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Responses of net ecosystem CO_2 exchange to nitrogen fertilization in experimentally manipulated grassland ecosystems

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ABSTRACT

Nitrogen (N) addition enhances primary productivity of terrestrial ecosystems. However, the effects of N fertilization and/or deposition on net ecosystem CO_2 exchange (NEE) are not fully understood. The effects of N on NEE were investigated in two experimental cheatgrass ecosystems in Ecologically Controlled Enclosed Lysimeter Laboratories (EcoCELLs), Reno, Nevada. In this experiment, no N fertilization was added to the two EcoCELLs in the first year and two different N fertilization regimes were applied in the second year. N fertilizer was applied once to one EcoCELL (pulse fertilization, PF), and the same total amount of N in biweekly increments to the other EcoCell (gradual fertilization, GF). NEE, photosynthetically active radiation (PAR) and canopy green leaf area index (LAI) were continuously measured in the two EcoCELLs during the pretreatment and N-fertilized years. Plant N content and biomass were measured at the end of the growing season in each year. Radiation-use efficiency (RUE_{CO2}) was calculated as the ratio of gross ecosystem photosynthesis (GEP) to the intercepted photosynthetically active radiation (IPAR). The responses of NEE to IPAR were used to estimate the maximum ecosystem photosynthetic capacity (F_{max}). N fertilization stimulated canopy LAI, plant N content, F_{max}, RUE_{CO2}, NEE and biomass in both methods of N supply applications. PF led to higher LAI, F_{max} and NEE than GF, but both had a similar RUE_{CO2} during the early growing season. GF maintained higher LAI, F_{max}, RUE_{CO2} and NEE than PF during the late growing season. At the ecosystem level, N fertilization stimulated daily NEE directly by increasing canopy LAI, plant N content, shoot/root ratio and the maximum ecosystem photosynthetic capacity, and increased the seasonally accumulated NEE indirectly by extending the growing season. PF differed significantly from GF in its effects on NEE and RUE_{CO2}, possibly due to differential rates and timing of N availability. Our study suggested that these changes in the canopy RUE_{CO2} and growing season under N fertilization or N deposition regimes should be considered in modeling studies of ecosystem C sequestration.

1. Introduction

Nitrogen (N) is a major limiting nutrient to primary productivity of terrestrial ecosystems. N fertilization has been found to stimulate plant photosynthesis by increasing Rubisco (ribulose-1,5-bisphosphate carboxylase/oxygenase) enzyme concentration, light interception and radiation-use efficiency (RUE) at leaf- and plant levels (e.g., Weerakoon et al., 2000; Allen et al., 2005; Maier et al., 2008). N fertilization and/or deposition have also been shown to increase leaf area index (LAI) and stimulate plant productivity (e.g., Gough et al., 2004; Bubier et al., 2007; LeBauer and Treseder, 2008). However, how net ecosystem CO_2 exchange (NEE) responds to N addition is still controversial (e.g., Aeschlimann et al., 2005; Bubier et al., 2007; Harpole et al., 2007; Xia et al., 2009; Niu et al., 2009). For instance, Xia et al. (2009) have showed that N fertilization resulted in increases in NEE in a temperate grassland of Inner Mongolia, China, primarily due to stimulated gross ecosystem photosynthesis (GEP). Conversely, N addition has no significant effect on NEE unless additional water was applied in a

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southern California grassland (Harpole et al., 2007). In contrast, nutrient addition in a bog, Ontario, Canada lowered rates of maximum NEE after 5 years of fertilization treatments, largely due to loss of moss photosynthesis (Bubier et al., 2007).

NEE, an important parameter for estimating ecosystem C sequestration, is determined by the balance between GEP and ecosystem respiration (ER) (e.g., Verburg et al., 2004; Aeschlimann et al., 2005). N fertilization potentially affects NEE by altering GEP or ER or both. Usually, N fertilization stimulates gross photosynthesis at ecosystem level based on the leaf- and plant-level responses mentioned above, but plant canopies exhibit variation in response to light from the topmost leaves to the lower leaves (Springer et al., 2005) and to N supply among different species in ecosystems (Reich et al., 2003). This may ultimately cause some uncertainties in how N affects GEP. On the other hand, the N effects on ER can vary greatly in individual ecosystems, mostly owing to the variable responses of autotrophic and heterotrophic respiration in ER, and alterations in ER through changes in litter supply and quality (e.g., Lutze et al., 2000; Craine et al., 2001; Billings and Ziegler, 2008). Thus, it is not sufficient to predict responses of ER and NEE to N fertilization based only on leaf-level and/or plot-level measurements. Understanding the effects of N fertilization on the whole-ecosystem CO_2 exchange requires large scale and well-designed manipulative experiments.

Besides photosynthesis and respiration, canopy radiation-use efficiency (RUE_{CO2}) is another important factor determining ecosystem gas exchange. Ecosystem RUE_{CO2} is defined as the ratio of GEP to the intercepted photosynthetically active radiation (IPAR) and represents the photosynthic capacity. N fertilization can increase photosynthetic capacity by increasing carboxylation capacity (e.g., Evans, 1989) and LAI (e.g., Vose and Allen, 1988). It is well known that N fertilization increases canopy RUE_{CO2} at leaf- and plant levels (e.g., Weerakoon et al., 2000; Allen et al., 2005). Several modeling studies have applied RUE_{CO2} to estimate ecosystem productivity (e.g., Medlyn, 1998; Nichol et al., 2000) and to predict net primary productivity (NPP) as affected by elevated CO_2 and N deposition (Medlyn and Dewar, 1996). However, the impacts of N fertilization on canopy RUE_{CO2} at whole-ecosystem scales are still unclear, with extant studies reporting increased (e.g., Allen et al., 2005), decreased (e.g., Olesen et al., 2000), or unchanged (Bange et al., 1997) canopy RUE_{CO2} after N addition.

Accurate estimation of the impacts of N on NEE is challenging. Currently, several approaches have been used to quantify whole-ecosystem CO_2 exchange, including eddy-covariance techniques (e.g., Jassal et al., 2008), modeling (e.g., Griffis et al., 2000; Lai et al., 2002), environmentally controlled facilities (e.g., Griffin et al., 1996), and whole-ecosystem measurement chambers (Johnson et al., 2000). Among the numerous approaches, 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; Verburg et al., 2004).

In this study, we measured the NEE of experimental ecosystems with cheatgrass (*Bromus tectorum* L.) under pulse vs. gradual N application using Ecologically Controlled Enclosed Lysimeter Laboratories (EcoCELLs) (Griffin et al., 1996; Verburg et al., 2004). We used cheatgrass grassland because it is an important exotic species and has taken over millions of hectares in the intermountain western United States since its introduction in the late 1800s (Mack, 1981). The successful expansion of cheatgrass has been related to several factors, including grazing, fire and fertilization. In particular, cheatgrass establishment and growth is highly dependent on soil N availability (Kay, 1966; Mazzola et al., 2008). Human activities have led to large increases in deposition of atmospheric N to the terrestrial biosphere of North America, where rates of deposition are currently an order of magnitude greater than in pre-industrial times (e.g., Phoenix et al., 2006). Thus, increased atmospheric N deposition and/or N fertilization should greatly enhance cheatgrass establishment and growth (Mazzola et al., 2008) and hence increase cheatgrass grassland productivity and C storage and further affect the local and global carbon cycle.

Additionally, NEE is strongly affected by the amount and the timing of N supply at the ecosystem scale (Aeschlimann et al., 2005; Bubier et al., 2007; Niu et al., 2009). For example, Aeschlimann et al. (2005) have reported that low N leads to a higher NEE than did high N supply in a managed grassland. Further, 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. Thus, it is desirable to know if N fertilization (mimicked by pulse N fertilization, i.e., N applied to one EcoCELL in one application) and/or N deposition (mimicked by gradual N fertilization, i.e., the same amount of N fertilizer applied to another EcoCELL in 15 biweekly additions) would have different effects on NEE. We hypothesized that (1) N fertilization would increase the daily NEE (NEE_d) directly by improving RUE_{CO2} via increasing LAI, 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 delayed plant senescence, and (3) pulse fertilization (PF) and gradual fertilization (GF) would result in different seasonal dynamics of NEE, and different biomass accumulation by altering plant N content, canopy LAI, F_{max} and RUE_{CO2}. To test these hypotheses, we compared NEE, PAR, LAI, plant N content, shoot/root ratio, RUE_{CO2}, light response curves of NEE and the estimated F_{max} between the pretreatment year and N-fertilized year, and between PF and GF, respectively.

2. Materials and methods

2.1. Experimental system

Two EcoCELLs (EcoCELL1 and EcoCELL2) established in Desert Research Institute (Reno, NV, USA) were used for growth facilities in this study. The EcoCELLs have been described in detail by Griffin et al. (1996) and successfully used in several ecosystem-level studies (Luo et al., 2000; Obrist et al., 2003; Verburg et al., 2004; Arnone et al., 2008). In brief, the EcoCELLs are large open-flow mass balance systems using the same principles as leaf-level gas exchange measurements. An EcoCELL is a large environmentally controlled and naturally lit plant growth chamber (7.3 m × 5.5 m × 2.4 m). There are three soil containers (2.85 m × 1.3 m × 1.8 m) within each EcoCELL. Environmental control includes temperature, CO_2 concentration and relative humidity.

Starting in July 1998, each of the three adjacent containers in the two EcoCELLs was filled with a 1-m layer of washed pea gravel, which served as a space holder, 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 containers for 8 months. During the experiment, water was applied using polyethylene irrigation lines put on top of the soil with a spacing of 15 cm to maintain soil water content at field capacity. Daytime and nighttime temperatures in the EcoCELLs were maintained at 28 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.

Three monoculture stands of cheatgrass (*B. tectorum* L.) were established in February 1999 in each of the two EcoCELLs. 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 either EcoCELL 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 the two EcoCELLs, respectively). Prior to the second sowing on 31 January 2000, soils were left fallow without disturbance for 6 months. Beginning 25 February 2000, N fertilizer as $(NH_4)_2SO_4$ was applied as liquid by using a sprayer to each of the EcoCELLs: the equivalent of 88 kg N ha^{-1} in one application to EcoCELL1 (i.e., pulse N fertilization, PF) and the same amount to EcoCELL2 (i.e., gradual N fertilization, GF) in 15 biweekly additions of 5.87 (totaling 88) kg N ha^{-1}. These rates are typical of commonly applied fertilizer unit based on agricultural fertilizer and an order of magnitude greater than atmospheric N deposition ranging from 2 to 12 kg N ha^{-2} y^{-1} in North America (Bubier et al., 2007). Our intent was not to simulate a particular scenario of N deposition under climate change, but rather to see the impacts of N fertilization (mimicked by pulse N fertilization) and N deposition (mimicked by gradual N fertilization) on NEE in cheatgrass grassland ecosystems. Aboveground biomass was harvested 128 days after sowing on 8 June 2000.

2.2. Measurements

NEE in the EcoCELLs was measured continuously using infrared gas analyzers (IRGAs) (Li-Cor, Lincoln, NE, USA) during the experimental period. The measurements were made using the same theoretical basis as leaf- and plant-level photosynthesis measurements. Air flowed continuously through each chamber with CO_2 concentration measured at the inlet and outlet. The accuracy of the NEE measurements was routinely verified by injecting known amounts of CO_2 into each EcoCELL at night when photosynthetic CO_2 uptake was absent. These tests typically lasted only 1 or 2 h and the nighttime respiration was interpolated during this period. The NEE data were corrected for IRGA drift occurring between instrument spans and for variation in airflow meter performance. Fluxes and environmental parameters, including temperature, humidity and photosynthetically active radiation (PAR) were measured every 10 s and stored as 15-min 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 Luo and Zhou (2006) and expressed on a ground surface area basis:

$$\mathrm{NEE} = \frac{\mu_e c_e - \mu_o c_o}{A} \quad (1)$$

where μ_e is the air flow entering the chamber (mol s^{-1}); c_e is the mole fraction of CO_2 in the air entering the chamber (mol of CO_2 - mol^{-1} of air); μ_o is the air flow leaving the chamber (mol s^{-1}); c_o is the mole fraction of CO_2 in the air leaving the chamber (mol of CO_2 mol^{-1} of air); and A is the ground area (m^2).

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. Aboveground biomass was separated into live (green) and dead (brown) biomass. Root biomass was measured by washing roots out of six replicate soil columns (25 cm diameter and 80 cm depth) over a sieve (63 mm mesh size) to avoid loss of roots during washing. Mineral material adhering to roots after washing was removed with tweezers. The biomasses of live, dead, total shoots and total roots were measured by weighing the materials after they had been dried at 70 °C for 48 h. Approximately 20 mg of live and dead shoot subsamples were ground to pass through 20-mesh sieves for N analysis. The N concentration was analyzed using a PerkinElmer CHN analyzer (PerkinElmer, Wellesley, MA, USA). Acetanilide standard was used to verify the accuracy of the analysis instrument. We calculated the plant N content based on biomass (N content = N concentration × biomass).

2.3. 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. In this study, we used each EcoCELL as an experimental unit, creating a pseudo-replicated design (Hurlbert, 1984) to examine the N pulse vs. gradual fertilization effect on NEE. Originally, we did not intend to compare N effects on NEE in the treatment year (2000) to the pre-N treatment year (1999) because of the 24-day sowing difference in the two growing seasons, which could affect the NEE. However, by comparing the weekly averaged PAR between the two growing seasons (Fig. 3(a) vs. (e)), we found that the PAR levels in 1999 were very close to those in 2000 during most time periods except for the first 4 weeks. Therefore, we believe, light might not cause large differences in NEE between the 2 years after the N treatments (see Section 3). In addition, to assess the potential disturbance effects of soil handling, related investigations conducted in the same system at same periods have found that the leachate C and N concentrations and soil respiration were stable prior to the two sowings, meanwhile, the leaching concentrations and soil respiration rates prior to the second sowing were the same as prior to the first seeding (Verburg et al., 2004). Thus, we were able to compare NEE in the N treatment year (2000) to the pre-treatment year (1999) to provide further support to the comparative results of PF and GF. Even if we could not do conventional statistical analyses due to lack of replicates, the quantification of NEE 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 a 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 exchange. Furthermore, it is a common practice in biophysical studies that measurements are made with fewer or no replicates if the instruments have high accuracy. For example, ecosystem flux measurements made by Wofsy et al. (1993) in Harvard Forest using eddy-covariance instruments were not replicated.

In order to compare the measurements between the two EcoCELLs and between the 2 years, we averaged the daily data over one week (7 days). Ecosystem RUE_{CO2} was calculated using RUE_{CO2} = GEP/IPAR. GEP was estimated by integrating daytime measurements of NEE plus ecosystem dark respiration. Dark respiration was plant and soil respiration during the daytime, estimated from nighttime ecosystem respiration (NER) corrected by Q_{10} = 1.5 for the temperature difference between day and night

(6 °C). IPAR was estimated using IPAR = PAR × $(1 - e^{(-LAI \times k)})$ (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 NEE was calculated by integrating 24-h measurements. NER was calculated by averaging nighttime measurements from 19:00 PST to 06:45 PST.

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

$$\mathrm{NEE} = \frac{F_{\max}\alpha I}{F_{\max} + \alpha I} + F_0 \quad (2)$$

where $F_{\max}$ is the maximum value of gross photosynthesis, α is the canopy quantum yield, I is IPAR, and F_0 is the NEE when $I = 0$.

Analysis of variance (ANOVA) was applied to examine the differences in LAI, plant biomass, shoot/root ratio and plant N content between PF and GF and between the 2 years. t-Tests were used to compare the weekly averaged daily PAR, NEE, NER, and ecosystem RUE_{CO2} between PF and GF. Nonlinear regression analysis was used to fit the NEE response curve to IPAR. The statistical analyses were carried out using the SAS 8.0 software (SAS Institute Inc., Cary, NC).

3. Results

3.1. Canopy development, biomass, shoot/root ratio and plant N content

There was no difference in LAI between the two EcoCELLs, and the highest canopy LAI was around 3.4 $m^2\ m^{-2}$ for both EcoCELLs in 1999 (Fig. 1(a)). LAI in 2000 was higher than that in 1999 after the 40th day after emergence (Fig. 1(a) vs. (b)). PF differed greatly from GF in seasonal pattern of LAI, with the highest canopy LAI 5.6 $m^2\ m^{-2}$ for PF and 8.1 $m^2\ m^{-2}$ for GF in 2000 (Fig. 1(b)). LAI values in PF averaged 39% higher than those in GF before the 84th day, whereas LAI values in PF averaged 58% lower than those in GF after the 84th day (Fig. 1(b)).

The total shoot biomass within the two EcoCELLs ranged from 213.1 to 229.6 g m^{-2} in 1999, whereas the total shoot biomass were about 406.2 and 469.7 g m^{-2} under PF and GF, respectively in 2000 (Fig. 2(a)). Similarly, green shoot biomasses, shoot/root ratios and plant N contents in PF and GF in 2000 were higher than those in 1999 (Fig. 2; $p < 0.05$). The total biomasses at harvest in GF (469.7) were 16% higher than that in PF (406.3) (Fig. 2(a)). Green shoot biomasses and green shoot N content were 34% and 46.6% higher in GF than those in PF (Fig. 2(a) and (c)).

3.2. PAR, daily NEE, NER, and canopy RUE_{CO2} during canopy development

PAR, daily NEE, NER and canopy RUE_{CO2} in the two EcoCELLs were similar in 1999 (Fig. 3(a)–(d)). PAR values before week 4 in both EcoCELLs in 1999 were significantly higher than those in 2000 (Fig. 3(a) vs. (e)). However, this light shift pattern was not consistent after week 4, with the PAR levels in 1999 being very close to those in 2000 during most time periods until the end of the first growing season (Fig. 3(a) vs. (e)). Furthermore, the seasonally

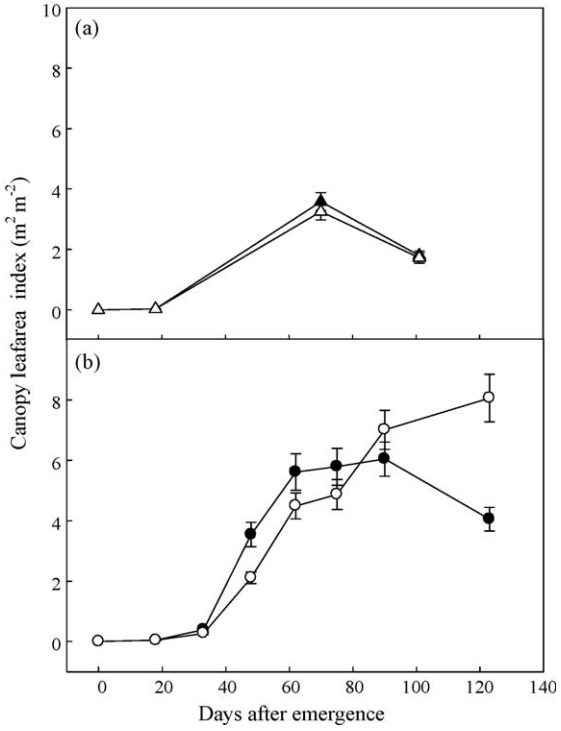

Fig. 1. Canopy LAI in EcoCELL1 (solid triangle) vs. EcoCELL2 (open triangle) in 1999 (a) and EcoCELL1 (PF) (solid circle) vs. EcoCELL2 (GF) (open circle) in 2000 (b). The error bars represent the standard error of means of three replicates (one-way ANOVA, $p < 0.05$).

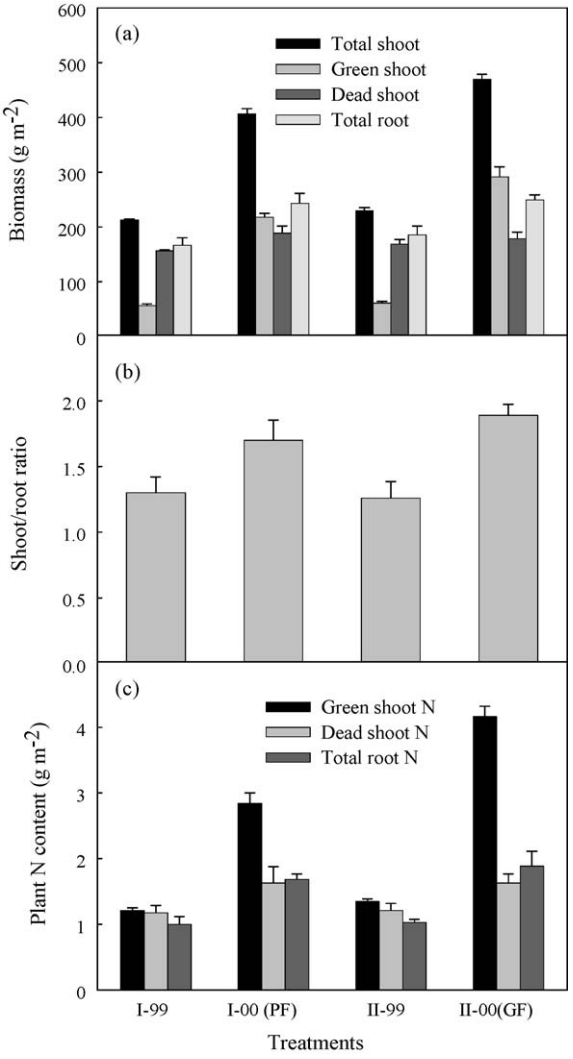

Fig. 2. Biomass of different components (a), shoot/root ratio (b), and N content (c) between the two EcoCELLs in different years. The error bars represent the standard error of means of three replicates (one-way ANOVA, $p < 0.05$).

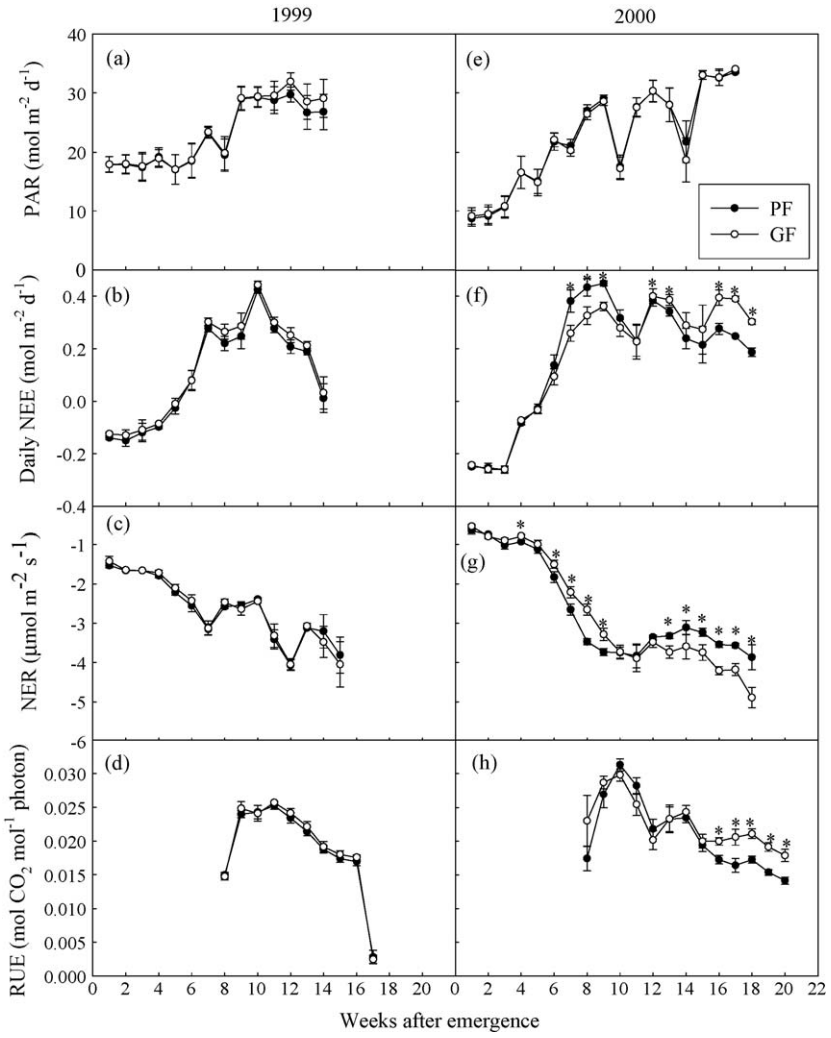

Fig. 3. The weekly averaged PAR levels, daily NEE, NER and canopy RUE_{CO2} in two EcoCELLs in 1999 (a–d) vs. 2000 (e–h). The error bars represent the standard error of the weekly mean. "*" indicates significant difference between PF and GF at the level of $p < 0.05$ (t-test).

accumulated PAR in 2000 (20.1 mol m^{-2} d^{-1}) was lower than that in 1999 (23.2 mol m^{-2} d^{-1}) by the end of the first growing season.

N fertilization enhanced daily NEE in 2000 from week 8 compared with their respective EcoCELLs in 1999 except at week 11. The weekly average daily NEE (NEE_d) in 2000 (0.17 mol m^{-2} d^{-1}) was higher than in 1999 (0.11 mol m^{-2} d^{-1}) (Fig. 3(b) vs. (f)). NEE in PF was higher than that in GF during the weeks 7–9 but lower than in GF after week 11 (Fig. 3(f)). NER in 2000 was significantly lower before week 7, higher at weeks 8, 9, 10 and 13, and lower at week 12 compared to 1999 (Fig. 3(c) vs. (g)). PF averaged 16.0% higher NER compared with GF during weeks 4–9, whereas GF had 14.9% more NER than PF during weeks 13–18 (Fig. 3(g)).

RUE_{CO2} was higher during weeks 9–11 and 13–17 in 2000 than that in 1999. The weekly averaged RUE_{CO2} in 2000 (0.022 mol CO_2 mol^{-1} photon) was higher than 1999 (0.019 mol CO_2 mol^{-1} photon) (Fig. 3(b) vs. (f)). There was no significant difference in ecosystem RUE_{CO2} between PF and GF before week15, but GF enhanced RUE_{CO2} averaged 22.8% greater than PF from weeks 16 to 20 (Fig. 3(h)).

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

There were typical curvilinear relationships between NEE and IPAR in both years 1999 and 2000 (Fig. 4). A rectangular hyperbolic equation was fitted for NEE (Table 1). The initial slopes of the light response curves of the two EcoCELLs were similar in 1999, while PF and GF differentially altered the initial slopes during different growth periods in 2000. The initial slopes of the light response curves in 2000 were steeper than those in 1999 after the 41st day after emergence (DAE) (Fig. 4).

The initial slope in PF was steeper than in GF before 72 DAE, but became lower than GF during the remaining period in 2000 (Fig. 4(e)–(i)). In correspondence to the initial slopes, N fertilization in 2000 also enhanced estimated F_{max} compared with 1999 after 43–49 DAE (Table 1). On the average, PF and GF increased the estimated F_{max} by 67.8% and 48.5% compared with their respective EcoCELLs in 1999 by the end of the first sowing. In addition, PF differed in altering the estimated F_{max} from GF during different growth periods (Table 1). PF enhanced the estimated F_{max} by up to 50.9% compared to GF during the early growth period, whereas GF enhanced the estimated F_{max} by 33.4% compared to PF during the very late growth period (Table 1).

4. Discussion

As previously found at leaf- and plant levels (e.g., Weerakoon et al., 2000; Allen et al., 2005), our results showed that N fertilization increased the weekly averaged daily NEE (NEE_d) (0.11 mol m^{-2} d^{-1} vs. 0.17 mol m^{-2} d^{-1}; Fig. 3(b) vs. (f)) by

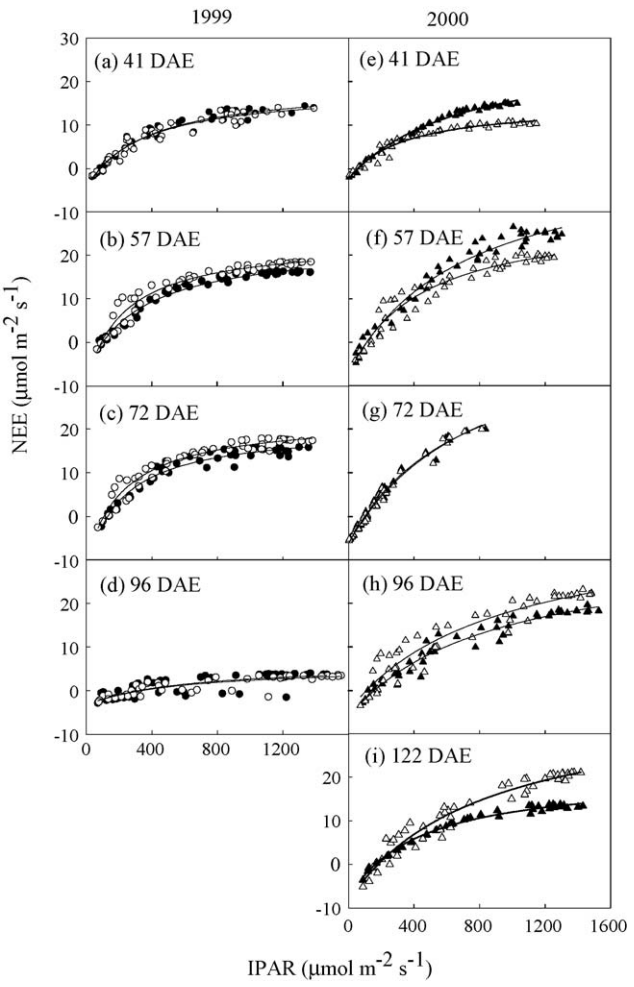

Fig. 4. Response of NEE to IPAR in EcoCELL1 (solid circle) vs. EcoCELL2 (open circle) in 1999 (a–d) and EcoCELL1 (PF) (solid triangle) vs. EcoCELL2 (GF) (open triangle) in 2000 (e–i) at different days after emergence (DAE).

increasing ecosystem RUE_{CO2} via increasing canopy LAI (Fig. 1), plant N content, shoot/root ratio (Fig. 2) and maximum ecosystem photosynthetic capacity (F_{max}) (Table 1). N fertilization increased GEP more than IPAR (data not shown), resulting in higher ecosystem RUE_{CO2} (Fig. 3(d) vs. (h)). The increased GEP may be caused by the increased Rubisco enzyme concentration under N fertilization at the leaf- and plant levels (e.g., Evans, 1989; Gough et al., 2004). Weerakoon et al. (2000) have indicated that the canopy RUE increases curvilinearly with both leaf N concentration and leaf N content, the increased plant N content under N fertilization in 2000 (Fig. 2(c)) led to higher RUE_{CO2} (Fig. 3(h)) through its effect on the GEP and the estimated F_{max} (Table 1). Additionally, canopy biomass and structure allocation may affect canopy RUE_{CO2} and NEE by changing LAI because leaves in sparse canopies are more likely to be light saturated than those in denser canopies (e.g., Gough et al., 2004; Springer et al., 2005). N fertilization can shift biomass allocation from root to leaf components (Gough et al., 2004), which might result in high shoot/root ratio and change canopy structure. We found that N fertilization in 2000 greatly increased canopy LAI (Fig. 1) and shoot/root ratio (Fig. 2(b)), which may have partly resulted in increased canopy RUE_{CO2} (Bange et al., 1997). Thus, our results demonstrated that N fertilization increased ecosystem RUE_{CO2} and hence NEE_d through a combination of three factors by increasing LAI, maximum photosynthetic capacity, and absorption of diffuse light under the canopy.

Table 1
Responses of net ecosystem CO_2 exchange (NEE) to intercepted photosynthetically active radiation (IPAR) in EcoCELL1-1999, EcoCELL2-1999, EcoCELL1-2000 (PF) 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 Fig. 4.

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

DAE: days after emergence; F_{max}: maximum gross photosynthesis; 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.

Another observation was that N fertilization increased the seasonally accumulated NEE and biomass accumulation by extending the growing season (Figs. 2 and 3). We found that the early senescence of plants in 1999 greatly reduced canopy LAI (Fig. 1) and F_{max} (Table 1) and hence reduced RUE_{CO2} compared with year 2000 (Fig. 3(d) vs. (h)). A similar pattern was also observed for PF and GF in 2000 (Figs. 1(b), 3(f)–(h)). Since GF continuously supplied N to plants while PF only stimulated plant growth during the early growing season, GF delayed plant senescence and maintained higher LAI, RUE_{CO2}, biomass and NEE than PF possibly by maintaining high plant N content, Rubisco enzyme activity and photosynthetic capacity. These results are consistent with studies showing that N fertilization can enhance photosynthetic capacity and improve growth with high late-season LAI values (e.g., Scholberg et al., 2000; Gough et al., 2004). In the present study, N fertilization in 2000 delayed plant senescence and extended the growing season by an average of 20 days (calculated using time difference in DAE at which peak LAI occurred) compared to 1999 (Fig. 1). Furthermore, we found that NEE during the extended growing season in 2000 contributed 28.6% and 47.5% to seasonal NEE accumulation, for PF and GF, respectively, compared to 1999 (Fig. 3(b) vs. (f)). Schulze et al. (1994) have reported that plant N content and the length of the growing season largely determine ecosystem gas exchange across different vegetation types of the world. Thus, our results support the evidence that the extension of growing season made a substantial contribution to increase in NEE_{SA}, RUE_{CO2} and biomass accumulation under N fertilization (Schulze et al., 1994; Gough et al., 2004).

The response of NEE to N fertilization was regulated by the different N availability rates and the timing of N additions (Aeschlimann et al., 2005; Bubier et al., 2007; Niu et al., 2009). Although our results showed that N fertilization enhanced NEE, the effects of PF and GF on NEE were significantly different during the growing season in 2000 (Fig. 3(f)). In order to look at the difference of soil N availability between PF and GF during different growth periods, soil inorganic N (NH_4^+-N, and NO_3^--N) were measured *in situ* in 2000 (Evans, unpublished data). Results showed that inorganic N in PF (22.9 mg kg^{-1}) was higher than in GF (16.5 mg kg^{-1}) between March and May of 2000, whereas inorganic N in GF (0.92 mg kg^{-1}) was higher than in PF (0.61 mg kg^{-1}) between May and June of 2000 (Evans, unpublished data). Thus, the different rates of N availability and the different timing of N additions will likely affect NEE by affecting NER, RUE_{CO2} (Fig. 3(g) and (h)) and plant N (Fig. 2(c)). Previous studies have indicated that high N supply may have increased respiration by increasing biomass, plant N and microbial activity (Casella and Soussana, 1997; Aeschlimann et al., 2005). In the present study, N fertilization increased total biomass and leaf N content (Fig. 2), which most probably enhanced ecosystem respiration during the peak and late growing seasons (Fig. 3(c) vs. (g)). Moreover, we found that the high N level in PF stimulated NER during the early growing season, but adequate N in GF maintained high NER during the late growing season (Fig. 3(g)). In addition, the significantly different effects of N regime on canopy RUE_{CO2} between PF and GF were only observed during the late growing season (Fig. 3(h)). RUE_{CO2} may not increase much under adequate N conditions if further N fertilizer is added (N in PF was more than in GF during the early growing season) whereas it may increase dramatically when N becomes limiting (N was limiting in PF, but still adequate in GF during the late growing season). Similar results have been observed by Bange et al. (1997): the major effects of N on early growth of sunflower were mediated by canopy leaf area and leaf N rather than by direct effects of canopy leaf N on RUE_{CO2} alone, and the responses of RUE_{CO2} to leaf N are greater during late growth. Some authors have also indicated that leaf N declines during the early growing season (spring), and starts to recover in mid-summer due to continuous N uptake (e.g., Arain et al., 2006). Being consistent with previous studies (Bange et al., 1997; Arain et al., 2006), higher plant N content in GF than PF during the second harvest (Fig. 2(c)) might lead to higher RUE_{CO2} in GF than PF during the late growing season. Overall, our results highlight the fact that the impacts of N fertilization on NEE are controlled by N availability rates and the timing of N additions through affecting respiration, RUE_{CO2} and plant N.

5. Conclusions

The EcoCELL facility allowed us to study mechanisms underlying NEE responses to N fertilization and deposition. Our study demonstrated that N fertilization has the potential to increase NEE by increasing canopy RUE_{CO2} and extending the growing season in cheatgrass grassland. More importantly, changes in N availability with PF and GF N treatments during different growth periods resulted in different seasonal dynamics of NEE 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. Although the lack of replications in this study could not permit rigorous statistical tests of N effects on NEE, the continuous, high-precision measurements of carbon fluxes over a long time period provide enough quantitative evidence on changes in NEE under different N treatments. Our results thus suggest that changes in the canopy RUE_{CO2} and growing season should be considered in modeling NEE and NPP as affected by N deposition and/or fertilization. To predict the long-term effects of N deposition and/or fertilization on ecosystem processes, we need more long-term field studies and use models to extrapolate experimental results of the N deposition and/or fertilization effect on NEE combined to project future changes in ecosystem carbon cycles in response to multifactor global change.

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