

Ecosystem CO₂ Exchange in Response to Nitrogen and Phosphorus Addition in a Restored, Temperate Grassland

Author(s): L. M. Huff and D. L. PottsE. P. Hamerlynck

Source: The American Midland Naturalist, 173(1):73-87. 2015.

Published By: University of Notre Dame

DOI: <http://dx.doi.org/10.1674/0003-0031-173.1.73>

URL: <http://www.bioone.org/doi/full/10.1674/0003-0031-173.1.73>

BioOne (www.bioone.org) is a nonprofit, online aggregation of core research in the biological, ecological, and environmental sciences. BioOne provides a sustainable online platform for over 170 journals and books published by nonprofit societies, associations, museums, institutions, and presses.

Your use of this PDF, the BioOne Web site, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at www.bioone.org/page/terms_of_use.

Usage of BioOne content is strictly limited to personal, educational, and non-commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

Ecosystem CO₂ Exchange in Response to Nitrogen and Phosphorus Addition in a Restored, Temperate Grassland

L. M. HUFF AND D. L. POTTS¹

SUNY-Buffalo State, Biology Department SAMC 213, New York 14222

AND

E. P. HAMERLYNCK

USDA-ARS, Eastern Oregon Agricultural Research Center, Burns, 97720

ABSTRACT.—Nitrogen (N) and phosphorus (P) affect the structure and function of grasslands by altering plant competitive interactions, shifting patterns of above and below ground biomass allocation, and increasing net primary production. However, the influence of N and P on net ecosystem CO₂ exchange (NEE) is poorly understood. In a field-based factorial N- and P-addition experiment, we measured shallow soil moisture, leaf area index, and component fluxes of midday ecosystem CO₂ exchange throughout the growing season in a restored temperate grassland near Buffalo, New York. Throughout the growing season, N-addition increased gross ecosystem CO₂ exchange (GEE) and correspondingly altered NEE to increase ecosystem CO₂ uptake. In contrast N-addition caused a seasonally dynamic decline in leaf area adjusted GEE, a pattern consistent with increased photosynthetic light limitation. P-addition did not significantly increase R_e, and N- and P-addition interacted to significantly weaken the ecosystem as a midday CO₂ sink. Moreover, water limitation and phenological constraints during the middle and late growing season appear to limit plant responses to nutrient addition. These results suggest influences of N- and P-addition on ecosystem processes are seasonally dynamic and by differentially influencing above and below ground components of ecosystems, the availability of N and P in soils may interact to influence ecosystem CO₂ exchange.

INTRODUCTION

Anthropogenic climate change, species introductions, changing patterns of land-use, and shifting patterns of nutrient cycling are altering ecosystem structure and function worldwide (Schlesinger, 2006; Vitousek *et al.*, 2010). Terrestrial ecosystems have both above and below ground components, which, though linked through litter inputs and the rhizosphere, respond differently to changes in the environment (Wardle *et al.*, 2004; Kardol and Wardle, 2010). For example nitrogen deposition has increased greatly due to a combination of anthropogenic factors associated with the burning of fossil fuels and the use of industrial fertilizers in agriculture (Vitousek *et al.*, 1997). Nitrogen deposition has a wide range of effects in terrestrial ecosystems (Pardo *et al.*, 2011). These effects may include altered species richness, increased photosynthesis and above ground net primary productivity, and shifted above and below ground plant biomass allocation as well as increased emissions of biogenic greenhouse gases (Stevens *et al.*, 2004; LeBauer & Treseder, 2008; Liu & Greaver, 2009; Phoenix *et al.*, 2012). In turn these changes may modify the biogeochemistry of water, carbon, and phosphorus in terrestrial ecosystems (Hessen *et al.*, 2004; Elser *et al.*, 2007; Peñuelas *et al.*, 2012).

¹Corresponding author: phone: (716) 878-9831; fax: (716) 878-4028; e-mail: pottsdl@buffalostate.edu

Nitrogen and phosphorus availability influences the structure and function of grassland ecosystems (Tilman, 1987; Chapin *et al.*, 1987; Menge and Field, 2007), and may co-limit temperate grassland productivity (Niinemets and Kull, 2005; Craine *et al.*, 2003a; Harpole *et al.*, 2011). N-limitation to above ground productivity is primarily photosynthetic in nature; N-addition is associated with increasing concentrations of ribulose-1, 5-bisphosphate carboxylase oxygenase (Rubisco), the enzyme responsible for primary carboxylation in C_3 photosynthesis, and, hence, photosynthetic capacity (Field and Mooney, 1986; Evans, 1989). However, increasing phosphorus availability may indirectly increase shoot length by shifting patterns of above and below ground allocation in response to soil P availability (Marschner *et al.*, 1996). Below ground enhanced N can directly result in greater plant P-limitation, as higher biomass and physiological demand can out-strip plant capacity for P-uptake, especially in fast growing species such as grasses (Craine *et al.*, 2003b). N-addition may also indirectly influence soil microbial communities by increasing plant productivity and thereby reducing labile carbon limitation through increased litter and root exudate production (de Gaaft *et al.*, 2010; Metcalfe *et al.*, 2011). Conversely, N- and P-addition may influence soil microbes directly by altering community composition, biomass, and microbial respiration (Bardgett *et al.*, 1999; Piceno and Lovell, 2000; Kennedy *et al.*, 2004). While the direct effects of P-limitation on microbial communities are expected to be strongest in deeply weathered tropical and subtropical soils (Liu *et al.*, 2012), at least occasional limitation of microbial respiration by P availability has been noted in temperate soils (Allen and Schlesinger, 2004).

Nutrient-influenced shifts in ecosystem productivity may mediated by altered plant phenology (Harpole *et al.*, 2007) as well as the availability of soil moisture driven by precipitation characteristics (Knapp *et al.*, 2008) and feedbacks associated with plant canopies themselves (McLaren *et al.*, 2004). Whereas the effects of rainfall variability on temperate grassland structure and function is well characterized (Knapp *et al.*, 2001; Knapp *et al.*, 2008), the interplay between nutrient availability, soil moisture, and grassland ecosystem function is less well known (Harpole *et al.*, 2007; Xu and Wan, 2008). Adding to this complexity, plants and soil microbes have differing metabolic sensitivity to resource availability depending on antecedent environmental conditions and phenology (Hessen *et al.*, 2004; Potts *et al.*, 2006; Harpole *et al.*, 2007) while plants may feedback to increase soil moisture heterogeneity (McLaren *et al.*, 2004) to further constrain metabolic processes.

By integrating above and below ground biological activity, net ecosystem CO_2 exchange (NEE) represents an important ecosystem function (Potts *et al.*, 2006). NEE represents the balance of ecosystem photosynthesis (gross ecosystem CO_2 exchange, GEE) and ecosystem respiration (R_e) and is sensitive to above or below ground factors such as soil moisture, resource availability, or disturbance that can differentially influence NEE constituent fluxes (Xu and Baldocchi, 2004; Niu *et al.*, 2009; Yan *et al.*, 2011; Potts *et al.*, 2012). By differentially influencing above and below ground processes, N and P availability may mediate ecosystem CO_2 exchange and in turn alter terrestrial carbon cycle source-sink dynamics (Hessen *et al.*, 2004). Our objective was to determine how N- and P-addition influence seasonal patterns of shallow soil moisture, canopy development, ecosystem CO_2 exchange, and above ground net primary productivity (ANPP). In the context of an N- and P-addition experiment in a restored temperate grassland, we predicted N-addition would stimulate canopy development and photosynthesis, therefore increasing net ecosystem CO_2 exchange (NEE) and depletion of shallow soil moisture. As P-limitation to grassland processes usually follow relaxation of N-limitations (Chapin *et al.*, 1987; Phoenix *et al.*, 2003a), we did not expect to P-amendment alone to alter canopy development or ecosystem gas exchange fluxes. Though soil microbial respiration and populations are often P-limited (Bardgett *et al.*, 1999;

Piceno and Lovell, 2000; Kennedy *et al.*, 2004), R_e in grasslands follow soil contributions primarily determined by plant root respiration (Knapp *et al.*, 1998) and above ground plant biomass (Flanagan *et al.*, 2002; Flanagan and Johnson, 2005). Therefore, we expected canopy development, NEE, GEP, and R_e to be enhanced further with the addition of P with N-enrichment.

MATERIALS AND METHODS

SITE DESCRIPTION

This research was conducted at Tiff Nature Preserve (TNP) in the city of Buffalo, New York (42.87°N, 78.87°W; elev. 181 m), located on a former industrial site south of downtown Buffalo on the shore of Lake Erie. TNP is managed by the Buffalo Museum of Science as a nature preserve that contains wetlands, riparian forest, and grassland areas. The experimental site is located on the gently sloping east face of a former municipal landfill that was capped in 1973. Soils at the experimental site are shallow (~45 cm deep) and are situated on top of an impermeable layer of bentonite clay. Soils at the site originated as pond dredging during excavation of surrounding wetlands. This organic-rich silty-clay soil was subsequently compacted during the capping of the landfill (D. Spiering, pers. comm.).

Climate at site is humid continental-type, strongly influenced by the presence of the Great Lakes. The nearest climate data is recorded approximately 16 km away at the Buffalo Niagara International Airport in Cheektowaga, New York (42.56°N, 78.43°W, elev. 222 m) where the mean annual temperature is 9 C with mean annual precipitation of 924 mm. Precipitation during the 2010 Apr.–Nov. growing season was 617.98 mm (Fig. 1A).

The site was seeded in 1973 with a mix of nonnative grasses and forbs. Presently, the plant community is dominated by the cool-season grasses *Festuca rubra* (red fescue) and *Bromus inermis* (smooth brome). Other, less abundant species include the cool-season grass, *Agrostis gigantea* (red top) and the nonnative forbs, *Coronilla varia* (crown vetch) and *Lotus corniculatus* (bird's foot trefoil).

EXPERIMENTAL DESIGN

Five experimental blocks, each containing four 1 × 1 m plots, were delineated in May 2009. Block locations were selected based on similar slope position, proximity to one another, and contained similar representative vegetation. Within each experimental block, a 1 m aisle separated experimental plots to facilitate access and to provide a buffer between experimental treatments. Plots corners were demarcated using short lengths of 0.5 in PVC pipe and were randomly assigned as a control (C), nitrogen-addition (N), phosphorus-addition (P), or nitrogen and phosphorus-addition (N + P). The plots were fertilized for 2 y beginning in 2009; nutrients were applied on Apr. 1, Jun. 14, Aug. 1, and Sep. 14. Nitrogen was applied by hand in the form of calcium nitrate (CaNO₃) pellets totaling a rate of 10 g N/m²/yr, and phosphorus was applied by hand in the form of triple super phosphate (TSP) pellets at a rate of 8.6 g P/m²/yr, consistent with similar nutrient addition experiments (Baddeley *et al.*, 1994; Phoenix *et al.*, 2003a, b; Harpole *et al.*, 2007; Niu *et al.*, 2010).

MEASUREMENTS OF SOIL MOISTURE, CANOPY DEVELOPMENT

Volumetric soil moisture (θ) was measured every 7 to 10 d using a portable meter fit with 12 cm probes (Hydrosense Campbell Scientific, Logan, Utah) between Apr. 22 and Oct. 8, 2010 using the instrument's factory calibration. Probes were inserted vertically into the soil in three representative locations in each plot, and measurements of θ were averaged. Seasonal patterns of leaf area index (LAI) were estimated by measuring canopy interception

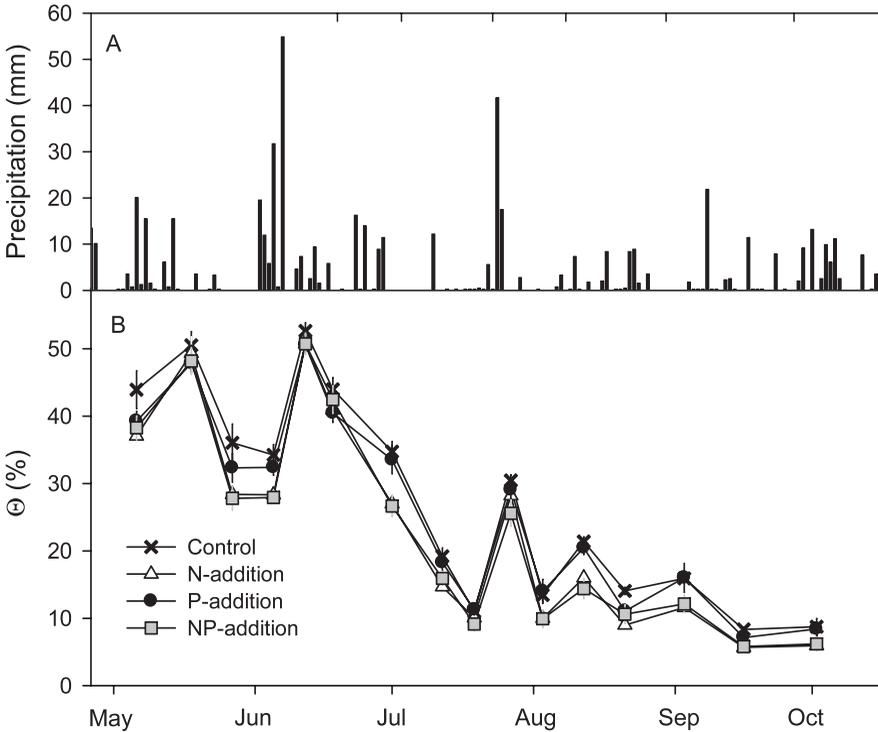


FIG. 1(A).—Daily precipitation during the 2010 growing season. (B) Mean \pm SE volumetric soil moisture (Θ) taken using a 12 cm soil moisture probe in control, N-addition, P-addition, and NP-addition plots during the 2010 growing season in a restored, temperate grassland near Buffalo, NY

of photosynthetically active radiation (PAR) using a line ceptometer (Accupar, Decagon Devices, Pullman, Washington) every 7 to 10 d between Apr. 22 and Oct. 8, 2010 during the midday under clear sky conditions. For above-canopy measurements of PAR, the line ceptometer was oriented level and positioned 25 cm above the canopy, facing south. For below-canopy measurements, three measurements of PAR were made at even intervals, positioned level above the soil litter layer, facing south and averaged. Leaf area index was calculated from measurements of canopy light interception using the line ceptometer's built-in algorithm.

MEASUREMENTS OF ECOSYSTEM CO₂ EXCHANGE

We measured midday ecosystem CO₂ exchange using a portable static chamber measuring 1.0 m by 1.0 m by 1.5 m, constructed of PVC piping, and covered by a tightly sewn translucent polyethylene cover (Durashield 6000, Thor Tarp, Oconomowoc, Wisconsin). These measurements were repeated eight times over the course of the 2010 growing season (May 10, May 25, Jun. 18, Jul. 2, Jul. 22, Aug. 7, Aug. 29, and Sep. 26) under clear sky conditions between the hours of 1100–1300. To measure net ecosystem CO₂ exchange (NEE), the chamber was positioned over a plot and secured to the ground by a heavy chain to minimize leaks. Inside the chamber, two 23 cm diameter electric fans mounted on a portable tripod mixed the chamber atmosphere while a pump connected to

flexible tubing cycled chamber atmosphere to and from an infrared gas analyzer (Li-840, Licor Environmental, Lincoln, Nebraska) positioned outside of the tent. After an initial mixing period of 30 s, changes in CO₂ concentration inside the tent were logged to a portable computer for 90 s.

To measure ecosystem respiration (R_e), the chamber was ventilated, resealed, and the heavy chain was replaced. Then, an opaque cover was placed over the chamber, the chamber was allowed to mix for 30 s, and the increase in chamber CO₂ concentration was logged for 90 s. Air temperature inside the chamber was recorded with a fine-wire thermocouple attached to a portable reader (Fluke 51–54 Series II Thermometer, Everett, Washington) at the beginning and end of each measurement of NEE and R_e . During the 120 s that would elapse during a typical measurement of NEE, air temperature would increase ~ 2 C over ambient. In addition light conditions inside the chamber were recorded with a photosynthetically active radiation (PAR) sensor connected to a bubble-level and attached to the portable tripod (Decagon Devices, Pullman Washington) during each measurement. We calculated NEE and R_e using air temperature and change in CO₂ concentration with time according to Jasoni *et al.* (2005) and estimated gross ecosystem CO₂ exchange (GEE) as the difference between NEE and R_e . Leaf area within plots varied greatly throughout the growing season and between experimental treatments. To better understand seasonal and treatment mediated shifts in GEE per unit leaf area, we standardized GEE measurements with LAI measurements to calculate leaf area normalized GEE (GEE_{LA}) as:

$$GEE_{LA} = GEE / LAI$$

where LAI was taken within ± 2 d of the ecosystem CO₂ exchange measurements.

MEASUREMENTS OF ABOVE GROUND NET PRIMARY PRODUCTIVITY

We estimated above ground net primary productivity (ANPP) and litter abundance by clipping a 10 cm \times 100 cm strip in each experimental plot during the second week of Oct. 2010. Clipped biomass was sorted in the field as grasses, forbs, and litter; returned to the laboratory; oven dried for 48 h at 50 C; and weighed. We report ANPP as the sum of area-adjusted live grass and forb biomass and expressed on a per square meter basis.

STATISTICAL ANALYSIS

Data were examined to see if they met the normality assumption of parametric statistics. In the case of ANPP, data were log transformed and in the case of θ , data were arcsine transformed (Zar, 1999). A three-factor repeated measures ANOVA (JMP, SAS Institute, Cary, North Carolina) was used to test for the significance of N-addition, P-addition, time, and their interactions using NEE, GEE, R_e , θ and LAI as response variables. We used a two-factor ANOVA to test the effect of N-addition, P-addition, and their interaction on ANPP. To better understand the effects of N- and P-addition on the linkage between above and below ground processes, we compared plot-averaged GEE and R_e across all dates using linear regression and tested for separate slopes.

RESULTS

Across nutrient addition treatments, volumetric soil moisture (θ) reflected seasonal patterns of precipitation (Figs. 1A, B). Early in the growing season, frequent rainfall was associated with greater θ . As the growing season progressed, smaller, less frequent storms were associated with declining θ across treatments (Table 1, Time). Throughout the

TABLE 1.—Degrees of freedom (*df*) and F-statistics (*F*) of a repeated-measures ANOVA of arcsine transformed volumetric soil moisture (Θ) and leaf area index (LAI) during the 2010 growing season in factorial nitrogen (N) and phosphorus (P) addition field experiment in a restored temperate grassland near Buffalo, New York. * indicates significance at $P < 0.05$; ** indicates significance at $P < 0.01$

Factors	Θ		LAI	
	<i>df</i>	<i>F</i>	<i>df</i>	<i>F</i>
Nitrogen	1, 16	24.9**	1, 16	59.3**
Phosphorus	1, 16	0.98	1, 16	0.003
N \times P	1, 16	1.19	1, 16	0.34
Time	16, 1	235.7*	14, 3	139.9**
Time \times N	16, 1	47.8	14, 3	2.60
Time \times P	16, 1	1.74	14, 3	0.31
Time \times N \times P	16, 1	2.35	14, 3	0.31

growing season, N-addition influenced a decline in θ in comparison with untreated controls ($0.25 \text{ m}^3 \text{ m}^{-3} \pm 0.86$ and $0.29 \text{ m}^3 \text{ m}^{-3} \pm 0.72$ SE, respectively; Table 1, Nitrogen). In contrast P-addition did not influence θ .

Beginning in mid-Apr. and continuing through early Oct., continuous grassland canopy development was reflected in increasing leaf area index (LAI) throughout the growing season (Fig. 2; Table 1, Time). This seasonal long trend was temporarily interrupted during

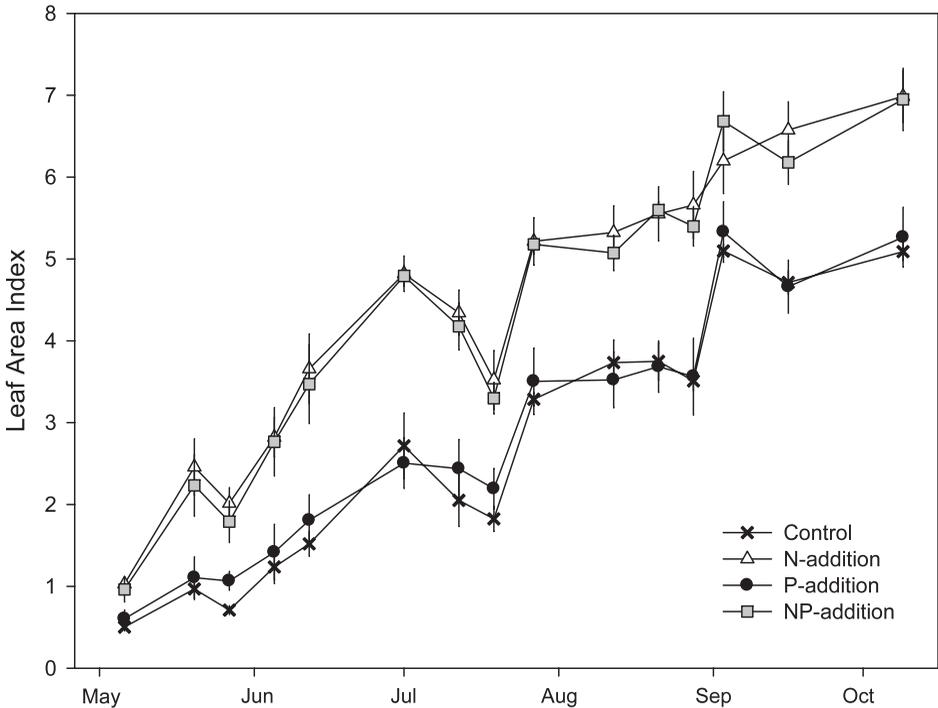


FIG. 2.—Mean \pm SE leaf area index (LAI) in control, N-addition, P-addition, and NP-addition plots during the 2010 growing season in a restored, temperate grassland near Buffalo, NY

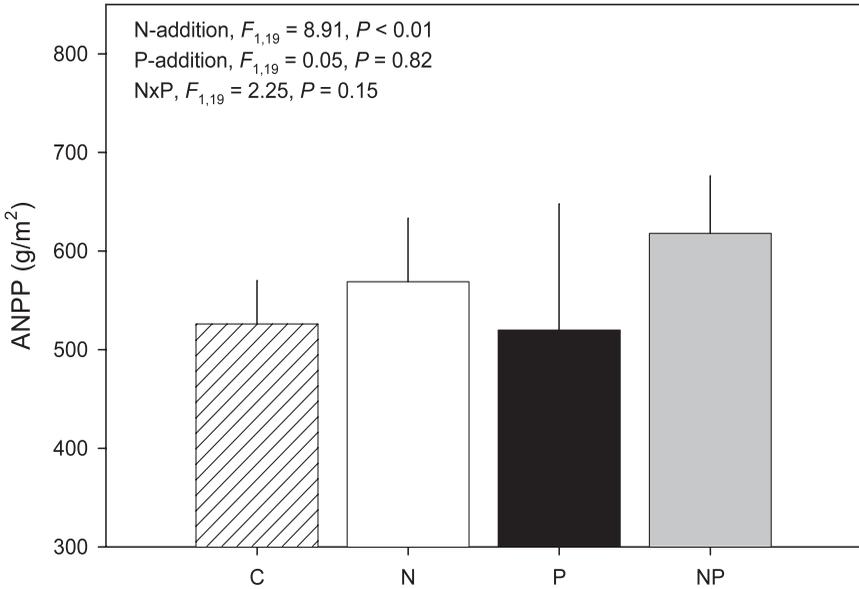


FIG. 3.—Aboveground net primary productivity (ANPP; g/m²) in control (C), N-addition (N), P-addition (P) and NP-addition (NP) plots in a restored, temperate grassland near Buffalo, NY. Error bars indicate SE

early and mid-Jul., a period of rapidly declining soil moisture (Fig. 1A). Similar to θ , N-addition influenced increased LAI throughout the growing season (Table 1, N). The positive effect of N-addition on LAI was reflected in the significant positive effect of N-addition on ANPP (Fig. 3). In contrast P did not significantly increase ANPP nor did the positive effect of N-addition depend on the addition of P (Fig. 3).

Net ecosystem CO₂ exchange (NEE) varied throughout the growing season (Table 2; Time, Fig. 4A). The greatest rates of midday net CO₂ uptake (indicated by large negative values of NEE) occurred during the early growing season. As the growing season progressed,

TABLE 2.—Degrees of freedom (*df*) and F-statistics (*F*) of a repeated-measures ANOVA of net ecosystem CO₂ exchange (NEE), gross ecosystem CO₂ exchange (GEE), ecosystem respiration (R_c) and leaf-area adjusted GEE (GEE_{LA}) during the 2010 growing season in factorial nitrogen (N) and phosphorus (P) addition field experiment in a restored temperate grassland near Buffalo, New York. * indicates significance at P < 0.05; ** indicates significance at P < 0.01

Factors	<i>df</i>	NEE	GEE	R _c	GEE _{LA}
		<i>F</i>	<i>F</i>	<i>F</i>	<i>F</i>
Nitrogen	1, 16	8.61**	17.24**	10.34**	15.75**
Phosphorus	1, 16	0.01	0.25	0.43	0.85
N × P	1, 16	4.73*	1.60	0.14	0.05
Time	7, 10	32.2**	47.86**	113.8**	71.2**
Time × N	16, 1	10.68**	4.12*	3.08*	8.83**
Time × P	16, 1	0.94	2.12	1.05	1.16
Time × N × P	16, 1	1.87	0.58	1.13	0.77

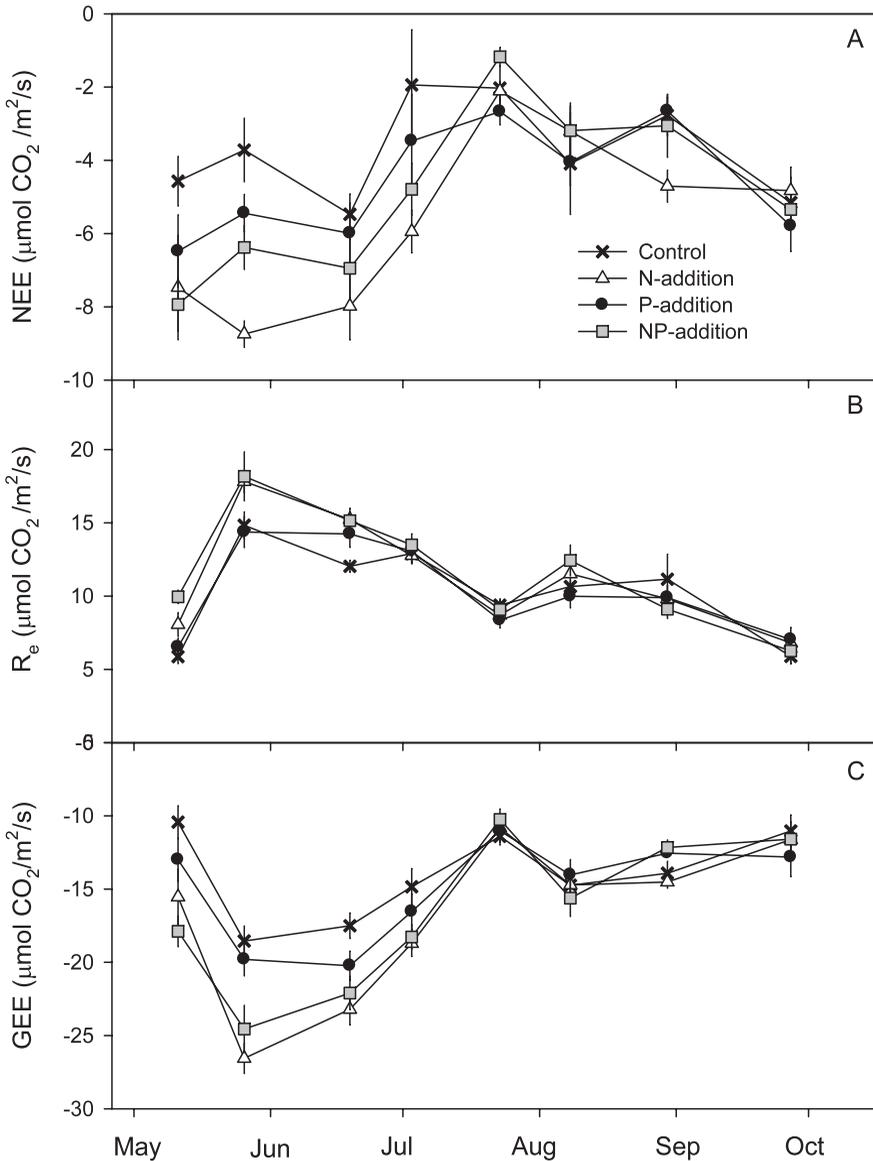


FIG. 4.—Components of mean midday ecosystem CO₂ exchange in control, N-addition, P-addition, and NP-addition plots during the 2010 growing season in a restored, temperate grassland near Buffalo, NY. Error bars indicate \pm SE. (A) Net ecosystem CO₂ exchange (NEE; $\mu\text{mol CO}_2/\text{m}^2/\text{s}$); greater negative values indicate increasing ecosystem CO₂ assimilation. (B) Ecosystem respiration (R_e ; $\mu\text{mol CO}_2/\text{m}^2/\text{s}$); positive values indicate increasing ecosystem CO₂ loss. (C) Gross ecosystem CO₂ exchange (GEE; $\mu\text{mol CO}_2/\text{m}^2/\text{s}$); greater negative values indicate increasing ecosystem CO₂ assimilation. Note the difference in y-axis scale in panel A

midday NEE became less negative (indicating a weakening of the ecosystem as a midday C-sink) and became more negative again during the late-growing season.

Consistent with our prediction, N-addition influenced greater negative values of NEE (Table 2; Nitrogen, Fig. 4A). The effect of N-addition was greatest during the early-growing season and declined as the growing season progressed (Fig. 4; Table 2, Time \times N). In contrast P-addition did not influence NEE (Fig. 4A). However, N and P in combination were associated with a weakening of the ecosystem as a midday CO₂ sink (as indicated by values of NEE closer to zero) compared those that received only N-addition (Table 2, NxP).

Ecosystem respiration (R_e) varied throughout the growing season (Fig. 4B; Table 2, Time). During the early growing season, R_e increased rapidly, with large positive values indicating increased respiratory losses of CO₂ by plants and soil microbes, and then declined steadily through Jul. A slight increase in R_e was observed during Aug. followed by a gradual decline in R_e through late Sep. Contrary to prediction, P-addition did not significantly increase R_e (Fig. 4B). Similar to NEE, the influence of N-addition on R_e was seasonally dynamic such that the positive influence of N on R_e was greatest during the earliest part of the growing season (Table 2, Time \times N).

Like the other component fluxes of NEE, gross ecosystem CO₂ exchange (GEE) rapidly became more negative (indicating greater ecosystem CO₂ assimilation) during the early-growing season followed by a middle growing season nadir and a late growing season recovery (Fig. 4C). Moderate temperatures and abundant soil moisture during the early growing season created an ideal environment for C₃ photosynthesis (Fig. 1B). However, in the middle portion of the growing season, high temperatures and low soil moisture (Fig. 1B) may explain the drop-off in photosynthesis (Fig. 4C). The most negative GEE values occurred in the early growing season (May 10, May 25, Jun. 18) and the greatest differences between nutrient addition treatments also occurred at these times (Fig. 4C). Similar to ecosystem respiration, N-addition significantly increased gross ecosystem CO₂ exchange (GEE) as predicted (Table 2, Nitrogen). The treatment effect of N on GEE was seasonally dynamic, being pronounced in the early portion of the growing season and later declining (Fig. 4C; Table 2, Time \times N). Consistent with the responses of NEE and R_e , P-addition did not influence GEE.

To better understand the influence of N- and P-addition on the relationship between GEE and R_e , we used least squares linear regression to compare treatment averaged GEE and R_e from each sampling date throughout the growing season (Fig. 5). Regardless of treatment, increasingly positive R_e (indicating increasing CO₂ efflux) was correlated with increasingly negative GEE (indicating increasing CO₂ uptake by the ecosystem; Fig. 5). However, the 95% confidence interval of slope of best-fit line included 1 suggesting that throughout the growing season changes in midday GEE are consistently matched by changes in R_e of the same magnitude (mean = -1.16 , 95% CI = -1.37 – -0.96 , $n = 32$). Treatment-specific regressions did not explain significant additional variance, suggesting nutrient addition does not alter the fundamental relationship between these component ecosystem carbon-balance fluxes.

In contrast with seasonal patterns of GEE, leaf area-adjusted GEE (GEE_{LA}) declined throughout the growing season (Fig. 6). A seasonal decline in GEE_{LA} reflects a steady increase in LAI coupled with more consistent values of GEE throughout the growing season (Figs. 2, 4C respectively). Interestingly, N-addition had the effect of reducing GEE_{LA} (less negative values) compared to other plot types (Table 2; Nitrogen) and the effect of N-addition tended to decline through the growing season (Table 2; Time \times N). This pattern of response suggests a complex interplay between plant N allocation, leaf level photosynthetic capacity, and light limitation.

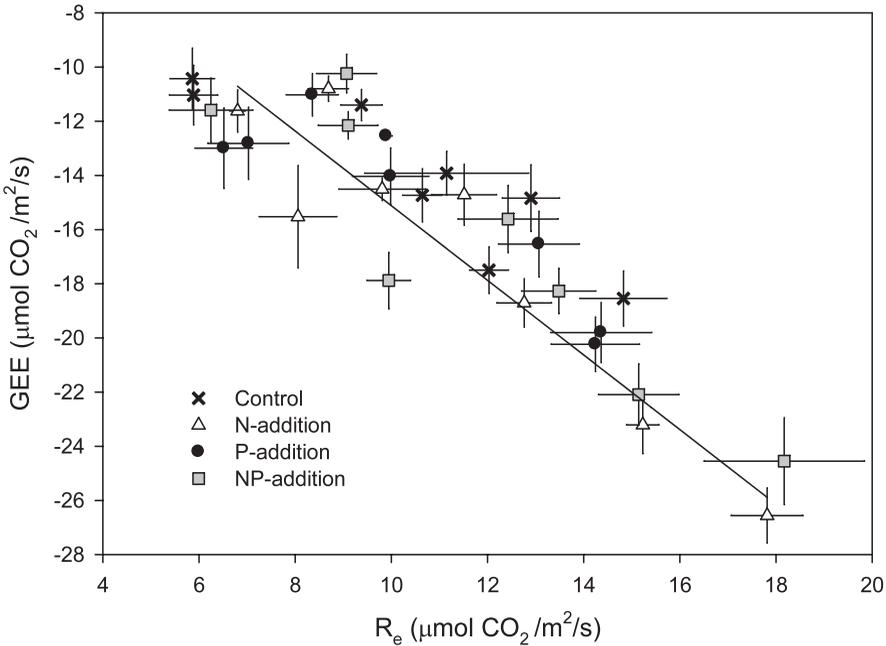


FIG. 5.—Midday ecosystem respiration (R_e ; $\mu\text{mol CO}_2/\text{m}^2/\text{s}$) versus midday gross ecosystem CO_2 exchange (GEE; $\mu\text{mol CO}_2/\text{m}^2/\text{s}$) for all sampling days in control, N-addition, P-addition, and NP-addition plots during the 2010 growing season in a restored, temperate grassland near Buffalo, NY. Symbols follow Figure 1B. Increasing negative values of GEE note increasing ecosystem CO_2 assimilation

DISCUSSION

Differential responses of above and below ground components of grassland ecosystems to N- and P-addition influence ecosystem CO_2 exchange and hence, ecosystem carbon balance. Moreover, these responses are seasonally dynamic reflecting plant phenology and allocation plasticity as well as the limitation of temperature and soil moisture on photosynthesis and soil microbial activity. In the present study, the mid-season decline in midday NEE we observed may be attributed to a decline in GEE (values closer to zero) associated with high midday temperatures and water limitation leading to stomatal closure and high rates of photorespiration in this plant community dominated by the C_3 photosynthetic pathway (Sharkey, 1988). While several studies have experimentally established the linkage between soil moisture and ecosystem CO_2 exchange in semi-arid (Potts *et al.*, 2006; Patrick *et al.*, 2007) and Mediterranean-type grasslands (Harpole *et al.*, 2007; Potts *et al.*, 2012), much remains to be learned regarding the role of seasonal water deficit in constraining ecosystem CO_2 exchange in grassland ecosystems not typically associated with water limitation (Novick *et al.*, 2004; Xia *et al.*, 2009).

While mid-season high temperatures and water limitation may have worked together to constrain ecosystem C-uptake, N-addition strengthened ecosystem C-uptake (Fig. 4A). Whereas Flanagan *et al.* (2002) observed close correlation between changes in GEE and changes in LAI in a temperate shortgrass steppe with relatively low LAI (<1), we observed the most negative GEE during the early growing season, well before peak LAI (~ 7 ; Fig. 2). Increasing light limitation with increasing LAI may explain the observed weak linkage

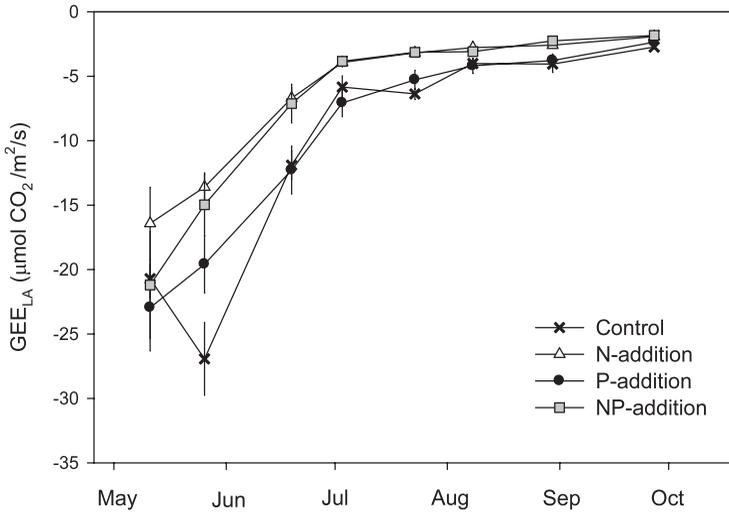


FIG. 6.—Leaf area adjusted midday gross ecosystem CO₂ exchange (GEE_{LA}; μmol CO₂/m²/s) in control, N-addition, P-addition, and NP-addition plots during the 2010 growing season in a restored, temperate grassland near Buffalo, NY. Error bars indicate SE. Increasing negative values note increasing uptake of CO₂ by the ecosystem

between LAI and GEE. Indeed, canopy light limitation has been implicated in altering the structure of tallgrass prairie and meadow communities (Knapp and Steastedt, 1986; Hautier *et al.*, 2009) though its influence on grassland CO₂ exchange remains poorly studied (Niu *et al.*, 2010).

We observed that N-addition was associated with a decline in leaf area adjusted GEE (GEE_{LA}) suggesting the positive effect of supplemental N on leaf-level photosynthesis is offset by canopy-scale light limitation. Increasing LAI may have reduced incident photon flux densities to inner-canopy leaves thereby reducing maximum leaf-level photosynthesis (Anten *et al.*, 1995). In addition N-amendment accelerated canopy development (Fig. 2), which, coupled with light-attenuation, may have increased inner-canopy leaf senescence. Attached senescent leaves would contribute to total LAI, but not to GEE, thereby reducing GEE_{LA}. If we had partitioned LAI into green and standing dead biomass, it may be that GEE scaled to green leaf area index were similar, or even higher, in N-amended grassland plots (Hamerlynck *et al.*, 2010).

In addition to influencing a decline in GEE_{LA}, N-addition positively influenced R_e. Enhanced R_e in N-addition plots may be explained in part by the greater plant biomass that these plots supported (Flanagan and Johnson, 2005; Potts *et al.*, 2008; Xu and Wan, 2008; Xia *et al.*, 2009). Also, if the senescent LAI fraction increased proportionally with increased canopy development in N-addition plots, decomposition of this aboveground pool may have contributed to enhanced R_e (Zhou *et al.*, 2009). Moreover, N-addition may have indirectly increased soil microbial respiration through the increased availability of labile carbon in the rhizosphere (de Graaff *et al.*, 2010). The close correlation between GEE and R_e across experimental treatments is consistent with the idea that these important ecosystem functions are tightly coupled to one another though plant productivity (Fig. 5; Metcalfe *et al.*, 2011).

Contrary to our prediction, P-addition had little influence on R_e and NEE. Instead, the effect of P-addition on ecosystem CO₂ exchange depended on nitrogen availability (Table 2,

N × P). By stimulating plant productivity, N-addition may have indirectly increased labile carbon supply to the soil through enhanced root activity and increased root exudates (Högberg and Read, 2006) in turn shifting microbial activity from C- to P-limitation. However, while this explanation is supported by the response of NEE, the interactive effects of N- and P-addition on R_e are less consistent suggesting the possibility that the interactive N + P effect is mediated by other environmental conditions such as temperature or soil moisture. Alternatively, a lack of R_e response to P-addition may be related to a decrease in plant allocation to fine roots and root exudates with increased P-availability (Marschner *et al.*, 1996). By reducing below ground allocation of carbon by plants, increasing labile carbon limitation to microbial activity may have offset P-mediated increases in soil microbial respiration.

Driven by increasing LAI, N-addition significantly increased growing season GEE, R_e and NEE. In contrast the effect of P-addition on NEE depended on the availability of N and influenced a weakening of the ecosystem as a carbon sink. The significant interaction of these factors reinforces the critical role of plant-mediated labile carbon allocation to the rhizosphere (Metcalf *et al.*, 2011) and the close coupling of nitrogen and phosphorus availability in controlling carbon balance in terrestrial ecosystems (Kardol and Wardle, 2010).

Acknowledgments.—D. Huff, M. Socha, J. Hirtreiter, C. Holmgren, J. Louie, E. Snyder, N. Wood and J. Wuerstle assisted in the field and in the laboratory. We thank the Buffalo Museum of Science for providing access Tift Nature Preserve as well as the generous cooperation and support of D. Spiering, managing ecologist of Tift Nature Preserve. C. Pennuto and E. Standora provided thoughtful comments on an earlier draft of this manuscript. Financial support was provided by the Buffalo State Research Foundation, the Buffalo State School of Natural and Social Sciences and by a grant from the Buffalo State Community Partnerships Office.

LITERATURE CITED

- ALLEN, A. S. AND W. H. SCHLESINGER. 2004. Nutrient limitations to soil microbial biomass and activity in loblolly pine forests. *Soil Biol. & Biochem.*, **36**:581–589.
- ANTEN, N. P. R., F. SCHIEVING, AND M. J. A. WERGER. 1995. Patterns of light and nitrogen distribution in relation to whole canopy carbon gain in C_3 and C_4 mono- and dicotyledonous species. *Oecologia*, **101**:504–51.
- BADDELEY, J. A., S. J. WOODIN, AND I. J. ALEXANDER. 1994. Effect of increased nitrogen and phosphorus availability on the photosynthesis and nutrient relations of three arctic dwarf shrubs from Svalbard. *Func. Ecol.*, **8**:676–685.
- BARDGETT, R. D., J. L. MAWDSLEY, S. EDWARDS, P. J. HOBBS, J. S. RODWELL, AND W. J. DAVIES. 1999. Plant species and nitrogen effects on soil biological properties of temperate upland grasslands. *Func. Ecol.*, **13**:650–660.
- CHAPIN, F. S., A. J. BLOOM, C. B. FIELD, AND R. H. WARING. 1987. Plant responses to multiple environmental factors. *BioScience*, **37**:49–57.
- CRAINE, J. M., C. MORROW, AND W. D. STOCK. 2008. Nutrient concentration ratios and co-limitation in South African grasslands. *New Phyt.*, **179**:829–836.
- DE GAAFF, M., A. T. GLASSEN, H. F. CASTRO, AND C. W. SCHADT. 2010. Labile soil carbon inputs mediate the soil microbial community composition and plant residue decomposition rates. *New Phyt.*, **188**:1055–1064.
- ELSER, J. J., M. E. S. BRACKEN, E. E. CLELAND, D. S. GRUNER, W. S. HARPOLE, H. HILLEBRAND, J. T. NGAI, E. W. SEABLOOM, J. B. SHURIN, AND J. E. SMITH. 2007. Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. *Ecol. Lett.* doi: 10.1111/j. 1461-0248.2007.01113.x
- EVANS, J. R. 1989. Photosynthesis and nitrogen relationships in leaves of C_3 plants. *Oecologia*, **78**:9–19.

- FIELD, C. AND H. A. MOONEY. 1986. The photosynthesis-nitrogen relationship in wild plants, p. 25–54. In: T. I. Givnish (ed.). *On the economy of plant form and function*. Cambridge University Press, Cambridge.
- FLANAGAN, L. B. AND B. G. JOHNSON. 2005. Interacting effects of temperature, soil moisture and plant biomass production on ecosystem respiration in a northern temperate grassland. *Ag. For. Met.*, **130**:237–253.
- , L. A. WEVER, AND P. J. CARLSON. 2002. Seasonal and interannual variation in carbon dioxide exchange and carbon balance in a northern temperate grassland. *Glob. Change Biol.*, **8**:599–615.
- HAMERLYNCK, E. P., R. L. SCOTT, M. S. MORAN, T. O. KEEFER, AND T. E. HUXMAN. 2010. Growing season ecosystem and leaf-level gas exchange of an exotic and native semiarid bunchgrass. *Oecologia*, **163**:561–570. doi 10.1007/s00442-009-1560-1
- HARPOLE, W. S., J. T. NGAI, E. E. CLELAND, E. W. SEABLOOM, E. T. BORER, M. E. S BRACKEN, J. J. ELSER, D. S. GRUNER, H. HILLEBRAND, J. B. SHURIN, AND J. E. SMITH. 2011. Nutrient co-limitation of primary producer communities. *Ecol. Let.* doi: 10.1111/j.1461-0248.2011.01651.x
- , D. L. POTTS, AND K. N. SUDING. 2007. Ecosystem responses to water and nitrogen amendment in a California grassland. *Glob. Change Biol.*, **13**:2341–2348.
- HAUTIER, Y., P. A. NIKLAUS, AND A. HECTOR. 2009. Competition for light causes plant biodiversity loss after eutrophication. *Science*, **324**:636–638.
- HESSEN, D. O., G. ÅGREN, T. R. ANDERSON, J. J. ELSER, AND P. C. DE RUITER. 2004. Carbon sequestration in ecosystems: the role of stoichiometry. *Ecology*, **85**:1179–1192.
- HÖGBERG, P. AND D. J. READ. 2006. Towards a more plant physiological perspective on soil ecology. *Trends in Ecol. and Evol.*, **21**:548–554.
- JASONI, R. L., S. D. SMITH, AND J. A. ARNONE, III. 2005. Net ecosystem CO₂ exchange in Mojave Desert shrublands during the eighth year of exposure to elevated CO₂. *Glob. Change Biol.*, **11**:749–756.
- KARDOL, P. AND D. A. WARDLE. 2010. How understanding aboveground–belowground linkages can assist restoration ecology. *Trends in Ecol. and Evol.*, **25**:670–679.
- KENNEDY, N., E. BRODIE, J. CONNOLLY, AND N. CLIPSON. 2004. Impact of lime, nitrogen and plant species on bacterial community structure in grassland microcosms. *Environ. Microbiol.*, **6**:1070–1080.
- KNAPP, A. K., C. BEIER, D. D. BRISKE, A. T. CLASSEN, Y. LUO, M. REICHSTEIN, M. D. SMITH, S. D. SMITH, J. E. BELL, P. A. FAY, J. L. HEISLER, S. W. LEAVITT, R. SHERRY, B. SMITH, AND E. WENG. 2008. Consequences of more extreme precipitation regimes for terrestrial ecosystems. *BioScience*, **58**:1–11.
- , J. M. BRIGGS, AND J. K. KOELLIKER. 2001. Frequency and extent of water limitation to primary production in a mesic temperate grassland. *Ecosystems*, **4**:19–28.
- , J. L. CONARD, AND J. M. BLAIR. 1998. Determinants of soil CO₂ efflux from a sub-humid grassland: effect of fire and fire history. *Ecol. Apps.*, **8**:760–770.
- AND T. R. SEASTEDT. 1986. Detritus accumulation limits productivity of tallgrass prairie. *Bioscience*, **36**:662–668.
- LEBAUER, D. S. AND K. K. TRESEDER. 2008. Nitrogen limitation of net primary productivity in terrestrial ecosystems is globally distributed. *Ecology*, **89**:371–379.
- LIU, L. AND T. L. GREAVER. 2009. A review of nitrogen enrichment effects on three biogenic GHGs: the CO₂ sink may be largely offset by stimulated N₂O and CH₄ emission. *Ecol. Let.*, **12**:1103–1117.
- , P. GUNDERSEN, T. ZHANG, AND J. MO. 2012. Effects of phosphorus addition on soil microbial biomass and community composition in three forest types in tropical China. *Soil Biol. & Biochem.*, **44**:31–38.
- MARSCHNER, H., E. A. KIRKBY, AND I. ÇAKMAK. 1996. Effect of mineral nutritional status on shoot-root partitioning of photoassimilates and cycling of mineral nutrients. *J. of Exp. Bot.*, **47**:1255–1263.
- MCLAREN, J. R., S. D. WILSON, AND D. A. PELTZER. 2004. Plant feedbacks increase the temporal heterogeneity of soil moisture. *Oikos*, **107**:199–205.
- MENGE, D. M. AND C. B. FIELD. 2007. Simulated global changes alter phosphorus demand in annual grassland. *Glob. Change Biol.*, **13**:2582–2591.
- METCALFE, D. B., FISHER, R. A., AND WARDLE, D. A. 2011. Plant communities as drivers of soil respiration: pathways, mechanisms, and significance for global change. *Biogeosciences*, **8**:2047–2061.

- NIINEMETS, U. AND K. KULL. 2005. Co-limitation of plant primary productivity by nitrogen and phosphorus in a species-rich wooded meadow on calcareous soils. *Acta Oecologica*, **28**:345–356.
- NIU, S., M. WU, Y. HAN, J. XIA, Z. ZHANG, H. YANG, AND S. WAN. 2010. Nitrogen effects on net ecosystem carbon exchange in a temperate steppe. *Glob. Change Biol.*, **16**:144–155.
- , H. YANG, Z. ZHANG, M. WU, Q. LU, L. LI, X. HAN, AND S. WAN. 2009. Non-additive effects of water and nitrogen addition on ecosystem carbon exchange in a temperate steppe. *Ecosystems*, **12**:915–926.
- NOVICK, K. A., P. C. STROY, G. G. KATUL, D. S. ELLSWORTH, M. B. S. SIQUEIRA, J. JUANG, AND R. OREN. 2004. Carbon dioxide and water vapor exchange in a warm temperate grassland. *Oecologia*, **138**:259–274.
- PARDO, L. H., M. FENN, C. L. GOODALE, L. H. GEISER, C. T. DRISCOLL, E. ALLEN, J. BARON, R. BOBBINK, W. D. BOWMAN, C. CLARK, B. EMMETT, F. S. GILLIAM, T. GREAVES, S. J. HALL, E. A. LILLESKOV, L. LIU, J. LYNCH, K. NADELHOFFER, S. PERAKIS, M. J. ROBIN-ABBOTT, J. STODDARD, K. WEATHERS, AND R. L. DENNIS. 2011. Effects of nitrogen deposition and empirical critical loads for nitrogen from ecoregions of the United States. *Ecol. Apps.*, **21**:3049–3082.
- PATRICK, L., J. CABLE, D. POTTS, D. IGNACE, G. BARRON-GAFFORD, A. GRIFFITH, H. ALPERT, N. VAN GESTEL, T. ROBERTSON, T. E. HUXMAN, J. ZAK, M. E. LOIK, AND D. TISSUE. 2007. Effects of an increase in summer precipitation on leaf, soil, and ecosystem fluxes of CO₂ and H₂O in a sotol grassland in Big Bend National Park, Texas. *Oecologia*, **151**:704–718.
- PEÑUELAS, J., J. SARDANS, A. RIVAS-UBACH, AND I. A. JANSSENS. 2012. The human-induced imbalance between C, N and P in Earth's life system. *Glob. Change Biol.*, **18**:3–6.
- PICENO, Y. M. AND C. R. LOVELL. 2000. Stability in natural bacterial communities: I. Nutrient addition effects on rhizosphere diazotroph assemblage composition. *Micro. Ecol.*, **39**:32–40.
- PHOENIX, G. K. 2003b. Simulated pollutant nitrogen deposition increases P stress and enhances root-surface phosphatase activities of three plant functional types in a calcareous grassland. *New Phyt.*, **161**:279–289.
- , R. E. BOOTHE, J. R. LEAKE, D. J. READ, P. GRIME, AND J. A. LEE. 2003a. Effects of enhanced nitrogen deposition and phosphorus limitation on nitrogen budgets of semi-natural grasslands. *Glob. Change Biol.*, **9**:1309–1321.
- , B. A. EMMETT, A. J. BRITTON, S. J. M. CAPORN, N. B. DISE, R. HELLIWELL, L. JONES, J. R. LEAKE, I. D. LEITH, L. J. SHEPPARD, A. SOWERBY, M. G. PILKINGTON, E. C. ROWE, M. R. ASHMORE, AND S. A. POWER. 2012. Impacts of atmospheric nitrogen deposition: responses of multiple plant and soil parameters across contrasting ecosystems in long-term field experiments. *Glob. Change Biol.*, **18**:1197–1215.
- POTTS, D. L., T. E. HUXMAN, J. M. CABLE, N. B. ENGLISH, D. D. IGNACE, J. A. EILTS, M. J. MASON, J. F. WELTZIN, AND D. G. WILLIAMS. 2006. Antecedent moisture and seasonal precipitation influence the response of canopy-scale carbon and water exchange to rainfall pulses in a semi-arid grassland. *New Phyt.*, **170**:849–860.
- , K. N. SUDING, G. C. WINSTON, A. V. ROCHA, AND M. L. GOULDEN. 2012. Ecological effects of experimental drought and prescribed fire in a southern California coastal grassland. *J. of Arid Env.*, **81**:59–66.
- SCHLESINGER, W. H. 2006. Global change ecology. *Trends in Ecol. & Evol.*, **21**:348–351.
- SHARKEY, T. D. 1988. Estimating the rate of photorespiration in leaves. *Phys. Plantarum*, **73**:147–152.
- STEVENS, C. J., N. B. DISE, J. O. MOUNTFORD, AND D. J. GOWING. 2004. Impact of nitrogen deposition on the species richness of grasslands. *Science*, **303**:1876–1879.
- TILMAN, D. 1987. Secondary succession and the pattern of plant dominance along experimental nitrogen gradients. *Ecol. Mono.*, **57**:189–214.
- VITOUSEK, P. M., J. D. ABER, R. W. HOWARTH, G. E. LIKENS, P. A. MATSON, D. W. SCHINDLER, W. H. SCHLESINGER, AND D. G. TILMAN. 1997. Human alteration of the global nitrogen cycle: sources and consequences. *Ecol. Apps.*, **7**:737–750.
- , S. PORDER, B. Z. HOULTON, AND O. A. CHADWICK. 2010. Terrestrial phosphorus limitation: mechanisms, implications, and nitrogen-phosphorus interactions. *Ecol. Apps.*, **20**:5–15.
- WARDLE, D. A., R. D. BARDGETT, J. N. KLIRONOMOS, H. SETALA, W. H. VAN DER PUTTEN, AND D. H. WALL. 2004. Ecological linkages between aboveground and belowground biota. *Science*, **304**:629–1633.

- XIA, J., S. NIU, AND S. WAN. 2009. Response of ecosystem carbon exchange to warming and nitrogen addition during two hydrologically contrasting growing seasons in a temperate steppe. *Glob. Change Biol.*, **15**:1544–1556.
- XU, L. AND D. D. BALDOCCHI. 2004. Seasonal variation in carbon dioxide exchange over a Mediterranean annual grassland in California. *Ag. For. Met.*, **123**:79–96.
- AND S. WAN. 2008. Water- and plant-mediated responses of soil respiration to topography, fire, and nitrogen fertilization in a semiarid grassland in northern China. *Soil Biol. Biochem.*, **40**:679–687.
- YAN, L., S. CHEN, J. HUANG, AND G. LIN. 2011. Increasing water and nitrogen availability enhanced net ecosystem CO₂ assimilation of a temperate steppe. *Plant and Soil*, **349**:227–240.
- ZAR, J. H. 1999. *Biostatistical analysis*, 4th ed. Prentice Hall, New Jersey. 663 p.
- ZHOU, X., M. TALLEY, AND Y. LUO. 2009. Biomass, litter, and soil respiration along a precipitation gradient in southern Great Plains, USA. *Ecosystems*, **12**:1369–1380.

SUBMITTED 24 MAY 2013

ACCEPTED 4 AUGUST 2014