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# Effects of addition of nitrogen on soil fungal and bacterial biomass and carbon utilisation efficiency in a city lawn soil

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**Abstract.** The aim of this study was to investigate the effects of nitrogen (N) addition on soil microbial (fungal and bacterial) biomass and carbon utilisation efficiency (CUE) in a city lawn soil. A field experiment was conducted with three N treatments (kg N $ha^{-1}$ $year^{-1}$): the control (0), low-N (100), and high-N (200). Soil biogeochemical properties including pH, C : N, CUE, microbial biomass C (MBC), fungal and bacterial biomass, microbial C uptake rates, and soil respiration (SR) rates were determined during a 500-day experiment. The low- and high-N treatments significantly decreased soil pH, MBC, and CUE. Available N and soil acidification caused a decline in soil MBC. Soil acidification was not beneficial for microbial biomass growth, especially for bacteria. The treatments with N changed soil biomass from bacterial-dominant to fungal-dominant.

The results also showed that the CUE of bacterial-dominant soil was higher than that of fungal-dominant soil, which is contrary to previous studies. However, SR did not increase with decreased CUE under N treatments, because the addition of N limited soil microbial C uptake rates and significantly decreased soil microbial biomass. The CUE showed a negative correlation with soil temperature for the control treatment but not for the N treatments, which suggested that added N played a more important role in CUE than did soil temperature. Our results showed that addition of further N significantly alters soil biogeochemical properties, alters the ratio of bacteria to fungi, and decreases microbial carbon utilisation, which should provide important information for model-based prediction of soil C-cycling.

**Additional keywords:** carbon utilisation efficiency, microbial biomass, N treatment, soil acidification, soil carbon, soil respiration.

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## Introduction

Carbon utilisation efficiency (CUE) represents the fraction of assimilated C allocated to microbial growth (Micks *et al*. 2004; López-Urrutia and Morán 2007; Allison *et al*. 2010), and is an important determinant of C cycling in aquatic and terrestrial ecosystems. A higher CUE often indicates an increase in C storage in soils or slower soil organic C turnover. The CUE has been used as an important parameter in several carbon–climate models (Steinweg *et al*. 2008) for C and nitrogen (N) cycling predictions. For example, in the CENTURY model, a CUE value of 55% is assumed, suggesting that 45% of C taken up by microorganisms is lost as respiration (Parton *et al*. 1987). However, it has been reported that the CUE and pattern of soil microbial substrate use change with soil temperature (Allison *et al*. 2010), soil water content (Schimel *et al*. 1999; Devêvre and Horwáth 2000) and soil C : N (Keiblinger *et al*. 2010), which suggests that CUE is not a fixed value. Therefore, it is necessary to study variation in CUE under different environmental conditions.

The CUE is sensitive to environmental changes such as increases in N deposition. Human activities have significantly altered global N-cycling in the last several decades, resulting in several-fold increases in N deposition and soil N availability (Hobbie *et al*. 2002). In an N-limited soil, N addition usually increases the microbial CUE because a larger amount of available N should reduce the microbial energetic cost of N assimilation, so that more C uptake can be used for growth. Some short-term N-treatment experiments also showed that addition of N could stimulate soil CUE. For example, Thiet *et al*. (2006) reported that amendment with N increased the CUE by 20% in a 15-day laboratory experiment. Teklay *et al*. (2007) inferred that the absence of a pronounced respiratory response to the added N in a 60-day field experiment might be attributed to increased microbial CUE. However, excessive N input can result in soil acidification and changes in soil C : N, which may significantly affect soil microbial biomass, and this implies that CUE may not increase with N addition.

The issue of the relative contributions of fungal and bacterial biomass to soil microbial CUE has been controversial for many years. Some studies have reported that fungal CUE was higher than bacterial CUE because fungi have more biomass C per unit biomass N, and soils dominated by fungi may sequester

 

more C than soils with lower fungal abundance (Parton *et al.* 1987; Zak *et al.* 1996). However, Thiet *et al.* (2006) did not find any difference between fungal and bacterial CUEs in a direct comparison based on a 14-day incubation experiment. However, 14 days may be insufficient time for soil microbes to respond to soil property changes under different treatments. For example, treatments with excessive N commonly cause soil acidification (Krusche *et al.* 2003). According to the results of Rousk *et al.* (2009), lower soil pH led to a decrease in bacterial growth and an increase in fungal growth, which suggested that the CUEs of fungi and bacteria both changed and there may be a difference between fungal and bacterial CUEs under soil acidification. Boyle (1998) and (Boberg *et al.* 2008) also showed that N or C addition could increase or decrease individual fungal CUE, which suggests that fungal or bacterial CUEs are not consistent values and can be changed under N treatments. In this way, treatments with N over longer periods might change fungal and bacterial CUEs and cause shifts in soil CUE between different treatments.

Relative amounts of soil fungal and bacterial biomass commonly vary with soil pH and C : N ratio (Hobbie 2000; Rousk *et al.* 2009; Nilsson *et al.* 2012). Ammonium hydrolysis after application of ammonium-based fertilisers is considered a critical factor influencing soil acidification (Krusche *et al.* 2003; Zhao and Xing 2009). Additional N incorporated into soil organic matter increased soil N content and reduced soil C : N ratio (Hobbie and Gough 2004). In this way, both pH and C : N ratio may change under N treatments. According to previous studies, lower soil pH values generally decrease bacterial competition and thus favour fungal growth (Rousk *et al.* 2008, 2009). Soil fungi were found to be more sensitive to additional N than bacteria because soil organic matter with lower C : N ratios resulting from the N addition is less favourable to fungi (Bossuyt *et al.* 2001).

We examined the response of soil microbial CUE to N treatments for 500 days in a subtropical city lawn. In south China, city lawns or grasslands represent about one-third of the land use in metropolitan areas (Jim and Chen 2006). In other regions around the world, such as the United States, ~8% of the land is urban, and 41% of this area is classified as residential, most of which is made up of lawns (Groffman and Pouyat 2009). Recently, atmospheric N deposition in metropolitan areas has significantly increased, which strongly affects $CO_2$ emission from soils in these areas (Groffman *et al.* 2009). Given that lawns occupy a large proportion of metropolitan areas and can be greatly affected by increasing atmospheric N deposition, there is concern that N deposition could affect regional $CO_2$ budgets. Furthermore, previous N treatments at the same experimental site showed that soil pH significantly decreased under N treatments. Therefore, we aimed to investigate: (*i*) whether N treatments could significantly change soil fungal and bacterial biomass; (*ii*) how soil fungal and bacterial CUE respond to N treatments.

## Materials and methods

### Site description

A city lawn at Sun Yat-sen University, Guangzhou, China (23°06′13″N, 113°17′41″E), was chosen as the experimental site. In 2008, an experimental station was established on the site, and several experiments related to soil respiration (SR) have been carried out since then (Lin *et al.* 2011). The botanical composition of the site includes *Axonopus affinis*, *Eleusine indica*, and *Oxalis corniculata*. With a subtropical monsoon climate in this region, the average annual temperature and rainfall are 21.9°C and 1735 mm, respectively. Most rainfall occurs between April and September. Detailed information about the experimental site is available in Lin *et al.* (2011). The soil texture of the study site is loamy with 43% sand, 35% silt, and 22% clay. The soil organic C content in the top 10 cm soil layer was 1.04%. The soil pH (soil to water 1 : 2.5) was 6.81.

### Nitrogen addition experiment

Prior to N addition, nine treatment plots (3 m by 3 m) were established within the experimental site, and the distance between any two plots was >1 m. Three N addition levels were established: control (no addition), low-N (100 kg N $ha^{-1}$ $year^{-1}$ or 8.33 kg N $ha^{-1}$ $month^{-1}$), and high-N (200 kg N $ha^{-1}$ $year^{-1}$ or 16.67 kg N $ha^{-1}$ $month^{-1}$). Currently, atmospheric N deposition in southern China is in the range 30–73 kg N $ha^{-1}$ $year^{-1}$ and expected to increase by 50% in 2030 (Mo *et al.* 2007). Three treatment plots were randomly assigned to each N treatment. For each plot, four soil chamber collars (diameter 23 cm) were installed 10 cm deep into soil before the N treatments; one was used for measurement of SR rate and three for soil sampling. Before the N treatments and each measurement of SR rate, all grass on the soil surface within the chamber collars was removed by cutting. The N treatments were started on 1 July 2010 (day 0) and lasted for 18 months. Based on rates of 8.33 or 16.67 kg N $ha^{-1}$ $month^{-1}$, appropriate amounts of $NH_4NO_3$ was added to 100-L deionised water and the solution was applied to each plot with a hand-held sprayer at the beginning of each month. The control plots received the same amount of deionised water as the N treatments. Measurements of SR rates, CUE, and chemical and biological properties were conducted on days 0, 30, 45, 90, 180, 360, 450, and 500 after the N treatments.

### Measurements of soil respiration rates

Rates of SR were measured using the Automatic Soil $CO_2$ Exchange System (ACE; ADC BioScientific Ltd, Hoddesdon, UK). Prior to measurements of SR rate, the ACE station was placed on, and sealed to, the chamber collar. The chamber of each ACE station was 40 mm above the soil surface. Rates of SR were measured every 20 min during each measuring period of 24 h. Soil temperature ($T$) at 5 cm depth was measured synchronously using soil temperature sensors (ADC BioScientific Ltd). The measurement system was controlled and data were recorded by a computer. Averaged $T$ and SR rates in each measuring time were used for the following analysis and discussion. At each sampling time, soil moisture contents of three treatments were determined using the gravimetric method (Dalal and Mayer 1986).

### Soil analyses

On the dates of SR rate measurement, soil samples from the top 10 cm (diameter 10 cm) were taken from one chamber collar of each treatment plot. Each soil sample was divided into two

subsamples. One was used to determine the CUE and the other to analyse the following properties. Soil total organic C (TOC) was determined using the potassium dichromate method, in which soil organic C was oxidised by potassium dichromate and then residual potassium dichromate was titrated using sodium thiosulfate (Yeomans and Bremner 1988). Total N (TN) was measured using an azotometer (Kjeltec 2300; Foss, Hillerød, Sweden). The C : N ratio was estimated as the quotient of TOC to TN. Soil pH was measured in 1 : 2.5 soil (g) : water (mL) using a pH electrode (FE20; Mettler Toledo, Switzerland).

Soil microbial biomass C (MBC) was determined with the fumigation–extraction method. Concentrations of TOC of the soil $K_2SO_4$ extract were determined by a TOC analyser (TOC-V CSH; Shimadzu Corporation, Kyoto, Japan) (Vance *et al*. 1987).

Fungal biomass was estimated by fluorescence microscopy. Fluorescent brightener 28 (FB28) was used to determine the total hypha bio-volume (Söderström 1977; Ingham and Klein 1984). Hyphal extraction was conducted following the method of Busse *et al*. (2009). Soil (10 g) was suspended in 95 mL of dilute Ringers solution (2.25 g NaCl, 0.11 g KCl, 0.12 g $CaCl_2$, 0.05 g $NaHCO_3$, and 1.0 L filter-sterilised-$H_2O$). This suspension was shaken for 10 min on a wrist-action shaker. Then the suspension was diluted in 0.3 M phosphate buffer to a final dilution of 1 mg soil $mL^{-1}$. All diluted samples (1 mL each) were stained with 0.5 mL FB28 (0.002 mg $mL^{-1}$ filter-sterilised $H_2O$ clarified with 1 M NaOH) for 30 min at 24°C in the dark. After staining, the samples were filtered onto 0.2-μm blackened polycarbonate filters and viewed using an fluorescent microscope at 400× magnification under UV excitation. Hyphal length and width were measured based on 20 fields per sample and converted to biomass using the method of Bottomley (1994).

Bacterial biomass was determined with a similar procedure. Bacteria were extracted from soil (as before) and stained using 4,6-diamidino-2-phenylindole for 30 min at 24°C in the dark. Fluorescence microscopy was used to determine cell volumes (Bottomley 1994).

### *Determination of CUE*

The CUE was determined according to Thiet *et al*. (2006) and Steinweg *et al*. (2008). Each soil sample collected from the study site was partially dried for 6 h and then placed into 12 bottles (40 g of soil in each bottle). Sealing rings with gas tubes were placed on the bottles. Six bottles were used for amended samples and the rest for non-amended samples. For each of the amended samples, 1.8 mL cellobiose solution (20 mg $mL^{-1}$) was added (equivalent to 420 μg C $g^{-1}$ soil), and then deionised water was added to maintain the soil water content at the same level as at sampling time. For the non-amended samples, only deionised water was added to maintain the soil moisture content at the same level as in the amended samples. At the beginning, and then every 3 h after the addition of the cellobiose solution or deionised water, 1 g soil was collected from each of three amended samples and three non-amended samples to measure cellobiose concentrations using the sulfuric acid–anthrone method for water-soluble carbohydrates (Brink *et al*. 1960).

The other three amended samples and three non-amended samples were used to measure $CO_2$ concentrations in the bottles, using an infrared gas analyser (LI-840; LI-COR Inc., Lincoln, NE, USA) with gas tubes on the bottles. The incubation period lasted for 48 h. Remaining cellobiose and cellobiose-derived $CO_2$ were determined by subtracting the results of the non-amended samples from those of the amended samples. CurveExpert v1.38 (D. G. Hyams, Starkville, MS, USA; www.curveexpert.net/) was used to fit the cellobiose concentration data to obtain the estimated time ($t_e$) at which the cellobiose concentration was close to zero. CurveExpert v1.38 was also used to fit $CO_2$ concentrations to obtain the cumulative $CO_2$ emission at $t_e$. The CUE was calculated as follows (Steinweg *et al*. 2008):

$$\mathrm{CUE} = 100\%\left[1 - \left(\frac{C_R}{C_0}\right)\right] \quad (1)$$

where $C_R$ is the cumulative $CO_2$-C emission at $t_e$ (μg $g^{-1}$ soil), and $C_0$ is the concentration of cellobiose-C measured at the beginning of cellobiose addition (μg $g^{-1}$ soil). Concentration of $CO_2$ was multiplied by 0.273 to convert $CO_2$ to C. Cellobiose concentration was multiplied by 0.421 to convert cellobiose to C. Quotients of the added cellobiose-C to $t_e$ were used to represent the soil microbial C uptake rates.

### *Data analysis*

Statistics analysis was carried out using the SPSS software package (2003; SPSS Inc., Chicago, IL, USA). Two-way analysis of variance (ANOVA) was used on different soil biogeochemical properties (soil temperature, moisture content, pH, C : N, SR rates, microbial C uptake rate, CUE, MBC, fungal biomass, bacterial biomass, and fungal : bacterial biomass) with N addition levels and sampling time as fixed variables. Pearson correlation analysis was used to test the correlation among the different properties at $P = 0.01$ and $P = 0.05$. One-way ANOVA with Tukey's HSD test was used to test differences of the properties among the treatments for each sampling time at $P = 0.05$.

## Results

### *Soil pH, C : N, SR rates, and microbial C uptake rates*

Soil temperature and moisture content showed no significant difference between three treatments during the whole experiment (Fig. 1). The two-way ANOVA analysis showed that N addition significantly decreased soil pH during the whole experiment ($P < 0.05$). After 500 days of the N treatments, pH values of the control, low-N, and high-N treatments changed from 6.81 to 6.78, 5.96, and 5.16, respectively (Fig. 2*a*). As shown in Fig. 2*b*, the C : N values were significantly lower in the N addition treatments than in the control ($P < 0.05$), and the lowest C : N occurred in the high-N treatment. After 500 days of N treatments, C : N values of the control, low-N, and high-N treatments were 11.26, 10.38, and 9.86, respectively. In general, the N addition treatments significantly decreased SR rates compared with the control (Fig. 2*c*, $P < 0.01$). Except for day 180, SR rates were significantly higher in the control than in the N treatments. On day 0, differences in microbial C uptake rates among the three treatments were not significant (Fig. 2*d*, $P > 0.05$). For the control, the microbial C uptake rates declined within the first 180 days, then increased after reaching the lowest values of $17.54 \pm 0.74$ μg $h^{-1}$. For the low-N and

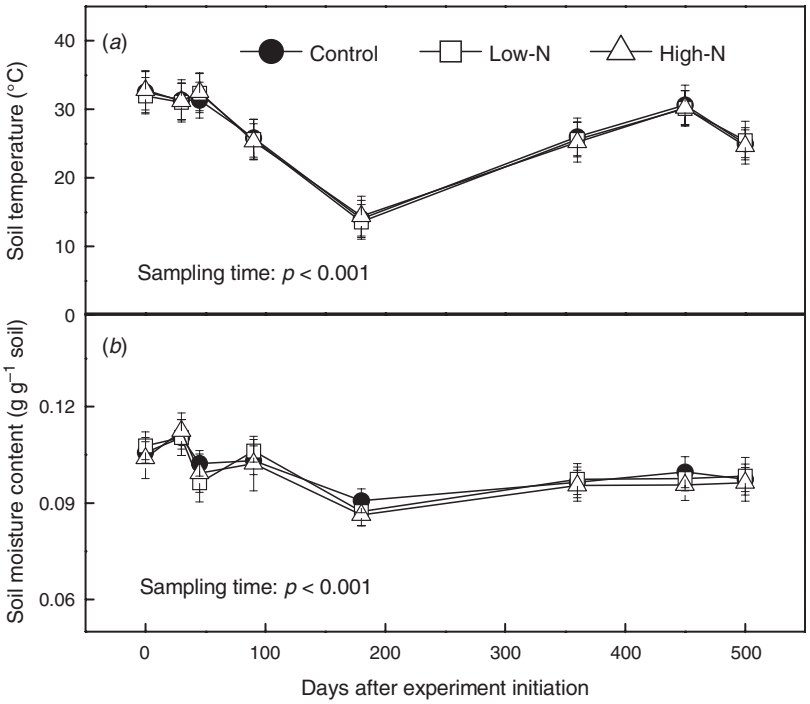


**Fig. 1.** Variation of (*a*) soil temperature (°C), and (*b*) soil moisture content ($g\,g^{-1}$ soil) for the control, low-N, and high-N treatments. Bars represent means ± s.e.

high-N treatments, C uptake rates decreased from $30.49 \pm 0.46$ to $21.05 \pm 0.98\,\mu g\,h^{-1}$ and from $33.90 \pm 0.46$ to $17.83 \pm 0.58\,\mu g\,h^{-1}$, respectively, during 500 days of incubation. After 360 days, N addition significantly decreased microbial C uptake rates compared with the control.

### *Carbon utilisation efficiency, and fungal and bacterial biomass*

As shown in Fig. 3, soil microbial CUEs on day 0 were not significantly different among the three treatments ($P > 0.05$). From days 90 to 360, CUE was significantly lower in the low-N treatment than in the other two treatments ($P < 0.05$), while CUE of the control treatment was the highest among the treatments. Differences in CUE among the treatments on day 450 were negligible, and on day 500, CUE was significantly higher in the control than in the other two treatments ($P < 0.05$).

Differences of the MBC values among the three treatments were not significant on day 0 (Fig. 4*a*, $P > 0.05$). During the whole experiment, the N addition treatments significantly decreased MBC compared with the control ($P < 0.01$). As shown in Fig. 4*b*, effects of the N treatments on fungal biomass were generally not significant during the 500 days of the experiment. Differences in bacterial biomass values among the three treatments were not significant on day 0 ($P > 0.05$). However, N addition significantly decreased bacterial biomass over the course of the experiment ($P < 0.05$). Bacterial biomass values of the control varied from 70.5 to $75.6\,\mu g\,g^{-1}$ soil, which was significantly higher than those of other two treatments (Fig. 4*c*). Ratios of fungal to bacterial biomass were significantly higher for the high-N treatment than for the other two treatments after 90 days of the N treatment ($P < 0.01$). For the control, ratios of fungal to bacterial biomass varied from 0.67 to 0.77 during the experiment. Ratios of fungal to bacterial biomass were >1 after 90 days for the high-N treatment and after 450 days for the low-N treatment (Fig. 4*d*).

### *Correlations among soil biogeochemical properties*

Based on the results of correlation analysis, a significant correlation between pH and MBC was found ($r = 0.658$, $n = 72$, $P < 0.01$). The correlation between bacterial biomass and pH was also significant ($r = 0.739$, $n = 72$, $P < 0.01$), while the correlation between fungal biomass and pH was not significant ($r = –0.315$, $n = 72$, $P > 0.05$). The correlation between C : N and MBC was not significant ($r = 0.412$, $n = 72$, $P > 0.05$). The SR rates were positively correlated with bacterial biomass ($r = 0.865$, $n = 72$, $P < 0.01$), while the correlation between SR rates and fungal biomass was not significant ($r = –0.206$, $n = 72$, $P > 0.05$).

Values of CUE were positively correlated with the fungal biomass for the low-N ($r = 0.797$, $n = 24$, $P < 0.05$) and high-N ($r = 0.835$, $n = 24$, $P < 0.05$) treatments. However, the correlation between fungal biomass and CUE for the control treatment was not significant ($r = 0.428$, $n = 24$, $P > 0.05$). A significantly negative correlation between CUE and soil temperature was observed for the control treatment ($r = –0.897$, $n = 24$, $P < 0.01$).

## Discussion

Previous studies showed that addition of a new C source resulted in enhanced degradation of old soil organic C due to the priming effect (Magid *et al.* 1999; Bell *et al.* 2003). In this study, part of the $CO_2$ emitted after the cellobiose addition might come from the soil organic C, which likely made the CUE results lower

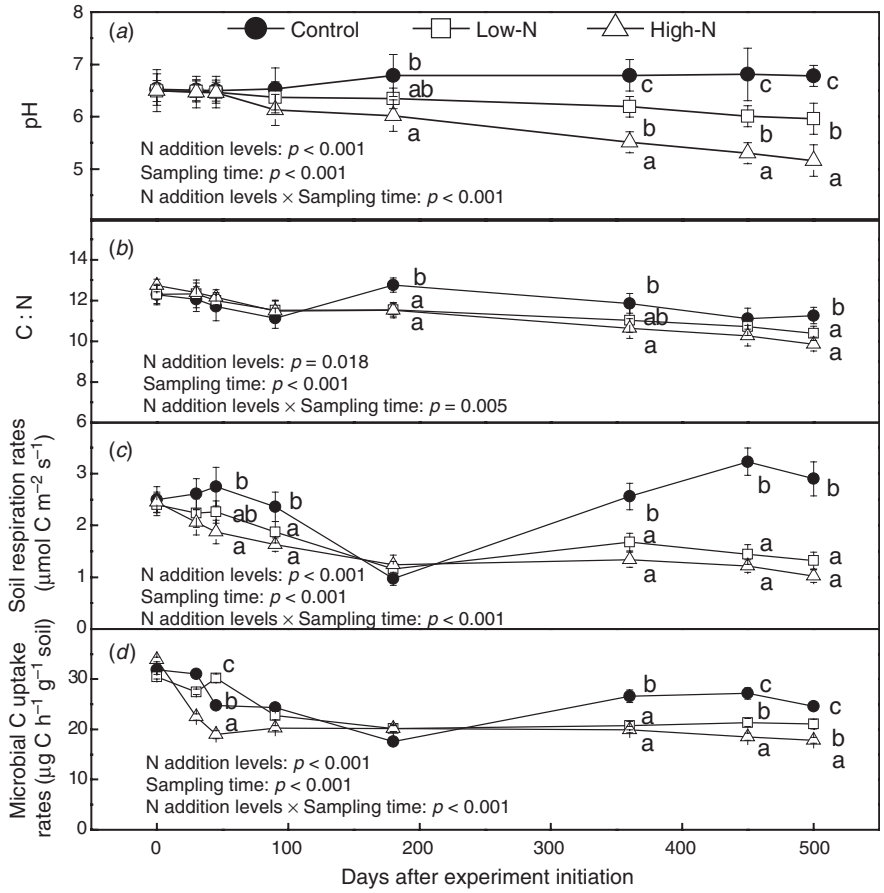


**Fig. 2.** Variation of (*a*) soil pH, (*b*) soil C : N, (*c*) soil respiration rates (μmol C $m^{-2}$ $s^{-1}$), and (*d*) microbial C uptake rates (μg C $h^{-1}$ $g^{-1}$ soil) for the control, low-N, and high-N treatments. Bars represent means ± s.e. Significant effects from two-way ANOVA are shown on each panel. Letters indicate the one-way ANOVA of significant difference between the three treatments for each sampling time; means with the same letter are not significantly different at $P = 0.05$.

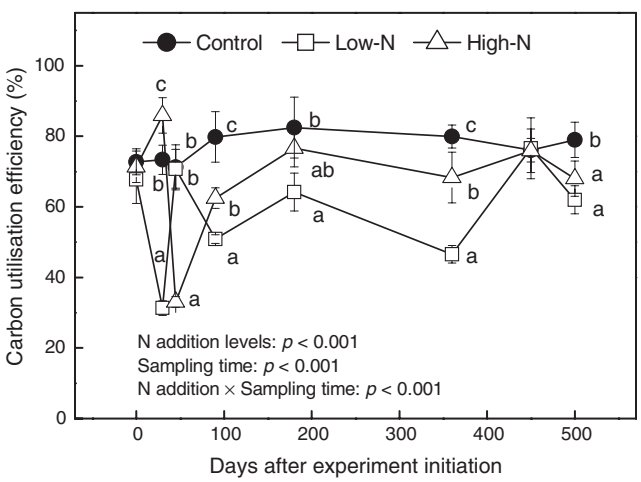


**Fig. 3.** Variation of carbon utilisation efficiency (CUE) for the control, low-N, and high-N treatments. Bars represent means ± s.e. Significant effects from two-way ANOVA are shown on each panel. Letters indicate the one-way ANOVA of significant difference between the three treatments for each sampling time; means with the same letter are not significantly different at $P = 0.05$.

than those determined using the $^{13}C$ isotope method. However, because of the lag time between microbial uptake of $^{13}C$-glucose and actual mineralisation of the glucose, it is difficult to determine whether $CO_2$ evolved at a specific time is solely from $^{13}C$-glucose and not from microbial biomass turnover (Thiet *et al*. 2006). Non-labelled substrates have been used to study CUEs with various treatments (Frey *et al*. 2001; Steinweg *et al*. 2008). As shown by Thiet *et al*. (2006), non-labelled and labelled substrates provided the same results for CUEs in fungi- and bacteria-dominant soil. In our study, our focus was the response of the estimate of CUE to N treatments. Non-labelled substrate should be suitable to investigate differences in CUE between different N treatments, although the results for CUEs in our study would be likely lower than those determined using $^{13}C$ isotope-labelled substrate.

Soil microbial biomass commonly varies with soil available N and pH (Johnson *et al*. 1998; Aciego Pietri and Brookes 2009). Previous studies showed that N addition stimulated microbial biomass in grassland or pasture (Rousk and Bååth 2007; Hamer *et al*. 2009). However, decreases in microbial biomass with increasing N application in steppe have also been reported (Zhang *et al*. 2008; Bi *et al*. 2012), which is consistent

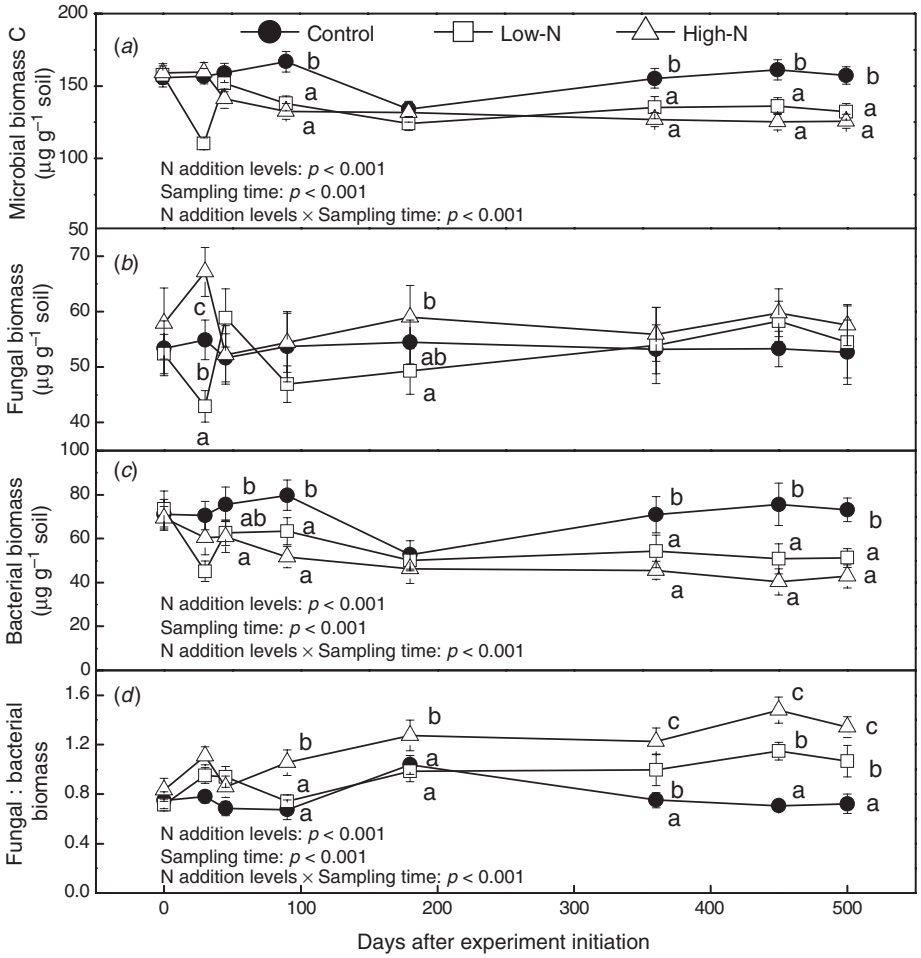


**Fig. 4.** Variation of (*a*) microbial biomass C (MBC, μg $g^{-1}$ soil), (*b*) fungal biomass, (*c*) bacterial biomass, and (*d*) fungal: bacterial biomass for the control, low-N, and high-N treatments. Bars represent means ± s.e. Significant effects from two-way ANOVA are shown on each panel. Letters indicate the one-way ANOVA of significant difference between the three treatments for each sampling time; means with the same letter are not significantly different at $P = 0.05$.

with our results. The soil acidification caused by N addition in this study was more severe than that experienced in other studies (Magill *et al*. 1997; Lu *et al*. 2009). Excessive N addition commonly causes accumulation of soil $NH_4^+$. Ammonium hydrolysis is the main process for soil acidification (Li *et al*. 2010; Fang *et al*. 2012). Soil acidification can lead to mobilisation of Al or cause Al toxicity to soil microbes, which can greatly affect soil microbial biomass (Xu and Ji 2001).

Both N addition and pH can affect soil fungal or bacterial biomass. Previous studies showed that soil fungi were more sensitive to N addition and soil acidification than bacteria (Bardgett *et al*. 1999; Bardgett and McAlister 1999; Bossuyt *et al*. 2001). However, in our study, fungal biomass did not change significantly, whereas bacterial biomass decreased significantly under N addition, suggesting that soil bacteria were more sensitive to N addition. Soil fungal biomass and bacterial biomass was also correlated with soil pH (Aciego Pietri and Brookes 2009). The significant correlation between bacterial biomass and pH and the insignificant correlation between fungal biomass and pH in our study indicated that bacteria were more sensitive than fungi to soil acidification. Our results suggested that a decline in soil pH was not beneficial for soil microbial growth.

Our results did not support the theory that ecosystems with soils dominated by fungi may sequester more C than systems with lower fungal abundance. Addition of N changed the soil microbial composition from bacterial dominance to fungal dominance but decreased soil CUE in our field experiment. For the control treatment, microbial biomass was bacteria-dominant. For the N addition treatments, bacterial biomass was significantly lower than fungal biomass (Fig. 4), indicating that microbial biomass became fungi- dominant. The CUE of the control treatment was higher than those of the N addition treatments (Fig. 3), suggesting that CUE in the bacteria-dominant soil was higher than in the fungi-dominant soil. Therefore, the theory that soil dominated by fungi sequesters more C than bacteria-dominated soil could not be proved in our study. In general, fungi have a high biomass C : N ratio and a high CUE, with a positive relationship between C : N and CUE (Katharina *et al*. 2010). In our study, N treatments

significantly decreased soil C : N and, similarly, lower soil C : N led to decline in soil fungal CUE.

Commonly, N addition may decrease microbial energetic costs of N assimilation (Bowden *et al*. 2004), so that more C uptake can be used for microbial growth, leading to higher CUE, especially in N-limited soils. We also observed a transitory increase of CUE under the high-N treatment from day 0 to day 45. From day 0 to day 45, soil acidification was not significant; therefore, the increase in CUE should be attributed to N addition in this short period. However, soil microbial CUE significantly decreased after 90 days for both the low-N and high-N treatments (Fig. 3), which suggested that there should be other, more important, factors controlling soil CUE beside available N. Interestingly, soil acidification and a decrease in CUE occurred simultaneously. As mentioned above, soil acidification limited soil microbial biomass growth, especially for the soil bacteria, which likely caused more microbial C uptake to be used for respiration rather than growth. Therefore, N addition played a key role in the increase in CUE in the short term (from day 0 to day 45), whereas soil pH was the major factor causing a decline in soil microbial CUE in the longer term.

Presumably, lower CUE means more C to be used for respiration, resulting in a higher SR rate. However, SR rates under the N treatments did not increase in our study. Previous studies showed that excessive N inputs could decrease soil microbial C uptake capability and limit soil microbial biomass growth (Compton *et al*. 2004; Follett *et al*. 2007). In our study, addition of N significantly decreased soil MBC and soil microbial C uptake rates compared with the control treatment (Fig. 2*d*). These two aspects likely prevented stimulation of SR rates and C loss from the soil.

The CUE is negatively correlated with incubation temperature (Steinweg *et al*. 2008). Therefore, an increase in the global temperature may lead to lower CUE, resulting in decline of C storage in the soil. In our study, CUE was negatively correlated with soil temperature for the control treatment. However, since N treatments change the soil microbial community structure and metabolism pattern (Hobbie and Gough 2004), the temperature-dependence of CUE disappeared for the N addition treatments. We also observed that SR rates for the three treatments were significantly lower on day 180 than at other sampling times. The lowest soil temperature on day 180 during the whole year led to decline of soil microbial activities, resulting in the lowest SR rates.

## Conclusions

Our result confirmed the hypotheses: N treatments resulted in significant soil acidification, which changed the soil from bacterial dominance to fungal dominance, and then N treatments greatly reduced soil microbial CUE. Our results showed that the CUE of fungi-dominant soil was lower than that of bacteria-dominant soil and N treatments might decrease soil fungal CUE. It was difficult to compare fungal and bacterial CUE directly in our study because the CUEs of both fungi and bacteria might change under N treatments.

However, our results did not support the theory that fungal and bacterial CUEs were similar. If the CUEs of fungi and bacteria were similar, fungal and bacterial biomass should show similar varied patterns under N treatments; however, N treatments did not affect fungal biomass significantly but greatly decreased bacterial biomass. Contrary to previous theory that N addition may decrease microbial energetic costs of N assimilation and stimulate microbial growth, we found that, with available N and soil acidification, soil bacteria were more sensitive than fungi, and soil microbial biomass decreased under N treatments. Decline in soil CUE under N treatments did not lead to higher soil respiration rates and more C loss from soil, because N treatments also decreased soil microbial biomass and microbial C uptake rates. These issues warrant further research.

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