Nitrogen addition mediates the effect of soil microbial diversity on microbial carbon use efficiency under long-term tillage practices

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Abstract

Tillage practices can influence soil microbial carbon use efficiency (CUE), which is critical for carbon cycling in terrestrial ecosystems. The effect of tillage practices could also be regulated by nitrogen (N) addition. However, the soil microbial mechanism about N fertilizer effect on microbial CUE under no-tillage is still unclear. We investigated how N fertilizer regulates the effect of tillage management on microbial CUE through changing microbial properties and further assessed the impact of microbial CUE on particulate (POC) and mineral-associated organic matter carbon (MAOC) using a 16-yr field experiment with no-tillage (NT) and conventional tillage (CT), both of which combined with 105 (N1), 180 (N2), and 210 kg N ha^{-1} (N3) N application. We found that microbial CUE increased with increasing N application rate. NT increased microbial CUE compared with CT under N1. The bacterial and fungal diversities of NT was higher than CT and N application decreased their diversities in the 0-10 cm layer. The partial least squares path model showed that bacteria diversity, fungal diversity, and fungal community structure played more critical roles in increasing microbial CUE. Furthermore, POC and MAOC under NT were higher than CT and they also increased with increasing N application rate. This could be explained by the finding that increasing microbial CUE induced by N application had the potential to increase POC and MAOC. Overall, N addition is an important pathway to influence microbial CUE, which is mainly regulated by bacterial and fungal diversities rather than their biomass under no-tillage.

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Tillage practices can influence soil microbial carbon use efficiency (CUE), which is critical for carbon cycling in terrestrial ecosystems. The effect of tillage practices could also be regulated by nitrogen (N) addition. However, the soil microbial mechanism about N fertilizer effect on microbial CUE under no-tillage is still unclear. We investigated how N fertilizer regulates the effect of tillage management on microbial CUE through changing microbial properties and further assessed the impact of microbial CUE on particulate (POC) and mineral-associated organic matter carbon (MAOC) using a 16-yr field experiment with no-tillage (NT) and conventional tillage (CT), both of which combined with 105 (N1), 180 (N2), and 210 kg N ha^{-1} (N3) N application. We found that microbial CUE increased with increasing N application rate. NT increased microbial CUE compared with CT under N1. The bacterial and fungal diversities of NT was higher than CT and N application decreased their diversities in the 0-10 cm layer. The partial least squares path model showed that bacteria diversity, fungal diversity, and fungal community structure played more critical roles in increasing microbial CUE. Furthermore, POC and MAOC under NT were higher than CT and they also increased with increasing N application rate. This could be explained by the finding that increasing microbial CUE induced by N application had the potential to increase POC and MAOC. Overall, N addition is an important pathway to influence microbial CUE, which is mainly regulated by bacterial and fungal diversities rather than their biomass under no-tillage.

Keywords

Microbial community; Microbial carbon use efficiency; Nitrogen; No-tillage; Soil organic carbon

1. Introduction

Soil biodiversity loss induced by agricultural practices threatened the provision of soil ecosystem functions (De valença *et al.* , 2017; Huang *et al.* , 2019). One of the functions is soil organic carbon (SOC) storage (Chen *et al.* , 2017; Novara *et al.* , 2017), which is crucial to the determination of carbon (C) cycling in ecological systems. The C stock is susceptible to microbial carbon use efficiency (CUE) that is the fraction of C taken up by microbial cells and retained in biomass as opposed to being respired (Li et al., 2019; Li et al., 2014; Zhou et al., 2020). Furthermore, tillage practices could influence soil microbial CUE by changing some soil properties (e.g., temperature and moisture) (Domeignoz-Horta*et al.* , 2020; Manzoni *et al.* , 2012). Soil microbial CUE can also be regulated by nitrogen (N) addition (Kallenbach *et al.* , 2019; Widdig *et al.* , 2020). Previous studies further found that microbial community structure and compositions are critical factors influencing microbial CUE (Nunes *et al.* , 2020; Sinsabaugh*et al.* , 2016; Wang *et al.* , 2020). Therefore, it is essential to study the soil microbial mechanism responsible for the effect of N application on microbial CUE to better understanding carbon sequestration under tillage management.

No-tillage is one of the main conservation tillage practices and numerous studies have investigated its effect on microbial CUE (Kallenbach *et al.* , 2019; Mo *et al.* , 2021; Yang *et al.* , 2020a). Some studies have shown that no-tillage increased microbial CUE compared with conventional tillage (Kallenbach *et al.* , 2019; Mo *et al.* , 2021; Sauvadet *et al.* , 2018), but no effect was also found (Van Groenigen *et al.* , 2013). A possible reason for the different effects is that N application could influence microbial CUE (Kallenbach *et al.* , 2019; Mo *et al.* , 2021; Van Groenigen *et al.* , 2013) and its application rate is different among these studies. N application can also affect microbial growth and respiration by changing soil nutrient availability, particularly

for N, because decomposer cells need to maintain balanced compositions of C and N (Manzoni *et al.* , 2012). In addition, the limitation of N increases over-flow respiration or C excretion rather than microbial growth, which further decreases microbial CUE (Qiao *et al.* , 2019). Previous studies showed that no-tillage with straw retention could decrease soil N availability (Gentile *et al.* , 2011; Thierfelder *et al.* , 2018). These findings indicate that N application is a promising way to induce no-tillage systems to increase microbial CUE.

Microbial CUE can be influenced by microbial populations that have different rates of organic matter decomposition and absorption (Waldrop & Firestone, 2004). Adu and Oades (1978) found that fungi played a more important role than bacteria on microbial CUE. The main reason is that the C:N variation range of fungi is generally wider than that of bacteria and fungi have a higher demand for C element than bacteria (Keiblinger *et al.* , 2010). However, other studies showed no significant difference in the effect of microbial CUE induced by fungi and bacteria (Six *et al.* , 2006; Thiet *et al.* , 2006). One reason for these conflicting results is that N application could also influence microbial CUE by stimulating microbial activity and decreasing microbial respiration metabolism (Lee & Schmidt, 2014; Liu *et al.* , 2018; Thiet *et al.* , 2006) and the difference N application rates under these studies could contribute to the discrepancy. Another reason is that these studies only focused on the influence of microbial populations and biomass on microbial CUE (Keiblinger *et al.* , 2010; Waldrop & Firestone, 2004) and ignore the key role of microbial diversity on microbial CUE (Domeignoz-Horta *et al.* , 2020). Hence, studying the impact of N application on microbial CUE based on its effects on microbial diversity and community structure could provide a comprehensive perspective to reveal the effect of N addition on C cycling.

Furthermore, the increase of microbial CUE is an effective means of increasing SOC sequestration (Bradford *et al.* , 2013; Haddix*et al.* , 2016). SOC fractions, especially for particulate organic matter carbon (POC) and mineral-associated organic matter carbon (MAOC), are more sensitive to microbial CUE than total SOC (Averill & Waring, 2018; Chen*et al.* , 2018; Ye *et al.* , 2018). Averill and Waring (2018) found that substrate use efficiency can also directly affect C cycling through changing POC and MAOC. In addition, N addition significantly influenced on soil POC and MAOC (Chen*et al.* , 2020b, 2019; Ye *et al.* , 2018). However, it remains unclear how N application regulates the effect of soil microbial CUE on POC and MAOC under tillage management. Therefore, studying the effects of N application is essential to understanding the role of soil microbial CUE on carbon sequestration potential.

This study was conducted to investigate the influence of N application on microbial CUE under tillage practices from a microbiological perspective. We hypothesized that: (i) the responses of soil microbial diversity, community structure, biomass, and CUE to N application under CT and NT were different, and (ii) microbial diversity plays a more important role than microbial biomass in microbial CUE. The objectives of this study were to (i) evaluate the effects of tillage management and N application on soil microbial diversity, community compositions, and soil microbial CUE, (ii) reveal how N application influences soil microbial CUE by regulating microbial diversity, community structure, and biomass, and (iii) assess the influence of microbial CUE on soil POC and MAOC under tillage management with different N application rates.

2. Materials and methods

2.1 Study site

We conducted a continuous field experiment from 2003 to 2019 at a Dryland Farming Experimental Station in Shouyang (112-113°E, 37-38°N), Shanxi Province in northern China. The climate of the station is continental monsoon and its average annual potential precipitation and evaporation is 484 mm and 1750 mm, respectively (Wang *et al.* , 2019). The annual frost-free season lasts on average 131 days. The sandy loam cinnamon soil in experimental site was classified as Calcaric-Fluvic Cambisols (Li *et al.* , 2020). Table 1 shows soil chemical and physical properties initially.

2.2 Experimental design

The long-term experiment was set up in 2003 using a randomized complete block design with three replicates.

Each plot size was 5 m × 5 m, the crop was continuous spring maize and there was a fallow period from November to March.

Three N fertilizer rates were applied under two tillage treatments in this study and the two tillage practices were CT (conventional tillage with maize stalk removed after harvesting, plowed twice to about 25 cm depth using moldboard plow after harvesting and before seeding, and fertilized before plow in April) and NT (no-tillage with the maize stalk mulched after harvesting, then seeded with a no-till planter and N fertilizer were applied in small holes between two maize in each row in April). The three N fertilizer rates were N1 (105 kg N ha^{-1}), N2 (180 kg N ha^{-1}), and N3 (210 kg N ha^{-1}) using urea. The row and plant spacings were 60 and 30 cm, respectively.

2.3 Soil sampling

We collected soil samples from depths of 0–10 cm and 10–25 cm using a 10 cm diameter soil auger on 1 August 2019. The sampling date corresponded to the tasseling stage. The soil samples were stored in airtight polypropylene bags and placed in a cool box at 4°C during transportation to the laboratory. Litter, roots, and gravel in soil samples were removed and the soil was divided into several samples for further analyses.

2.4 Soil analysis

2.4.1 biochemical analysis

We assessed the soil microbial biomass carbon (MBC) and nitrogen (MBN) by the chloroform fumigation extraction method (Cleveland & Liptzin, 2007). Fresh soil samples transported in an ice-cooled box were separated into two aliquots (15 g on a dry weight basis). One set of soil subsamples was extracted using 0.5 M K_2SO_4 to measure the MBC and MBN. Organic C in the K_2SO_4extracted solution was analyzed using a TOC analyzer (Vario TOC, Elementar, Germany). Both MBC and MBN concentrations were corrected for unrecovered biomass using a k factor of 0.45 (Jenkinson *et al.* , 2004).

Microplate-scale fluorometric procedures were employed to assay the activity of the following hydrolases (Sinsabaugh *et al.* , 1997): β-1, 4-glucosidase (BG), β-1, 4-N-acetyl-glucosaminidase (NAG), and leucine aminopeptidase (LAP). We prepared substrates and buffer solutions in sterile deionized water. In this study, 1 g of fresh soil sample was homogenized in 125 mL 50 mM sodium acetate buffer. The 50 μl of 50 mM buffer was dispensed into 16 replicate sample wells (sample solution + substrate), eight blank wells (sample solution + buffer), eight reference standard wells (buffer + standard), and eight negative control wells (buffer + substrate). The prepared microplates were then placed in a dark microcosm for 4 h at 20 °C. Finally, the reaction was stopped by adding 1 μl of 1 M NaOH to each well. The fluorescence was measured using an automated fluorometer (BioTek Synergy H1 microplate reader, Winooski, VT, USA) with an excitation wavelength of 365 nm and an emission wavelength of 450 nm. After correction of the assay wells' fluorescence measurements for the negative controls, blanks, and quench standard wells, the enzymatic activities were expressed as nanomoles of substrate released per hour per gram of dry soil (Saiya-Cork *et al.* , 2002).

2.4.2 Ecoenzymatic stoichiometry and CUE estimation

We used the activities of the enzymes, the C and N contents of the soil microbial biomass, and labile organic matter to calculate the CUE according to the previous studies (Geyer *et al.* , 2019; Sinsabaugh *et al.* , 2016; Sinsabaugh & Shah, 2012). The labile nutrient content was also replaced with soil organic matter (Sinsabaugh *et al.* , 2016)(Zhou *et al.* , 2020). Previous study also found that the CUE calculated from stoichiometric models was similar to it according to direct measurements of bacterial and fungal growth and respiration (Sinsabaugh *et al.* , 2016).

The microbial carbon use efficiency was calculated using the following equation:

$$CUE_{C:N} = CUE_{MAX} \left[S_{C:N} / (S_{C:N} + k_N) \right] \quad (1)$$

where $S_{C:N} = (1/EEA_{C:N})(B_{C:N} / L_{C:N})$, $S_{C:N}$ is a scalar that reflects the ability of the microorganisms to adjust the imbalance between the elemental composition of the available resources and the composition of the

microbial biomass through the allocation of enzymatic activities. K_N is the half-saturation constant with a value of 0.5. Based on the thermodynamic constraints, CUE_{max} is assumed to be 0.6 for the highest microbial growth efficiency. $EEA_{C:N}$ is the ratio of the C-acquiring activity to the N-acquiring activity,$EEA_{C:N} = BG /(NAG + LAP)$. $B_{C:N}$ represents the molar ratio of C to N of the soil microbial biomass. $L_{C:N}$represents the molar ratio of SOC to TN for the soil substrate that is consumed.

The threshold element ratios (TER) for C:N were estimated by the following function:

$TER_{C:N} = L_{C:N} \times EEA_{C:N}$ (2)

where $L_{C:N}$ and $EEA_{C:N}$ have the same meanings as in Eq. (1).

2.4.3 PLFA analysis

Total microbial biomass and microbial community structure were assessed using phospholipid fatty acid (PLFA) analysis. We used a modified Bligh and Dyer method to extract PLFAs (Börjesson *et al.* , 1998). Total lipids were extracted overnight from 5 g freeze-dried soil in a solvent phase of 3.0 ml 50 mM phosphate buffer (pH = 7.0), 3.8 ml chloroform, 7.6 ml methanol, and 4 ml Bligh and Dyer reagent (chloroform/methanol/phosphate buffer (1:2:0.8, v/v/v)). The extracted lipids were subsequently added to Discovery® DSC-Si SPE Tubes (Sigma-Aldrich), then separated into neutral lipids, glycolipid, and phospholipid by sequential addition of chloroform, acetone, and methanol solutions. We added PLFA 19:0 (Larodan Malmö, Sweden) to the phospholipid fraction as an internal standard. PLFAs were transesterified to fatty acid methyl esters using 1 ml 0.2 M methanolic-KOH (Chowdhury & Dick, 2012). We analyzed the extracts using a gas chromatograph equipped with a flame-ionization detector (Agilent 6890, Agilent Technologies, Palo Alto, CA, United States). Fungal biomass was the sum of PLFAs 18:2ω6c and 18:1ω9c (Frostegård & Bååth, 1996; White *et al.* , 1996). PLFAs a15:0, a17:0, i14:0, i15:0, i16:0, i17:0 were used as markers for Gram-positive bacteria, whereas PLFAs 16:1ω9c, 16:1ω11c, 18:1ω5c, 18:1ω7c, cy17:0, and cy19:0 were used as markers for Gram-negative bacteria (Brockett *et al.* , 2012; Frostegård & Bååth, 1996). Actinomycetes biomass was calculated based on the fatty acid: 10Me16:0 and 10Me18:0 (Willers *et al.* , 2015). Total bacterial biomass was the sum of G^+, G^-, and Actinomycetes biomass. We further calculated the ratio of fungal to bacterial biomass (F: B ratio) in soil samples using PLFAs data.

2.4.4 DNA extraction

Five grams aliquots of soil samples were mixed with 25 mL 0.1 mol/L Tris-HCl (pH 8.0), shaken and filtered through three layers of sterile gauze. The filtrate was then centrifuged at 10000 × *g* for 20 min at 4 °C. DNA was subsequently extracted from the pellets using a GMO food DNA Extraction Kit (Illumina MiSeq 250 PE, Auwigene Company, Beijing, China) according to the manufacturer's protocols. The total DNA concentration and quality were checked using a spectrophotometer (NanoDrop, ND2000, ThermoScientific, United States) and agarose gel electrophoresis.

2.4.5 16S rRNA gene amplicon sequencing and ITS amplicon sequencing

Variable regions V3-V4 on microbial 16S rRNA gene of bacteria and the ITS2 region of fungi were amplified using PCR (95 °C for 3 min, followed by 30 cycles at 98 °C for 20 s, 58 °C for 15 s, 72 °C for 20 s and a final extension at 72 °C for 5 min). The microbial 16S rRNA gene was amplified by forwarding primer 338F (5'-ACTCCTACGGGAGGCAGCAG-3') and reverse primer 806R (5'- GGACTACVVGGGTATCTAATC -3') (Lee *et al.* , 1993). The ITS was amplified with the following forward/reverse primer set: ITS1F/ITS2R (CTTGGTCATTTAG AGGAAGTAA/GCTG-CGTTCTTCATCGATGC) (Luan*et al.* , 2015). PCR reactions were performed in 30 μL mixture containing 15 μL of 2 × KAPA Library Amplification ReadyMix, 1 μL of each primer (10 μmol/L), 10 ng of template DNA, and ddH_2O. The PCR products were detected using 1% agarose gel electrophoresis, then purified with an AxyPrep DNA gel Extraction Kit (Axygen Biosciences, Union City, CA, United States). Amplicon libraries were quantified using a Fluorometer (Applied Biosystems 7500, Thermo Fisher Scientific, United States), after which amplicons were sequenced (Illumina MiSeq PE250, Allwegene Technologies, China).

2.4.6 Soil fractions separation

We used the soil wet-sieving method to separate different soil fractions (Curtin *et al.* , 2019; Fang *et al.* , 2019). To separate soil organic matter into labile and stable C fractions, we conducted a combined density and particle size fractionation (Herath *et al.* , 2014; Six *et al.* , 1998). The physical fractionation to separate two soil C fractions: light fraction, defined as f-POM, and the heavy fraction that contained aggregate protected organic matter (o-POM, > 53 μm fraction) and mineral protected organic matter (MAOM< 53 μm fraction) (Fang*et al.* , 2019). Density fractionation of the soils was then performed to isolate light fraction and heavy fraction using sodium polytungstate (SPT, IMBROS, Australia) (Herath *et al.* , 2014; Six*et al.* , 1998).

All fractions were dried (60 °C) and weighed to obtain the mass proportion of each fraction relative to the bulk soil. The soil fractions were ground to < 53 μm for C% analyses. Samples were then acidified with 1.0 M HCl to decompose the carbonate, after which they were dried for 8 hours at 60. After drying, the samples were ground (< 0.149 mm) with a mortar and pestle and the SOC was measured by dry combustion method using an elemental analyzer (Vario Macro C/N, Elementar, Germany).

Statistics

The data were analyzed by three-way ANOVA to compare the effects of soil depth, tillage management, nitrogen application rates, and their interaction on enzyme activities, microbial CUE, PLFAs, and microbial diversity. We compared the means by using the least significant difference with a significance level of $P < 0.05$. Statistical analyses were performed using the SPSS 18.0 software (SPSS Inc., Chicago, United States). Sequences were processed using Quantitative Insights Into Microbial Ecology (QIIME) version 1.9.1 (Caporaso, 2010). Operational taxonomic units clustering at 97% of identity were collected using UCLUST in QIIME software. Changes in the microbial community structures of the soil samples were evaluated by principal coordinate analysis (PCoA) in R (v. 3.4.1). The relationships among agricultural practices, soil microbial diversity and community structure, microbial biomass, soil microbial CUE, and soil POC were explored by using partial least squares path modeling (PLS-PM). Estimates of path coefficients and coefficients of determination (R^2) in our path model were validated by R (v.3.4.1) with the 'plspm' package (Ai *et al.* , 2018). The model was assessed using the Goodness of Fit (GoF) statistic, where the GoF value was set to 0.69.

3. Results

3.1 Changes in enzyme activities and microbial $CUE_{C:N}$

NT significantly increased BG and NAG activities on average relative to CT (Fig. 1). BG and NAG activities decreased with soil depth under NT, whereas soil depth had no influence under CT. Moreover, both variables of N2 was higher than N1 and N3. Compared with N2, N1 and N3 significantly decreased BG and NAG activities at 0-10 cm and 10-25 cm depths (Fig. 1). Moreover, the average value of LAP activity under CT treatment was higher than that of NT (Fig. 1c-d) and it was higher under N2 than under N1 and N3 for CT treatment.

The average value of $CUE_{C:N}$ under CT was increased by 16.2% compared with NT and soil depth did not influence it under the two tillage practices (Fig. 2g–h). The value of $CUE_{C:N}$increased with increasing N application. NT increased the $CUE_{C:N}$ compared with CT in the 0–10 cm and 10–25 cm layers under N1, whereas there was no significant difference between NT and CT under N3. These results showed that increasing N application rates under NT could enhance $CUE_{C:N}$.

3.2 Soil microbial community

The PLFA contents of the total and grouped soil microorganisms under tillage and N application treatments are shown in Fig. 3. All of the PLFA contents in the 0–10 cm layer were greater than in the 10–25 cm layer under NT, but the total PLFAs and bacterial PLFAs did not change with soil depth under CT. The average values of bacteria, fungi, and actinomycetes PLFAs were higher under NT than CT. In addition,

only fungi and the F:B ratio were significantly affected by N level (Table S3). Overall, the total PLFAs were increased by 19.2% under NT compared with CT and not significantly affected by N level (Fig. 3a–b). For each grouped soil microorganism, bacterial PLFAs and actinomycetes PLFAs under NT were increased by 21.2% and 24.4%, respectively, compared with CT, but not significantly affected by N level at both depths (Fig. 3c–d and Fig. 3g–h). Fungal PLFAs also increased under NT compared with under CT. The fungal PLFAs of N2 was the highest than N1 and N3 under NT, while there was no effect of N application rate under CT in the 0–10 cm layer (Fig. 3e–f). Moreover, the G^+:G^- ratio was insignificantly affected by soil depth, tillage treatments, and N application rates (Table S3). The G^+:G^- ratio increased with increasing N application rates under NT and there was no effect of N application rate under CT at 0–10 cm (Fig. 3i–j). In addition, the F:B ratio was not significantly affected by tillage management. N2 produced a higher F:B ratio than the other two N levels under NT, whereas there was no effect of N application rate on F:B ratio under CT in both depths (Fig. 3k–l).

3.3 Soil bacteria community compositions

According to 16S rRNA gene sequences, the number of sequences per sample ranged from 31458 to 172704 at a 97% sequence identity threshold. Overall, a total of 8232 OTUs were identified. Actinobacteria (14.5%–32.6% relative abundance), Proteobacteria (16.5%–28.7% relative abundance), Acidobacteria (15.5%–37.1% relative abundance), Chloroflexi (10.5%–21.6% relative abundance), and Gemmatimonadetes (4.0%–6.9% relative abundance) were considered the dominant phyla associated with residue decomposition (Fig. 4a–b). These five phyla accounted for 96.4% of all sequence reads (Fig. 4).

N application, tillage × soil depth, and N × tillage interaction significantly influenced the bacterial (16S) community compositions (Table S4). For the dominant phyla, the relative abundances of Acidobacteria, Planctomycetes, and Firmicutes increased with soil depth, while the relative abundances of Proteobacteria, Actinobacteria, Bacteroidetes, and Verrucomicrobia declined with soil depth (Fig. 4). Compared with CT, NT increased the relative abundances of Proteobacteria and Bacteroidetes in the 0–25 cm layer. The relative abundances of Bacteroidetes increased with an increasing in N application under NT, while N application had no effect on them under CT. Tillage management also had no influence on the relative abundances of Chloroflexi. Furthermore, N2 increased the relative abundances of Chloroflexi compared with N1 and N3 under CT, whereas the relative abundances of Chloroflexi of N1 were higher than N2 and N3 under NT in both layers.

3.4 Soil fungi community composition

Fungi were clustered at the phylum level. The histogram of community structure constructed according to OTU sequence abundance after clustering (Fig. 4c–d) revealed structural and abundance differences among N application rates and tillage treatments. There were five phyla of eumycota with an abundance $> 0.01\%$ in these treatments. Ascomycota and Mortierellomycota were the two most dominant, accounting for $> 60\%$ of all phyla.

N application, tillage, and soil depth significantly affected the fungal ITS community composition (Table S5). The abundance of Basidiomycota was higher under NT than under CT and showed a trend of first decreasing, then increasing with increasing N application rates under NT. We also found that the relative abundance of Mortierellomycota was higher under CT than NT in both layers.

3.5 Diversity of soil bacteria and fungi

NT significantly increased soil bacterial diversity on average compared with CT (Fig. 5). Its diversity decreased with soil depth under NT. N application also significantly affected bacterial diversity under NT, whereas N application and soil depth had no effect under CT. NT had higher bacterial diversity than CT in the 0–10 cm layer. Bacterial diversity decreased with an increase in N application rates under NT in the 0–10 cm layer, while N application had no influence in the 10–25 cm layer. Similarly, NT significantly enhanced the average value of soil fungi diversity compared with CT (Fig. 6). Soil fungal diversity decreased as the soil depth and N application increased under NT. However, fungal diversity of CT was not influenced by soil

depth and N application also had no influence on it in 10–25 cm layer.

Principal component analysis of bacterial composition at the phylum level showed that two principal components accounted for 47.7% and 42.4% of the overall variances among these treatments in the 0–10 cm and 10-25 cm layers, respectively (Fig. 7). We also found that PCoA of the fungal composition showed that two principal components accounted for 46.5% and 39.2%, respectively. We revealed that the two fractions (CT and NT) formed their clusters separated by PC1 in both layers. For fungi, the samples under the three N application rates of CT clustered closely, while samples within the NT differed more distinctly in the two layers.

3.6 Soil fractions

The POC and MAOC contents decreased with depth and were significantly affected by N application (Fig. 8a–b). NT increased the POC and MAOC contents by 12.1% and 10.1% compared with CT in the 0–10 cm layer, respectively. The POC and MAOC contents increased with increasing N application rates and the rate of increase under NT was higher than under CT in the 0–10 cm layer. However, tillage and N treatment had no influence on MAOC in the 10–25 cm layer.

3.7 PLS-PM analysis

To better integrate the interrelationships among N application, tillage practices, microbial communities, soil enzyme activities, soil microbial $CUE_{C:N}$, POC, and MAOC, we constructed a partial least squares path model (Fig. 9). The indirect effect of tillage treatments (0.38) on soil microbial $CUE_{C:N}$ was larger than that of N application (0.13). We further found that tillage management and N application affected microbial $CUE_{C:N}$ through changing soil bacterial diversity, fungal community structure, and fungus diversity more than bacterial and fungal biomass. The responses of microbial $CUE_{C:N}$ to bacterial and fungal diversity were also different (Fig. 9). Moreover, the results showed that microbial $CUE_{C:N}$ and soil enzyme activities had a direct effect on soil POC.

4. Discussion

4.1 Soil microbial diversity and community structure

Soil microbial communities are essential to maintaining soil ecosystem function and can be affected by tillage and N application (Bärlocher & Boddy, 2016; Keszthelyi *et al.* , 2008). We found that NT treatment increased bacterial and fungal diversity in 0-10 cm layer compared to CT treatment (Tables S6 and S7). The difference between CT and NT could be due to the reduction of soil physical disturbance and protection from fungal hyphae and their mycelial network under the no-tillage system (Ceja-Navarro *et al.* , 2010; Verbruggen & Toby Kiers, 2010; Wang*et al.* , 2017).

Furthermore, soil fungal and bacterial diversity decreased with increasing N application rates in the 0–10 cm layer and was higher under NT treatment than under CT (Figs. 5 and 9). One possible reason is that the straw in no-tillage has a wide C/N ratio (Thierfelder *et al.* , 2018), which leads to an N limitation under this tillage system because microbe needs more N under this condition. A previous meta-analysis showed that appropriate N addition (<100 kg N $ha^{-1}year^{-1}$) is essential to stimulate microbial growth in no-tillage systems because it regulates soil C/N (Thierfelder *et al.* , 2018; Zhou *et al.* , 2017). However, excessive N fertilization suppresses the diversity of soil microbes because of the toxic effect of urea (Omar & Ismail, 1999; Wang *et al.* , 2018). In this study, high N application rate (210 kg N ha^{-1}) could induce toxicity, resulting in lesser microbial diversity. In addition, CT had lower soil SOC (Li*et al.* , 2010; Liu *et al.* , 2021) and C/N ratio compared with NT (Fiorini *et al.* , 2020), which leads to carbon limiting for microorganisms. Hence, the effect of N application had a smaller effect on microbial diversity under CT than NT. Previous studies showed that low N application (35–140 kg N ha^{-1}) decreased soil bacterial diversity (Wang*et al.* , 2015) and our study highlighted that there was the same conclusion under high N application rate (105–210 kg N ha^{-1}), which extends our knowledge of the effect of N application on microbial diversity. In addition, increasing N application rates had a negative effect on some dominant flora such as Chloroflexi (Fig. 4), which also degrades SOM because Chloroflexi plays an important role in the decomposition of refractory C

compounds (Li *et al.* , 2019b; Piazza*et al.* , 2019). These results further indicate that N application needs to be considered when studying the effect of tillage management on microbial properties.

Tillage management could also influence the vertical distribution of soil microbial communities (Nunes *et al.* , 2020). We found no difference in enzyme activities, total PLFAs, and bacterial and fungal diversity among soil layers under CT treatment (Figs. 1, 2, 5, and 6). The main reason was that soil microbial communities in different soil layers would be similar to each other after homogenization induced by plowing under CT (Sun *et al.* , 2018). However, fungal and bacterial diversity decreased as soil depth increased under NT(Figs. 5 and 6), which was supported by the previous study (Jumpponen *et al.* , 2010). This was likely because no-tillage creates heterogeneous soil (Sun *et al.* , 2018). Moreover, the decrease rate of fungal and bacterial diversity with increasing soil depth was higher under N1 than N2 and N3 for NT treatment, indicating that a low N rate can enhance top soil bacterial and fungal diversity under NT. Hence, it is not sufficient to only consider the surface layer when investigating bacterial and fungal diversity response to N application rates in no-tillage systems.

4.2 Relationship of soil microbial characteristic and microbial $CUE_{C:N}$

Soil microbial CUE can affect soil C cycling (Spohn *et al.* , 2016). We found that NT increased the soil microbial $CUE_{C:N}$ on average compared with CT (Fig. 2) because no-tillage can decrease soil temperature by surface mulching and further increase microbial CUE (Apple *et al.* , 2006; Wetterstedt & Agren, 2011). In addition, higher residue production under NT is constantly supplying fresh and labile organic substrates for microbial activity and biomass thus explaining the greater CUE observed under NT compared with CT (Alvaro-Fuentes *et al.* , 2013). Microbial $CUE_{C:N}$increased with increasing N application under both tillage treatments (Fig. 2). The reason is that N addition can reduce microbial respiration metabolism (Liu*et al.* , 2018; Spohn *et al.* , 2016; Thiet *et al.* , 2006) and increase microbial biomass (Jha *et al.* , 2020), resulting in higher microbial $CUE_{C:N}$.

Furthermore, although a recent study showed that microbial diversity drives CUE in artificial soil (Domeignoz-Horta *et al.* , 2020), to the best of our knowledge, few experimental studies have directly demonstrated the interaction effect of tillage management and N application on microbial CUE in a field experiment. In this study, the PLS-PM showed that tillage and nitrogen influenced microbial $CUE_{C:N}$ through the microbial diversity and community structure (Fig. 9). We also found that the bacterial and fungal diversity had different influences on microbial $CUE_{C:N}$(Fig. 9) under two tillage and these relationships were regulated by N application (Fig. S1) under no-tillage. Bacterial diversity positively influenced microbial $CUE_{C:N}$, whereas fungal diversity had an adverse impact on microbial $CUE_{C:N}$ (Fig. 9). The difference points to the importance of studying the diversity of fungal and bacterial communities separately for predicting soil C cycling. In addition, microbial network complexity driving carbon cycling with direct feedback effects on multiple ecosystem functions (Morrien *et al.* , 2017; Wagg *et al.* , 2019; Zhou *et al.* , 2010), which could also influence microbial CUE. Further research should be undertaken to explore the effect of bacterial and fungal networks on microbial $CUE_{C:N}$.

4.3 The influence of microbial $CUE_{C:N}$ on soil POC and MAOC fractions

POC is a functional soil component for persistent soil organic carbon (Witzgall *et al.* , 2021). In contrast to POC, MAOC is more physically or chemically protected, which makes it less vulnerable to mineralization (Abramoff *et al.* , 2018). We found that high N application (210 kg N ha^{-1}) increased POC and MAOC content under two tillage practices (Fig. 9), which is similar to the previous study (Ye *et al.* , 2018). The possible reason was that plant biomass (Stewart*et al.* , 2016; Thomas *et al.* , 2010; Wang *et al.* , 2018) and microbial residues (Chen *et al.* , 2020a) increased with increasing N application. However, some discrepant findings showed that N addition decreased (Ye *et al.* , 2018) or had no significant influence on MAOC (Yuan *et al.* , 2020). The main reason for the inconsistent results could be that microbial residues controlled the changes of soil MAOC pool under N addition and the microbial residues were different due to different N application rates among these studies (Averill & Waring, 2018; Chen *et al.* , 2020a; Su *et al.* , 2020; Yang *et al.* , 2020b).

We further found that the $CUE_{C:N}$was significantly positively correlated with POC and MAOC and the increase rates were higher under NT than CT, which was influenced by nitrogen application (Fig. S2). Moreover, the POC also had a positive effect on MAOC (Fig. 9) because a portion of POC was degraded by microbes and then formed part of the MAOC (Su *et al.* , 2020). Therefore, these results highlight that nitrogen regulates the influence of microbial $CUE_{C:N}$ on soil organic carbon fractions under tillage practices.

5. Conclusions

N application could alter the effects of tillage practices on soil microbial diversity, community composition, biomass, and CUE. Bacterial and fungal diversities were more responsible for soil microbial $CUE_{C:N}$ than their biomass. Although microbial $CUE_{C:N}$ was more susceptible to tillage management than N application, it increased with an increasing in N application rate under the two tillage practices. Furthermore, soil microbial $CUE_{C:N}$ increased soil POC and MAOC contents and N application also increased the two SOC fractions. This research underscores the importance of N application to reveal the effect of tillage management on POC and MAOC from the perspective of soil microbial properties, which contributes to understanding the potential C sequestration benefits of N application under no-tillage.

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Zhou Z, Zhang H, Yuan Z, Gong R. 2020. The nutrient release rate accounts for the effect of organic matter type on soil microbial carbon use efficiency of a Pinus tabulaeformis forest in northern China.*Journal of Soils and Sediments* **20** : 352–364. DOI: 10.1007/s11368-019-02423-2**Table 1** Soil physical and chemical properties in 0-25 cm layer in 2003.

Soil layer (cm)	Soil particle size distribution (%)	Soil particle size distribution (%)	Soil particle size distribution (%)	Available soil nutrient (mg kg^{-1})	Available soil nutrient (mg kg^{-1})	Available soil nutrient (mg kg^{-1})	SOC (g kg^{-1})	Bulk density cm^{-3})
	>0.020 mm	0.002-0.020 mm	<0.002 mm	N	P	K		
0-10	58.5	35.7	5.8	58	8.3	96	22.7	1.06
10-25	59.6	34.6	5.8	52	6.9	93	19.8	1.2