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„ Does long-term soil warming affect microbial element limitation? A test by short-term assays of microbial growth responses to labile C, N and P additions "

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1. ABSTRACT

1.1 Abstract (English)

Human activities have caused global warming by 0.95 °C since the industrial revolution, and average temperatures in Austria have risen by almost 2 °C since 1880. Increased global mean temperatures have been reported to accelerate carbon (C) cycling, but also to promote nitrogen (N) and phosphorus (P) dynamics in terrestrial ecosystems. However, the extent of warming-induced increases in soil C, N and P processes can differ, causing an eventual uncoupling of biogeochemical C, N and P cycles, and leading to altered elemental imbalances between available plant and soil resources and soil microbial communities. The altered dynamics in soil C and nutrient availability caused by increased soil temperature could shift the growth-limiting element for soil microorganisms, with strong repercussions on the decomposition, mineralization and sequestration of organic C and nutrients. The latter relates to the conservative cycling of limiting elements while elements in excess are mineralized and released at greater rates by microbial communities.

Despite the many laboratory and in situ studies investigating factors that limit soil microbial activity, most of them explored nutrient addition effects on soil respiration or soil enzyme activities. A critical assessment, however, clearly indicated the inappropriateness of these measures to deduce growth-limiting nutrients for soil microbes. Similar to studies of plant nutrient limitation, unequivocal assessment of soil microbial element limitation can only be derived from the response of microbial growth to element amendments. To our knowledge this has not been performed on soils undergoing long-term soil warming.

In this study, we therefore investigated the effect of long-term soil warming on microbial nutrient limitation based on microbial growth measurements in a temperate calcareous forest soil. Soil samples were taken from two soil depths (0-10, 10-20 cm) in both control and heated plots in the Achenkirch soil warming project (>15 yrs soil warming by + 4 °C). Soil samples were pre-incubated at their corresponding field temperature after sieving and removal of visible roots. The soils were amended with different combinations of glucose-C, inorganic/organic N and inorganic/organic P in a full factorial design, the nutrients being dissolved in ^{18}O-water. After 24 hours of incubation, microbial growth was measured based on the ^{18}O incorporation into genomic DNA. Nutrient (co)limitation was determined by comparing microbial growth responses upon C and nutrient additions relative to unamended controls. Basal respiration was also measured based on the increase in headspace CO_2, allowing to estimate microbial C use efficiency (CUE). The fate of C and nutrient amendments was finally traced by measurements of inorganic and organic extractable and microbial biomass C, N and P. This study will thereby provide key insights into potential shifts in limiting nutrients for microbial growth under long-term soil warming, and into concomitant effects on soil C and nutrient cycles.

We hypothesize the following: (i) soil microorganisms at two field temperatures (control soils: 16 °C, heated soils: 20 °C) and from two soil depths (0-10 cm, 10-20cm) are primarily limited by C. (ii) Previous studies at the same site displayed accelerated microbial processing of SOM indicated by stable positive response (~40% increase) of soil respiration to warming (Schindlbacher et al., 2012). Due to less tight coupling between the supply of soil available C and P compared to C and N, >15 yrs soil warming could potentially enhance soil N availability to a larger extent, leading to microbial P limitation. (iii) As the efficiency of microbial substrate assimilation depends on the activity of specific transporters on cell membrane and microbial element limitation, microbial growth would respond differently to organic and inorganic nutrient additions of the same molar C to nutrient ratios.

Our result showed that warming-induced dynamics in soil C, N and P processes since 2005 caused soil microorganisms from being mainly C-limited in control soils to CP co-limited in heated soils at both soil depths. In addition to that, C still primarily limited microbial growth in both heated and control soils at both soil depth. Microbes showed stronger growth stimulation under combined glucose and inorganic nutrient amendments compared to organic nutrient additions. Lower activity of organic nutrient transporters could explain this finding. Moreover, glucosamine was mineralized more efficiently in glucosamine-6-P (contains C, N and P) compared to glucosamine (contains C and N) treated soils, which coincides with the direct evidence of strong P limitation in heated plots, as the alleviation of P limitation would make microbes more active in mining C by mineralizing glucosamine. Our results confirmed that C:P ratio within microbial biomass was non-homeostatic when the soil was susceptible to high labile P availability, irrespective of native nutrient status of the soil (as long as the soil is not over-saturated with P). We also found that organic P mineralization was triggered primarily by microbial P demand in both C limited and CP co-limited temperate forest soils.

1.2 Abstract (Deutsch)

Seit der industriellen Revolution wurde durch das Einwirken der Menschen eine globale Erwärmung um 0, 95 °C verursacht und die Durchschnittstemperatur in Österreich ist seit 1880 um fast 2 °C gestiegen. Es wurde berichtet, dass die erhöhten globalen Durchschnittstemperaturen nicht nur den Kohlenstoff (C) Zyklus sondern auch die Dynamik von Stickstoff (N)- und Phosphor (P) in terrestrischen Ökosystemen beschleunigen. Das Ausmaß der durch die Erwärmung verursachten Zunahme der C-, N- und P Prozessen in der Erde kann jedoch unterschiedlich sein, was zu einer eventuellen Entkopplung der biogeochemischen C-, N- und P Zyklen und zu veränderten elementaren Ungleichgewichten zwischen verfügbaren Pflanzen-, Bodenressourcen- und bodenmikrobiellen Gemeinschaften führt. Die veränderte Dynamik von Boden C- und Nährstoffverfügbarkeit, die durch eine erhöhte Bodentemperatur verursacht wird, könnte das wachstumslimitierende Element für Bodenmikroorganismen ändern und sich stark auf die Zersetzung, Mineralisierung und Sequestrierung von organischem C und Nährstoffen auswirken.

Letzteres bezieht sich auf die konservative Verarbeitung und den Kreislauf von limitierenden Elementen durch Mikroben während überschüssige Elemente mineralisiert und von mikrobiellen Gemeinschaften freigesetzt werden.

Trotz der vielen Labor- und In-situ Studien in denen Faktoren untersucht wurden, die die mikrobielle Aktivität im Boden beschränken, untersuchten die meisten von ihnen die Auswirkungen der Nährstoffzugabe auf die CO_2 Produktion oder die Enzymaktivitäten. Eine kritische Bewertung zeigte jedoch deutlich, dass diese Maßnahmen unangemessen sind, um wachstumslimitierende Nährstoffe für Bodenmikroben zu identifizieren. Ähnlich wie bei Studien über wachstumslimitierenden Nährstoffen für Pflanzen kann eine eindeutige Bewertung der wachstumslimitierenden Elementen für Mikroorganismen in der Erde nur aus der Reaktion des mikrobiellen Wachstums auf Nährstoffzugabe abgeleitet werden. Nach unserer Kenntnis wurde dies bisher nicht in Erden durchgeführt, welche einer langfristigen Bodenerwärmung ausgesetzt waren.

In dieser Studie untersuchten wir daher den Einfluss der langfristigen Bodenerwärmung auf die mikrobielle Nährstofflimitation anhand von Messungen des mikrobiellen Wachstums in einem gemäßigten kalkhaltigen Waldboden. Im Achenkirch-Bodenerwärmungsprojekt (>15 Jahre Bodenerwärmung um + 4 °C) wurden Bodenproben aus zwei Bodentiefen (0-10 cm, 10-20 cm) sowohl in Kontroll- als auch in beheizten Parzellen entnommen. Die Proben wurden nach dem Sieben und Entfernen der sichtbaren Wurzeln bei ihrer entsprechenden Feldtemperatur inkubiert. Verschiedene Kombinationen von Glucose-C, anorganischem/organischem N und anorganischem/organischem P in einem vollständig faktoriellen Design wurden anschließend den Erden zugeführt, wobei diese Substrate vorher in ^{18}O-Wasser gelöst wurden. Nach 24 Stunden Inkubation wurde das mikrobielle Wachstum basierend auf dem ^{18}O-Einbau in genomischer DNA gemessen. Die wachstumslimitierten Elemente wurden durch Vergleichen des mikrobiellen Wachstums in den C und Nährstoffe zugeführte Erden mit Erden welcher nichts zugegeben wurde, bestimmt. Die CO_2 Produktion durch Bodenmikroorganismen wurde beruhend auf den Anstieg des Headspace-CO_2 gemessen, wodurch die Effizienz der mikrobiellen C-Nutzung (CUE) abgeschätzt werden konnte. Das Schicksal vom zugegebenem C- und den Nährstoffen wurde schließlich durch Messungen der anorganischen und organischen extrahierbaren und mikrobiellen Biomasse C, N und P verfolgt. Diese Studie wird damit wichtige Einblicke in mögliche Wechselungen von limitierenden Elementen für das mikrobielle Wachstum unter langfristiger Bodenerwärmung liefern und gleichzeitig in deren Auswirkungen auf Boden C und Nährstoffkreisläufe.

Wir nehmen folgende Hypothese an: (i) Das Wachstum von Bodenmikroorganismen von zwei Feldtemperaturen und Bodentiefen sind hauptsächlich durch C begrenzt. (ii) Frühere Studien vor Ort zeigten eine beschleunigte mikrobielle Verarbeitung von SOM, was durch eine stabile positive Reaktion von CO_2 Produktion des Bodens (~ 40% Zunahme) aufgrund der Erwärmung angezeigt wird. (Schindlbacher et al., 2012). Anlässlich der im Vergleich zu C und N weniger engen Kopplung zwischen der

Versorgung der verfügbarem Boden C und P könnte eine Bodenerwärmung von über 15 Jahren möglicherweise die Verfügbarkeit von N in größerem Maße verbessern, was zu einer mikrobiellen P-Limitation führen könnte. (iii) Das mikrobielle Wachstum wird unterschiedlich auf die Zugabe von organischem und anorganischem Nährstoffen reagieren, da die Effizienz der Assimilation von Substrat von der Aktivität spezifischer Transporter auf die mikrobielle Zellmembran und die elementare Limitation von Bodenmikroorganismen abhängt.

Unser Ergebnis zeigte, dass die Erwärmung-induzierte Dynamik in Boden C-, N- und P Prozessen seit 2005 zu einer starken CP Co-Limitation des mikrobiellem Wachstum geführt hat, während Mikroben in ungeheizten Parzellen hauptsächlich durch C begrenzt waren. Darüber hinaus begrenzte C immer noch hauptsächlich das mikrobielle Wachstum in beiden Bodentiefen und Bodentemperaturen. Mikroben zeigten eine stärkere Wachstumsreaktion unter Zugabe von kombiniertem Glukose- und anorganischen Nährstoffen im Vergleich zur organischen Nährstoffzugabe mit dem gleichen molaren Verhältnis von C zu Nährstoffen. Eine schwächere Aktivität der Transporter von spezifischen organischen Nährstoffen auf die mikrobielle Zellmembran könnte diesen Befund erklären. Darüber hinaus wurde Glucosamine in Glucosamine-6-P (enthält C, N und P) effizienter mineralisiert als Glucosamine (enthält C und N) behandelte Erden, was mit dem direkten Nachweis einer starken P-Limitation in beheizten Parzellen zusammenfällt, da das Ausfallen einer P-Limitation mikrobielle Mineralisation von Glucosamine effizienter machen würde (Growth rate Hypothese). Unsere Ergebnisse bestätigten, dass das C:P-Verhältnis in der mikrobiellen Biomasse nicht homöostatisch war, als die Erde für eine hohe Verfügbarkeit von labilem P anfällig waren (solange der Boden nicht mit P übersättigt ist). Wir fanden auch heraus, dass die organische P-Mineralisierung hauptsächlich durch den mikrobiellen P-Bedarf sowohl in C-limitierten als auch in CP-co-limitierten gemäßigten Waldböden ausgelöst wurde.

2. GENERAL INTRODUCTION

Forest ecosystem stores more than double the amount of carbon (C) in the atmosphere in their vegetation, soil, and detritus (Sabina et al., 2004). Temperate and boreal forests occupy most of the land surface of the northern hemisphere and contain 46% of all trees on earth (Crowther et al., 2015). Therefore, understanding C, nitrogen (N) and phosphorous (P) dynamics in these ecosystems under the changing climate becomes crucial due to their global extension and role as strong C sink.

Characterized by high C to nutrient ratios, temperate forest ecosystems are more susceptible to warming-induced C loss through enhanced SOM decomposition compared to other ecosystems. Atmospheric CO_2 concentration has increased sharply since the beginning of the industrial revolution due to anthropogenic fossil fuel combustion, as well as land use and land cover change (Marland et al., 2008). Concerns have been raised on the role of forest ecosystems as C sink, as forest soil C, N and P processes might be affected by warming to a level where forests would potentially become net sources of CO_2 (Allison & Treseder, 2011).

Microorganisms are at the core of the terrestrial ecosystem C balance; while plants take up C from the atmosphere, microorganisms consume organic C for maintenance, growth, and enzyme production. Decomposer C metabolism contributes either to C sequestration through growth and necromass production or to soil C loss through respiration. This partitioning of microbial C uptake between growth and respiration is described as microbial carbon use efficiency (CUE) (Manzoni et al., 2012), which is regulated by many environmental factors (e.g. temperature and nutrient availability). Understanding the controls of microbial C metabolism is thus of great importance for predicting potential feedbacks of soil C decomposition to climate change. Generally, as temperature increases, respiration increases more strongly than growth, so soil microbial CUE tends to decrease with temperature (Allison, Wallenstein & Bradford, 2010), but with a stronger effect above the optimum temperature threshold (Apple et al., 2006).

Nutrient availability is another key regulator of microbial CUE through affecting primary production, microbially mediated SOM decomposition and stabilization. Generally, the relative availability of C to nutrients determines whether C and nutrients are immobilized in microbial biomass or mineralized and released to the environment by microorganisms. Improved nutrient availability, whether through anthropogenic inputs or by soil warming, has been reported to influence the ecosystem C balance through enhancing microbial activity and SOM decomposition (Hartley et al., 2010) and primary production (Shaver & Chapin, 1995). A meta-analysis carried out on north temperate forests revealed that enhanced N input increased soil C storage significantly (Nave et al., 2009). Moreover, the effect of increased N input on soil C stocks may depend on soil P availability. Street et al (2018) reported reduced soil C stocks after 20 years of N fertilization, whereas combined N and P addition increased the soil C stock significantly,

pointing out the importance of the relative availability of N and P and of the elemental balance on processes governing terrestrial ecosystem C balance.

Globally, both N and P are nutrients most commonly limiting primary production and soil C cycling (Aerts & Chapin, 1999). The release of both labile N and P is biologically controlled through SOM mineralization. However, unlike N, which is derived primarily from biological processes, P availability is mainly controlled geochemically through mineral weathering, adsorption/desorption and precipitation/dissolution processes (Vitousek et al., 2010, Barrow, 1983, Frossard et al., 2000). N and P in soils are readily lost through biogeochemical pathways (Nordin et al., 2001, Shen et al., 2011) and hydrological processes (Dise & Wright, 1995, Perakis & Hedin, 2002). While N can be supplied naturally through biological N fixation and atmospheric N deposition, under natural conditions, the loss of P is mainly replenished by the release of P from minerals, resulting in a gradual decrease of total P in the soil with time (Filippelli, 2008, Vitousek, 2010). It was thus hypothesized by Walker and Syers (1976) that as soil ecosystems develop, it would eventually reach a "terminal steady state" of profound P limitation.

What elements are limiting microbial growth is not trivial. An organism is limited if a factor is increased and no positive effect on growth is observed, then the factor is also not limiting (Gibson, 1971). According to Liebig's Law of the Minimum, growth at organismal level is restricted by the nutrient whose availability is at the lowest.

Lack of C has been assumed to be the most common limiting element for microbial growth in soil (Nordgren, 1992, Joergensen & Scheu, 1999, Aldén et al., 2001, Ekblad & Nordgren, 2002, Ilstedt & Singh, 2005). Apart from C, some extent of nutrient limitation of microbial biomass synthesis is likely also ubiquitous, since N is needed to build proteins and P is needed for ribosome synthesis (Hartman & Richardson, 2013). Temperate forests are typically thought to be N limited in terms of primary production (Galloway et al., 2008). This could be attributed to the following: (i) glaciation was frequent enough in temperate and boreal ecosystems to remove soils by glacial abrasion and restart soil development. This clearly avoids the so called "terminal steady state" of P limitation (Vitousek & Sanford, 1986), which occurs mostly in highly weathered tropical soils where low pH and intense precipitation facilitates strong P sorption/precipitation and leaching that lowers soil P availability. Moreover, near-neutral pH favours maximum P availability (Penn & Camberato, 2019), and soils developing on carbonate bedrock such as at our study site are characterized by high carbonate content and near neutral pH (Schnecker et al., 2016), at which availability of P in soil solution should be sufficient. (ii) Due to high C/N ratios of organic matter in temperate forests, microorganisms might be co-limited by both C and N, or even become N limited (Demoling, Figueroa & Bååth, 2007, Kamble & Bååth, 2014).

Nevertheless, due to human perturbations, atmospheric N emissions are estimated to have increased N deposition in parts of Europe between 5- and 50-fold higher compared to preindustrial levels (Dentener et al., 2006), which might alter limiting elements for microbial growth in soil and even lead to N saturation of soils. N saturation can be defined as increase in N availability that causes the alleviation of N limitations on rates of biological functions (Agren & Bossata, 1988, Stoddard, 1994). In many temperate forests, such as our study site, the system was found approaching N saturation as the N inputs (wet and dry N deposition; canopy ammonium uptake) exceeded the outputs (gaseous N emission; deep percolation) (Herman et al., 2002).

Human-induced warming reached approximately 1 °C (likely between 0.8 °C and 1.2 °C) above pre-industrial levels in 2017, increasing at 0.2 °C (likely between 0.1 °C and 0.3 °C) per decade (IPCC, 2018). Elevated temperature increases soil microbial metabolism and enzyme activity (Koch et al., 2007, Cookson et al., 2007). This has been empirically found to increase soil organic matter (SOM) decomposition which releases N bound to soil organic matter and to promote biological N fixation, and thereby eventually to increase soil N availability (Galloway et al., 2004, Hartley et al., 1999, Ma et al., 2011, Rustad et al., 2001). Moreover, a meta-analysis revealed that although the soil total N pool is not affected by warming (as soil total N is a large pool that needs more time to show any response to warming), net N mineralization and nitrification increased by 52.2 and 32.2%, respectively, due to soil warming (Bai et al., 2013), suggesting increased labile N availability with warming. In contrast to N, P has not received as much attention in climate change research and the impacts of warming on the element imbalance between microbial demand and soil resources remained undefined.

The available P pool represents a small portion of soil total P. The majority of soil P is therefore not readily available but is associated with soil primary minerals, secondary minerals, organic constituents or is occluded by soil minerals (Frossard et al., 2000, Hou et al., 2016, Condron & Newman, 2011). These soil mineral phase-associated P pools are not readily available to biota but regulate soil P availability by biological and geochemical processes (Frossard et al., 2000, Hou et al., 2016, Vitousek et al., 2010). Temperature can affect soil P availability through its control on biological and geochemical processes that contribute to the soil labile P pool. For example, warming increased available P by promoting the mineralization of P-containing SOM (Liu et al., 2017). However, the transformation of available P and secondary mineral P to occluded P were found to increase under warming (Barrow, 1983, Siebers et al., 2017), which may offset the positive effect of warming on the soil available P pool from accelerated SOM decomposition.

Many other factors also have significant impact on soil P availability. The soil organic matter content affects P supply to microbes in two aspects: (i) SOM mineralization releases easily available forms of P into the soil, and (ii) adsorption of dissolved organic matter releases phosphates adsorbed on soil surfaces

by competing with them for binding sites (Maluf et al., 2018, Yu et al., 2013). Since phosphates tend to bind to mineral surfaces (Spohn, 2020), soil mineralogy and soil pH control both, directly and indirectly, soil P availability. At neutral pH, maximum P availability is expected, because at low pH, greater amounts of aluminum and iron oxides form strong bonds with phosphate, and at high pH such as in calcareous soils, high content of calcium tends to cause precipitation of calcium phosphates (Penn & Camberato, 2019).

Terrestrial ecosystem C, N and P cycles are tightly coupled. The availability of nutrients is constrained by their different sources, cycles, and dynamics. Any warming-induced process that increases the availability of one element could make other elements to become limiting (Vitousek et al., 2010). Decoupling of soil available C, N and P dynamics has been reported with warming (Geng et al., 2017, Jiao et al., 2016) due to different level of controls exerted by warming on biogeochemical processes that contribute to N and P availability. Labile C and N pools are more tightly coupled due to the following mechanisms: (i) The supply of labile C and N are more coupled to each other as labile C and N originates primarily from biological processes, whereas soil available P is supplied mainly geochemically through mineral weathering and desorption (Vitousek et al., 2010). (ii) It is hypothesized that in contrast to N, organic P mineralization is decoupled from C mineralization (McGill & Cole, 1981). s

As microbes need to maintain a balanced composition of C and nutrients (biomass homeostasis hypothesis), microbial C metabolism depends on the relative abundance of key nutrients in soils, where more limiting elements are expected to be utilized by microbes for growth, and non-limiting elements that are in excess compared to other elements, must be disposed of and may be recycled (Hessen et al., 2004). For example, the ability of ecosystems to sequester CO_2 and how they respond to additional N input depends on whether N, P, or both limit ecosystem biological functions. N limitation would cause the ecosystem to absorb additional N and CO_2 (Reich et al., 2006), whereas P limitation constrains the uptake of additional N and CO_2 (Menge & Field, 2007). Therefore, identification of elements that limit microbial growth is mandatory since the alteration in their supply has the potential to transform the structure and functioning of ecosystems (Vitousek et al., 2010).

Methods used to identify element limitation of soil microorganisms either (i) involve adding substrates to the soil and monitoring whether there is an increase in microbial activity and biomass, or (ii) based on soil enzyme measurements, their stoichiometry and vector analysis reflecting microbial limitation if they invest into the production of enzymes mining elements in short supply. According to (i), upon the addition of a limiting element, microbes will show an increased rate of activities including respiration and growth. Respiration is not an ideal indicator for detecting limiting elements because an increase in respiration does not necessarily correspondent to increased growth but merely shows microbial use of C for energy production (Aldén, Demoling & Bååth, 2001) or even overflow respiration to get rid of excess labile C. Soil enzyme stoichiometry has also been shown not to directly reflect microbial element limitation, growth

based approaches therefore being the gold standard to determine microbial element limitation (Rosinger, Rousk & Sandén, 2019). Thymidine and leucine incorporation techniques have been widely used to estimate bacterial activity and growth rate. The principle of the method is based on the incorporation of labelled [^{3}H] thymidine and [^{14}C] leucine into DNA and bacterial protein, respectively. However, fungi do not incorporate thymidine due to the lack of the key enzyme thymidine kinase, and not all bacteria can incorporate thymidine (e.g. nitrifiers) (Bloem et al., 2008), causing an underestimation of microbial growth rates on a community level. Moreover, protein can be continuously degraded (Kirchman et al., 1985) during shift in the growth rate (Ingraham et al., 1983), which would also underestimate bacterial protein synthesis rates. A novel approach based on ^{18}O incorporation into genomic DNA (Spohn et al., 2016) was used in this study to detect limiting elements for microbial growth. Since genomic DNA is only synthesized when cells are growing, the incorporation of ^{18}O from soil water into DNA can be used to calculate microbial growth rates (Schwartz, 2007, Blazewicz & Schwartz, 2011). According to a linear relationship between microbial DNA and microbial biomass C (Anderson & Martens, 2013), microbial growth rates based on C incorporation into microbial biomass can be calculated.

In conclusion, mounting evidence has elucidated the threat of climate change in committing the world to long-term irreversible changes (Lenton et al., 2019). Warming could alter the coupling between soil available C, N and P pools by exerting different levels of controls over biogeochemical processes contributing to these pools, potentially shifting limiting element(s) for microbial growth. As microbes are at the core of soil ecosystem C cycling (Manzoni et al., 2012) and nutrient availability regulates the global forest C cycle (Fernández-Martínez et al., 2014), it is of great importance to investigate if long-term soil warming would alter microbial growth-limiting element(s) (C, N and P) to get a better understanding of the response of terrestrial ecosystem C balance to warming.

3. MANUSCRIPT

"Does long-term soil warming affect microbial element limitation? A test by short-term assays of microbial growth responses to labile C, N and P additions"

3.1 Introduction

Containing 14% of the carbon (C) stored in forests worldwide (Pan et al., 2011), temperate forests strongly affect climate due to its role as strong C sink by taking up large amounts of CO_2 in the atmosphere. With human-induced warming reaching approximately 1°C above pre-industrial levels in 2017 and continuously increasing at 0.2°C per decade (IPCC report, 2018), forest soil C, nitrogen (N) and phosphorous (P) processes might be affected by warming to a level where forests would potentially become net sources of CO_2 (Allison & Treseder, 2011).

Microorganisms are one of the major players in the terrestrial ecosystem C balance, where plants take up C from the atmosphere and soil, microorganisms consume organic C for maintenance, growth, and

enzyme productions. Decomposer C metabolism contributes either to soil C sequestration through growth and necromass productions or to soil C losses through soil organic matter (SOM) decomposition and respiration. The microbial C allocation of C taken up between growth and respiration is described as microbial carbon use efficiency (CUE) (Manzoni et al., 2012), which is regulated by many environmental factors (e.g. temperature and nutrient availability).

Generally, microbial respiration increases more than growth as a function of temperature, causing soil microbial CUE to decrease with temperature (Allison, Wallenstein & Bradford, 2010), with a stronger effect above the optimum temperature threshold (Apple et al., 2006). Nutrient availability is another key regulator of microbial CUE. Improved nutrient availability, whether through anthropogenic inputs or soil warming, has been reported to influence the soil C balance in through promoting microbial activity and SOM decomposition (Hartley et al., 2010) and by increasing vegetation productivity (Shaver & Chapin, 1995). A meta-analysis carried out on north temperate forests revealed that enhanced N input increased soil C storage significantly (Nave et al., 2009). Moreover, the effect of increased N input on soil C stocks may depend on P availability. For example, Street et al (2018) reported reduced C stocks after 20 years of N fertilization, whereas combined N and P addition increased the soil C stocks significantly, pointing out the importance of the relative availability of N and P and of the elemental balance on processes governing terrestrial ecosystem C sequestration.

Warming can directly or indirectly affect soil nutrient availability, in addition to its control on the soil C balance, by exerting different levels of controls on soil biogeochemical processes. Elevated temperature increases microbial metabolism and enzyme activity (Koch et al., 2007, Cookson et al., 2007), and this has been empirically found to increase SOM decomposition and biological N fixation that could eventually increase soil N availability (Galloway et al., 2004, Hartley et al., 1999, Ma et al., 2011). In contrast to N, soil P dynamics have not received as much attention in climate change research. In general, temperature can affect soil P availability through its control on (i) soil properties such as pH and organic matter content, and (ii) plant and microbial activities. For example, warming increases available P by accelerating the mineralization of P-containing SOM (Liu et al., 2017). However, greater transformation of available P and secondary mineral P to occluded P was found under warming (Barrow, 1983, Siebers et al., 2017), which may offset the positive effect of warming on soil available P from accelerated SOM decomposition. Since the impact of warming on the relative availability of C with respect to nutrients remains uncertain, it is crucial to identify element(s) that constrain the growth of soil microorganisms, critical players of the feedback of soil C decomposition to climate change.

Carbon has been assumed to be the most common limiting element for microbial growth in soils (Nordgren, 1992, Joergensen & Scheu, 1999, Aldén et al., 2001, Ekblad & Nordgren, 2002, Ilstedt & Singh, 2005). Soil microbes in temperate forests are typically thought to be N limited (Galloway et al.,

2008). Microbial P limitation is ubiquitous in old tropical soils (Camenzind et al., 2018) due to the non-renewable nature of soil P storage (Walker & Syers, 1976) and to the occlusion of soil P through sorption to aluminum and iron oxides in highly weathered environments (Cross & Schlesinger, 1995). Nevertheless, due to human perturbations, atmospheric N emissions are estimated to have increased N deposition up to 50-fold compared to preindustrial levels in parts of Europe (Dentener et al., 2006), eventually causing forest ecosystems approaching N saturation as the N inputs (wet and dry N deposition; canopy ammonium uptake) exceed the outputs (gaseous N emission; deep percolation) (Herman et al., 2002). Moreover, decoupling of P cycling from C and N cycling in response to warming has been reported (Geng et al., 2017, Jiao et al., 2016). This could be attributed to the following: (i) The supply of labile C and N are more coupled to each other as labile C and N originates primarily from biological processes, whereas soil available P is supplied mainly geochemically through mineral weathering and desoprtion (Vitousek et al., 2010). (ii) It has long been hypothesized that in contrast to N, organic P mineralization is generally decoupled from that of C mineralization (McGill & Cole, 1981). Most of organic P compounds in soil are phosphate esters, meaning that the P can be cleaved off without necessarily decomposing soil C (Condron et al., 2005). Therefore, long-term soil warming together with increased N deposition could make N more available than P, potentially leading to a shift in element(s) that constrain microbial growth.

The approaches used to identify microbial element limitation in soils are either (i) based on amending labile substrates to the soil followed by measuring an eventual increase in microbial activity or biomass, or (ii) on soil enzyme measurements, using enzyme stoichiometry or vector analysis as proxy of microbial element limitation, reflecting higher microbial production of enzymes mining elements in short supply. According to (i), upon the addition of a limiting element, microbes show increased activity such as respiration and growth. However, respiration is not an ideal proxy to detect limiting elements because an increase in respiration does not necessarily correspondent to increased growth but merely shows microbial use of C for energy production or even overflow respiration (Aldén, Demoling & Bååth, 2001). Soil enzyme stoichiometry has also been shown not to directly reflect microbial element limitation, growth based approaches therefore being the gold standard to determine microbial element limitation (Rosinger, Rousk & Sandén, 2019). Thymidine and leucine incorporation techniques have been widely used to estimate microbial growth rate. The principle of the method is based on the incorporation of labelled [^{3}H] thymidine and [^{14}C] leucine into DNA and bacterial protein, respectively. However, fungi do not incorporate thymidine due to the lack of the key enzyme thymidine kinase, and not all bacteria can incorporate thymidine (e.g. nitrifiers) (Bloem et al., 2008), causing an underestimation of microbial growth rates on a community level. Moreover, protein are constantly degraded when the microbial growth rates changes (Ingraham et al., 1983). This might underestimate bacterial protein synthesis rates. A novel

approach based on ^{18}O incorporation into genomic DNA (Spohn et al., 2016) was used in this study to detect limiting elements for microbial growth. Due to the fact that genomic DNA is only synthesized when the cell is dividing, microbial growth rates can be calculated based on the incorporation of ^{18}O from soil water into DNA (Schwartz 2007; Blazewicz & Schwartz, 2011).

In the present study we investigated if long-term soil warming (> 15 years, + 4 °C) would shift elements (C, N and P) that limit microbial growth at two soil depths (0-10 cm; 10-20 cm) in a temperate forest ecosystem. As reported by many studies (Yu et al., 2013, Maluf et al., 2018), forest soils even with high soil C:N ratio are primarily limited by C (energy), hence, we hypothesized that C primarily limits microbial growth at both soil depths and field temperatures. Since C and N availability derive primarily from biological processes, whereas available P is linked mainly to mineral weathering and to a lesser extent, from SOM decomposition, we hypothesized to find a shift in secondary limiting elements from N to P in warmed soils. The effect of nutrient quality (inorganic/organic) on microbial growth was also tested in this study by amendment of organic and inorganic nutrients.

3.2 Material and Methods

Site characteristics and soil sampling

In August 2019, soils were sampled in the northern limestone Alps at 910 m a.s.l. in a temperate calcareous forest, Achenkirch, Austria (47°34' 50"N; 11°38' 21"E). The dominant vegetation at the site are spruce (*Picea abies*) and European beech (*Fagus syvatica*). The bedrock is dolomite. The soils represent a mosaic of shallow Chromic Cambisols and Rendzic Leptosols, and are characterized by high carbonate content and near neutral pH. Local mean annual air temperature and precipitation were 6.9 °C and 1506 mm (1992-2012), respectively (Achenkirch village, data from ZAMG). The depths of the litter and O-layer reach from 0 to 5 cm, A-horizons ranges from 10 to 40 cm and the root density is highest in the O-, and A-horizons and few roots are found down to a depth of 60 cm (Schindlbacher et al., 2009).

The warming experiment was set up in 2004 (Schindlbacher et al., 2009). In 2005, soil warming started with three paires of heated and adjacent control plots. In 2008, another three paired heated and control plots were established. Soils were warmed (+ 4 °C) throughout the growing seasons (from April/May to November/December) by inserting dummy cables (control treatments) or resistant heating cables (heated treatments) in 3 cm deep slots with a spacing of 7-8 cm.

Soil samples were taken from each the six warmed (+ 4 °C) and control plots at two soil depth (0-10cm, 10-20cm) using a root corer (diameter 2.5cm, Eijkelkamp, Netherlands). 7-8 cores were taken from each plot and subsequently bulked for each sampling depth.

Soil preparation

Soils were sieved (<2 mm), and stones and visible roots in the soil were removed at the site. Afterwards, soils were brought back to the laboratory at the university of Vienna and stored in polyethylene bags at their corresponding mean field temperatures in August, namely 16 °C and 20 °C.

Soil physical and chemical analysis

Before start of the short-term substrate addition experiment, the soil water content (SWC) was measured by weighing a soil aliquot before and after drying at 105 °C for two days. Water holding capacity (WHC) was determined by saturating fresh soil (5 g) in a funnel with an ash-free filter paper, drainage of water-saturated soil for 2.5 h, and dividing the water retained in the soil by soil dry mass.

Determination of elements limiting microbial growth

Substrates were added to quadruplicate soils to determine microbial growth-limiting element(s) in control and warmed soils at their respective mean field temperatures (16 °C and 20 °C) in August, for both soil depths (0-10 cm; 10-20 cm). C (glucose), inorganic N (NH_4Cl) and inorganic P (KH_2PO_4) were added to the soils in a full factorial design. To test nutrient quality (inorganic/organic) effects on microbial growth, glucosamine (contains C and N), glucose-6-phosphate (contains C and P) and glucosamine-6-phosphate (contains C, N and P) were also applied to the soil. The amount of substrate added is illustrated in Table 1.The amount of C and nutrients added were set so that microbial assimilation of the added substrate can be ensured. The amount of labile C added equaled the amount of microbial biomass C in each soil treatment and each soil depth, which was directly measured before the start of the experiment. The molar C:N, C:P and N:P ratios are 6:1 in glucosamine, 6:1 in glucose-6-P and 6:1:1 in glucosamine-6-P, thus the substrate C:N:P stoichiometry was controlled at the same ratio when glucose-C and inorganic nutrient(s) were added to the soils.

Microbial respiration and microbial growth rate measurements after substrate addition

Since microbial growth rates were measured here based on ^{18}O incorporation into genomic DNA, all substrates were dissolved in a mixture of $H_2{}^{18}O$ (97.0 at%, Campro Scientific, Germany) and Milli-Q water. The ^{18}O enrichment and volume of the added $H_2{}^{18}O$ was determined by targeting the at% of ^{18}O in final soil water of 20 at%^{18}O and a final WHC of 70%, respectively.

Soil aliquots (0.4 gram) were weighted in 2 mL screw cap vials, and in two sets of 5 mL Greiner tubes (Greiner bio-one, Cellstar PP, conical). Afterwards, substrate solutions were added to the srew cap vials and the Greiner tubes. Directly after substrate addition, the soil aliquots in 2 mL screw cap vials were transferred to 50 mL glass serum bottles (Supelco, Sigma-Aldrich Chemie GmbH, Germany) and sealed with a crimp cap and butyl rubber stoppers (Supelco, Sigma-Aldrich Chemie GmbH, USA). 5 mL headspace gas was sampled immediately thereafter by a syringe and was directly measured for CO_2 concentration by connecting the syringe to an infrared gas analyzer (EGM-4, PP Systems, Amesbury, MA, USA). To keep the environment in the vials at the same atmospheric pressure, 5 mL of air with known CO_2 concentration was injected back into all vials. Afterwards, all samples were incubated for 24 h at their respective mean field temperatures. At the end of the incubation period (24 h after substrate addition), 5 mL gas sample was collected and determined for CO_2 concentration using the same infrared gas analyzer. The incubation experiment was then stopped by closing and shock freezing the screw cap vials in liquid nitrogen, and storing at -80 °C until further DNA extraction, quantification and oxygen isotope analysis. Total soil DNA was extracted with a DNA extraction kit (FastDNA™ SPIN Kit for Soil, MP Biomedicals, Germany) according to Spohn et al., (2016). DNA concentrations were quantified by the Picogreen fluorescence assay using a microplate spectrophotometer (Infinite M200, Tecan, Austria). Aliquots of DNA extracts were pipetted into silver capsules and dried at 60 °C for two days. Subsequently, the ^{18}O:^{16}O isotope composition of the soils were analyzed using a Thermochemical Elemental Analyzer coupled to an Isotope Ratio Mass Spectrometer.

Substrate addition	Total addition (g g soil C_{mic}^{-1})		
	C	N	P
No	0	0	0
Glucose	1	0	0
NH_4Cl	0	0.19	0
KH_2PO_4	0	0	0.43
Glucose + NH_4Cl	1	0.19	0
Glucose + KH_2PO_4	1	0	0.43
Glucose + NH_4Cl + KH_2PO_4	1	0.19	0.43

$NH_4Cl + KH_2PO_4$	0	0.19	0.43
Glucosamine	1	0.19	0
Glucose-6-P	1	0	0.43
Glucosamine-6-P	1	0.19	0.43

Table 1 Substrates added to soils at two field temperatures (control soils: 16 °C, heated soils: 20 °C) from two soil depths (0-10 cm; 10-20 cm). Glucose-C, NH4Cl-N and KH2PO4-P were added to soils in a full factorial design. Organic N and P were added as glucosamine (contains C and N), glucose-6-P (contains C and P) and glucosamine-6-P (contains C, N and P) to serve as organic nutrient additions. No indicates the no addition control.

Soil chemical and microbiological analysis after substrate addition

In parallel to microbial growth and respiration analysis, 24 hours after substrate addition, one set of soil aliquots in 5mL Greiner tubes was extracted with 1M KCl and the other set with 1M KCl plus 50 µl chloroform (the soil: solution ratio was 1:12.5 (w:v)), which were subsequently put on a shaker for 60 min at 200 rpm and centrifuged at 10,000 *g* for 5 min. Lastly, supernatants were retrieved and stored in scintillation vials (Sarstedt) for further soil chemical and microbiological analyses. The extraction with 1 M incl. liquid chloroform enables to estimate microbial biomass, similar to the chloroform-fumigation extraction (CFE) procedure, but is faster (1 h) than CFE (24-48 h).

To get rid of the chloroform, the second set of soil extracts was freeze dried. Dissolved organic C (DOC) and total dissolved nitrogen (TDN) were analyzed for both sets of KCl extracts using a TOC/TN analyzer (TOC-VCPH Total organic carbon analyzer, Shimadzu, Japan). To calculate microbial biomass C (C_{mic}) and microbial biomass N (N_{mic}), the concentration of DOC and TDN in the KCl extract without chloroform were subtracted from the concentration of the same soluble components in the soil extracted with KCl and chloroform, and by applying a conversion factor of 2.22 for the calculation according to Jenkinson et al., (2004). Ammonium and nitrate in 1M KCl extracts were quantified by colorimetric methods (Hood-Nowotny et al., 2010).

Soil total P (TP) was measured in 0.5M H_2SO_4 extracts of ignited (450 °C, 4 h) soil by malachite green measurements of reactive phosphate (Robertson et al., 1999). Dissolved inorganic P (DIP) was determined by applying the malachite green method in 1M KCl extracts. Soil dissolved organic P (DOP) was calculated as the difference between total dissolved P (TDP) and DIP. Acid persulfate digestion (Robertson et al., 1999) was applied to measure total dissolved P (TDP) and allowed calculation of dissolved organic P (DOP). Microbial biomass P (P_{mic}) was calculated as the difference in soil TDP between the two sets of KCl extracts, corrected with a conversion factor of 2.5 (Jenkinson et al., 2004).

$\bar{x}$ ± STD	Soil depth: 0-10 cm		Soil depth: 10-20 cm		Anova test					
	Control	Heated	Control	Heated	Warming		Depth		Warming × Depth	
					P	F	P	F	P	F
Water content (% of fresh soil)	48.6% ± 7.0%	42.9% ± 3.4%	39.0% ± 5.9%	34.0% ± 5.7%	*	4.5	**	13.5	n.s	0.0
DOC [µg C/g DW]	85.7 ± 13.8	69.2 ± 12.6	118.7 ± 29.3	47 ± 12.1	***	23.0	n.s	0.3	*	9.0
TDN [µg N/g DW]	52.3 ± 8.2	44.8 ± 3.3	24.7 ± 1.2	29.4 ± 1.6	n.s	0.3	***	67.4	*	5.4
TP [µg P/g DW]	25.6 ± 5.8	22 ± 6.5	20.1 ± 5.1	12.9 ± 3.1	**	11.3	**	13.1	n.s	0.2
Soil C:N ratio	13.7 ± 1.4	15.2 ± 1.3	15.1 ± 1	13.2 ± 0.4	n.s	0.0	n.s	0.7	***	54.3
Nitrate [µg N/g DW]	31.2 ± 3	35.9 ± 0.9	24.2 ± 0.7	24 ± 0.9	*	7.5	***	131.0	*	8.7
Ammonium [µg N/g DW]	6.3 ± 0.3	4.9 ± 0.2	5.7 ± 1	6.3 ± 0.1	n.s	2.1	n.s	2.0	**	13.1
DON [µg N/g DW]	7.3 ± 4.1	11.4 ± 8.5	0 ± 1.3	0 ± 1.6	n.s †	1.4	** †	23.7	n.s †	0.0
TDP [µg P/g DW]	0.9 ± 0	0.8 ± 0	0.7 ± 0	0.6 ± 0	*	5.7	***	53.7	n.s	1.8
DIP [µg P/g DW]	0.5 ± 0	0.4 ± 0	0.4 ± 0	0.3 ± 0	**	17.6	***	202.9	n.s	0.2
DOP [µg P/g SDW]	0.5 ± 0	0.4 ± 0	0.3 ± 0	0.3 ± 0	n.s	2.3	***	19.2	n.s	1.6
C_{mic} [µg C/g DW]	1079.9 ± 92.1	898.1 ± 41.5	409.6 ± 57	529.8 ± 87.8	n.s	0.7	***	203.9	**	17.2
N_{mic} [µg N/g DW]	122.3 ± 10.8	77.5 ± 18.6	51.8 ± 5.2	47.7 ± 8.6	***	42.5	***	304.2	***	21.54
P_{mic} [µg P/g DW]	0.7 ± 0	0.5 ± 0	0.3 ± 0	0.2 ± 0	***	59.1	***	329.5	***	25.4
Molar C_{mic} : N_{mic} ratio	10.8 ± 1	12 ± 1	8.6 ± 0.8	13 ± 1.3	***	28.0	n.s	1.1	**	9.6
Molar C_{mic} : P_{mic} ratio	51.3 ± 2	61.9 ± 9.8	50.5 ± 7.6	83.1 ± 10.2	***	28.6	*	6.4	*	7.4
Molar N_{mic} : P_{mic} ratio	4.8 ± 0.3	5.2 ± 0.6	5.9 ± 0.7	6.4 ± 0.4	n.s	3.2	***	21.0	n.s	0.0

*Table 2 Mean ± standard deviation of soil basic physiochemical and microbiological properties in heated (20 °C) and control (16 °C) subplots, from two soil depths (0-10 cm; 10-20 cm) and results for two-way Anova analysis of the factors warming and soil depth. Note that except for water content, soil C:N ratio and TP, all other data were measured parallel to substrate addition experiment after adjustment of soil water content to 70%. Variance homogeneity applied to all data, n.s indicates a non-significant result, * corresponds to a P < 0.05, ** to a P < 0.01, and *** to a P < 0.001. † Indicates results for log transformed data.*

Statistical analysis

Statistical analysis was performed in 'R' software version 3.6.1. Data were log-transformed if necessary, to fit normal distribution of residuals and homogeneity of variance. Outliers were identified (if more than 1.5 (IQR) above the upper quartile or more than 1.5 (IQR) below the lower quartile, IQR – interquartile range) and substituted by the average value of the other replicates. Five-way ANOVA followed by Tukey-HSD tests were used to test for effects of element addition (C, N and P), warming and soil depth on microbial C processes. Four-way ANOVA and Tukey-HSD tests were used to test for effects of element addition (C, N and P) and warming on microbial C processes separately for the two soil depths. Two-way ANOVA tests were used for testing the effects of warming and soil depth on basic soil physicochemical and microbiological properties.

3.3 Results

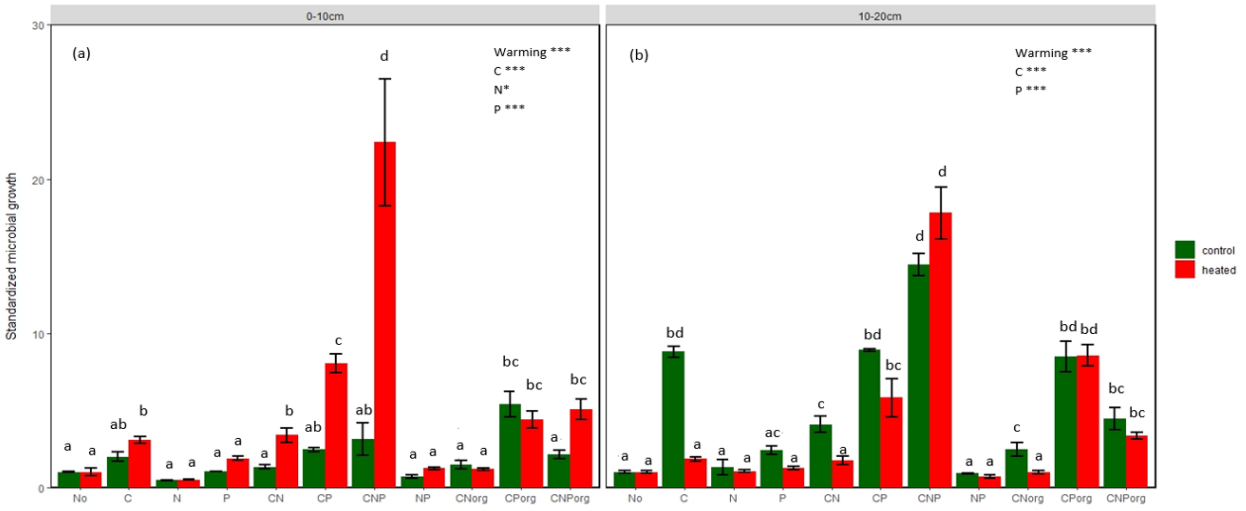

Figure 1 Soil microbial growth rates 24h after adding nutrients soils at their respective two field temperatures (control soils: 16°C; heated soils: 20°C) and from two soil depths (0-10 cm; 10-20 cm). Carbon (C), nitrogen (N) and phosphorus (P) were added in a full factorial design (designated as C, N, P, CN, CP, NP and CNP). CN_{org}, CP_{org} and CNP_{org} treatments represent the addition of glucosamine, glucose-6-P and glucosamine-6-P treatments, respectively. No indicates the no addition control. The data were standardized by dividing by the microbial growth rates of no addition controls for substrate addition. Significant main effects from four-way ANOVA (warming x C x N x P) are given in the graph.

Microbial growth limitation

When C, inorganic N or inorganic P were added separately, we found a strong growth stimulation by C amendment, and overall microbial C limitation increased with warming and from topsoils to subsoils (Table S6). Control and warmed topsoils showed 2- and 3.9-fold growth stimulation in response to C addition (Fig. 1), while in subsoils, C addition caused 8.8- and 1.8-fold growth stimulation in control and heated soils (Fig. 1), indicating that microbial growth was primarily limited by C with control subsoil being most C-limited.

N alone triggered no growth response in subsoils and decreased microbial growth in control and warmed topsoils. Compared to the C-only treatment, when C and N were added together, microbial growth responses either were not significant (in all topsoils and in heated subsoils) or decreased (control subsoils; $P<0.05$).

Notably, P addition alone stimulated microbial growth response in heated topsoils ($P<0.1$). Combined CP treatment further increased microbial growth by 160% and 216% in heated topsoils and subsoils compared to the C-only treatment (Fig. 1), whereas in control soils at both soil depths, microbial growth was not further triggered by combined CP amendment compared to single C addition, indicating CP co-limitation in heated plots but not in control plots at both soil depths.

Except for control topsoils, where growth responses were not further increased by CNP addition compared to that of CP or C-alone additions, CNP treatment was found most pronounced in boosting microbial growth across all treatments ($P<0.001$), with a 22-fold growth stimulation in heated topsoils.

Microbial use of inorganic- versus organic nutrients

CP and CP_{org} treatments did not differ significantly in affecting microbial growth rates (Fig. 1). CN and CNP treatments in heated topsoils outcompeted CN_{org} and CNP_{org} treatments in boosting microbial growth. In subsoils, CNP treatments triggered stronger growth response than CNP_{org} additions, but no difference in CN and CN_{org} treatments was observed.

Most of the glucosamine (CN_{org}) added was not mineralized within 24 h as shown by high DON concentrations and low ammonium and nitrate concentrations (Table 3), and only 21%-32% of glucosamine added was mineralized in heated and control top- and subsoils. Higher N mineralization was observed in glucosamine-6-phosphate (CNP_{org}) treated soils, in which 44%-78% of glucosamine-6-P added was mineralized. Phosphate released from mineralization of glucose-6-P (CP_{org}, 74%-85% of added glucose-6-P contributed to increased phosphate and P_{mic} concentrations) and glucosamine-6-P (CNP_{org}, 66%-88% of added glucose-6-P contributed to increased phosphate and P_{mic} concentrations) was therefore higher (Table 4) compared to N mineralized from glucosamine and glucosamine-6-P. Notably, glucosamine-6-P addition stimulated nitrification as displayed by significant increases in nitrate concentrations ($P<0.001$) compared to no addition controls (Table 3).

No difference in C uptake and respiration was triggered by CP compared to CP_{org}, while CN and CNP treatments had a larger positive effect on C uptake and respiration compared to CN_{org} and CNP_{org} amendments (Fig. 2; Fig. 3).

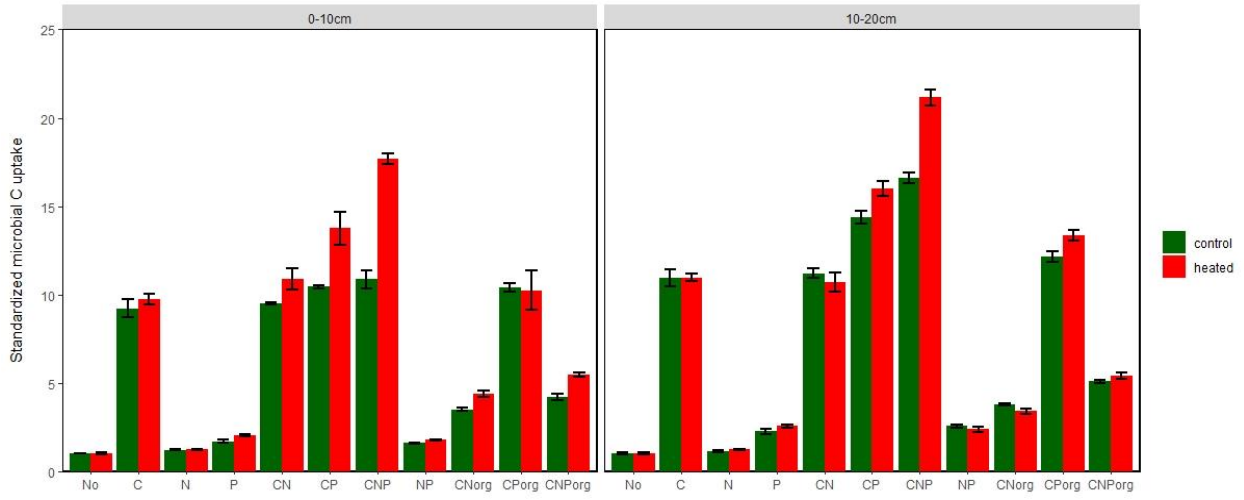

Figure 2 Soil microbial C uptake (sum of microbial growth and respiration) of two field temperatures (16°C; 20°C) and two soil depths (0-10 cm; 10-20 cm) 24h after nutrient addition. The data were standardized by dividing by the value of the no addition control for each soil.

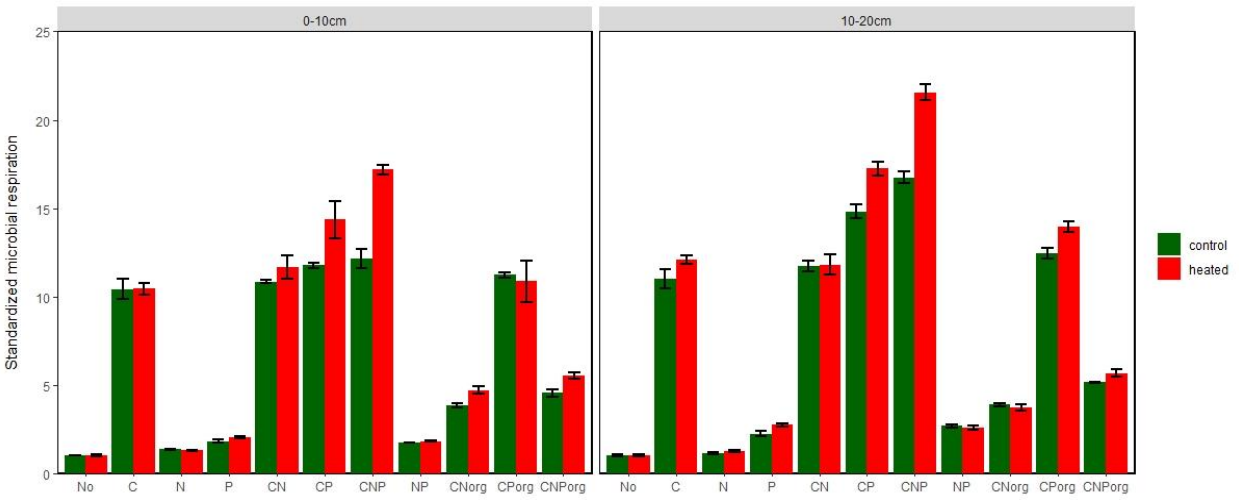

Figure 3 Soil microbial respiration of two field temperatures (16°C; 20°C) and two soil depths (0-10 cm; 10-20 cm) 24h after nutrient addition. The data were standardized by dividing by the value of the no addition control for each soil.

Short-term substrate fertilization effects on microbial C metabolism

Microbial C uptake

The patterns for microbial C uptake were similar for warmed and control plots at both soil depths, which is different from microbial growth response patterns. A strong C uptake response upon C addition was further increased by CP and CNP amendments while N addition alone or combined CN addition did not or weakly stimulate C uptake (Fig. 2). Therefore, microbial C uptake was primarily limited by labile C availability and secondarily co-limited by P.

Microbial respiration

Microbial respiration shared a similar response to nutrient amendment as microbial C uptake (Fig. 3). Respiration and C uptake were therefore not perfectly indicative of microbial growth-limitations as they captured no difference in process rates between control and heated plots.

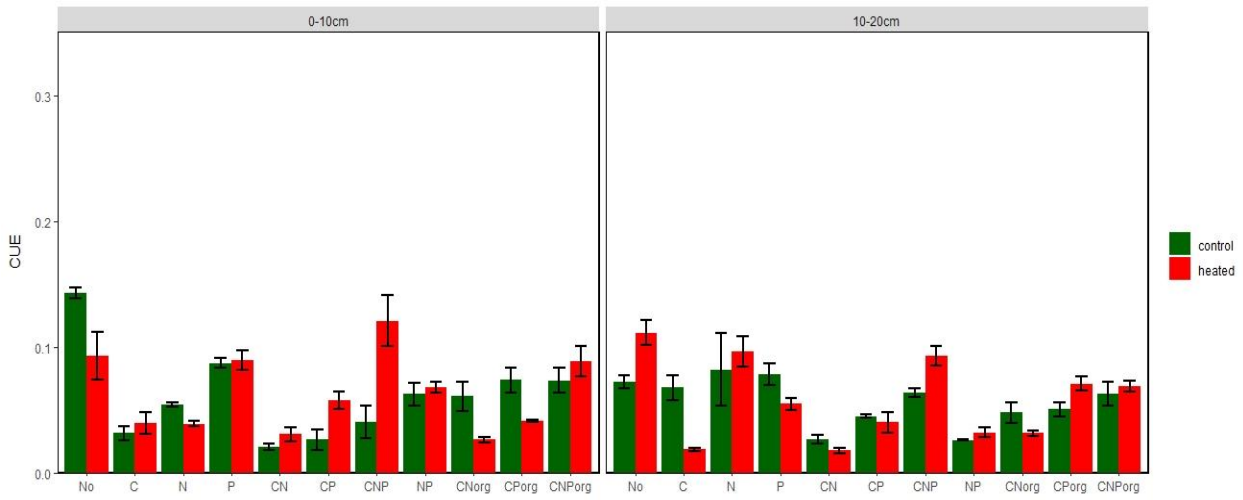

Figure 4 Microbial carbon use efficiency (CUE) at two mean field temperatures (control soils: 16°C; heated soils: 20°C) from two soil depths (0-10 cm; 10-20 cm) 24h after substrate addition.

Microbial carbon use efficiency

C addition stimulated respiration more strongly than microbial growth as indicated by a decline of microbial CUE in C, CN and CP amended soils. CNP addition, on the other hand, stabilized microbial CUE except in control topsoils, which is indicative of similar responses of microbial growth and respiration to CNP addition. Single N addition decreased CUE in both control- and heated topsoils (Fig. 4), which was explained by a negative effect to N addition on microbial growth in topsoils (Fig. 1).

3.4 Discussion

Elements limiting microbial growth

The significant stimulation of microbial growth (Fig. 1.) in glucose-C amended treatments compared to the no addition controls reveals that microbial decomposers at both field temperatures (control and heated soils) and from both soil depths were primarily limited by a lack of C, as reported in the few studies targeting soil microbial element limitation via growth stimulation assessments (Aldén et al., 2001, Demoling et al., 2007, Kamble & Bååth, 2014, Reischke, Rousk & Bååth, 2014). What element(s) limit microbial growth is determined by relative availability of elements. Greater stimulation of microbial growth by C addition in heated soils further demonstrates increased microbial C limitation in warmed soils, which was reflected in lower labile C availability in heated than in control soils as indicated by lower DOC concentrations in the former. Moreover, SOC and DOC decreased with soil depth, triggering greater microbial C-limitation in subsoils than in topsoils. The by far strongest C limitation in control subsoils could be explained by higher nutrient availability, namely significantly higher TDP and DIP concentrations in control subsoils (Table 2), that made C relatively more limiting compared to heated soils.

Forests in temperate regions are typically thought to be N limited in terms of primary production (Perakis & Hedin, 2002), and soil P availability has been widely viewed to be lower in hot and wet tropical regions than in cold temperate regions (Zhang et al., 2005). In this study, decadal soil warming has shifted soil microbes from being mainly C limited (control soils) to being strongly CP co-limited (heated soils). Even P addition alone stimulated microbial growth in heated topsoils. Notably, no significant warming effects on microbial community structure or composition were found at the same site (Kuffner et al., 2012). Hence, the shift in microbial growth-limiting element from C to CP could be due to:

(1) Accelerated SOM decomposition in response to warming has increased the availability of N which has made P less available. McGill and Cole (1981) proposed that N mineralization is more coupled to C mineralization. Thus, consistently accelerated microbial processing of SOC as indicated by the continued positive response (~40% increase) of soil CO_2 efflux to warming (Schindlbacher et al., 2012) at the site indicates higher labile N supply in soils compared to P, leading to elemental imbalances between available soil resources and microbial community.

(2) Decadal (12-15 years) warming decreased the SOC content which indirectly lowered soil P availability. A parallel experiment during the same sampling event in this study (Ye Tian, unpublished results) showed that warming tended to decrease SOC content which can affect P supply in two aspects: (i) SOM mineralization releases easily available forms of P to the soil, (ii) and organic matter releases phosphate adsorbed on soil surfaces by competing with them for binding sites (Yu et al., 2013, Brucker et al., 2020). Hence, reduced SOC and DOC content with warming could indirectly reduce soil P availability through

decreased microbially-mediated P supply via SOM decomposition and an enhanced Pi sorption capacity of the soil matrix.

(3) Warming potentially reduced soil P availability through its negative influence on microbial biomass production. It is estimated that P held within microorganisms generally accounts for 2% to 10% of total soil P (Oberson & Joner, 2005), which represents a significant pool of immobilized P that over longer term, through microbial biomass turnover, regulates the P availability in soil solution (Seeling & Zasoski, 1993). Moreover, microbial biomass competes with the soil matrix in immobilizing P by maintaining P in labile forms that is temporally protected from soil P sorption (Olander & Vitousek, 2004). Hence, reduced C_{mic} concentrations due to soil warming since 2005 may decrease soil P availability to a certain extent, by shifting from microbial towards geochemical P immobilization.

(4) Direct negative effect of warming on abiotic Pi supply. In a parallel experiment to this study, microbes and plants across all seasons responded to warming by increasing phosphatases production (Ye Tian, unpublished results), which suggests that microbes were investing more energy in P acquisition in warmer soils through microbial P demand in heated soils was not met fully through this supply pathway.

Since soil P availability is less affected by warming-induced changes in biological processes but more influenced by abiotic P input (Jiao et al., 2015), warming could have negatively affected abiotic P supply pathways. A rise in temperature can directly enhance soil P sorption rates (Barrow, 1983) as well as the migration rate of phosphate into fines pores of hydrous aluminum and iron oxides (Niskanen, 1990), and thereby dampen abiotic Pi mobilization and reduce P availability in heated soils. For example, the transformation of available P and of secondary P to occluded P is facilitated by warming (Barrow, 1983, Siebers et al., 2017). In our experiment, from the same sampling event as this study, by applying ^{33}P pool dilution method, abiotic Pi immobilization and Pi sorption was accelerated by warming while decreasing microbial P uptake and overall gross Pi mobilization (Ye Tian, unpublished results). It seems that warming has enhanced soil Pi sorption (that outcompeted microbial Pi immobilization) more than Pi mobilization processes. Moreover, our data show that in all substrate treatments that contained P, that the extractable phosphate fraction in heated soils was smaller than the control, and that more P was routed to the non-recoverable P fraction in heated soils (Fig. S2), while P_{mic} varied little between heated and control soils. This provides further evidence that abiotic Pi immobilization was facilitated by warming.

Microbial use of organic nutrients

Lower stimulation of microbial C uptake in CN_{org} and CNP_{org} treated soils compared to CN and CNP treatments but equivalent stimulation of C uptake by CP_{org} and CP additions indicate less active and available uptake transporters for glucosamine and glucosamine-6-phosphate than for glucose and glucose-6-P. Since soils at the study site were not N limited, microbial processing of glucosamine should

be driven by microbial search for C (energy), however, due to the limited ability of microbes for intact uptake of glucosamine and P limitation that restricted microbial activity, glucosamine mineralization was restricted. Higher organic N mineralization was found in the glucosamine-6-P treatment, probably due to alleviation of P limitation that stimulated higher microbial activity, leading to a larger extent of glucosamine being mineralized which has also made more C available for microbial C uptake.

P mineralization was hypothesized to be controlled mainly by microbial P demand (McGill & Cole, 1981). Contradictorily, P mineralization was found to become activated by microbial need for C in both C and P limited soils (Spohn et al., 2013, Heuck et al., 2015). In this study, by comparing CNP and CNP_{org} treatments, sufficient phosphate was supplied from glucosamine-6-P (CNP_{org}) which increased P_{mic} to an equivalent level as in CNP treated soils, whereas C uptake and microbial growth response were found significantly lower in CNP_{org} treated soils, indicating that organic P mineralization was triggered mainly by microbial P demand.

Substrate addition effects on microbial biomass C:N:P stoichiometry

Changes in microbial biomass C:N:P stoichiometry upon element amendments is due to microbial uptake of C, N and P in excess or in other words, storage of these elements by microbes. Excess microbial P uptake could explain the increase in P_{mic} with no corresponding increase in C_{mic}. Since glucose is readily available for microbial uptake, it was most likely used by microbes to build up C storage molecules or was invested into growth, resulting in higher C_{mic} in some of the C-treated soils.

As R- strategists is able to take up high amounts of P disproportionally to synthesize ribosomal RNA (Elser et al., 2000), an activation of R-strategists could explain higher microbial growth rates in heated compared to control soils upon addition of P-containing substrates. This is to some extent supported by our data showing simultaneous increases of P_{mic} and decreases of C_{mic}:P_{mic} and N_{mic}:P_{mic} ratios, however in both, control and heated soils, when P-containing substrates were added. Since oligotrophic microbial species can still grow when exposed to limited C and nutrient supplies while copiotrophic organisms are specialized in thriving on high C concentrations under optimal nutrient conditions (Fierer et al., 2007), a shift in dominant microbial taxa from oligotrophic to copiotrophic ones could have taken place.

Our results also displayed homeostatic behavior of microbes under nutrient amendment without P, and non-homeostasis of microbes in soils receiving P addition in both control (mainly C-limited) and heated soils (CP co-limited).

Considering the prevailing microbial element limitations at the study site, it can be argued that in temperate forest soil microbes suffering from CP co-limitation, P addition would stimulate microbial P uptake in excess that could subsequently increase P_{mic} and decrease microbial biomass C:P and N:P ratios. The fact that organic/inorganic CP and CNP treatments also decreased microbial biomass C:P and N:P

ratios could indirectly indicate higher microbial P use efficiency than microbial CUE, even though the soil microbes are primarily C-limited.

3.5 Conclusion

Decadal forest soil warming has altered microbial growth-limiting elements, from C limitation to CP co-limitation. While N is supplied rapidly enough through SOM decomposition with warming, P supply via weathering and other pathways did not keep up. In contrast, abiotic sorption of P increased in warmed soils, decreasing soil P availability. Our results confirmed that C:N:P ratios of microbial biomass are non-homeostatic when the soil is susceptible to high P availability, irrespective of the native nutrient status of the soil. We also found that organic P mineralization was primarily triggered by microbial P demand, in both C limited and CP co-limited temperate forest soils.

Increased temperature together with concomitant decreases in soil moisture can remove fine, nutrient-rich particles such as clay (Reynolds et al., 2007) and increase soil sand content (Hou et al., 2018). However, the influence of long-term soil warming on soil mineralogy and the interaction between soil biotic and abiotic processes is understudied. Future climate change research should focus on integrating perspectives from broader scientific communities and exploring the interactions between multiple soil biogeochemical processes.

3.6 Supplementary material

Supplementary results

Labile C, N and P fractions after substrate addition

In topsoils, both microbial growth and respiration responses appeared more pronounced in heated than in control soils, however, substrates containing C decoupled microbial catabolism from growth in subsoils (higher respiration stimulation but less growth response in heated soils compared to control soils), except for the CNP treatment. This may be attributed to more severe nutrient limitation in heated subsoils and

a larger extent of microbial C limitation in control subsoils (Fig. 1). The labile C pool (DOC) was increased in all C amended soils (Table S3). In heated topsoils, DOC increased less as in control topsoils, likely due to stronger growth limitation of heated microbial communities that consumed DOC more effectively (Table S3). DOC increased the least in the CNP treatments where microbial C uptake was highest among all amendments (Table S3; Fig. 2). Inorganic N and P additions also increased the soil labile C pool (Table S3) which could be attributed to desorption effects of N and P on DOC mobilization.

At both soil depths, highest DON contents were found in glucosamine (CN_{org}) amended samples due to low mineralization of glucosamine (Fig. S1). On the other hand, glucosamine-6-P (CNP_{org}) was mineralized more efficiently as glucosamine as shown by higher nitrate and ammonium concentrations (Fig. S1; Table S1). P addition alone had no effect on nitrate concentration, but C addition alone and CP addition both decreased nitrate concentrations at both soil depths and temperatures (Table S1, $P<0.001$), likely due to N uptake for increased microbial growth.

At both soil depths, in all single or combined P treatments, phosphate fractions in heated soils were smaller than in control soils, and more P was routed to the non-recoverable P fraction. As indicated before, growth of microbes in heated soils was more susceptible to CP co-limitation, and thus more P in heated soils was expected to be immobilized by microbes, resulting in higher P_{mic}. However, no significant difference was found in P_{mic} between control and heated soils. Soil warming therefore seems to accelerate abiotic immobilization which was revealed by higher non-recoverable P fractions in heated soils across all treatments (Fig. S2).

Substrate addition effects on C_{mic}, N_{mic} and P_{mic}, and their stoichiometry

Substrates containing P all decreased C_{mic}:P_{mic} and N_{mic}:P_{mic} ratios at both temperatures and soil depths (Table S5). C_{mic}:N_{mic} ratio varied little under all substrate additions in topsoils. Single C addition led to larger C_{mic}:P_{mic} ratios ($P<0.001$) and C_{mic}:N_{mic} ratios ($P<0.01$) in control subsoils, while combined CN addition increased C_{mic}:N_{mic} ratios at both temperatures and increased C_{mic}:P_{mic} ratios in heated subsoils. Moreover, the only substrate without P which decreased C_{mic}:P_{mic} and N_{mic}:P_{mic} ratios was CN_{org}, which decreased microbial C:P ratios in heated top- and subsoils and microbial N:P ratios in heated subsoils (Table S5).

Only CNP additions had a significant positive effect on C_{mic} in heated topsoils ($P<0.01$, Table S5), where C_{mic} increased by a factor of 2.2 compared to no addition controls. C_{mic} in control subsoils was enhanced by C, N, CN and CP additions (Table S5). CN addition increased C_{mic} in heated subsoils by a factor of 2.08 ($P<0.001$), and CNP addition also had a positive effect on C_{mic} ($P<0.05$) in heated subsoils. N_{mic} concentrations remained stable upon all substrate additions. Except for CP-treated topsoils, CNP-treated control topsoils, CP_{org}- and CNP_{org} treated heated topsoils, substrates containing P either alone or in combination with other elements all increased P_{mic} concentration.

Supplementary figures

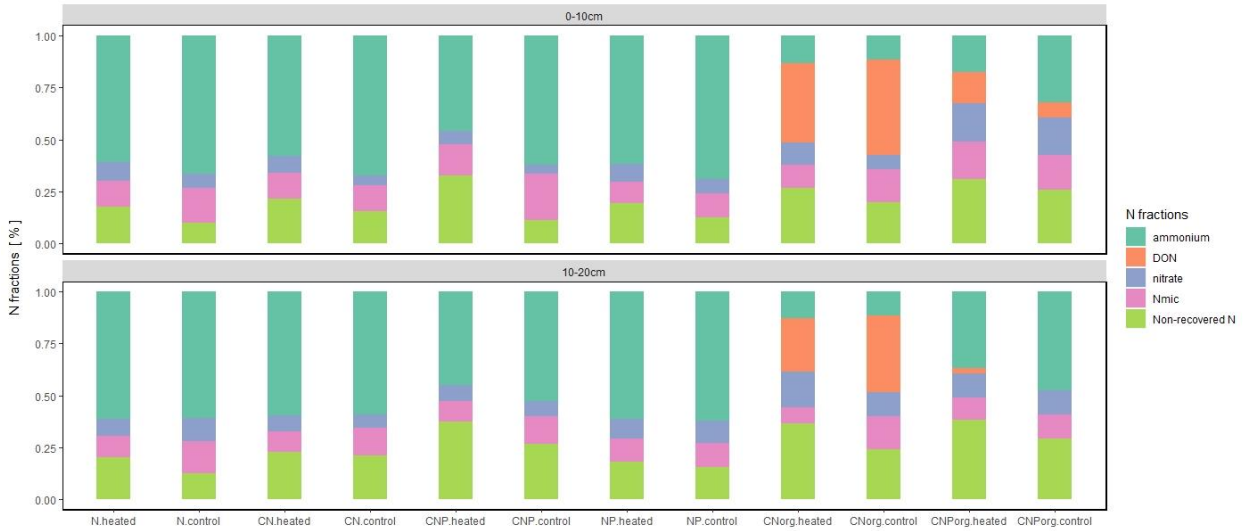

Figure S1 Labile soil N fractions expressed as a percentage of total labile soil N 24h after substrate addition at two mean field temperatures (control soils: 16°C; heated soils: 20°C) from two soil depths (0-10 cm; 10-20 cm).

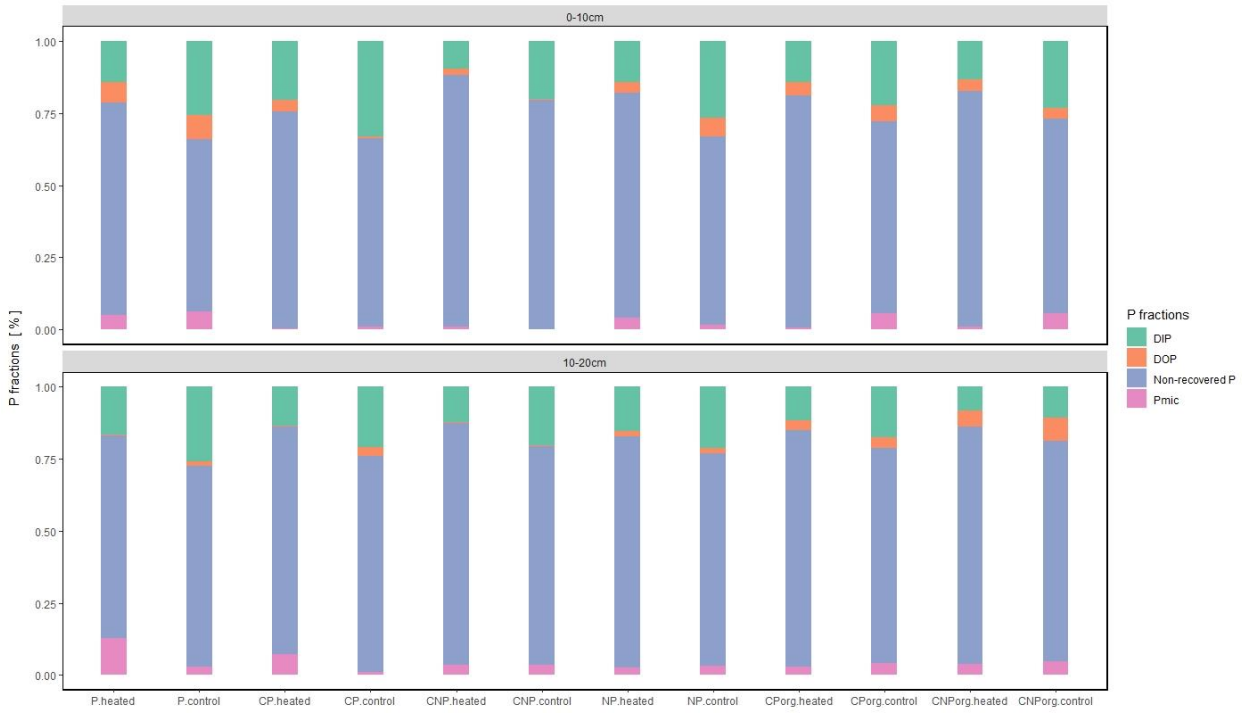

Figure S2 Labile soil P fractions expressed as a percentage of total labile soil P 24h after substrate addition at two mean field temperatures (control soils: 16°C; heated soils: 20°C) from two soil depths (0-10 cm; 10-20 cm).

Supplementary tables

a)

Treatment	Soil depth: 0-10 cm							
	Ammonium		Nitrate		N_{mic}		DON	
	Control	Heated	Control	Heated	Control	Heated	Control	Heated
No	6.3 ± 0.3	4.9 ± 0.2	31.2 ± 3	35.9 ± 0.9	122.3 ± 10.8	77.5 ± 18.6	7.3 ± 4.1	11.4 ± 8.5
C	6 ± 0.9	5.7 ± 0.6	13.5 ± 0.7	15.3 ± 1.7	124.4 ± 3.6	96.4 ± 27	0.4 ± 4.2	0 ± 7.5
N	449.4 ± 8.9 ***	391.1 ± 15.1 ***	46.5 ± 6.6 *	59.7 ± 12.6	124.6 ± 28.6	100.4 ± 28.7	0 ± 9.9	0 ± 14.8
P	4 ± 0.3	4.2 ± 0.3	34.8 ± 4.1	44.2 ± 6.8	109.8 ± 5.4	91.9 ± 22.5	12 ± 5.9	10.9 ± 7.2
CN	462.1 ± 2.8 ***	380 ± 8.2 ***	33.5 ± 1.1	58.6 ± 17.6	86.1 ± 23.4	87.4 ± 23.7	0 ± 5.6	0 ± 12.7
CP	6.2 ± 0.5	5.8 ± 1.5	3.9 ± 2.3 **(-)	0.3 ± 0.2 ***(-)	87.1 ± 15.3	86.3 ± 24.3	12.5 ± 4	19.6 ± 9.1
CNP	416.2 ± 4.6 ***	292.8 ± 8.6 ***	27.4 ± 4.2	40.1 ± 6.6	195.2 ± 99.9	87.9 ± 28.6	0 ± 14.8	0 ± 6.4
NP	471 ± 13.4 ***	407 ± 10.2 ***	46.5 ± 5.2	57.6 ± 8.4	76.4 ± 29.8	87.9 ± 54.7	0 ± 9.5	0 ± 46
Org.CN	72.1 ± 1.1 ***	78.4 ± 2.4 ***	43.2 ± 2.4	63.3 ± 16.5 **	99.8 ± 13.8	62.3 ± 12.2	289.5 ± 7.6	228.7 ± 10.9
Org.CP	10.7 ± 4.4	37.3 ± 4.3 ***	9.1 ± 5.3	3.7 ± 7.4 **(-)	142.2 ± 40.3	113.9 ± 6.9	7.7 ± 11.8	0 ± 8.9
Org.CNP	72.2 ± 6.9 ***	59.8 ± 8.7 ***	203 ± 15.6 ***	104.7 ± 22.6 ***	111.9 ± 20.7	111 ± 23.4	83.9 ± 32.2	139.8 ± 12.5

b)

Treatment	Soil depth: 10-20 cm							
	Ammonium		Nitrate		N_{mic}		DON	
	Control	Heated	Control	Heated	Control	Heated	Control	Heated
No	5.7 ± 1	6.3 ± 0.1	24.2 ± 0.7	24 ± 0.9	51.8 ± 5.2	47.7 ± 8.6	0 ± 1.3	0 ± 1.6
C	1.9 ± 0.2 *(-)	2.2 ± 0.1 *(-)	17.7 ± 1.5	9.8 ± 2.5 **(-)	66.5 ± 13	50.2 ± 5	4.8 ± 3.1	4.7 ± 1.7
N	236.8 ± 11.2 ***	223.6 ± 3.1 ***	44.2 ± 3.3 ***	30.1 ± 3.6	61.6 ± 12.3	35.2 ± 12.4	0 ± 10.6	0 ± 3.3
P	3.7 ± 0.4	4.1 ± 0.9	27.2 ± 4.4	19.9 ± 1.4	57 ± 3.8	45.4 ± 2.7	5.7 ± 5.7	7.7 ± 2.4
CN	236.6 ± 7.9 ***	218.4 ± 6.2 ***	34.8 ± 17.8	24.4 ± 6.4	40.8 ± 12.1	35.3 ± 8.9	0 ± 19.2	0 ± 9.9
CP	5.2 ± 1.2	3.5 ± 0.4	12.9 ± 1.5	1.6 ± 0.5 **(-)	43.2 ± 7.1	38.3 ± 7	4.3 ± 2.9	5.9 ± 1.7
CNP	193.9 ± 4.2 ***	159.5 ± 3.4 ***	26.2 ± 1.5	26.5 ± 4.4	48 ± 11.5	29.6 ± 17.1	0 ± 5.7	0 ± 0.8
NP	240.6 ± 4.8 ***	229.4 ± 10.2 ***	41.6 ± 2.8 ***	34.9 ± 4.7	52.2 ± 20	44.3 ± 13.9	0 ± 3.7	0 ± 6.7
Org.CN	38.5 ± 2 ***	41.5 ± 1 ***	38.4 ± 5.6 **	47.6 ± 18.6 ***	50.6 ± 19.1	27.8 ± 6.7	122.8 ± 2.9	90.2 ± 18.3
Org.CP	5.1 ± 0.7	4.8 ± 0.6	14.8 ± 2.1	3.9 ± 1.1 ***(-)	69.1 ± 4.5	68 ± 7.9	2.4 ± 4.3	0 ± 1.6
Org.CNP	35.7 ± 3.5 ***	38.3 ± 0.7 ***	182.3 ± 12 ***	118.6 ± 6.8 ***	44.6 ± 24.1	37.2 ± 5.6	0 ± 10.4	11.7 ± 3.6

*Table S1 Mean ± standard deviation (n=4) of labile soil N fractions at two mean field temperatures (control soil: 16°C; heated soil: 20°C) from two soil depths (0-10 cm; 10-20 cm) 24 h after nutrients were added: ammonium, nitrate, microbial biomass nitrogen (N_{mic}) and dissolved organic nitrogen (DON) in µg N g^{-1} dry weight. Significant differences compared to no addition controls are marked by asterisks (data for N_{mic} were log transformed). Levels of significance were * p < 0.05, ** p < 0.01, *** p < 0.001. (-) indicates significant negative effects of adding substrates.*

a)

Treatment	Soil depth: 0-10 cm					
	DOP		P_{mic}		Phosphate	
	Control	Heated	Control	Heated	Control	Heated
No	0.1 ± 0	0.1 ± 0	0.7 ± 0	0.5 ± 0	0.4 ± 0	0.4 ± 0
C	0.1 ± 0	0.1 ± 0	0.6 ± 0.2	0.6 ± 0.1	0.5 ± 0	0.4 ± 0
N	0.1 ± 0	0.1 ± 0	0.5 ± 0.1	0.3 ± 0	0.5 ± 0	0.5 ± 0
P	83.1 ± 18.7 ***	74.1 ± 2.4 ***	64.9 ± 22.7 ***	51 ± 11.8 ***	260.8 ± 22.2 ***	143.5 ± 3.8 ***
CN	0.1 ± 0	0 ± 0.1	0.6 ± 0.1	0.3 ± 0.1	0.5 ± 0	2.6 ± 0.179 ***
CP	5.7 ± 2.9	41.2 ± 26.8 ***	11.8 ± 10*	0 ± 11.3	335.4 ± 10.5 ***	209.6 ± 32.2 ***
CNP	0 ± 9.1	22.2 ± 4.2	0 ± 1.8	13.2 ± 6.4 *	205.7 ± 7.0 ***	98.7 ± 5.3 ***
NP	64.7 ± 12.9 ***	39.5 ± 5 ***	17.3 ± 7.1 **	42.7 ± 15.9 ***	271.1 ± 19.5 ***	144.2 ± 6.6 ***
Org.CN	0.1 ± 0	0.1 ± 0	1.4 ± 0.2	0.9 ± 0.1	0.5 ± 0	0.4 ± 0
Org.CP	57.3 ± 9.1 ***	48.6 ± 13.7 ***	70.6 ± 13.8 ***	0 ± 20.7	225.6 ± 44.6 ***	144.6 ± 3.5 ***
Org.CNP	37.6 ± 15.5 ***	42.5 ± 4.8 ***	57.9 ± 28 ***	11.8 ± 9.5 *	235.5 ± 8.2 ***	134.7 ± 6.9 ***

b)

Treatment	Soil depth: 10-20 cm					
	DOP		P_{mic}		Phosphate	
	Control	Heated	Control	Heated	Control	Heated
No	0.1 ± 0	0.1 ± 0	0.3 ± 0	0.2 ± 0	0.4 ± 0	0.3 ± 0
C	0.1 ± 0	0.1 ± 0	0.2 ± 0.1	0.3 ± 0	0.4 ± 0	0.4 ± 0
N	0.1 ± 0	0.1 ± 0	0.2 ± 0.1	0.2 ± 0.1	0.4 ± 0	0.4 ± 0
P	8.6 ± 4.9 ***	2.6 ± 2.4	16.8 ± 12 ***	70.2 ± 8.3 ***	142.8 ± 4.4 ***	91.5 ± 10.6 ***
CN	0.1 ± 0	0.1 ± 0	0.4 ± 0.1	0.1 ± 0	0.5 ± 0.1	0.4 ± 0
CP	17.2 ± 3.5 ***	0 ± 6.4	7.3 ± 1.2 ***	40.1 ± 11.5 ***	116.5 ± 6.5 ***	75.2 ± 4.7 ***
CNP	0 ± 4.4	0 ± 2.6	20.0 ± 7.8 ***	19.5 ± 12 ***	113.5± 2.4 ***	68.8 ± 6.3 ***
NP	9.6 ± 4.8 **	10.2 ± 8.9 **	22.8 ± 2.2 ***	18.2 ± 6.5 ***	118.3 ± 10.6 ***	84.9 ± 9.8 ***
Org.CN	0.1 ± 0	0.2 ± 0	0.8 ± 0.2	0.9 ± 0.3	0.4 ± 0	0.4 ± 0
Org.CP	20.5 ± 3.8 ***	17.5 ± 7.7 ***	23.4 ± 5.9 ***	15.3 ± 7.9 ***	97.3 ± 4 ***	65.5 ± 6.7 ***
Org.CNP	44 ± 10.2 ***	29.9 ± 9.1 ***	26 ± 5 ***	21.1 ± 6.1 ***	59.8 ± 7.8 ***	46.2 ± 4.8 ***

*Table S2 Mean ± standard deviation (n=4) of labile soil P fractions at two mean field temperatures (control soil: 16°C; heated soil: 20°C) from two soil depths (0-10 cm; 10-20 cm) 24 h after nutrients were added: dissolved inorganic phosphorus (DIP), organic phosphorous (DOP) and microbial biomass phosphorous (P_{mic}) in µg P g^{-1} dry weight. Significant differences compared to no addition controls are marked by asterisks (data for P_{mic} and phosphate were sqrt and log transformed, respectively). Levels of significance were * p < 0.05, ** p < 0.01, *** p < 0.001.*

Treatment	Soil depth: 0-10 cm				Soil depth: 10-20 cm			
	C_{mic}		DOC		C_{mic}		DOC	
	Control	Heated	Control	Heated	Control	Heated	Control	Heated
No	1079.9 ± 92.1	898.1 ± 41.5	85.7 ± 13.8	69.2 ± 12.6	409.6 ± 57	529.8 ± 87.8	118.7 ± 29.3	47 ± 12.1
C	1750 ± 592.8	1330.2 ± 514.5	1602.4 ± 191.8 ***	1371.1 ± 228.5 ***	1422.8 ± 392 ***	696.7 ± 92.4	258.8 ± 18.3	558 ± 11.9
N	857.9 ± 23.1	726.2 ± 100.5	414.9 ± 30.3	485.7 ± 282.6	854.6 ± 262.3 **	470.7 ± 35	179.4 ± 110.9	228.1 ± 21.2 *
P	897.5 ± 53.6	804.1 ± 79.6	273.7 ± 13.6	238.6 ± 27.1	639.8 ± 76.7	501.5 ± 34	394.4 ± 69.1 ***	260.7 ± 125.9 ***
CN	1079.1 ± 92.3	1199.7 ± 339.3	1897 ± 198.8 ***	1303.6 ± 144.1 ***	844.2 ± 340.2 ***	1104.6 ± 77.2 ***	953 ± 120 ***	602.1 ± 35.2 ***
CP	1471.2 ± 766.3	1566.5 ± 333.2	1810.7 ± 139.7 ***	1213.1 ± 301.9 ***	812.5 ± 34.8	561.5 ± 129.7	866.6 ± 43.3 ***	637.8 ± 30.3 ***
CNP	1490.7 ± 156.4	1996.7 ± 906 **	2289.3 ± 136.6 ***	1125.1 ± 188.3 ***	632.9 ± 51.8	894.9 ± 94.2 *	587.1 ± 19.8 ***	298.6 ± 35.3 ***
NP	812.9 ± 265.3	791.3 ± 49.4	261.7 ± 84.9	195.7 ± 19.6	533.3 ± 25	458.9 ± 93.5	239 ± 104.1	236 ± 31.3 **
Org.CN	1309.6 ± 274.2	646.7 ± 107.6	1983.5 ± 157.5 ***	1791.4 ± 40.8 ***	646.9 ± 214.4	375.3 ± 40.5	882.9 ± 12.3 ***	769.2 ± 15.5 ***
Org.CP	1372.2 ± 446.6	650.3 ± 99.9	1065.1 ± 173.2 ***	1102 ± 187.3 ***	524.2 ± 136.9 **	652.4 ± 94.1	500.9 ± 32 ***	271.7 ± 16.9 ***
Org.CNP	672.7 ± 247.9	984.3 ± 316.6	1873.1 ± 102.2 ***	1357.2 ± 34 ***	482.5 ± 146.7	418.3 ± 78.8	811.6 ± 45.2 ***	813.3 ± 101.3 ***

*Table S3 Mean ± standard deviation (n=4) of labile soil C fractions at two mean field temperatures (control soil: 16°C; heated soil: 20°C) from two soil depths (0-10 cm; 10-20 cm) 24 h after nutrients were added: microbial biomass C (C_{mic}) and dissolved organic carbon (DOC) in µg C g^{-1} dry weight. Significant differences compared to no addition controls are marked by asterisks (data for C_{mic} were log transformed). Levels of significance were * p < 0.05, ** p < 0.01, *** p < 0.001.*

a)

Treatment	Soil depth: 0-10 cm					
	C_{mic}		N_{mic}		P_{mic}	
	Control	Heated	Control	Heated	Control	Heated
No	1079.9 ± 92.1	898.1 ± 41.5	122.3 ± 10.8	77.5 ± 18.6	0.7 ± 0	0.5 ± 0
C	1750 ± 592.8	1330.2 ± 514.5	124.4 ± 3.6	96.4 ± 27	0.6 ± 0.2	0.6 ± 0.1
N	857.9 ± 23.1	726.2 ± 100.5	124.6 ± 28.6	100.4 ± 28.7	0.5 ± 0.1	0.3 ± 0
P	897.5 ± 53.6	804.1 ± 79.6	109.8 ± 5.4	91.9 ± 22.5	64.9 ± 22.7 ***	51 ± 11.8 ***
CN	1079.1 ± 92.3	1199.7 ± 339.3	86.1 ± 23.4	87.4 ± 23.7	0.6 ± 0.1	0.336 ± 0.1
CP	1471.2 ± 766.3	1566.5 ± 333.2	87.1 ± 15.3	86.3 ± 24.3	11.8 ± 10	0 ± 11.3
CNP	1490.7 ± 156.4	1996.7 ± 906 **	195.2 ± 99.9	87.9 ± 28.6	0 ± 1.8	13.23 ± 6.4 *
NP	812.9 ± 265.3	791.3 ± 49.4	76.4 ± 29.8	87.9 ± 54.7	17.3 ± 7.1 ***	42.7 ± 15.9 ***
Org.CN	1309.6 ± 274.2	646.7 ± 107.6	99.8 ± 13.8	62.3 ± 12.2	1.4 ± 0.2	0.9 ± 0.1
Org.CP	1372.2 ± 446.6	650.3 ± 99.9	142.2 ± 40.3	113.9 ± 6.9	70.6 ± 13.8 ***	0 ± 20.7
Org.CNP	672.7 ± 247.9	984.3 ± 316.6	111.9 ± 20.7	111 ± 23.4	57.9 ± 28 ***	11.8 ± 9.5

b)

Treatment	Soil depth: 10-20 cm					
	C_{mic}		N_{mic}		P_{mic}	
	Control	Heated	Control	Heated	Control	Heated
No	409.6 ± 57	529.8 ± 87.8	51.8 ± 5.2	47.7 ± 8.6	0.3 ± 0	0.2 ± 0
C	1422.8 ± 392 ***	696.7 ± 92.4	66.5 ± 13	50.2 ± 5	0.2 ± 0.1	0.3 ± 0
N	854.6 ± 262.3 **	470.7 ± 35	61.6 ± 12.3	35.2 ± 12.4	0.2 ± 0.1	0.2 ± 0.1
P	639.8 ± 76.7	501.5 ± 34	57 ± 3.8	45.4 ± 2.7	16.8 ± 12 ***	70.2 ± 8.3 ***
CN	844.2 ± 340.2 **	1104.6 ± 77.2 ***	40.8 ± 12.1	35.3 ± 8.9	0.4 ± 0.1	0.1 ± 0
CP	812.5 ± 34.8 **	561.5 ± 129.7	43.2 ± 7.1	38.3 ± 7	7.3 ± 1.2 **	40.1 ± 11.5 ***
CNP	632.9 ± 51.8	894.9 ± 94.2 *	48 ± 11.5	29.6 ± 17.1	20.0 ± 7.8 ***	19.5 ± 12 ***
NP	533.3 ± 25	458.9 ± 93.5	52.2 ± 20	44.3 ± 13.9	22.8 ± 2.2 ***	18.2 ± 6.5 ***
Org.CN	646.9 ± 214.4	375.3 ± 40.5	50.6 ± 19.1	27.8 ± 6.7	0.8 ± 0.2	0.9 ± 0.3
Org.CP	524.2 ± 136.9	652.4 ± 94.1	69.1 ± 4.5	68 ± 7.9	23.4 ± 5.9 ***	15.3 ± 7.9 ***
Org.CNP	482.5 ± 146.7	418.3 ± 78.8	44.6 ± 24.1	37.2 ± 5.6	26 ± 5 ***	21.1 ± 6.1 ***

*Table S4 Microbial biomass C in µg C g-1 dry weight, N in µg N g-1 dry weight and P in µg P g-1 dry weight at two mean field temperatures (control soils: 16°C, heated soils: 20°C) from two soil depths (0-10 cm, 10-20 cm) 24h after substrate addition. Values are given as mean with standard deviation (n=4), and significant differences compared to no addition controls are marked by asterisks. Levels of significance were * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.*

a)

Treatment	Soil depth: 0-10 cm					
	C/N ratio		C/P ratio		N/P ratio	
	Control	Heated	Control	Heated	Control	Heated
No	10.8 ± 1	12 ± 1	51.3 ± 2	61.9 ± 9.8	4.8 ± 0.3	5.2 ± 0.6
C	16.5 ± 6	11.8 ± 0.8	95.5 ± 55.5	60.6 ± 8.6	5.7 ± 1.8	5.1 ± 0.6
N	8.3 ± 1.6	8.7 ± 3.1	53.2 ± 10.2	68.6 ± 16.8	6.7 ± 2.3	8.3 ± 2.3
P	9.6 ± 0.7	10.5 ± 1.8	0.5 ± 0.2 ***	0.5 ± 0.1 ***	0.1 ± 0 ***	0.1 ± 0 ***
CN	14.5 ± 5.4	14.7 ± 4.8	49.3 ± 4.2	96.3 ± 5.2	3.7 ± 1.2	7.1 ± 2
CP	14.6 ± 8	15.8 ± 2.7	4 ± 1.3 ***	4.3 ± 1.9 ***	0.3 ± 0.2 ***	0.3 ± 0.1 ***
CNP	9 ± 7.4	16.2 ± 6.3	12.3 ± 1.3 ***	5.8 ± 2.6 ***	1.9 ± 1 ***	0.4 ± 0.2 ***
NP	9.1 ± 2.1	9.7 ± 5.8	1.4 ± 0.6 ***	0.5 ± 0.1 ***	0.2 ± 0 ***	0.1 ± 0.1 ***
Org.CN	10 ± 2.7	9 ± 2.8	27.7 ± 7.2	23.2 ± 4.2 *	2.8 ± 0.4	2.7 ± 0.8
Org.CP	9.1 ± 3.5	4.8 ± 0.7	0.6 ± 0.2 ***	1.7 ± 0.9 ***	0.1 ± 0 ***	0.4 ± 0.3 ***
Org.CNP	5 ± 1.6	7.4 ± 1.3	0.4 ± 0.2 ***	3.2 ± 1.6 ***	0.1 ± 0.1 ***	0.4 ± 0.2 ***

b)

Treatment	Soil depth: 10-20 cm					
	C/N ratio		C/P ratio		N/P ratio	
	Control	Heated	Control	Heated	Control	Heated
No	8.6 ± 0.8	13 ± 1.3	50.5 ± 7.6	83.1 ± 10.2	5.9 ± 0.7	6.4 ± 0.4
C	22.4 ± 5 **	16.4 ± 3.1	191.4 ± 51.7 ***	64.2 ± 9	8.7 ± 1.8	4 ± 0.5
N	14.2 ± 6.6	18.4 ± 2.3	140.2 ± 55.8 *	103.7 ± 36.2	10.1 ± 1.2	5.8 ± 2.4
P	13.2 ± 2.6	12.2 ± 1.3	1.4 ± 0.6 ***	0.2 ± 0 ***	0.1 ± 0 ***	0 ± 0 ***
CN	22 ± 6.1 **	39.3 ± 13.1***	86.2 ± 41.4	252 ± 47.4 **	3.8 ± 0.8	6.9 ± 2.3
CP	15.9 ± 2.2	12.4 ± 3.3	4.9 ± 1.2 ***	0.5 ± 0.3 ***	0.3 ± 0.1 ***	0 ± 0 ***
CNP	12.2 ± 1.5	33.8 ± 20.9	1.1 ± 0.3 ***	1.4 ± 0.4 ***	0.1 ± 0 ***	0.1 ± 0 ***
NP	7.4 ± 0.6	9.2 ± 2.8	0.7 ± 0.1 ***	1 ± 0.5 ***	0.1 ± 0 ***	0.1 ± 0.1 ***
Org.CN	12.6 ± 8.3	10.1 ± 0.9	26.6 ± 12	14.3 ± 6.7 ***	2.4 ± 1	1.4 ± 0.7 ***
Org.CP	7.1 ± 0.7	8.2 ± 2	0.9 ± 0.2 ***	1.8 ± 1.1 ***	0.1 ± 0 ***	0.2 ± 0.1 ***
Org.CNP	13.1 ± 7.7	9.5 ± 2.3	0.7 ± 0.1 ***	0.6 ± 0.2 ***	0.1 ± 0 ***	0.1 ± 0 ***

*Table S5 Molar ratios of microbial biomass C:N, C:P and N:P at two mean field temperatures (control soils: 16°C; heated soils: 20°C) from two soil depths (0-10 cm; 10-20 cm) 24 h after substrate addition. Values are given as mean with standard deviation (n=4), and significant differences compared to no addition controls are marked by asterisks. Levels of significance were * p < 0.05, ** p < 0.01, *** p < 0.001.*

		Microbial growth	Microbial respiration	Microbial C uptake	Microbial CUE
C	P	***	***	***	***
	F	858.1	21724.4	22038.2	112.2
N	P	*	***	***	***
	F	6.9	48.2	34.0	21.4
P	P	***	***	***	**
	F	171.5	1147.8	1214.0	10.8
Warming	P	*	***	***	n.s
	F	6.6	42.5	53.9	1.128
Depth	P	***	***	***	n.s
	F	44.8	99.8	145.0	2.6
C x N	P	***	n.s	n.s	***
	F	37.7	0.2	1.6	29
C x P	P	***	***	***	***
	F	49.1	140.1	99.7	93.4
C x N x P	P	***	***	***	***
	F	35.4	30.1	40.8	23.5
N x P	P	**	*	n.s	***
	F	10.6	5.1	0.2	15.2
C x Warming	P	*	**	***	n.s
	F	4.4	9.8	13.8	1.1
N x Warming	P	***	n.s	n.s	***
	F	15.3	0.0	0.6	14.7
P x Warming	P	***	***	***	***
	F	26.7	20.5	30.0	12.5
C x N x Warming	P	**	*	*	n.s
	F	7.4	4.2	6.5	3.4
C x P x Warming	P	***	*	**	***
	F	19.5	4.4	8.8	14.0
C x N x P x Warming	P	n.s	**	**	n.s
	F	0.1	8.7	9.8	0.1
C x Depth	P	*	n.s	n.s	n.s
	F	6.0	0.9	1.8	3.6
N x Depth	P	n.s	n.s	n.s	n.s
	F	0.9	0.1	0.6	1.0
P x Depth	P	n.s	***	***	**
	F	0.4	89.3	77.7	9.6
C x N x Depth	P	n.s	n.s	n.s	n.s
	F	0.7	0.1	0.9	0.4
C x P x Depth	P	**	***	**	***
	F	9.0	13.4	10.2	17.6
C x N x P x Depth	P	***	n.s	n.s	***
	F	16.6	1.3	0.1	18.3
Warming x Depth	P	***	n.s	***	**
	F	129.3	0.0	13.1	8.5

*Table S6 P and F values of 5-way ANOVA test. Data was log transformed to fit for normal distribution of residual and homogeneity of variance. Main effects of single factors and their interactive effects are displayed for C: carbon, N: nitrogen, P: phosphorous, warming and soil depth. Significance levels: ***. P < 0.001; **. P < 0.01; *. P < 0.05; n.s. not significant.*

		Microbial growth	Microbial respiration	Microbial C uptake	Microbial CUE
CN	P	***	***	***	***
	F	219.1	52.0	89.3	163.6
CP	P	***	***	***	***
	F	39.9	1939.9	1817.4	41.9
CNP	P	***	***	***	*
	F	267.9	4108.7	4046.4	5.3
Warming	P	**	***	***	n.s
	F	8.4	42.0	52.4	1.1
organic/inorganic	P	***	***	***	***
	F	193.2	5563.9	5450.0	65.2
Depth	P	***	***	***	n.s
	F	35.2	33.3	51.9	0.8
CN x Warming	P	***	*	**	***
	F	27.1	5.1	9.5	20.8
CP x Warming	P	**	n.s	*	**
	F	11.2	2.6	5.2	8.1
CNP x Warming	P	*	**	***	n.s
	F	6.2	10.7	14.3	2.1
CN x Depth	P	n.s	***	***	n.s
	F	3.5	28.3	27.2	0.1
CP x Depth	P	n.s	n.s	n.s	n.s
	F	1.1	2.0	3.3	0.3
CNP x Depth	P	n.s	*	*	n.s
	F	2.9	4.1	5.9	1.2
CN x organic/inorganic	P	n.s	***	***	**
	F	0.0	100.5	94.5	8.8
CP x organic/inorganic	P	***	***	***	n.s
	F	70.6	491.8	522.5	3.4
CNP x organic/inorganic	P	-	-	-	-
	F	-	-	-	-
Warming x Depth	P	***	n.s	***	n.s
	F	75.5	3.0	19.0	0.0
Warming x organic/inorganic	P	***	**	***	***
	F	27.7	10.1	16.9	18.5
Depth x organic/inorganic	P	*	***	***	n.s
	F	4.5	17.4	19.0	0.8

*Table S7 P and F values of 6-way ANOVA test. Data was log transformed to fit for normal distribution of residual and homogeneity of variance. Main effects of single factors and their interactive effects are displayed for CN: substrate addition containing C and N addition, CP: substrate addition containing C and P addition, CNP: substrate addition containing C, N and P, warming, depth and substrate quality: organic/inorganic. Significance levels: ***. P < 0.001; **. P < 0.01; *. P < 0.05; n.s. not significant.*

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