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RESEARCH ARTICLE

# Long-term N addition decreases microbial carbon use efficiency in poplar plantations in eastern coastal China

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**Abstract** Soil microbial carbon use efficiency (CUE) plays a critical role in carbon (C) cycling and ecosystem functioning, yet its response to nitrogen (N) deposition remains poorly understood, particularly in planted forests. This study investigates how N addition affects microbial CUE and its underlying mechanisms in *Populus deltoides* plantations in coastal eastern China. Using a long-term field experiment with five levels of N addition (0–30 g $N \cdot m^{-2} \cdot yr^{-1}$), we measured microbial CUE, soil chemical properties, enzyme activities, and microbial community composition from 2018 to 2020. We found that N addition significantly reduced microbial CUE, primarily through N-induced stoichiometric imbalances and soil acidification. Excess N increased available N and decreased the DOC:AN ratio, driving microbial carbon limitation and reducing metabolic efficiency. Furthermore, N addition suppressed bacterial diversity and shifted microbial communities toward taxa with lower CUE. Model selection identified soil pH, available N, and DOC:AN as key predictors of microbial CUE. These findings highlight the dominant role of soil environmental factors—particularly nutrient stoichiometry and pH—in regulating microbial CUE. Our results suggest that excessive N deposition may compromise soil C sequestration in poplar plantations by altering microbial resource allocation and reducing microbial metabolic efficiency. Managing nutrient balance and maintaining microbial diversity are thus critical for sustaining soil health and carbon storage in forest ecosystems under increasing N deposition.

**Keywords** microbial carbon use efficiency, nitrogen addition, soil stoichiometry, poplar plantation, soil acidification

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## 1 Introduction

Soil microbes are key "engines" driving the Earth's biogeochemical cycles, with these microbially driven changes in soil carbon (C) dynamics being closely linked to microbial catabolism and anabolism (Wieder et al., 2013; Feng et al., 2022b; Guo et al., 2024; Diao et al., 2025). Microbial C use efficiency (CUE) is a measure of the proportion of C that is incorporated into microbial biomass during the process of organic matter decomposition (Cruz-Paredes and Rousk, 2024; Jiang et al., 2025). It is a critical determinant of C cycling and can have cascading effects on ecosystem functioning in forest ecosystems (Shi et al., 2025). Efficient microbial CUE promotes the retention of C in soil organic matter, contributing to soil fertility and ecosystem stability. Models with incorporation of CUE could largely improve the prediction accuracy of global C distribution (Bradford and Crowther, 2013; Wieder et al., 2013). As one important environmental change, N deposition has increased significantly in the past decades and is predicted to continue to increase (Eastman et al., 2021). It could cause pervasive impacts on soil nutrient availability, pH and microbial community, potentially

affecting microbial CUE (e.g., Malik et al., 2020). However, the connection between microbial CUE and N deposition remains unclear and our understanding of it is therefore essential for the accurate prediction of forest C dynamics under N deposition.

Many studies have been conducted to explore the effects of N addition to mimic N deposition on microbial CUE, but the results have been equivocal (Li et al., 2021b; Yang et al., 2023; Diao et al., 2025). The addition of N could enhance microbial CUE by increasing N availability, thereby reducing the need for microbes to allocate C to metabolic costs associated with N acquisition (Manzoni et al., 2012; Malik et al., 2020). Meanwhile, microbes may allocate more C to biomass to maintain a balanced biomass C:N ratio, with anticipated increases in CUE according to the theory of stoichiometric homeostasis (Manzoni and Porporato, 2009; Manzoni et al., 2017). But others indicate a decrease in CUE as microbial community become nutrient limited (Manzoni et al., 2012; Riggs and Hobbie, 2016). Excess N can result in nutrient imbalances, particularly other essential elements like phosphorus (P). These imbalances can stress microbes and reduce their CUE as expend more energy to acquire limiting nutrients. In some cases, increased N availability may stimulate plant growth, leading to increased root exudates and litter inputs that could enhance microbial growth (Feng et al., 2023). When the additional N makes the environment more conducive to microbial metabolism, CUE could increase. But high N levels can accelerate the decomposition of labile C substrates (e.g., root exudates and litter), which could also lead to greater C loss via respiration, thus reducing CUE (Bicharanloo et al., 2020; Li et al., 2021a). Moreover, N addition could increase the recalcitrance of plant-derived substrate (Liu et al., 2016), which reduces CUE due to higher microbial energy consumption. Studies on the effects of N addition on microbial CUE have yielded mixed results. This highlights the complexity of microbial responses to N addition and underscores the need for context-specific investigations.

In addition to changing the availability of soil nutrients, N addition often causes soil acidification (Chen et al., 2023a; Wang et al., 2023b). Acidic conditions can induce stress on microbial growth, activity and diversity, potentially having significant impacts on soil microbial CUE (Shi et al., 2025). First, N addition can significantly influence the production of extracellular enzymes by soil microbes (Chen et al., 2025). These enzymes are crucial for the breakdown of complex organic compounds into simpler forms that microbes can assimilate. For instance, increased N can stimulate the production of certain enzymes, like cellulases, that break down complex carbohydrates (Luo et al., 2019). This can lead to a more efficient decomposition process, increasing the availability of simpler C compounds for microbial uptake and conversion into biomass. Conversely, some enzymes may be inhibited under high N conditions (Xiao et al., 2018; Chen et al., 2023b). For example, lignin-degrading enzymes might be suppressed, reducing the breakdown of more recalcitrant C sources (Wang et al., 2022). This imbalance can affect the overall CUE of microbes, as they may not fully utilize all available C sources efficiently. Second, elevated N levels can shift the composition of the microbial community (Zhou et al., 2017; Craig et al., 2021), often favoring nitrophilic species while suppressing those less adapted to high N conditions. This shift can reduce overall microbial diversity, which has several implications. In a diverse community, multiple species can perform similar functions. Loss of diversity can reduce this redundancy, making the ecosystem more vulnerable to disturbances and less efficient at C utilization (Duan et al., 2023; Dang and Morrissey, 2024). Moreover, unique functional traits: different microbial species possess unique enzymes and metabolic pathways that contribute to efficient C cycling. A less diverse community lacks this variety, potentially leading to less efficient decomposition and C use. Third, increased N can change how microbes interact within the soil ecosystem (Zhang et al., 2022; Ye et al., 2025). Microbes often engage in cooperative interactions, such as cross-feeding, where one species breaks down a compound into a form that another can use (Mmotla et al., 2025). High N levels might reduce the need for such cooperation, leading to more competitive interactions through increased competition (Liu et al., 2025b). With more N available, microbes may compete more intensely for other limited resources, such as P or C. This competition can lead to higher energy expenditures on survival and maintenance rather than growth and biomass production, reducing overall CUE (Lian et al., 2024). Overall, these highlight a current knowledge gap in understanding the complex interactions between N deposition, microbial CUE, and C cycling in forest ecosystems. Further research is thus needed to elucidate the underlying mechanisms and their implications for ecosystem C dynamics.

Poplar is a tree species that is cultivated industrially in China, covering an area of more than 7.5 million ha. Poplar plantations play important roles in C sequestration in China and mitigating climate change (Geng et al., 2021; Jiang et al., 2021a). However, increasing N deposition, especially in the eastern coast of China (Yu et al., 2019), could substantially affect soil microbial CUE and subsequently the potential of C storage of poplar plantations. Compared to the natural forest ecosystem, we still know little of the CUE in response to N deposition in planted forests. We thus conducted an experiment to explore the effects of N addition on soil microbial CUE and its underlying regulating mechanisms in poplar plantations at the Dongtai Forest Farm. Based on previous studies, we hypothesized that (i) N addition

will decrease microbial CUE due to direct effects of N-induced nutrient imbalances and indirect effects of N-induced soil acidification on microbial communities, and (ii) the soil environment plays a more dominant role in regulating variations in CUE compared to soil microbes, as microbial changes are ultimately driven by environmental factors.

## 2 Materials and methods

### 2.1 Experimental site and design

Our experimental site is located at the Dongtai Forest Farm on the coast of the Yellow Sea in Yancheng, Jiangsu Province, China (120º49′E, 32º52′N). The forest farm is established in 1965 and covers an area of approximately 3000 ha. The climate in this region is classified as a humid subtropical monsoon climate (Cfa) according to the Köppen classification. The mean annual air temperature and precipitation from 1960 to 2020 are 14.92°C and 1056.6 mm, respectively. Forest cover is approximately 85% and poplar (*Populus deltoids*) and dawn redwood (*Metasequoia glyptostroboides*) are major tree species planted at the farm. The soil at the farm is desalinated meadow and sandy with alkaline soil pH values (Geng et al., 2023).

In May 2012, an N addition experiment was established in an 8-year-old pure poplar (*Populus deltoids* cv. 'I-35') plantation. Four randomly distributed blocks (25 m × 190 m) with similar site and management history were selected. We then applied a randomized block design with a five-level gradient of N addition (N0: 0, N1: 5, N2: 10, N3: 15, and N4: 30 g $N \cdot m^{-2} \cdot yr^{-1}$) in each replicate block (25 m × 190 m). The ambient N deposition rate is approximately 5 g $N \cdot m^{-2} \cdot yr^{-1}$ for this area (Zhu et al., 2016) and Liu et al. (2013) predicted that critical loads of N deposition in Jiangsu Province could exceed 20 g $N \cdot m^{-2} \cdot yr^{-1}$. Each N treatment plot was approximately 25 m × 30 m with a 10 m buffer zone between any adjacent two plots and a minimum 500 m between any two replicate blocks. In total, we had 20 plots (4 blocks × 5 N addition levels) in this experiment. N deposition was simulated using ammonium nitrate ($NH_4NO_3$) as reported by previous studies at the same site (Xu et al., 2014; Zhou et al., 2015). For each plot, the experimental quantity of $NH_4NO_3$ was dissolved in 20 L water and added using a backpack sprayer monthly to the ground during the growing season from May to October each year. 20 L of water was sprayed as a control in the N0 plots. Detailed description of the experimental design can be found in Geng et al. (2021).

### 2.2 Soil sampling and property measurement

Soil samples were collected in August (growing season) and November (non-growing season) each year from 2018 to 2020. In each of the 20 plots, we collected soil samples at the same soil depth (0–20 cm) using a six-point "S" shape sampling from which soils were pooled as a single replicate. Soil samples were sealed in air-tight plastic bags and transported in coolers to our laboratory. In the laboratory, all the soil samples were sieved (< 2 mm) to remove soil fauna, rocks, and fine roots, air-dried or refrigerated at 4°C and −80°C for further analyses. We disinfected the soil corer and sieves with 75% ethanol after each field sampling and laboratory sieving of soil samples.

Soil temperature ($T_{soil}$) of the 0–20 cm soils was measured using a thermocouple connected to a Li-Cor 6400 portable infrared gas analyzer (IRGA) (LI-COR Inc., Lincoln, NE, USA). Soil water content ($W_{soil}$) was measured by oven-drying 20 g of fresh soil at 105°C for 48 h. Soil pH was measured with a pH meter in deionized water suspension with a 1:2.5 (w:v) soil: water ratio (Model PHS-25, INESA Instruments, Shanghai, China). Dissolved organic C (DOC) was extracted with 0.5 M $K_2SO_4$, and then measured using TOC/TN (Multi/C2100 TOC/TN, Jena, Germany). Soil available N (AN), the sum of $NH_4^+$ and $NO_3^-$, was determined with the alkaline dispersion method (Kielak et al., 2008). Soil available P (AP) was extracted using microwave digestion and a $NaHCO_3$ solution then measured using the Mo-Sb colorimetric method (Qin et al., 2011). Soil microbial biomass C (MBC), N (MBN) and P (MBP) was determined using the chloroform fumigation-extraction method (Brookes et al., 1985). A portion of the air-dried soil was sieved through a 0.212 mm sieve and then dipped into 0.5 M hydrochloric acid to remove carbonates. Soil organic C (SOC) and TN were then measured using an elemental analyzer (EA 6000, Leeman Instruments, Shenyang, China). Total P (TP) was determined following $H_2SO_4$-$H_2O_2$ digestion (Jiang et al., 2021b) and measured using a micro-plate reader (TECAN infinite 200pro, Tecan Group, Switzerland).

### 2.3 Soil microbial CUE estimation

We assessed the activities of four extracellular hydrolytic enzymes involved in soil carbon (*β*-1, 4-glucosidase, BG), nitrogen (N-acetyl-*β*-D-glucosaminidase, NAG; leucine aminopeptidase, LAP), and phosphorus (alkaline phosphatase, AKP) cycling. Enzyme activities ($nmol \cdot h^{-1} \cdot g^{-1}$) were quantified using a fluorometric assay in 96-well microplates, following protocols adapted from German et al. (2011) and Bach et al. (2013). In brief, 1 g of fresh soil was homogenized in 100 mL of 200 mM sodium acetate buffer (pH ~7.5) using microwave-assisted shaking for 3 min. Then, 150 µL of the resulting soil slurry was pipetted into each well, followed by the addition of 50 µL of 200 µM fluorogenic substrate. The plates were incubated in the dark at 30°C for 3 h.

Fluorescence was measured at an excitation wavelength of 360 nm and an emission wavelength of 460 nm using a microplate reader (TECAN Infinite 200 Pro, Tecan Group Ltd., Switzerland). Soil microbial CUE was estimated from ecological stoichiometry according to Sinsabaugh et al. (2016) using the following equations:

$$\mathrm{CUE} = \mathrm{CUE}_{\max} \times (S_{\mathrm{C:N}} \times S_{\mathrm{C:P}}) / [(S_{\mathrm{C:N}} + K_x) \times (S_{\mathrm{C:P}} + K_x)]^{0.5}, \quad (1)$$

$$S_{\mathrm{C:N}} = (1/\mathrm{EEA}_{\mathrm{C:N}}) \times (B_{\mathrm{C:N}}/L_{\mathrm{C:N}}), \quad (2)$$

$$S_{\mathrm{C:P}} = (1/\mathrm{EEA}_{\mathrm{C:P}}) \times (B_{\mathrm{C:P}}/L_{\mathrm{C:P}}), \quad (3)$$

$$\mathrm{EEA}_{\mathrm{C:N}} = \mathrm{BG}/(\mathrm{NAG} + \mathrm{LAP}), \quad (4)$$

$$\mathrm{EEA}_{\mathrm{C:P}} = \mathrm{BG}/\mathrm{AKP}, \quad (5)$$

$$B_{\mathrm{C:N}} = \mathrm{MBC}/\mathrm{MBN}, \quad (6)$$

$$B_{\mathrm{C:P}} = \mathrm{MBC}/\mathrm{MBP}, \quad (7)$$

$$L_{\mathrm{C:N}} = \mathrm{SOC}/\mathrm{TN}, \quad (8)$$

$$L_{\mathrm{C:P}} = \mathrm{SOC}/\mathrm{TP}, \quad (9)$$

where CUE is the $\mathrm{CUE}_{\max}$ is the upper limit for microbial growth efficiency based on thermodynamic constraints. We set $\mathrm{CUE}_{\max}$ = 0.60 as a conservative upper bound for soil heterotrophic microorganisms, consistent with syntheses indicating maximal CUE rarely exceeds ~0.6 under C-replete conditions (Sinsabaugh et al., 2016). The half-saturation constant is fixed at $K_x$ = 0.5, following Sinsabaugh et al. (2016). EEA is ecoenzymatic activities ($\mathrm{nmol \cdot g^{-1} \cdot h^{-1}}$). $B_{\mathrm{C:N}}$ and $B_{\mathrm{C:P}}$ is the elemental C:N and C:P ratio of soil microbial biomass. $L_{\mathrm{C:N}}$ and $L_{\mathrm{C:P}}$ is elemental composition of the substrate consumed, is the ratio of SOC/TN and SOC/TP.

### 2.4 DNA extraction, amplification, and Illumina MiSeq sequencing

The total soil DNA was extracted from 0.25 g soil samples using a Magen Hipure Soil DNA Kit (Guangzhou Magen Biotechnology Co., Ltd. Guangzhou, China) according to the manufacturer's instructions. The extracted DNA was evaluated using a 1.5% agarose gel and quantified DNA concentration using a Equalbit 1xdsDNA HS Assay Kit (Vazyme, Nanjing, China). To profile soil fungal communities, we amplified the internal transcribed spacer 2 (ITS2) region with the primers sets ITS2-F (5′-GTGAATCATCGARTC-3′) and ITS2-R (5′-TCCTCCGCTTATTGAT-3′) designed by GENEWIZ (GENEWIZ, Inc., South Plainfield, NJ, USA). For bacterial communities, we amplified the V3-V4 hypervariable region of 16S rRNA gene with the primer pair (F: 5′-CCTACGGRRBGCASCAGKVRVGAA-3′ and R: 5′-GGACTACNVGGGTWTCTAATCC-3′) designed by GENEWIZ (GENEWIZ, Inc., South Plainfield, NJ, USA). Polymerase chain reaction (PCR) amplification was performed with 25 μL reaction volumes: 2.5 μL of TransStart Buffer, 2 μL of dNTPs, 1 μL of Forward primer, 1 μL of Reverse primer, 0.5 μL of TransStart Taq DNA, 20 ng of DNA Template, and dd$H_2O$. For the ITS2 region, the PCR thermocycler conditions were as follows: an initial denaturation at 94°C for 5 min, followed by 25 cycles of 94°C for 30 s, 57°C for 30 s, and 72°C for 30 s, and a final extension of 72°C for 5 min. For the 16S rRNA gene, the PCR thermal cycling condition was 94°C for 3 min, followed by 24 cycles of 94°C for 5 s, 57°C for 90 s, and 72°C for 10 s with a final extension at 72°C for 5 min. PCR products were extracted from 1.5% agarose gels and purified using a DNA gel extraction kit, and quantified on the Qubit 3.0 Fluorometer (Invitrogen, Carlsbad, CA) with Qubit dsDNA HS reagents. The purified PCR products were sequenced on the Illumina MiSeq platform (Illumina, San Diego, CA, USA) using the 2 × 250 bp paired-end sequencing according to standard protocols.

### 2.5 Soil microbial diversity calculation

Paired reads were merged to form consensus sequences using the fastq_mergepairs command in VSEARCH (minOverlap = 20) (Rognes et al., 2016). Next, the reads were trimmed from their barcode and primer sequences, and the sequences with lengths shorter than 200 bp, as well as low-quality bases with Phred score shorter than 20, were removed and low using CUTADAPT (Martin, 2011) to retain only biologically relevant sequences. The remaining sequences were clustered into biologically relevant operational taxonomic units (OTUs) at a 97% similarity level using the VSEARCH (Rognes et al., 2016), and chimeras sequence were eliminated during this procedure. Taxonomic annotations of OTUs were determined using the Ribosomal Database Project Classifier tool (Wang et al., 2007) with a confidence threshold of 0.8 against the UNITE 8.0 database (Tedersoo et al., 2018; Nilsson et al., 2019) for fungi and the Silva 132 database (Quast et al., 2013) for bacteria. The singleton OTUs and OTUs that were not classified into fungi were removed before subsequent analyses (Hu et al., 2021). The OTUs tables were subsampled to a minimum number of sequences from each sample (20,489 for fungi and 80,218 for bacteria) for subsequent analyses. Rarefaction analysis was carried out at the OTUs level using the function rarecurve in the '*vegan*' package to assess the sequencing depth, ensuring rarefaction-curve plateau. OTUs richness and Shannon diversity index of the fungal and bacterial communities

for each sample were calculated using the diversity function in '*vegan*' package (Oksanen et al., 2019). Faith's phylogenetic diversity index was used to measure phylogenetic α-diversity (PD) using the '*Picante*' package.

### 2.6 Statistical analyses

All statistical analyses were performed using R 4.3.0. We used repeated-measures linear mixed models to test the effects of N addition, sampling times, year and all interactions of these factors on microbial CUE and related variables using the "nlme" package (Pinheiro et al., 2021), with block as a random term. N addition and year were treated as categorical variables in the model. Data were log-transformed to meet the assumptions of normality and homogeneity of variances when necessary. Additionally, random forest analysis was performed to identify the most important factors predicting the CUE using the "randomForest." Predictors included soil pH, $T_{soil}$, $W_{soil}$, DOC, AN, AP, DOC:AN, DOC:AP, AN:AP and the $\alpha$–diversity indices (including OTU richness, Shannon diversity, and PD) of both bacteria and fungi. Simple correlations of CUE with the predictor with test using the "cor" function. All the figures were prepared using Sigmaplot 14.0 (SYSTAT Software Inc., CA, USA).

## 3 Results

### 3.1 Effects of N addition soil and microbial properties

N addition decreased soil pH ($p < 0.01$, Table 1, Fig. 1(a)) and showed no significant impacts on soil temperature and moisture (all $p > 0.05$, Table 1, Figs. 1(b) and 1(c)). Both soil temperature and moisture varied greatly across the sampling times with lower soil temperature observed in November and lower soil moisture in 2019 (all $p < 0.01$, Table 1, Figs. 1(b) and 1(c)). Interactions between N addition and sampling time did not significantly affect either soil pH or soil temperature or moisture (all $p > 0.05$, Table 1, Figs. 1(b) and 1(c)).

For soil labile C, N, P, and their stoichiometric ratios, we found N addition increased DOC and AN (all $p < 0.01$, Table 1, Figs. 2(a) and 2(b)) but had no significant impacts on AP ($p > 0.05$, Table 1, Fig. 2(c)). DOC, AN, and AP varied significantly across sampling times, with all lower values found in 2020. N addition decreased DOC:AN and increased AN:AP (all $p < 0.01$, Table 1, Figs. 2(d)−2(f)) but had no significant impacts on DOC:AP ($p > 0.05$, Table 1, Fig. 2(e)). DOC:AN, DOC:AP, and AN:AP all significantly varied across sampling times with higher values observed in 2020, 2019, and 2019, respectively (all $p < 0.05$, Table 1, Figs. 2(d)−2(f)).The interactions between N addition and sampling time did not significantly affect DOC, AN, AP or their stoichiometric ratios (all $p > 0.05$, Table 1, Fig. 2).

**Table 1** Results of linear mixed-effect model ANOVA (*F*-tests) for the effects of nitrogen addition (N), sampling time (T), and their interactions (N × T) on soil temperature and moisture ($T_{soil}$ and $W_{soil}$), pH, dissolved organic carbon (DOC), available nitrogen and phosphorus (AN and AP), the ratios of DOC:AN, DOC:AP, and AN:AP, soil bacterial and fungal diversity (including richness, Shannon diversity and Phylogenetic diversity (PD)), and microbial carbon use efficiency (CUE) from 2018 to 2020. Significance is indicated by: *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$, otherwise $p > 0.05$. *df* = degree of freedom

| Variables | | N | T | N × T |
|---|---|---|---|---|
| *df* | | 4, 87 | 5, 87 | 20, 87 |
| Soil pH | | **22.02*** ** | 1.13 | 0.87 |
| $T_{soil}$ | | 1.85 | **9020.24*** ** | 1.49 |
| $W_{soil}$ | | 0.77 | **23.70*** ** | 0.37 |
| DOC | | **3.92** * | **28.45*** ** | 1.30 |
| AN | | **7.11*** ** | **8.49*** ** | 1.08 |
| AP | | 0.60 | **5.32*** ** | 0.96 |
| DOC:AN | | **3.68** * | **2.67** * | 1.21 |
| DOC:AP | | 1.48 | **9.26*** ** | 1.23 |
| AN:AP | | **3.97** * | **6.64*** ** | 0.91 |
| Bacteria | Richness | **2.69** * | **27.90*** ** | 1.24 |
| | Shannon diversity | **5.45*** ** | **64.60*** ** | 1.31 |
| | PD | 2.04 | **16.89*** ** | **2.32** * |
| Fungi | Richness | 1.01 | **47.64*** ** | 1.07 |
| | Shannon diversity | 0.23 | **5.51** * | 0.81 |
| | PD | 0.88 | **67.60*** ** | 1.46 |
| CUE | | **8.62*** ** | **40.78*** ** | 1.33 |

N addition decreased the richness and Shannon diversity of bacteria (all $p < 0.01$, Table 1, Figs. 3(a) and 3(b)) and had non-significant impacts either on PD of bacteria or the richness, Shannon diversity and PD of fungi (all $p > 0.05$, Table 1, Figs. 3(c), 3(d)−3(f)). All of the diversity indices of both bacteria and fungi varied greatly across sampling times (all $p < 0.01$, Table 1, Fig. 3). The interactions between N addition and sampling time generally had non-significant effects on all diversity indices of both bacteria and fungi, except for the PD of bacteria, which initially increased with N addition before subsequent decreases. This pattern likely reflected transient alleviation of N limitation at low additions, followed by diversity losses at higher N driven by soil acidification.

### 3.2 Effects of N addition on soil microbial CUE and its driving factors

N addition reduced soil microbial CUE, with a

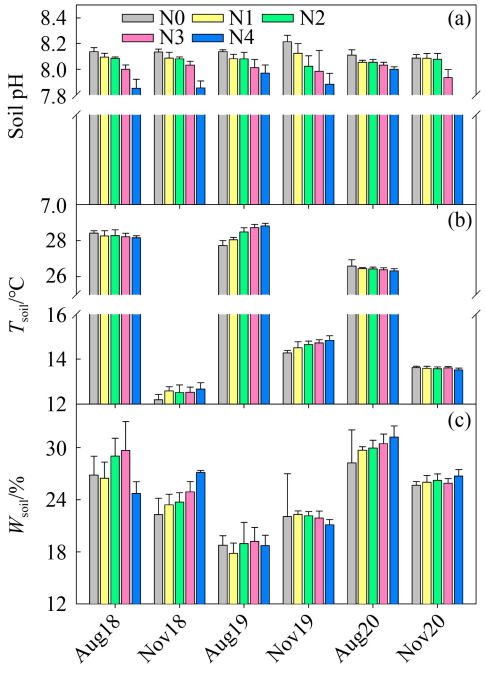


**Fig. 1** Effects of N addition on soil pH values (a), soil temperature ($T_{soil}$, b) and moisture ($W_{soil}$, c) over the study period. Values are in mean ± standard error (SE).

significantly lower value observed in 2018 (all $p < 0.01$, Table 1, Fig. 4). The interaction between N addition and sampling time had no significant effect on CUE ($p > 0.05$, Table 1, Fig. 4). Model selection analysis identified soil pH, AN, and DOC:AN as the key factors influencing soil microbial CUE (all $p < 0.05$, Fig. 5(a)). CUE was positively correlated with soil pH and DOC:AN but negatively correlated with AN (all $p < 0.01$, Figs. 5(b)−5(d)).

## 4 Discussion

### 4.1 N addition decreased CUE

Balanced C, N, and P ratios are essential for optimal microbial functioning. In line with previous findings (Manzoni et al., 2012; Riggs and Hobbie, 2016; Li et al., 2025), we found N addition decreased CUE associated with increases in AN, DOC, and AN:AP but decreases in DOC:AN. Under N addition, the relative abundance of C can become a limiting factor. This is observed at the same experimental site when N inputs are not balanced with C inputs (Li et al., 2023). According to ecological stoichiometry theory, the C:N ratio of soil microbial biomass is typically maintained within a specific range to ensure internal elemental homeostasis, allowing microbes to sustain efficient metabolic processes (Sterner and Elser, 2002; Cleveland and Liptzin, 2007; Cui et al., 2022a). However, elevated N deposition can disrupt this balance, forcing microbes to allocate additional C and energy to compensate for the imbalance, ultimately affecting nutrient cycling and organic matter decomposition efficiency. In our study, we found that a decrease in the DOC:AN ratio below the critical range of 7–8.6 under N addition resulted in a significant decline in microbial CUE. Since both DOC and AN are directly utilized by microbes, an imbalance between these two resources likely contributed to this reduction in CUE (Adingo et al., 2021; Feng et al., 2022a; Liu et al., 2025a). If the C:N ratio drops too low due to excessive N addition, microbial metabolic processes may shift toward prioritizing N assimilation over C metabolism. This shift increases the energy demand for maintaining intracellular homeostasis, reducing metabolic efficiency and further

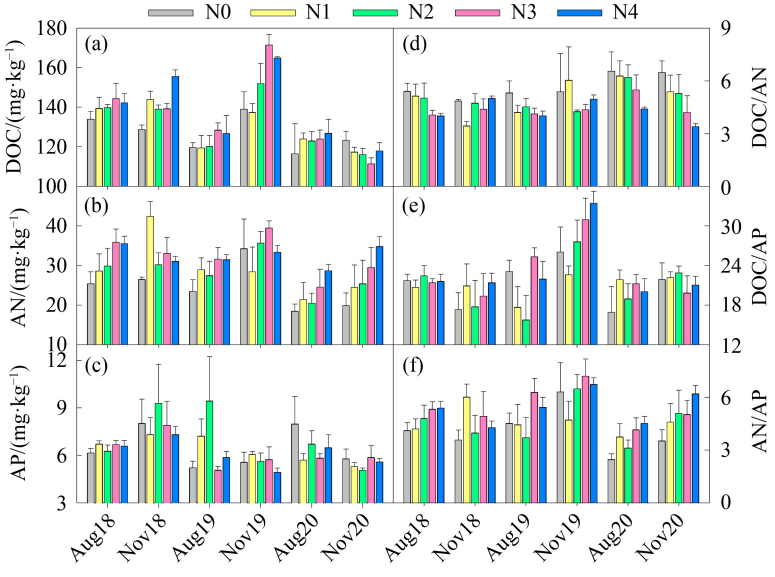


**Fig. 2** Effects of N addition on soil dissolved organic carbon (DOC, a), available nitrogen (AN, b), available phosphorus (AP, c) and their stoichiometric ratios (d–f) over the study period. Values are in mean ± standard error (SE).

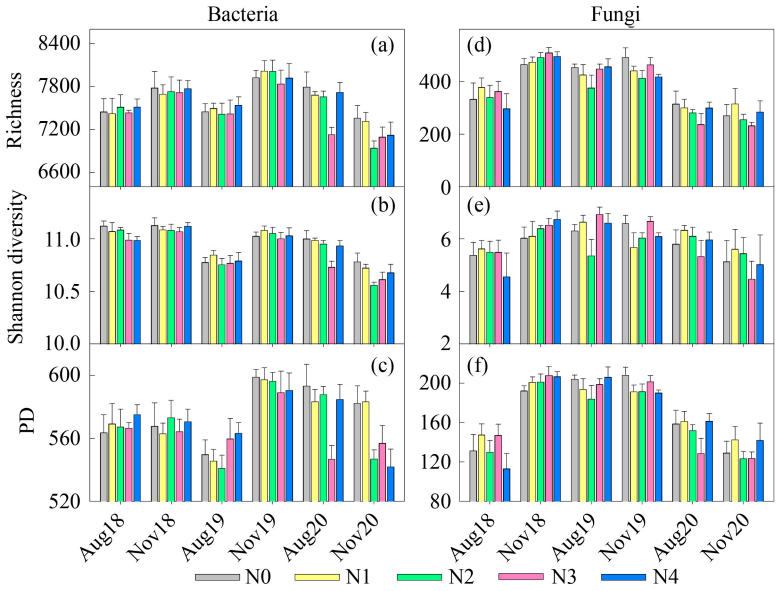


**Fig. 3** Effects of N addition on soil bacterial (a–c) and fungal diversity (d–f), including richness (a, d), Shannon diversity (b, e) and phylogenetic diversity (PD, c and f) over the study period. Values are in mean ± standard error (SE).

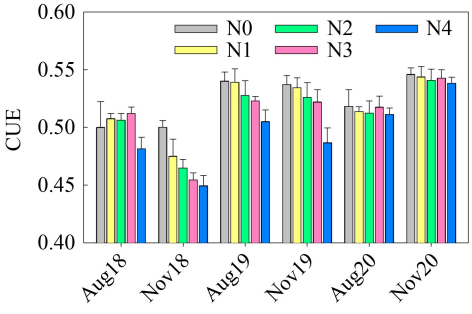


**Fig. 4** Effects of N addition on soil microbial carbon use efficiency (CUE) over the study period. Values are in mean ± standard error (SE).

lowering CUE.

Additionally, under N addition, the relative scarcity of phosphorus (P) can become more pronounced, imposing additional constraints on microbial growth and further reducing CUE. Since microbial metabolism relies on a balanced supply of C, N, and P, an excess of N without sufficient P can disrupt microbial stoichiometric homeostasis. In response, microbes may reallocate energy toward scavenging for P through enzymatic production, such as increasing phosphatase activity, or adjusting metabolic pathways to optimize nutrient acquisition (Gao et al., 2024). However, these compensatory mechanisms come at an energetic cost, diverting resources away from biomass production and leading to a decline in microbial CUE. Moreover, soil stoichiometric imbalances have been shown to trigger overflow respiration, a process where microbes metabolize excess C inefficiently due to nutrient limitations, ultimately leading to increased $CO_2$ emissions and lower microbial CUE (Yuan et al., 2019; Liao et al., 2024). Under such conditions, microbes may prioritize energy dissipation over biomass synthesis, exacerbating SOC losses rather than promoting stabilization. These effects are particularly pronounced in at our site where natural P availability is low, where microbial inefficiencies driven by nutrient imbalances are more severe. Prolonged stoichiometric imbalances caused by N enrichment can create suboptimal microbial growth conditions, inducing microbial stoichiometric stress. This stress not only reduces microbial efficiency in utilizing C but may also lead to shifts in community composition. Such shifts can further influence decomposition dynamics, nutrient cycling, and long-term soil C storage.

Microbial community composition plays a fundamental role in regulating CUE, as different microbial taxa contribute distinct functional traits to C cycling. In our study, we found that N addition significantly reduced bacterial richness and Shannon diversity, while having no significant impact on fungal diversity. This shift in microbial diversity may explain the observed decline in microbial CUE, as reduced bacterial diversity could limit functional redundancy and metabolic flexibility within the microbial community, ultimately impairing C processing efficiency (Domeignoz-Horta et al., 2020; Bastida et al., 2021). One potential mechanism linking bacterial diversity loss to lower CUE is the reduction in metabolic complementarity within the microbial community. Diverse bacterial communities often exhibit functional redundancy, where multiple species contribute to key metabolic pathways, ensuring stable and efficient decomposition and assimilation of organic C (Jia and Whalen, 2020). However, under N enrichment, the decline in bacterial richness may have reduced the range of functional traits available for efficient substrate utilization, leading to higher respiratory losses relative to biomass production. A decrease in microbial diversity can also result in reduced cross-feeding interactions, where

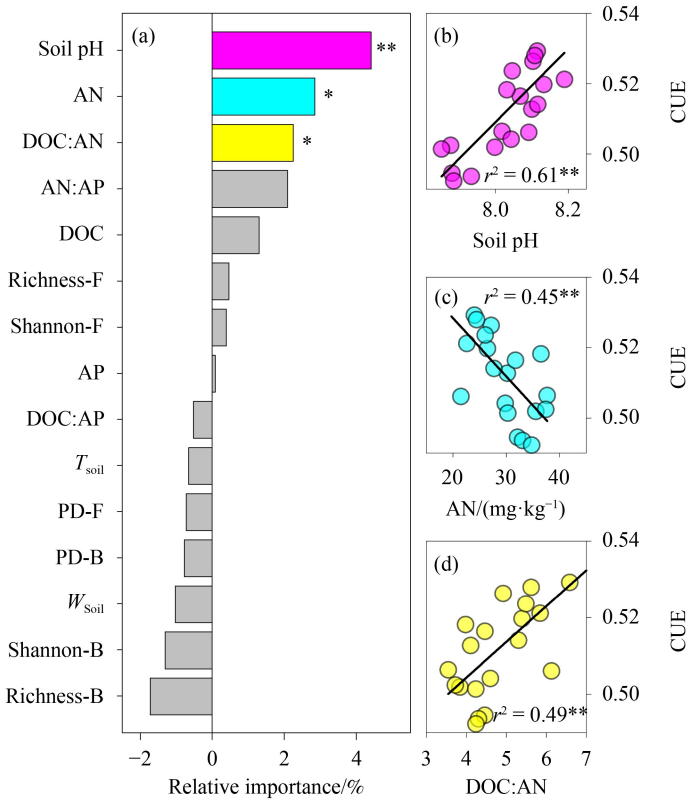


**Fig. 5** The relative importance of variables for predicting variations in soil microbial carbon use efficiency (CUE, a) and significant relationships of CUE with soil pH (b), AN (c), and DOC:AN (d). In ecological terms, predictors with higher relative importance are interpreted as the dominant controls on CUE in our data set. Predictors included soil pH, soil temperature and moisture ($T_{soil}$ and $W_{soil}$), dissolved organic carbon (DOC), soil available nitrogen and phosphorus (AN and AP), DOC:AN, DOC:AP, AN:AP and the $\alpha$–diversity indices (including OTU richness, Shannon diversity, and phylogenetic diversity (PD)) of both bacteria (B) and fungi (F).

metabolic byproducts from one species serve as substrates for another, further decreasing the efficiency of organic C transformation and retention in microbial biomass (Giri et al., 2021). Additionally, the selective pressure imposed by N enrichment may have favored specific microbial taxa with lower CUE. Previous studies suggest that under high N availability, bacterial communities shift toward fast-growing, r-strategist species that prioritize rapid resource acquisition and high respiration over biomass production (Wang et al., 2021; Wang et al., 2024; Zheng et al., 2024). This shift can result in a community dominated by taxa with inherently lower CUE, further contributing to the observed reduction in microbial efficiency. Meanwhile, fungi—key decomposers of recalcitrant organic matter—remained unaffected by N addition, likely reflecting hyphal foraging and enzyme-based resource strategies and a higher tolerance to N-induced pH shifts. Accordingly, the bacterial-driven component of soil C cycling appears more sensitive to changes in nutrient availability (Ramirez et al., 2012; Wang et al., 2023a). Furthermore, a decline in bacterial diversity may disrupt microbial regulatory mechanisms that optimize resource use. In diverse microbial communities, competition among taxa can drive more efficient substrate utilization, as microbes must optimize their metabolic pathways to maximize C assimilation while minimizing unnecessary losses (Zhang et al., 2025). However, under N enrichment, the loss of competitive interactions due to reduced diversity may lead to a breakdown of these regulatory mechanisms, resulting in less efficient C metabolism and increased respiratory losses. Overall, our results suggest that N-induced reductions in bacterial diversity may play a key role in decreasing microbial CUE by limiting functional redundancy, altering community composition toward lower-CUE taxa, and disrupting microbial resource optimization. These findings highlight the importance of microbial diversity in maintaining efficient soil C cycling and suggest that biodiversity loss under N deposition could have significant consequences for soil organic C dynamics. While the ecoenzymatic stoichiometric approach provides a valuable ecosystem-scale estimate of microbial CUE for large field studies, it is proxy-based rather than a direct measurement; future work coupling this method with isotopic tracers (e.g., $^{18}O$-$H_2O$ or $^{13}C$-acetate incorporation) would help validate and refine the patterns reported here.

### 4.2 Drivers of CUE in poplar plantations under N addition

Environmental conditions exert a fundamental influence on biological communities by shaping species

distributions, interactions, and functional traits (Åkesson et al., 2021; Liu et al., 2024; Nizamani et al., 2024). Niche theory provides a useful framework for understanding how environmental factors, such as soil pH and nutrient availability, shape microbial community composition and function, ultimately influencing CUE. According to niche differentiation, microbial species occupy distinct functional and physiologic niches based on their resource preferences and environmental tolerances (Bahram et al., 2018; Nuccio et al., 2020; Lin et al., 2021). Environmental factors, particularly those influencing nutrient availability and habitat conditions, act as filters that select for microbial taxa best suited to prevailing conditions (Finlay and Clarke, 1999; Su et al., 2020). In the context of N addition, shifts in soil pH and nutrient stoichiometry can alter microbial niche space, favoring certain taxa while constraining others (Tian et al., 2025). These changes in microbial community composition can, in turn, influence microbial metabolic processes, including CUE, by modifying microbial resource allocation strategies, enzyme activity, and interactions among microbial functional groups.

Our results indicate that microbial CUE increased with increasing soil pH, suggesting that acidification imposes constraints on microbial metabolism that reduce efficiency in C utilization. Soil pH is a fundamental regulator of microbial activity, influencing enzyme function, nutrient availability, and community composition, all of which play crucial roles in determining CUE (Wang et al., 2019; Kerfahi et al., 2024; Xiang et al., 2024). Acidic conditions can inhibit the activity of key extracellular enzymes involved in organic matter decomposition, reducing the efficiency of C acquisition and increasing metabolic costs (Feng et al., 2021). Many microbial enzymes operate optimally within a specific pH range, and deviations from this range can suppress enzyme activity, leading to slower decomposition and increased respiratory losses relative to biomass production (Nigam, 2013; Puissant et al., 2019). Additionally, soil acidification alters nutrient availability, often reducing the bioavailability of essential elements such as P while increasing the solubility of toxic metals like aluminum ($Al^{3+}$) and manganese ($Mn^{2+}$) (Barrow and Hartemink, 2023; Bolan et al., 2023). P limitation can further constrain microbial growth and metabolic efficiency, while increased metal toxicity imposes additional physiologic stress, forcing microbes to divert energy toward stress adaptation rather than biomass production, ultimately lowering CUE (Cui et al., 2022b; Chukwu and Gulser, 2025). Furthermore, acidification can drive shifts in microbial community composition, often favoring acid-tolerant fungal taxa over bacteria. Since bacteria generally exhibit higher CUE than fungi due to their resource-use strategies, a shift toward fungal dominance may further reduce microbial efficiency in C assimilation. These compositional changes can also disrupt metabolic interactions and functional complementarity within microbial communities, exacerbating declines in CUE. Given that N addition frequently leads to soil acidification, understanding how pH dynamics regulate microbial metabolism is critical for predicting the long-term effects of N deposition on soil C cycling.

In addition to soil pH, our results identified AN and the ratio of DOC:AN as key drivers of microbial CUE. Consistent with niche theory, shifts in nutrient availability following N addition can modify microbial resource competition and niche partitioning, favoring species with specific physiologic adaptations (Aanderud et al., 2018; Lin et al., 2021). The negative correlation between CUE and AN suggests that excessive N availability may reduce microbial efficiency by disrupting nutrient balance and altering microbial metabolic strategies (Chen et al., 2018; Chen et al., 2024). Elevated AN levels can lead to stoichiometric imbalances that force microbes to reallocate energy toward N assimilation at the expense of C processing, decreasing CUE. This pattern is further supported by the positive correlation between CUE and DOC:AN, indicating that a sufficient supply of organic C relative to N availability is essential for maintaining microbial efficiency. When DOC:AN decreases beyond a critical threshold, microbes may experience C limitation, leading to increased respiratory losses as they attempt to acquire additional C through overflow respiration (Wu et al., 2025). Furthermore, shifts in microbial community composition in response to N enrichment may favor copiotrophic bacteria that exhibit high N demand but lower CUE due to increased metabolic costs associated with rapid growth and resource acquisition (Ma et al., 2023; Stone et al., 2023). Collectively, these findings suggest that microbial CUE is strongly influenced by nutrient stoichiometry, with both excess N availability and reduced C:N balance contributing to lower microbial efficiency in poplar plantation soils under N addition.

In summary, our findings underscore the importance of managing N inputs in poplar plantations to sustain soil microbial efficiency and C sequestration. Prolonged N enrichment can alter microbial resource allocation, metabolic efficiency, and community composition, potentially destabilizing SOC and accelerating its turnover. Given the strong interactions among C, N, and P availability, maintaining a balanced nutrient supply is essential for optimizing microbial function and soil health. Management strategies such as controlled fertilization, organic matter amendments, or soil pH regulation (e.g., liming) may help mitigate N-induced microbial inefficiencies and enhance SOC stability (e.g., Bertolino et al., 2025; Jiang et al., 2025). Future research should focus on integrating microbial processes into forest management practices to improve nutrient cycling and long-term C storage in poplar plantations.

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