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Exogenous nitrogen input skews estimates of microbial nitrogen use efficiency by ecoenzymatic stoichiometry

Lifei Sun[1*], Daryl L. Moorhead[2], Yongxing Cui[3*], Wolfgang Wanek[4], Shuailin Li[1] and Chao Wang[1]

Abstract

Background Ecoenzymatic stoichiometry models (EEST) are often used to evaluate microbial nutrient use efficiency, but the validity of these models under exogenous nitrogen (N) input has never been clarified. Here, we investigated the effects of long-term N addition (as urea) on microbial N use efficiency (NUE), compared EEST and ^{18}O-labeling methods for determining NUE, and evaluated EEST's theoretical assumption that the ratios of standard ecoenzymatic activities balance resource availability with microbial demand.

Results We found that NUE estimated by EEST ranged from 0.94 to 0.98. In contrast, estimates of NUE by the ^{18}O-labeling method ranged from 0.07 to 0.30. The large differences in NUE values estimated by the two methods may be because the sum of β-N-acetylglucosaminidase and leucine aminopeptidase activities in the EEST model was not limited to microbial N acquisition under exogenous N inputs, resulting in an overestimation of microbial NUE by EEST. In addition, the acquisition of carbon by N-acquiring enzymes also likely interferes with the evaluation of NUE by EEST.

Conclusions Our results demonstrate that caution must be exercised when using EEST to evaluate NUE under exogenous N inputs that may skew standard enzyme assays.

Keywords Extracellular enzyme, Resource allocation, Nitrogen addition, Microbial metabolism limitation, Isotope labeling

Background

Microbial nitrogen use efficiency (NUE) describes the proportion of N taken up by microorganisms that is allocated to biomass synthesis, and is a key characteristic of microbial metabolism that plays an important role in soil N cycling (Mooshammer et al. 2014; Sun et al. 2023; Zhang et al. 2019). NUE has mostly been evaluated by isotope labeling methods. For example, ^{15}N-labeled amino acids have been used to trace the uptake of organic N by microbes (Wild et al. 2013). However, microbes can assimilate other N sources such as inorganic N and amino sugars, apart from amino acids. Hence, this method represents amino acid use efficiency but not a fully integrated NUE (Andresen et al. 2015). Recently, ^{18}O-labeled water has been used to determine microbial growth and NUE, because microbes can assimilate

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multiple N substrates, and the use of ^{18}O-water avoids substrate addition effects (Zhang et al. 2019). Alternatively, Sinsabaugh et al. (2016) proposed a novel model based on the ecoenzymatic stoichiometric theory (EEST) and on the mass balance principle to evaluate NUE from the elemental stoichiometry of organic matter and microbial biomass, given the ratio of activities of enzymes that target carbon (C) vs. N acquisition. A growing body of studies have used the ecoenzyme model to evaluate microbial resource use efficiency in many ecosystems, including forest, farmland and grassland (Auwal et al. 2023; Chen et al. 2018b; Li et al. 2023; Lv et al. 2022; Shen et al. 2023; Sun et al. 2022; Wang et al. 2022), partly because parameter determination is rapid, relatively inexpensive and easy to evaluate (Schimel et al. 2022).

The ecoenzymatic theory argues that the resource requirements of soil microbes are reflected by the activities of specific extracellular enzymes targeting different resources in organic polymers; i.e., β-1,4-glucosidase (BG) for C acquisition, β-1,4-N-acetylglucosaminidase (NAG) and leucine aminopeptidase (LAP) for N, and acid/alkaline phosphatase (AP) for phosphorus (P), and predicts that microbes would regulate the ratios of ecoenzymatic activities to compensate for the imbalances between resource availability and microbial demand (Sinsabaugh et al. 2008). However, exogenous N input can affect microbial N-acquiring enzyme activities because microbial use of soluble resources that do not require enzyme action to acquire, including mineral forms of N, can affect resource allocation for extracellular enzyme production (Allison 2005; Moorhead et al. 2023; Schimel et al. 2022). Also, N added such as urea fertilizer would not require the action of standard indicator enzymes (NAG + LAP) and similarly affect estimates of N acquisition unless urease was assayed. Therefore, exogenous N input can skew estimates of NUE by standard ecoenzymatic stoichiometry.

The aim of this study was to test predictions of NUE by the ecoenzyme model under conditions of N addition and to compare these to measurements of NUE based on the ^{18}O-approach. We hypothesized that the ecoenzyme model would be less applicable to estimating NUE with external N input because NAG + LAP would not accurately reflect microbial N acquisition.

Methods

Study site and soil sample collection

The study site is located in the Changbai Mountain Natural Nature Reserve in northeastern China (42.70° N, 127.63° E). This region is characterized by a typical temperate climate, with warm summers and long and cold winters. The mean annual temperature and precipitation are 4 °C and 750 mm, respectively. The ambient atmospheric N deposition rate in this area is about ~ 27 kg N ha^{-1} year^{-1}. The N addition experiment consists of 12 experimental plots (50 × 50 m) in a Korean pine and broadleaf mixed forest with at least a 20-m buffer zone between plots. The dominant coniferous species are *Pinus koraiensis* Siebold & Zucc., and *Abies holophylla* Maxim., and the broad-leaved species are *Corylus mandshurica* Maxim., *Tilia amurensis* Rupr., *Acer rufinerve* Siebold & Zucc., and *Acer pseudosieboldianum* (Pax) Kom. in the plots. Starting in 2014, each of these plots was randomly assigned to the following treatments (three replicates per treatment): Control (0 kg N ha^{-1} year^{-1}), low N treatment (25 kg N ha^{-1} year^{-1}), medium N treatment (50 kg N ha^{-1} year^{-1}), and high N treatment (75 kg N ha^{-1} year^{-1}). Urea was used as N fertilizer, which was spread once annually in May or June on the forest floor (Li et al. 2021). The quantity of N added in the medium N treatment and high N treatment is equivalent to about twofold and threefold of the atmospheric N deposition rate, respectively.

Soil samples were collected at the beginning of June, July, August, September, and October 2021 after eight years of experimental treatment. Before soil samples were collected, soil temperature was determined with a PT100 thermometer. Fifteen subsamples were collected from the surface soil (0–10 cm, 2.5 cm diameter cores) in each plot. Then, the subsamples in each plot were pooled and mixed to form a composite sample and transported to the laboratory in cooling boxes on ice. Each composite sample was sieved (2 mm) and then divided into three subsamples. The subsamples were stored at − 20 °C, 4 °C, or were air-dried, for extracellular enzyme activity analysis, the incubation experiment, and for the analysis of soil physicochemical properties, respectively. The incubation experiment was carried out within one week after sample collection. Soil samples in October were used to analyze soil properties.

Soil gross N mineralization analysis

We conducted two separate incubation experiments to determine microbial growth and gross N mineralization rates. Soil samples collected in June, July, and October were used to evaluate NUE by the ^{18}O-approach. Soil temperatures in June, July, and October were 16, 21, and 7 °C, respectively. For each composite soil stored at 4 °C, we prepared three conical flasks with fresh soil (20 g oven-dry base), two of which were used for the analysis of gross N mineralization and one for determination of microbial growth and microbial biomass C and N. Soil moisture was adjusted to 60% water holding capacity (WHC) and then pre-incubated at the respective in situ

soil temperature of the collection month for 24 h. For N mineralization determinations, flasks were amended with 1 ml $^{15}NH_4NO_3$ (at 10 atom% ^{15}N) solution at a rate of 20 μg NH_4^+-N g^{-1} soil, respectively, after pre-incubation. These flasks were sealed with parafilm with five pinholes and incubated for 0.5 h and 48 h at the respective in situ soil temperature. Soil extractions then were carried out with 2 M KCl (1:5 (w:v)) for 1 h to terminate isotope pool dilutions assays and to measure the concentrations and ^{15}N enrichments of NH_4^+-N at 0.5 h (first flask) and 48 h (second flask) after tracer addition.

Microbial growth rate and NUE analysis

After pre-incubation, we determined microbial growth rates and calculated NUE by the ^{18}O-H_2O tracer technique (Zhang et al. 2019). For each composite soil, subsamples of 1 g soil from the remaining conical flask were weighed into 2 ml brown chromatographic vials in duplicates. One was amended with ^{18}O-H_2O (98 atom%) to reach 20 atom% of ^{18}O in final soil water and the other one was amended with an equal volume of non-labeled water serving as a control. Then, the vials were transferred to 20-ml headspace bottles, closed with butyl rubber stoppers, and incubated at the respective in situ soil temperature for 48 h. After incubation, soil samples were stored at − 20 °C for DNA extraction and ^{18}O abundance analysis. The rest of the pre-incubated soil was used for determination of microbial biomass (see below).

Soil physical and chemical properties

Soil pH was determined by a pH meter in a 2.5:1 (v:w) water to soil ratio. Soil organic matter (SOC), total N, and soil C/N ratio were determined by an elemental analyzer (vario MACRO cube, Germany). Soil texture was assayed by the pipette-sedimentation method. Soil water content was measured gravimetrically after oven drying for three days at 85 °C. Soil water holding capacity was analyzed by repeated saturation of soil in a funnel with filter paper for 2 h and draining for 8 h to approximate the water retained in soil at field capacity. Soil microbial biomass C (MBC) and microbial biomass N (MBN) were measured by the chloroform fumigation method (Vance et al. 1987). MBC and MBN were calculated as the differences in dissolved organic C and total N in extracts between non-fumigated subsamples and fumigated subsamples using conversion factors of 0.54 and 0.45, respectively. Soil NH_4^+-N content was assayed with a continuous flow analyzer (Skalar Analytical, Breda, The Netherlands) after extracting NH_4^+-N from soil with a 2 M KCl solution. The ^{15}N abundance of NH_4^+-N was determined by the diffusion method (Brooks et al. 1989). NH_4^+-N in the KCl extracts was isolated for ^{15}N abundance measurements by adding MgO. The liberated NH_3 was trapped by acidified glass fiber filters wrapped in Teflon tape. The ^{15}N abundance of NH_4^+-N was evaluated with an Elemental Analyzer (Thermo-Element Flash EA 1112, USA) coupled with an Isotope Ratio Mass Spectrometer (IRMS; Thermo Fisher MAT 253, USA).

Soil extracellular enzyme activities

We determined the activities of β-glucosidase (BG), β-N-acetylglucosaminidase (NAG), leucine aminopeptidase (LAP), and acid phosphatase (AP) according to Saiya-Cork et al. (2002), following modifications by Allison et al. (2009) and German et al. (2011). We used a multi-functional microplate reader (Synergy^{H4} Hybrid Reader, Synergy^{H4} BioTek, USA) with 365-nm excitation and 450-nm emission filter to evaluate fluorescence after adding 1 M NaOH to stop the reaction. The (NAG + LAP)/BG ratio indicated the ratio of N-acquiring enzyme activity to C-acquiring enzyme activity.

Soil DNA extraction and ^{18}O abundance analysis

Total DNA in ^{18}O-labeled and non-labeled soils was extracted using a DNeasy PowerSoil Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The quantity of extracted DNA was evaluated by the Picogreen fluorescence assay (Quanti-iT™ PicoGreen dsDNA Reagent, Thermo Fisher, USA) using a multi-functional microplate reader (Synergy^{H4} Hybrid Reader, Synergy^{H4} BioTek, USA). Then, aliquots (70 μl) of the DNA extracts were added to silver capsules and dried at 45 °C for 5 h. The concentration and abundance of O originating from the DNA were analyzed by a TC/EA-IRMS system (Thermo Scientific, USA).

Calculations

Soil gross N mineralization rates (M) (ng N g^{-1} soil h^{-1}) were calculated based on Eq. (1) (Kirkham & Bartholomew 1954):

$$M = \frac{C_{t2} - C_{t1}}{t_2 - t_1} \times \frac{\ln(\mathrm{APE}_{t1}/\mathrm{APE}_{t2})}{\ln(C_{t2}/C_{t1})}, \quad (1)$$

where t_1 and t_2 represent soil extraction time, C_{t1} and C_{t2} represent soil NH_4^+-N content (μg N g^{-1} soil) at t_1 and t_2 respectively, and APE_{t1} and APE_{t2} represent ^{15}N atom% excess of NH_4^+-N at t_1 and t_2, respectively.

The DNA produced ($DNA_{produced}$), microbial N growth rate (N_{growth}), and NUE were calculated as follows, with details given in Zhang et al. (2019). The DNA produced (ng) during the 48 h incubation was calculated based on Eq. (2):

$$\mathrm{DNA_{produced}} = \mathrm{O_{total}} \times \frac{\mathrm{at\%_{excess}}}{100} \times \frac{100}{\mathrm{at\%_{label}}} \times \frac{100}{31.21}, \quad (2)$$

where $\mathrm{at\%_{excess}}$ represents the atom% of ^{18}O in the labeled samples minus the atom% of ^{18}O in control samples, 31.21 is the mean weight% of O in DNA, $\mathrm{O_{total}}$ represents the O content of the dried DNA extracts (ng), and $\mathrm{at\%_{label}}$ is ^{18}O atom% of soil water at the beginning of the incubation, which was 20 atom% in this study.

Microbial N growth rate (ng N g^{-1} soil h^{-1}) was calculated based on Eq. (3):

$$\mathrm{N_{growth}} = \frac{\mathrm{DNA_{produced}} \times f_{\mathrm{DNA}}}{t \times \mathrm{DW}}, \quad (3)$$

where t is incubation time (h) and DW is soil dry mass (g). The f_{DNA} represents the conversion factor, which was calculated separately at each site to represent the ratio of MBN to soil DNA content.

NUE was calculated as given below:

$$\mathrm{NUE} = \frac{\mathrm{N_{growth}}}{\mathrm{N_{growth}} + M}. \quad (4)$$

We also estimated NUE based on the ecoenzyme model for each soil sample (Sinsabaugh et al. 2016):

$$\mathrm{NUE} = \mathrm{NUE_{max}}[S_{N:C}/(S_{N:C} + K_C)], \quad (5)$$

$$S_{N:C} = (1/\mathrm{EEA}_{N:C})(B_{N:C}/L_{N:C}), \quad (6)$$

where $\mathrm{EEA_{N:C}}$ is (NAG+LAP)/BG ratio, $B_{N:C}$ and $L_{N:C}$ are the molar ratios of MBN/MBC and soil TN/SOC, respectively. K_C is set to 0.5 and $\mathrm{NUE_{max}}$ is set to 1. $S_{N:C}$ is a scalar that represents the extent to which the allocation of enzyme activities balances the disparity between elemental compositions of available resources and microbial biomass.

Statistical analysis

Repeated measures ANOVA followed by a Tukey multiple-comparison test was conducted to evaluate the effect of N addition on NUE, microbial growth rate, gross N mineralization rate, $B_{N:C}/L_{N:C}$, and $\mathrm{EEA}_{N:C}$ across months. A one-way ANOVA followed by a Tukey multiple-comparison test was performed to evaluate the effects of N addition on soil properties. Prior to statistical analysis, the homogeneity of variances was checked by Levene's test and data were transformed if necessary. Pearson correlation analysis was used to assess relationships among variables. $P < 0.05$ was considered significant. All statistical analyses were performed in R 4.1.2 (R Core Team 2021). Figures were produced in R with the "ggplot2" package (Valero-Mora 2010).

Results

Soil pH (ranging from 4.67 to 5.02) decreased with N addition (Table 1). The contents of clay (ranging from 22.3% to 35.7%), silt (ranging from 32.3 to 38.6%), and sand (ranging from 26.88 to 45.4%) did not change with N addition. The SOC and TN contents were lowest in the low N treatments, while soil C/N remained constant across N addition treatments.

NUE estimated by the ecoenzyme model ranged from 0.94 to 0.98 and did not change with N addition (Fig. 1 and Additional file 1: Table S1). NUE estimated by the ^{18}O-approach ranged from 0.07 to 0.30 and tended to decrease with N addition (Fig. 2 and Additional file 1: Table S1). Microbial N growth rate showed a weak tendency to decrease with N addition, while gross N mineralization rate did not change (Additional file 1: Fig. S2 and Table S1). $\mathrm{EEA_{N:C}}$ and $\mathrm{B_{N:C}/L_{N:C}}$ did not change with N addition (Additional file 1: Fig. S1 and Table S1). No relationship was found between $\mathrm{EEA_{N:C}}$ and $\mathrm{B_{N:C}/L_{N:C}}$ (Additional file 1: Fig. S3).

Discussion

The NUE values estimated by the ^{18}O-approach (ranging from 0.07 to 0.30) were within the previously reported range of 0.02 to 0.73 in forest ecosystems (Sun et al. 2023; Zhang et al. 2019). These reported NUE values likely varied greatly because they are affected by temperature, moisture, soil properties, and other factors (Zhang et al. 2019).

In contrast, the NUE values estimated by the ecoenzyme model were much higher than those estimated by the ^{18}O-approach (Fig. 2). We believe that this difference

Table 1 Soil properties under control, low N addition, medium N addition, and high N addition

Treatments	SOC (mg g^{-1})	TN (mg g^{-1})	C/N	pH	Clay (%)	Silt (%)	Sand (%)
CK	96.69 ± 9.53 a	5.54 ± 0.26 ab	17.55 ± 2.59 a	4.99 ± 0.04 a	30.76 ± 1.36 a	35.13 ± 1.76 a	34.12 ± 2.94 a
LN	81.60 ± 1.45 b	5.05 ± 0.01 b	16.17 ± 0.31 a	4.96 ± 0.01 a	29.29 ± 2.81 a	35.77 ± 1.55 a	34.94 ± 3.88 a
MN	89.59 ± 1.85 ab	5.53 ± 0.10 ab	16.20 ± 0.09 a	4.79 ± 0.02 b	32.16 ± 2.75 a	36.73 ± 2.29 a	31.11 ± 4.12 a
HN	93.12 ± 1.98 ab	5.67 ± 0.30 a	16.43 ± 0.58 a	4.67 ± 0.01 c	28.80 ± 6.74 a	35.02 ± 2.61 a	36.18 ± 9.34 a

SOC soil organic carbon, *TN* total nitrogen, *C/N* SOC/TN. Different letters indicate a significant difference among N treatments. Data presented are mean and standard error ($n = 3$). Soil properties were measured using samples collected in October

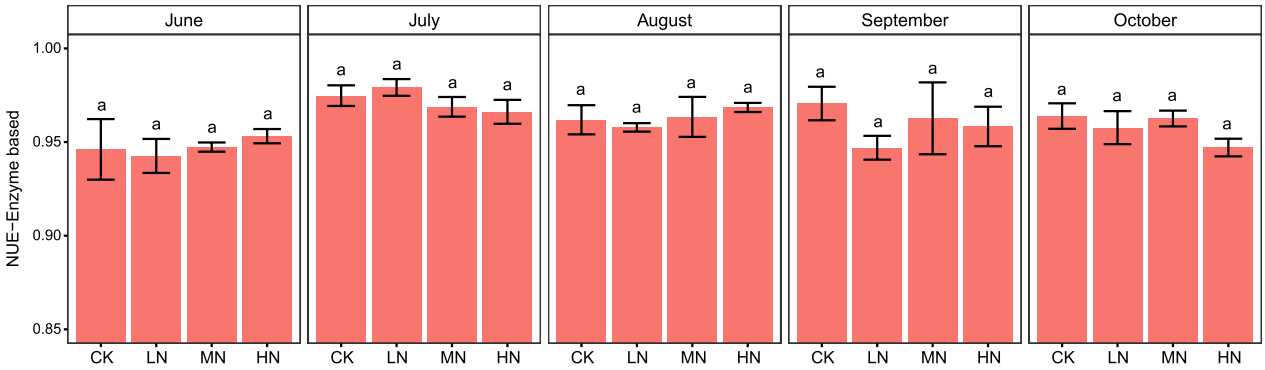

Fig. 1 Estimates of soil microbial NUE by the enzyme stoichiometry method for N addition treatments. *CK* control (0 kg N ha^{-1} $year^{-1}$), *LN* low N addition (25 kg N ha^{-1} $year^{-1}$), *MN* medium N addition (50 kg N ha^{-1} $year^{-1}$), *HN* high N addition (75 kg N ha^{-1} $year^{-1}$). Different letters indicate a significant difference among N addition treatments in a specific month. Data presented are mean and standard error ($n = 3$)

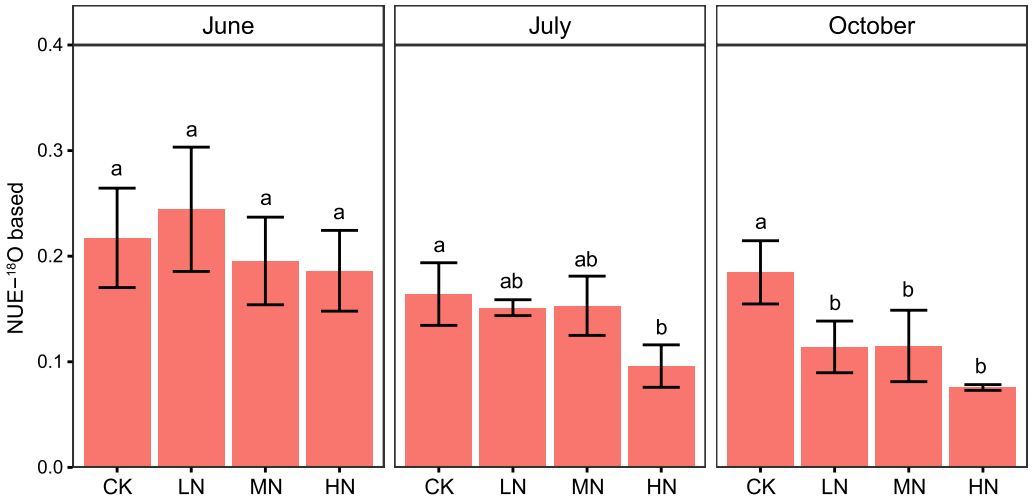

Fig. 2 Estimates of soil microbial NUE by the ^{18}O-based method for N addition treatments. CK, control (0 kg N ha^{-1} $year^{-1}$); *LN* low N addition (25 kg N ha^{-1} $year^{-1}$), *MN* medium N addition (50 kg N ha^{-1} $year^{-1}$), *HN* high N addition (75 kg N ha^{-1} $year^{-1}$). Different letters indicate a significant difference among N addition treatments in a specific month. Data presented are mean and standard error ($n = 3$)

was the result of reduced contributions of NAG + LAP to microbial N acquisition under N addition, which led to an overestimation of NUE by the EEST method. We found that the ratios of N-acquiring enzyme activity to C-acquiring enzyme activity ($EEA_{N:C}$) were low (< 0.5) (Additional file 1: Fig. S1), which overestimates NUE by the ecoenzyme model (Eqs. 5 and 6). Possibly other N-acquiring enzymes were important for microbial N acquisition under N addition (such as urease), however, these enzymes were not included in the calculations (Eqs. 5 and 6). These results imply that β-N-acetylglucosaminidase and leucine aminopeptidase activities cannot accurately indicate microbial N acquisition under this external N input which decouples the expected relationships between N:C in microbial biomass and soil resources as balanced by N- to C-acquiring enzyme activities (Additional file 1: Fig. S3) (Sinsabaugh & Follstad Shah 2012). In addition, soluble resources (such as mineral N deposition) can be assimilated directly by microbes without enzyme catalysis, which similarly affects the estimation of microbial NUE by enzyme model. Taken together, we argue that ecoenzyme stoichiometry does not accurately estimate NUE with external N inputs that affect microbial N acquisition outside the framework of the ecoenzyme stoichiometry model based on the standard indicator enzymes (NAG + LAP), consistent with our hypothesis.

N-acquiring enzymes may be produced for C-liberation under exogenous N inputs, which also interferes with the estimation of NUE by the enzyme model. The

ecoenzymatic theory predicts that $EEA_{N:C}$ should decrease with N addition because the relative activities of NAG and LAP should decrease compared to BG (Sinsabaugh & Follstad Shah 2012; Sinsabaugh et al. 2008). However, we did not find significant changes in the (NAG+LAP)/BG ratio ($EEA_{N:C}$) with N addition (Additional file 1: Fig. S1). This is inconsistent with ecoenzyme stoichiometry theory, but reported by previous meta-analyses that N addition had negligible effects on the ratio of total C- to N-acquiring enzyme activities (Chen et al. 2018a; Jian et al. 2016). This may be because microbes can utilize N-acquiring enzymes for acquiring C under C-poor and/or N-rich conditions (Mori 2020). N addition can stimulate microbial activity and growth by increasing the acquisition of C from organic N substrates, that reduces NUE but in turn increases the production of microbial residues containing chitin, peptidoglycans, and proteins (Hu et al. 2022; Liang et al. 2019; Mori et al. 2021; Zheng et al. 2022). As a result, N-acquiring enzymes may be increasingly important to obtain C under N addition.

The ^{18}O-approach estimated microbial growth by tracing the incorporation of ^{18}O from water into DNA; however, the concurrent mortality of growing microorganisms and the growth of cell size (without DNA replication) can cause an underestimation of microbial growth and therefore of element use efficiencies. To decrease the effect of microbial turnover, the ^{18}O-approach is usually applied only over short-term measurement periods (1–3 days). In addition, microbial extracellular products, which may contain C and N or both, are currently not included in estimations of microbial element use efficiencies due to methodological limitations (Geyer et al. 2016). This causes uncertainty in NUE estimation, which needs to be resolved in the future.

Conclusions

NUE values estimated by the ecoenzyme model were significantly higher than those estimated by the ^{18}O-labeling method and did not change with N addition. This is because the NAG+LAP cannot accurately reflect microbial N acquisition under conditions with external N inputs. In summary, our results suggest that the ecoenzyme model should be used with caution in managed ecosystems.

Abbreviations

MBC	Microbial biomass carbon
MBN	Microbial biomass nitrogen
$B_{N:C}$	MBN/MBC ratio
SOC	Soil organic carbon
TN	Total nitrogen
$L_{N:C}$	TN/SOC ratio
EEST	Ecoenzymatic stoichiometry models
NUE	Nitrogen use efficiency
BG	β-Glucosidase
NAG	β-N-acetylglucosaminidase
LAP	Leucine aminopeptidase
$EEA_{N:C}$	(NAG+LAP)/BG ratio
N_{growth}	Microbial nitrogen growth rate
M	Gross nitrogen mineralization rate

Supplementary Information

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Additional file 1: Table S1. Results of repeated-measures analysis of variance (*F* values) showing effects of N addition and month on microbial metabolism parameters. **Fig. S1** Effects of N addition on $EEA_{N:C}$ (A) and $B_{N:C}/L_{N:C}$ (B). $EEA_{N:C}$, the ratio of enzymatic N (LAP+NAG) vs. C (BG) acquisition activities; $B_{N:C}/L_{N:C}$, N:C ratio of microbes vs. soil; BG, β-1,4-glucosidase (nmol g^{-1} soil h^{-1}); NAG, β-1,4-N-acetylglucosaminidase (nmol g^{-1} soil h^{-1}); LAP, leucine aminopeptidase (nmol g^{-1} soil h^{-1}); CK, control (0 kg N ha^{-1} $year^{-1}$); LN, low N addition (25 kg N ha^{-1} $year^{-1}$); MN, medium N addition (50 kg N ha^{-1} $year^{-1}$); HN, high N addition (75 kg N ha^{-1} $year^{-1}$). Different letters indicate a significant difference among N addition treatments in a specific month. Data presented are mean and standard error ($n = 3$). **Fig. S2** Effects of N addition on microbial N growth rate (A) and gross N mineralization rate (B). CK, control (0 kg N ha^{-1} $year^{-1}$); LN, low N addition (25 kg N ha^{-1} $year^{-1}$); MN, medium N addition (50 kg N ha^{-1} $year^{-1}$); HN, high N addition (75 kg N ha^{-1} $year^{-1}$). Different letters indicate a significant difference among N addition treatments in a specific month. Data presented are mean and standard error ($n = 3$). **Fig. S3** Relationship between $EEA_{N:C}$ and $B_{N:C}/L_{N:C}$. $EEA_{N:C}$, the ratio of enzymatic N (LAP+NAG) and C (BG) acquisition activities; $B_{N:C}/L_{N:C}$, N:C ratio of microbes vs. soil; BG, β-1,4-glucosidase (nmol g^{-1} soil h^{-1}); NAG, β-1,4-N-acetylglucosaminidase (nmol g^{-1} soil h^{-1}); LAP, leucine aminopeptidase (nmol g^{-1} soil h^{-1}).

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Author contributions

L.S. and C.W. designed the study. L.S., Y.C. and S.L. performed the experiment. L.S., D.M. and W.W. wrote the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no conflict of interest or competing interests.

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