcr.us[c]	18.1 ± 11.3^{b}	0.8 ± 0.1^{a}	12.0 ± 6.1^{b}	2.1 ± 0.2^{a}	10.9 ± 3.3^{b}	1.4 ± 0.1^{a*}	9.6 ± 4.7^{b}	2.7 ± 0.6^{ab}
	cbgb.us	10.8 ± 3.0^{b}	2.1 ± 0.2^{a}	4.9 ± 1.5^{ab}	2.3 ± 0.6^{a}	10.0 ± 4.7^{a}	5.9 ± 1.6^{a}	8.8 ± 3.5^{a}	5.6 ± 1.9^{a}
	rook.uk[a]	2.0 ± 0.5^{a}	2.3 ± 0.3^{ab}	4.6 ± 0.6^{c}	4.0 ± 0.1^{bc}	5.1 ± 1.9^{a*}	4.8 ± 0.2^{a*}	8.1 ± 1.7^{a*}	5.4 ± 1.1^{a}
	hero.uk	2.7 ± 0.4^{a}	2.1 ± 0.3^{a}	4.0 ± 1.1^{a}	4.2 ± 2.3^{a}	2.6 ± 1.0^{a}	2.5 ± 0.2^{a}	2.5 ± 0.6^{a}	3.5 ± 0.7^{a}
	ukul.za	18.2 ± 0.7^{b}	3.0 ± 0.5^{a}	17.8 ± 7.3^{b}	5.3 ± 1.2^{a}	27.0 ± 1.5^{b*}	14.8 ± 5.5^{a*}	20.3 ± 3.5^{ab}	13.0 ± 1.4^{a*}
	summ.za[b]	22.9 ± 0.6^{b}	6.0 ± 1.1^{a}	23.2 ± 1.6^{b}	7.5 ± 1.2^{a}	NA	NA	NA	NA
DIP (mg P kg^{-1})	cdcr.us[a]	0.16 ± 0.12^{a}	0.13 ± 0.06^{a}	13.37 ± 2.76^{b}	12.29 ± 3.55^{b}	0.06 ± 0.03^{a*}	0.04 ± 0.01^{a*}	4.19 ± 0.55^{b*}	5.01 ± 0.89^{b*}
	cbgb.us[a]	0.37 ± 0.08^{a}	0.25 ± 0.19^{a}	7.67 ± 1.23^{b}	11.1 ± 3.18^{b}	0.13 ± 0.08^{a*}	0.11 ± 0.05^{a}	7.02 ± 3.11^{b}	6.96 ± 1.91^{b}
	rook.uk[a]	0.02 ± 0.01^{a}	0.03 ± 0.01^{a}	1.25 ± 0.88^{b}	1.22 ± 1.20^{b}	0.02 ± 0.01^{a}	0.02 ± 0.01^{a}	0.04 ± 0.03^{a*}	0.06 ± 0.02^{a*}
	hero.uk[a]	0.04 ± 0.01^{a}	0.04 ± 0.01^{a}	1.64 ± 0.85^{b}	1.15 ± 0.72^{b}	0.02 ± 0.01^{a*}	0.03 ± 0.01^{ab}	0.04 ± 0.01^{ab*}	0.06 ± 0.01^{b*}
	ukul.za[a]	0.10 ± 0.07^{a}	0.12 ± 0.04^{a}	1.43 ± 0.19^{b}	1.10 ± 0.25^{b}	0.13 ± 0.05^{a}	0.05 ± 0.05^{a*}	2.09 ± 1.58^{b}	0.92 ± 0.24^{b}
	summ.za[a,b]	0.05 ± 0.04^{a}	0.10 ± 0.02^{ab}	0.25 ± 0.09^{b}	0.27 ± 0.12^{b}	NA	NA	NA	NA
MBC (mg C kg^{-1})	cdcr.us	163 ± 63^{a}	235 ± 44^{a}	193 ± 9^{a}	169 ± 99^{a}	119 ± 103^{a}	100 ± 47^{a*}	46 ± 31^{a*}	134 ± 124^{a}
	cbgb.us	175 ± 61^{a}	121 ± 23^{a}	168 ± 51^{a}	148 ± 100^{a}	61 ± 15^{a*}	119 ± 35^{a}	69 ± 43^{a}	142 ± 118^{a}
	rook.uk	651 ± 35^{a}	588 ± 49^{a}	507 ± 210^{a}	746 ± 88^{a}	351 ± 46^{a*}	317 ± 94^{a*}	248 ± 176^{a*}	342 ± 248^{a*}
	hero.uk	662 ± 116^{a}	603 ± 70^{a}	638 ± 51^{a}	591 ± 85^{a}	526 ± 8^{b}	363 ± 61^{ab*}	298 ± 73^{a*}	352 ± 76^{ab*}
	ukul.za	1005 ± 134^{a}	767 ± 196^{a}	819 ± 157^{a}	852 ± 71^{a}	488 ± 93^{a*}	444 ± 85^{a*}	502 ± 113^{a*}	549 ± 66^{a*}
	summ.za[b]	843 ± 133^{a}	769 ± 111^{a}	928 ± 278^{a}	699 ± 16^{a}	NA	NA	NA	NA

[a] Data were LOG10 transformed.
[b] One-Way ANOVA and Tukey Test were performed.
[c] Reciprocally transformed (1/x).

2018).

3. Results

3.1. Site characteristics and soil chemistry

The analyzed sites span broad abiotic and biotic gradients, for instance MAT ranged from 6 °C at one site in the USA to 18 °C at the sites in South Africa, MAP ranged from 678 mm at a site in the UK to 891 mm at a site in the USA, and soil texture was diverse, ranging from sand at a site in the USA to silty clay at a site in South Africa (Table 1). Further, ANPP ranged from 178 g m^{-2} yr^{-1} at a prairie site in the USA to 509 g m^{-2} yr^{-1} at a site in the UK (Table 1).

Soil TOC and TN concentration did not change significantly due to N, P, and NP addition (except for one UK site and one South African site, Table 2). Topsoil TP concentrations increased under P and NP addition at one site in South Africa, the USA, and the UK. Soil pH did not change significantly in response to nutrient addition (except for one site in the USA, Table 2).

The different sites also responded differently towards nutrient addition (Tables 2, 3). Topsoil DN concentrations were higher under N and NP addition at all sites except for the sites in the UK, and topsoil DIP concentrations were higher under P and NP addition compared to control at all sites. Addition of N and NP decreased the DOC:DN ratio in most topsoils, except for one site in the UK (Table 3). On average, the decrease in the DOC:DN ratio due to N and NP addition amounted to −64% and −57%, respectively, across all soils and depth increments compared to the control. The decrease in the DOC:DN ratio under N and NP addition was mainly caused by increased DN concentrations under N and NP addition by +164% and +106%, respectively, across all soils and depth increments.

3.2. Carbon use efficiency

Addition of N, P, and NP did not significantly change CUE across all grassland soils at either depth increment (Fig. 1, Table S1). Soil microbial CUE ranged between 25% at one site in the USA and 57% at a UK site (Fig. 2a, Table S2), with a mean of 40% across all sites.

A linear mixed-effects model of all treatments and sites based on sand content, MAP, DOC:DN ratio, and MAT accounted for 70% of the variability in CUE across all sites (Fig. 3a, Table S3). Soil microbial CUE decreased with DOC:DN ratio and MAP and increased with MAT and sand content. When considering exclusively the control plots, these factors explained 89% of the variation in CUE ($p < 0.001$, Fig. 3b). Further, ANPP had no significant effect on CUE in multi-model selection. There was a significant correlation between ANPP and soil microbial CUE ($p = 0.007$), however the R^2 was 0.10 (data not shown).

In addition, CUE was negatively correlated with the activities of BG ($R^2 = 0.31$, $p = 0.02$), NAG ($R^2 = 0.40$, $p = 0.006$), and LAP ($R^2 = 0.50$, $p = 0.002$) in topsoils (Fig. 4). Considering the topsoil of the control plots, CUE increased with DN concentration ($R^2 = 0.41$, $p = 0.004$, data not shown) and was negatively related with the DOC:DN ratio ($R^2 = 0.27$, $p = 0.03$, data not shown). When considering only topsoils (except Summerveld), the negative correlation between CUE and DOC:DN in the

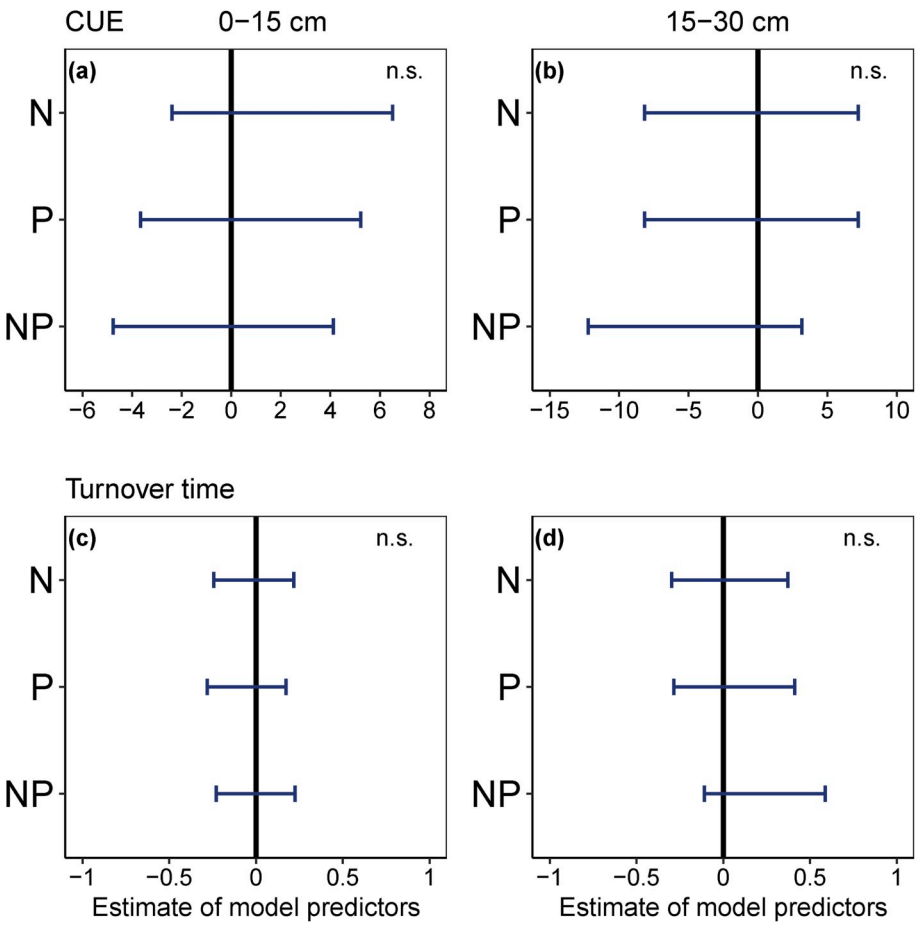


Fig. 1. Effect of nutrient addition (N, P, NP) on (a, b) microbial carbon use efficiency (CUE) and (c, d) microbial biomass turnover across all six sites in (a, c) 0–15 cm depth and (b, d) 15–30 cm depth. The vertical intercept (position zero) corresponds to the control. Linear mixed-effects models were calculated with treatment as fixed factor and random intercepts for site (n = 18 in 0–15 cm depth and n = 15 in 15–30 cm depth). Dots represent the mean value of the model predictor while error bars represent the range of 95% confidence intervals. Predictors are considered significant, if error bars do not overlap with zero, indicated by asterisks (* significant at $p < 0.05$, ** significant at $p < 0.01$, *** significant at $p < 0.001$). Model predictors display original data in panel a and b and transformed data in panel c and d.

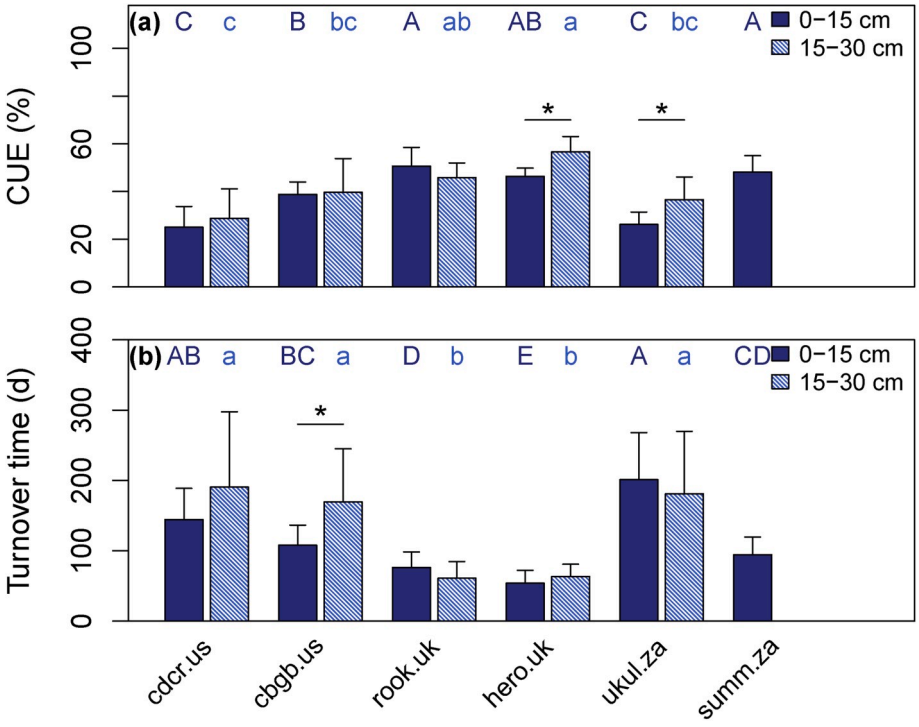


Fig. 2. Mean (a) carbon use efficiency (CUE) and (b) microbial biomass turnover time of all treatments at six grassland sites in two soil depth increments across all treatments. Error bars indicate standard deviations (n = 12). Upper-case letters indicate significant differences between sites tested separately for 0–15 cm depth. Lower-case letters indicate significant differences between sites tested separately for 15–30 cm depth. Asterisks indicate significant differences between both depth increments tested separately for each site.

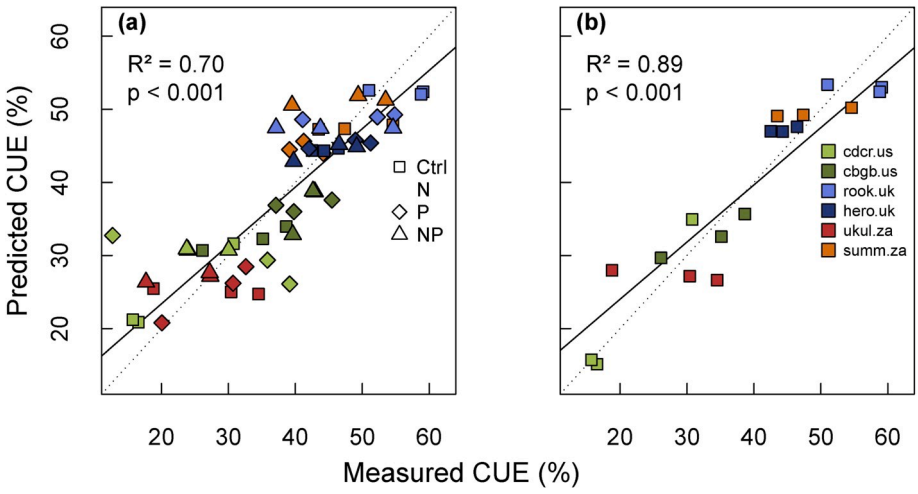


Fig. 3. Linear mixed-effects model of (a) carbon use efficiency (CUE) in all treatments and (b) CUE in the control at all six grassland sites in 0–15 cm depth. Measured CUE is shown on the x-axis and predicted CUE is shown on the y-axis. Best model predictors were sand content, mean annual precipitation, dissolved organic carbon-to-dissolved nitrogen ratio, and mean annual temperature (Table S3). The linear mixed-effects model was calculated after multi-model selection. R^2 was calculated as conditional R^2 according to Nakagawa and Schielzeth (2013), the standard line is dashed (intercept = 0, slope = 1), and the fitted line of the model is solid.

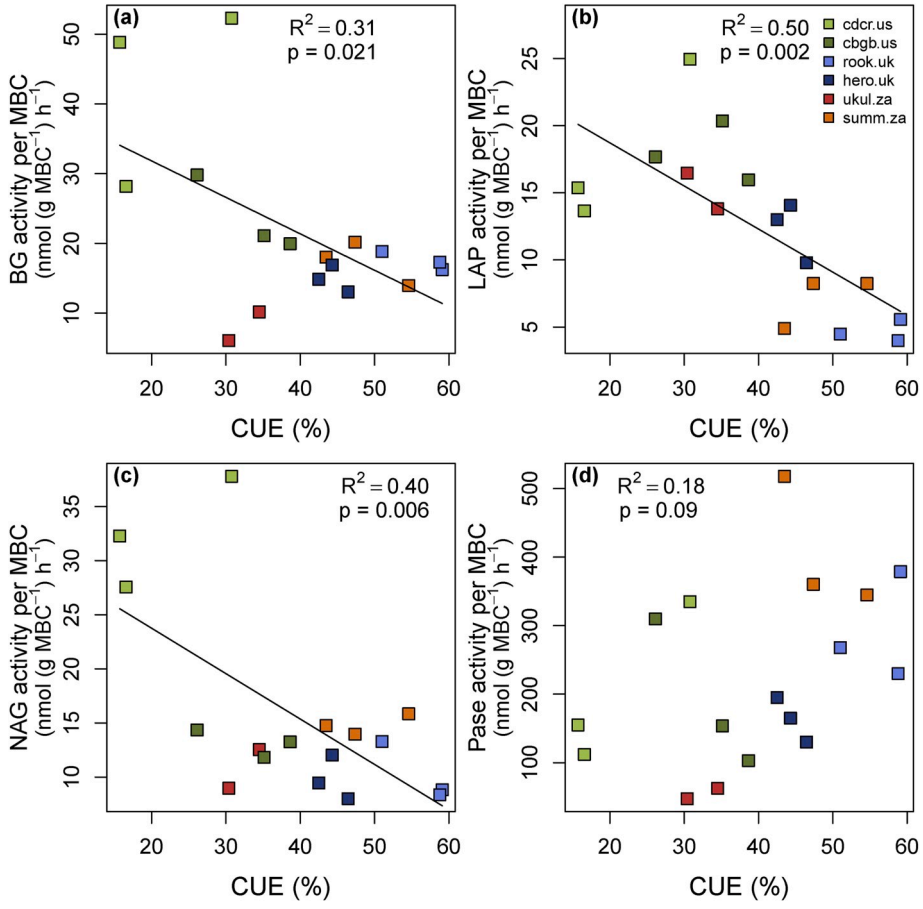


Fig. 4. Correlation of (a) beta-glucosidase (BG) activity, (b) leucine-aminopeptidase (LAP) activity, (c) N-acetylglucosaminidase (NAG) activity, and (d) phosphatase (Pase) activity per unit microbial biomass carbon (MBC) with microbial carbon use efficiency (CUE) in 0–15 cm depth in the control treatments. BG, LAP, and NAG activities were sqrt-transformed to achieve normal distribution before running the correlation analyses.

control plots was highly significant ($R^2 = 0.71$, $p < 0.001$, Fig. S1).

3.3. Microbial biomass carbon, nitrogen, and microbial community structure

Mean microbial biomass C concentrations in the topsoil of all treatments ranged between 156 mg kg soil^{-1} at a site in the USA and 848 mg kg soil^{-1} at a South African site (Table 2). Nutrient addition did not significantly change microbial biomass C in either depth increment at any of the sites compared to the control. Mean molar microbial biomass C:N ratios across all sites did not change in response to nutrient addition at either depth (Fig. S3). Similarly, the bacterial and fungal communities of all sites were significantly different from each other (Fig. S4), except for the bacterial communities of Cedar Creek and Rookery and the fungal communities of the two South African sites. Neither the bacterial nor the fungal community differed among nutrient addition treatments

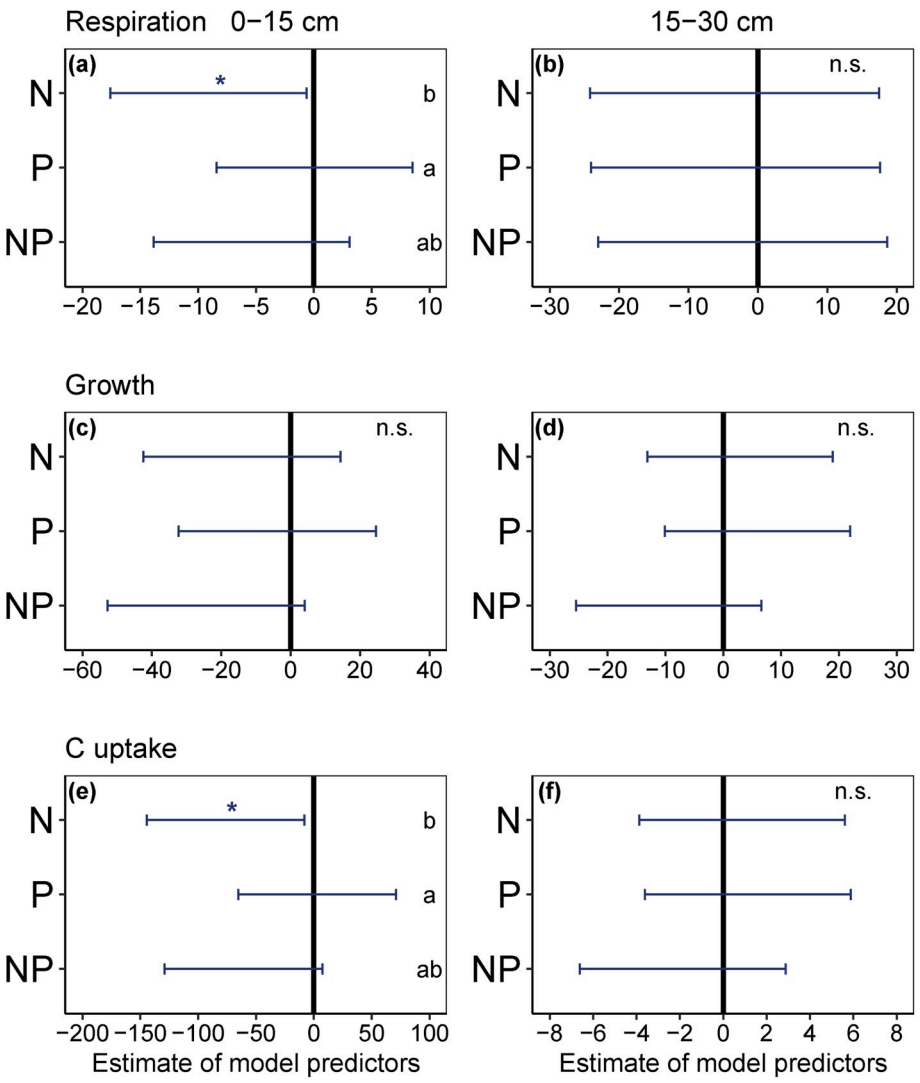


Fig. 5. Effect of nutrient addition (N, P, NP) on (a, b) soil microbial respiration, (c, d) microbial growth, and (e, f) microbial C uptake across all six sites in (a, c, e) 0–15 cm depth and (b, d, f) 15–30 cm depth. Linear mixed-effects models were calculated with treatment as fixed and site intercepts as random factor (n = 18 in 0–15 cm depth and n = 15 in 15–30 cm depth). Dots represent the mean value of the model predictor while error bars represent the range of 95% confidence intervals. Predictors are considered significant, if error bars do not overlap with zero, indicated by asterisks (* significant at $p < 0.05$, ** significant at $p < 0.01$, *** significant at $p < 0.001$). The vertical intercept (position zero) corresponds to the control. Significant differences between treatments (N, P, and NP) are indicated by lower-case letters at the right side of the subplot. Model predictors display original data in panel b, c, d, and e and transformed data in panel a and f.

at each site (data not shown) and across all sites (Fig. S5).

3.4. Microbial biomass turnover time

Nutrient addition (N, P, and NP) did not significantly affect microbial biomass turnover time at either depth increment (Fig. 1c and d), because small and not significant changes in microbial biomass C and C_{Growth} cancelled each other out. Mean microbial biomass turnover time ranged between 54 days at one of the sites in the UK and 201 days at a South African site, with an average of 122 days across all sites, treatments, and depth increments (Fig. 2b).

3.5. Microbial respiration, growth, and C uptake

Soil microbial respiration declined significantly in response to N addition by −23% according to the estimated predictor of the linear mixed-effects model in the topsoils across all six grassland sites (Fig. 5a). Addition of P and NP did not change mean topsoil microbial respiration. Additionally, microbial growth (Fig. 5c and d) did not change in response to nutrient addition. Topsoil C uptake was significantly lower under N addition (−14%) than in the control and P addition treatments across all soils (Fig. 5e). In the second depth increment, C uptake did not change significantly in response to nutrient addition (Fig. 5f).

Microbial respiration in the topsoil across all treatments was highest at the two sites in South Africa and at one site in the UK (Fig. 6a). Microbial growth in the topsoil was lowest at a site in the USA and highest at a South African site (Fig. 6b). Microbial growth and respiration were positively correlated ($R^2 = 0.45$, $p < 0.001$, Fig. S2). Further, microbial C uptake (the sum of growth and respiration) was smallest at the sites in the USA. C uptake was highest at a site in South Africa (Fig. 6c). Microbial respiration, growth, and C uptake of all treatments were significantly higher in topsoils compared to subsoils (Fig. 6).

4. Discussion

4.1. Microbial carbon use efficiency robust to nutrient addition

We found that soil microorganisms across all sites invested 40% of the C they took up into growth (mean CUE of 40%), which is close to CUE estimates based on kinetic and metabolic considerations (Sinsabaugh et al., 2013). Several studies found similar results based on the same method used here with mean soil microbial CUE ranging between 25 and 45% (Spohn et al., 2016a, 2016b; Walker et al., 2018; Poeplau et al., 2019; Zheng et al., 2019).

Our finding that soil microbial CUE was not affected by changes in nutrient supply contrasts previous studies that found increases in CUE under N addition (Ziegler and Billings, 2011; Spohn et al., 2016b; Poeplau et al., 2019). However, our findings are in line with Riggs and Hobbie (2016) reporting that N addition did not increase soil microbial CUE in three North American grassland soils. Similarly, Lee and Schmidt

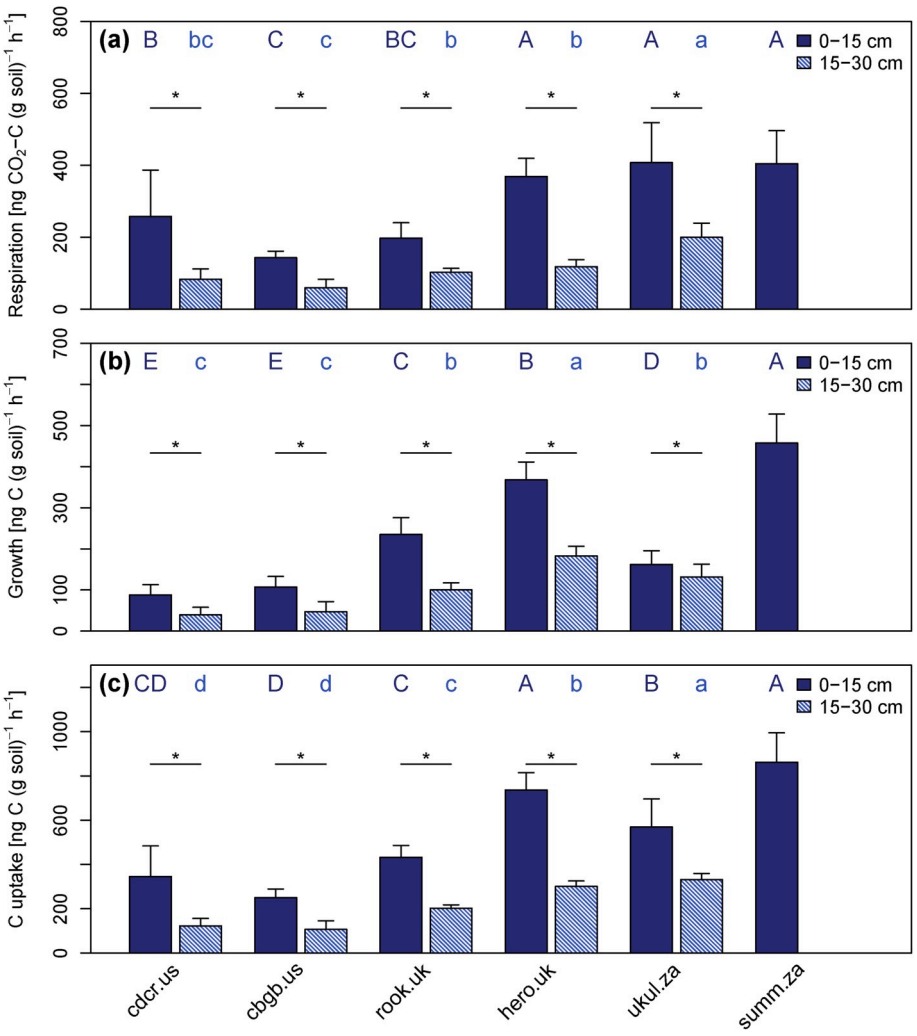


Fig. 6. Mean (a) microbial respiration, (b) growth, and (c) carbon (C) uptake of all treatments at the six sites in two depth increments. Error bars indicate standard deviations (n = 12). Upper-case letters indicate significant differences between sites tested separately for 0–15 cm depth. Lower-case letters indicate significant differences between sites tested separately for 15–30 cm depth. Asterisks indicate significant differences between both depth increments tested separately for each site.

(2014) found no change in microbial CUE due to N-amendment in a cropland soil.

4.2. Microbial respiration and growth under nutrient addition

We found that microorganisms regulated both respiration and growth similarly (Fig. S2), explaining the unresponsiveness of CUE to increased N availability. Our data suggest that microbes did not uncouple respiration from growth (performing overflow respiration) in response to nutrient addition. Overflow respiration, i.e. the disposal of C, has been very critically discussed recently, and seems rather unlikely to occur under natural conditions (Hessen and Anderson, 2008; Spohn, 2015). Soil microbes are most commonly C limited in most mineral soils (Alden et al., 2001; Demoling et al., 2007; Heuck et al., 2015) and, in case C is available in excess, it could be stored or used to establish defense mechanisms or symbiosis (Hessen and Anderson, 2008). Further, a strong increase in microbial growth at the expense of respiration is unlikely to occur because microorganisms need to uphold maintenance respiration and respiration to support anabolic energy requirements for biosynthesis (Geyer et al., 2016) to enable cellular functioning and growth. Hence, our work indicates that respiration and growth of the microbial community under nutrient addition may be coupled more tightly than previously thought (Mooshammer et al., 2014; Manzoni et al., 2017) leading to unaltered CUE.

Addition of N to soil reduced both microbial respiration and C uptake (Fig. 5). Reduced microbial respiration under N addition was found before (Söderström et al., 1983; Treseder, 2008; Rousk et al., 2011; Zhou et al., 2014) and there might be several mechanisms leading to this effect. First, a high availability of inorganic N has been shown to inhibit oxidative enzymes, which hinders microbial C uptake because complex C compounds are decomposed more slowly (Fog, 1988; Carreiro et al., 2000; Sinsabaugh, 2010). Consequently, the amount of internally processed C is reduced, affecting both respiration and growth (Saiya-Cork et al., 2002; Sinsabaugh, 2010). Second, high N availability might intermittently prevent microbes from decomposing soil organic matter, when mining organic matter for N (Moorhead and Sinsabaugh, 2006; Craine et al., 2007). Third, abundant inorganic N can lead to soil acidification, which can reduce microbial biomass (Riggs and Hobbie, 2016; Schleuss et al., 2019) and change microbial community composition (Treseder, 2008; Rousk et al., 2011), both of which can be associated with reduced microbial respiration. However, here, nutrient addition only led to changes in soil pH at a single grassland site (Table 2) and did not alter microbial biomass (Table 3) and bacterial and fungal community composition. Therefore, the decreased respiration and C uptake in response to N addition observed here is more likely caused by the inhibition of oxidative enzymes combined with reduced N mining by soil microbes. Since microbial growth, soil microbial CUE, and soil microbial biomass turnover time were not significantly changed by N addition, also microbial biomass C concentrations did not decrease under N addition (Table 3).

Our finding that P addition did not have a significant effect on all variables considered (Figs. 1 and 5) indicates that P is not critical for

microbial C processing across this broad range of grassland sites. Although P addition significantly increased soil DOC concentrations in two out of six sites, probably due to increased plant litter inputs (Elser et al., 2007) or desorption of organic compounds from the soil solid phase (Spohn and Schleuss, 2019), it did not influence CUE. However, P addition in combination with N mitigated the effect on microbial respiration and C uptake found in response to single N addition. A reason for this might be that addition of P in combination with N leads to microbial immobilization of N, which prevents N from inhibiting oxidative enzymes. Our findings are in accordance with previous work showing that the addition of P in combination with N counteracted the effect of N addition on soil C sequestration (Fornara et al., 2013). Further, the relative abundance of microbial genes associated with metabolism strongly decreased with N addition, but P added in combination with N attenuated this effect (Leff et al., 2015).

4.3. Substrate stoichiometry and microbial CUE

Our finding that soil microbial CUE and the DOC:DN ratio were negatively correlated (Fig. 3) indicates that the availability of organic C relative to N is a key factor shaping CUE. Soil DOC:DN ratios mostly exceed C:N ratios of the microbial biomass (Mooshammer et al., 2014; Spohn, 2016), forcing microbes to adapt their foraging strategies to the available substrate in order to maintain their biomass C:N stoichiometry (Sinsabaugh et al., 2013). The microbial biomass has a relatively constrained C:N stoichiometry (Cleveland and Liptzin, 2007; Xu et al., 2013) and maintains its biomass stoichiometry independent of the stoichiometry of its environment, which was also confirmed in our study. Consequently, as the DOC:DN ratio approaches that of microbial biomass, less C and energy needs to be invested by soil microbes into nutrient acquisition to compensate for stoichiometrically imbalanced substrates, and thus CUE increases. Our data demonstrate that the relationship between soil microbial CUE and DOC:DN ratios assumed in models (Sinsabaugh et al., 2013, 2016; Manzoni et al., 2017) holds true across grasslands spanning a wide range of locations and conditions, but was unaffected by nutrient addition.

4.4. Enzyme activities and microbial CUE

Extracellular enzyme activities are commonly interpreted as indicators of microbial nutrient demand as they mediate nutrient acquisition from organic matter (Sinsabaugh et al., 2008; Schimel and Weintraub, 2003). It has been proposed that if DOC:DN ratios are large, microorganisms invest into nutrient acquisition, which reduces microbial CUE. Vice versa, high nutrient availability reduces the energy investment of microbial communities into nutrient acquisition via the production of extracellular enzymes, and therefore increases their CUE (Manzoni et al., 2012; Sinsabaugh et al., 2016; Spohn et al., 2016b). However, the relationship between microbial CUE and extracellular enzymes has rarely been studied. Our finding that LAP and NAG activities per unit microbial biomass C were negatively correlated with CUE (Fig. 4b and c) confirms this concept. In addition, our findings show that microbial CUE was negatively correlated with BG activity (Fig. 4a), indicating that microorganisms do not only produce more nutrient acquiring enzymes, but also invest more into C acquisition when they run at low CUE. If microorganisms are well supplied with C and nutrients, they do not need to invest into C- and N-acquiring enzymes, and thus microbial CUE increases. In contrast, under C and nutrient deficiency, they invest into C- and N-acquiring enzymes, and thus soil microbial CUE is decreased. Phosphatase activity was not related to CUE (Fig. 4d), which confirms our finding that P alone was not critical for microbial C processing in the studied soils (Figs. 1 and 5). The negative correlations between CUE and DOC:DN ratios and CUE and enzyme activities could point to a positive correlation of DN concentrations and N-acquiring enzyme activities. However, the correlations between DOC:DN ratios and N-acquiring enzymes were mainly driven by changes in DOC rather than DN concentrations. A global meta-analysis confirms the relation of increasing hydrolytic enzyme activities with soil organic matter concentrations (Sinsabaugh et al., 2008).

4.5. Environmental conditions and microbial CUE

Besides substrate stoichiometry, climatic variables and soil texture were related to soil microbial CUE (Fig. 3). A negative relationship between MAP and CUE (Fig. 3) was similarly reported by Takriti et al. (2018) and Herron et al. (2009). Further, CUE increased with MAT (Fig. 3) as found previously (Zheng et al., 2019), which can be explained by increased microbial growth but constant maintenance respiration under higher temperatures. The positive relationship between CUE and sand content (Fig. 3) is consistent with previous work suggesting that soil texture may influence CUE (Zheng et al., 2019). A high clay content could negatively influence CUE because it reduces the accessibility of C and nutrients to microbes due to sorption and soil aggregation (Mikutta et al., 2006; Cotrufo et al., 2013). Further, it needs to be considered that the nutrient addition treatments had variable effects on nutrient availability in the different soils. Taken together, our study suggests that changes in climatic conditions may impact soil microbial CUE due to its dependence on MAT and MAP.

4.6. Microbial biomass turnover time

Our finding that microbial biomass turnover was not affected by nutrient addition is confirmed by another study that also observed no change in turnover time with nutrient addition (Spohn et al., 2016b). We found that mean microbial biomass turnover time was 122 days (Fig. 2), which is in the range of previously reported microbial turnover times (Kouno et al., 2002; Perelo and Munch, 2005; Cheng, 2009; Spohn et al., 2016a, 2016b). The effect of a low microbial C uptake rate on soil microbial biomass can be compensated either by high CUE or by long turnover time (Spohn et al., 2016a). We found that a reduced microbial C uptake, as found in the soils in the USA (Fig. 6), was accompanied by a relatively long microbial turnover time (Fig. 2). Similarly, low microbial C uptake due to reduced C availability was mirrored by relatively long turnover times in forest soils (Spohn et al., 2016a), showing that microbial communities exhibiting slow turnover rates have low C uptake rates.

4.7. Conclusion

Here we showed that soil microbial CUE was not affected by changes in N and P supply in six grassland soils, representing widely differing biotic and abiotic conditions, in contrast to our first hypothesis. Soil microbial respiration and growth decreased similarly in response to N addition, which explains the non-responsiveness of soil microbial CUE to N addition. Microbial CUE across all sites was negatively related to the DOC:DN ratio, confirming our second hypothesis. Together, the DOC:DN ratio, sand content, MAP, and MAT explained 70% of the variability in CUE across all six sites, suggesting that climate is likely to be an important predictor of soil microbial CUE. Neither N nor P addition changed microbial biomass turnover time, in contrast to our third hypothesis. Taken together, the study demonstrates that high N inputs to grassland soils decreased microbial respiration and C uptake but did not significantly affect soil microbial CUE. Thus, our finding that microbial growth and respiration are homeostatically coupled with respect to nutrient additions is validating assumptions of constant soil microbial CUE in most Earth system models.

Declaration of competing interest

The authors declare no conflict of interest.

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). We thank Giovanni Pastore for his helpful comments on a previous version of the manuscript. We thank Renate Krauss, Uwe Hell, and Karin Söllner for technical assistance and thank the chemical analytics of the Bayreuth Center of Ecological and Environmental Research for performing parts of the chemical analyses and the Centre for Stable Isotope Research and Analysis of the University of Göttingen for measuring ^{18}O isotopes.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.soilbio.2020.107815.

References

Ågren, G., Bosatta, E., Magill, A., 2001. Combining theory and experiment to understand effects of inorganic nitrogen on litter decomposition. Oecologia 128, 94–98.
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., Wallenstein, M.D., Bradford, M.A., 2010. Soil-carbon response to warming dependent on microbial physiology. Nature Geoscience 3, 336–340.
Anderson, T.-H., Martens, R., 2013. DNA determinations during growth of soil microbial biomasses. Soil Biology and Biochemistry 57, 487–495.
Barton, K., 2018. MuMIn: Multi-Model Inference. R Package, version 1.42.1.
Blazewicz, S.J., Schwartz, E., 2011. Dynamics of ^{18}O incorporation from $H_2^{18}O$ into soil microbial DNA. Microbial Ecology 61, 911–916.
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.
Bradford, M.A., Fierer, N., Jackson, R.B., Maddox, T.R., Reynolds, J.F., 2008. Nonlinear root-derived carbon sequestration across a gradient of nitrogen and phosphorous deposition in experimental mesocosms. Global Change Biology 14, 1113–1124.
Bray, R.H., Kurtz, L.T., 1945. Determination of total, organic, and available forms of phosphorus in soils. Soil Science 95, 39–46.
British Geological Survey, 1999. Windsor. England and Wales Sheet 269. Solid and Drift Geology. 1:50 000. British Geological Survey, Keyworth, Nottingham.
Brookes, P.C., Landman, A., Pruden, G., Jenkinson, D.S., 1985. Chloroform fumigation and the release of soil nitrogen: a rapid direct extraction method to measure microbial biomass nitrogen in the soil. Soil Biology and Biochemistry 17, 837–842.
Brookes, P.C., Powlson, D.S., Jenkinson, D.S., 1982. Measurement of microbial biomass phosphorus in soil. Soil Biology and Biochemistry 14, 319–329.
Cardinale, M., Brusetti, L., Quatrini, P., Borin, S., Puglia, A.M., Rizzi, A., Zanardini, E., Sorlini, C., Corselli, C., Daffonchio, D., 2004. Comparison of different primer sets for use in automated ribosomal intergenic spacer analysis of complex bacterial communities. Applied and Environmental Microbiology 70, 6147–6156.
Carreiro, M.M., Sinsabaugh, R.L., Repert, D.A., Parkhurst, D.F., 2000. Microbial enzyme shifts explain litter decay responses to simulated nitrogen deposition. Ecology 81, 2359–2365.
Cheng, W., 2009. Rhizosphere priming effect: its functional relationships with microbial turnover, evapotranspiration, and C-N budgets. Soil Biology and Biochemistry 41, 1795–1801.
Clark, C.M., Tilman, D., 2008. Loss of plant species after chronic low-level nitrogen deposition to prairie grasslands. Nature 451, 712–715.
Clarke, K.R., Gorley, R.N., 2015. PRIMER V7: User Manual/Tutorial. Plymouth.
Cleveland, C.C., Liptzin, D., 2007. C:N:P stoichiometry in soil: is there a "Redfield ratio" for the microbial biomass? Biogeochemistry 85, 235–252.
Cotrufo, M.F., Wallenstein, M.D., Boot, C.M., Denef, K., Paul, E., 2013. The Microbial Efficiency-Matrix Stabilization (MEMS) framework integrates plant litter decomposition with soil organic matter stabilization: do labile plant inputs form stable soil organic matter? Global Change Biology 19, 988–995.
Craine, J.M., Morrow, C., Fierer, N., 2007. Microbial nitrogen limitation increases decomposition. Ecology 88, 2105–2113.
Crowther, T.W., Riggs, C., Lind, E.M., Borer, E.T., Seabloom, E.W., Hobbie, S.E., Wubs, J., Adler, P.B., Firn, J., Gherardi, L., Hagenah, N., Hofmockel, K.S., Knops, J. M.H., McCulley, R.L., McDougall, A., Peri, P.L., Prober, S.M., Stevens, C.J., Routh, D., 2019. Sensitivity of global soil carbon stocks to combined nutrient enrichment. Ecology Letters 936–945.
del Giorgio, P.A., Cole, J.J., 1998. Bacterial growth efficiency in natural aquatic systems. Annual Review of Ecology and Systematics 29, 503–541.
Demoling, F., Figueroa, D., Bååth, E., 2007. Comparison of factors limiting bacterial growth in different soils. Soil Biology and Biochemistry 39, 2485–2495.
Devêvre, O.C., Horwáth, W.R., 2000. Decomposition of rice straw and microbial carbon use efficiency under different soil temperatures and moistures. Soil Biology and Biochemistry 32, 1773–1785.
Eliasson, P.E., Ågren, G.I., 2011. Feedback from soil inorganic nitrogen on soil organic matter mineralisation and growth in a boreal forest ecosystem. Plant and Soil 338, 193–203.
Elser, J.J., Bracken, M.E.S., Cleland, E.E., Gruner, D.S., Harpole, W.S., Hillebrand, H., Ngai, J.T., Seabloom, E.W., Shurin, J.B., Smith, J.E., 2007. Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. Ecology Letters 10, 1135–1142.
Fay, P.A., Prober, S.M., Harpole, W.S., Knops, J.M.H., Bakker, J.D., Borer, E.T., Lind, E. M., MacDougall, A.S., Seabloom, E.W., Wragg, P.D., Adler, P.B., Blumenthal, D.M., Buckley, Y.M., Chu, C., Cleland, E.E., Collins, S.L., Davies, K.F., Du, G., Feng, X., Firn, J., Gruner, D.S., Hagenah, N., Hautier, Y., Heckman, R.W., Jin, V.L., Kirkman, K.P., Klein, J., Ladwig, L.M., Li, Q., McCulley, R.L., Melbourne, B.A., Mitchell, C.E., Moore, J.L., Morgan, J.W., Risch, A.C., Schütz, M., Stevens, C.J., Wedin, D.A., Yang, L.H., 2015. Grassland productivity limited by multiple nutrients. Nature Plants 1, 15080.
Fisher, M.M., Triplett, E.W., 1999. Automated approach for ribosomal intergenic spacer analysis of microbial diversity and its application to freshwater bacterial communities. Applied and Environmental Microbiology 65, 4630–4636.
Fog, K., 1988. The effect of added nitrogen on the rate of decomposition of organic matter. Biological Reviews 63, 433–462.
Fornara, D.A., Banin, L., Crawley, M.J., 2013. Multi-nutrient vs. nitrogen-only effects on carbon sequestration in grassland soils. Global Change Biology 19, 3848–3857.
Fornara, D.A., Tilman, D., 2012. Soil carbon sequestration in prairie grasslands increased by chronic nitrogen addition. Ecology 93, 2030–2036.
Fynn, R.W.S., O'Connor, T.G., 2005. Determinants of community organization of a South African mesic grassland. Journal of Vegetation Science 16, 93–102.
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.
Gelman, A., Su, Y.-S., Yajima, M., Hill, J., Pittau, M.G., Kerman, J., Zheng, T., Dorie, V., 2018. Arm: Data Analysis Using Regression and Multilevel/Hierarchical Models, pp. 10–11. R package version 1.
German, D.P., Weintraub, M.N., Grandy, A.S., Lauber, C.L., Rinkes, Z.L., Allison, S.D., 2011. Optimization of hydrolytic and oxidative enzyme methods for ecosystem studies. Soil Biology and Biochemistry 43, 1387–1397.
Geyer, K.M., Kyker-Snowman, E., Grandy, A.S., Frey, S.D., 2016. Microbial carbon use efficiency. Accounting for population, community, and ecosystem-scale controls over the fate of metabolized organic matter. Biogeochemistry 127, 173–188.
Grueber, C.E., Nakagawa, S., Laws, R.J., Jamieson, I.G., 2011. Multimodel inference in ecology and evolution: challenges and solutions. Journal of Evolutionary Biology 24, 699–711.
Hagerty, S.B., van Groenigen, K.J., Allison, S.D., Hungate, B.A., Schwartz, E., Koch, G.W., Kolka, R.K., Dijkstra, P., 2014. Accelerated microbial turnover but constant growth efficiency with warming in soil. Nature Climate Change 4, 903–906.
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.
Herold, N., Schöning, I., Berner, D., Haslwimmer, H., Kandeler, E., Michalzik, B., Schrumpf, M., 2014. Vertical gradients of potential enzyme activities in soil profiles of European beech, Norway spruce and Scots pine dominated forest sites. Pedobiologia 57, 181–189.
Herron, P.M., Stark, J.M., Holt, C., Hooker, T., Cardon, Z.G., 2009. Microbial growth efficiencies across a soil moisture gradient assessed using ^{13}C-acetic acid vapor and ^{15}N-ammonia gas. Soil Biology and Biochemistry 41, 1262–1269.
Hessen, D.O., Anderson, T.R., 2008. Excess carbon in aquatic organisms and ecosystems: physiological, ecological, and evolutionary implications. Limnology & Oceanography 53, 1685–1696.
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.
Janssens, I.A., Dieleman, W., Luyssaert, S., Subke, J.-A., Reichstein, M., Ceulemans, R., Ciais, P., Dolman, A.J., Grace, J., Matteucci, G., Papale, D., Piao, S.L., Schulze, E.-D., Tang, J., Law, B.E., 2010. Reduction of forest soil respiration in response to nitrogen deposition. Nature Geoscience 3, 315–322.
Jenkinson, D.S., Brookes, P.C., Powlson, D.S., 2004. Measuring soil microbial biomass. Soil Biology and Biochemistry 36, 5–7.
Joergensen, R.G., Mueller, T., 1996. The fumigation-extraction method to estimate soil microbial biomass: calibration of the k_{en} value. Soil Biology and Biochemistry 28, 33–37.
Köhn, M., 1928. Bemerkungen zur mechanischen Bodenanalyse. III. Ein neuer Pipettapparat. Zeitschrift für Pflanzenernährung. Düngung und Bodenkunde A 364–369. Wissenschaftlicher Teil XI.
Kouno, K., Wu, J., Brookes, P.C., 2002. Turnover of biomass C and P in soil following incorporation of glucose or ryegrass. Soil Biology and Biochemistry 34, 617–622.

Kurtz, L.T., 1942. Elimination of fluoride interference in the molybdenum blue reaction. Industrial & Engineering Chemistry Analytical Edition 14, 855.
Lee, Z.M., Schmidt, T.M., 2014. Bacterial growth efficiency varies in soils under different land management practices. Soil Biology and Biochemistry 69, 282–290.
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.
Li, J., Wang, G., Mayes, M.A., Allison, S.D., Frey, S.D., Shi, Z., Hu, X.-M., Luo, Y., Melillo, J.M., 2018. Reduced carbon use efficiency and increased microbial turnover with soil warming. Global Change Biology 900–910.
Lu, M., Zhou, X., Luo, Y., Yang, Y., Fang, C., Chen, J., Li, B., 2011. Minor stimulation of soil carbon storage by nitrogen addition: a meta-analysis. Agriculture, Ecosystems & Environment 140, 234–244.
Manzoni, S., Čapek, P., Mooshammer, M., Lindahl, B.D., Richter, A., Šantrůčková, H., 2017. Optimal metabolic regulation along resource stoichiometry gradients. Ecology Letters 20, 1182–1191.
Manzoni, S., Taylor, P., Richter, A., Porporato, A., Agren, G.I., 2012. Environmental and stoichiometric controls on microbial carbon-use efficiency in soils. The New Phytologist 196, 79–91.
Mikutta, R., Kleber, M., Torn, M.S., Jahn, R., 2006. Stabilization of soil organic matter: association with minerals or chemical recalcitrance? Biogeochemistry 77, 25–56.
Moorhead, D.L., Sinsabaugh, R.L., 2006. A theoretical model of litter decay and microbial interaction. Ecological Monographs 76, 151–174.
Mooshammer, M., Wanek, W., Zechmeister-Boltenstern, S., Richter, A., 2014. Stoichiometric imbalances between terrestrial decomposer communities and their resources: mechanisms and implications of microbial adaptations to their resources. Frontiers in Microbiology 5, 22.
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.
Nakagawa, S., Schielzeth, H., 2013. A general and simple method for obtaining R^2 from generalized linear mixed-effects models. Methods in Ecology and Evolution 4, 133–142.
Perelo, L., Munch, J., 2005. Microbial immobilisation and turnover of C labelled substrates in two arable soils under field and laboratory conditions. Soil Biology and Biochemistry 37, 2263–2272.
Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., R Core Team, 2018. Nlme: linear and nonlinear mixed effects models. R package version 3, 1–137.
Poeplau, C., Helfrich, M., Dechow, R., Szoboszlay, M., Tebbe, C.C., Don, A., Greiner, B., Zopf, D., Thumm, U., Korevaar, H., Geerts, R., 2019. Increased microbial anabolism contributes to soil carbon sequestration by mineral fertilization in temperate grasslands. Soil Biology and Biochemistry 130, 167–176.
Prior, J.C., 1991. Landforms of Iowa. University of Iowa Press.
R Core Team, 2018. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/.
Riggs, C.E., Hobbie, S.E., 2016. Mechanisms driving the soil organic matter decomposition response to nitrogen enrichment in grassland soils. Soil Biology and Biochemistry 99, 54–65.
Rousk, J., Brookes, P.C., Bååth, E., 2011. Fungal and bacterial growth responses to N fertilization and pH in the 150-year 'Park Grass' UK grassland experiment. FEMS Microbiology Ecology 76, 89–99.
Saiya-Cork, K.R., Sinsabaugh, R.L., Zak, D.R., 2002. The effects of long term nitrogen deposition on extracellular enzyme activity in an Acer saccharum forest soil. Soil Biology and Biochemistry 34, 1309–1315.
Sandaa, R.-A., Enger, Ø., Torsvik, V., 1998. Rapid method for fluorometric quantification of DNA in soil. Soil Biology and Biochemistry 30, 265–268.
Schimel, J.P., Weintraub, M.N., 2003. The implications of exoenzyme activity on microbial carbon and nitrogen limitation in soil: a theoretical model. Soil Biology and Biochemistry 35, 549–563.
Schlesinger, W.H., 2009. On the fate of anthropogenic nitrogen. Proceedings of the National Academy of Sciences of the United States of America 106, 203–208.
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.
Schwartz, E., 2007. Characterization of growing microorganisms in soil by stable isotope probing with $H_2^{18}O$. Applied and Environmental Microbiology 73, 2541–2546.
Scurlock, J.M.O., Hall, D.O., 1998. The global carbon sink: a grassland perspective. Global Change Biology 4, 229–233.
Sinsabaugh, R.L., 2010. Phenol oxidase, peroxidase and organic matter dynamics of soil. Soil Biology and Biochemistry 42, 391–404.
Sinsabaugh, R.L., Lauber, C.L., Weintraub, M.N., Ahmed, B., Allison, S.D., Crenshaw, C., Contosta, A.R., Cusack, D., Frey, S., Gallo, M.E., Gartner, T.B., Hobbie, S.E., Holland, K., Keeler, B.L., Powers, J.S., Stursova, M., Takacs-Vesbach, C., Waldrop, M. P., Wallenstein, M.D., Zak, D.R., Zeglin, L.H., 2008. Stoichiometry of soil enzyme activity at global scale. Ecology Letters 11, 1252–1264.
Sinsabaugh, R.L., Manzoni, S., Moorhead, D.L., Richter, A., 2013. Carbon use efficiency of microbial communities: stoichiometry, methodology and modelling. Ecology Letters 16, 930–939.
Sinsabaugh, R.L., Turner, B.L., Talbot, J.M., Waring, B.G., Powers, J.S., Kuske, C.R., Moorhead, D.L., Follstad Shah, J.J., 2016. Stoichiometry of microbial carbon use efficiency in soils. Ecological Monographs 86, 172–189.
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.
Söderström, B., Bååth, E., Lundgren, B., 1983. Decrease in soil microbial activity and biomasses owing to nitrogen amendments. Canadian Journal of Microbiology 29, 1500–1506.
Spohn, M., 2015. Microbial respiration per unit microbial biomass depends on litter layer carbon-to-nitrogen ratio. Biogeosciences 12, 817–823.
Spohn, M., 2016. Element cycling as driven by stoichiometric homeostasis of soil microorganisms. Basic and Applied Ecology 17, 471–478.
Spohn, M., Klaus, K., Wanek, W., Richter, A., 2016a. Microbial carbon use efficiency and biomass turnover times depending on soil depth – implications for carbon cycling. Soil Biology and Biochemistry 96, 74–81.
Spohn, M., Pötsch, E.M., Eichorst, S.A., Woebken, D., Wanek, W., Richter, A., 2016b. Soil microbial carbon use efficiency and biomass turnover in a long-term fertilization experiment in a temperate grassland. Soil Biology and Biochemistry 97, 168–175.
Spohn, M., Schleuss, P.-M., 2019. Addition of inorganic phosphorus to soil leads to desorption of organic compounds and thus to increased soil respiration. Soil Biology and Biochemistry 130, 220–226.
Spohn, M., Widdig, M., 2017. Turnover of carbon and phosphorus in the microbial biomass depending on phosphorus availability. Soil Biology and Biochemistry 113, 53–59.
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.
Takriti, M., Wild, B., Schnecker, J., Mooshammer, M., Knoltsch, A., Lashchinskiy, N., Eloy Alves, R.J., Gentsch, N., Gittel, A., Mikutta, R., Wanek, W., Richter, A., 2018. Soil organic matter quality exerts a stronger control than stoichiometry on microbial substrate use efficiency along a latitudinal transect. Soil Biology and Biochemistry 121, 212–220.
Treseder, K.K., 2008. Nitrogen additions and microbial biomass: a meta-analysis of ecosystem studies. Ecology Letters 11, 1111–1120.
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.
Walker, T.W.N., Kaiser, C., Strasser, F., Herbold, C.W., Leblans, N.I.W., Woebken, D., Janssens, I.A., Sigurdsson, B.D., Richter, A., 2018. Microbial temperature sensitivity and biomass change explain soil carbon loss with warming. Nature Climate Change 8, 885–889.
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.
Weig, A.R., Peršoh, D., Werner, S., Betzlbacher, A., Rambold, G., 2013. Diagnostic assessment of mycodiversity in environmental samples by fungal ITS1 rDNA length polymorphism. Mycological Progress 12, 719–725.
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.
Wragg, P.D., 2017. Multiple nutrients control threatened grassland vegetation in eastern South Africa. South African Journal of Botany 112, 225–236.
Wu, J., Joergensen, R.G., Pommerening, B., Chaussod, R., Brookes, P.C., 1990. Measurement of soil microbial biomass C by fumigation-extraction - an automated procedure. Soil Biology and Biochemistry 22, 1167–1169.
Xu, X., Thornton, P.E., Post, W.M., 2013. A global analysis of soil microbial biomass carbon, nitrogen and phosphorus in terrestrial ecosystems. Global Ecology and Biogeography 22, 737–749.
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.
Zeng, D.-H., Li, L.-J., Fahey, T.J., Yu, Z.-Y., Fan, Z.-P., Chen, F.-S., 2010. Effects of nitrogen addition on vegetation and ecosystem carbon in a semi-arid grassland. Biogeochemistry 98, 185–193.
Zheng, Q., Hu, Y., Zhang, S., Noll, L., Böckle, T., Richter, A., Wanek, W., 2019. Growth explains microbial carbon use efficiency across soils differing in land use and geology. Soil Biology and Biochemistry 128, 45–55.
Zhou, L., Zhou, X., Zhang, B., Lu, M., Luo, Y., Liu, L., Li, B., 2014. Different responses of soil respiration and its components to nitrogen addition among biomes: a meta-analysis. Global Change Biology 20, 2332–2343.
Ziegler, S.E., Billings, S.A., 2011. Soil nitrogen status as a regulator of carbon substrate flows through microbial communities with elevated CO_2. Journal of Geophysical Research 116.

Supporting information

Microbial carbon use efficiency in grassland soils subjected to nitrogen and phosphorus additions

Meike Widdig[1*], Per-M. Schleuss[1], Lori A. Biederman[2], Elizabeth T. Borer[3], Michael J. Crawley[4], Kevin P. Kirkman[5], Eric W. Seabloom[3], Peter D. Wragg[6], Marie Spohn[1]

[1]Department of Soil Biogeochemistry and Soil Ecology, University of Bayreuth, Germany
[2]Department of Ecology, Evolution and Organismal Biology, Iowa State University, USA
[3]Department of Ecology, Evolution, and Behavior, University of Minnesota, USA
[4]Department of Life Sciences, Imperial College London, England
[5]School of Life Sciences, University of KwaZulu-Natal, South Africa
[6]Department of Forest Resources, University of Minnesota, USA

Supplement Tables and Figures

Table S1: Soil microbial carbon use efficiency (CUE) as affected by N and P addition and their interaction. Linear mixed-effects models were calculated with treatments split into two main factors (N addition with levels 0 or 1 and P addition with levels 0 or 1) and their interaction.

	CUE 0-15 cm depth				CUE 15-30 cm depth		
	Estimate	Std. Error	p-value		Estimate	Std. Error	p-value
(Intercept)	38.57	4.53	<0.001	Intercept	42.87	4.97	<0.001
N	2.06	2.29	0.37	N	-0.48	3.97	0.91
P	0.78	2.29	0.73	P	-0.48	3.97	0.90
Interaction (N*P)	-3.16	3.24	0.33	Interaction (N*P)	-3.58	5.62	0.53

Table S2: Soil microbial carbon use efficiency (CUE), soil microbial turnover time, microbial respiration and growth in 0-15 and 15-30 cm soil depth in the control, N, P, and NP treatment at the six grassland sites. The soil at the site Summerveld (summ.za) was only sampled in 0-15 cm depth because of limited soil depth. Numbers depict means ± standard deviations (n=3). One-way ANOVA was conducted followed by Tukey-Test for multiple comparisons. Lower-case letters indicate significant differences between treatments tested separately for each site and depth increment.

		0-15				15-30			
	Site	Ctrl	N	P	NP	Ctrl	N	P	NP
CUE (%)	cdcr.us	21.02 ± 6.91[a]	23.97 ± 8.53[a]	29.23 ± 11.76[a]	25.93 ± 2.91[a]	29.73 ± 9.25[a]	36.12 ± 16.16[a]	31.60 ± 8.71[a]	17.52 ± 0.88[a]
	cbgb.us	33.30 ± 5.27[a]	39.48 ± 4.60[a]	40.79 ± 3.50[a]	41.72 ± 1.49[a]	46.49 ± 17.25[a]	35.03 ± 10.22[a]	35.01 ± 16.93[a]	42.17 ± 3.76[a]
	rook.uk	56.30 ± 3.75[a]	51.74 ± 8.94[a]	49.39 ± 5.96[a]	45.16 ± 7.21[a]	50.56 ± 7.99[a]	46.24 ± 4.31[a]	43.58 ± 4.10[a]	43.05 ± 3.92[a]
	hero.uk	44.42 ± 1.62[a]	48.37 ± 1.95[a]	47.42 ± 3.91[a]	45.15 ± 3.97[a]	53.21 ± 4.28[a]	58.22 ± 2.08[a]	62.02 ± 5.80[a]	52.99 ± 7.37[a]

	ukul.za	27.91 ± 6.64[a]	25.04 ± 1.19[a]	27.79 ± 5.52[a]	24.07 ± 4.51[a]	34.35 ± 11.40[a]	36.35 ± 8.05[a]	39.74 ± 10.65[a]	35.93 ± 6.20[a]
	summ.za	48.48 ± 4.60[a]	55.16 ± 5.96[a]	41.51 ± 2.07[a]	47.46 ± 5.86[a]	NA	NA	NA	NA
Turnover time (d)	cdcr.us	152.89 ± 20.98[a]	145.06 ± 27.99[a]	93.89 ± 20.88[a]	185.61 ± 43.49[a]	128.18 ± 4.16[a]	218.46 ± 136.90[a]	123.32 ± 4.02[a]	292.15 ± 87.49[a]
	cbgb.us	132.16 ± 12.66[a]	118.28 ± 33.54[a]	86.20 ± 22.50[a]	95.19 ± 12.06[a]	189.40 ± 32.57[a]	176.46 ± 54.82[a]	206.48 ± 103.24[a]	105.38 ± 46.64[a]
	rook.uk	59.67 ± 10.37[a]	85.16 ± 3.71[a]	96.87 ± 27.02[a]	63.93 ± 12.12[a]	35.00 ± 9.01[a]	58.93 ± 19.09[a]	75.45 ± 9.02[a]	74.84 ± 25.17[a]
	hero.uk	52.68 ± 19.02[a]	40.27 ± 6.39[a]	48.56 ± 10.83[a]	74.37 ± 13.33[a]	74.28 ± 13.68[a]	53.94 ± 19.61[a]	51.54 ± 3.44[a]	73.87 ± 13.70[a]
	ukul.za	247.41 ± 11.93[a]	213.64 ± 40.33[a]	207.41 ± 57.76[a]	136.12 ± 79.13[a]	213.88 ± 143.60[a]	129.84 ± 23.88[a]	186.31 ± 56.74[a]	194.11 ± 57.31[a]
	summ.za	81.99 ± 21.12[a]	74.30 ± 9.55[a]	112.39 ± 27.27[a]	109.47 ± 10.60[a]	NA	NA	NA	NA
Respiration (ng CO_2−C g $soil^{-1}$ h^{-1})	cdcr.us	346.03 ± 126.25[a]	239.58 ± 77.78[a]	278.61 ± 165.43[a]	167.24 ± 75.00[a]	96.80 ± 35.42[a]	61.62 ± 13.95[a]	82.02 ± 31.98[a]	91.21 ± 19.45[a]
	cbgb.us	140.69 ± 10.27[a]	142.92 ± 19.21[a]	139.72 ± 19.35[a]	147.97 ± 24.04[a]	45.23 ± 16.29[a]	80.56 ± 29.77[a]	64.06 ± 7.40[a]	47.97 ± 19.04[a]
	rook.uk	164.23 ± 13.80[b]	183.60 ± 42.17[ab]	184.24 ± 18.90[ab]	257.59 ± 22.47[a]	93.01 ± 8.04[a]	103.39 ± 4.57[a]	110.24 ± 15.05[a]	101.43 ± 9.09[a]
	hero.uk	378.49 ± 37.18[a]	321.47 ± 24.70[a]	391.17 ± 49.41[a]	384.33 ± 58.69[a]	135.63 ± 27.11[a]	113.75 ± 11.67[a]	102.41 ± 8.65[a]	119.38 ± 12.12[a]
	ukul.za	415.50 ± 131.65[a]	328.15 ± 31.79[a]	471.92 ± 155.57[a]	413.75 ± 17.33[a]	202.34 ± 40.35[a]	196.87 ± 40.48[a]	198.11 ± 47.43[a]	202.00 ± 33.84[a]
	summ.za	471.19 ± 63.74[a]	327.46 ± 46.51[a]	491.19 ± 45.13[a]	327.24 ± 66.20[a]	NA	NA	NA	NA
Growth (ng C g $soil^{-1}$ h^{-1})	cdcr.us	81.08 ± 5.17[a]	74.26 ± 28.63[a]	89.17 ± 12.26[a]	57.21 ± 21.51[a]	36.77 ± 6.65[a]	42.16 ± 26.44[a]	34.23 ± 3.57[a]	19.38 ± 4.30[a]
	cbgb.us	71.16 ± 16.26[a]	93.08 ± 13.15[a]	97.96 ± 22.51[a]	107.13 ± 22.91[a]	38.14 ± 12.26[a]	43.78 ± 19.00[a]	41.23 ± 29.73[a]	37.17 ± 19.04[a]
	rook.uk	213.78 ± 34.02[a]	195.06 ± 34.38[a]	180.68 ± 26.25[a]	215.56 ± 47.92[a]	97.45 ± 22.46[a]	89.92 ± 14.14[a]	84.23 ± 2.14[a]	76.52 ± 6.66[a]
	hero.uk	302.97 ± 35.77[a]	303.81 ± 44.78[a]	351.03 ± 30.96[a]	312.55 ± 14.66[a]	151.88 ± 12.72[a]	158.12 ± 10.97[a]	170.47 ± 30.02[a]	137.11 ± 28.20[a]
	ukul.za	149.34 ± 8.02[a]	109.31 ± 8.20[a]	170.07 ± 24.08[a]	132.45 ± 29.77[a]	104.88 ± 32.24[a]	109.56 ± 17.68[a]	128.51 ± 33.59[a]	111.71 ± 15.08[a]
	summ.za	444.05 ± 62.92[a]	402.32 ± 45.22[ab]	350.30 ± 46.36[ab]	291.03 ± 31.03[b]	NA	NA	NA	NA

Table S3: Regression coefficients (sand content, mean annual precipitation (MAP), dissolved organic carbon-to-dissolved nitrogen (DOC:DN) ratio, and mean annual temperature (MAT)), R^2, intercept, and slope of the linear mixed-effects model of microbial carbon use efficiency (CUE) of all treatments in 0-15 cm depth. Regression coefficients were selected by multi-model selection. R^2 is the conditional R^2 according to Nakagawa and Schielzeth (2013). Estimation, standard error, and p-value of each standardized regression coefficient are displayed.

Linear mixed-effects model of CUE of all treatments			
Coefficients	Estimation	Std. error	p-value
(Intercept)	39.20	1.00	<0.001
Sand	30.01	4.21	<0.001
MAP	-11.94	2.12	<0.001
DOC:DN ratio	-6.82	2.14	0.001
MAT	33.52	4.40	<0.001
R^2	0.70		
Intercept	7.43		
Slope	0.80		

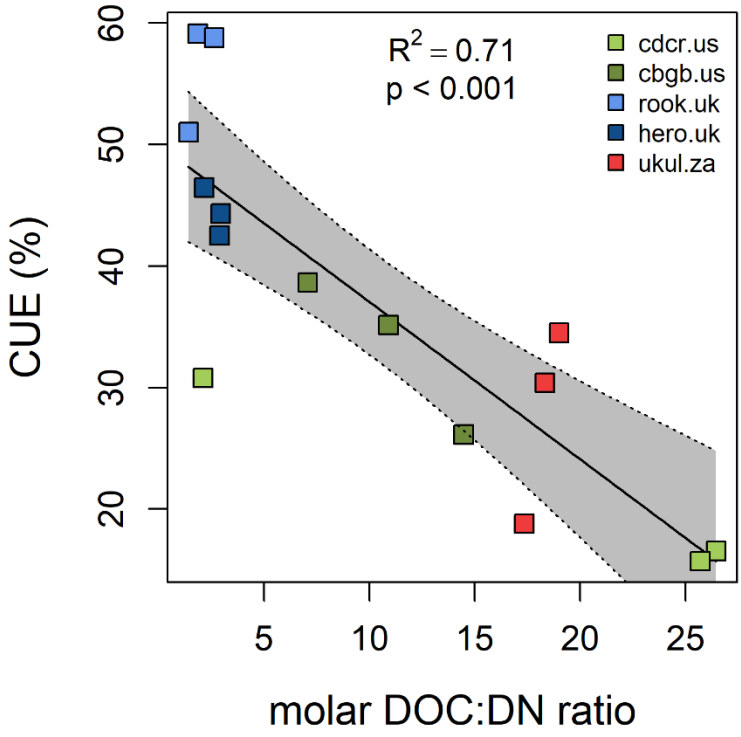


Figure S1: Correlation of molar dissolved organic carbon-to-dissolved nitrogen ratio and soil microbial carbon use efficiency (CUE) in the topsoils of the control plots of all sites with a soil depth > 20 cm.

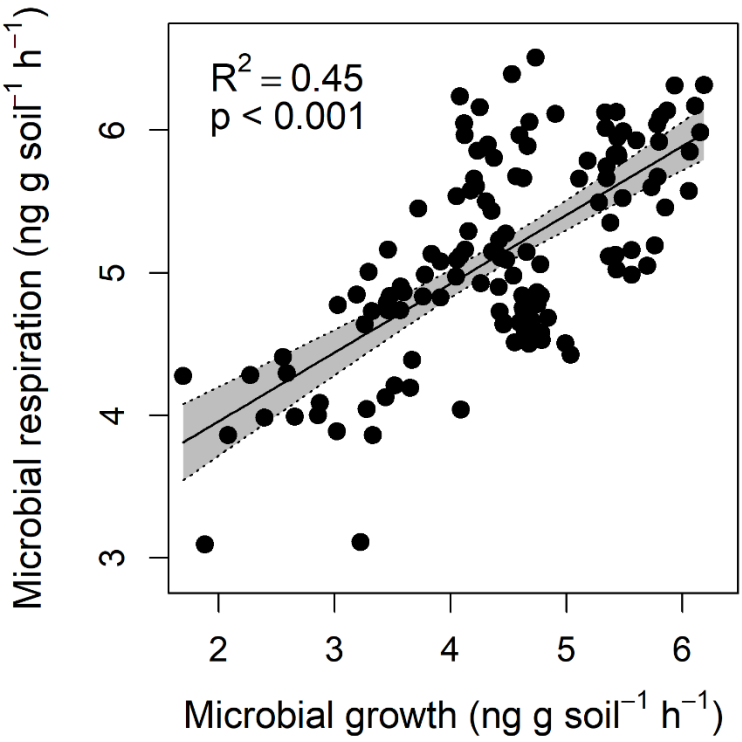
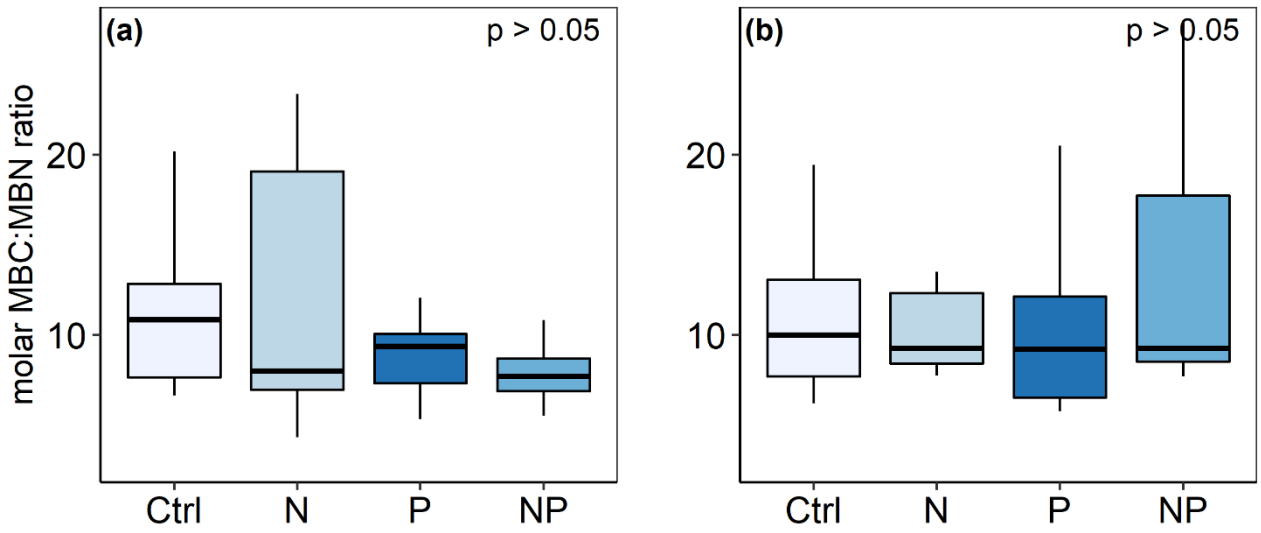


Figure S2: Correlation of microbial growth and microbial respiration across all soils, treatments, and soil depths. Prior to correlation analysis, microbial growth and respiration data were log-transformed to achieve normal distribution.



40

41 **Figure S3:** Molar microbial biomass carbon-to-nitrogen ratio (MBC:MBN) in (a) 0-15 cm depth
42 and (b) 15-30 cm depth in the control, N, P, and NP treatments across all six sites. Subsoil
43 MBC:MBN ratios of one site (rook.uk) were excluded, because MBN values were below detection
44 limit.

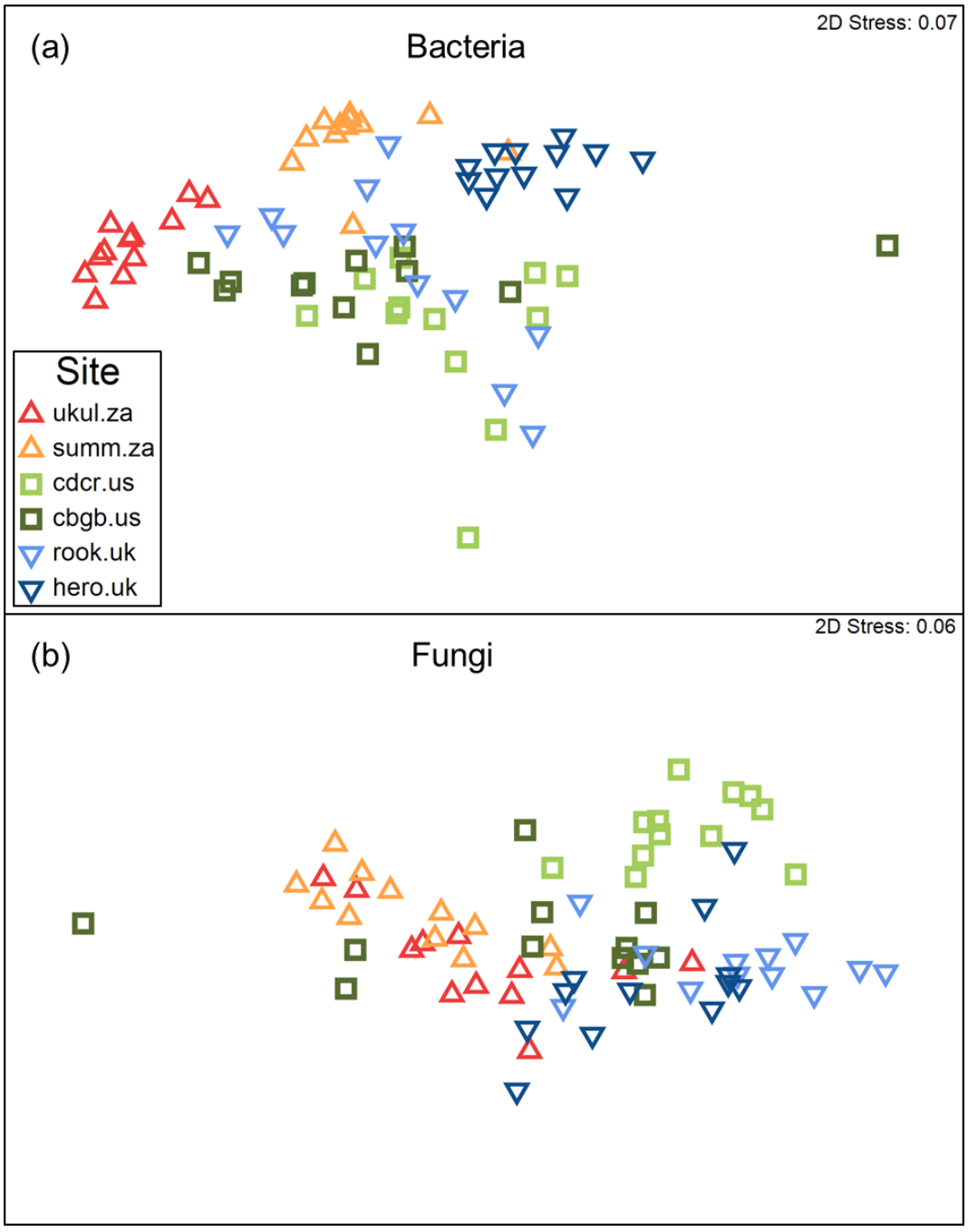


Figure S4: Community composition of (a) bacteria and (b) fungi shown via non-metric multi-dimensional scaling (nMDS) based on ARISA analyses of topsoils of all six sites. One-way ANOSIM with 999 permutations was used to test if microbial communities at the different sites differ significantly from each other.

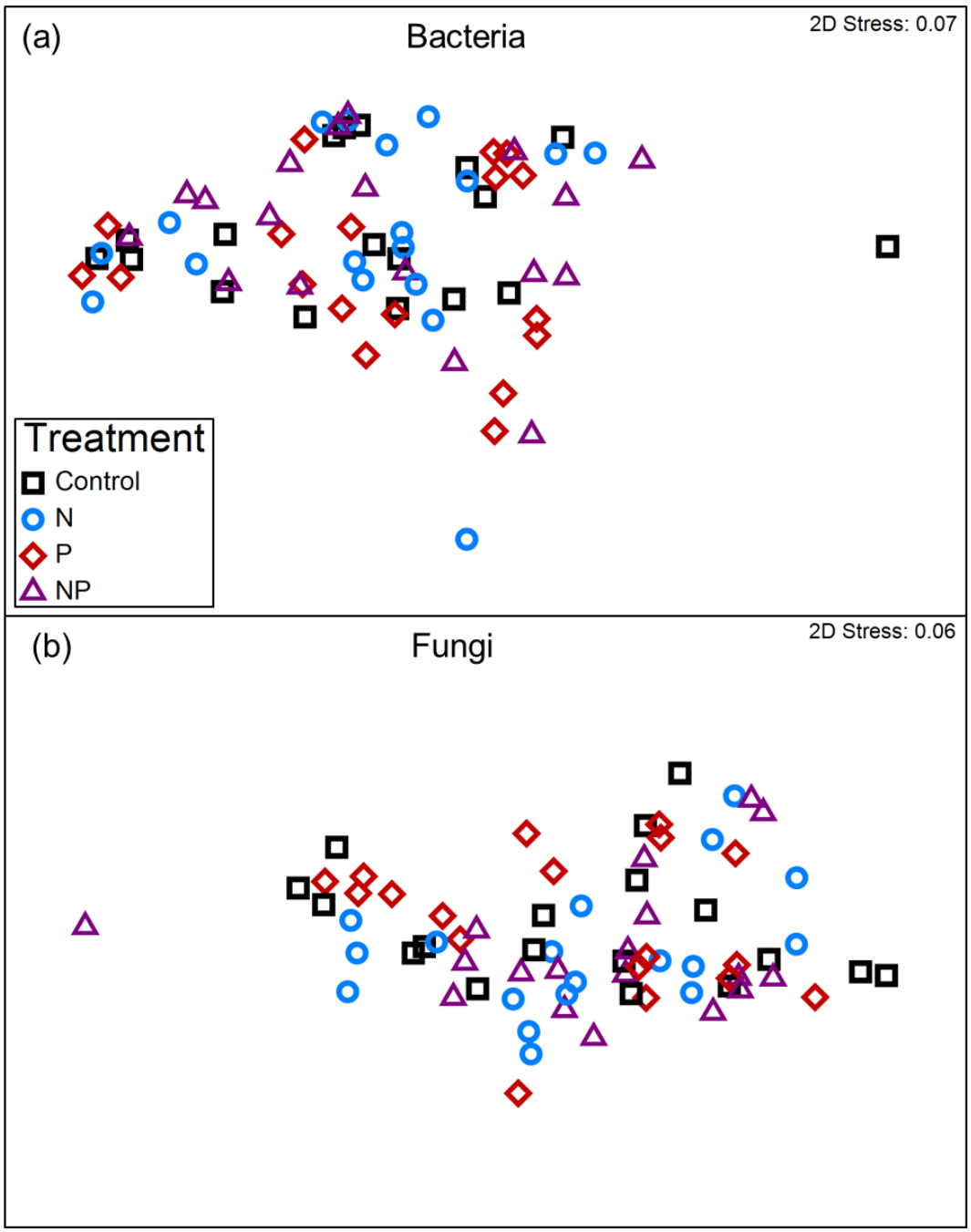


Figure S5: Effect of nutrient addition on (a) bacterial community composition and (b) fungal community composition shown via non-metric multi-dimensional scaling (nMDS) based on ARISA analyses of topsoils of all six sites. One-way ANOSIM with 999 permutations was used to test for significant effects of nutrient addition.