

# Nitrogen effects on net ecosystem carbon exchange in a temperate steppe

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## Abstract

It has widely been documented that nitrogen (N) enrichment stimulates plant growth and net primary production. However, there is still dispute on how N addition affects net ecosystem CO<sub>2</sub> exchange (NEE), which represents the balance between ecosystem carbon (C) uptake and release. We conducted an experimental study to examine effects of N addition on NEE in a temperate steppe in northern China from 2005 to 2008. N was added at a rate of 10 g N m<sup>-2</sup> yr<sup>-1</sup> with NH<sub>4</sub>NO<sub>3</sub> alone or in combination with phosphorous (P, 5 g P<sub>2</sub>O<sub>5</sub> m<sup>-2</sup> yr<sup>-1</sup>) in both clipped and unclipped plots. Over the 4 years, N addition significantly stimulated growing-season NEE, on average, by 27%. Neither the main effects of P addition or clipping nor their interactions with N addition were statistically significant on NEE in any of the 4 years. However, the magnitude of N stimulation on NEE declined over time. N addition significantly increased NEE by 60% in 2005 and 21% in 2006, but its effect was not significant in 2007 and 2008. N-induced shift in species composition was primarily responsible for the declined N stimulation over time. The gradually increasing coverage of the upper canopy species (*Stipa krylovii*) and standing litter accumulation induced light limitation on the lower canopy species (*Artemisia frigida*). Thus, N-induced shifts in plant species composition strongly regulated the direct effects of N addition on C sequestration in the temperate steppe.

**Keywords:** biofuel feedstock production, carbon sequestration, clipping, grassland, interannual variability, land use, net ecosystem carbon exchange, nitrogen, phosphorus, photosynthesis, plant cover

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## Introduction

The accelerating industrialization and use of nitrogen (N) fertilizer now make N deposition significant in most parts of the world (Galloway *et al.*, 2008). It is estimated that about 100 Tg N yr<sup>-1</sup> was emitted as NO<sub>x</sub> and NH<sub>3</sub> and then deposited to the Earth's surface in 1995, and will increase to 200 Tg N yr<sup>-1</sup> by 2050 (Galloway *et al.*, 2008). N enrichment will potentially influence regional and global carbon (C) budgets and regulate response and feedback of biosphere to climate change. Nevertheless, the C sequestration potential in terrestrial ecosystems is one of the largest uncertainties in projection of climate-C feedbacks, largely caused by the negligence of N limitation to terrestrial C sequestration in

model simulation (Hungate *et al.*, 2003; Luo *et al.*, 2004; Heimann & Reichstein, 2008). Better understanding of the interaction between N input and ecosystem C dynamics is crucial to reduce this uncertainty (Reay *et al.*, 2008).

Most of the previous studies on the responses of ecosystem C cycling to N enrichment focused on gross primary productivity (GPP) and net primary productivity (NPP, Elser *et al.*, 2007; LeBauer & Treseder, 2008). N enrichment usually increases GPP and NPP by stimulating plant growth and biomass production (Xia & Wan, 2008). Nevertheless, the majority of ecosystem C uptake through plant photosynthesis is eventually released back to the atmosphere via ecosystem respiration (ER) (Luo & Zhou, 2006), which can also be stimulated by N enrichment (Bragazza *et al.*, 2006; Cleveland & Townsend, 2006; Xu & Wan, 2008). As a consequence, N effect on C sequestration, which is determined by relative responses of NPP and ER, remains highly

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controversial (Nadelhoffer *et al.*, 1999; Mack *et al.*, 2004; Magnani *et al.*, 2007; Bradford *et al.*, 2008; Reay *et al.*, 2008).

Some indirect methods have been used to examine responses of ecosystem C sequestration to N addition, such as model simulations (Lai *et al.*, 2002; Pepper *et al.*, 2005), transect study (Hyvönen *et al.*, 2007), temporal variability (Sievering *et al.*, 2007), and estimation of components (e.g., soil C content) (Hyvönen *et al.*, 2008; Reay *et al.*, 2008). A few manipulative experiments have been conducted to directly measure NEE responses to N addition. For example, the N responses of NEE has been examined in a wet sedge tundra (Shaver *et al.*, 1998), a boreal mire (Saarnio *et al.*, 2003), a bog (Bubier *et al.*, 2007), an annual grassland (Harpole *et al.*, 2007), and a temperate grassland (Xia *et al.*, 2009). The results in these experiments are conflicting, showing either positive (Shaver *et al.*, 1998; Xia *et al.*, 2009) or no significant (Saarnio *et al.*, 2003; Bubier *et al.*, 2007; Harpole *et al.*, 2007) responses of NEE to N enrichment.

The inconsistent findings reported in previous studies may result from the impacts of experimental duration and other factors on the N responses of ecosystem C processes. For example, a 30-years' N addition experiment showed initially positive N response of tree growth but the N effect decreased with time (Högberg *et al.*, 2006). The short-term positive effect of N fertilization has also been noted on soil C and N cycling in a poplar ecosystem (Moscatelli *et al.*, 2008). In addition, other environmental factors can further compromise N effects on ecosystem C sequestration. Ecosystems may change toward P limitation as a consequence of ameliorated N limitation (Menge & Field, 2007), which will further restrain the N effect. In addition, land use practice (such as mowing for hay) in grasslands also affects ecosystem C cycling and their response to climate change (Jones & Donnelly, 2004). All these factors contribute to the variation in ecosystem responses to N addition. Therefore, it is necessary to explore interactive effects of N addition and other factors on NEE.

In order to examine the effects of N enrichment on NEE, we conducted a manipulative experiment in a temperate steppe in northern China, which is a typical vegetation type in the Eurasian continent. This study area is in general N deficient as indicated by the strong stimulation of gross ecosystem primary productivity after N addition (Xia *et al.*, 2009). We applied  $\text{NH}_4\text{NO}_3$  at a rate of  $10 \text{ g N m}^{-2} \text{ yr}^{-1}$  from 2005 to 2008. This paper primarily focused on N effects in an experiment with additional phosphorus and clipping treatments largely because either the main effects of P addition and clipping or their interactions with N addition were statistically not significant on NEE.

## Materials and methods

### Study site

This study was conducted at the Duolun Restoration Ecology Station of the Institute of Botany, Chinese Academy of Sciences, which was located in a typical temperate steppe in Duolun County ( $42^\circ 02' \text{N}$ ,  $116^\circ 17' \text{E}$ , 1324 m a.s.l.), Inner Mongolia, China. Long-term (1953–2006) mean annual precipitation is 382.2 mm with 91% distributing from May to October. Mean annual temperature is  $2.1^\circ \text{C}$  with monthly mean temperature ranging from  $18.9^\circ \text{C}$  in July to  $-17.5^\circ \text{C}$  in January. The sandy soil at the study site is classified as chestnut according to the Chinese classification, or Haplic Calcisols according to the FAO classification, with  $62.75 \pm 0.04\%$  sand,  $20.30 \pm 0.01\%$  silt, and  $16.95 \pm 0.01\%$  clay. Mean soil bulk density is  $1.31 \text{ g cm}^{-3}$ . The soil organic C concentration is  $12.28 \text{ g kg}^{-1}$  and pH is 7.12. The soil N and P concentrations are 0.17% and  $0.28 \text{ g kg}^{-1}$ , respectively. N deposition in this area was estimated at about  $20 \text{ kg ha}^{-1} \text{ yr}^{-1}$  in 2005–2006 (Zhang *et al.*, 2008). The study site has been fenced to exclude grazing disturbance since 2001. The native vegetation is a typical steppe community, characterized by the dominance of perennial species *Artemisia frigida* Willd., *Stipa krylovii* Roshev., *Potentilla acaulis* L., *Cleistogenes squarrosa* (Trin.) Keng, *Allium bidentatum* Fisch. ex Prokh., and *Agropyron cristatum* (L.) Gaertn.

### Experimental design

This study was a part of Duolun Global Change Multi-factor Experiment (GCME). The whole experimental area is  $199 \text{ m} \times 265 \text{ m}$ . Eight  $92 \text{ m} \times 60 \text{ m}$  plots were arranged into four rows and two columns. The eight plots were randomly assigned to unclipping and clipping treatments with four replicates. There were 5 m-wide buffer zone between any two plots. The clipped plots were mowed annually at a height of 10 cm above soil surface on August 15, 2005; August 20, 2006; August 18, 2007; and August 19, 2008 and the harvested plant material was removed immediately after clipping.

Each plot was divided into four  $44 \text{ m} \times 28 \text{ m}$  subplots with 1 m-wide buffer zone. The four subplots within each plot were randomly assigned with four treatments of nutrient addition, including no nutrient addition (Control), nitrogen addition (N) treated with urea in 2005 and  $\text{NH}_4\text{NO}_3$  in 2006–2008 ( $10 \text{ g N m}^{-2} \text{ yr}^{-1}$ ), phosphorus addition (P) treated with calcium superphosphate ( $5 \text{ g P}_2\text{O}_5 \text{ m}^{-2} \text{ yr}^{-1}$ ), and combined additions of both N and P (NP, with the same form and amount of N and P with those in the N and P treatments). Fertilizer

treatments were broadcasted once a year on July 20, 2005; July 21, 2006; July 13, 2007; and July 12, 2008.

#### *Ecosystem gas exchange measurement*

In April 2005, one square aluminum frame (0.5 m × 0.5 m) was inserted into the soil at 3-cm depth in each subplot, which was about 1–1.5 m away from edges of the plots. The frames provided a flat base between the soil surface and the CO<sub>2</sub> sampling chamber.

We measured ecosystem C exchange with an infrared gas analyzer (IRGA; LI-6400, LiCor Inc., Lincoln, NE, USA) attached to a transparent chamber (0.5 m × 0.5 m × 0.5 m), which covered all the vegetation within the aluminum frames (also see the detail information in Niu *et al.*, 2008). During the measurement, the chamber was sealed to the frame surface. Two small electric fans were running continuously to promote air mixing within the chamber during the measurement. Nine consecutive recordings of CO<sub>2</sub> and water vapor concentrations were taken on each frame at 10-s intervals during a 90-s period after steady-state conditions were achieved within the chamber. Increases in air temperatures within the chamber during the measuring time period were <0.2 °C. CO<sub>2</sub> concentrations were allowed to build up or draw down over time, from which flux rates were determined from the time-course of the concentration to calculate net ecosystem CO<sub>2</sub> exchange (NEE). The details on these static-chamber flux calculations can be found in the soil-flux calculation procedure on the LI-6400 manual (LiCor Inc., 2004). This static-chamber method has been used and validated in many previous studies (Huxman *et al.*, 2004; Welker *et al.*, 2004; Bubier *et al.*, 2007; Xia *et al.*, 2009). Following the measurements of NEE, the chamber was vented, replaced on each frame, and covered with an opaque cloth. Then the CO<sub>2</sub> exchange measurements were repeated. Because the second set of measurements eliminated light (and hence photosynthesis), the values obtained represented ER (Welker *et al.*, 2004). The difference between NEE and ER was considered to represent gross ecosystem productivity (GEP) for the vegetation within the chamber. By convention, negative NEE values refer net C uptake by the ecosystem, while positive NEE values represent net C loss from the ecosystem.

Ecosystem gas exchange was measured at 2-week intervals (8:00–11:00 hours) from May to October. Some measurements were missing because of the machine malfunction, such as that in the early May 2006 and the early August in 2007. C fluxes values and the seasonal dynamics measured by the canopy chamber were similar to that measured using eddy covariance technique which was set up adjacent to our study site (<200 m in distance, Zhang, 2007), illustrating the validity of the

canopy chamber method for ecosystem gas exchange measurements. For example, the maximum NEE value measured by both eddy covariance and chamber was nearly 8 μmol m<sup>-2</sup> s<sup>-1</sup> in the middle growing season in 2006 (Zhang, 2007).

#### *Vegetation sampling*

In June 2005, one permanent 1 m × 1 m quadrat was established in each subplot. Plant species composition was recorded in each quadrat at the end of August with peak biomass from 2005 to 2008 by counting species number and estimating percent cover of each plant species. A 1 m × 1 m frame with 100 equally distributed grids (10 cm × 10 cm) was put above the canopy in each quadrat during the measurement. The percent cover of each species was recorded in all the grids and summed as the species cover in each quadrat.

#### *Leaf gas exchange measurement*

An open gas-exchange system (Li-6400; LiCor Inc) with a 6 cm × 6 cm clamp-on leaf cuvette was used to measure leaf photosynthesis rate of dominant plant species *S. krylovii*. In each subplot, three fully expanded leaves of *S. krylovii* were selected and measured in middle growing season once a year in 2005, 2007, and 2008. Leaf gas exchange was measured in the morning between 8:00 to 11:00 hours (local time) on clear days. During the measurement, leaves were illuminated at 1500 μmol m<sup>-2</sup> s<sup>-1</sup> using the LED light system.

#### *Estimate seasonal integrals of ecosystem C exchange*

Seasonal integrals of NEE, ER, and GEP over the growing season (May–October) were calculated by multiplying daily values of NEE, ER, and GEP, respectively, by the number of days between measurements. Our measurements, collected between 8:00 and 10:00 hours local time, were 8.73 and 1.47 times of the observed daily mean values based on the 24 h diurnal measurement in the control plots (unpublished data) for NEE and ER, respectively. This up-scaling method has been used in previous studies (Bremer *et al.*, 1998; Zhou *et al.*, 2007). Although the method of scaling up from snapshot to the season could cause bias, the seasonal integrals of NEE values estimated by this method were similar to that measured by an adjacent eddy covariance tower (Zhang, 2007), suggesting that bias was minor in this study.

#### *Statistical analyses*

Repeated measures analysis of variances (ANOVAS) were used to examine interannual variability in seasonal integrals of ecosystem C fluxes when combined with

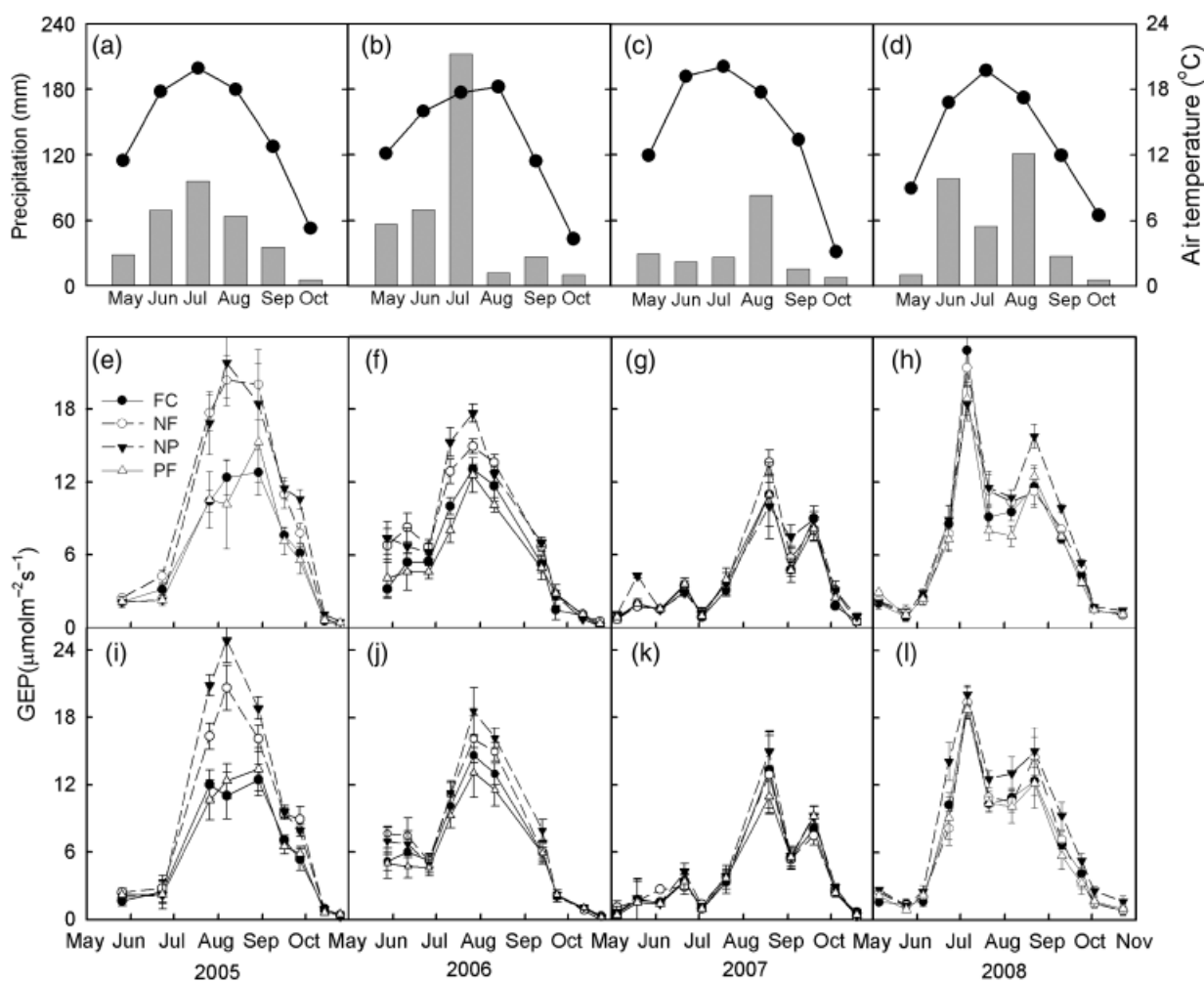
N and P addition and clipping treatments. Since there were significant interactions between year and N addition for all the measured parameters (i.e.,  $P < 0.001$  for the year effects), repeated measures ANOVAs were used again to examine treatment effects on ecosystem C fluxes in each year. Between-subject effects were evaluated as N and P addition, clipping, and their interactions and within-subject effects were time-of-season and its interactions with N and P addition and clipping. Three-way ANOVAs were used to examine the effects of N and P addition and clipping on the maximum leaf photosynthesis and plant cover in each year. Because the main effects of clipping and its interaction with N and P addition were not significant for any parameters in any year, we calculated the effects of N, P, and NP across both clipped and unclipped plots. Simple linear regression was used to determine relationships between plants cover response and C flux response to N addi-

tion. Stepwise multiple linear regressions were used to examine relationships of N-induced changes in C fluxes with the N-induced changes in soil temperature, soil moisture, soil pH, and plant cover. All statistical analyses were conducted with SPSS software (SPSS 11.0 for windows, SPSS Inc., Chicago, IL, USA).

## Results

### *Seasonal and interannual variability in ecosystem C exchange*

Precipitation in the growing season (May–October) changed substantially in the 4 years, with 298.2 mm in 2005, 387.2 mm in 2006, 185.6 mm in 2007, and 318.0 mm in 2008 (Fig. 1a–d). The seasonal dynamics of ecosystem C exchange followed one-peak pattern with higher values in the middle growing season and lower values



**Fig. 1** Monthly mean air temperature (lines) and precipitation (bars a, b, c, d) and seasonal dynamics of gross ecosystem productivity (GEP) under the unclipping (e, f, g, h) and clipping (i, j, k, l) treatments from 2005 to 2008. FC, control; NF, nitrogen fertilization; PF, phosphorus fertilization; NP, combined additions of both nitrogen and phosphorus.

in the early and late growing seasons (Figs 1–3), which was similar to the seasonal dynamics of precipitation (Fig. 1a–d). Some dips during the growing season were corresponding to lower precipitation prior to the measurements (Fig. 1). In the control plots, seasonal integrals of NEE changed from the maximum ( $-73 \text{ g C m}^{-2}$ ) in 2006 to the minimum ( $-22 \text{ g C m}^{-2}$ ) in 2007 (Table 1). The maximum seasonal integrals of ER and GEP occurred in 2008, which was 89% and 95% higher than the minimum in 2007, respectively (Table 1). Yearly N addition increased seasonal and interannual variability of ecosystem C exchange by strengthening the maximum values (Figs 1–3).

#### Effects of N addition on ecosystem C exchange

N addition did not change the bell-like shape of seasonal dynamics of ecosystem C exchange, but increased peak values of GEP, ER, and NEE (Figs 1–3). For example, in the unclipped plots, N addition increased peak values of GEP, ER, and NEE by 59%, 54%, and 95% in 2005, and 15%, 29%, and 18% in 2006, respectively (all  $P < 0.05$ , Figs 1–3). N addition did not significantly increase peak values of GEP, ER, and NEE in 2007 or 2008 ( $P > 0.05$ , Figs 1–3). Across the growing seasons of the 4 years, N addition stimulated GEP, ER, and NEE, on average, by 27.1%, 27.1%, and 26.5%, respectively (all  $P < 0.05$ , Tables 1 and 2). However, there was significant interaction between year and N addition

(Table 2). Over the growing season, the main effects of N addition on NEE were significant in 2005 and 2006, but not in 2007 or 2008 (Table 3). N addition stimulated GEP and ER in all the 4 years (all  $P < 0.05$ , except  $P = 0.055$  for GEP in 2007, Table 3). The magnitudes of N stimulation on GEP, ER, and NEE depended on year, which were diminishing over time. Across both the clipped and unclipped plots, N addition increased seasonal integrals of GEP, ER, and NEE by 35–60% in 2005, 16–27% in 2006, and 1–10% in 2007 ( $P < 0.05$  only in 2005 and 2006, Fig. 4). In 2008, GEP and ER increased by 7–8% but NEE decreased by 5% under N addition. Similar to the N treatment, the NP treatment stimulated GEP, ER, and NEE by 46–66% in 2005, 13–42% in 2006, 13–21% in 2007, and 11–22% in 2008 over the growing season ( $P < 0.05$  only in 2005 and 2006, Fig. 4). The NP effects on ecosystem C exchange also declined with year (Fig. 4). None of the C exchange variables in the NP subplots was significantly different from those in the N subplots in any year ( $P > 0.05$ , Figs 1–3). The main effects of clipping or P addition or their interactions with each other or with N addition were not significant on any of the C variables in any year (Tables 3).

#### N addition, species composition, and ecosystem C exchange

*S. krylovii* and *A. frigida* are the two predominant species in the study site. N addition increased *S. krylovii* cover

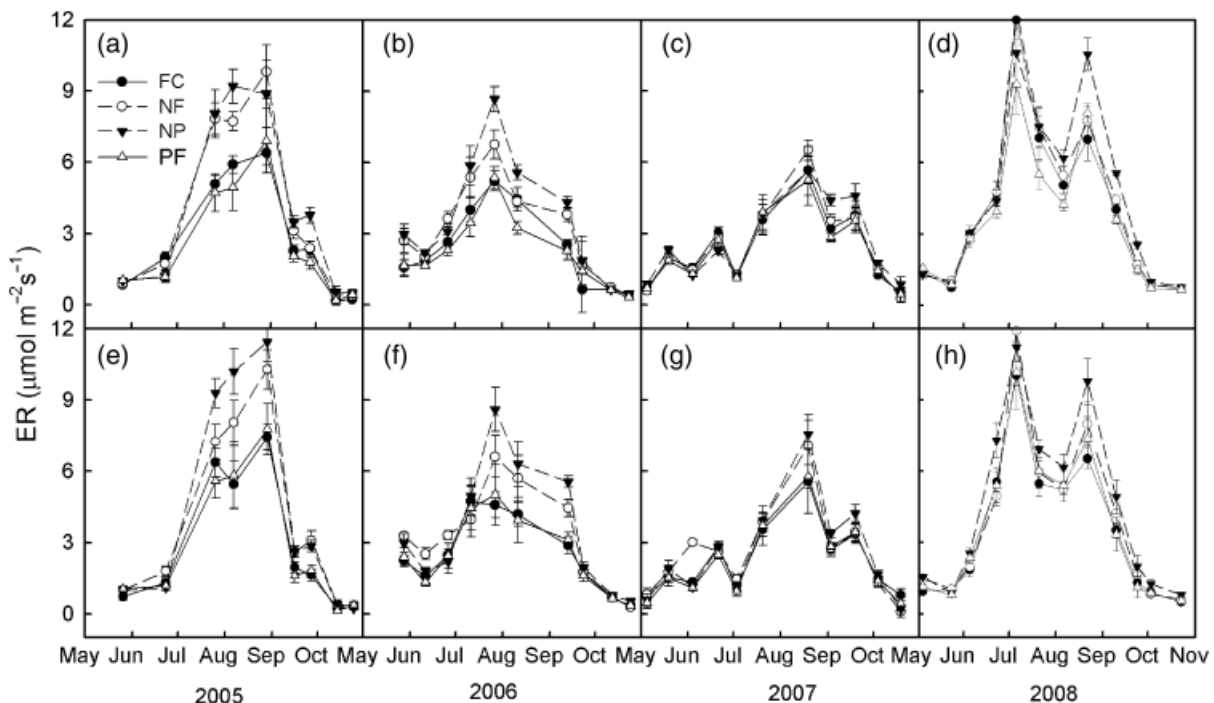
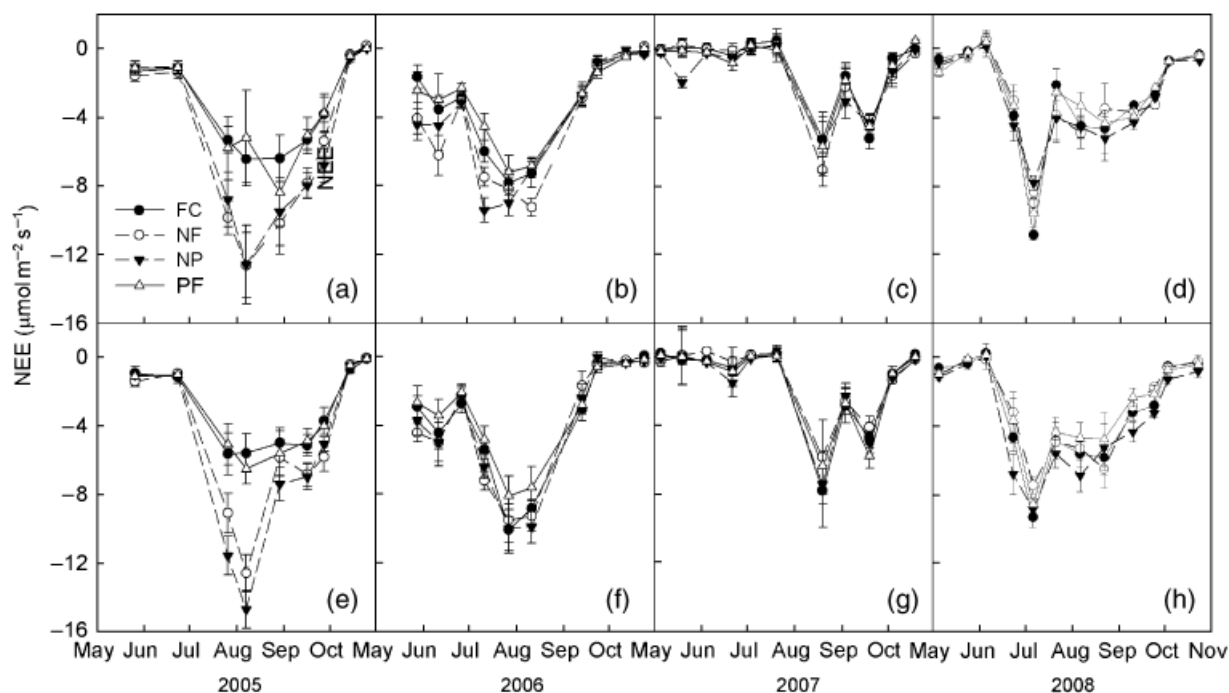


Fig. 2 Seasonal dynamics of ecosystem respiration (ER) in the unclipped (a, b, c, d) and clipped (e, f, g, h) plots from 2005 to 2008. See Fig. 1 for abbreviations.



**Fig. 3** Seasonal dynamics of net ecosystem CO<sub>2</sub> exchange (NEE) under the unclipping (a, b, c, d) and clipping (e, f, g, h) treatments from 2005 to 2008. See Fig. 1 for abbreviations.

**Table 1** Seasonal integrals (mean ± SE) of net ecosystem CO<sub>2</sub> exchange (NEE), ecosystem respiration (ER), and gross ecosystem productivity (GEP) under the eight treatments (CCFC = unclipping and fertilization control, CCNF = unclipping and nitrogen addition, CCPF = unclipping and phosphorus addition, CCNP = unclipping and added both nitrogen and phosphorus, CLFC = clipping and fertilization control, CLNF = clipping and nitrogen addition, CLPF = clipping and phosphorus addition, CLNP = clipping and added both nitrogen and phosphorus) from 2005 to 2008

	CCFC	CCNF	CCNP	CCPF	CLFC	CLNF	CLNP	CLPF
<b>NEE</b>								
2005	-62 <sup>b</sup> ± 7	-101 <sup>a</sup> ± 12	-99 <sup>a</sup> ± 13	-63 <sup>b</sup> ± 16	-56 <sup>b</sup> ± 4	-88 <sup>a</sup> ± 5	-98 <sup>a</sup> ± 4	-59 <sup>b</sup> ± 5
2006	-73 <sup>bc</sup> ± 8	-95 <sup>a</sup> ± 7	-88 <sup>abc</sup> ± 8	-69 <sup>c</sup> ± 10	-85 <sup>abc</sup> ± 8	-90 <sup>ab</sup> ± 10	-91 <sup>ab</sup> ± 9	-73 <sup>bc</sup> ± 4
2007	-22 <sup>a</sup> ± 7	-28 <sup>a</sup> ± 8	-31 <sup>a</sup> ± 7	-25 <sup>a</sup> ± 8	-29 <sup>a</sup> ± 7	-24 <sup>a</sup> ± 8	-31 <sup>a</sup> ± 8	-30 <sup>a</sup> ± 7
2008	-61 <sup>ab</sup> ± 6	-61 <sup>ab</sup> ± 7	-65 <sup>ab</sup> ± 6	-58 <sup>b</sup> ± 7	-70 <sup>ab</sup> ± 7	-64 <sup>ab</sup> ± 8	-81 <sup>ab</sup> ± 8	-60 <sup>a</sup> ± 9
<b>ER</b>								
2005	326 <sup>b</sup> ± 7	426 <sup>a</sup> ± 23	450 <sup>a</sup> ± 36	292 <sup>b</sup> ± 31	315 <sup>b</sup> ± 25	439 <sup>a</sup> ± 30	485 <sup>a</sup> ± 27	321 <sup>b</sup> ± 27
2006	301 <sup>bc</sup> ± 25	378 <sup>ab</sup> ± 41	437 <sup>a</sup> ± 40	271 <sup>c</sup> ± 17	316 <sup>bc</sup> ± 28	404 <sup>a</sup> ± 44	440 <sup>a</sup> ± 44	309 <sup>bc</sup> ± 49
2007	281 <sup>a</sup> ± 25	294 <sup>a</sup> ± 18	311 <sup>a</sup> ± 24	268 <sup>a</sup> ± 25	265 <sup>a</sup> ± 27	305 <sup>a</sup> ± 28	305 <sup>a</sup> ± 19	257 <sup>a</sup> ± 19
2008	530 <sup>abc</sup> ± 37	559 <sup>ab</sup> ± 29	607 <sup>a</sup> ± 37	477 <sup>bc</sup> ± 32	468 <sup>c</sup> ± 32	527 <sup>abc</sup> ± 30	609 <sup>a</sup> ± 26	490 <sup>bc</sup> ± 27
<b>GEP</b>								
2005	389 <sup>b</sup> ± 9	527 <sup>a</sup> ± 33	549 <sup>a</sup> ± 48	355 <sup>b</sup> ± 45	371 <sup>b</sup> ± 27	527 <sup>a</sup> ± 31	584 <sup>a</sup> ± 28	380 <sup>b</sup> ± 27
2006	374 <sup>c</sup> ± 23	473 <sup>ab</sup> ± 42	524 <sup>a</sup> ± 43	340 <sup>c</sup> ± 23	401 <sup>bc</sup> ± 35	494 <sup>a</sup> ± 44	531 <sup>a</sup> ± 44	382 <sup>c</sup> ± 49
2007	303 <sup>a</sup> ± 19	322 <sup>a</sup> ± 15	342 <sup>a</sup> ± 22	293 <sup>a</sup> ± 24	294 <sup>a</sup> ± 30	330 <sup>a</sup> ± 34	336 <sup>a</sup> ± 27	287 <sup>a</sup> ± 26
2008	591 <sup>bc</sup> ± 35	619 <sup>abc</sup> ± 27	673 <sup>ab</sup> ± 32	535 <sup>c</sup> ± 27	539 <sup>c</sup> ± 28	592 <sup>bc</sup> ± 34	690 <sup>a</sup> ± 32	550 <sup>c</sup> ± 32

The units for NEE, ER, and GEP are g C m<sup>-2</sup> yr<sup>-1</sup>. Different letters (a, b, c) indicate statistical differences at  $P < 0.05$  among the eight treatments in each year.

in all the 4 years and *A. frigida* cover in 2005 and 2006, but decreased *A. frigida* cover in 2007 and 2008 (Fig. 5). Across the 4 years, N-induced changes in NEE and GEP were negatively correlated with the N-induced % changes in *S. krylovii* cover but positively correlated

with the N-induced % changes in *A. frigida* cover (all  $P < 0.05$ , Fig. 5).

Stepwise multiple regression analyses were conducted with N-induced changes in soil moisture, soil temperature, soil pH, and dominant plant cover as the

independent variables and N-induced changes in seasonal integrals of NEE, ER, and GEP as the dependent variables across the 4 years. Only one variable, changes in *A. frigida* cover, was retained in the stepwise multiple regression model with NEE and GEP ( $P < 0.01$ ).

#### N addition effect on leaf level C exchange

In agreement with the C fluxes at the ecosystem level, the maximum photosynthetic rate ( $P_{\max}$ ) of the domi-

**Table 2** Results (i.e.,  $P$  values) of repeated measures ANOVAS on the effects of nitrogen addition (N), phosphorus addition (P), clipping (C), year (Y), and their interactions on seasonal integrals of net ecosystem CO<sub>2</sub> exchange (NEE), ecosystem respiration (ER), and gross ecosystem productivity (GEP)

	ER	GEP	NEE
Y	<0.001	<0.001	<0.001
Y × C	0.26	0.41	0.15
Y × N	<0.001	<0.001	<0.001
Y × P	0.73	0.73	0.34
Y × C × N	0.92	0.85	0.66
Y × C × P	0.53	0.44	0.89
Y × N × P	0.58	0.39	0.54
Y × C × N × P	0.99	0.99	0.93
C	0.81	0.71	0.46
N	<0.001	<0.001	<0.001
P	0.32	0.33	0.79
C × N	0.76	0.88	0.49
C × P	0.42	0.39	0.61
N × P	0.03	0.03	0.18
C × N × P	0.50	0.71	0.21

**Table 3** Results ( $P$  values) of repeated measures ANOVAS on the effects of nitrogen addition (N), phosphorus addition (P), clipping (C), measuring date (D), and their interactions on net ecosystem CO<sub>2</sub> exchange (NEE), ecosystem respiration (ER), and gross ecosystem productivity (GEP) from 2005 to 2008

	2005			2006			2007			2008		
	NEE	ER	GEP	NEE	ER	GEP	NEE	ER	GEP	NEE	ER	GEP
D	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
D × C	0.002	0.099	0.210	0.002	0.202	0.021	0.43	0.04	0.09	0.004	<0.001	<0.001
D × N	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.71	0.01	0.49	0.16	0.001	0.08
D × P	0.92	0.71	0.61	0.72	0.11	0.51	0.85	0.15	0.38	0.89	<0.001	0.12
D × C × N	0.84	0.98	0.98	0.94	0.18	0.41	0.77	0.31	0.57	0.98	0.95	0.91
D × C × P	0.69	0.91	0.54	0.48	0.98	0.93	0.44	0.49	0.48	0.64	0.08	0.13
D × N × P	0.96	0.36	0.43	0.77	0.17	0.15	0.95	0.38	0.86	0.41	0.62	0.17
D × C × N × P	0.43	0.64	0.19	0.28	0.98	0.93	0.11	0.89	0.01	0.90	0.88	0.90
C	0.21	0.48	0.58	0.61	0.38	0.51	0.53	0.68	0.71	0.16	0.43	0.41
N	<0.001	<0.001	<0.001	0.002	<0.001	<0.001	0.60	0.03	0.05	0.27	<0.001	0.002
P	0.45	0.53	0.40	0.19	0.39	0.82	0.38	0.93	0.57	0.59	0.28	0.28
C × N	0.83	0.93	0.84	0.35	0.59	0.25	0.36	0.62	0.74	0.69	0.82	0.62
C × P	0.47	0.59	0.34	0.63	0.95	0.98	0.94	0.86	0.97	0.69	0.12	0.22
N × P	0.72	0.24	0.36	0.69	0.15	0.19	0.65	0.46	0.35	0.07	0.03	0.008
C × N × P	0.48	0.99	0.54	0.70	0.66	0.71	0.68	0.75	0.63	0.29	0.65	0.49

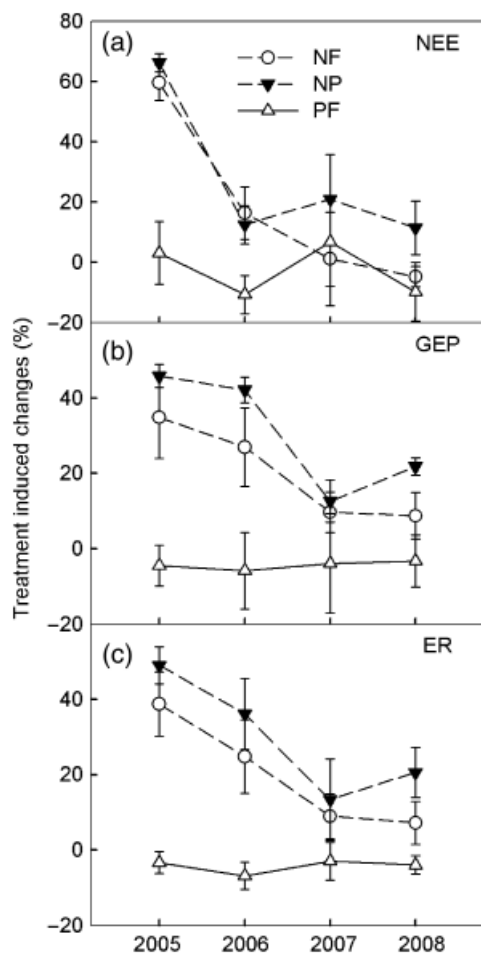
nant species *S. krylovii* showed little response to clipping and P addition in any year ( $P > 0.05$ , Fig. 6). N addition increased  $P_{\max}$  significantly in 2005 and 2007 ( $P < 0.05$ ) and marginally in 2008 ( $P = 0.09$ , Fig. 6). Comparing with that in the subplots without N addition,  $P_{\max}$  in the subplots with N addition was 20%, 54%, 20% higher, in 2005, 2007, and 2008, respectively.

## Discussion

#### Effects of N addition on net ecosystem C exchange (NEE)

N addition significantly stimulated NEE in the first 2 years, largely due to N-induced increases in GEP more than those in ER (Figs 1–3). The N-induced increases in canopy C uptake were partly caused by the stimulation of leaf photosynthesis (Fig. 6) and leaf area as reflected by relative species cover (Fig. 5). Stimulation in plant productivity provides more substrate for soil and plant respiration, leading to increases in ER under N enrichment (Figs 2 and 4). The positive N response of NEE in this study is consistent with that in a *Lolium perenne* community (Aeschlimann *et al.*, 2005), but not with those in a wet sedge tundra (Shaver *et al.*, 1998), a bog (Bubier *et al.*, 2007), and an annual grassland (Harpole *et al.*, 2007). N stimulation of gross ecosystem production in the temperate steppe (Fig. 1) is in accordance with the meta-analysis results of positive N responses of grassland productivity at regional and global scales (Elser *et al.*, 2007; LeBauer & Treseder, 2008).

Although N stimulation of NEE was transient, the N use efficiency in term of the stimulated net C sequestra-



**Fig. 4** Changes in net ecosystem CO<sub>2</sub> exchange (NEE, a), gross ecosystem productivity (GEP, b), and ecosystem respiration (ER, c) induced by nitrogen addition (NF), phosphorus addition (PF), and combined additions of both (NP) across the clipped and unclipped plots from 2005 to 2008.

tion per unit of applied N in this study is in a comparable range with other studies. N load of 40 g N m<sup>-2</sup> during 4 years to this temperate steppe stimulated an additional net C sequestration by 48.3 g C m<sup>-2</sup> based on integration of NEE over the growing seasons from May to October, which was almost equal to the annual NEE values ( $NEE_{(May-Oct)}/NEE_{annual} = 1.07$ ) at our study site (Zhang, 2007). Thus, the N use efficiency was 1.2 g C g<sup>-1</sup> N, which was within the low range of -0.8 to 61 g C g<sup>-1</sup> N in N addition experiments in European forests over 14–30 years (Högberg *et al.*, 2006; Hyvönen *et al.*, 2008).

Although the diminishing N stimulation of NEE over time has not been reported in literatures, a similar phenomenon was observed in boreal forest ecosystems (Högberg *et al.*, 2006), where N stimulation of tree growth was high in the first 3 years and then declined

from the fourth to 10th year. After 10 years of N addition at the rate of 68 or 108 kg N ha<sup>-1</sup> yr<sup>-1</sup>, tree growth in the N addition plots was not significantly different from that in the control plots (Högberg *et al.*, 2006). In our study, N stimulation of NEE was 60% in the first year and declined from the second year down to no stimulation in the fourth year (Fig. 4a). Our results, together with those in Högberg *et al.* (2006), suggest acclimation to N enrichment in plant growth and ecosystem C exchange.

#### *Causes for the diminishing N stimulation over time*

Many processes may cause the diminishing N stimulation on NEE over time. It has been known that N fertilization could cause adverse effects on ecosystems, such as soil acidification (Högberg *et al.*, 2006), and/or push ecosystems to limitation by P and water availability (Aber *et al.*, 1989; Menge & Field, 2007). We conducted a stepwise regression analysis, which showed that N-induced changes in soil pH were not significantly correlated with those in NEE over years ( $P = 0.6$ ), although soil pH slightly decreased from 7.39 to 6.77 (W. X. Liu, unpublished results) over the experimental period. In addition, we did not observe any biological symptom to indicate adverse effects of soil acidification. Growth of the dominant plant species, *S. krylovii*, was still stimulated even after 4-year of N addition (Fig. 6). Högberg *et al.* (2006) have also demonstrated that soil acidification and losses of base cations under N loading are not the causes for diminishing N stimulation. A modeling analysis suggests that increasing N deposition can push the ecosystem toward P limitation in many ecosystems (Menge & Field, 2007). N saturation may occur when other factors such as water and P becoming limiting (Aber *et al.*, 1989). However, P addition and higher precipitation in 2006 did not change the diminishing trend of N stimulation (Fig. 4).

Our study provided evidence that N-induced shifts in species composition and the subsequent light limitation were likely a main cause leading to the diminishing N stimulation of NEE. N addition stimulated the growth of the dominant species *S. krylovii* with a height of 17–28 cm (Figs 5 and 6). After senescence at the end of growing seasons, the standing litter of *S. krylovii* stayed erect for years. As a consequence, light availability was reduced by 67–81% (unpublished data) for the lower canopy species *A. frigida* with a height of <8 cm. Its biomass growth and cover accordingly decreased in the N addition plots in comparison with that in the control plots. The divergent N responses between the two species were reflected by a negative correlation between the N-induced changes in *S. krylovii* cover and those in



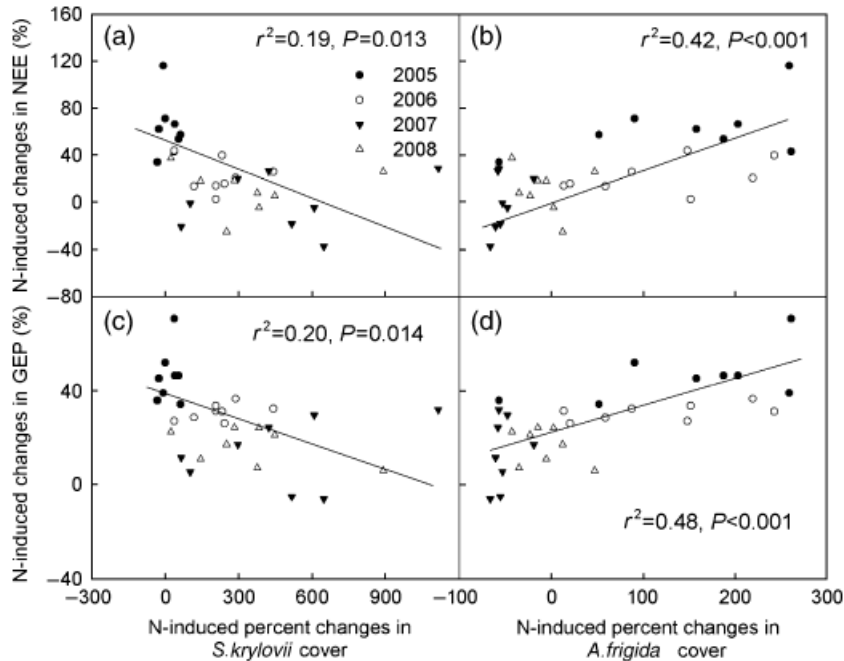


Fig. 5 Dependence of the N-induced changes in net ecosystem CO<sub>2</sub> exchange (NEE) and gross ecosystem productivity (GEP) on the N-induced percent changes in *Stipa krylovii* (a, c) or *Artemisia frigida* (b, d) cover across the 4 years.

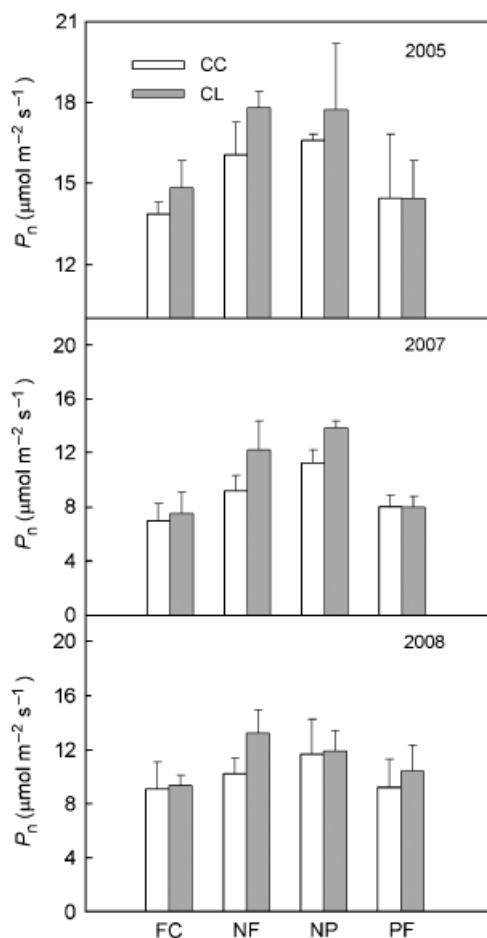
*A. frigida* cover ( $P < 0.05$ ). In addition, the negative dependence of the N-induced changes in GEP and NEE on the N-induced changes in *S. krylovii* cover and the positive dependence on those of *A. frigida* cover across the 4 years (Fig. 5) suggest that shift in species composition likely contributes to the declining N effects over time. Although photosynthesis and plant cover of *S. krylovii* were still stimulated by N enrichment in 2007 and 2008 (Figs 5a, c and 6), the negative N effects on *A. frigida* cover offset the positive N effect on *S. krylovii*, leading to no significant changes in NEE in 2007 and 2008. Our results indicate that NEE could acclimate to N enrichment by shifting species composition in a medium or long-term.

#### Effects of phosphorus and clipping on net ecosystem C exchange

The anticipated positive P effects on GEP and NEE were not observed in our study (Figs 1–4), largely due to little change in plant photosynthesis (Fig. 6) and plant cover. Newbery *et al.* (2002) also showed similar results with no change in plant productivity under P addition. However, the positive P effects were observed on net ecosystem CO<sub>2</sub> exchange in Arctic tundra (Shaver *et al.*, 1998). The contrasting observations may result from the different ecosystem types (grassland vs. tundra) and soil P content in the soil. In our study site, soil P concentration is 0.28 g kg<sup>-1</sup> at the upper limit of the

typical alpine grassland ecosystem (0.09–0.28 g kg<sup>-1</sup>, Thiel-Egenter *et al.*, 2007). Although the main effects of P addition were not significant on ecosystem C exchange over the experimental period (Table 2), the higher NP effect than N effect on ER and GEP (Fig. 4b and c) suggests that P fertilization produces, to some extent, positive effects on ecosystem C exchange when combining with N enrichment. The significant N × P interactions on ER and GEP were observed in the 4th year (Table 3), suggesting P fertilization produced effect in that year. We therefore speculate that significant P effect may be detectable in the long-term.

Clipping has also little effect on NEE itself and its response to N addition in this study (Tables 2 and 3), which is consistent with Blum *et al.*'s (1997) study in a *L. perenne* grassland. Luo *et al.* (2009) also observed minor effects of clipping on peak aboveground biomass. The minor effects of clipping on NEE and its responses to N addition probably result from three aspects. First, clipping was treated in the late growing season when plants began to senesce, and thus had little effect on plant growth and ecosystem C fluxes. Second, clipping at a height of 10 cm above the soil surface did not impact the growth of the dominant species *A. frigida* and other lower canopy species. Finally, possible negative clipping effects on the upper canopy species might have been compensated by the stimulated growth of the lower-canopy species due to improved light condition after clipping. However, in a longer term, clipping may



**Fig. 6** Responses of the maximum photosynthetic rate ( $P_{\max}$ ) of the dominant species *Stipa krylovii* to clipping and nitrogen (N) and phosphorus (P) addition in 2005, 2007, and 2008. CC, clipping control; CL, clipping. See Fig. 1 for other abbreviations.

potentially impact ecosystem C fluxes because of the long-term influence on the dynamics of litter and soil C (Luo *et al.*, 2009).

#### Seasonal and interannual variability

In the temperate steppe of northern China, greater ecosystem C uptake (GEP) than C release (ER) leads to a net C sink (positive NEE) during the growing seasons in all the 4 years (Figs 1–3) although the N stimulation of NEE was not statistically significant in the last 2 years. The maximum NEE values in this grassland ( $5\text{--}8 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) are comparable to those in a temperate grassland of Canada (Flanagan *et al.*, 2002), but higher than values observed in a central Mongolia steppe (Li *et al.*, 2005) and lower than that in a tall grass prairie in Kansas (Ham & Knapp, 1998) and a mixed-grass prairie in North Dakota (Polley *et al.*, 2008). Seasonal integrals of NEE ranged from  $-73$  to

$-22 \text{ g C m}^{-2} \text{ yr}^{-1}$  over the study period (Table 2). The large seasonal and interannual variations in C fluxes (Figs 1–3) indicate that the temperate steppe is sensitive to environmental change. Both  $T_{\text{air}}$  and precipitation was positively correlated with NEE (all  $P < 0.01$ ) across the growing season, suggesting that  $T_{\text{air}}$  and precipitation are important controlling factors over ecosystem C exchange in the study site. This can be reflected also by the similar seasonal changes (one peak pattern) of  $T_{\text{air}}$  and ecosystem C exchanges over the growing season (Fig. 1). Some dips occurred was due to low precipitation (Fig. 1). The strong seasonal and interannual variability in ecosystem C exchange has also been reported in many other grassland ecosystems such as a tall-grass prairie (Suyker *et al.*, 2003), a mixed-grass prairie (Polley *et al.*, 2008), and a California grassland (Xu & Baldocchi, 2004; Ma *et al.*, 2007).

#### Conclusions

Our results showed that N addition stimulated NEE in the first 2 years of our experiment, suggesting that N deposition under global climate change will likely increase C sequestration in the temperate steppe in a short term. The magnitude of N stimulation on NEE declined over time. We conclude that results from short-term experiments may not provide a solid base for predicting the long-term impacts of N deposition on ecosystem processes. The temporal decline in the N stimulation of NEE resulted from the N-induced shift in species composition. It is yet to be examined whether our conclusion on diminishing N stimulation on C sequestration could be generalized across other ecosystems.

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