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Plant carbon substrate supply regulated soil nitrogen dynamics in a tallgrass prairie in the Great Plains, USA: results of a clipping and shading experiment

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Abstract

Aims
Land use management affects plant carbon (C) supply and soil environments and hence alters soil nitrogen (N) dynamics, with consequent feedbacks to terrestrial ecosystem productivity. The objective of this study was to better identify mechanisms by which land-use management (clipping and shading) regulates soil N in a tallgrass prairie, OK, USA.

Methods
We conducted 1-year clipping and shading experiment to investigate the effects of changes in land-use management (soil microclimates, plant C substrate supply and microbial activity) on soil inorganic N ($NH_4^+ - N$ and $NO_3^- - N$), net N mineralization and nitrification in a tallgrass prairie.

Important Findings
Land-use management through clipping and/or shading significantly increased annual mean inorganic N, possibly due to lowered plant N uptake and decreased microbial N immobilization into biomass growth. Shading significantly increased annual mean mineralization rates ($P < 0.05$). Clipping slightly decreased annual mean N nitrification rates whereas shading significantly increased annual mean N nitrification rates. Soil microclimate significantly explained 36% of the variation in $NO_3^- - N$ concentrations ($P = 0.004$). However, soil respiration, a predictor of plant C substrate supply and microbial activity, was negatively correlated with $NH_4^+ - N$ concentrations ($P = 0.0009$), net N mineralization ($P = 0.0037$) and nitrification rates ($P = 0.0028$) across treatments. Our results suggest that change in C substrate supply and microbial activity under clipping and/or shading is a critical control on $NH_4^+ - N$, net N mineralization and nitrification rates, whereas clipping and shading-induced soil microclimate change can be important for $NO_3^- - N$ variation in the tallgrass prairie.

Keywords: plant C substrate supply • N mineralization • N nitrification • soil microclimate • soil respiration

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INTRODUCTION

Nitrogen (N) is a major limiting nutrient for primary productivity in terrestrial ecosystems (e.g. LeBauer and Treseder 2008; Vitousek and Howarth 1991). Numerous studies have been done to relate soil N dynamics to terrestrial productivity and environmental changes (e.g. Booth *et al.* 2005; Cookson *et al.* 2006; Craine *et al.* 2002). Conversely, soil N dynamics

are also influenced by plant function and environmental changes (Carrera *et al.* 2009; Updegraff *et al.* 1995). For example, plant C substrate inputs to the soil are a primary resource of microbial growth (Magill and Aber 2000; Zak *et al.* 1994). Meanwhile, soil N transformations (i.e. mineralization and nitrification) are microbial processes often limited by C substrate and soil temperature and moisture (Aranibar *et al.* 2004; Zak *et al.* 1994; Theodose and Martin 2003). Thus, any change in available C substrates and soil microclimates through changes in land-use management may substantially affect soil N dynamics by influencing microbial activity.

Here we focus on the consequences of land-use management for soil N dynamics related to change in plant carbon substrate supply and soil microclimates. Soil N dynamics processes are strongly regulated by the interactions of biotic (e.g. plant C substrate supply) and abiotic (e.g. soil temperature and moisture) factors (Binkley and Giardina 1998; Schimel and Bennett 2004). For instance, plant can affect net N mineralization and nitrification rates primarily by altering plant labile C input, plant N uptake and microbial N immobilization and activity (e.g. Johnson and Matchett, 2001; Magill and Aber 2000; Reynolds and Hunter 2001; Schaeffer *et al.* 2003). Although several studies have demonstrated that soil C substrate plays a crucial and even more important role in controlling soil N dynamics than soil microclimate (Nadelhoffer *et al.* 1991; Updegraff *et al.* 1995), soil temperature and moisture are closely associated with both spatial and temporal variations in soil N cycling processes (e.g. Aranibar *et al.* 2004; Dijkstra *et al.* 2008; Theodose and Martin 2003). Theodose and Martin (2003) have, e.g. reported that microclimate is a more significant predictor of N dynamics during growing season compared with C substrate. Meanwhile, Dijkstra *et al.* (2008) have demonstrated that soil moisture under elevated CO_2 plays a key role in N mineralization in semi-arid grassland ecosystems. Yet plant C substrates that influence soil N dynamics can differently interact with microclimate among many terrestrial ecosystems (Booth *et al.* 2005). Thus, understanding the response of soil N dynamics to change in land-use management is critical for accurate prediction of long-term ecosystem N cycling in future global change.

Land-use management has great impact on soil C substrate and then can potentially affect soil N dynamics (e.g. McKinley and Blair 2008; Pocewicz *et al.* 2006). Land use practices through biomass removal can affect plant C substrate input to soil and soil microclimates (Belay-Tedla *et al.*, 2009; Klein *et al.* 2005; Wan *et al.* 2002). Thus, clipping for hay harvest, a major agricultural land use practice in the Southern Great Plains of the USA, can affect soil N dynamics primarily through altering soil microclimate and plant C substrate input to soil (Belay-Tedla *et al.*, 2009; Wan and Luo, 2003). For example, clipping significantly decreases soil labile C and N which potentially affects soil microbial N contents (Belay-Tedla *et al.*, 2009) and reduces soil respiration. Other studies have reported that land-use change by grazing or fire affect ecosystem N cycles in ecosystems by changing plant C substrate input, root growth and soil environments (Bardgett and Wardle 2003; Johnson and Matchett 2001). Moreover, shading, another pathway of reducing labile C (photosynthates) supply to the ecosystem, can alter soil N dynamics by lowering root N uptake and microbial activity (Jonasson *et al.* 1999; Wan and Luo 2003). Thus, the interaction of clipping and shading can affect soil N dynamics by reducing plant C substrate input, altering microbial activity and soil microclimate. Although a few studies have reported the impacts of biomass removal by grazing on soil N dynamics (e.g. Holland and Detling 1990; Johnson and Matchett 2001), little experimental evidence exists on how clipping and shading interactively affect C substrate supply, which in turn regulate soil N dynamics under controlled field conditions.

In this study, we conducted a 1-year clipping and shading experiment in a tallgrass prairie to examine the interactive regulation of plant C substrate supply, microbial activity and soil microclimates on N dynamics. The field manipulative experiment was also conducted to examine effects of reduced plant C substrates on soil respiration (Wan and Luo 2003). Wan and Luo (2003) have reported that both clipping and shading primarily reduce photosynthates to roots and rhizosphere microbes, clipping also cuts off labile C to microbes from aboveground litter. Thus, clipping and shading suppress rhizosphere respiration and microbial respiration from aboveground litter (Wan and Luo 2003). A similar relationship between photosynthates and soil respiration has been observed by tree girdling in forest ecosystem (Högberg *et al.* 2001; Scott-Denton *et al.* 2006). Moreover, previous studies have correlated soil respiration to soil net N mineralization and nitrification rates under changing C substrate conditions (e.g. Johnson and Matchett 2001; Rhoades and Coleman 1999). Thus, we used rhizosphere respiration plus microbial respiration from aboveground litter data obtained by Wan and Luo (2003) as a predictor of plant C substrate supply and microbial activity in this study. The specific objective of this work was to test the following hypotheses: (i) clipping and/or shading increase inorganic N concentrations by limiting plant N uptake, microbial biomass growth and microbial N immobilization; (ii) clipping and/or shading increase net N mineralization and nitrification rates by decreasing microbial N immobilization; and (iii) soil respiration, a predictor of plant C substrate supply and microbial activity, could be more important than soil microclimate in regulating soil N dynamics.

MATERIALS AND METHODS

Site description and experimental design

This study was conducted at a tallgrass prairie ecosystem, 3 km east of the Norman campus of the University of Oklahoma (35.2°N, 97.4°W). The climate is characterized by an annual precipitation of 911.4 mm (Oklahoma Meteorological Survey) and annual mean temperature of 16.0°C with monthly mean temperature of 3.1°C in January and 28.0°C in July. The soil type is Vernon clay loam. The dominant plant species in study area are *Panicum virgatum*, *Schizachyrium scoparium*, *Andropogon*

gerardii, Sorghastrum nutans, Ambrosia psilostachyia, Xanthocephalum texanum, Bromus japonicus, and *Eragrostis* spp.

A randomized complete block was selected on 21 June 2001with four treatments (details given in Wan and Luo 2003): (i) control (C); (ii) clipping (CL), we completely removed the aboveground live and dead plant biomass (including surface litter) to the soil surface and cut the re-growth once a week; (iii) shading (S), we used double layer black shade cloth to wrap a wood frame (1.2 × 1.2 × 1 m) over the 1 × 1-m plot; and (iv) clipping plus shading (CL + S). Each treatment replicated five times. Plot size was 1 × 1 m^2, and the interval distance among plots was 1.5 m.

Field measurements

Soil net N mineralization and nitrification rates were measured six times from July 2001 to July 2002. Namely, these six incubations were from 04 July 2001 to 19 July 2001, 19 July 2001 to 28 August 2001, 28 August 2001 to 18 November 2001, 18 November 2001 to 18 January 2002, 18 January 2002 to 04 June 2002 and 04 June 2002 to 19 July 2002, respectively. Soil net N mineralization and nitrification rates were measured using the Polyvinyl chloride (PVC) tube closed top sequential incubation method modified from Raison *et al.* (1987). Soil incubation chambers consisted of 4 cm in inner diameter PVC tubes driven into the top 15 cm of mineral soil. Within each tube, changes in inorganic-N content during incubation period represent net N mineralized from organic source (Rhoades and Coleman 1999). The initial and final soil $NO_3^- - N$ and $NH_4^+ - N$ concentration for each incubation period were extracted with 1 M KCl solution and quantified using the cadmium reduction method.

$$\text{Net N mineralization/nitrification rate} = (N_1 - N_0)/d$$

where d was the number of incubation days. For net mineralization rate, N_1 was the final total inorganic N concentration ($NH_4^+ + NO_3^- - N$, N_0 was the initial total inorganic N concentration ($NH_4^+ - N + NO_3^- - N$. For net nitrification rate, N_1 was the final $NO_3^- - N$ concentration and N_0 was the initial $NO_3^- - N$ concentration.

Soil respiration was measured twice a month with an LI-6400 portable photosynthesis system (LI-COR Inc., Lincoln, NE, USA) attached with an LI-6400-09 soil CO_2 flux chamber (See Wan and Luo 2003). The rhizosphere respiration plus microbial respiration from aboveground litter accounted for 44% of total soil respiration (Wan and Luo 2003). The soil respiration (refer to rhizosphere respiration plus microbial respiration from aboveground litter in this present study) data of each N incubation period obtained by Wan and Luo (2003) were used as a proxy for plant C substrate availability as mentioned in the introduction. Soil temperature was measured using a thermocouple connected to the LI-6400 at the 0- to 5-cm soil layer as soil respiration was being measured. Soil moisture (% volumetric) at the 0- to 15-cm layer was measured twice a month using time domain reflectometry.

The soil samples collected in July 2002, shortly after the completion of clipping and shading experiment, were measured for labile nutrients and microbial biomass C and N. Labile C and N pools were determined using the two-step acid hydrolysis method of Oades *et al.* (1970). Microbial biomass C and N was measured using chloroform ($CHCL_3$) fumigation–extraction (Brookes *et al.* 1985; Vance *et al.* 1987). C and N in fumigated and non-fumigated soil samples were extracted by 0.5 M K_2SO_4 and the differences in extractable C and N between the fumigated and non-fumigated soils were converted to soil microbial biomass C and N using extraction factors of 2.64 and 1.46, respectively (Brookes *et al.* 1985; Vance *et al.* 1987).

Statistical analysis

Before analysis of variance, the data sets were checked for normality and log or cube root transformed to meet the assumptions for statistical analysis. The data analyses were performed using SAS software package (SAS Institute Inc., NC, USA). Statistical significance of treatment effects was evaluated with repeated-measures ANOVA, where multiple measurements on a given treatment through time represented the repeated variables. Stepwise regressions were carried out to correlate soil inorganic N, net N mineralization and nitrification rates with soil microclimate and/or soil respiration. Simple regression was further performed to correlate soil inorganic N, net N mineralization and nitrification rates with soil respiration.

RESULTS

Soil microclimates and general soil properties

Seasonal soil temperature and moisture was altered by clipping and shading. Overall, clipping did not significantly change annual mean soil temperature, whereas shading and clipping plus shading significantly decreased soil temperature by 0.9 and 0.7°C, respectively. Clipping slightly reduced annual mean soil moisture, whereas shading and clipping plus shading significantly increased annual mean soil moisture (Wan and Luo, 2003).

Treatments decreased labile C and N pools without statistical difference (Table 1, $P > 0.05$). However, clipping significantly decreased microbial biomass C and N content ($P < 0.05$), and shading and clipping plus shading slightly decreased microbial biomass C and N content (Table 1).

Inorganic N concentrations, net N mineralization and nitrification

Soil inorganic N concentrations exhibited clear seasonal variations across treatments (Fig. 1a and b). In general, soil inorganic N concentrations were lower in growing season than those in non-growing season (Fig. 1a and b). There were significant treatment effects on annual mean inorganic N concentrations (Fig. 1c and d, $NH_4^+ - N$: $P = 0.0187$, $NO_3^- - N$: $P = 0.0028$). Clipping significantly increased $NH_4^+ - N$ concentration by averaged 15.2% ($P < 0.05$), shading slightly

increased $NH_4^+ - N$ concentration, whereas clipping plus shading slightly decreased $NH_4^+ - N$ concentration (Fig. 1c). Clipping, shading and clipping plus shading significantly increased $NO_3^- - N$ concentrations by averaged 78.4, 37.3 and 42.7%, respectively (Fig. 1d, $P < 0.05$). As a result, clipping and shading significantly increased the total inorganic N concentrations by averaged 29.1 and 19.3%, respectively (Fig. 1c and d, $P = 0.0008$).

Similarly, there were clear seasonal variations in net N mineralization and nitrification rates and treatment effects during incubation periods (Fig. 2a and b). Significant treatment effects were also observed in annual mean net N mineralization and nitrification rates (Fig. 2c and d, N mineralization: $P < 0.0001$, nitrification: $P = 0.0019$). Clipping, shading and clipping plus shading increased annual mean net N mineralization, but only shading effect was statistically significant ($P < 0.05$; Fig. 2c). Clipping slightly decreased annual mean nitrification rates, whereas shading significantly increased annual mean nitrification rates (Fig. 2d, $P < 0.01$).

Factors affecting the seasonal changes of soil N dynamics

Soil microclimate accounted for 18% of the variation in $NH_4^+ - N$ concentrations ($P = 0.09$), but significantly explained 36% of the variation in $NO_3^- - N$ concentrations ($P = 0.004$) across all treatments and dates (Table 2). Soil microclimate did not show significant relationships with net N mineralization and nitrification rates. However, after combining soil microclimate with soil respiration in the multiple regression analysis, soil T, M and R together accounted for 45, 37, 37 and 38% of the variations in $NH_4^+ - N$, $NO_3^- - N$, net N mineralization and nitrification rates, respectively ($P < 0.05$, Table 2). Furthermore, $NH_4^+ - N$ concentrations decreased exponentially as soil respiration increased ($P = 0.0009$, Fig. 3a), but $NO_3^- - N$ concentration did not show significant relationship with soil respiration ($P > 0.05$, Fig. 3b). Both net N mineralization and nitrification rates decreased linearly with increasing soil respiration ($P = 0.0037$, $P = 0.0028$, Fig. 3c and d).

Table 1: soil chemical properties under four treatments at the end of the experiment

Treatments	Control	Clipping	Shading	Clipping + shading
Labile C (mg kg^{-1})	3797 ± 292^a	3409 ± 184^a	3232 ± 93^a	3410 ± 179^a
Labile N (mg kg^{-1})	154 ± 12^a	143 ± 9^a	143 ± 10^a	141 ± 10^a
Microbial biomass C (mg kg^{-1})	551 ± 77^a	365 ± 64^b	476 ± 82^{ab}	399 ± 100^{ab}
Microbial biomass N (mg kg^{-1})	150 ± 12^a	98 ± 6^b	125 ± 9^{ab}	112 ± 11^{ab}

Values are mean ($n = 5$) with standard error. Different superscript letters indicate significant differences among treatments (one-way ANOVA, $P < 0.05$).

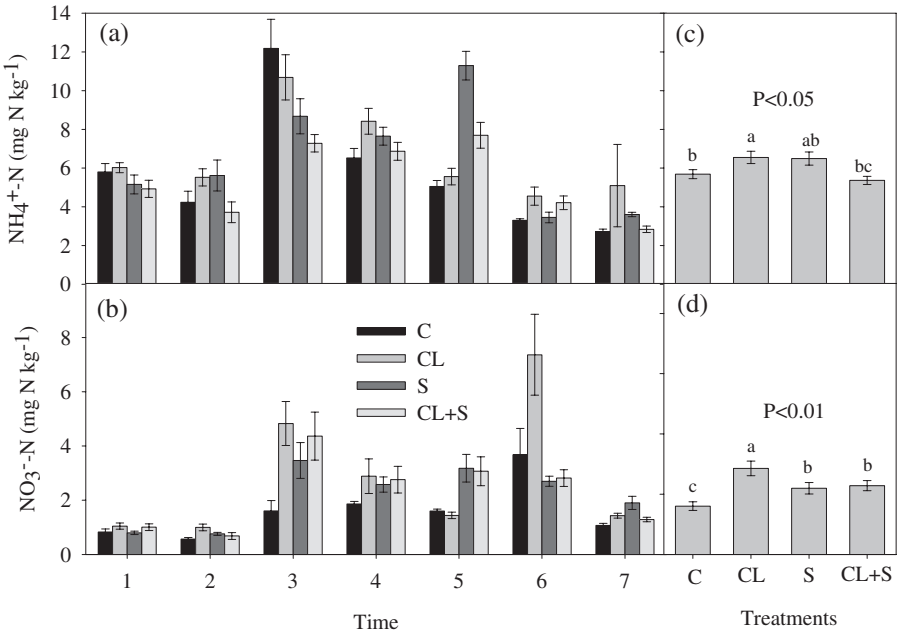

Figure 1: seasonal variations of $NH_4^+ - N$ and $NO_3^- - N$ concentrations (**a**, **b**), and annual averages of $NH_4^+ - N$ and $NO_3^- - N$ concentrations (**c**, **d**) (mean ± SE) under four treatments (C = control; CL = clipping; S = shading; CL + S = clipping + shading). Different letters over the bars (c, d) indicate statistically significant differences at $\alpha = 0.05$ level between treatments. Notes: Data: 1 = 4 July 2001; 2 = 19 July 2001; 3 = 28 August 2001; 4 = 18 November 2001; 5 = 18 January 2002; 6 = 4 June 2002; 7 = 19 July 2002.

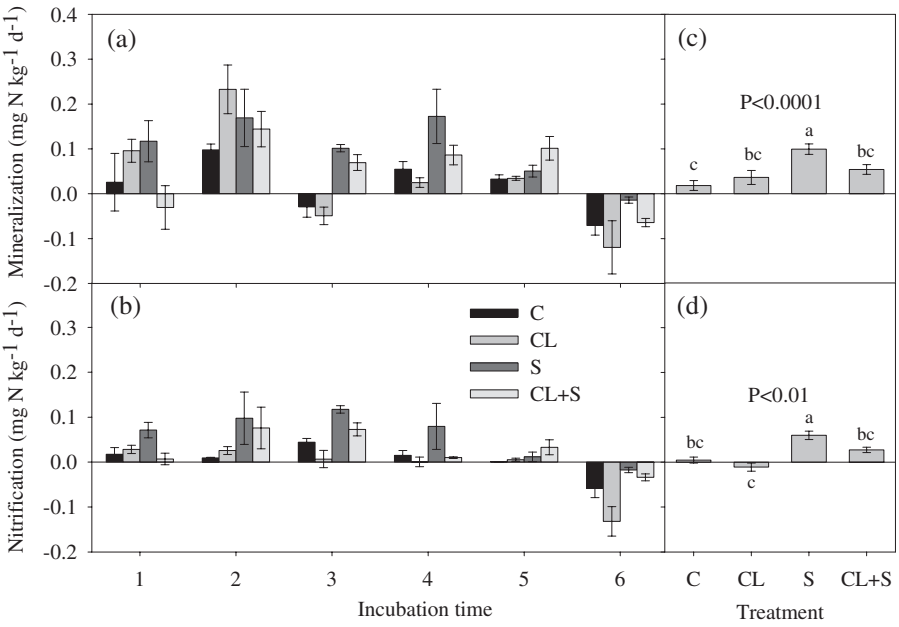

Figure 2: seasonal variations of net N mineralization and nitrification rates (**a**, **b**) and annual averages of net N mineralization and nitrification rates (**c**, **d**) under four treatments (mean ± SE). Different letters over the bars (c, d) indicate statistically significant differences at $\alpha = 0.05$ level between treatments. Notes: Incubation periods: 1 = 4 July to 19 July 2001; 2 = 19 July to 28 August 2001; 3 = 28 August to 18 November 2001; 4 = 18 November 2001 to 18 January 2002; 5 = 18 January to 4 June 2002; 6 = 4 June to 19 July 2002. See Fig. 1 for abbreviations.

Table 2: stepwise regression analysis of inorganic N concentrations, net N mineralization and nitrification rates against soil temperature (T, °C), moisture (M, %) and/or soil respiration (R, μmol m^{-2} s^{-1}) across the treatments

	Regression analysis with T and M			Regression analysis with T, M and R		
	Equation	R^2	*P*	Equation	R^2	*P*
$NH_4^+ - N$ (mg kg^{-1})	Y = 0.990 + 0.083T +0.089M	0.175	0.090	Y = −1.858 + 0.282T + 0.118M − 1.024R	0.448	0.002
$NO_3^- - N$ (mg kg^{-1})	Y = −6.809 + 0.245T + 0.116M	0.358	0.004	Y = −6.461 + 0.220T + 0.113M + 0.125R	0.372	0.010
Mineralization rate (mg kg^{-1} day^{-1})	Y = 0.082 − 0.001T + 0.0003M	0.001	0.994	Y = −0.116 + 0.009T + 0.002M − 0.043R	0.366	0.025
Nitrification rate (mg kg^{-1} day^{-1})	Y = 0.019 − 0.0001T + 0.0001M	0.003	0.973	Y = −0.103 + 0.006T + 0.002M − 0.026R	0. 378	0.021

DISCUSSION

Land-use management though clipping and shading clearly affected N dynamics and did so in slightly different ways from that we originally hypothesized in this present study. One-year clipping and/or shading increased soil inorganic N concentrations (Fig. 1), primarily by reducing microbial biomass C and microbial N immobilization, and/or by lowering plant N uptake (Table 1; Fig. 1). Meanwhile, we found that shading caused higher net N mineralization and nitrification rates, whereas clipping increased net N mineralization rates, but decreased N nitrification rates (Fig. 2). The results might be related to interactions of plant C substrate supply, microbial activity and soil microclimate in regulating soil N dynamics (Nadelhoffer *et al.* 1991; Saetre and Stark 2005).

Alterations in plant C substrate in response to land-use management (clipping and shading) can have large effects on soil N dynamics. Plants can affect soil N dynamics directly through root N uptake and indirectly through regulating C input into the soil. Changes in soil C substrate can have large effects on microbial activity (Zak *et al.* 1994). The microbial activity potentially plays an important role in assimilating and retaining N mineralized following change in plant C substrate input (Davidson *et al.* 1992). Both clipping and shading significantly reduced total root biomass by 24 and 38%, respectively, in comparison with the control (Wan and Luo 2003), causing lower roots and microbial activity and lower plant N uptake. Thus, the lowered plant N uptake and reduced microbial N immobilization led to the higher inorganic N pools under clipping and shading in the present study (Table 1; Fig. 1). Additionally, we found that inorganic N pools

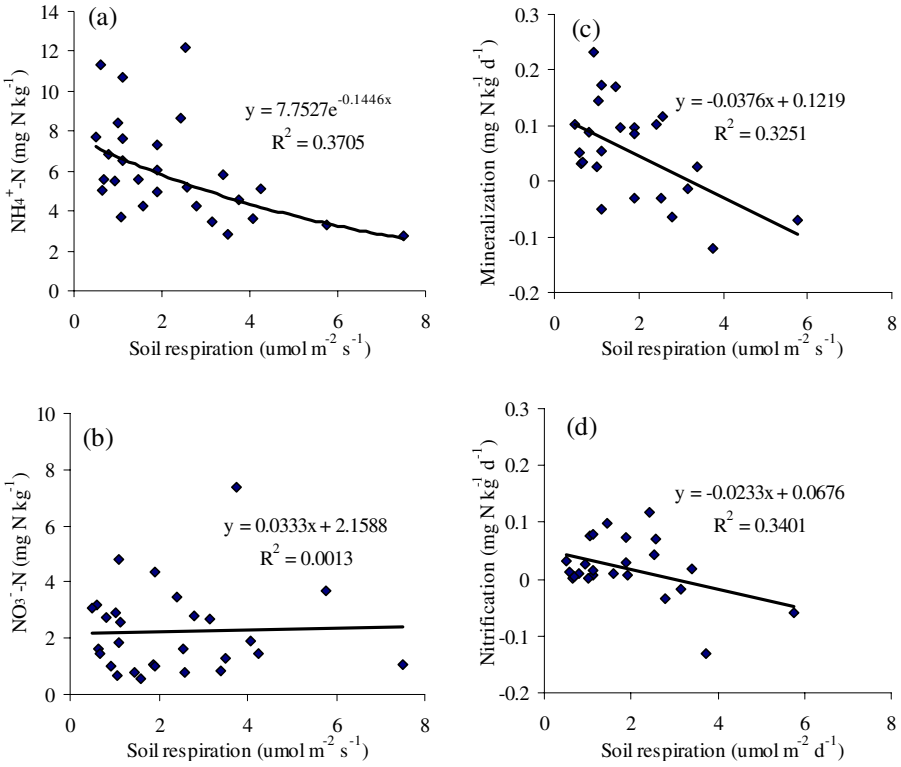

Figure 3: the relationships of soil respiration rates with (**a**) $NH_4^+ - N$ concentration, (**b**) $NO_3^- - N$ concentration, (**c**) net N mineralization and (**d**) nitrification rates across the treatments.

were lower in growing season compared to non-growing season (Fig. 1). Our results was consistent with other studies that reduced plant N requirements are correlated with increased soil $NH_4^+ - N$ and/or $NO_3^- - N$ concentrations by shading (Jonasson *et al.* 1999), litter removal (Reynolds and Hunter 2001) and lowering plant N uptake (Wan and Luo 2003). The reduced microbial N immobilization under biomass removal and/or shading might also be the major reason for the higher net N mineralization and nitrification rates. Similar to the reduced C substrates by grazing (Holland and Detling 1990; Johnson and Matchett 2001) and vegetation removal (Ohtonen *et al.* 1992), shading significantly increased annual net N mineralization and nitrification rates, possibly due to reduced microbial N immobilization (Table 1; Fig. 2). Being consistent with the fact that shading only cut-off substrate supply from photosynthesis, where clipping plus shading cut off substrate from both photosynthesis and above-ground litter (Wan and Luo 2003), we found that shading increased $NH_4^+ - N$, total inorganic N concentrations, net N mineralization and nitrification rates compared with clipping plus shading (Figs 2 and 3), further supporting that plant C substrate plays an important role in determining the major soil N pools and fluxes.

Change in soil microclimates (i.e. soil temperature and moisture) under land-use management can be an important factor in regulating certain soil N cycling processes (e.g. Aranibar *et al.* 2004; Dijkstra *et al.* 2008). By comparing the soil N pools and fluxes between clipping and clipping plus shading, we were able to isolate the soil microclimate effect on soil N dynamics. The inorganic N concentration was higher under clipping than those under clipping plus shading (Fig. 1), suggesting that soil microclimate might determine $NO_3^- - N$ concentration under similarly low C substrate levels. Indeed, soil microclimate explained the major variations in $NO_3^- - N$ concentration across treatments (Table 2). The interactive effects of soil substrate and microclimate were also reported in other ecosystems (Nadelhoffer *et al.* 1991; Theodose and Martin 2003). Our results indicated that clipping slightly decreased C substrate (Table 1) and significantly decreased annual mean soil moisture (Wan and Luo, 2003). Several studies have showed that nitrification may respond positively to soil moisture up to −0.01 MPa (Stark and Firestone 1995; Low *et al.* 1997). Thus, clipping decreased N nitrification rates, possibly due to the interaction of low soil moisture (Fig. 1d) and the depletion of readily available substrate (Table 1, Saetre and Stark 2005; Steltzer and Bowman 1998).

It has been argued that the available substrate can influence soil N dynamics by affecting microbial activity more importantly than soil microclimate (Nadelhoffer *et al.* 1991; Nunan *et al.* 2000). Our stepwise regression analysis showed that soil microclimate and soil respiration together explained more additional variations in $NH_4^+ - N$, net N mineralization and nitrification rates than soil microclimate alone (Table 2). This result suggested that changes in plant C substrate supply and microbial activity represented as soil respiration is more

important in determining the temporal N dynamics than soil microclimate (Nadelhoffer *et al.* 1991). Wan and Luo (2003) have reported that clipping and/or shading cause great C substrate limitations for microbial and plant root activities, resulting in lower root and microbial respiration and higher soil N availability. Consequently, negative correlations between soil respiration and soil inorganic N concentrations, net N mineralization and nitrification rates across the treatments were observed during the entire experimental period in this present study (Fig. 3). Similar negative relationships between concomitantly measured soil respiration and soil N dynamics have been observed in arctic (Nadelhoffer *et al.* 1991), forest (Ohtonen *et al.* 1992), tropic (Rhoades and Coleman 1999) and grassland (Johnson and Matchett 2001) ecosystems. For example, Johnson and Matchett (2001) have reported that grazing increases net N mineralization and nitrification rates but reduces soil respiration, possibly because continuous grazing reduces the labile C input to the rhizosphere and then induces microbial C limitation to both soil respiration and N immobilization. Thus, our results, together with other ecosystem studies, further suggest that the coupled C and N cycles are not only controlled by microbial activity (e.g. Updegraff *et al.* 1995; Magill and Aber 2000; Schaeffer *et al.* 2003) but also determined by plant growth and labile C substrate input (Carrera *et al.* 2009; Johnson and Matchett 2001; Nadelhoffer *et al.* 1991; Ohtonen *et al.* 1992).

CONCLUSIONS

In summary, we found that land-use management (clipping and/or shading) increased soil inorganic N concentration primarily by reducing plant C substrate supply, microbial N immobilization and plant N uptake. Shading accelerated net N mineralization and nitrification rates, whereas clipping increased net N mineralization but decreased N nitrification rates. Clipping and shading-induced changes in C substrate, microbial activity and soil microclimates interactively influenced inorganic N, net N mineralization and nitrification rates. Plant C substrate and microbial activity caused by clipping and shading appeared to be more important in determining $NH_4^+ - N$, net N mineralization and nitrification rates than soil microclimate, whereas soil microclimate induced by clipping and shading was very important in determining $NO_3^- - N$ production. Finally, we acknowledged that soil N dynamics is controlled by multiple factors, including soil moisture, soil temperature, substrate, vegetation and rhizodeposition. One-year clipping and/or shading experiment might not sufficiently address the possible land-use management effects on terrestrial ecosystem N cycling. Our results suggest that changes in plant C supply and soil microclimate in response to land-use management could potentially modify soil N dynamics and consequently affect ecosystem functions. To predict the potential for environmental change to influence soil N dynamics, we need more long-term field studies to extrapolate of effect of the plant C substrate on soil N dynamics combined to microbial activity and soil microclimate, which is likely and could contribute to a better understanding of N *in situ* cycling and feedback to net primary productivity in terrestrial ecosystems.

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Conflict of Interest statement. None declared.

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