UNIVERSITÄT BAYREUTH

Element cycling in grassland soils as driven by stoichiometric homeostasis of microorganisms

DISSERTATION

zur Erlangung des akademischen Grades einer Doktorin
der Naturwissenschaften (Dr. rer. nat.)
an der Fakultät für Biologie, Chemie und Geowissenschaften
der Universität Bayreuth

vorgelegt von

Meike Widdig
geboren in Schwalmstadt

Bayreuth, Juli 2020

Die vorliegende Arbeit wurde in der Zeit von Dezember 2016 bis Juli 2020 in Bayreuth am Lehrstuhl für Bodenökolgie, Arbeitsgruppe Boden-Biogeochemie, unter Betreuung von Frau Prof. Dr. Marie Spohn angefertigt.

Vollständiger Abdruck der von der Fakultät für Biologie, Chemie und Geowissenschaften der Universität Bayreuth genehmigten Dissertation zur Erlangung des akademischen Grades einer Doktorin der Naturwissenschaften (Dr. rer. nat.).

Dissertation eingereicht am: 24.07.2020
Zulassung durch die Promotionskommission: 29.07.2020
Wissenschaftliches Kolloquium: 11.11.2020

Amtierender Dekan: Prof. Dr. Matthias Breuning

Prüfungsausschuss:

Prof. Dr. Marie Spohn	(Gutachterin)
Prof. Dr. Werner Borken	(Gutachter)
Prof. Dr. Martin Obst	(Vorsitz)
Prof. Dr. Bettina Engelbrecht	

Summary

An unprecedented increase in nitrogen (N) emissions since the 1970s has changed soils N-to-phosphorus (P) stoichiometry, i.e. the relation of N and P in soil. Yet, the consequences of increased nutrient supply on soil microbial communities and element cycling driven by microorganisms are poorly understood. Relatively constrained element ratios in the microbial biomass have been found, indicating stoichiometric homeostasis of microbial biomass. Thus, independent of their surroundings, microorganisms keep a relatively constant biomass carbon (C):N:P ratio, whereas the resources they feed on can have much larger and highly variable element ratios. The concept of Ecological Stoichiometry explains ecological processes based on their elemental ratios and thus by acknowledging chemical constraints on organismal functioning. Yet, it is unclear if Ecological Stoichiometry can be used as a framework predicting element cycling in terrestrial ecosystems. A main research question of this thesis was if microorganisms largely drive C, N, and P cycling in grassland soils by maintaining their biomass stoichiometry. To do the latter, microorganisms are thought to adjust processes of element partitioning and turnover, as well as of element acquisition. Novel isotopic methods enabled us to study microbial element partitioning and turnover, two processes that largely determine element cycling.

To understand element cycling in grasslands on a global scale, we studied six experimental sites on three continents. Seven to nine years of N, P, and NP additions allowed us to study the consequences of changing element inputs on soil microbial element partitioning, turnover, and acquisition. We used novel isotopic methods based on ^{18}O and ^{33}P labelling of the microbial DNA to study carbon use efficiency (CUE) and element turnover times in the microbial biomass. We determined microbial biomass C and N with chloroform-fumigation, net C and N mineralization in incubation experiments, extracellular enzyme activities with fluorogenic substrates, and non-symbiotic N_2 fixation in a ^{15}N labelled atmosphere. Further, we used physiological assays to screen for P-solubilizing bacteria (PSB) and we analyzed the PSB and the microbial community via Sanger and Illumina Sequencing, respectively.

After years of nutrient addition, ratios of available soil elements strongly changed, whereas microbial biomass stoichiometry was unaffected confirming the concept of stoichiometric homeostasis. Microbial C partitioning, the ratio between C allocated to growth and C taken up, called soil microbial CUE, correlated with substrate stoichiometry. Microorganisms respired less when substrate stoichiometry was closer to their own biomass stoichiometry, whereas they respired more when thriving on substrate with a more unfavorable stoichiometry. Besides element partitioning, microorganisms adjusted

turnover times of elements in their biomass. For the first time, we showed that with decreasing P availability, the mean residence time of P in the microbial biomass increased likely because microorganisms recycled P more efficiently internally. Besides C partitioning and P turnover, microorganisms adjusted processes of element acquisition to their stoichiometric demands. Non-symbiotic N_2 fixation was correlated with soil N:P ratios showing that the energy-consuming process of N_2 fixation depended on sufficient P to enable ATP production and at the same time on low N availabilities. Microbial release of N, net N mineralization, was highly dependent on substrate stoichiometry in the way that microorganisms released more N, when N compared to C availability was high and vice versa. However, the activity of leucine-aminopeptidase, a N-acquiring enzyme, was not related to substrate stoichiometry. Further, the relative abundance of PSB was related to soil C:P ratios indicating that the production of organic acids, that solubilize P, needs sufficient C sources. Organic P can be mineralized through phosphatases, extracellular enzymes. Phosphatase activity increased with rising N availability indicating that (i) microbial P demand increased to maintain biomass stoichiometry and that (ii) more N enabled the production of enzymes. Further, element availabilities were the main drivers of element cycling as opposed to microbial community change at one site in the USA confirming the importance of element availabilities on element cycling in grasslands.

In conclusion, Ecological Stoichiometry has proven to be a promising tool for explaining and predicting various element cycling rates in grasslands. However, it needs to be considered that besides element ratios climatic variables, soil pH, and soil texture impacted element cycling rates. Further, not all processes, such as leucine-aminopeptidase activity, were driven by microbial homeostasis. Nevertheless, based on stoichiometric homeostasis of soil microorganisms, many element cycling rates can be understood and predicted in more detail. Thus, Ecological Stoichiometry should be considered as a key concept in terrestrial ecology.

Zusammenfassung

Ein nie dagewesener Anstieg der Stickstoff (N)-Emissionen seit den 1970ern hat die N-zu-Phosphor (P)-Stöchiometrie der Böden verändert, d. h. das Verhältnis von N zu P im Boden. Dennoch sind die Auswirkungen gestiegener Nährstoffeinträge auf mikrobielle Gemeinschaften und Stoffkreisläufe im Boden kaum verstanden. In der mikrobiellen Biomasse wurden konstante Elementverhältnisse gefunden, die auf stöchiometrische Homöostase der mikrobiellen Biomasse hindeuten. Dies bedeutet, dass Mikroorganismen, unabhängig von der Umgebung, ein relativ stabiles Kohlenstoff (C):N:P-Verhältnis in ihrer Biomasse aufweisen, wohingegen ihre Nahrungsquellen viel größere und variable Elementverhältnisse haben. Das Konzept der Ökologischen Stöchiometrie erklärt ökologische Prozesse basierend auf Elementverhältnissen und erkennt somit chemische Einschränkungen auf das Funktionieren der Organismen an. Dennoch ist unklar, ob das Konzept der Ökologischen Stöchiometrie als Basis zur Prognose von zentralen Stoffumsetzungsprozessen in terrestrischen Ökosystemen verwendet werden kann. Eine Hauptfrage dieser Arbeit war, ob Mikroorganismen durch die Aufrechterhaltung ihrer Biomasse-Stöchiometrie C-, N- und P-Kreisläufe in Grasländern bestimmen. Um ihre Biomasse-Stöchiometrie zu bewahren, wird angenommen, dass Mikroorganismen die Element-Partitionierung, die Verweilzeiten von Elementen in der Biomasse und die Raten der Nährstoffakquise anpassen. Erst neue Isotopenmethoden haben es uns ermöglicht, die Element-Partitionierung und die Verweilzeit von Elementen in der Biomasse zu erforschen.

Um Stoffkreisläufe in Grasländern zu verstehen, haben wir sechs Experimente auf drei Kontinenten erforscht. Sieben bis neun Jahre der N-, P- und NP-Zugabe ermöglichten es uns die Auswirkungen veränderter Elementverfügbarkeiten auf mikrobielle Element-Partitionierung, die Verweilzeit von Elementen in der Biomasse und die Nährstoffakquise zu studieren. Um die C-Nutzungseffizienz und die Element-Verweilzeiten in der mikrobiellen Biomasse zu analysieren, haben wir neuartige Isotopenmethoden basierend auf ^{18}O- und ^{33}P-Markierung der mikrobiellen DNA verwendet. Wir haben C und N in der mikrobiellen Biomasse mittels der Chloroform-Fumigation, netto C- und N-Mineralisierung in Inkubationsexperimenten, extrazelluläre Enzymaktivitäten mit fluoreszierenden Substraten und nicht-symbiotische N_2-Fixierung in einer ^{15}N-markierten Atmosphäre bestimmt. Außerdem haben wir physiologische Assays benutzt, um P-Solubilisierende-Bakterien (PSB) zu finden und die PSB- und mikrobielle Gemeinschaft wurde via Sanger und Illumina Sequenzierung erfasst.

Nach Jahren der Nährstoffzugabe haben sich die Verhältnisse der verfügbaren Elemente im Boden stark verändert, wohingegen die Elementverhältnisse in der mikrobiellen

Biomasse gleichblieben, welches das Konzept der stöchiometrischen Homöostase von Mikroorganismen bestätigt. Die mikrobielle C-Partitionierung, das Verhältnis von dem für Wachstum bereit-gestelltem C und dem aufgenommenen C, auch C-Nutzungseffizienz, war mit der Substrat-Stöchiometrie korreliert. Mikroorganismen haben weniger respiriert, wenn die Substrat-Stöchio-metrie näher an ihrer eigenen Biomasse-Stöchiometrie lag, wohingegen sie mehr in Nährstoff-akquise investierten und damit mehr atmeten, wenn sie auf Substrat mit unvorteilhafterer Stöchiometrie wuchsen. Neben der Element-Partitionierung haben Mikroorganismen die Verweilzeit von Elementen in ihrer Biomasse angepasst. Zum ersten Mal haben wir gezeigt, dass mit abnehmender P-Verfügbarkeit die mittlere Verweilzeit von P in der mikrobiellen Biomasse zunahm, vermutlich, weil die Mikroorganismen P effizienter intern wiederverwendet haben. Neben der C-Partitionierung und den Element-Verweilzeiten, haben die Mikroorganismen Prozesse der Nährstoffakquise an ihre stöchiometrischen Bedürfnisse angepasst. Die nicht-symbiotische N_2-Fixierung war mit den Boden-N:P-Verhältnissen korreliert, was zeigt, dass die energieaufwändige N_2-Fixierung auf genügend P für die ATP-Produktion und gleichzeitig auf geringe N-Verfügbarkeit angewiesen war. Die mikrobielle N-Freisetzung, die N-Mineralisierung, war stark abhängig von der Substrat-Stöchiometrie, insofern, dass Mikroorganismen mehr N freisetzten, wenn die N- im Vergleich zur C-Verfügbarkeit hoch war. Die Aktivität der Leucin-Aminopeptidase, ein N-akquirierendes Enzym, war jedoch nicht mit der Substrat-Stöchiometrie verknüpft. Weiterhin war die relative Abundanz der PSB mit den C:P-Verhältnissen im Boden verknüpft, was andeutet, dass die Produktion von organischen Säuren, die zur P-Solubilisierung beitragen, auf C-Quellen angewiesen ist. Organischer P kann durch das extrazelluläre Enzym Phosphatase mineralisiert werden, und die Phosphatase-Aktivität nahm mit zunehmender N-Verfügbarkeit zu. Dies deutet an, dass (a) der mikrobielle P-Bedarf zunahm, um die Biomasse-Stöchiometrie auszugleichen und, dass (b) mehr N die Produktion von N-reichen Enzymen ermöglichte. Im Gegensatz zur mikrobiellen Artenzusammensetzung, waren Elementverfügbarkeiten die wesentlichen Triebkräfte der Stoffumsetzungsprozesse in einem Grasland in den USA.

Zusammengefasst hat sich das Konzept der Ökologischen Stöchiometrie als vielversprechendes Werkzeug erwiesen, welches diverse Stoffumsetzungsprozesse in Grasländern erklären und vorhersagen kann. Jedoch muss beachtet werden, dass neben den Elementverhältnissen klimatische Bedingungen, Boden-pH und Bodentextur diese Prozesse beeinflussten. Außerdem wurden nicht alle Prozesse, wie die Leucin-Aminopeptidase-Aktivität, von mikrobieller Homöostase angetrieben. Nichtsdestotrotz können basierend auf der stöchiometrischen Homöostase von Bodenmikroorganismen viele Stoffumsetzungsprozesse verstanden und besser vorhergesagt werden. Deshalb sollte das Konzept der Ökologischen Stöchiometrie als ein Kernkonzept der terrestrischen Ökologie betrachtet werden.

Table of Contents

1 Synopsis

1.1 Introduction

1.1.1 Atmospheric nutrient deposition and grasslands

The input of nutrients to ecosystems has been greatly increased through anthropogenic activities (Galloway et al., 2004; Schlesinger, 2009; Wang et al., 2015). Since 1970, the emission of reactive nitrogen (N) has more than doubled (Galloway et al., 2008; Gruber and Galloway, 2008). Around 200 Tg N per year enter the biosphere through burning of fossil fuels, fertilizer inputs, and biological fixation of atmospheric N by cultivated legumes and rice (Peñuelas et al., 2012), whereas annual phosphorus (P) inputs by industrial fertilizers are currently estimated at 17 Tg P. Thus, the increases in N exceed the increases in P in most regions of the world, changing soils N:P stoichiometry (Peñuelas et al., 2013). The N emissions result in atmospheric N deposition > 1 g N m^{-2} yr^{-1} in large regions of the world (Galloway et al., 2004) and it has been predicted that in 2050 some regions may receive up to 5 g N m^{-2} yr^{-1} (Galloway et al., 2004). Currently, 50–80% of terrestrial N is deposited in natural and semi-natural ecosystems (Dentener et al., 2006) such as grasslands.

Grasslands are among the largest ecosystems of the world and their area is estimated at 40.5% of the terrestrial surface excluding Greenland and Antarctica (Suttie et al., 2005). Grasslands are critically endangered biomes because of extensive habitat loss (Hoekstra et al., 2005), they harbor large parts of the terrestrial biodiversity (White et al., 2000), and account for about 30% of the world's terrestrial net primary production (Fay et al., 2015). Further, they play an important role in mitigating climate change because they store large amounts of carbon (C) and can also sequester additional C (O'Mara, 2012). Global grassland soil C stocks are estimated at approximately 343 Gt C, about double the amount stored in forests (Conant, 2010).

The effects of increasing N and P inputs on plant growth (Fay et al., 2015; Stevens et al., 2015), diversity (Clark and Tilman, 2008; Harpole et al., 2016), and community composition (Cleland and Harpole, 2010) in grassland ecosystems are well studied. However, the consequences of increased nutrient inputs on soil element cycling driven by microorganisms and on microbial communities are less well understood. Understanding changes in soil element cycling under nutrient inputs is pivotal for Earth System Model projections of future climate scenarios (Crowther et al., 2019), especially considering the high grassland C stocks.

1.1.2 Stoichiometric homeostasis of soil microorganisms

Microorganisms largely drive the cycling of C, N, and P in soils (Vitousek et al., 2010), hence they are assumed to govern ecosystem responses to the globally increased N and P availabilities (Galloway et al., 2008; Wang et al., 2015; Peñuelas et al., 2013). One dominant factor shaping microbial C, N, and P cycling is the stoichiometric relationship between microorganisms and their environment (Hall et al., 2011; Zechmeister-Boltenstern

et al., 2015; Spohn, 2016).

In 1958, Alfred Redfield found well-constrained C:N:P ratios in marine planktonic biomass around the world (Redfield, 1958). This homeostatic element ratio in phytoplankton was called "Redfield ratio" thereafter. A new theory called "Ecological Stoichiometry" has evolved thereof trying to understand the stoichiometric relation between organisms and their environment (Sterner and Elser, 2002). It uses elemental ratios to explain ecological processes by taking chemical constraints on metabolic and physiologic functions of organisms into account (Mooshammer et al., 2014). The theory of Ecological Stoichiometry has been increasingly used in terrestrial ecosystems. It is also of special interest there, because microorganisms recycle C and N mainly as CO_2 and ammonium that in the end contribute to global warming and plant nutrition, respectively (Mooshammer et al., 2014).

Even though element concentrations of individual phylogenetic strains may vary (Mouginot et al., 2014), on average well-constrained element ratios have been found for soil microorganisms (Cleveland and Liptzin, 2007), showing that they maintain stoichiometric homeostasis. Stoichiometric homeostasis means that independent of their surroundings, organisms keep a relatively constant biomass C:N:P ratio. In global meta-analyses, microbial biomass C:N:P ratios of 60:7:1 (Cleveland and Liptzin, 2007) and 42:6:1 (Xu et al., 2013) have been found. In contrast, the resources the microbial biomass feeds on, can be highly variable in their stoichiometry and have much larger element ratios. For instance, wood can have average C:N:P ratios of 14,103:40:1 (Weedon et al., 2009), leaf litter of 3,055:43:1 (Yuan and Chen, 2009), and soil of 186:13:1 (Cleveland and Liptzin, 2007).

According to the theory of consumer-driven nutrient recycling, homeostatic organisms are expected to regulate their element stoichiometry by releasing excess elements and retaining limited elements (Sterner and Elser, 2002; Zechmeister-Boltenstern et al., 2015). More detailed, depending on microbial element demand and supply, microorganisms are thought to maintain their biomass stoichiometry by (i) element acquisition (mobilization of missing elements), by (ii) adjusting partitioning and turnover times of elements in their biomass, and by (iii) element release (production of CO_2, inorganic N, and P, Figure 1) (Spohn, 2016). Consequently, stoichiometric relationships play a pivotal role in shaping processes such as decomposition of organic material, non-symbiotic N_2 fixation, and P solubilization (Figure 1). Understanding controls and constraints on microbial biomass stoichiometry in more detail, can help to predict when the microbial biomass acts as sink or source of C and mineral nutrients in a wide range of ecosystems (Bardgett et al., 2008; Hall et al., 2011).

1.1.3 Microbial element partitioning and turnover time

As a measure to cope with stoichiometric imbalances between substrate and microbial biomass, microorganisms can adjust element partitioning and turnover times (Spohn,

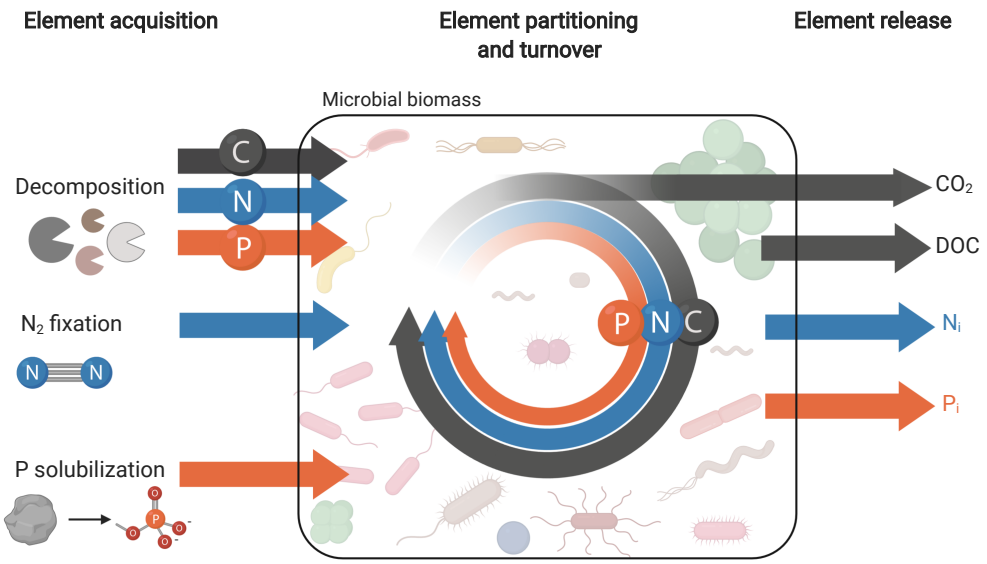


Figure 1: Processes of element acquisition, partitioning and turnover, and release driven by soil microorganisms (adapted from Spohn, 2016, created with BioRender.com).

2016). Elements taken up into the microbial biomass are partitioned between growth and release. The ratio of an element invested into growth over total element uptake is called element use efficiency.

1.1.3.1 C partitioning

Soil microbial carbon use efficiency (CUE) is defined as ratio between C allocated to growth and C taken up by microorganisms (del Giorgio and Cole, 1998). Even though soil microbial CUE shapes soil C cycling, it is poorly understood how increasing N and P availabilities affect it. It is assumed that if the stoichiometry of the substrate microorganisms feed on is much larger than the stoichiometry of the microbial biomass, CUE decreases because microorganisms need to invest more C and energy into nutrient acquisition (Manzoni et al., 2012; Sinsabaugh et al., 2013). The stoichiometry of the substrate on which soil microorganisms feed is reflected by the C:N ratio of dissolved organic matter (DOC:DN ratio). In accordance with this concept, soil microbial CUE decreased with rising DOC:DN ratios in natural soils (Sinsabaugh et al., 2013; Sinsabaugh et al., 2016; Manzoni et al., 2017). However, other studies could not confirm this relation in fertilized soils (Manzoni et al., 2012) and in rice straw (Devêvre and Horwáth, 2000).

1.1.3.2 Element turnover time in the microbial biomass

Further, microorganisms can maintain their biomass stoichiometry by adjusting the turnover time or mean residence time of individual elements in the microbial biomass. The turnover time can indicate how long an element remains in the microbial biomass (Spohn, 2016). With decreasing availability of an element, it is assumed that the mean

residence time of this element in the microbial biomass increases because microorganisms recycle this element more efficiently internally (Spohn, 2016). This adaption might allow microorganisms to thrive on nutrient-poor substrate whose stoichiometry is much larger than that of the microbial biomass.

1.1.4 Microbial element acquisition

Microorganisms can also adjust processes of element acquisition to maintain their biomass stoichiometry (Sinsabaugh et al., 2008; Spohn, 2016). They acquire C, N, and P for example through the decomposition of organic matter. This process largely depends on extracellular enzymes exuded by microorganisms and plants that, for instance, catalyze the hydrolysis of organic molecules. By adjusting the production of extracellular enzymes, microorganisms can try to acquire missing elements (Sinsabaugh et al., 2008). Further, to acquire N and P, microorganisms are expected to adjust N_2-fixation rates and the solubilization of bound inorganic P.

1.1.4.1 N acquisition and release

It has been shown that microorganisms invest more into N acquisition and immobilize N in their biomass under low N availability (Saiya-Cork et al., 2002; Nemergut et al., 2008; Schleuss et al., 2019), whereas they increase N release under high N availability (Mooshammer et al., 2014; Spohn, 2016) and thereby affect plant-available nutrients (Manzoni et al., 2008). For instance, non-symbiotic N_2 fixation likely depends on N and P availability since it is energetically more favorable to take up soil mineral N than to fix atmospheric N (Reed et al., 2011) because non-symbiotic N_2 fixation is one of the metabolically most costly processes on earth (Smith, 1992). Besides the presence of organic C as an energy source, sufficient P needs to be available to synthesize ATP supporting N_2 fixation (Reed et al., 2011). Similarly, the production of leucine-aminopeptidase, an N-acquiring enzyme that catalyzes the depolymerization of peptides, is thought to decrease with increasing N availabilities (Ramirez et al., 2012; Schleuss et al., 2019).

Further, whether net release of inorganic N through net N mineralization or immobilization of N in the microbial biomass prevails, might depend on the DOC:DN ratio. With increasing DOC:DN ratios, microorganisms likely retain N in their biomass, resulting in N immobilization. In contrast, with decreasing DOC:DN ratio, microorganisms likely release excess N and net N mineralization increases (Manzoni et al., 2008; Heuck and Spohn, 2016; Schleuss et al., 2019). Thus, different N cycling processes such as non-symbiotic N_2 fixation, the production of leucine-aminopeptidase, and net N mineralization might be affected by changing soil element availabilities.

1.1.4.2 P acquisition

Microorganisms can acquire missing P by solubilizing bound inorganic P or by recycling organic P through the exudation of enzymes. Soil P solubilization is defined as mobiliza-

tion of bound inorganic P from soil minerals by plants and microorganisms (Hinsinger, 2001) and it can be stimulated through the release of protons, organic acids, exopolysaccharides, and siderophores (Jones and Oburger, 2011). Since the production of organic acids and exopolysaccharides that solubilize P needs organic compounds as a C source and N for transcription and translation (Spohn, 2016), P solubilization is thought to underlie stoichiometric constraints. However, stoichiometric theory has never been applied to P solubilization.

Further, microorganisms and plants can mineralize organic P through the exudation of phosphatases, extracellular enzymes. They catalyze the hydrolysis of organic P to plant available phosphate. Several factors influence the production of phosphatases, such as soil organic matter content, plant and microbial nutrient demand, soil pH, and N availability (Olander and Vitousek, 2000; Sinsabaugh et al., 2008). Since microorganisms need N and organic C to produce N-rich phosphatases, their production is thought to underlie stoichiometric constraints too. Yet, the effect of N and P availability on phosphatase activity, and especially on the abundance and community of P-solubilizing bacteria (PSB) has rarely been investigated (Mander et al., 2012; Lepleux et al., 2013; Nicolitch et al., 2016).

1.1.5 Microbial communities and element cycling

Nutrient inputs not only affect soil element cycling but also microbial community composition and microbial community changes might again influence element cycling processes. For instance, N addition altered microbial diversity (Allison et al., 2007; Ramirez et al., 2010) and changed microbial community composition in grassland soils (Ramirez et al., 2010; Ramirez et al., 2012; Fierer et al., 2012; Leff et al., 2015). These changes in microbial community structure can be caused by different factors such as altered nutrient availabilities, soil pH, and 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). Yet, the dominant factors of microbial community change under nutrient addition remain uncertain.

Altered microbial community composition has shown to affect soil element cycling (Philippot et al., 2013; Delgado-Baquerizo et al., 2016; Strickland et al., 2009), whereas other studies found unaffected biogeochemical processes indicating functional redundancy of the microbial community (Louca et al., 2018; Nannipieri et al., 2003). Functional redundancy means that a microbial taxon can carry out the same process as another and thus loss of species may not impact ecosystem functioning (Allison and Martiny, 2008; Philippot et al., 2013; Louca et al., 2018). Understanding how nutrient inputs affect microbial communities and if and how changes in microbial communities are linked to element cycling, is pivotal for the prediction of global C, N, and P cycles and ecosystem response to nutrient addition.

1.2 Objectives and Hypotheses

The aim of this work was to understand the consequences of changing element inputs on processes of microbial element partitioning, turnover, and acquisition, and thus to take a step forward in understanding soil element cycling in grasslands on a global scale. Further, we wanted to understand if the property of the microbial biomass to maintain stoichiometric homeostasis controls the mentioned processes. All in all, our objective was to test to what extent the concept of Ecological Stoichiometry can be used as a framework to predict element cycling in terrestrial ecosystems. For this purpose, we studied six nutrient addition experiments on three continents.

The topic was divided into five studies: We studied the effect of nutrient addition on microbial C partitioning in study I, on P turnover time in study II, on N acquisition and release in study III, and on P acquisition in study IV (Figure 2). In study V, we analyzed drivers of microbial community composition changes and element cycling under nutrient addition at one site in the USA.

Our general hypothesis was that N, P, and NP additions alter nutrient availabilities and ratios of available elements but do not change the C:N ratio of the microbial biomass based on the assumption that microorganisms maintain stoichiometric homeostasis despite changes in substrate stoichiometry.

Study I –In the first study we investigated the effect of N and P additions on soil microbial C partitioning. Our main hypothesis was that soil microbial CUE increases with decreasing availability of organic C relative to N.

Study II – In the second study, the turnover time of P in the microbial biomass depending on P availability was studied. Our main hypothesis was that the mean residence time of P in the microbial biomass increases with decreasing P availability.

Study III – In the third study, N cycling processes under N and P additions were studied. Our main hypotheses were that (i) non-symbiotic N_2 fixation rates increase with decreasing availability of N relative to P. Further, we assumed that (ii) net N mineralization increases with decreasing availability of organic C relative to N and that (iii) leucine-aminopeptidase activity increases with decreasing N availability.

Study IV – The fourth study dealt with the question of how N and P additions affect indicators of microbial P acquisition processes. Our main hypotheses were that (i) the abundance of PSB increases with decreasing availability of P relative to N and that (ii) phosphatase activity increases with increasing N availability.

Study V – In the fifth study we analyzed changes in the microbial community composition under N and P additions, the drivers of these changes, and the connection to

element cycling rates at one site in the USA. Our main hypotheses were that (i) soil pH and plant community are the dominant drivers of microbial community change under nutrient addition and that (ii) changes in the microbial community determine element cycling processes (net N mineralization, non-symbiotic N_2 fixation, microbial respiration).

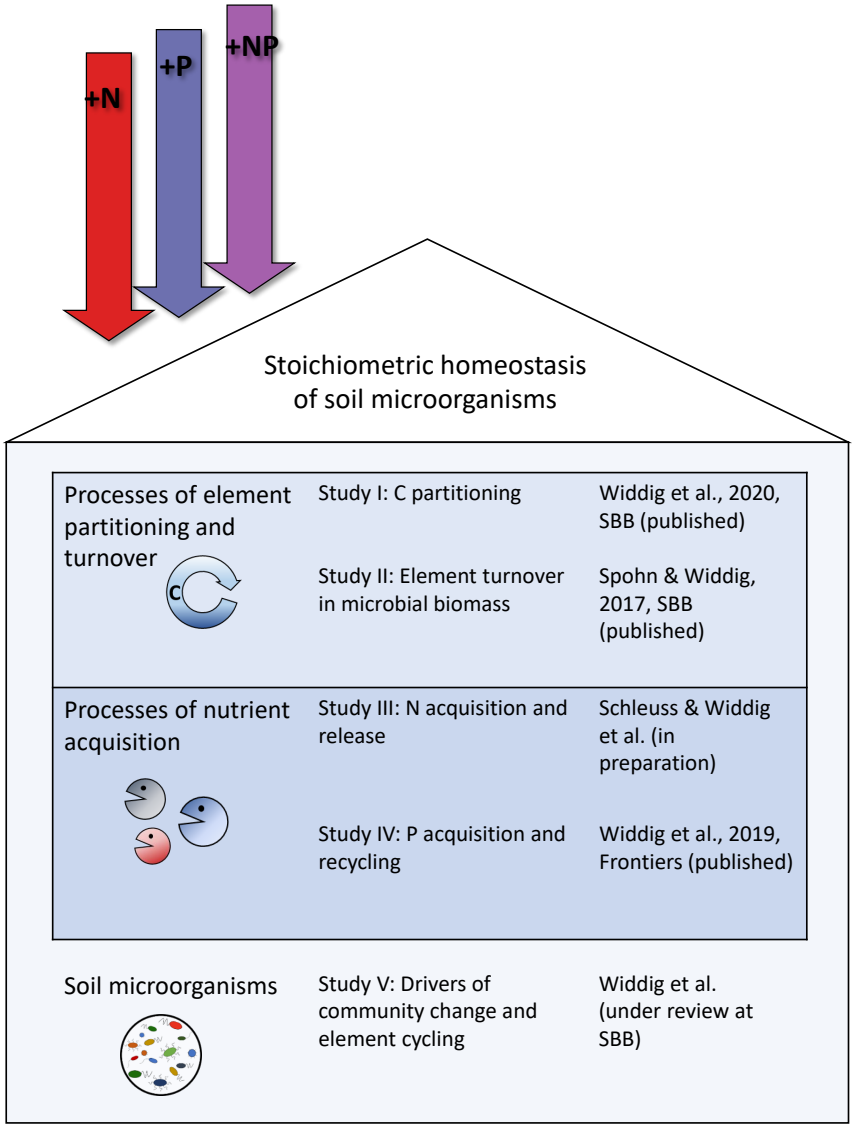


Figure 2: Conceptual outline and structure of this PhD thesis, included studies, and their publication status. Blue-shaded boxes show studies on processes of element partitioning and turnover, on nutrient acquisition, and on the soil microbial community as affected by nutrient addition to grassland soils.

1.3 Material and Methods

1.3.1 Study sites

We sampled soil at six grassland sites (Figure 3, Table 1) belonging to a globally-coordinated research network (Nutrient Network, Borer et al., 2014). Two sites, Cedar Creek and Chichaqua Bottoms, are located in the Central Plains, USA, and are both vegetated by tallgrass prairie. The two sites Rookery and Heron's Brook are located in Silwood Park, UK, and are mesic grasslands. The other two sites, Ukulinga and Summerveld, are located in KwaZulu-Natal, South Africa, and are mesic grasslands as well (Figure 3). The sites were chosen because they represent some of the major types of grasslands that exist globally and span large biogeographical gradients (Table 1). For instance, mean annual temperature ranges from 6 °C to 18 °C, topsoil pH values range from 3.8 to 5.9 and soil texture is diverse, ranging from sand to silty clay (Table 1). The sites of the Nutrient Network follow an identical experimental set-up and standardized full-factorial nutrient addition treatments (Figure 3). Further details on the experimental set-up and methods can be found in Borer et al. (2014).

To understand interactions between N and P supply, we analyzed independent and interactive effects of N and P additions. We sampled plots (5 x 5 m) with and without N and P additions (control (Ctrl), N, P, and NP), which were replicated across three blocks (randomized block design) at each site (Figure 3). Nutrients had been added annually at the beginning of the growing season for at least seven years as 10 g m^{-2} yr^{-1} slow-release urea ($(NH_2)_2CO$) and 10 g m^{-2} yr^{-1} triple-super phosphate ($Ca(H_2PO_4)_2$).

These amounts of nutrients were added because, first, nutrient addition needed to be higher than actual atmospheric deposition to enable identification of differences between control plots and nutrient addition treatments. Second, isolating the effect of nutrient addition on grassland ecosystem functioning can only be achieved when nutrient addition treatments are high enough to level out heterogeneity in soil properties immanent in field experiments across ecosystems (Crowther et al., 2019).

For study II, soil samples from the Botanical Garden of the University of Bayreuth were taken. The soil was a Cambisol with a sandy loam texture. The site hosted a semi-natural grassland community dominated by tall grasses. Further details on sampling, sample preparation, experimental design, and analyses are described in study II.

Study V involved the soils from Cedar Creek. In addition to the sampled nutrient addition treatments (as described above), we sampled two more levels of N addition at the site Cedar Creek. In total, four different levels of N addition (0, 1, 5, and 10 g N m^{-2} yr^{-1}), a P (10 g P m^{-2} yr^{-1}) and a combined NP (10 g N and P m^{-2} yr^{-1}) addition treatment were sampled (Ctrl, N1, N5, N10, N10P, and P).

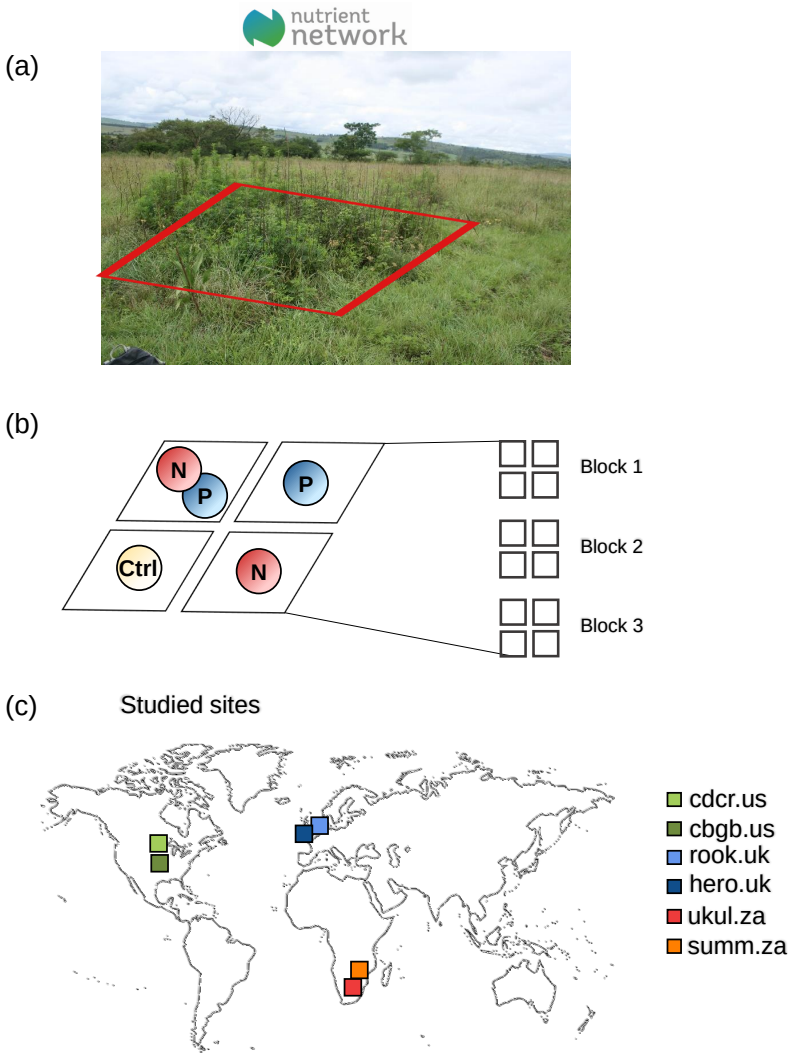


Figure 3: The experimental design of the Nutrient Network (NutNet) and the studied sites. Panel (a) shows an example of a NutNet plot in Ukulinga, panel (b) shows the experimental design of the nutrient addition treatments and blocks (adapted from Borer et al., 2014). Panel (c) shows the studied sites Cedar Creek (cdcr.us) and Chichaqua Bottoms (cbgb.us) in the USA, Rookery (rook.uk) and Heron's Brook (hero.uk) in the UK, and Ukulinga (ukul.za) and Summerveld (summ.za) in South Africa.

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. See also study I and III.

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[1,2]	*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[2](%)	94	74	45	104	116	120
Forb cover[2](%)	21	42	61	26	44	56
Legume cover[2](%)	1	6	0	19	12	10
ANPP[2](g m^{-2} yr^{-1})	178	397	180	509	498	349

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

1.3.2 Sampling and sample preparation

At all sites, samples were collected at the end of the vegetation period in 2017 (February in South Africa, September in the USA, and October in the UK). Soil samples were collected from two depth increments from 0-15 (termed "topsoil") and from 15-30 cm depth (termed "subsoil"), both depth increments were located in the A horizon of each soil. In Summerveld, only the topsoil was sampled because of limited soil depth. One mixed sample consisting of six individual samples from each plot and soil depth was collected using a 3.5 cm diameter soil corer. Undisturbed soil cores (100 cm^3) were taken adjacent to the experiment for bulk density determination. Samples were shipped to the University of Bayreuth within one week after sampling. Soil samples were sieved (< 2 mm) and stones and roots were removed.

1.3.3 Summary of main methods

1.3.3.1 Soil physical and chemical analyses

Soil pH was measured in deionized water and in 1 M KCl in a soil:solution ratio of 1:2.5 using air-dried soil. Soil samples were dried at 60 °C and milled to determine total organic C (TOC), total N (TN), and total P (TP). 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 filtered through 0.45 µm filters, before dissolved element concentrations were quantified. Total organic phosphorus (TOP) was determined by the ignition method according to Saunders and Williams (1955) modified by Walker and Adams (1958). Phosphorus fractions were measured according to the Hedley fractionation (Hedley et al., 1982) modified by Tiessen and Moir (2007). Details on TOP determination and Hedley fractionation can be found in study IV.

1.3.3.2 Soil microbial biomass carbon and nitrogen

Soil 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. 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. Microbial biomass C and N were calculated by subtracting the concentration of the fumigated sample from the non-fumigated sample and multiplying the difference with a conversion factor of 2.22 for microbial biomass C (Jenkinson et al., 2004; Wu et al., 1990) and 1.85 for microbial biomass N (Brookes et al., 1985; Joergensen and Mueller, 1996).

1.3.3.3 Enzyme activities

Phosphatase, β-1,4-glucosidase, β-1,4-N-acetyl-glucosaminidase, and L-leucine aminopeptidase activities were determined using fluorogenic substrates 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 for 20 min. Four replicates of the soil homogenates were pipetted into black polystyrene 96-well microplates and substrate solution was added to each sample well. The microplates were covered, 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. 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.

1.3.3.4 Soil microbial carbon use efficiency

Microbial CUE was determined based on the incorporation of ^{18}O from ^{18}O-labeled water into microbial DNA (Spohn et al., 2016). Soil samples were split into two aliquots, one aliquot received ^{18}O labeled water (97 at%) and one non-labeled Millipore water. Both samples were incubated for 24 h at 15 °C. Subsequently, samples were frozen until DNA extraction was done. DNA concentration was measured with the picogreen assay (Sandaa et al., 1998). 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. 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., 2016) and on a correlation between microbial DNA and microbial biomass C concentrations. Finally, CUE was computed based on growth and respiration rate (Manzoni et al., 2012; Sinsabaugh et al., 2013). Further details on CUE measurements and calculations can be found in study I.

1.3.3.5 Soil 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 of accumulated C-CO_2 over time (Heuck and Spohn, 2016).

1.3.3.6 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 in N-NH_4^+ and N-NO_3^- over time.

1.3.3.7 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 was filled into 12 ml exetainers. All exetainers were closed, flushed with argon, carefully evacuated, finally filled with 7.2 ml $^{15}N_2$ (99.8 at% $^{15}N_2$, Sigma Aldrich) and 0.8 ml O_2, and incubated in the dark at 15 °C for 72 h in the ^{15}N enriched artificial atmosphere. Samples exposed to ^{15}N-N_2 as well as samples not exposed to ^{15}N-N_2 (natural abundance) were dried at 50 °C, milled and analyzed for ^{15}N. The ^{15}N at% was calculated using the isotope ratio of each sample and the $^{15}N_2$ fixation was calculated using an isotope mixing model (Zechmeister-Boltenstern, 1996). Further details on determination of N_2 fixation can be found in study III and V.

1.3.3.8 Relative abundance of P-solubilizing bacteria

For the determination of PSB, 0.5 g of soil from the topsoil were mixed with 49.5 ml of sterile water and shaken for 45 min. An aliquot of the soil suspension was added to an agar plate containing Pikovskaya medium (Pikovskaya, 1948). Plates were incubated for seven days at 20 °C before colonies were counted. At least 100 colony-forming units (CFU) per soil sample were screened. If a CFU can solubilize P from the hydroxyapatite in the medium, a clear zone around the CFU becomes visible. Details on how we determined the abundance of PSB can be found in study IV.

1.3.3.9 Sequencing of P-solubilizing bacteria and processing of sequence data

Colonies of PSB were selected for sequencing and picked using sterile toothpicks. Genomic DNA of the bacterial colonies was extracted, and 16S fragments covering variable regions (V5 to V8) were amplified using the primers 799F (Chelius and Triplett, 2001) and 1391R (Walker and Pace, 2007). Sequence data were analyzed in the Geneious sequence analysis software. The list of the 20 best matching sequence hits for each isolate was uploaded into PRIMER 7 (Clarke and Gorley, 2015), standardized, cumulated, and clustered. Isolates, which had a consensus of more than 99% in the cluster analysis, were considered as identical isolates. Then, a consensus name was assigned for each isolate cluster according to Peršoh et al. (2010) called operational taxonomic unit (OTU) in the following. More details on PSB sequencing and sequence data processing can be found in study IV.

1.3.3.10 Illumina sequencing of prokaryotic 16S rRNA gene and fungal ITS2 region

The DNeasy PowerSoil Kit 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, modified with heterogeneity spacers according to Cruaud et al. (2017) and sequenced using the Nextera XT kit on an Illumina MiSeq with 2 x 300 bp. Sequences were taxo-

nomically 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). More details on Illumina sequencing and sequence processing can be found in study V.

1.3.4 Statistics

In all studies, the threshold of significance was set at $p < 0.05$. To test differences between nutrient addition treatments and soil depths at each site, ANOVA followed by a Tukey post-hoc test for multiple comparisons was used. Previously, data was 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.

Linear mixed-effects models were used in study I, III, and IV to test the effect of nutrient addition across all sites. This approach was chosen because it allows to show treatment effects across a set of sites spanning large biogeographical gradients controlling for between-site variation. Nutrient addition treatment was set as fixed factor and random intercepts were included for sites to adjust for variability among them.

In study I, III, and IV, multi-model selection according to Grueber et al. (2011) was performed to assess the relative importance of climatic, soil physical, chemical, and microbial variables on element cycling processes. A global model was fitted with random intercepts for treatments at each site to adjust for variations caused by them. A full submodel set was generated and out of all possible models, the best ones were selected using the AICc. Model variables having the highest relative importance (> 0.90) were selected to fit a linear mixed-effects model, for which a conditional R^2 according to Nakagawa and Schielzeth (2013) and a p-value obtained by likelihood ratio test was calculated.

For prokaryotic, fungal, and plant community composition analyses, non-metric multidimen-sional scaling was applied as described in further detail in study V. One-way-ANOSIM with 999 permutations was used to test for significant effects of either nutrient addition or site on bacterial, fungal, and plant community composition.

Further, multiple backward regression analyses were applied in study V for the identification of main controls on element cycling processes. The initial linear model contained different abiotic and biotic variables. Variance inflation factors were used to check for multicollinearity and highly collinear variables were dropped. All statistical analyses were done using R version 3.3.1 (R Core Team, 2018).

1.4 Results and Discussion of the Main Findings

1.4.1 Altered element availabilities under nutrient addition

We measured soil pH and total as well as dissolved element concentrations after seven to nine years of N, P, and NP additions at six grassland sites (see also study I, III, and IV). Soil pH was only decreased by N and NP additions at Cedar Creek, whereas N, P, and NP additions did not change soil pH at the other sites (Table 2). Despite high amounts of N and P added, TOC and TN concentrations did not change with nutrient addition in most soils (Table 2). In contrast, TP concentrations increased with single P and combined NP additions at half of the sites (Table 2).

The concentrations of dissolved soil elements responded more strongly to nutrient addition than total element concentrations, especially in the topsoil (Table 3). Addition of N, P, and NP significantly increased topsoil DOC concentrations at some sites (Table 3) and calculated across all sites, NP addition increased topsoil DOC concentrations by +17%. Further, N and NP additions raised DN concentrations across all soils by +164% and +106%, respectively, compared to the soils that did not receive N (Table 3). Changes in DOC and DN concentrations led to decreased DOC:DN ratio in most topsoils, with the exception of Heron's Brook (Table 3). On average, the DOC:DN ratio was by -64% and -57% lower under N and NP additions, respectively, compared to control. At all six sites, P and NP additions significantly increased topsoil DIP concentrations by +3,307% and +3,508%, respectively, compared to control (Table 3).

As expected, bioavailable N and P concentrations were increased by N and P additions, respectively. Our finding that DOC concentrations increased significantly under nutrient addition (at some sites) is likely related to higher plant biomass and thus higher above- and belowground C inputs to soil (LeBauer and Treseder, 2008; Xia and Wan, 2008; Lu et al., 2011). A meta-analysis has shown that C inputs from aboveground, belowground, and litter were significantly increased with N addition, resulting in increased DOC concentrations (Lu et al., 2011). Especially the strong increases in DN concentrations decreased DOC:DN ratios changing the stoichiometry of the substrate microorganisms thrive on.

1.4.2 Stoichiometric homeostasis of soil microorganisms

To test the concept of stoichiometric homeostasis, we measured microbial biomass C:N ratios after seven to nine years of nutrient addition (see also study I, III, and IV). Nutrient addition did not influence microbial biomass C concentrations at any of the sites (exception: subsoil in Heron's Brook, Table 3). Mean topsoil microbial biomass C concentrations in the control plots varied widely across the sites with 163 mg kg soil^{-1} in Cedar Creek and up to 1005 mg kg soil^{-1} in Ukulinga (Table 3). Despite large changes in element availabilities due to nutrient addition, seven to nine years of nutrient addition did not alter microbial biomass C:N ratios across all sites (Figure 4).

Table 2: Total organic carbon (TOC), total nitrogen (TN), total phosphorus (TP), and soil pH in 0-15 and 15-30 cm soil depth of 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 tested separately for each site and depth increment. Asterisks indicate significant differences between depth increments tested individually for each site and treatment. See also study I, III, and IV.

	Site	0-15 cm				15-30 cm			
		Ctrl	+N	+P	+NP	Ctrl	+N	+P	+NP
TOC (g C kg^{-1})	cdcr.us[1]	9.4 ± 1.1a	15.7 ± 8.8a	9.0 ± 0.3a	11.0 ± 3.4a	5.2 ± 1.1a	10.4 ± 7.8a	4.4 ± 0.5a*	5.8 ± 2.1a
	cbgb.us	7.2 ± 2.8a	8.2 ± 0.5a	6.9 ± 2.3a	7.4 ± 2.4a	4.1 ± 0.5a*	5.1 ± 0.2a*	4.0 ± 1.2a	4.1 ± 0.9a*
	hero.uk	36.7 ± 6.8a	36.7 ± 6.1a	36.5 ± 1.8a	37.0 ± 7.7a	24.4 ± 3.5a*	24.5 ± 4.2a*	25.6 ± 4.7a*	23.9 ± 4.4a*
	rook.uk	24.3 ± 2.6a	28.7 ± 3.2a	26.9 ± 1.2a	24.9 ± 2.2a	10.5 ± 1.5a*	12.8 ± 1.3a*	11.6 ± 1.3a*	10.1 ± 0.7a*
	ukul.za	42.0 ± 2.0a	42.5 ± 0.8a	44.4 ± 0.5a	45.7 ± 0.8a	37.5 ± 3.6a	3.2.0 ± 4.4a*	34.8 ± 5.3a*	36.4 ± 0.6a*
	summ.za[2]	49.1 ± 3.0a	51.1 ± 2.2a	51.7 ± 3.0a	51.7 ± 1.9a	NA	NA	NA	NA
TN (g N kg^{-1})	cdcr.us[1]	0.7 ± 0.1a	1.1 ± 0.6a	0.6 ± 0.1a	0.8 ± 0.3a	0.3 ± 0.1a*	0.7 ± 0.5a	0.3 ± 0.1a	0.4 ± 0.1a*
	cbgb.us	0.6 ± 0.2a	0.8 ± 0.1a	0.6 ± 0.2a	0.7 ± 0.2a	0.4 ± 0.1a*	0.5 ± 0.1a*	0.4 ± 0.1a*	0.4 ± 0.2a*
	hero.uk	3.1 ± 0.7a	3.1 ± 0.5a	3.0 ± 0.2a	3.1 ± 0.8a	2.1 ± 0.3a*	2.1 ± 0.3a	2.1 ± 0.4a	2.0 ± 0.4a*
	rook.uk	2.1 ± 0.2ab	2.4 ± 0.3b	2.2 ± 0.1ab	2.0 ± 0.1a	1.0 ± 0.1a*	1.2 ± 0.1a**	1.0 ± 0.2a*	0.9 ± 0.1a*
	ukul.za	2.9 ± 0.3a	3.1 ± 0.3ab	3.3 ± 0.1ab	3.4 ± 0.1b	2.6 ± 0.2a	2.4 ± 0.2a*	2.6 ± 0.3a*	2.7 ± 0.1a*
	summ.za[2]	2.8 ± 0.2a	3.0 ± 0.4a	2.9 ± 0.3a	3.0 ± 0.1a	NA	NA	NA	NA
TP (g P kg^{-1})	cdcr.us[1]	0.31 ± 0.03a	0.46 ± 0.24a	0.58 ± 0.09a	0.56 ± 0.11a	0.24 ± 0.03a	0.40 ± 0.19a	0.36 ± 0.04a	0.38 ± 0.06a
	cbgb.us	0.28 ± 0.02a	0.24 ± 0.05a	0.43 ± 0.04b	0.41 ± 0.06b	0.24 ± 0.03a	0.24 ± 0.02a	0.34 ± 0.03b*	0.35 ± 0.07b*
	hero.uk	0.62 ± 0.16a	0.57 ± 0.07a	0.93 ± 0.13a	0.96 ± 0.28a	0.54 ± 0.15a	0.48 ± 0.06a	0.55 ± 0.17a*	0.62 ± 0.25a*
	rook.uk	0.38 ± 0.01a	0.38 ± 0.04a	0.60 ± 0.06b	0.61 ± 0.14b	0.27 ± 0.01a*	0.31 ± 0.04a	0.32 ± 0.03a*	0.31 ± 0.04a*
	ukul.za[1]	0.45 ± 0.02a	0.46 ± 0.08a	1.33 ± 0.12b	1.20 ± 0.15b	0.39 ± 0.02a	0.38 ± 0.04a	0.74 ± 0.25b*	0.58 ± 0.01b*
	summ.za[2]	0.37 ± 0.01a	0.49 ± 0.18a	0.60 ± 0.33a	0.83 ± 0.7a	NA	NA	NA	NA
pH in H_2O	cdcr.us	5.27 ± 0.09b	4.70 ± 0.17a	5.27 ± 0.10b	4.84 ± 0.10a	5.36 ± 0.10b	5.17 ± 0.19ab*	5.45 ± 0.23b	4.96 ± 0.23a
	cbgb.us	5.73 ± 0.50a	5.68 ± 0.72a	5.86 ± 0.47a	5.72 ± 0.43a	5.40 ± 0.66a	5.56 ± 0.92a	5.58 ± 0.79a	5.34 ± 0.63a
	hero.uk	5.12 ± 0.21a	5.18 ± 0.11a	5.08 ± 0.08a	5.09 ± 0.15a	5.24 ± 0.30a	5.30 ± 0.09a	5.20 ± 0.15a	5.22 ± 0.10a
	rook.uk	3.76 ± 0.04a	3.78 ± 0.02a	3.91 ± 0.02a	3.87 ± 0.03a	4.10 ± 0.13a*	4.08 ± 0.09a*	4.12 ± 0.10a*	4.06 ± 0.06a*
	ukul.za	5.89 ± 0.08a	5.58 ± 0.42a	5.94 ± 0.09a	5.63 ± 0.11a	5.83 ± 0.10a	5.79 ± 0.33a	5.72 ± 0.16a	5.62 ± 0.16a
	summ.za[2]	5.20 ± 0.04a	5.03 ± 0.09a	5.01 ± 0.12a	4.97 ± 0.13a	NA	NA	NA	NA

[1] Data were LOG10 transformed
[2] 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 of 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. See also study I, III, and IV.

	Site	0-15 cm				15-30 cm			
		Ctrl	+N	+P	+NP	Ctrl	+N	+P	+NP
DOC (mg C kg^{-1})	ukul.za	115 ± 7a	108 ± 15a	127 ± 6a	152 ± 34a	127 ± 33a	201 ± 52ab*	238 ± 14b*	217 ± 66b*
	summ.za[1]	98 ± 3a	98 ± 9a	102 ± 10a	100 ± 1a	NA	NA	NA	NA
	cdcr.us	15 ± 0.4a	20 ± 4.8b	20 ± 1.1b	24 ± 2.6b	11 ± 0.6a	12 ± 2.0a*	12 ± 0.3a*	15 ± 1.3a*
	cbgb.us	18 ± 2.7a	22 ± 4.1a	18 ± 2.1a	22 ± 1.6a	15 ± 1.6a	22 ± 3.4a	20 ± 5.6a	20 ± 1.6a
	rook.uk	23 ± 2.7a	29 ± 7.5ab	44 ± 8.3c	36 ± 3.5bc	15 ± 1.5a	16 ± 3.1a*	19 ± 1.0a*	18 ± 1.0a*
	hero.uk	29 ± 2.6a	30 ± 3.4a	36 ± 1.2ab	40 ± 4.1b	19 ± 1.2a*	22 ± 4.2a*	23 ± 3.0a*	25 ± 3.9a*
DN (mg N kg^{-1})	ukul.za	7.3 ± 0.3a	43.2 ± 11.2b	9.9 ± 5.0a	34.4 ± 5.7b	5.5 ± 1.1a	17.3 ± 4.9ab*	14.1 ± 3.3ab	19.5 ± 5.3b*
	summ.za[1]	5.0 ± 0.1a	19.8 ± 5.0b	5.1 ± 0.2a	15.9 ± 3.1b	NA	NA	NA	NA
	cdcr.us[2]	3.2 ± 4.4a	32.9 ± 15.6b	2.7 ± 2.1a	13.4 ± 2.9b	1.8 ± 1.5a	10.0 ± 1.0b	2.1 ± 1.5a	7.0 ± 2.1ab
	cbgb.us	4.1 ± 3.1a	12.0 ± 1.4b	4.7 ± 1.9a	12.1 ± 3.9b	2.6 ± 2.2a	5.0 ± 2.8a*	2.9 ± 1.1a	4.8 ± 2.1a*
	rook.uk	14.2 ± 2.7ab	14.7 ± 3.2b	10.9 ± 0.4ab	10.3 ± 1.2a	4.2 ± 2.4a*	3.8 ± 1.0a*	2.9 ± 0.8a*	4.0 ± 0.8a*
	hero.uk	12.9 ± 3.6a	17.1 ± 3.9a	11.1 ± 3.5a	14.6 ± 8.7a	9.7 ± 3.8a	10.3 ± 0.8a	11.9 ± 4.4a	8.9 ± 3.2a
DOC:DN ratio	ukul.za	18.2 ± 0.7b	3.0 ± 0.5a	17.8 ± 7.3b	5.3 ± 1.2a	27.0 ± 1.5b*	14.8 ± 5.5a*	20.3 ± 3.5ab	13.0 ± 1.4a*
	summ.za[1]	22.9 ± 0.6b	6.0 ± 1.1a	23.2 ± 1.6b	7.5 ± 1.2a	NA	NA	NA	NA
	cdcr.us[3]	18.1 ± 11.3b	0.8 ± 0.1a	12.0 ± 6.1b	2.1 ± 0.2a	10.9 ± 3.3b	1.4 ± 0.1a*	9.6 ± 4.7b	2.7 ± 0.6ab
	cbgb.us	10.8 ± 3.0b	2.1 ± 0.2a	4.9 ± 1.5ab	2.3 ± 0.6a	10.0 ± 4.7a	5.9 ± 1.6a	8.8 ± 3.5a	5.6 ± 1.9a
	rook.uk[2]	2.0 ± 0.5a	2.3 ± 0.3ab	4.6 ± 0.6c	4.0 ± 0.1bc	5.1 ± 1.9a*	4.8 ± 0.2a*	8.1 ± 1.7a*	5.4 ± 1.1a
	hero.uk	2.7 ± 0.4a	2.1 ± 0.3a	4.0 ± 1.1a	4.2 ± 2.3a	2.6 ± 1.0a	2.5 ± 0.2a	2.5 ± 0.6a	3.5 ± 0.7a
DIP (mg P kg^{-1})	ukul.za[2]	0.10 ± 0.07a	0.12 ± 0.04a	1.43 ± 0.19b	1.10 ± 0.25b	0.13 ± 0.05a	0.05 ± 0.05a*	2.09 ± 1.58b	0.92 ± 0.24b
	summ.za[1,2]	0.05 ±0.04a	0.10 ± 0.02ab	0.25 ± 0.09b	0.27 ± 0.12b	NA	NA	NA	NA
	cdcr.us[2]	0.16 ± 0.12a	0.13 ± 0.06a	13.37 ± 2.76b	12.29 ± 3.55b	0.06 ± 0.03a*	0.04 ± 0.01a*	4.19 ± 0.55b*	5.01 ± 0.89b*
	cbgb.us[2]	0.37 ± 0.08a	0.25 ± 0.19a	7.67 ± 1.23b	11.1 ± 3.18b	0.13 ± 0.08a*	0.11 ± 0.05a	7.02 ± 3.11b	6.96 ± 1.91b
	rook.uk[2]	0.02 ± 0.01a	0.03 ± 0.01a	1.25 ± 0.88b	1.22 ± 1.20b	0.02 ± 0.01a	0.02 ± 0.01a	0.04 ± 0.03a*	0.06 ± 0.02a*
	hero.uk[2]	0.04 ± 0.01a	0.04 ± 0.01a	1.64 ± 0.85b	1.15 ± 0.72b	0.02 ± 0.01a*	0.03 ± 0.01ab	0.04 ± 0.01ab*	0.06 ± 0.01b*
MBC (mg C kg^{-1})	ukul.za	1005 ± 134a	767 ± 196a	819 ± 157a	852 ± 71a	488 ± 93a*	444 ± 85a*	502 ± 113a*	549 ± 66a*
	summ.za[1]	843 ± 133a	769 ± 111a	928 ± 278a	699 ± 16a	NA	NA	NA	NA
	cdcr.us	163 ± 63a	235 ± 44a	193 ± 9a	169 ± 99a	119 ± 103a	100 ± 47a*	46 ± 31a*	134 ± 124a
	cbgb.us	175 ± 61a	121 ± 23a	168 ± 51a	148 ± 100a	61 ± 15a*	119 ± 35a	69 ± 43a	142 ± 118a
	rook.uk	651 ± 35a	588 ± 49a	507 ± 210a	746 ± 88a	351 ± 46a*	317 ± 94a*	248 ± 176a*	342 ± 248a*
	hero.uk	662 ± 116a	603 ± 70a	638 ± 51a	591 ± 85a	526 ± 8b	363 ± 61ab*	298 ± 73a*	352 ± 76ab*

[1] One-Way ANOVA and Tukey test were performed
[2] Data were LOG10 transformed
[3] Data were reciprocally transformed (1/x)

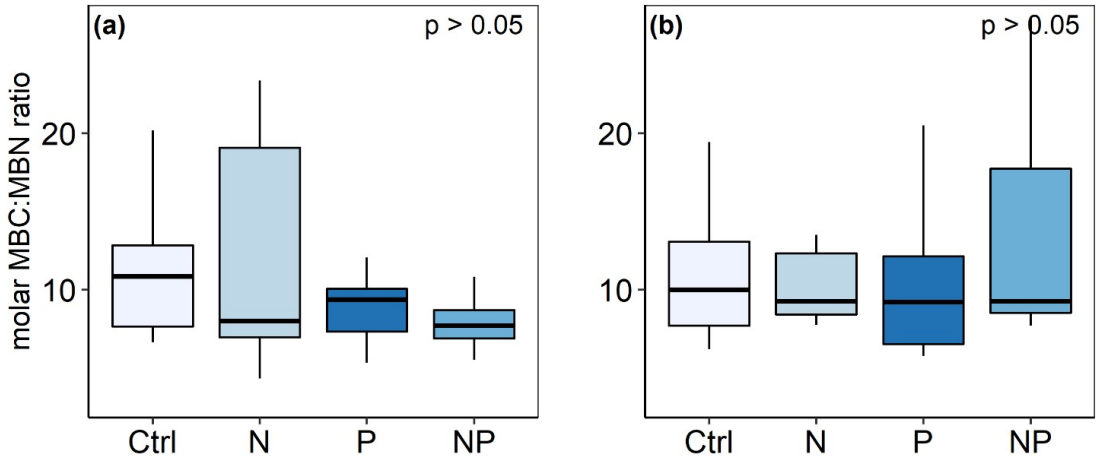


Figure 4: Molar microbial biomass carbon-to-nitrogen ratio (MBC:MBN) in (a) 0-15 cm depth and (b) 15-30 cm depth of the control and under N, P, and NP additions across all six sites. Subsoil MBC:MBN ratios of one site (rook.uk) were excluded, because MBN values were below detection limit. See also study I.

Since we found unaffected microbial biomass C:N ratios under nutrient addition, microorganisms maintained their biomass stoichiometry independently of that of their environment confirming the concept of stoichiometric homeostasis. Homeostatic behavior of the microbial biomass has been found in several studies (Cleveland and Liptzin, 2007; Xu et al., 2013). Even under nutrient addition microorganisms kept their biomass stoichiometry (Griffiths et al., 2012; Schleuss et al., 2019; Joergensen and Scheu, 1999). For instance, microbial biomass stoichiometry was unaffected by 66 years of N and P additions in a South African grassland (Schleuss et al., 2019).

1.4.3 Microbial element partitioning and turnover time

1.4.3.1 C partitioning

One measure to maintain biomass stoichiometry despite changed substrate stoichiometry is the adjustment of element partitioning in the microbial biomass. In this study we investigated soil microbial C partitioning in growth and respiration (CUE) under N, P, and NP additions. Further, we studied if microorganisms adjust CUE to substrate stoichiometry (see also study I).

Addition of N significantly decreased topsoil microbial respiration by -23% across all sites, whereas microbial growth did not significantly change upon nutrient addition (Figure 5). Consequently, topsoil C uptake (the sum of respiration and growth) was -14% lower under N addition than in the control and under P addition (Figure 5).

Soil microbial CUE was unaffected by nutrient addition at either soil depth increment and varied between 25 and 57% with a mean value of 40% across all sites, soil depth increments, and nutrient addition treatments (Figure 6). Sand content, mean annual precipitation and temperature, and DOC:DN ratio explained most of the site variability

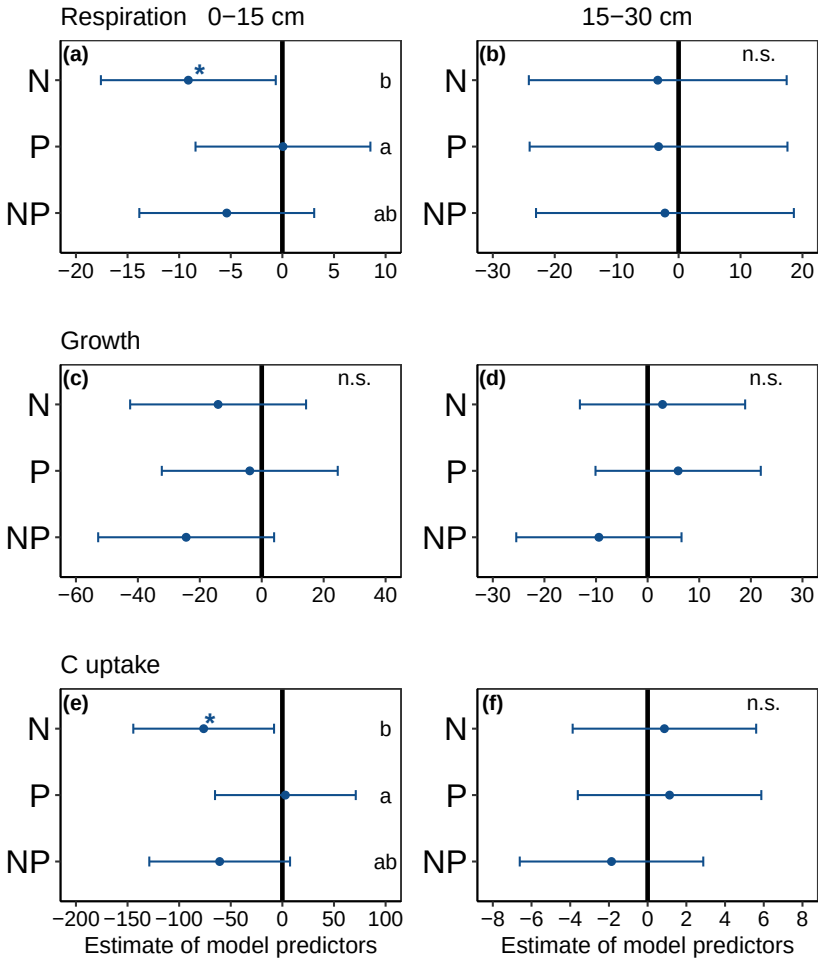


Figure 5: Effect of nutrient addition on (a, b) soil microbial respiration, (c, d) microbial growth, and (e, f) microbial C uptake across all six sites in (a, c, e) 0-15 cm depth and (b, d, f) 15-30 cm depth. Linear mixed-effects models were calculated with treatment as fixed and site intercepts as random 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. The vertical intercept (position zero) corresponds to the control. Significant differences between nutrient addition treatments are indicated by lower-case letters at the right side of the subplot. See also study I.

in microbial CUE. Soil microbial CUE and DOC:DN ratios of the topsoil control plots were negatively related ($R^2 = 0.27$, $p = 0.03$) and excluding data of the site Summerveld, the correlation became highly significant ($R^2 = 0.71$, $p < 0.001$, Figure 7). In addition, topsoil CUE was negatively correlated with the activity of beta-glucosidase ($R^2 = 0.31$, $p = 0.02$), a C-acquiring enzyme. Further, topsoil CUE was negatively correlated with activities of the N-acquiring enzymes N-acetyl-glucosaminidase ($R^2 = 0.40$, $p = 0.006$) and leucine-aminopeptidase ($R^2 = 0.50$, $p = 0.002$, data not shown).

Our finding that nutrient addition did not affect soil microbial CUE can be explained by the fact that microorganisms regulated both respiration and growth similarly. Consequently, microorganisms neither performed overflow respiration, i.e. the disposal of C via

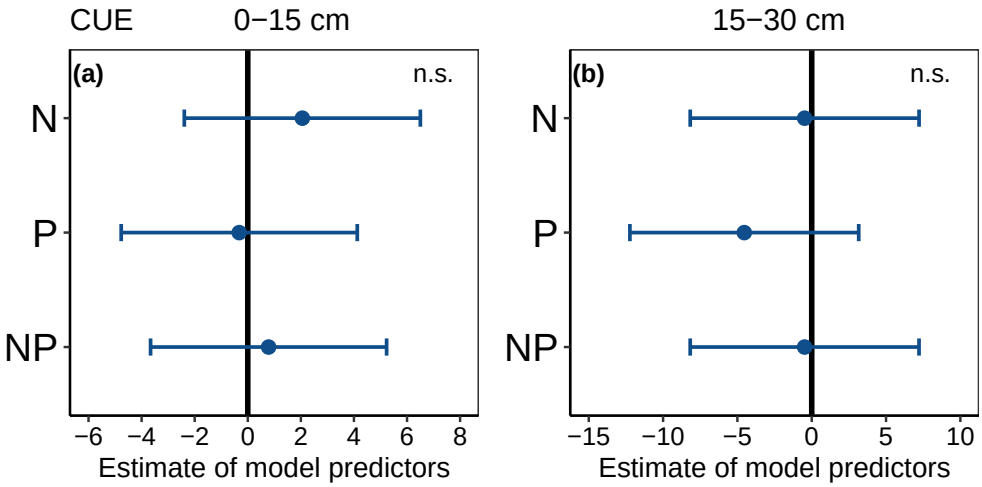


Figure 6: Effect of nutrient addition on microbial carbon use efficiency (CUE) across all six sites in (a) 0-15 cm depth and (b) 15-30 cm depth. The vertical intercept (position zero) corresponds to the control. Linear mixed-effects models were calculated with treatment as fixed factor and random intercepts 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. See also study I.

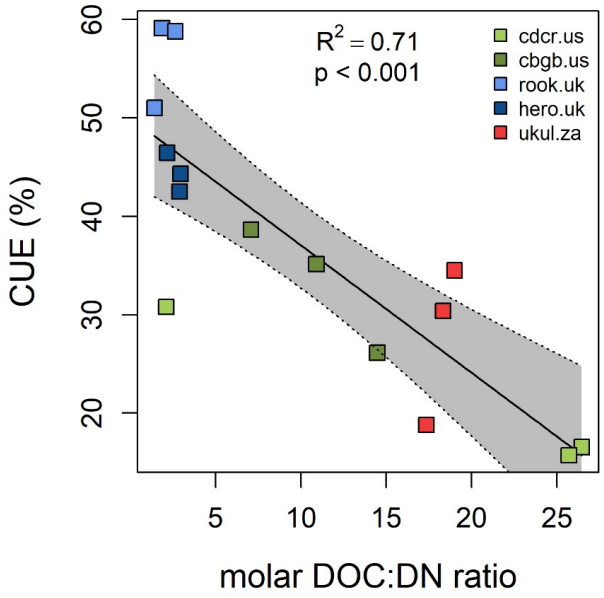


Figure 7: Correlation of molar dissolved organic carbon-to-dissolved nitrogen (DOC:DN) ratio and soil microbial carbon use efficiency (CUE) in the topsoils of the control plots of all sites with a soil depth > 20 cm. See also study I.

respiration, nor strongly increased growth at the cost of respiration. The disposal of C through overflow respiration seems rather unlikely under natural conditions (Hessen and Anderson, 2008; Spohn, 2015) for microorganisms that are most commonly C limited (Demoling et al., 2007; Heuck et al., 2015). Further, microorganisms need to uphold respiration for maintenance and anabolic energy requirements (Geyer et al., 2016), thus a strong increase in growth at the expense of respiration is questionable.

Our finding of a negative correlation between soil microbial CUE and DOC:DN ratio confirms the dependency of microbial element partitioning on substrate stoichiometry and our hypothesis. To maintain their biomass stoichiometry, microorganisms need to adapt their foraging strategies to the available substrate, since DOC:DN ratios mostly exceed microbial biomass C:N ratios (Sinsabaugh et al., 2013). If DOC:DN ratios are large, microorganisms need to invest into nutrient acquisition, which reduces their CUE. Vice versa, as the DOC:DN ratio gets closer to that of the microbial biomass, less C and energy needs to be invested into nutrient acquisition and CUE increases.

It is assumed that microorganisms invest into nutrient acquisition via the production of extracellular enzymes at the expense of CUE (Manzoni et al., 2012; Sinsabaugh et al., 2016). Yet, direct measurements of the relationship between microbial CUE and extracellular enzymes are rare. Extracellular enzyme activities mediate nutrient acquisition from organic matter and are thus commonly interpreted as indicators of microbial nutrient demand (Sinsabaugh et al., 2008; Schimel and Weintraub, 2003). Our finding that activities of N-acquiring enzymes were negatively related with CUE confirms the assumption that microorganisms invest into N acquisition when less N is available, which decreases their CUE (Manzoni et al., 2012; Sinsabaugh et al., 2016). In contrast, microbial CUE increases when microorganisms are well supplied with nutrients and do not need to invest into the production of extracellular enzymes. Not only the activities of the N-acquiring enzymes were negatively correlated with CUE, but also the activity of beta-glucosidase, a C-acquiring enzyme, indicating that microorganisms also invest more into C acquisition when they run at low CUE. We showed that CUE was related to substrate stoichiometry. Consequently, microorganisms adjusted C partitioning to maintain their biomass stoichiometry and cope with stoichiometrically unfavorable substrate.

1.4.3.2 Element turnover time in the microbial biomass

Besides the partitioning of elements, microorganisms might adjust element turnover times to maintain their biomass stoichiometry. In this study, we tested for the first time, if microorganisms adjust the turnover time of P in their biomass to P availabilities (see also study II).

Addition of P did not affect microbial DNA, and microbial biomass C concentrations. Using ^{33}P labelling, we showed that with increasing levels of P addition the mean residence time of P in the microbial biomass significantly decreased (Figure 8). The mean residence time of P in the microbial biomass amounted to 39 days in the control and

decreased by -54% to 18 days under the highest level of P addition (Figure 8).

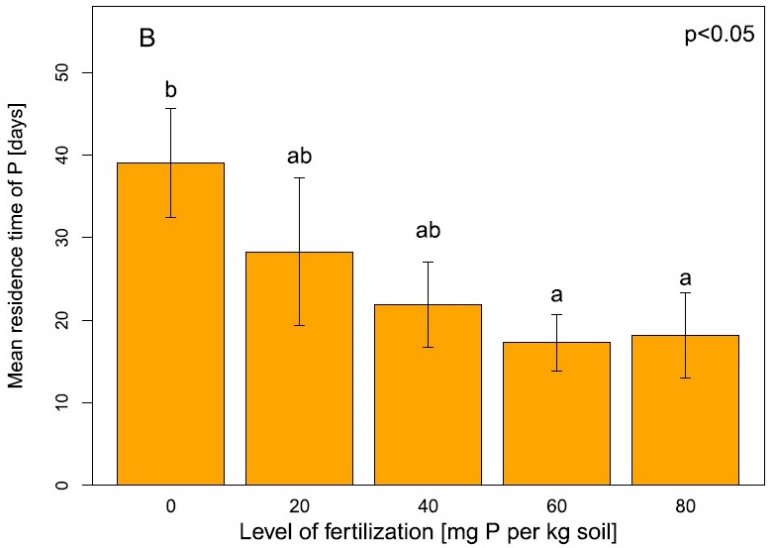


Figure 8: Mean residence time (MRT) of phosphorus (P) in the microbial biomass. Bars represent means, and error bars show standard deviation (n = 3). Letters indicate significant differences between P addition treatments. See also study II.

For the first time, ^{33}P labelling was used to determine P turnover time in the microbial biomass and P addition caused a shorter mean residence time of P in the microbial biomass compared to control. This finding indicates that microorganisms adjust turnover times of elements in their biomass, a process often overlooked in the debate of stoichiometric homeostasis (Mooshammer et al., 2014). According to our hypothesis, the mean residence time of P in the microbial biomass increased with decreasing P availability. An explanation can be that microorganisms recycled P already present in the cell more efficiently under low compared to high P levels. Accordingly, soil microorganisms adjusted P turnover times to bioavailable P concentrations, which seems to be an important adjustment to nutrient-poor environments (Spohn, 2016). The adjustment of turnover times of individual elements in the microbial biomass can be a measure to maintain stoichiometric homeostasis when thriving on substrate with an unfavorable stoichiometry i.e. substrate with high C-to-nutrient ratios.

1.4.4 Microbial element acquisition

1.4.4.1 N acquisition and release

We further investigated if microorganisms, besides element partitioning and turnover, also adjust processes of N acquisition and release such as non-symbiotic N_2 fixation, leucine-aminopeptidase (LAP) activity, and net N mineralization towards their stoichiometric demands (see also study III).

Topsoil non-symbiotic N_2 fixation rates increased by 96% under NP addition compared to control (Figure 9). Further, topsoil non-symbiotic N_2 fixation was -55% lower under N

compared to P addition, whereas subsoil N_2 fixation rates were not affected by nutrient addition (Figure 9). Non-symbiotic N_2 fixation was negatively correlated with soil TN:TP ratios in the topsoil ($R^2 = 0.20$, $p = 0.005$) and subsoil ($R^2 = 0.08$, $p = 0.04$, Figure 10). Further, TOC:TP, TN:TP, and DOC:DIP ratios were the best predictors of N_2 fixation. Since TN:TP ratio was intercorrelated with TOC:TP ratio, we calculated the model with TOC:TP and DOC:DIP ratio and these variables could predict 83% of variability in N_2 fixation.

LAP activities were not affected by any nutrient addition treatment in neither soil depth increment (Figure 9). However, topsoil LAP activities were negatively correlated with DN concentrations ($R^2 = 0.15$, $p = 0.002$, Figure 10). The best predictors for LAP activity were mean annual temperature and precipitation, and soil pH ($R^2 = 0.61$).

Single N and combined NP additions increased net N mineralization rates in the topsoil by 134% and 138%, respectively, compared to control and P addition across all six sites (Figure 9).

We found a highly significant positive correlation between net N mineralization and soil DN concentration in the topsoil ($R^2 = 0.42$) and subsoil ($R^2 = 0.56$) and a highly significant negative correlation between net N mineralization and DOC:DN concentration in the topsoil ($R^2 = 0.61$) and subsoil ($R^2 = 0.67$, Figure 10). Accordingly, multi-model selection revealed that DN concentration, plant biomass, and soil pH could explain 89% of variability in N mineralization. Considering DOC:DN ratios instead of DN concentrations, 84% of variation in N mineralization could be explained by the model.

In accordance with our hypothesis, non-symbiotic N_2 fixation was negatively correlated with soil N:P ratio suggesting that microorganisms regulate N_2 fixation based on soil stoichiometry. Both low N and sufficient P availability have shown to increase non-symbiotic N_2 fixation. It is energetically more efficient to take up mineral N, when available, than to fix atmospheric N_2 (Reed et al., 2011) and sufficient P needs to be available to support ATP production (Vitousek et al., 2002). This finding confirms chemical constraints on element cycling and that microorganisms adjust element acquisition to meet their nutritional demands as previously assumed (Mooshammer et al., 2014; Zechmeister-Boltenstern et al., 2015; Spohn, 2016).

Our result that, besides soil N:P ratio, C:P ratios explained a large proportion of non-symbiotic N_2 fixation across the six grassland sites might indicate that N_2 fixation is C limited. Microorganisms need available C substrates as energy sources for nitrogenase production. Between 5 and up to 117 g C are needed in different microorganisms to fix 1g of N_2 (Hill, 1992).

Another way to acquire N is the exudation of extracellular enzymes such as the peptide-degrading LAP. In accordance with our hypothesis that LAP activities increase with decreasing N availability, we found a negative correlation of LAP activity with DN concentrations in the topsoil indicating that microorganisms invested less into the production of N-acquiring enzymes when more N was available. However, LAP activity did not change upon nutrient addition as similarly found in three contrasting grasslands in the USA and

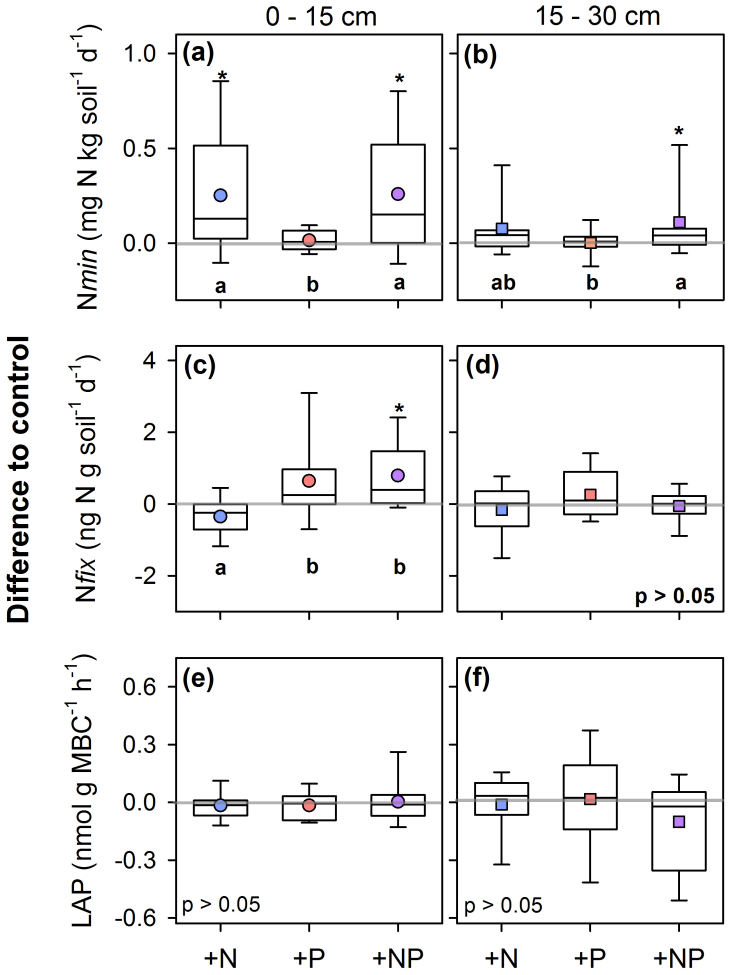


Figure 9: Changes in net N mineralization rate (N*min*) (a), non-symbiotic N_2 fixation rate (N*fix*) (b), and leucine-aminopeptidase activity (LAP) (c) for the two soil depth increments depending on N and P additions across all six sites. Changes in variables were calculated by subtracting the control from each treatment. Colored circles and squares represent the mean value ± SD for each treatment in 0-15 cm (n=18) and in 15-30 cm (n=15). Linear mixed-effects models were performed with treatment as fixed factor and random intercepts for sites. Significant differences between treatments are indicated by lower-case letters, while asterisks indicate significant differences between treatments and the control (illustrated by the grey line). See also study III.

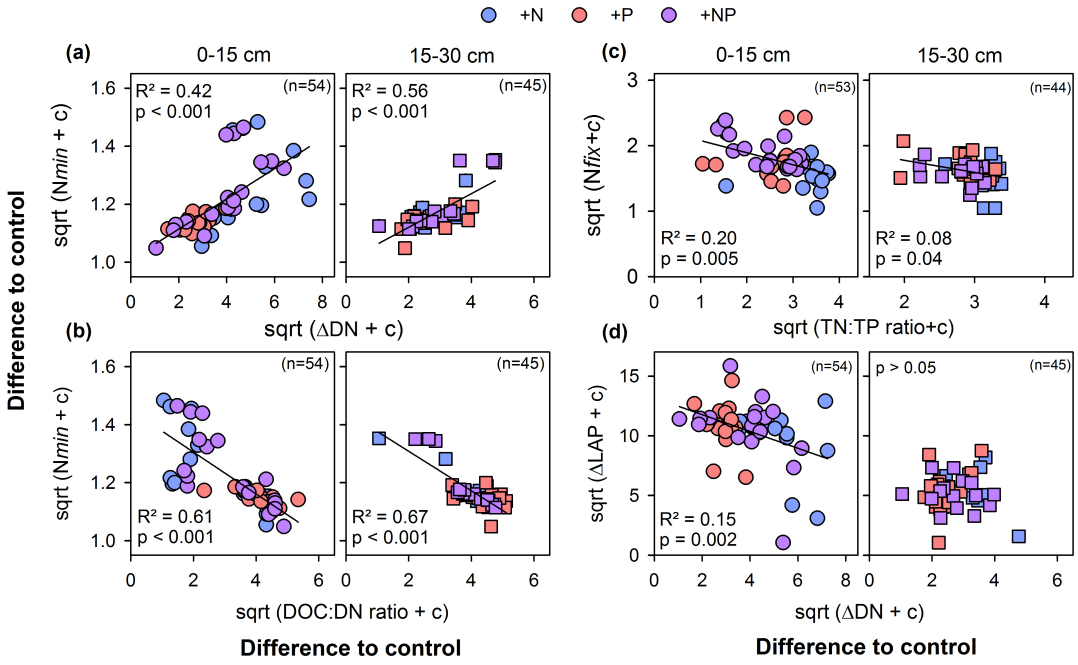


Figure 10: Relationships between changes in soil N cycling processes and changing element availabilities due to N and P additions across all sites in 0-15 cm and 15-30 cm soil depth. Correlations between N mineralization rate (N*min*) and dissolved N concentrations (DN) (a), correlation between N*min* and dissolved organic matter C:N ratios (DOC:DN ratio) (b), correlations between non-symbiotic N_2 fixation (Nfix) and total N:total P ratio (TN:TP ratio) (c), and correlations between leucine-aminopeptidase activity (LAP) and dissolved N concentrations (DN) (d). Changes in variables were calculated by subtracting the control from each treatment. To avoided negative values for subsequent square root-transformations changes in variables were adjusted by adding a constant (c = minimum of data frame + 1.1). See also study III.

South Africa (Zeglin et al., 2007), in a semiarid grassland in New Mexico (Stursova et al., 2006), and in a recent meta-analysis (Chen et al., 2018). Reasons for inconsistencies regarding LAP activities and N addition might be, first, that N can be obtained from different molecules and thus several enzymes and N-acquisition strategies exist (Sinsabaugh et al., 2008). Second, N-acquisition can be linked to C-acquisition since peptides can be N and C sources (Sinsabaugh et al., 2008; Sinsabaugh and Follstad Shah, 2012). Third, in our study and a global meta-analysis (Sinsabaugh et al., 2008), LAP activities were mainly controlled by soil pH.

Our finding that N addition increased net N mineralization indicates that inorganic N is released by microorganisms once their N demands are covered and N is in excess (Nave et al., 2009; Ma et al., 2011; Zhang et al., 2012). Net N mineralization was negatively related to the DOC:DN ratio demonstrating the importance of substrate stoichiometry on microbial N release (Manzoni et al., 2008; Heuck and Spohn, 2016). Microorganisms immobilize N in their biomass when DOC:DN ratios increase and less N compared to C is available, whereas they release excess N under decreasing DOC:DN ratios and thus N mineralization increases. Consequently, microorganisms adjusted processes of N acquisition and N release to their nutritional demands and likely to their biomass stoichiometry.

1.4.4.2 P acquisition

Here, we studied if, besides N acquisition, P acquisition (assessed by phosphatase activity and abundance and community of PSB) is affected by nutrient addition and stoichiometric constraints of microorganisms (see also study IV).

We found that across all sites the relative abundance of PSB in the topsoil was significantly lower under N (-18 %) and NP additions (-41%, Figure 11a), whereas in the subsoil it was significantly lower under NP addition (-60%) as compared to control (Figure 11b). Soil TOC:TP ratio, percentage of sand, pH, and water-extractable P predicted the variance of the relative abundance of PSB in the topsoil with an R^2 of 0.71 ($p < 0.01$). Based on a model only considering the control plots, the variance of the relative PSB abundance was predicted with an R^2 of 0.83 ($p = 0.012$). The PSB community across all sites was significantly different in the N and NP compared to control and P addition ($p = 0.002$, Figure 12).

Phosphatase activity was significantly higher under N addition (+17%) compared to control across all six topsoils (Figure 13a). In the subsoil, differences in phosphatase activity between nutrient addition treatments and control were not statistically significant (Figure 13b).

Our finding that a higher TOC in relation to P concentration enhanced the abundance of PSB shows the impact of the soil stoichiometry on the abundance of PSB. An explanation could be that microorganisms need organic C to build and release organic acid anions that largely contribute to P solubilization (Jones and Oburger, 2011). Further, C addition enhanced P solubilization in laboratory studies (Hameeda et al., 2006; Patel et

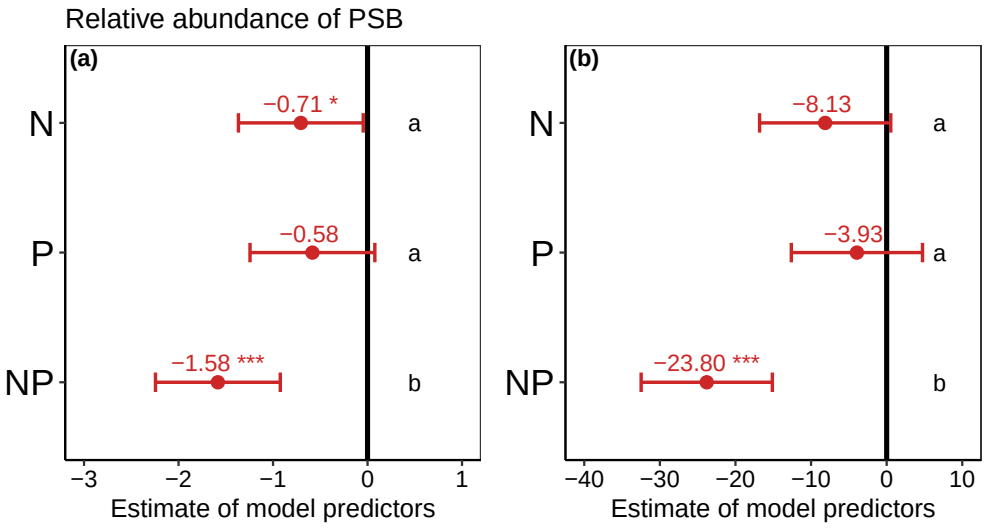


Figure 11: Effect of nutrient addition (N, P, NP) on the relative abundance of phosphorus-solubilizing bacteria (PSB) across all six sites in (a) 0-15 cm depth and (b) 15-30 cm depth. A linear mixed-effects model was calculated with treatment as fixed factor and random intercepts for sites. Points represent 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 with asterisks. The vertical intercept (position zero) corresponds to the control. Significant differences between treatments are indicated by lower-case letters at the right side of the subplot. See also study IV.

al., 2008), P solubilization was strongly C limited in an incubation experiment (Pastore et al., 2020), and soil TOC:TP ratio was positively correlated with the relative abundance of PSB in pastures in New Zealand (Mander et al., 2012). This finding contradicts our hypothesis that the abundance of PSB increases with increasing TN:TP ratios because microorganisms need N for transcription and translation to produce organic acids and siderophores that solubilize P. Instead, it emphasizes the role of soil C for P solubilization.

Our observation that N and NP additions decreased the relative abundance of PSB and altered their community composition indicates first, that N inputs may affect the functional traits of the microbial community since a lower number of PSB might also solubilize less P. Second, PSB likely have an advantage under nutrient-poor conditions and are poor competitors at high N and NP availability. A reason could be that microorganisms adapted to N- and NP-rich soils invest less into nutrient acquisition and have more energy left for growth and other fitness-related processes. Several authors have described similar functional adaptions of microbial communities to nutrient availability (Calvaruso et al., 2007; Mander et al., 2012; Lepleux et al., 2013; Nicolitch et al., 2016). For instance, P solubilization efficacy was lower in nutrient-rich than in nutrient-poor beech forest soils (Nicolitch et al., 2016).

In accordance with our hypothesis, phosphatase activity was significantly higher under N addition indicating, first, that increased N availability increased P demand and microorganisms invested into the production of P-acquiring enzymes. By this measure microorganisms likely maintain their biomass stoichiometry despite changed element

availabilities (Spohn, 2016). Second, increased N availability likely enables phosphatase production, since enzymes contain large amounts of N. A meta-analysis and previous studies have shown that plants and microorganisms invest into P acquisition if N availability increases (Marklein and Houlton, 2012; Olander and Vitousek, 2000; Margalef et al., 2017). Our findings that the abundance of PSB was related to substrate stoichiometry and that N availability strongly impacted phosphatase activity indicate the importance of element availability and stoichiometry not only for N, but also for P acquisition.

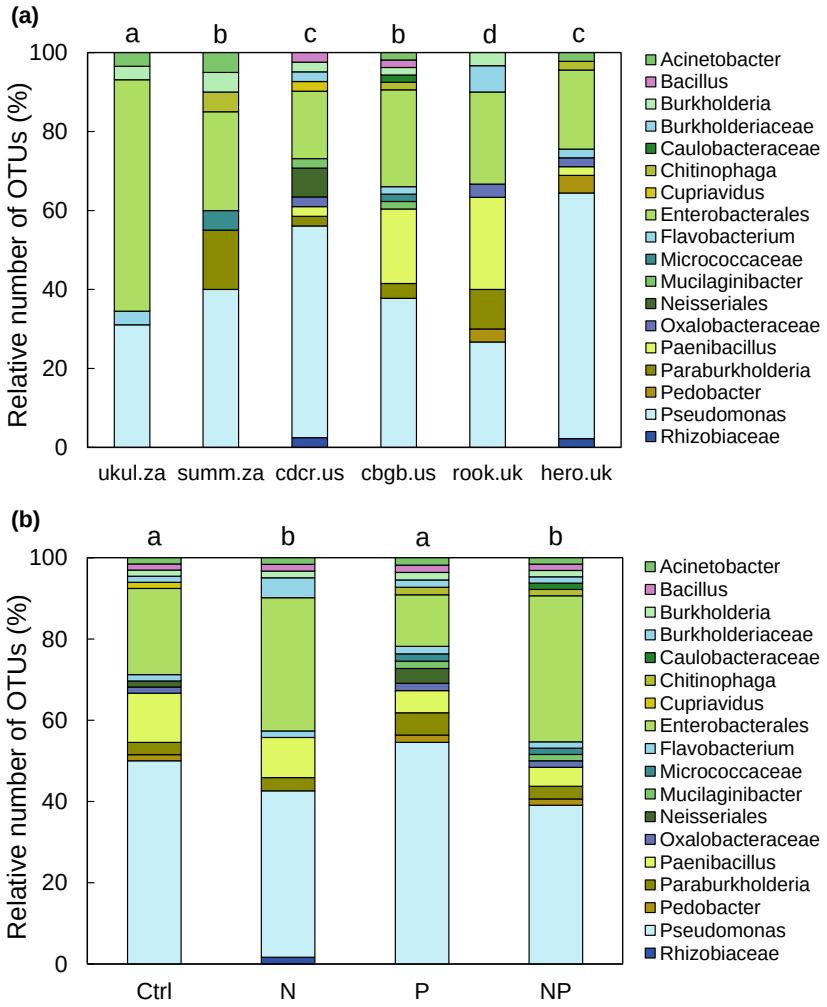


Figure 12: Relative number of operational taxonomic units (OTUs) of phosphorus-solubilizing bacteria in 0-15 cm soil depth sorted by nutrient addition treatments. Different letters indicate significant differences among treatments. See also study IV.

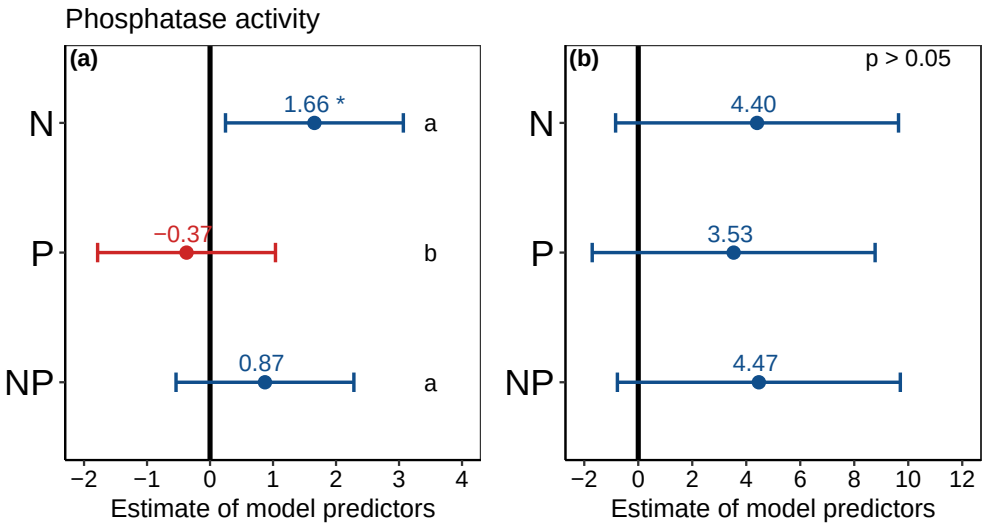


Figure 13: Effect of nutrient addition (N, P, NP) on soil phosphatase activity across all six sites in (a) 0-15 cm depth and (b) 15-30 cm depth. A linear mixed-effects model was calculated with treatment as fixed factor and random intercepts for sites. Points represent the mean value of the model predictor while error bars represent the range of 95% confidence intervals. Predictors are considered significant if error bars do not overlap with zero, indicated with asterisks. The vertical intercept (position zero) corresponds to the control. Significant differences between nutrient addition treatments are indicated by lower-case letters at the right side of the subplot. See also study IV.

1.4.5 Microbial communities and element cycling

Besides studying the effects of nutrient addition on element cycling processes driven by stoichiometric homeostasis of soil microorganisms, we studied how nutrient addition affects microbial communities and how the changes in microbial community composition can be linked to element cycling (see also study V).

Under N addition, microbial community composition changed with increases in the relative abundance of Alphaproteobacteria and Actinobacteria and decreases in Deltaproteobacteria and Acidobacteria. Although variation in prokaryotic community was highly correlated with plant community (Mantel test r = 0.48, p = 0.001), plant community composition accounted for only 10% of variance in prokaryotic community composition. Instead, soil pH and TOC concentration accounted for a larger proportion of variance in prokaryotic community composition with 23% and 20%, respectively (Figure 14). In the fungal community, the relative abundance of Basidiomycota decreased under N addition, whereas the relative abundance of Ascomycota increased. The variation in the fungal community was highly correlated with the plant community (Mantel test r = 0.46, p = 0.001). However, plant community composition accounted for only 9% of variation in fungal community composition, whereas the DOC:DN ratio accounted for 21% (Figure 14).

Microbial biomass C concentrations and microbial respiration rates were not significantly affected by nutrient addition. Topsoil net N mineralization rates increased under

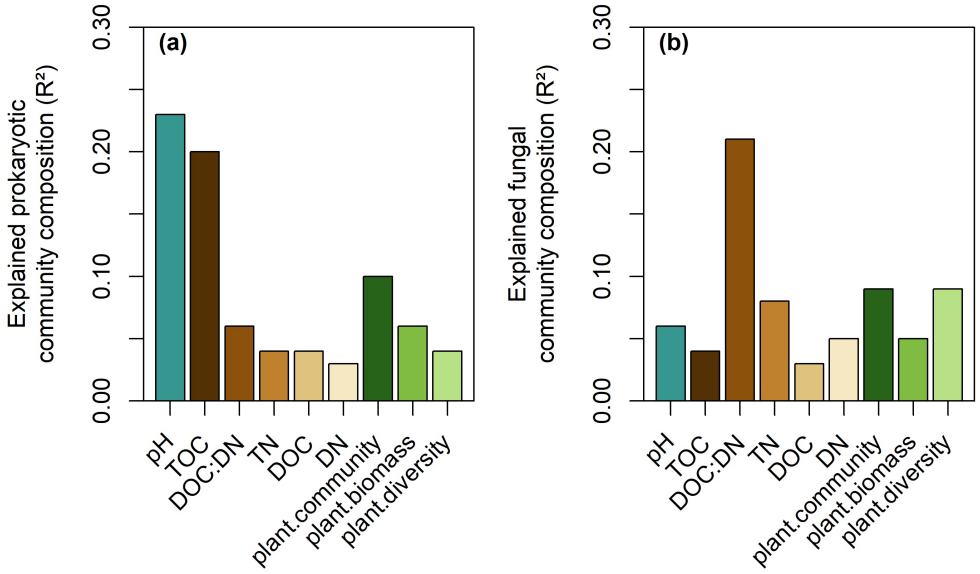


Figure 14: Proportion of variation of prokaryotic (a) and fungal (b) community composition explained by the displayed soil and plant factors in 0-15 cm soil depth. Results are based on permutational multivariate analyses of variance (PERMANOVA) using Jensen-Shannon divergence of microbial communities at OTU level, 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 first axis of principal coordinates analysis, plant biomass, and plant diversity (Shannon diversity). See also study V.

any level of N and NP additions compared to control. Subsoil non-symbiotic N_2 fixations rates were higher under the lowest level of N and P addition compared to control. Instead of plant or microbial variables, soil variables accounted for a larger proportion of variance in microbial respiration, non-symbiotic N_2 fixation, and net N mineralization. DOC:DN ratio accounted for the largest proportion of variation in microbial respiration (Figure 15). Further, DN and DOC concentrations were the best explaining variables of net N mineralization rates and TOC concentration was the best explaining variable for non-symbiotic N_2 fixation (Figure 15).

Our finding that under high levels of N addition Actinobacteria, Alphaproteobacteria, and Ascomycota abundances increased corroborates previous findings that fast-growing, copiotrophic organisms are more abundant under elevated nutrient inputs than slow-growing, oligotrophic organisms (Fierer et al., 2012; Leff et al., 2015). Copiotrophs likely need more N per unit biomass C to support their rapid growth and short biomass turnover times (Kaiser et al., 2014) and thus have a competitive advantage under N addition.

Soil pH was a main driver of prokaryotic community composition as found in other studies (Ramirez et al., 2010; Lauber et al., 2009; Fierer and Jackson, 2006; Rousk et al., 2010). Most soil prokaryotes have a relatively narrow pH tolerance (Rousk et al., 2010) and thus soil pH can induce physiological stress impairing growth or competitiveness (Fernández-Calviño and Bååth, 2010). Further, TOC concentrations were important

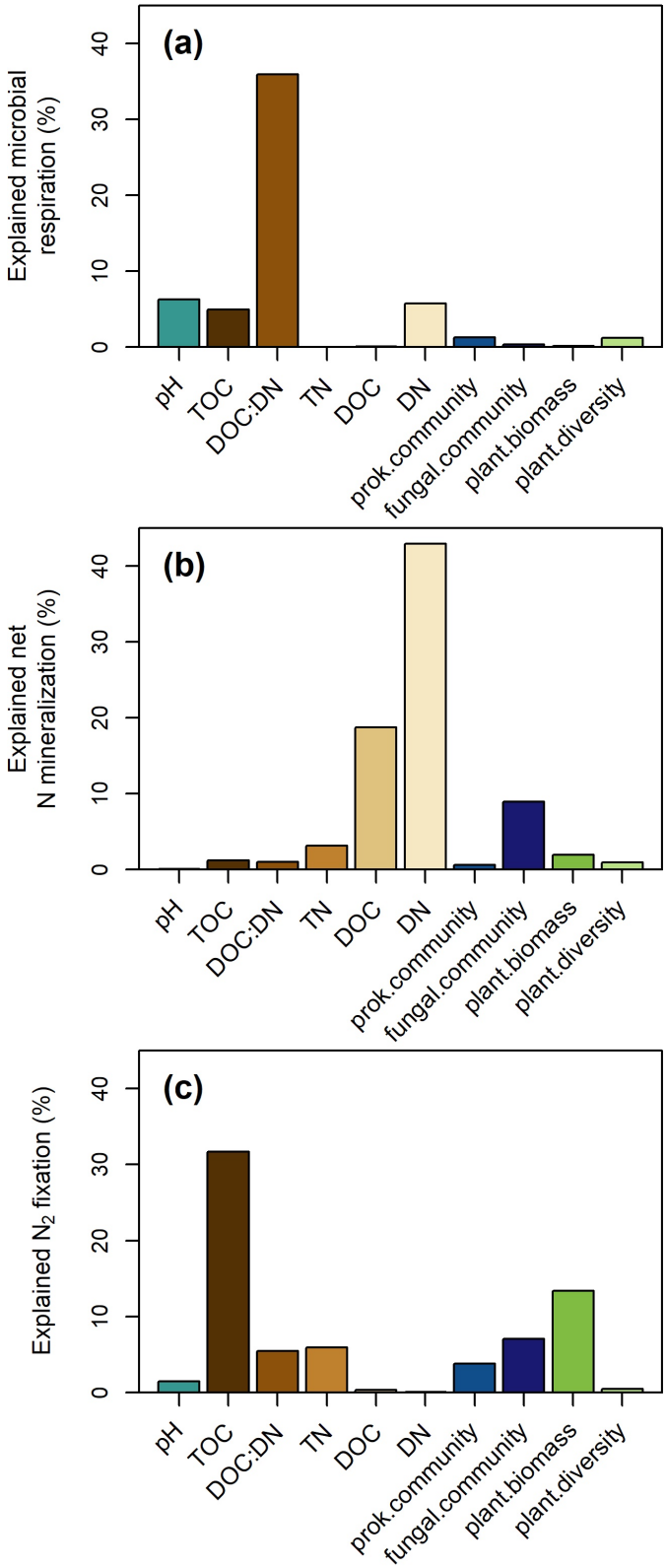


Figure 15: Proportion of microbial respiration (a), net nitrogen (N) mineralization (b), and non-symbiotic N_2 fixation rates (c) in 0-15 cm soil depth explained by the displayed soil, microbial, and plant factors. Soil factors include soil pH, total organic carbon (TOC), dissolved organic carbon-to-dissolved nitrogen ratio (DOC:DN), total nitrogen (TN), dissolved organic carbon (DOC), and dissolved nitrogen (DN). Microbial factors include prokaryotic and fungal community composition at OTU level based on first axis of principal coordinates analysis. Plant factors include plant biomass and diversity (measured as Shannon diversity). See also study V.

drivers of prokaryotic community composition, reflecting the importance of C as limiting resource, supporting and structuring microbial communities (Alden et al., 2001; Heuck et al., 2015; Sul et al., 2013).

The predominant driver of fungal community composition was the DOC:DN ratio likely because some fungi, mainly Basidiomycota, are specialists in decomposing complex C sources with high C:N ratios (de Boer et al., 2005), whereas fast-growing fungi in the Ascomycota, such as molds (Lundell et al., 2014), also benefit from smaller DOC:DN ratios. Thus, smaller DOC:DN ratios affect fungal communities explaining the shift from Basidiomycota to Ascomycota under N addition.

Although microbial community composition changed under nutrient addition, microbial respiration remained unaffected indicating functional redundancy of the microbial community (Banerjee et al., 2016; Rousk et al., 2009; Wertz et al., 2006). Instead, the DOC:DN ratio explained a large proportion of microbial respiration demonstrating the importance of substrate stoichiometry on microbial respiration (Spohn, 2015; Spohn and Chodak, 2015). 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. The coupling of N cycling to soil C and N concentrations is an often observed phenomenon (Colman and Schimel, 2013; Liu et al., 2017; Schleuss et al., 2019; Nelson et al., 2016). Specialized functions, such as non-symbiotic N_2 fixation by free-living microorganisms (Dixon and Kahn, 2004), might be more affected by microbial community change than broader functions (Schimel, 1995; Reed et al., 2010). However, we found that TOC concentrations were the major driver of non-symbiotic N_2 fixation rates likely because non-symbiotic N_2 fixation is one of the most energy-costly processes on earth (Smith, 1992; Hill, 1992). In conclusion, element cycling was mainly mediated by soil factors as opposed to plant and microbial community shifts emphasizing the control of element availabilities on element cycling rates.

1.5 Summary, Conclusion, and Outlook

Here, we evaluated the influence of nutrient inputs on microbial biomass stoichiometry and on processes by which microorganisms can maintain biomass stoichiometry in six grassland soils across three continents. Moreover, we added details to the current question if and to what extent the concept of Ecological Stoichiometry can be used to explain element cycling rates by assessing processes of C, N, and P cycling and biotic and abiotic controls on them. It is of special interest to understand if and how stoichiometric relationships regulate element cycling to predict responses of terrestrial ecosystems to global change.

Seven to nine years of nutrient addition to six different grassland soils around the world did not change microbial biomass stoichiometry despite large changes in element availabilities. This finding confirms the assumed stoichiometric homeostasis of soil microorganisms in terrestrial ecosystems also after years of nutrient addition. To maintain homeostatic stoichiometry, microorganisms adjusted processes of element partitioning and turnover and processes of element acquisition.

The partitioning of C into growth and respiration, measured by a novel isotopic method, was highly dependent on substrate stoichiometry. Under higher DOC:DN ratios, microorganisms had a lower CUE. Thus, they respired more, indicating that they needed to invest into nutrient acquisition. As substrate stoichiometry gets closer to microbial stoichiometry, microorganisms invest less into nutrient acquisition and have a higher CUE. The relation between nutrient demand and substrate stoichiometry was also expressed by microbial enzyme activities: N-acquiring enzymes were negatively correlated with CUE showing that the investment into N acquisition decreases CUE.

Further, for the first time, we used ^{33}P labeling of microbial DNA to determine the turnover time of P in the microbial biomass. We showed that microorganisms increased the mean residence time of P in their biomass with decreasing P availability indicating that microorganisms adjust turnover times of elements in their biomass according to element availabilities.

Besides element partitioning and turnover, microorganisms also adjusted processes of element acquisition to element availabilities. Non-symbiotic fixation of atmospheric N_2 was highly related to TOC:TP and TN:TP ratios. A high N availability suppresses N_2 fixation since the uptake of reactive N from soil is less energy consuming than fixing atmospheric N_2. Non-symbiotic N_2 fixation is one of the most energy-consuming processes on earth and sufficient P for ATP production needs to be available. However, C availability seemed to control N_2 fixation more strongly than P availability as indicated by the positive relation between N_2 fixation and TOC:TP ratios. Nutrient addition did not directly affect LAP activities, whereas they were negatively related with DN concentrations indicating that high N availabilities prevent microorganisms from investing into N-acquiring enzymes. Further, N release highly depended on substrate stoichiometry. The decrease in net N mineralization rates with increasing DOC:DN ratios indicates

that the more N in relation to C is available, the more N is released.

In addition, P acquisition processes are possibly altered by N availability as indicated by changes in phosphatase activity and the abundance of PSB under N addition. High N availability increased phosphatase activity but decreased the abundance and altered the community of PSB indicating a switch from solubilization to mineralization of P under increasing N inputs. Phosphatase activity was enhanced with increasing N availability, reflecting on the one hand, increased microbial P demand as an attempt to balance biomass stoichiometry, and on the other hand, the capacity to produce enzymes, which are generally rich in N. For the first time, the concept of Ecological Stoichiometry was applied to the abundance of PSB. We found that the abundance of PSB was related to TOC:TP ratios likely because microorganisms need organic C to produce organic acids that enable P solubilization.

At one of the studied sites, nutrient inputs strongly shifted microbial community composition without affecting microbial biomass and respiration. Net N mineralization, non-symbiotic N_2 fixation, and microbial respiration under nutrient addition were largely explained by element availabilities rather than by plant or microbial community shifts against our expectation and that of many sequencing studies. This finding confirms that nutrient availabilities exert a strong control on element cycling rates, even stronger than plant and microbial community shifts.

Using the concept of Ecological Stoichiometry as a predictor of element cycling rates is appealing because soil and dissolved organic matter stoichiometry is relatively easy to access. However, this approach also has limitations. The activities of LAP, a N-acquiring enzyme, were not directly related to substrate stoichiometry indicating that not all element cycling processes can be based on stoichiometric relationships. Further, multi-model selection reveled in many cases that stoichiometric relations cannot explain element cycling alone, rather climatic variables, soil pH, and soil texture were important predictors as well. Yet, microbial homeostasis can be applied to predict many processes of element acquisition, partitioning, turnover, and release in grassland ecosystems and thus Ecological Stoichiometry has proven as an important concept not only for aquatic but also terrestrial ecology.

Based on our results, we can roughly estimate the consequences of long-term atmospheric N deposition on soil element cycling in grassland ecosystems driven by stoichiometric homeostasis of soil microorganisms (Figure 16). Acquisition of N via non-symbiotic N_2 fixation likely decreases under elevated N inputs, whereas N-acquiring enzymes (in this case LAP) might not respond. The microbial release of N via net N mineralization might increase. With increasing N inputs, P solubilization from inorganic P might decrease paralleled by an increase in organic P mineralization changing P cycling in grassland soils. Even though soil microbial CUE might remain unaffected by increasing N inputs, C uptake and microbial respiration and thus soil CO_2 emissions might decrease (Figure 16). The microbial community composition likely shifts towards more copiotrophic organisms, whereas rather element inputs than changes in microbial

communities might drive element cycling. This preliminary framework might help to predict how grassland ecosystems respond to elevated nutrient inputs. However, it needs to be considered that our results are based on six grasslands sites and that added N amounts were higher than actual atmospheric N deposition in most regions of the world.

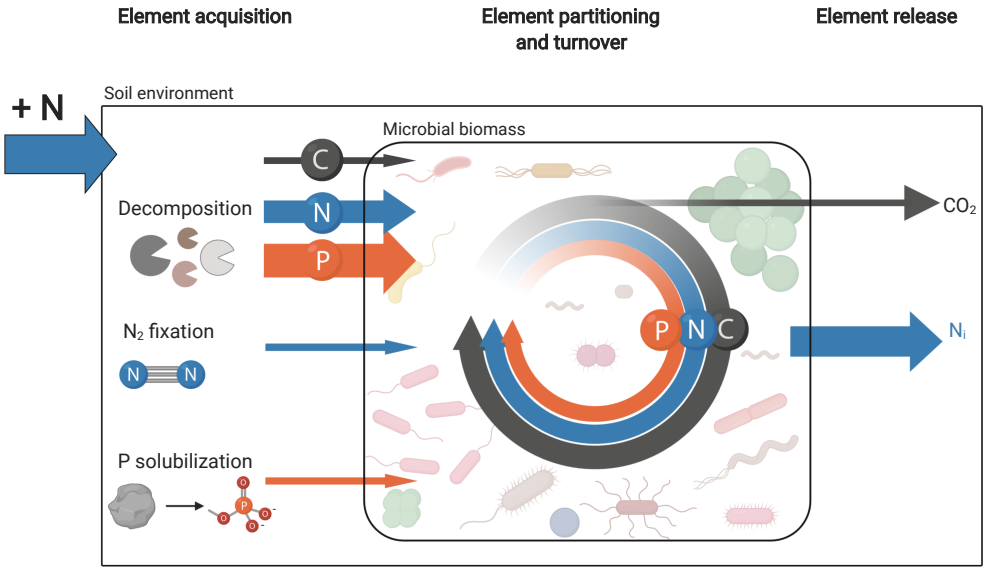


Figure 16: Conceptual framework on how N inputs can affect processes of element acquisition, partitioning, turnover, and release in grassland soils based on the results of this work (compare with Figure 1, changed from Spohn, 2016, created with BioRender.com).

To develop a detailed conceptual framework of soil element cycling in grasslands under nutrient addition, more studies are necessary to enhance the statistical power and to test the effects of nutrient addition in further grassland ecosystems. To understand the contribution of the different processes regulating biomass stoichiometry and to further understand the relevance of stoichiometric homeostasis for element cycling, modelling of the different processes using path models would be helpful. In addition, studying long-term nutrient addition experiments would enable to apply the concept to plant and microbial communities adapted to changed element availabilities over a long period of time. Moreover, it is worth studying element cycling related to stoichiometric homeostasis of microorganisms not only in grasslands but also in other terrestrial ecosystems such as forests, deserts, etc. and to compare ecosystem behavior.

The concept of Ecological Stoichiometry may contribute to the highly topical challenge of predicting soil CO_2 emissions and nutrient availabilities for plant nutrition since it enables a more profound understanding of drivers of element cycling on a global scale. Thus, it should be implemented in biogeochemical or climate models and might, in general, support predictions of ecological responses to global change.

1.6 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.

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.

Bardgett, R.D., Freeman, C., Ostle, N.J., 2008. Microbial contributions to climate change through carbon cycle feedbacks. The ISME Journal 2, 805–814.

Blazewicz, S.J., Schwartz, E., 2011. Dynamics of 18O incorporation from H218O 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.

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.

Calvaruso, C., Turpault, M.-P., Leclerc, E., Frey-Klett, P., 2007. Impact of ectomycorrhizosphere on the functional diversity of soil bacterial and fungal communities from a forest stand in relation to nutrient mobilization processes. Microbial Ecology 54, 567–577.

Chelius, M.K., Triplett, E.W., 2001. The Diversity of Archaea and Bacteria in Association with the Roots of Zea mays L. Microbial Ecology 41, 252–263.

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, H., Li, D., Zhao, J., Xiao, K., Wang, K., 2018. Effects of nitrogen addition on activities of soil nitrogen acquisition enzymes:A meta-analysis. Agriculture, Ecosystems & Environment 252, 126–131.

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.

Cleland, E.E., Harpole, W.S., 2010. Nitrogen enrichment and plant communities. Annals of the New York Academy of Sciences 1195, 46–61.

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.

Colman, B.P., Schimel, J.P., 2013. Drivers of microbial respiration and net N mineralization at the continental scale. Soil Biology and Biochemistry 60, 65–76.

Conant, R.T., 2010. Challenges and opportunities for carbon sequestration in grassland systems. A technical report on grassland management and climate change mitigation. Food and Agriculture Organization of the United Nations, Rome.

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.

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.

de Boer, W., Folman, L.B., Summerbell, R.C., Boddy, L., 2005. Living in a fungal world: impact of fungi on soil bacterial niche development. FEMS Microbiology Reviews 29, 795–811.

del Giorgio, P.A., Cole, J.J., 1998. Bacterial Growth Efficiency in Natural Aquatic Systems. Annual Review of Ecology and Systematics 29, 503–541.

Delgado-Baquerizo, M., Giaramida, L., Reich, P.B., Khachane, A.N., Hamonts, K., Edwards, C., Lawton, L.A., Singh, B.K., Brophy, C., 2016. Lack of functional redundancy in the relationship between microbial diversity and ecosystem functioning. Journal of Ecology 104, 936–946.

Demoling, F., Figueroa, D., Bååth, E., 2007. Comparison of factors limiting bacterial growth in different soils. Soil Biology and Biochemistry 39, 2485–2495.

Dentener, F., Drevet, J., Lamarque, J.F., Bey, I., Eickhout, B., Fiore, A.M., Hauglustaine, D., Horowitz, L.W., Krol, M., Kulshrestha, U.C., Lawrence, M., Galy-Lacaux, C., Rast, S., Shindell, D., Stevenson, D., van Noije, T., Atherton, C., Bell, N., Bergman, D., Butler, T., Cofala, J., Collins, B., Doherty, R., Ellingsen, K., Galloway, J., Gauss, M., Montanaro, V., Müller, J.F., Pitari, G., Rodriguez, J., Sanderson, M., Solmon, F., Strahan, S., Schultz, M., Sudo, K., Szopa, S., Wild, O., 2006. Nitrogen and sulfur deposition on regional and global scales: A multimodel evaluation. Global Biogeochemical Cycles 20, GB4003.

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.

Dixon, R., Kahn, D., 2004. Genetic regulation of biological nitrogen fixation. Nature

Reviews. Microbiology 2, 621–631.

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.

Fierer, N., Jackson, R.B., 2006. The diversity and biogeography of soil bacterial communities. Proceedings of the National Academy of Sciences of the United States of America 103, 626–631.

Fierer, N., Lauber, C.L., Ramirez, K.S., Zaneveld, J., Bradford, M.A., Knight, R., 2012. Comparative metagenomic, phylogenetic and physiological analyses of soil microbial communities across nitrogen gradients. The ISME Journal 6, 1007–1017.

Galloway, J.N., Dentener, F.J., Capone, D.G., Boyer, E.W., Howarth, R.W., Seitzinger, S.P., Asner, G.P., Cleveland, C.C., Green, P.A., Holland, E.A., 2004. Nitrogen cycles: past, present, and future. Biogeochemistry 70, 153–226.

Galloway, J.N., Townsend, A.R., 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.

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.

Griffiths, B.S., Spilles, A., Bonkowski, M., 2012. C:N:P stoichiometry and nutrient limitation of the soil microbial biomass in a grazed grassland site under experimental P limitation or excess. Ecological Processes 1.

Gruber, N., Galloway, J.N., 2008. An Earth-system perspective of the global nitrogen cycle. Nature 451, 293–296.

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.

Hall, E.K., Maixner, F., Franklin, O., Daims, H., Richter, A., Battin, T., 2011. Linking Microbial and Ecosystem Ecology Using Ecological Stoichiometry: A Synthesis of Conceptual and Empirical Approaches. Ecosystems 14, 261–273.

Hameeda, B., Harish Kumar Reddy, Y., Rupela, O.P., Kumar, G.N., Reddy, G., 2006. Effect of carbon substrates on rock phosphate solubilization by bacteria from

composts and macrofauna. Current Microbiology 53, 298–302.

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.

Hedley, M.J., Stewart, J.W.B., Chauhan, B.S., 1982. Changes in inorganic and organic soil phosphorus fractions by cultivation practices and by laboratory incubations. Soil Science Society of America Journal 46, 970–976.

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.

Hessen, D.O., Anderson, T.R., 2008. Excess carbon in aquatic organisms and ecosystems: Physiological, ecological, and evolutionary implications. Limnology and 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.Hill, S., 1992. Physiology of nitrogen fixation in free-living heterotrophs. Biological nitrogen fixation, 87–134.

Hinsinger, P., 2001. Bioavailability of soil inorganic P in the rhizosphere as affected by root-induced chemical changes: a review. Plant and Soil, 173–195.

Hoekstra, J.M., Boucher, T.M., Ricketts, T.H., Roberts, C., 2005. Confronting a biome crisis: global disparities of habitat loss and protection. Ecology Letters 8, 23–29.

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.

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 ken value. Soil Biology and Biochemistry 28, 33–37.

Joergensen, R.G., Scheu, S., 1999. Response of soil microorganisms to the addition of carbon, nitrogen and phosphorus in a forest Rendzina. Soil Biology and Biochemistry 31, 859–866.

Jones, D.L., Oburger, E., 2011. Solubilization of Phosphorus by Soil Microorganisms, in: Bünemann, E.K., Oberson, A., Frossard, E. (Eds.), Phosphorus in Action.

Biological Processes in Soil Phosphorus Cycling. Springer, Berlin Heidelberg, pp. 169–198.

Kaiser, C., Franklin, O., Dieckmann, U., Richter, A., 2014. Microbial community dynamics alleviate stoichiometric constraints during litter decay. Ecology Letters 17, 680–690.

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.

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.

LeBauer, D.S., Treseder, K.K., 2008. Nitrogen limitation of net primary productivity in terrestrial ecosystems is globally distributed. Ecology 89, 371–379.

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.

Lepleux, C., Uroz, S., Collignon, C., Churin, J.-L., Turpault, M.-P., Frey-Klett, P., 2013. A short-term mineral amendment impacts the mineral weathering bacterial communities in an acidic forest soil. Research in microbiology 164, 729–739.

Liu, Y., Wang, C., He, N., Wen, X., Gao, Y., Li, S., Niu, S., Butterbach-Bahl, K., Luo, Y., Yu, G., 2017. A global synthesis of the rate and temperature sensitivity of soil nitrogen mineralization: latitudinal patterns and mechanisms. Global Change Biology 23, 455–464.

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.

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.

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.

Mander, C., Wakelin, S., Young, S., Condron, L., O'Callaghan, M., 2012. Incidence and diversity of phosphate-solubilising bacteria are linked to phosphorus status in grassland soils. Soil Biology and Biochemistry 44, 93–101.

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., Jackson, R.B., Trofymow, J.A., Porporato, A., 2008. The Global Stoichiometry of Litter Nitrogen Mineralization. Science (New York, N.Y.) 321, 684–686.

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.

Margalef, O., Sardans, J., Fernández-Martínez, M., Molowny-Horas, R., Janssens, I.A., Ciais, P., Goll, D., Richter, A., Obersteiner, M., Asensio, D., Peñuelas, J., 2017. Global patterns of phosphatase activity in natural soils. Scientific Reports 7, 1337.

Marklein, A.R., Houlton, B.Z., 2012. Nitrogen inputs accelerate phosphorus cycling rates across a wide variety of terrestrial ecosystems. The New Phytologist 193, 696–704.

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.

Mouginot, C., Kawamura, R., Matulich, K.L., Berlemont, R., Allison, S.D., Amend, A.S., Martiny, A.C., 2014. Elemental stoichiometry of Fungi and Bacteria strains from grassland leaf litter. Soil Biology and Biochemistry 76, 278–285.

Nakagawa, S., Schielzeth, H., 2013. A general and simple method for obtaining R2 from generalized linear mixed-effects models. Methods in Ecology and Evolution 4, 133–142.

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.

Nave, L.E., Vance, E.D., Swanston, C.W., Curtis, P.S., 2009. Impacts of elevated N inputs on north temperate forest soil C storage, C/N, and net N-mineralization. Geoderma 153, 231–240.

Nelson, M.B., Martiny, A.C., Martiny, J.B.H., 2016. Global biogeography of microbial nitrogen-cycling traits in soil. Proceedings of the National Academy of Sciences of the United States of America 113, 8033–8040.

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.

Nicolitch, O., Colin, Y., Turpault, M.-P., Uroz, S., 2016. Soil type determines the distribution of nutrient mobilizing bacterial communities in the rhizosphere of beech trees. Soil Biology and Biochemistry 103, 429–445.

Olander, L.P., Vitousek, P.M., 2000. Regulation of soil phosphatase and chitinase activity by N and P availability. Biogeochemistry, 175–190.

O'Mara, F.P., 2012. The role of grasslands in food security and climate change. Annals of Botany 110, 1263–1270.

Pastore, G., Kaiser, K., Kernchen, S., Spohn, M., 2020. Microbial release of apatite- and goethite-bound phosphate in acidic forest soils. Geoderma 370, 114360.

Patel, D.K., Archana, G., Kumar, G.N., 2008. Variation in the nature of organic acid secretion and mineral phosphate solubilization by Citrobacter sp. DHRSS in the presence of different sugars. Current Microbiology 56, 168–174.

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.

Peršoh, D., Melcher, M., Flessa, F., Rambold, G., 2010. First fungal community analyses of endophytic ascomycetes associated with Viscum album ssp. austriacum and its host Pinus sylvestris. Fungal Biology 114, 585–596.

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.

Pikovskaya, R.I., 1948. Mobilization of phosphorus in soil in connection with the vital activity of some microbial species. Mikrobiologiya 17, 362–370.

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.

Redfield, A.C., 1958. The Biological Control of Chemical Factors in the Environment. American Scientist 46, 205–221.

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., 2010. 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.

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.

Saunders, W.M.H., Williams, E.G., 1955. Observations on the determination of total organic phosphorus in soils. Journal of Soil Science 6, 254–267.

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.

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.

Schloss, P.D., Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M., Hollister, E.B., Lesniewski, R.A., Oakley, B.B., Parks, D.H., Robinson, C.J., Sahl, J.W., Stres, B., Thallinger, G.G., van Horn, D.J., Weber, C.F., 2009. Introducing mothur: Open-source, platform-independent, community-supported software for describing and comparing microbial communities. Applied and Environmental Microbiology 75, 7537–7541.

Schwartz, E., 2007. Characterization of growing microorganisms in soil by stable isotope probing with H218O. Applied and Environmental Microbiology 73, 2541–2546.

Sinsabaugh, R.L., Follstad Shah, J.J., 2012. Ecoenzymatic Stoichiometry and Ecological Theory. Annual Review of Ecology, Evolution, and Systematics 43, 313–343.

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.

Smith, V.H., 1992. Effects of nitrogen: phosphorus supply ratios on nitrogen fixation in agricultural and pastoral ecosystems. Biogechemistry, 19–35.

Spohn, M., 2015. Microbial respiration per unit microbial biomass depends on litter layer carbon-to-nitrogen ratio. Biogeosciences 12, 817–823.

Spohn, M., 2016. Element cycling as driven by stoichiometric homeostasis of soil microorganisms. Basic and Applied Ecology 17, 471–478.

Spohn, M., Chodak, M., 2015. Microbial respiration per unit biomass increases with carbon-to-nutrient ratios in forest soils. Soil Biology and Biochemistry 81, 128–133.

Spohn, M., Klaus, K., Wanek, W., Richter, A., 2016. Microbial carbon use efficiency and biomass turnover times depending on soil depth – Implications for carbon cycling. Soil Biology and Biochemistry 96, 74–81.

Sterner, R.W., Elser, J.J., 2002. Ecological stoichiometry: The biology of elements from molecules to the biosphere. Princeton University Press.

Stevens, C.J., Lind, E.M., Hautier, Y., Harpole, W.S., Borer, E.T., Hobbie, S., Seabloom, E.W., Ladwig, L., Bakker, J.D., Chu, C., Collins, S., Davies, K.F., Firn, J., Hillebrand, H., La Pierre, K.J., MacDougall, A., Melbourne, B., McCulley, R.L., Mor-

gan, J., Orrock, J.L., Prober, S.M., Risch, A.C., Schuetz, M., Wragg, P.D., 2015. Anthropogenic nitrogen deposition predicts local grassland primary production worldwide. Ecology 96, 1459–1465.

Strickland, M.S., Lauber, C.L., Fierer, N., Bradford, M.A., 2009. Testing the functional significance of microbial community composition. Ecology 90, 441–451.

Stursova, M., Crenshaw, C.L., Sinsabaugh, R.L., 2006. Microbial responses to long-term N deposition in a semiarid grassland. Microbial Ecology 51, 90–98.

Sul, W.J., Asuming-Brempong, S., Wang, Q., Tourlousse, D.M., Penton, C.R., Deng, Y., Rodrigues, J.L.M., Adiku, S.G.K., Jones, J.W., Zhou, J., Cole, J.R., Tiedje, J.M., 2013. Tropical agricultural land management influences on soil microbial communities through its effect on soil organic carbon. Soil Biology and Biochemistry 65, 33–38.

Suttie, J.M., Reynolds, S.G., Batello, C., 2005. Grasslands of the world. Food and Agriculture Organization of the United Nations, Rome.

Tiessen, H., Moir, J.O., 2007. Characterization of Available P by Sequential Extraction, in: Carter, M.R., Gregorich, E.G. (Eds.), Soil sampling and methods of analysis, 2nd ed. Taylor & Francis Group, Boca Raton, pp. 293–306.

Vance, E.D., Brookes, P.C., Jenkinson, D.S., 1987. An extraction method for measuring soil microbial biomass C. Soil Biology and Biochemistry 19, 703–707.

Vestgarden, L.S., Selle, L.T., Stuanes, A.O., 2003. In situ soil nitrogen mineralisation in a Scots pine (Pinus sylvestris L.) stand: effects of increased nitrogen input. Forest Ecology and Management 176, 205–216.

Vitousek, P.M., Cassman, K., Cleveland, C., Crews, T., Field, C.B., Grimm, N.B., Howarth, R.W., Marino, R., Martinelli, L., Rastetter, E.B., Sprent, J.I., 2002. Towards an Ecological Understanding of Biological Nitrogen Fixation. Biogeochemistry 57/58, 1–45.

Vitousek, P.M., Porder, S., Houlton, B.Z., Chadwick, O.A., 2010. Terrestrial phosphorus limitation: mechanisms, implications, and nitrogen-phosphorus interactions. Ecological Applications 20, 5–15.

Vourlitis, G.L., Zorba, G., Pasquini, S.C., Mustard, R., 2007. Chronic Nitrogen Deposition Enhances Nitrogen Mineralization Potential of Semiarid Shrubland Soils. Soil Science Society of America Journal 71, 836.

Walker, J.J., Pace, N.R., 2007. Phylogenetic composition of Rocky Mountain endolithic microbial ecosystems. Applied and Environmental Microbiology 73, 3497–3504.

Walker, T.W., Adams, A.F.R., 1958. Studies on soil organic matter: I. Influence of phosphorus content of parent materials on accumulations of carbon, nitrogen, sulfur, and organic phosphorus in grassland soils. Soil Science 85, 307–318.

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.

Weedon, J.T., Cornwell, W.K., Cornelissen, J.H.C., Zanne, A.E., Wirth, C., Coomes,

D.A., 2009. Global meta-analysis of wood decomposition rates: a role for trait variation among tree species? Ecology Letters 12, 45–56.

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, R.P., Murray, S., Rohweder, M., Prince, S.D., Thompson, K.M., 2000. Grassland ecosystems. World Resources Institute Washington, DC.

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.

Xia, J., Wan, S., 2008. Global response patterns of terrestrial plant species to nitrogen addition. The New Phytologist 179, 428–439.

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.

Yuan, Z., Chen, H.Y.H., 2009. Global trends in senesced-leaf nitrogen and phosphorus. Global Ecology and Biogeography 18, 532–542.

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.

Zechmeister-Boltenstern, S., Keiblinger, K.M., Mooshammer, M., Peñuelas, J., Richter, A., Sardans, J., Wanek, W., 2015. The application of ecological stoichiometry to plant–microbial–soil organic matter transformations. Ecological Monographs 85, 133–155.

Zeglin, L.H., Stursova, M., Sinsabaugh, R.L., Collins, S.L., 2007. Microbial responses to nitrogen addition in three contrasting grassland ecosystems. Oecologia 154, 349–359.

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.

Zhang, X., Wang, Q., Gilliam, F.S., Bai, W., Han, X., Li, L., 2012. Effect of nitrogen fertilization on net nitrogen mineralization in a grassland soil, northern China. Grass and Forage Science 67, 219–230.

2 Manuscripts

2.1 Study I

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

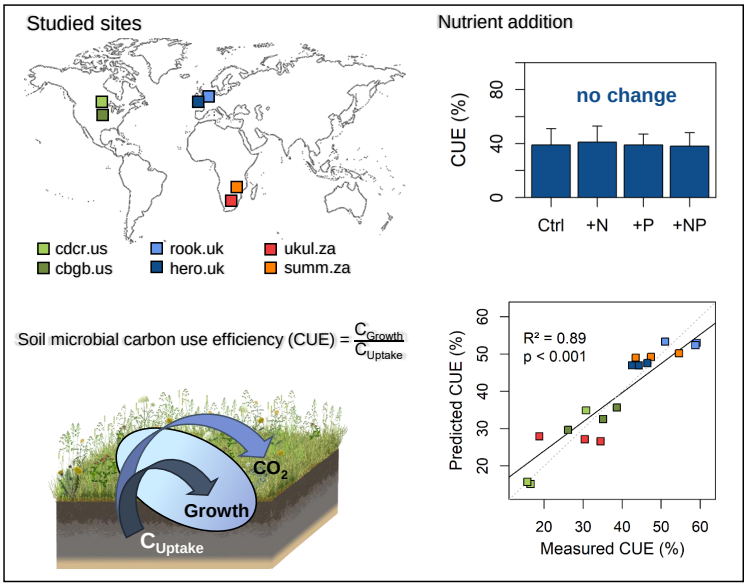
Meike Widdig, Per-M. Schleuss, Lori A. Biederman, Elizabeth T. Borer, Michael J. Crawley, Kevin P. Kirkman, Eric W. Seabloom, Peter D. Wragg, Marie Spohn (2020),

published in *Soil Biology and Biochemistry* 146, 107815,
DOI: https://doi.org/10.1016/j.soilbio.2020.107815

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.

Graphical Abstract



2.1.1 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; 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; Sinsabaugh et al., 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 18O 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.

2.1.2 Materials and Methods

2.1.2.1 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 (Table 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.

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[1,2]	*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[2](%)	94	74	45	104	116	120
Forb cover[2](%)	21	42	61	26	44	56
Legume cover[2](%)	1	6	0	19	12	10
ANPP[2](g m^{-2} yr^{-1})	178	397	180	509	498	349

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

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 x 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 ($Ca(H_2PO_4)_2$).

2.1.2.2 Sampling and Sample Preparation

Soils were sampled in two depth increments, from 0-15 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 one 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.

2.1.2.3 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 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).

2.1.2.4 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 hours) and respiration rates were calculated based on the linear increase in accumulated C-CO_2 over time (Heuck and Spohn, 2016).

2.1.2.5 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; Spohn et al., 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):

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

2.1.2.6 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).

2.1.2.7 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.

2.1.2.8 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-methyl-umbelliferyl-phosphate, 4-methylumbelliferyl-β-D-glucoside, 4-methyl-umbelliferyl-N-acetyl-β-D-glucosaminide, and L-leucine-7-amino-4-methylcoumarine 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.

2.1.2.9 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, 2018).

2.1.3 Results

2.1.3.1 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 (Table 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.

2.1.3.2 Carbon use efficiency

Addition of N, P, and NP did not significantly change CUE across all grassland soils at either depth increment (Figure 1, Table S1). Soil microbial CUE ranged between 25% at one site in the USA and 57% at a UK site (Figure 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 (Figure 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 ($p < 0.001$, Figure 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 (Figure 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 control plots was highly significant ($R^2 = 0.71$, $p < 0.001$, Figure S1).

2.1.3.3 Microbial biomass turnover time

Nutrient addition (N, P, and NP) did not significantly affect microbial biomass turnover time at either depth increment (Figure 1c, 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 (Figure 2b).

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[1]	9.4 ± 1.1a	15.7 ± 8.8a	9.0 ± 0.3a	11.0 ± 3.4a	5.2 ± 1.1a	10.4 ± 7.8a	4.4 ± 0.5a*	5.8 ± 2.1a
	cbgb.us	7.2 ± 2.8a	8.2 ± 0.5a	6.9 ± 2.3a	7.4 ± 2.4a	4.1 ± 0.5a*	5.1 ± 0.2a*	4.0 ± 1.2a	4.1 ± 0.9a*
	hero.uk	36.7 ± 6.8a	36.7 ± 6.1a	36.5 ± 1.8a	37.0 ± 7.7a	24.4 ± 3.5a*	24.5 ± 4.2a*	25.6 ± 4.7a*	23.9 ± 4.4a*
	rook.uk	24.3 ± 2.6a	28.7 ± 3.2a	26.9 ± 1.2a	24.9 ± 2.2a	10.5 ± 1.5a*	12.8 ± 1.3a*	11.6 ± 1.3a*	10.1 ± 0.7a*
	ukul.za	42.0 ± 2.0a	42.5 ± 0.8a	44.4 ± 0.5a	45.7 ± 0.8a	37.5 ± 3.6a	3.2.0 ± 4.4a*	34.8 ± 5.3a*	36.4 ± 0.6a*
	summ.za[2]	49.1 ± 3.0a	51.1 ± 2.2a	51.7 ± 3.0a	51.7 ± 1.9a	NA	NA	NA	NA
TN (g N kg^{-1})	cdcr.us[1]	0.7 ± 0.1a	1.1 ± 0.6a	0.6 ± 0.1a	0.8 ± 0.3a	0.3 ± 0.1a*	0.7 ± 0.5a	0.3 ± 0.1a	0.4 ± 0.1a*
	cbgb.us	0.6 ± 0.2a	0.8 ± 0.1a	0.6 ± 0.2a	0.7 ± 0.2a	0.4 ± 0.1a*	0.5 ± 0.1a*	0.4 ± 0.1a*	0.4 ± 0.2a*
	hero.uk	3.1 ± 0.7a	3.1 ± 0.5a	3.0 ± 0.2a	3.1 ± 0.8a	2.1 ± 0.3a*	2.1 ± 0.3a	2.1 ± 0.4a	2.0 ± 0.4a*
	rook.uk	2.1 ± 0.2ab	2.4 ± 0.3b	2.2 ± 0.1ab	2.0 ± 0.1a	1.0 ± 0.1a*	1.2 ± 0.1a**	1.0 ± 0.2a*	0.9 ± 0.1a*
	ukul.za	2.9 ± 0.3a	3.1 ± 0.3ab	3.3 ± 0.1ab	3.4 ± 0.1b	2.6 ± 0.2a	2.4 ± 0.2a*	2.6 ± 0.3a*	2.7 ± 0.1a*
	summ.za[2]	2.8 ± 0.2a	3.0 ± 0.4a	2.9 ± 0.3a	3.0 ± 0.1a	NA	NA	NA	NA
TP (g P kg^{-1})	cdcr.us[1]	0.31 ± 0.03a	0.46 ± 0.24a	0.58 ± 0.09a	0.56 ± 0.11a	0.24 ± 0.03a	0.40 ± 0.19a	0.36 ± 0.04a	0.38 ± 0.06a
	cbgb.us	0.28 ± 0.02a	0.24 ± 0.05a	0.43 ± 0.04b	0.41 ± 0.06b	0.24 ± 0.03a	0.24 ± 0.02a	0.34 ± 0.03b*	0.35 ± 0.07b*
	hero.uk	0.62 ± 0.16a	0.57 ± 0.07a	0.93 ± 0.13a	0.96 ± 0.28a	0.54 ± 0.15a	0.48 ± 0.06a	0.55 ± 0.17a*	0.62 ± 0.25a*
	rook.uk	0.38 ± 0.01a	0.38 ± 0.04a	0.60 ± 0.06b	0.61 ± 0.14b	0.27 ± 0.01a*	0.31 ± 0.04a	0.32 ± 0.03a*	0.31 ± 0.04a*
	ukul.za[1]	0.45 ± 0.02a	0.46 ± 0.08a	1.33 ± 0.12b	1.20 ± 0.15b	0.39 ± 0.02a	0.38 ± 0.04a	0.74 ± 0.25b*	0.58 ± 0.01b*
	summ.za[2]	0.37 ± 0.01a	0.49 ± 0.18a	0.60 ± 0.33a	0.83 ± 0.7a	NA	NA	NA	NA
pH in H_2O	cdcr.us	5.27 ± 0.09b	4.70 ± 0.17a	5.27 ± 0.10b	4.84 ± 0.10a	5.36 ± 0.10b	5.17 ± 0.19ab*	5.45 ± 0.23b	4.96 ± 0.23a
	cbgb.us	5.73 ± 0.50a	5.68 ± 0.72a	5.86 ± 0.47a	5.72 ± 0.43a	5.40 ± 0.66a	5.56 ± 0.92a	5.58 ± 0.79a	5.34 ± 0.63a
	hero.uk	5.12 ± 0.21a	5.18 ± 0.11a	5.08 ± 0.08a	5.09 ± 0.15a	5.24 ± 0.30a	5.30 ± 0.09a	5.20 ± 0.15a	5.22 ± 0.10a
	rook.uk	3.76 ± 0.04a	3.78 ± 0.02a	3.91 ± 0.02a	3.87 ± 0.03a	4.10 ± 0.13a*	4.08 ± 0.09a*	4.12 ± 0.10a*	4.06 ± 0.06a*
	ukul.za	5.89 ± 0.08a	5.58 ± 0.42a	5.94 ± 0.09a	5.63 ± 0.11a	5.83 ± 0.10a	5.79 ± 0.33a	5.72 ± 0.16a	5.62 ± 0.16a
	summ.za[2]	5.20 ± 0.04a	5.03 ± 0.09a	5.01 ± 0.12a	4.97 ± 0.13a	NA	NA	NA	NA

[1] Data were LOG10 transformed
[2] 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})	ukul.za	115 ± 7a	108 ± 15a	127 ± 6a	152 ± 34a	127 ± 33a	201 ± 52ab*	238 ± 14b*	217 ± 66b*
	summ.za[1]	98 ± 3a	98 ± 9a	102 ± 10a	100 ± 1a	NA	NA	NA	NA
	cdcr.us	15 ± 0.4a	20 ± 4.8b	20 ± 1.1b	24 ± 2.6b	11 ± 0.6a	12 ± 2.0a*	12 ± 0.3a*	15 ± 1.3a*
	cbgb.us	18 ± 2.7a	22 ± 4.1a	18 ± 2.1a	22 ± 1.6a	15 ± 1.6a	22 ± 3.4a	20 ± 5.6a	20 ± 1.6a
	rook.uk	23 ± 2.7a	29 ± 7.5ab	44 ± 8.3c	36 ± 3.5bc	15 ± 1.5a	16 ± 3.1a*	19 ± 1.0a*	18 ± 1.0a*
	hero.uk	29 ± 2.6a	30 ± 3.4a	36 ± 1.2ab	40 ± 4.1b	19 ± 1.2a*	22 ± 4.2a*	23 ± 3.0a*	25 ± 3.9a*
DN (mg N kg^{-1})	ukul.za	7.3 ± 0.3a	43.2 ± 11.2b	9.9 ± 5.0a	34.4 ± 5.7b	5.5 ± 1.1a	17.3 ± 4.9ab*	14.1 ± 3.3ab	19.5 ± 5.3b*
	summ.za[1]	5.0 ± 0.1a	19.8 ± 5.0b	5.1 ± 0.2a	15.9 ± 3.1b	NA	NA	NA	NA
	cdcr.us[2]	3.2 ± 4.4a	32.9 ± 15.6b	2.7 ± 2.1a	13.4 ± 2.9b	1.8 ± 1.5a	10.0 ± 1.0b	2.1 ± 1.5a	7.0 ± 2.1ab
	cbgb.us	4.1 ± 3.1a	12.0 ± 1.4b	4.7 ± 1.9a	12.1 ± 3.9b	2.6 ± 2.2a	5.0 ± 2.8a*	2.9 ± 1.1a	4.8 ± 2.1a*
	rook.uk	14.2 ± 2.7ab	14.7 ± 3.2b	10.9 ± 0.4ab	10.3 ± 1.2a	4.2 ± 2.4a*	3.8 ± 1.0a*	2.9 ± 0.8a*	4.0 ± 0.8a*
	hero.uk	12.9 ± 3.6a	17.1 ± 3.9a	11.1 ± 3.5a	14.6 ± 8.7a	9.7 ± 3.8a	10.3 ± 0.8a	11.9 ± 4.4a	8.9 ± 3.2a
DOC:DN ratio	ukul.za	18.2 ± 0.7b	3.0 ± 0.5a	17.8 ± 7.3b	5.3 ± 1.2a	27.0 ± 1.5b*	14.8 ± 5.5a*	20.3 ± 3.5ab	13.0 ± 1.4a*
	summ.za[1]	22.9 ± 0.6b	6.0 ± 1.1a	23.2 ± 1.6b	7.5 ± 1.2a	NA	NA	NA	NA
	cdcr.us[3]	18.1 ± 11.3b	0.8 ± 0.1a	12.0 ± 6.1b	2.1 ± 0.2a	10.9 ± 3.3b	1.4 ± 0.1a*	9.6 ± 4.7b	2.7 ± 0.6ab
	cbgb.us	10.8 ± 3.0b	2.1 ± 0.2a	4.9 ± 1.5ab	2.3 ± 0.6a	10.0 ± 4.7a	5.9 ± 1.6a	8.8 ± 3.5a	5.6 ± 1.9a
	rook.uk[2]	2.0 ± 0.5a	2.3 ± 0.3ab	4.6 ± 0.6c	4.0 ± 0.1bc	5.1 ± 1.9a*	4.8 ± 0.2a*	8.1 ± 1.7a*	5.4 ± 1.1a
	hero.uk	2.7 ± 0.4a	2.1 ± 0.3a	4.0 ± 1.1a	4.2 ± 2.3a	2.6 ± 1.0a	2.5 ± 0.2a	2.5 ± 0.6a	3.5 ± 0.7a
DIP (mg P kg^{-1})	ukul.za[2]	0.10 ± 0.07a	0.12 ± 0.04a	1.43 ± 0.19b	1.10 ± 0.25b	0.13 ± 0.05a	0.05 ± 0.05a*	2.09 ± 1.58b	0.92 ± 0.24b
	summ.za[1,2]	0.05 ±0.04a	0.10 ± 0.02ab	0.25 ± 0.09b	0.27 ± 0.12b	NA	NA	NA	NA
	cdcr.us[2]	0.16 ± 0.12a	0.13 ± 0.06a	13.37 ± 2.76b	12.29 ± 3.55b	0.06 ± 0.03a*	0.04 ± 0.01a*	4.19 ± 0.55b*	5.01 ± 0.89b*
	cbgb.us[2]	0.37 ± 0.08a	0.25 ± 0.19a	7.67 ± 1.23b	11.1 ± 3.18b	0.13 ± 0.08a*	0.11 ± 0.05a	7.02 ± 3.11b	6.96 ± 1.91b
	rook.uk[2]	0.02 ± 0.01a	0.03 ± 0.01a	1.25 ± 0.88b	1.22 ± 1.20b	0.02 ± 0.01a	0.02 ± 0.01a	0.04 ± 0.03a*	0.06 ± 0.02a*
	hero.uk[2]	0.04 ± 0.01a	0.04 ± 0.01a	1.64 ± 0.85b	1.15 ± 0.72b	0.02 ± 0.01a*	0.03 ± 0.01ab	0.04 ± 0.01ab*	0.06 ± 0.01b*
MBC (mg C kg^{-1})	ukul.za	1005 ± 134a	767 ± 196a	819 ± 157a	852 ± 71a	488 ± 93a*	444 ± 85a*	502 ± 113a*	549 ± 66a*
	summ.za[1]	843 ± 133a	769 ± 111a	928 ± 278a	699 ± 16a	NA	NA	NA	NA
	cdcr.us	163 ± 63a	235 ± 44a	193 ± 9a	169 ± 99a	119 ± 103a	100 ± 47a*	46 ± 31a*	134 ± 124a
	cbgb.us	175 ± 61a	121 ± 23a	168 ± 51a	148 ± 100a	61 ± 15a*	119 ± 35a	69 ± 43a	142 ± 118a
	rook.uk	651 ± 35a	588 ± 49a	507 ± 210a	746 ± 88a	351 ± 46a*	317 ± 94a*	248 ± 176a*	342 ± 248a*
	hero.uk	662 ± 116a	603 ± 70a	638 ± 51a	591 ± 85a	526 ± 8b	363 ± 61ab*	298 ± 73a*	352 ± 76ab*

[1] One-Way ANOVA and Tukey test were performed
[2] Data were LOG10 transformed
[3] Data were reciprocally transformed (1/x)

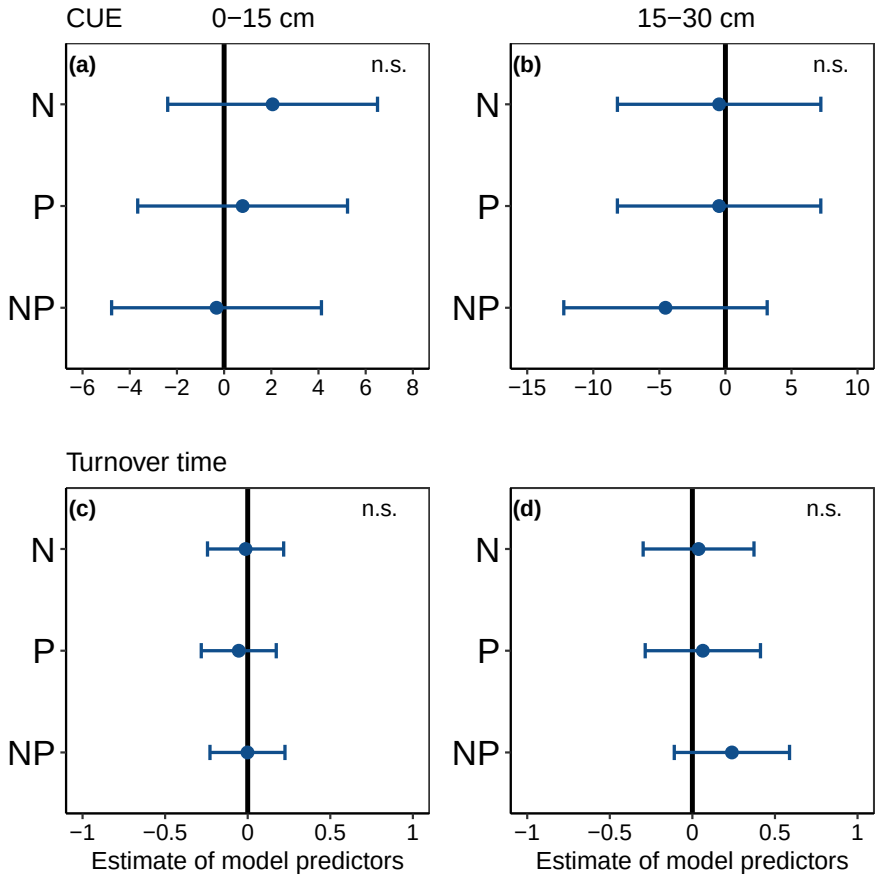


Figure 1: Effect of nutrient addition (N, P, NP) on (a, b) microbial carbon use efficiency (CUE) and (c, d) microbial biomass turnover across all six sites in (a, c) 0-15 cm depth and (b, d) 15-30 cm depth. The vertical intercept (position zero) corresponds to the control. Linear mixed-effects models 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.

2.1.3.4 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 (Figure S3). Similarly, the bacterial and fungal communities of all sites were significantly different from each other (Figure 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 at each site (data not shown) and across all sites (Figure S5).

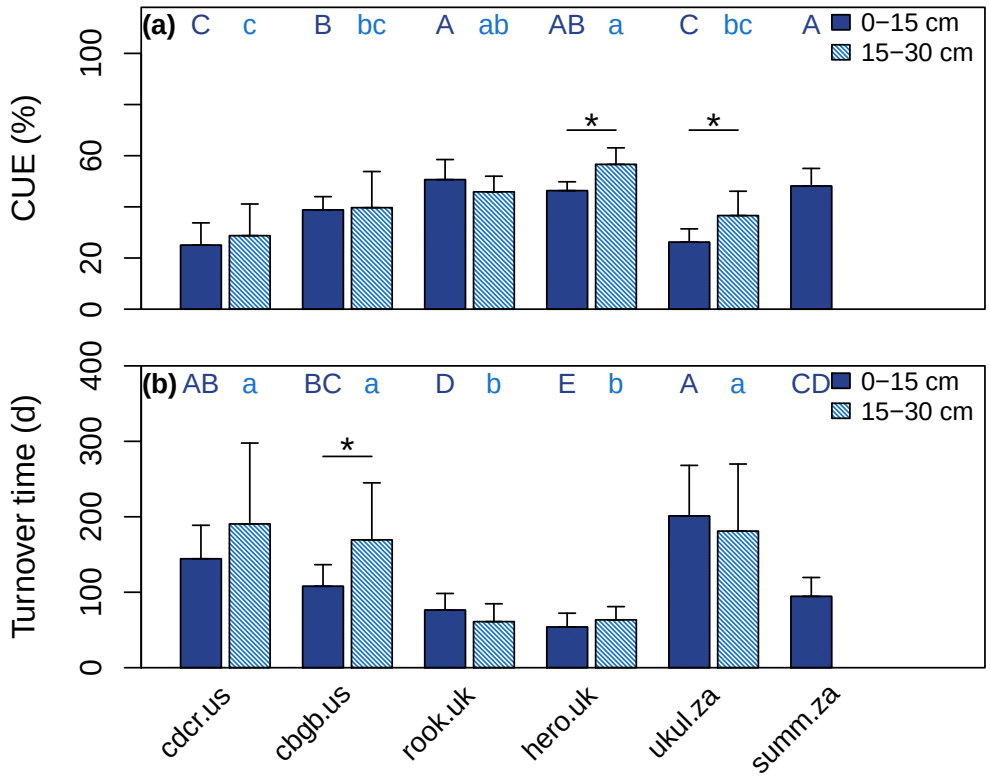


Figure 2: Mean (a) carbon use efficiency (CUE) and (b) microbial biomass turnover time of all treatments at six grassland sites in two soil depth increments across all treatments. Error bars indicate standard deviations (n = 12). Upper-case letters indicate significant differences between sites tested 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.

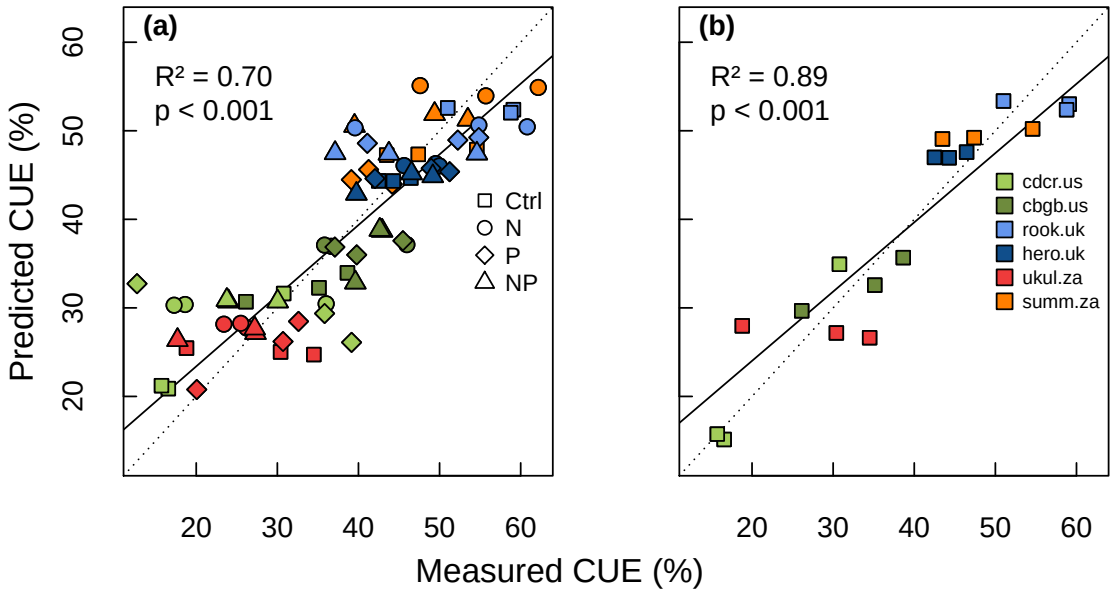


Figure 3: Linear mixed-effects model of (a) carbon use efficiency (CUE) in all treatments and (b) CUE in the control at all six grassland sites in 0-15 cm depth. Measured CUE is shown on the x-axis and predicted CUE is shown on the y-axis. Best model predictors were sand content, mean annual precipitation, 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 (2013), the standard line is dashed (intercept = 0, slope = 1), and the fitted line of the model is solid.

2.1.3.5 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 (Figure 5a). Addition of P and NP did not change mean topsoil microbial respiration. Additionally, microbial growth (Fig 5c, 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 (Figure 5e). In the second depth increment, C uptake did not change significantly in response to nutrient addition (Figure 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 (Figure 6a). Microbial growth in the topsoil was lowest at a site in the USA and highest at a South African site (Figure 6b). Microbial growth and respiration were positively correlated ($R^2 = 0.45$, $p < 0.001$, Figure 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 (Figure 6c). Microbial respiration, growth, and C uptake of all treatments were significantly higher in topsoils compared to subsoils (Figure 6).

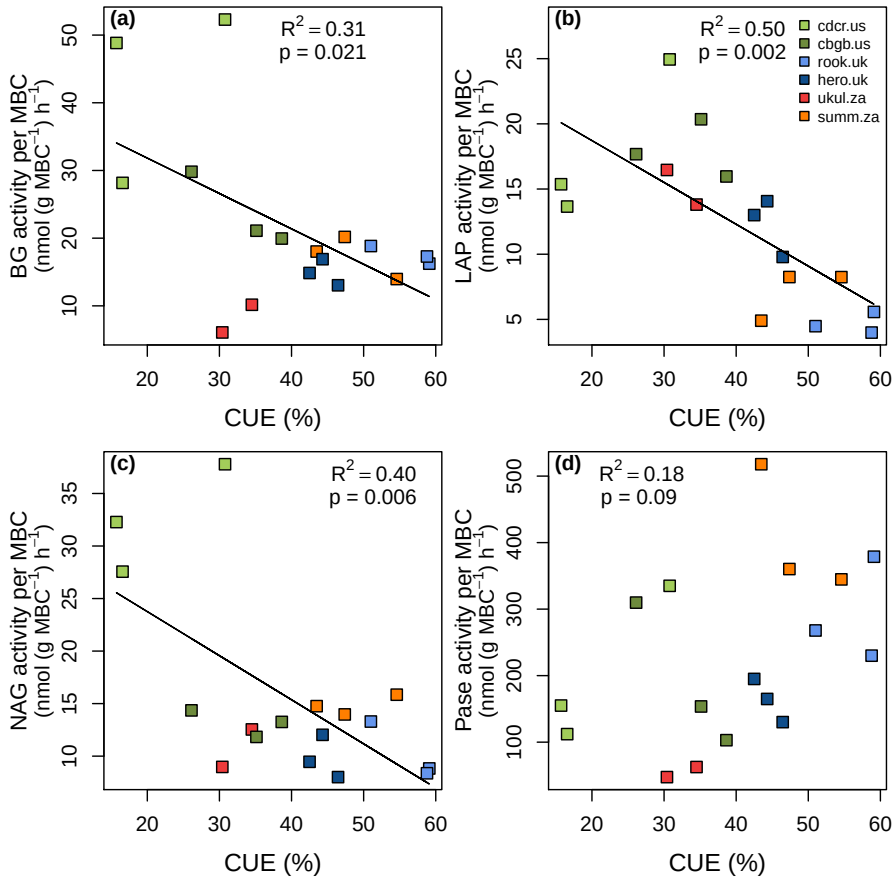


Figure 4: Correlation of (a) beta-glucosidase (BG) activity, (b) leucine-aminopeptidase (LAP) activity, (c) N-acetylglucosaminidase (NAG) activity, and (d) phosphatase (Pase) activity per unit microbial biomass carbon (MBC) with microbial carbon use efficiency (CUE) in 0-15 cm depth in the control treatments.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

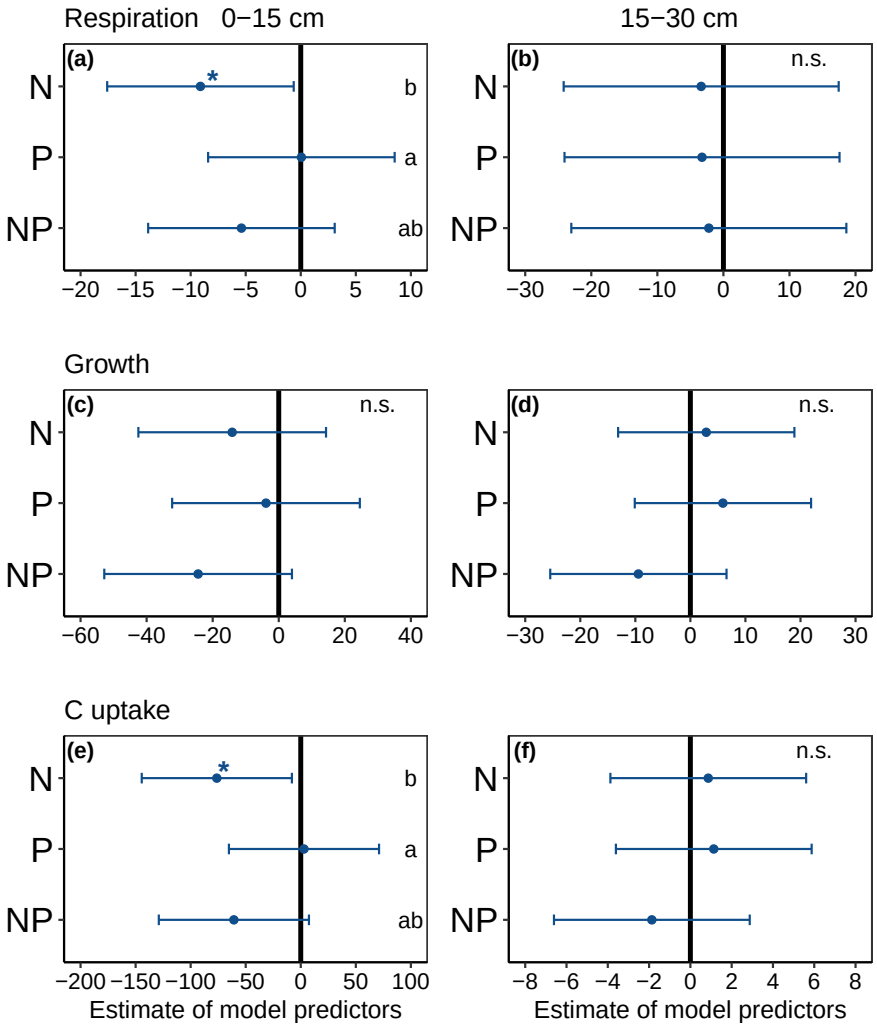


Figure 5: Effect of nutrient addition (N, P, NP) on (a, b) soil microbial respiration, (c, d) microbial growth, and (e, f) microbial C uptake across all six sites in (a, c, e) 0-15 cm depth and (b, d, f) 15-30 cm depth. Linear mixed-effects models were calculated with treatment as fixed and site intercepts 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.

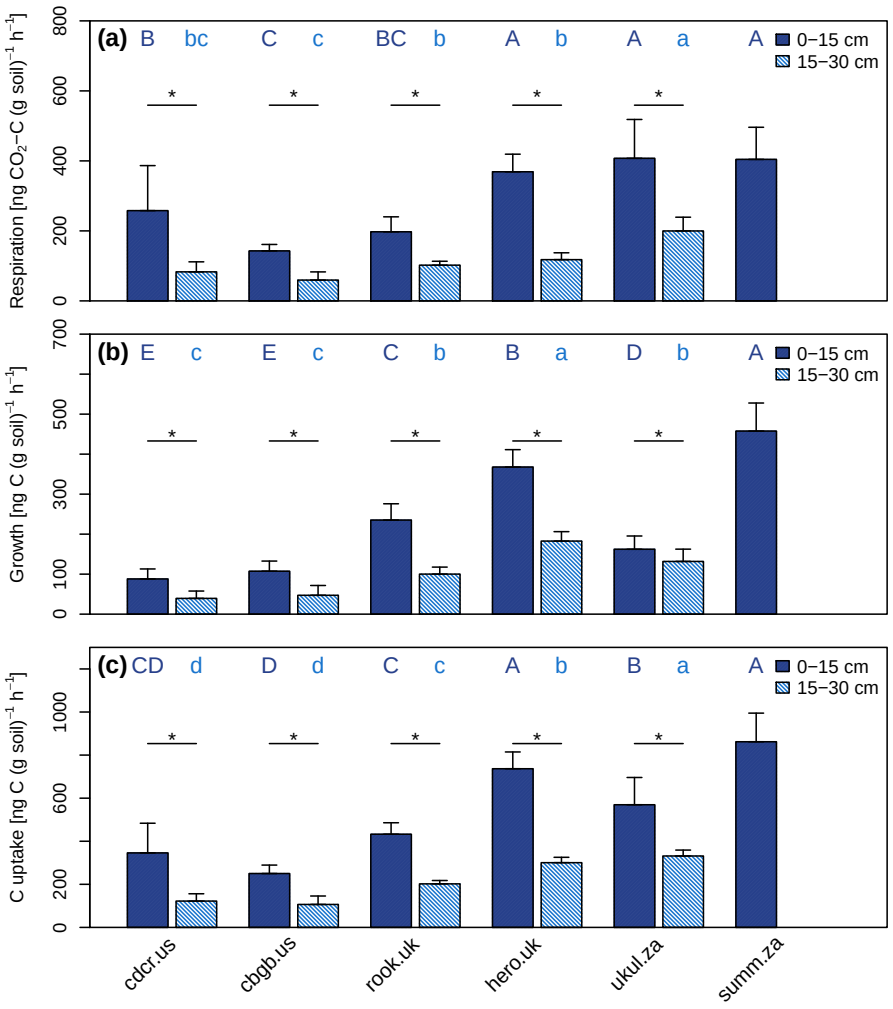


Figure 6: Mean (a) microbial respiration, (b) growth, and (c) carbon (C) uptake of all treatments at the six sites in two depth increments. Error bars indicate standard deviations (n = 12). Upper-case letters indicate significant differences between sites tested separately for 0-15 cm depth. Lower-case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

2.1.4 Discussion

2.1.4.1 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; Spohn et al., 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 (2014) found no change in microbial CUE due to N-amendment in a cropland soil.

2.1.4.2 Microbial respiration and growth under nutrient addition

We found that microorganisms regulated both respiration and growth similarly (Figure 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 (Figure 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 (Figure 1, 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).

2.1.4.3 Substrate stoichiometry and microbial CUE

Our finding that soil microbial CUE and the DOC:DN ratio were negatively correlated (Figure 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 demon-

strate that the relationship between soil microbial CUE and DOC:DN ratios assumed in models (Sinsabaugh et al., 2013; Sinsabaugh et al., 2016; Manzoni et al., 2017) holds true across grasslands spanning a wide range of locations and conditions, but was unaffected by nutrient addition.

2.1.4.4 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 (Figure 4b, c) confirms this concept. In addition, our findings show that microbial CUE was negatively correlated with BG activity (Figure 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. In contrast, phosphatase activity was not related to CUE (Figure 4d), which confirms our finding that P alone was not critical for microbial C processing in the studied soils (Figure 1, 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).

2.1.4.5 Environmental conditions and microbial CUE

Besides substrate stoichiometry, climatic variables and soil texture were related to soil microbial CUE (Figure 3). A negative relationship between MAP and CUE (Figure 3) was similarly reported by Takriti et al. (2018) and Herron et al. (2009). Further, CUE increased with MAT (Figure 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 (Figure 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.

2.1.4.6 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 (Figure 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; Spohn et al., 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 (Figure 6), was accompanied by a relatively long microbial turnover time (Figure 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.

2.1.4.7 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.

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.

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 18O incorporation from H218O 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. R package version 1.10-1.

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 13C-acetic acid vapor and 15N-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 and 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 ken 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. Wissenschaftlicher Teil XI, 364–369.

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 R2 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. URL http://www.R-project.org/, Vienna, Austria.

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 H218O. 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 CO2. Journal of Geophysical Research 116.

Supporting Information

Table S1: 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.

	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) 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.

		0-15				15-30			
	Site	Ctrl	+N	+P	+NP	Ctrl	+N	+P	+NP
CUE (%)	cdcr.us	21.02 ± 6.91a	23.97 ± 8.53a	29.23 ± 11.76a	25.93 ± 2.91a	29.73 ± 9.25a	36.12 ± 16.16a	31.60 ± 8.71a	17.52 ± 0.88a
	cbgb.us	33.30 ± 5.27a	39.48 ± 4.60a	40.79 ± 3.50a	41.72 ± 1.49a	46.49 ± 17.25a	35.03 ± 10.22a	35.01 ± 16.93a	42.17 ± 3.76a
	rook.uk	56.30 ± 3.75a	51.74 ± 8.94a	49.39 ± 5.96a	45.16 ± 7.21a	50.56 ± 7.99a	46.24 ± 4.31a	43.58 ± 4.10a	43.05 ± 3.92a
	hero.uk	44.42 ± 1.62a	48.37 ± 1.95a	47.42 ± 3.91a	45.15 ± 3.97a	53.21 ± 4.28a	58.22 ± 2.08a	62.02 ± 5.80a	52.99 ± 7.37a
	ukul.za	27.91 ± 6.64a	25.04 ± 1.19a	27.79 ± 5.52a	24.07 ± 4.51a	34.35 ± 11.40a	36.35 ± 8.05a	39.74 ± 10.65a	35.93 ± 6.20a
	summ.za	48.48 ± 4.60a	55.16 ± 5.96a	41.51 ± 2.07a	47.46 ± 5.86a	NA	NA	NA	NA
Turnover time (d)	cdcr.us	152.89 ± 20.98a	145.06 ± 27.99a	93.89 ± 20.88a	185.61 ± 43.49a	128.18 ± 4.16a	218.46 ± 136.90a	123.32 ± 4.02a	292.15 ± 87.49a
	cbgb.us	132.16 ± 12.66a	118.28 ± 33.54a	86.20 ± 22.50a	95.19 ± 12.06a	189.40 ± 32.57a	176.46 ± 54.82a	206.48 ± 103.24a	105.38 ± 46.64a
	rook.uk	59.67 ± 10.37a	85.16 ± 3.71a	96.87 ± 27.02a	63.93 ± 12.12a	35.00 ± 9.01a	58.93 ± 19.09a	75.45 ± 9.02a	74.84 ± 25.17a
	hero.uk	52.68 ± 19.02a	40.27 ± 6.39a	48.56 ± 10.83a	74.37 ± 13.33a	74.28 ± 13.68a	53.94 ± 19.61a	51.54 ± 3.44a	73.87 ± 13.70a
	ukul.za	247.41 ± 11.93a	213.64 ± 40.33a	207.41 ± 57.76a	136.12 ± 79.13a	213.88 ± 143.60a	129.84 ± 23.88a	186.31 ± 56.74a	194.11 ± 57.31a
	summ.za	81.99 ± 21.12a	74.30 ± 9.55a	112.39 ± 27.27a	109.47 ± 10.60a	NA	NA	NA	NA
Respiration (ng CO_2-C g $soil^{-1}$ h^{-1})	cdcr.us	346.03 ± 126.25a	239.58 ± 77.78a	278.61 ± 165.43a	167.24 ± 75.00a	96.80 ± 35.42a	61.62 ± 13.95a	82.02 ± 31.98a	91.21 ± 19.45a
	cbgb.us	140.69 ± 10.27a	142.92 ± 19.21a	139.72 ± 19.35a	147.97 ± 24.04a	45.23 ± 16.29a	80.56 ± 29.77a	64.06 ± 7.40a	47.97 ± 19.04a
	rook.uk	164.23 ± 13.80b	183.60 ± 42.17ab	184.24 ± 18.90ab	257.59 ± 22.47a	93.01 ± 8.04a	103.39 ± 4.57a	110.24 ± 15.05a	101.43 ± 9.09a
	hero.uk	378.49 ± 37.18a	321.47 ± 24.70a	391.17 ± 49.41a	384.33 ± 58.69a	135.63 ± 27.11a	113.75 ± 11.67a	102.41 ± 8.65a	119.38 ± 12.12a
	ukul.za	415.50 ± 131.65a	328.15 ± 31.79a	471.92 ± 155.57a	413.75 ± 17.33a	202.34 ± 40.35a	196.87 ± 40.48a	198.11 ± 47.43a	202.00 ± 33.84a
	summ.za	471.19 ± 63.74a	327.46 ± 46.51a	491.19 ± 45.13a	327.24 ± 66.20a	NA	NA	NA	NA
Growth (ng C g $soil^{-1}$ h^{-1})	cdcr.us	81.08 ± 5.17a	74.26 ± 28.63a	89.17 ± 12.26a	57.21 ± 21.51a	36.77 ± 6.65a	42.16 ± 26.44a	34.23 ± 3.57a	19.38 ± 4.30a
	cbgb.us	71.16 ± 16.26a	93.08 ± 13.15a	97.96 ± 22.51a	107.13 ± 22.91a	38.14 ± 12.26a	43.78 ± 19.00a	41.23 ± 29.73a	37.17 ± 19.04a
	rook.uk	213.78 ± 34.02a	195.06 ± 34.38a	180.68 ± 26.25a	215.56 ± 47.92a	97.45 ± 22.46a	89.92 ± 14.14a	84.23 ± 2.14a	76.52 ± 6.66a
	hero.uk	302.97 ± 35.77a	303.81 ± 44.78a	351.03 ± 30.96a	312.55 ± 14.66a	151.88 ± 12.72a	158.12 ± 10.97a	170.47 ± 30.02a	137.11 ± 28.20a
	ukul.za	149.34 ± 8.02a	109.31 ± 8.20a	170.07 ± 24.08a	132.45 ± 29.77a	104.88 ± 32.24a	109.56 ± 17.68a	128.51 ± 33.59a	111.71 ± 15.08a
	summ.za	444.05 ± 62.92a	402.32 ± 45.22ab	350.30 ± 46.36ab	291.03 ± 31.03b	NA	NA	NA	NA

Table 3: 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		

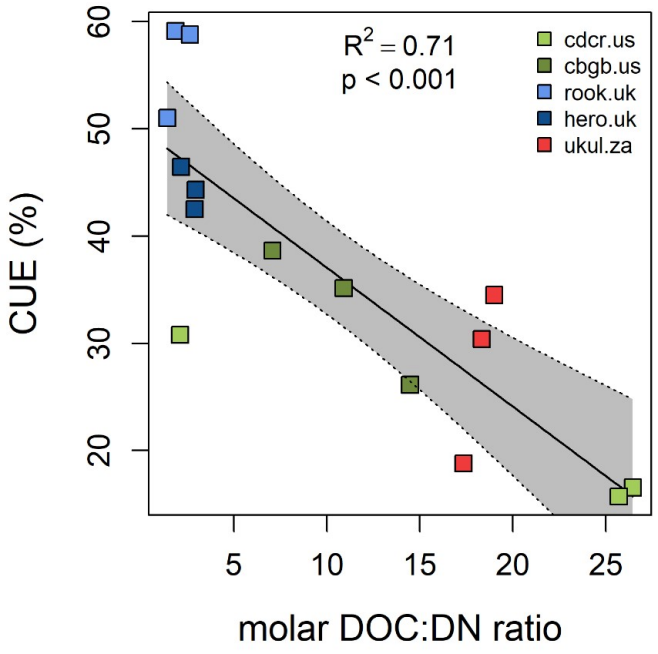


Figure S1: Correlation of molar dissolved organic carbon-to-dissolved nitrogen ratio and soil microbial carbon use efficiency (CUE) in the topsoils of the control plots of all sites with a soil depth > 20 cm.

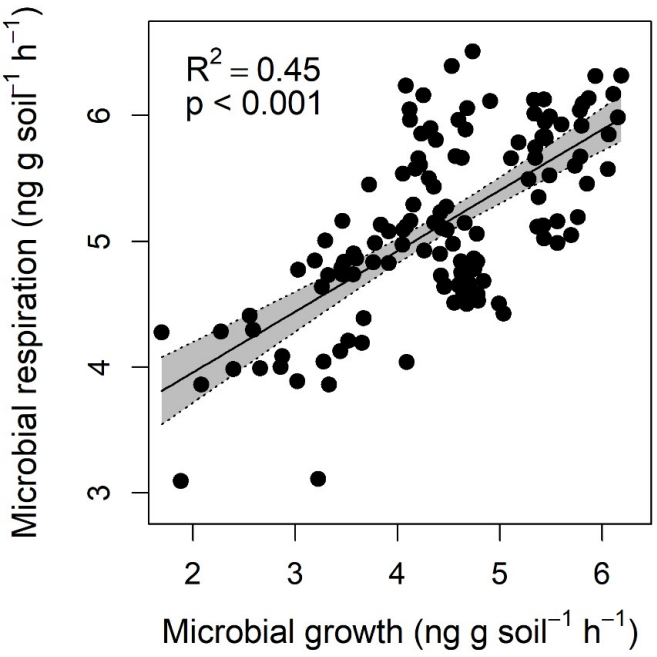


Figure S2: Correlation of microbial growth and microbial respiration across all soils, treatments, and soil depths. Prior to correlation analysis, microbial growth and respiration data were log-transformed to achieve normal distribution.

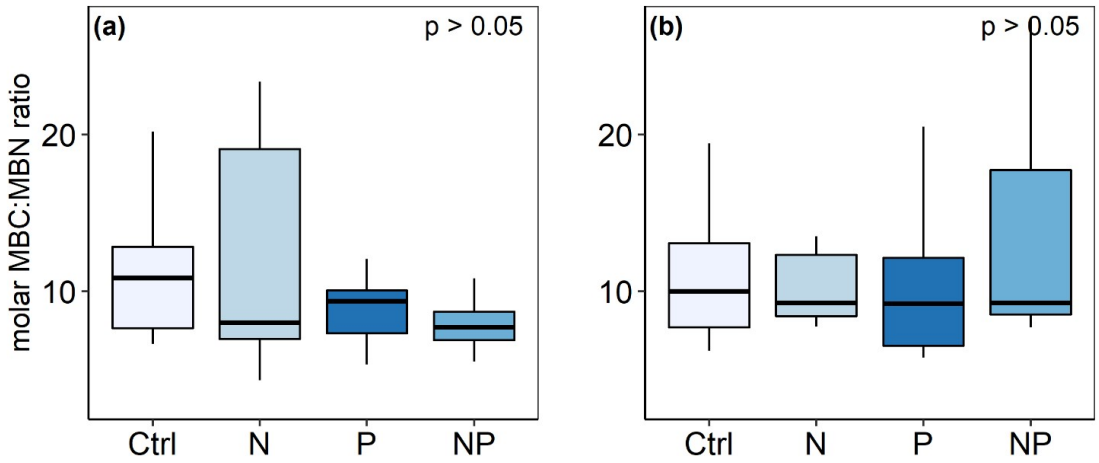


Figure S3: Molar microbial biomass carbon-to-nitrogen ratio (MBC:MBN) in (a) 0-15 cm depth and (b) 15-30 cm depth in the control, N, P, and NP treatments across all six sites. Subsoil MBC:MBN ratios of one site (rook.uk) were excluded, because MBN values were below detection limit.

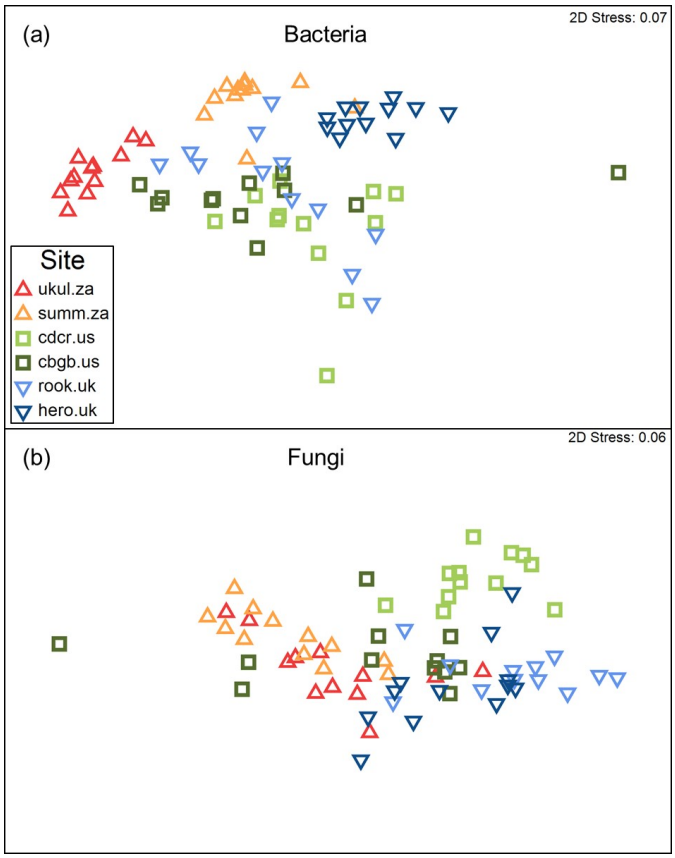


Figure S4: Community composition of (a) bacteria and (b) fungi shown via non-metric multi-dimensional scaling (nMDS) based on ARISA analyses of topsoils of all six sites. One-way ANOSIM with 999 permutations was used to test if microbial communities at the different sites differ significantly from each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

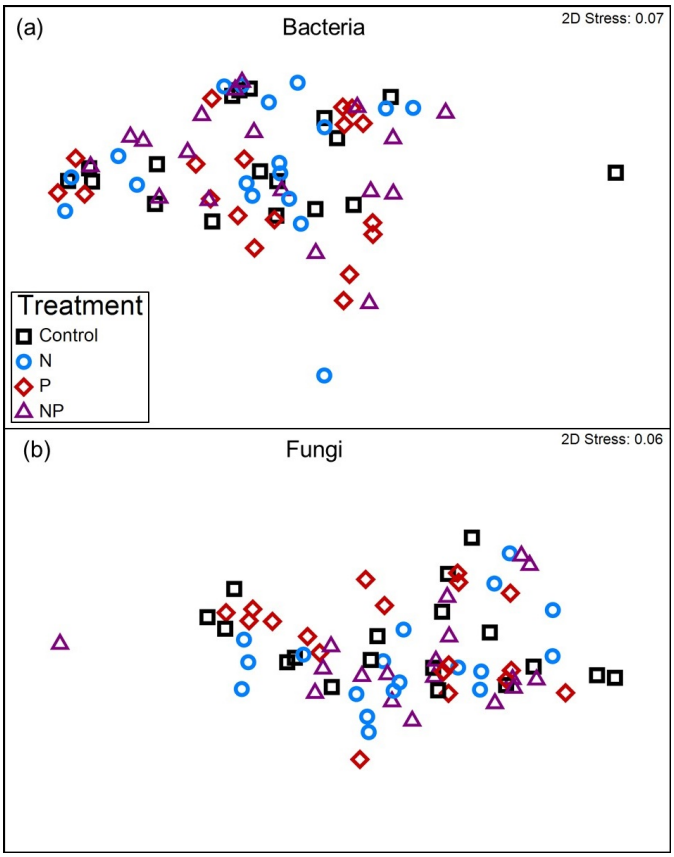


Figure S5: Effect of nutrient addition on (a) bacterial community composition and (b) fungal community composition shown via non-metric multi-dimensional scaling (nMDS) based on ARISA analyses of topsoils of all six sites. One-way ANOSIM with 999 permutations was used to test for significant effects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

2.2 Study II

Turnover of carbon and phosphorus in the microbial biomass depending on phosphorus availability

Marie Spohn and Meike Widdig (2017),
published in *Soil Biology and Biochemistry* 113, 53-59, DOI:
https://doi.org/10.1016/j.soilbio.2017.05.017

Abstract

Adjustment of the mean residence time (MRT) of elements in the soil microbial biomass (SMB) might be an important process by which microbial communities adapt to nutrient-poor environments and maintain their element biomass ratio. Yet, little is known about the turnover of elements such as carbon (C) and phosphorus (P) in the SMB, which is partly due to methodological challenges. This is the first study to determine the MRT of C and P in SMB based on labeling of microbial DNA with ^{14}C and ^{33}P. We studied the MRT of C and P in the SMB of topsoil from a temperate, mesic grassland fertilized with five different amounts of P. For this purpose, soil was labeled with ^{14}C-glucose and ^{33}P-phosphate and incubated for 24 h, and then, ^{14}C and ^{33}P in the soil microbial DNA were determined. The MRT of C and P was calculated based on the C and P content of DNA, and the observation that soil microbial biomass C and microbial DNA are linearly correlated. The SMB-C and the MRT of C in the SMB were not affected by P availability, whereas the microbial community significantly increased the MRT of P in its biomass from 18.1 to 39.0 days with decreasing P availability. Our results indicate that the adjustment of the MRT of individual elements in the SMB is an important process by which microbes adapt to nutrient poor environments and are able to maintain their biomass element ratio when decomposing substrate with a very high C-to-nutrient ratio.

2.2.1 Introduction

Microorganisms can immobilize large amounts of nutrients in their biomass, which strongly affects the concentrations of plant available nutrients (Van der Heijden et al., 2008; Richardson et al., 2009). While there are many publications dealing with soil microbial biomass C, N and P, and biomass stoichiometry (for reviews see Cleveland and Liptzin, 2007; Xu et al., 2013), very little is known about the mean residence time (turnover time) of individual elements in the microbial biomass, and how it changes with element availability (Spohn, 2016).

The mean residence time (MRT) of an element in the microbial biomass likely increases with decreasing availability of the element (Oberson and Joner, 2005; Kaiser et al., 2014; Spohn, 2016), similar to the MRT of nutrients in plants (Aerts and Chapin, 2000). The reason for this seems to be that the efficiency of internal element recycling by microorganisms increases with decreasing element availability. This might be important especially during the decomposition of nutrient-poor substrates as for example dead wood and forest litter, in which the C-to-nutrient ratios exceed by far the C-to-nutrient ratios of the microbial biomass (Spohn, 2016). In an arable soil of the temperate zone, C in the microbial biomass had a MRT of 82-95 days, while P had a MRT of 37-42 days (Kouno et al., 2002), indicating that C was more efficiently retained in the microbial biomass than P. This finding is in accordance with the generally observed C limitation of microorganisms in mineral soil (De Nobili et al., 2001; Demoling et al., 2007). Cheng (2009) reported MRTs of the microbial biomass C of 48 and 35 days in planted soils, and

97 days in unplanted soils, suggesting that the presence of roots stimulated the turnover of microbial biomass C, likely due to the release of exudates, i.e. easily available C. Other studies on P in the microbial biomass determined P based on labeling of soil with ^{33}P in the field (McLaughlin et al., 1988; Oehl et al., 2001a). However, in these studies the period of time between labeling and determination of the isotope in the microbial biomass was very long, which likely led to an overestimation of the MRT.

So far, there is little reliable data on the MRT of individual elements in the microbial biomass pool because of several methodological difficulties. Most studies that investigated MRT of elements in the microbial biomass pool did so by tracing isotopes in the microbial biomass based on the chloroform-fumigation-extraction method (Kouno et al., 2002; Oberson and Joner, 2005). However, this method has the serious limitation that it is a differential method (based on the difference between two samples), which leads to relatively large errors because errors from the extraction of the two samples sum up. Kouno et al. (2002) determined the turnover of C and P based on isotope dilution. For this purpose, they labeled soil with ^{14}C and ^{33}P and determined the decrease in recovery of the tracer in the SMB over several months. Besides the previously mentioned shortcomings, this approach likely leads to an overestimation of the MRTs of elements in the SMB because once the tracer is in the soil, it might be constantly used for the build-up of new SMB. Achat et al. (2010) determined the turnover of microbial biomass P in forest soils combining an isotopic dilution method with a modelling approach. They found that a large part of the microbial P turned over in just a few days. In contrast, when the turnover time of microbial P was estimated from the seasonal fluctuations of microbial P, it was found to range between 224 and 293 days (Liebisch et al., 2014). This comparatively longer time is most likely due to the approach used, which likely overestimates the MRT.

The aim of this study was to overcome these methodological difficulties by determining the MRT of C and P in the SMB based on short-term incubation and isotopic labeling of microbial DNA. We hypothesized that microbes recycle an element more efficiently internally when little of the element is available than when it is highly available. Therefore, the MRT of P but not of C in the SMB increases with decreasing P availability.

2.2.2 Material and Methods

2.2.2.1 Soil and experimental setup

We sampled the A horizon of a mesic grassland in the Botanical Garden of the University of Bayreuth (49°55'18 N, 11°35'03 E). The soil is a Cambisol and the A horizon has the following features: 14.1 g kg^{-1} total organic C, 1.0 g kg^{-1} total N, pH 4.5, and 0.32 mg g^{-1} total P (extractable in 0.5 M H_2SO_4, determined by ICP OES).

The soil was sieved (<2000 mm), and all fine roots visible with the naked eye were removed using a tweezer. In total 15 PE bottles received each 30.0 g of fresh soil, and

each represented one experimental unit. The water content was adjusted to 50% of the water holding capacity in all bottles. Simultaneously, five different levels of P fertilizer were added in the form of dissolved NaH_2PO_4 to reach P fertilization levels of 0, 20, 40, 60 and 80 mg P per g dry soil, each three times replicated. The fertilized soils were pre-incubated for 19 days at 20 °C in order to allow the microbial biomass to adjust to the altered P availability. Subsequently, two subsamples of each experimental unit (400 mg of fresh soil corresponding to 337 mg dry mass) were each placed in a 2 ml screw caps and were labeled with either ^{33}P or ^{14}C. For this purpose, 4 kBq ^{14}C-U-glucose (Hartmann Analytics) was added to one subsample (corresponding to 7.53 nmol C per g dry soil) and 20 kBq ^{33}P-orthophosphate (Hartmann Analytics) to the other subsample (corresponding to 0.52 pmol P per g dry soil), both dissolved in 50 ml milliporewater. It should be noted that the amounts of P and C added were negligible, and ^{33}P and ^{14}C served only as a tracer without affecting the C or P availability. The soils were further incubated for 24 h at 20 °C, and subsequently they were frozen at -25 °C. This short incubation time after addition of the label was chosen in order to prevent an underestimation of the rate of DNA formation due to the decomposition of labeled DNA (see Discussion).

2.2.2.2 Analyses

The DNA was extracted from labeled samples using a DNA extraction kit (FastDNA™ SPIN Kit for Soil, MP Biomedicals) which, compared to other protocols, has been shown to extract microbial DNA from soil with a high efficiency (Vishnivetskaya et al., 2014). The cells in the soil samples were lysed by bead beating in the FastPrep Instrument for 40 s at a speed setting of 6.0. Soil particles, colloids and cell debris was sedimented by centrifugation for 15 min at 14,000 x g. Subsequently, the supernatant was collected and proteins were denatured by adding the protein precipitation solution, and were sedimented by centrifugation for 5 min at 14,000 x g. The resulting supernatant was gently shaken for 2 min together with the binding matrix in order to allow the DNA to bind, and subsequently, the total volume of the mix was loaded on a spin filter that was inserted into a 2 ml cup. The DNA bound onto the matrix was washed with the washing solution containing mostly ethanol. The matrix on the filter was centrifuged to remove residues of the washing solution and air-dried. At last, the DNA was eluted from the binding matrix in 200 ml DNase-free water. Our DNA extracts were transparent, indicating that no humic substances were co-extracted. However, this might be different when studying soils that contain more humic substances, which might require washing the samples previous to DNA extraction according to He et al. (2005). The weight of the DNA extract was determined gravimetrically, and the DNA concentration in each extract was then determined fluorimetrically on a 5 ml aliquot of each extract by the picogreen assay (Sandaa et al., 1998) using a kit (Quant-iT™ PicoGreen® dsDNA Reagent, Life Technologies). The ^{14}C activity and the ^{33}P activity were determined by a Perkin-Elmer scintillation counter (Tri-Carb TR/SL 3180, Waltham, MA, USA) using scintillation cocktail (Rotiszint eco

plus, Roth, Karlsruhe, Germany), and the signal was corrected for radioactive decay.

Inorganic P (Pi) and organic C, as well as the ^{33}P activity in the soil solution were determined using a different subsample from each experimental unit. For this purpose, 400 mg of fresh soil (corresponding to 337 mg dry mass) of each experimental unit were labeled with the same amount of ^{33}P as the samples used for DNA-extraction (20 kBq) and incubated at 20 °C. After 24 h, the soils were extracted in 20 ml distilled water by placing them on a horizontal shaker for 20 min. Subsequently, the samples were filtered through whatman filters. The concentration of Pi in the extracts was determined by the molybdenum blue assay using a multiplate reader (Infinite® M200 PRO, Tecan). The concentration of organic C in the water extracts was determined using a TOC/TN analyzer (Multi N/C 2100S, Analytik Jena AG, Jena, Germany), and the ^{33}P activity was determined by a Perkin-Elmer scintillation counter (Tri-Carb TR/SL 3180, Waltham, MA, USA) using scintillation cocktail (Rotiszint eco plus, Roth, Karlsruhe, Germany), and the signal was corrected for radioactive decay. We assumed that all glucose added to the soil was available for the microorganisms because glucose does not sorb to the soil matrix (Fransson and Jones, 2007) and is quickly taken up by microorganisms. The uptake of ^{14}C glucose into the SMB is so fast (Gunina and Kuzyakov, 2015) that one would strongly underestimate the bioavailability of ^{14}C-glucose if assessed by ^{14}C activity in water extracts.

Soil microbial biomass C and P (SMB-C and SMB-P) concentrations were determined by the chloroform fumigation extraction method (Brookes et al., 1982; Vance et al., 1987). Exactly 4.0 g of fresh soil were fumigated with chloroform for 24 h. Dissolved P was extracted from the fumigated and the non-fumigated soil in Bray-1 solution (0.03 M NH_4F - 0.025 M HCl) following Khan and Joergensen (2012), and quantified by the molybdenum blue assay using a multiplate reader (Infinite® M200 PRO, Tecan). A conversion factor of 2.5 was used to calculate SMB-P (Brookes et al., 1982).

Dissolved C was extracted from the fumigated and the nonfumigated soil in 40 ml 0.5 M K_2SO_4 and measured using a TOC/ TN analyzer (Multi N/C 2100S, Analytik Jena AG, Jena, Germany). A conversion factor of 2.22 was used to calculate SMB-C (Joergensen, 1996).

2.2.2.3 Calculations and statistics

The MRTs of C and P were estimated from the MRTs of C and P in microbial DNA. The amount of DNA-P formed from P in the soil solution taken up during 24 h was calculated based on the total ^{33}P incorporated into DNA during 24 h and the specific ^{33}P activity of the water extract as follows, assuming that the inorganic P (Pi) of the water extract represents the bioavailable P:

$$DNA - P\ formed\ (\mu g) = \frac{Total\ ^{33}P\ in\ DNA\ (Bq)}{Specific\ ^{33}P\ activity\ (Bq\ (\mu g\ P)^{-1})}$$

where DNA-P formed is DNA-P formed from P taken up from the soil solution during 24 h.
The specific ^{33}P activity of the water extractable P is defined as follows:

$$Specific\ ^{33}P\ activity\ (Bq\ (\mu gP)^{-1}) = \frac{^{33}P_{water\ extract}\ (Bq\ (g\ soil)^{-1})}{P_{water\ extract}\ (\mu g\ (g\ soil)^{-1})}$$

The specific ^{33}P activity of water extractable P was determined at the end of the incubation, i.e., 24 h after tracer addition. The specific ^{33}P activity in the water extracts is mostly affected by sorption of ^{33}P to the soil matrix upon tracer addition. However, this sorption would be largely complete within a few minutes after tracer addition (Oehl et al., 2001b; Bünemann et al., 2007; Spohn et al., 2013; Bünemann, 2015 and references therein). Therefore, we assume that the specific ^{33}P activity of the water extractable P determined after 24 h was representative for the specific ^{33}P activity of the water extractable P during most time of the incubation.

Calculating the total amount of C incorporated into DNA during incubation is more complicated because, in contrast to inorganic P in the water extracts, we cannot assume that all organic C in the water extract was bioavailable due to the recalcitrance of many substances. Therefore, we calculated three different scenarios for the incorporation of total C into DNA based on the assumptions that 15, 10 or 5% of the organic C in the water extracts were bioavailable, and hence were taken up with the same probability as the ^{14}C. The specific ^{14}C activity of the available C was calculated for the three scenarios under the assumption that all ^{14}C-glucose added was available as follows:

$$Specific\ ^{14}C\ activity\ (Bq\ (\mu g\ C)^{-1}) = \frac{^{14}C_{added}\ (Bq\ (g\ soil)^{-1})}{y\ \times\ C_{water\ extract}\ (\mu g\ (g\ soil)^{-1})}$$

where y is 0.15, 0.10 or 0.05. The amount of DNA-C formed from the soil solution during 24 h was calculated in the same way as the amount of DNA-P formed. In order to calculate the MRT of C and P in the microbial biomass, we assumed that all four nucleotides were equally abundant in the microbial DNA, resulting in a C content of 34.9% (w/w) and a P content of 9.5% (w/w) in DNA. The MRT of C and P in the microbial DNA was calculated as the pool size divided by the influx to the pool as follows:

$$MRT\ of\ X\ (days)\ = \frac{DNA-X\ (ng\ (g\ soil)^{-1})}{DNA-X\ formed\ (ng\ (g\ soil)^{-1}\ day^{-1})}$$

where X stands for C or P. Homogeneity of variance was tested by the Levene test. Differences between fertilization treatments were tested by ANOVA followed by Tukey

post-hoc test using R (R Core Team, 2013).

2.2.3 Results

The concentrations of microbial DNA, SMB-C, SMB-P and water extractable organic C were not affected by P fertilization ($p > 0.05$; Table 1). The water-extractable P concentration increased by a factor of 4.6 from the non-fertilized soil to the soil that had received 80 mg P g soil^{-1} of fertilizer. The ^{33}P activity in the water extracts also increased with P fertilization, while the specific ^{33}P activity decreased with P fertilization (Table 1).

Table 1: Total soil microbial DNA, soil microbial biomass carbon (SMB-C) and soil microbial biomass P (SMB-P), along with properties of the water extracts. Numbers are means (n = 3), ± represent standard deviations, and letters indicate significant differences ($p < 0.05$) between fertilizer treatments.

	Level of fertilization (mg P (g soil)$^{-1}$)				
	0	20	40	60	80
DNA [mg (g soil)$^{-1}$]	35.93a (±3.16)	35.98ab (±13.63)	31.08b (±1.01)	28.02b (±2.33)	38.44c (±16.13)
SMB-C [mg (g soil)$^{-1}$]	399.51 (±44.65)	424.12 (24.18±)	464.47 (±10.58)	466.16 (±34.85)	471.93 (±32.05)
SMB-P [mg (g soil)$^{-1}$]	36.6 (±7.8)	19.7 (±7.6)	14.8 (±7.4)	21.7 (±8.9)	37.4 (±3.2)
Water extractable ^{33}P [kBq (g soil)$^{-1}$]	4.20a (±0.15)	5.02b (±0.26)	5.49b (±6.49)	6.39c (±0.29)	7.19d (±0.45)
Water-extractable Pi [mg (g soil)$^{-1}$]	2.5a (±0.3)	5.0b (±0.5)	6.1b (±0.5)	8.8c (±0.6)	11.6d (±1.0)
Specific ^{33}P water extract [kBq (mg P)$^{-1}$]	1.69c (±0.18)	1.00b (±0.11)	0.90ab (±0.07)	0.73ab (±0.06)	0.62a (±0.04)
Water-extractable OC [mg (g soil)$^{-1}$]	259.8 (±24.4)	299.9 (44.0±)	279.3 (14.1±)	307.7 (14.8±)	308.7 (±11.7)
Water-extractable N [mg (g soil)$^{-1}$]	26.3 (±1.9)	30.2 (±3.4)	29.8 (±0.4)	30.9 (±2.7)	30.5 (±0.5)

The relationship between SMB-C and total DNA was in agreement with a previously described DNA/SMB-C ratio across sites and soil horizons (Supplement Figure S1). The total amount of DNA-P formed from Pi of the soil solution during the 24 h incubation (Fig. 1A) increased significantly ($p < 0.05$) from the non-fertilized soil to the highest level of fertilization by a factor of 1.8. In contrast, the total amount of DNA-C formed from C of the soil solution did not change with P fertilization (Fig. 2A). The calculation of the total amount of DNA-C formed from OC in the soil solution is associated with a high uncertainty, because we do not know the size of the bioavailable C pool that is taken up with the same probability as the ^{14}C tracer. For this reason, we calculated three different scenarios, assuming that either 15, 10 or 5% of the water extractable organic carbon (OC) was taken up with the same probability as the ^{14}C-glucose (Fig. 2A). The MRT of C in the DNA did not differ significantly between the treatments ($p > 0.05$),

and it was on average 49.7, 74.6 and 149.2 days assuming that 15, 10 or 5% of the OC was bioavailable (Fig. 2A). In contrast, the MRT of P decreased significantly ($p < 0.05$) with the level of P fertilization by a factor of 2.2, from 39.0 days in the control to 18.1 days in the soil fertilized with 80 mg P g^{-1} (Fig. 2B). C and P had the same MRT in the SMB in the non-fertilized treatment (39.0 days), under the assumption that 18.4% of the water-extractable OC was bioavailable, i.e., was taken up with the same probability as the ^{14}C-glucose by the microbial biomass. In the non-fertilized treatment, 0.7 mg P per g soil and per day cycled through the microbial biomass pool, whereas in the treatment fertilized with 80 mg P per g soil, 1.4 mg P per g soil and per day moved through the SMB pool. The amount of C that cycled through the SMB C pool differed between 9.0 and 3.0 mg C per g soil and per day in all treatments, depending on the assumption made about the size of the available C pool.

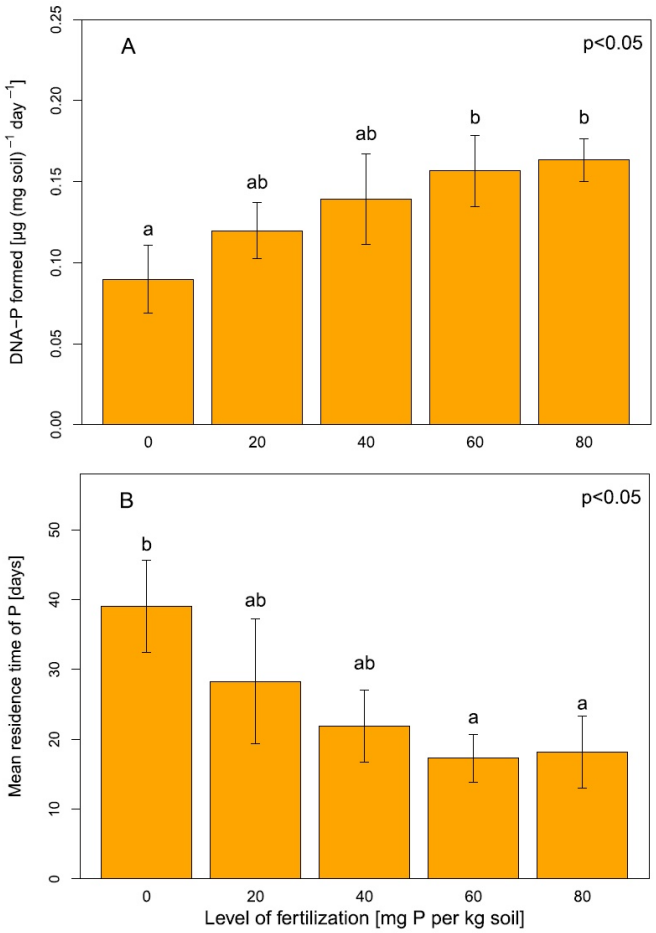


Figure 1: Total amount of DNA-P formed from Pi taken up from the soil solution during 24 h (A) and the mean residence time (MRT) of P in the microbial biomass (B). Bars represent means, and error bars show standard deviation (n = 3). Letters indicate significant differences ($p < 0.05$) between fertilizer treatments.

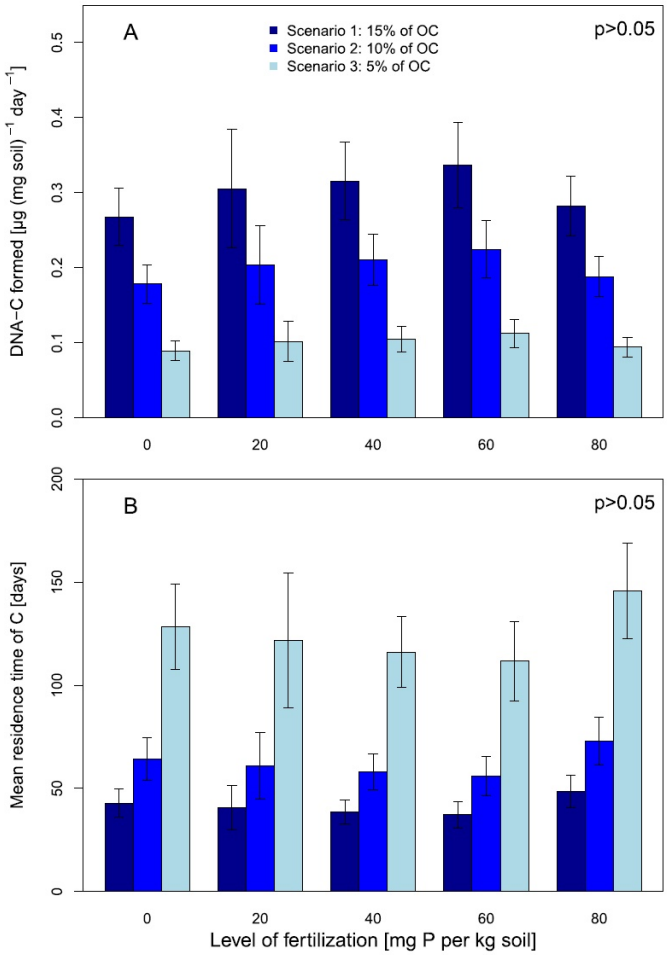


Figure 2: Total amount of DNA-C formed from organic C (OC) taken up from of the soil solution during 24 h (A) and the mean residence time (MRT) of C in the microbial biomass (B). Bars represent means, and error bars show standard deviation, considering different estimates of the bioavailability of the OC in the soil solution (Scenario 1: 15% of the water extractable OC is bioavailable; Scenario 2: 10% of the water extractable OC is bioavailable; and Scenario 3: 15% of the water extractable OC is bioavailable). Bars represent means, and error bars show standard deviation. The p value is given for the comparison between the five different levels of fertilization (n = 3).

2.2.4 Discussion

We estimated the turnover time of C and P in the SMB based on the turnover time of C and P in microbial DNA. Microbial growth occurs along with formation of genomic DNA, and the turnover time of elements in the DNA reflect their turnover in the SMB (Anderson and Martens, 2013; Schwartz, 2007; Blazewicz and Schwartz, 2011; Spohn et al., 2016a, b). For this reason, microbial growth and SMB turnover have previously been determined based on the incorporation of ^{18}O into microbial DNA (Schwartz, 2007; Blazewicz and Schwartz, 2011; Spohn et al., 2016a, b). A short incubation period after addition of the label was chosen here in order to prevent an underestimation of the rate of DNA formation due to decomposition of labeled DNA. Blazewicz and Schwartz (2011) showed that 72 days after the addition of ^{18}O, the incorporation rate of ^{18}O into DNA

stopped to be linearly correlated with the incubation time, likely due to decomposition of labeled DNA.

This is the first study to show that soil microbes adjusted the P turnover in their biomass to the concentration of bioavailable P in their environment, which seems to be an important process by which microorganisms adapt to a nutrient-poor environment. In fertilized soils, more DNA was formed from P in the soil solution compared to non-fertilized soils (Fig. 1A), leading to a shorter MRT of P in the microbial biomass (Fig. 1B). Under the assumption that microbes in all treatments produced DNA at the same rate, this means that microorganisms in the non-fertilized soil used a larger percentage of P already present in the cell, i.e., they recycled P more efficiently internally than in the fertilized soil. Thus, instead of changing the C:P ratio of their biomass as an adaptation to the ratio of available elements (Mooshammer et al., 2015), they adapted the turnover time of P in the microbial biomass. Quantitatively this seems to be a much more powerful adaptation to a nutrient poor environment, than the adjustment of the biomass stoichiometry, which is only possible in a relatively narrow range (Mooshammer et al., 2015). The adjustment of MRTs of individual elements in the SMB could be an important process, which allows microbes to thrive on substrate with unfavorable stoichiometry (Spohn, 2016). This has often been disregarded in studies on microbial element cycling so far.

Our results show that concentrations of SMB-C and DNA, as well as the MRT of C in the microbial biomass were not affected by P availability. This is in accordance with previous studies reporting that SMB-C (Heuck et al., 2015) and microbial total DNA (Spohn et al., 2016b) did not change upon soil P fertilization. Assuming that 18.4% of the organic C of the soil solution was bioavailable, C and P in our experiment both had the same MRT in the microbial biomass in the non-fertilized treatment (39.0 days). However, the experiment also suggests that C and P in many soils do not have the same MRT in the microbial biomass. The regulation of the MRT of individual elements in the SMB might be an important adaptation that allows microbes to live on substrate whose stoichiometry is very different from the microbial biomass stoichiometry, which is fairly constant (Spohn, 2016).

The MRTs of C and P estimated here are in the same range as those reported in previous labeling studies. In an arable soil of the temperate zone, C in the microbial biomass had a MRT of 82-95 days, while P had a MRT in the microbial biomass of 37-42 days (Kouno et al., 2002). Moreover, Cheng (2009) reported MRTs of the microbial biomass C of 48 and 35 days in planted soils, and 97 days in unplanted soils. Yet, it has to be taken into account that the MRTs given in the previous studies are associated with a large uncertainty because of the way the isotopic labeling was performed (see Introduction).

In conclusion, this is the first study to determine the turnover time of C and P in SMB based on labeling of microbial DNA with ^{14}C and ^{33}P. Our results show that the MRT of C in the SMB was not affected by P availability, whereas the microbial

community increased the MRT of P in its biomass with decreasing P availability. Our results indicate that the adjustment of MRT of individual elements in the SMB could be an important process by which microbes adapt to nutrient poor environments and maintain their biomass stoichiometry when decomposing substrate with a very high C-to-nutrient ratio.

Acknowledgements

We thank the Laboratory of Radioactive Isotopes (LARI) of the University of Goettingen.

References

Achat, D.L., Morel, C., Bakker, M.R., Augusto, L., Pellerin, S., Gallet-Budynek, A., Gonzalez, M., 2010. Assessing turnover of microbial biomass phosphorus: combination of an isotopic dilution method with a mass balance model. Soil Biology and Biochemistry 42, 2231e2240.

Aerts, R., Chapin, F.S., 2000. The mineral nutrition of wild plants revisited: a reevaluation of processes and patterns. Advances in Ecological Research 30, 1e67.

Anderson, T.H., Martens, R., 2013. DNA determinations during growth of soil microbial biomasses. Soil Biology and Biochemistry 57, 487e495.

Blazewicz, S.J., Schwartz, E., 2011. Dynamics of 18O Incorporation from H2 18O into soil microbial DNA. Microbial Ecology 61, 911e916.

Brookes, P.C., Powlson, D.S., Jenkinson, D.S., 1982. Measurement of microbial biomass phosphorus in soil. Soil Biology and Biochemistry 14, 319e329.

Bünemann, E.K., 2015. Assessment of gross and net mineralization rates of soil organic phosphorus - A review. Soil Biology and Biochemistry 89, 82e98.

Bünemann, E.K., Marschner, P., McNeill, A.M., McLaughlin, M.J., 2007. Measuring rates of gross and net mineralisation of organic phosphorus in soils. Soil Biology and Biochemistry 39, 900e913.

Cheng, W., 2009. Rhizosphere priming effect: its functional relationships with microbial turnover, evapotranspiration, and C-N budgets. Soil Biology and Biochemistry 41, 1795e1801.

Cleveland, C.C., Liptzin, D., 2007. C: N: P stoichiometry in soil: is there a "Redfield ratio" for the microbial biomass? Biogeochemistry 85, 235e252.

De Nobili, M., Contin, M., Mondini, C., Brookes, P.C., 2001. Soil microbial biomass is triggered into activity by trace amounts of substrate. Soil Biology and Biochemistry 33, 1163e1170.

Demoling, F., Figueroa, D., Bååth, E., 2007. Comparison of factors limiting bacterial growth in different soils. Soil Biology and Biochemistry 39, 2485e2495.

Fransson, A.M., Jones, D.L., 2007. Phosphatase activity does not limit the microbial use of low molecular weight organic-P substrates in soil. Soil Biology and Biochemistry 39, 1213e1217.

Gunina, A., Kuzyakov, Y., 2015. Sugars in soil and sweets for microorganisms: review of origin, content, composition and fate. Soil Biology and Biochemistry 90, 87e100.

He, J., Xu, Z., Hughes, J., 2005. Pre-lysis washing improves DNA extraction from a forest soil. Soil Biology and Biochemistry 37, 2337e2341.

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, 119e129.

Joergensen, R.G., 1996. The fumigation-extraction method to estimate soil microbial biomass: calibration of the k EC value. Soil Biology and Biochemistry 28, 25e31.

Kaiser, C., Franklin, O., Dieckmann, U., Richter, A., 2014. Microbial community dynamics alleviate stoichiometric constraints during litter decay. Ecology Letters 17, 680e690.

Khan, K.S., Joergensen, R.G., 2012. Relationships between P fractions and the microbial biomass in soils under different land use management. Geoderma 173, 274e281.

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, 617e622.

Liebisch, F., Keller, F., Huguenin-Elie, O., Frossard, E., Oberson, A., Bünemann, E.K., 2014. Seasonal dynamics and turnover of microbial phosphorus in a permanent grassland. Biology and Fertility of Soils 50, 465e475.

McLaughlin, M.J., Alston, A.M., Martin, J.K., 1988. Phosphorus cycling in wheat pasture rotations. II. The role of the microbial biomass in phosphorus cycling. Soil Research 26, 333e342.

Mooshammer, M., Wanek, W., Zechmeister-Boltenstern, S., Richter, A., 2015. Stoichiometric imbalances between terrestrial decomposer communities and their resources: mechanisms and implications of microbial adaptations to their resources. Frontiers in Microbiology 4, 22.

Oberson, A., Joner, E.J., 2005. In: Turner, B. (Ed.), Microbial Turnover of Phosphorus in Soil. Organic Phosphorus in the Environment. CABI, Wallingford, pp. 133e164.

Oehl, F., Oberson, A., Probst, M., Fliessbach, A., Roth, H.R., Frossard, E., 2001a. Kinetics of microbial phosphorus uptake in cultivated soils. Biology and Fertility of Soils 34, 31e41.

Oehl, F., Oberson, A., Sinaj, S., Frossard, E., 2001b. Organic phosphorus mineralization studies using isotopic dilution techniques. Soil Science Society of America Journal 65, 780e787.

R Core Team R, 2013. A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Richardson, A.E., Barea, J.M., McNeill, A.M., Prigent-Combaret, C., 2009. Acquisition of phosphorus and nitrogen in the rhizosphere and plant growth promotion by microorganisms. Plant and Soil 321, 305e339.

Sandaa, R.A., Enger, Ø., Torsvik, V., 1998. Rapid method for fluorometric quantification of DNA in soil. Soil Biology and Biochemistry 30, 265e268.

Schwartz, E., 2007. Characterization of growing microorganisms in soil by stable isotope probing with H_2 ^{18}O. Applied and Environmental Microbiology 73, 2541e2546.

Spohn, M., 2016. Element cycling as driven by stoichiometric homeostasis of soil microorganisms. Basic and Applied Ecology 17, 471e478.

Spohn, M., Ermak, A., Kuzyakov, Y., 2013. Microbial gross organic phosphorus mineralization can be stimulated by root exudates - a ^{33}P isotopic dilution study. Soil Biology and Biochemistry 65, 254e263.

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, 74e81.

Spohn, M., Poetsch, 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, 168e175.

Van der Heijden, M.G., Bardgett, R.D., Van Straalen, N.M., 2008. The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. Ecology Letters 11, 296e310.

Vance, E.D., Brookes, P.C., Jenkinson, D.S., 1987. An extraction method for measuring soil microbial biomass C. Soil Biology and Biochemistry 19, 703e707.

Vishnivetskaya, T.A., Layton, A.C., Lau, M.C., Chauhan, A., Cheng, K.R., Meyers, A.J., Pfiffner, S.M., 2014. Commercial DNA extraction kits impact observed microbial community composition in permafrost samples. FEMS Microbiology Ecology 87 (1), 217e230.

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, 737e749.

Supporting Information

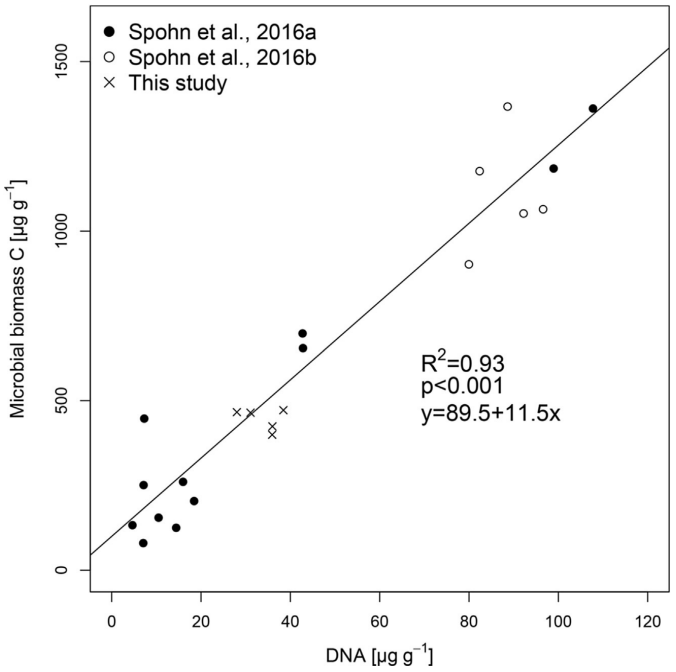


Figure S1: Correlation of microbial DNA and microbial biomass C concentrations of this study and of previous studies.

2.3 Study III

Microbial substrate stoichiometry governs nutrient effects on nitrogen cycling in grassland soils

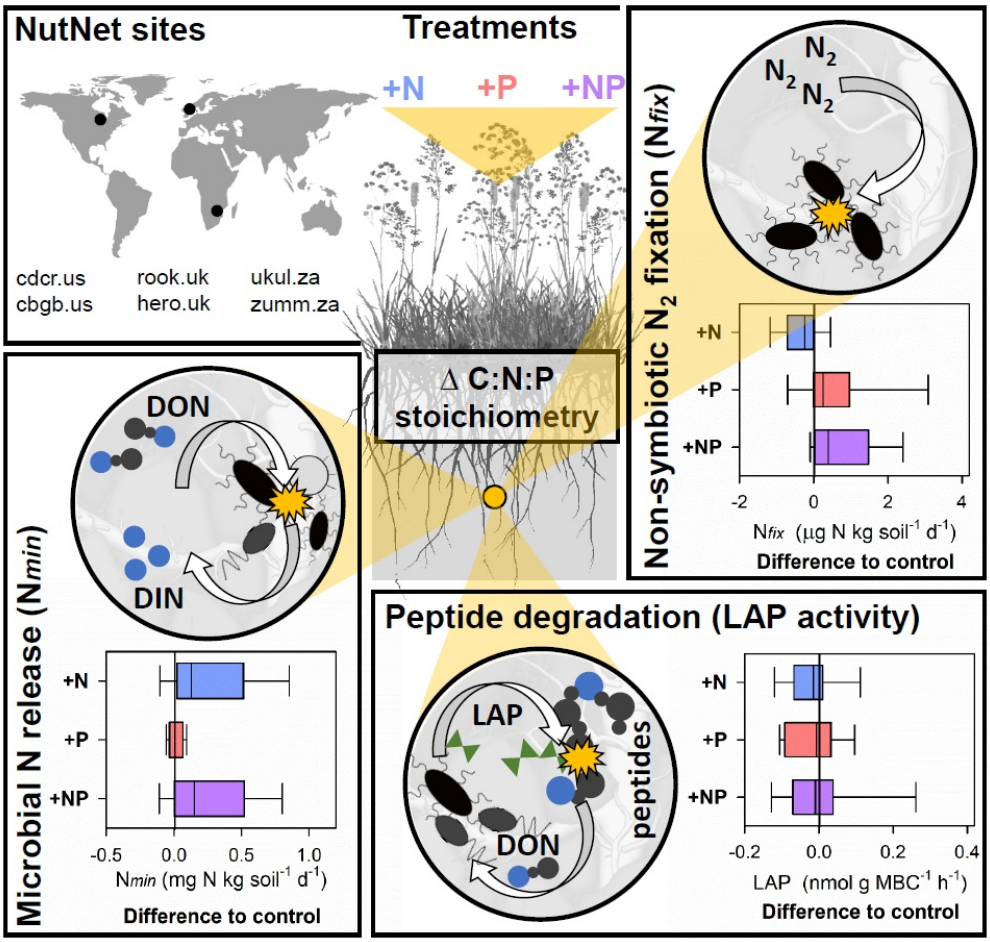
Per-M. Schleuss[§], Meike Widdig[§], Lori A. Biederman, Elizabeth T. Borer, Michael J. Crawley, Kevin P. Kirkman, Eric W. Seabloom, Peter D. Wragg, Marie Spohn (2021),
published in *Soil Biology and Biochemistry* 115, 108168,
DOI: https://doi.org/10.1016/j.soilbio.2021.108168

[§]Equal contribution, shared first-authorship

Abstract

Human activities have increased nitrogen (N) and phosphorus (P) inputs in terrestrial ecosystems and altered carbon (C) availability, shifting the stoichiometry of microbial substrates in soils, such as the C:N:P ratios of the dissolved organic matter pool. These stoichiometric deviations between microbial biomass and its substrate may control microbial processes of N cycling. We studied the effects of this stoichiometric mismatch using a full factorial N and P addition experiment replicated in six grassland ecosystems in South Africa, the USA, and the UK. We found that N and P addition changed the dissolved organic matter C:N ratio, but not the C:N ratio of the soil microbial biomass. Compared to P addition, N addition decreased microbial N acquisition via non-symbiotic N_2 fixation by -55% and increased microbial N release via net N mineralization by +134%. A possible explanation is that the dissolved elements, e.g., dissolved organic C (DOC) and dissolved total N (DN), serve as the main microbial substrate and its C:N ratio defines whether N is scarce or abundant with respect to microbial demands. If N is available in excess relative to microbial demands, net N mineralization increases. In contrast, when N is scarce, immobilization outweighs release decreasing net N mineralization. However, the activity of leucine aminopeptidases, which decompose peptides, was not affected by nutrient additions. Further, C rather than P availability may control the rates of non-symbiotic N_2 fixation in the six studied grassland sites. In conclusion, globally increasing nutrient inputs change processes of microbial N acquisition and release in grassland ecosystems and these changes are largely driven by shifts in substrate stoichiometry.

Graphical Abstract



2.3.1 Introduction

Nitrogen (N) inputs to terrestrial ecosystems have strongly increased through human activities during the last century. At present, fossil fuel burning and industrial production of N fertilizer contribute to 60 % of the global N inputs to terrestrial ecosystems with approximately 295 Gt N yr^{-1} (Schlesinger, 2009). Compared to 100 years ago, anthropogenic N inputs have increased by a factor of 10 (Galloway et al., 2008), and the global N deposition rate is predicted to double by 2050 relative to the present deposition rate (Galloway et al., 2004; Galloway et al., 2008). In contrast, anthropogenic phosphorus (P) inputs increased to a much smaller extent (Peñuelas et al., 2013). As a consequence, carbon (C), N and P availabilities in soil have changed and have affected the soil C:N:P stoichiometry (Sardans et al., 2012).

Changes in bioavailable element ratios are important components of soil element cycling processes (Manzoni et al., 2008; Mooshammer et al., 2014; Spohn, 2016; Schleuss et al., 2019). Soil microorganisms thrive mostly on dissolved organic matter (DOM)

(Marschner and Kalbitz, 2003), and the C:N ratio of this organic substrate determines whether N is scarce or abundant with respect to their demands. If nutrient cycling is consumer driven, microorganisms meet their nutrient demands by acquiring and immobilizing limiting elements and releasing those elements supplied in excess (Elser et al., 2007; Zechmeister-Boltenstern et al., 2015). Thus, the stoichiometry of the microbial substrate can mediate microbial processes of element acquisition and release (Mooshammer et al., 2014; Zechmeister-Boltenstern et al., 2015; Spohn, 2016).

Nitrogen additions alter the availabilities of N relative to C in the pool of available elements either directly through inputs of inorganic N forms (e.g. due to fertilizers that contain NO_3^- and NH_4^+), or indirectly by altering the quantity and quality (e.g. the stoichiometric composition) of above- and belowground plant residuals (Elser et al., 2007; Sardans et al., 2012; Yuan and Chen, 2012). Changes in soil element availabilities (i.e. C, N, and P) or element stoichiometry (i.e. C:N:P ratio) affect different biochemical processes by which microorganisms acquire or release N. For instance, several prokaryotes have developed mechanisms to turn the almost inert atmospheric N_2 into reactive N forms (Dixon and Kahn, 2004). This process, non-symbiotic N_2 fixation, is driven by the nitrogenase enzyme complex and is one of the metabolically most costly processes on Earth (Smith, 1992). The fixation of N_2 requires 16 moles of ATP (Simpson and Burris, 1984) and depends on the presence of easily-available C compounds as energy source, as well as on sufficient P to synthesize large amounts of ATP (Eisele et al., 1989; Vitousek et al., 2002; Reed et al., 2011; Bürgmann et al., 2005). High N availability has been shown to decrease N_2 fixation rates likely because of its high energetic cost, as it is less energy-consuming for microorganisms to use available reactive N than to fix N_2. Further, the abundance and diversity of the N_2 fixing bacterial community decreases with increasing N availability in soils (Smith, 1992; Reed et al., 2011; Fani et al., 2000; Hartley and Schlesinger, 2002).

Another important process providing bioavailable N in soil is the breakdown of N-containing organic polymers (Schimel and Bennett, 2004). In terrestrial ecosystems, most N is stored in organic forms, such as living and dead organic matter. The decomposition of N-containing compounds and the mineralization of organic N is catalyzed by microbial- and plant-derived enzymes (Kandeler et al., 2011). An important group of enzymes for the decomposition of N-containing compounds are leucine aminopeptidases (LAP) that hydrolyze peptides containing leucine and other hydrophobic amino acids (Sinsabaugh et al., 2008). According to resource allocation theory, N addition increases the activities of C- and P-acquiring enzymes and decreases the activities of enzymes degrading organic N compounds (Allison et al., 2007). Several studies have found a decrease in LAP activity in soil in response to elevated N inputs (Saiya-Cork et al., 2002; Nemergut et al., 2008; Ramirez et al., 2012; Schleuss et al., 2019), although others have found that LAP activity was unaffected by N addition (Chen et al., 2018).

N is released from N-containing organic compounds as ammonium (N mineralization) and is often quickly turned into nitrate by microorganisms (Geisseler et al., 2010). In

addition, it is immobilized, i.e. taken up, by the soil microbial biomass (Schimel and Bennett, 2004). The difference between gross N mineralization and microbial N immobilization determines how much inorganic N is released into the soil (defined as net N mineralization rate). Usually, the net N mineralization rate increases with elevated N inputs (Vestgarden et al., 2003; Ma et al., 2011; Andersson et al., 2001; Nave et al., 2009; Zhang et al., 2012) and decreases with increasing substrate C:N ratio (Manzoni et al., 2008; Heuck and Spohn, 2016). The lowest substrate C:N ratio at which mineralization levels out immobilization is termed critical C:N ratio or critical threshold C:N ratio (Bosatta and Berendse, 1984; Manzoni et al., 2008; Heuck and Spohn, 2016), which is an indication for C or N deficiency of specific substrate with respect to the requirements of the homeostatic decomposer community (Bosatta and Berendse, 1984).

In this study, we aim to understand how changes in bioavailable element concentrations, in response to chronic N and P additions, affect the processes of microbial N cycling. Further, we explore which biotic and abiotic factors control processes of microbial N acquisition and release to identify key drivers of N cycling processes in grassland soils. For this purpose, we studied six different grasslands located in the USA, the UK, and South Africa that are part of a globally coordinated and standardized experimental research cooperative (Nutrient Network). Altogether, these sites cover broad biogeographic gradients including soil chemistry and plant communities.

We hypothesized that long-term N and P additions would change the availabilities of C, N, and P on which the soil microorganisms thrive; specifically, the concentrations of dissolved organic C (DOC), dissolved N (DN) and dissolved inorganic P (DIP) (Hypothesis 1). Second, we hypothesized that P addition increases microbial N acquisition through elevated N_2 fixation (Hypothesis 2). Third, we hypothesized that processes of N acquisition, such as the non-symbiotic N_2 fixation and the leucine-aminopeptidase activity, would decline with higher N availability (Hypothesis 3). Further, we expected that microorganisms would release excess N into the soil once their N demands are covered, and finally we hypothesized that net N mineralization would increase with elevated reactive N supply (Hypothesis 4).

2.3.2 Materials and Methods

2.3.2.1 Experimental sites

We studied six grassland soils on three different continents. Two sites were located in the United Kingdom (hero.uk and rook.uk), two sites were located in South Africa (ukul.za and summ.za), and two sites were located in the United States of America (cdcr.us and cbgb.us, Table 1) as described in Widdig et al. (2019) and Widdig et al. (2020b). All sites are imbedded in the Nutrient Network (NutNet), a global research cooperative using standardized nutrient additions experiments (Borer et al., 2014). In this study, four treatments were investigated, namely a control treatment (Ctrl), a N addition treatment

(+N), a P addition treatment (+P), and a combined N and P addition treatment (+NP). Both N and P were added annually for more than seven years at all sites at doses of 10 g m^{-2} as time-release urea ($(NH_2)_2CO$) and as triple-super phosphate ($Ca(H_2PO_4)_2$), respectively. The treatments were replicated in three 5 x 5 m plots that were arranged in randomized block design (Borer et al., 2014).

2.3.2.2 Sampling and sample preparation

We collected soil samples of all six sites at the time of peak biomass in 2017. The South African sites were sampled in February 2017, the American sites in September 2017, and the English sites in October 2017. Two soil depth increments were sampled (0-15 cm and 15-30 cm) with exception of the summ.za grassland site, where extremely shallow soil profiles prevented the sampling of the second depth increment (15-30 cm).

Six soil samples were taken per plot, and all samples were combined in one mixed sample. Samples were shipped to the University of Bayreuth and prepared within one week after sampling. Soil was sieved (<2 mm) and roots were carefully removed. For elemental analyses, soil material was dried at 50°C and subsequently milled. Other material was adjusted to 60% of the maximal water holding capacity and incubated at 15°C for subsequent measurements (i.e. for the net N mineralization rate (Nmin), the non-symbiotic N_2 fixation rate (Nfix), and leucine aminopeptidase (LAP) activity; see description below).

2.3.2.3 Element concentrations in soil and soil water extracts

Total soil organic C (TOC) and total N (TN) concentrations were analyzed using an element analyzer (Vario Max Elementar, Hanau, Germany) after samples had been milled. Total P (TP) concentration of milled soil was determined using an inductively coupled plasma-optical emission spectroscopy (ICP-OES, Vista-Pro radia, Varian) after a pressure digestion in concentrated nitric acid.

For measurements of element concentrations in soil water extracts, a dry-mass equivalent of 20 g soil was extracted in 80 ml distilled water and filtrated (0.45µm) using an under-pressure device. The filtrated water extracts were measured for total dissolved nitrogen (DN; TOC-TN Analyser, Jena Analytics), dissolved organic carbon (DOC; TOC-TN Analyser, Jena Analytics) and dissolved inorganic phosphorus (DIP; UV 1800, Shimadzu).

Table 1: Site name, code, country, latitude, longitude, mean annual precipitation (MAP), mean annual temperature (MAT), year of first nutrient addition, management, soil texture, soil pH, total organic carbon (TOC), total nitrogen (TN), total phosphorus (TP) concentration, habitat, time of biomass sampling, dominant plant species, and aboveground net primary productivity (ANPP) of the six grassland sites.

Site name	Ukulinga	Summerveld	Cedar Creek	Chichaqua Bottoms	Rookery	Heron's Brook
Site code	ukul.za	summ.za	cdcr.us	cbgb.us	rook.uk	hero.uk
Country	South Africa	South Africa	USA	USA	UK	UK
Latitude	-29.67	-29.81	45.43	41.79	51.41	51.41
Longitude	30.4	30.72	-93.21	-93.21	-0.64	-0.64
MAP (mm)	838	809	800	891	678	678
MAT (°C)	18	18	6	9	10	10
First nutrient addition	2010	2011	2008	2010	2008	2008
Management	regularly burned	regularly burned	not burned	regularly burned	not burned	not burned
Soil texture (sand%, silt%, clay%)[1]	(4, 43, 53)	(50, 33, 17)	(90, 7, 3)	(85, 10, 5)	(75, 18, 7)	(65, 25, 10)
soil pH (H_2O)[1]	5.9	5.2	5.3	5.7	3.8	5.1
TOC (g C kg soil^{-1})[1]	42	49	9	7	24	37
TN (g N kg soil^{-1})[1]	2.9	2.8	0.7	0.6	2.0	3.2
TP (g P kg soil^{-1})[1]	0.45	0.37	0.31	0.28	0.38	0.62
Habitat	Mesic grassland	Mesic grassland	Tallgrass prairie	Tallgrass prairie restored	Mesic grassland	Mesic grassland
Biomass sampling	Nov. – Mar.	Feb. – Mar.	Jul. – Aug.	Aug.	Aug.	Aug.
Dominant plant species[2,3]	*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*	*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*
ANPP (g m^{-2} yr^{-1})[3]	498	349	178	397	180	509

[1] Data refer to the upper 15 cm of soil
[2] Taxa are in alphabetic order
[3] Data refer to the control (without any addition of N and P)

2.3.2.4 Microbial biomass C and N

Microbial biomass C and N concentrations (MBC and MBN) were determined using the chloroform fumigation-extraction method (Brookes et al., 1982; Vance et al., 1987). Fumigation was performed for 24 h in the dark in a desiccator. 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 and N using a TOC/TN analyzer (multi N/C 2100, Analytik Jena, Jena, Germany). Microbial biomass C and N were calculated by subtracting the concentrations of the extracts of the non-fumigated samples from the ones of the fumigated samples and multiplying the difference with a conversion factor of 2.22.

2.3.2.5 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 soil was incubated within a ^{15}N enriched artificial atmosphere containing 99.8 atom% $^{15}N_2$ (Sigma Aldrich). Fresh soil (60% WHC) was filled into 12 ml exetainer (Labco). All exetainers were gas-tightly closed, flushed with argon (Ar), carefully evacuated, and finally filled with 7.2 ml $^{15}N_2$ and 0.8 ml O_2. 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%, respectively. Fresh soil samples were incubated in this atmosphere in the dark at 15°C for 72 h and afterwards dried at 50°C. Subsequently, soil exposed to $^{15}N_2$ (^{15}N labeled) and soil not exposed (natural abundance) was 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 rate (in ng N g soil^{-1} h^{-1}) was estimated using an isotope mixing model (Zechmeister-Boltenstern, 1996):

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

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

2.3.2.6 Leucine aminopeptidase activity

LAP activity was determined according to Marx et al. (2001), German et al. (2011) and Herold et al. (2014). One gram of moist soil was homogenized in 50 ml of sterile

water by shaking for 20 min. The soil homogenates were pipetted into microplates and fluorescent substrate solution was added. Samples were preincubated in the dark at 15°C for 30 min, and subsequently measured fluorometrically after 0, 30, 60, and 180 min using a microplate reader (Infinite® 200 PRO, TECAN). Fluorescence was corrected for quenching of the soil as well as for the fluorescence of substrate and soil (German et al., 2011). LAP activity was calculated from the slope of net fluorescence over incubation time in nmol g^{-1} h^{-1} according to German et al. (2011).

2.3.2.7 Net N mineralization

Approximately 150 g of soil was adjusted to 60% of the maximal water holding capacity and incubated at 15°C in a 2 l PET-bottle. After 0, 14, 28, and 42 days of incubation, subsamples of 20 g soil dry-mass equivalent were extracted in 80 ml distilled water on an overhead shaker for one hour and filtrated as stated above. The extraction-filtration procedure was repeated after 0, 14, 28, and 42 days of soil incubation. 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-NH_4^+ plus N-NO_3^- (dissolved inorganic N, DIN) over time.

2.3.2.8 Aboveground net primary productivity (ANPP)

At peak biomass in 2017 at all sampled sites, all aboveground plant biomass was clipped in two 0.1 x 1 m strips within each 5 x 5 m plot, sorted to functional group, dried to a constant mass at 60 °C, and weighted to the nearest 0.01 g. ANPP was estimated by summing the combined live biomass from these functional groups.

2.3.2.9 Statistical approaches

Statistical analyses, data transformation, and data presentation were carried out using SigmaPlot 13 (SYSSTAT) and R version 3.3.1 (R Core Team, 2018). We used different statistical approaches to identify effects of nutrient addition within and across sites for element concentrations in soil and soil water extracts, C:N ratios of microbial biomass and DOM, net N mineralization, leucine-aminopeptidase activity and non-symbiotic N_2 fixation.

Effects of N and P addition within sites

For all six sites, two-way ANOVA was used for each variable to indicate effects of single and combined N and P addition in both depth increments, separately (with "treatment" and "depth" defining the two factors in the model). The ANOVAs were followed by post-hoc test for multiple comparisons using the Tukey-test ($p < 0.05$). Previously, data were checked for normality (Shapiro-Wilk-test, $p > 0.05$) and homogeneity of variance (Levene-test, $p > 0.05$). If necessary, data were log- or square root-transformed and retested.

Effects of N and P addition across sites

To identify changes across sites, linear mixed-effects models were performed using the "nlme" package (Pinheiro et al., 2018) and the "multcomp" package (Hothorn et al., 2008) for post-hoc test in R. Treatment was set as fixed factor in the model. Additional variance coming from different geographical locations was eliminated by selecting "site" as random factor. LME was followed by Tukey-post-hoc test for multiple comparisons ($p < 0.05$). In some cases, data were log- or square root-transformed to meet requirements of normality (Shapiro-Wilk-test, $p > 0.05$) and homogeneity of variance (Levene-test, $p > 0.05$). Concurrent with the LME-output, all variables were based on the control by subtracting the average of the control from each treatment (+N, +P, +NP). Thus, values higher or lower than zero indicate a positive or negative treatment effect, respectively.

Multivariate statistics

To access biotic and abiotic drivers of N cycling processes, we performed multi-model selections (Grueber et al., 2011). Multi-model selection allows assessing the relative importance of a range of covarying factors and thus can help to examine the effect of soil chemical and physical factors as well as climatic factors on N cycling processes. A global model, containing TOC, TN, TP, DOC, DN, and DIP concentrations, TOC:TN, TOC:TP, TN:TP, DOC:DN, and DOC:DIP ratios, soil pH, sand content, mean annual temperature (MAT), mean annual precipitation (MAP), and ANPP, was used to predict changes in soil N cycling processes; e.g. net N mineralization rates, non-symbiotic N_2 fixation rates and leucine-aminopeptidase activities across all sites and treatments. All input variables in this model were standardized prior to analysis using the standardize function within the arm R library package (Gelman et al., 2018) to allow interpreting the parameter estimates after model averaging. Further, random intercepts for site and treatment were included to correct for site- and treatment-specific differences. All possible models were run using the dredge function in MuMIn R library package (Barton, 2018) and a full submodel set was generated. Of these models, the best ones were selected based on their AIC corrected for small sample size (AICc). The models within four AICc units of the model with the lowest AICc were selected and averaged. For each model variable, a relative variable importance was calculated by dividing the sum of the AIC weights of all models in which the variable appears by all generated models. The relative variable importance ranges between 0 and 1. Variables that appear in all models are assigned an importance of 1, indicating the highest explanatory weight. The variables, which showed the highest relative importance (> 0.80), were used to fit linear mixed-effects models. For these models, p-values were obtained by likelihood ratio test of the full model with the effect in question against the model without the effect in question. Further, R^2 were calculated as conditional R^2 according to Nakagawa and Schielzeth (2013).

2.3.3 Results

2.3.3.1 Element concentrations in soil and soil water extracts

Soil TOC, TN, and TP concentrations did not significantly change in response to single and combined N and P addition across all sites (Figure S1). However, analyzing the effect of nutrient addition on soil element concentrations within each site (each site tested independently) showed that the TP concentration increased in response to single P and combined NP addition in half of the sites under study in the upper depth increment (e.g. in cbgb.us, root.uk, ukul.za; for more information see supplement text 1, Table S1).

The effects of N and P addition were more pronounced for dissolved soil element concentrations than for total element concentrations, with strongest responses in the upper 15 cm (Table S1). Across all sites, combined NP addition increased DOC concentrations in 0-15 cm by +27 % ($p < 0.05$) as compared to the control (Figure 1). The DOC concentration in 0-15 cm ranged between 98 and 115 mg C kg soil^{-1} in the South African sites, while it was considerably lower in the UK sites (23-29 mg C kg soil^{-1}) and US sites (15-18 mg C kg soil^{-1}) (Table S2).

Compared to the control, single N addition increased DN concentrations by 199% and 96%, and combined NP addition by 116% and 86% in 0-15 cm and 15-30 cm depth, respectively (Figure 1). In contrast, P addition had no effect on the DN concentration in both depth increments of all six sites (Figure 1). DN concentrations in 0-15 cm ranged between 3.2-14.2 mg N kg soil^{-1} across all six sites and were 25-70% lower in 15-30 cm (range: 1.8-9.7 mg N kg soil^{-1}) (Table S2). The main part of the DN pool is formed by N-NO_3^- concentrations, which showed very similar patterns as revealed for the DN pool (Table S2).

N and P addition changed the C:N ratio of the DOM. The DOC:DN ratio was significantly lower by 71% and 43% in treatments with than without N addition in 0-15 cm and 15-30 cm, respectively, across all six sites (Figure 2). The molar DOC:DN ratio in the controls of all six sites ranged between 2-18 and 3-27 in 0-15 cm and 15-30 cm, respectively (Table S2). We did not find any significant effect of P addition on the DOC:DN ratio in both soil depths across all six sites. However, in all six sites, P addition consistently increased DIP concentrations as compared to the control, while N addition had no effect on DIP in both soil depths (Table S2). Overall, DIP concentrations in 0-15 cm and 15-30 cm were 3300% and 3900% higher due to single P addition and 3500% and 3700% higher due to NP addition as compared to the control, respectively (Figure 1).

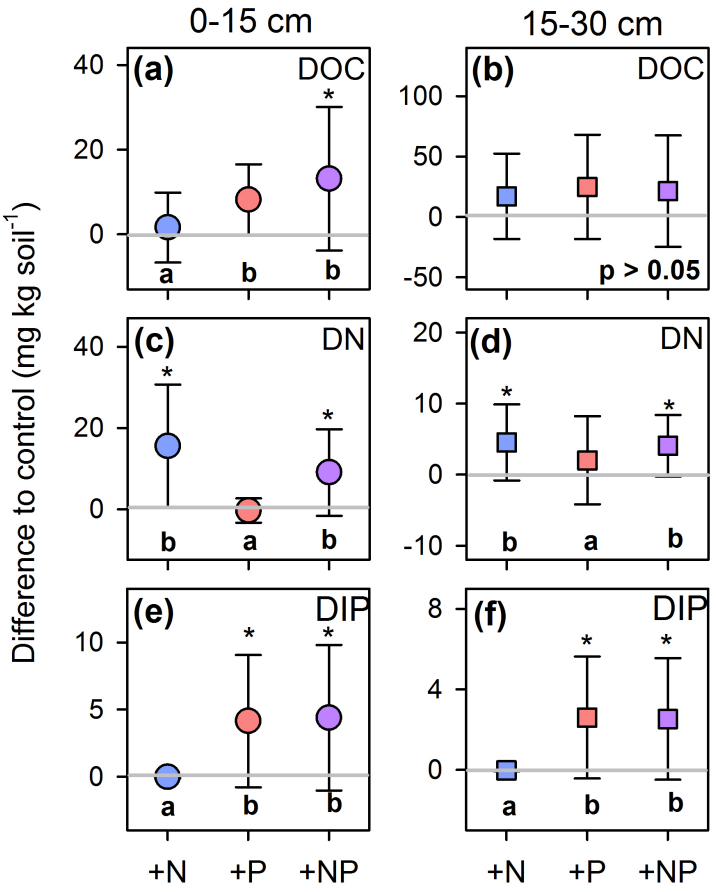


Figure 1: Changes in dissolved element concentrations in 0-15 cm (n=54) and 15-30 cm (n=45) due to N and P addition across all six sites. Changes in variables were calculated by subtracting the control from the treatment. Element concentrations include dissolved organic C (DOC) (a & b), dissolved total N (DN) (c & d), and dissolved inorganic P (DIP) (e & f). Linear mixed-effects models were performed with treatment as fixed factor and random intercepts for sites followed by Tukey-post-hoc test for multiple comparisons. Significant differences ($p < 0.05$) between treatments (N, P, and NP) are indicated by lower-case letters, while asterisks (*) indicate a significant difference ($p < 0.05$) between a treatment and the control. The mean value of the control is illustrated with the grey line (Ctrl = 0).

2.3.3.2 Aboveground net primary productivity (ANPP)

All nutrient addition treatments increased ANPP compared to the control considering all six grassland sites. Across all sites, N addition increased ANPP by 23%, P addition by 27% and NP addition by 37% as compared to the control, respectively (Figure S2a). However, no significant changes were found among N, P and NP addition treatments across all sites. Across all sites, we found a positive correlation between DOC concentrations and ANPP (Figure S2b).

2.3.3.3 Microbial biomass

We found no significant difference between nutrient addition treatments for microbial biomass carbon (MBC) across all six sites (Figure 2). Separate analyses within each site also revealed no significant effect of N and P addition on MBC in both soil depths (Table S3). The MBC in 0-15 cm was highest in soils of the South-African sites (843-1005 mg C kg soil^{-1}), slightly lower in soils of the UK sites (651-661 mg C kg soil^{-1}), and lowest in soils of the US sites (175-163 mg C kg soil^{-1}, Table S3).

Although N and P addition strongly changed the C:N ratio of the DOM, the microbial C:N ratio (MBC:MBN ratio) did not change significantly with nutrient additions in both soil depths across all six sites (Figure 2). The mean MBC:MBN ratio was 9.22 and 9.89 considering all treatments and sites in 0-15 and 15-30 cm, respectively (the second depth increment in one UK site, Rookery, was not considered because MBN data were below the detection limit).

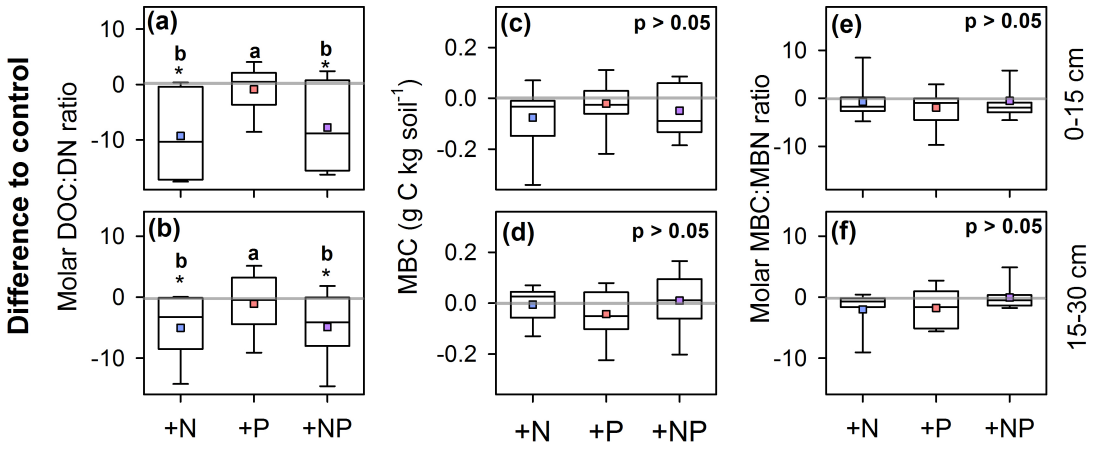


Figure 2: Changes in the molar DOC:DN ratio (a & b), microbial biomass carbon (MBC) concentration (c & d), and microbial biomass C:N stoichiometry (MBC:MBN ratio) (e & f) for two soil increments (0-15 cm and 15-30 cm) in response to N and P addition across all six sites. The substrate C:N ratio is measured as DOC:DN ratio and the microbial C:N ratio is measured as MBC:MBN ratio. Changes in variables were calculated by subtracting the control from the treatment. Box plots show the median (black line), the mean (colored squares), the 25^th and the 75^th percentile (box edges) and the 10^th and the 90^th percentile (error bars). Linear mixed-effects models were performed with treatment as fixed factor and random intercepts for sites followed by Tukey-post-hoc test for multiple comparisons. MBN values in the second soil depth increment in Rookery were below detection limit and thus excluded from the analysis (0-15 cm: n = 18; 15-30 cm: n = 12). Significant differences ($p < 0.05$) between treatments (N, P, and NP) are indicated by lower-case letters, while asterisks (*) indicate a significant difference ($p < 0.05$) between a treatment and the control. The mean value of the control is illustrated with the grey line (Ctrl = 0).

2.3.3.4 Non-symbiotic N_2 fixation

Non-symbiotic N_2 fixation rates were significantly higher under NP addition than in the control. Further, they were significantly higher under P and NP addition than under N addition in 0-15 cm depth, whereas N_2 fixation rates were not significantly changed in response to nutrient addition in 15-30 cm depth (Figure 3). In 0-15 cm depth, under P and NP addition, N_2 fixation rates were 122% and 179% higher than under N addition, respectively. Further, under NP addition N_2 fixation rates in 0-15 cm depth were 96% higher than in the control (Figure 3). Non-symbiotic N_2 fixation was negatively correlated with the TN:TP ratios in both soil depth increments (0-15 cm depth: $R^2 = 0.20$, $p = 0.005$, 15-30 cm depth: $R^2 = 0.08$, $p = 0.04$, Figure 4). Mean non-symbiotic N_2 fixation rates were highest in the South African soils (2.58- 3.85 ng N g soil^{-1} d^{-1}), intermediate in the US soils (0.78-1.36 ng N soil^{-1} d^{-1}), and lowest in the UK soils (0.29-1.01 ng N g soil^{-1} d^{-1}, Table S3).

2.3.3.5 Leucine aminopeptidase activity

LAP activities per unit MBC were not significantly changed by any nutrient addition treatment in either the 0-15 or 15-30 cm soil depth (Figure 3). They were negatively correlated with DN concentrations in 0-15 cm soil depth ($R^2 = 0.15$, $p = 0.002$, Figure 4). In the control plots in 0-15 cm soil depth, LAP activities per unit MBC ranged from 22 and 53 nmol g MBC^{-1} h^{-1} in Rookery (UK) and Summerveld (South Africa), respectively, to 327 and 348 nmol g MBC^{-1} h^{-1} in the soils of the US sites, with intermediate LAP activity in Heron's Brook (UK) and Ukulinga (South Africa, Table S3).

2.3.3.6 Net N mineralization

Net N mineralization rates were significantly higher in treatments with N addition than without N addition in both soil depths across all six sites (Figure 3). Net N mineralization was 134% and 74% higher with single N addition and 138% and 108% higher with combined NP addition in 0-15 cm and 15-30 cm, respectively, compared to the control. P addition did not significantly change net N mineralization in both soil depths as compared to the control. Net N mineralization was highly positively correlated with DN concentrations in both soil depth increments (0-15 cm depth: $R^2 = 0.42$, 15-30 cm depth: $R^2 = 0.56$) and highly negatively correlated with DOC:DN ratios (0-15 cm depth: $R^2 = 0.61$, 15-30 cm depth: $R^2 = 0.67$, Figure 4). Net N mineralization was lowest in soils at the South African sites (0-0.03 mg N kg soil^{-1} d^{-1}), slightly higher in soils at the US sites (0.09-0.15 mg N kg soil^{-1} d^{-1}), and highest in soils at the UK sites (0.38-0.48 mg N kg soil^{-1} d^{-1}, Table S3).

2.3.3.7 Biotic and abiotic controls on N cycling processes

Multi-model selection revealed that DN concentration, ANPP, and soil pH were the best predictors of net N mineralization (Figure 5, Table S4). Together, these three variables accounted for 89% of variability in net N mineralization. Another model with DOC:DN ratios instead of DN concentrations explained 84% of variability in net N mineralization (data not shown). TOC:TP ratio and DOC:DIP ratio were the best predictors of N_2 fixation and explained 83% of variability (Figure 5, Table S4). The proportion of explained variability of N_2 fixation was larger when the TN:TP ratio was included ($R^2 = 0.85$). However, we excluded TN:TP ratio because it was intercorrelated with TOC:TP ratio. Soil pH, MAT, and MAP were the best predictors of leucine aminopeptidase activity ($R^2 = 0.61$, Figure 5, Table S4).

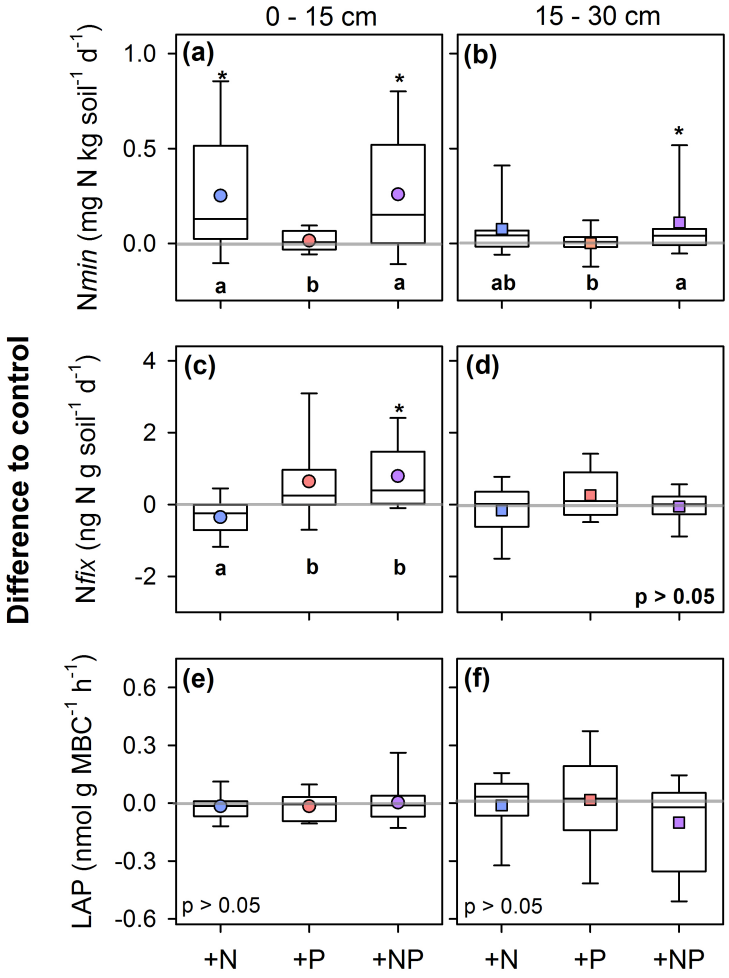


Figure 3: Changes in net N mineralization rate (N*min*) (a & b), non-symbiotic N_2 fixation rate (Nfix) (c & d) and leucine aminopeptidase activity (LAP) (e & f) in the two soil depth increments depending on N and P addition across all six sites. Changes in variables (N*min*, N*fix* and LAP) were calculated by subtracting the control from each treatment. Colored circles and squares (+N in blue, +P in red, and +NP in purple) represent the mean value ± SD for each treatment in 0-15 cm (n=18) and in 15-30 cm (n=15). Box plots show the median (black line), the 25^th and the 75^th percentile (box edges) and the 10^th and the 90^th percentile (error bars). Linear mixed-effects models were performed with treatment as fixed factor and random intercepts for sites. Significant differences ($p < 0.05$) between treatments (N, P, and NP) are indicated by lower-case letters, while asterisks (*) indicate a significant difference ($p < 0.05$) between a treatment and the control (indicated by the grey line).

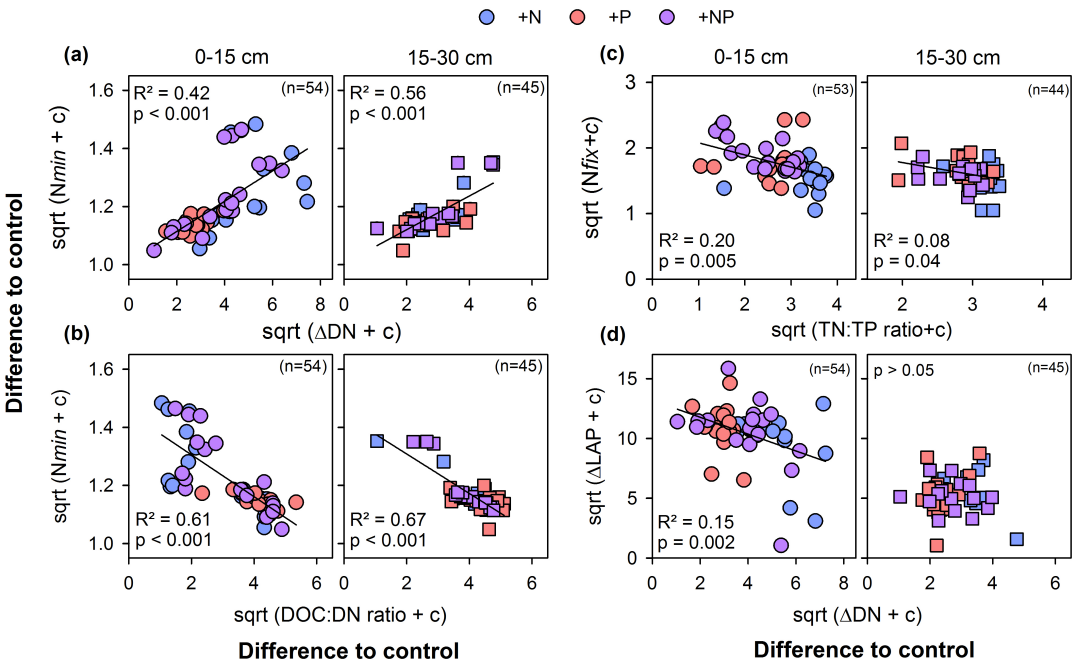


Figure 4: Relationships between changes in soil N cycling processes and changing element availabilities due to N and P additions across all sites in 0-15 cm and 15-30 cm soil depth. (a) Correlations between N mineralization rate (N*min*) and dissolved N concentrations (DN), (b) correlation between N mineralization rate and dissolved organic matter C:N ratios (DOC:DN ratio), (c) correlations between non-symbiotic N_2 fixation (N*fix*) and total N:total P ratio (TN:TP ratio) and (d) correlations between leucine aminopeptidase activity per MBC (LAP) and dissolved N concentrations (DN). Changes in variables were calculated by subtracting the control from each treatment (+N, +P, +NP). In order to avoided negative values for subsequent square root-transformations changes in variables were adjusted by adding a constant (c = minimum of data frame + 1.1).

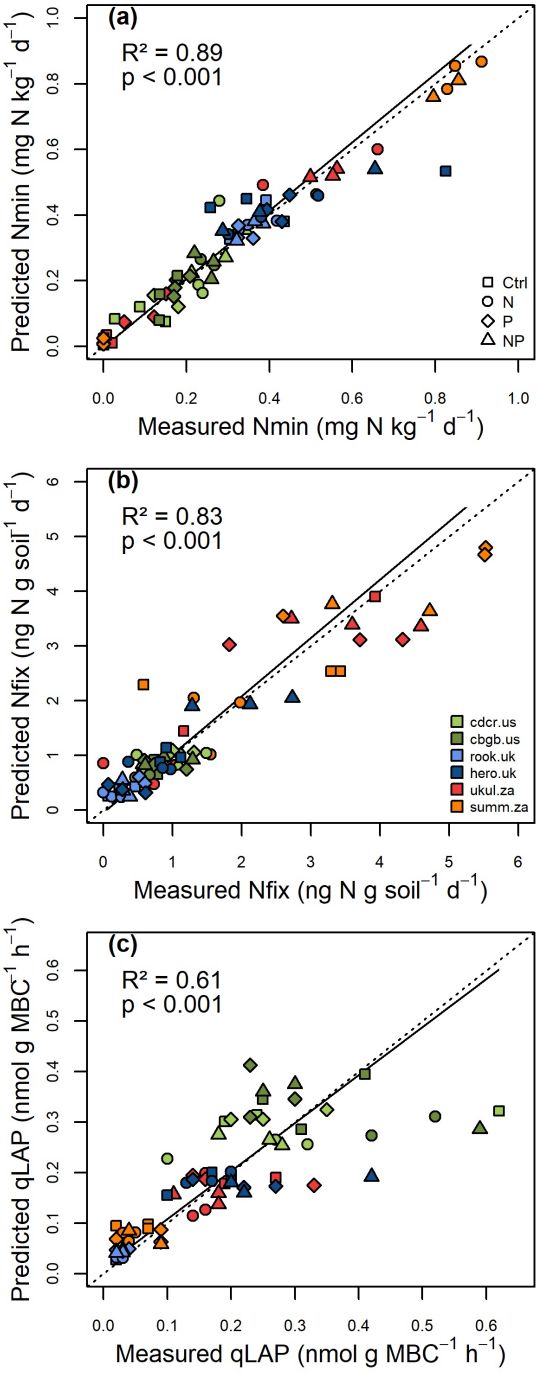


Figure 5: Performance of linear mixed-effects models of (a) N mineralization (Nmin), (b) N fixation (Nfix), and (c) leucine aminopeptidase activity per unit microbial biomass carbon (qLAP) of all treatments at all six grassland sites in 0-15 cm depth. Measured values are shown on the x-axes and predicted values are shown on the y-axes. Best model predictors for Nmin were dissolved nitrogen, soil pH, and ANPP. Best model predictors for Nfix were total organic carbon-to-total phosphorus ratio and dissolved organic carbon-to-dissolved inorganic phosphorus ratio. Best model predictors for qLAP were mean annual temperature, soil pH, and mean annual precipitation (Table S4). The linear mixed-effects models were 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.

2.3.4 Discussion

2.3.4.1 Soil and microbial element concentrations

Although N and P additions caused strong changes in the DOC:DN ratio, the C:N ratio of the soil microbial biomass (MBC:MBN ratio) was not affect by N and P addition. While some studies show that the microbial stoichiometry adapts to changes of their substrate after nutrient additions (Li et al., 2012; Khan and Joergensen, 2019), our finding builds on previous studies indicating that the microbial biomass C:N ratio is not affected by nutrient additions (Joergensen and Scheu, 1999; Heuck et al., 2015; Schleuss et al., 2019; Tapia-Torres et al., 2015). These results suggest that the microbial community adjusts processes of element acquisition, uptake, turnover, partitioning, and release according to its stoichiometric demands, which allows soil microorganisms to maintain their biomass stoichiometry despite changes in the ratio of bioavailable elements in its environment (Sterner and Elser, 2002; Zechmeister-Boltenstern et al., 2015; Spohn, 2016).

Consistent with our first hypothesis, N addition increased DN while P addition increased DIP concentrations. Nutrient additions also elevated DOC concentrations at some sites, and DOC concentration was positively correlated with ANPP across all sites (Figure S2). A possible reason for this is that the added nutrients elevated plant productivity (Figure S2), which increased above- and belowground C inputs (Zak et al., 1994; Dias et al., 2010; Peterson and Lajtha, 2013). This explanation is consistent with a previous meta-analysis showing that N addition enhances soil C inputs and leads to higher DOC concentrations in soils of various ecosystems (Lu et al., 2011). An alternative explanation might be that P and NP additions have changed biochemical processes affecting the C availability in soil. For instance, the addition of N or P could have affected the production of fine roots and mycorrhizal fungi (Leff et al., 2015), or inorganic P might have desorbed organic C compounds from soil minerals (Spohn and Schleuss, 2019).

N addition did not increase soil TN concentrations in most sites under study, even though about 70-100 g N m^{-2} was added throughout the last seven to nine years. Although some of the added N might have been removed due to specific grassland management practices (e.g. annual biomass harvest or burning), it is likely that the majority of added N has been translocated to deeper soil depths (> 30 cm) or was even leached below the rooting zone. This seems plausible because N is directly added at the beginning of the vegetation period and most of the urea-fertilizer is dissociated to inorganic N species (e.g. NO_3^- or NH_4^+). While NH_4^+ is preferentially taken up by most plants (Bloom et al., 1992; Gazzarrini et al., 1999; Wiren et al., 2000), large parts of NO_3^-, which is the main compartment of the total dissolved N pool (DN), may have been leached due to its high mobility, and thus did not increase soil N concentrations.

2.3.4.2 Processes of N acquisition depending on nutrient addition

The non-symbiotic N_2 fixation rate was lower under N than under P addition across all six sites, which is partly consistent with our second hypothesis. The reason why P addition might lead to a higher non-symbiotic N_2 fixation rate relative to N addition is that the fixation of atmospheric N_2 is highly energy-consuming and requires high levels of ATP in the microbial cell. Further, the negative correlation with soil TN:TP ratio suggests that besides a sufficient P supply, a low N availability favors non-symbiotic N_2 fixation. This is probably because it is less energy demanding for microorganisms to take up mineral N than to fix atmospheric N_2 (Reed et al., 2011). Consequently, microorganisms likely regulate N_2 fixation based on soil stoichiometry to meet their nutritional demands (Mooshammer et al., 2014; Zechmeister-Boltenstern et al., 2015; Spohn, 2016).

Combined NP addition, in contrast to single P addition, significantly enhanced N_2 fixation rates. This finding agrees with studies showing a larger N_2 fixation under combined NP than under single P addition (Reed et al., 2011). The strong increase in non-symbiotic N_2 fixation under NP addition might be associated with the higher DOC concentrations, because the fixation of N_2 is a process with a high energy demand and microorganisms may use available C substrates as an energy source to enable nitrogenase production (Reed et al., 2011; Schleuss et al., 2020). For instance, between 5 and 117 g C are needed in different microorganisms to fix 1g of N_2 (Hill, 1992), and thus the C-input of fresh plant detritus likely facilitates non-symbiotic N_2 fixation in the NP treatments. Thus, our finding, based on multi-model selection, that the TOC:TP and DOC:DIP ratios accounted for a large proportion of variance in non-symbiotic N_2 fixation across the six grasslands might also indicate a C limitation of N_2 fixation (Widdig et al., 2020a). Taken together, in accordance with our hypothesis, N acquisition by microorganisms via non-symbiotic N_2 fixation was lower under N compared to P and NP addition and the soil stoichiometry turned out to be an important driver controlling non-symbiotic N_2 fixation rate. However, C rather than P availability seems to determine the non-symbiotic N_2 fixation rates in the six studied grassland soils.

Besides fixation of atmospheric N_2, microorganisms can acquire organic N through exudation of extracellular enzymes, e.g. through the decomposition of N-containing compounds. For instance, they can hydrolyze peptides by releasing the enzyme leucine-aminopeptidase. Given the observed effects of N availability on N mineralization, it seems likely that N addition also affects LAP activity. However, LAP activity did not change upon nutrient addition and multi-model selection indicated no main influence of nutrient availability on LAP activity. Similarly, LAP activity did not change upon N addition in three contrasting grasslands (Zeglin et al., 2007), in a semiarid grassland in New Mexico (Stursova et al., 2006), and a recent meta-analysis (Chen et al., 2018). Thus, some studies have argued that resource allocation theory might not apply to N-acquiring enzymes such as LAP (Jian et al., 2016; Sinsabaugh and Follstad Shah, 2012). However, we found a negative correlation of LAP activity with DN concentrations in

the topsoil across all sites and treatments, which indicated that microorganisms facing a relative high N availability invested less into the production of N-acquiring enzymes, consistent with resource allocation theory. Explanations for the independent response of LAP activity might be, first, that several enzymes besides LAP are involved in peptide degradation (Landi et al., 2011). Second, peptides are only one group of organic N pools in soils. There are several other classes of N-containing compounds, which alternatively could be degraded to meet the microbial N demands (Sinsabaugh et al., 2008; McGill and Cole, 1981). Third, peptides can also serve as important C sources (Farrell et al., 2014), thus LAP activity might be linked to microbial C rather than N demand (Sinsabaugh et al., 2008; Sinsabaugh and Follstad Shah, 2012). Finally, it might be that LAP activity is to a substantial extent produced by meiofauna (Abad et al., 2008), which has slightly different nutritional needs than the soil microbial biomass. Even though our results suggest that N acquisition through LAP activity was independent of nutrient addition, a more comprehensive knowledge of stoichiometric regulations including also other N degrading enzymes would help to better understand processes of microbial N cycling in response to elevated N and P inputs.

2.3.4.3 Processes of N release depending on nutrient addition

The net N mineralization rate significantly increased in response to N addition, both in the N and combined NP treatment. This finding indicates that soil microorganisms release inorganic N once available N is present in excess relative to the microbial demand. This result is consistent with our third hypothesis and with previous studies of grassland and forest soils (Nave et al., 2009; Ma et al., 2011; Zhang et al., 2012). Moreover, we found that the net N mineralization rate was negatively related to the DOC:DN ratio. This finding indicates that not only the available N but also the C:N stoichiometry of the DOM affects microbial N release as previously reported (Manzoni et al., 2008; Heuck and Spohn, 2016). If DOC:DN ratios are high, less N in contrast to C is available for soil microorganisms resulting in microbial N immobilization. Alternatively, if DOC:DN ratios are low, microorganisms facilitate net N mineralization because they are well-supplied with bioavailable N sources and release excessive N back to the soil. Multi-model selection confirmed the importance of DN concentrations and DOC:DN ratios for net N mineralization in the studied grassland soils.

The finding that besides nutrient availabilities, plant biomass was negatively related to net N mineralization across all sites might be due to a positive relationship between DOC concentration and ANPP.

Overall, the net N mineralization rate of the upper 15 cm in control treatments ranged between 0 and 0.48 mg N kg^{-1} d^{-1} for all sites. This range was very similar to values reported (a) by Risch et al. (2020) for 22 grasslands sites of the Nutrient Network on five different continents (range of net Nmin: 0-0.84 mg N kg^{-1} d^{-1}, mean net Nmin: $\approx$ 0.27 mg N kg^{-1} d^{-1}) and (b) also by Zhang et al. (2012) for different temporal measurements

in 2005 and 2006 at an Inner Mongolian grassland (range of Net Nmin: 0-0.51 mg N kg^{-1} d^{-1}).

2.3.4.4 Conclusion

Our study demonstrates that soil microorganisms adjusted processes of N acquisition and N release in response to nutrient additions. In line with our hypothesis, elevated N inputs altered the concentrations and ratios of bioavailable elements but had no effect on the microbial biomass C:N ratio across the studied grassland sites. Our study has important implications as it reinforces ecological stoichiometry as important concept linking biogeochemistry and microbial element cycling. Specifically, we show that soil microorganisms facing low N availability (e.g. a high DOC:DN ratio) invested into N acquisition via non-symbiotic N_2 fixation and increased N release through net N mineralization when they were exposed to high N availability (e.g. a low DOC:DN ratio) confirming our hypotheses. The decrease of symbiotic N_2 fixation and increase net N mineralization likely allowed the microbial community to maintain its biomass stoichiometry according to their nutritional demands. However, against our expectation, peptide degradation through leucine aminopeptidase was independent of nutrient additions demonstrating that likely other N-containing compounds have been degraded alternatively to meet the microbial N demand. Based on these findings we conclude that processes of microbial N cycling in grasslands are largely governed by microbial substrate stoichiometry. Our study demonstrates the importance of stoichiometric regulation for microbial element cycling in grassland soils, in particular under changing N and P availabilities.

Acknowledgements

The study was funded by the German Research Foundation within 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 (NSF-DEB-1234162 to Cedar Creek LTER) programs, and the Institute on the Environment (DG-0001-13). We further 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 chemical analyses, and the Centre for Stable Isotope Research and Analysis (KOSI) of the University of Göttingen for measuring ^{15}N isotopes.

References

Abad, P., Gouzy, J., Aury, J.-M., Castagnone-Sereno, P., Danchin, E.G.J., Deleury, E., Perfus-Barbeoch, L., Anthouard, V., Artiguenave, F., Blok, V.C., Caillaud, M.-C., Coutinho, P.M., Dasilva, C., Luca, F. de, Deau, F., Esquibet, M., Flutre, T.,

Goldstone, J.V., Hamamouch, N., Hewezi, T., Jaillon, O., Jubin, C., Leonetti, P., Magliano, M., Maier, T.R., Markov, G.V., McVeigh, P., Pesole, G., Poulain, J., Robinson-Rechavi, M., Sallet, E., Ségurens, B., Steinbach, D., Tytgat, T., Ugarte, E., van Ghelder, C., Veronico, P., Baum, T.J., Blaxter, M., Bleve-Zacheo, T., Davis, E.L., Ewbank, J.J., Favery, B., Grenier, E., Henrissat, B., Jones, J.T., Laudet, V., Maule, A.G., Quesneville, H., Rosso, M.-N., Schiex, T., Smant, G., Weissenbach, J., Wincker, P., 2008. Genome sequence of the metazoan plant-parasitic nematode Meloidogyne incognita. Nature biotechnology 26, 909–915.

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.

Andersson, P., Berggren, D., Johnsson, L., 2001. 30 Years of N Fertilisation in a Forest Ecosystem - The Fate of Added N and Effects on N Fluxes. Water, Air, and Soil Pollution 130, 637–642.

Barton, K., 2018. MuMIn: Multi-Model Inference. R package version 1.42.1.

Bloom, A.J., Sukrapanna, S.S., Warner, R.L., 1992. Root respiration associated with ammonium and nitrate absorption and assimilation by barley. Plant Physiology 99, 1294–1301.

Borer, E.T., Harpole, W.S., Adler, P.B., Lind, E.M., Orrock, J.L., Seabloom, E.W., Smith, M.D., 2014. Finding generality in ecology: a model for globally distributed experiments. Methods in ecology and evolution 5, 65–73.

Bosatta, E., Berendse, F., 1984. Energy or Nutrient Regulation of Decomposition: Implications for the Mineralization-Immobilization Response to Perturbations. Soil Biology and Biochemistry 16, 63–67.

Brookes, P.C., Powlson, D.S., Jenkinson, D.S., 1982. Measurement of microbial biomass phosphorus in soil. Soil Biology and Biochemistry 14, 319–329.

Bürgmann, H., Meier, S., Bunge, M., Widmer, F., Zeyer, J., 2005. Effects of model root exudates on structure and activity of a soil diazotroph community. Environmental Microbiology 7, 1711–1724.

Chen, H., Li, D., Zhao, J., Xiao, K., Wang, K., 2018. Effects of nitrogen addition on activities of soil nitrogen acquisition enzymes: A meta-analysis. Agriculture, Ecosystems & Environment 252, 126–131.

Dias, A.T.C., van Ruijven, J., Berendse, F., 2010. Plant species richness regulates soil respiration through changes in productivity. Oecologia 163, 805–813.

Dixon, R., Kahn, D., 2004. Genetic regulation of biological nitrogen fixation. Nature Reviews. Microbiology 2, 621–631.

Eisele, K.A., Schimel, D.S., Kapustka, L.A., Parton, W.J., 1989. Effects of available P and N:P ratios on non-symbiotic dinitrogen fixation in tallgrass prairie soils. Oecologia 79, 471–474.

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.

Fani, R., Gallo, R., Liò, P., 2000. Molecular evolution of nitrogen fixation: the evolutionary history of the nifD, nifK, nifE, and nifN genes. Journal of Molecular Evolution 51, 1–11.

Farrell, M., Prendergast-Miller, M., Jones, D.L., Hill, P.W., Condron, L.M., 2014. Soil microbial organic nitrogen uptake is regulated by carbon availability. Soil Biology and Biochemistry 77, 261–267.

Galloway, J.N., Dentener, F.J., Capone, D.G., Boyer, E.W., Howarth, R.W., Seitzinger, S.P., Asner, G.P., Cleveland, C.C., Green, P.A., Holland, E.A., 2004. Nitrogen cycles: past, present, and future. Biogeochemistry 70, 153–226.

Galloway, J.N., Townsend, A.R., 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.

Gazzarrini, S., Lejay, L., Gojon, A., Ninnemann, O., Frommer, W.B., Wiren, N. von, 1999. Three Functional Transporters for Constitutive, Diurnally Regulated, and Starvation-Induced Uptake of Ammonium into Arabidopsis Roots. The Plant Cell 11, 937.

Geisseler, D., Horwath, W.R., Joergensen, R.G., Ludwig, B., 2010. Pathways of nitrogen utilization by soil microorganisms – A review. Soil Biology and Biochemistry 42, 2058–2067.

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. R package version 1.10-1.

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.

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.

Hartley, A.E., Schlesinger, W.H., 2002. Potential environmental controls on nitrogenase activity in biological crusts of the northern Chihuahuan Desert. Journal of Arid Environments 52, 293–304.

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.

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.

Hothorn, T., Bretz, F., Westfall, P., 2008. Simultaneous Inference in General Parametric Models. Biometrical Journal 50, 346–363.

Jian, S., Li, J., C., J., Wang, G., Mayes, M.A., Dzantor, K.E., Hui, D., Luo, Y., 2016. Soil extracellular enzyme activities, soil carbon and nitrogen storage under nitrogen fertilization: A meta-analysis. Soil Biology and Biochemistry 101, 32–43.

Joergensen, R.G., Scheu, S., 1999. Response of soil microorganisms to the addition of carbon, nitrogen and phosphorus in a forest Rendzina. Soil Biology and Biochemistry 31, 859–866.

Kandeler, E., Poll, C., Frankenberger, W.T., Ali Tabatabai, M., 2011. Nitrogen Cycle Enzymes, in: Dick, R.P. (Ed.), Methods of soil enzymology. Soil Science Society of America, Madison Wis., Madison, Wisconsin, pp. 211–245.

Khan, K.S., Joergensen, R.G., 2019. Stoichiometry of the soil microbial biomass in response to amendments with varying C/N/P/S ratios. Biology and Fertility of Soils 5, 571.

Landi, L., Renella, G., Giagnoni, L., Nannipieri, P., 2011. Activities of Proteolytic Enzymes, in: Dick, R.P. (Ed.), Methods of Soil Enzymology. American Society of Agronomy, Crop Science Society of America, and Soil Science Society of America, Madison, WI, USA, pp. 247–260.

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, Y., Wu, J., Liu, S., Shen, J., Huang, D., Su, Y., Wei, W., Syers, J.K., 2012. Is the C:N:P stoichiometry in soil and soil microbial biomass related to the landscape and land use in southern subtropical China? Global Biogeochemical Cycles 26.

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.

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.

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.

Marschner, B., Kalbitz, K., 2003. Controls of bioavailability and biodegradability of dissolved organic matter in soils. Geoderma 113, 211–235.

Marx, M.-C., Wood, M., Jarvis, S.C., 2001. A microplate fluorimetric assay for the study of enzyme diversity in soils. Soil Biology and Biochemistry 33, 1633–1640.

McGill, W.B., Cole, C.V., 1981. Comparative aspects of cycling of organic C, N, S and P through soil organic matter. Geoderma 26, 267–286.

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.

Nakagawa, S., Schielzeth, H., 2013. A general and simple method for obtaining R2 from generalized linear mixed-effects models. Methods in Ecology and Evolution 4, 133–142.

Nave, L.E., Vance, E.D., Swanston, C.W., Curtis, P.S., 2009. Impacts of elevated N inputs on north temperate forest soil C storage, C/N, and net N-mineralization. Geoderma 153, 231–240.

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.

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.

Peterson, F.S., Lajtha, K.J., 2013. Linking aboveground net primary productivity to soil carbon and dissolved organic carbon in complex terrain. Journal of Geophysical Research: Biogeosciences 118, 1225–1236.

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.

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.

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.

Risch, A.C., Zimmermann, S., Moser, B., Schütz, M., Hagedorn, F., Firn, J., Fay, P.A., Adler, P.B., Biederman, L.A., Blair, J.M., Borer, E.T., Broadbent, A.A.D., Brown, C.S., Cadotte, M.W., Caldeira, M.C., Davies, K.F., Di Virgilio, A., Eisenhauer, N., Eskelinen, A., Knops, J.M.H., MacDougall, A.S., McCulley, R.L., Melbourne, B.A., Moore, J.L., Power, S.A., Prober, S.M., Seabloom, E.W., Siebert, J., Silveira, M.L., Speziale, K.L., Stevens, C.J., Tognetti, P.M., Virtanen, R., Yahdjian, L., Ochoa-Hueso, R., 2020. Global impacts of fertilization and herbivore removal on soil net nitrogen mineralization are modulated by local climate and soil properties. Global Change Biology 26, 7173—7185.

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.

Sardans, J., Rivas-ubach, A., Peñuelas, J., 2012. The C:N:P stoichiometry of organisms and ecosystems in a changing world: A review and perspectives. Perspectives in Plant Ecology, Evolution and Systematics 14, 33–47.

Schimel, J.P., Bennett, J., 2004. Nitrogen mineralization: challenges of a changing paradigm. Ecology 85, 591–602.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., Kirkman, K., Spohn, M., 2020. Interactions of nitrogen and phosphorus cycling promote P acquisition and explain synergistic plant-growth responses. Ecology, e03003.

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.

Simpson, F.B., Burris, R.H., 1984. Redox-Dependence of the Shethna Iron-Sulfur Protein II-Nitrogenase Interaction and the Effect of High pN2 on H2 Evolution by Nitrogenase, in: Advances in Nitrogen Fixation Research. Springer, p. 243.

Sinsabaugh, R.L., Follstad Shah, J.J., 2012. Ecoenzymatic Stoichiometry and Ecological Theory. Annual Review of Ecology, Evolution, and Systematics 43, 313–343.

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.Smith, V.H., 1992. Effects of nitrogen: phosphorus supply ratios on nitrogen fixation in agricultural and pastoral ecosystems. Biogechemistry, 19–35.

Spohn, M., 2016. Element cycling as driven by stoichiometric homeostasis of soil microorganisms. Basic and Applied Ecology 17, 471–478.

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.

Sterner, R.W., Elser, J.J., 2002. Ecological stoichiometry: The biology of elements from molecules to the biosphere. Princeton University Press.

Stursova, M., Crenshaw, C.L., Sinsabaugh, R.L., 2006. Microbial responses to long-term N deposition in a semiarid grassland. Microbial Ecology 51, 90–98.

Tapia-Torres, Y., Elser, J.J., Souza, V., García-Oliva, F., 2015. Ecoenzymatic stoichiometry at the extremes: How microbes cope in an ultra-oligotrophic desert soil. Soil Biology and Biochemistry 87, 34–42.

Vance, E.D., Brookes, P.C., Jenkinson, D.S., 1987. An extraction method for measuring soil microbial biomass C. Soil Biology and Biochemistry 19, 703–707.

Vestgarden, L.S., Selle, L.T., Stuanes, A.O., 2003. In situ soil nitrogen mineralisation in a Scots pine (Pinus sylvestris L.) stand: effects of increased nitrogen input. Forest Ecology and Management 176, 205–216.

Vitousek, P.M., Cassman, K., Cleveland, C., Crews, T., Field, C.B., Grimm, N.B., Howarth, R.W., Marino, R., Martinelli, L., Rastetter, E.B., Sprent, J.I., 2002. Towards an Ecological Understanding of Biological Nitrogen Fixation. Biogeochemistry 57/58, 1–45.

Widdig, M., Heintz-Buschart, A., Schleuss, P.-M., Guhr, A., Borer, E.T., Seabloom, E.W., Spohn, M., 2020a. Effects of nitrogen and phosphorus addition on microbial community composition and element cycling in a grassland soil. Soil Biology and Biochemistry 151, 108041.

Widdig, M., Schleuss, P.-M., Biederman, L.A., Borer, E.T., Crawley, M.J., Kirkman, K.P., Seabloom, E.W., Wragg, P.D., Spohn, M., 2020b. Microbial carbon use efficiency in grassland soils subjected to nitrogen and phosphorus additions. Soil Biology and Biochemistry 146, 107815.

Widdig, M., Schleuss, P.-M., Weig, A.R., Guhr, A., Biederman, L.A., Borer, E.T., Crawley, M.J., Kirkman, K.P., Seabloom, E.W., Wragg, P.D., Spohn, M., 2019. Nitrogen and Phosphorus Additions Alter the Abundance of Phosphorus-Solubilizing Bacteria and Phosphatase Activity in Grassland Soils. Frontiers in Environmental Science 7, 185.

Wiren, N. von, Gazzarrini, S., Gojon, A., Frommer, W.B., 2000. The molecluar physiology of ammonium uptake and retrieval. Current Opinion in Plant Biology 3, 254–261.

Yuan, Z.Y., Chen, H.Y.H., 2012. A global analysis of fine root production as affected by soil nitrogen and phosphorus. Proceedings of the Royal Society B 279, 3796–3802.

Zak, D.R., Tilman, D., Parmenter, R.R., Rice, C.W., Fisher, F.M., Vose, J., Milchunas, D., Martin, C.W., 1994. Plant Production and Soil Microorganisms in Late-Successional Ecosystems: A Continental-Scale Study. Ecology 75, 2333.

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.

Zechmeister-Boltenstern, S., Keiblinger, K.M., Mooshammer, M., Peñuelas, J., Richter, A., Sardans, J., Wanek, W., 2015. The application of ecological stoichiometry to plant–microbial–soil organic matter transformations. Ecological Monographs 85, 133–155.

Zeglin, L.H., Stursova, M., Sinsabaugh, R.L., Collins, S.L., 2007. Microbial responses to nitrogen addition in three contrasting grassland ecosystems. Oecologia 154, 349–359.

Zhang, X., Wang, Q., Gilliam, F.S., Bai, W., Han, X., Li, L., 2012. Effect of nitrogen fertilization on net nitrogen mineralization in a grassland soil, northern China. Grass and Forage Science 67, 219–230.

Supporting Information

Table S1: Total organic carbon (TOC), total nitrogen (TN), total phosphorus (TP) and their molar element ratios 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[1]	9.4 ± 1.1a	15.7 ± 8.8a	9.0 ± 0.3a	11.0 ± 3.4a	5.2 ± 1.1a	10.4 ± 7.8a	4.4 ± 0.5a*	5.8 ± 2.1a
	cbgb.us	7.2 ± 2.8a	8.2 ± 0.5a	6.9 ± 2.3a	7.4 ± 2.4a	4.1 ± 0.5a*	5.1 ± 0.2a*	4.0 ± 1.2a	4.1 ± 0.9a*
	hero.uk	36.7 ± 6.8a	36.7 ± 6.1a	36.5 ± 1.8a	37.0 ± 7.7a	24.4 ± 3.5a*	24.5 ± 4.2a*	25.6 ± 4.7a*	23.9 ± 4.4a*
	rook.uk	24.3 ± 2.6a	28.7 ± 3.2a	26.9 ± 1.2a	24.9 ± 2.2a	10.5 ± 1.5a*	12.8 ± 1.3a*	11.6 ± 1.3a*	10.1 ± 0.7a*
	summ.za[2]	49.1 ± 3.0a	51.1 ± 2.2a	51.7 ± 3.0a	51.7 ± 1.9a	NA	NA	NA	NA
	ukul.za	42.0 ± 2.0a	42.5 ± 0.8a	44.4 ± 0.5a	45.7 ± 0.8a	37.5 ± 3.6a	3.2.0 ± 4.4a*	34.8 ± 5.3a*	36.4 ± 0.6a*
TN (g N kg^{-1})	cdcr.us[1]	0.7 ± 0.1a	1.1 ± 0.6a	0.6 ± 0.1a	0.8 ± 0.3a	0.3 ± 0.1a*	0.7 ± 0.5a	0.3 ± 0.1a	0.4 ± 0.1a*
	cbgb.us	0.6 ± 0.2a	0.8 ± 0.1a	0.6 ± 0.2a	0.7 ± 0.2a	0.4 ± 0.1a*	0.5 ± 0.1a*	0.4 ± 0.1a*	0.4 ± 0.2a*
	hero.uk	3.1 ± 0.7a	3.1 ± 0.5a	3.0 ± 0.2a	3.1 ± 0.8a	2.1 ± 0.3a*	2.1 ± 0.3a	2.1 ± 0.4a	2.0 ± 0.4a*
	rook.uk	2.1 ± 0.2ab	2.4 ± 0.3a	2.2 ± 0.1ab	2.0 ± 0.1b	1.0 ± 0.1a*	1.2 ± 0.1a**	1.0 ± 0.2a*	0.9 ± 0.1a*
	summ.za[2]	2.8 ± 0.2a	3.0 ± 0.4a	2.9 ± 0.3a	3.0 ± 0.1a	NA	NA	NA	NA
	ukul.za	2.9 ± 0.3a	3.1 ± 0.3ab	3.3 ± 0.1ab	3.4 ± 0.1b	2.6 ± 0.2a	2.4 ± 0.2a*	2.6 ± 0.3a*	2.7 ± 0.1a*
TP (g P kg^{-1})	cdcr.us[1]	0.31 ± 0.03a	0.46 ± 0.24a	0.58 ± 0.09a	0.56 ± 0.11a	0.24 ± 0.03a	0.40 ± 0.19a	0.36 ± 0.04a	0.38 ± 0.06a
	cbgb.us	0.28 ± 0.02a	0.24 ± 0.05a	0.43 ± 0.04b	0.41 ± 0.06b	0.24 ± 0.03a	0.24 ± 0.02a	0.34 ± 0.03b*	0.35 ± 0.07b*
	hero.uk	0.62 ± 0.16a	0.57 ± 0.07a	0.93 ± 0.13a	0.96 ± 0.28a	0.54 ± 0.15a	0.48 ± 0.06a	0.55 ± 0.17a*	0.62 ± 0.25a*
	rook.uk	0.38 ± 0.01a	0.38 ± 0.04a	0.60 ± 0.06b	0.61 ± 0.14b	0.27 ± 0.01a*	0.31 ± 0.04a	0.32 ± 0.03a*	0.31 ± 0.04a*
	summ.za[2]	0.37 ± 0.01a	0.49 ± 0.18a	0.60 ± 0.33a	0.83 ± 0.7a	NA	NA	NA	NA
	ukul.za[1]	0.45 ± 0.02a	0.46 ± 0.08a	1.33 ± 0.12b	1.20 ± 0.15b	0.39 ± 0.02a	0.38 ± 0.04a	0.74 ± 0.25b*	0.58 ± 0.01b*
TOC:TN ratio	cdcr.us[1]	15.6 ± 0.7a	16.3 ± 3.1a	17.1 ± 0.8a	15.6 ± 0.2a	17.6 ± 2.5a	17.5 ± 2.9a	15.6 ± 1.2a	16.4 ± 2.4a
	cbgb.us	12.7 ± 1.1a	12.4 ± 0.5a	13.1 ± 1.2a	12.7 ± 0.7a	12.5 ± 0.4a	12.0 ± 0.4a	13.1 ± 1.1a	12.5 ± 0.3a
	hero.uk	13.7 ± 0.6a	13.8 ± 0.2a	14.4 ± 0.1a	14.2 ± 0.1a	13.4 ± 0.3a	13.5 ± 0.4a	14.0 ± 0.4a	13.7 ± 0.5a
	rook.uk	13.8 ± 0.4a	14.0 ± 0.3a	14.4 ± 0.3a	14.3 ± 0.7a	12.8 ± 0.9a	13.0 ± 0.3a	13.0 ± 0.7a	13.6 ± 0.9a
	summ.za[2]	20.5 ± 0.7a	20.0 ± 2.1a	20.9 ± 1.3a	20.3 ± 0.1a	NA	NA	NA	NA
	ukul.za	17.0 ± 0.9a	15.9 ± 1.0a	15.7 ± 0.7a	15.7 ± 0.1a	16.8 ± 0.6a	15.4 ± 1.5a	15.8 ± 0.8a	15.9 ± 0.4a
TN:TP ratio	cdcr.us	5.0 ± 0.3a	5.5 ± 0.9a	2.4 ± 0.5b	3.2 ± 0.6b	3.2 ± 0.4a*	3.4 ± 1.2a*	2.0 ± 0.3a	2.3 ± 0.3a
	cbgb.us	5.1 ± 1.9ab	7.1 ± 1.1a	3.1 ± 0.8b	3.7 ± 1.0b	3.6 ± 0.5a	4.7 ± 0.4a*	2.3 ± 0.9a	2.5 ± 1.0a
	hero.uk	11.3 ± 1.7a	11.9 ± 0.9a	7.1 ± 1.0b	7.2 ± 1.0b	3.0 ± 1.7a*	9.7 ± 0.9a	8.8 ± 0.9a*	7.6 ± 1.8a*
	rook.uk	12.1 ± 0.7a	14.2 ± 0.8a	8.1 ± 0.9b	7.7 ± 1.9b	7.8 ± 1.1a*	8.2 ± 0.4a*	7.2 ± 1.6a	6.7 ± 0.7a
	summ.za[2]	16.7 ± 0.7a	14.9 ± 5.7a	12.5 ± 4.9a	8.0 ± 0.7a	NA	NA	NA	NA
	ukul.za	14.2 ± 0.9a	15.3 ± 1.5a	5.5 ± 0.3b	6.3 ± 0.8b	14.8 ± 0.4a	14.2 ± 2.2a	8.0 ± 1.6b*	10.1 ± 0.2b*

[1] Data were LOG10 transformed
[2] One-Way ANOVA and Tukey test were performed

Table S2: Dissolved organic carbon (DOC), dissolved nitrogen (DN), dissolved inorganic phosphorus (DIP) concentrations, molar DOC:DN ratio, and N-NO_3 concentrations 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 ± 0.4a	20 ± 4.8b	20 ± 1.1b	24 ± 2.6b	11 ± 0.6a	12 ± 2.0a*	12 ± 0.3a*	15 ± 1.3a*
	cbgb.us	18 ± 2.7a	22 ± 4.1a	18 ± 2.1a	22 ± 1.6a	15 ± 1.6a	22 ± 3.4a	20 ± 5.6a	20 ± 1.6a
	hero.uk	29 ± 2.6a	30 ± 3.4a	36 ± 1.2ab	40 ± 4.1b	19 ± 1.2a*	22 ± 4.2a*	23 ± 3.0a*	25 ± 3.9a*
	rook.uk	23 ± 2.7a	29 ± 7.5ab	44 ± 8.3c	36 ± 3.5bc	15 ± 1.5a	16 ± 3.1a*	19 ± 1.0a*	18 ± 1.0a*
	summ.za[1]	98 ± 3a	98 ± 9a	102 ± 10a	100 ± 1a	NA	NA	NA	NA
	ukul.za	115 ± 7a	108 ± 15a	127 ± 6a	152 ± 34a	127 ± 33a	201 ± 52ab*	238 ± 14b*	217 ± 66b*
DN (mg N kg^{-1})	cdcr.us[2]	3.2 ± 4.4a	32.9 ± 15.6b	2.7 ± 2.1a	13.4 ± 2.9b	1.8 ± 1.5a	10.0 ± 1.0b	2.1 ± 1.5a	7.0 ± 2.1ab
	cbgb.us	4.1 ± 3.1a	12.0 ± 1.4b	4.7 ± 1.9a	12.1 ± 3.9b	2.6 ± 2.2a	5.0 ± 2.8a*	2.9 ± 1.1a	4.8 ± 2.1a*
	hero.uk	12.9 ± 3.6a	17.1 ± 3.9a	11.1 ± 3.5a	14.6 ± 8.7a	9.7 ± 3.8a	10.3 ± 0.8a	11.9 ± 4.4a	8.9 ± 3.2a
	rook.uk	14.2 ± 2.7ab	14.7 ± 3.2b	10.9 ± 0.4ab	10.3 ± 1.2a	4.2 ± 2.4a*	3.8 ± 1.0a*	2.9 ± 0.8a*	4.0 ± 0.8a*
	summ.za[1]	5.0 ± 0.1a	19.8 ± 5.0b	5.1 ± 0.2a	15.9 ± 3.1b	NA	NA	NA	NA
	ukul.za	7.3 ± 0.3a	43.2 ± 11.2b	9.9 ± 5.0a	34.4 ± 5.7b	5.5 ± 1.1a	17.3 ± 4.9ab*	14.1 ± 3.3ab	19.5 ± 5.3b*
DIP (mg P kg^{-1})	cdcr.us [2]	0.16 ± 0.12a	0.13 ± 0.06a	13.37 ± 2.76b	12.29 ± 3.55b	0.06 ± 0.03a*	0.04 ± 0.01a*	4.19 ± 0.55b*	5.01 ± 0.89b*
	cbgb.us[2]	0.37 ± 0.08a	0.25 ± 0.19a	7.67 ± 1.23b	11.1 ± 3.18b	0.13 ± 0.08a*	0.11 ± 0.05a	7.02 ± 3.11b	6.96 ± 1.91b
	hero.uk[2]	0.04 ± 0.01a	0.04 ± 0.01a	1.64 ± 0.85b	1.15 ± 0.72b	0.02 ± 0.01a*	0.03 ± 0.01ab	0.04 ± 0.01ab*	0.06 ± 0.01b*
	rook.uk[2]	0.02 ± 0.01a	0.03 ± 0.01a	1.25 ± 0.88b	1.22 ± 1.20b	0.02 ± 0.01a	0.02 ± 0.01a	0.04 ± 0.03a*	0.06 ± 0.02a*
	summ.za[1]	0.05 ±0.04a	0.10 ± 0.02ab	0.25 ± 0.09b	0.27 ± 0.12b	NA	NA	NA	NA
	ukul.za[2]	0.10 ± 0.07a	0.12 ± 0.04a	1.43 ± 0.19b	1.10 ± 0.25b	0.13 ± 0.05a	0.05 ± 0.05a*	2.09 ± 1.58b	0.92 ± 0.24b
DOC:DN ratio	cdcr.us	18.1 ± 13.1a	0.8 ± 0.2b	12.2 ± 7.5a	2.1 ± 0.2b	10.0 ± 5.5a	1.4 ± 0.1b	9.6 ± 5.7ab*	2.7 ± 0.8b*
	cbgb.us	8.1 ± 6.0a	2.1 ± 0.2b	4.9 ± 1.8ab	2.3 ± 0.7b	10.1 ± 5.8a	5.9 ± 2.0a	8.8 ± 4.3a	5.6 ± 2.3a
	hero.uk	2.7 ± 0.4a	2.1 ± 0.3a	4.0 ± 1.3a	4.2 ± 2.8a	2.6 ± 1.2a	2.5 ± 0.3a	2.5 ± 0.8a	3.5 ± 0.9a
	rook.uk	2.0 ± 0.6 a	2.3 ± 0.3a	4.6 ± 0.7b	4.0 ± 0.1ab	5.1 ± 2.3a*	4.8 ± 0.3a*	8.1 ± 2.1a*	5.4 ± 1.4a
	summ.za[1]	22.9 ± 0.8a	6.0 ± 1.4b	23.2 ± 1.9a	7.5 ± 1.5b	NA	NA	NA	NA
	ukul.za	18.2 ± 0.8a	3.0 ± 0.6b	17.8 ± 9.0a	5.3 ± 1.5b	27.0 ± 1.9a*	14.8 ± 6.8b*	20.3 ± 4.3a	13.0 ± 1.7ab*
N-NO_3 (mg N kg^{-1})	cdcr.us	0.3 ± 0.5a	29.7 ± 12.9c	1.5 ± 2.1a	11.6 ± 2.8b	1.4 ± 1.4a	8.8 ± 0.9a	1.5 ± 1.4a	5.9 ± 1.9a
	cbgb.us	3.0 ± 2.9a	10.3 ± 1.3b	3.4 ± 1.9a	10.4 ± 3.9ab	2.0 ± 2.0a	3.8 ± 2.5a*	1.8 ± 1.1a	3.5 ± 1.9a*
	hero.uk	11.3 ± 3.2a	16.4 ± 4.2a	9.5 ± 4.1a	13.5 ± 9.7a	8.7 ± 3.7a	9.1 ± 0.8a	10.8 ± 4.3a	7.6 ± 3.1a
	rook.uk	14.1 ± 3.1ab	14.5 ± 3.1a	9.9 ± 0.8b	9.3 ± 1.1b	3.7 ± 2.4a*	3.2 ± 0.8a*	2.2 ± 0.9a*	3.3 ± 0.9a*
	summ.za[1]	0.4 ± 0.0a	12.4 ± 2.7b	0.4 ± 0.0a	9.4 ± 2.1b	NA	NA	NA	NA
	ukul.za	3.8 ± 0.1a	38.5 ± 10.0b	5.3 ± 4.3a	28.5 ± 4.5b	1.8 ± 0.8a	10.6 ± 6.4a*	4.5 ± 2.9a	10.5 ± 2.5a*

[1] One-Way ANOVA and Tukey test were performed
[2] Data were LOG10 transformed

Table S3: Microbial biomass carbon (MBC), net N mineralization (Nmin), non-symbiotic N_2 fixation (Nfix) and leucine-aminopeptidase activity per unit MBC (qLAP) 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. 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
MBC (mg C kg^{-1})	cdcr.us	163 ± 63a	235 ± 44a	193 ± 9a	169 ± 99a	119 ± 103a	100 ± 47a*	46 ± 31a*	134 ± 124a
	cbgb.us	175 ± 61a	121 ± 23a	168 ± 51a	148 ± 100a	61 ± 15a*	119 ± 35a	69 ± 43a	142 ± 118a
	hero.uk	662 ± 116a	603 ± 70a	638 ± 51a	591 ± 85a	526 ± 8a	363 ± 61ab*	298 ± 73b*	352 ± 76ab*
	rook.uk	651 ± 35a	588 ± 49a	507 ± 210a	746 ± 88a	351 ± 46a*	317 ± 94a*	248 ± 176a*	342 ± 248a*
	summ.za[1]	843 ± 133a	769 ± 111a	928 ± 278a	699 ± 16a	NA	NA	NA	NA
	ukul.za	1005 ± 134a	767 ± 196a	819 ± 157a	852 ± 71a	488 ± 93a*	444 ± 85a*	502 ± 113a*	549 ± 66a*
Net N min (mg N kg^{-1} d^{-1})	cdcr.us	0.09 ± 0.06a	0.25 ± 0.03b	0.16 ± 0.03a	0.28 ± 0.07b	0.06 ± 0.01a	0.11 ± 0.02a*	0.07 ± 0.02a*	0.13 ± 0.01a*
	cbgb.us	0.15 ± 0.02a	0.24 ± 0.03bc	0.18 ± 0.02ab	0.25 ± 0.03c	0.07 ± 0.02a*	0.10 ± 0.04a*	0.10 ± 0.02a*	0.10 ± 0.02a*
	hero.uk	0.48 ± 0.31a	0.40 ± 0.11a	0.42 ± 0.03a	0.44 ± 0.19a	0.21 ± 0.06a	0.18 ± 0.04a	0.18 ± 0.17a*	0.18 ± 0.02a
	rook.uk	0.38 ± 0.07a	0.36 ± 0.05a	0.34 ± 0.02a	0.36 ±0.03a	0.07 ± 0.05a*	0.09 ± 0.06a*	0.06 ± 0.05a*	0.04 ± 0.04a*
	summ.za[1]	0	0.86 ± 0.04	0	0.81 ± 0.04	NA	NA	NA	NA
	ukul.za[2]	0.03 ± 0.04a	0.52 ± 0.14b	0.09 ± 0.08a	0.54 ± 0.03b	0.11 ± 0.06a	0.41 ± 0.23b	0.13 ± 0.09a	0.62 ±0.01b
N_2 fixation (ng N g^{-1} d^{-1})	cdcr.us	0.69 ± 0.11c	1.02 ± 0.51a	1.08 ± 0.20a	0.79 ± 0.26a	1.05 ± 0.25ab	1.74 ± 0.23ab*	1.87 ± 0.34b*	0.80 ± 0.69a
	cbgb.us	0.69 ± 0.12a	0.50 ± 0.16a	0.84 ± 0.32a	0.92 ± 0.35a	1.03 ± 0.16a	0.95 ± 0.47a	1.32 ± 0.60a	1.21 ± 0.19a
	hero.uk	0.95 ± 0.15a	0.73 ± 0.32a	0.32 ± 0.27a	2.04 ± 0.73b	0.78 ± 0.09a	0.32 ± 0.30a	0.36 ± 0.14a	0.24 ± 0.25a*
	rook.uk	0.25 ± 0.19a	0.13 ± 0.13a	0.47 ± 0.17a	0.31 ± 0.06a	0.55 ± 0.15a*	0.50 ± 0.16a*	0.61 ± 0.09a	0.58 ± 0.17a*
	summ.za[1]	2.43 ± 1.61a	1.61 ± 0.34a	4.55 ± 1.69a	5.71 ± 3.01a	NA	NA	NA	NA
	ukul.za	1.70 ± 2.02ab	0.59 ± 0.54a	3.29 ± 1.31b	3.64 ± 0.94ab	1.53 ± 0.46ab	0.64 ± 1.07a	4.66 ± 4.38b	1.83 ± 0.58ab
qLAP (mol g MBC^{-1} h^{-1})	cdcr.us	348 ± 238a	230 ± 119a	266 ± 78a	242 ± 52a	354 ± 152a	502 ± 42ab*	724 ± 160b*	294 ± 160a
	cbgb.us	327 ± 81a	470 ± 73a	253 ± 41a	383 ± 185a	221 ± 83a	213 ± 61a*	339 ± 43a	148 ± 19a*
	hero.uk	154 ± 53a	167 ± 32a	213 ± 64a	277 ± 121a	135 ± 12a	218 ± 22a	348 ± 80a*	270 ± 71a
	rook.uk	22 ± 8a	25 ± 5a	31 ± 13a	25 ± 8a	31 ± 9a	47 ± 25ab	70 ± 35b*	44 ± 30ab
	summ.za[1]	53 ± 25a	40 ± 14a	65 ± 37a	68 ± 27a	NA	NA	NA	NA
	ukul.za[3]	231 ± 57a	157 ± 11a	207 ± 102a	153 ± 41a	141 ± 42a	131 ± 50a	133 ± 57a	111 ± 9a

[1] One-Way ANOVA and Tukey test were performed
[2] Data were LOG10 transformed
[3] Data were reciprocally transformed (1/x)

Table S4: Regression coefficients, model R^2, and p-value of the linear mixed-effects models of net N mineralization, non-symbiotic N2 fixation, and leucine-aminopeptidase activity per unit microbial biomass carbon (qLAP) of all treatments and sites in 0-15 cm depth. Regression coefficients were selected by multi-model selection and standardized prior to analysis. 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.

	Net N mineralization				N_2 fixation				qLAP		
Coefficients	Estimation	Std. Error	p-value	Coefficients	Estimation	Std. Error	p-value	Coefficients	Estimation	Std. Error	p-value
(Intercept)	0.32	0.04	<0.001***	(Intercept)	1.41	0.25	<0.001***	(Intercept)	0.18	0.01	<0.001***
DN	0.21	0.04	<0.001***	TOC:TP ratio	1.12	0.36	0.002**	MAT	-0.16	0.02	<0.001***
Plant biomass	-0.09	0.04	0.015*	DOC:DIP ratio	0.69	0.22	0.002**	pH	0.15	0.03	<0.001***
pH	-0.18	0.05	<0.001***					MAP	0.06	0.03	0.057.
Conditional R^2	0.89			0.83				0.61			
Model p-value	<0.001			<0.001				<0.001			

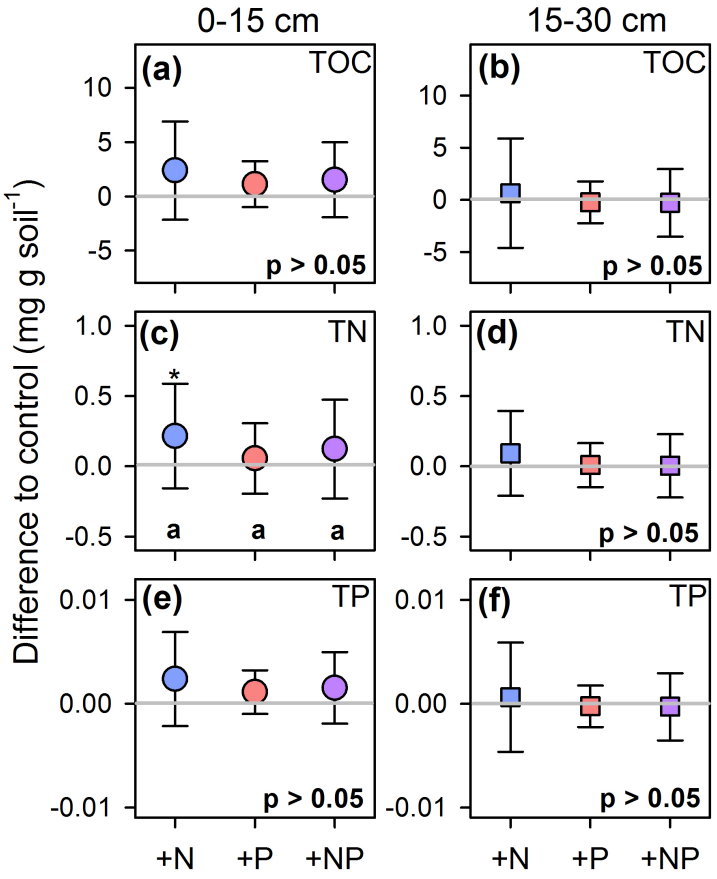


Figure S1: Changes in total soil element concentrations in 0-15 cm (n=54) and 15-30 cm (n=45) in response to N and P addition across all six sites. Changes in variables were calculated by subtracting the control from the treatment. Soil element concentrations include total organic C (TOC) (a b), total N (TN) (c d), and total P (TP) (e f). Colored circles and squares (+N in blue, +P in red, and +NP in purple) represent the mean value ± SD for each treatment in 0-15 cm and in 15-30 cm. Linear mixed-effects models were performed with treatment as fixed factor and random intercepts for sites followed by Tukey-post-hoc test for multiple comparisons. Significant differences ($p < 0.05$) between treatments (N, P, and NP) are indicated by lower-case letters, while asterisks (*) indicate a significant difference ($p < 0.05$) between treatments and the control. The mean value of the control is illustrated with the grey line (Ctrl = 0).

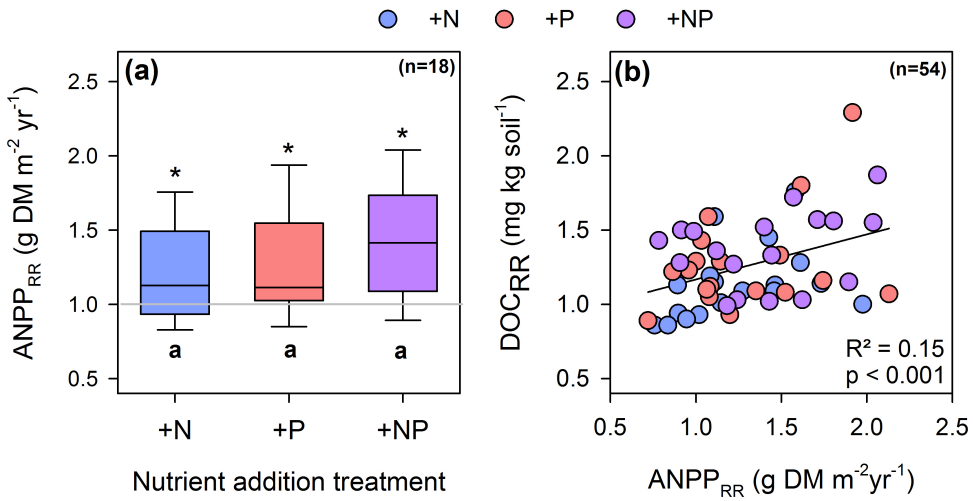


Figure S2: Changes in aboveground net primary productivity (ANPP) (a) and relationship between dissolved organic C (DOC) concentrations and ANPP (b) depending on single and combined N and P addition in the six studied grassland sites. Variables (DOC and ANPP) were calculated as response ratios (RR: treatment/control). For panel (a): Box plots show the median (black line), the 25th and the75th percentile (box edges) and the 10th and the 90th percentile (error bars). Linear mixed-effects models were performed with treatment as fixed factor and random intercepts for sites. Significant differences ($p < 0.05$) between treatments (N, P, and NP) are indicated by lower-case letters, while asterisks (*) indicate a significant difference ($p < 0.05$) between a treatment and the control (indicated by the grey line).

2.4 Study IV

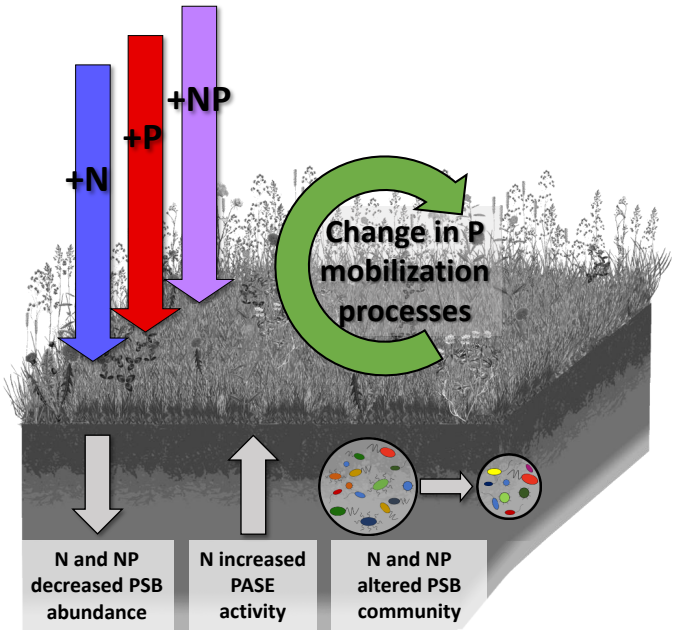
Nitrogen and phosphorus additions alter the abundance of phosphorus-solubilizing bacteria and phosphatase activity in grassland soils

Meike Widdig, Per-M. Schleuss, Alfons R. Weig, Alexander Guhr, Lori A. Biederman, Elizabeth T. Borer, Michael J. Crawley, Kevin P. Kirkman, Eric W. Seabloom, Peter D. Wragg, Marie Spohn (2019),
published in *Frontiers of Environmental Science* 7, 185,
DOI: https://doi.org/10.3389/fenvs.2019.00185

Abstract

Microorganisms mobilize phosphorus (P) in soil by solubilizing bound inorganic P from soil minerals and by mineralizing organic P via phosphatase enzymes. Nitrogen (N) inputs are predicted to increase through human activities and shift plants to be more P limited, increasing the importance of P mobilization processes for plant nutrition. We studied how the relative abundance of P-solubilizing bacteria (PSB), PSB community composition, and phosphatase activity respond to N and P addition (+N, +P, +NP) in grassland soils spanning large biogeographic gradients. The studied soils are located in South Africa, USA, and UK and part of a globally coordinated nutrient addition experiment. We show that the abundance of PSB in the topsoil was reduced by 18% in the N and by 41% in the NP treatment compared to the control. In contrast, phosphatase activity was significantly higher in the N treatment than in the control across all soils. Soil C:P ratio, sand content, pH, and water-extractable P together explained 71% of the variance of the abundance of PSB across all study sites and all treatments. Further, the community of PSB in the N and NP addition treatment differed significantly from the control. Taken together, this study shows that N addition reduced the relative abundance of PSB, altered the PSB community, and increased phosphatase activity, whereas P addition had no impact. Increasing atmospheric N deposition may therefore increase mineralization of organic P and decrease solubilization of bound inorganic P, possibly inducing a switch in the dominant P mobilization processes from P solubilization to P mineralization.

Graphical Abstract



2.4.1 Introduction

The availability of reactive nitrogen (N) has strongly increased during the last century through anthropogenic activities (Schlesinger, 2009) and is not paralleled by a similar increase in phosphorus (P) inputs to terrestrial ecosystems (Peñuelas et al., 2013). This imbalance between N and P inputs might shift plant productivity in grasslands towards P limitation (Elser et al., 2007). Therefore, it is important to understand processes of microbial P mobilization such as mineralization of organic P and solubilization of bound inorganic P, which maintain plant P nutrition. A key knowledge gap is the role that N supply plays in P mobilization processes and how N affects the community composition of P-solubilizing bacteria (PSB).

Plants and microorganisms mineralize organic P through the release of phosphatase enzymes that catalyze the hydrolysis of organic P, and turn organic P into inorganic P, which can be taken up by plants. The production of phosphatase enzymes depends on soil organic matter content, soil pH, plant and microbial nutrient demand, and, in particular, on N availability (Olander and Vitousek, 2000; Sinsabaugh et al., 2008). Hence, N addition often increases phosphatase activity (Heuck et al., 2018; Marklein and Houlton, 2012). In contrast, inorganic P addition can reduce phosphatase activity

in soils because organisms stop producing phosphatase when supplied with inorganic P (Marklein and Houlton, 2012; Olander and Vitousek, 2000; Sinsabaugh et al., 2008).

Soil P solubilization is defined as the mobilization of inorganic P bound to soil minerals, of which most are apatites, leading to a release of plant available P into the soil solution (Hinsinger, 2001; Walpola and Yoon, 2012). Different mechanisms for P solubilization are known, which include the release of protons, chelation, exchange reactions, and production of various acids (Jones and Oburger, 2011; Walpola and Yoon, 2012). One of the main processes of P solubilization is acidification through the release of protons, which can be accelerated by plant and microorganisms (Hinsinger, 2001; Jones and Oburger, 2011). Furthermore, plants and microorganisms can stimulate P dissolution by the release of organic acids, exopolysaccharides, and siderophores (Jones and Oburger, 2011; Reid et al., 1985; Yi et al., 2008).

Both P mineralization and P solubilization likely underlie stoichiometric constraints, because microbes need N and organic C to produce phosphatases. Further they need organic compounds as a C source to produce acids and exopolysaccharides that solubilize P (Spohn, 2016). Moreover, the production of these compounds requires N for transcription and translation. Yet, the effect of N and P availability on P solubilization and the abundance of PSB has rarely been studied (Lepleux et al., 2013; Mander et al., 2012; Nicolitch et al., 2016). In a temperate beech forest, PSB were significantly more abundant in nutrient-poor than in nutrient-rich soils (Nicolitch et al., 2016), and PSB were significantly more abundant in unfertilized soils than in soils fertilized with P in three long-term grassland fertilizer trials in New Zealand (Mander et al., 2012). These studies suggest that the abundance of PSB is enhanced in nutrient-poor soils and suppressed by P addition, however, it is not clear yet how increasing N supply with and without P influences the abundance of PSB. Since N addition might increase P demand and N is required for the production of solubilizing compounds, we expect an increase of PSB under N addition.

While it has frequently been reported that N and P addition can shift the overall microbial community composition (Fierer et al., 2012; Leff et al., 2015), few studies have been conducted with regard to PSB communities. Several bacterial genera such as *Pseudomonas*, *Burkholderia*, *Bacillus*, *Enterobacter*, *Erwinia*, *Serratia*, *Acinetobacter*, and *Rhizobium* are able to solubilize P (Rodrıguez and Fraga, 1999). Under nutrient deficiency, PSB belonging to the genera *Collimonas*, *Burkholderia*, and *Kitasatospora* were more abundant than under high nutrient supply (Nicolitch et al., 2016). Furthermore, long-term P fertilization altered the abundance of *Actinobacteria*, Pseudomonadaceae, and Moraxellaceae (Mander et al., 2012). However, little is known about PSB community changes under single and combined N and P addition.

Here, we use a standardized nutrient-addition experiment at six grassland sites in South Africa, the USA, and the UK to investigate the effects of N and P additions on P mobilization in soil. Specifically, we used a dual approach investigating indicators of P solubilization from minerals (abundance of PSB) and P mineralization from organic

matter (phosphatase activity). We studied how single and combined addition of N and P affects the relative abundance of PSB, community composition of PSB, and phosphatase activity. We hypothesized that i) P addition decreases the relative abundance of PSB and phosphatase activity ii) N addition increases the relative abundance of PSB and phosphatase activity, and iii) N, P, and NP additions alter the community composition of PSB.

2.4.2 Materials and Methods

2.4.2.1 Sampling Sites and Experimental Design

We sampled soil at six grassland sites located in South Africa, the USA, and the UK (Table 1 and Supplement A) belonging to a globally replicated experiment (Nutrient Network, Borer et al., 2014). The sites were chosen because they span large biogeographical gradients (Tables 1, 2) and represent some of the major types of grasslands that exist globally. To understand interactions between N and P supply and to test whether P availability influences P mobilization processes, we analyzed not only effects of N addition, but also independent and interactive effects of N and P addition. We sampled the soil in four different treatments, namely control, +N, +P, and +NP. Nutrients were applied annually to the experimental plots at the beginning of the growing season: 10 g m^{-2} yr^{-1} as time-release urea ($(NH_2)_2CO$), and 10 g m^{-2} yr^{-1} as triple-super phosphate ($Ca(H_2PO_4)_2$), and the nutrient addition treatment had been in place for at least 7 years at the time of sampling (2017). The added amount of N and especially P in our study is higher than current levels of atmospheric deposition based on two reasons. First, to identify differences between the control and the treatments, the nutrient additions needed to be significantly larger than current atmospheric deposition. Second, the nutrient addition treatments needed to be high enough to identify changes in a field experiment against a background that changes due to heterogeneity in soil properties. The experimental design is a randomized block design and each treatment was applied to 5×5m plots and replicated three times at each site. Further details of the experimental design are explained in Borer et al. (2014).

2.4.2.2 Sampling and Sample Preparation

At all sites, samples were collected at the end of the vegetation period in 2017 (February in South Africa, September in the USA, and October in the UK). Soil samples were collected from two depth increments (0–15 cm, 15–30 cm, both located in the A horizon of each soil), except for Summerveld (summ.za), where only the first depth increment was sampled because the soil was very shallow. Six soil samples were taken per plot using a 3.5 cm diameter soil corer, and the samples were combined into one mixed sample per plot. Samples were shipped to the University of Bayreuth within 1 week after sampling. Soils were sieved ($<$ 2mm) and stones and roots were removed.

Table 1: Site name, site code, country, habitat, elevation, latitude, longitude, mean annual precipitation (MAP), mean annual temperature (MAT), and the duration of the nutrient addition treatments of the six grassland sites.

Site name	Site code	Country	Habitat	Elevation (m)	Latitude	Longitude	MAP (mm)	MAT (°C)	Nutrient addition (years)
Ukulinga	ukul.za	South Africa	Mesic grassland	843	-29.67	30.4	838	18	7
Summerveld	summ.za	South Africa	Mesic grassland	679	-29.81	30.72	809	18	7
Cedar Creek	cdcr.us	USA	Tallgrass prairie	270	45.43	-93.21	800	6	9
Chichaqua Bottoms	cbgb.us	USA	Tallgrass prairie restored	275	41.79	-93.39	891	9	7
Rookery	rook.uk	UK	Mesic grassland	60	51.41	-0.64	678	10	9
Heron's Brook	hero.uk	UK	Mesic grassland	60	51.41	-0.64	678	10	9

To determine water content and water holding capacity, an aliquot of the samples was weighted, soaked with water, drained for 24 h in a sand bath and weighted again before and after drying at 105 °C. After sieving, water holding capacity was adjusted to 60% and samples were pre-incubated for 1 week at 15 °C for subsequent measurements (i.e., soil water extracts, and phosphatase activity). Samples for total C, N, and P concentration analyses were dried at 60 °C and subsequently milled using a ball mill.

2.4.2.3 Soil Chemical Parameters

Soil pH was measured in deionized water in a soil:water ratio of 1:2.5. Soil samples were dried at 60 °C and milled before measuring total organic C (TOC) and total N (TN) using an element analyzer (Vario Max Elementar, Hanau, Germany) and total P (TP) using ICP-OES (Vista-Pro radial, Varian) after a pressure digestion in aqua regia. Dissolved organic carbon (DOC), dissolved nitrogen (DN), and dissolved inorganic phosphorus (DIP) were extracted from 20 g of soil (dry-mass equivalent) in 80ml deionized water by shaking for 1 h. Subsequently, the extracts were passed through 0.45 μm cellulose acetate filters and quantified (TOC, TN: TOC:TN Analyzer, multi N/C 2100, Jena Analytics, Germany, and DIP: UV 1800, Shimadzu). Adsorbed P (Bray-1 P) was extracted from 4 g of soil (dry-mass equivalent) in 40 ml Bray-1 solution (0.03 M NH_4F + 0.025 M HCl) (Bray and Kurtz, 1945) and was determined colorimetrically by a multi-plate reader (Infinite R 200 PRO, TECAN), using the molybdenum blue method (Murphy and Riley, 1962). Total organic phosphorus (TOP) was determined by the ignition method according to Saunders and Williams (1955) modified by Walker and Adams (1958). An aliquot of each sample was ignited at 550 °C in a muffle furnace. Both ignited and non-ignited aliquots were extracted in 0.5 M H_2SO_4 for 16 h on a horizontal shaker followed by centrifugation at 1,500 x g for 15 min. TOP was calculated as the difference between ignited and non-ignited samples. Phosphorus fractions were measured according to the Hedley fractionation (Hedley et al., 1982) modified by Tiessen and Moir (2007). Dried soil samples of the control were milled and 0.5 g soil and 30 ml deionized water were shaken for 16 h on an overhead shaker and centrifuged at 4,100 x g for 15 min. Inorganic P in water extracts was measured colorimetrically by a multi-plate reader using the molybdenum blue method. The remaining soil was subsequently extracted in 30 ml 0.5 M $NaHCO_3$ (labile P), followed by an extraction with 30 ml 0.1 M NaOH (occluded P), and 30 ml 1 M HCl (apatite P). Total P of $NaHCO_3$, NaOH, and HCl extracts were measured using ICP-OES. Residual P was calculated by subtracting all P fractions from TP.

2.4.2.4 Soil Microbial Biomass C and Microbial Community Composition

Microbial biomass C was determined using the chloroform fumigation-extraction method (Vance et al., 1987). Each soil sample was split into two samples, of which one was fumigated. Fumigated and non-fumigated samples were extracted in 0.5 M K_2SO_4 in a

ratio of 1:5 (soil:extractant, m/v). Total C was measured by a TOC:TN Analyzer (multi N/C 2100, Analytik Jena, Jena, Germany). To calculate the concentration of microbial biomass C, the concentration of C in the non-fumigated sample was subtracted from the one in the fumigated sample and multiplied by a conversion factor of 2.22 (Jenkinson et al., 2004).

For analysis of microbial community composition, DNA was extracted from 250 to 500 mg soil samples using the Nucleo-Spin Soil kit (No. 740780, Macherey-Nagel) as described in Heuck et al. (2015). Automated ribosomal intergenic spacer analysis (ARISA; Fisher and Triplett, 1999) was adopted to analyze bacterial and fungal communities in parallel as follows: 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; White et al., 1990; Weig et al., 2013), respectively. Five nanogram metagenomic DNA was used in a 12.5 μl PCR volume as previously described (Weig et al., 2013). The ITSF forward primer was labeled with fluorescent dye BMN-6 and the ITS1F-Z forward primer was labeled with fluorescent dye BMN-5 (Biomers, Ulm, Germany). 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 μl of ARISA PCR products were used for the double-stranded DNA kit DNF-910 (Agilent) and separated on the Fragment Analyzer using default specifications defined in the DNF-910 method file accompanying the instrument. 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 further analyses. For statistical analyses of the ARISA data, only fragments between 200 and 1,000 bp in size were selected and analyzed by the Primer7 software (v 7.0.13, Primer-E Ltd.). PCR fragment profiles were compared between samples by the shape of cumulative frequency curves independently for bacterial and fungal ITS amplification products: A matrix of the peak table (size in bp vs. peak intensity/RFU) for all samples was imported in Primer 7 for bacterial and fungal data, respectively. Each sample was then standardized to its own total value (total RFU) and a cumulative profile (adding standardized peak values along ascending fragment size) was calculated for each sample. Finally, a resemblance matrix was calculated from the cumulative profile matrix using Manhattan distance (implemented in Primer 7) as resemblance measure. Differences between microbial community signatures of different samples and sites were tested by ANOSIM (One-way-analysis of similarities with 999 permutations).

2.4.2.5 Relative Abundance of P-Solubilizing Bacteria

To determine the relative abundance of PSB, 0.5 g of soil from the topsoil was mixed with 49.5 ml of sterile water and shaken for 45 min. The relative abundance of PSB is the proportion of colony-forming units (CFU, colonies that grow on the medium) able to

solubilize P divided by the total number of CFU multiplied by 100. The soil suspension was diluted by a factor of either 1,000 or 10,000 according to cell density assessed in pre-experiments. An aliquot of the soil suspension was added to an agar plate containing Pikovskaya (PVK) medium [10 g glucose, 5 g hydroxyapatite, 0.5 g $(NH_4)_2SO_4$, 0.2 g NaCl, 0.1 g $MgSO_4$ * 7 H_2O, 0.2 g KCl, 0.5 g yeast extract, 0.002 g $MnSO_4$ * H_2O, 0.002 g $FeSO_4$ * 7 H_2O, and 15 g agar per liter (Pikovskaya, 1948)]. Plates were incubated for 7 days at 20 °C before colonies were counted. At least 100 colony-forming units (CFU) per soil sample were screened. If CFU are able to solubilize P from the hydroxyapatite in the medium, a clear zone (halo) around the CFU becomes visible. If a halo with a diameter > 1 mm was visible around a CFU, the isolate was classified as a strong PSB. If a CFU produced a halo < 1 mm it was classified as weak PSB. However, it has to be taken into account that the condition in our physiological assay favored fast-growing bacteria. Thus, we might underestimate the P solubilization of slow-growing bacteria.

2.4.2.6 Sequencing of P-Solubilizing Bacteria and Processing of Sequence Data

Colonies of PSB were selected for sequencing and picked from the agar plate using sterile toothpicks. Genomic DNA of the bacterial colonies was extracted using the "NucleoMag 96 Tissue" kit (No. 744300, Macherey-Nagel) on a KingFisher liquid handling platform (Thermo Scientific). Genomic DNA was diluted 100-fold with nuclease free water and 16S fragments covering variable regions (V5–V8) were amplified using primers 799F (Chelius and Triplett, 2001) and 1391R (Walker and Pace, 2007). PCR products were purified using the "NucleoMag 96 PCR" cleanup kit (No. 744100, Macherey-Nagel) on a KingFisher liquid handling platform. PCR products were sequenced (Sanger sequencing, GATC Biotech) using primer 799F. Sequence data were analyzed in Geneious sequence analysis software (v. 11; Biomatters Ltd.). The sequences were searched for PCR primer sequences (799F and 1391R) and for low quality bases, which were excluded from sequence database searches. Fragment size after quality trimming was 580 bp, some fragments with high quality in most parts were shorter. Sequence similarity searches were conducted against the nr/nt nucleotide database at NCBI as well as the 16S sequences of the "NCBI RefSeq Targeted Loci Project". 16S sequence alignments were calculated by MAFFT (v. 7.388, Geneious plugin; Katoh and Standley, 2013). Sequences were aligned in phylogenetic trees, which were edited using Evolview (He et al., 2016). In total, 903 PSB isolates were sequenced and used for further analyses. The list of the 20 best matching sequence hits for each isolate was uploaded into PRIMER 7 (Clarke and Gorley, 2015), standardized, cumulated, and clustered. Isolates, which had a consensus of more than 99% in the cluster analysis, were considered as identical isolates. After identification of identical isolates, a "consensus name" was assigned for each isolate cluster according to Peršoh et al. (2010) called operational taxonomic unit (OTU) in the following. For OTU assignment, we chose a taxonomic level that unified all names under which the 20 best matching sequences with a consensus 97% to the respective

isolate sequence were deposited. To this end, the name of the lowest common rank in the nomenclatural hierarchy was chosen (Peršoh et al., 2010). Names for OTU assignment were used as indicated in the NCBI database, although recently so-called environmental bacteria of the genus *Burkholderia* have been transferred to the genus *Paraburkholderia* (Dobritsa and Samadpour, 2016). Analyses of similarities (ANOSIM) based on the number of OTUs grouped by taxon were conducted in PRIMER 7 to check first, if sites have a significant effect on PSB community and second, if treatments have a significant effect on PSB community.

2.4.2.7 Phosphatase Activity

Phosphatase activity was determined using the fluorogenic substrate 4-methylumbelliferyl-phosphate following German et al. (2011) and Herold et al. (2014). To this end, 1 g of moist soil was mixed with 50 ml of sterile water and shaken for 20 min. Four replicates of the soil homogenates were pipetted into black polystyrene 96-well microplates and substrate solution was added. Microplates were covered and pre-incubated in the dark at 15 °C for 30 min and measured fluorometrically 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 R 200 PRO, TECAN). Enzyme activities were calculated according to German et al. (2011), except that we used the slope of net fluorescence over time to calculate enzyme activities. Fluorescence values were corrected for quenching of the soil, fluorescence of the substrate, and fluorescence of the homogenate. Since we added a substrate to our samples, potential enzyme activities were measured. Each time we mention enzyme activities, potential enzyme activities are meant.

2.4.2.8 Statistics

To test whether differences between the treatments at one site and in one soil depth increment were statistically significant, ANOVA followed by Tukey post-hoc test ($p < 0.05$) for multiple comparisons was used, if residuals were normally distributed (tested with Shapiro-Wilks test, $p > 0.05$) and variances were homogenous (tested with Levene test, $p>0.05$). If necessary, data were log-, or square root-transformed and retested.

To show the element concentrations and the P fractions of the different sites, we first normalized element and P fraction data of all sites (TOC, TN, TP, TOC:TN ratio, TOC:TP ratio, TN:TP ratio, DOC, DN, DIP, DOC:DN ratio, TOP, Bray-1 P, water-extractable P, labile P, occluded P, apatite P, residual P, and soil pH) and calculated an Euclidean distance matrix in PRIMER 7 (Clarke and Gorley, 2015) with 999 permutations before nonmetric multi-dimensional scaling (nMDS) was perfomed. The nMDS plot was overlaid by circles whose sizes reflect values of the TOC:TP ratios of all soils and with vectors of the Pearson correlations of the variables, where the arrows show in the direction of the highest correlation with the variables. Finally, one-way-ANOSIM with 999 permutations was used to test, if nutrient concentrations significantly differ by

site.

To test the effects of nutrient addition on relative PSB abundance and phosphatase activity across all sites, a linear mixed-effects model as implemented in the R package nlme (Pinheiro et al., 2018) was used. This approach was chosen because it allows to show treatment effects among a set of sites spanning large biogeographical gradients. Treatment was set as fixed factor and random intercepts were included for sites to adjust for variability among them.

Finally, to examine the effects of climate and soil physical and chemical parameters as predictors for the relative abundance of PSB at site level, multi-model selection according to Grueber et al. (2011) was done to assess the relative importance of a range of covarying factors. The global model was fitted with random intercepts for treatments at each site to adjust for variations caused by them. Explanatory variables included in the global model were TOC, TN, TP, soil TOC:TN ratio, soil TOC:TP ratio, soil TN:TP ratio, DOC, DN, DIP, Bray-1 P, TOP, water-extractable P, labile P, occluded P, apatite P, residual P, pH, sand, MAT, and MAP. 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. Prior to analysis, input variables were standardized using the arm function within arm R library (Gelman et al., 2018). A full submodel set was generated with the dredge function in MuMIn R library (Barton, 2018). The models within the top four AICc (AIC corrected for small sample size) units of the model with the lowest AICc were averaged using the model.avg function of the MuMIn R library. The term "importance" represents the relativized sum of the AIC weights summed across all the models in which the parameter appears and can range between 0 (parameter has no explanatory weight) and 1 (parameter is present in all topmodels). The model variables with a relative variable importance of 1 were selected to fit an optimal linear mixed-effects model containing the most important variables, of which R^2 and p-value were calculated. The model R^2 was calculated as the conditional R^2 according to Nakagawa and Schielzeth (2013), and the model p-value was obtained by likelihood ratio test of the full model with the effect in question against the model without the effect in question. All statistical analyses were done using R version 3.3.1 (RCore Team, 2018).

2.4.3 Results

2.4.3.1 Site Characteristics and Soil Chemical Parameters

The analyzed sites span broad biogeographic gradients that could control P dynamics. Mean annual temperature ranged from 6 °C at Cedar Creek (cdcr.us) to 18 °C at Ukulinga and Summerveld (ukul.za, summ.za), mean annual precipitation ranged from 678 mm at Rookery (rook.uk) to 891 mm at Chichaqua Bottoms (cbgb.us) (Table 1), and pH values in the topsoil controls ranged from 3.8 at Rookery (rook.uk) to 5.9 at Ukulinga (ukul.za) (Table 2). In addition, soil texture was diverse, ranging from sand at Cedar Creek (cdcr.us) to silty clay at Ukulinga (ukul.za, Table 2). Further, there

was a pronounced gradient of TOC and TN concentrations across all sites (Table 2). Mean TOC concentrations in the topsoil control reached from 7 g kg soil^{-1} at Chichaqua Bottoms (cbgb.us) to 49 g kg soil^{-1} at Summerveld (summ.za) and mean TN concentrations from 0.5 g kg soil^{-1} at Chichaqua Bottoms (cbgb.us) to 3.7 g kg soil^{-1} at Herons Brook (hero.uk). In the control, TOC and TN in both depth increments were highest in the soils in South Africa and significantly lower in the soils in UK (-44 and -25%, respectively) and the USA (-85 and -81%, respectively) (Table 2). Mean TP concentrations in the topsoil control ranged between 0.28 g kg^{-1} in Chichaqua Bottoms (cbgb.us) and 0.62 g kg^{-1} in Heron's Brook (hero.uk, Table 2). The soils in UK contained the highest TP concentrations in the control in both depth increments, followed by the soils in South Africa (-11%) and in the USA (-41%). Topsoil TOC:TP ratios in the controls ranged from 40 in the soils from Chichaqua Bottoms (cbgb.us) to 359 in the soils from Summerveld (summ.za, Figure S1) and topsoil TN:TP ratios in the control soils ranged from 3.4 in Chichaqua Bottoms (cbgb.us) to 17.4 in Summerveld (summ.za). P fractions varied among the sites as well. For instance, apatite P was below 1% of TP in the soils in South Africa, amounted to 3 - 5% in the soils in the UK, and to 14 - 22% of TP in the soils in the USA (Figure S2). DOC, DN, and DIP formed a pronounced gradient among all soils as well (Table 3). Mean DOC concentrations in the topsoil control ranged from 15 mg kg soil^{-1} in Cedar Creek (cdcr.us) to 115 mg kg soil^{-1} in Ukulinga (ukul.za) and mean DN concentrations were lowest in Cedar Creek (cdcr.us, 3 mg kg soil^{-1}) and highest in Rookery (rook.uk, 14 mg kg soil^{-1}) compared to the other sites (Table 3). The resembled element concentrations and P fractions of all sites were significantly different from each other (Figure S1).

2.4.3.2 Treatment Effects on Soil pH and Element Concentrations

Addition of N and NP did not significantly decrease soil pH in most soils. Only at Cedar Creek (cdcr.us), soil pH decreased significantly in response to N addition in the topsoil and due to N andNP addition in the second soil depth increment (Table S1). Despite high amounts of N and P added, TOC, and TN concentrations did not change significantly with nutrient addition (an exception was the topsoil at Rookery (rook.uk), where N addition increased TN concentrations compared to NP addition and at Ukulinga (ukul.za), where NP addition increased TN concentrations compared to control, Table S1). Addition of P and NP significantly increased topsoil TP concentrations in Ukulinga (ukul.za), Chichaqua Bottoms (cbgb.us), and Rookery (rook.uk, Table S1). Mean TOC:TP ratios in the control soils of all sites were 147.0 and P addition reduced mean TOC:TP ratios to 96.0 (Figure S1). Topsoil TN:TP ratios in all treatments ranged between 1.9 and 18.8 (Table S1) and topsoil DN:DIP ratios in all treatments ranged even between 0.2 and 1604.6 (Table 3). In the NP treatment, TN:TP ratios ranged between 2.5 and 9.8 (Table S1), and DN:DIP ratios ranged between 1.2 and 236.5 (Table 3). Addition of N, P, and NP significantly increased topsoil DOC concentrations at Cedar Creek (cdcr.us),

Table 2: Soil texture, pH, total organic carbon (TOC), total nitrogen (TN), and total phosphorus (TP) in 0-15 cm and 15-30 cm depth in the soils of the control treatment at the six sites. The soil at the site Summerveld (summ.za) was only sampled in 0-15 cm depth. Numbers depict means ± standard deviations (n=3). Details on soil pH, TOC, TN, and TP concentrations under nutrient addition can be found in the Supplement A (Table S1).

Site code	Depth (cm)	Sand (%)	Silt (%)	Clay (%)	pH[1]	TOC	TN (g kg soil^{-1})	TP
ukul.za	0-15	4.2	42.5	53.3	5.89	41.97 ±1.7	2.89 ±0.2	0.45 ±0.02
	15-30	5.1	39.8	55.1	5.83	37.53 ±3.0	2.60 ±0.1	0.39 ±0.02
summ.za	0-15	49.6	33.5	16.9	5.20	49.10 ±2.5	2.80 ±0.1	0.37 ±0.01
cdcr.us	0-15	90.1	6.7	3.1	5.27	9.44 ±0.9	0.71 ±0.1	0.31 ±0.03
	15-30	85.8	10.8	3.4	5.36	5.17 ±0.9	0.35 ±0.1	0.24 ±0.03
cbgb.us	0-15	85.3	10.3	4.5	5.73	7.15 ±2.2	0.64 ±0.2	0.28 ±0.02
	15-30	83.4	11.3	5.3	5.40	4.10 ±0.4	0.38 ±0.1	0.24 ±0.03
rook.uk	0-15	74.9	18.4	6.7	3.76	24.33 ±2.1	2.06 ±0.1	0.38 ±0.01
	15-30	75.7	19.0	5.3	4.10	10.52 ±1.2	0.96 ±0.1	0.27 ±0.01
hero.uk	0-15	65.2	25.2	9.6	5.12	36.70 ±5.6	3.15 ±0.6	0.62 ±0.13
	15-30	65.5	24.9	9.7	5.24	24.36 ±2.9	2.11 ±0.2	0.54 ±0.12

[1] measured in deionized H_2O

P and NP addition increased topsoil DOC concentrations at Rookery (rook.uk), and NP addition increased topsoil DOC concentrations at Heron's Brook (hero.uk) compared to control (Table 3). N and NP addition also raised DN concentrations compared to control and P addition in the topsoil at all sites except of Rookery (rook.uk) and Heron's Brook (hero.uk, Table 3). On average, N and NP addition raised DN concentrations across all soils by +164 and +106%, respectively, compared to the soils that did not receive N (Table 3). In the topsoils, P and NP additions significantly increased DIP concentrations compared to control and N addition at all sites (Table 3).

2.4.3.3 Soil Microbial Biomass C and Microbial Community Composition

The mean topsoil control concentration of microbial biomass C varied widely between 163 mg kg soil^{-1} in Cedar Creek (cdcr.us) and 1,005 mg kg soil^{-1} in Ukulinga (ukul.za, Table 3). The microbial biomass C concentrations were significantly lower in soils of the American sites compared to the concentrations in the soils of South Africa and the UK. Topsoil microbial biomass C did not vary significantly among treatments at each of the sites ($p > 0.05$, Table 3). The bacterial and fungal community did not differ significantly between treatments in either depth increment among each site (data not shown) and at all sites ($p > 0.05$, Figure S3). The bacterial community composition was significantly different at each of the six sites, except for the sites Cedar Creek (cdcr.us) and Rookery (rook.uk), which did not differ in their bacterial community composition (Figure S4a). The fungal community composition at each site was significantly different from each other (Figure S4b).

Table 3: Dissolved organic carbon (DOC), dissolved nitrogen (DN), 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})	ukul.za	115 ± 7a	108 ± 15a	127 ± 6a	152 ± 34a	127 ± 33a	201 ± 52ab*	238 ± 14b*	217 ± 66b*
	summ.za[1]	98 ± 3a	98 ± 9a	102 ± 10a	100 ± 1a	NA	NA	NA	NA
	cdcr.us	15 ± 0.4a	20 ± 4.8b	20 ± 1.1b	24 ± 2.6b	11 ± 0.6a	12 ± 2.0a*	12 ± 0.3a*	15 ± 1.3a*
	cbgb.us	18 ± 2.7a	22 ± 4.1a	18 ± 2.1a	22 ± 1.6a	15 ± 1.6a	22 ± 3.4a	20 ± 5.6a	20 ± 1.6a
	rook.uk	23 ± 2.7a	29 ± 7.5ab	44 ± 8.3c	36 ± 3.5bc	15 ± 1.5a	16 ± 3.1a*	19 ± 1.0a*	18 ± 1.0a*
	hero.uk	29 ± 2.6a	30 ± 3.4a	36 ± 1.2ab	40 ± 4.1b	19 ± 1.2a*	22 ± 4.2a*	23 ± 3.0a*	25 ± 3.9a*
DN (mg N kg^{-1})	ukul.za	7.3 ± 0.3a	43.2 ± 11.2b	9.9 ± 5.0a	34.4 ± 5.7b	5.5 ± 1.1a	17.3 ± 4.9ab*	14.1 ± 3.3ab	19.5 ± 5.3b*
	summ.za[1]	5.0 ± 0.1a	19.8 ± 5.0b	5.1 ± 0.2a	15.9 ± 3.1b	NA	NA	NA	NA
	cdcr.us[2]	3.2 ± 4.4a	32.9 ± 15.6b	2.7 ± 2.1a	13.4 ± 2.9b	1.8 ± 1.5a	10.0 ± 1.0b	2.1 ± 1.5a	7.0 ± 2.1ab
	cbgb.us	4.1 ± 3.1a	12.0 ± 1.4b	4.7 ± 1.9a	12.1 ± 3.9b	2.6 ± 2.2a	5.0 ± 2.8a*	2.9 ± 1.1a	4.8 ± 2.1a*
	rook.uk	14.2 ± 2.7ab	14.7 ± 3.2b	10.9 ± 0.4ab	10.3 ± 1.2a	4.2 ± 2.4a*	3.8 ± 1.0a*	2.9 ± 0.8a*	4.0 ± 0.8a*
	hero.uk	12.9 ± 3.6a	17.1 ± 3.9a	11.1 ± 3.5a	14.6 ± 8.7a	9.7 ± 3.8a	10.3 ± 0.8a	11.9 ± 4.4a	8.9 ± 3.2a
DOC:DN ratio	ukul.za	18.2 ± 0.7b	3.0 ± 0.5a	17.8 ± 7.3b	5.3 ± 1.2a	27.0 ± 1.5b*	14.8 ± 5.5a*	20.3 ± 3.5ab	13.0 ± 1.4a*
	summ.za[1]	22.9 ± 0.6b	6.0 ± 1.1a	23.2 ± 1.6b	7.5 ± 1.2a	NA	NA	NA	NA
	cdcr.us[3]	18.1 ± 11.3b	0.8 ± 0.1a	12.0 ± 6.1b	2.1 ± 0.2a	10.9 ± 3.3b	1.4 ± 0.1a*	9.6 ± 4.7b	2.7 ± 0.6ab
	cbgb.us	10.8 ± 3.0b	2.1 ± 0.2a	4.9 ± 1.5ab	2.3 ± 0.6a	10.0 ± 4.7a	5.9 ± 1.6a	8.8 ± 3.5a	5.6 ± 1.9a
	rook.uk[2]	2.0 ± 0.5a	2.3 ± 0.3ab	4.6 ± 0.6c	4.0 ± 0.1bc	5.1 ± 1.9a*	4.8 ± 0.2a*	8.1 ± 1.7a*	5.4 ± 1.1a
	hero.uk	2.7 ± 0.4a	2.1 ± 0.3a	4.0 ± 1.1a	4.2 ± 2.3a	2.6 ± 1.0a	2.5 ± 0.2a	2.5 ± 0.6a	3.5 ± 0.7a
DIP (mg P kg^{-1})	ukul.za[2]	0.10 ± 0.07a	0.12 ± 0.04a	1.43 ± 0.19b	1.10 ± 0.25b	0.13 ± 0.05a	0.05 ± 0.05a*	2.09 ± 1.58b	0.92 ± 0.24b
	summ.za[1,2]	0.05 ±0.04a	0.10 ± 0.02ab	0.25 ± 0.09b	0.27 ± 0.12b	NA	NA	NA	NA
	cdcr.us[2]	0.16 ± 0.12a	0.13 ± 0.06a	13.37 ± 2.76b	12.29 ± 3.55b	0.06 ± 0.03a*	0.04 ± 0.01a*	4.19 ± 0.55b*	5.01 ± 0.89b*
	cbgb.us[2]	0.37 ± 0.08a	0.25 ± 0.19a	7.67 ± 1.23b	11.1 ± 3.18b	0.13 ± 0.08a*	0.11 ± 0.05a	7.02 ± 3.11b	6.96 ± 1.91b
	rook.uk[2]	0.02 ± 0.01a	0.03 ± 0.01a	1.25 ± 0.88b	1.22 ± 1.20b	0.02 ± 0.01a	0.02 ± 0.01a	0.04 ± 0.03a*	0.06 ± 0.02a*
	hero.uk[2]	0.04 ± 0.01a	0.04 ± 0.01a	1.64 ± 0.85b	1.15 ± 0.72b	0.02 ± 0.01a*	0.03 ± 0.01ab	0.04 ± 0.01ab*	0.06 ± 0.01b*
MBC (mg C kg^{-1})	ukul.za	1005 ± 134a	767 ± 196a	819 ± 157a	852 ± 71a	488 ± 93a*	444 ± 85a*	502 ± 113a*	549 ± 66a*
	summ.za[1]	843 ± 133a	769 ± 111a	928 ± 278a	699 ± 16a	NA	NA	NA	NA
	cdcr.us	163 ± 63a	235 ± 44a	193 ± 9a	169 ± 99a	119 ± 103a	100 ± 47a*	46 ± 31a*	134 ± 124a
	cbgb.us	175 ± 61a	121 ± 23a	168 ± 51a	148 ± 100a	61 ± 15a*	119 ± 35a	69 ± 43a	142 ± 118a
	rook.uk	651 ± 35a	588 ± 49a	507 ± 210a	746 ± 88a	351 ± 46a*	317 ± 94a*	248 ± 176a*	342 ± 248a*
	hero.uk	662 ± 116a	603 ± 70a	638 ± 51a	591 ± 85a	526 ± 8b	363 ± 61ab*	298 ± 73a*	352 ± 76ab*

[1] One-Way ANOVA and Tukey test were performed
[2] Data were LOG10 transformed
[3] Data were reciprocally transformed (1/x)

2.4.3.4 Relative Abundance of P-Solubilizing Bacteria

Across all sites, the relative abundance of PSB in the topsoil was significantly lower in the N (-18%) and NP treatment (-41%) than in the control (Figure 1A) according to the estimated model predictors of the linear mixed-effects model. In the second depth increment, the relative abundance of PSB in the NP treatment was also significantly lower (-60%) than in the control (Figure 1B).

Site level-differences in the relative abundance of PSB were more pronounced than treatment effects on the relative abundance of PSB (Figure 2). The South African site Summerveld showed the highest relative abundance of PSB with 31% of all CFU being PSB, whereas the site Rookery in the UK showed the lowest relative abundance of PSB with 4% in the topsoil (Figure S5 and Table S3). Mean numbers of CFUs ranged from 5.6×10^4 to 8.9×10^6 per g soil (Table S3). Considering the sites separately, the relative abundance of PSB did not differ significantly between the treatments, except for the sites Cedar Creek (cdcr.us) and Summerveld (summ.za, Figures S5b, d). The tendency of decreased topsoil PSB abundance under NP addition was similar at all sites. However, differences between nutrient addition treatments were mostly not significant at single site level, mainly because of high standard deviations. After multi-model selection, we found that at site level, soil TOC:TP ratio, percentage of sand, pH, and water-extractable P significantly predicted the variance of the relative abundance of PSB in the topsoil with an R^2 of 0.71 ($p < 0.01$, Figure 2A and Table S2). A model based only on the control treatments with the same variables, predicted the variance of the relative PSB abundance with an R^2 of 0.83 (all variables were significant, $p = 0.012$, Figure 2B and Table S2).

2.4.3.5 Community Composition and Activity of P-Solubilizing Bacteria

The 903 sequenced PSB belonged to four phyla (Proteobacteria, Firmicutes, Bacteroidetes, Actinobacteria) and eight different classes (Actinobacteria, Alphaproteobacteria, Bacilli, Betaproteobacteria, Chitinophagia, Flavobacteriia, Gammaproteobacteria, Sphingobacteriia). Pseudomonas and Enterobacterales were present in all soils (Figure 3). The sequenced colonies were assigned to 146 OTUs and the most abundant OTUs were Pseudomonas (56 OTUs), Enterobacterales (50 OTUs), and Paenibacillus (14 OTUs).

The PSB communities at all sites were significantly different from each other ($p < 0.05$, occurrence of OTUs of different taxa was tested), except for the sites Summerveld (summ.za) and Chichaqua Bottoms (cbgb.us) as well as Cedar Creek (cdcr.us) and Heron's Brook (hero.uk) that were not different from each other (Figure 4A). Across all sites, the PSB community in the N and NP treatment was significantly different from the community in the control and P treatment ($p = 0.002$) (Figure 4B). The soils of the control and P treatment contained more different Pseudomonas OTUs than the soils of the N and NP treatments and the number of different Enterobacterales OTUs was higher in soils of the N and NP treatments than in the soils of the control and P treatment (Figure 4B). Between 16 and 24% of all PSB at the different sites were classified as strong P

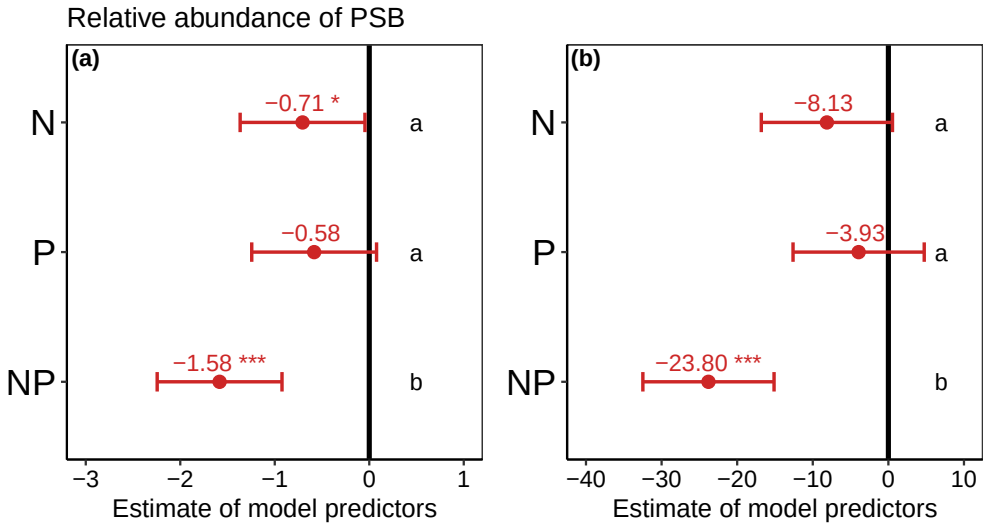


Figure 1: Effect of nutrient addition (N, P, NP) on the relative abundance of phosphorus-solubilizing bacteria (PSB) across all six sites in (a) 0-15 cm depth and (b) 15-30 cm depth. A linear mixed-effects model was calculated with treatment as fixed factor and random intercepts for sites. Points represent 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 with asterisks. The vertical intercept (position zero) corresponds to the control. Significant differences between treatments are indicated by lower-case letters at the right side of the subplot.

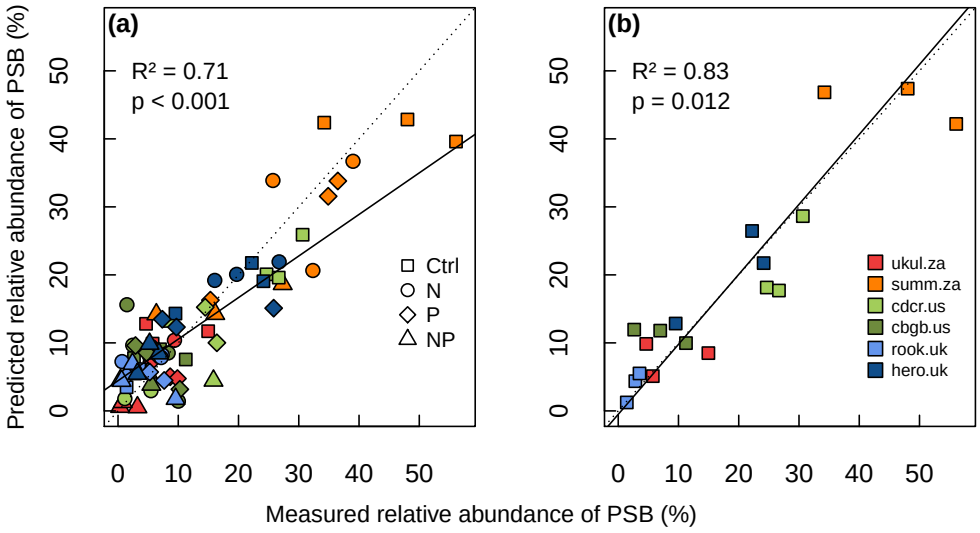


Figure 2: Linear mixed-effects model of (a) relative abundance of phosphorus-solubilizing bacteria (PSB) in all treatments, and (b) relative abundance of PSB in the control at all six grassland sites in 0-15 cm depth. Measured relative abundance of PSB is shown on the x-axis and predicted relative abundance of PSB (best model predictors: total organic carbon-to-total phosphorus ratio, sand content, soil pH, and water extractable phosphorus, Table S2) is shown on the y-axis. 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.

solubilizers according to their halo size. The proportions of PSB that were classified as strong solubilizers across all sites differed among treatments, with the greatest proportion (25%) in the control, 21% in both the N and P treatment, and 18% in the NP treatment.

2.4.3.6 Phosphatase Activity

Phosphatase activity in the N treatment was significantly higher (+17%) than in the control across all six topsoils (Figure 5A), according to the estimated model predictor of the linear mixedeffects model. Phosphatase activity also tended to be higher in the NP treatment (+ 9%) than in the control in the topsoil, but this difference was not statistically significant. Similarly, in the P treatment, phosphatase activity tended to be lower (-4%) than in the control in the topsoil, albeit the differences were not statistically significant. In the second depth increment, the differences in phosphatase activity between treatments and control were not statistically significant (Figure 5B).

Considering the sites separately, phosphatase activity did not differ significantly among the treatments at any site and in any depth increment (Figure S6), mainly because of high standard deviations. However, a tendency of increased phosphatase activity under N addition was visible at most sites. Phosphatase activity in the control treatment ranged from 17 nmol g^{-1} h^{-1} at Chichaqua Bottoms (cbgb.us) to 352 nmol g^{-1} h^{-1} at Summerveld (summ.za) across all sites and depth increments (Figure S6). Comparing the mean controls, the soils in the USA had the lowest phosphatase activities of all six soils (Figure S6). The specific phosphatase activity (phosphatase activity per microbial biomass C) showed no significant difference ($p > 0.05$) among treatments (data not shown).

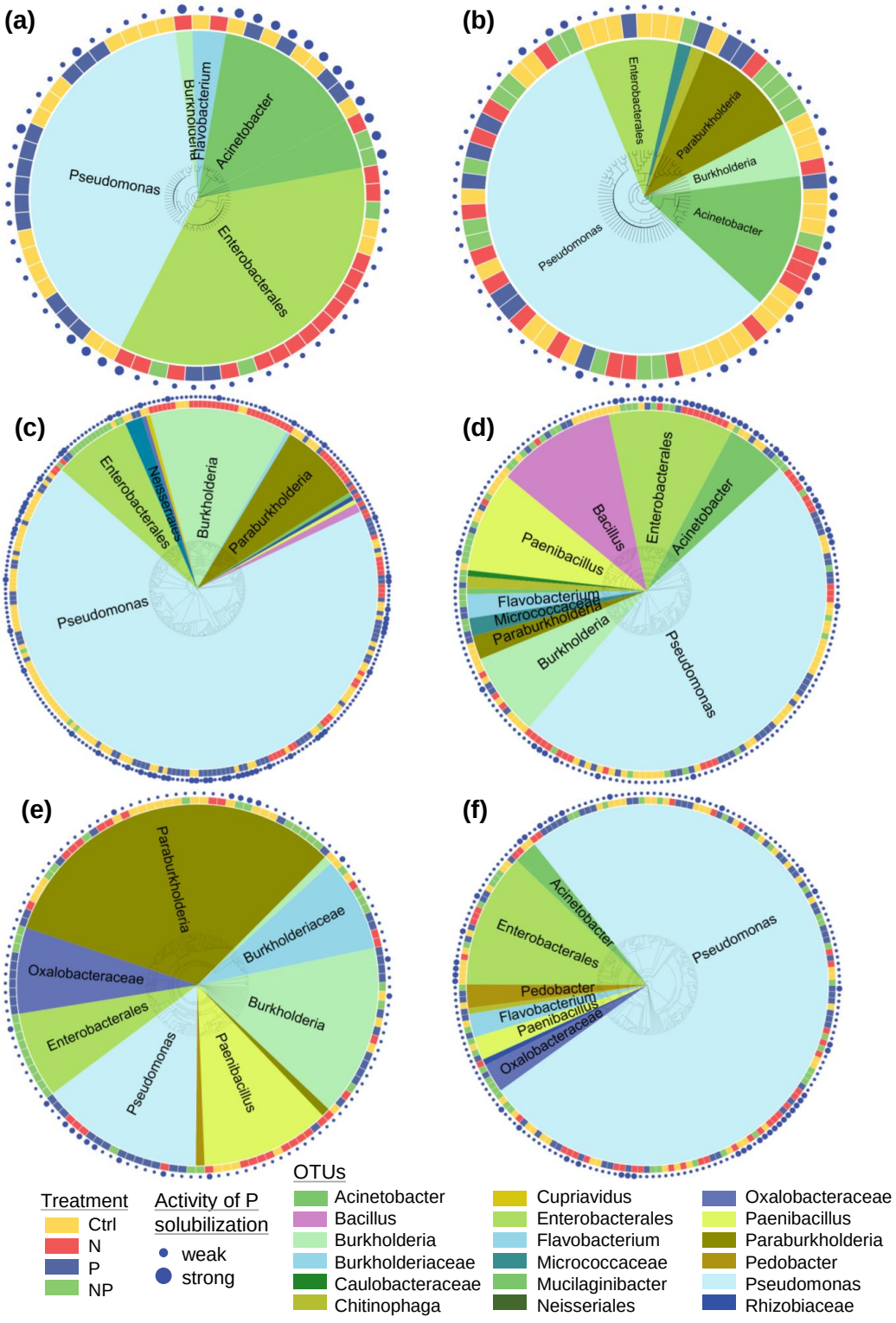


Figure 3: Phylogenetic tree of all sequenced phosphorus-solubilizing bacteria at the site (a) Ukulinga (ukul.za), (b) Summerveld (summ.za), (c) Cedar Creek (cdcr.us), (d) Chichaqua Bottoms (cbgb.us), (e) Rookery (rook.uk), and (f) Heron's Brook (hero.uk) in 0-15 cm soil depth. At least 100 colony-forming units per sample were screened. Colors around the tree indicate the nutrient addition treatments. Colors inside the tree show the assigned name (OTU). The size of the dots around the tree indicates the activity of P solubilization (weak or strong).

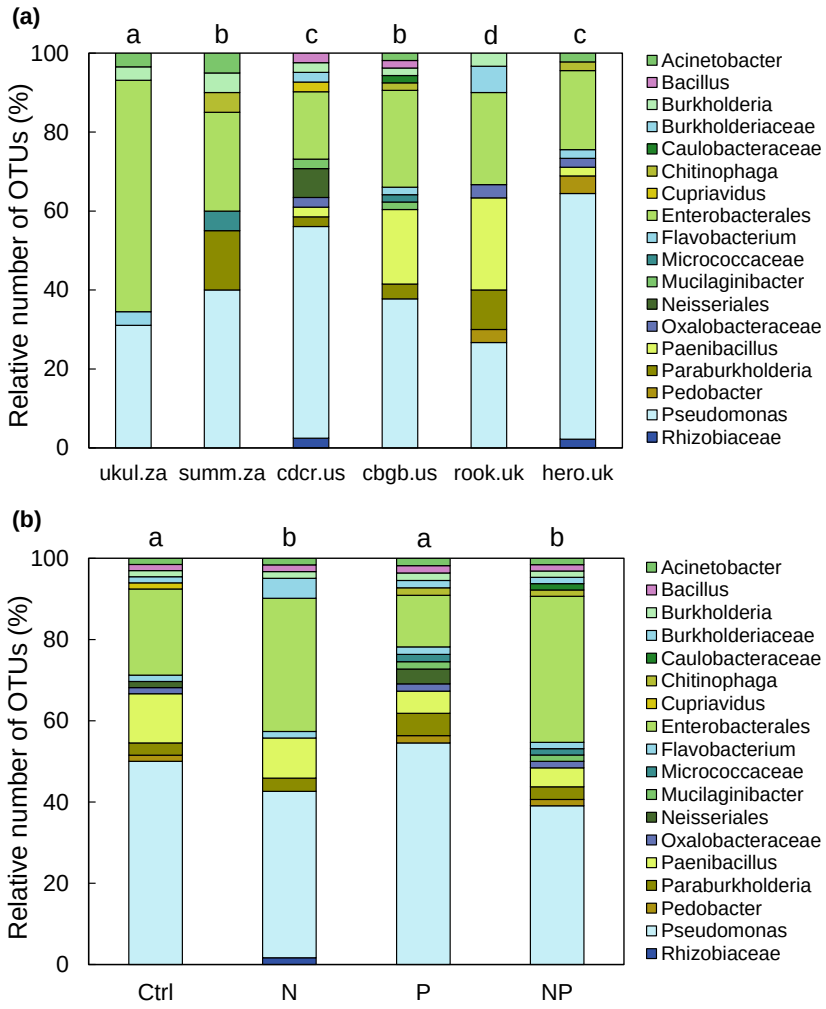


Figure 4: Relative number of operational taxonomic units (OTUs) of phosphorus-solubilizing bacteria in 0-15 cm soil depth sorted by (a) sites and (b) treatments. Different letters indicate significant differences ($p < 0.05$) among sites (panel a) and among treatments (panel b).

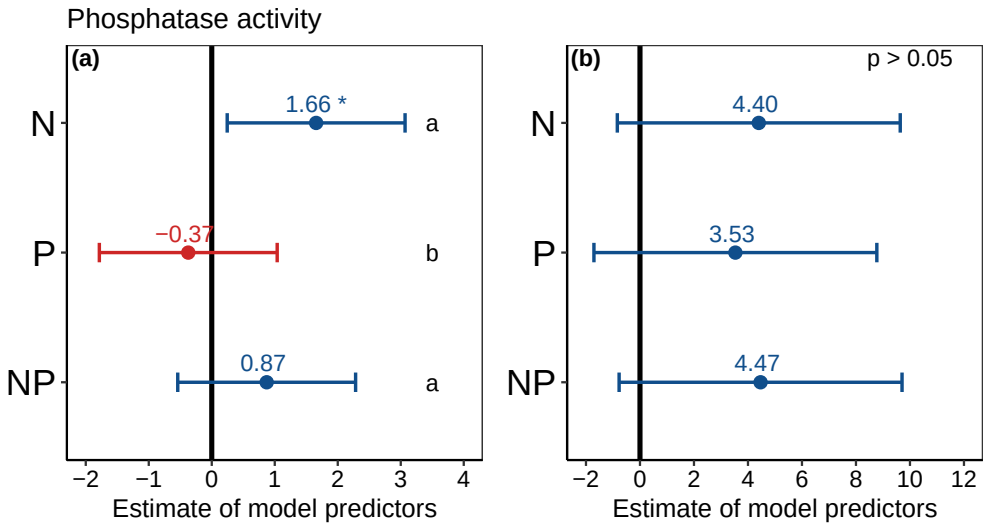


Figure 5: Effect of nutrient addition (N, P, NP) on soil phosphatase activity across all six sites in (a) 0-15 cm depth and (b) 15-30 cm depth. A linear mixed-effects model was calculated with treatment as fixed factor and random intercepts for sites. Points represent the mean value of the model predictor while error bars represent the range of 95 % confidence intervals. Predictors are considered significant, if error bars do not overlap with zero, indicated with asterisks. 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.

2.4.4 Discussion

Here we found that N addition led to a significant decrease in the abundance of PSB (Figure 1). Furthermore, N addition in combination with P addition decreased the abundance of PSB even further, but when P was added alone, the abundance of PSB was not affected. Addition of N and NP changed the PSB community composition relative to the control and the P addition treatment (Figure 4). Furthermore, N addition significantly increased soil phosphatase activity across all topsoils and P addition tended to decrease phosphatase activity, albeit not significantly (Figure 5).

2.4.4.1 Relative Abundance and Activity of P-solubilizing Bacteria

Our observation that the relative abundance of PSB was significantly lower in the N and NP treatments than in the control across all six topsoils (Figure 1) indicates that N availability may affect the functional traits of the microbial community since a reduced abundance of PSB might also result in a decreased P solubilization. Microbes that can solubilize P may have an advantage under nutrient-poor conditions, leading to an increase in their relative abundance. The reason for this might be that microorganisms adapted to N- and NP-rich soil do not invest organic C and N into processes of nutrient acquisition, and thus have more energy left for growth and other fitness-related processes such as defense or the formation of symbiosis. Similar functional adaptions of microbial communities to nutrient availability have been described before (Calvaruso et al., 2007; Lepleux et al., 2013; Mander et al., 2012; Nicolitch et al., 2016). For example, in the

rhizosphere of a temperate beech forest, it has been shown that P solubilization efficacy was lower in nutrient-rich soils than in soils with low nutrient availability (Nicolitch et al., 2016). The finding that N and NP addition decreased the relative abundance of PSB is against our hypothesis that PSB need N for the processes to produce organic acids and exopolysaccharides that solubilize P. In contrast, PSB are likely sensitive to soil nutrient conditions, and their abundance decreases under elevated nutrient availability, suggesting that they are poor competitors when N and NP availability is high.

We found that the total number of strong PSB was lowest under NP addition (-28% in comparison to control), suggesting that the ability to strongly solubilize P is rewarding under low-nutrient conditions, but not when N and P availability is high. Our results build on a previous study of a beech forest in France, reporting that the percentage of strong P solubilizers was significantly lower in nutrient-rich soils than in nutrient-poor soils (Nicolitch et al., 2016). Taken together, our results show that across a wide range of soil biotic and abiotic conditions, NP addition not only reduces the relative abundance of PSB but also the abundance of PSB with a specifically high capacity to solubilize P from minerals.

One reason why P addition alone did not significantly reduce the relative abundance of PSB compared to the control (Figure 1) might be that PSB do not only contribute to weathering of apatite but also to weathering of other minerals, leading to mobilization of K, Mg, Ca, and Fe (Hinsinger, 2001). Thus, even though the ability to mobilize P might not be beneficial in soils with high P availability, PSB might remain competitive in a high P environment since they can also mobilize other nutrients. In addition it could be that weathering is a side-effect of other metabolic processes that do not fulfill the primary function of mobilizing nutrients. This is supported by studies showing that the abundance of PSB was not related to levels of total and available P in Canadian cropland and pasture soils (Kucey, 1983), and in Irish agricultural soils (Browne et al., 2009). Still, it also needs to be considered that the amount of P added in our study (100 kg P ha^{-1} yr^{-1}) may have been too small to cause significant changes in the relative abundance of PSB. Mander et al. (2012) reported a reduced abundance of PSB caused by additions as high as 367 kg P ha^{-1} yr^{-1} in a New Zealand pasture. Taken together, P addition did not reduce the relative abundance of PSB, which contrasts our hypothesis.

Our finding that the relative abundance of PSB in the control across all sites ranged from 2.6% in Rookery (rook.uk) to 46% in Summerveld (summ.za) of all colonies matches with previous studies that found abundances of PSB of 0.5% (Kucey, 1983) and between 47 and 53% (Browne et al., 2009). We found that the abundance of PSB across all sites was mostly affected by the TOC:TP ratio, sand content, soil pH, and water-extractable P content of the soils (Figure 2). The high relative abundance of PSB in Summerveld (summ.za) can thus largely be explained by the high TOC:TP ratios (Figure S1) and low concentrations of water-extractable P (Figure S2) found at this site compared to the other sites. We did not find a direct effect of P addition on the abundance of PSB, but our results (Table S2) indicate that the P concentration relative to the TOC

concentration affects the abundance of PSB and the explanatory value of TOC:TP ratios for PSB abundance across all sites was higher compared to TN:TP ratios. The reason for this might be that a main mechanism of P solubilization is the production of organic acid anions (Jones and Oburger, 2011), and microbes need organic C to build and to release them. In addition, C addition has been shown to enhance P solubilization in laboratory studies (Hameeda et al., 2006; Patel et al., 2008), and a positive correlation between the soil TOC:TP ratio and the relative abundance of PSB was found by Mander et al. (2012). The sand content was positively correlated with the relative abundance of PSB (Table S2). The reason for this might be that across our sites, the sand content was correlated with low nutrient concentrations (TOC, TN, TP, DOC, DN) and those conditions favored the abundance of PSB. Further, PSB increased with soil pH (Table S2), in agreement with Mander et al. (2012), likely because the ability of PSB to release protons only results in P solubilization in alkaline to moderately acid soils, but much less in acid soils (Hinsinger, 2001). Furthermore, the relative abundance of PSB was negatively related to water-extractable P (easily-available P) in our model (Table S2). In lab studies, P solubilization was also suppressed by very high levels of easily-available P in the cultivation medium (Chabot et al., 1998; Goldstein, 1986).

2.4.4.2 Community Composition of P-solubilizing Bacteria

Our finding that the OTU Pseudomonas, Enterobacterales, Acinetobacter, Burkholderia, Para-burkholderia, Bacillus, and Paenibacillus were both present and highly abundant in most samples (Figure 3) corroborates previous studies reporting that these bacterial groups are able to solubilize P (Lepleux et al., 2013; Liu et al., 2015; Oliveira et al., 2009). Pseudomonas was one of the most abundant and most diverse OTU. They are known to efficiently contribute to P solubilization because many Pseudomonas species can oxidize glucose to gluconic acid in the periplasm and release it into soil (Goldstein, 2007).

In addition to the decreased relative abundance of PSB under N and NP addition, we found a significant change in PSB community composition due to N and NP addition as hypothesized (Figure 4). For the whole bacterial community, it was already shown that N addition shifted the community composition in grassland soils (Fierer et al., 2012; Leff et al., 2015; Ramirez et al., 2010). Against our hypothesis, P addition did not change the PSB community composition confirming studies showing that the bacterial community was less responsive to P addition than to N addition (Coolon et al., 2013; Pan et al., 2014). However, to our knowledge, there is no previous study describing shifts in the PSB community and general PSB community pattern under N and NP addition in grasslands.

The addition of N and NP lowered the number of Pseudomonas OTUs and increased the number of Enterobacterales OTUs. Our results indicate that certain Pseudomonas OTUs are hampered by N and NP addition and seem to be better adapted to nutrient-poorer conditions. This agrees with studies reporting that the abundance and diversity

of Pseudomonads was higher on a nutrient-poor than on nutrient-rich medium (Aagot et al., 2001) and that most PSB were Pseudomonads in non-fertilized soils (Mander et al., 2012). A reason for the higher number of Enterobacterales than Pseudomonas OTUs under N and NP addition could be that they respond differently to changing amounts and composition of root exudates as can be expected by the reported strong changes in plant community composition in response to the nutrient additions (Badri and Vivanco, 2009; Rengel and Marschner, 2005). This speculation is based on the observation that Enterobacterales and Pseudomonads prefer different substrates: Enterobacterales prefer the amino acid glycine, while Pseudomonas prefer sugars (Goldfarb et al., 2011).

We screened for PSB with a culture-dependent approach using PVK medium, which is commonly used to determine the abundance of PSB (Mehta and Nautiyal, 2001) but discriminates against non-cultivable microorganisms. Currently, there are no metagenomic approaches available for determining the abundance of PSB since many processes are involved in P solubilization, which makes it impossible to determine PSB by targeting one specific gene. However, the sequenced PSB reflect the dominant taxa found in the same grassland soils (Leff et al., 2015). Further, previous studies showed the PSB isolated based on culture-dependent screening techniques were representative for the total bacterial community as analyzed by pyrosequencing (Nicolitch et al., 2016). Besides bacteria, plants and fungi also contribute to P solubilization by the release of protons, organic acids and other chelating agents (Hinsinger, 2001; Jones and Oburger, 2011). However, in this study we focused on the abundance of P-solubilizing bacteria, since between 1 - 50% of soil bacteria (Kucey et al., 1989) can be classified at PSB, whereas only 0.1 - 3% of soil fungi (Banik and Dey, 1982; Kucey, 1983) can be classified as P-solubilizing fungi. However, rates of P solubilization by bacteria and fungi on single species level can be similar (e.g. (Vazquez et al., 2000).

2.4.4.3 Phosphatase Activity

Our result that phosphatase activity was significantly higher under N addition in the topsoil than in the control (Figure 5) indicates that microorganisms and plants invested into the production of phosphatases when provided with additional N, suggesting that increased N availability might increase P demand. This is in accordance with a meta-analysis showing that plants and microbes invest into P acquisition, if N availability increases (Marklein and Houlton, 2012). This might allow them to maintain their biomass stoichiometry despite changes in element availability in their environment (Spohn, 2016). In addition, increased N availability likely facilitates the production of phosphatases, since phosphatases contain large amounts of N that is lost for the organisms when phosphatases are released into the soil. Our finding is in accordance with our hypothesis and with previous studies, reporting an increase in phosphatase activity in response to N addition (Margalef et al., 2017; Marklein and Houlton, 2012; Olander and Vitousek, 2000).

Phosphatase activity was likely lower in the P than in the N addition treatment because plants and microbes reduce the production of phosphatases when provided with inorganic P (Oshima et al., 1996). Yet, contrary to our hypothesis, phosphatase activity was not significantly reduced in the P addition treatment compared to the control (Figure 5). Our results are counter to the findings of a meta-analysis (Marklein and Houlton, 2012) and further studies showing that inorganic P addition inhibits phosphatase activities in soil (Moscatelli et al., 2005; Olander and Vitousek, 2000). However, the absence of a suppressive effect of P addition on phosphatases has also been reported (Allison and Vitousek, 2005; Criquet and Braud, 2008; Schneider et al., 2001). An explanation for the less pronounced decrease in phosphatase activity due to P addition could be that microbial phosphatase activity is also controlled by microbial need for C, and thus rather depends on C than on P availability (Heuck et al., 2015; Spohn and Kuzyakov, 2013). Further, depending on the type of nutrient limitation of the plants, P and especially NP addition can increase aboveground net primary productivity (Elser et al., 2007) and thus increase organic matter inputs to soil making it more rewarding for microorganisms to mineralize organic P.

2.4.4.4 Conclusions

Taken together, our study indicates that N addition to soil strongly affects P mobilization processes in grassland soils. Our results suggest that microorganisms invest preferentially into mobilizing organic P in response to high N inputs to soil. In contrast, under N-poor conditions, the microbial community has a high capacity to mobilize P from inorganic sources as indicated by a high abundance of PSB. Consequently, increased atmospheric N deposition may decrease solubilization of bound inorganic P and increase mineralization of organic P. These findings have important implications for element cycling because they indicate that high chronic N inputs could induce a shift from P mobilization from minerals to increased recycling of organically bound P in grassland soils.

Data availability statement

The partial 16S rDNA sequences of the 903 colonies were deposited in GenBank (https://www.ncbi.nlm.nih.gov/genbank/) under the accession numbers MK488507-MK489408.

Acknowledgements

MS 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 EB and ES from the National Science Foundation Research Coordination Network (NSF-DEB-1042132) and Long Term Ecological Research (NSF-DEB-1234162 to Cedar Creek LTER) programs, and the Institute

on the Environment (DG-0001-13). This publication was funded by the German Research Foundation (DFG) and the University of Bayreuth in the funding programme Open Access Publishing. We thank Anita Gössner, Uwe Hell, Renate Krauss, Ralf Mertel, and Karin Söllner for technical assistance and thank the chemical analytics (CAN) of the Bayreuth Center of Ecological and Environmental Research (BayCEER) for performing parts of the chemical analyses and the Keylab for Genomics and Bioinformatics of the BayCEER for sequencing the PSB and for the bacterial and fungal community analyses (ARISA).

References

Aagot, N., Nybroe, O., Nielsen, P., Johnsen, K., 2001. An altered Pseudomonas diversity is recovered from soil by using nutrient-poor Pseudomonas-selective soil extract media. Applied and Environmental Microbiology 67, 5233–5239.

Allison, S.D., Vitousek, P.M., 2005. Responses of extracellular enzymes to simple and complex nutrient inputs. Soil Biology and Biochemistry 37, 937–944.

Badri, D.V., Vivanco, J.M., 2009. Regulation and function of root exudates. Plant, Cell Environment 32, 666–681.

Banik, S., Dey, B.K., 1982. Available phosphate content of an alluvial soil as influenced by inoculation of some isolated phosphate-solubilizing micro-organisms. Plant and Soil 69, 353–364. Barton, K., 2018. MuMIn: Multi-Model Inference. R package version 1.42.1.

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.

Bray, R.H., Kurtz, L.T., 1945. Determination of total, organic, and available forms of phosphorus in soils. Soil Science 95, 39–46. Browne, P., Rice, O., Miller, S.H., Burke, J., Dowling, D.N., Morrissey, J.P., O'Gara, F., 2009. Superior inorganic phosphate solubilization is linked to phylogeny within the Pseudomonas fluorescens complex. Applied Soil Ecology 43, 131–138.

Calvaruso, C., Turpault, M.-P., Leclerc, E., Frey-Klett, P., 2007. Impact of ectomycorrhizosphere on the functional diversity of soil bacterial and fungal communities from a forest stand in relation to nutrient mobilization processes. Microbial Ecology 54, 567–577.

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.

Chabot, R., Beauchamp, C.J., Kloepper, J.W., Antoun, H., 1998. Effect of phosphorus on root colonization and growth promotion of maize by bioluminescent mutants

of phosphate-solubilizing Rhizobium leguminosarum biovar phaseoli. Soil Biology and Biochemistry 30, 1615–1618.

Chelius, M.K., Triplett, E.W., 2001. The Diversity of Archaea and Bacteria in Association with the Roots of Zea mays L. Microbial Ecology 41, 252–263.

Clarke, K.R., Gorley, R.N., 2015. PRIMER v7: User Manual/Tutorial, Plymouth.

Coolon, J.D., Jones, K.L., Todd, T.C., Blair, J.M., Herman, M.A., 2013. Long-term nitrogen amendment alters the diversity and assemblage of soil bacterial communities in tallgrass prairie. PLoS ONE 8.

Criquet, S., Braud, A., 2008. Effects of organic and mineral amendments on available P and phosphatase activities in a degraded Mediterranean soil under short-term incubation experiment. Soil and Tillage Research 98, 164–174.

Dobritsa, A.P., Samadpour, M., 2016. Transfer of eleven species of the genus Burkholderia to the genus Paraburkholderia and proposal of Caballeronia gen. nov. to accommodate twelve species of the genera Burkholderia and Paraburkholderia. International journal of systematic and evolutionary microbiology 66, 2836–2846.

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.

Fierer, N., Lauber, C.L., Ramirez, K.S., Zaneveld, J., Bradford, M.A., Knight, R., 2012. Comparative metagenomic, phylogenetic and physiological analyses of soil microbial communities across nitrogen gradients. The ISME Journal 6, 1007–1017.

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.

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. R package version 1.10-1.

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.

Goldfarb, K.C., Karaoz, U., Hanson, C.A., Santee, C.A., Bradford, M.A., Treseder, K.K., Wallenstein, M.D., Brodie, E.L., 2011. Differential growth responses of soil bacterial taxa to carbon substrates of varying chemical recalcitrance. Frontiers in Microbiology 2, 94.

Goldstein, A.H., 1986. Bacterial solubilization of mineral phosphates: Historical perspective and future prospects. American Journal of Alternative Agriculture 1, 51–57.

Goldstein, A.H., 2007. Future trends in research on microbial phosphate solubilization: one hundred years of insolubility, in: Velázquez, E., Rodríguez-Barrueco, C. (Eds.), First International Meeting on Microbial Phosphate Solubilization. Devel-

opments in Plant and Soil Sciences. Springer, Dordrecht.

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.

Hameeda, B., Harish Kumar Reddy, Y., Rupela, O.P., Kumar, G.N., Reddy, G., 2006. Effect of carbon substrates on rock phosphate solubilization by bacteria from composts and macrofauna. Current Microbiology 53, 298–302.

He, Z., Zhang, H., Gao, S., Lercher, M.J., Chen, W.-H., Hu, S., 2016. Evolview v2: an online visualization and management tool for customized and annotated phylogenetic trees. Nucleic Acids Research 44, W236-41.

Hedley, M.J., Stewart, J.W.B., Chauhan, B.S., 1982. Changes in inorganic and organic soil phosphorus fractions by cultivation practices and by laboratory incubations. Soil Science Society of America Journal 46, 970–976.

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.

Heuck, C., Smolka, G., Whalen, E.D., Frey, S., Gundersen, P., Moldan, F., Fernandez, I.J., Spohn, M., 2018. Effects of long-term nitrogen addition on phosphorus cycling in organic soil horizons of temperate forests. Biogeochemistry 39, 378.

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.

Hinsinger, P., 2001. Bioavailability of soil inorganic P in the rhizosphere as affected by root-induced chemical changes: a review. Plant and Soil, 173–195.

Jenkinson, D.S., Brookes, P.C., Powlson, D.S., 2004. Measuring soil microbial biomass. Soil Biology and Biochemistry 36, 5–7.

Jones, D.L., Oburger, E., 2011. Solubilization of Phosphorus by Soil Microorganisms, in: Bünemann, E.K., Oberson, A., Frossard, E. (Eds.), Phosphorus in Action. Biological Processes in Soil Phosphorus Cycling. Springer, Berlin Heidelberg, pp. 169–198.

Katoh, K., Standley, D.M., 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Molecular Biology and Evolution 30, 772–780.

Kucey, R.M.N., 1983. Phosphate-solubilizing Bacteria and Fungi in Various Cultivated and Virgin Alberta Soils. Canadian Journal of Soil Science 63, 671–678.

Kucey, R.M.N., Janzen, H.H., Leggett, M.E., 1989. Microbially mediated increases in plant-available phosphorus, in: Advances in agronomy. Elsevier, pp. 199–228.

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.

Lepleux, C., Uroz, S., Collignon, C., Churin, J.-L., Turpault, M.-P., Frey-Klett, P., 2013. A short-term mineral amendment impacts the mineral weathering bacterial communities in an acidic forest soil. Research in microbiology 164, 729–739.

Liu, Z., Li, Y.C., Zhang, S., Fu, Y., Fan, X., Patel, J.S., Zhang, M., 2015. Characterization of phosphate-solubilizing bacteria isolated from calcareous soils. Applied Soil Ecology 96, 217–224.

Mander, C., Wakelin, S., Young, S., Condron, L., O'Callaghan, M., 2012. Incidence and diversity of phosphate-solubilising bacteria are linked to phosphorus status in grassland soils. Soil Biology and Biochemistry 44, 93–101.

Margalef, O., Sardans, J., Fernández-Martínez, M., Molowny-Horas, R., Janssens, I.A., Ciais, P., Goll, D., Richter, A., Obersteiner, M., Asensio, D., Peñuelas, J., 2017. Global patterns of phosphatase activity in natural soils. Scientific Reports 7, 1337.

Marklein, A.R., Houlton, B.Z., 2012. Nitrogen inputs accelerate phosphorus cycling rates across a wide variety of terrestrial ecosystems. The New Phytologist 193, 696–704.

Mehta, S., Nautiyal, C.S., 2001. An efficient method for qualitative screening of phosphate-solubilizing bacteria. Current Microbiology 43, 51–56.

Moscatelli, M.C., Lagomarsino, A., Angelis, P.D., Grego, S., 2005. Seasonality of soil biological properties in a poplar plantation growing under elevated atmospheric CO2. Applied Soil Ecology 30, 162–173.

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 R2 from generalized linear mixed-effects models. Methods in Ecology and Evolution 4, 133–142.

Nicolitch, O., Colin, Y., Turpault, M.-P., Uroz, S., 2016. Soil type determines the distribution of nutrient mobilizing bacterial communities in the rhizosphere of beech trees. Soil Biology and Biochemistry 103, 429–445.

Olander, L.P., Vitousek, P.M., 2000. Regulation of soil phosphatase and chitinase activity by N and P availability. Biogeochemistry, 175–190.

Oliveira, C.A., Alves, V.M.C., Marriel, I.E., Gomes, E.A., Scotti, M.R., Carneiro, N.P., Guimarães, C.T., Schaffert, R.E., Sá, N.M.H., 2009. Phosphate solubilizing microorganisms isolated from rhizosphere of maize cultivated in an oxisol of the Brazilian Cerrado Biome. Soil Biology and Biochemistry 41, 1782–1787.

Oshima, Y., Ogawa, N., Harashima, S., 1996. Regulation of phosphatase synthesis in Saccharomyces cerevisiae - a review. Gene 179, 171–177.

Pan, Y., Cassman, N., de Hollander, M., Mendes, L.W., Korevaar, H., Geerts, R. H. E. M., van Veen, J.A., Kuramae, E.E., 2014. Impact of long-term N, P, K, and NPK fertilization on the composition and potential functions of the bacterial community in grassland soil. FEMS Microbiology Ecology 90, 195–205.

Patel, D.K., Archana, G., Kumar, G.N., 2008. Variation in the nature of organic acid secretion and mineral phosphate solubilization by Citrobacter sp. DHRSS in the presence of different sugars. Current Microbiology 56, 168–174.

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.

Peršoh, D., Melcher, M., Flessa, F., Rambold, G., 2010. First fungal community analyses of endophytic ascomycetes associated with Viscum album ssp. austriacum and its host Pinus sylvestris. Fungal Biology 114, 585–596.

Pikovskaya, R.I., 1948. Mobilization of phosphorus in soil in connection with the vital activity of some microbial species. Mikrobiologiya 17, 362–370.

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.

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., 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.

Reid, R.K., Reid, C.P.P., Szaniszlo, P.J., 1985. Effects of synthetic and microbially produced chelates on the diffusion of iron and phosphorus to a simulated root in soil. Biology and Fertility of Soils 1, 45–52.

Rengel, Z., Marschner, P., 2005. Nutrient availability and management in the rhizosphere: exploiting genotypic differences. The New Phytologist 168, 305–312.

Rodrıguez, H., Fraga, R., 1999. Phosphate solubilizing bacteria and their role in plant growth promotion. Biotechnology Advances 17, 319–339.

Saunders, W.M.H., Williams, E.G., 1955. Observations on the determination of total organic phosphorus in soils. Journal of Soil Science 6, 254–267.

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.

Schneider, K., Turrion, M.-B., Grierson, P., Gallardo, J., 2001. Phosphatase activity, microbial phosphorus, and fine root growth in forest soils in the Sierra de Gata, western central Spain. Biology and Fertility of Soils 34, 151–155.

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.

Spohn, M., 2016. Element cycling as driven by stoichiometric homeostasis of soil microorganisms. Basic and Applied Ecology 17, 471–478.

Spohn, M., Kuzyakov, Y., 2013. Phosphorus mineralization can be driven by microbial need for carbon. Soil Biology and Biochemistry 61, 69–75.

Tiessen, H., Moir, J.O., 2007. Characterization of Available P by Sequential Extraction, in: Carter, M.R., Gregorich, E.G. (Eds.), Soil sampling and methods of analysis, 2nd ed. Taylor Francis Group, Boca Raton, pp. 293–306.

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.

Vazquez, P., Holguin, G., Puente, M.E., Lopez-Cortes, A., Bashan, Y., 2000. Phosphate-solubilizing microorganisms associated with the rhizosphere of mangroves in a semiarid coastal lagoon. Biology and Fertility of Soils 30, 460–468.

Walker, J.J., Pace, N.R., 2007. Phylogenetic composition of Rocky Mountain endolithic microbial ecosystems. Applied and Environmental Microbiology 73, 3497–3504.

Walker, T.W., Adams, A.F.R., 1958. Studies on soil organic matter: I. Influence of phosphorus content of parent materials on accumulations of carbon, nitrogen, sulfur, and organic phosphorus in grassland soils. Soil Science 85, 307–318.

Walpola, B.C., Yoon, M.-H., 2012. Prospectus of phosphate solubilizing microorganisms and phosphorus availability in agricultural soils: A review. African Journal of Microbiology Research 6, 6600–6605.

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.

Yi, Y., Huang, W., Ge, Y., 2008. Exopolysaccharide: a novel important factor in the microbial dissolution of tricalcium phosphate. World Journal of Microbiology and Biotechnology 24, 1059–1065.

Supporting Information

Site descriptions

The Ukulinga site is located at the Ukulinga Research station near Pietermaritzburg, KwaZulu Natal, South Africa (-29.67 N, 30.4 E). It was established in 2009 and nutrients were added for the first time in 2010. Mean annual precipitation (MAP) amounts to 838 mm, occurring mostly during summer (September to April). The mean annual temperature (MAT) is 18 °C. The experiment is located at an elevation of 843 m a.s.l on top of a plateau formed by Ecca group shales (Fynn O'Connor 2005). The soil has a silty clay to clay texture (5 % sand, 41 % silt, 54 % clay) and pH_{H2O} values are approximately 5.8. The site is burned annually in early August, but not mown.

The Summerveld site is located in Summerveld, Kwa-Zulu Natal, South Africa (-29.81 N, 30.72 E). It was established in 2009 and nutrients were added for the first time in 2010. MAP amounts to 809 mm per year and MAT is 18 °C. The site is situated on a sandstone plateau on a south-facing slope at an elevation of 679 m a.s.l. (Wragg 2017). The soil has a loamy texture (50 % sand, 33 % silt, and 17 % clay) and pH_{H2O} values amount to approximately 5.1. The soil is shallow with an average depth of 16.75 cm. The site is annually burned in winter (June-August).

The Cedar Creek site is located in the Central plains at the Cedar Creek Ecosystem Science Reserve, Minnesota, USA (45.43 N, -93.21 E). It was established in 2007, and nutrients were added for the first time in 2008. The MAT is 6 °C and MAP amounts to 800 mm per year. The site is situated 270 m a.s.l. on the Anoka Sand Plain, an outwash plain of the Wisconsin Glacial Episode, and is underlain by bedrock of Cambrian and Ordovician dolomite, sandstone, and shale (Blinnikov, Bagent Reyerson 2013). The site is vegetated by tallgrass prairie. The soil has a sandy texture (88 % sand, 9 % silt, and 3 % clay) and pH_{H2O} values amount to 5.1. The site is neither burned nor mown.

The Chichaqua Bottoms site is situated in the Central Plains near Ames, Iowa, USA (41.78 N, -93.38 W). It was established in 2009 and nutrients were added for the first time in 2010. MAP amounts to 891 mm and MAT is 9 °C. The site is located at 275 m a.s.l. on Pleistocene till of the Dows Formation (Prior 1991). The texture of the soil is loamy sand (84 % sand, 11 % silt, 5 % clay) and the pH_{H2O} value amounts to 5.7. The vegetation is a restored tallgrass prairie, which was planted in 2001 (at the first block) and 2003 (at the second and third block). The site is burned every second to third year, but not mown.

The Rookery and Heron's Brook sites are both located at Silwood Park, Ascot, UK (51.41 N, -0.64 E). They were established in 2007 and nutrients were added for the first time in 2008. The MAT is 10 °C and MAP amounts to 678 mm at both sites with little seasonal pattern. The sites are situated 60 m a.s.l. on sands of the Bagshot Formation (British Geological Survey 1999). The texture is sandy loam (Rookery: 75 % sand, 19 % silt, and 6 % clay; Heron's Brook: 65 % sand, 25 % silt, and 10 % clay) with pH_{H2O} values amount to 4.0 in Rookery and 5.2 in Heron's Brook. The mesic grasslands are

neither burned nor mown.

References

Blinnikov, M.S., Bagent, C.M. Reyerson, P.E. (2013) Phytolith assemblages and opal concentrations from modern soils differentiate temperate grasslands of controlled composition on experimental plots at Cedar Creek, Minnesota. Quaternary International, 287, 101–113. British Geological Survey (1999) Windsor. England and Wales Sheet 269. Solid and Drift Geology. 1:50 000. British Geological Survey, Keyworth, Nottingham.

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.

Nakagawa, S. Schielzeth, H. (2013) A general and simple method for obtaining R2 from generalized linear mixed-effects models. Methods in Ecology and Evolution, 4, 133–142.

Prior, J.C. (1991) Landforms of Iowa. University of Iowa Press. Wragg, P.D. (2017) Multiple nutrients control threatened grassland vegetation in eastern South Africa. South African Journal of Botany, 112, 225–236.

Tables and Figures

Table S1: 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 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
TOC (g C kg^{-1})	cdcr.us[1]	9.4 ± 1.1a	15.7 ± 8.8a	9.0 ± 0.3a	11.0 ± 3.4a	5.2 ± 1.1a	10.4 ± 7.8a	4.4 ± 0.5a*	5.8 ± 2.1a
	cbgb.us	7.2 ± 2.8a	8.2 ± 0.5a	6.9 ± 2.3a	7.4 ± 2.4a	4.1 ± 0.5a*	5.1 ± 0.2a*	4.0 ± 1.2a	4.1 ± 0.9a*
	hero.uk	36.7 ± 6.8a	36.7 ± 6.1a	36.5 ± 1.8a	37.0 ± 7.7a	24.4 ± 3.5a*	24.5 ± 4.2a*	25.6 ± 4.7a*	23.9 ± 4.4a*
	rook.uk	24.3 ± 2.6a	28.7 ± 3.2a	26.9 ± 1.2a	24.9 ± 2.2a	10.5 ± 1.5a*	12.8 ± 1.3a*	11.6 ± 1.3a*	10.1 ± 0.7a*
	ukul.za	42.0 ± 2.0a	42.5 ± 0.8a	44.4 ± 0.5a	45.7 ± 0.8a	37.5 ± 3.6a	3.2.0 ± 4.4a*	34.8 ± 5.3a*	36.4 ± 0.6a*
	summ.za[2]	49.1 ± 3.0a	51.1 ± 2.2a	51.7 ± 3.0a	51.7 ± 1.9a	NA	NA	NA	NA
TN (g N kg^{-1})	cdcr.us[1]	0.7 ± 0.1a	1.1 ± 0.6a	0.6 ± 0.1a	0.8 ± 0.3a	0.3 ± 0.1a*	0.7 ± 0.5a	0.3 ± 0.1a	0.4 ± 0.1a*
	cbgb.us	0.6 ± 0.2a	0.8 ± 0.1a	0.6 ± 0.2a	0.7 ± 0.2a	0.4 ± 0.1a*	0.5 ± 0.1a*	0.4 ± 0.1a*	0.4 ± 0.2a*
	hero.uk	3.1 ± 0.7a	3.1 ± 0.5a	3.0 ± 0.2a	3.1 ± 0.8a	2.1 ± 0.3a*	2.1 ± 0.3a	2.1 ± 0.4a	2.0 ± 0.4a*
	rook.uk	2.1 ± 0.2ab	2.4 ± 0.3b	2.2 ± 0.1ab	2.0 ± 0.1a	1.0 ± 0.1a*	1.2 ± 0.1a**	1.0 ± 0.2a*	0.9 ± 0.1a*
	ukul.za	2.9 ± 0.3a	3.1 ± 0.3ab	3.3 ± 0.1ab	3.4 ± 0.1b	2.6 ± 0.2a	2.4 ± 0.2a*	2.6 ± 0.3a*	2.7 ± 0.1a*
	summ.za[2]	2.8 ± 0.2a	3.0 ± 0.4a	2.9 ± 0.3a	3.0 ± 0.1a	NA	NA	NA	NA
TP (g P kg^{-1})	cdcr.us[1]	0.31 ± 0.03a	0.46 ± 0.24a	0.58 ± 0.09a	0.56 ± 0.11a	0.24 ± 0.03a	0.40 ± 0.19a	0.36 ± 0.04a	0.38 ± 0.06a
	cbgb.us	0.28 ± 0.02a	0.24 ± 0.05a	0.43 ± 0.04b	0.41 ± 0.06b	0.24 ± 0.03a	0.24 ± 0.02a	0.34 ± 0.03b*	0.35 ± 0.07b*
	hero.uk	0.62 ± 0.16a	0.57 ± 0.07a	0.93 ± 0.13a	0.96 ± 0.28a	0.54 ± 0.15a	0.48 ± 0.06a	0.55 ± 0.17a*	0.62 ± 0.25a*
	rook.uk	0.38 ± 0.01a	0.38 ± 0.04a	0.60 ± 0.06b	0.61 ± 0.14b	0.27 ± 0.01a*	0.31 ± 0.04a	0.32 ± 0.03a*	0.31 ± 0.04a*
	ukul.za[1]	0.45 ± 0.02a	0.46 ± 0.08a	1.33 ± 0.12b	1.20 ± 0.15b	0.39 ± 0.02a	0.38 ± 0.04a	0.74 ± 0.25b*	0.58 ± 0.01b*
	summ.za[2]	0.37 ± 0.01a	0.49 ± 0.18a	0.60 ± 0.33a	0.83 ± 0.7a	NA	NA	NA	NA
pH in H_2O	cdcr.us	5.27 ± 0.09b	4.70 ± 0.17a	5.27 ± 0.10b	4.84 ± 0.10a	5.36 ± 0.10b	5.17 ± 0.19ab*	5.45 ± 0.23b	4.96 ± 0.23a
	cbgb.us	5.73 ± 0.50a	5.68 ± 0.72a	5.86 ± 0.47a	5.72 ± 0.43a	5.40 ± 0.66a	5.56 ± 0.92a	5.58 ± 0.79a	5.34 ± 0.63a
	hero.uk	5.12 ± 0.21a	5.18 ± 0.11a	5.08 ± 0.08a	5.09 ± 0.15a	5.24 ± 0.30a	5.30 ± 0.09a	5.20 ± 0.15a	5.22 ± 0.10a
	rook.uk	3.76 ± 0.04a	3.78 ± 0.02a	3.91 ± 0.02a	3.87 ± 0.03a	4.10 ± 0.13a*	4.08 ± 0.09a*	4.12 ± 0.10a*	4.06 ± 0.06a*
	ukul.za	5.89 ± 0.08a	5.58 ± 0.42a	5.94 ± 0.09a	5.63 ± 0.11a	5.83 ± 0.10a	5.79 ± 0.33a	5.72 ± 0.16a	5.62 ± 0.16a
	summ.za[2]	5.20 ± 0.04a	5.03 ± 0.09a	5.01 ± 0.12a	4.97 ± 0.13a	NA	NA	NA	NA

[1] Data were LOG10 transformed
[2] One-Way ANOVA and Tukey test were performed

Table S2: Regression coefficients and R^2 of linear mixed-effects model of the relative abundance of phosphorus-solubilizing bacteria (PSB) of all treatments in 0-15 cm depth. R^2 is the conditional R^2 according to Nakagawa & Schielzeth (2013). Estimation and p-value of each regression coefficient are displayed.

Linear mixed-effects model of relative abundance of PSB		
Coefficients	Estimation	p-value
(Intercept)	-69.244	0.000 ***
TOC:TP ratio	0.098	0.000 ***
Sand	0.311	0.000 ***
pH	10.611	0.000 ***
H_2O-P	-1.901	0.003 **
$R^2 = 0.71$		

Table S3: Mean number of colony-forming units (CFU) per g soil and relative abundance of phosphorus-solubilizing bacteria (% PSB) in 0-15 and 15-30 cm depth at each site.

Site	ukul.za		summ.za	cdcr.us		cbgb.us		rook.uk		hero.uk	
Depth	0-15 cm	15-30 cm	0-15 cm	0-15 cm	15-30 cm	0-15 cm	15-30 cm	0-15 cm	15-30 cm	0-15 cm	15-30 cm
CFU g^{-1}	1.34E+06	2.46E+06	4.86E+06	1.22E+06	1.40E+06	5.63E+04	2.48E+05	1.71E+05	1.46E+05	3.49E+06	2.40E+06
% PSB	6.16 ± 3.91	15.7 ± 9.63	30.99 ± 13.4	12.79 ± 9.93	20.01 ± 9.68	5.97 ± 3.21	11.71 ± 9.98	4.16 ± 2.98	4.61 ± 2.84	14.71 ± 8.32	15.71 ± 13.45

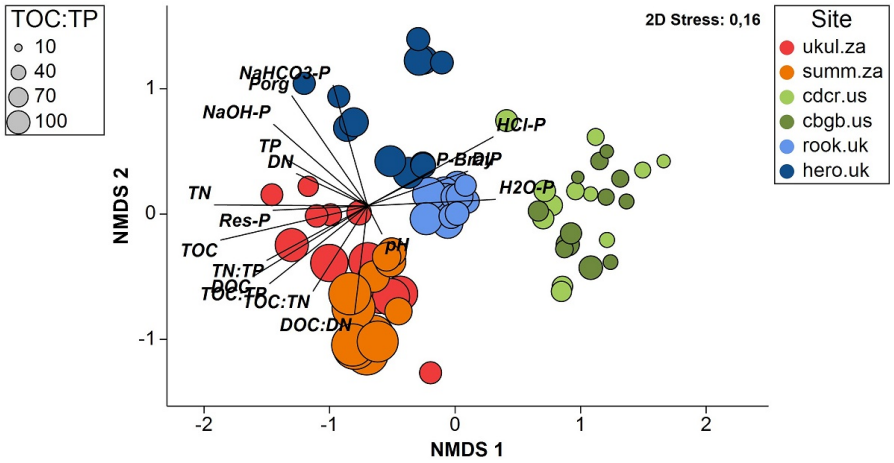


Figure S1: Soil pH, element concentrations, and P fractions of all topsoil nutrient addition treatments of the sites Ukulinga (ukul.za), Summerveld (summ.za), Cedar Creek (cdcr.us), Chichaqua Bottoms (cbgb.us), Rookery (rook.uk), and Herons Brook (hero.uk) displayed via non-metric multidimensional scaling (nMDS) of Euclidean distances. The included variables are total organic carbon (TOC), total nitrogen (TN), total phosphorus (TP), TOC:TN ratio, TOC:TP ratio, TN:TP ratio, dissolved organic carbon (DOC), dissolved nitrogen (DN), dissolved inorganic phosphorus (DIP), DOC:DN ratio, total organic phosphorus (TOP), Bray-1 P, water-extractable P (H_2O-P), labile P ($NaHCO_3$-P), occluded P (NaOH-P), apatite P (HCl-P), residual P (Res-P), and soil pH. The nMDS plot was overlaid by circles whose sizes reflect values of the TOC:TP ratios of all soils and with vectors of the Pearson correlations of the variables.

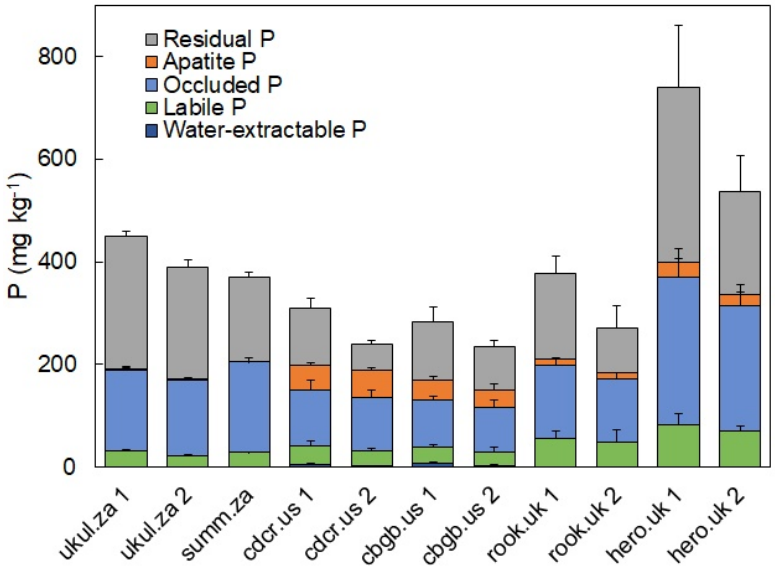


Figure S2: Soil phosphorus (P) fractions determined by Hedley fractionation in 0-15 cm (1) and 15-30 cm (2) soil depth in the control of the six sampled soils at the site Ukulinga (ukul.za), Summerveld (summ.za), Cedar Creek (cdcr.us), Chichaqua Bottoms (cbgb.us), Rookery (rook.uk), and Heron's Brook (hero.uk). The soil at the site Summerveld (summ.za) was only sampled in 0-15 cm depth because of limited soil depth. Error bars indicate standard deviations (n=3).

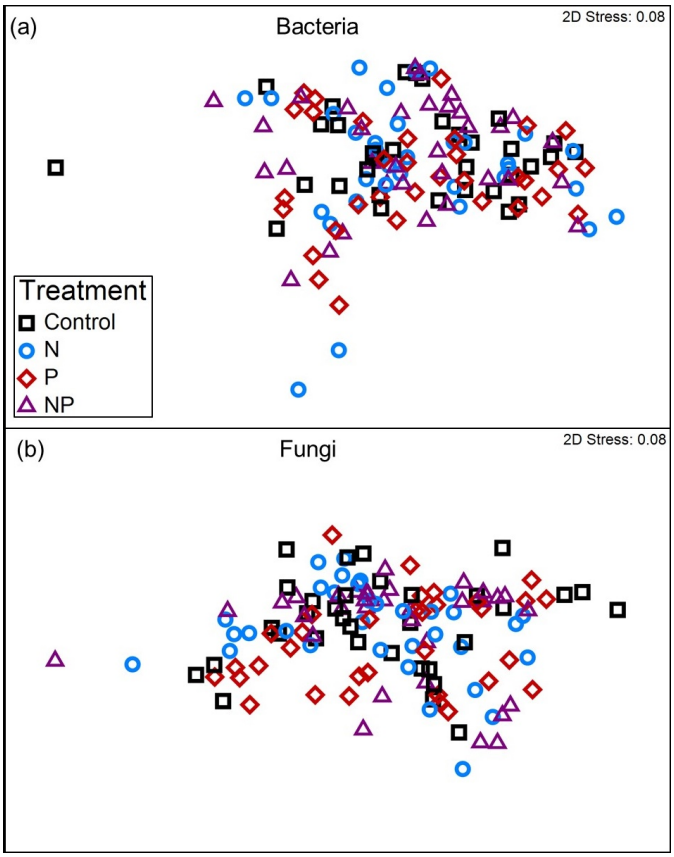


Figure S3: Effect of nutrient addition (+N, +P, +NP) on bacterial community composition (a) and fungal community composition (b) shown via non-metric multi-dimensional scaling (nMDS) of all six sites and both depth increments (0-15 and 15-30 cm).

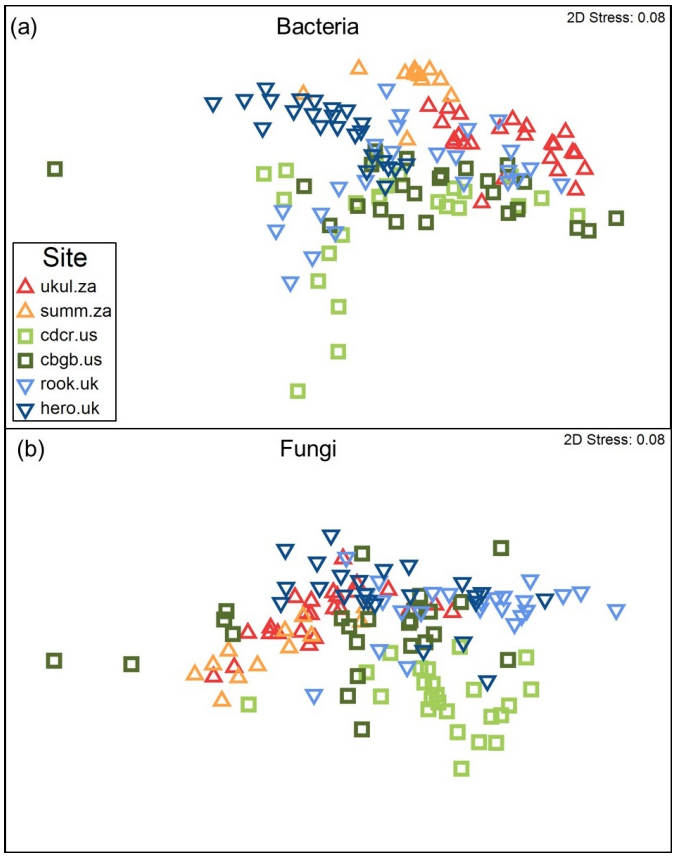


Figure S4: Bacterial community composition (a) and fungal community composition (b) shown via non-metric multi-dimensional scaling (nMDS) of the six sites Ukulinga (ukul.za), Summerveld (summ.za), Cedar Creek (cdcr.us), Chichaqua Bottoms (cbgb.us), Rookery (rook.uk), and Heron's Brook (hero.uk) in both depth increments (0-15 and 15-30 cm).

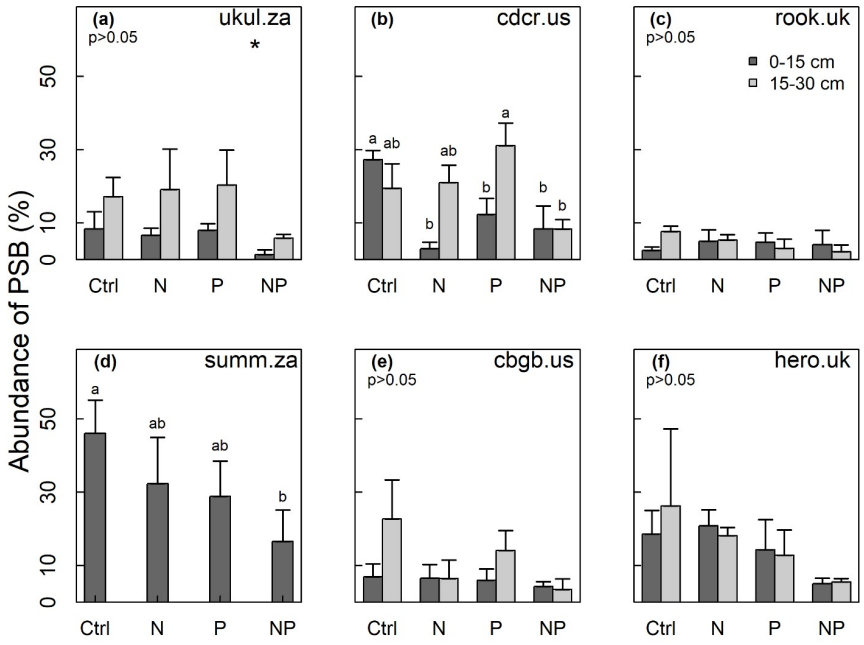


Figure S5: Relative abundance of phosphorus solubilizing bacteria (PSB) in two soil depth increments at the site (a) Ukulinga in South Africa, (b) Cedar Creek in the USA, (c) Rookery in the UK, (d) Summerveld in South Africa, (e) Chichaqua Bottoms in the USA and (f) Heron's Brook in the UK in 0-15 and 15-30 cm depth. The soil at the site Summerveld was only sampled in 0-15 cm depth because of limited soil depth. Error bars indicate standard deviations (n=3). Different lower-case letters above the bars indicate significant differences ($p < 0.05$) between treatments tested separately for each site and depth increment. Significant differences between depth increments separately tested for each site across all treatments are indicated by an asterisk at the right side of the subplot.

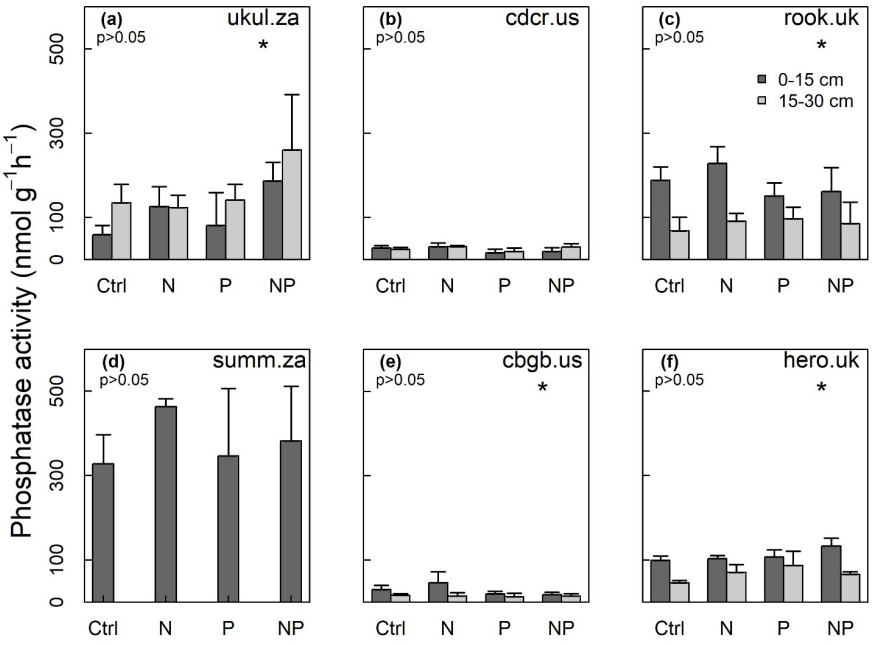


Figure S6: Phosphatase activity in two soil depth increments at the site (a) Ukulinga in South Africa, (b) Cedar Creek in the USA, (c) Rookery in the UK, (d) Summerveld in South Africa, (e) Chichaqua Bottoms in the USA and (f) Heron's Brook in the UK. The soil at the site Summerveld was only sampled in 0-15 cm depth because of limited soil depth. Error bars indicate standard deviations (n=3). Different lower-case letters above the bars indicate significant differences ($p < 0.05$) between treatments tested separately for each site and depth increment. Significant differences between depth increments separately tested for each site across all treatments are indicated by an asterisk at the right side of the subplot.

2.5 Study V

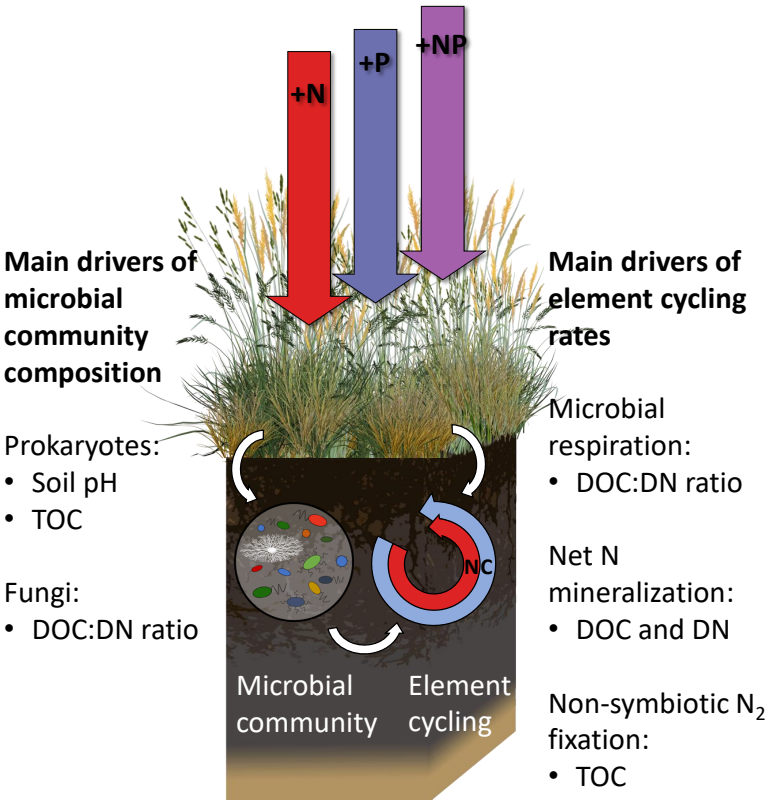
Effects of nitrogen and phosphorus addition on microbial community composition and element cycling in a grassland soil

Meike Widdig, Anna Heintz-Buschart, Per-M. Schleuss, Alexander Guhr, Elizabeth T. Borer, Eric W. Seabloom, Marie Spohn (2020),
published in *Soil Biology and Biochemistry* 151, 108041,
DOI: https://doi.org/10.1016/j.soilbio.2020.108041

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.

Graphical Abstract



2.5.1 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.

2.5.2 Material and Methods

2.5.2.1 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}.

2.5.2.2 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.

2.5.2.3 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).

2.5.2.4 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).

2.5.2.5 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).

2.5.2.6 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-NH_4^+ and N-NO_3^- (dissolved inorganic N, DIN) over time.

2.5.2.7 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-N_2 as well as the samples that were not exposed to ^{15}N-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):

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

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).

2.5.2.8 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-GTGARTCATCGA-ATCTTTG 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.

2.5.2.9 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.

2.5.2.10 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.

2.5.2.11 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 $\sim$ N * P. To test if plant beta-diversities correlated with beta-diversities of prokaryotes and fungi, Mantel tests were performed using the mantel.rtest function from the R-package ade4 (Dray and Dufour, 2007).

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

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

Plant diversity was calculated using Shannon's Diversity ($H' = -\sum (p\mathrm{i} * \ln (p\mathrm{i}))$, where pi 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).

2.5.3 Results

2.5.3.1 Soil physical and chemical parameters

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

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

2.5.3.2 Microbial biomass carbon and element cycling processes

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

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

[1] Data were log10 transformed for statistical tests
[2] Data were 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 ± 0.05a	0.69 ± 0.09
	1	0	155.81 ± 35.26	3.41 ± 0.27	22.73 ± 3.83	0.20 ± 0.03bc	1.13 ± 0.20
	5	0	163.70 ± 47.23	3.80 ± 1.29	23.11 ± 4.43	0.32 ± 0.07d	1.28 ± 0.13
	10	0	235.22 ± 36.29	5.75 ± 1.87	25.97 ± 12.03	0.25 ± 0.02bcd	1.02 ± 0.41
	0	10	192.71 ± 7.12	6.69 ± 3.97	34.73 ± 20.79	0.16 ± 0.03ab	1.08 ± 0.17
	10	10	168.81 ± 80.65	4.01 ± 1.80	29.32 ± 13.13	0.28 ± 0.05cd	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 ± 0.21a
	1	0	113.07 ± 66.81	2.03 ± 0.97	39.34 ± 26.35	0.09 ± 0.03*	1.78 ± 0.27b*
	5	0	95.29 ± 37.12	1.62 ± 0.48	17.85 ± 2.47	0.12 ± 0.01*	1.50 ± 0.29ab
	10	0	100.30 ± 38.56*	1.48 ± 0.33*	16.74 ± 5.44	0.11 ± 0.01*	1.74 ± 0.19ab*
	0	10	45.87 ± 21.92*	1.97 ± 0.77*	39.49 ± 17.56	0.07 ± 0.02*	1.87 ± 0.28b*
	10	10	133.81 ± 87.67	2.19 ± 0.47	27.75 ± 14.93	0.13 ± 0.01*	1.20 ± 0.03ab

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), 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			

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

2.5.3.3 Plant diversity, biomass, and community composition

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

2.5.3.4 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,

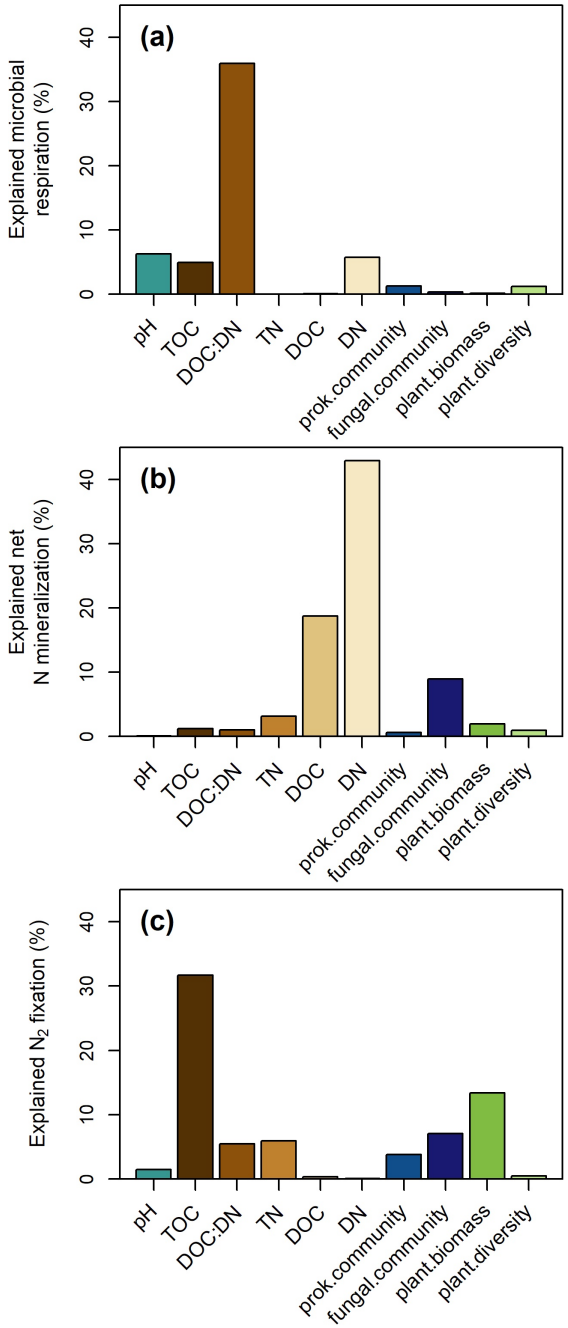


Figure 1: Proportion of microbial respiration (a), net nitrogen (N) mineralization (b), and non-symbiotic N2 fixation rates (c) in 0-15 cm soil depth explained by the displayed soil, microbial, and plant factors. Soil factors include soil pH, total organic carbon (TOC), dissolved organic carbon-to-dissolved nitrogen ratio (DOC:DN), total nitrogen (TN), dissolved organic carbon (DOC), and dissolved nitrogen (DN). Microbial factors include prokaryotic and fungal community composition at OTU 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.

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).

2.5.3.5 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).

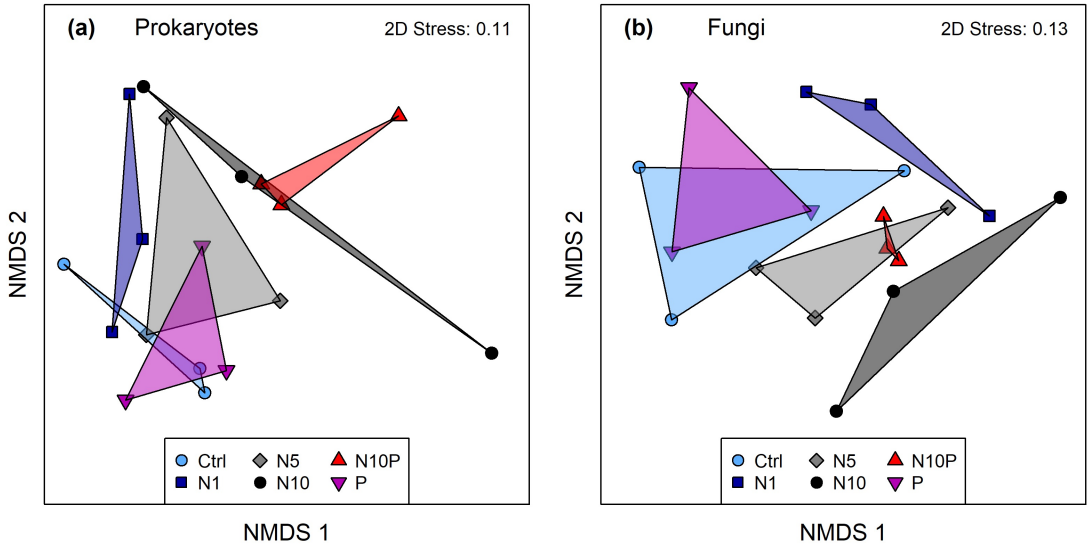


Figure 2: Prokaryotic (a) and fungal (b) community composition at ASV level displayed via non-metric multidimensional scaling (NMDS) of Jensen-Shannon divergences for different treatments in 0-15 cm soil depth. To test for significant differences in community profiles of all treatments, analyses of similarity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

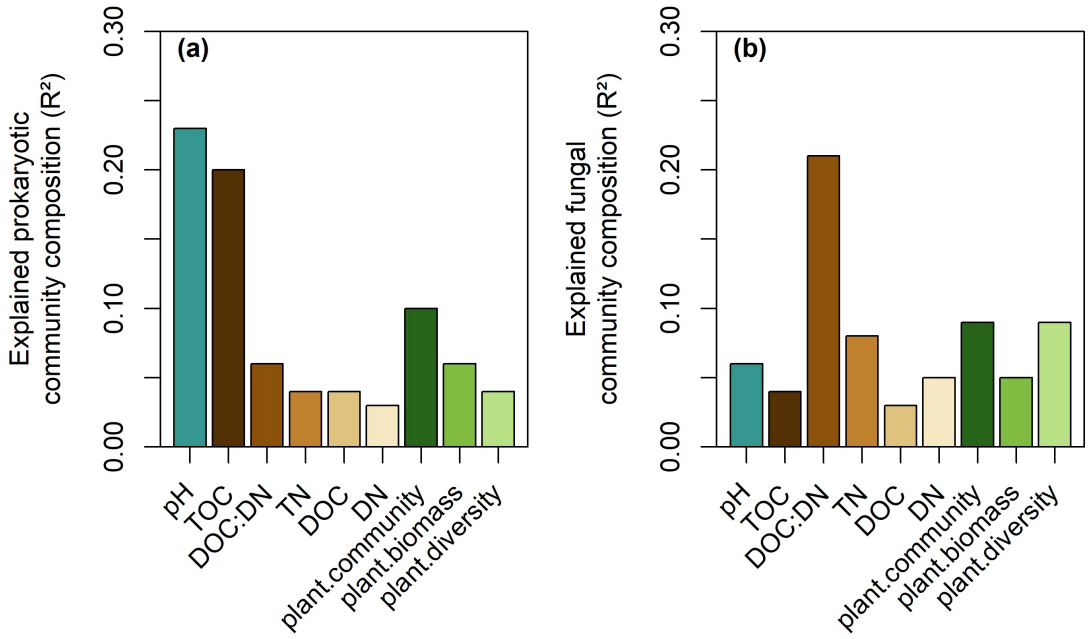


Figure 3: Proportion of variation (R^2) of prokaryotic (a) and fungal (b) community composition explained by the displayed soil and plant factors in 0-15 cm soil depth. Results are based on permutational multivariate analyses of variance (PERMANOVA) using Jensen-Shannon divergence of microbial communities at OTU 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.

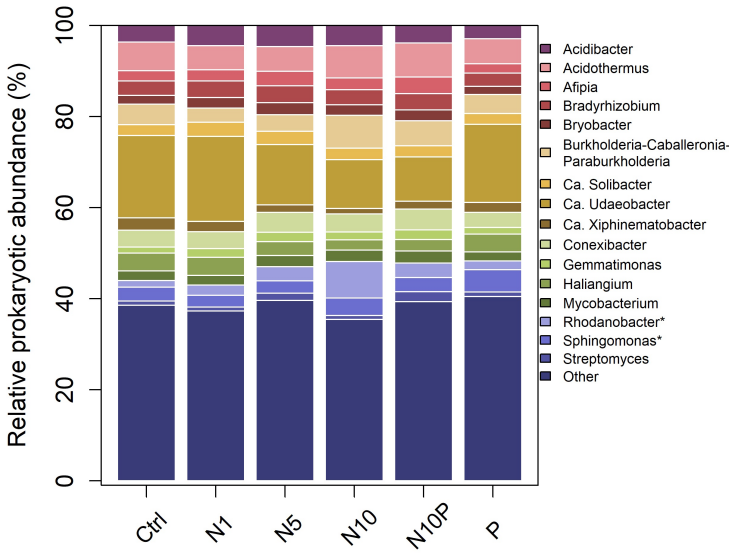


Figure 4: Relative abundances of prokaryotic genera in 0-15 cm soil depth. Displayed are prokaryotic genera that made up $> 2\%$ of relative abundance. Prokaryotic genera $< 2\%$ relative abundance were grouped as "Other". Differentially abundant genera were detected from a data matrix containing the 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.

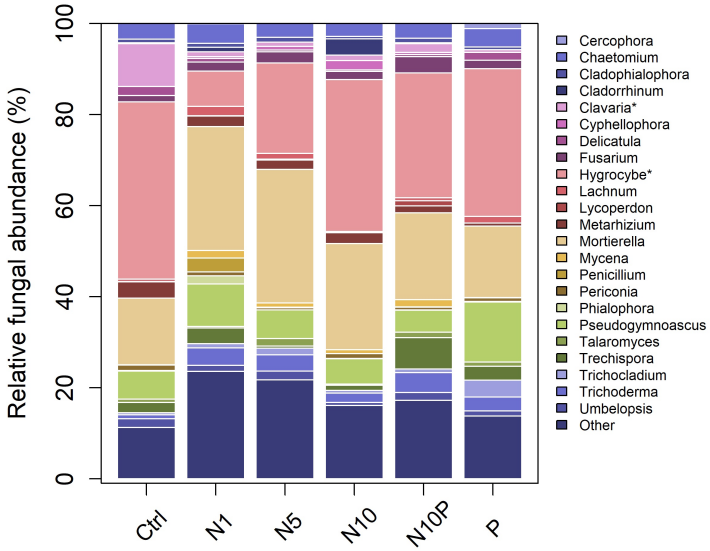


Figure 5: Relative abundances of fungal genera in 0-15 cm soil depth. Displayed are genera with $> 1\%$ relative abundance. Unclassified fungi and fungal genera $< 1\%$ abundance were grouped as "Other". Differentially abundant genera were detected from a data matrix containing the samples from the Ctrl, 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.

2.5.4 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.

2.5.4.1 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.

2.5.4.2 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.

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

Nemergut et al., 2008; Klaubauf et al., 2010; Morrison et al., 2016).

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

2.5.4.4 Conclusion

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

Cui, J., Wang, J., Xu, J., Xu, C., Xu, X., 2017. Changes in soil bacterial communities in an evergreen broad-leaved forest in east China following 4 years of nitrogen addition. Journal of Soils and Sediments 17, 2156–2164.

de Boer, W., Folman, L.B., Summerbell, R.C., Boddy, L., 2005. Living in a fungal world: impact of fungi on soil bacterial niche development. FEMS Microbiology Reviews 29, 795–811.

De Deyn, G.B., Cornelissen, J.H.C., Bardgett, R.D., 2008. Plant functional traits and soil carbon sequestration in contrasting biomes. Ecology Letters 11, 516–531.

Delgado-Baquerizo, M., Giaramida, L., Reich, P.B., Khachane, A.N., Hamonts, K., Edwards, C., Lawton, L.A., Singh, B.K., Brophy, C., 2016. Lack of functional redundancy in the relationship between microbial diversity and ecosystem functioning. Journal of Ecology 104, 936–946.

Dixon, R., Kahn, D., 2004. Genetic regulation of biological nitrogen fixation. Nature Reviews. Microbiology 2, 621–631.Dray, S., Dufour, A.-B., 2007. The ade4 Package: Implementing the Duality Diagram for Ecologists. Journal of Statistical Software 22, 1–20.

Falkowski, P.G., Fenchel, T., Delong, E.F., 2008. The Microbial Engines That Drive Earth's Biogeochemical Cycles. Science 320, 1034–1039.

Fierer, N., Jackson, R.B., 2006. The diversity and biogeography of soil bacterial communities. Proceedings of the National Academy of Sciences of the United States of America 103, 626–631.

Fierer, N., Lauber, C.L., Ramirez, K.S., Zaneveld, J., Bradford, M.A., Knight, R., 2012. Comparative metagenomic, phylogenetic and physiological analyses of soil microbial communities across nitrogen gradients. The ISME Journal 6, 1007–1017.

Fierer, N., Strickland, M.S., Liptzin, D., Bradford, M.A., Cleveland, C.C., 2009. Global patterns in belowground communities. Ecology Letters 12, 1238–1249.

Freedman, Z.B., Romanowicz, K.J., Upchurch, R.A., Zak, D.R., 2015. Differential responses of total and active soil microbial communities to long-term experimental N deposition. Soil Biology and Biochemistry 90, 275–282.

Galloway, J.N., Dentener, F.J., Capone, D.G., Boyer, E.W., Howarth, R.W., Seitzinger, S.P., Asner, G.P., Cleveland, C.C., Green, P.A., Holland, E.A., 2004. Nitrogen cycles: past, present, and future. Biogeochemistry 70, 153–226.

Galloway, J.N., Townsend, A.R., 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 phosphorus addition in a mesic grassland in South Africa. Soil Biology and Biochemistry 135, 294–303.

Schloss, P.D., Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M., Hollister, E.B., Lesniewski, R.A., Oakley, B.B., Parks, D.H., Robinson, C.J., Sahl, J.W., Stres, B., Thallinger, G.G., van Horn, D.J., Weber, C.F., 2009. Introducing mothur: Open-source, platform-independent, community-supported software for describing and comparing microbial communities. Applied and Environmental Microbiology 75, 7537–7541.

Semenov, M.V., Manucharova, N.A., Stepanov, A.L., 2016. Distribution of Metabolically Active Prokaryotes (Archaea and Bacteria) throughout the Profiles of Chernozem and Brown Semidesert Soil. Eurasian Soil Science 49, 217–225.

Six, J., Frey, S.D., Thiet, R.K., Batten, K.M., 2006. Bacterial and Fungal Contributions to Carbon Sequestration in Agroecosystems. Soil Science Society of America Journal 70, 555–569.

Smith, V.H., 1992. Effects of nitrogen: phosphorus supply ratios on nitrogen fixation in agricultural and pastoral ecosystems. Biogechemistry, 19–35.

Spohn, M., 2015. Microbial respiration per unit microbial biomass depends on litter layer carbon-to-nitrogen ratio. Biogeosciences 12, 817–823.

Spohn, M., Chodak, M., 2015. Microbial respiration per unit biomass increases with carbon-to-nutrient ratios in forest soils. Soil Biology and Biochemistry 81, 128–133.

Sterner, R.W., Elser, J.J., 2002. Ecological stoichiometry: The biology of elements from molecules to the biosphere. Princeton University Press.

Stevens, C.J., Lind, E.M., Hautier, Y., Harpole, W.S., Borer, E.T., Hobbie, S., Seabloom, E.W., Ladwig, L., Bakker, J.D., Chu, C., Collins, S., Davies, K.F., Firn, J., Hillebrand, H., La Pierre, K.J., MacDougall, A., Melbourne, B., McCulley, R.L., Morgan, J., Orrock, J.L., Prober, S.M., Risch, A.C., Schuetz, M., Wragg, P.D., 2015. Anthropogenic nitrogen deposition predicts local grassland primary production worldwide. Ecology 96, 1459–1465.

Strickland, M.S., Lauber, C.L., Fierer, N., Bradford, M.A., 2009. Testing the functional significance of microbial community composition. Ecology 90, 441–451.

Strickland, M.S., Rousk, J., 2010. Considering fungal:bacterial dominance in soils - Methods, controls, and ecosystem implications. Soil Biology and Biochemistry 42, 1385–1395.

Sul, W.J., Asuming-Brempong, S., Wang, Q., Tourlousse, D.M., Penton, C.R., Deng, Y., Rodrigues, J.L.M., Adiku, S.G.K., Jones, J.W., Zhou, J., Cole, J.R., Tiedje, J.M., 2013. Tropical agricultural land management influences on soil microbial communities through its effect on soil organic carbon. Soil Biology and Biochemistry 65, 33–38.

Tedersoo, L., Bahram, M., Põlme, S., Kõljalg, U., Yorou, N.S., Wijesundera, R., Villarreal Ruiz, L., Vasco-Palacios, A.M., Thu, P.Q., Suija, A., Smith, M.E., Sharp, C., Saluveer, E., Saitta, A., Rosas, M., Riit, T., Ratkowsky, D., Pritsch, K., Põldmaa, K., Piepenbring, M., Phosri, C., Peterson, M., Parts, K., Pärtel, K., Otsing,

E., Nouhra, E., Njouonkou, A.L., Nilsson, R.H., Morgado, L.N., Mayor, J., May, T.W., Majuakim, L., Lodge, D.J., Lee, S.S., Larsson, K.-H., Kohout, P., Hosaka, K., Hiiesalu, I., Henkel, T.W., Harend, H., Guo, L.-d., Greslebin, A., Grelet, G., Geml, J., Gates, G., Dunstan, W., Dunk, C., Drenkhan, R., Dearnaley, J., Kesel, A. de, Dang, T., Chen, X., Buegger, F., Brearley, F.Q., Bonito, G., Anslan, S., Abell, S., Abarenkov, K., 2014. Global diversity and geography of soil fungi. Science (New York, N.Y.) 346, 1256688.

van den Heuvel, R.N., van der Biezen, E., Jetten, M.S.M., Hefting, M.M., Kartal, B., 2010. Denitrification at pH 4 by a soil-derived Rhodanobacter-dominated community. Environmental Microbiology 12, 3264–3271.

Vance, E.D., Brookes, P.C., Jenkinson, D.S., 1987. An extraction method for measuring soil microbial biomass C. Soil Biology and Biochemistry 19, 703–707.

Vestgarden, L.S., Selle, L.T., Stuanes, A.O., 2003. In situ soil nitrogen mineralisation in a Scots pine (Pinus sylvestris L.) stand: effects of increased nitrogen input. Forest Ecology and Management 176, 205–216.

Vourlitis, G.L., Zorba, G., Pasquini, S.C., Mustard, R., 2007. Chronic Nitrogen Deposition Enhances Nitrogen Mineralization Potential of Semiarid Shrubland Soils. Soil Science Society of America Journal 71, 836.

Wang, R., Balkanski, Y., Boucher, O., Ciais, P., Peñuelas, J., Tao, S., 2015. Significant contribution of combustion-related emissions to the atmospheric phosphorus budget. Nature Geoscience 8, 48–54.

Wardle, D.A., Gundale, M.J., Jäderlund, A., Nilsson, M.-C., 2013. Decoupled long-term effects of nutrient enrichment on aboveground and belowground properties in subalpine tundra. Ecology 94, 904–919.

Weand, M.P., Arthur, M.A., Lovett, G.M., McCulley, R.L., Weathers, K.C., 2010. Effects of tree species and N additions on forest floor microbial communities and extracellular enzyme activities. Soil Biology and Biochemistry 42, 2161–2173.

Wertz, S., Degrange, V., Prosser, J.I., Poly, F., Commeaux, C., Freitag, T., Guillaumaud, N., Le Roux, X., 2006. Maintenance of soil functioning following erosion of microbial diversity. Environmental Microbiology 8, 2162–2169.

White, T.J., Bruns, T., Lee, S., Taylor, J., 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics, in: Innis, M.A., Gelfand, D.H., Sninsky, J.J., White, T.J. (Eds.), PCR Protocols: A Guide to Methods and Applications. Academic Press, San Diego, pp. 315–322.

Yamamoto, E., Muramatsu, H., Nagai, K., 2014. Vulgatibacter incomptus gen. nov., sp. nov. and Labilithrix luteola gen. nov., sp. nov., two myxobacteria isolated from soil in Yakushima Island, and the description of Vulgatibacteraceae fam. nov., 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.

Supporting Information

Table S1: Statistical differences in prokaryotic (Prok.) and fungal community composition between nutrient addition treatments. Shown are p-values (bottom triangle). Analyses of similarity were performed on Jensen-Shannon divergences of community profiles at OTU level stratified for sampling depth.

	Prok.	Fungi	Prok.	Fungi	Prok.	Fungi	Prok.	Fungi	Prok.	Fungi
Treatment	Ctrl		N1		N5		N10		N10P	
Ctrl	x	x								
N1	0.116	0.046*	x	x						
N5	0.106	0.326	0.206	0.188	x	x				
N10	0.057	0.014*	0.142	0.141	0.236	0.192	x	x		
N10P	0.003**	0.005**	0.006**	0.048*	0.055	0.040*	0.010*	0.029*	x	x
P	0.189	0.703	0.100	0.043*	0.084	0.153	0.016*	0.010*	0.004**	0.005**

Table S2: Changes in microbial beta-diversity depending on N and P addition. Results of permutational multivariate analysis of variance using weighted UniFrac and Jensen-Shannon divergence (JSD) matrices with sample depth as strata are displayed.

Community	Taxonomic rank	Distance measure	R^2 N addition	p N addition	R^2 P addition	p P addition	R^2 interaction term	p interaction term	total variance explained
prokaryotes	OTU	weighted UniFrac	0.12	0.0001	0.06	0.003	0.02	0.5	19 %
prokaryotes	OTU	JSD	0.10	0.0001	0.05	0.006	0.03	0.2	18 %
prokaryotes	genus[1]	JSD	0.26	0.0001	0.06	0.02	0.00	0.8	32 %
prokaryotes	family[1]	JSD	0.26	0.0001	0.06	0.02	0.00	0.9	32 %
prokaryotes	order[1]	JSD	0.25	0.0001	0.07	0.01	0.00	0.9	31 %
prokaryotes	class[1]	JSD	0.28	0.0001	0.07	0.01	0.00	1	35 %
prokaryotes	phylum[1]	JSD	0.20	0.0001	0.08	0.004	0.00	1	28 %
bacteria	OTU	weighted UniFrac	0.12	0.0001	0.06	0.003	0.02	0.4	20 %
bacteria	OTU	JSD	0.10	0.0001	0.05	0.006	0.03	0.2	18 %
bacteria	genus[1]	JSD	0.25	0.0001	0.06	0.02	0.00	0.8	31 %
bacteria	family[1]	JSD	0.26	0.0001	0.06	0.02	0.00	0.9	32 %
bacteria	order[1]	JSD	0.25	0.0001	0.07	0.01	0.00	0.9	31 %
bacteria	class[1]	JSD	0.28	0.0001	0.07	0.02	0.00	1	35 %
bacteria	phylum[1]	JSD	0.20	0.0001	0.08	0.007	0.00	1	28 %
archaea	OTU	weighted UniFrac	0.32	0.0002	0.01	0.5	0.01	0.7	34 %
archaea[2]	OTU	JSD	0.17	0.0001	0.03	0.1	0.01	0.9	21 %
fungi	OTU	JSD	0.12	0.0002	0.03	0.2	0.03	0.2	19 %
fungi	genus[1]	JSD	0.40	0.0001	0.04	0.1	0.01	0.5	45 %
fungi	family[1]	JSD	0.42	0.0001	0.04	0.1	0.01	0.6	46 %
fungi	order[1]	JSD	0.44	0.0001	0.04	0.1	0.00	1	48 %
fungi	class[1]	JSD	0.35	0.0004	0.05	0.1	0.00	1	40 %
fungi	division[1]	JSD	0.46	0.0003	0.14	0.02	0.00	1	59 %

[1] Analysis is based on classifiable proportion of the community
[2] Too few OTUs of kingdom archaea were classified to genus, family, class and order level for multivariate analysis

Table S3: Permutational multivariate analysis of variance (PERMANOVA) using Jensen-Shannon divergence (JSD) of microbial communities at OTU level. Shown are the results of models that include all factors with significant explanatory value by single-factor PERMANOVA.

Community	PERMANOVA results[1] Factor	R^2	p-value
prokaryotes	pH	0.23	0.0001
	TOC	0.20	0.0001
	plant community (PC1)	0.10	0.0004
	plant biomass	0.06	0.02
	DOC:DN ratio	0.06	0.02
	plant diversity	0.04	0.2
	TN	0.04	0.2
	DOC	0.04	0.2
	DN	0.03	0.5
fungi	DOC:DN ratio	0.21	0.0001
	plant diversity	0.09	0.02
	plant community (PC1)	0.09	0.02
	TN	0.08	0.02
	pH	0.06	0.1
	plant biomass	0.05	0.1
	DN	0.05	0.2
	TOC	0.04	0.3
	DOC	0.03	0.6

[1] Soil and treatment factors: pH_{H2O}, TOC, TN, TOC:TN ratio, DOC, DN, DOC:DN ratio, plant diversity, plant biomass, plant community composition (axis 1, 2, and 3 of PCoA, Plants used for PCoA: *Achillea millefolium, Agrostis scabra, Andropogon gerardii, Carex sp., Conyza canadensis, Elymus repens, Lespedeza capitata, Panicum oligosanthes, Poa pratensis, Rumex acetosella, Schizachyrium scoparium*)

Table S4: Changes in relative microbial abundances depending on N and P addition (top soil and only the highest N addition compared to no N addition). The results of DESeq2 analysis are displayed.

Community	Taxonomic rank	Number of taxa	Identity of differential taxa				
			decreasing with N	increasing with N	decreasing with P	increasing with P	interaction P:N
prokaryotes	genus[1]	315	*Aeromicrobium, Anaeromyxobacter, Enterobacter, Ferruginibacter, Labilithrix, Microbacterium, Microvirga, Pelomonas, Polycyclovorans*	*Actinospica, Candidatus Nitrocosmicus, Catenulispora, Chujaibacter, Devosia, Granulicella, Nitrosospira, Occallatibacter, Pseudolabrys, Rhodanobacter, and Rhodopseudomonas*	*Enterobacter, Acidisphaera*		*Enterobacter, Sphingomonas*
fungi	genus[1]	325	*Archaeorhizomyces, Clavaria, Hygrocybe, Myxocephala*	*Scutellinia*			

[1] analysis is based on classifiable portion of the community

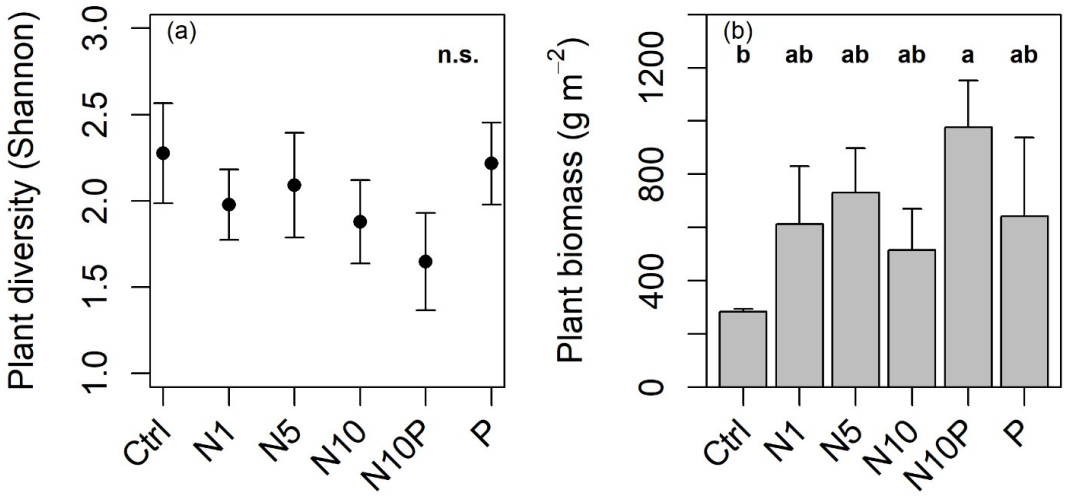


Figure S1: Plant diversity (Shannon Index) (a) and plant biomass (b) under nutrient addition. One-Way-ANOVA was conducted followed by Tukey Post Hoc Test for multiple comparisons to test significant differences between nutrient addition treatments. Shown are means, and error bars depict standard deviations (n=3).

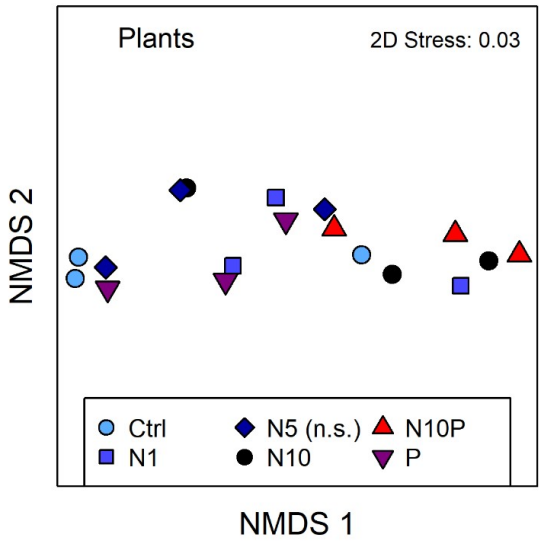


Figure S2: Plant community composition displayed via non-metric multidimensional scaling (NMDS) of Bray-Curtis similarities for different treatments. Differences between nutrient addition treatments were tested using one-way-ANOSIM with 999 permutations.

Contributions to the Included Manuscripts

Study I

Title: Microbial carbon use efficiency in grassland soils subjected to nitrogen and phosphorus additions.
Authors: Meike Widdig, Per-Marten Schleuss, Lori Biederman, Elizabeth Borer, Michael Crawley, Kevin Kirkman, Eric Seabloom, Peter Wragg, Marie Spohn
Status: published in 2020 in *Soil Biology and Biochemistry* 146, 107815.

M. Widdig	study design, soil sampling, laboratory analyses, data analyses, manuscript writing (60%)
P.-M. Schleuss	soil sampling, laboratory analyses, manuscript contribution (10%)
L. Biederman, E. Borer, M. Crawley, K. Kirkman, E. Seabloom, P. Wragg	maintenance of the study sites, manuscript contribution (together 15%)
M. Spohn	research concept, study design, soil sampling, manuscript contribution (15%)

Study II

Title: Turnover of carbon and phosphorus in the microbial biomass depending on phosphorus availability.
Authors: Marie Spohn and Meike Widdig
Status: published in 2017 in *Soil Biology and Biochemistry*, 113, 53-59

M. Spohn	research concept, study design, soil sampling, laboratory analyses, data analyses, manuscript writing (80%)
M. Widdig	soil sampling, laboratory analyses, manuscript contribution (20%)

Study III

Title: Microbial substrate stoichiometry governs nutrient effects on nitrogen cycling in grassland soils.
Authors: Per-Marten Schleuss[§], Meike Widdig[§] , Lori Biederman, Elizabeth Borer, Michael Crawley, Kevin Kirkman, Eric Seabloom, Peter Wragg, Marie Spohn
[§]Equal contribution, shared first-authorship
Status: published in 2021 in *Soil Biology and Biochemistry* 155, 108168

P.-M. Schleuss	study design, soil sampling, laboratory analyses, data analyses, manuscript writing (35%)
M. Widdig	study design, soil sampling, laboratory analyses, data analyses, manuscript writing (35%)
L. Biederman, E. Borer, M. Crawley, K. Kirkman, E. Seabloom, P. Wragg	maintenance of the study sites, manuscript contribution (together 15%)
M. Spohn	research concept, study design, manuscript contribution (15%)

Study IV

Title: Nitrogen and Phosphorus Additions Alter the Abundance of Phosphorus-Solubilizing Bacteria and Phosphatase Activity in Grassland Soils.
Authors: Meike Widdig, Per-Marten Schleuss, Alfons Weig, Alexander Guhr, Lori Biederman, Elizabeth Borer, Michael Crawley, Kevin Kirkman, Eric Seabloom, Peter Wragg, Marie Spohn
Status: published in 2019 in *Frontiers in Environmental Science* 7, 185

M. Widdig	study design, soil sampling, laboratory analyses, data analyses, manuscript writing (45%)
P.-M. Schleuss	study design, soil sampling, laboratory analyses, manuscript contribution (15%)
A. Weig	laboratory analyses, data analyses (5%)
A. Guhr	data analyses (5%)
L. Biederman, E. Borer, M. Crawley, K. Kirkman, E. Seabloom, P. Wragg	maintenance of the study sites, manuscript contribution (together 15%)
M. Spohn	research concept, study design, soil sampling, manuscript contribution (15%)

Study V

Title: Effects of nitrogen and phosphorus addition on microbial community composition and element cycling in a grassland soil.
Authors: Meike Widdig, Anna Heintz-Buschart, Per-Marten Schleuss, Alexander Guhr, Elizabeth Borer, Eric Seabloom, Marie Spohn
Status: published in 2020 in *Soil Biology and Biochemistry*, 151, 108041

M. Widdig	study design, soil sampling, laboratory analyses, data analyses, manuscript writing (45%)
A. Heintz-Buschart	laboratory analyses, data analyses, manuscript contribution (25%)
P.-M. Schleuss	soil sampling, laboratory analyses, manuscript contribution (10%)
A. Guhr	data analyses, manuscript contribution (5%)
E. Borer, E. Seabloom	maintenance of study site, manuscript contribution (together 5%)
M. Spohn	research concept, manuscript contribution (10%)

Acknowledgements

First of all, I want to thank everybody who supported me during the time of my PhD whether in a scientific or personal way.

I thank Prof. Dr. Marie Spohn for the chance to work in her Emmy-Noether grant on soil microbial element cycling and the possibility to take all the soil samples in South Africa, the USA, and England. I thank her for her support and continuous motivation to further improve. She showed me to critically question my own work over and over again. Further, I want to thank her for the permission to conduct a research stay at ETH Zurich, which was funded by the University of Bayreuth Graduate School. Thank you, Federica Tamburini, for introducing δ^{18}O-P analysis to me and a special thanks goes to Emanuel Frossard and his group at the ETH Zurich for including me so fast in all their group activities.

Further, I highly appreciate the support of all our cooperation partners and their contribution to the manuscripts: Eric Seabloom, Elizabeth Borer, Lori Biederman, Kevin Kirkman, Peter Wragg, and Michael Crawley who maintained all the study sites and Anna Heintz-Buschart and Alfons Weig who contributed a lot with sequencing and sequence data analysis.

Thank you to the members of the Soil Ecology department and Soil Biogeochemistry group at University of Bayreuth for all the nice lunch breaks, summer and winter trips. Thank goes to Prof. Matzner, Prof. Werner Borken, and Prof. Eva Lehndorff for scientific discussion and feedback. Further, I want to thank Renate Krauß and Karin Söllner for the valuable support in the lab and for their motivation. Thanks to Uwe Hell for his technical knowledge on experimental questions, texture analyses, help with tire changes and secret mushroom spots. Thank you, Ingeborg Vogler, for the endless supply with coffee and chats.

Further, I would like to thank all my colleagues: Ulrike Schwerdtner, Christine Heuck, Emanuel Brucker, Giovanni Pastore, Andrea Scheibe, Katerina Diakova, Nele Meyer, Sascha Scherer, Manuel Vergara-Sosa, and especially my office mates, Per-Marten Schleuss, Alexander Guhr, Mai Van Dinh, Cynthia Minnich, and Eduardo Vazquez. I think about endless coffee breaks, vegetable cultivation, cooking sessions, after work beers and barbecues. My time in Bayreuth would have been only half as good without you!

A special thanks goes to Per-Marten Schleuss who joined the field trips, gave advise in the lab, with data analysis, writing and lots more, to Alexander Guhr for his statistical

support, and to Isabell Zeißig and Renate Krauß for watering plants at the weekends and their support in every situation and especially their supply with chocolate.

I also thank Ralf Mertel and Anita Gößner from the ecological microbiology group for autoclaving tons of glassware and Birgit Luyven, Tanja Gonter, and Christine Stöcker from the BayCEER Central Analytics for measuring hundreds of samples, especially all the nasty K_2SO_4 samples.

I thank my roommate Colleen Rafferty for all the nice kitchen talks and gossip. Being in exactly the same situation was always helpful and supporting. Finally, I thank my parents and my sister for their support and love they give in every situation. Constantin, without your constant support, motivation in bad times, and patience during three years of a long-distance relationship this dissertation would not have come to an end. Thank you!

This work was supported by the German Research Foundation (grant SP1389/6-1) to whom I am very grateful.

Peer-reviewed Publications

Publications included in the thesis

Schleuss, P.-M., **Widdig, M.**, Biederman, L.A., Borer, E.T., Crawley, M.J., Kirkman, K.P., Seabloom, E.W., Wragg, P.D., Spohn, M., 2021. Microbial substrate stoichiometry governs nutrient effects on nitrogen cycling in grassland soils. *Soil Biology and Biochemistry* 115, 108168.

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.

Widdig, M., Heintz-Buschart, A., Schleuss, P.-M., Guhr, A., Borer, E.T., Seabloom, Spohn, M., 2020. Effects of nitrogen and phosphorus addition on microbial community composition and element cycling in a grassland soil. *Soil Biology and Biochemistry* 151, 108041.

Widdig, M., Schleuss, P.-M., Biederman, L.A., Borer, E.T., Crawley, M.J., Kirkman, K.P., Seabloom, E.W., Wragg, P.D., Spohn, M., 2020. Microbial carbon use efficiency in grassland soils subjected to nitrogen and phosphorus additions. *Soil Biology and Biochemistry* 146, 107815.

Widdig, M., Schleuss, P.-M., Weig, A.R., Guhr, A., Biederman, L.A., Borer, E.T., Crawley, M.J., Kirkman, K.P., Seabloom, E.W., Wragg, P.D., Spohn, M., 2019. Nitrogen and Phosphorus Additions Alter the Abundance of Phosphorus-Solubilizing Bacteria and Phosphatase Activity in Grassland Soils. *Frontiers in Environmental Science* 7, 185.

Other publications

Klotzbücher, A., Schunck, F., Klotzbücher, T., Kaiser, K., Glaser, B., Spohn, M., **Widdig, M.**, Mikutta, R., 2020. Goethite-associated phosphorus is not available to beech (Fagus sylvatica L.). *Frontiers in Forests and Global Change* 3, 94.

Schleuss, P.M., **Widdig, M.**, Heintz-Buschart, A., Kirkman, K., Spohn, M., 2020. Interactions of nitrogen and phosphorus cycling promote P acquisition and explain synergistic plant-growth responses. *Ecology*, e03003.

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.

Spohn, M., Zeißig, I., Brucker, E., **Widdig, M.**, Lacher, U., Aburto, F., 2020. Phosphorus solubilization in the rhizosphere in two saprolites with contrasting phosphorus fractions. *Geoderma* 366, 114245.

Further Scientific Contributions (talks and poster presentations)

Widdig, M., Schleuss, P.-M., Weig, A.R., Guhr A., Biederman L.A., Borer, E.T., Crawley, M.J., Kirkman, K.P., Seabloom, E.W., Wragg, P.D., Spohn, M. (2019): Soil carbon and phosphorus cycling as dependent on nitrogen and phosphorus addition in grassland soils. Conference of the German and Swiss Soil Science Society, Bern, Switzerland, August 25-28, 2019 (oral)

Widdig, M., Heintz-Buschart, A., Schleuss, P.-M., Biederman, L.A., Borer, E.T., Seabloom, E.W., Spohn, M. (2019): Effect of nitrogen and phosphorus addition on soil microbial activities, diversity and communities in two grassland soils. NutNet workshop, Minneapolis, USA, August 5-9, 2019 (oral)

Widdig, M., Schleuss, P.-M., Weig, A.R., Guhr A., Biederman L.A., Borer, E.T., Crawley, M.J., Kirkman, K.P., Seabloom, E.W., Wragg, P.D., Spohn, M. (2019): Phosphorus-solubilizing bacteria and phosphatase activity show deviating responses towards nitrogen and phosphorus addition in grassland soils. International Phosphorus Workshop 9, Zurich, Switzerland, July 8-12, 2019 (oral)

Widdig, M., Schleuss, P.-M., Biederman L.A., Borer, E.T., Crawley, M.J., Kirkman, K.P., Seabloom, E.W., Wragg, P.D., Spohn, M. (2019): Microbial carbon use efficiency in grassland soils on three continents as dependent on nitrogen and phosphorus addition. European Geoscience Union General Assembly 2019, Vienna, Austria, April 7-12, 2019 (oral)

Widdig, M., Schleuss, P.-M., Weig, A.R., Guhr A., Biederman L.A., Borer, E.T., Crawley, M.J., Kirkman, K.P., Seabloom, E.W., Wragg, P.D., Spohn, M. (2018): Nitrogen and phosphorus inputs affect phosphorus solubilizing bacteria and phosphatase activity in six grassland soils in South Africa, USA, and UK. BayCEER workshop 2018, University of Bayreuth, Germany, October 11, 2018 (oral) - Workshop Award for the best talk

Widdig, M., Schleuss, P.-M., Weig, A.R., Guhr, A., Biederman, L.A., Borer, E.T., Crawley, M.J., Kirkman, K.P., Seabloom, E.W., Wragg, P.D., Spohn, M. (2018): Ef-

fects of element inputs on phosphorus-solubilizing bacteria and on phosphatase activity in six grassland soils in South Africa, USA and England. 6th Symposium on Phosphorus in Soils and Plants - From Molecular Scale to Ecosystems, Leuven, Belgium, September 10-13, 2018 (poster)

Widdig, M., Schleuss, P.-M., Weig, A.R., Guhr, A., Kirkman, K.P., Wragg, P.D., Spohn, M. (2018): Effects of element inputs on P-solubilizing bacteria and on phosphatase activity in two grassland soils in South Africa. 3rd Conference on Ecology of Soil Microorganisms - Digging deeper, Helsinki, Finland, June 17-21, 2018 (poster)

Widdig, M., Roelcke, M., Gao, Z.L., Ma, W.Q., Michalczyk, A., Kersebaum, C., Nieder, R. (2017): Ammonia volatilization from irrigated and non-irrigated winter wheat plots in the North China Plain - Quantification and modeling. German Soil Science Society, Annual Meeting 2017, Göttingen, Germany, September 2-7, 2017 (poster)

Widdig, M., Roelcke, M., Tong, B.X., Xin, S.Y., Zhou, Y.Y., Gao. Z.L., Ma. W.Q., Michalczyk, A., Kersebaum, C., Nieder, R. (2017): Quantification and modeling of ammonia volatilization from irrigated and non-irrigated winter wheat plots in the North China Plain. 15th International Symposium on Soil and Plant Analysis - The Roles of Soil, Plant, Water and Waste Analyses in Food Security and Environmental Quality, Nanjing, China, May 14-18, 2017 (poster)

(Eidesstattliche) Versicherungen und Erklärungen

(§ 8 Satz 2 Nr. 3 PromO Fakultät)
Hiermit versichere ich eidesstattlich, dass ich die Arbeit selbstständig verfasst und keine anderen als die von mir angegebenen Quellen und Hilfsmittel benutzt habe (vgl. Art. 64 Abs. 1 Satz 6 BayHSchG).

(§ 8 Satz 2 Nr. 3 PromO Fakultät)
Hiermit erkläre ich, dass ich die Dissertation nicht bereits zur Erlangung eines akademischen Grades eingereicht habe und dass ich nicht bereits diese oder eine gleichartige Doktorprüfung endgültig nicht bestanden habe.

(§ 8 Satz 2 Nr. 4 PromO Fakultät)
Hiermit erkläre ich, dass ich Hilfe von gewerblichen Promotionsberatern bzw. –vermittlern oder ähnlichen Dienstleistern weder bisher in Anspruch genommen habe noch künftig in Anspruch nehmen werde.

(§ 8 Satz 2 Nr. 7 PromO Fakultät)
Hiermit erkläre ich mein Einverständnis, dass die elektronische Fassung der Dissertation unter Wahrung meiner Urheberrechte und des Datenschutzes einer gesonderten Überprüfung unterzogen werden kann.

(§ 8 Satz 2 Nr. 8 PromO Fakultät)
Hiermit erkläre ich mein Einverständnis, dass bei Verdacht wissenschaftlichen Fehlverhaltens Ermittlungen durch universitätsinterne Organe der wissenschaftlichen Selbstkontrolle stattfinden können.

. . . . . .
Ort, Datum, Unterschrift