Effects of nitrogen addition on soil oxidisable organic carbon fractions in the rhizospheric and bulk soils of Chinese pines in north-western China

Hongfei Liu, Sha Xue, Guoliang Wang, and Guobin Liu

The authors of the above mentioned paper regret to inform that the affiliation for Guobin Liu was incorrect as published. At the time of publication, this author was affiliated with the following two institutions:

Chinese Academy of Sciences, Institute of Soil & Water Conservation, Yangling 712100, Shaaxi, P. R. China.

Ministry of Water Resources, Yangling 712100, Shaaxi, P. R. China.

Effects of nitrogen addition on soil oxidisable organic carbon fractions in the rhizospheric and bulk soils of Chinese pines in north-western China

Hongfei Liu[A,*], *Sha Xue*[A,B,*], *Guoliang Wang*[A,B], *and Guobin Liu*[C]

[A]College of Forestry, State Key Laboratory of Soil Erosion and Dryland Farming on the Loess Plateau,
 Northwest A&F University, Yangling 712100, Shaanxi, P. R. China.
[B]Institute of Soil and Water Conservation, Chinese Academy of Sciences and Ministry of Water Resources,
 Yangling 712100, Shaaxi, P. R. China.
[C]Corresponding author. Email: gbliu@ms.iswc.ac.cn

Abstract. Increased atmospheric nitrogen (N) deposition caused by human activities has potentially important effects on ecosystem carbon (C) dynamics and different effects on C fractions with different stabilities and chemical compositions. A better understanding of the responses of different C fractions to N addition is vital for maintaining soil quality and protecting vegetation. In order to investigate the differential effects of N addition on total soil organic carbon (SOC) and four SOC fractions with increasing degrees of oxidisability in *Pinus tabuliformis* rhizospheric and bulk soils, a 6-year pot experiment was performed testing the effects of the addition of N at rates of 2.8, 5.6, 11.2, 22.4 and 44.8 g m^{-2} year^{-1} compared with a control (CK) group (no N addition). Addition of N addition had significant ($P < 0.05$) effects on SOC fractions of very labile C (C1) and recalcitrant C (C4), but negligible effects on total SOC (TOC) and SOC fractions of labile C (C2) and less labile C (C3). The C1 content and ratio of C1 to TOC in rhizospheres decreased following the addition of low levels (N2.8–N5.6) of N, but increased after the addition of high levels (N11.2–N44.8) of N, with minimum values obtained after the addition of 11.2 N g m^{-2} year^{-1}. Low rates (N2.8–N5.6) of N addition considerably increased C4 and the ratio of C4 to TOC in the rhizosphere, whereas addition of high rates (N11.2–N44.8) of N decreased these parameters. The responses of C1 and C4 in the bulk soil to N addition were opposite. The SOC fraction was significantly higher in the rhizosphere than in the bulk soil, indicating large rhizospheric effects. However, increased N addition weakened these effects. These findings suggest that low rates (N2.8–N5.6) of N addition stabilise SOC against chemical and biological degradation, whereas increased rates of N addition increase the lability of SOC in the bulk soil. Thus, the rhizosphere plays a vital role in soil carbon stability and sequestration in response to N addition.

Introduction

Global emissions of reactive nitrogen (N), such as NH$_3$, N$_2$O, urea, amines and proteins, have increased three- to fivefold over the past century because of anthropogenic activities such as the burning of fossil fuels and the use agricultural fertilisers (Galloway *et al*. 2008; Reay *et al*. 2008; Lu *et al*. 2010; Fang *et al*. 2011). N deposition rates in many areas of the world are much higher than they would otherwise have been in the absence of these human activities (Galloway *et al*. 2008). Increased N deposition in terrestrial ecosystems has a profound effect on global carbon (C) and N cycles and may induce long-term changes to natural ecosystems (Vitousek *et al*. 1997; Galloway *et al*. 2004; Phoenix *et al*. 2012). Thus, N is a common limiting nutrient in terrestrial ecosystems (Vitousek and Matson 1991).

Some studies have suggested that N addition increases C storage by alleviating N limitation, promoting tree productivity (Gruber and Galloway 2008; Schlesinger 2009), decreasing litter decomposition and soil respiration (Gruber and Galloway 2008; Hobbie *et al*. 2012) and inhibiting microbial enzymes, particularly those that degrade lignin (Waldrop *et al*. 2004). However, some studies have shown that the effects of N addition on C cycling can be transient (Bowden *et al*. 2004; Hagedorn *et al*. 2012), and others have reported that the effect of N addition on soil C storage is negligible (Lovett *et al*. 2013; Zeng *et al*. 2010). Thus, the role of long-term N addition on soil C pools and stabilisation remains uncertain.

Walkley and Black (1934) proposed a method for determining the C content of soil and Chan *et al*. (2001)

---

*These authors contributed equally to this study.

modified the Walkley–Black method to separate soil organic carbon (SOC) into four fractions with different labilities and oxidisabilities: C1, very labile C; C2, labile C; C3, less labile C; and C4, recalcitrant C. Different SOC fractions play different roles in SOC dynamics and soil C sequestration. The C1 and C2 fractions are primarily composed of light fraction C, which belongs to the labile C (LC) pool and is mostly associated with the availability of nutrients and formation of macroaggregates (Janzen 1987; Maia *et al*. 2007). The C3 and C4 fractions are associated with compounds with high chemical stability that are slowly altered by microbial activities; these fractions comprise the recalcitrant C (RC) pool (Chan *et al*. 2001; Sherrod *et al*. 2005). The LC pool, with short turnover times, can be easily mineralised and is a more sensitive indicator of changes in soil fertility than total SOC (Yang *et al*. 2009; Datta *et al*. 2010). Meanwhile, the RC pool is a stable C sink in the soil cycle and dominates long-term C storage (Neff *et al*. 2002; Jiang *et al*. 2014). The LC and RC pools are significantly affected by changes in plant inputs, including aboveground biomass, fine root biomass and dissolved organic C (Carrillo *et al*. 2011). N addition can alter these inputs and thus indirectly affects the turnover of the LC and RC pools. Positive (Chen *et al*. 2012*a*; Jiang *et al*. 2014), negative (Rodriguez *et al*. 2014) and even neutral (Chen *et al*. 2012*b*) responses of soil lability to N addition have been reported. These apparent discrepancies could be explained by differences in climate conditions, plant species, soil type, the amount and chemical composition of N fertilisers and the timing and duration of N addition in the different studies. However, the role of long-term N addition in the allocation of soil C between LC and RC pools in a temperate semi-arid climate remains unclear and thus requires investigation.

N addition at low levels ($<25\,kg\,h\,m^{-2}\,y^{-1}$) can alleviate N shortages and promote plant growth, thereby increasing C storage. However, excessive N (i.e. N in excess of biotic demands) has negative effects on ecosystems. For example, an N input of $>25\,kg\cdot h\,m^{-2}$ has been reported to cause N saturation in European forests (Emmett *et al*. 1998). Excessive N addition can increase the intensity of unfavourable changes in the shoot/root ratio, soil acidification, nutrient imbalances, insects and pathogenic pests, nutrient deficiencies, aluminium toxicity due to acidification by nitrification and assimilation of inorganic N stealing C that could otherwise be used for maintenance and growth (Fenn *et al*. 1998; Nadelhoffer *et al*. 1999; Andersson *et al*. 2001). Thus, excessive N addition has a negative effect on long-term soil C storage (Song *et al*. 2013). However, information regarding the mechanisms involved in the effects of long-term excessive N addition on stabilisation of C in the soil is limited and thus requires further investigation.

The rhizosphere is commonly defined as the area where root activity significantly affects the biological properties of the soil (Zoysa *et al*. 1999; Chen *et al*. 2001). It is a biologically active zone where complex interactions among plant roots, soil particles and microbes occur (Puglisi *et al*. 2008). Compared with bulk soil, plant rhizospheres generally have higher C availability (Cheng *et al*. 2003) and a higher biomass of soil micro-organisms (Griffiths 1994). In addition, the activity of extracellular enzymes involved in soil organic matter (SOM) decomposition and nutrient cycling are often higher in the

rhizosphere than in the bulk soil (Hinsinger *et al*. 2009). These effects are primarily attributed to rhizodeposition, which may account for as much as 25% of belowground allocated C (Jones *et al*. 2009) and comprises water-soluble root exudates, such as sugars, amino acids, organic acids and hormones (Grayston *et al*. 1997; Dennis *et al*. 2010). In addition, moisture dynamics and nutrient availability in the rhizosphere are affected by root uptake of water and nutrients (Zhu *et al*. 2014). Thus, the rhizosphere functions as a central site of microbial activity and biogeochemical cycling. Increased soil fertility due to N addition can reduce relative belowground C allocation and thus lower the effects of the rhizosphere (Phillips and Fahey 2008; Fontaine *et al*. 2011; Ai *et al*. 2012). However, some studies have found that N addition has a neutral or even positive effect on the actions of the rhizosphere (Phillips and Fahey 2008; Zhu *et al*. 2014). These conflicting results are due primarily to differences in plant species, soil type, climate condition, the amount and chemical composition of fertilisers and the duration of N addition in different studies. Therefore, more mechanistic studies on the responses of the rhizosphere with regard to soil C lability following N addition are needed.

The Chinese pine *Pinus tabuliformis* is a prominent species in the forest plantations of Shaanxi Province, China, and plays an important role in the ecology of forest regeneration and forestry in warm temperate regions. Previous studies indicated that N addition decreases the labile C pool in soils under tree species with high lignin litter (Rodriguez *et al*. 2014). In the present study we investigated changes in total SOC, as well as in the concentration and distribution of oxidisable organic carbon fractions in rhizospheric and bulk soils of Chinese pine following N addition in pot experiments. Based on previous studies (Lv *et al*. 2017), we formulated the following hypotheses to be tested: (1) low levels ($<5.6\,g\,kg^{-1}\,y^{-1}$) of N addition reduce soil C allocation to the labile oxidisable SOC fractions and increase soil C stability in both rhizospheric and bulk soils; (2) excessive N addition promotes soil C allocation to the labile oxidisable SOC fractions and increases soil C lability in rhizospheric and bulk soils; and (3) N addition weakens the rhizospheric effect on SOC, total nitrogen (TN) and SOC fraction contents.

Materials and methods

Study site

The study was conducted in experimental plots of the Institute of Soil and Water Conservation, Chinese Academy of Sciences and Ministry of Water Resources, Yangling, Shaanxi, China. The area has a temperate continental monsoon climate, a mean annual temperature of 13.2°C, mean annual precipitation of 674.3 mm, a mean frost-free period of 225 days and 1993.7 h annual sunshine. Chinese pine seeds were planted on a seedbed (5 m × 10 m) in March 2007. Then, 1-year-old seedlings were individually transplanted in March 2008 to polyvinyl chloride (PVC) experimental pots (35 cm diameter, 40 cm deep). Soil was collected from the 0–30 cm soil horizon in a Chinese pine forest plantation in Yichuan, Shaanxi Province (Lv *et al*. 2017), and a huangmian soil was developed on wind-deposited loessial parental material in the absence of bedding, with a loose silty texture, macroporosity and wetness-induced collapsibility.

Although the pH of the huangmian soil is weakly alkaline (8.5–8.8), it has a low N content (the soil used in the present study had an N content of 0.724 mg kg^{-1}). The soil was sieved through a 2-mm plastic mesh and was homogenised before the experiment, with 18 kg soil added to each experimental pot.

Experimental design and soil sampling

In the present study, the effects of N addition on different fractions of SOC were investigated. There were six treatment groups in the experiment: a control (CK) group, with no added N, and five treatment groups in which N was added at a rate of 2.8, 5.6, 11.2, 22.4 and 44.8 g m^{-2} year^{-1} (N2.8, N5.6, N11.2, N22.4 and N44.8 respectively). The current rate of N deposition on the Loess Plateau in China is 2.06 g^{-1} year^{-1}. This experiment not only simulated the current rate of N deposition on the Loess Plateau, but also simulated the extremely excessive N deposition on the Loess Plateau. According to the single-factor randomised block design, each treatment had 68 pots. Urea was dissolved in 10 mL water and was added once to each experimental pot from late March to early April every year from 2008 to 2014 before the seasonal rains. This experiment was conducted in the open.

Six 5-year-old Chinese pines of medium height and basal diameter were selected from each treatment for measurement on 11 July 2014. The roots of the Chinese pines were gently shaken to remove loosely adhering soil particles as that described by Butler *et al*. (2003). Selected plants were removed from their pots and weighed. The root–soil systems were sliced down the middle and shaken in plastic bags until approximately four-fifths of the initial weight was in the bag. The portion obtained was considered bulk soil. The remaining one-fifth that was still attached to the root system was considered rhizosphere soil. The rhizosphere soil was then carefully removed from the roots with a probe and forceps. Plant material and gravel were removed from the soil samples. One subsample each of plant rhizosphere and bulk soil was air dried, crushed and passed through a 0.25-mm sieve for the measurement of total SOC (TOC) and oxidisable SOC. Another subsample was passed through a 1-mm sieve for the measurement of TN, NH$_4^+$-N, and NO$_3^-$-N in the plant rhizosphere and bulk soil. The remaining soil samples were stored at 4°C for the measurement of pH and microbial biomass C in the plant rhizosphere and bulk soil. Plant shoots and roots were harvested separately and were oven dried at 65°C to a constant weight, which was regarded as the aboveground biomass and belowground biomass respectively.

Laboratory analysis

TOC was determined using the Walkley–Black method (Nelson *et al*. 1996) and TN was determined using the Kjeldahl method (Bremner and Mulvaney 1982). Soil pH was measured using a pH electrode in a solution with a soil (in grams) to water (in millilitres) ratio of 1 : 2.5. NH$_4^+$ and NO$_3^-$ concentrations in soil samples were analysed using a continuous flow analyser.

Different oxidisable SOC fractions were estimated using the modified Walkley–Black method described by Chan *et al*. (2001). Briefly, 0.5 g ground soil (0.25 mm) was placed in a 500-mL Erlenmeyer flask, to which 10 mL of 0.167 M K$_2$CrO$_7$ was added. The resulting solution was then added to 5, 10 or 20 mL concentrated H$_2$SO$_4$ (18 M) to produce three solutions

with acid–aqueous ratios of 0.5 : 1, 1 : 1 and 2 : 1 respectively (corresponding to 6, 9 and 12 M H$_2$SO$_4$). The samples were oxidised with dichromate in an acidic medium (Yeomans and Bremner 1988) at different H$_2$SO$_4$ concentrations without external heating. Residual dichromate was determined by titrating against 0.5 M FeSO$_4$. The amount of oxidisable SOC was determined and separated into the following four fractions with decreasing lability: Fraction 1 (C1), SOC oxidisable at 6 M H$_2$SO$_4$, corresponding to the very labile SOC fraction; Fraction 2 (C2), the difference between oxidisable SOC extracted with 9 and 6 M H$_2$SO$_4$, corresponding to the labile fraction; Fraction 3 (C3), the difference between oxidisable SOC extracted with 12 and 9 M H$_2$SO$_4$, corresponding to the less labile fraction; and Fraction 4 (C4), the residual SOC after reaction with 12 M H$_2$SO$_4$ compared with TOC, corresponding to the non-labile SOC fraction. Oxidisable organic carbon (OC) was determined by summing C1 + C2 + C3 (Chan *et al*. 2001). The rhizospheric effect was calculated as follows:

$$\text{Rhizospheric effect} = (C_{iR} - C_{iB})/C_{iR}$$

where C_{iR} is the content of the C$_i$ fraction in the rhizosphere and C_{iB} is the content of the C$_i$ fraction in the bulk soil.

Statistical analysis

One-way analysis of variance (ANOVA) was used to assess the effects of N addition on SOC, TOC, TN, NO$_3^-$-N, NH$_4^+$-N, C : N ratio and pH. If significant effects were detected ($P < 0.05$), Duncan's multiple-range test was used to compare means. Pearson linear correlation analysis and Redundancy analysis (RDA) were performed to analyse the responses of the SOC fractions to N addition. ANOVA and Pearson correlation analyses were performed using SPSS version 21.0 (IBM Corp., Armonk, NY, USA), whereas the RDA was performed using CANOCO for Windows 4.5 (Biometris, Wageninggen, Netherlands). Figures were drawn using SigmaPlot 10.0 (Systat Software, Inc., San Jose, CA, USA).

Results

SOC and TN responses to N addition

TOC contents in the plant rhizospheres increased in N2.8 and N5.6, but not significantly (Fig. 1), and were highest in N5.6 and N44.8.There were no significant increases in the TOC content of plant rhizospheres in the N2.8 and N5.6 treatment groups (Fig. 1). The highest TOC content was observed in the N5.6 and N44.8 groups. Conversely, there were no significant decreases in TOC in the bulk soil in the N2.8–N5.6 groups, but TOC in the bulk soil increased in the N11.2–N44.8 groups. Maximum TOC in bulk soil was found in the N5.6 treatment group. TOC content was significantly higher in the rhizosphere than in the bulk soil. The rhizospheric effect on TOC increased in the N2.8 and N5.6 groups, whereas excessive N addition in the N11.2, N22.4 and N44.8 groups had a negligible effect on this rhizospheric effect.

TN content in both rhizosphere and bulk soils tended to increase with N content, with highest TN content seen in the rhizosphere in the N44.8 treatment group (Fig. 1). TN content was significantly higher in the rhizosphere than in the bulk soil. Compared with CK, N addition had a negligible effect on the rhizospheric effect on TN. However, the rhizospheric effect on

TN was significantly higher in the N5.6 and N44.8 groups than in the N22.4 group.

C : N ratios decreased with increasing N content in both the rhizosphere and bulk soils (Table 1).

Responses of oxidisable SOC fractions to N addition

The addition of N significantly altered the oxidisable SOC content in the very labile (C1) and recalcitrant (C4) C fractions in the rhizosphere and bulk soil, but had no significant effect on labile (C2) and less labile (C3) C fractions (Fig. 2). N2.8, N5.6, N11.2 and N22.4 treatments decreased C1 content in the rhizosphere, but increased the C1 fraction in bulk soil. Rhizospheric C1 content was highest in the N44.8 treatment group (Fig. 2). In contrast, rhizospheric C4 content increased in the N2.8, N5.6 and N11.2 treatment groups. Although C4

content was significantly lower in bulk soil in all treatments with added N compared with the CK group, there were no significant differences among the different N treatment groups. The OC content in rhizosphere decreased in the N2.8, N5.6, N11.2 treatment groups, but increased in the N22.4 and N44.8 treatment groups, and was highest in the N44.8 group. The OC content in bulk soil increased with N addition, although this increase did not reach statistical significance. The content of the SOC fractions was significantly higher in the rhizosphere than in bulk soil, except for C4 content in the CK group.

The C1, C4 and OC content in the rhizosphere was 53.2–62.6%, 5.8–17.2% and 82.8–94.2% respectively (Fig. 2). The C1 and OC fractions in the rhizosphere decreased following the addition of N at low levels (i.e. in the N2.8, N5.6, N11.2 groups), but increased at high levels (in the N44.8 group). The lowest C1 and OC content was observed in the N5.6
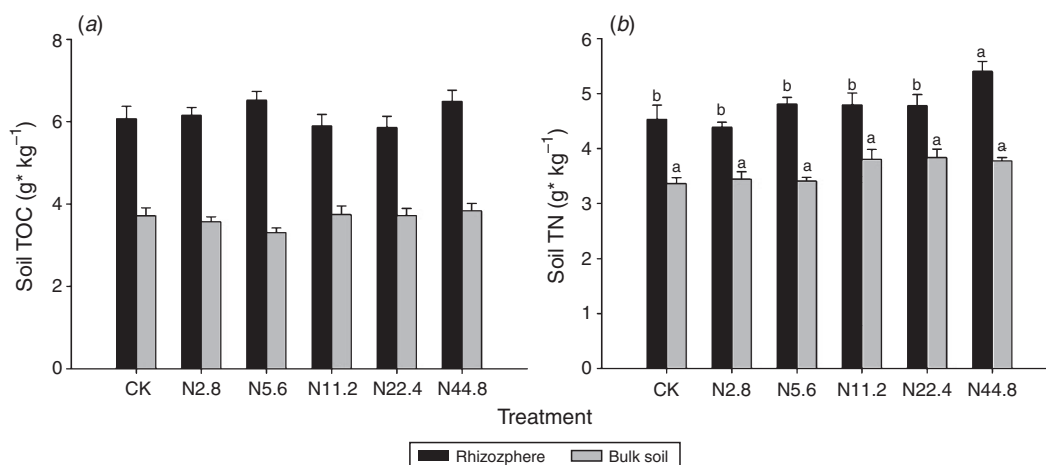

Fig. 1. Effects of N addition on (*a*) total organic C (TOC) and (*b*) total nitrogen (TN) in rhizosphere and bulk soil. N was added at rates of 2.8, 5.6, 11.2, 22.4 and 44.8 g m^{-2} year^{-1} (N2.8, N5.6, N11.2, N22.4 and N44.8 respectively). CK, control treatment. Data are the mean ± s.e.m. Different letters indicate significant differences in TOC or TN within rhizosphere or bulk soil between N treatment groups.

Table 1. Effects of the addition of N on properties of plant rhizosphere and bulk soil

Data are given as the mean ± s.e.m. Within columns values with different letters differ significantly ($P < 0.05$). N was added at rates of 2.8, 5.6, 11.2, 22.4 and 44.8 g m^{-2} year^{-1} (N2.8, N5.6, N11.2, N22.4 and N44.8 respectively). CK, control treatment

| Treatment group	NH$_4^+$-N (mg kg^{-1})	NO$_3^-$-N (mg kg^{-1})	C : N	pH
Rhizosphere soil				
CK	11.437 ± 0.579b	6.628 ± 0.563d	13.459 ± 0.473a	8.585 ± 0.015a
N2.8	13.103 ± 0.763a	10.592 ± 1.684d	14.014 ± 0.532a	8.578 ± 0.016a
N5.6	10.413 ± 0.208c	14.870 ± 1.616cd	13.561 ± 0.312a	8.533 ± 0.018a
N11.2	11.568 ± 0.734b	19.462 ± 4.052c	12.293 ± 0.288a	8.513 ± 0.048a
N22.4	12.667 ± 0.281ab	28.770 ± 2.946b	12.251 ± 0.242b	8.411 ± 0.033b
N44.8	13.083 ± 0.659a	38.172 ± 1.893a	12.000 ± 0.480b	8.252 ± 0.040c
Bulk soil				
CK	11.088 ± 0.501b	7.632 ± 1.098c	11.047 ± 0.423a	8.675 ± 0.024a
N2.8	13.053 ± 0.747a	8.853 ± 1.592c	10.446 ± 0.229ab	8.673 ± 0.032a
N5.6	13.823 ± 0.531a	15.493 ± 1.751bc	9.707 ± 0.175b	8.602 ± 0.017ab
N11.2	12.833 ± 0.846ab	21.753 ± 2.778b	9.830 ± 0.212b	8.563 ± 0.021b
N22.4	12.587 ± 0.375ab	31.010 ± 4.096a	9.684 ± 0.177b	8.445 ± 0.031c
N44.8	12.597 ± 0.562ab	36.617 ± 2.331a	10.154 ± 0.118ab	8.350 ± 0.034d
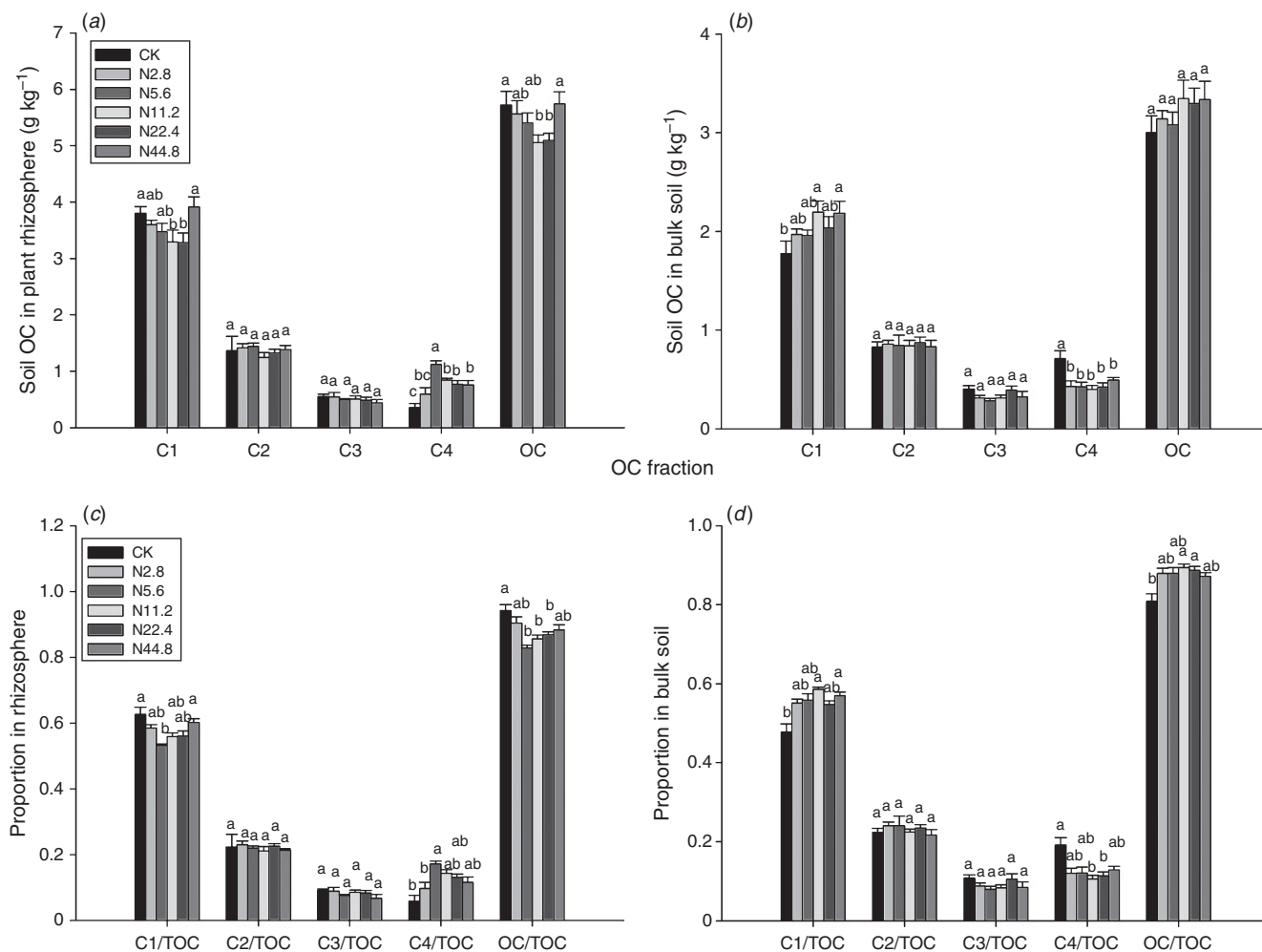
Fig. 2. (*a*, *b*) Soil oxidisable organic carbon (OC) fractions in the rhizosphere (*a*) and bulk soil (*b*) in the control (CK) group and treatment groups after N addition. N was added at rates of 2.8, 5.6, 11.2, 22.4 and 44.8 g m^{-2} year^{-1} (N2.8, N5.6, N11.2, N22.4 and N44.8 respectively). (*c*, *d*) Proportion of different OC fractions relative to total organic carbon (TOC) in the rhizosphere (*c*) and bulk soil (*d*) in the control and treatment groups. Data are the mean ± s.e.m. Different letters indicate significant differences between N treatment groups. C1, very labile C; C2, labile C; C3, less labile C; and C4, recalcitrant C; OC, oxidisable organic carbon.

treatment group. However, the amount of organic C in the C4 fraction in the rhizosphere increased following the addition of low levels of N (i.e. in the N2.8 and N5.6 groups), but decreased after the addition of high levels (in the N11.2, N22.4 and N44.8 groups). Maximum C4 content was observed in the N5.6 group. The organic C content as C1, C4 and OC fractions in bulk soil was 47.8–58.6%, 10.6–19.1% and 80.9–89.4% respectively. Conversely, the addition of N increased the amount of organic C in the C1 and OC fractions in bulk soil, but decreased the amount in the C4 fraction compared with the CK group. The percentage of C1 and the OC:TOC ratio were significantly higher in the rhizosphere than bulk soil in the CK group, whereas the ratio of C4 to TOC was significantly lower in the rhizosphere than bulk soil. However, there were no significant differences between the rhizosphere and bulk soil in terms of the amount of organic C in the C1, C4 and OC fractions in the N2.8, N5.6, N11.2 and N22.4 and N44.8 treatment groups. Moreover, the rhizospheric effect on C1

and OC decreased at low levels of N addition (i.e. in the N2.8, N5.6 and N11.2 groups), but increased at high levels (in the N22.4 and N44.8 groups). The lowest effects were observed in the N11.2 treatment group (Fig. 2). In contrast, the rhizospheric effect on C4 exhibited an opposite trend, with the maximum effect observed in the N5.6 treatment group (Fig. 3).

The RDA and correlation analysis investigated the effects of soil properties on various C fractions. The first two canonical axes accounted for 31.6% and 6.5% of the total variance respectively across all data for rhizospheric soil of Chinese pine. These axes also accounted for 45.4% of the total variance in the bulk soil (35.2% for RDA1 and 10.2% for RDA2; Fig. 4). TOC and TN had a greater effect on the various SOC fractions than the other parameters, in both the rhizospheric and bulk soils. TOC and TN had stronger effects on very labile (C1) and labile (C2) C fractions than on less labile (C3) and recalcitrant (C4) C fractions in the rhizospheric and bulk soils. In both soil
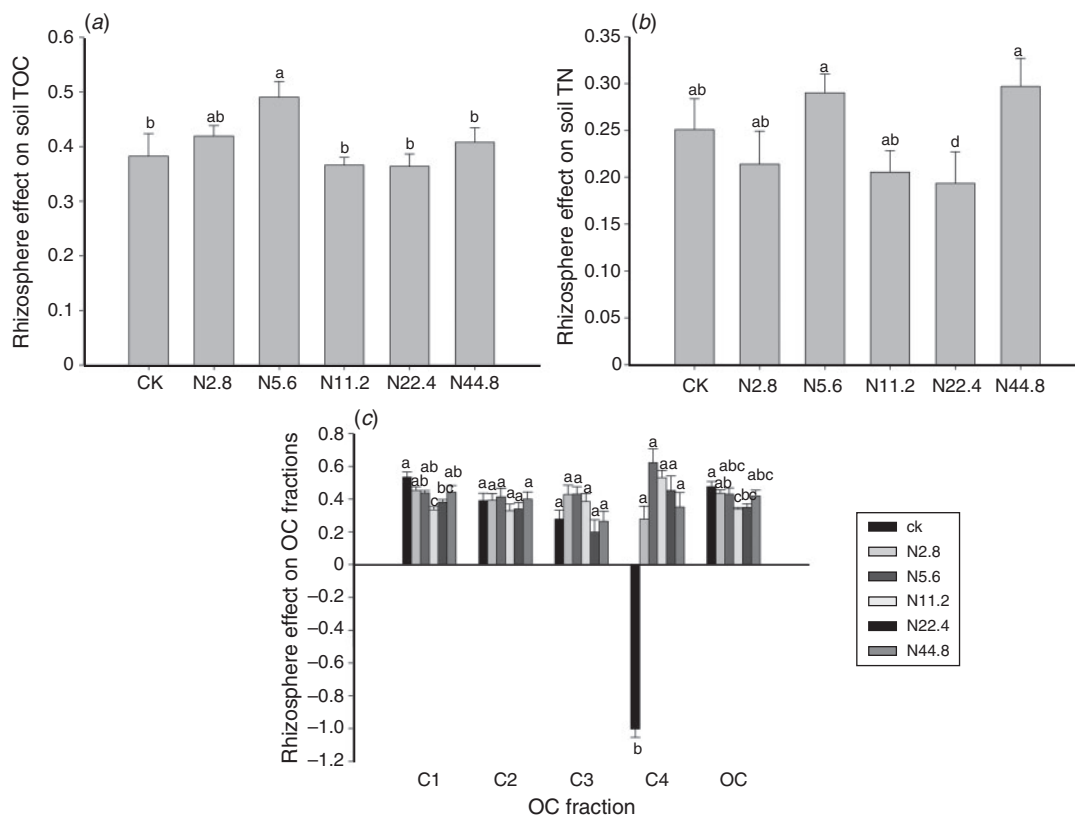
Fig. 3. Rhizosphere effects on (*a*) soil total organic carbon (TOC), (*b*) soil total nitrogen (TN) and (*c*) organic carbon fractions. N was added at rates of 2.8, 5.6, 11.2, 22.4 and 44.8 g m^{-2} year^{-1} (N2.8, N5.6, N11.2, N22.4 and N44.8 respectively). CK, control group; C1, very labile C; C2, labile C; C3, less labile C; and C4, recalcitrant C. Data are the mean ± s.e.m. Different letters indicate significant differences between the treatment groups.
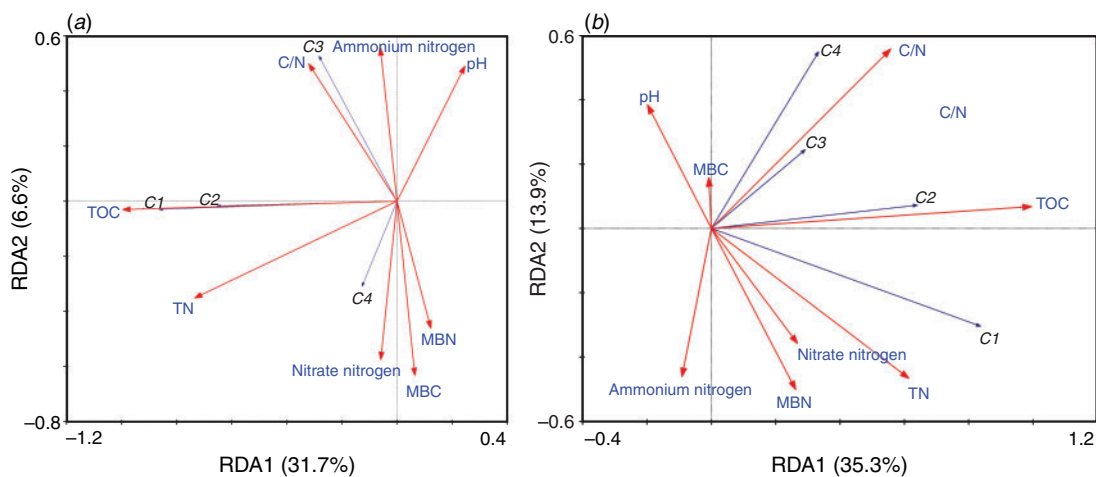

Fig. 4. Redundancy analysis (RDA) of soil organic carbon fractions to soil total organic carbon (TOC), total nitrogen (TN), ammonium nitrogen, nitrate nitrogen, the C : N ratio and pH in the (*a*) rhizosphere and (*b*) bulk soil. C1, very labile C; C2, labile C; C3, less labile C; and C4, recalcitrant C; MBN, microbial biomass nitrogen; MBC, microbial biomass nitrogen.

types, the C : N ratio and microbial biomass carbon (MBC) had the highest correlations with the C3 and C4 fractions respectively. These results were supported by the correlation matrix, which identified a significantly positive correlation

among C1, C2 and TOC content in both soil types; these parameters were all positively correlated with TN (*P* < 0.01). In contrast, the correlations among C3, C4 and TOC content were not significant in the rhizosphere, and these fractions were

not significantly correlated with either the rhizospheric or bulk soil (Tables 2 and 3).

Discussion

Although rhizospheric and bulk soil SOC fractions responded differently to N addition, the rhizospheric and bulk soil TOC was relatively unchanged after N addition. The addition of N decreased the C1 fraction in the N11.2–N22.4 groups and increased the C4 fraction in the rhizospheres of the N5.6–N44.8 groups. The C1 fraction in the rhizosphere was significantly lower in the N44.8 than N11.2–N22.4 groups, whereas the C4 fraction in the rhizosphere was significantly higher in the N5.6 N11.2–N44.8 groups. However, N addition, increased the C1 fraction in the N11.2 and N44.8 groups, but decreased the C4 fraction in the bulk soil in the N2.8–N44.8 groups. Soil SOC fractions and their content depend on the balance between OC inputs and outputs (Mack *et al.* 2004). Plant residues, fine roots and rhizodeposition inputs to the soil may be factors controlling the accumulation of SOC fractions in the rhizosphere and bulk soils under atmospheric N deposition. In the present study, low levels of N addition (i.e. in the N2.8 and N5.6 groups) increased aboveground biomass, but had no

significant effect on belowground plant biomass. In contrast, high levels of N addition (i.e. in the N22.4 and N44.8 groups) decreased aboveground biomass and increased belowground biomass (Fig. 5). Chinese pine is a coniferous tree, and its leaf litter generally contains a higher lignin content and has a lower decomposition rate than leaf litter of broad-leaved trees (Zhang *et al.* 2008). Low levels of N addition (3–8 kg N h^{-1} y^{-1}) can promote plant growth by alleviating N shortages, which commonly occur in most terrestrial ecosystems (Vitousek *et al.* 1997; Fenn *et al.* 2003). High levels of N addition can cause N saturation in the soil, thereby inducing a change from N to P limitation (Britton *et al.* 2008; Han *et al.* 2011), and increases in soil inorganic N concentrations can lead to N pollution, particularly in water (Han *et al.* 2011), inhibition of plant growth and an increased root shoot ratio. Meanwhile, the increase in belowground plant biomass induced by excessive N addition promotes fine root and rhizodeposition inputs to the soil, thus contributing to the accumulation of recalcitrant carbon in the rhizosphere. However, in the present study, the N44.8 treatment facilitated the accumulation of labile carbon compared with the N11.2 and N22.4 treatments. Extremely excessive N addition in the present study seriously inhibited plant growth as a result of increased P limitation in the soil

Table 2. Correlation Coefficients (Pearson's r) among C1, very labile C; C2, labile C; C3, less labile C; and C4, recalcitrant C; OC, oxidisable organic carbon; TOC, total organic carbon; TN, total nitrogen in rhizosphere soil. Correlations among different organic carbon fractions and soil chemical properties in rhizosphere soil

P < 0.05 level, ** *P* < 0.01 (two-tailed). C1, very labile C; C2, labile C; C3, less labile C; and C4, recalcitrant C; OC, oxidisable organic carbon; TOC, total organic carbon; TN, total nitrogen

	C1	C2	C3	C4	OC	TOC	TN	NH_4^+-N	NO_3^--N	pH
C1	1									
C2	0.377*	1								
C3	0.213	0.142	1							
C4	−0.022	−0.200	−0.438	1						
OC	0.882**	0.730**	0.405*	−0.192	1					
TOC	0.882**	0.670**	0.263	0.140	0.945**	1				
TN	0.726**	0.437**	0.030	0.209	0.689**	0.765**	1			
NH_4^+-N	0.036	−0.014	0.323	−0.166	0.082	0.027	0.181	1		
NO_3^--N	0.163	−0.039	−0.338	0.139	0.025	0.071	0.548**	0.283	1	
pH	−0.359*	−0.083	0.271	−0.115	−0.224	−0.265	−0.650**	−0.235	−0.919**	1

Table 3. Correlation Coefficients (Pearson's r) among C1, very labile C; C2, labile C; C3, less labile C; and C4, recalcitrant C; OC, oxidisable organic carbon; TOC, total organic carbon; TN, total nitrogen in bulk soil. Correlations among different organic carbon fractions and chemical soil properties in bulk soil

P < 0.05 level, ** *P* < 0.01 (two-tailed). C1, very labile C; C2, labile C; C3, less labile C; and C4, recalcitrant C; OC, oxidisable organic carbon; TOC, total organic carbon; TN, total nitrogen

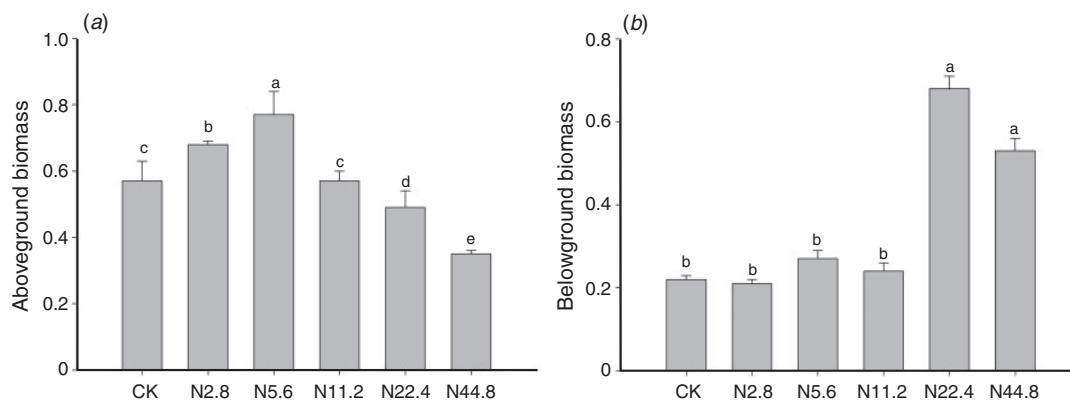|	C1	C2	C3	C4	OC	TOC	TN	NH_4^+-N	NO_3^--N	pH
C1	1									
C2	0.382*	1								
C3	0.048	0.078	1							
C4	−0.068	0.001	0.146	1						
OC	0.904**	0.674**	0.304	−0.016	1					
TOC	0.820**	0.630**	0.338*	0.355*	0.929**	1				
TN	0.641**	0.423*	0.005	−0.077	0.639**	0.569**	1			
NH_4^+-N	0.010	0.019	0.024	−0.360*	0.021	−0.114	−0.101	1		
NO_3^--N	−0.427**	−0.106	0.194	0.174	−0.314	−0.229	−0.531**	0.362	1	
pH	−0.293	−0.080	0.048	0.164	−0.238	−0.162	−0.532**	−0.361	−0.255	1

Fig. 5. (*a*) Aboveground and (*b*) belowground biomass in the control (CK) group and treatment groups after N addition. N was added at rates of 2.8, 5.6, 11.2, 22.4 and 44.8 g m^{-2} year^{-1} (N2.8, N5.6, N11.2, N22.4 and N44.8 respectively). Data are the mean ± s.e.m. Different letters indicate significant differences between the treatment groups.

(Fig. 5). Thus, Chinese pines must increase their bioavailable C inputs to ectomycorrhiza in exchange for P in order to increase soil labile carbon and subsequently P (Read and Perez-Moreno 2003; Hobbie and Hobbie 2006). The very labile C (C1) fraction in the bulk soil accumulates primarily from plant residues (Maia *et al*. 2007). Increased inputs of plant residues to soil with N addition may increase the C1 fraction in bulk soil.

N addition can frequently have a negative effect on the decomposition of recalcitrant substrates because of an inhibition of microbial enzymes, such as phenol oxidase, which degrades lignin, and an increase in the activity of hydrolases in carbohydrate decomposition (DeForest *et al*. 2004; Frey *et al*. 2004; Zak *et al*. 2008; Whittinghill *et al*. 2012). N addition can also suppress heterotrophic respiration, and this effect is consistent with reduced phenol oxidase activity (Rodriguez *et al*. 2014). The rhizosphere is a zone of high microbial activity in the vicinity of growing plants relative to the bulk soil (Butler *et al*. 2003), and soil enzyme activities may not be equally distributed between the rhizosphere and bulk soil, with higher activity in the former (Ai *et al*. 2012). In the present study, N addition increased the microbial biomass in the rhizosphere (Lv *et al*. 2017), whereas it had minimal effect on the microbial biomass in bulk soil (Fig. 6), suggesting that the microbial activity in the rhizosphere was sensitive to added N. Thus, the rhizospheric C1 fraction was likely decreased following the addition of N primarily because of an increased rate of carbohydrate decomposition. Decreased phenol oxidase activity following N addition, which lowered the decomposition rate of RC, likely contributed to the accumulation of the recalcitrant C (C4) fraction in the rhizosphere. Although the microbial biomass in bulk soil was less affected by N addition, it was also significantly stimulated by the addition of excessive N (i.e. in the N22.4 and N44.8 groups). The amount of organic C in the C1 and OC fractions was significantly higher in the rhizosphere than in bulk soil in the CK group, indicating that the availability of C substrates for soil microbes was low. The increased microbial activity in the bulk soil induced by N addition likely promoted RC decomposition to increase bioavailable C substrates for soil microbes and thus contributed to LC accumulation.

We also tested whether N addition altered the TOC proportions of the SOC fractions. The amount of organic C in the C1 and OC fractions in the rhizosphere decreased after the addition of low levels of N (i.e. in the N2.8, N5.6 and N11.2 groups), but increased in the N44.8 group, with minimum C1 and OC content observed in the N11.2 group. The amount of organic C found in the C4 fraction in the rhizosphere showed an opposite trend following N addition. This indicates that the addition of low levels of N (i.e. in the N2.8 and N5.6 groups) enhanced SOC allocation to recalcitrant rhizospheric C, whereas excessive N addition (i.e. in the N44.8 group) enhanced SOC allocation to labile rhizospheric C. This finding is in agreement with our hypotheses 1 and 2. In contrast, the addition of N had an opposite effect on the labile and recalcitrant C pools in bulk soil. These results suggest that rhizosphere soil is important for long-term soil C storage under low levels of N addition, whereas bulk soil plays a vital role in maintaining long-term soil C storage under excessive N addition. A previous study on temperate forest soil also confirmed that increased N addition significantly reduced SOM decomposition rates and soil lability (Rodriguez *et al*. 2014); these effects are partly consistent with the results of the present study. However, some studies in a subtropical monsoon climate found that N addition increased the LC pool in the soil (Chen *et al*. 2012*a*; Jiang *et al*. 2014). Thus, climate condition and soil quality affect how the lability of soils responds to increased N addition. Excessive N addition plays a totally opposite role in the allocation of soil LC and RC pools to that of low levels of N addition.

Most SOC fractions and TOC content were higher in the rhizosphere than in the bulk soil. The C4 fraction was higher in the bulk soil in the CK group. Approximately 40% of plant primary production is exuded by roots into the soil by rhizodeposition, which contributes to the high availability of soil C in the rhizosphere (Lynch and Whipps 1990). N is a common limiting nutrient in natural ecosystems (Vitousek and Howarth 1991). Some studies have suggested that low N availability can induce positive rhizospheric priming effects (Fontaine *et al*. 2011; Zhang and Wang 2012; Dijkstra *et al*. 2013), thus promoting the mineralisation of SOM for the provision of nutrients used for plant growth. In the CK group, the enhanced RC decomposition rate was induced by low
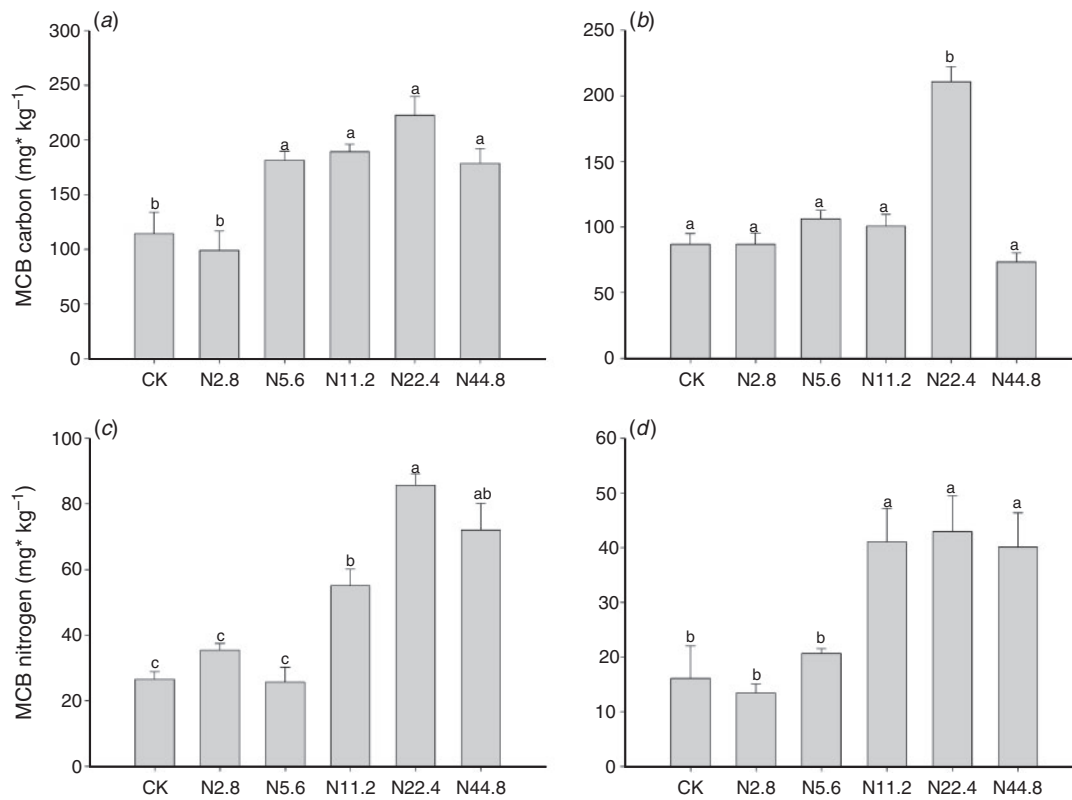
Fig. 6. (*a*, *b*) Microbial biomass (MCB) carbon and (*c*, *d*) MCB nitrogen content in the rhizosphere (*a*, *c*) and bulk soil (*b*, *d*) in the control (CK) group and treatment groups after N addition. N was added at rates of 2.8, 5.6, 11.2, 22.4 and 44.8 g m^{-2} year^{-1} (N2.8, N5.6, N11.2, N22.4 and N44.8 respectively). Data are the mean ± s.e.m. Different letters indicate significant differences between the treatment groups.

N availability, which offsets the effects of rhizodeposition on C accumulation and consequently decreases the C4 fraction in the rhizosphere.

TN content tended to increase with N addition in both the rhizosphere and bulk soils. This trend is in agreement with other studies (Ochoa-Hueso *et al.* 2013) that evaluated the effects of N addition on soil TN content. In the present study, excess N addition significantly increased soil TN content in the rhizosphere. The positive affects of N addition on the accumulation of soil N in the present study were probably associated with N limitation, which is common in these ecosystems, and with the ability of ectomycorrhizal fungi to acquire N and compete with free-living decomposers (Waldrop *et al.* 2004; Reid *et al.* 2012; Averill *et al.* 2014). In addition, TN content was significantly higher in the rhizosphere than in the bulk soil because of the increased plant C supply to the rhizosphere and subsequent enhanced microbial N immobilisation (Cheng *et al.* 2011; Laungani and Knops 2012).

The amount of organic C found in the C1, C4 and OC fractions differed significantly between the rhizosphere and bulk soils in the CK group, but not in the groups in which N was added. Increased amounts of added N weakened the rhizospheric effects on C1 and OC content (Fig. 3). The rhizosphere is a zone of high microbial activity in the vicinity of growing plants (Butler *et al.* 2003). Micro-organisms preferentially colonise the rhizosphere because root exudates

are a major source of nutrients in the soil, making the rhizosphere an area of intense activity with specific biological, chemical and physical characteristics (Lynch and Whipps 1990; Reynolds *et al.* 1998). N addition increased N availability and may have reduced the demand for roots to invest C in nutrient-absorbing systems, thereby reducing the allocation of C to root systems and the allocation of proportionally less C to belowground growth (Treseder 2008). The microbial composition of the soil changed with N addition because of the altered nutrient conditions (Treseder 2008; Liu and Greaver 2010). Furthermore, N addition suppressed microbial activities (Liu and Greaver 2010) that have a negative effect on rhizospheric priming effect (Kuzyakov *et al.* 2002). A previous study found that long-term addition of N fertilisers to a wheat–maize rotation field reduced the rhizospheric effect on most extracellular enzyme activities (Ai *et al.* 2012). N addition also induced lower rhizospheric effects on SOM decomposition (Liljeroth *et al.* 1994).

Our redundancy and correlation analyses showed that TOC and TN contents had more effect than the other parameters on the SOC fractions in the rhizospheric and bulk soils. TOC and TN had stronger effects on very labile C (C1) and labile C (C2) than on less labile C (C3) and recalcitrant C (C4) fractions. The most easily oxidised C fraction (C1) responded sensitively to environmental changes. This finding is consistent with the hypothesis that LC is more sensitive to management practices

(Blair *et al*. 1995; Maia *et al*. 2007; Yang and Kay 2001). Thus, the C1 fraction can be an indicator of changes, caused by atmospheric N deposition, in the quality of soil organic material.

Conclusions

The results of the present 6-year N-addition pot study reveal that the amount of organic C found in the C1 and OC fractions of the rhizosphere decreased at low levels of N addition (i.e. in the N2.8, N5.6, N11.2 groups) and increased at high levels (i.e. in the N44.8 group). Minimum C1 and OC content was observed in the N11.2 group. The amount of organic C found in the C1 and OC fractions of bulk soil exhibited an opposite response to N addition. This suggests that the SOC in the rhizosphere became more recalcitrant at low levels of N addition (i.e. in the N2.8 and N5.6 groups), but addition of high levels of N resulted in labile C accumulation that was less stable against chemical and biological degradation. The SOM in the bulk soil exhibited an opposite response to N addition. The rhizosphere and bulk soils had distinctly different chemical properties, but N addition weakened the rhizospheric effect. The results of the present study also indicate that the most easily oxidised C fraction (i.e. C1) was sensitive to environmental changes. These results can provide a theoretical basis for C dynamics and long-term C turnover in a changing global environment. Nevertheless, the microbial processes involved in C stabilisation and transposition to different C pools following the application of N require further investigation.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgements

References

Ai C, Liang G, Sun J, Wang X, Zhou W (2012*a*) Responses of extracellular enzyme activities and microbial community in both the rhizosphere and bulk soil to long-term fertilization practices in a fluvo-aquic soil. *Geoderma* **173–174**, 330–338. doi:10.1016/j.geoderma.2011.07.020

Andersson P, Berggren D, Johnsson L (2001) 30 years of N fertilisation in a forest ecosystem – the fate of added N and effects on N fluxes. *Water, Air, and Soil Pollution* **130**, 637–642. doi:10.1023/A:1013896410589

Averill C, Turner BL, Finzi AC (2014) Mycorrhiza-mediated competition between plants and decomposers drives soil carbon storage. *Nature* **505**, 543–545. doi:10.1038/nature12901

Blair GJ, Lefroy RDB, Lise L (1995) Soil carbon fractions based on their degree of oxidation, and the development of a carbon management index for agricultural systems. *Australian Journal of Agricultural Research* **46**, 1459–1466. doi:10.1071/AR9951459

Bowden RD, Davidson E, Savage K, Arabia C, Steudler P (2004) Chronic nitrogen additions reduce total soil respiration and microbial respiration in temperate forest soils at the Harvard Forest. *Forest Ecology and Management* **196**, 43–56. doi:10.1016/j.foreco.2004.03.011

Bremner JM, Mulvaney CS (1982) Nitrogen-total. Agronomy monograph 9. In 'Methods of soil analysis, part 2, chemical and microbial properties'. (Eds AL Page, RH Miller, DR Keeney) pp. 595–624. (Agronomy Society of America: Madison, WI)

Britton AJ, Helliwell RC, Fisher JM, Gibbs S (2008) Interactive effects of nitrogen deposition and fire on plant and soil chemistry in an alpine heathland. *Environmental Pollution* **156**, 409–416. doi:10.1016/j.envpol.2008.01.029

Butler JL, Williams MA, Bottomley PJ, Myrold DD (2003) Microbial community dynamics associated with rhizosphere carbon flow. *Applied and Environmental Microbiology* **69**, 6793–6800. doi:10.1128/AEM.69.11.6793-6800.2003

Carrillo Y, Pendall E, Dijkstra FA, Morgan JA, Newcomb JM (2011) Response of soil organic matter pools to elevated CO_2 and warming in a semi-arid grassland. *Plant and Soil* **347**, 339–350. doi:10.1007/s11104-011-0853-4

Chan KY, Bowman A, Oates A (2001) Oxidizible organic carbon fractions and soil quality changes in an Oxic Paleustalf under different pasture leys. *Soil Science* **166**, 61–67. doi:10.1097/00010694-200101000-00009

Chen MC, Wang MK, Chiu CY, Huang PM, King HB (2001) Determination of low molecular weight dicarboxylic acids and organic functional groups in rhizosphere and bulk soils of Tsuga and Yushania in a temperate rain forest. *Plant and Soil* **231**, 37–44. doi:10.1023/A:1010347421351

Chen X, Li Y, Mo J, Otieno D, Tenhunen J, Yan J, Liu J, Zhang D (2012*a*) Effects of nitrogen deposition on soil organic carbon fractions in the subtropical forest ecosystems of S China. *Journal of Plant Nutrition and Soil Science* **175**, 947–953. doi:10.1002/jpln.201100059

Chen X, Liu J, Deng Q, Yan J, Zhang D (2012*b*) Effects of elevated CO_2 and nitrogen addition on soil organic carbon fractions in a subtropical forest. *Plant and Soil* **357**, 25–34. doi:10.1007/s11104-012-1145-3

Cheng WX, Johnson DW, Fu SL (2003) Rhizosphere effects on decomposition: controls of plant species, phenology, and fertilization. *Soil Science Society of America Journal* **67**, 1418–1427. doi:10.2136/sssaj2003.1418

Cheng XL, Luo YQ, Su B, Wan SQ, Hui DF, Zhang QF (2011) Plant carbon substrate supply regulated soil nitrogen dynamics in a tallgrass prairie in the Great Plains, USA: results of a clipping and shading experiment. *Journal of Plant Ecology* **4**, 228–235. doi:10.1093/jpe/rtr024

Datta SP, Rattan RK, Chandra S (2010) Labile soil organic carbon, soil fertility, and crop productivity as influenced by manure and mineral fertilizers in the tropics. *Journal of Plant Nutrition and Soil Science* **173**, 715–726. doi:10.1002/jpln.200900010

DeForest JL, Zak DR, Pregitzer KS, Burton AJ (2004) Atmospheric nitrate deposition, microbial community composition, and enzyme activity in northern hardwood forests. *Soil Science Society of America Journal* **68**, 132–138. doi:10.2136/sssaj2004.1320

Dennis PG, Miller AJ, Hirsch PR (2010) Are root exudates more important than other sources of rhizodeposits in structuring rhizosphere bacterial communities? *FEMS Microbiology Ecology* **72**, 313–327. doi:10.1111/j.1574-6941.2010.00860.x

Dijkstra FA, Carrillo Y, Pendall E, Morgan JA (2013) Rhizosphere priming: a nutrient perspective. *Frontiers in Microbiology* **4**, 216. doi:10.3389/fmicb.2013.00216

Emmett BA, Reynolds B, Silgram M, Sparks TH, Woods C (1998) The consequences of chronic nitrogen additions on N cycling and soilwater chemistry in a Sitka spruce stand, North Wales. *Forest Ecology and Management* **101**, 165–175. doi:10.1016/S0378-1127(97)00133-3

Fang YT, Yoh M, Koba K, Zhu W, Takebayashi Y, Xiao Y, Lei C, Mo J, Zhang W, Lu X (2011) Nitrogen deposition and forest nitrogen cycling along an urban–rural transect in southern China. *Global Change Biology* **17**, 872–885. doi:10.1111/j.1365-2486.2010.02283.x

Fenn ME, Poth MA, Aber JD, Baron JS, Bormann BT, Johnson DW, Lemly AD, McNulty SG, Ryan DF, Stottlemyer R (1998) Nitrogen excess in North American ecosystems: predisposing factors, ecosystem responses, and management strategies. *Ecological Applications* **8**, 706–733. doi:10.1890/1051-0761(1998)008[0706:NEINAE]2.0.CO;2

Fenn ME, Baron JS, Allen EB, Rueth HM, Nydick KR, Geiser L, Bowman WD, Sickman JO, Meixner T, Johnson DW, Neitlich P (2003) Ecological effects of nitrogen deposition in the western United States. *Bioscience* **53**, 404–420. doi:10.1641/0006-3568(2003)053[0404:EEONDI]2.0.CO;2

Fontaine S, Henault C, Aamor A, Bdioui N, Bloor JMG, Maire V, Mary B, Revaillot S, Maron PA (2011) Fungi mediate long term sequestration of carbon and nitrogen in soil through their priming effect. *Soil Biology & Biochemistry* **43**, 86–96. doi:10.1016/j.soilbio.2010.09.017

Frey SD, Knorr M, Parrent JL, Simpson RT (2004) Chronic nitrogen enrichment affects the structure and function of the soil microbial community in temperate hardwood and pine forests. *Forest Ecology and Management* **196**, 159–171. doi:10.1016/j.foreco.2004.03.018

Galloway JN, Dentener FJ, Capone DG, Boyer EW, Howarth RW, Seitzinger SP, Asner GP, Cleveland CC, Green PA, Holland EA, Karl DM, Michaels AF, Porter JH, Townsend AR, Vöosmarty CJ (2004) Nitrogen cycles: past, present, and future. *Biogeochemistry* **70**, 153–226. doi:10.1007/s10533-004-0370-0

Galloway JN, Townsend AR, Erisman JW, Bekunda M, Cai ZC, Freney JR, Martinelli LA, Seitzinger SP, Sutton MA (2008) Transformation of the nitrogen cycle: Recent trends, questions, and potential solutions. *Science* **320**, 889–892. doi:10.1126/science.1136674

Grayston SJ, Vaughan D, Jones D (1997) Rhizosphere carbon flow in trees, in comparison with annual plants: the importance of root exudation and its impact on microbial activity and nutrient availability. *Applied Soil Ecology* **5**, 29–56. doi:10.1016/S0929-1393(96)00126-6

Griffiths BS (1994) Microbial-feeding nematodes and protozoa in soil – their effects on microbial activity and nitrogen mineralization in decomposition hotspots and the rhizosphere. *Plant and Soil* **164**, 25–33. doi:10.1007/BF00010107

Gruber N, Galloway JN (2008) An Earth-system perspective of the global nitrogen cycle. *Nature* **451**, 293–296. doi:10.1038/nature06592

Hagedorn F, Kammer A, Schmidt MWI, Goodale CL (2012) Nitrogen addition alters mineralization dynamics of ^{13}C-depleted leaf and twig litter and reduces leaching of older DOC from mineral soil. *Global Change Biology* **18**, 1412–1427. doi:10.1111/j.1365-2486.2011.02603.x

Han XW, Tsunekawa A, Tsubo M, Li SQ (2011) Aboveground biomass response to increasing nitrogen deposition on grassland on the northern Loess Plateau of China. *Acta Agriculturae Scandinavica, Section B – Soil & Plant Science* **61**, 112–121. doi:10.1080/09064710903544201

Hinsinger P, Bengough AG, Vetterlein D, Young IM (2009) Rhizosphere: biophysics, biogeochemistry and ecological relevance. *Plant and Soil* **321**, 117–152. doi:10.1007/s11104-008-9885-9

Hobbie JE, Hobbie EA (2006) N-15 in symbiotic fungi and plants estimates nitrogen and carbon flux rates in Arctic tundra. *Ecology* **87**, 816–822. doi:10.1890/0012-9658(2006)87[816:NISFAP]2.0.CO;2

Hobbie SE, Eddy WC, Buyarski CR, Adair EC, Ogdahl ML, Weisenhorn P (2012) Response of decomposing litter and its microbial community to multiple forms of nitrogen enrichment. *Ecological Monographs* **82**, 389–405. doi:10.1890/11-1600.1

Janzen H (1987) Soil organic matter characteristics after long-term cropping to various spring wheat rotations. *Canadian Journal of Soil Science* **67**, 845–856. doi:10.4141/cjss87-081

Jiang XY, Cao LX, Zhang RD (2014) Changes of labile and recalcitrant carbon pools under nitrogen addition in a city lawn soil. *Journal of Soils and Sediments* **14**, 515–524. doi:10.1007/s11368-013-0822-z

Jones DL, Nguyen C, Finlay RD (2009) Carbon flow in the rhizosphere: carbon trading at the soil–root interface. *Plant and Soil* **321**, 5–33. doi:10.1007/s11104-009-9925-0

Kuzyakov Y, Biryukova OV, Kuznetzova TV, Molter K, Kandeler E, Stahr K (2002) Carbon partitioning in plant and soil, carbon dioxide fluxes and enzyme activities as affected by cutting ryegrass. *Biology and Fertility of Soils* **35**, 348–358. doi:10.1007/s00374-002-0480-6

Laungani R, Knops JMH (2012) Microbial immobilization drives nitrogen cycling differences among plant species. *Oikos* **121**, 1840–1848. doi:10.1111/j.1600-0706.2011.20434.x

Liljeroth E, Kuikman P, Vanveen JA (1994) Carbon translocation to the rhizosphere of maize and wheat and influence on the turnover of native soil organic-matter at different soil-nitrogen levels. *Plant and Soil* **161**, 233–240. doi:10.1007/BF00046394

Liu LL, Greaver TL (2010) A global perspective on belowground carbon dynamics under nitrogen enrichment. *Ecology Letters* **13**, 819–828. doi:10.1111/j.1461-0248.2010.01482.x

Lovett GM, Arthur MA, Weathers KC, Fitzhugh RD, Templer PH (2013) Nitrogen addition increases carbon storage in soils, but not in trees, in an eastern US deciduous forest. *Ecosystems* **16**, 980–1001. doi:10.1007/s10021-013-9662-3

Lu XK, Mo JM, Gilliam FS, Zhou GY, Fang YT (2010) Effects of experimental nitrogen additions on plant diversity in an old-growth tropical forest. *Global Change Biology* **16**, 2688–2700. doi:10.1111/j.1365-2486.2010.02174.x

Lv F, Xue S, Wang G, Zhang C (2017) Nitrogen addition shifts the microbial community in the rhizosphere of *Pinus tabuliformis* in northwestern China. *PLoS One* **12**, e0172382. doi:10.1371/journal.pone.0172382

Lynch JM, Whipps JM (1990) Substrate flow in the rhizosphere. *Plant and Soil* **129**, 1–10. doi:10.1007/BF00011685

Mack MC, Schuur EAG, Bret-Harte MS, Shaver GR, Chapin FS (2004) Ecosystem carbon storage in arctic tundra reduced by long-term nutrient fertilization. *Nature* **431**, 440–443. doi:10.1038/nature02887

Maia SMF, Xavier FAS, Oliveira TS, Mendonça ES, Araújo Filho JA (2007) Organic carbon pools in a Luvisol under agroforestry and conventional farming systems in the semi-arid region of Ceará, Brazil. *Agroforestry Systems* **71**, 127–138. doi:10.1007/s10457-007-9063-8

Nadelhoffer KJ, Downs MR, Fry B (1999) Sinks for N-15-enriched additions to an oak forest and a red pine plantation. *Ecological Applications* **9**, 72–86. doi:10.1890/1051-0761(1999)009[0072:SFNEAT]2.0.CO;2

Neff JC, Townsend AR, Gleixner G, Lehman SJ, Turnbull J, Bowman WD (2002) Variable effects of nitrogen additions on the stability and turnover of soil carbon. *Nature* **419**, 915–917. doi:10.1038/nature01136

Nelson DW, Sommers LE, Sparks D, Page A, Helmke P, Loeppert R, Soltanpour P, Tabatabai M, Johnston C, Sumner M (1996) Total carbon, organic carbon, and organic matter. Methods of soil analysis Part 3 (Eds Nelson DW and Sommers LE)-chemical methods: 961-1010 (Soil Science Society of America and American Society of Agronomy: Madison, WI).

Ochoa-Hueso R, Stevens CJ, Ortiz-Llorente MJ, Manrique E (2013) Soil chemistry and fertility alterations in response to N application in a semiarid Mediterranean shrubland. *The Science of the Total Environment* **452–453**, 78–86. doi:10.1016/j.scitotenv.2013.02.049

Phillips RP, Fahey TJ (2008) The influence of soil fertility on rhizosphere effects in northern hardwood forest soils. *Soil Science Society of America Journal* **72**, 453–461. doi:10.2136/sssaj2006.0389

Phoenix GK, Emmett BA, Britton AJ, Caporn SJM, Dise NB, Helliwell R, Jones L, Leake JR, Leith ID, Sheppard LJ, Sowerby A, Pilkington MG, Rowe EC, Ashmore MR, Power SA (2012) Impacts of atmospheric nitrogen deposition: responses of multiple plant and soil parameters across contrasting ecosystems in long-term field experiments. *Global Change Biology* **18**, 1197–1215. doi:10.1111/j.1365-2486.2011.02590.x

Puglisi E, Fragoulis G, Del Re AAM, Spaccini R, Piccolo A, Gigliotti G, Said-Pullicino D, Trevisan M (2008) Carbon deposition in soil rhizosphere following amendments with compost and its soluble fractions, as evaluated by combined soil–plant rhizobox and reporter gene systems. *Chemosphere* **73**, 1292–1299. doi:10.1016/j.chemosphere.2008.07.008

Read DJ, Perez-Moreno J (2003) Mycorrhizas and nutrient cycling in ecosystems – a journey towards relevance? *New Phytologist* **157**, 475–492. doi:10.1046/j.1469-8137.2003.00704.x

Reay DS, Dentener F, Smith P, Grace J, Feely RA (2008) Global nitrogen deposition and carbon sinks. *Nature Geoscience* **1**, 430–437. doi:10.1038/ngeo230

Reid JP, Adair EC, Hobbie SE, Reich PB (2012) Biodiversity, nitrogen deposition, and CO_2 affect grassland soil carbon cycling but not storage. *Ecosystems* **15**, 580–590. doi:10.1007/s10021-012-9532-4

Reynolds B, Wilson EJ, Emmett BA (1998) Evaluating critical loads of nutrient nitrogen and acidity for terrestrial systems using ecosystem-scale experiments (NITREX). *Forest Ecology and Management* **101**, 81–94. doi:10.1016/S0378-1127(97)00127-8

Rodriguez A, Lovett GM, Weathers KC, Arthur MA, Templer PH, Goodale CL, Christenson LM (2014) Lability of C in temperate forest soils: assessing the role of nitrogen addition and tree species composition. *Soil Biology & Biochemistry* **77**, 129–140. doi:10.1016/j.soilbio.2014.06.025

Schlesinger WH (2009) On the fate of anthropogenic nitrogen. *Proceedings of the National Academy of Sciences of the United States of America* **106**, 203–208. doi:10.1073/pnas.0810193105

Sherrod L, Peterson G, Westfall D, Ahuja L (2005) Soil organic carbon pools after 12 years in no-till dryland agroecosystems. *Soil Science Society of America Journal* **69**, 1600–1608. doi:10.2136/sssaj2003.0266

Song CC, Liu DY, Song YY, Mao R (2013) Effect of nitrogen addition on soil organic carbon in freshwater marsh of northeast China. *Environmental Earth Sciences* **70**, 1653–1659. doi:10.1007/s12665-013-2252-z

Treseder KK (2008) Nitrogen additions and microbial biomass: a meta-analysis of ecosystem studies. *Ecology Letters* **11**, 1111–1120. doi:10.1111/j.1461-0248.2008.01230.x

Vitousek PM, Howarth RW (1991) Nitrogen limitation on land and in the sea: how can it occur? *Biogeochemistry* **13**, 87–115. doi:10.1007/BF00002772

Vitousek P, Matson P (1991) 'Gradient analysis of ecosystems.' (Springer: New York, NY)

Vitousek PM, Aber JD, Howarth RW, Likens GE, Matson PA, Schindler DW, Schlesinger WH, Tilman D (1997) Human alteration of the global nitrogen cycle: sources and consequences. *Ecological Applications* **7**, 737–750.

Waldrop MP, Zak DR, Sinsabaugh RL, Gallo M, Lauber C (2004) Nitrogen deposition modifies soil carbon storage through changes in microbial enzymatic activity. *Ecological Applications* **14**, 1172–1177. doi:10.1890/03-5120

Walkley A, Black I (1934) An examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. *Soil Science* **37**, 29–38.

Whittinghill KA, Currie WS, Zak DR, Burton AJ, Pregitzer KS (2012) Anthropogenic N deposition increases soil C storage by decreasing the extent of litter decay: analysis of field observations with an ecosystem model. *Ecosystems* **15**, 450–461. doi:10.1007/s10021-012-9521-7

Yang XM, Kay BD (2001) Impacts of tillage practices on total, loose- and occluded-particulate, and humified organic carbon fractions in soils within a field in southern Ontario. *Canadian Journal of Soil Science* **81**, 149–156. doi:10.4141/S00-015

Yang Y, Guo J, Chen G, Yin Y, Gao R, Lin C (2009) Effects of forest conversion on soil labile organic carbon fractions and aggregate stability in subtropical China. *Plant and Soil* **323**, 153–162. doi:10.1007/s11104-009-9921-4

Yeomans J, Bremner JM (1988) A rapid and precise method for routine determination of organic carbon in soil 1. *Communications in Soil Science and Plant Analysis* **19**, 1467–1476. doi:10.1080/00103628809368027

Zak DR, Holmes WE, Burton AJ, Pregitzer KS, Talhelm AF (2008) Simulated atmospheric NO_3^- deposition increases soil organic matter by slowing decomposition. *Ecological Applications* **18**, 2016–2027. doi:10.1890/07-1743.1

Zeng DH, Li LJ, Fahey TJ, Yu ZY, Fan ZP, Chen FS (2010) Effects of nitrogen addition on vegetation and ecosystem carbon in a semi-arid grassland. *Biogeochemistry* **98**, 185–193. doi:10.1007/s10533-009-9385-x

Zhang WD, Wang SL (2012) Effects of NH_4^+ and NO_3^- on litter and soil organic carbon decomposition in a Chinese fir plantation forest in South China. *Soil Biology & Biochemistry* **47**, 116–122. doi:10.1016/j.soilbio.2011.12.004

Zhang DQ, Hui DF, Luo YQ, Zhou GY (2008) Rates of litter decomposition in terrestrial ecosystems: global patterns and controlling factors. *Journal of Plant Ecology* **1**, 85–93. doi:10.1093/jpe/rtn002

Zhu B, Gutknecht JLM, Herman DJ, Keck DC, Firestone MK, Cheng W (2014) Rhizosphere priming effects on soil carbon and nitrogen mineralization. *Soil Biology & Biochemistry* **76**, 183–192. doi:10.1016/j.soilbio.2014.04.033

Zoysa AKN, Loganathan P, Hedley MJ (1999) Phosphorus utilisation efficiency and depletion of phosphate fractions in the rhizosphere of three tea (*Camellia sinensis* L.) clones. *Nutrient Cycling in Agroecosystems* **53**, 189–201. doi:10.1023/A:1009706508627