Soil Biology and Biochemistry 156 (2021) 108207

Contents lists available at ScienceDirect

Soil Biology and Biochemistry

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

Stoichiometric imbalance and microbial community regulate microbial elements use efficiencies under nitrogen addition

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ARTICLE INFO

Keywords:
Microbial carbon use efficiency
Nitrogen use efficiency
Global N deposition
Microbial community
Stoichiometry

ABSTRACT

Microbial elements use efficiencies are the important parameters in regulating soil carbon (C) and nitrogen (N) mineralization processes. Microbial C use efficiency (CUE) describes the proportion of C used for growth relative to the total organic C uptake. As such, high CUE values mean relatively less CO_2 emission and more C retention in microbial biomass. Similarly, a higher microbial N use efficiency (NUE) indicates efficient biomass N sequestration and less N mineralization. However, very little is known how the microbial CUE and NUE are affected by N enrichment in forest soils. Here, we studied soil microbial CUE and NUE simultaneously using ^{18}O-water tracer approach in a long-term N addition experiment comprising control (atmospheric N deposition, 2.7 g N m^{-2} yr^{-1}), low N addition (atmospheric N deposition + 2.5 g N m^{-2} yr^{-1}) and high N addition (atmospheric N deposition + 7.5 g N m^{-2} yr^{-1}) in a temperate forest. We found microbial CUE responses to N addition were dependent on N addition rates and soil horizons. Specifically, low N addition significantly increased the microbial CUE by 45.12% while high N addition significantly reduced it by 27.84% in organic soil. Further, mineral soil microbial CUE did not change under low N addition but significantly increased by 133.18% under high N addition. We also found microbial NUE decreased with increasing N addition rate in organic soil but showed an opposite pattern in mineral soil. The stoichiometric imbalances associated with phosphorus between microbial biomass and resources and the microbial community changes under N addition were correlated with microbial CUE and NUE. Further, N addition decreased microbial biomass turnover in organic soil but accelerated it in mineral soil. Altogether, our results indicated that N addition could control soil C and N cycling processes by affecting microbial elements use efficiencies (i.e. CUE and NUE), which may consequently impact C and N sequestration in this temperate forest soil.

1. Introduction

Reactive nitrogen (N) deposition on the Earth's surface is increasing since the industrial revolution (Vitousek et al., 1997; Galloway et al., 2008). Because N is an essential element that can limit the growth of living organisms in the terrestrial ecosystems (Vitousek and Howarth, 1991; Du et al., 2020), the increase of N deposition could alleviate N limitation and increase net primary productivity as well as carbon storage in some ecosystems (Quinn Thomas et al., 2009). However, excessive N input may cause some adverse ecological effects, such as reducing plant diversity (Bobbink et al., 2010), decreasing microbial biomass (Treseder, 2008), acidifying aquatic and terrestrial ecosystems (Tian and Niu, 2015). Importantly, the abundance and diversity of microbial communities could also be depressed under excess N deposition (Wang et al., 2018; Xia et al., 2020; Zhou et al., 2020), which may impact the fundamental community and ecosystem processes (Jones et al., 2014; Crowther et al., 2019). But how microorganisms will respond and adapt to the new N rich environment, for instance, by adjusting their activities and anabolic processes, is not well understood.

Microbial growth and elements use efficiencies have been considered as the critical physiological parameters in regulating soil carbon storage (Frey et al., 2013; Kallenbach et al., 2016; Liang et al., 2017). Since some recent studies indicated that a substantial proportion of soil organic carbon (SOC) is derived from microbial products (Miltner et al., 2012;

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https://doi.org/10.1016/j.soilbio.2021.108207
Received 12 October 2020; Received in revised form 27 February 2021; Accepted 2 March 2021
Available online 6 March 2021

Kallenbach et al., 2016; Wang et al., 2020), an increase in microbial biomass could potentially result in more C sequestration in the soil (Cotrufo et al., 2013). The partitioning of C consumed by soil microbes for growth and energy production can be described by carbon use efficiency (CUE) (Manzoni et al., 2012). Studies have shown that microbial communities with high CUE generally have lower C loss and more C converted to biomass, which is potentially beneficial for the storage of C derived from microbial necromass in soils (Kallenbach et al., 2016; Prommer et al., 2019). Some C cycling models that consider microbial CUE have shown better SOC prediction performance under global changes than the ones without this parameter (Allison et al., 2010; Frey et al., 2013).

In addition, microbial N use efficiency (NUE), which is also crucial for soil organic matter and nutrient cycling, has not been well studied (Mooshammer et al., 2014a; Zhang et al., 2019). Microbial NUE describes the proportion of organic N allocated to growth relative to acquired N (Mooshammer et al., 2014a). Based on the theory of ecological stoichiometry, the soil microbial biomass C:N ratio is generally constrained within a range to keep elemental homeostasis compared to the resources used for growth (Sterner and elser, 2002; Cleveland and Liptzin, 2007). Thus, microbial CUE and NUE are expected to interrelate to the microbial biomass C:N ratio ($B_{C:N}$) and the substrate C:N ratio ($S_{C:N}$) by the mass balance equation $B_{C:N} = S_{C:N}\frac{CUE}{NUE}$ (Mooshammer et al., 2014a; Takriti et al., 2018). This equation suggests that microbes may adjust their CUE, NUE or both when they encounter C or N limitation in soil substrates. However, microbial CUE and NUE may not necessarily change to the same extent because soil phosphorus or other elements could constrain microbial growth.

Changes in soil N availability through atmospheric N deposition could affect microbial CUE (Riggs and Hobbie, 2016; Spohn et al., 2016b; Widdig et al., 2020). Most studies found that increasing N enhanced microbial CUE which could be explained by several potential hypotheses (Fig. S1). First, decline in soil pH induces by N addition may decrease microbial respiration rate and thus increase microbial CUE if microbial biomass remains constant or has few changes (Silva-Sánchez et al., 2019). Second, microorganisms may need less C for metabolic costs associated with N acquisition under N addition, resulting in more C allocated to growth and increased microbial CUE (Manzoni et al., 2012). Third, the N addition can shift the abundance and composition of microbial communities, indirectly changing the microbial community CUE (Manzoni et al., 2012; Wang et al., 2018; Domeignoz-Horta et al., 2020; Xia et al., 2020). For example, soil microbial communities dominated by bacteria could have a higher CUE than ones dominated by fungi (Silva-Sánchez et al., 2019; Soares and Rousk, 2019). Since N addition may increase the proportion of bacteria in soil microbial community (Zhang et al., 2018), the community CUE could increase due to the changes in microbial community structure. Further, bacterial taxa have significant CUE variations, and the changes in each taxon's relative abundance in bacterial community under N addition may shift the community CUE (Yang et al., 2019; Pold et al., 2020).

Moreover, the N addition to the soil could greatly change soil N mineralization process (Li et al., 2019), possibly affecting microbial NUE and microbial N retention. Since soil microorganisms are generally believed to be C limited for their growth based on the threshold element ratio (TER) concept (Soong et al., 2019), microbial NUE is expected to be unchanged or reduced under N addition, and more inorganic N would be exuded to the soil environment (Mooshammer et al., 2014a). Studies have also suggested that soil phosphorus cycling is changed by N addition (Deng et al., 2017), which could alter the C:N:P stoichiometry of soil substrates. Suppose microbial communities require strict homeostasis. In that case, a possible way to cope with the stoichiometric differences in soil substrates (i.e. stoichiometric imbalance) under N addition is by adjusting microbial elements use efficiencies, which is expected to cause a strong correlation between the extent of microbial elements imbalance and microbial elements use efficiencies. In summary, there are still open questions on the changes of microbial CUE and NUE under N addition as well as the underlying mechanisms.

Here we used a long-term N deposition experiment in a temperate forest ecosystem, which has been running for six years, to study the effects of N addition on soil microbial CUE and NUE. We analyzed the soil microbial community composition, enzyme activities, microbial biomass elemental stoichiometric imbalances under N addition, and their relationships with CUE and NUE in organic and mineral soils. We hypothesized that i) soil microbial CUE in this temperate forest will increase under N addition, while NUE will not change or decrease slightly, ii) due to the differences in microbial community composition and nutrient condition between organic and mineral soils, the responses of microbial CUE and NUE to N addition in these two layers should be different, and iii) microbial community structure and elemental stoichiometric imbalances of microbial biomass would covary with the changes in microbial CUE and NUE.

2. Materials and methods

2.1. Study site and experimental design

The N addition experiment was established in 2014 in a natural Korean pine and broadleaf mixed forest in Jilin Province, northern China (42.70° N,127.63° E). This region is characterized by a typical temperate monsoon climate, and climatic records from a weather station near the study site show that the mean annual precipitation and air temperature were 750 mm and 4 °C in 2018, respectively. The long-term N addition experiment consists of nine experimental plots, each with an area of 2500 m^2 (50 m by 50 m) and a buffer zone between them of at least 20 m. The numbers of species within the 9 plots vary from 20 to 24, and the predominant coniferous species were *Abies holophylla Maxim* and *Pinus koraiensis,* and the broad-leaved species are *Acer barbinerve*, *Corylus mandshurica Maxim, Acer pseudosieboldianum, and Tilia amurensis Rupr* in those plots. The soil type in all plots was dark brown soil developed from volcanic ash (Albic Luvisol); soil pH varied from 5.1 to 5.3. The averaged sand, silt and clay content of the studied soil were 16.9%, 35.6% and 47.5%, respectively (Table S1). Each of these plots (n = 3) was randomly assigned to the following treatments: control (0 g N m^{-2} yr^{-1}), low N addition (2.5 g N m^{-2} yr^{-1}) and high N addition (7.5 g N m^{-2} yr^{-1}). The current N deposition rate in this study region is ~2.7 g N m^{-2} yr^{-1}, which means that the quantity of low N addition and high N addition is equivalent to about one-fold and three folds of the atmospheric N deposition rate, respectively. Urea was applied as the N fertilizer and it was spread on the soil surface in the treatment plots one time in May or June in each year.

Soil samples were collected in August 2019 after six years of the experimental treatment. Soil from organic horizon and mineral horizon (0–10 cm) was collected after removing the plant litter on the soil surface. Twenty random soil samples were collected using a soil corer (5 cm diameter, volume = 196.25 cm^3) and were homogenized manually and mixed as one composite sample. The soil was transported to the laboratory in the incubator with ice bags. The soil was then sieved through a 2 mm sieve and stored in a refrigerator under 4 °C until microbial measurements took place (less than one week). The soil was divided into two parts: one subsample was air-dried at room temperature for soil physical and chemical properties analyses and the other one was used for soil microbial community composition and elements use efficiencies (CUE and NUE) analyses.

2.2. Soil physical and chemical properties

Soil water content was measured gravimetrically after oven drying fresh soil in aluminum dishes for 24 h at 105 °C. Soil pH was measured at a ratio of fresh soil to water ratio of 1:2.5 (w:v) by a pH electrode (Leici, Shanghai, China). Soil organic carbon (SOC) and total nitrogen (TN) were determined by an elemental analyzer (Elementar Analysis system,

Germany). Soil total P (TP) content was extracted by sulfuric acid and perchloric acid digestion and then determined by an automated discrete analyzer (SmartChem140, AMS, Italy).

2.3. Microbial biomass and enzymes

Soil microbial biomass C (MBC) and N (MBN) were determined by the fumigation extraction method described by Vance et al. (1987) and Brookes et al. (1985), respectively. Dissolved organic C (DOC) and dissolved nitrogen (DN) were extracted in 0.5 M K_2SO_4 from fumigated and non-fumigated soil samples. The conversion factor used to calculate the MBC and MBN was 0.45 (Wu et al., 1990) and 0.54 (Brookes et al., 1985), respectively. Soil microbial biomass P (MBP) was determined by the fumigation extraction method described by Brookes et al. (1982) and the dissolved phosphorus (DP) was extracted in 0.5 M $NaHCO_3$ from fumigated and non-fumigated soil samples. A conversion factor used to calculate the MBP was 0.40 (Brookes et al., 1982). The C, N and P concentrations in non-fumigated extraction were considered as soil DOC, DN and DP, respectively. The DOC and DN concentrations were analyzed on a TOC analyzer (TOC-L CPH, Shimadzu, Japan) and the concentration of DP was determined using an automated discrete analyzer (Smartchem 140, AMS, Italy).

The activities of β-glucosidase (BG, hydrolysis of cellulose), β-N-acetylglucosaminidase (NAG, hydrolysis of chitooligosaccharides), leucine aminopeptidase (LAP, cleaving of peptide bonds in proteins) and acid phosphatase (AP, cleaving of PO_4 from P-containing organic matter) were measured as described by Cenini et al. (2015). Sample suspensions were prepared for all enzymes by adding fresh soil 2 g to 125 ml sodium acetate buffer (50 mM, pH = 5.0) for the extraction of enzymes. The sample suspensions were placed on a magnetic stirrer and stirred 1 min to make slurries. BG activity was measured using 4-MUB-β-D-glucoside as the substrate, NAG activity was assayed using 4-MUB-N-acetyl-β-D-glucosaminide as the substrate, LAP activity was tested using L-Leucine-7-amido-4-methylcoumarin hydrochloride as the substrate, and AP activity was quantified using 4-methylumbelliferyl phosphate as the substrate. The reactions were terminated with 10 μl NaOH (1 M), and fluorescence was measured using a Microplate Reader (SynergyH1, USA) set at 365 nm excitation and 450 nm emission. We calculated enzymatic activity as the rate of substrate converted in nmol g^{-1} dry soil h^{-1}. The stoichiometry of enzymatic activity was calculated to reflect the equilibria between the elemental composition of microbial biomass and organic matter (Sinsabaugh et al., 2009). The ratios of C:P, C:N and N:P acquisition enzymes activity were indicated by ratios of BG: AP, BG: (LAP + NAG) and (LAP + NAG): AP, respectively.

2.4. Microbial community analysis

Soil DNA was extracted from 250 mg freeze-dried soil using a Mobio PowerSoil DNA Isolation Kit (MoBio Laboratories, Carlsbad, USA) according to the manufacturer's instructions. The quantity and quality of extracted DNA were estimated by a Nanodrop Spectrophotometer (Thermo Fisher Scientific, USA). The primer pairs 515F(5′- GTG CCA GCM GCC GCG GTA A -3′)/806R(5′- GGA CTA CHV GGG TWT CTA AT -3′) and ITS1(5′- CTT GGT CAT TTA GAG GAA GTA A -3′)/ITS2(5′- TGC GTT CTT CAT CGA TGC-3′) with 8-bp barcodes at the 5′-end of them were used to amplify the V4–V5 region of bacterial 16S rRNA genes, and the ITS1 region of fungal ITS genes, respectively. PCR amplification was conducted in triplicate 25 μl mixtures, which contained 12.5 μl of 2 × Taq Plus Master Mix, 1 μl of 5 μM of each primer, 3 μl of 2 ng μl^{-1} BSA, 30 ng of template DNA and ddH_2O filled to 25 μl. The high-throughput sequencing was performed on an Illumina Miseq system (Illumina, San Diego, CA, USA) at Allwegene technology company (Beijing, China). The detailed analytical processes of microbial community can be found in Xia et al. (2020). The sequencing data of soil bacteria and fungi have been deposited on the figshare (https://doi.org/10.6084/m9.figshare.13040756.v1).

2.5. Microbial growth and respiration

We applied the $^{18}O–H_2O$ tracer incubation approach to determine soil microbial growth rate (Spohn et al., 2016a; Zheng et al., 2019). Briefly, fresh soil (10 g) for each sample was pre-incubated under 60% of water holding capacity (WHC) at 15 °C for 24 h. After pre-incubation, duplicates of each soil sample (1 g) were placed in 2 ml screw sample vials. For the half of soil replicates, 50–60 μl ^{18}O-labeled water (97.0 atom% ^{18}O) were injected into soil to adjust soil final water atom% ^{18}O to 20. For the other half, Milli-Q water was injected with the same volume as the $^{18}O–H_2O$ added in the treatments. The screw vials were transferred into 20 ml headspace vials, capped, and flushed with CO_2-free air for 5 min and then incubated at 15 °C for 24 h.

After 24 h, the CO_2 production (soil respiration, Rs, ng C g^{-1} h^{-1}) in 20 ml headspace vials was measured by gas chromatograph (GC-7890B, Agilent, USA). Then the screw vials containing soil were retrieved and capped, immediately frozen in a lyophilizer. Total soil DNA was extracted using a DNA extraction kit (MoBio, Powersoil) following the manufacturer's procedures. The DNA concentration was determined by the Picogreen fluorescence assay (PicoGreen, Thermo Fisher) using a Microplate spectrophotometer (Infinite M200, Tecan, Austria). The remaining DNA extract was dried in a silver capsule at 45 °C for 5 h to remove water. Subsequently, the ^{18}O abundance and the total O content were measured using an IRMS-TC/EA (Thermo Scientific, TX, USA).

The rate of C (C_{growth}) and N (N_{growth}) uptake for microbial growth were calculated according to the production of DNA during soil incubation time (Spohn et al., 2016b; Zheng et al., 2019). The DNA production ($DNA_{produced}$, μg) was calculated based on the difference in ^{18}O-DNA between labeled and unlabeled soil samples:

$$DNA_{produced} = O_{total} * \frac{at\%_{excess}}{100} * \frac{100}{at\%_{final}} * \frac{100}{31.21} \quad (1)$$

where O_{total} is the total O content (μg) of the dried DNA extract, $at\%_{excess}$ is the difference between at% ^{18}O of the labeled sample and at% ^{18}O of the non-labeled sample, and 31.21 is the average percentage of O in DNA ($C_{39}H_{44}O_{24}N_{15}P_4$). The $at\%_{final}$ is the ^{18}O atom % of soil water at the beginning of incubation (20% in this study). Here we assumed the O in new DNA only derived from water (Qu et al., 2020). Because of the short incubation time, the mortality of newly produced ^{18}O-labeled microbial cells is negligible in the study.

Then microbial C_{growth} rate (ng C g^{-1} h^{-1}) and N_{growth} rate (ng N g^{-1} h^{-1}) were calculated by multiplying the $DNA_{produced}$ and a conversion factor ($f_{DNA\text{-}MBC}$ and $f_{DNA\text{-}MBN}$) as the following (Zhang et al., 2019; Zheng et al., 2019):

$$C_{growth} = \frac{f_{DNA-MBC} \times DNA_{produced} \times 1000}{DW \times t} \quad (2)$$

$$N_{growth} = \frac{f_{DNA-MBN} \times DNA_{produced} \times 1000}{DW \times t} \quad (3)$$

The $f_{DNA\text{-}MBC}$ was calculated at each specific sample to represent the ratio of soil MBC to soil DNA content while $f_{DNA\text{-}MBN}$ was calculated at each specific sample to represent the ratio of soil MBN to soil DNA content. Additionally, microbial respiration rate (Rs, ng C g^{-1} h^{-1}) was calculated according to the following equation:

$$Rs = \frac{R_{con}}{DW*t} * \frac{Pre*n}{R*T} *V*1000 \quad (4)$$

where Pre is the atmosphere pressure (kPa), *n* is the molecular mass of the element C (12.01 g mol^{-1}), R is the ideal gas constant (8.314 J mol^{-1} K^{-1}), and T is the absolute temperature of the gas (295.15 K). V is the headspace volume (L) of the vials. R_{con} (ppm) is the CO_2 concentration produced during the 24 h incubation period.

2.6. Microbial gross N mineralization

Soil microbial gross N mineralization (GNM) was estimated using the isotope pool dilution technique (^{15}N-IPD) as modified by Wanek et al. (2010). To determine GNM, duplicate of 20 g (dry weight) soil samples were added into polypropylene vials pre-incubated under 60% of water hold capacity (WHC) at 15 °C for 24 h, then evenly dropwise labeled with 1 ml $^{15}NH_4NO_3$ (10 atom% ^{15}N), and the vials were incubated at 15 °C in an incubator. The soil incubation was terminated after 0.5 and 24 h, respectively, and soils were extracted using 100 ml of 2M KCl. The N content in extraction was determined using an automated discrete analyzer (SmartChem140, AMS, Italy) and isotope composition in extraction was determined using the micro-diffusion method. The GNM (ng N g^{-1} h^{-1}) was calculated according to the following equation:

$$GNM = \frac{C_{t_1} - C_{t_0}}{t_1 - t_0} \times \frac{\ln\left(\frac{APE_{t_0}}{APE_{t_1}}\right)}{\ln\left(\frac{C_{t_1}}{C_{t_0}}\right)} \quad (5)$$

where C_{t0} and C_{t1} are the N concentration in soil extraction at t_0 (0.5 h) and t_1 (24 h), respectively. APE_{t0} and APE_{t1} are ^{15}N atom percent excess (%) in soil extraction at t_0 (0.5 h) and t_1 (24 h), respectively.

2.7. Microbial CUE, NUE and turnover calculation

Soil microbial community CUE was calculated as the ratio of microbial C_{growth} over microbial total C uptake rate (C_{growth} + Rs) and microbial community NUE was calculated as the ratio of microbial N_{growth} over microbial organic N uptake rate (N_{growth} + GNM). Both of CUE and NUE are dimensionless and within the range 0–1.

$$CUE = \frac{C_{growth}}{C_{uptake}} = \frac{C_{growth}}{C_{growth} + R_s} \quad (6)$$

$$NUE = \frac{N_{growth}}{N_{uptake}} = \frac{N_{growth}}{N_{growth} + GNM} \quad (7)$$

Microbial biomass turnover rate (yr^{-1}) was calculated using the following equation:

$$Turnover = \frac{DNA_{produced} * 24}{DNA_{content} * t} * 365 \quad (8)$$

where $DNA_{content}$ is the DNA content (μg) in each soil and t is incubation time in hours.

2.8. Statistical analysis

All data were transformed to meet model assumptions when necessary. $Imbalance_{SOM}$ was calculated as the C:N (N:P or C:P) ratio of soil over C:N (N:P or C:P) ratio of the microbes. $Imbalance_{extr}$ was calculated as the C:N (N:P or C:P) ratio of the extractable soil fraction over the C:N (N:P or C:P) ratio of the microbes. A two-way ANOVA was used to test the effects of N addition rates, soil horizons and their interactions on soil elemental concentration, stoichiometry, and microbial parameters (enzymes, microbial growth, R_S, GNM, CUE and NUE). Pearson's correlation was used to assess the relations between microbial processes and soil elemental stoichiometry as well as microbial elemental stoichiometry. A redundancy analysis (RDA) was conducted to elucidate the relationships between the soil microbial community and chemical properties in the different N treatments. In order to test the relationship between microbial community and microbial CUE (or NUE), distance matrix for microbial community and CUE (or NUE) were calculated using Bray-Curtis dissimilarity and Euclidean distance, respectively. Then the Mantel test was used to analyze the correlation between microbial community matrix and the CUE and NUE matrices (Delgado-Baquerizo et al., 2018).

3. Results

3.1. Microbial elements use efficiencies and turnover

Microbial respiration rate in the organic soil decreased significantly under low N addition relative to control, but it was unchanged in mineral soil (Fig. 1a). High N addition depressed microbial growth in organic soil but greatly increased microbial growth in mineral soil (Fig. 1b). Consequently, microbial CUE did not show any consistent effects with increasing N addition rates, significantly increasing under low N addition but decreasing under high N addition in organic soil (Fig. 1c). In mineral soil, the highest CUE was found under the high N addition (Fig. 1c).

Gross N mineralization rate (GNM) in organic soil didn't show any consistent effects with increasing N addition (Fig. 1d). But GNM was clearly declined under high N addition in mineral soil (Fig. 1d). Besides, the high N addition significantly slowed down the rate of N uptake for microbial growth in organic soils but increased it in mineral soils (Fig. 1e). Further, microbial NUE decreased from 0.65 under the control to 0.49 and 0.35 under low and high N addition in organic soil, respectively. Microbial NUE significantly increased in the mineral soil by 297.76% under high N addition (Fig. 1f).

Considering the organic and mineral soil together, there was a polynomial correlation between microbial CUE and NUE with the maximum NUE at CUE = 0.40 (Fig. 2). Moreover, we found that microbial CUE significantly decreased with microbial biomass-specific respiration rate (qCO_2) in organic soil and positively correlated with microbial biomass-specific growth C (qGrowth C) in mineral soil (Fig. 3). Additionally, microbial NUE showed a positive correlation with qGrowth N and a negative correlation with microbial biomass-specific gross N mineralization (qGNM) in organic and mineral soils (Fig. 3).

We calculated the threshold elemental ratio ($TER_{C:N}$) of microbial growth, which indicates the elemental ratio that reflects when an ecological process changes from C limitation to N limitation. In organic soil, the $TER_{C:N}$ declined under N addition but increased in mineral soil under high N addition (Fig. 4). Moreover, microbial biomass turnover significantly decreased under high N addition in organic soil, while it significantly increased under both low and high N addition in mineral soil (Fig. 5).

3.2. Elements in microbial biomass and soil

Soil pH decreased by 0.4 and 0.5 units under low and high N addition in organic soil, respectively, but it was not changed in mineral soil (Table S2). Soil organic C and TN were not significantly affected by the treatments compared with the control (Table S2). However, N addition significantly increased soil TP concentration under low N addition (Table S2), and consequently the TN:TP ratio declined significantly in this horizon. Moreover, soil DOC and DN were higher under high N addition than control in mineral soil, but organic samples did not show any consistent effects with increasing N addition rates. Nitrogen addition did not affect soil DOC:DN, DOC:DP and DN:DP ratios in the present study (Table S2).

Soil MBC and MBN declined significantly in organic soil under high N addition, and they also declined under low N addition in mineral soil (Table S3). Soil MBP had no change under N addition in organic and mineral soils. MBC:MBP ratio and MBN:MBP ratio, thus, significantly decreased under high N addition in organic and mineral soils (Table S3). LAP activity declined from 1.01 nmol h^{-1} g^{-1} under the control to 0.55 and 0.25 nmol h^{-1} g^{-1} under low and high N addition in mineral soil, respectively. Besides, AP activity increased by ~46% under low N addition relative to the control in organic soil (Table S3).

3.3. Microbial stoichiometric imbalance

We found that stoichiometric imbalances of C:P or N:P between SOM

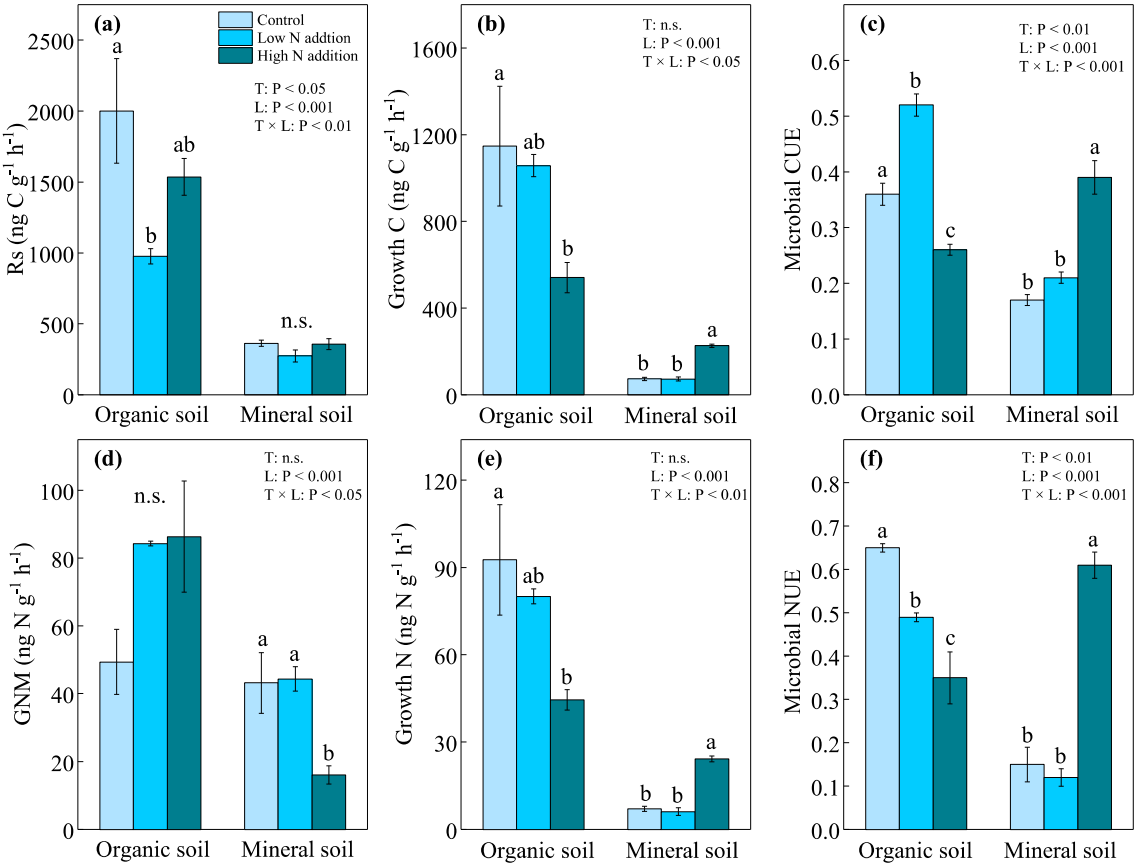

Fig. 1. Effects of N addition on (**a**) microbial respiration rate (Rs, ng C g^{-1} h^{-1}), (**b**) microbial growth rate (ng C g^{-1} h^{-1}), (**c**) microbial carbon use efficiency (CUE), (**d**) microbial gross N mineralization rate (GNM, ng N g^{-1} h^{-1}), (**e**) microbial growth rate (ng N g^{-1} h^{-1}), and (**f**) microbial nitrogen use efficiency (NUE) in organic and mineral soils. The effects of treatment (T), soil layer (L), and their interaction (T × L) are shown. Lower-case letters indicate significant differences between treatments tested separately for each soil ($P < 0.05$). Data are presented as the mean and standard error (n = 3). n.s, not significant.

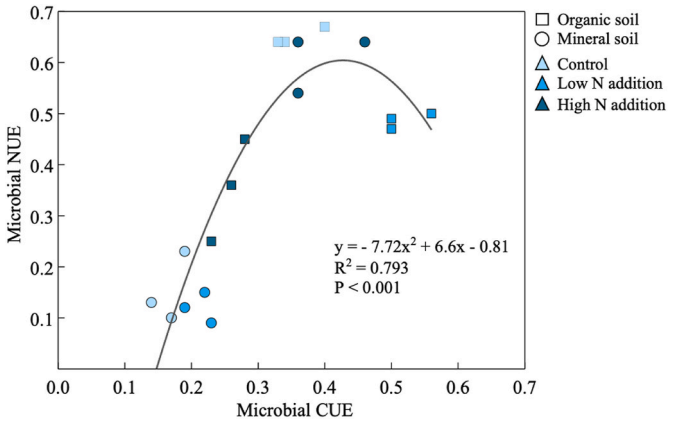

Fig. 2. Polynomial correlation between soil microbial carbon use efficiency (CUE) and microbial nitrogen use efficiency (NUE) across different nitrogen addition rates and soil layers.

and microbial biomass (imbalanceCP_{SOM} or imbalanceNP_{SOM}) was higher under high N addition than that under low N addition in organic soil (Table 1). This pattern occurred in mineral soil although the differences were not statistically significant. Moreover, high N addition increased imbalances of C:P or N:P between extractable soil fraction and microbial biomass (imbalanceCP_{extr} or imbalanceNP_{extr}) in organic soil. In mineral soil, high N addition significantly increased imbalanceCN_{extr} and imbalanceCP_{extr} (Table 1).

3.4. Microbial community composition

The redundancy analysis (RDA) plot revealed that the bacterial community structure in mineral soil was shifted marginally under N addition (Fig. S2, PERMANOVA test, $R^2 = 0.38$, $P = 0.08$). Specifically, the bacterial community under high N addition was marginally different from that under low N addition, and the RDA 1 accounted for 49.59% and the RDA 2 for 9.79% of the variations. Soil N:P, C:P and DN:DP ratios were the most important three variables in regulating bacterial community under N addition in mineral soil. However, the N addition did not change bacterial community in organic soil. Further, the fungal community structure was significantly changed under N addition in mineral soil (Fig. S3, PERMANOVA test, $R^2 = 0.34$, $P < 0.05$), and the fungal communities under the high N addition and control treatments were generally separated into two groups along RDA 1, which can account for 19.85% of the variances. Moreover, fungal diversity was marginally affected by N addition in organic soil ($R^2 = 0.31$, $P = 0.08$). The dominant bacteria phyla did not change in organic soil under N addition, but the relative abundance of Proteobacteria, Acidobacteria and Actinobacteria in mineral soil was altered (Fig. S4). In the fungal communities, high N addition significantly increased the relative abundance of dominant fungal class of Mortierellomycetes in organic and mineral soils (Fig. S5).

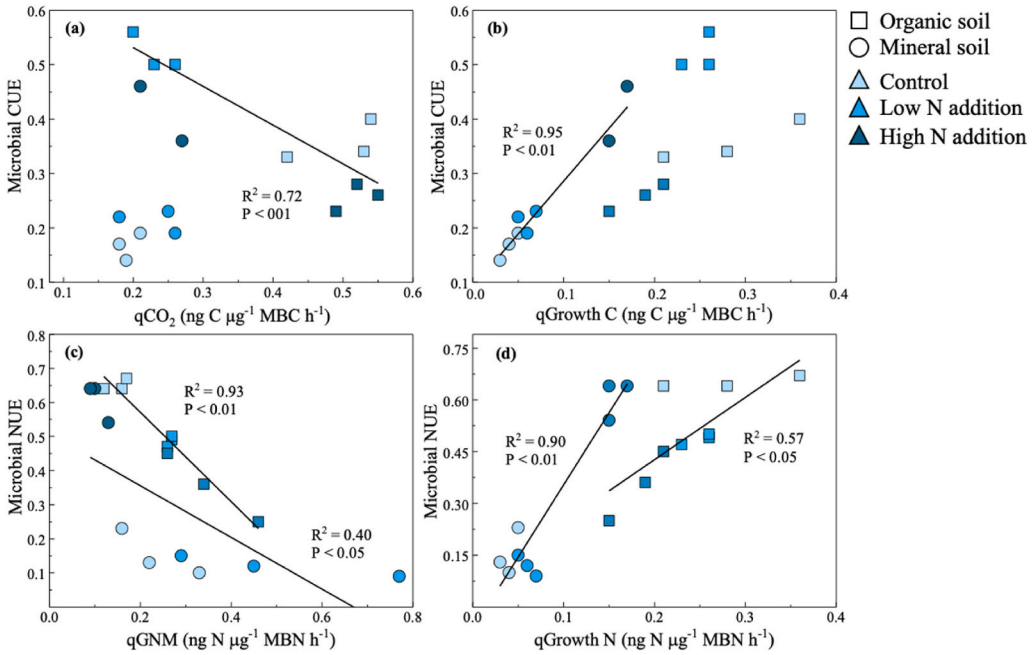

Fig. 3. Regressions between (**a**) microbial CUE and microbial biomass-specific respiration rate (qCO_2, ng C μg^{-1} MBC h^{-1}), (**b**) microbial CUE and microbial biomass-specific growth (qGrowth C, ng C μg^{-1} MBC h^{-1}), (**c**) microbial NUE and microbial biomass-specific gross N mineralization (qGNM, ng N μg^{-1} MBN h^{-1}), and (**d**) microbial NUE and microbial biomass-specific growth (qGrowth N, ng N μg^{-1} MBN h^{-1}) in organic and mineral soils.

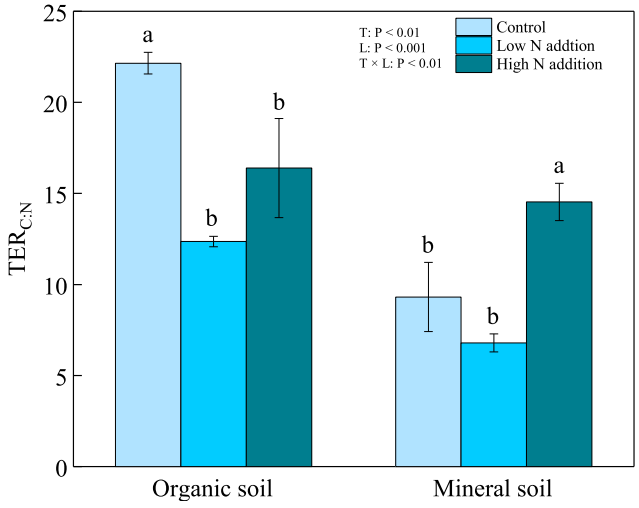

Fig. 4. Effects of N addition on the threshold elemental ratio ($TER_{C:N}$) in organic and mineral soils. The effects of treatment (T), soil layer (L), and their interaction (T × L) are shown. Lower-case letters indicate significant differences between treatments tested separately for each soil ($P < 0.05$). Data presented are mean and standard error (n = 3).

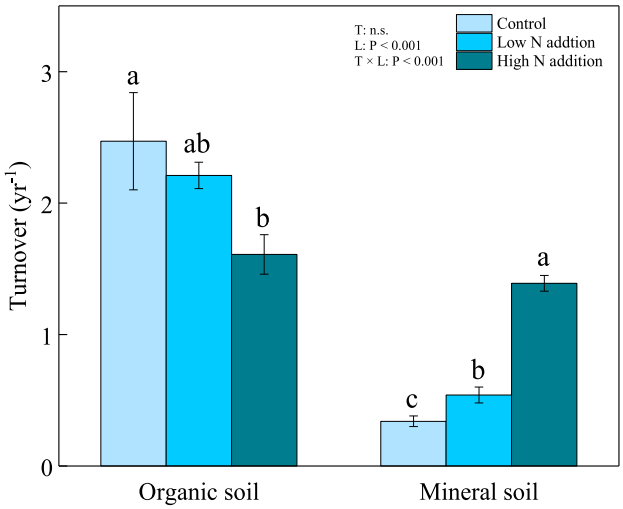

Fig. 5. Effects of N addition on microbial turnover in organic and mineral soils. The effects of treatment (T), soil layer (L), and their interaction (T × L) are shown. Lower-case letters indicate significant differences between treatments tested separately for each soil ($P < 0.05$). Data are presented as the mean and standard error (n = 3).

3.5. *Influencing factors for microbial elements use efficiencies*

In organic soil, microbial CUE increased with increasing MBC:MBP ratio and increasing MBN:MBP ratio, but decreased with imbalanceC-P_{SOM}, imbalanceNP_{SOM}, imbalanceCP_{extr}, and imbalanceNP_{extr} (Table 2). RDA analysis also showed a similar pattern, and the RDA1 can explain 66.7% and 95.68% of the variances in CUE and NUE for organic layer and mineral layer, respectively (Fig. S6). Additionally, a positive correlation between organic soil microbial CUE and the fungal community was observed. In mineral soil, microbial CUE decreased with increasing MBC:MBP ratio, but increased with increasing BG:(NAG + LAP) ratio and imbalanceCP_{extr}. Further, bacterial community composition positively impacted microbial CUE in mineral soil (Table 2). There was no correlation between soil, microbial, and enzyme activity stoichiometry, stoichiometric imbalances, and microbial NUE in organic soil (Table 2). In mineral soil, microbial NUE was negatively correlated with the MBC:MBP ratio but positively correlated with imbalanceCP_{SOM}, imbalanceCN_{extr}, imbalanceCP_{extr}, and bacterial community composition (Table 2).

Table 1
Microbial element stoichiometric imbalance under N addition in organic and mineral soils. The effects of treatment (T), soil layer (L), and their interaction (T × L) are shown in the right columns. $Imbalance_{SOM}$ was calculated as the ratio of CN (NP or CP) of soil over CN (NP or CP) of microbe; $Imbalance_{extr}$ was calculated as the ratio of CN (NP or CP) of extractable soil fraction over CN (NP or CP) of microbe. Bold lower-case letters indicate significant differences between treatments tested separately for each soil. *, $P < 0.05$; **, $P < 0.01$; n.s., not significant. Data are presented as the mean and standard error (n = 3).

	Organic soil			Mineral soil			T	L	T × L
	Control	Low N addition	High N addition	Control	Low N addition	High N addition			
$ImbalanceCN_{SOM}$	1.15 ± 0.13	0.94 ± 0.04	1.09 ± 0.16	1.34 ± 0.03	1.07 ± 0.15	1.37 ± 0.11	n.s.	n.s.	n.s.
$ImbalanceCP_{SOM}$	**1.98 ± 0.45^{ab}**	**0.99 ± 0.08^{b}**	**2.55 ± 0.54^{a}**	0.39 ± 0.05	0.50 ± 0.05	1.42 ± 0.64	*	*	n.s.
$ImbalanceNP_{SOM}$	**1.68 ± 0.19^{ab}**	**0.93 ± 0.09^{b}**	**2.20 ± 0.46^{a}**	0.33 ± 0.04	0.48 ± 0.06	1.02 ± 0.44	*	**	n.s.
$ImbalanceCN_{extr}$	0.83 ± 0.09	0.60 ± 0.02	0.73 ± 0.09	**1.09 ± 0.02^{ab}**	**0.86 ± 0.08^{b}**	**1.28 ± 0.08^{a}**	*	**	n.s.
$ImbalanceCP_{extr}$	**1.67 ± 0.11^{a}**	**1.07 ± 0.11^{b}**	**1.75 ± 0.19^{a}**	**1.20 ± 0.04^{b}**	**1.10 ± 0.28^{b}**	**1.97 ± 0.18^{a}**	**	n.s.	n.s.
$ImbalanceNP_{extr}$	**2.04 ± 0.10^{b}**	**1.79 ± 0.11^{b}**	**2.41 ± 0.06^{a}**	1.10 ± 0.05	1.26 ± 0.30	1.57 ± 0.22	*	**	n.s.

Table 2
Coefficient of correlations between microbial elements use efficiencies (CUE and NUE) and soil elemental stoichiometry, microbial elemental stoichiometry, element stoichiometric imbalance, microbial enzyme stoichiometry and microbial community structure in organic and mineral soils. Bold lower-case letters indicate significant differences between treatments tested separately for each soil. *, $P < 0.05$; **, $P < 0.01$.

	Organic soil		Mineral soil	
	CUE	NUE	CUE	NUE
Soil elemental stoichiometry				
SOC:TN	−0.279	0.214	−0.661	−0.427
SOC:TP	−0.523	0.232	0.443	0.596
TN:TP	−0.662	0.316	0.454	0.633
DOC:DN	−0.184	0.451	0.352	0.526
DOC:DP	0.090	0.236	−0.140	−0.110
DN:DP	0.313	−0.116	−0.205	−0.217
Microbial elemental stoichiometry				
MBC:MBN	0.559	0.020	−0.288	−0.530
MBC:MBP	**0.843****	−0.021	**−0.762***	**−0.793***
MBN:MBP	**0.748***	−0.028	−0.634	−0.567
Elemental stoichiometric imbalance				
$ImbalanceCN_{SOM}$	−0.478	0.061	0.096	0.386
$ImbalanceCP_{SOM}$	**−0.732***	0.069	0.510	**0.676***
$ImbalanceNP_{SOM}$	**−0.732***	0.091	0.501	0.638
$ImbalanceCN_{extr}$	−0.428	0.326	0.457	**0.712***
$ImbalanceCP_{extr}$	**−0.795***	0.115	**0.761***	**0.860****
$ImbalanceNP_{extr}$	**−0.868****	−0.175	0.604	0.548
Microbial enzyme stoichiometry				
BG:(NAG + LAP)	0.350	−0.264	**0.674***	0.613
BG:AP	0.004	−0.060	−0.464	−0.505
(NAG + LAP):AP	−0.387	0.292	−0.216	−0.430
Microbial community structure				
Bacterial community	0.053	−0.276	**0.438***	**0.617***
Fungal community	**0.407***	−0.243	−0.086	0.095

4. Discussion

4.1. Effects of nitrogen addition on microbial CUE

We used a novel ^{18}O-water tracer approach to estimate forest soil microbial CUE (Spohn et al., 2016a; Zheng et al., 2019) and found that the microbial CUE responses to N addition were strongly influenced by the N application rates and soil horizons. Specifically, we observed that the CUE in organic soil increased by 0.16 under low N addition (2.5 g N m^{-2} yr^{-1}) (Fig. 1), which supported our first hypothesis that the N addition increased microbial CUE and was in line with previous studies (Thiet et al., 2006; Spohn et al., 2016b; Poeplau et al., 2019). The main reason for the increase in CUE is due to the decreasing microbial respiration rate under N addition (Treseder, 2008; Yuan et al., 2019). But we also found microbial CUE decreased more substantially under high N addition (7.5 g N m^{-2} yr^{-1}) in the organic soil horizon (Fig. 1). Similar negative effects of N addition on soil microbial CUE have been reported in North American grasslands (Riggs and Hobbie, 2016). We suspect that the microbial CUE decrease under N addition could be due to the microbial community's composition shifting towards dominance by organisms with lower CUEs (discuss later). Our findings also showed that mineral soil microbial CUE was unaffected by N supply changes under low N addition (2.5 g N m^{-2} yr^{-1}) but clearly increased under high N addition (7.5 g N m^{-2} yr^{-1}). These opposite and inconsistent microbial CUE responses to N addition rates between soil layers indicate that soil N status changes could significantly impact the microbial C allocation between growth and respiration in forest soil.

Our results suggest that increased N availability in soil could decouple microbial respiration and growth processes. The negative effects of N addition on soil microbial respiration are well documented (Ramirez et al., 2010; Zhang et al., 2018), and it was observed in this study in organic soil, the magnitude of this decrease varied between low N addition (−51%) and high N addition (−23%). Microbial growth changed slightly at low N addition (−8%) but showed a great decline at high N addition (−53%). This decoupling between respiration and growth also happened in mineral soil (Fig. 1). Microbial respiration changes under N addition could be attributed to microbial biomass changes (Treseder, 2008; Liu and Greaver, 2010; Riggs and Hobbie, 2016), as a positive relationship between microbial biomass and microbial respiration under N addition had been observed (Treseder, 2008). Nitrogen addition might reduce microbial biomass due to soil acidification, declining soil base cations and inhibiting microbial extracellular enzyme activity, while the latter could lead to a decrease in microbial access to C (Waldrop et al., 2004; Ramirez et al., 2012; Tian and Niu, 2015). Our results partly support these mechanisms that decreasing pH and microbial biomass are responsible for the decline in microbial respiration. However, the microbial growth responses to N addition may not be accounted for by the changes in microbial biomass. The standing microbial biomass did not necessarily indicate growth rate and some studies even found N addition had positive effects on microbial growth rate but did not change the standing biomass (Stapleton et al., 2005; Demoling et al., 2008). Overall, our results indicate that microbial growth and respiration were decoupled in mineral soil, where nutrients were limited. This is also supported by the positive relationship between biomass-specific growth rate (qGrowth) and microbial CUE in mineral soil (Fig. 3b). For the organic soil, microbial respiration rate per biomass C (qCO_2) was strongly and negatively related to CUE in organic soil (Fig. 3a), which suggests the overflow metabolism was occurring in the soils under N addition, causing a trade-off between microbial growth, respiration and CUE (Manzoni et al., 2012; Lipson, 2015; Zheng et al., 2019).

4.2. Effects of nitrogen addition on microbial NUE

In addition to microbial CUE changes, N enrichment also altered microbial N use efficiency (NUE) that strongly regulated soil inorganic N cycling and microbial N retention (Mooshammer et al., 2014a, 2014b; Zhang et al., 2019). We demonstrated that low and high N addition reduced microbial NUE by 25% and 46% in organic soil, respectively (Fig. 1f). This result supports our hypothesis and is consistent with

studies that observed that microbial NUE decreased along the soil profile from plant litter to organic soil and mineral soil, showing a N availability gradient (Mooshammer et al., 2014a; Wild et al., 2015). However, we also found high N addition could promote microbial N uptake for growth and therefore increase microbial NUE by 297.76% in mineral soils (Fig. 1f). Since N addition did not change microbial uptake N (sum of growth N and gross N mineralization) in both soil horizons (Fig. S7), the relative shift between growth N and gross N mineralization meant that microorganisms just redistributed, allocating less N to growth (or more N was mineralized to NH_4^+) in organic soil under N addition. In contrast, more N was allocated to growth in mineral soil under high N addition. These results indicate that organic soil microorganisms were not N limited and increase in microbial GNM after N addition was not to meet their demand for N, but for mining C (Knorr et al., 2005; Spohn et al., 2016b). Consequently, soil N cycles would be more open in organic soil, while more N would be retained in mineral soil after N addition in this temperate forest.

4.3. Stoichiometric imbalances and microbial elements use efficiencies

Contrary to our expectations, N addition did not affect the soil C:N ratio and dissolved soil fraction C:N ratio in the current study (Table S2 and S3). Although microbial biomass C and N were changed, microbial biomass C:N ratio kept constant under N addition (Table S3). However, the high N addition greatly decreased the bulk soil N:P ratio and microbial biomass N:P ratio as well as microbial biomass C:P ratio, meaning that soil P availability and P cycling processes were altered under N addition, consistent with previous findings (Deng et al., 2017). The possible reasons for the altered availability of soil P under N addition include the changes in soil pH, microbial P enzymes, microbial immobilization of dissolved P, plant P uptake, and litter decomposition (Lu et al., 2012). It was hard to identify which of these processes occurred in this study; however, our results suggest the decrease in soil pH and increase in acid phosphatase likely contribute to the changes in P cycling (Tables S2 and S3). We also found the N:P or C:P imbalance between SOM and the microbial biomass decreased under low N addition and increased under high N addition (Table 1), and these changes were significantly negatively correlated with microbial CUE in organic soil and positively correlated with microbial NUE in mineral soil (Table 2). The imbalance of C:P and N:P ratios between the dissolved soil fraction and microbial biomass also can explain the variations in microbial CUE and NUE (Wild et al., 2015; Yuan et al., 2019). This possible reason is that, on the one hand, high availability of P could alleviate microbial stoichiometric constrain and reduce the C and energy investment for enzyme production, potentially increasing the microbial utilization efficiency of C (Manzoni et al., 2012). One the other hand, soil P availability could shift microbial functions associated with the degradation of aromatic compound and chitinolysis (Xia et al., 2020), which could change the microbial substrates and indirectly affects microbial growth and CUE (Yuan et al., 2019). Altogether, our results therefore suggest the microbial element imbalances of P relative to C and N are more important to shape microbial CUE in C-rich soil (organic soil in this study) and microbial NUE in C poor soil (mineral soil in this study) after N addition.

4.4. Microbial community and microbial elements use efficiencies

Microbial community structure can be shifted after N addition (Wang et al., 2018; Zhou et al., 2020), but whether these changes are associated with microbial CUE and NUE remains unexplored. We found N addition affected fungal and bacterial community structure in organic and mineral soils in this study (Fig. S2 and S3), and those changes were associated with microbial CUE and NUE (Table 2). In organic soil, the fungal growth rate may increase faster than respiration under N addition, leading to a significant positive relationship between fungal community and microbial CUE. A previous study using sustained inhibitor application and phospholipid fatty acid analysis found that the microbial CUE tended to increase with increasing fungal dominance (Bonner et al., 2018). This may be because the soil fungi have enzymatic advantages in decomposing organic matter under N addition in organic soil, which could help fungi to access more C and P and then increase microbial growth and CUE (Treseder, 2008; Bonner et al., 2018). Regarding to the mineral soil, we found the bacterial community was positively correlated with soil CUE and NUE (Table 2), which may be due to bacterial dominance of the microbial composition in mineral soil after six years of N addition (Zhang et al., 2018). This possibility is supported by a positive correlation between bacterial community composition and microbial growth rate in the mineral soil (Table S4). A recent study using a soil-mimicking system suggested that bacterial community and diversity are the best drivers of microbial CUE (Domeignoz-Horta et al., 2020). Because different microbial strains vary in their CUE (Pold et al., 2020), thereby the changes in community members under N addition could alter microbial community CUE and therefore affect soil functions, such as respiration, decomposition and denitrification (Liu and Greaver, 2010; Ramirez et al., 2010). Taken together, our results provide an empirical link between microbial community structure and microbial CUE and NUE, which helps explain the interaction between microbial community composition and soil C and N cycles.

4.5. Implications for soil C and N cycling

We explored the relationship between microbial CUE and NUE (Fig. 2) and found microbial CUE was positively correlated with microbial NUE when CUE <0.40, above which CUE became negatively correlated with NUE. This pattern indicated that the assimilation of C and N by microorganisms could be coupled before CUE <0.40 (or NUE < 0.70). Behind this threshold, microbial community in this studied soil could mineralize more N even as microbial biomass increased, which might promote N losses, e.g. by gaseous emissions or nitrate leaching (Lu et al., 2011). High microbial N release may indicate high N availability for plants, possibly increasing plant primary production and root C exudates that alleviate C limitation in microorganisms (Xiong et al., 2020). Indeed, we found the threshold element ratio of C:N ($TER_{C:N}$) declined in organic soil after N addition but increased under high N addition in mineral soil (Fig. 4). By comparing soil C:N ratio under each treatment with $TER_{C:N}$, we speculate that N addition alleviates the C limitation of microbial community in the organic soil but shifts from N limitation to N and C limitation in the mineral soil. Further, the increase in microbial turnover in mineral soil also suggests that more C would be stored in mineral soil under N addition through pumping microbial residuals into soils (Liang et al., 2017). In summary, understanding microbial CUE and NUE responses to N addition could provide a powerful approach to integrate shifts in microbial metabolic pathways into models of ecosystem C and nutrient exchanges.

5. Conclusions

In summary, we measured soil microbial CUE and NUE simultaneously based on a novel substrate-independent approach in a temperate forest and found that the microbial CUE and NUE responses to N addition depended on N addition rates and soil horizons. The decoupling of microbial growth and respiration was responsible for the changes in microbial CUE under N addition. Microbial CUE and NUE were strongly linked to elemental stoichiometric imbalances, such as imbalances in C:P and N:P ratio, suggesting soil P was likely to be an important predictor in regulating microbial growth, respiration and N mineralization rate in this study. Our results also highlight that shifts in microbial communities can impact microbial CUE and NUE. In conclusion, this study suggests that N addition can control microbial activities and therefore regulate soil C and N storage in temperate forest soils.

Author contributions

C.W. designed the study. J.L., C.P.S., J.Y.Y., Z.W.X. and C.W. conducted the field and laboratory analyses. Data analysis was conducted by J.L., C.P.S. and C.W. The paper was written by C.W. and J.L. with input from the other authors.

Data availability

All data are available in the main text or the supplementary materials and raw data are available upon request to corresponding author. The raw sequences of bacteria and fungi have been deposited on the figshare (https://doi.org/10.6084/m9.figshare.13040756.v1).

Declaration of competing interest

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

Acknowledgements

We thank Dr. Ember Morrissey, Dr. Weiwei Dai and two anonymous reviewers for their comments on a previous draft of this manuscript. C. Wang would like to thank Dr. Edith Bai for her help in the experimental design and her support in C. Wang's career development. This work was financially supported by the National Natural Science Foundation of China (31830015), the Key Research Program of Frontier Sciences, CAS (QYZDB-SSW-DQC006), the Key Laboratory of Geographical Processes and Ecological Security of Changbai Mountains, Ministry of Education (GPES201902), the Youth Innovation Promotion Association CAS to Chao Wang (2018231), and K. C. Wong Education Foundation of China.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.soilbio.2021.108207.

References

Allison, S.D., Wallenstein, M.D., Bradford, M.A., 2010. Soil-carbon response to warming dependent on microbial physiology. Nat. Geosci. 3, 336–340.
Bobbink, R., Hicks, K., Galloway, J., Spranger, T., Alkemade, R., Ashmore, M., et al., 2010. Global assessment of nitrogen deposition effects on terrestrial plant diversity: a synthesis. Ecol. Appl. 20, 30–59.
Bonner, M.T.L., Shoo, L.P., Brackin, R., Schmidt, S., 2018. Relationship between microbial composition and substrate use efficiency in a tropical soil. Geoderma 315, 96–103.
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 soil. Soil Biol. Biochem. 17, 837–842.
Brookes, P.C., Powlson, D.S., Jenkinson, D.S., 1982. Measurement of microbial biomass phosphorus in soil. Soil Biol. Biochem. 14, 319–329.
Cenini, V.L., Fornara, D.A., McMullan, G., Ternan, N., Lajtha, K., Crawley, M.J., 2015. Chronic nitrogen fertilization and carbon sequestration in grassland soils: evidence of a microbial enzyme link. Biogeochemistry 126, 301–313.
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? Glob. Chang. Biol. 19, 988–995.
Crowther, T.W., Hoogen, J.v.d., Wan, J., Mayes, M.A., Keiser, A.D., Mo, L., et al., 2019. The global soil community and its influence on biogeochemistry. Science 365 eaav0550.
Delgado-Baquerizo, M., Fry, E.L., Eldridge, D.J., de Vries, F.T., Manning, P., Hamonts, K., et al., 2018. Plant attributes explain the distribution of soil microbial communities in two contrasting regions of the globe. New Phytologist 219, 574–587.
Demoling, F., Ola Nilsson, L., Bååth, E., 2008. Bacterial and fungal response to nitrogen fertilization in three coniferous forest soils. Soil Biol. Biochem. 40, 370–379.
Deng, Q., Hui, D., Dennis, S., Reddy, K.C., 2017. Responses of terrestrial ecosystem phosphorus cycling to nitrogen addition: a meta-analysis. Glob. Ecol. Biogeogr. 26, 713–728.
Domeignoz-Horta, L.A., Pold, G., Liu, X.A., Frey, S.D., Melillo, J.M., DeAngelis, K.M., 2020. Microbial diversity drives carbon use efficiency in a model soil. Nature Communications 11, 3684.
Du, E., Terrer, C., Pellegrini, A.F.A., Ahlström, A., van Lissa, C.J., Zhao, X., et al., 2020. Global patterns of terrestrial nitrogen and phosphorus limitation. Nat. Geosci. 13, 221–226.
Frey, S.D., Lee, J., Melillo, J.M., Six, J., 2013. The temperature response of soil microbial efficiency and its feedback to climate. Nat. Clim. Chang. 3, 395–398.
Galloway, J.N., Townsend, A.R., Erisman, J.W., Bekunda, M., Cai, Z., Freney, J.R., et al., 2008. Transformation of the nitrogen cycle: recent trends, questions, and potential solutions. Science 320, 889–892.
Jones, L., Provins, A., Holland, M., Mills, G., Hayes, F., Emmett, B., et al., 2014. A review and application of the evidence for nitrogen impacts on ecosystem services. Ecosyst. Serv. 7, 76–88.
Kallenbach, C.M., Frey, S.D., Grandy, A.S., 2016. Direct evidence for microbial-derived soil organic matter formation and its ecophysiological controls. Nat. Commun. 7, 13630.
Knorr, M., Frey, S.D., Curtis, P.S., 2005. Nitrogen additions and litter decomposition: a meta-analysis. Ecology 86, 3252–3257.
Li, Z., Tian, D., Wang, B., Wang, J., Wang, S., Chen, H.Y.H., et al., 2019. Microbes drive global soil nitrogen mineralization and availability. Glob. Chang. Biol. 25, 1078–1088.
Liang, C., Schimel, J.P., Jastrow, J.D., 2017. The importance of anabolism in microbial control over soil carbon storage. Nat. Microbiol. 2, 17105.
Lipson, D.A., 2015. The complex relationship between microbial growth rate and yield and its implications for ecosystem processes. Front. Microbiol. 6, 615.
Liu, L., Greaver, T.L., 2010. A global perspective on belowground carbon dynamics under nitrogen enrichment. Ecol. Lett. 13, 819–828.
Lu, M., Yang, Y., Luo, Y., Fang, C., Zhou, X., Chen, J., et al., 2011. Responses of ecosystem nitrogen cycle to nitrogen addition: a meta-analysis. New Phytologist 189, 1040–1050.
Lu, X., Mo, J., Gilliam, F.S., Fang, H., Zhu, F., Fang, Y., et al., 2012. Nitrogen addition shapes soil phosphorus availability in two reforested tropical forests in southern China. Biotropica 44, 302–311.
Manzoni, S., Taylor, P., Richter, A., Porporato, A., Agren, G.I., 2012. Environmental and stoichiometric controls on microbial carbon-use efficiency in soils. New Phytologist 196, 79–91.
Miltner, A., Bombach, P., Schmidt-Brucken, B., Kastner, M., 2012. SOM genesis: microbial biomass as a significant source. Biogeochemistry 111, 41–55.
Mooshammer, M., Wanek, W., Hämmerle, I., Fuchslueger, L., Hofhansl, F., Knoltsch, A., et al., 2014a. Adjustment of microbial nitrogen use efficiency to carbon:nitrogen imbalances regulates soil nitrogen cycling. Nat. Commun. 5, 3694.
Mooshammer, M., Wanek, W., Zechmeister-Boltenstern, S., Richter, A., 2014b. Stoichiometric imbalances between terrestrial decomposer communities and their resources: mechanisms and implications of microbial adaptations to their resources. Front. Microbiol. 5, 22.
Poeplau, C., Helfrich, M., Dechow, R., Szoboszlay, M., Tebbe, C.C., Don, A., et al., 2019. Increased microbial anabolism contributes to soil carbon sequestration by mineral fertilization in temperate grasslands. Soil Biol. Biochem. 130, 167–176.
Pold, G., Domeignoz-Horta, L.A., Morrison, E.W., Frey, S.D., Sistla, S.A., DeAngelis, K.M., 2020. Carbon use efficiency and its temperature sensitivity covary in soil bacteria. mBio 11 e02293-02219.
Prommer, J., Walker, T.W.N., Wanek, W., Braun, J., Zezula, D., Hu, Y., et al., 2019. Increased microbial growth, biomass, and turnover drive soil organic carbon accumulation at higher plant diversity. Glob. Chang. Biol. 26, 669–681.
Qu, L., Wang, C., Bai, E., 2020. Evaluation of the ^{18}O-H_2O incubation method for measurement of soil microbial carbon use efficiency. Soil Biol. Biochem. 145, 107802.
Quinn Thomas, R., Canham, C.D., Weathers, K.C., Goodale, C.L., 2009. Increased tree carbon storage in response to nitrogen deposition in the US. Nat. Geosci. 3, 13–17.
Ramirez, K.S., Craine, J.M., Fierer, N., 2010. Nitrogen fertilization inhibits soil microbial respiration regardless of the form of nitrogen applied. Soil Biol. Biochem. 42, 2336–2338.
Ramirez, K.S., Craine, J.M., Fierer, N., 2012. Consistent effects of nitrogen amendments on soil microbial communities and processes across biomes. Glob. Chang. Biol. 18, 1918–1927.
Riggs, C.E., Hobbie, S.E., 2016. Mechanisms driving the soil organic matter decomposition response to nitrogen enrichment in grassland soils. Soil Biol. Biochem. 99, 54–65.
Silva-Sánchez, A., Soares, M., Rousk, J., 2019. Testing the dependence of microbial growth and carbon use efficiency on nitrogen availability, pH, and organic matter quality. Soil Biol. Biochem. 134, 25–35.
Sinsabaugh, R.L., Hill, B.H., Follstad Shah, J.J., 2009. Ecoenzymatic stoichiometry of microbial organic nutrient acquisition in soil and sediment. Nature 462, 795–798.
Soares, M., Rousk, J., 2019. Microbial growth and carbon use efficiency in soil: links to fungal-bacterial dominance, SOC-quality and stoichiometry. Soil Biol. Biochem. 131, 195–205.
Soong, J.L., Fuchslueger, L., Maranon-Jimenez, S., Torn, M.S., Janssens, I.A., Penuelas, J., et al., 2019. Microbial carbon limitation: the need for integrating microorganisms into our understanding of ecosystem carbon cycling. Glob. Chang. Biol. 26, 1953–1961.
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 Biol. Biochem. 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 Biol. Biochem. 97, 168–175.
Stapleton, L.M., Crout, N.M.J., Säwström, C., Marshall, W.A., Poulton, P.R., Tye, A.M., et al., 2005. Microbial carbon dynamics in nitrogen amended Arctic tundra soil: measurement and model testing. Soil Biol. Biochem. 37, 2088–2098.
Sterner, R.W., elser, J.J., 2002. Ecological Stoichiometry: Biology of Elements from Molecules to the Biosphere. Priceton Uniersity Press, Princeton, NJ, USA.
Takriti, M., Wild, B., Schnecker, J., Mooshammer, M., Knoltsch, A., Lashchinskiy, N., et al., 2018. Soil organic matter quality exerts a stronger control than stoichiometry on microbial substrate use efficiency along a latitudinal transect. Soil Biol. Biochem. 121, 212–220.
Thiet, R.K., Frey, S.D., Six, J., 2006. Do growth yield efficiencies differ between soil microbial communities differing in fungal:bacterial ratios? Reality check and methodological issues. Soil Biol. Biochem. 38, 837–844.
Tian, D., Niu, S., 2015. A global analysis of soil acidification caused by nitrogen addition. Environ. Res. Lett. 10, 024019.
Treseder, K.K., 2008. Nitrogen additions and microbial biomass: a meta-analysis of ecosystem studies. Ecol. Lett. 11, 1111–1120.
Vance, E.D., Brookes, P.C., Jenkinson, D.S., 1987. An extraction method for measuring soil microbial biomass C. Soil Biol. Biochem. 19, 703–707.
Vitousek, P.M., Aber, J.D., Howarth, R.W., Likens, G.E., Matson, P.A., Schindler, D.W., et al., 1997. Human alteration of the global nitrogen cycle: sources and consequences. Ecol. Appl. 7, 737–750.
Vitousek, P.M., Howarth, R.W., 1991. Nitrogen limitation on land and in the sea: how can it occur? Biogeochemistry 13, 87–115.
Waldrop, M.P., Zak, D.R., Sinsabaugh, R.L., Gallo, M., Lauber, C., 2004. Nitrogen deposition modifies soil carbon storage through changes in microbial enzymatic activity. Ecol. Appl. 14, 1172–1177.
Wanek, W., Mooshammer, M., Blöchl, A., Hanreich, A., Richter, A., 2010. Determination of gross rates of amino acid production and immobilization in decomposing leaf litter by a novel ^{15}N isotope pool dilution technique. Soil Biol. Biochem. 42, 1293–1302.
Wang, C., Liu, D., Bai, E., 2018. Decreasing soil microbial diversity is associated with decreasing microbial biomass under nitrogen addition. Soil Biol. Biochem. 120, 126–133.
Wang, C., Wang, X., Pei, G., Xia, Z., Peng, B., Sun, L., et al., 2020. Stabilization of microbial residues in soil organic matter after two years of decomposition. Soil Biol. Biochem. 141, 107687.
Widdig, M., Schleuss, P.-M., Biederman, L.A., Borer, E.T., Crawley, M.J., Kirkman, K.P., et al., 2020. Microbial carbon use efficiency in grassland soils subjected to nitrogen and phosphorus additions. Soil Biol. Biochem. 146, 107815.
Wild, B., Schnecker, J., Knoltsch, A., Takriti, M., Mooshammer, M., Gentsch, N., et al., 2015. Microbial nitrogen dynamics in organic and mineral soil horizons along a latitudinal transect in western Siberia. Glob. Biogeochem. Cycles 29, 567–582.
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 Biol. Biochem. 22, 1167–1169.
Xia, Z., Yang, J., Sang, C., Wang, X., Sun, L., Jiang, P., et al., 2020. Phosphorus reduces negative effects of nitrogen addition on soil microbial communities and functions. Microorganisms 8, 1828.
Xiong, D., Huang, J., Yang, Z., Cai, Y., Lin, T.-C., Liu, X., et al., 2020. The effects of warming and nitrogen addition on fine root exudation rates in a young Chinese-fir stand. For. Ecol. Manag. 458, 117793.
Yang, Y., Cheng, H., Gao, H., An, S., 2019. Response and driving factors of soil microbial diversity related to global nitrogen addition. Land Degrad. Develop. 31, 190–204.
Yuan, X., Niu, D., Gherardi, L.A., Liu, Y., Wang, Y., Elser, J.J., et al., 2019. Linkages of stoichiometric imbalances to soil microbial respiration with increasing nitrogen addition: evidence from a long-term grassland experiment. Soil Biol. Biochem. 138, 107580.
Zhang, S., Zheng, Q., Noll, L., Hu, Y., Wanek, W., 2019. Environmental effects on soil microbial nitrogen use efficiency are controlled by allocation of organic nitrogen to microbial growth and regulate gross N mineralization. Soil Biol. Biochem. 135, 304–315.
Zhang, T., Chen, H.Y.H., Ruan, H., 2018. Global negative effects of nitrogen deposition on soil microbes. ISME J. 12, 1817–1825.
Zheng, Q., Hu, Y., Zhang, S., Noll, L., Böckle, T., Richter, A., et al., 2019. Growth explains microbial carbon use efficiency across soils differing in land use and geology. Soil Biol. Biochem. 128, 45–55.
Zhou, Z., Wang, C., Luo, Y., 2020. Meta-analysis of the impacts of global change factors on soil microbial diversity and functionality. Nat. Commun. 11, 3072.