

Long-term nitrogen addition increased soil microbial carbon use efficiency in subalpine forests on the eastern edge of the Qinghai–Tibet Plateau

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Research Article

Keywords: microbial carbon use efficiency, long-term nitrogen addition, soil stoichiometry, subalpine forests, Qinghai–Tibet Plateau

Posted Date: May 25th, 2022

DOI: <https://doi.org/10.21203/rs.3.rs-1624611/v1>

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Abstract

Aims Nitrogen (N) deposition increased forest carbon (C) sink significantly, hence exploring the microscopic mechanisms is critical to predicting future global ecosystem C cycle, especially the effects of enhanced N deposition on soil microbial carbon use efficiency (CUE), which still unclear.

Methods We evaluated the responses of soil microbial CUE to long-term (5 years) N addition in an evergreen broad-leaved forest and a mature coniferous forest by using a ^{13}C isotope tracing method.

Results The results showed that the soil microbial CUE ranged from 0.38 to 0.51, which was smaller than the results obtained from the previous studies based the same method and forest type. In evergreen broad-leaved forest, the microbial CUE had no significant changes in the low N-addition treatment, but it was increased by 9.23% and 12.69% in medium and high N-addition treatments compared to the control. In coniferous forest, soil microbial CUE was increased by 14.64%, 21.89% and 24.34% in low, medium and high N-addition treatments, respectively. Moreover, the soil C:P and N:P are negatively relate to soil microbial CUE.

Conclusions Our findings indicate that the enhancing N deposition can increase soil microbial CUE and ultimately promote C sequestration, especially in coniferous forest. The imbalance of soil stoichiometry is the main impact factor of CUE under N addition. However, we speculate that the key to enhance forest soil C sequestration is to promote the decomposition rate of litter and thus increase the available carbon content.

Introduction

N deposition plays an important role in terrestrial ecosystem C cycle. It was estimated that the global forest C sink caused by N deposition was about $0.72 \text{ Pg C yr}^{-1}$ in the 2010s (Gurmessa et al. 2022). Previous studies have shown that N deposition regulates the microbial C cycle of forest soil by changing the ratio of soil nutrient elements, therefore, N deposition may have effects on forest soil C sequestration (Wamelink et al. 2009). In addition to affecting the growth of vegetation, soil N content also plays a decisive role in the growth and metabolism of soil microbial communities (Cleveland and Liptzin 2007). Researchers have carried out soil N addition experiments, and well understand the changes of soil microbial metabolism in grassland and cultivated land (Fang et al. 2018; Luo et al. 2020).

However, the responses of microbial C cycle process to N fertilization in forest soil remain controversial. Soil microbial CUE, defined as the ratio of microbial C allocated to growth, is an important synthetic representation of the microbial community metabolism. Despite it is vital for the forecast of global C cycle, mutability in CUE for terrestrial microorganisms is still poorly understood, and excluded from most biogeochemical models due to the varying methods and spatial heterogeneity (Manzoni et al. 2012). It has been speculated that the imbalance of soil stoichiometry, such as high C:N, can trigger overflow respiration, leading to relatively low microbial CUEs (Oliver et al. 2021; Pei et al. 2021; Subedi et al. 2021). Based on the ecological stoichiometry theory, the C:N ratio of soil microbial biomass is constrained within the range of 7-8.6 to keep internal balance of the elementals. It means that microorganisms must allocate larger proportions of C and energy to acquire C or nutrients in the case that the C:N ratio goes beyond this range, leading to a decrease in microbial CUE (Manzoni et al. 2012; Sinsabaugh et al. 2013). Manzoni et al. (2010) found that microbial CUE was reckoned to be about half of the maximum CUE (0.6) carried out at low C:N ratio. Hence, assessing the effects of N addition on forest soil microbial CUE can help us better understand the mechanisms of N deposition affecting soil C sink and enhance the predictive accuracy of relevant models.

It is generally accepted that N addition increases soil microbial CUE. For instance, Spohn et al. (2016) found that the long-term addition of N fertilizer to grassland soils gave resulted in a decrease of microbial C uptake and respiration rates, while the growth rates remained unchanged, resulting in an increase in microbial CUE. Soares and Rousk (2019) observed that the higher the N availability, the higher the CUE, but they believed that site-specific differences overrode the effect of N-availability. However, it is found from recent studies that N addition may have no effect in specific contexts. Li et al.(2021)conducted a six-year N deposition experiment to evaluate the response of microbial CUE to N addition in a temperate forest, and the results showed that microbial CUE was increased by 133.18% with high N ($7.5 \text{ g N m}^{-2} \text{ yr}^{-1}$) addition, while remained unchanged under low N

addition ($2.5 \text{ g N m}^{-2} \text{ yr}^{-1}$) in mineral soil. Taken together, there are two speculations for the enhanced soil microbial CUE induced by N addition. Firstly, the N-fertilizer in soils acts as an inhibitor for oxidative enzymes involved in the mineralization of complex compounds, leading to a decrease of microbial respiration and a reduction of the energy requirement of microbial N acquisition, and a further, increase of microbial CUE (Gallo et al. 2004; Manzoni et al. 2010; Spohn et al. 2016). Secondly, higher N availability generated a lower fungal-to-bacterial ratio, resulting in a relative high CUE due to the fact that bacteria had a higher CUE than fungi (Maynard et al. 2017).

In this study, we planned to study the effects of N deposition on subalpine forests soil microbial CUE by artificially simulating N deposition. Therefore, our research objectives are as follows: 1) Estimate the effects of long-term N addition on subalpine soil microbial CUE, compare the sensitivity of soil microbial CUE to long-term N addition between evergreen broad-leaf forest and coniferous forest on Gongga Mountain; 2) Speculate the underlying mechanisms and influencing factors of the changes of forest soil microbial CUE under N addition.

Material And Methods

Study area

The N-addition experiment was conducted in Mt. Gongga ($29^{\circ}20' - 30^{\circ}20' \text{ N}$, $101^{\circ}30' - 102^{\circ}15' \text{ E}$), which is on the southeastern fringe of the Tibetan Plateau. Mt. Gongga has concentrated N deposition, an ideal area for studying the changes in forest soil microbial CUE at different concentrations of N deposition. The N-addition experiment was established in an evergreen broad-leaf forest (the soil type is yellow brown soil, 2257 m) and a coniferous forest (the soil type is dark brown soil, 2839 m), these two vegetation types are typical and widely distributed in Mt. Gongga. In the evergreen broad-leaf forest, the predominant species is *Lithocarpus cleistocarpus* and is about 25 ~ 30m high, and the stand density is about 525 trees per hectare. In the coniferous forest, the predominant species is *Abies fabri* and is about 20 ~ 30m high, and the stand density is about 800 trees per hectare. East Asian monsoon is the dominant climate in Gongga Mountain, and the mean annual precipitation was 1403 mm at 2200 m and 1938 mm at 2900 m. The mean annual temperature was 13–14 °C at 2200 m and 3.5–5.0 °C at 2900 m. The soil is mostly acid soil with a pH value ranging from 4.5 to 6.

Nitrogen addition experiment

The N addition experiment was established in 2015, and we designed three gradients of N-addition rate (applied 10 kg N $\text{ha}^{-1} \text{ yr}^{-1}$, 20 kg N $\text{ha}^{-1} \text{ yr}^{-1}$ and 40 kg N $\text{ha}^{-1} \text{ yr}^{-1}$, respectively) and a control (0 kg N $\text{ha}^{-1} \text{ yr}^{-1}$), marked as N₁₀, N₂₀, N₄₀ and N₀, respectively. There are 3 uniform plots (1 m×2 m) per treatment for each forest type (24 plots in total). We applied the N fertilizer (urea) once a month from May to October on yearly basis (6 times a year in total), by mixing it in 500 ml deionized water, and then spreading it on the soil surface.

Samples collection

Soil samples were collected in August 2020, and the litters and humus were removed before sampling at each site. The samples were taken with cutting rings randomly (diameter = 5 cm, volume = 100 cm³) ranging from 0–10 cm in each of the replicate for measuring several soil physical indicators (e.g., water content, bulk density, and porosity, n = 3). Given the acreage of the plots, we collected four samples in S-shape from 0–10 cm in each of the replicate and sieved the (2 mm) immediately. Ice bags were used to keep the samples at a lower temperature during the transportation. Each sample was divided into three parts: i) For incubation, the subsample was stored in the frozen state (-20°C) in Ziploc bags until further use; ii) For microbial properties testing, it was stored under 4°C until further use; iii) For soil physical and chemical properties testing, the subsample was air-dried at room temperature.

Analysis of soil properties

Soil water holding capacity was determined by gravimetric method. In brief, weighed the samples after soaking in water for 12 hours (soil samples were in the cutting rings), and then calculate the water-holding capacity. Take a part of each soil sample,

weighed it and dry it to a consistent weight at 105°C, then calculate the soil density based on its proportion to the total soil sample and the volume of the cutting ring. A pH meter (Precision and Scientific Corp, China) was used to measuring soil pH (soil: water = 1:2.5). An elemental analyzer (Elementar Vario MACRO cube, Germany) was used to determine soil organic carbon (SOC) and total nitrogen (TN). Soil total phosphorus (TP) was extracted into sulfuric acid (98%) and then measured by an Auto Discrete Analyzer (Smartchem 200, AMS, Italy).

Soil microbial biomass carbon (MBC), phosphorus (MBP) and nitrogen (MBN) were determined by fumigation extraction method. K_2SO_4 solution (0.5 M) was used to extract dissolved carbon (DOC) and dissolved nitrogen (DON) in fumigated (fumigated with chloroform for 24 h) and non-fumigated soil (Brookes et al. 1985; Vance et al. 1987). The conversion factors of calculating the MBC and MBN were 0.45 and 0.54, respectively (Wu et al. 1990). Dissolved phosphorus (DOP) was extracted into $NaHCO_3$ solution (0.5 M) from fumigated (fumigated with chloroform for 24 h) and non-fumigated soil, and the conversion factor was 0.40 for calculating MBP (Brookes et al. 1985).

Determination of soil microbial CUE

For the understanding of the metabolic process of soil microorganisms, we applied the ^{13}C isotope tracing method to determine soil microbial ^{13}C -respiration and ^{13}C -growth rate following Davey L. Jones et al. (2018). Before incubating, samples were kept for 3 days at 15°C (average soil temperature at the sites during the growing season) to reactive the microorganisms and the water content of soils was kept at 60% of soil's water holding capacity to maintain an optimal conditions for microbial activity. At the end of the pre-incubation stage, 5g of the pre-incubated soil was placed into a polypropylene tube (50cm³) and then received a quantified glucose solution (0.2 mg glucose- ^{13}C g⁻¹ soil) that included other reagents (0.1% $MgCl_2$, 0.2% KH_2PO_4 , and 0.1% K_2SO_4) and sodium nitrate to make it C:N ratio = 40 to mimic the circumstances of natural environment (Wadsö 2009). 2 ml sodium hydroxide solution (NaOH, 1M) was injected into a polypropylene scintillation vial, and placed the scintillation vial upright on the soil surface to catch the respired $^{13}CO_2$, sealed the tubes and put them into a thermostat with a temperature of 15°C. After 72 h, the polypropylene tubes and scintillation vials were harvested and the remaining ^{13}C -glucose was extracted in ice-cold 1M NaCl (25 ml), the cultivation time was selected based on Glanville et al. (2016) that most of the glucose have been consumed by the microorganisms. The content of ^{13}C in NaCl and NaOH was determined by Delta V Advantage (Thermo, America) and ISOPRIME100 (Elementar, German), respectively.

Microbial ^{13}C -uptake ($^{13}C_u$) can be estimated as follows:

$$^{13}C_u = ^{13}C_t - ^{13}C_{NaCl}$$

where $^{13}C_t$ is the total amount of ^{13}C -glucose added to each of the sample, $^{13}C_{NaCl}$ is the amount of ^{13}C extracted in the 1M NaCl. Microbial CUE for ^{13}C -glucose was estimated by:

$$CUE = \left(^{13}C_u - ^{13}C_r \right) / ^{13}C_u$$

where $^{13}C_r$ is the amount of ^{13}C trapped by the 1M NaOH which is represents the ^{13}C -respiration. The threshold (TER) of C:N ratio was estimated by (Sterner and Elser 2002; Soong et al. 2020):

$$TER \approx C:N_{MB} \times \frac{NUE}{CUE}$$

where $C:N_{MB}$ is microbial biomass C:N ratio, NUE is the microbial N efficiency and it can be estimated by (Zhong et al. 2015):

$$\frac{CUE}{NUE} = \frac{C:N_{MB}}{C:N_{soil}}$$

Where $C:N_{soil}$ is C:N ratio of the soil.

Statistical analysis

The data were checked for normality and homogeneity of variance, and transformed in the case that they were not normally distributed. After that, a one-way ANOVA was performed to test the differences of the properties of soil (soil bulk density, water-holding capacity, pH, SOC, TN and TP) and the microorganisms (MBC, MBN and MBP) in the treatments, which was followed by a Tukey post-hoc test for multiple comparisons. The significant differences were calculated at the confidence interval of 95%. Similarly, the differences of the soil microbial metabolic parameters (^{13}C -uptake, ^{13}C -growth, ^{13}C -respiration and CUE) at varying N-addition rates and soil types was analyzed by using a two-way ANOVA. Furthermore, a liner regression model was established to describe the relationships between soil C:P (or N:P) ratio and microbial CUE. Besides, a redundancy analysis (RDA) was performed to reveal the influences of soil properties on microbial biomass and parameters in metabolic process. The one-way and two-way ANOVA analyses were carried out by using SPSS 25.0 software, and the RDA analysis was conducted using with the vegan package (version 2.5.7) of R software (version 4.4.1).

Results

Effects of N addition on soil properties in the two subalpine forest types

Soil bulk density and water-holding capacity remained almost unchanged under long-term N addition. Soil pH slightly decreased with the increase of N addition rates, and evergreen broad-leaf forest had greater average soil pH than coniferous forest. Soil SOC and TN in evergreen broad-leaf forest first increased and then decreased with the increase of N addition rates, while SOC and TN in the two forest types showed no consistent changes after N fertilizer was added. Soil C:P (SOC:TP) ratio in N addition treatments were generally less than that in controls in both coniferous forest and evergreen broad-leaf forest. Moreover, total phosphorous (TP), C:N (SOC:TN) and N:P (TON:TP) and N:P (TN:TP) did not show significant differences.

In general, there was no significant differences in terms of the content of microbial biomass C, N, and P in the two types of forest soil. N addition decreased MBC and MBN content in evergreen broad-leaf forest, but had no significant effects on that in coniferous forest. Furthermore, N addition can increase the soil microbial phosphorus content in coniferous forests.

Table 1

Soil properties of the N addition and control treatments in two soil types. "R": the effects of N-addition rate; "T": the effects of forest type; "R*T": the interaction of N-addition rate and forest type. Different lowercase letters indicate that significant differences in soil and soil microbial properties in the same forest type. "##" indicate that the soil microbial properties of different forest types have highly significant differences. "n.s." means there was no significant differences.

Properties	Evergreen broad-leaf forest				Coniferous forest				R	T	R*T
	N ₀	N ₁₀	N ₂₀	N ₄₀	N ₀	N ₁₀	N ₂₀	N ₄₀			
Soil bulk density	0.69 ± 0.05	0.75 ± 0.02	0.89 ± 0.04	0.75 ± 0.12	0.84 ± 0.04	0.78 ± 0.05	0.85 ± 0.10	0.78 ± 0.08	n.s.	n.s.	n.s.
Water-holding capacity (g/kg)	413.87 ± 22.59	391.86 ± 37.22	332.18 ± 79.30	476.34 ± 53.77	396.81 ± 38.89	251.64 ± 75.99	372.66 ± 106.13	393.59 ± 76.34	n.s.	n.s.	n.s.
pH	6.44 ± 0.42	6.03 ± 0.30	5.74 ± 0.034	5.91 ± 0.25	5.89 ± 0.30	5.90 ± 0.44	5.66 ± 0.37	5.63 ± 0.21	n.s.	n.s.	n.s.
SOC(g/kg)	26.78 ± 4.95	32.68 ± 9.07	31.3 ± 8.11	20.59 ± 6.54	20.89 ± 4.73	16.77 ± 5.60	27.14 ± 5.60	15.85 ± 4.59	n.s.	n.s.	n.s.
TN(g/kg)	2.3 ± 0.24	3.19 ± 0.75	2.30 ± 0.37	1.93 ± 0.50	1.56 ± 0.65	1.31 ± 0.33	3.15 ± 0.40	2.19 ± 0.46	n.s.	n.s.	n.s.
TP(mg/kg)	850.39 ± 61.46	947.75 ± 45.86	1092.77 ± 52.16	1039.95 ± 73.61	939.96 ± 48.43	914.63 ± 33.37	1097.37 ± 188.24	855.28 ± 39.33	n.s.	n.s.	n.s.
C:N	11.47 ± 1.19	10.04 ± 0.47	13.49 ± 2.94	10.39 ± 0.56	13.04 ± 0.16	11.47 ± 1.05	12.44 ± 0.24	11.83 ± 0.48	n.s.	n.s.	n.s.
C:P	34.25 ± 6.92ab	32.14 ± 8.38ab	28.60 ± 7.01ab	19.16 ± 54.63b	43.45 ± 4.02a	18.36 ± 6.35b	25.36 ± 4.66ab	18.45 ± 5.14b	n.s.	n.s.	
N:P	2.77 ± 0.45	3.35 ± 0.70	2.13 ± 0.43	1.81 ± 0.33	3.34 ± 0.35	1.72 ± 0.75	2.03 ± 0.35	1.53 ± 0.36	n.s.	n.s.	n.s.
MBC(mg/kg)	293.67 ± 23.07	233.10 ± 27.40	210.87 ± 44.47	208.47 ± 9.57	175.70 ± 21.94	171.07 ± 13.06	162.10 ± 46.54	179.13 ± .27	n.s.	n.s.	n.s.
MBN(mg/kg)	50.67 ± 2.47a	37.53 ± 2.93ab	35.13 ± 5.93b	35.67 ± 0.97b	43.87 ± 5.78ab	35.10 ± .52b	35.10 ± 4.52b	39.90 ± 1.57ab	n.s.	n.s.	
MBP(mg/kg)	0.96 ± 0.12	0.79 ± 0.14	0.86 ± 0.22	0.91 ± 0.35	1.06 ± 0.53	1.74 ± 0.07	3.11 ± 1.59**	2.74 ± 0.41	n.s.		n.s.

Effects of N addition on soil microbial CUE in the two subalpine forest types

N addition treatments had no significant influences on microbial ¹³C-uptake rate in evergreen broad-leaf forest in which ¹³C-growth rates were enhanced in N₂₀ and N₄₀ treatments, but showed no significant changes in N₁₀ treatments. However, all N addition treatments are available to promote the microbial ¹³C-growth in coniferous forest. Besides, N addition decreased microbial ¹³C-respiration rate to different degrees, but the amplitude of the decrease was not significant in N₁₀ treatments in both soil types. We demonstrated that soil microbial ¹³C-respiration was decreased by 1.97%, 11.00% and 6.99% in N₁₀ and N₂₀ and N₄₀ treatment compared with the controls respectively in evergreen broad-leaf forest, and in coniferous forest, the decreasing amplitude was 10.01%, 14.14% and 11.18%, respectively.

Consequently, microbial CUE shows different responses to N addition treatments in the two forest types. In evergreen broad-leaf forest, microbial CUE was not affected in N₁₀ treatment, which, however, was significantly increased in N₂₀ and N₄₀ treatments. In that case, in coniferous forest, all the N addition treatments had increased microbial CUE significantly compared to the

control. The microbial CUE of N₁₀, N₂₀ and N₄₀ treatments were increased 1.13%, 9.23% and 12.69% respectively in evergreen broad-leaf forest. While in coniferous forest, the microbial CUE increments were 14.64%, 21.89% and 24.34%, respectively. Thus, the microbial CUE in coniferous forest was more sensitive to long-term N addition than that in evergreen broad-leaf forest.

Relations of soil properties and microbial CUE under N addition

The redundancy analysis (RDA) plot shows the relationships between soil properties and microbial metabolic parameters (Fig. 2), revealing that N addition increases microbial CUE by decreasing soil C:N ratio. Constrained axes RDA1 and RDA2 accounted for 67.68% and 23.57% of total variations, respectively. The pH value has the most significant influence on microbial community physiological characteristics under N addition, and it is positively correlated with MBC and MBN. The microbial ¹³C-growth and CUEs are negatively correlated with soil C:N ratios, but microbial ¹³C-reparation is positively correlated with soil C:N ratio. Although the SOC has a positive effect on soil microbial respiration, it is negatively related to microbial growth. As a result, the SOC has a negative effect on microbial CUE under N addition. Furthermore, we observed that soil phosphorus content (P) and water-holding capacity had little effect on the growth of soil microorganisms.

Although soil P has little effect on the process of microbial heterooxygen respiration, the imbalance of C, N and P in the soil has a great impact on the CUE. It can be seen from the results of regression analysis among soil C:P and N:P ratio with microbial CUE that both the C:P ($R^2 = 0.534$, $P < 0.01$) and the N:P ($R^2 = 4.999$, $P < 0.01$) ratios were inversely related to the microbial CUE across the sites (Fig. 3a and Fig. 3b). However, soil C:N ratio has no significant relationship with microbial CUE ($R^2 = 0.085$, $P = 0.09$).

Discussion

Effects of N addition on soil microbial CUE

We found that the soil microbial CUE across all the sites ranges from 0.38 to 0.51 with an average of 0.44, the results were lower than those of other studies using the same method (Sauvadet et al. 2018; Takriti et al. 2018). Our results suggest that N additions can lead to an increase of soil microbial CUE, which is in line with the previous studies (Spohn et al. 2016; Poeplau et al. 2019). However, our finding contrasts with Widdig et al. (2020) and Riggs and Hobbie (2016), their researches showed that N addition had no impact on soil microbial CUE, and Silva-Sánchez et al. (2019) observed that N availability was negatively correlated with microbial CUE. We observed that the microbial CUE stopped growing when the N addition was increased to 20 kg·ha⁻¹·yr⁻¹, therefore, we can speculate that the enhancement of N addition on soil microbial CUE peaked at nearly 20 kg·ha⁻¹·yr⁻¹.

According to our observation, the increased soil microbial CUE was due to the declined microbial respiration and the advanced growth (Yuan et al. 2019; Li et al. 2021a). Our finding coincides with the previous work that microbial ¹³C-respiraion was decreased and ¹³C-growth was increased under N addition treatments (Söderström et al. 1983; Treseder 2008; Widdig et al. 2020) and there may be two mechanisms leading to this effect. On the one hand, microorganisms may consume less adenosine triphosphate (ATP) for the metabolism associated with N acquisition in N enriched soils, therefore, the excrecent C was allocated to growth, leading to the increase of microbial CUE (Manzoni et al. 2012). On the other hand, it has been confirmed that N addition can alter decomposer community composition to have more labile substrates (Allison and Vitousek 2005; Liu and Zhang 2019). Subedi et al. (2021b) observed that greater pine litter mass was decomposed under N + P addition treatment compared with the control treatment in a long-term silvicultural study area. Moreover, previous studies demonstrated that microbial uptake was normally declined, which was induced by the decline of microbial biomass in N addition experiments (Wu et al. 2019; Soong et al. 2020; Widdig et al. 2020), but our result was different. According to our observation, in general, the soil microbial ¹³C-uptake in N addition treatments were have no significant differences compared with that in control treatments.

Interestingly, however, the increasing ranges of soil microbial CUE in our study were relatively low compared with the previous researches (Spohn et al. 2016; Li et al. 2021a). The most likely explanation is that the C limitation restrained the increase amplitude of soil microbial CUE under N addition (as explained in detail below). In summary, the increase of N availability indeed

improved the microbial CUE, but the limitation of available C was the key factor for it. Therefore, the relatively low increments of microbial CUE were induced by N addition.

We found that coniferous forest was more sensitive to N addition than evergreen broad-leaved forests. Although there was no significant difference in terms of the soil C:N ratios between the two forest types, the C:N ratios of the litter in the coniferous forest was greater than that in the broad-leaved forest. It can be inferred that the N limitation in the coniferous forest is more serious, thus, the microbial CUE improvement effect was more obvious after N addition.

The influencing factors of microbial CUE under N addition

We supposed that the relatively low pHs were responsible for the low average values of microbial CUE. The pHs ranging from 4.4 to 6.5 with an average of 5.9, and some studies have shown that pH is positively correlated with microbial CUE in forest soil (Silva-Sánchez et al. 2019; Li et al. 2021b). Inorganic N fertilizer can result in soil acidification (Table 1), contributing to the reduction of soil base cations and the inhibition of microbial enzyme activities (Schleuss et al. 2019). Moreover, our results of RDA showed that pH value is one of the strongest predictors driving the observed variation in microbial metabolic and physiological characteristics (Soares and Rousk 2019). In contrast to the previous studies, we found that pH was negatively correlated with microbial CUE (Silva-Sánchez et al. 2019). The most likely reason is that the soil microbial CUE is more sensitive to N addition than pH in the study area. After all, the decrease of pH at the sites was caused by the addition of N (Liu and Zhang 2019).

We found that SOC had a negative relationship with microbial growth, resulting in an adverse impact on microbial CUE, implying that CUE is inversely related to soil C:N when there is a colimitation of C and N in the context, which is consistent with the inference by Sinsabaugh et al. (2013). Whether soil P has an effect on soil microbial CUE has been controversial. Widdig et al. (2020) came to a conclusion that soil P was not critical for microbial C cycling across a broad range of grassland sites, while Li et al. (2021a) believed that high availability of P will indirectly affect microbial process by reducing the energy and C investment for enzyme production. Moreover, the increase of availability of P can enhance soil microbial performance on the retrogradation of aromatic compounds (Manzoni et al. 2012; Chen et al. 2020). According to the results of our dataset analysis, soil P had no significant influence on microbial metabolism and CUE across the sites.

The soil C: N and C:P ratios within and across terrestrial ecosystems have a very large span and therefore microbial communities forced to adapt their foraging strategies to the available substrates by regulating the rates of respiration and growth. Despite high N fertilizer inputted, we found that soil C:N ratios and microbial biomass C:N ratios have no significant differences across all of the treatments, which is in accordance with the general observations that N addition have no influences on both of them (Cleveland and Liptzin 2007; Xu et al. 2013), indicating that microbial communities will adjust the rates of the processes of C and N cycling to make their internal biomass stoichiometry independent of the soil substrate stoichiometry. Although soil C and N remained almost unchanged after long-term N addition, however, C:P ratios decreased largely in coniferous forest, indicating that the P availability was influenced by N addition, which is consistent with previous findings (Deng et al. 2017; Liu et al. 2021). Furthermore, soil C:P and N:P ratios were negatively correlated with microbial CUE, and C:N had no obvious relationship with microbial CUE. Overall, our results showed that the soil element imbalance of C and N relative to P has a significant impact on microbial CUE in the study area.

Therefore, combining the above two points of view, we think it is hard to find the relationships between individual elements and microbial C cycling, because the microorganisms regulated their community composition, metabolic processes and intracellular elements cycling to adapt the imbalanced stoichiometry, thereby resulting in a varying microbial CUE.

Moreover, C limitation is severe for microbial growth in our study area according to the theory of threshold (TER) elements ratio (especially C:N ratio), which was introduced to evaluate the C:N ratio of soil and limit the growth of microorganisms (Sterner and Elser 2002). We found that the average TER_{C:N} (≈ 22.42) across the sites was slightly higher than the global average TER_{C:N} (≈ 21), and the average soil C:N ratio in our study was 11.62, thus, the growth of soil microorganisms were clearly C limited and it may constraint soil microbial CUE (Soong et al. 2020). We also found that the TER_{C:N} was decreased in the three N addition treatments compared with the controls, so it can be speculated that the C limitation of microbial growth can be mitigated by N

addition (Schleuss et al. 2019; Li et al. 2021a). Nevertheless, the mitigation effect stopped when N addition rate approached 20 kg·ha⁻¹·yr⁻¹ and once again, the C limitation became the major factor for the increase of CUE. In that case, we supposed that C limitation is the major factor for microbial C sequestration in the forests, which is similar to the condition in the current study area, therefore, the increase of C availability might be the most effective way to enhance microbial C sequestration.

Conclusions

In summary, we estimated soil microbial CUE in the two forest types based on a ¹³C-glucose isotope tracing method and found that long-term N addition can inhibit microbial respiration and enhance microbial growth, resulting the increase of microbial CUE. Coniferous forest is more sensitive to N addition than evergreen broad-leaf forest due to the difference of the C:N ratios. Therefore, we speculate that N addition may promote soil C sequestration in forests, especially in coniferous forest. It is also emphasized by our results that it is not the individual element but the stoichiometry imbalance that regulates soil microbial C cycling, such as C:P and N:P ratios. Furthermore, we believed that accelerating litter decomposition to increase the availability of SOC is the most effective way to increase soil microbial CUE, leading to an increase of soil C storage.

Declarations

Acknowledgements

We thank Dr. Weikai Bao for advices on writing, Xiaoquan Lan for the help of field sampling. Additional gratitude goes to Zhijie Long for the suggestions of the manuscript.

Founding

This work was supported by the Major scientific and technological projects of environmental governance and ecological protection of Sichuan Province (2018SZDZX0031), National key research and development program of China (2017YFC0505004) and 135 Strategic Program of the Institute of Mountain Hazards and Environment, CAS (SDS-135-1707).

Author contributions

Wenwu Wang conducted the laboratory experiments and wrote the manuscript, Wanze Zhu designed the study and participated in paper revision, Xia Li and Shenglan Ma conducted the field sampling.

Data availability

Any data related to this study are available upon request.

Declarations & Competing interests

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Figures

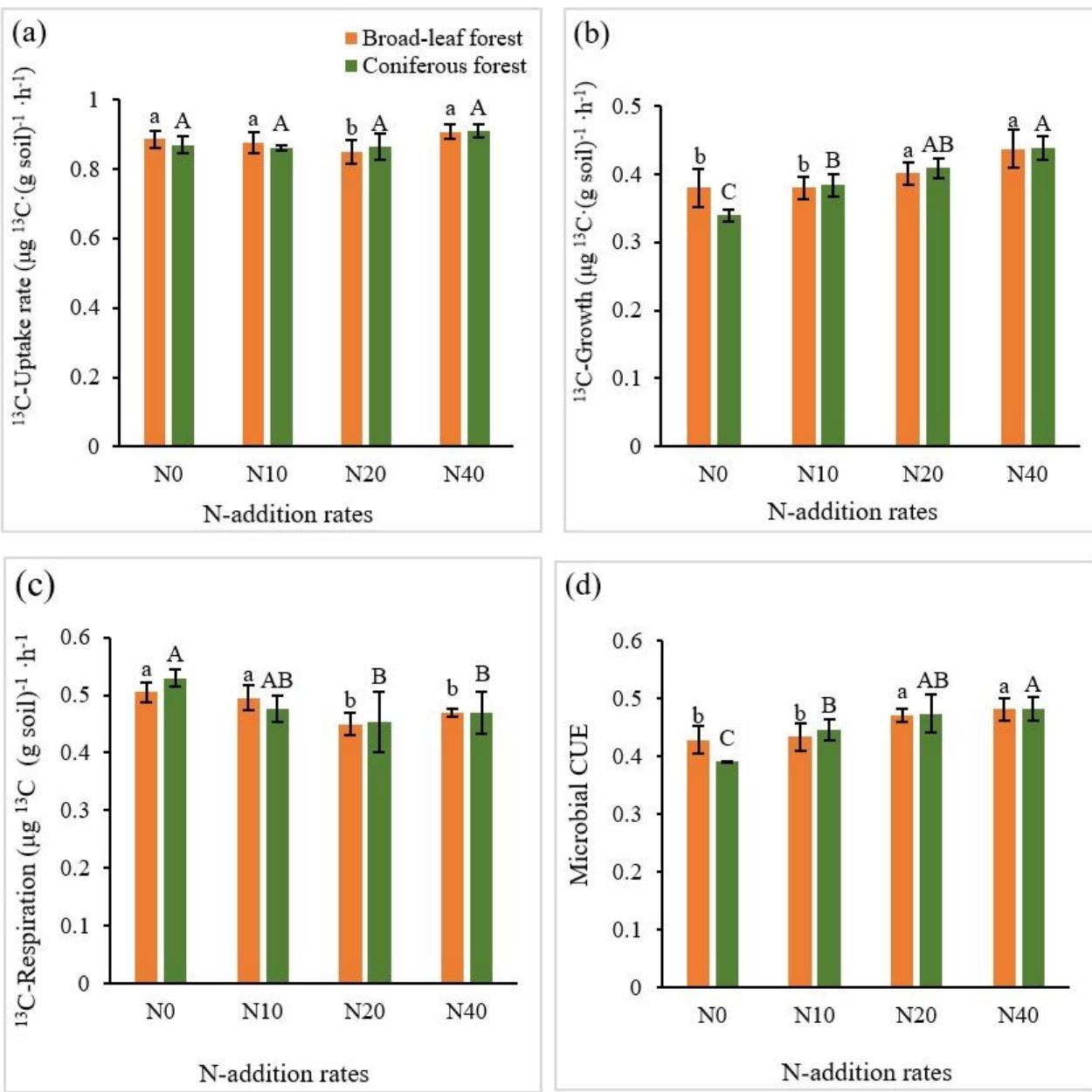


Figure 1

Mean microbial ^{13}C -uptake rates (a), ^{13}C -growth rates (b), ^{13}C -respiration rates (c) and microbial CUE (d) of the three N-addition and control treatments in evergreen broad-leaf forest and coniferous forest for 0-10 cm depth.

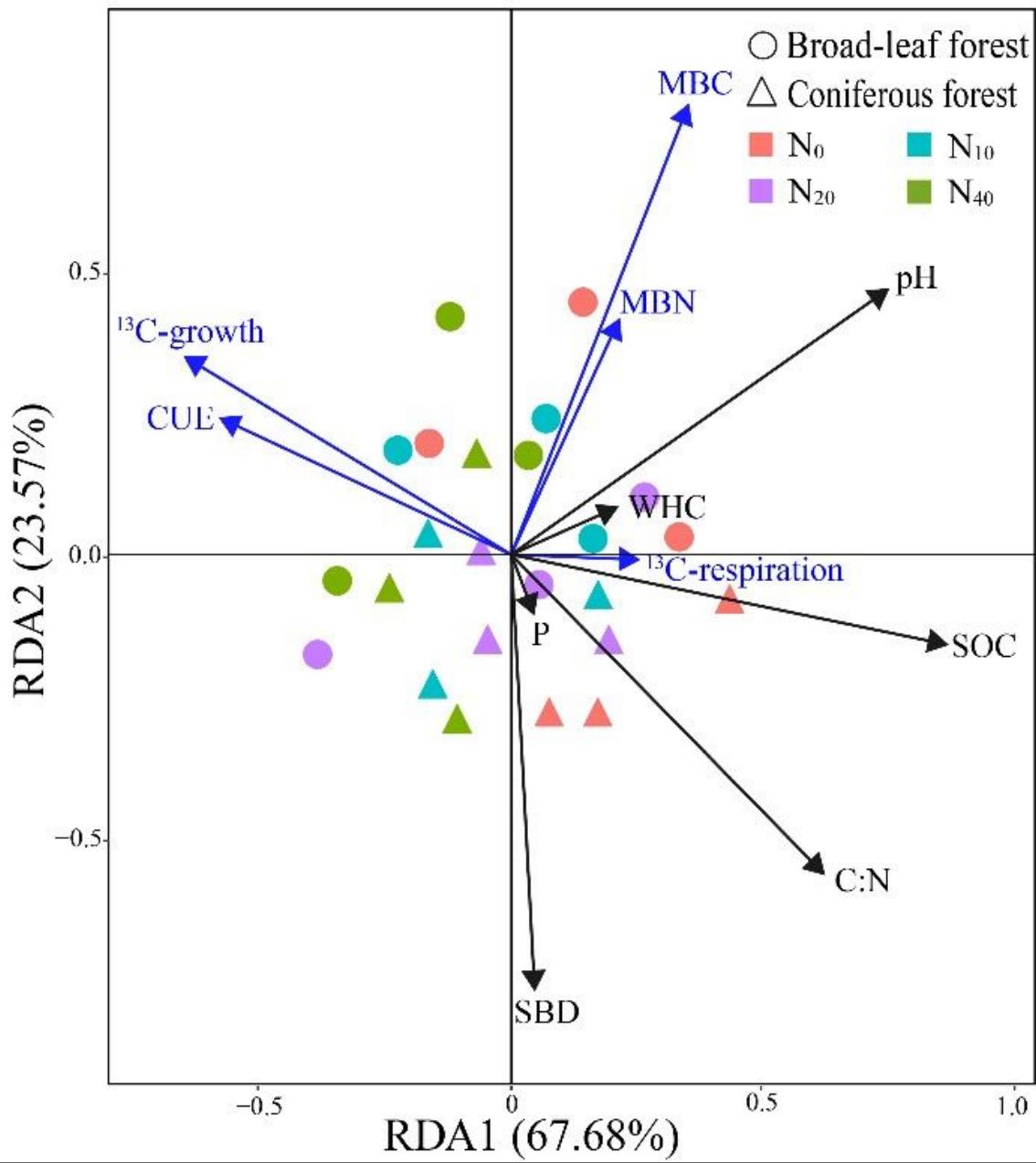


Figure 2

Correlation triplot based on redundancy analysis (RDA) depicting the relationship between soil properties and microbial CUE and physiological characteristics. The position of the scatters in the figure correspond to the mapped values of its original values.

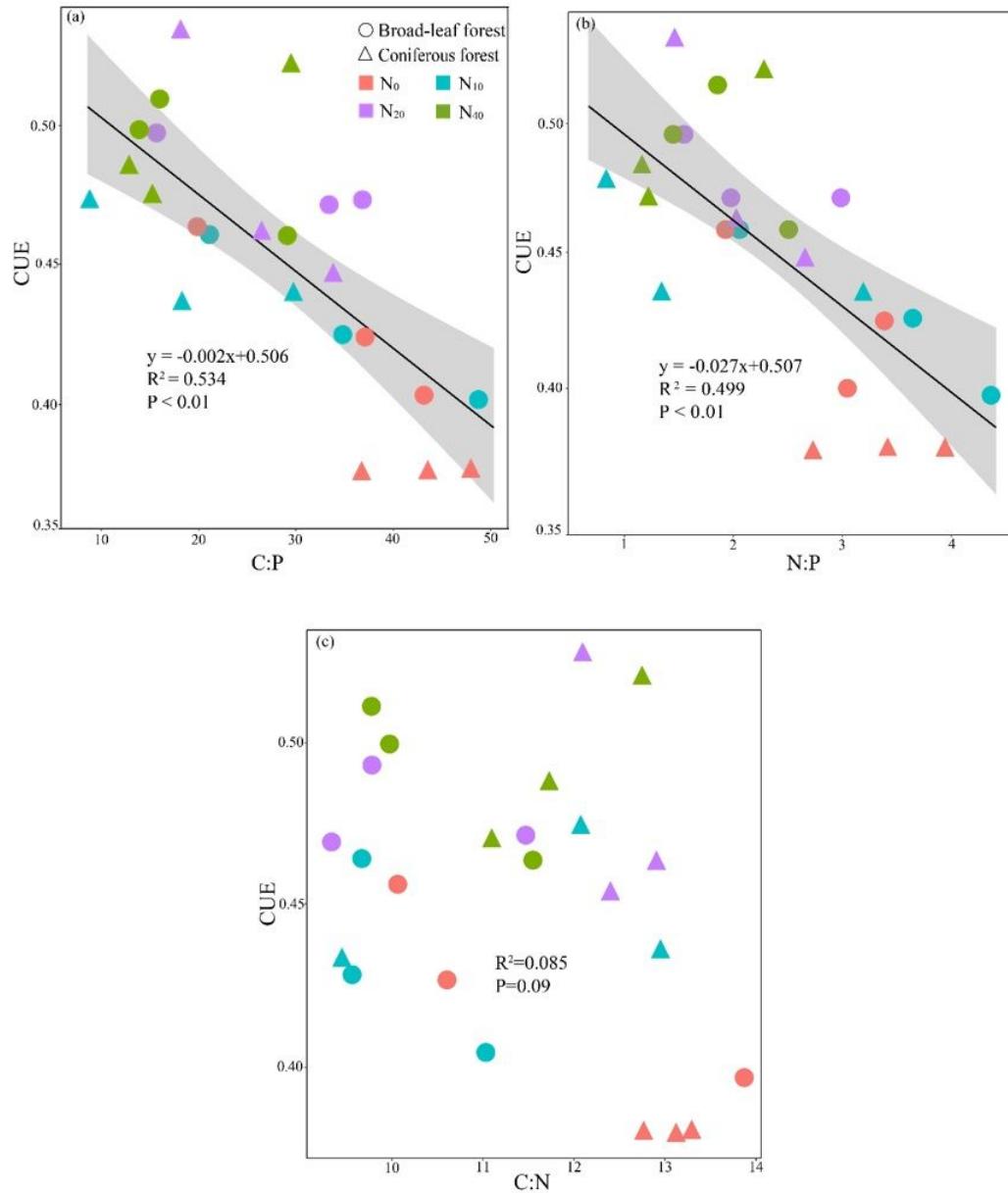


Figure 3

Correlations of C:P (a) ratio, N:P (b) ratio and C:N (c) ratio with microbial CUE in the three N-addition and the control treatments for 0-10 cm depth. The shaded areas are confidence intervals (confidence level = 90%).