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INTERACTIONS BETWEEN ECOSYSTEM CARBON, NITROGEN AND WATER CYCLES UNDER GLOBAL CHANGE: RESULTS FROM FIELD AND MESOCOSM EXPERIMENTS

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INTERACTIONS BETWEEN ECOSYSTEM CARBON, NITROGEN AND WATER CYCLES UNDER GLOBAL CHANGE: RESULTS FROM FIELD AND MESOCOSM EXPERIMENTS

A Dissertation APPROVED FOR THE

DEPARTMENT OF BOTANY AND MICROBIOLOGY

BY

_____

Dr. Yiqi Luo (chair)

_____

Dr. Linda L. Wallace

_____

Dr. Lee Krumholz

_____

Dr. Mariëlle Hoefnagels

_____

Dr. Bruce Hoagland

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ABSTRACT

Little is known about how climate warming, land-use change and N fertilization/deposition affect ecosystem carbon (C), nitrogen (N) and water cycles. By reducing plant C substrate input via cutting aboveground biomass and shading, I investigated how plant C supply affects soil N dynamics in a tallgrass prairie. By incubating litter bags in the field, I studied the effects of warming and clipping on litter decomposition and N dynamics of 3 litter species. By measuring whole-ecosystem CO_2 and water fluxes of a model grassland ecosystem, I investigated whether N fertilization (simulated by pulse N fertilization) and deposition (mimicked by gradual N fertilization) increase NEE, radiation-use efficiency (RUE), ET, and water -use efficiency (WUE).

Biomass removal (BR) and shading (S) increased soil inorganic N, likely due to the severed plant N uptake and reduced microbial N immobilization. Shading increased net N mineralization and nitrification probably by reducing microbial N immobilization. Soil respiration, together with soil microclimate, accounted for 27-38% additional variations in NH_4^+-N, net N mineralization and nitrification rates than soil microclimate alone, suggesting an important role of plant C supply in regulating NH_4^+-N, net N mineralization and nitrification rates.

Warming and clipping together significantly altered the mass remaining percentage of *S. scoparium* shoot litter, with the treatment effects being mainly caused by clipping. Warming and clipping did not affect the net nitrogen (N) immobilization/release of the 3 litter types. However, the decay constant k and N remaining percentages differed among the 3 litter types due to their different initial litter quality. Warming reduced whereas

clipping increased the *in situ* litter quality, suggesting a potential impact on the long-term ecosystem C and N cycles.

N additions increased NEE by enhancing light interception, RUE, and extending growing seasons. Different N application methods (PF versus GF) caused differential seasonal dynamics of LAI, NEE and RUE due to their different N supply doses during different growth periods. N fertilization stimulated evapotranspiration primarily by extending growing season. N fertilization enhanced photosynthesis more than evapotranspiration, causing higher ecosystem WUE. Different fertilization methods changed the seasonal dynamics of WUE due to their differential effects on gross ecosystem photosynthesis.

CHAPTER I

Introduction

Since the industrial revolution, the earth has experienced dramatic changes, which are altering our way of life. The increases in the concentrations of greenhouse gases, including CO_2, N_2O and CH_4, have caused an increase in the global surface temperature by more than 0.5 °C over the past century and is predicted to increase by 1.4-5.8 °C over the period from 1990 to 2100 (Wigley and Raper, 2001). In the mean time, the explosive increase in the world population has resulted in more and more disturbances on the natural ecosystems on earth. Huge amounts of natural forest and grassland ecosystems have been converted into different land-use types, including cropland, pasture ranch, and economic plantations (e.g., Reiners et al., 1994; Fernandes and Stanford, 1995). Furthermore, with the increase in the nitrogen (N) application in agriculture and the emission of polluted air to the atmosphere, such as N_2O, NO and SO_2, large areas of natural ecosystems (especially forests) have been polluted and even destroyed (Norby, 1998). Therefore, climate warming, land-use changes, N fertilization and deposition have become the major anthropogenically-induced factors affecting the ecosystem functioning and the health of our planet. In order to maintain the sustainable development of our human society, it is necessary to study the effects of those anthropogenic factors on ecosystem functions and what measures we can take to minimize the negative effects of those factors.

Global climate change, land-use change, and N fertilization/deposition have substantially altered ecosystem carbon (C), N and water cycles.

For C cycles, elevated CO_2 has been reported to increase plant photosynthesis (Groninger et al., 1996; Tissue et al., 1997), the major C fixation process of terrestrial ecosystems. Soil respiration and microbial respiration are also reported to be higher under elevated CO_2 primarily due to higher labile C substrate and root respiration (Zak et al., 2000). Artificial warming in manipulated growth chambers reportedly increases plant photosynthesis (Zhu et al., 1999), whereas warming in field conditions either increases, decreases, or does not change (Loik et al., 2000) plant photosynthesis and other C processes, including litter decomposition and soil CO_2 efflux (Shaver et al., 2000; Shaw and Harte, 2001; Rustad et al., 2001). Land-use changes, such as clipping for hay (or haying) in grassland regions, may change ecosystem C cycles by changing plant substrate input and hence the soil microbial activities (Zhang et al., 2004). N fertilization and deposition is another big environmental issue across the world, especially in Northeast America, and North European countries (Norby, 1998). Numerous studies have examined the effects of N additions on plant-level CO_2 (Weerakoon et al., 2000; Caviglia and Sadras, 2001) and water flux (Gimenez et al., 1994; Kubiske et al., 1998; Shangguan et al., 2000), however, very few have studied the effects of N additions via agricultural N fertilization and N deposition on whole-ecosystem level CO_2 and H_2O fluxes. Since it is difficult to extrapolate the C and water fluxes from plant-level measurements to ecosystem-level measurements (Leuning et al., 1995; Hui et al., 2001), predictions of N effects on net primary productivity (NPP) require accurate measurements of whole-ecosystem CO_2 and water fluxes.

Elevated CO_2 either increases (Zak et al., 1993) or decreases (Diaz et al., 1993) soil N availability to plants due to different methodologies and ecosystems studied (Zak et al.,

2000; Hungate et al., 1996). Warming either increases, decreases, or does not change litter decomposition and N dynamics (Nadelhoffer et al., 1991; Peterjohn et al., 1994; Harte and Shaw, 1995; Hobbie, 1996). A meta-analysis reported that warming substantially increased soil net N mineralization and nitrification rates (Rustad et al., 2001). Land-use change, such as clipping for hay, may alter soil N availability by changing both plant and microbial activities (Zhang et al., 2004). N fertilization and deposition may increase the soil N availability and N losses from leaching, NH_3 volatilization and denitrification (Aber and Driscoll, 1997).

Water is one of the most important environmental factors that mediate ecosystem C and N cycles (Gower et al., 1992; Germino and Wraith, 2003). Therefore, it is important to study how C and N cycles interact with water dynamics in order to better understand the ecosystem responses to climate change and human perturbations. Under elevated CO_2 conditions, soil water content was reported to be higher than under control, primarily resulting from higher plant water-use efficiency and less evapotranspiration (Zhu et al., 1999). Likewise, N fertilization and deposition may also alter soil water content by affecting plant transpiration and soil evaporation (Gimenez et al., 1994; Kubiske et al., 1998). Global warming may reduce soil moisture by increasing evapotranspiration and plant water uptake (Loik et al., 2000). Land-use change, such as clipping for hay, may also alter soil moisture by reducing vegetation cover and plant water uptake. The changes in ecosystem water cycles under different climate change scenarios will eventually affect many ecosystem C and N cycling processes.

No matter how global climate changes, land-use change, and N fertilization/deposition affect ecosystem C, N and water cycles, one thing is common.

That is, they ultimately change the plant carbon substrate quantity and quality by changing plant photosynthesis, plant nutrient uptake, and plant litter materials input. Therefore, any changes in plant C substrate will have great impact on C, N and water cycles. Similarly, any changes in soil N conditions will affect C fixation and water fluxes at both plant- and ecosystem-levels. All in all, ecosystem C, N and water cycles will interactively respond to human perturbations, such as elevated CO_2, warming, land-use change, and N fertilization/deposition. Studying how ecosystem C, N and water cycles interact with each other will help better understand their responses to different climate change scenarios.

Here I propose a conceptual model to describe the interactions between ecosystem C, N and water cycles at both plot- and ecosystem levels (Fig. 1).

At the plot-level, plants supply C substrate to the ecosystem by two major pathways: photosynthesis and litterfall. Plant photosynthesis continuously supplies labile C (photosynthates) to plant roots and soil microbes, providing C energy for rhizosphere respiration, plant N uptake and microbial N immobilization. Litterfall, both aboveground and belowground, is the major source of soil organic matter (SOM). The organic C in SOM can be utilized by soil microbes to form microbial biomass, immobilize nutrients and respire CO_2. The organic N in SOM can be converted into inorganic N by microbial mineralization and nitrification processes. The sum of root respiration, rhizosphere microbial respiration and microbial respiration from decomposition of litter and SOM leads to the conventionally measured soil respiration. In addition to the plant C substrate

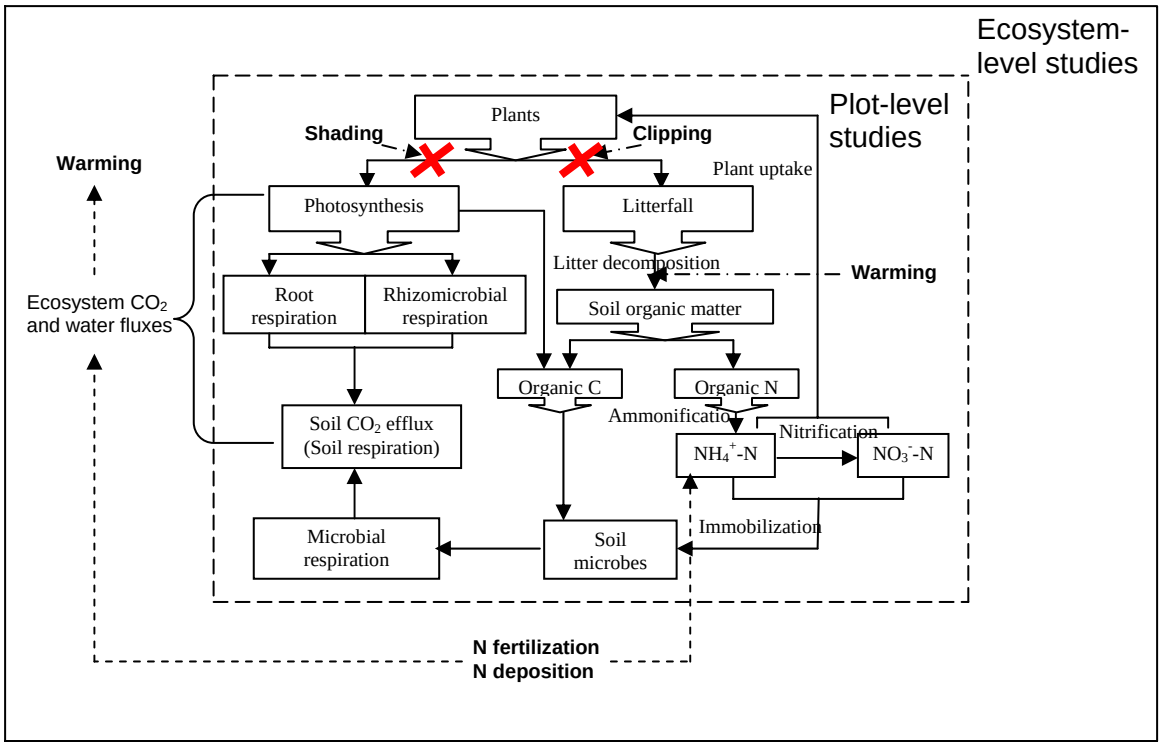


Figure 1. A conceptual model to show how ecosystem C, N and water cycles interact at both plot- and ecosystem-levels, and how human perturbations, including warming, shading, clipping, N fertilization and/or deposition affect ecosystem C, N and water cycles.

supply, plant N uptake may also affect soil N dynamics by competing for inorganic N with soil microbes. It appears from this conceptual model that any changes in plant C substrate supply, either photosynthesis or litterfall, will affect both C and N dynamics simultaneously. Therefore, any changes in these two C input pathways under different perturbations, such as warming, shading and clipping, and their subsequent effects on soil microbes may alter the ecosystem C, N and water dynamics. For example, warming may affect ecosystem C and N cycles by affecting plant physiology and eventually plant substrate input into the ecosystems. Clipping may alter ecosystem C and N dynamics by

reducing plant litterfall input. Shading may alter ecosystem C and N dynamics by cutting plant photosynthesis. A few studies have recognized the potential effects of plant substrate changes in affecting ecosystem C and N cycles (Zak et al., 1994). However, few field experimental studies have specifically investigated how the changes in plant substrate quantity and quality affect soil carbon and nitrogen cycles and their interactions.

At the ecosystem level, N fertilization/deposition may alter ecosystem C, N and water cycles by increasing soil available N and plant photosynthesis, and hence stimulating gross ecosystem photosynthesis, ecosystem respiration, and net ecosystem CO_2 exchanges (NEE). While affecting CO_2 flux, N additions will also alter ecosystem water fluxes by changing stomatal conductance of plants and canopy boundary layer. At leaf- and plant-level studies, N additions may increase plant photosynthesis by increasing Rubisco enzyme concentration, light interception and radiation-use efficiency (Weerakoon et al., 2000; Caviglia and Sadras, 2001). However, little is known about how N fertilization affects NEE although N fertilization is likely to increase net primary productivity (NPP) (Nadelhoffer et al., 1999). By altering ecosystem CO_2 and water fluxes, N fertilization and deposition may eventually feed back to the global climate change because both CO_2 concentration and water flux may change the atmospheric temperature and global water cycles.

In this dissertation, I mainly focus on the following four topics:

1. How reducing plant C substrate input by shading and clipping regulates *in situ* soil N dynamics in a tallgrass prairie.

Great efforts have been made in studying how N additions alter ecosystem C dynamics, such as litter decomposition, and soil respiration. However, little is known

about how plant C substrate affects ecosystem N dynamics in the field conditions. By reducing plant C substrate via clipping and shading a tallgrass prairie, we investigated how reducing plant C substrate altered net N mineralization and nitrification rates in the field conditions for one year.

2. How changes in site quality under warming and clipping would affect litter decomposition and N dynamics in a tallgrass prairie ecosystem.

Warming and clipping may change both soil microclimate and site quality, and hence affect ecosystem C and N dynamics, such as litter decomposition, the most critical process for C and N cycles. However, little is known about the relative importance of soil microclimate changes and site quality changes in determining litter decomposition under warming and land-use change, such as clipping for hay, in the central US. We investigated this issue by measuring the litter decomposition and N dynamics of two dominant plant species, the site quality and soil microbial biomass in a tallgrass prairie under warming and clipping.

3. How N fertilization and/or deposition would affect NEE in a model grassland ecosystem with cheatgrass.

Little is known about how N fertilization and/or deposition affect CO_2 fluxes at ecosystem level, although quite a lot of studies have investigated the N effects on plant photosynthesis at leaf- and plant-levels. By continuously measuring the ecosystem CO_2 and water fluxes in an environmentally controlled growth facility, we tested whether N additions also increase CO_2 flux by increasing canopy radiation-use efficiency (RUE) as often reported at leaf- and plant-levels. Since modeling studies at large scales often assume a constant canopy RUE, our results would provide some

ecosystem-level experimental evidence and some useful suggestions for modeling studies.

4. How N fertilization and/or deposition affect evapotranspiration and water-use efficiency (WUE) of a model grassland ecosystem with cheatgrass.

Little is known about how changes in soil N availability affect evapotranspiration (ET) at the ecosystem level. By continuously measuring the ecosystem water flux of a model grassland ecosystem, we investigated how N additions affected ET and WUE, and compared the effects of pulse and gradual N fertilization on the seasonal dynamics of ET and WUE. Ultimately, we intended to identify major processes that regulate responses of ecosystem water and CO_2 fluxes to N additions.

The objectives of this dissertation were to (1) investigate how plant substrate quality changes affect ecosystem N dynamics and C-N interactions; (2) examine how warming and clipping affect litter decomposition of dominant plant species in tallgrass prairie ecosystems; and (3) study how N fertilization/deposition affect ecosystem CO_2 and water fluxes at ecosystem level in model grassland ecosystems.

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CHAPTER II

Plant carbon supply regulates soil nitrogen availability and dynamics in a tallgrass prairie: implications for global climate change [*]

[*] This part has been submitted to Global Biogeochemical Cycles (in review)

Abstract

Substantial research efforts have been made to examine how nitrogen (N) influences ecosystem carbon (C) processes. However, our understanding of the regulation of plant C supply on soil N pools and dynamics is limited. A 1-year field experiment (June 2001 to July 2002) was conducted to examine how the *in situ* soil N pools and dynamics change with reduced plant C supply in a tallgrass prairie ecosystem in Oklahoma, USA. Plant C substrate were decreased by completely removing live and dead aboveground biomass (biomass removal, BR), shading (S), and BR+S. The results showed that reduction in plant C substrate generally led to greater inorganic N, net N mineralization and nitrification in soils. Both BR and S increased soil inorganic N, net N mineralization and nitrification rates, likely due to the reduced plant N uptake and/or microbial N immobilization compared to the control. In comparison with BR+S, S had more C substrate from aboveground biomass, resulting in higher NH_4^+-N, total inorganic N, net N mineralization and nitrification rates but similar NO_3^- -N. Biomass-removal caused higher NH_4^+-N but lower net nitrification rate than BR+S, which was mainly attributable to the difference in microclimate since these two treatments had similar plant C supply. Over the 1-year experimental period, NH_4^+-N, net N mineralization and nitrification rates were negatively correlated with soil respiration across the treatments, but not for NO_3^--N. Adding soil respiration as an independent variable to soil microclimate accounted for 27-38% of the additional variations in NH_4^+-N, net N mineralization and nitrification rates but only 1% for NO_3^--N. Thus, our results indicate that plant C supply is a main factor regulating NH_4^+-N, net N mineralization and nitrification rates whereas soil microclimate plays an important role in determining NO_3^--N production. The results observed in this

study suggest that the potential changes in plant growth and belowground C allocation induced by the changing climate may substantially influence the *in situ* soil N pools and dynamics, feeding back to net primary productivity in terrestrial ecosystems.

Keywords: ammonium; biomass-removal; nitrate; nitrification; nitrogen mineralization; plant carbon substrate; shading; soil respiration

1. Introduction

As the major limiting nutrient to the primary productivity in natural ecosystems [*Vitousek and Howarth*, 1991], nitrogen (N) plays a critical role in regulating responses of terrestrial C storage to global climate changes [*Hungate et al*., 1996; *Giardina et al.*, 2001; *Hungate et al.*, 2003; *Luo et al.*, 2004]. The N release processes, i.e. mineralization and nitrification, and their controlling factors have been extensively studied during the past decades. Soil temperature and moisture are closely associated with both spatial and temporal variations in soil N cycling processes [*Schimel and Parton*, 1986; *Burke*, 1989; *Ruess and Seagle*, 1994]. In addition, soil organic substrate also plays a crucial and even more important role in controlling soil N dynamics than soil microclimate [*Burke*, 1989; *Nadelhoffer et al.*, 1991; *Updegraff et al.*, 1995]. By manipulating different types of C substrate, many studies have reported that C substrate affected net N mineralization and nitrification rates primarily by altering microbial N immobilization via changing microbial activity [e.g., *Magill and Aber*, 2000; *Johnson and Matchett*, 2001; *Reynolds and Hunter*, 2001; *Schaeffer et al.*, 2003]. In a laboratory incubation study, *Zak et al.* [1994] suggested that plant production across a continental gradient may affect N availability by altering plant C substrate input to soil and the amount of energy available

for heterotrophic metabolism. Consequently, changes in plant growth and belowground C allocation induced by global climate change can influence soil N pools and dynamics, put feedbacks to plant growth and net primary productivity in terrestrial ecosystems. Therefore, better understanding of the effects of plant C supply and its interaction with soil microclimate on soil N dynamics *in situ* will facilitate the projections of the responses of terrestrial C sequestration to global change.

To study C substrate effects on soil N dynamics, ecologists usually add different types of C substrate to soils and/or remove certain plant C materials, and then measure the N dynamics of soil samples incubated in the laboratory [e.g., *Ruess and Seagle*, 1994; *Beltran-Hernandez et al.*, 1999; *Magill and Aber*, 2000; *Schaeffer et al.*, 2003]. However, most studies have failed to examine the effects of plant C substrate on *in situ* soil N dynamics because they either excluded the plant C substrate effects by measuring N dynamics in the laboratory or only studied the effects of certain parts of plant C substrate such as litter and foliage. Essentially, laboratory incubation eliminates the contributions of plant growth to belowground labile C input, plant N uptake, and the seasonal microclimate changes in the field situations [*Zak et al.*, 1993a; *Zak et al.*, 1994; *Ruess and Seagle*, 1994; *Weintraub and Schimel*, 2003], and cannot adequately reflect the important controls over soil activity in the field [*Vance and Chapin*, 2001]. Well-designed field experiments are desirable to better understand the effects of plant C substrate supply on *in situ* soil N dynamics.

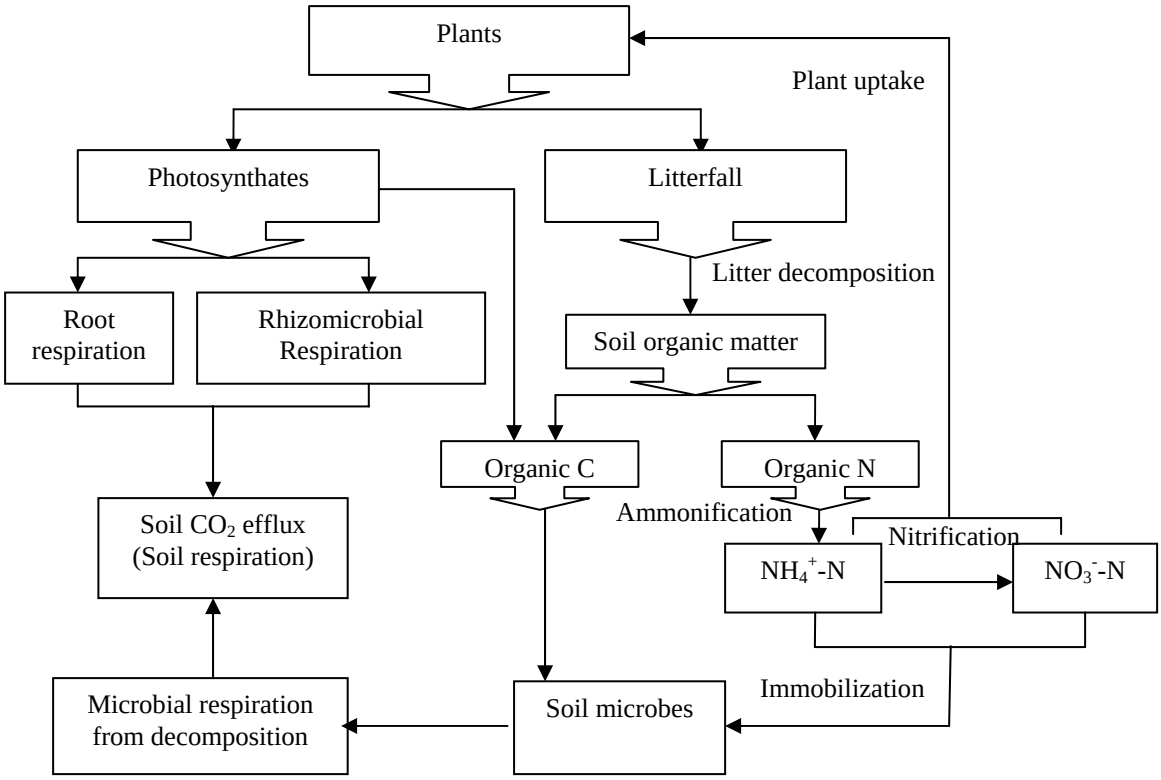


Figure 1. A conceptual diagram of the coupled C and N cycles to demonstrate how plant C substrate affects soil N dynamics.

Here we propose a stoichiometrical conceptual model to show how plants may affect soil N dynamics in natural ecosystems (Fig. 1). Plants supply C substrate to the ecosystem by two major pathways: photosynthesis and litterfall. Plant photosynthesis continuously supplies labile C (photosynthates) to plant roots and soil microbes, providing C energy for rhizosphere respiration, plant N uptake and microbial N immobilization. Litterfall is the major source of soil organic matter (SOM). The organic C in SOM can be utilized by soil microbes to form microbial biomass, immobilize nutrients and to respire CO_2 in the mean time. The organic N in SOM can be converted into inorganic N by microbial mineralization and nitrification processes. The sum of root

respiration, rhizosphere microbial respiration and microbial respiration from decomposition of litter and SOM leads to the conventionally measured soil respiration. In addition to the plant C substrate supply, plant N uptake may also affect soil N dynamics by competing for inorganic N with soil microbes. It appears from this conceptual model that any changes in plant C substrate supply, either photosynthesis or litterfall, will affect both C and N dynamics simultaneously. Therefore, in order to fully understand the plant C substrate effects on soil N dynamics, the two C substrate input pathways need to be manipulated in the field conditions for at least one growing season.

By reducing plant C substrate input via biomass-removal and/or shading, we have studied the effects of plant C substrate supply on *in situ* soil N dynamics in a tallgrass prairie ecosystem in Central Oklahoma, USA. By completely removing the aboveground live and dead biomass we cut off the C substrate supply from photosynthesis and litterfall. By shading we removed the C substrate supply from photosynthesis. The reduced plant C substrate has been reported to decrease soil respiration irrespective of the changes of soil microclimate in a concomitant study [*Wan and Luo*, 2003]. Since soil respiration consists of both root respiration and microbial respiration, we used the soil respiration data obtained by *Wan and Luo* [2003] as a proxy for plant and microbial activities and hence plant C substrate availability in this study. We hypothesized that (1) biomass-removal and/or shading would increase inorganic N concentrations by limiting plant N uptake and microbial N immobilization; (2) biomass-removal and/or shading would increase net N mineralization and nitrification rates by decreasing microbial N immobilization; and (3) plant C substrate supply (as indicated by soil respiration) could be more important than soil microclimate in regulating soil N dynamics. To test these hypotheses, we measured

the inorganic N concentrations, and net N mineralization and nitrification rates under different treatments. We regressed inorganic N, net N mineralization and nitrification rates against soil microclimate and/or soil respiration in order to see how much variation in the inorganic N and net N mineralization and nitrification rates can be explained by soil microclimate and plant C substrate. We also plotted soil respiration against inorganic N concentrations, net N mineralization and nitrification rates during the same time period to examine the possible relationships between soil C and N dynamics.

2. Materials and Methods

2.1. Site description and experimental design

The study site is located in a tallgrass prairie ecosystem, 3 km east of the Norman campus of the University of Oklahoma (35.2° N, 97.4° W). The soil type is Vernon clay loam. The dominant plant species are *Panicum virgatum*, *Schizachyrium scoparium, Andropogon gerardii, Sorghastrum nutans, Ambrosia psilostachyia, Gutierrezia texana, Bromus japonicus*, and *Eragrostis spp*.

This experiment was established on 21 June 2001. A randomized complete block design was applied in this study with four treatments, biomass-removal (BR), complete light elimination by shading (S), BR plus S (BR+S), and control. Each treatment was replicated five times. The plot size was 1 m × 1 m. The interval distance between adjacent plots was 1.5 m. The BR treatment was set up by completely removing the aboveground live and dead plant biomass (including surface litter) to the soil surface and cutting the re-growth once a week. For the S treatment, we used double layer black shade

cloth to wrap a wood frame (1.2 m × 1.2 m × 1 m) over the 1 m × 1 m plot. The shade cloth reduced the light intensity to zero in the plot.

2.2. Field measurements

Soil net N mineralization and nitrification rates were measured utilizing the PVC-tube closed top sequential incubation method modified from Raison et al. (1987). The soil samples were taken using a PVC tube (15 cm in length, 4 cm in inner diameter). One 0-15 cm soil core was collected from each plot as the initial soil sample for inorganic N concentration (N_0) measurement. A second soil core was collected from the space very close to the first soil core, covered with a plastic film on the top, returned to the original hole and incubated *in situ* for 15 days from 07/04/2001 to 07/19/2001 (incubation period 1). The same procedure was repeated in other five incubation periods: period 2: 07/19/2001-08/28/2001, period 3: 08/28/2001-11/18/2001, period 4: 11/18/2001-01/18/2002, period 5: 01/18/2002-06/04/2002, and period 6: 06/04/2002-07/19/2002. The incubated soil samples during each period were collected and used for analysis of the post-incubation inorganic N concentration (N_1). Net N mineralization rate during each incubation period was calculated by the following formula: $(N_1 – N_0) / d$, where N1 was the post-incubation total inorganic N concentration ($NH_4^+ + NO_3^-$-N), N_0 was the initial total inorganic N concentration ($NH_4^+ + NO_3^-$-N), and d was the number of incubation days. For net nitrification rate, N_1 was the post-incubation NO_3^--N concentration; N_0 was the initial NO_3^--N concentration. Soil samples for NO_3^--N and NH_4^+-N measurements were extracted with 1 M KCl solution and quantified using the cadmium reduction method [*LACHAT*, 1994].

During the time period of measuring net N mineralization and nitrification, soil respiration was measured once a month with a LI-6400 portable photosynthesis system (LI-COR Inc., Lincoln, Nebraska, USA) attached with a Li-Cor 6400-09 soil CO_2 flux chamber [*Wan and Luo*, 2003]. The soil respiration data obtained by *Wan and Luo* [2003] were used as a proxy for plant C substrate availability as mentioned in the introduction. In order to correlate soil respiration with soil inorganic N pools, we used the soil respiration rates on the day (or a few days) when we collected the soil samples for inorganic N measurements. In order to correlate soil respiration with net N mineralization and nitrification rates, we used the averaged soil respiration rates during the time period when we measured the net N mineralization and nitrification rates. Soil temperature was measured using a thermocouple connected to the Li-Cor 6400 at the 0-5 cm soil layer as soil respiration was being measured. Soil moisture (% volumetric) at the 0-15 cm layer was measured twice a month using Time Domain Reflectometry.

Labile nutrients and microbial biomass C were determined for initial soil samples collected on 18 January 2002. Labile C and N pools were determined using the two-step acid hydrolysis method of *Oades et al.* [1970]. Microbial biomass C was measured using chloroform-fumigation method [*TSBF*, 1993].

2.3. Statistical analysis

Statistical significance of treatment effects was evaluated by analysis of variance (ANOVA). The relationships of soil inorganic N, net N mineralization and nitrification rates with soil microclimate and/or soil respiration were established using regression analysis. Both ANOVA and regression analysis were performed with SAS software package (SAS Institute Inc., NC, USA).

3. Results

3.1. Soil microclimate and other soil properties

Soil microclimate, i.e., soil temperature and moisture, was altered by BR and S (Fig. 2). Shading and BR+S significantly decreased soil temperature by 1 °C and 2 °C compared with the control in July of the two years ($p<0.05$). Biomass-removal increased soil temperature in both November 2001(1.5 °C) and January 2002 (1 °C), while BR+S increased soil temperature by 1 °C in January 2002. There was no difference in soil moisture between treatments during the first growing season ($p > 0.05$). However, BR significantly reduced soil moisture ($p < 0.05$) compared with the control and the shaded plots in winter of 2001 possibly because BR increased soil temperature, reduced water holding capacity and increased soil evaporation. In summer of 2002, S and BR+S significantly ($p < 0.05$) increased soil moisture compared with the control and BR primarily due to the reduced soil evaporation under shading.

In addition to soil microclimate, soil labile nutrients and microbial biomass C were also altered by the treatments (Table 1). Compared with the control, Shading caused a 565 mg kg^{-1} (14.9%) decrease in labile C content while BR and BR+S reduced the labile C content by about 390 mg kg^{-1} (10%). However, neither the effects of BR nor BR+S were statistically significant ($p > 0.05$). There were no significant treatment effects on labile N content ($p>0.05$). Compared with the control, S, BR and BR+S reduced the microbial biomass C content by 13.6%, 33.8% and 27.6%, respectively. However, only the BR effect was statistically significant ($p<0.05$) (Table 1).

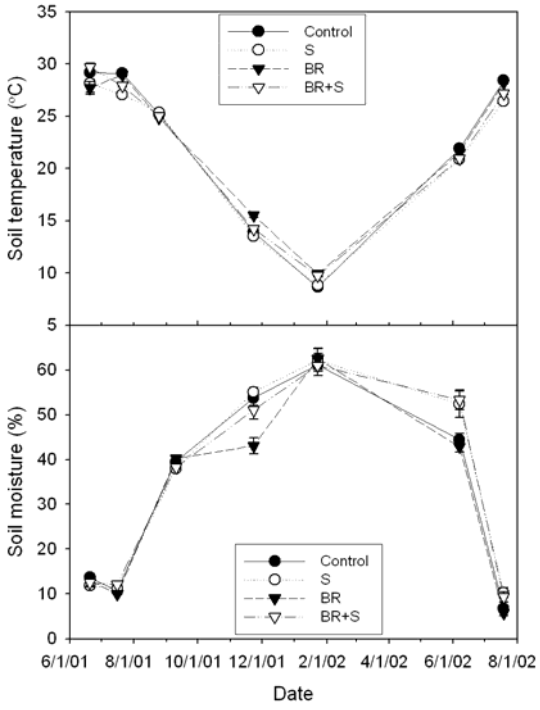


Figure 2. The seasonal changes of soil temperature (upper panel) and soil moisture (lower panel) under different treatments.

Table 1. Soil labile nutrient pools and microbial biomass C content under the treatments. Numbers are mean values with standard error of the mean in parenthesis. Different letters in the same row indicate statistical difference at α=0.05 level among treatments.

Treatments	Control	Shading	Biomass-removal	Biomass-removal+Shading
Labile C (mg kg^{-1})	3797(292)a	3232(93)a	3409(184)a	3410(179)a
Labile N (mg kg^{-1})	154(12)a	143(10)a	143(9)a	141(10)a
Microbial biomass C (mg kg^{-1})	551(77)a	476(82)ab	365(64)b	399(100)ab

3.2. Inorganic N concentrations and net N mineralization and nitrification rates

There were clear seasonal variations in both NH_4^+-N and NO_3^--N concentrations and treatment effects on certain sampling dates (Fig. 3). To show the treatment effects more clearly, we averaged the inorganic N concentrations of different sampling dates for each plot and then averaged them for each treatment (Fig. 4).

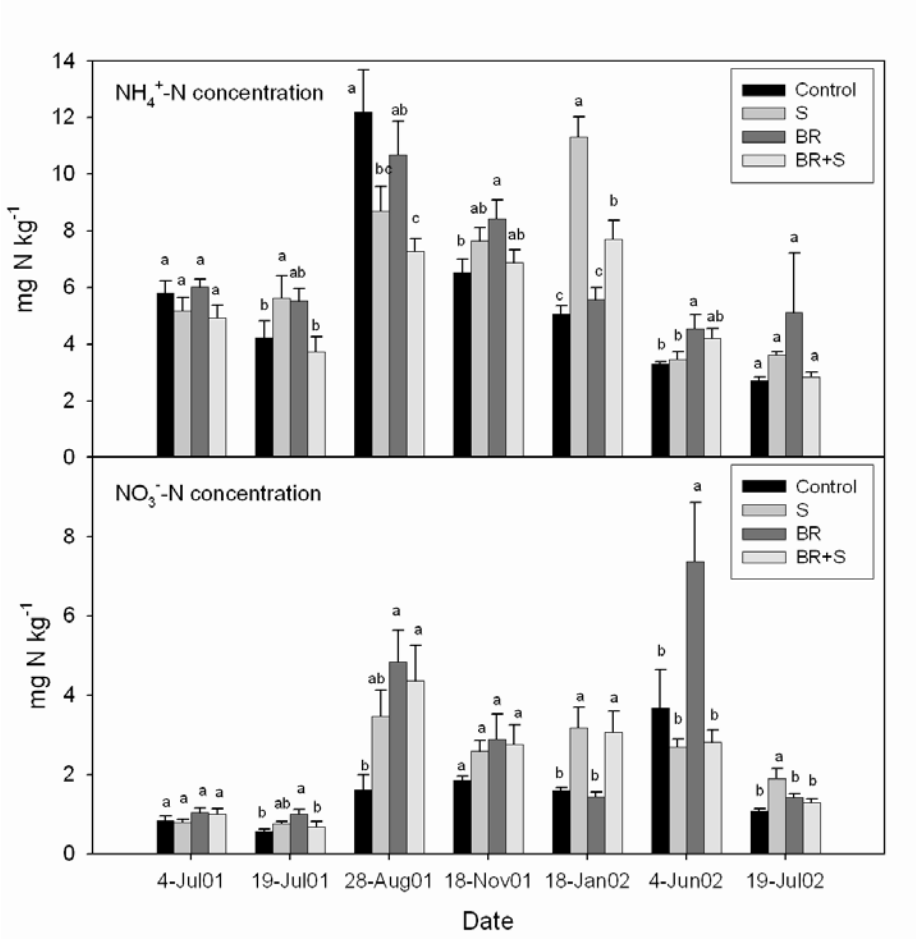


Figure 3. The seasonal changes of NH_4^+-N (upper panel) and NO_3^--N (lower panel) concentrations under different treatments. Different letters over the bars indicate statistically significant differences at α=0.05 level between treatments on different sampling dates.

Results showed that there were significant treatment effects on inorganic N concentrations (NH_4^+-N, p=0.0187; NO_3^--N, p=0.0028; total inorganic N, p=0.0008). Compared with the control, BR and S significantly increased NH_4^+-N concentration by 15.2% ($p<0.05$) and 14.2% ($p<0.05$), respectively. NO_3^--N concentrations in BR, S and BR+S plots were 78.4% ($p<0.05$), 37.3% ($p<0.05$), and 42.7% ($p<0.05$) higher than that in the control plots, respectively. As a result, BR and S significantly increased the total inorganic N concentrations by 29.1% ($p<0.05$) and 19.3% ($p<0.05$), respectively. Biomass-removal caused higher NO_3^--N concentrations than S ($p<0.05$).

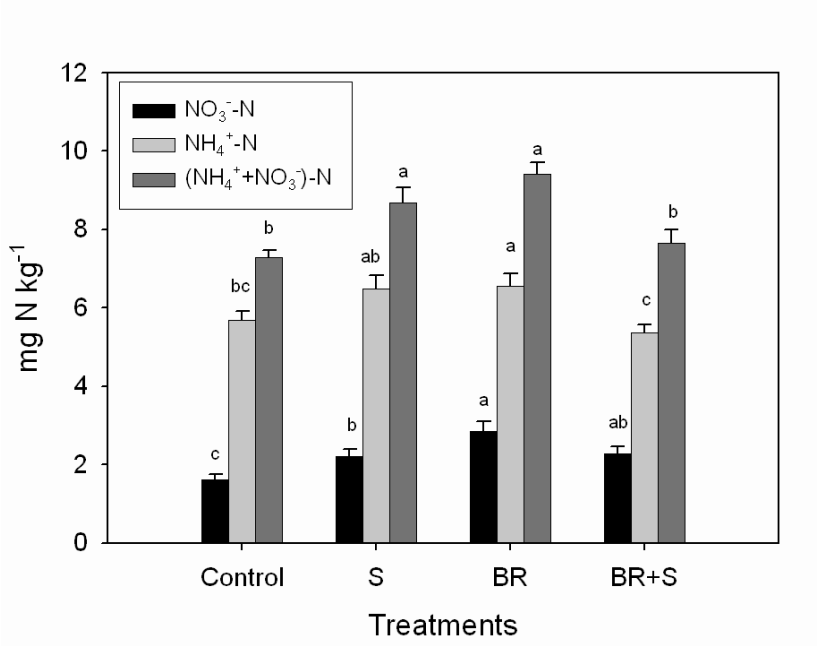


Figure 4. Comparisons of the yearly averaged NH_4^+-N, NO_3^--N and total inorganic N concentrations under different treatments. Different letters over the bars indicate statistically significant differences at α=0.05 level between treatments.

There were also obvious seasonal variations in net N mineralization and nitrification rates and treatment effects during certain incubation periods (Fig. 5). We also averaged

the net N mineralization and nitrification rates using the same method as averaging inorganic N concentrations (Fig. 6). Significant treatment effects were observed in net N mineralization and nitrification rates (N mineralization, $p<0.0001$; nitrification, $p=0.0019$).

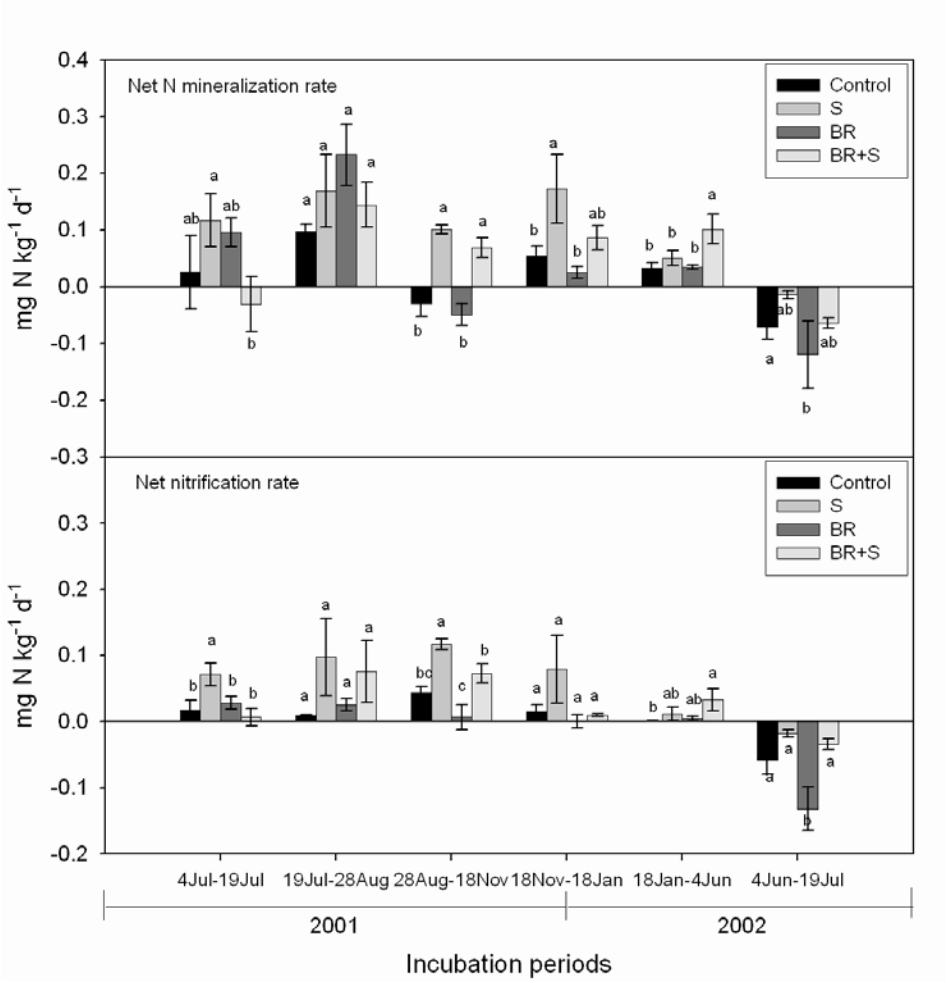


Figure 5. The seasonal changes of net N mineralization (upper panel) and nitrification rates (lower panel) under different treatments. Different letters over the bars indicate statistically significant differences at $\alpha=0.05$ level between treatments during different incubation periods.

Compared with the control, BR, S and BR+S increased the averaged net N mineralization rate by 444%, 106%, and 206%, respectively. The nitrification rates were 900% and 400% higher under S and BR+S compared with the control, whereas the nitrification rate under BR was 167% lower than that under control. Nevertheless, only the S effects on both mineralization and nitrification rates were significant ($p<0.05$). In addition, S resulted in higher net N mineralization and nitrification rates than BR and BR+S ($p<0.05$). Biomass-removal+S caused higher net nitrification rates than BR ($p<0.05$).

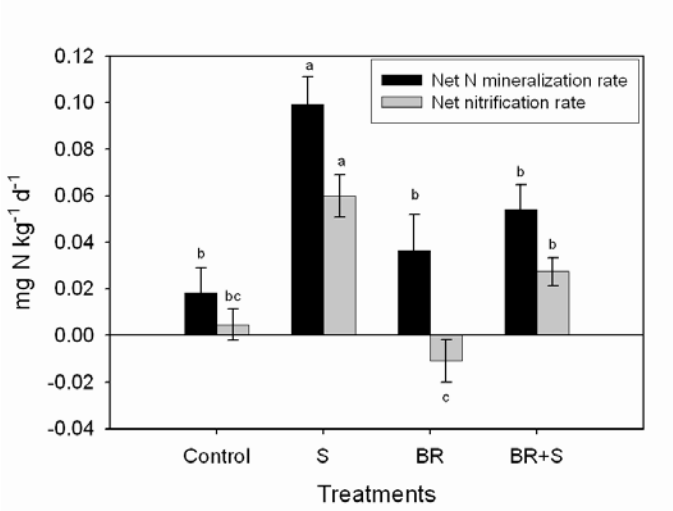


Figure 6. Comparisons of the yearly averaged net N mineralization and nitrification rates under different treatments. Different letters over the bars indicate statistically significant differences at $\alpha=0.05$ level between treatments.

3.3. Factors affecting the seasonal changes of soil N dynamics

In order to examine how soil microclimate affects the seasonal dynamics of soil N, regression analyses were carried out to explore the relationships between soil microclimate (temperature, T, and moisture, M) and inorganic N concentrations, net N

mineralization and nitrification rates (Table 2). Results showed that soil microclimate marginally accounted for only 18% of the variation in NH_4^+-N concentrations, but significantly explained 36% of the variation in NO_3^--N concentrations across all treatments and dates. Soil microclimate did not show significant relationships with net N mineralization and nitrification rates. However, after combining soil microclimate with soil respiration (R) in the multiple regression analysis, soil T, M and R together accounted for 45%, 37%, 37% and 38% of the variation in NH_4^+-N, NO_3^--N, net N mineralization and nitrification rates, respectively.

Table 2. Multiple regression equations 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}).

	Regression analysis with T and M			Regression analysis with T, M and R		
	Equation	R^2	P value	Equation	R^2	P value
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 ($mgkg^{-1}d^{-1}$)	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 ($mgkg^{-1}d^{-1}$)	Y=0.019-0.0001T+0.0001M	0.003	0.973	Y=-0.103+0.006T+0.002M-0.026R	0.378	0.021

3.4. Relationship between seasonally coupled C and N dynamics

To examine the temporal relations between the coupled soil C and N dynamics across the treatments, we plotted inorganic N concentrations, net N mineralization and nitrification rates against soil respiration. NH_4^+-N concentrations decreased exponentially as soil respiration increased (p=0.0009), but NO_3^--N concentration did not show a significant relationship with soil respiration (p>0.05) (Fig. 7). Both net N mineralization and nitrification rates showed negative linear correlations with soil respiration (p=0.0037; p=0.0028) (Fig. 7).

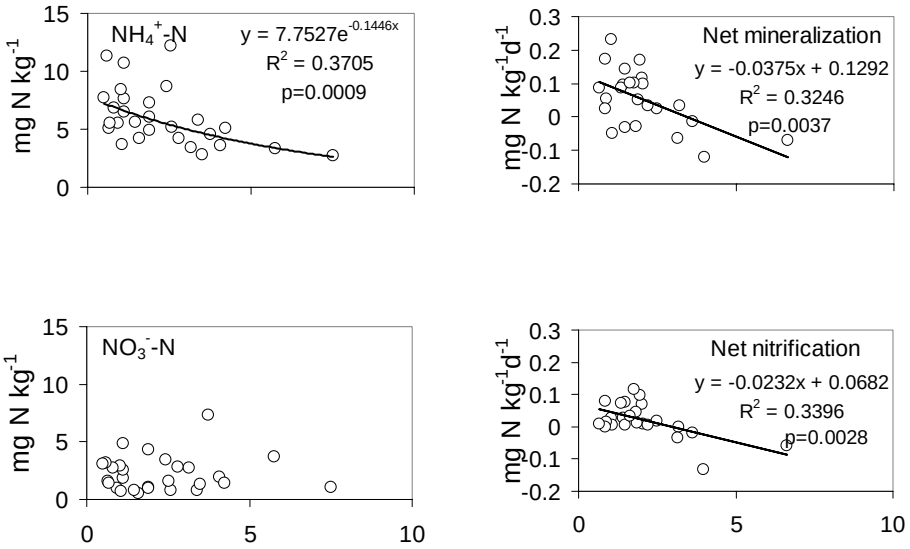


Figure 7. The relationships of soil respiration rates with NH_4^+-N concentration, NO_3^--N concentration, net N mineralization and nitrification rates across the treatments.

4. **Discussion**

This study provides the direct field experimental evidence that plant production affects N availability by altering plant C substrate input to soil and the amount of energy

available for heterotrophic metabolism as suggested by *Zak et al.* [1994]. Reduction in plant C substrate through BR and/or S increased soil inorganic N concentrations (Figs. 3 and 4) primarily by reducing microbial biomass C (Table 1) and hence microbial N immobilization, and by eliminating plant N uptake. Likewise, BR and/or S caused higher net N mineralization and nitrification rates than the control (Figs. 5 and 6) likely due to the lower microbial N immobilization with reduced microbial biomass C content. These results, together with those of *Wan and Luo* [2003], suggest that BR and/or S caused 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 (Fig. 7). Soil respiration, the proxy for plant C substrate availability, accounted for a substantial part of the seasonal variations in NH_4^+-N, net N mineralization and nitrification rates, while soil microclimate explained the major variations in NO_3^--N concentration (Table 2). Overall, our results indicate that plant C substrate determines the major soil N pool and fluxes by limiting microbial activity and plant N uptake, and that soil microclimate is an important factor affecting NO_3^--N production.

4.1. Plant C substrate effects on soil N dynamics

Plants may affect soil N dynamics directly through root N uptake and indirectly through regulating C input into the soil. Studies have shown that removing organic matter and reducing plant activity may directly or indirectly limit microbial growth [*Zak et al.*, 1990; *Ohtonen et al.*, 1992]. Thus, the higher soil inorganic N concentrations under BR

and/or S observed in the present study may have been caused by the reduced plant root N uptake and microbial N immobilization under the low C substrate levels (Fig. 5, Table 1). Reduced plant N requirements and higher soil NH_4^+-N and/or NO_3^--N concentrations have also been observed in other C substrate reduction experiments, including shading [*Jonasson et al.*, 1999] and litter removal [*Reynolds and Hunter*, 2001] studies. C substrate reduction by BR and/or S also increased net N mineralization and nitrification rates compared to the control (Fig. 6), primarily due to the reduced microbial N immobilization under BR and S. Previous studies have reported that reducing C substrate by grazing [*Holland and Detling*, 1990; *Johnson and Matchett*, 2001] and vegetation removal [*Ohtonen et al*., 1992] increased net N mineralization and nitrification rates by decreasing microbial N immobilization.

In addition to comparing the control with the 3 treatments, the differences found between S and BR+S in both soil N pools and fluxes provided further support for the regulation of plant C substrate on soil N dynamics. Compared with BR+S, S increased NH_4^+-N, total inorganic N concentrations, net N mineralization and nitrification rates (Figs. 5 and 7), primarily due to the more plant C materials under shading because the soil microclimate was quite similar under BR+S and S. Since soil microclimate was also altered by BR and/or S (Fig. 2), it could affect soil N dynamics by changing soil microbial and faunal activities. By comparing the soil N pools and fluxes between BR+S and BR, we were able to isolate the soil microclimate effect on soil N dynamics. Our results that NH_4^+-N concentration was higher while net nitrification rates were lower under BR than under BR+S indicate that soil microclimate mainly determines net nitrification process under similarly low C substrate levels. It is likely that BR+S

provided a more favorable microclimate for nitrifying bacteria than BR, resulting in more NH_4^+-N consumption by nitrifiers and higher net nitrification rates. Nitrifiers have been reported to be more sensitive to soil microclimate than ammonifers [*Richards et al.*, 1985]. Overall, our study suggests that plant C substrate determines the major soil N pools and fluxes while soil microclimate plays an important role in regulating net nitrification rate. The interactive effects of soil substrate and microclimate were also reported in other ecosystems [*Burke*, 1989; *Nadelhoffer et al.*, 1991].

4.2. Factors affecting temporal soil N dynamics

Although soil temperature and moisture are usually considered to be the main factors determining the temporal soil N dynamics, the temporal soil N dynamics is also closely related to the labile organic substrate available to the actively mineralizing microbial populations during the growing season [*Nunan et al.*, 2000; *Corre et al.*, 2002]. For example, in studying the spatial and seasonal variation in gross N transformations and microbial biomass in a Northeastern US grassland, *Corre et al.* [2002] reported that the seasonal patterns of NH_4^+ were not directly related to moisture and temperature, but possibly indirectly through the effects of these seasonal environmental factors on flushes of available organic matter which, in turn, affect the microbial biomass. By measuring soil N dynamics and using soil respiration as a proxy of plant C availability under BR and S, we were able to examine the plant C substrate effects on temporal soil N dynamics. Multiple regression analyses 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 per se (Table 2). This result suggests that plant C substrate availability reflected from soil respiration is more important in determining the temporal

N dynamics of NH_4^+-N concentration, net N mineralization and nitrification rates than soil microclimate, which generally supports the findings of *Nadelhoffer et al.* [1991] and *Corre et al.* [2002].

4.3. Coupled soil C and N dynamics during growing season

Since most organic N in soil is covalently bound to soil C, soil C and N dynamics are closely coupled [*Schimel et al.*, 1994; *Paul and Clark*, 1996, *Murphy et al.*, 2003, also see Fig. 1]. Many studies have examined the coupled C and N dynamics by incubating soil samples in the laboratory, and reported positive relationships between microbial respiration and potential net N mineralization and nitrification rates [*Nadelhoffer et al.*, 1991; *Zak et al.*, 1993a; *Zak et al.*, 1994; *Ruess and Seagle*, 1994; *Updegraff et al.*, 1995]. A recent study examined the gross N mineralization and nitrification rates after adding straw to mixed bare soil using ^{15}N pool dilution method [e.g., *Recous et al.*, 1999], which also reported positive relationships between mineralized C and mineralized N. However, given that all those studies did not measure the coupled C and N dynamics with live growing plants in the field, these positive relationships could be different with the effects of live growing plants on soil N dynamics because plant roots and mycorrhizae probably compete for NH_4^+-N and supply labile C to microbial heterotrophs during the growing season [*Hart et al.*, 1994].

Our results suggest that the coupled *in situ* soil C and N cycles when the live plants were present were negatively correlated across the treatments temporally (Fig. 7) rather than positively correlated as reported by laboratory incubation studies. Both BR and S obviously cut off the plant C substrate input pathways and reduced plant root activity, causing lower root and microbial respiration [*Wan and Luo*, 2003], and lower plant N

uptake. The lowered plant N uptake and reduced microbial N immobilization might have led to the higher inorganic N pools under BR and S (Fig. 4), while the reduced microbial N immobilization and/or higher gross N cycling rates under S may have caused the higher net N mineralization and nitrification rates (Fig. 7). Similar inverse relationships between concomitantly measured soil respiration and soil N dynamics have been observed in arctic [*Nadelhoffer et al.*, 1991], forest [*Ohtonen et al.*, 1992], and grassland [*Johnson and Matchett*, 2001] ecosystems. For example, *Johnson and Matchett* [2001] reported that grazing increased net N mineralization and nitrification rates but reduced soil respiration, probably because continuous grazing reduced the labile C input to the rhizosphere and induced microbial C limitation to both soil respiration and N immobilization. This negative relationship between soil respiration and soil N dynamics provides evidence that the coupled C and N cycles are not only controlled by microbial activity as suggested by laboratory incubation studies, but also determined by plant growth activity and plant labile C substrate input in the field conditions. Therefore, plant growth effects via labile C input and N uptake should be carefully considered when the coupled C and N cycles are to be simulated in ecological modeling.

5. Conclusions

Plant C substrate reduction by BR and/or S increased soil inorganic N concentration primarily by reducing plant N uptake and microbial N immobilization, whereas S accelerated net N mineralization and nitrification rates likely by reducing microbial N immobilization. Comparisons between treatments, the negative relationships of soil respiration with soil N pools and fluxes, and multiple regression analyses all suggest that plant C substrate is more important in determining NH_4^+-N, net N mineralization and

nitrification rates than soil microclimate; soil microclimate appears very important in determining NO_3^--N production. The most important finding of this study is that it provides field evidence that plant C substrate, rather than only soil microbes as suggested by laboratory incubation studies, is the ultimate determining factor of soil N dynamics and the coupled C and N cycles in the field conditions.

Our results potentially have important implications for ecosystems undergoing plant growth and plant C substrate changes under the changing global climate. Since plant production affects N availability by altering plant C substrate input as suggested by *Zak et al.* [1994] and evidenced by the present study, changes in plant growth and productivity and their effects on soil microbes under global warming [*Shaver et al.*, 2000], elevated CO_2 [*Zak et al.*, 1993b; *Diaz et al.*, 1993; *Hungate et al.*, 1996], and N deposition [*Aber et al.*, 1993] may substantially affect the N response and its feedbacks to global climate change. Therefore, plant growth effects on *in situ* soil N dynamics, either via plant C substrate input or plant N uptake, should be carefully considered when measuring and predicting ecosystem C and N responses to different driving factors of global change.

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CHAPTER III

Litter decomposition of standard litter, *in situ* litter quality and soil microbial biomass under warming and clipping in a tallgrass prairie [*]

[*] This part has been submitted to Global Change Biology (in review)

Abstract

Little is known about how global warming and land-use change interactively affect litter decomposition. In this study, I used standard litter materials of two dominant plant species to investigate the effects of warming and clipping on litter decomposition and nitrogen (N) dynamics in a tallgrass prairie in the central US. The two dominant plant species were little bluestem (*S. scoparium*, shoot and root) and western ragweed (*A. psilostachya*, shoot). Overall, warming and clipping together significantly altered the decomposition rate of *S. scoparium* shoot litter alone among the 3 litter types. However, the treatment effects were mainly caused by clipping, with the mass remaining percentage of *S. scoparium* shoot litter under clipping being lower than that under the control. Warming and clipping did not affect the net N immobilization/release of the 3 litter types. However, there were significant differences in the decay constant k and N remaining percentages among the 3 litter types due to their different initial litter quality. *S. scoparium* shoot litter decomposed slower than *A. psilostachya* shoot litter and *S. scoparium* root litter. *S. scoparium* shoot litter did not release any N while *A. psilostachya* shoot and *S. scoparium* root litter released about 30% and 5% of the original N. In addition to the standard litter, I also measured the *in situ* litter quality and soil microbial biomass in order to look at how warming and clipping would affect the long-term ecosystem C and N cycles by affecting *in situ* litter quality. Warming increased *in situ* litter C/N ratio, whereas clipping reduced *in situ* litter C/N ratio and microbial biomass N content of the dominant plant species, suggesting that warming and clipping may alter the long-term ecosystem C and N cycles by changing the *in situ* litter quality and microbial biomass nutrients. Our standard litter decomposition results indicate that

land-use change may have more impacts than global warming on litter decomposition. The *in situ* measurements indicate that warming and clipping might affect the long-term ecosystem C and N cycles by changing *in situ* litter quality and microbial biomass nutrients via changes in both microclimate and plant substrate input.

Keywords: clipping, global warming, grassland, litter decomposition, microclimate, nitrogen dynamics, tallgrass prairie.

Introduction

The global surface temperature has increased by more than 0.5 °C over the past century and is predicted to increase by 1.4-5.8 °C over the period from 1990 to 2100 (Wigley & Raper, 2001). As a major process determining ecosystem C and N cycles (Aerts & De Caluwe, 1997), litter decomposition has been reported to increase under elevated temperature (Melillo *et al.*, 1990; Raich & Schlesinger, 1992). Increased litter decomposition under warming may back feed on global climate changes by (1) increasing CO_2 release to the atmosphere (Schimel *et al.*, 1990; Townsend *et al.*, 1992), and (2) stimulating nutrient availability for plants to increase net primary productivity (NPP) of N-limited ecosystems (Schimel *et al.*, 1990; Melillo *et al.*, 1993). Land-use change is another important driving factor of global climate change (Shaver *et al.*, 2000). Given that warming and land-use change happens concurrently, climate warming and land-use change may interactively affect litter decomposition by altering soil microbial activity and community structure (Zhang *et al.*, in press). Therefore, accurate prediction of climate change effects on long-term ecosystem C and N cycles requires better

understanding of litter decomposition under both warming and land-use changes (Shaver *et al.*, 2000; Rustad *et al.*, 2001).

Litter decomposition rates are controlled by soil temperature, moisture, litter quality, and the composition and dynamics of the decomposer community (Swift *et al.*, 1979). Thus, climate warming may affect litter decomposition directly by changing soil temperature and moisture via their effects on soil microbial activity (Nadelhoffer *et al.*, 1992; Carreiro & Koske, 1992), and indirectly by altering litter quantity and quality (Meentemeyer, 1978; Cornelissen, 1996), nutrient availability (Vitousek & Sanford, 1986; Lavelle *et al.*, 1993; Schimel *et al.*, 1994) and plant species composition (Harte & Shaw 1995). Similarly, clipping for hay (or haying), a major agricultural land-use type in central US grasslands, may affect soil microbes by reducing plant C substrate input and altering soil microclimate (Wan *et al.*, 2002; Wan & Luo, 2003), and hence litter decomposition. Although a few studies have reported warming effects on litter decomposition (e.g., Shaw and Harte, 2001), little experimental evidence exists on how warming and land-use change interactively affect litter decomposition.

Early studies on warming effects on litter decomposition have been carried out under environmentally controlled conditions (e.g., Hobbie, 1996), which eliminate the confounding effects of soil moisture changes under warming (Kirschbaum, 2000; Shaw & Harte, 2001). Quite a few studies have tried to reveal the interactive effects of temperature and moisture, among other factors, on litter decomposition along environmental gradients (Scowcroft *et al.*, 2000; Murphy *et al.*, 1998). However, very few have investigated this issue under field warming conditions (Shaw & Harte, 2001). In a subalpine ecosystem, Shaw & Harte (2001) reported that warming increased one-year

leaf litter decomposition in the mesic zone and reduced one-year leaf litter decomposition in the xeric zone of their study site.

In order to study the effects of warming and land-use change on ecosystem structure and function, a long-term experimental warming and clipping (mimicking haying) field experiment was initiated in a tallgrass prairie ecosystem since 21 November 1999 in Central Oklahoma, USA (Luo *et al.*, 2001). Previous studies in this field experiment have reported that warming caused higher percentage of C_4-grass in the increased aboveground biomass (Wan & Luo, unpublished data). Given that, we reasoned (1) that changes in *in situ* litter quality and soil microbial biomass, caused by both microclimate change and plant substrate changes under warming and clipping would exert strong impact on the long-term ecosystem C and N cycles via changes in plant community structure. The objectives of this study were: (1) to determine how warming and clipping affect litter decomposition of standard litter of two dominant plant species; (2) to examine whether warming and clipping would affect the in situ litter quality and surface microbial biomass and hence potentially influencing the long-term ecosystem C and N cycles. To obtain these goals, we measured litter decomposition of standard litter of two dominant plant species, one C_4 grass and one C_3 forb, in a tallgrass prairie under experimental warming and clipping. We also measured the *in situ* litter quality of the two dominant plant species, and surface soil microbial biomass C and N contents.

Materials and Methods

Site description

The experimental site is located at the Great Plain Apiaries (34°58'54"N, 97°31'14"W), 40 km from the Norman campus of the University of Oklahoma, USA. Detailed description of the site characteristics and design of the experiment have been reported else where (Luo *et al*. 2001; Wan *et al.*, 2002). Briefly, the site is a tallgrass prairie primarily dominated by C_4 grasses including little bluestem (*S. scoparium* (Michx.) Nash-Gould), Indian grass (*Sorghastrum nutans* (L.) Nash.), and weeping lovegrass (*Eragrostis curvula* (Schrad.) Nees), and a few C_3 forbs, including western ragweed (*A. psilostachya* DC.), common boomweed (*Hemiachyris dracunuloides*), and bottomland aster (*Aster ontarionis* Wieg.). The percent coverage of *S. scoparium* is higher than 80% of the entire community, and *A. psilostachya* is the most dominant C_3 forb in this community (Bowdish, 2002). Mean annual temperature is 16.0 °C with monthly mean temperature of 3.1 °C in January and 28.0 °C in July. Mean annual precipitation is 911.4 mm (Oklahoma Meteorological Survey). The soil is part of the Nash-Lucien complex, which is characterized by having a low permeability, high available water capacity, and deep, moderately penetrable root zone (USDA Soil Conservation Service and Oklahoma Agricultural Experiment Station, 1963).

Experimental design

This experiment was designed as a split-plot design with warming as the main factor and clipping as the secondary factor. Six pairs of 2m × 2m plots were chosen to establish the warming and control treatments, respectively. Each 2m × 2m plot was divided into four 1m × 1m subplots under both warming and control. Two diagonal subplots in each

main plot were clipped 10 cm above the ground in July or August of every year, which removed about 85% of the aboveground biomass (Luo *et al.*, 2001). The other two diagonal subplots were the unclipped subplots. Totally, there were six replicates for each treatment, i.e., unclipped control (UC), clipped control (CC), unclipped warming (UW), and clipped warming (CW). An infrared radiator (Kalglo Electronics, Bethlehem, Pennsylvania) suspended at 1.5 m above the ground in each warmed plot was used as the heating device. A "dummy heater" with the same shape and size as the infrared heater was suspended at the same height in the control plots to simulate the shading effect of the heater on the plant canopy. The plots have been warmed continuously since November 21, 1999.

Soil temperature and moisture

At the centers of one clipped and one unclipped subplot in each plot, thermocouples were installed at the depth of 2.5 cm to measure soil temperature. All the thermocouples were connected to a CR10 datalogger (Campbell Scientific Inc., Utah, USA). Soil temperature was measured every ten minutes, and then averages within one hour were stored in an SM196 Storage Module. In addition, soil temperature at the depth of 5 cm was measured adjacent to each PVC collar at the time of the soil respiration measurement using a thermocouple connected to Li-Cor 6400 (Luo *et al.*, 2001). Soil moisture (% volumetric) at the depth of 15 cm was measured twice a month using Time Domain Reflectometry (Soilmoisture Equipment Corp., Santa Barbara, CA).

Litter decomposition

Litter decomposition was measured using the litter-bag method. The most dominant C_4 grass, *S. scoparium*, and the most dominant C_3 forb, *A. psilostachya*, were chosen to

be the standard litter materials. The dead standing shoots (leaf plus stem) of *S. scoparium* and *A. psilostachya* grown under ambient temperature conditions out of but very close to the plots, were collected in February 2001. Since it was difficult to collect the senesced root materials, we collected the live root of *S. scoparium* from the dead standing *S. scoparium* as in King *et al.* (1997). The root samples were then washed and cleaned using tap water in the laboratory. Before filling the bags with litter, all the litter materials were air-dried for four months at room temperature. The moisture content of the litter was measured to calibrate the dry weight of the litter materials from air-dried to oven-dried weight before filling the bags.

The litter bags were made of fiberglass mesh (1.5 × 1.0 mm). One 15 cm × 20 cm bag was sewed into two halves, one half for *S. scoparium* sample (~5g), another half for *A. psilostachya* sample (~4g). Another small litter bag (10 cm × 15 cm) was made to accommodate the root sample (~2g). The initial weights of litter bag contents were recorded for future use. On 1 June 2001, the litter bags for *S. scoparium* and *A. psilostachya* were anchored on the soil surface with iron wire to make sure complete contact between the litter bags and the surface soil. The litter bags for root litter were buried vertically within the 0-15 cm soil layer. In each of the four subplots within one plot, we put 3 bags of *S. scoparium* and *A. psilostachya* litter on the soil surface, and 3 bags of root litter in the soil. In this study, we only used five of the six pairs of plots in order to reduce the cost and labor. Therefore, we totally had 120 shoot and 120 root litter bags in the plots. We retrieved one shoot litter bag and one root litter bag each time for 6 times after 2, 4, 8, 12, 17 and 24 months of decomposition on 1 August 2001, 3 October 2001, 3 February 2002, 3 June 2002, 6 November 2002, and 2 June 2003, respectively.

Given the limited space of the plots, we were not able to put replicated litter bags for each collection date and to retrieve more than one litter bag each time. However, since we managed to minimize the spatial heterogeneity of the plots when we chose this site, we assumed that one bag each time for both shoot and root litter should be adequate to reflect the real situation.

After the litter bags were collected, the debris and dust on the litter surface were gently removed by soft brush. Litter contents in the bags were then oven dried at 65 °C for 48 hrs. After drying, the masses of the litter bag contents were weighed. All the samples were milled, and sent to the Water and Forage Analytical Laboratory of the Oklahoma State University for analyses of C and N concentrations. Plant C concentration was determined using a dry combustion carbon analyzer (Nelson & Sommers, 1996), and N concentration using a dry combustion Nitrogen Analyzer (LECO 428, National Forage Testing Association, 1993). The subsamples of the initial litter materials for each litter types were also analyzed for total C and N. In addition, the lignin content of the initial litter materials was analyzed using the methods formulated by the Association of Official Analytical Chemists (Association of Official Analytical Chemists, 1990) in A&L laboratories, Inc. (Memphis, TN).

The decay constant, k, was determined by fitting a single exponential decay function to each species for each treatment after different decomposition time periods (Weider & Lang, 1982):

$$\ln(M_t/M_0) = -kt + c,$$

Where M_t is the mass at time t, M_0 is the initial mass, t is the time in days, k is the slope of the regression, and c is the intercept of the regression. This model was used to

calculate a single variable (k) of litter decomposition to facilitate comparisons across species and treatments, and to correlate decomposition rate with soil microclimate and site quality indices.

In situ litter quality and microbial biomass

The *in situ* litter (the litter materials within the plots, not the litter used for decomposition) of *S. scoparium* and *A. psilostachya* were collected from inside the plots in May 2003. Litter materials of *S. scoparium* and *A. psilostachya* were cleaned by soft brush in the laboratory right after collection. All the litter materials were oven-dried at 65°C for 48 hours and then milled. Total C and N concentrations of the litter materials were analyzed using the same methods as mentioned previously in the Water and Forage Analytical Laboratory of the Oklahoma State University.

Surface soil microbial biomass C and N contents in the 0-3 cm surface soil close to the litter bags were analyzed using the chloroform fumigation-extraction method (Vance *et al.*, 1987). The soil samples were collected in June 2003, shortly after the completion of the litter decomposition experiment. Carbon 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). In order to see how surface soil microbial biomass affected litter decomposition rates of the litter of the two dominant species, we correlated the microbial biomass C and N contents with the decay constant k and N remaining percentages of *S. scoparium* and *A. psilostachya* after 24 months of decomposition. Since root litter was buried in the 0-15 cm of soil layer, k and N remaining percentage of root were much less

affected by surface soil microbial activity, we did not include the k of root in the correlation analyses.

Statistical analyses

The treatment effects on *in situ* litter quality, soil microbial biomass, mass remaining percentages during different decomposition periods were analyzed with the analysis of variance (ANOVA) of split-plot design. The treatment effects on the overall mass remaining percentages and N remaining percentages were analyzed with the repeated-measures ANOVA. The differences in initial litter quality among the 3 litter types were analyzed using one-way ANOVA. All the statistical analyses were carried out with SAS software package (SAS institute, Cary, NC).

Results

Soil microclimate changes

On average, warming increased soil temperature by about 1.0 ºC and 1.4 ºC under unclipped and clipped subplots, respectively (Fig. 1a). Clipping increased soil temperature by 1.8 ºC and 2.2 ºC under warmed and unwarmed plots, respectively (Fig. 1a). The averaged soil moistures under warmed unclipped and warmed clipped plots were 1.1% and 1% lower than the control, respectively. Clipping reduced soil moisture less than warming, with the averaged soil moisture being 0.8% and 0.7% lower under clipped subplots with and without warming than under unclipped subplots, respectively (Fig. 1b).

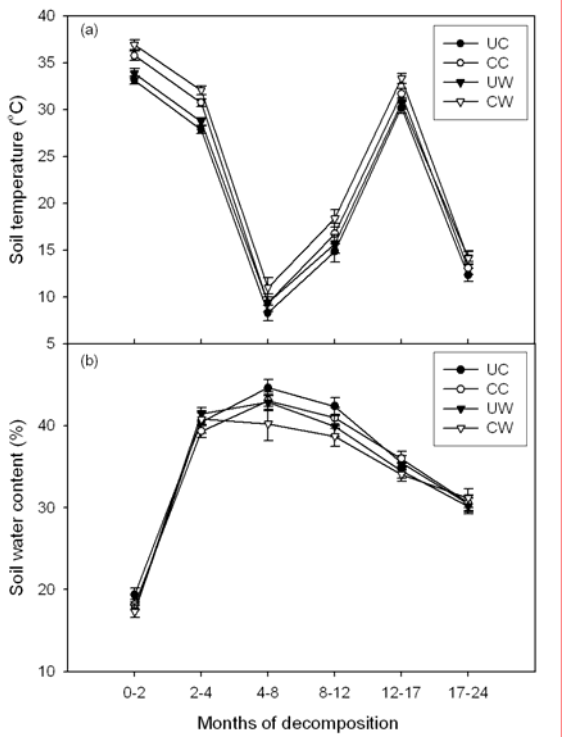


Figure 1. The averaged soil temperature (a) and soil moisture (b) during different decomposition periods. 0-2 months of decomposition (MOD): June 1, 2001-August 1, 2001; 2-4 MOD: August 1, 2001-October 3, 2001; 4-8 MOD: October 3, 2001-February 3, 2002; 8-12 MOD: February 3, 2002-June 3, 2002; 12-17 MOD: June 3, 2002-November 6, 2002; 17-24 MOD: November 6, 2002-June 2, 2003.

Initial litter quality

The initial litter quality of the 3 standard litter types differed significantly ($p<0.05$) (Table 1). No significant differences were observed for total C concentrations among the 3 standard litter types. In contrast, total N concentration of *A. psilostachya* was the highest, followed by root and *S. scoparium*. C/N ratio was the highest for *S. scoparium* and lowest for *A. psilostachya*, with root being in the middle. There were no differences in lignin concentration between root and *A. psilostachya*, and between *S. scoparium* and

A. psilostachya, but lignin concentration of root was significantly higher than that of *S. scoparium*. Lignin/N ratios of *S. scoparium* and its root were very close, being significantly higher than that of *A. psilostachya*.

Table 1. Initial litter chemistry of *S. scoparium*, *A. psilostachya*, and root of *S. scoparium*. Each value is the mean (with SE reported in parentheses) of five samples and reported on a percentage dry mass basis. Different letters following the values indicate significant difference in litter quality between litter types (One-way ANOVA).

Litter type	Total C (%)	Total N (%)	C/N ratio	Lignin (%)	Lignin/N ratio
S. scoparium	48.10 (0.38)a	0.35 (0.01)c	139.6 (4.3)a	13.4 (1.0)b	38.5 (2.1)a
A. psilostachya	47.77 (0.40)a	0.77 (0.02)a	62.3 (1.2)c	15.6 (0.7)ab	20.4 (1.1)b
Root of *S. scoparium*	46.71 (0.94)a	0.60 (0.02)b	78.5 (2.3)b	17.2 (0.8)a	29.0 (1.8)a

Litter mass loss

Overall, there were significant treatment effects on the mass remaining percentage of *S. scoparium* shoot litter ($p=0.0218$) (Fig. 2). However, the significant treatment effects were mainly caused by clipping. The mass remaining percentages of *S. scoparium* shoot litter were significantly lower under clipped control plots than under unclipped control plots ($p=0.0347$), suggesting higher decomposition rate under clipping treatment. By contrast, warming did not significantly affect the mass remaining percentage of *S. scoparium* shoot litter ($p>0.05$). However, significantly interactive effects of warming

and clipping on the mass remaining percentage of *S. scoparium* shoot litter were observed after 4 months of decomposition (ANOVA of split-plot design, $p=0.0259$), with clipped warmed plots having higher mass remaining percentage than unclipped warmed plots ($p<0.05$). No significant overall treatment effects on the mass remaining percentages were observed for *A. psilostachya* shoot litter and *S. scoparium* root litter ($p>0.05$). However, clipping significantly increased the mass remaining percentage of root litter after 2 months of decomposition (ANOVA of split-plot design, $p=0.0121$), whereas warming reduced the mass remaining percentage of root litter (ANOVA of split-plot design, $p=0.0122$) after 17 months of decomposition.

Litter nitrogen changes

There were no significant treatment effects on net N immobilization/release (as indicated by the N concentration percent changes) of the 3 litter types ($p>0.05$) (Fig. 3). However, there was a clear trend that net N immobilization of both *S. scoparium* and *A. psilostachya* was lower under warmed plots after two years of decomposition, suggesting that litter under warming tended to immobilize less N from the soil. In contrast, clipping tended to increase net N immobilization of both *S. scoparium* and *A. psilostachya* after two years of decomposition.

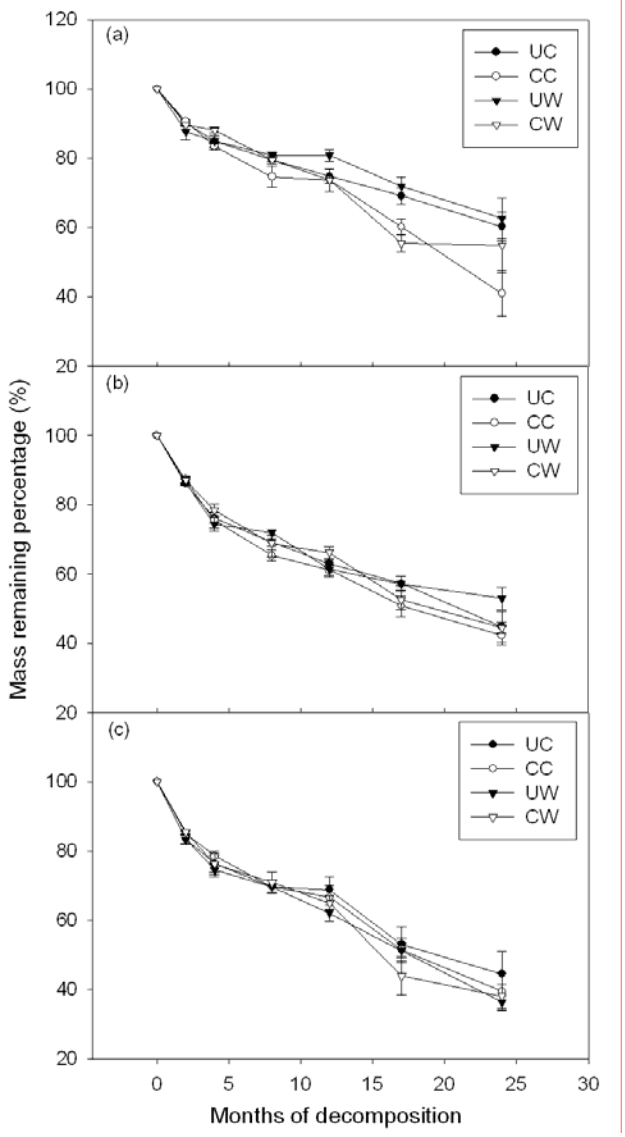


Figure 2. The mass remaining percentages of *S. scoparium* (a), *A. psilostachya* (b) and root of *S. scoparium* (c) under different treatments.

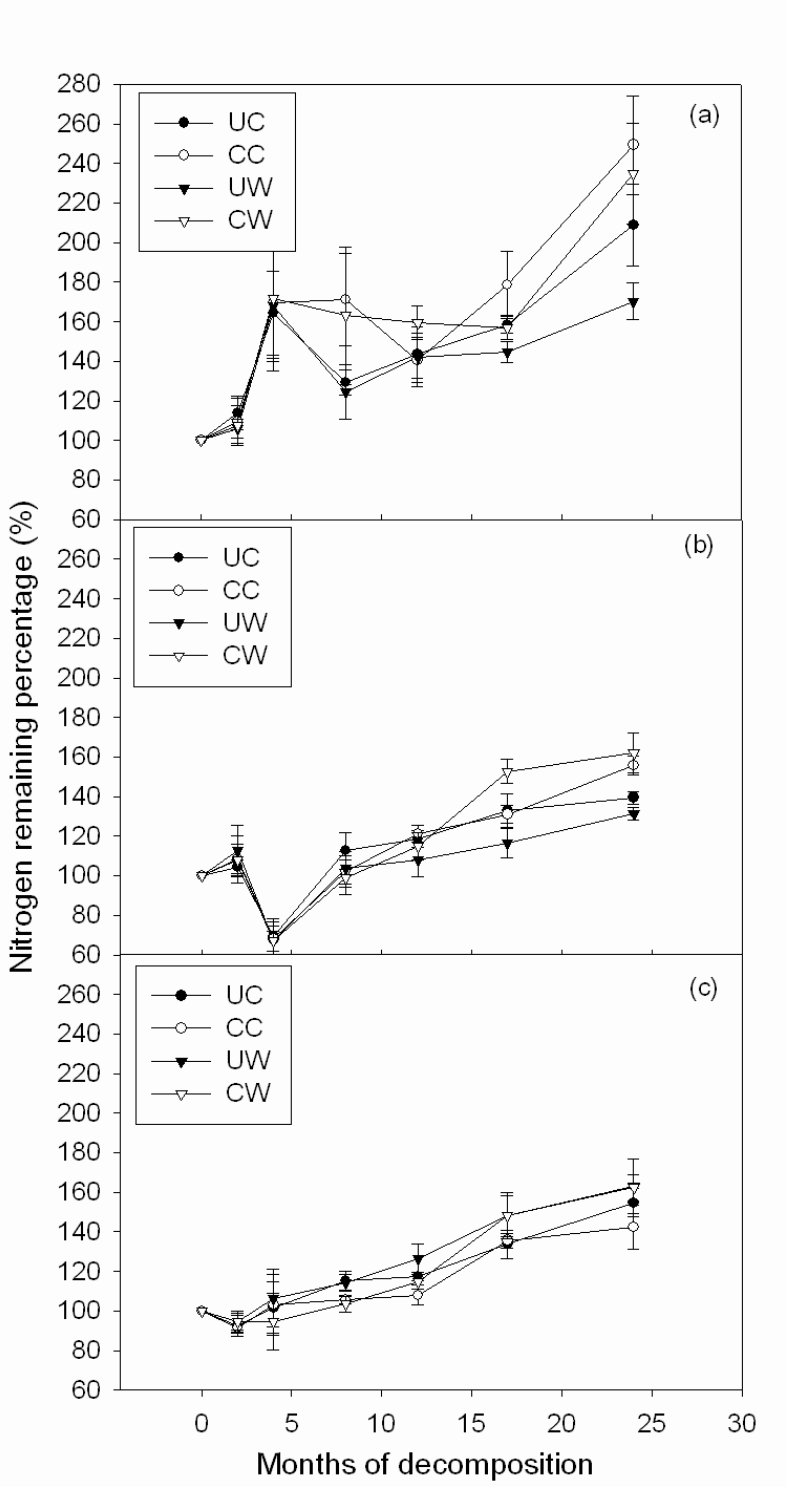


Figure 3. The N remaining percentages of *S. scoparium* (a), *A. psilostachya* (b) and root of *S. scoparium* (c) under different treatments.

Comparisons of k and N remaining percentages among the three litter types

Primarily due to the different initial litter quality, the decay constant k differed significantly among the 3 litter types during different decomposition stages ($p<0.05$) (Fig. 10). The decay constant k of *A. psilostachya* was generally higher than that of *S. scoparium* during most of the decomposition periods across the treatments ($p<0.05$, Fig. 4). Decay constant k of root was always higher than that of *A. psilostachya* during the first 2 months of decomposition, but either similar to or lower than that of *A. psilostachya* under all but the unclipped warmed treatments during the later decomposition periods.

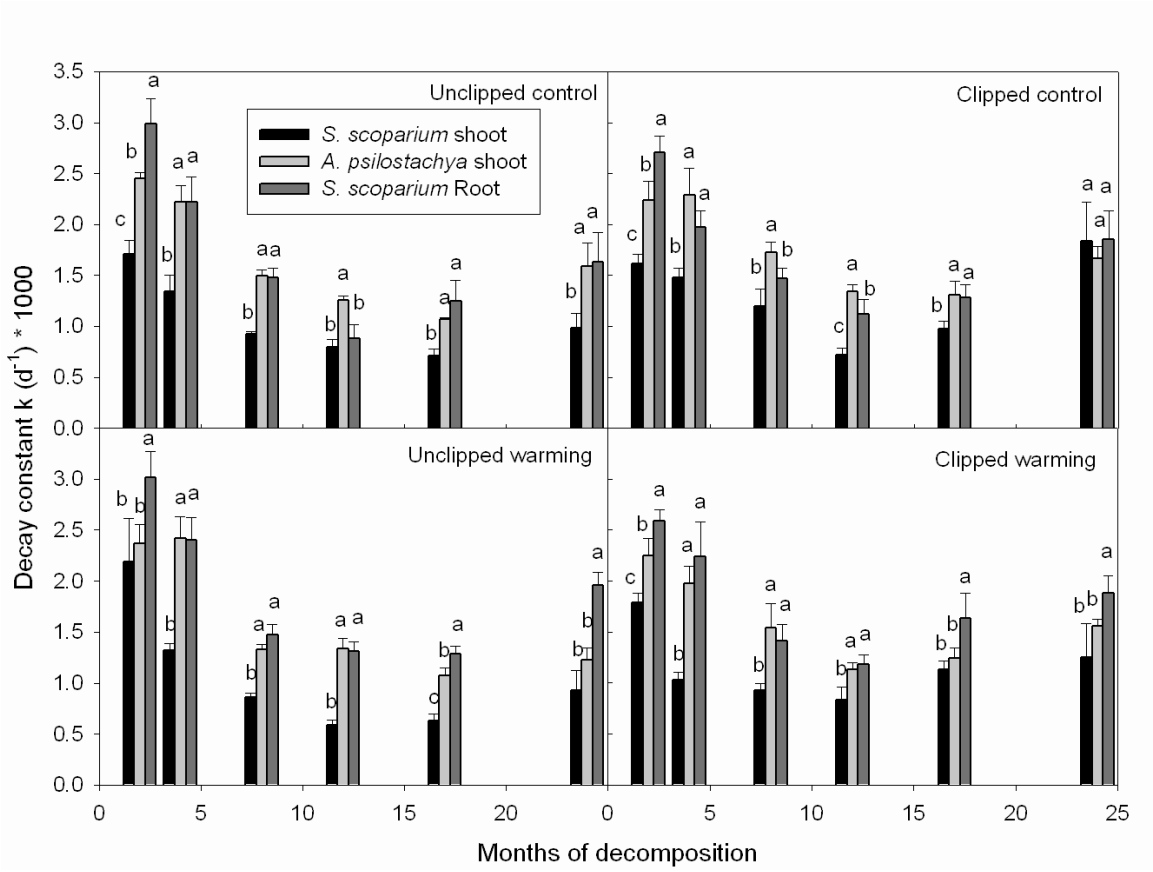


Figure 4. Comparisons of decay constant k values of *S. scoparium*, *A. psilostachya* and root of *S. scoparium* under unclipped control (a), clipped control (b), unclipped warming (c) and clipped warming (d) treatments.

As with the decay constant k, the 3 litter types also differed in their timing and pattern of N release. *S. scoparium* shoot litter did not release any N after two years of decomposition, whereas *A. psilostachya* and root of *S. scoparium* released about 30% and 5% of their original N concentrations, respectively, during early decomposition periods (Fig. 3). The final N remaining percentage of *S. scoparium* (170-250%) was much higher than those of *A. psilostachya* (120-160%) and root (130-160%), suggesting more N being immobilized by *S. scoparium* than by *A. psilostachya* and root.

In situ litter quality

The *in situ* litter mentioned here was the litter produced within the treatment plots, which may reflect the accumulating treatment effects of plant physiology and nutrient use efficiency to a great extent. Our results showed that warming and clipping together significantly altered the *in situ* litter quality of *S. scoparium* ($p<0.0001$) and *A. psilostachya* ($p<0.05$) (Fig. 5). Warming reduced total N ($p=0.0026$), increased total C ($p=0.0131$) and C/N ratio ($p=0.0032$) of the *in situ* litter of *S. scoparium*. By contrast, clipping increased total N ($p=0.0335$), reduced total C ($p<0.0001$) and C/N ratio ($p=0.0048$) of the *in situ* litter of *S. scoparium*. There were significant warming and clipping interactive effects on the total N ($p=0.0388$) and C/N ratio ($p=0.025$) of the *in situ* litter of *S. scoparium*. For *A. psilostachya*, warming significantly reduced total N concentration ($p<0.0001$), and increased C/N ratio ($p<0.0001$); clipping only reduced total C concentration ($p=0.0214$), but did not affect total N and C/N ratio.

Surface soil microbial biomass

On average, warming did not alter the surface soil microbial biomass C and N contents, but tended to increase them by about 14% and 21%, respectively (Fig. 6).

Clipping reduced microbial biomass C and N contents by 18% and 30%, respectively, but only significantly for microbial biomass N (p=0.0011) (Fig. 6). As a result, warming did not affect microbial biomass C/N ratio while clipping tended to increase it.

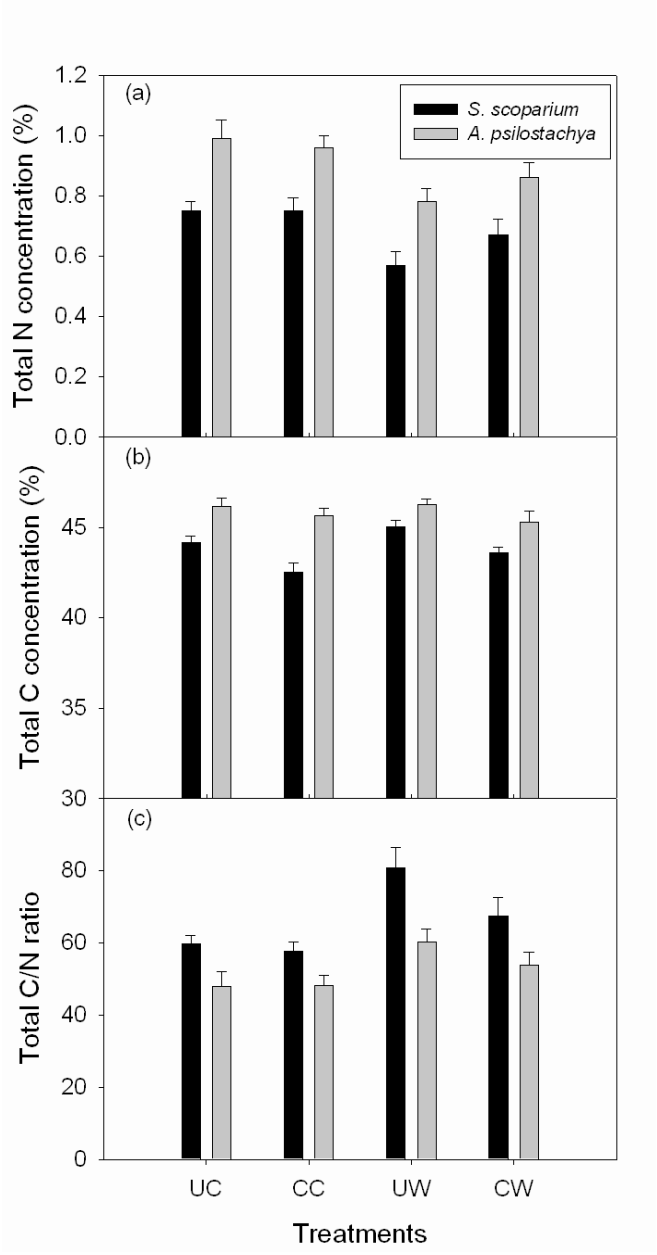


Figure 5. The total C concentration (a), total N concentration (b) and C/N ratio (c) of the *in situ* litter of *S. scoparium* and *A. psilostachya*.

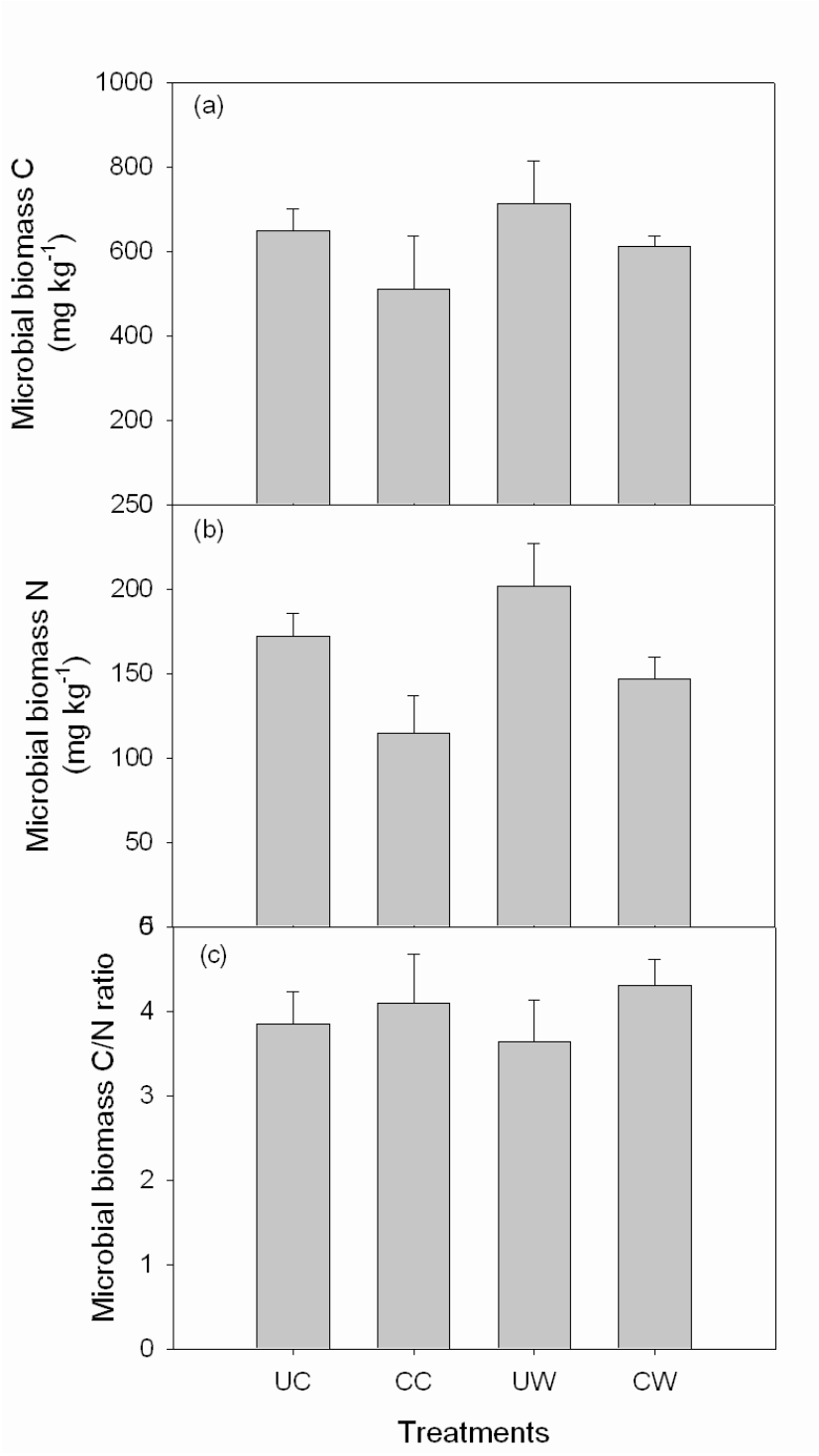


Figure 6. Soil microbial biomass C (a) and N (b) contents, and microbial biomass C/N ratio (c) in the surface soil (0-3 cm) under different treatments.

Discussion

Little is known about how warming and land-use change interactively affect litter decomposition (Rustad et al., 2001). Our results showed that warming and clipping significantly altered the two-year decomposition rate of shoot litter of *S. scoparium*, the most dominant species, but not those of *A. psilostachya* shoot and *S. scoparium* root litter (Fig. 2). However, the significant treatment effects were mainly caused by clipping, with litter decomposition under clipped treatment being higher than that under unclipped treatment (Fig. 2). The little warming effects were likely caused by the small temperature difference between warming and control, by warming and clipping interactions, and by the severe moisture limitation during the summer drought of 2001 (Fig. 1). The significant clipping effects were possibly due to the higher soil temperature and stronger substrate limitation to soil microbes under clipping, which caused soil microbes to be more active in exploring new substrates on the soil surface. In addition to the standard litter, warming and clipping significantly altered the *in situ* litter quality and microbial biomass (Figs. 5 and 6), potentially affecting the long-term ecosystem C and N cycles. Specifically, warming increased whereas clipping reduced the C/N ratio of the *in situ* litter of both *S. scoparium* shoot and *A. psilostachya* shoot. These results suggest that (1) land-use change (clipping in this case) may have more impact on litter decomposition than warming; (2) warming and clipping may affect the long-term ecosystem C and N cycles by altering the *in situ* litter quality and microbial biomass of the dominant plant species; (3) the greater dominance of C_4 grasses (Wan and Luo, unpublished data) and fungi (Zhang *et al.*, 2005) under warming may provide further evidence for the effects of

in situ litter quality and microbial biomass on the long-term ecosystem C and N cycles in tallgrass prairie.

Soil microclimate controls over litter decomposition

Soil microclimate is one of the major factors affecting litter decomposition rates (Swift et al., 1979). In field conditions, soil moisture usually confounds warming effects on litter decomposition due to its great impact on soil microbial activity (Griffin, 1981; Shaw & Harte, 2001). Our results suggested that the lower soil moisture under warming may have depressed litter decomposition during dry seasons and offset the potential stimulating warming effects on litter decomposition during wet seasons. Lower litter decomposition rates in dry habitats and dry seasons or under soil warming have been reported in other ecosystems (Cornejo *et al.*, 1994; Robinson *et al.*, 1995; Murphy *et al.*, 1998; Shaw & Harte, 2001; O'Neill *et al.*, 2003). In the present study, although warming did not affect the overall two years of litter decomposition rate, it did marginally reduce litter decomposition after 4 months of decomposition ($p=0.072$) when the soil just experienced a severe summer drought (Fig. 2). Moreover, the litter decomposition rate of *S. scoparium* shoot after 8 (or 12) months of decomposition was higher under the clipped (or unclipped) control treatment than under the clipped (or unclipped) warming treatment ($p<0.05$). The decrease in litter decomposition under warming was mainly caused by the lower soil moisture during these time periods (Fig. 1). Therefore, soil moisture changes mainly determined the litter decomposition rate during different decomposition stages and hence the overall warming effects.

Similar to warming, clipping also reduced the litter decomposition of *S. scoparium* during the summer drought of 2001 due to the soil drying effect ($p<0.05$). However,

clipping increased the litter decomposition rate during other periods, resulting in consistently positive effect on the overall two years of decomposition rate. Our results suggest that climate warming by about 1.2 °C may not be warm enough to cause dramatic differences in litter decomposition due to the moisture effects during dry seasons while changes in plant C substrate and higher temperature (about 2 °C) caused by land-use change (clipping in this case) may substantially affect litter decomposition.

In situ litter quality, microbial biomass and litter decomposition

Warming significantly reduced total N, increased total C and C/N ratio of the *in situ* litter of *S. scoparium* and *A. psilostachya* shoot litter (Fig. 5), resulting in lower *in situ* litter quality. This result was consistent with that of Jonasson *et al.* (1999), which reported that air warming reduced the N and phosphorus (P) concentrations of vascular plants in heath in an arctic ecosystem. The reduced nutrient concentrations of plants were primarily attributed to the nutrient dilution effects of extra growth of plants (Jonasson *et al.*, 1999), and possibly due to the higher nutrient resorption rate of plants under warming. A concomitant study in our experiment reported that warming significantly increased the total aboveground biomass, the majority of which being C_4 grasses (Wan & Luo, unpublished data), which could have substantially diluted the nutrients in the plant parts and caused lower nutrient concentrations. Another study reported that warming consistently increased N resorption rate of the dominant plant species in the same experiment for 4 years from 2000 to 2003 (An *et al.*, unpublished data).

In contrast to warming, clipping significantly increased total N, reduced total C and C/N ratio of *S. scoparium* and/or *A. psilostachya* shoot litter (Fig. 5), causing high-quality *in situ* litter. The increased total N concentration of *in situ* litter under clipping might be

resulted from the following two reasons. First, clipping might have reduced the total plant N uptake and left more available N for the standing live plants, causing higher N concentration in the plant materials. Second, clipping might have reduced the competition between plants and soil microbes in N nutrition, leaving more N for uptake by the standing live plants. In a clipping and shading experiment in the small tallgrass prairie, Su *et al.* (in review) found that clipping increased the extractable inorganic N primarily due to the less plant N uptake and lower microbial N immobilization.

Due to the opposite effects of warming and clipping on *in situ* litter quality, warming and clipping altered the surface microbial biomass C and N contents in opposite directions. Warming increased surface soil microbial biomass C and N contents by 14% and 21%, respectively, while clipping reduced microbial biomass C and N contents (Fig. 3), which was consistent with the results of 0-15 cm soil microbial biomass reported by Zhang *et al.* (in press). The increased microbial biomass under warming was mainly caused by the increases in the C input and in the total C concentration of the *in situ* litter while the reduced microbial biomass under clipping was primarily due to the decreased C input and the decrease in total C concentration of the *in situ* litter.

Implications and conclusions

Similar to Shaw and Harte (2001), no significant warming effects on two years of litter decomposition were observed in this study. However, warming may eventually reduce the long-term ecosystem litter decomposition through three mechanisms. Firstly, warming may reduce litter decomposition by lowering the *in situ* litter quality of the dominant plant species. Secondly, warming may reduce litter decomposition by increasing the amount of low-quality litter (C_4 grass) materials than high-quality litter (C_3

forb) given that C_4 grasses were more dominant both in coverage (Bowdish, 2002) and biomass (Wan & Luo, unpublished data) than C_3 forbs under warming. Thirdly, warming may reduce litter decomposition by reducing soil and litter moisture. The present study adds further evidence to the opinion that the increase of low-quality litter materials under warming may cause higher ecosystem C and N storage and slower long-term C and N cycling rates (Hobbie, 1996; Shaw & Harte, 2001).

Similar mechanisms existed for the clipping treatment. Clipping stimulated litter decomposition by increasing higher soil temperature and by reducing plant C substrate, causing substrate limitation to soil microbes. Clipping increased total N, reduced total C and C/N ratio of the *in situ* litter materials, resulting in high litter quality. Clipping also tended to increase the component of high-quality litter (C_3 forbs) compared with the control (Bowdish, 2002). All these results suggest that clipping may eventually increase the litter decomposition and ecosystem C and N turnover rates by altering soil microclimate, *in situ* litter quality and plant species composition.

In conclusion, warming and clipping together altered the decomposition rate of *S. scoparium* (the most dominant C_4 grass), but the effects were mainly caused by clipping. Warming and clipping reduced and increased the *in situ* litter quality, respectively, potentially having impact on the long-term ecosystem C and N cycles. Together with the previous studies reporting higher dominance of *S. scoparium* biomass (Wan and Luo, unpublished data) and fungi (Zhang *et al.*, in press), our results indicate that changes in plant and microbial community structure and changes in site quality may eventually affect the long-term bulk litter decomposition and ecosystem C and N cycles under climate change in tallgrass prairie ecosystem.

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CHAPTER IV

Nitrogen stimulates net ecosystem CO_2 exchange by enhancing canopy radiation-use efficiency and extending the growing season: experimental evidence from a mesocosm study[*]

[*] This part has been submitted to Acta Oecologia (in review)

Abstract

It is well established that nitrogen (N) fertilization stimulates photosynthesis by increasing light interception and plant radiation-use efficiency (RUE) at leaf- and plant-levels. However, little is known about how N affects net ecosystem CO_2 exchanges (NEE). We conducted a mesocosm experiment to investigate this issue by continuously measuring NEE of model grassland ecosystems with cheatgrass in two Ecologically Controlled Enclosed Lysimeter Laboratories (EcoCELLs). In 1999, we measured NEE without N additions to the two EcoCELLs; in 2000, we measured NEE with two N fertilization treatments, i.e., applying N fertilizer once to one EcoCELL (pulse fertilization, PF), and same amount of N biweekly to another (gradual fertilization, GF). We also measured canopy green leaf area index (LAI), plant N content and biomass, and calculated canopy RUE as the ratio of gross ecosystem photosynthesis to the intercepted photosynthetically active radiation (IPAR). The responses of NEE to IPAR were used to estimate the maximum ecosystem photosynthetic capacity (F_{max}). Canopy LAI, plant N content, F_{max}, RUE, NEE and biomass in 2000 were higher than those in 1999, suggesting dramatic N fertilization effects given that the environmental factors were quite similar between the two years. PF caused higher LAI, F_{max} and NEE than but similar RUE to GF during early growing season, whereas GF maintained higher LAI, F_{max}, RUE and NEE than PF during late growing season. Our results suggest that N fertilization stimulates the daily NEE directly by increasing RUE, and increases the seasonally accumulated NEE indirectly by extending growing season; PF and GF differentially altered NEE and RUE during different growth periods due to their different N supply doses. Our study also provides whole-mesocosm gas exchange evidence that canopy RUE varies with N supply, which should be considered for modeling ecosystem C sequestration as affected by N

fertilization and/or deposition. Moreover, modeling studies may need to consider the extended growing season under N fertilization.

Keywords: *Bromus tectorum*; Cheatgrass; Ecosystem respiration; Grassland ecosystem; Net ecosystem CO_2 exchange; Nitrogen fertilization; Radiation-use efficiency

Introduction

Nitrogen (N) is a major limiting nutrient to primary productivity of terrestrial ecosystems (Peterson et al., 1999). As a result, N fertilization and/or deposition are likely to increase ecosystem uptake of CO_2 from the atmosphere, and hence slow global warming (Norby, 1998; Pussinen et al., 2002). It has been well established that N fertilization stimulates plant photosynthesis by increasing Rubisco enzyme concentration, leaf area index (LAI), light interception and radiation-use efficiency (RUE) at leaf- and plant-levels (Gimenez et al., 1994; Weerakoon et al., 2000; Caviglia and Sadras, 2001). However, little is known about how N fertilization affects net ecosystem CO_2 exchanges (NEE) although N fertilization is likely to increase net primary productivity (NPP) (Nadelhoffer et al., 1999; Jenkinson et al., 1999; Neff et al., 2002).

NEE is the difference between ecosystem gross photosynthesis and ecosystem respiration. Thus, the effects of N on these two components mainly determine the N effects on NEE. However, there are great uncertainties how N affects these two components. On one hand, it is reasonably expected that N fertilization would stimulate gross photosynthesis at ecosystem level based on the leaf- and plant-level studies.

However, the light condition under a canopy is quite different from that of a single leaf or plant, which may cause some uncertainties on how N affects ecosystem gross photosynthesis. On the other hand, the N effects on ecosystem respiration are not clear due to the variable responses of each components of ecosystem respiration to N additions, including plant root/rhizosphere respiration (e.g., Lutze et al., 2000; but see Zogg et al., 1996), soil respiration (e.g., Vance and Chapin, 2001; Craine et al., 2001; but see Williams and Silcock, 1997; Shan et al., 2001), and decomposition (Carreiro et al., 2000; Saiya-Cork et al., 2002). Therefore, it is difficult to predict responses of ecosystem respiration and hence NEE to N fertilization based on those plot-level studies. Well-designed manipulative experiments are urgently needed to quantify the whole ecosystem CO_2 exchanges to probe the N effects on NEE.

Canopy RUE is another important factor determining ecosystem gas exchanges. However, it is unknown if N fertilization also increases NEE by improving canopy RUE at whole-ecosystem level as reported at the leaf- and plant-level studies. In agricultural studies, N fertilization always increases crop yield and biomass accumulation, however, its effect on canopy RUE, i.e., the ratio of crop biomass and the intercepted solar radiation (Muchow and Sinclair, 1994; Medlyn, 1998) varies, either increasing (Wang et al., 1991; Fischer, 1993; Abbate et al., 1995), decreasing (Olesen et al., 2000), or unchanging (Garcia et al., 1988; Bange et al., 1997). The inconsistent effects of N on canopy RUE may be partly due to the use of aboveground instead of total biomass accumulation in some studies to calculate RUE (Wang et al., 1991). However, no matter whether total or aboveground biomass are used in calculating RUE, canopy RUE based on biomass ($RUE_{biomass}$ hereafter) does not account for the allocation of fixed C to plant

and soil respiration (Rochette et al., 1995). As a result, canopy $RUE_{biomass}$ does not match canopy RUE calculated using ecosystem CO_2 fluxes (i.e., canopy RUE_{CO2} hereafter) (Rochette et al., 1995).

Whether N fertilization increases canopy RUE is very important for modeling studies. Light use efficiency (or RUE) models have been widely used to estimate crop yield and ecosystem productivity (Potter et al., 1993; Medlyn, 1998; Nichol et al., 2000) and to predict NPP as affected by elevated CO_2 and N deposition (Medlyn and Dewar, 1996). Models predicting how N fertilization and/or deposition affect NPP often assume that N stimulates NPP through an increased canopy light absorption but not through increased canopy RUE (e.g., Dewar, 1996; Medlyn and Dewar, 1996). However, this assumption of constant RUE has been proved to be flawed or questionable by several modeling studies (Medlyn, 1998; Gamon et al., 2001). Given that, experimental information about whether and to what extent canopy RUE changes with N additions is desirable in order to accurately predict N effects on NPP (Medlyn and Dewar, 1996). Since RUE_{CO2} accounts for both ecosystem C gains and losses, it may be more accurate and more suitable than $RUE_{biomass}$ as a parameter in modeling N effects on NPP. However, little experimental evidence is available on how N affects canopy RUE_{CO2}.

The lack of experimental evidence of N effects on NEE and RUE_{CO2} is mainly due to the technical limitations. Currently, several approaches are used to quantify whole-ecosystem CO_2 exchanges, including eddy covariance technique (e.g., Grace et al. 1998), modeling (e.g., Griffs et al., 2000), environmentally controlled facilities (e.g., Griffin et al., 1996), and whole-ecosystem measurement chambers (Johnson et al., 2000). However, not all methods are equally suitable for studying N effects on ecosystem CO_2 fluxes. For

example, the eddy covariance technique allows continuous measurements of ecosystem gas exchanges in the field but requires large-scale N applications and replicated eddy-flux towers. Modeling can scale up leaf- or plant-level measurements under N fertilization but depends on experiments to determine critical processes and parameter values (Leuning et al., 1995; Hui et al., 2001). By contrast, environmentally controlled facilities can continuously make accurate measurements of whole ecosystem CO_2 fluxes, and have the potential to explore the mechanisms underlying ecosystem responses to perturbations by manipulating other environmental factors (e.g. Griffin et al., 1996).

In this study, an environmentally controlled growth facility, called Ecologically Controlled Enclosed Lysimeter Laboratory (EcoCELL) (Griffin et al., 1996) was used to investigate how N additions affect NEE. By using the same facility, Verburg et al. (2004) investigated the whole-ecosystem C budget and its components under two growing seasons and two fertilization regimes in model grassland ecosystems. The essential difference between Verburg et al. (2004) and the present study was that they quantified the ecosystem C sequestration of the model grassland whereas we mainly focused on the physiological mechanisms of N effects on NEE and RUE. We hypothesized that 1) N fertilization would increase the daily NEE (NEE_d) directly by improving RUE_{CO2} via increasing light interception, plant N content, shoot/root ratio and the maximum ecosystem photosynthetic capacity (F_{max}); (2) N fertilization would increase the seasonally accumulated NEE (NEE_{SA}) and biomass accumulation indirectly by extending growing season via delaying plant senescence, and (3) PF and GF would result in different seasonal dynamics of NEE, and different biomass accumulation by differentially affecting plant N content, canopy LAI, F_{max} and RUE_{CO2}. These hypotheses were tested

by comparing NEE, LAI, plant N content, shoot/root ratio, RUE_{CO2}, light response curves of NEE and the estimated F_{max} between years with and without N fertilization and between PF and GF.

Materials and Methods

Plant material and experimental facility

This study used two EcoCELLs established in the Desert Research Institute (Reno, NV, USA) as the growth facilities. The EcoCELLs have been described in detail by Griffin et al. (1996) and successfully used in several ecosystem-level studies (Sims et al., 1999; Luo et al., 2000; Hui et al., 2001; Obrist et al., 2003; Verburg et al., 2004). In brief, the EcoCELLs are big open-flow mass balance systems using the same principles as leaf-level gas exchange measurements. The total volume of each EcoCELL is 183.5 m^3 of which 20.1 m^3 is occupied by three lysimeters that can be filled with soil. The lysimeter [2.85m (L) x 1.3m (W) x 1.8m (D)] is mounted on four truck scales which can measure a weight of 5,000 kg with a precision of 250 g. The environmental control includes temperature, CO_2 concentration, and relative humidity. The light is from natural solar radiation.

Starting in July 1998, each of the three adjacent lysimeters in the two EcoCELLs was filled with a 1-m layer of washed pea gravel, which served as a space holder; the gravel was then covered with root-impermeable landscape fabric. A 40-cm layer of washed, non-calcareous coarse sand was layered on top of the fabric, followed by a 40-cm layer consisting of 1:2 mixture of topsoil (Mollisol) from the Konza Prairie Long-term

Ecological Research site near Manhattan, Kansas, USA (39° 05' N, 96° 35' W). All roots were removed from the prairie soil before mixing it with the sand. In order to ensure that the disturbance effects on microbial respiration and N mineralization from soil handling had disappeared before the start of our study, the soils were allowed to sit in the lysimeters for 8 months.

Three monoculture stands of cheatgrass (*Bromus tectorum*) were established in February 1999 in each of the two EcoCELLs. Cheatgrass was chosen to represent a major anthropogenic disturbance in the Great Basin, USA. Cheatgrass is the major invasive species in this region and has been shown to alter soil N dynamics of the cheatgrass invaded ecosystems (Evans et al., 2001). In addition, the competitive ability of cheatgrass can be enhanced by increased N availability (Kay, 1966). Therefore, measuring ecosystem CO_2 exchanges of cheatgrass ecosystem under N fertilization may help better understand the effects of this invasive species on ecosystem C dynamics in the US Great Plains. On 23 February 1999, we sowed cheatgrass seeds (70 seeds m^{-2}) in six rows (20 cm apart) per soil container with 20 cm spacing between individual plants (84 plants per soil container; 252 plants per EcoCELL). No N was applied to the EcoCELLs during the first growing cycle. Aboveground biomass of the first crop was harvested 108 days after sowing (10 June 1999), soon after peak green LAI (LAI=4.2) was attained and the plants became apparently senescent (LAI=1.8 and 1.7 for two EcoCELLs, respectively).

Prior to the second sowing on 31 January 2000, soils were left fallow for six months. On 25 February 2000, $(NH_4)_2SO_4$ was applied to each of the EcoCELLs: the equivalent of 88 kg N/ha in one application to EcoCELL 1 (i.e., pulse N fertilization, PF) and the same amount to EcoCELL 2 (i.e., gradual N fertilization, GF) in 15 times of weekly

additions of 5.87 (totaling 88) kg N/ha. Aboveground biomass was harvested 128 days after sowing on 8 June 2000. In order to look at the difference of soil N availability between PF and GF during different growth periods, net N mineralization rates were measured using the *in situ* incubation method in 2000 (R. D. Evans, personal communication). Results showed that net N mineralization rate in PF (15.3 $mg.kg^{-1}d^{-1}$) was higher than in GF (0.69 $mg.kg^{-1}d^{-1}$) between April and May of 2000, whereas net N mineralization rate in GF (2.83 $mg.kg^{-1}d^{-1}$) was higher than in PF (-0.02 $mg.kg^{-1}d^{-1}$) between May and June of 2000 (R. D. Evans, personal communication). Therefore, we were able to consider PF and GF as high and low N treatments during the early growing season, while considering GF and PF as low and high N treatments during the late growing season.

During the experiment, water was applied using polyethylene irrigation lines to maintain soil water content at field capacity. Daytime and nighttime temperatures in the EcoCELLs were maintained at 28 °C and 22 °C, respectively, with daytime temperatures starting at 05:00 PST and ending at 19:00 PST. The maintenance of relatively constant soil water content and temperature allowed us to examine the N effects on C processes without complications of water and temperature interactions.

Gas exchange measurements

NEE in the EcoCELLs was measured continuously using Li-Cor 6262 infrared gas analyzers (IRGAs) during the experimental period. The NEE measurements were made using the same theoretical basis with the leaf- and plant-level photosynthesis measurements. The accuracy of gas exchange measurements was routinely verified by injecting known amounts of CO_2 into each EcoCELL. These standard addition tests were

performed at night when no photosynthetic uptake of CO_2 occurred. Fluxes and environmental factors, including temperature, humidity and light were measured every minute and stored as 15-minute averages. Data points affected by the presence of people inside the chambers were removed. Calculations of NEE were made as open system differential measurements as described by Field et al. (1991), and expressed on a ground surface area basis.

Canopy development and biomass measurement

Live leaf area index (LAI) was determined by counting the number of live leaves in each of the three experimental plots within each EcoCELL, and then multiplying by the mean leaf area for individual leaves in the corresponding experimental plots. At each date of leaf area determination, we counted the number of live leaves on a subset of 5 plants out of the 42 plants in each experimental plot. Subsequently, the mean leaf areas were measured by subsampling 20 live leaves randomly selected in each experimental plot and analyzing their leaf areas using an imaging analysis program (Image Pro Plus, Version 1.3.2).

Aboveground biomass was harvested by clipping at ground level on 10 June 1999 and 8 June 2000, respectively. Aboveground biomass was separated into live (green) and dead (brown) biomass. Root samples were taken using two soil cores per lysimeter, down to an 80-cm depth. Roots were isolated from soils by washing soil samples with tap water. We did not include the root crowns in the biomass measurement at the first harvest, but included the root crowns at the second harvest, causing underestimated root biomass in 1999. The biomasses of live, dead, total shoot and root were measured by weighing the

materials after they had been dried under 70ºC for 48 hours. The N concentrations of live and dead shoots were analyzed using a Perkin Elmer CHN analyzer.

Pseudo-replication and data analysis

Due to the limitation of the number of EcoCELLs and operation costs, it was not practical to set replicates of N treatments at the ecosystem level. Therefore, we used each EcoCELL as an experimental unit creating a pseudo-replicated design (Hurlbert, 1984) to examine the N effects. By comparing the measurements between PF and GF, we were able to see the N fertilization effects because they provided different available N to the plants during different growth periods (see the previous content). Originally, we did not intend to look at the N effects by comparing year 1999 and 2000 because there was 24 days of difference in the sowing dates of the two growing seasons, which could affect the ecosystem gas exchanges to some extent. However, after we compared the weekly averaged PAR of the two growing seasons (Fig. 3), we only observed consistent light shifts during first 4 weeks after emergence between the two years, which, we believe, were not big enough to cause the large difference in ecosystem CO_2 flux between the two years after the N treatments (see the Results section). Therefore, we were also able to look at the N fertilization effects by comparing the measurements between 1999 and 2000 to provide further support to the comparative results of PF and GF. Even though we could not do the conventional statistical analyses due to lack of replicates, the quantification of ecosystem CO_2 fluxes is valid because we used time series of high-precision data for quality control (Luo et al., 2000). We quantified the accuracy of system-level measurements and found that more than 95% of 96 data points over 24 h period varied within ± 0.5 μmol m^{-2} s^{-1} in both the EcoCELLs. This variation is extremely

small compared to the magnitude of ecosystem CO_2 exchanges. Furthermore, it is also a common practice in biophysical studies that measurements are made with no or few replicates if the instruments have high accuracy. For example, ecosystem flux measurements using eddy-covariance instruments, which are much less accurate than EcoCELLs, are usually not replicated. Eddy-flux data reported in the literature, even without replicated towers, are extremely useful in illustrating diurnal, seasonal, and interannual variability in ecosystem exchanges.

In order to compare the measurements between the two EcoCELLs and between the two years, we averaged the daily data within one week (7 days). Ecosystem RUE_{CO2} was defined as the ratio of gross ecosystem photosynthesis to IPAR. Gross ecosystem photosynthesis was estimated by integrating the 24-h measurements of NEE plus ecosystem dark respiration. Dark respiration was estimated from nighttime NEE (i.e., ecosystem respiration) corrected for the temperature difference between day and night with Q_{10}=1.5, which was calculated from the relationship between nighttime ecosystem respiration and temperature. IPAR was estimated using $IPAR=PAR \times (1-e^{(-LAI \times k)})$ (Campbell and Norman, 1998), where k is the canopy extinction coefficient, LAI is the leaf area index, and PAR is the measured photosynthetic photon flux density (PPFD). In this study, we used 0.48 as the canopy light extinction coefficient for cheatgrass, which is equal to the extinction coefficient of the C_3 grasses used in the BIOME-BGC model (White *et al.*, 2000). Daily IPAR was calculated by integrating 24-h measurements. Nighttime NEE was calculated by averaging nighttime measurements from 1900 PST to 0645 PST.

The relationship between net ecosystem C flux and IPAR was analyzed with a rectangular hyperbolic equation (Luo et al., 2000):

$$F_c = (F_{max}\alpha I)/(F_{max} + \alpha I) + F_0$$

Where F_c is the net ecosystem C flux, F_{max} is the maximum net ecosystem CO_2 flux, α is the canopy quantum yield, I is IPAR, and F_0 is net ecosystem C flux when I=0.

One-way analysis of variance (ANOVA) was applied to examine the differences in plant biomass, shoot/root ratio and plant N content between PF and GF and between the two years. T-tests were used to compare the weekly averaged daily NEE, nighttime NEE, and ecosystem RUE_{CO2} between PF and GF. Nonlinear regression analysis was used to fit the light response curve of net ecosystem CO_2 flux. The statistical analyses were carried out using the SAS package (SAS Institute Inc., Cary, NC).

Results

Canopy development

In 1999, the highest canopy LAI values within the two EcoCELLs were around 4, whereas those within the two EcoCELLs in 2000 were around 6 and 8 for PF and GF, respectively (Fig. 1). This result suggests that N fertilization in 2000 may have substantially enhanced the canopy development in comparison to 1999 given that all the environmental factors were quite similar between the two years, including the light levels (see Fig. 3b). There was no difference in LAI between the two EcoCELLs in 1999. The seasonal pattern of LAI differed substantially between PF and GF in 2000, however. LAI values in PF before 84 DAE were 39% higher on average than those in GF, with the

biggest difference (68%) occurring at 48 DAE. By contrast, LAI values in GF were 58% higher on average than that in PF after 84 DAE, with the biggest difference (99%) occurring at 123 DAE (Fig. 1).

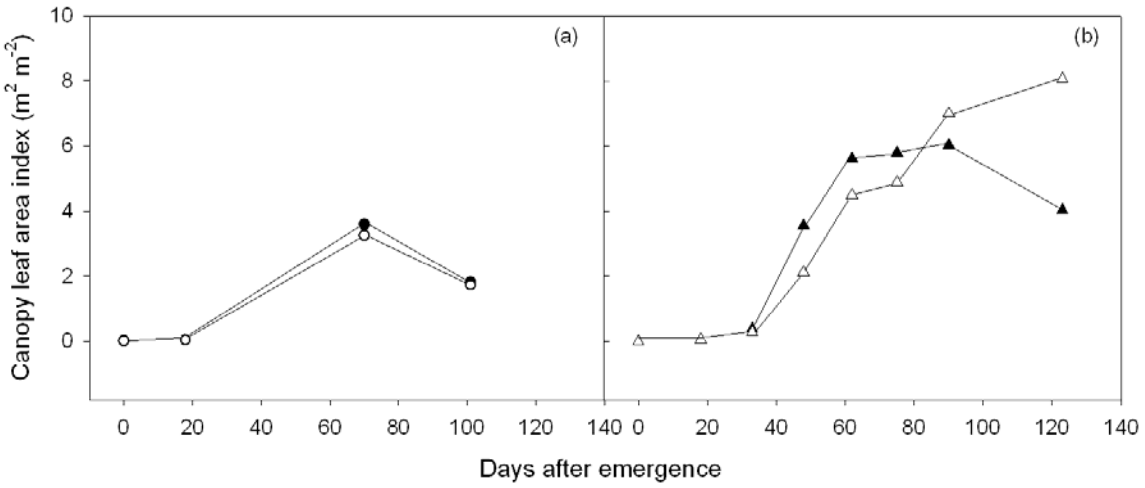


Figure 1. Canopy LAI in EcoCELL1-99 (solid circle) vs. EcoCELL2-99 (open circle) (a) and EcoCELL1-00 (PF) (solid triangle) vs. EcoCELL2-00 (GF) (open triangle) (b)

Biomass, shoot/root ratio and plant N content

In 1999, the total shoot biomass within the two EcoCELLs was around 200 g m^{-2}, whereas the total shoot biomass in 2000 was about 400 and 450 g m^{-2} under PF and GF, respectively, being 90.7% and 104.6% higher than those in 1999 (Fig. 2). Similarly, the green shoot biomasses, shoot/root ratios and plant N contents in PF and GF were higher than those in 1999 (Fig. 2), further suggesting that N fertilization in 2000 may have increased the canopy development and biomass accumulation by increasing plant nutrition and photosynthesis. Although the total biomass in 1999 was underestimated by about 10% because root crowns were not included in the biomass measurement (Verburg

et al., 2004), the substantial increase in total shoot biomass in PF and GF still warranted a great increase in total biomass compared with the first growing season. In addition to the differences between the two years, PF and GF also resulted in different biomass and plant N content because of their different sustainability to supply N to plants. The total and green shoot biomasses at harvest in GF (469.7 and 291.5 g m^{-2}) were 16% and 34% higher than those in PF (406.3 and 217.9 g m^{-2}), respectively. The green shoot N content was 46.6% higher in GF than in PF.

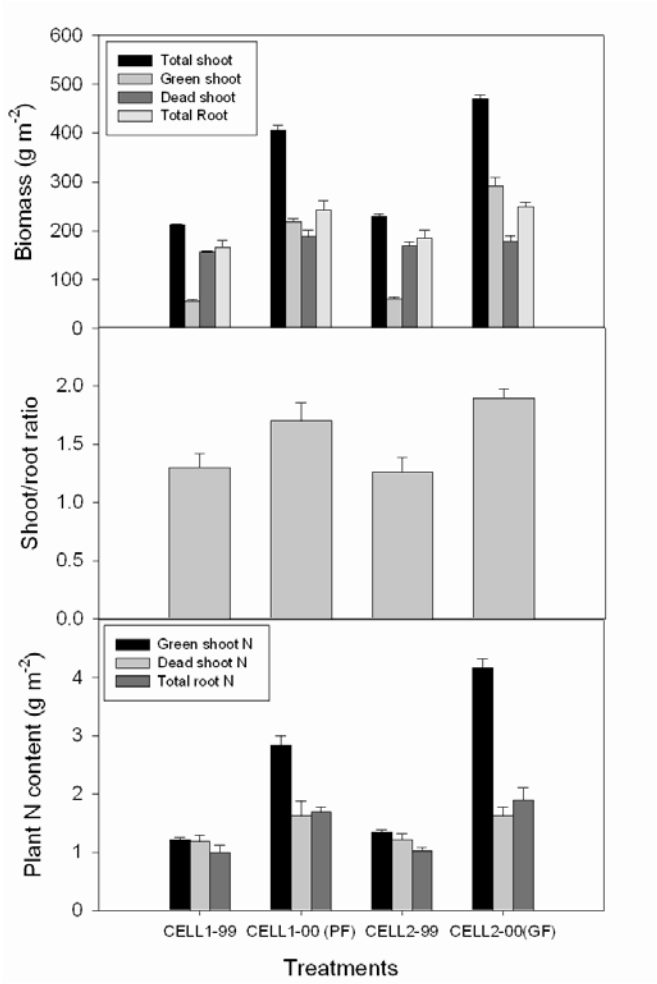


Figure 2. Comparisons of biomasses of different components (a), shoot/root ratio (b), and N content (c) between the two EcoCELLs in different years. The error bars represent the standard error of means of 3 replicates.

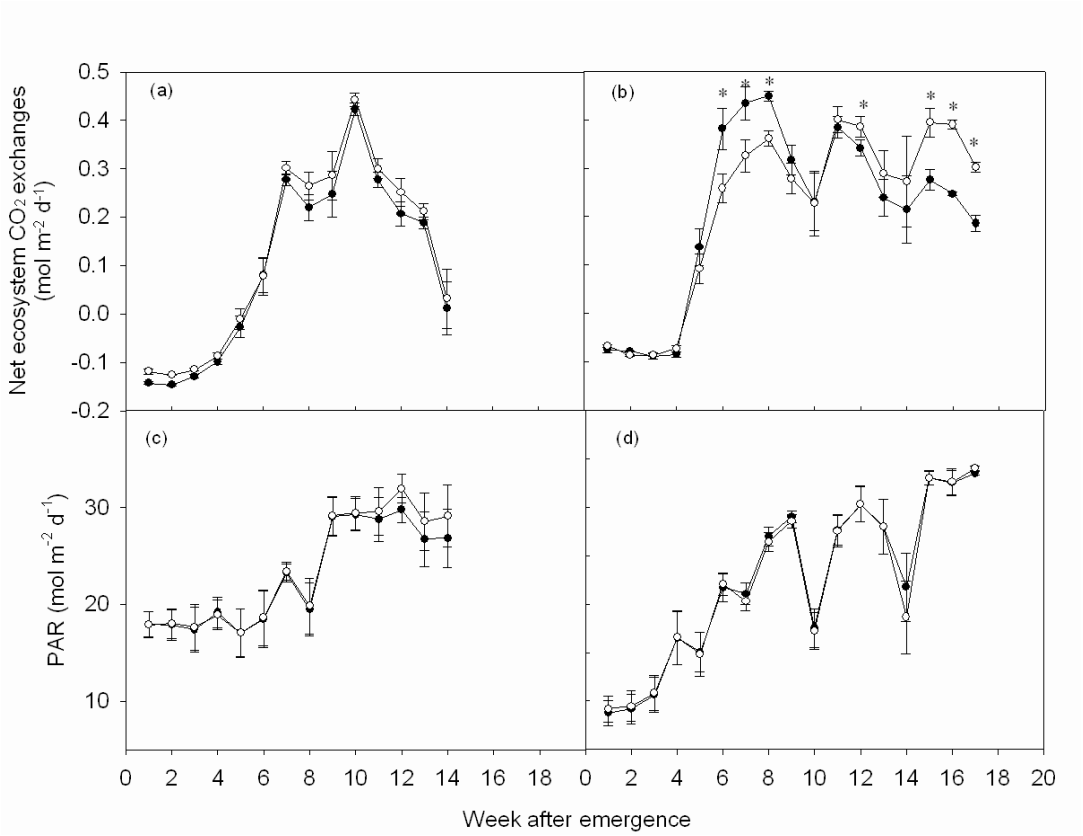


Figure 3. The weekly averaged daily NEE and PAR levels in EcoCELL1-1999 (solid circle) vs. EcoCELL2-1999 (open circle) (a, c) and EcoCELL1-2000 (PF) (solid triangle) vs. EcoCELL2-2000 (GF) (open triangle) (b, d). The error bars represent the standard error of the weekly mean. "*" indicates significant difference between PF and GF at the level of $p<0.05$ (t-test).

PAR, daily and nighttime NEE during canopy development

Because there were 24 days of difference in the sowing dates (23 February 1999 v.s. 31 January 2000) between the two years, PAR values before 4 weeks after emergence in both EcoCELLs in 1999 were significantly higher than those in 2000 (Fig. 3). However, this light shift pattern did not consistently exist after 4 weeks after emergence, with the PAR levels in 1999 being very close to those in 2000 during most time periods (at 4, 5, 6,

9, 11, 12, 13, and 14 weeks after emergence for EcoCELL1, and at 4, 5, 6, 9, 11, 12 and 13 weeks after emergence for EcoCELL2) until the end of the first growing season. This result suggested that the 24 days of difference in the sowing dates did not significantly affect the light levels during the most time periods of the two growing seasons. Furthermore, the seasonally accumulated PAR in 2000 was consistently lower than that in 1999 (data not shown), further suggesting that N fertilization in 2000 did increase NEE and could have increased NEE more than it did. Therefore, it is valid to look at the N effects on NEE by comparing the two years' data.

In 1999, the daily NEE was similar between the two EcoCELLs. Compared with their respective EcoCELLs in 1999, both PF and GF had lower NEE (more negative) before 4 weeks after emergence, likely caused by the higher PAR levels in 1999 than in 2000 during this time period (Fig. 3a). PF enhanced daily NEE between 7 and 9 weeks after emergence whereas GF stimulated daily NEE at 9 weeks after emergence compared with their respective EcoCELLs in 1999. At 10 weeks after emergence, both PF and GF had lower NEE than their respective EcoCELLs in 1999 possibly due to the lower PAR level in 2000 than in 1999 during this period (Fig. 3b). However, NEE in both PF and GF became higher than their respective EcoCELLs in 1999 starting from 12 weeks after emergence until the end of the experiment (Fig. 3) although the PAR levels in 2000 were very close to and even lower than those in 1999. This suggests that the increase in daily NEE in 2000 was not proportional to the change in PAR during most time periods of the two growing seasons, and that N fertilization must have played a major role in determining NEE because other factors were all controlled in the EcoCELLs. Although the light levels between PF and GF were the same (Fig. 3b), PF and GF differentially

affected the seasonal dynamics of the daily NEE, suggesting dramatic N effects on NEE. NEE in PF was 35.0% higher than in GF during the period between 7 and 9 week after emergence, but lower than in GF after 11 week after emergence. The daily NEE in GF was 54.4% higher than in PF during the period between 16 and 18 weeks after emergence. Since ecosystem respiration was also increased during the peak growing season under N treatments (see below), the sum of NEE and ecosystem respiration, i.e., gross ecosystem photosynthesis, was increased even more than NEE (data not shown).

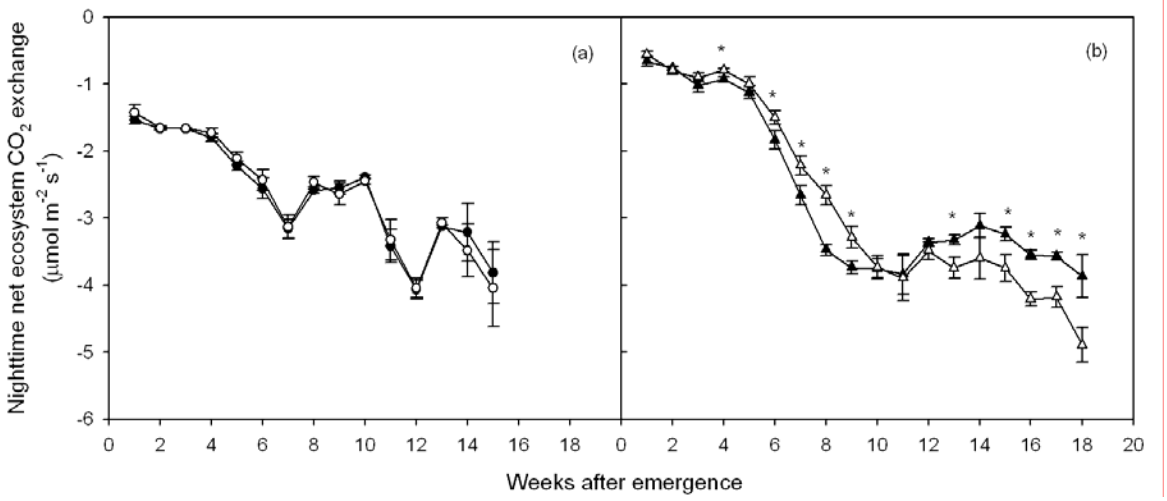


Figure 4. The weekly averaged daily nighttime NEE in EcoCELL1-1999 (solid circle) vs. EcoCELL2-1999 (open circle) (a) and EcoCELL1-2000 (PF) (solid triangle) vs. EcoCELL2-2000 (GF) (b). The error bars represent the standard error of the weekly mean. "*" indicates significant difference between PF and GF at the level of $p<0.05$ (t-test).

Nighttime NEE (i.e., ecosystem respiration) was also similar between the two EcoCELLs in 1999 (Fig. 4). Compared with year 1999, ecosystem respiration in PF and GF were significantly lower before 8 and 7 week after emergence, respectively, primarily caused by the lower PAR levels in 2000 than in 1999 before 4 weeks after emergence

(Fig. 3b). The higher PAR before 4 weeks after emergence in 1999 could have stimulated more gross photosynthesis than that in 2000, resulting in higher labile C input to the soil and hence ecosystem respiration. Because there was often a time lag between the labile C allocation and CO_2 release, the ecosystem respiration continued to be higher in 1999 than in 2000 until 7 and/or 8 weeks after emergence. Ecosystem respiration rates under PF and GF were higher than those in 1999 at 8, 9, 10 and 13 weeks after emergence likely because of the significant increase in photosynthesis under PF and GF during the period from 7 to 9 weeks after emergence and from 12 to 13 weeks after emergence (Fig. 3). Ecosystem respiration rates under PF and GF were lower at 12 weeks after emergence ($p<0.05$) likely due to the lower photosynthesis during the period at 10 week after emergence. Ecosystem respiration was differentially affected by PF and GF (Fig. 4) although the light levels were the same between PF and GF. Compared with GF, PF resulted in 16.0% more ecosystem respiration on average during the period between 4 and 9 week after emergence. By contrast, GF caused 14.9% more ecosystem respiration than PF during the period between 13 and 18 week after emergence (20.9% at 18 week after emergence). In addition to nighttime NEE, the seasonal values of NEE when IPAR was zero (F_0) (Table 1), which can reflect ecosystem respiration, also showed similar N effects.

Canopy RUE_{CO2} during canopy development

The seasonal dynamics of canopy RUE_{CO2} was similar between the two EcoCELLs in 1999 (Fig. 5). Compared with year 1999, RUE_{CO2} under PF and GF was higher during the period between 8 and 11 and between 8 and 10 weeks after emergence, respectively, likely due to the higher gross ecosystem photosynthesis (NEE + respiration) under PF

and GF in 2000 than in 1999 during this period (Figs. 3 and 4). There was no significant difference in RUE_{CO2} between 1999 and 2000 at 13 week after emergence. During the period between 14 and 17 weeks after emergence, RUE_{CO2} in both PF and GF were higher than in 1999 ($p<0.05$). Before 15 weeks after emergence, there was no obvious difference in ecosystem RUE_{CO2} between PF and GF, but GF enhanced RUE_{CO2} more than PF until the end of the second growing season. During the period between 16 and 20 weeks after emergence, the averaged RUE_{CO2} in GF was 22.8% higher than that in PF. Similar N effects on the seasonal changes of RUE_{CO2} were also reflected from the estimated canopy quantum yield (α) (Table 1), which was another form of expression of RUE_{CO2}.

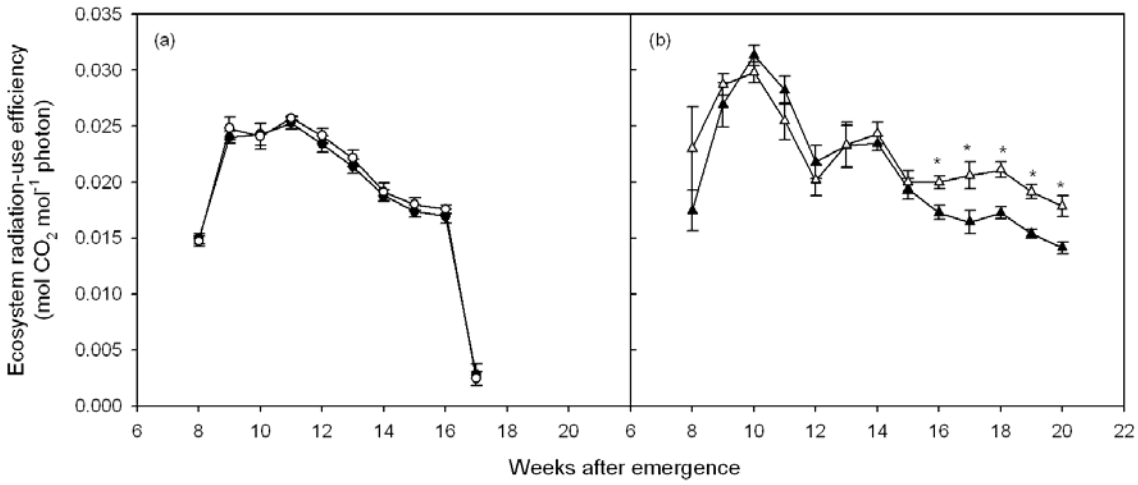


Figure 5. The weekly averaged canopy RUE in EcoCELL1-1999 (solid circle) vs. EcoCELL2-1999 (open circle) (a) and EcoCELL1-2000 (PF) (solid triangle) vs. EcoCELL2-2000 (GF) (b). "*" indicates significant difference between PF and GF at the level of $p<0.05$ (t-test).

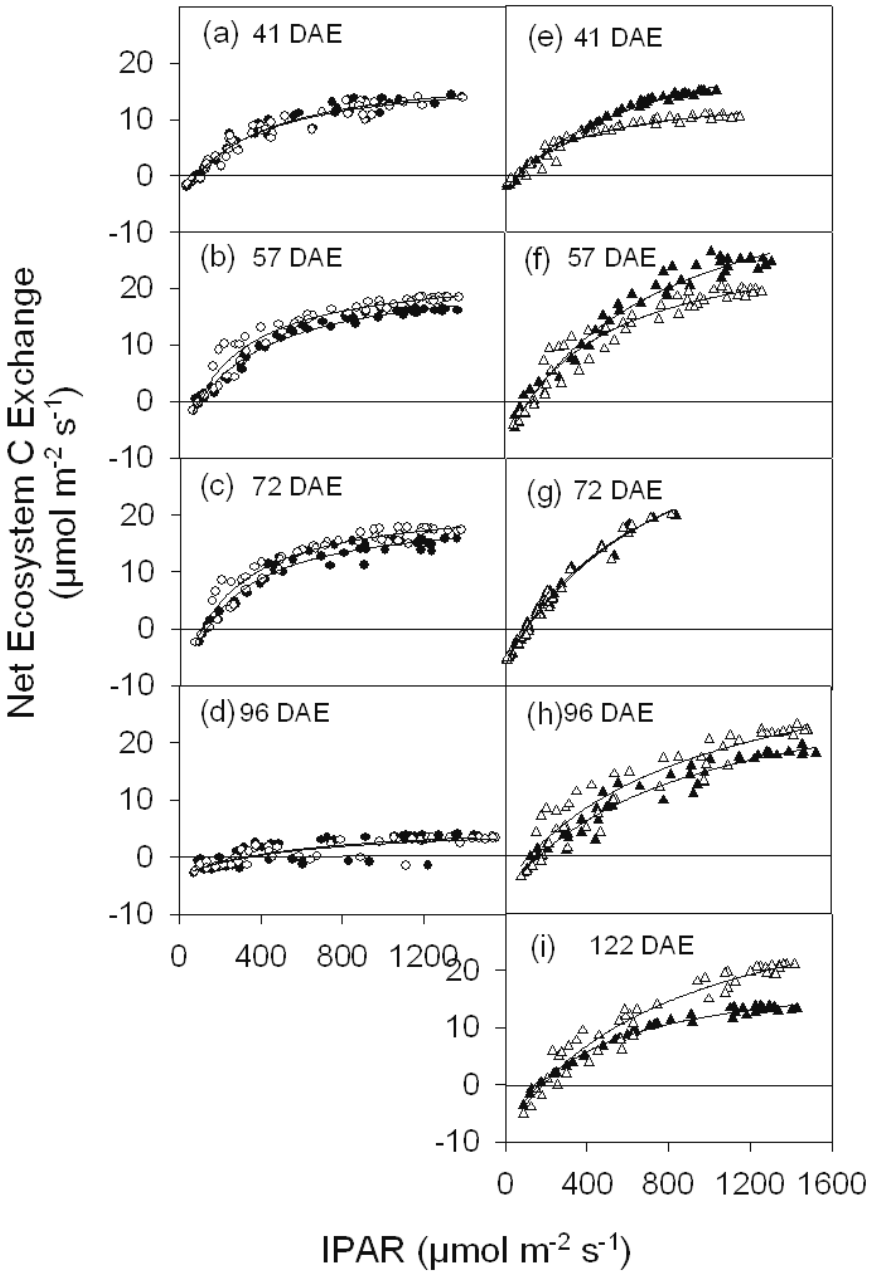


Figure 6. Response of NEE to IPAR in EcoCELL1-1999 (solid circle) vs. EcoCELL2-1999 (open circle) (from (a) to (d)) and EcoCELL1-2000 (PF) (solid triangle) vs. EcoCELL2-2000 (GF) (from (e) to (i)) at different days after emergence (DAE).

Table 1. Responses of Net Ecosystem CO_2 Exchange (NEE) (F) to intercepted photosynthetically active radiation (IPAR) in EcoCELL1-1999, EcoCELL1-2000 (PF) EcoCELL2-1999 and EcoCELL2-2000 (GF). Values are estimates ± standard errors. For the purpose of simplicity, this table only shows the estimated parameters for the curves shown in Figure 6.

	EcoCELL1-1999			EcoCELL1-2000 (PF)		
DAE	F_{max}	F_0	α ($\times 10^{-3}$)	F_{max}	F_0	α ($\times 10^{-3}$)
43-49	29.1±0.4	-3.7±0.1	56.2±1.3	51.9±7.0	-3.1±0.2	58.5±6.5
57-63	30.8±1.0	-3.2±0.0	53.9±1.7	47.4±2.2	-4.5±0.1	56.7±2.1
71-77	30.3±0.4	-4.1±0.2	48.4±1.5	44.3±2.1	-4.4±0.2	59.9±1.5
92-98	22.8±8.2	-3.4±0.3	20.7±5.1	46.1±7.3	-3.9±0.1	39.0±2.2
113-119				34.6±0.8	-4.3±0.1	34.6±0.8
	EcoCELL2-1999			EcoCELL2-2000 (GF)		
DAE	F_{max}	F_0	α ($\times 10^{-3}$)	F_{max}	F_0	α ($\times 10^{-3}$)
43-49	29.5±1.3	-3.2±0.4	54.6±5.0	34.4±2.9	-2.6±0.2	45.8±5.1
57-63	33.1±1.2	-3.6±0.1	63.9±2.6	36.7±0.9	-4.1±0.2	54.2±2.3
71-77	30.7±0.5	-4.2±0.2	58.3±1.4	45.3±3.0	-4.6±0.2	62.4±2.2
92-98	15.4±4.5	-3.6±0.3	23.0±6.1	45.5±3.1	-4.7±0.1	54.2±3.4
113-119				45.6±1.2	-5.7±0.1	51.8±1.4

DAE, days after emergence; F_{max}, Maximum NEE; F_0, NEE when IPAR=0; α, canopy quantum yield; determinant coefficients are mostly above 0.96 except for EcoCELLs data between 92 and 98 DAE with 0.80 and 0.85.

Responses of NEE to IPAR and the estimated F_{max}

There were typical curvilinear relationships between NEE and IPAR in both year 1999 and 2000 (Fig. 6). A rectangular hyperbolic equation was fitted for NEE (Table 1). In 1999, the initial slopes of the light response curves of the two EcoCELLs were similar, while PF and GF differentially altered the initial slopes during different growth periods. Compared with year 1999, the initial slopes of the light response curves in 2000 were steeper after 41 DAE due to N fertilization. In 2000, the initial slope in PF was steeper than that of GF before 84 DAE, but became lower than that in GF during the remaining period. In correspondence to the initial slopes, N fertilization in 2000 also enhanced the estimated F_{max} compared with year 1999 after the period of 43-49 DAE (Table 1). PF and GF increased the estimated F_{max} by 66.8% and 58.5%, respectively, on average compared with their respective EcoCELLs in 1999. In addition, PF and GF differentially altered the estimated F_{max} during different growth periods (Table 1). During the early growth period, PF enhanced the estimated F_{max} by up to 50.9% (at 43-49 DAE) relative to GF. By contrast, GF enhanced the estimated F_{max} by 33.4% (at 106-112 DAE) in comparison to PF during the late growth period.

Discussion

Being consistent with the results of leaf- and plant-level studies (e.g., Weerakoon et al., 2000; Caviglia and Sadras, 2001), our results show that N fertilization and/or deposition increase the weekly averaged daily NEE (NEE_d) by increasing ecosystem RUE_{CO2} (Fig. 6) while increasing light interception (Fig. 2). Scaling studies also suggest improved canopy RUE under N fertilization (Leuning et al., 1995). Furthermore, our

study suggests that N fertilization may increase the seasonally accumulated NEE (NEE_{SA}) by extending growing season (Figs. 2 and 4), which is consistent with Schulze et al. (1994). More importantly, the present study provides experimental evidence that the assumption of a constant canopy RUE in models predicting the effects of elevated CO_2 and N deposition on NPP (e.g., Medlyn and Dewar, 1996) may not be justified as reported by other modeling studies (e.g., Medlyn et al., 1998; Gamon et al., 2001).

Mechanisms of N effects on ecosystem CO_2 exchanges

N fertilization stimulated the weekly averaged NEE_d (Fig. 4) by improving ecosystem RUE_{CO2} (Fig. 5) via increasing canopy LAI (light interception) (Fig. 1), plant N content (Fig. 2), shoot/root ratio and the maximum ecosystem photosynthetic capacity (F_{max}) (Table 1), which support our hypothesis 1 (also see Fig. 7). Although N fertilization also increased gross ecosystem photosynthesis while increasing IPAR (data not shown), N fertilization apparently increased gross ecosystem photosynthesis more than IPAR, resulting in higher ecosystem RUE_{CO2} (Fig. 5). Since canopy $RUE_{biomass}$ increases with specific leaf N content (SLN) (Weerakoon et al., 2000), the increased plant N content observed under N fertilization in 2000 (Fig. 2) may have caused higher RUE_{CO2} through its effect on the estimated F_{max} (Table 1) and gross ecosystem photosynthesis. The increased estimated F_{max} may be caused by the increased Rubisco enzyme concentration under N fertilization as with the leaf- and plant-level studies (e.g., Evans, 1989; Weerakoon et al., 2000; Caviglia and Sadras, 2001). In addition to canopy light interception and the estimated F_{max}, canopy structure may also affect canopy RUE and NEE because leaves in sparse canopies are more likely to be light saturated than in denser canopies (Gimenez et al., 1994; Hollinger et al., 1994; Leuning et al., 1995). In the

present study, since N fertilization in 2000 greatly increased canopy LAI (Fig. 1), it might have caused great shading effects on the leaves under the canopy and increased the diffuse component of the whole canopy, partly resulting in increased canopy RUE (Bange et al., 1997; Dreccer et al., 2000). Therefore, N fertilization may have increased ecosystem RUE_{CO2} and hence NEE_d by a combination of three factors: (1) the increased canopy light interception (Fig. 2), (2) the increased maximum photosynthetic capacity (Fig. 6, Table 1), and (3) the increased absorption of diffuse light under the canopy.

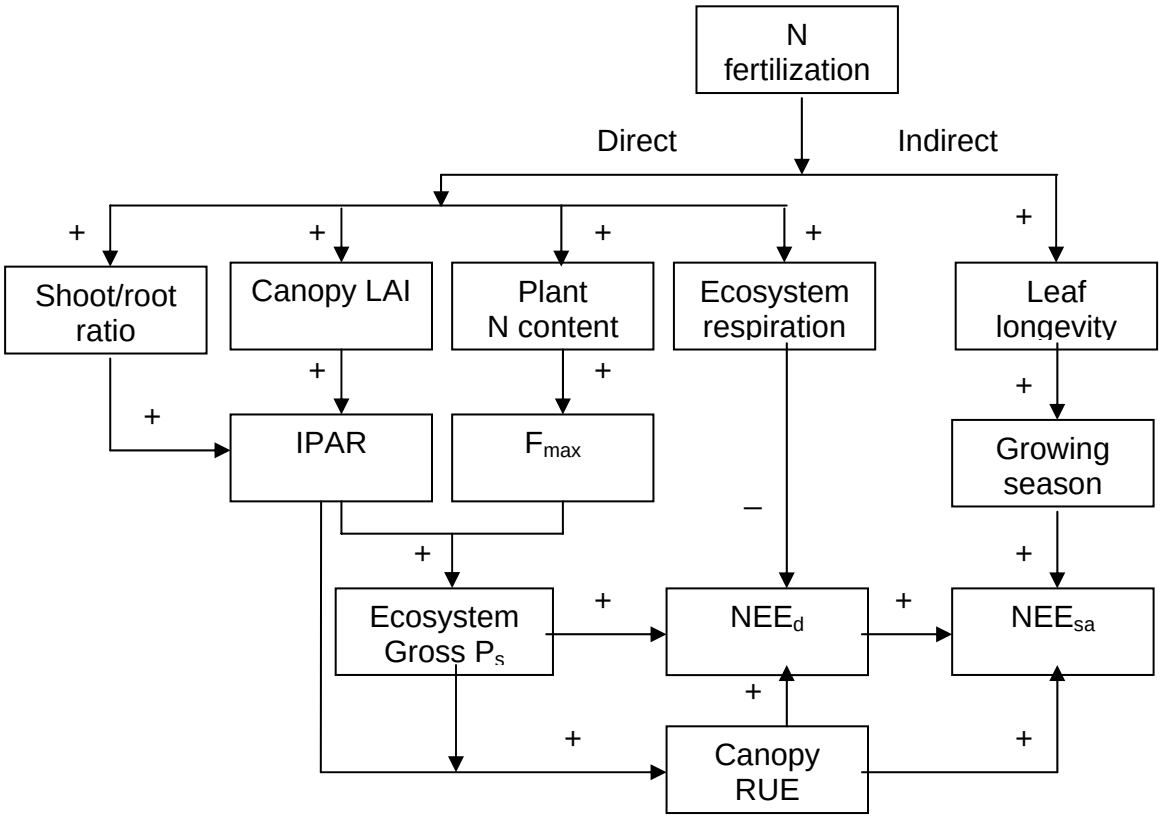


Figure 7. A conceptual model illustrating how N fertilization affects NEE. "+" denotes positive effect. "–" denotes negative effect. "P_s" stands for "photosynthesis". "F_{max}" is the estimated maximum ecosystem photosynthetic capacity. "NEE_d" stands for the daily NEE. "NEE_{SA}" represents seasonally accumulated NEE.

N fertilization also increased the seasonally accumulated NEE (NEE_{SA}) and biomass accumulation by extending the growing season via delaying plant senescence, also supporting our hypothesis (Fig. 7). In this study, the early senescence of plants in 1999 greatly reduced canopy LAI (Fig. 1) and light interception, F_{max} (Table 1) and hence RUE_{CO2} (Fig. 6) compared with year 2000. A similar pattern was also observed for PF and GF (Fig. 2). Since GF continuously supplied N to plants while PF only stimulated plant growth during early growing season, GF delayed plant senescence and maintained higher LAI, RUE_{CO2} and NEE than PF possibly by maintaining high plant N content, Rubisco enzyme activity and photosynthetic capacity. Other studies have reported delayed canopy senescence and higher late-season LAI values under N fertilization (e.g., Scholberg et al., 2000). In the present study, N fertilization in 2000 delayed plant senescence and extended the growing season by about 20 days (calculated using time difference in DAE at which peak LAI occurred) on average compared with year 1999 (Fig. 1). In order to estimate how much the extension of growing season contributed to the NEE increase, we calculated the percentage of the accumulated NEE during the extended growing season (from 102 to 124 DAE) within the total NEE_{sa}. Results showed that of the 60.8% and 31.9% increase in daily NEE in PF and GF compared with year 1999 (see the Results section), 28.6% and 47.5% were contributed by the extension of growing season in PF and GF, respectively. Therefore, the extension of growing season made a substantial contribution to increase in NEE_{SA}, RUE_{CO2} and biomass accumulation under N fertilization, which generally supported the conclusion of Schulze et al. (1994). Schulze et al. (1994) reported that plant N concentration and the length of growing

season mainly determined ecosystem gas exchanges across different vegetation types of the world.

In order to examine whether N fertilization also increased canopy $RUE_{biomass}$, we calculated canopy RUE as the ratio of total accumulated biomass to the total accumulated IPAR over the two growing seasons. Similar to the results of Olesen et al. (2000) on winter wheat, N fertilization reduced canopy $RUE_{biomass}$ by 18.2% and 14.3% for PF and GF, respectively (data not shown) although canopy LAI and RUE_{CO2} increased under N fertilization (Figs. 1 and 5). Olesen et al. (2000) attributed the reduced canopy $RUE_{biomass}$ partly to the increased growth and maintenance respiration under N fertilization. In the present study, the higher ecosystem respiration observed under N fertilization during the peak and late growing seasons (Fig. 4), which was mainly caused by the increased plant-associated respiration and reduced soil organic matter decomposition (Verburg et al., 2004), may have contributed a major part to the reduced canopy $RUE_{biomass}$. Given the opposite N effects on RUE_{CO2} and $RUE_{biomass}$, both the present study and Olesen et al. (2000) further suggest that it may be problematic to calculate canopy RUE based on biomass, and that canopy RUE needs to be calculated using ecosystem CO_2 fluxes because ecosystem respiration can be incorporated when calculating canopy RUE.

N fertilization versus N deposition in affecting ecosystem CO_2 exchange

One of the major differences between N fertilization and N deposition is the timing of N additions. Since different timing of N additions will likely affect the seasonal dynamics of soil N availability and plant growth by providing different N doses during different periods, it is desirable to know if N fertilization (mimicked by pulse N fertilization) would have different effects from N deposition (mimicked by gradual N fertilization) on

ecosystem CO_2 exchanges. Our results suggest that the two methods of N additions (PF and GF) differentially affect the seasonal dynamics of NEE, but only differentially stimulate canopy RUE_{CO2} during the late growing season (Figs. 3 and 5). This result suggests that under adequate N conditions, RUE_{CO2} may not increase much if further N fertilizer is added (N in PF was more than in GF during early growing season) whereas it may increase dramatically when N becomes limiting (N was limited in PF, but still adequate in GF during late growing season). Similar results were observed by Bange et al. (1997), which reported that the major effects of N on early growth of sunflower was mediated by leaf area and the distribution of specific leaf N (SLN) in the canopy rather than direct effects of canopy SLN on RUE alone, and that greater responses of RUE to SLN are more evident later in growth. This pattern is likely caused by the curvilinear relationship between canopy RUE and SLN so that at high SLN, RUE was high but increased little in response to a further increase in SLN while at low SLN, canopy RUE increased markedly with increases in SLN (Bange et al., 1997). Our plant N content data showed that at the second harvest, plant N content in GF was higher than PF (Fig. 3), which might have increased RUE_{CO2} in GF than PF. Unfortunately, we did not measure the SLN in both PF and GF during the early growing season, but we may reasonably believe that the SLN in PF and GF could be similarly high, resulting in similar RUE_{CO2} between PF and GF during the early growing season.

Conclusions

In summary, our study provides experimental evidence of how N fertilization and/or deposition affect NEE and whether N additions stimulate NEE by increasing canopy RUE_{CO2}. N fertilization enhanced the weekly averaged daily NEE by increasing canopy

RUE_{CO2} via increases in canopy LAI (light interception), plant N content, shoot/root ratio, F_{max} and possibly diffuse component of incident radiation under the canopy; N fertilization also increased the seasonally accumulated NEE by extending the growing season via delaying plant senescence. With the same mechanisms, relative changes in N availability with pulse (PF) and gradual (GF) N treatments during different growth periods resulted in different seasonal dynamics of ecosystem CO_2 exchanges and canopy RUE_{CO2}. Due to the continuous supply of available N to plants, GF resulted in more seasonally accumulated NEE than PF by maintaining higher RUE_{CO2} and delaying plant senescence. The uniqueness of this study is that we experimentally observed higher canopy RUE, calculated using ecosystem CO_2 flux, under N additions, which was usually assumed to be constant with N additions in modeling studies (e.g., Medlyn and Dewar, 1996). Our results suggest that future modeling studies on ecosystem responses to N fertilization and/or deposition need to carefully consider the dependence of canopy RUE on N nutrition. Given that N fertilization also extended the growing season by delaying plant senescence, modeling studies may also need consider the length of growing season in modeling NEE and NPP as affected by N deposition and/or fertilization.

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CHAPTER V

Evapotranspiration and water-use efficiency of a model ecosystem as affected by pulse and gradual nitrogen fertilization[*]

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Abstract

We investigated nitrogen (N) effects on evapotranspiration and water-use efficiency (WUE) of one model ecosystem with cheatgrass in two environmentally controlled facilities. Whole-ecosystem water and CO_2 fluxes were measured without (in 1999) and with (in 2000) N fertilization. N was applied as pulse and gradual (i.e., applying the same amount N once and many times during early and entire growing season) N fertilization to the two facilities, respectively. N fertilization stimulated both photosynthesis and evapotranspiration primarily by sustaining high leaf area index and shoot N content, and extending the growing season. N fertilization enhanced photosynthesis more than evapotranspiration, causing higher ecosystem WUE. Different fertilization methods changed the seasonal dynamics of photosynthesis and evapotranspiration but altered photosynthesis more than evapotranspiration. Accordingly, WUE was higher early but lower late in the growing season under pulse than under gradual N fertilization. This study indicates that N additions affect ecosystem water flux primarily by extending the growing season and delaying plant senescence, and much less by changing ecosystem conductance as suggested in the literature; N additions affect ecosystem WUE mainly by altering ecosystem C fluxes.

Keywords: cheatgrass, ecosystem CO_2 exchange, evapotranspiration, model ecosystem, nitrogen fertilization, nitrogen deposition, water-use efficiency.

Introduction

Water vapor exchange between the biosphere and atmosphere through evapotranspiration (ET) is one crucial process in regulating global water balance,

biogeochemical cycles, and climatic dynamics (Dickinson et al., 2002, Schulze et al., 1994; Meroni et al., 2002). The past research on ET has primarily focused on its relationships with leaf area index (Hatton et al., 1992), leaf biomass (Jackson et al., 1998; Paruelo and Sala, 1995), stomatal and aerodynamic conductance of plant canopies (Baldocchi et al., 1987; Valentini et al., 1995), soil moisture and temperature (Baldocchi, 1997; Bunce et al., 1997), wind speed (Valentini et al., 1995), vapor pressure deficit (Jarvis and McNaughton, 1986; Bunce et al., 1997), and solar radiation (Hemakumara et al., 2003; Baldocchi et al., 1996). In addition to those environmental and biological factors, ecosystem ET may strongly vary with soil nitrogen (N) availability. The latter influences canopy development (thereby the dynamic of LAI) and perhaps canopy stomatal conductance (Schulze et al., 1994, Leuning et al., 1995; Ewers et al., 2001). However, few ecosystem-level experiments have been done to examine the relationships between nutrient availability, particularly nitrogen (N), and ET.

At the leaf and plant levels, many studies have been done to examine effects of N fertilization on photosynthesis, transpiration, stomatal conductance (Green and Mitchell, 1992; Chandler and Dale, 1993; Gimenez et al., 1994; Kubiske et al., 1998), and water-use efficiency (WUE) (Livingston et al., 1999; Shangguan et al., 2000). For example, Chandler and Dale (1993) found that N fertilization increased net photosynthesis rate and stomatal conductance of two-year-old Sitka spruce (*Picea sitchensis* (Bong.) Carr.) seedlings which were subjected to N deficiency for 1 year. Livingston et al. (1999) also observed higher net photosynthetic rates in N fertilized white spruce (*Picea glauca* (Moench) Voss) seedlings than in N stressed seedlings, but they found a reduced stomatal conductance and hence higher WUE under N fertilization. Shangguan et al. (2000)

reported that high-N treatment caused higher leaf photosynthesis of winter wheat than low-N treatment with no effects on leaf transpiration. As a result, high-N fertilization resulted in higher WUE of winter wheat than low-N fertilization. Overall, all the studies indicate that N fertilization appears to have more positive effects on photosynthesis than transpiration, resulting in higher WUE of plants.

It is relatively difficult to study N regulation of ecosystem water and carbon (C) fluxes, largely due to technical limitations. Ecosystem water and C fluxes can be quantified by the eddy covariance technique (Baldocchi, 1997; Arneth et al., 1998; Hutley et al., 2000), plant growth facilities (Baker et al., 1997; Stocker et al., 1997), closed canopy gas exchange systems (Kumar et al., 1999), open-top chambers (Bunce et al., 1997; Owensby et al., 1997; Maurer et al., 1999), soil water balance approaches (Hunsaker et al., 2000), sap-flux-based scaling methods (Wullschleger and Norby, 2001; Ewers et al., 2001; Wullschleger et al., 2002), and modeling (Leuning et al., 1995). The eddy covariance technique allows continuous measurements of canopy fluxes in the field but requires large-scale N applications and replicated eddy-flux towers to study the N effects. Modeling can scale up leaf- or plant-level measurements to estimate canopy conductance but depends on experiments to determine critical processes and parameter values (Leuning et al., 1995; Hui et al., 2001).

With limited availability of ecosystem-scale data, Schulze et al. (1994) proposed an approach to scaling up leaf- and/or plant-level studies to predict ecosystem water and C fluxes as regulated by plant N concentration. Their approach is based on linear correlations (1) between leaf N concentrations and maximum leaf stomatal conductance, (2) between maximum leaf stomatal conductance and ecosystem surface conductance,

and (3) between ecosystem conductance for ET and canopy CO_2 uptake. Based on the distribution of leaf nitrogen in different vegetation types, Schulze et al. (1994) predicted global-scale surface conductance of water fluxes, which was shown to be high in the northern mid latitudes and low in southern hemisphere. However, that scaling approach involves many assumptions. For example, ecosystem water fluxes include both soil evaporation and plant transpiration. The N effect on soil surface evaporation could be quite different from that on leaf transpiration. Thus, it is imperative to experimentally examine effects of N addition on the whole-ecosystem C and water fluxes.

In this study, we used a unique facility, Ecologically Controlled Enclosed Lysimeter Laboratory (EcoCELL), to quantify the C and water fluxes of a model ecosystem with cheatgrass (*Bromus tectorum*). We chose cheatgrass to represent another major anthropogenic factor. The grassland ecosystems in the Great Basin, USA, have experienced considerable anthropogenic influences during the past decades, which include wet and dry N deposition (NADP, 1998; Holland et al., 1999) and the expansion of invasive species (Evans et al., 2001). Cheatgrass is the major invasive species in this region and has been shown to alter N (Evans et al., 2001) and water cycling processes (Booth et al., 2003). However, how N additions affect C, N and water relations of cheatgrass invaded ecosystem has not been carefully studied. Since water is the major limiting factor of primary productivity in the Great Basin region, it is important to study the water relations of the cheatgrass invaded ecosystem as affected by N fertilization/deposition.

EcoCELLs have been successfully used in several ecosystem-level studies (Sims et al., 1999; Luo et al., 2000; Hui et al., 2001; Obrist et al., 2003; Verburg et al., 2004) with

continuous monitoring of C, water, and energy fluxes (Griffin et al., 1996). EcoCELLs were particularly useful in this study to document time courses of ecosystem responses to two N fertilization treatments since we hypothesized that the timing of fertilization could change temporal dynamics of C and water fluxes at the ecosystem level. We used pulse N fertilization to mimic conventional agricultural N fertilization because it is a common agronomic practice to apply N fertilizer once during the early growing season. We used gradual N fertilization to mimic N deposition because natural ecosystems continuously receive N from the atmospheric deposition. The objectives of this study were: 1) to examine the N effects on ecosystem ET and WUE, and 2) to compare the effects of pulse and gradual N fertilization on the seasonal dynamics of ET and WUE of the model ecosystem. Ultimately, we intended to identify major processes that regulate responses of ecosystem water and C fluxes to N addition.

Materials and Methods

Plant material and experimental facility

Two environmentally manipulated growth facilities, called EcoCELLs, at Desert Research Institute, Reno, NV, USA, were used in this study. The EcoCELLs are environmentally controlled, naturally lit, open-flow and mass-balanced systems (Griffin et al., 1996). In each EcoCELL, there are three adjacent lysimeters (3.7 m^2 in area, 1.8 m in depth). Starting in July 1998, each lysimeter was filled with a 1-m layer of washed pea gravel, which served as a space holder; the gravel was then covered with root-impermeable landscape fabric. A 40-cm layer of washed, non-calcareous coarse sand was layered on top of the fabric, followed by a 40-cm layer consisting of 1:2 mixture of

topsoil (Mollisol) from the Konza Prairie Long-term Ecological Research site near Manhattan, Kansas, USA (39° 05' N, 96° 35' W). Although this study was originally designed to study responses of a local ecosystem in the Great Basin, the use of Konza soil from the Great Plains, which has a strong ^{13}C signal from C_4 plants, was to facilitate isotopic tracing of carbon processes (Verburg et al., 2004). All roots were removed from the soil before mixing it with the sand. In order to ensure that the disturbance effects from soil handling had disappeared before the start of our study, the soils were allowed to sit in the lysimeters for 8 months.

We grew monoculture stands of cheatgrass (*Bromus tectorum*) in a model ecosystem. Two model ecosystems were established in 23 February 1999 by sowing cheatgrass (70 seeds/m^2) seeds in all three lysimeters in both EcoCELLs (EcoCELL1 and 2). We sowed seeds of the cheatgrass in six rows (20 cm apart) per soil container with 20 cm spacing between individual plants (84 plants per soil container; 252 plants per EcoCELL). No N was applied in either of the EcoCELLs. Aboveground biomass of the first crop was harvested 108 days after sowing (10 June 1999), soon after peak green LAI (LAI=4.2) was attained and the plants became apparently senescent (LAI=1.8 and 1.7 for two EcoCELLs, respectively). The study in 1999 was designed to examine performance of the facility with plants growing in the EcoCELLs. As shown in the Results section, water and C fluxes during the growing period were similar between the EcoCELLs. The similar performance of the two EcoCELLs in 1999 offered the possibility to examine effects of N treatments in 2000.

Prior to the second sowing on 31 January 2000, soils were left fallow for six months. On 25 February 2000, $(NH_4)_2SO_4$ was applied to each of the EcoCELLs: the equivalent

of 88 kg N/ha in one application to EcoCELL 1 (i.e., pulse N fertilization) and the same amount to EcoCELL 2 (i.e., gradual N fertilization) in 15 times of weekly additions of 5.87 (totaling 88) kg N/ha. The amount of 88 kg N/ha N fertilizer applied is within the range of the average N fertilization rate in the central grassland region of USA (Burke et al., 2002), and is at the upper level of the anthropogenic N deposition in terrestrial ecosystems (Berendse et al., 1993; Galloway et al., 1995). The reason we chose the upper level of N deposition was to see how the extreme N deposition affects ecosystem gas exchanges in the model ecosystem. Aboveground biomass was harvested 128 days after seeding on 8 June 2000.

During the experiment, water was applied using polyethylene irrigation lines to maintain soil water content at field capacity. Daytime and nighttime temperatures in the EcoCELLs were maintained at 28 °C and 22 °C, respectively, with daytime temperatures starting at 05:00 PST and ending at 19:00 PST. The maintenance of relatively constant soil water content and temperature allowed us to examine the N effects on carbon and water processes without complications of water and temperature interactions.

Gas exchange measurements

Ecosystem gas exchange in the EcoCELLs was measured continuously using Li-Cor 6262 infrared gas analyzers (IRGAs) during the experimental period. Fluxes and environmental factors including temperature, humidity and light were measured every minute and stored as 15-min averages. Data points affected by the presence of people inside the chambers were removed. Calculations of C and water fluxes were made as open system differential measurements as described by Field et al. (1991), and expressed on the ground surface area basis.

Canopy development measurement

Green leaf area index (LAI) was determined by counting the number of green leaves in each of the six experimental plots, and then multiplying by the mean leaf area for individual leaves in the corresponding experimental plots. At each date of leaf area determination, we counted the number of green leaves on a subset of 5 plants out of the 42 plants in each experimental plot. Then, the mean leaf areas were measured by subsampling 20 green leaves randomly selected in each experimental plot and analyzing their leaf areas using an imaging analysis program (Image Pro Plus, Version 1.3.2).

Pseudo-replication and data analysis

Due to the constraints of the number of EcoCELLs and operation costs, it was not practical to set replicates of treatments at the ecosystem level. Therefore, we designed the so-called pseudo-replicates (Hurlbert, 1984) to examine the N effects. Since pulse and gradual N fertilization supplied different available N during early and late growing seasons (R.D. Evans, personal communication), we considered pulse N fertilization as high and gradual N fertilization as low N treatment during the early growing season while gradual N fertilization as high and pulse N fertilization as low N treatment during the late growing season. By comparing the measurements between pulse and gradual N fertilization treatments, we were able to see the relative N effects. We did not intend to look at the N effects by comparing the unfertilized year (1999) and fertilized year (2000) because there was about 20 days of difference in the sowing date between the two years, which could somewhat affect the ecosystem gas exchanges. However, as shown in our results, the N treatment effects were so substantial that different sowing dates in 1999 and 2000 didn't compromise our experiment. Even though we did not have replicates for the

treatments, the quantification of ecosystem C and water fluxes is valid because we used time series of high-precision data for quality control. We quantified the accuracy of system-level measurements and found that more than 95% of 96 data points over 24 h period varied within ±0.5 μmol m^{-2} s^{-1} in both the EcoCELLs. This variation is extremely small compared to the magnitude of ecosystem CO_2 and water exchanges. It is a common practice in biophysical studies that measurements are made with no or few replicates if the instruments have high accuracy. For example, ecosystem flux measurements using eddy-covariance instruments, which are much less accurate than EcoCELLs, are usually not replicated. Eddy-flux data reported in the literature, even without replicated towers, are extremely useful in illustrating diurnal, seasonal, and interannual variability in ecosystem exchange (e.g., Saleska et al., 2003).

We analyzed the data from 21 days after emergence to the harvest of the aboveground biomass in June 1999 and 2000. In order to compare the measurements between the two years and the two treatments, we averaged the 7-day daily gross ecosystem photosynthesis, evapotranspiration and WUE as shown in the figures. Daily gross ecosystem photosynthesis was estimated by integrating 24-h measurements of ecosystem net C flux plus ecosystem dark respiration. Dark respiration was estimated from nighttime ecosystem respiration corrected for the temperature difference between day and night with Q_{10}=1.5, which was calculated from the relationship between nighttime ecosystem respiration and temperature. Ecosystem WUE was defined as the ratio of gross ecosystem photosynthesis to ET. Daily ecosystem WUE was calculated by using the daily gross ecosystem photosynthesis and ET. The differences in gross ecosystem photosynthesis, ET and WUE between pulse and gradual N fertilization treatments and

between year 1999 and 2000 were tested with one-way ANOVA. The linear relationships between ecosystem C and water fluxes were fitted using regression analysis. All the statistical analyses were carried out with the SAS software package (SAS Institute Inc., Cary, NC).

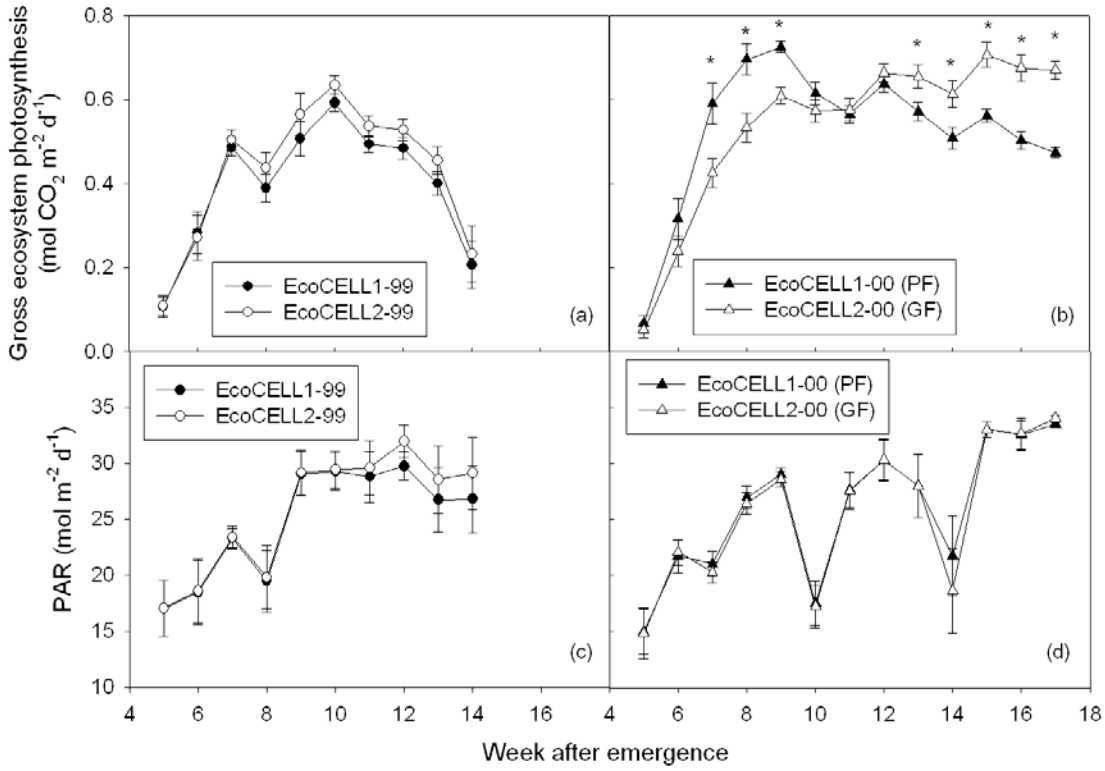


Figure 1. Gross ecosystem photosynthesis (a and b) and photosynthetically active radiation (PAR) (c and d) in EcoCELL1 (solid circle) vs. EcoCELL2 (open circle) in 1999 and EcoCELL1 (pulse N fertilization, solid triangle) vs. EcoCELL2 (gradual N fertilization, open triangle) in 2000. The data points are 7-day averaged daily value with standard error. "*" represents significant difference between pulse and gradual N fertilization at the level of $p<0.05$.

Results

Gross ecosystem photosynthesis, PAR and canopy development

In 1999, gross ecosystem photosynthesis was negligible before 25 days after emergence, and increased to about 0.6 mol m^{-2} d^{-1} at 65 days after emergence, and then declined to zero after harvest. Gross ecosystem photosynthesis was similar between the two EcoCELLs (Fig. 1a). In 2000, N fertilization enhanced daily gross ecosystem photosynthesis during canopy development and delayed foliage senescence for nearly 20 days (Fig. 1b). Specifically, pulse N fertilization accelerated canopy development and increased gross ecosystem photosynthesis during most of the growth periods whereas gradual N fertilization mainly enhanced gross ecosystem photosynthesis during the second half of the growing season. For example, gross ecosystem photosynthesis in pulse N fertilization was 40% higher than in gradual N fertilization at 7 weeks after emergence but 30% lower at 16 weeks after emergence.

Because there was 24 days of difference in the sowing dates (23 February 1999 v.s. 31 January 2000) between the two years, one major concern was that the light difference caused by the different sowing dates may have confounded the N fertilization effects in 2000. PAR in 1999 was significantly higher than that in 2000 within the same EcoCELLs before 4 weeks after emergence due to the different sowing date (data not shown). However, this light shift pattern did not consistently exist after 4 weeks after emergence (Fig. 1), with the PAR levels in 1999 being very close to those in 2000 during most time periods (at 5, 6, 9, 11, 12, 13, and 14 weeks after emergence for EcoCELL1, and at 5, 6, 9, 10, 11, 12 and 13 weeks after emergence for EcoCELL2) until the end of the growing season in 1999. This result suggests that the difference in sowing dates did not

significantly affect the light levels of the two growing seasons during most time periods. The difference in gross ecosystem photosynthesis between the two years was unlikely caused by light difference. Since all other environmental factors were similarly controlled for both EcoCELLs and growing seasons, N fertilization in 2000 became the only possible reason that resulted in the different gross ecosystem photosynthesis between the two years.

The seasonal dynamics of gross ecosystem photosynthesis and their response to N fertilization were related to changes in green leaf area index (LAI). In 1999, the measured LAI in the two EcoCELLs was similar, being 3.58 and 3.25 m^2/m^2 at 70 days after emergence, 1.80 and 1.72 m^2/m^2 at 101 days after emergence. In 2000, the measured LAI in the EcoCELL with pulse N fertilization was 0.04, 0.39, 3.54, 5.61, 5.79, 5.04, and 4.05 m^2/m^2 at 19, 33, 48, 62, 75, 90, and 123 days after emergence, respectively. The measured LAI in the EcoCELL with gradual N fertilization was 0.04, 0.27, 2.11, 4.49, 4.87, 7.01, and 8.06 m^2/m^2 at 19, 33, 48, 62, 75, 90, and 23 days after emergence, respectively. In short, LAI with N fertilization was higher than that without N fertilization; LAI with pulse N fertilization was higher than that with gradual N fertilization during the early growing season and lower than that with gradual N fertilization during the late growing season.

The N fertilization effect on gross ecosystem photosynthesis was also reflected from plant N content and biomass data. The total shoot biomass at harvest in 1999 was 213 g m^{-2} and 230 g m^{-2} for the two EcoCELLs. In 2000, the total shoot biomass was 406 g m^{-2} with pulse N fertilization and 470 g m^{-2} with gradual N fertilization. The green shoot biomass was 57 and 61 g m^{-2} for the two EcoCELLs in 1999, and 218 g m^{-2} for pulse N

fertilization and 291 g m^{-2} for gradual N fertilization in 2000. Gradual N fertilization enhanced the total and green shoot biomass by 16% and 34% relative to pulse N fertilization. The N content of green shoot biomass in 1999 was 1.21 g m^{-2} and 1.35 g m^{-2} in the two EcoCELLs while it was 2.84 and 4.16 g m^{-2} for pulse and gradual N fertilization, respectively, by the end of the growing season in 2000.

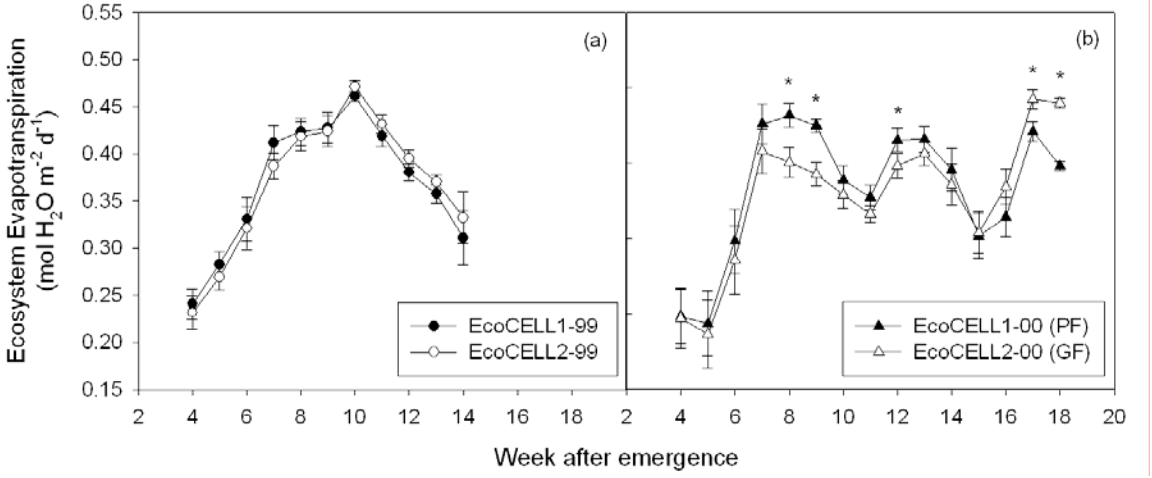


Figure 2. Ecosystem evapotranspiration (ET) in EcoCELL1 (solid circle) vs. EcoCELL2 (open circle) in 1999 (a) and EcoCELL1 (pulse N fertilization, solid triangle) vs. EcoCELL2 (gradual N fertilization, open triangle) in 2000 (b). The data points are 7-day averaged daily value with standard error. "*" represents significant difference between pulse and gradual N fertilization at the level of $p<0.05$.

Ecosystem evapotranspiration

In 1999, ecosystem evapotranspiration (ET) increased with the canopy development, reached the highest value at 10 weeks after emergence, and then decreased with the foliage senescence during the late growing season (Fig. 2a). In 2000, ET maintained lower values than in 1999 at 10-11 weeks after emergence, primarily due to the lower

light levels in 2000 than in 1999 during this period (Fig. 1). By contrast, although the light levels were similar during the late growing season of the two years (Fig. 1), ET in 2000 was higher than in 1999 during this time period (Fig. 2b), mainly resulting from the delayed plant senescence and extended growing season under N fertilization. Pulse and gradual N fertilization caused differential effect on the seasonal dynamics of ET. Before 15 weeks after emergence, pulse N fertilization generally enhanced ET more than gradual N fertilization, with ET being significantly higher under pulse N fertilization than under gradual N fertilization at 8, 9 and 12 weeks after emergence ($p<0.05$). ET under gradual N fertilization was significantly higher than that under pulse N fertilization at 17 and 18 weeks after emergence ($p<0.05$). Since the light levels between pulse and gradual N fertilization were quite close and other environmental factors were manipulated, the different N doses during different growth periods caused by the two N application methods mainly accounted for the difference between the two treatments.

Ecosystem water-use efficiency

Similar to the seasonal dynamics of gross ecosystem photosynthesis and ET, the time course of ecosystem water-use efficiency (WUE) over the growing season in 1999 followed a parabolic curve. In 2000, ecosystem WUE under pulse and gradual N fertilization were similar to that in 1999 during the early growing season (before 7 weeks after emergence), but consistently maintained at high levels until harvest ($p<0.05$) (Fig. 3). Pulse and gradual N fertilization differentially altered the seasonal dynamics of WUE. WUE in pulse N fertilization was higher than that in gradual N fertilization at 7, 8 and 9 weeks after emergence. WUE in gradual N fertilization became higher than that in pulse N fertilization at 13, 14, 15 16 and 17 weeks after emergence. For example, at 7 weeks

after emergence, WUE in gradual N fertilization was 30% higher than in pulse N fertilization. At 17 weeks after emergence, WUE in gradual N fertilization was about 30% higher than that in pulse N fertilization.

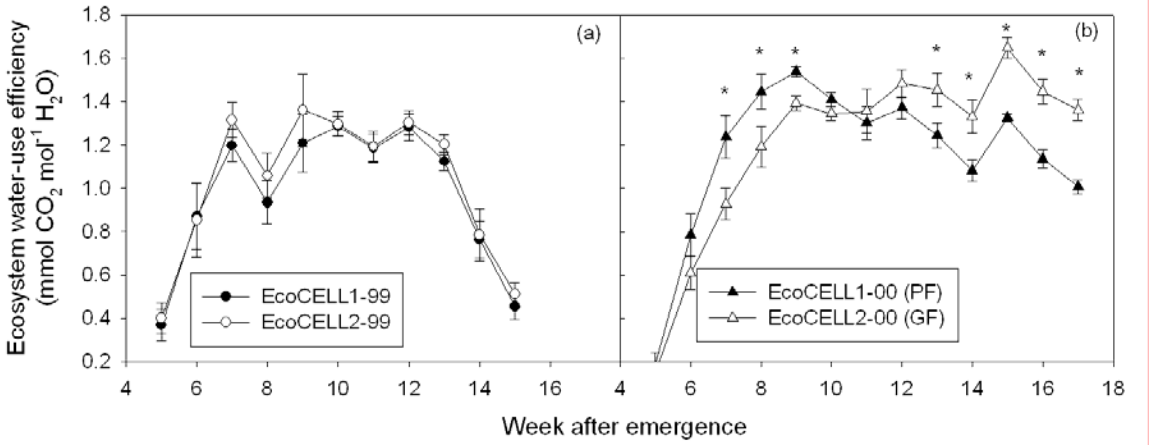


Figure 3. Ecosystem WUE in EcoCELL1 (solid circle) vs. EcoCELL2 (open circle) in 1999 (a) and EcoCELL1 (pulse N fertilization, solid triangle) vs. EcoCELL2 (gradual N fertilization, open triangle) in 2000 (b). The data points are 7-day averaged daily value with standard error. "*" represents significant difference between pulse and gradual N fertilization at the level of $p<0.05$.

The N effects on ecosystem WUE were also demonstrated by the linear relationships between daytime (from 09:00h to 16:45h) ecosystem net C flux and ET (Fig. 4), because the slopes of the curves can be also considered as WUE. Before 7 weeks after emergence, the slopes of the curves in 1999 were similar to those in 2000 for the two EcoCELLs. After this period, the slopes of the curves in 2000 became steeper than in 1999 until the end of the experiment, suggesting higher WUE in 2000 than in 1999. Before 10 weeks after emergence, the slope of the pulse N fertilization curve was steeper than that of

gradual N fertilization, but the slope of the gradual N fertilization curve gradually became steeper than that of pulse N fertilization after that period until the end of the experiment.

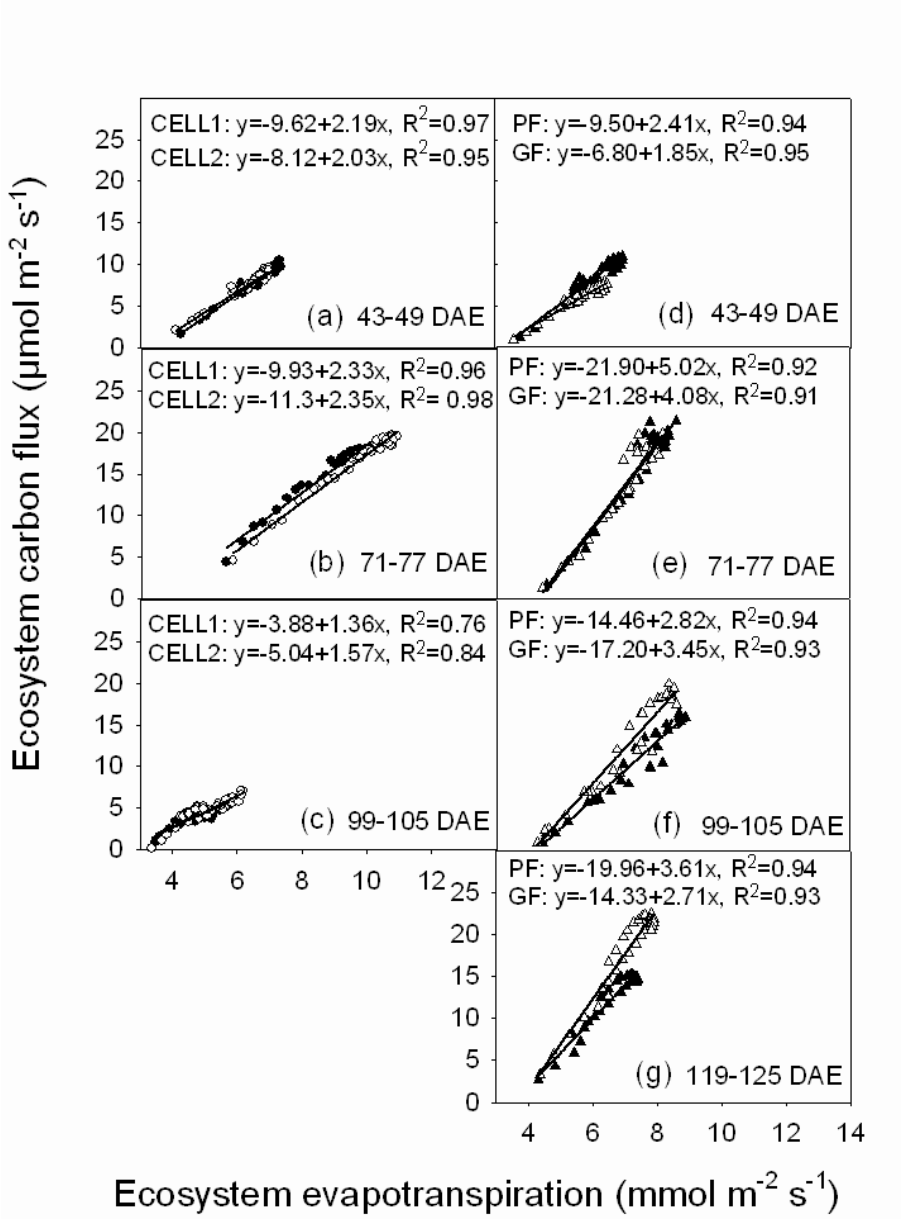


Figure 4. Relationships between ecosystem C flux and ET in EcoCELL1 (solid circle) vs. EcoCELL2 (open circle) in 1999 with no N addition (a - c) and EcoCELL1 (pulse N fertilization, PF, solid triangle) vs. EcoCELL2 (gradual N fertilization, GF, open triangle) in 2000 with N additions (d - g) during the periods of 43-49 (a, d), 71-77 (b, e), 99-105 (c, f) and 119-125 (g) days after emergence (DAE).

Discussion

Ecosystem evapotranspiration (ET) is a major determinant of land surface temperature and other features of climate (Dickinson et al., 2002). However, little is known about how N addition regulates ET and water use at ecosystem level. This study represents one of the first experimental investigations on how different N application methods (pulse and gradual N fertilization) affect ecosystem ET and WUE by utilizing large environmentally controlled growth facilities, EcoCELLs. Compared with the unfertilized year, both pulse and gradual N fertilization increased LAI, plant N content, and extended the growing season by delaying the foliage senescence, resulting in greater gross ecosystem photosynthesis (Fig. 1) during most of the growing season, and greater ET (Fig. 2) during the late growing season. Since N fertilization enhanced gross ecosystem photosynthesis more than ET, N fertilization resulted in higher ecosystem WUE (Fig. 3). The two N fertilization methods differentially affected the seasonal dynamics of gross ecosystem photosynthesis (Fig. 1) more than that of ecosystem ET (Fig. 2), resulting in substantial differences in the seasonal patterns of ecosystem WUE (Fig. 3).

Nitrogen and ecosystem evapotranspiration

Results from our EcoCELL experiment suggest that N influences ecosystem ET primarily through extension of growing season of the annual plants and unlikely through linear correlations between leaf N concentration and stomatal conductance as suggested by Schulze et al. (1994). The nitrogen additions resulted in N content of green shoot biomass being 2.84 g m^{-2} with pulse N fertilization and 4.16 g m^{-2} with gradual N fertilization by the end of growing season in 2000. In comparison, N content of green

shoot biomass was 1.21 and 1.35 g m^{-2} in the two EcoCELLs without N addition in 1999. The substantial increases in tissue N content under nitrogen fertilization in 2000 in comparison with that in 1999 without nitrogen addition did not correspond to proportional increases in ecosystem ET (Fig. 2). In another study on the phenological effects on transpiration and evaporation from the same experiment, Obrist et al. (2003) reported that plant transpiration nonlinearly increased with LAI to the peak value at LAI=3.5-4, and then leveled off. The other important component of ecosystem ET, soil evaporation, progressively declined with increase of LAI (Obrist et al., 2003). The increased LAI with N additions might have reduced solar radiation under the canopy and increased canopy boundary layer resistance, resulting in less water loss from soil surface. Similarly, other studies showed that N fertilization decreased soil evaporation while increasing transpiration in wheat crops (Caviglia and Sadras, 2001), maize fields (Ogola et al., 2002), and tropical forests (Schulze et al., 1994).

Our results illustrated that N additions delayed plant senescence, extended growing seasons, and increased late-season ET in 2000 in comparison to that in 1999. In 1999, plants started senescence at 10 weeks after emergence and completely died at about 14 weeks after emergence with plant photosynthesis and transpiration approaching zero. However, plants with N additions in 2000 maintained high photosynthesis and ET until 17 weeks after emergence. Thus N additions increased ecosystem ET by approximately 30% merely through extension of growing seasons. Other studies have also found that N applications delay plant senescence and extend growing seasons (McCabe et al., 2001).

By stimulating gross ecosystem photosynthesis more than ET, N additions substantially enhanced daily ecosystem WUE (Fig. 3). Similar results were found at leaf

and plant levels (Fredeen et al., 1991; Green and Mitchell, 1992; Lutze and Gifford, 1998; Livingston et al., 1999; Hunsaker et al., 2000). However, mechanisms underlying the enhanced WUE at the ecosystem level could be different from those at leaf- and plant-levels. At the plant level, N fertilization increases plant photosynthesis more than transpiration (Chandler and Dale, 1993; Kubiske et al., 1998), resulting in higher WUE than with N fertilization. This positive N effect on plant WUE is usually attributed to the improved root hydraulic conductivity, increased water storage and improved stomatal control (Hillerdal-Hagstromer et al., 1982). While the mechanism at the plant level primarily regulates WUE at the ecosystem level, soil surface evaporation results in water loss without carbon gain, reducing ecosystem WUE. N additions increased LAI, resulting in relatively less contributions of soil evaporation to the ecosystem ET. Thus, increased ecosystem WUE under N additions could be attributable to both increased plant-level WUE and decreased relative contribution of soil evaporation to ecosystem ET.

Invasive species, water use, and N relationships

Cheatgrass (*Bromus tectorum*) is native to Euroasia and Mediterranean. It was introduced into the intermountain west in the 1890s in impure seed (Mack and Pyke, 1983), and quickly spread through arid regions (Mack, 1981), including the Great Basin. A recent survey showed that *Bromus tectorum* dominates 3.3 millions of acres of public lands in the Great Basin regions (Pellant and Hall, 1994). Cheatgrass invasion may affect ecosystem C and N cycling processes by (1) altering species composition and litter quantity and quality by out-competing the native species (Evans et al., 2001), (2) altering soil temperature and soil water dynamics (Wright et al., 1979; Cline et al., 1977), and (3)

increasing the frequency of wildfires compared with the native species (Wright et al., 1979; Whisenant, 1990; Devine, 1998).

Besides ecosystem C and N cycles, cheatgrass invasion also affects ecosystem water processes in invaded ecosystems. Cheatgrass is a winter annual species, and depletes soil water faster than native species partly because the active growing period of cheatgrass coincides with the winter rainy season (Malek et al., 1997) and partly because cheatgrass was more efficient at using water to a soil depth of 0.5m than a native species community (Cline et al., 1977). It was also found that soil temperature in the cheatgrass-invaded grasslands is higher than that in the interspaces between sagebrush shrubs, causing more soil water depletion in cheatgrass invaded grasslands (Wright et al., 1979). By increasing soil temperature and depleting moisture, cheatgrass effectively depletes soil water resources that otherwise would be available for native plant species in spring and summer (Booth et al., 2003). This study demonstrated another potential mechanism that N fertilization/deposition stimulated growth of cheatgrass and extended growing season, which may likely exacerbate water depletion and further depress growth of native species in later seasons. Such alternations in resource availability and species composition could eventually affect the regional climates and biogeochemical cycles. Although we did not include native species and could not simulate the real situation in the field, we did observe the sole N effects by manipulating other environmental factors.

Nitrogen fertilization versus deposition in influencing ecosystem fluxes

Nitrogen deposition adds N to an ecosystem continuously while N fertilization usually adds a large amount of N to soil once or twice a year. While both theoretical (Luo and Reynolds, 1999) and experimental studies (Hui et al., 2002) have shown that a

step increase in CO_2 concentration has contrasting effects from a gradual CO_2 increase on ecosystem C and N dynamics, it is desirable to know if N fertilization (mimicked by pulse N fertilization) would have different effects from N deposition (mimicked by gradual N fertilization) on ecosystem fluxes. Results from this study suggest that the two methods of N additions differentially alter the seasonal patterns of C and water fluxes.

Pulse and gradual N fertilization added different N doses to the model ecosystems at different growth stages, resulting in differential effects on ecosystem ET and WUE. To examine N availability between pulse and gradual N fertilization at different growth stages, we measured net N mineralization rates using an *in situ* incubation method in 2000. Results showed that the soil N availability in pulse N fertilization (15.3 $mg.kg^{-1}d^{-1}$) was higher than in gradual N fertilization (0.69 $mg.kg^{-1}d^{-1}$) between April and May of 2000, whereas N availability in gradual N fertilization (2.83 $mg.kg^{-1}d^{-1}$) became higher than in pulse N fertilization (-0.02 $mg.kg^{-1}d^{-1}$) between May and June of 2000 (R. D. Evans, personal communications). As a consequence, gross ecosystem photosynthesis was enhanced more in pulse N fertilization than in gradual N fertilization during the early growth period. During the late growth period, gross ecosystem photosynthesis was enhanced more in gradual N fertilization than in pulse N fertilization (Fig. 1). Similar to C flux, ecosystem ET was also differentially altered by pulse and gradual N fertilization during different growth periods, but the extent of alteration was lower than that in C flux (Fig. 2). Thus, ecosystem WUE, the ratio of gross ecosystem photosynthesis to ET, was higher in pulse N fertilization than in gradual N fertilization during the early growing period and higher in gradual N fertilization than in pulse N fertilization during the late growing period (Fig. 2b). The enhanced ecosystem WUE under N treatments was

supported by the relationships between daytime ecosystem net C flux and ET (Fig. 4) and those observed in leaf- and plant-level studies (Fredeen et al., 1991; Cechin, 1998; Siegwolf et al., 2001).

Conclusions

The difficulty in making ecosystem-scale measurements has been a major impediment to advance our understanding of coupling of C, N and water cycles in terrestrial ecosystems. This mesocosm study with EcoCELLs provided unique data sets that offered insights into the biogeochemical coupling. Our results indicate that N fertilization/deposition increased ecosystem WUE primarily by enhancing gross ecosystem photosynthesis more than ET; N fertilization/deposition increased ET primarily by delaying plant senescence and extending growing seasons, much less to the extent by altering ecosystem conductance as suggested by Schulze et al. (1994). By extending growing seasons in a model invasive ecosystem, N fertilization/deposition also has the potential to stimulate invasive species to deplete more soil water resources, accelerating invasion and alternation of regional carbon and water cycles. Our comparative study of pulse N fertilization (mimicking agricultural N applications) and gradual N fertilization (mimicking atmospheric N deposition) suggests that the two methods of N additions differentially affect the seasonal dynamics of ecosystem C and water fluxes, but have minor effects on overall magnitudes of the fluxes, particularly the water flux.

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