Running Title: Nitrogen and Water Impacts on CO₂, CH₄, and N₂O

Interactive Impacts of Nitrogen Input and Water Amendment on Growing Season Fluxes of CO₂, CH₄, and N₂O in a Semiarid Grassland, Northern China

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Main points:

1. Nitrogen input stimulated CO₂ uptake and N₂O emission, while inhibited CH₄ uptake during growing season in a semiarid grassland.
2. Water amendment stimulated CO₂ uptake and N₂O emission, while inhibited CH₄ uptake during growing season in a semiarid grassland.
3. Background precipitation condition affects the interaction between nitrogen and water on GHG fluxes.

Abstract

Nitrogen and water are two important factors influencing GHG (primarily CO₂ - carbon dioxide; CH₄ – methane, and N₂O – nitrous oxide) fluxes in semiarid grasslands. However, the interactive effects of nitrogen and water on GHG fluxes remain elusive. A 3-year (2010-2012) manipulative experiment was conducted to investigate the individual and interactive effects of nitrogen and water additions on GHG fluxes during growing seasons (May to September) in a semiarid grassland in Northern China. Accumulated throughout growing seasons, nitrogen input stimulated CO₂ uptake by $3.3 \pm 1.0 \text{ g C m}^{-2} (\text{g N})^{-1}$, enhanced N₂O emission by $1.2 \pm 0.3 \text{ mg N m}^{-2} (\text{g N})^{-1}$, and decreased CH₄ uptake by $5.2 \pm 0.9 \text{ mg C m}^{-2} (\text{g N})^{-1}$; water amendment stimulated CO₂ uptake by $0.2 \pm 0.1 \text{ g C m}^{-2} (\text{mm H}_2\text{O})^{-1}$ and N₂O emission by $0.2 \pm 0.02 \text{ mg N m}^{-2} (\text{mm H}_2\text{O})^{-1}$, decreased CH₄ uptake by $0.3 \pm 0.1 \text{ mg C m}^{-2} (\text{mm H}_2\text{O})^{-1}$. A synergistic effect between nitrogen and water was found on N₂O flux in normal year while the additive effects of nitrogen and water additions were found on CH₄ and CO₂ uptakes during all experiment years, and on N₂O emission in dry years. The nitrogen addition had stronger impacts than water amendment on stimulating CH₄ uptake in the normal year, while water was the dominant factor affecting CH₄ uptake in dry years. For N₂O emission, the N-stimulating impact was stronger in un-watered than in watered plots, and the water-stimulating impact was stronger in non-fertilized than in fertilized treatments in dry years. The interactive impacts of nitrogen and water additions on GHG fluxes advance our understanding of GHG fluxes in responses to multiple environmental factors. This data source could be valuable for validating ecosystem models in simulating GHG fluxes in a multiple factors environment.

Key words: Greenhouse Gas, Interactive Impacts, Nitrogen Input, Water Amendment

1. Introduction

The semiarid grasslands play an important role in regulating the climate system through the land-atmosphere exchanges of greenhouse gases (GHGs), primarily carbon dioxide (CO₂), methane (CH₄), and nitrous oxide (N₂O) [Frey *et al.*, 2013; Friedlingstein *et al.*, 2014; Ringer *et al.*, 2011; Field *et al.*, 2014]. The GHG fluxes are controlled by many environmental factors including soil temperature, soil moisture, vegetation, management practices, etc. [Liu *et al.*, 2014; Merbold *et al.*, 2014; Tian *et al.*, 2012]. Over the past century, changes in climate system and human activities (e.g. changes in temperature, precipitation, and nitrogen inputs) have dramatically altered these environmental factors and thus the GHG fluxes in semiarid grasslands [Dermody, 2006; Norby and Luo, 2004; Tian *et al.*, 2015; Xu *et al.*, 2010, 2012]. Therefore, quantifying the GHG fluxes and their responses to environmental changes is critically important for projecting the feedback of semiarid grasslands to the climate system [Dermody, 2006; Norby and Luo, 2004; Poulter *et al.*, 2014].

For semiarid grasslands, nitrogen and water are two of the most important limiting factors which individually and interactively affect plant growth and GHG fluxes at the ecosystem scale [Lv *et al.*, 2014; Niu *et al.*, 2009]. A number of field experiments have been carried out to investigate the individual impacts of nitrogen and water on GHG fluxes in semiarid grasslands. For example, studies showed that nitrogen deposition stimulated N₂O emission [Liu *et al.*, 2014; Zhang *et al.*, 2014], which partially canceled out the stimulating impact on CO₂ uptake [Niu *et al.*, 2010; Niu *et al.*, 2009; Volk *et al.*, 2011]; few other studies found that nitrogen addition stimulated CH₄ flux in semiarid grasslands [Chen *et al.*, 2013; Li *et al.*, 2012; Louro *et al.*, 2013; Šimek *et al.*, 2014; Stiehl-Braun *et al.*, 2011; Zhang *et al.*, 2014]; manipulative experiments found that increased precipitation enhanced ecosystem CO₂ uptake through its stimulating effect

on gross primary production [Chimner *et al.*, 2010; Nagy *et al.*, 2007; Yan *et al.*, 2011]. For CH₄ uptake, the impact of water addition could be inhibition [Blankinship *et al.*, 2010b; Liu and Greaver, 2009], neutral [Liu and Greaver, 2009; Mariko *et al.*, 2007], or stimulating [Blankinship *et al.*, 2010a; Xu *et al.*, 2015]; while for N₂O emission, the impact of water addition could be stimulating [Horváth *et al.*, 2010; Zhang and Han, 2008] or neutral [Liu and Greaver, 2009; Unteregelsbacher *et al.*, 2013] in semiarid grasslands.

Although the individual effects of nitrogen input and water amendment on GHG fluxes have been extensively investigated [Chen *et al.*, 2013; Niu *et al.*, 2009; Blankinship *et al.*, 2010a,b], the interactive impacts between nitrogen and water inputs remain elusive [Woodward *et al.*, 2010]. The importance of interactive impacts between nitrogen and water has been widely recognized [Norby & Luo, 2004] and modeling approaches have been used to quantify the interaction [Xu *et al.*, 2012]. For instance, a number of modeling studies have concluded significant interactions between climate change, nitrogen input and land use change on CH₄ and N₂O fluxes; while the model validation is solely based on single factor field experiment [Xu *et al.*, 2010, 2012; Tian *et al.*, 2011, 2015]. Limited field experiments were carried out with two or more manipulated environmental factors [Xia *et al.*, 2009; Zhou *et al.*, 2006]. Additive and non-additive (synergistic or antagonistic) effects have been proposed for the impacts of multiple factors [Ball *et al.*, 2008; Blankinship *et al.*, 2010b; Dijkstra *et al.*, 2012]. In this aspect, how nitrogen interact with water affecting GHG fluxes is critically important to advance our understanding of ecosystem responses to multiple global change factors. Therefore, investigating the interactive impacts between nitrogen addition and water amendment is essential for predicting grassland responses to multi-factor global change [Ball *et al.*, 2008; Dijkstra *et al.*,

2012; *Blankinship et al.*, 2010 a, b].

The grassland in northern China has been proved to be typically representing semiarid grassland (Xia et al., 2009; Niu et al., 2009; Poulter et al., 2014); they play an important role in regulating the climate system. Recent studies have projected increases in nitrogen input and precipitation in northern and central Asia in the 21st century (IPCC 2013). Therefore, designing a manipulative experiment to investigate the nitrogen and water impacts on GHG fluxes in the semiarid grassland in northern China would be valuable to better understand the semiarid grasslands and their role on the terrestrial ecosystems-climate feedback (Poulter et al., 2014).

Theoretically, nitrogen stimulates CO₂ uptake by enhancing ecosystem productivity, stimulates N₂O emission by enlarging substrate pool, and decreases CH₄ uptake due to competition between NH₄⁺ and CH₄ for methanotrophy; meanwhile, water amendment stimulates CO₂ uptake by enhancing ecosystem productivity, stimulates N₂O emission by creating favorable condition for denitrifiers, and decreases CH₄ uptake by enhancing methanogenesis. Considering the complexity of the biological system, we expect an interactive impact between nitrogen and water on GHG fluxes. Thus, this study was designed to test three hypotheses about individual effects and interactive effects of nitrogen and water additions on three GHGs: (i) nitrogen deposition has primarily positive effects on CO₂ sequestration and N₂O emission but negative effects on CH₄ uptake; (ii) water addition has positive effects on CO₂ sequestration and N₂O emission but negative effects on CH₄ uptake; (iii) there are strong interactive impacts between nitrogen addition and water amendment in terms of affecting GHG fluxes in semiarid grasslands.

2. Materials and methods

2.1. Site description and experimental design

The study site is a typical semiarid temperate grassland in northern China, located in Duolun County (42°02'N, 116°17'E), Inner Mongolia. Long-term mean annual temperature is 2.11°C with monthly mean temperatures ranging from -17.5°C in January to 18.9°C in July. Mean annual precipitation is 383 mm yr⁻¹, with an obvious seasonality, peak rainfall occurring between May and October. Soil properties and plant species for the study site have been described in our previous publication [Zhang *et al.*, 2014]. The soil was composed of approximately 63% sand, 20% silt, and 17% clay at depths of 0-10cm. The site plants were mainly perennial plants such as *Stypa sylvii*, *Artemisa frigida*, *Potentilla acaulis*, *Cleistogenes squarrosa*, *Allium bidentatum*, and *Agropyron cristatum*.

The annual precipitation was 329 mm in 2010, 232 mm in 2011, and 269 mm in 2012, respectively, varied substantially both intra- and inter- annually (Fig. 1A-C). The long-term mean annual precipitation has been estimated at 383 mm [Yan *et al.*, 2010]; the years of 2011 and 2012 were climatically dry years compared to 2010 as a normal year. Apparent seasonal patterns were observed for soil temperature (ST) and moisture (SM), with peaks in July - August and nadirs in December – January (Fig. 1 D-I). Over the three years, nitrogen addition significantly increased ST by 3.4% ($p < 0.005$) and decreased SM by 9.0% ($p < 0.005$) and water addition has no significant impact on ST while significantly increased SM by 3.0% ($p < 0.005$).

[Insert Figure 1 here]

To assess the effects of nitrogen and water additions on GHG fluxes, we established a factorial manipulative experiment with two levels of nitrogen addition (control and 10 g N m⁻² year⁻¹) and two levels of water addition (control and 120 mm water m⁻² year⁻¹). The experiment

ran from 2010 to 2012. We used a complete block design, with three blocks in total, to deal with spatial heterogeneity. The four experimental treatments were each randomly assigned to the four 6×6 m plots in each block, with three replicates per treatment (Fig. S1). All plots were separated by a 3 m buffer zone. The four treatments were control (C), nitrogen addition (N), water addition (W), and concurrent additions of nitrogen and water (NW), respectively. Nitrogen and water addition treatments were applied every 2 weeks over the growing season (from May to September) during 2010 – 2012. The dose for nitrogen addition is $10 \text{ g N m}^{-2} \text{ year}^{-1}$ as NH_4NO_3 solution; this dose was applied because it meets the nitrogen required to sustain maximum vegetation productivity [Bai *et al.*, 2010]. The total amount of added water, $120 \text{ mm/m}^2/\text{year}$, equals approximately ~40% of the annual precipitation in this area. The plots with concurrent additions of nitrogen and water received the same amounts of nitrogen and water as in the single factor treatment; this design is used to better evaluate the interactive effects of nitrogen and water additions. Control plots were not fertilized, but rather watered with the same amount of water as used in the fertilizer solutions; the water used to dissolve nitrogen is ~500 ml, which is equivalent to 0.5 mm, a test of field experiment found that this small amount of water addition does not cause any significant changes in water supply, thus the ecosystem functions.

2.2. Gas sampling and measurements

The GHG fluxes were measured in each plot three times a month from May to September during the growing seasons of 2010 - 2012. Net ecosystem CO_2 exchange (NEE) was measured by using a static transparent chamber [Xia *et al.*, 2009 b]. CH_4 and N_2O fluxes in each plot were measured using an opaque static chamber and gas chromatography (GC). The method has been described in details in our previous publication [Zhang *et al.*, 2014]. Gas samples were taken every ten minutes within a thirty-minute duration. The linear regression of the gas concentrations

for the four data points was used to estimate the net changing rate of gas concentration in the chambers. Sample sets were rejected unless they yielded a linear regression value of r^2 greater than 0.9 for CO₂ and CH₄, and 0.7 for N₂O because of the high variability but low value of N₂O flux rate. The gas flux was calculated according to the following equation from Song *et al.* [2009]:

$$F = \frac{dc}{dt} \times \frac{M}{V_0} \times \frac{P}{P_0} \times \frac{T_0}{T} \times H$$

where dc/dt is the slope of the linear regression for gas concentration gradient through time; M is the molecular mass of each gas; P is the atmospheric pressure; T is the absolute temperature during sampling; V_0 , T_0 , and P_0 are the gas mole volume, absolute air temperature (K), and atmospheric pressure under standard conditions, respectively; and H is the height of chamber during sampling (m).

The fluxes of CO₂, CH₄, and N₂O were linearly interpolated and sequentially accumulated to estimate the total flux over the growing season [Nikiema *et al.*, 2011]. The linear interpolation was adopted primarily due to two reasons: (1) it has been widely used in previous studies and has been suggested to be effective for seasonal interpolation [Song *et al.*, 2009; Nikiema *et al.*, 2011]; (2) auxiliary data to assist seasonal interpolation was lacking. Soil temperature and vegetation properties are likely two widely used factors to assist seasonal interpolation of gas flux [Gomez-Casanovas *et al.*, 2013], yet this study recorded these data variables in association with observed gas flux, no data are available between measurements. The poor correlations between soil temperature and GHG fluxes prevent using empirical interpolation based on auxiliary data (particularly soil temperature) (Fig. S 2). To be consistent, the positive values indicate gas emission from the grassland to the atmosphere while the negative values indicate gas uptake by the grassland ecosystems.

2.3. Soil and plant sampling

Soil samples were taken to a 10 cm depth, once per month in 2010, and once per year in 2011 and 2012. Three soil cores were taken randomly in each plot and mixed completely; the mixed soil sample was then divided into two sub-samples, one was stored at 4°C and the other air-dried for measuring pH, soil total carbon (STC), nitrogen (STN), and phosphorus (STP) analyses. STC, STN, STP and ammonium ($\text{NH}_4^+\text{-N}$) and nitrate ($\text{NO}_3^-\text{-N}$) were measured by using a CNS elemental analyzer (*Variomax CNS Analyser, Elementar GmbH, Hanau, Germany*). For $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$, fresh soil samples were extracted with 2 M KCl [Searle, 1984] for 1 h at 200 rpm in a horizontal shaker (1:2 fresh soil:extractant ratio) and then filtered through Whatman No. 5 paper. The extracts were analyzed for $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ using the CNS elemental analyzer. Soil microbial biomass carbon and nitrogen (MBC and MBN) were determined using the chloroform fumigation–extraction method [Vance *et al.*, 1987] following the protocols described by Liu *et al.* [2007].

Aboveground plant biomass was measured at the end of August when aboveground biomass was at the peak. The above ground vegetation biomass within an area of 1 m² was clipped and the plant samples were oven-dried at 65°C for 48 h before being weighed; the vegetation materials reached a constant weight. Finally, the vegetation materials were grinded, and carbon and nitrogen content were measured with the CNS elemental analyzer. All these environmental factors were chosen because they have been suggested to be direct or indirect controlling factors on GHG processes [Conrad *et al.*, 1996; Tian *et al.*, 2011].

2.4. Statistical analyses

We used a two-way ANOVA analysis (ANOVA; SAS software, Version 9.1.3, 2002) to

examine the impacts of nitrogen and water additions and their interactions on seasonal cumulative CO₂, CH₄ uptake and N₂O emission during the growing season, and other environmental variables including soil temperature, soil moisture, above net primary production (peak above ground biomass), litter, total vegetation carbon (PTC), nitrogen (PTN), phosphorus (PTP), STC, STN, STP, soil MBC, and soil MBN. The *Bonferroni correction* was adopted in ANOVA analysis. Significant effects are deemed if $p < 0.05$. Both linear and nonlinear regressions were used to investigate the relationships between GHGs and environmental factors (i.e., soil temperature, soil moisture, ANPP, Litter, PTC, PTN, PTP, STC, STN, STP, soil MBC and MBN, NH₄⁺-N and NO₃⁻-N).

We classified the interactive effects of nitrogen and water according to a directional interaction classification system used in *Piggott, et al.*, [2015]:

1. An additive effect (no significant interaction in two-way ANOVA) represents the sum of the individual effect that may arise from concurrent effects of two environmental factors.
2. An interaction that deviates from additive (i.e. a significant two-factor interaction term in ANOVA) and is less than the sum of the individual effects is antagonistic.
3. A deviation from the additive that is greater than the sum of individual effects and greater than any individual effect is synergistic.

The effects of nitrogen and water additions were calculated for each year. Figures were produced with SigmaPlot 10.0 (Systat Software, Inc.). A multiple linear regression analysis was carried out by using “glm” package with R 3.2.3 on Windows.

3. Results

3.1. GHG flux in the semiarid grassland under different treatments

The instantaneous CO₂ flux varied substantially over the study period, ranging from

190.0±66.7 mg m⁻² h⁻¹ under the control treatment in 2012 to -2439.6±1549.3 mg m⁻² h⁻¹ under water-addition treatment in 2010 (Fig. 2 A-C). Over the three growing seasons, the grassland ecosystems acted as a typical sink of atmospheric CO₂ under all treatments (Fig. 3 B). An obvious seasonality of instantaneous CH₄ flux was observed with a maximum uptake in June for three growing seasons (Fig. 2 D-F); the maximum CH₄ flux (emission) was observed at 60.0±58.1 µg m⁻² h⁻¹ on 17 August 2011 under the NW treatment and minimum value (uptake) was observed at -325.2±111.5 µg m⁻² h⁻¹ on 10 June, 2011 under control treatment (Fig. 2 D-F).

[Insert Figure 2 here]

Similar temporal variations with different magnitudes of instantaneous N₂O flux were found among treatments in the growing seasons during 2010-2012 (Fig. 2 G-I). The instantaneous N₂O flux dramatically increased (more positive) immediately after nitrogen and water additions (Fig. 2 G-I). During the study period, the grassland ecosystem had a very small flux of N₂O under various treatments, except several pulses of N₂O fluxes right after nitrogen and water application (Fig. 2 G-I). The highest instantaneous N₂O flux occurred under NW treatment was 141.2 ± 36.9 µg m⁻² h⁻¹ on 1 June 2011, while the lowest instantaneous N₂O flux occurred under the nitrogen treatment was -26.9 ± 3.5 µg m⁻² h⁻¹ on 10 May 2011 (Fig. 2 G-I).

3.2. Individual impacts of nitrogen and water additions on GHG fluxes

Nitrogen addition and water amendment had dramatic impacts on GHG fluxes. Both water and nitrogen additions had significant effects on CO₂ uptake in 2011 and 2012 (Table 1). The NW treatment (concurrent nitrogen and water addition) significantly stimulated CO₂ uptake in 2011 and 2012 (p<0.05, Fig. 3 B), while the stimulating effect was not significant in 2010. Meanwhile, the impacts of nitrogen and water amendments on annual cumulative CO₂ uptake

varied among years. For example, CO₂ uptake during 2011-2012 (dry year) was lower than that in 2010 (normal year) under control, nitrogen and water treatment. The CO₂ uptake in NW treatment was not significantly different among three years (Fig. 3 B).

[Insert Figure 3 here]

[Insert Table 1 here]

The semiarid grassland ecosystem was a CH₄ sink under all treatments throughout the experimental period (Fig. 3 C). Water addition in 2010 and 2012 and nitrogen addition in 2011 had significant effects on seasonal cumulative CH₄ uptake (Table 1; Fig. 3 C). The impacts of concurrent nitrogen and water additions on cumulative CH₄ uptake varied among years. For example, NW significantly suppressed cumulative CH₄ uptake by 23.7% in 2010, 53.3% in 2011 and 40.3% in 2012, respectively ($p < 0.05$, Fig. 3 C). Water addition significantly inhibited seasonal cumulative CH₄ uptake by 26% in 2010 and 28.9% in 2012 ($p < 0.05$, Fig. 3 C). An insignificant, negative change in CH₄ uptake in response to NW treatment was observed across three years (Fig. 3 C). Meanwhile, the NW-induced cumulative CH₄ uptake was weaker in 2011 than in 2010 although no significant difference was observed among years under both control and nitrogen treatments (Fig. 3 C). The cumulative seasonal CH₄ flux ranged from -96.13 ± 26.9 mg C m⁻² in 2011 under the NW treatment to -211 ± 8.7 mg C m⁻² in 2010 under the control treatment (Fig. 3 C). Averaged across three years, nitrogen addition significantly inhibited CH₄ uptake by 25.1% ($p=0.03$) and water treatment marginally inhibited CH₄ uptake by 23.9% ($P = 0.07$).

All nitrogen and water treatments stimulated N₂O emission in all three years (Table 1; Fig. 3 D). Compared with the control, NW treatment increased cumulative N₂O emission over growing season by 115.8% in 2010, 270.2% in 2011, and 250.2% in 2012, respectively (Fig. 3

D); while water treatments increased cumulative N₂O emission over growing season by 120.7% in 2012 (Fig. 3 D). The treatments did not significantly alter the inter-annual variation of the N₂O emission. For example, the growing-season cumulated N₂O emission in 2011 was much larger than that in 2010 and 2012 under all treatments (Fig. 3 D). Averaged across three years, N₂O emission was significantly stimulated by nitrogen addition (76.6%, $p = 0.02$) and by water addition (81.7%, $p = 0.03$). The individual impacts of nitrogen addition and water amendment on GHG fluxes are consistent with our hypotheses 1 & 2.

3.3. Interactive impacts of nitrogen and water additions on GHG fluxes

There are complicated interactive impacts between nitrogen and water addition on CO₂, CH₄, and N₂O fluxes in growing season for a majority of the experimental years (Table 3). However, the interactive impacts are not always significant. Specifically, in this study, both water and nitrogen additions had significant effects on seasonal CO₂ uptake in dry years, but their interactions are not significant in all three years (Table 1); no significant interaction between nitrogen and water on seasonal CH₄ uptake was observed in all three years (Table 1). Nitrogen and water treatments stimulated N₂O emission in any of the three years, but the interaction between water and nitrogen additions on N₂O emission was observed only in normal year (Table 1; Fig. 3 D).

[Insert Table 2 here]

[Insert Table 3 here]

In addition, there was an interaction between nitrogen and water on GHG fluxes. The effect of nitrogen addition on the cumulative CO₂ uptake was higher in the watered than unwatered plots in 2011, and the effect of water addition was higher in the fertilized than unfertilized plots in 2012 (Fig. 4 A, B). The nitrogen addition tended to strengthen the positive

effect on CO₂ uptake in dry years due to water addition, and water addition tends to strengthen the positive effect on CO₂ uptake due to nitrogen addition in the third year. Moreover, the cumulative CO₂ uptake in dry years was lower than that in normal year across all treatments except under concurrent nitrogen and water addition treatment (Fig. 3 B). The inhibition impacts of nitrogen addition on cumulative CH₄ uptake varied between watered and unwatered conditions, and the impacts of water addition varied across fertilized and unfertilized conditions. Nitrogen-induced increase in cumulative CH₄ uptake was higher than the water induced changes in 2010, while water induced decreases in CH₄ uptake was higher than nitrogen induced changes in 2011 (Fig. 4 C, D). The additive impacts of nitrogen and water additions on CO₂ and CH₄ flux in all three years and N₂O flux in dry years partially rejected the hypothesis 3.

3.4. Environmental controls on GHG fluxes

The GHG flux was affected by various environmental factors. Significantly positive correlations were found between CO₂ flux and STN, STP, MBC, and PTP, whereas significantly negative correlations were found between CO₂ flux and NH₄⁺, ANPP, PTC and PTN (Fig. S 2). Multiple regression analysis showed that PTN and STN were the dominant factors controlling the variation in seasonal CO₂ uptake (partial R² = 0.27, P = 0.001; partial R² = 0.15, P = 0.007). A significantly negative correlation was observed between seasonal CH₄ flux and litter biomass (Fig. S 2), and significantly positive correlations were found between seasonal CH₄ flux and STP, NH₄⁺ and MBC (Fig. S 2). Multiple regression analysis showed that litter biomass was the dominant factor controlling the variation in seasonal CH₄ flux (R² = 0.29, P = 0.0007). Significantly positive correlations were observed between seasonal N₂O flux and STC, NO₃⁻-N and PTP, whereas significantly negative correlations were found between seasonal N₂O flux and litter biomass, non-legume perennial forbs (NF) biomass, PTC and PTN (Fig. S 2). Multiple

regression analysis showed that PTN (partial $R^2 = 0.27$, $P = 0.001$), soil MBC (partial $R^2 = 0.1$, $P = 0.03$), and PTP (partial $R^2 = 0.09$, $P = 0.03$) were the dominant factors controlling the seasonal variation of N_2O flux. In sum, the dominant factors varied across different gas fluxes.

4. Discussion

4.1. Mechanisms for factorial impacts on GHGs

The factorial impacts on GHGs are caused by the direct and indirect impacts of nitrogen and water on vegetation activities and substrate concentrations [Ambus and Robertson, 2006; Billings *et al.*, 2000; Borken *et al.*, 2006; Cheng *et al.*, 2014; Liu *et al.*, 2008]. The strong association between nitrogen addition and plant biomass condition (Table 2; Fig. 3 A, B) indicated the nitrogen-induced stimulation of vegetation productivity was the foundational mechanism for changes in CO_2 flux [Du *et al.*, 2014]. The nitrogen addition suppressed CH_4 uptake (Fig. 3 C), which might be explained by substrate competition theory [Ambus and Robertson, 2006] -- aerobic soil methanotrophs compete for NH_4^+ and CH_4 as substrate [Aronson and Helliker, 2010; Hanson and Hanson, 1996]. This could be confirmed by the significantly negative correlation between CH_4 uptake and NH_4^+ in our study (Fig. S 2). Furthermore, nitrogen addition enhanced the litter decomposition [Peng *et al.*, 2014], which might have suppressed microbial activities due to carbon limitation. As a result, the methanotrophy activity was reduced and less CH_4 was oxidized, expressed as lower CH_4 uptake under nitrogen treatment (Fig. 3 C). Nitrogen additions directly enlarged the nitrogen substrate for N_2O emission, explaining the nitrogen-stimulating impacts on N_2O emission [Kulkarni *et al.*, 2008; Scheer *et al.*, 2008].

That water addition significantly enhanced CO_2 uptake in dry years, N_2O emission in all three years, and CH_4 uptake in 2010 and 2012 (Table 1; Fig. 3) support our second hypothesis that water addition has positive effects on CO_2 sequestration and N_2O emission but negative

effects on CH₄ uptake. The strong association between CO₂ uptakes with precipitation gradients suggests that water addition stimulates CO₂ uptake in semiarid ecosystems (Fig. 1 A-C; Fig. 3 B). Decreasing CH₄ consumption with increasing soil moisture has been reported in numerous studies [Castro *et al.*, 1994; Rosenkranz *et al.*, 2006], in this study only the water addition in 2010 and 2012 had significantly inhibiting effects on the cumulative CH₄ uptake (Table 1; Fig. 3 C). These results suggested water addition exerted significant impacts on CH₄ uptake in normal year (2010, Fig. 1 A; Fig. 3 C) and the third year (2012, Fig. 1 C; Fig. 3 C) of the experiment treatment. Two alternative reasons may account for the observed water addition impact on CH₄ flux. First, semiarid grassland soils with low moisture (Fig. 1 G-I) showed steady CH₄ consumption (Fig. 2 D-F; Fig. 3 C), therefore water addition may reduce soil aeration, thus slowing down the diffusion of atmospheric CH₄ and further reduce CH₄ oxidation [Conrad, 2007]. Second, inhibition of CH₄ oxidation under dry conditions might be caused by water stress on methanotrophs [Xu *et al.*, 2015; Borken *et al.*, 2006]. Therefore, water addition might suppress the activity of CH₄-oxidizing bacteria [Aronson and Helliker, 2010], which reduced the CH₄ efflux to atmosphere in 2010 and 2012. But the added water in dry year may have not reach the threshold of moisture level for stimulating the methanotrophs activity, therefore, water addition did not affect the CH₄ sink strength in 2011 (dry year). The stimulation of water addition on N₂O emission has been attributed to the anaerobic condition, which is favorable for denitrification [Xu *et al.*, 2012].

4.2. How do nitrogen-water interactions on GHG fluxes occur?

The impacts of nitrogen and water on the fluxes of CO₂ and CH₄ were additive for all three years, and their impact on N₂O flux was additive in dry years. No interaction was inferred in these treatment-years; while there was a synergetic interaction between nitrogen and water

additions on N₂O flux in normal year. There are three potential mechanisms for this interaction. First, in the normal year, the water was not a serious limiting factor; therefore, both water and nitrogen additions had weaker stimulating impact on N₂O flux in normal years than that in dry years (Table 2). Second, both nitrogen and water were limiting factors for N₂O production, therefore, either of them stimulate N₂O emission; according to the law of mass action, the increase in overall reaction rate was much higher than that caused by individual substrate since both of them were stimulating impacts. While in dry years, both nitrogen and water were stimulating factors for the N₂O production, their impacts were very strong with high variance, their interaction was relatively small compared to the individual impacts.

[Insert Figure 4 here]

[Insert Figure 5 here]

Moreover, the cumulative CO₂ uptake in dry years (2011 and 2012) was lower than that in normal year (2010) across all treatments except under concurrent nitrogen and water addition treatment (Fig. 3 B), due to the higher precipitation amount in normal year (2010) (Fig. 1 A-C). The inhibition impacts of nitrogen addition on cumulative CH₄ uptake varied between watered and unwatered conditions, and the impacts of water addition varied across fertilized and unfertilized conditions. Nitrogen-induced increases in cumulative CH₄ uptake was higher than the water induced changes in normal year, while water induced decreases in CH₄ uptake was greater than nitrogen induced changes in dry year (Fig. 4 C, D), indicating that nitrogen is more important than water for stimulating CH₄ uptake in normal year and water is the dominant factor affecting CH₄ uptake in dry year. In sum, the relative importance of nitrogen and water effect on CH₄ uptake varies across years, in association with background climate condition.

4.3. Comparisons with other studies

The impacts of nitrogen and water additions on CO₂, CH₄, and N₂O fluxes in this study are comparable with other studies in semiarid grassland (Table 4 & Fig. 5). Nitrogen addition stimulated CO₂ uptake by 34.5%, which is consistent with other studies (19~30%) [Niu *et al.*, 2009; Xia *et al.*, 2009b; Yan *et al.*, 2011]. The water addition increased CO₂ uptake by 33%, in consistency with another study in same region (11~30%) [Yan *et al.*, 2011] (Table 4). The effects of combined nitrogen and water on CO₂ uptake fall in a larger range as shown in this study and previous studies; the impact was averaged at 72% in this study, which is consistent with other studies (30~153%) (Table 4). The average CH₄ uptake was significantly decreased 25.1% by nitrogen addition (p=0.03), which falls in the range of -34~19% derived from previous studies [Li *et al.*, 2012; Mosier *et al.*, 1991]. Water addition decreased CH₄ uptake by 23.9%, which is dramatically smaller than an inhibition impact of 61% from a grassland in California [Blankinship *et al.*, 2010 b] (Table 4). The reason is that this study site is much drier compared to California site (~300 vs. 652mm); therefore, our study has much larger CH₄ uptake rate ($-5.8 \pm 0.04 \text{ mg C m}^{-2} \text{ d}^{-1} \sim -0.29 \pm 0.03 \text{ mg C m}^{-2} \text{ d}^{-1}$), compared to $-0.45 \pm 0.14 \sim -0.06 \pm 0.09 \text{ mg C d}^{-1}$ as reported by Blankinship *et al* [2010 b]. A significant stimulation of N₂O emission (16.5-88.6%) was observed under nitrogen addition treatment (p=0.02), which is consistent with previously reported N-impact on N₂O (0-87%) [Horváth *et al.*, 2010; Li *et al.*, 2012; Liu *et al.*, 2014; Zhang and Han, 2008]. In addition, water addition had a significant stimulating impact (8.8%-120%) on N₂O emission (p = 0.03), consistent with 0-100% reported by previous studies [Chen *et al.*, 2013; Horváth *et al.*, 2010; Liu *et al.*, 2014; Zhang and Han, 2008] (Table 4).

[Insert Table 4 here]

Putting these impacts in semiarid grasslands into a global context, they are typical and

comparable to other biomes worldwide (Table 4). For example, the nitrogen addition impact on N_2O emission was 21% in a fallow land, which is consistent with those in semiarid grasslands summarized across the global grassland (0~87%), while there was an extremely large impact of nitrogen addition on N_2O emission by 375% from a barley land in Finland (Table 4). The water impacts on CH_4 in Amazonia were reported at 7% to -79% [Vasconcelos *et al*, 2004; Davidson *et al.*, 2004], which is consistent while in a broader range comparing to that in grassland (Table 4). The interactive impacts between nitrogen and water additions in agricultural land are in a broader range compared to those in grassland (-23% ~ 318% vs. 0 ~ 121%) (Table 4). In addition, modeled factorial impacts are in a much narrower range compared to the observational results (Table 4). For example, Lu *et al* [2012] modeled that nitrogen impacts on carbon uptake varied between 1.17% to 6.13% in various biomes in China, which is in consistency while in a much weaker impact compared to the observational results of -4% – 30%. This discrepancy of impact magnitude between model and observational results is likely caused by model assumption of simplifying reality [May, 1976], indicating a need for more mechanistic representation of soil biogeochemical processes in simulating GHG fluxes.

4.4. Uncertainty analysis

This study confirmed the interaction between nitrogen and water additions on GHG fluxes in a semiarid grassland; a number of issues need careful attentions when interpreting the results obtained in this study. First, the low frequency of gas measurements might have caused biases. The low frequency of gas flux would lead to bias in (1) the estimation of gas budget over the growing season, (2) quantification of the instant impacts of nitrogen or water on the gas flux; and (3) quantification of the interaction between nitrogen and water. Second, accurate measurements of gas fluxes would reduce the uncertainties in quantifying interaction between

nitrogen and water. The low flux of N_2O , in particular, requires more accurate measurement of gas concentration and fluxes. Third, the relatively small range of nitrogen input and water amendment might have caused biases in estimating factorial impacts and interaction; broader ranges of nitrogen and water inputs would be valuable for accurately quantifying the nitrogen and water impacts on gas fluxes. Fourth, the differences in gas fluxes between dry and wet years might have caused bias in quantifying the interactive impacts because comparing dry year and normal year is not the key purpose of this study; in this aspect, long-term manipulative experiments under various treatments guarantee a beneficial mechanistic understanding of climate change impacts on ecosystem functions.

5. Conclusion

A 3-year study of growing season CO_2 , CH_4 and N_2O fluxes in a semiarid grassland ecosystem was carried out to quantify the individual and interactive impacts of nitrogen and water amendments. The study reported the single factor impacts of nitrogen or water addition on GHGs fluxes, and further concluded there was an additive impact between nitrogen and water on CO_2 and CH_4 and N_2O fluxes while the interactive impact varied among years and background climate condition. The finding that multiple factor interaction varies across years and background climate conditions called for further mechanistic understanding through manipulative experiments studies.

The key findings of additive impacts between nitrogen addition and water amendment on CO_2 , CH_4 , and N_2O fluxes in a grassland ecosystem have two implications: (1) the analysis could serve as valuable data for validating ecosystem model for simulating GHGs fluxes in response to multiple environmental factors. Given the large uncertainties of nitrogen and water amendment, the results of this study provide valuable insights for parameter optimization to better simulate

nitrogen and climate change impacts on climate feedback of grassland ecosystems. (2) the synergetic impacts between nitrogen and water additions on N₂O flux indicate the complexity of multiple factors in terms of impacts on GHG, inferring the needs of more well-designed field experiments to understand ecosystem processes in a multiple-factor world. To investigate the additive impacts of nitrogen and water, a data-model integration approach to simulate the impacts of multiple factors and their interaction should be undertaken.

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Table 1. Two way ANOVA analysis of seasonal average soil temperature (ST) and moisture (SM), the seasonal fluxes of CO₂ (g C m⁻² seasonal⁻¹), CH₄ (mg C m⁻² seasonal⁻¹), and N₂O (mg N m⁻² seasonal⁻¹) under nitrogen (N) and Water (W) addition, and their interactions (n = 3). P values smaller than 0.05 and 0.10 are in bold and italic, indicating significant and marginally significant, respectively.

		ST	SM	CO ₂	CH ₄	N ₂ O
2010	W	0.004	0.3	0.9	0.03	0.01
	N	<0.001	0.004	0.9	0.45	<0.001
	W*N	0.002	0.32	0.15	0.3	0.03
2011	W	0.03	0.9	0.01	0.35	0.04
	N	0.74	0.002	0.03	0.05	0.02
	W*N	0.009	0.32	0.18	0.99	0.35
2012	W	0.84	0.1	0.01	0.04	0.01
	N	0.13	0.02	0.01	<i>0.08</i>	0.04
	W*N	0.46	0.03	0.18	0.49	0.59

Table 2 Soil and vegetation properties under different treatments [soil microbial biomass carbon (MBC, mg kg⁻¹) and nitrogen (MBN, mg kg⁻¹), NH₄-N (ppm), NO₃-N (ppm), pH, soil total carbon (STC, g kg⁻¹), soil total nitrogen (STN, g kg⁻¹), soil total phosphorus (STP, g kg⁻¹), plant total carbon (PTC, g kg⁻¹), plant total nitrogen (PTN, g kg⁻¹) and plant total phosphorus (PTP, g kg⁻¹) under four treatments in 2010, 2011 and 2012]

	MBC	MBN	NH ₄ ⁺	NO ₃ ⁻	pH	STC	PTC	STN	PTN	STP	PTP
2010											
C	58.8 ^b	8.9 ^b				15.6 ^a	501.6 ^a	1.4 ^a	18.6 ^c	0.5 ^a	1.6 ^c
N	73.8 ^b	18.7 ^a				14.0 ^a	485.4 ^a	1.2 ^b	26.8 ^a	0.5 ^a	1.9 ^b
W	80.1 ^b	9.7 ^b				14.9 ^a	473.4 ^a	1.3 ^{ab}	21.2 ^{bc}	0.7 ^a	1.9 ^b
NW	159.4 ^a	18.5 ^a				13.2 ^a	497.2 ^a	1.2 ^b	25.1 ^{ab}	0.7 ^a	2.5 ^a
2011											
C	107.5 ^b	10.4 ^b	5.5 ^b	18.4 ^a	8.1 ^c	23.0 ^a	461.1 ^a	1.3 ^{ab}	9.0 ^a	0.5 ^a	9.3 ^a
N	142.1 ^b	13.0 ^b	15.2 ^a	20.7 ^a	8.2 ^b	21.3 ^a	430.1 ^a	1.2 ^b	10.2 ^a	0.4 ^a	7.2 ^a
W	137.1 ^b	12.6 ^b	5.0 ^b	13.9 ^a	8.2 ^b	21.8 ^a	442.4 ^a	1.3 ^{ab}	12.2 ^a	0.5 ^a	6.0 ^a
NW	330.7 ^a	19.1 ^a	4.5 ^b	19.1 ^a	8.3 ^a	21.5 ^a	452.8 ^a	1.3 ^a	8.3 ^a	0.5 ^a	8.7 ^a
2012											
C	239.6 ^b	24.7 ^b	6.7 ^a	10.0 ^a		19.4 ^a	414.3 ^b	1.6 ^a	14.3 ^a	0.6 ^a	11.1 ^{ab}
N	246.8 ^b	19.3 ^b	7.2 ^a	7.8 ^a		13.7 ^b	476.0 ^a	1.5 ^{ab}	16.7 ^a	0.8 ^a	13.2 ^{ab}
W	235.3 ^b	13.6 ^b	2.3 ^b	6.5 ^a		14.9 ^b	419.3 ^{ab}	1.6 ^a	17.4 ^a	0.6 ^a	8.0 ^b
NW	440.2 ^a	62.3 ^a	4.9 ^a	9.3 ^a		13.3 ^b	399.6 ^b	1.3 ^b	16.3 ^a	0.9 ^a	15.4 ^a

Values represent the mean of three replicates. Different letters in one column mean significant difference among treatments at P<0.05. C, control plots; N, nitrogen addition; W, water addition; NW, concurrent water and nitrogen addition.

Table 3 Interaction and Additive effect of nitrogen and water additions on CO₂, CH₄ and N₂O fluxes (For NEE and CH₄, -indicated increased uptake, and + indicated decreased uptake; for N₂O, - indicated decreased emission, + indicate increased emission)

	Years	N impacts	Water impacts	Concurrent impacts induced by N and Water	Sum of N and Water impacts	Interactions
NEE (C-CO ₂) (g C m ⁻²)	2010	-32.0 (6.9)	-70.6(36.6)	-25.7(10.5)	-102.6(31.4)	Additive
	2011	-31.8(13.8)	-27.0(3.0)	-103.2(28.5)	-58.8(13.3)	Additive
	2012	-36.3(18.5)	-34.4(0.4)	-115.7(14.2)	-70.7(18.9)	Additive
CH ₄ (C-CH ₄) (mg C m ⁻²)	2010	29.9(24.7)	66.2(21.7)	59.3(4.1)	96.0(34.3)	Additive
	2011	74.5(49.6)	58.8(40.3)	197.6(24.1)	133.3(64.3)	Additive
	2012	52.8(49.6)	78.7(18.2)	136.4(11.5)	131.5(33.4)	Additive
N ₂ O (N-N ₂ O) (mg N m ⁻²)	2010	0.9(1.2)	1.8(1.0)	9.0(0.6)	2.7(0.3)	Synergistic
	2011	22.4(13.6)	37.2(2.8)	91.1(25.9)	60.0(14.5)	Additive
	2012	12.1(6.7)	28.8(1.0)	51.4(13.2)	40.9(7.3)	Additive

Table 4 Impacts of nitrogen and water additions on fluxes of CO₂, CH₄, and N₂O in grassland worldwide (for CO₂ flux, they are net ecosystem exchanges except stated; positive values indicate emission while negatives uptake; SR: soil respiration; ER: ecosystem respiration; For CH₄ flux, the negative values indicate enhanced uptake while positive values indicate enhance emission)

Treatment	Biomes	Location/Spatial coverage	Nitrogen inputs (g N m ⁻² y ⁻¹) or water amendment (mm W y ⁻¹)	Duration (years)	Effect (%)			References
					CO ₂	CH ₄	N ₂ O	
N	Grassland	Switzerland	0, 2, 6, 18, 54, 162	1.0		0		<i>Stiehl-Braun et al. [2011]</i>
N	Grassland	Inner Mongolia	5, 10, 20	0.5			increased	<i>Liu et al., [2014]</i>
N	Grassland			14.0		-34		<i>Mosier et al. [1991]</i>
N	Semiarid grassland	Hungarian	0.1, 1.38	3			26	<i>Horvath et al. [2010]</i>
N	Grassland	Inner Mongolia	0, 5, 10, 15	2			20-87	<i>Zhang et al. [2008]</i>
N	Grassland	Tianshan Mountains	0, 1, 3, 9, 15		10-18(SR)	-2~19	15-34	<i>Li et al. [2012]</i>
N	Steppe	Tibetan Plateau	0, 1	3	-8.2~0(ER)	0	0	<i>Wei et al. [2014]</i>
N	Steppe	Inner Mongolia	0, 10	4	19			<i>Niu et al. [2009]</i>
N	Steppe	Inner Mongolia	0, 10	2	23			<i>Xia et al. [2009]</i>
N	Steppe	Inner Mongolia	0, 28	2	-8~8(SR)			<i>Yan et al. [2010]</i>
N	Steppe	Inner Mongolia	0, 28	2	30			<i>Yan et al. [2011]</i>
N	Boreal forest	China	Dynamics over 105 years	1901-2005 (model)	1.17			<i>Lu et al.[2012]</i>
N	Temperate forest	China	Dynamics over 105 years	1901-2005 (model)	2.75			<i>Lu et al.[2012]</i>
N	Tropical forest	China	Dynamics over 105 years	1901-2005 (model)	1.76			<i>Lu et al.[2012]</i>
N	Broadleaf forest	China	Dynamics over 105 years	1901-2005 (model)	2.40			<i>Lu et al.[2012]</i>

			years					
N	Needleleaf forest	China	Dynamics over 105 years	1901-2005 (model)	2.66			<i>Lu et al.</i> [2012]
N	Forest	China	Dynamics over 105 years	1901-2005 (model)	2.53			<i>Lu et al.</i> [2012]
N	Shrubland	China	Dynamics over 105 years	1901-2005 (model)	6.13			<i>Lu et al.</i> [2012]
N	Grassland	China	Dynamics over 105 years	1901-2005 (model)	1.14			<i>Lu et al.</i> [2012]
N	Crop	China	Dynamics over 105 years	1901-2005 (model)	2.93			<i>Lu et al.</i> [2012]
N	Terrestrial ecosystem	US	Dynamics over 30 years	1979-2008 (model)		0.02		<i>Xu et al.</i> [2010]
N	Terrestrial ecosystem	Canada	Dynamics over 30 years	1979-2008 (model)		0.004		<i>Xu et al.</i> [2010]
N	Terrestrial ecosystem	Mexico	Dynamics over 30 years	1979-2008 (model)		-0.80		<i>Xu et al.</i> [2010]
N	Terrestrial ecosystem	North America	Dynamics over 30 years	1979-2008 (model)		0.02		<i>Xu et al.</i> [2010]
N	Shortgrass Steppe	USA		5	-4	-15.7	38.5	<i>Mosier et al.</i> [2003]
N	Fallow	Finland	0, 5	1			21	<i>Simojoki and Jaakkola</i> [2000]
N	Barley	Finland	0, 5	1			375	<i>Simojoki and Jaakkola</i> [2000]
N	Organic soil	Canada	0, 5, 10, 15	0.7			-63~42	<i>Rochette et al.</i> [2010]
N	Rice	China	0, 22, 30	1			239	<i>Wang et al.</i> [2011]
W	Steppe	Inner Mongolia	0, 95	0.5		0	100	<i>Chen et al.</i> [2013]
W	Grassland	California	0, 326	8.0		-61		<i>Blankinship et al.</i> [2010b]
W	Grassland	Inner Mongolia	0, 10	2			0	<i>Liu et al.</i> [2014]

W	Grassland	Inner Mongolia	0, 291	2		-22.5	28.6	<i>Liu et al.</i> , [2008]
W	Semiarid grassland	Hungarian		3			70	<i>Horvath et al.</i> 2010
W	Grassland	Inner Mongolia	0, 15	2			43	<i>Zhang et al.</i> 2008
W	Grassland	Arizona	-30%~36%	4		0		<i>Blankinship et al.</i> [2010a]
W	Steppe	Inner Mongolia	0, 120	4	11			<i>Niu et al.</i> [2009]
W	Steppe	Inner Mongolia	0, 120	2	16-60(SR)			<i>Yan et al.</i> [2009]
W	Steppe	Inner Mongolia	0, 120	2	11-30			<i>Yan et al.</i> [2011]
W	Regrowth forest	Amazonian	0, 665, 790	2	13-27(SR)	7	78.6	<i>Vasconcelos et al.</i> [2004]
W	Tropical forest	east-central Amazonia	58-61% of the total rain	4.2	-15(SR)	-79	42	<i>Davidson et al.</i> [2004]
W	Boreal forest	Alaska	0, 269	7.3		-54-0		<i>Billings et al.</i> [2000]
W	Coniferous forest	German	0, 152, 475	2.0		-46		<i>Borken et al.</i> [2000]
W	Deciduous forest	USA	0.46, 0.63, 0.77, 0.80 g H ₂ O g ⁻¹ soil	1.0		-6		<i>Borken et al.</i> [2006]
W	Fallow	Finland	0, 95	1			21	<i>Simojoki and Jaakkola</i> [2000]
W	Barley	Finland	0,95	1			609	<i>Simojoki and Jaakkola</i> [2000]
W	Organic soil	Canada	571, 71, 897, 128	0.7			-8~15	<i>Rochette et al.</i> [2010]
W	Rice	China		1			36	<i>Wang et al.</i> [2011]
N*W	Grassland	Inner Mongolia		2			increased	<i>Liu et al.</i> [2014]
N*W	Grassland	California		8.0		-93		<i>Blankinship et al.</i> [2010b]
N*W	Steppe	Inner Mongolia		0.5		0	65-94	<i>Chen et al.</i> [2013]
N*W	Grassland	Inner Mongolia		2			52-121	<i>Zhang et al.</i> [2008]
N*W	Steppe	Inner Mongolia		2	30-45(SR)			<i>Yan et al.</i> [2009]
N*W	Steppe	Inner Mongolia		2	42-153			<i>Yan et al.</i> [2011]
N*W	forest	China		1	75.5 (SR)	-11.2	16.2	<i>Yan et al.</i> [2014]
N*W	Fallow	Finland		1			111	<i>Simojoki and</i>

								<i>Jaakkola</i> [2000]
N*W	Barley	Finland		1			859	<i>Simojoki and Jaakkola</i> [2000]
N*W	Organic soil	Canada		0.7			-23~28	<i>Rochette et al.</i> [2010]
N*W	Rice	China		1			318	<i>Wang et al.</i> [2011]
N	Steppe	Inner Mongolia	0, 10	3	19~99	-36~-14	17~89	This study
W	Steppe	Inner Mongolia		3	21~91	-29~-17	9~121	This study
N*W	Steppe	Inner Mongolia		3	2~128	-53~-24	125~270	This study

Effect = 100% × (treatment – control)/control; “0” indicates no significant effect

Figure legend

Figure 1. Seasonal variations of monthly rainfall (pillar) and air temperature (dot) of the three years (A, B and C) and the seasonal variability of soil temperature at the depth of 10cm (D, E and F), volumetric soil moisture (V/V%) at the depth of 0–10cm (G, H, and I).

Figure 2. Seasonal variability of the instant net ecosystem CO₂ fluxes (A, B and C), CH₄ fluxes (D, E and F) and N₂O fluxes (G, H and I) under four different treatments in 2010 (left panels), 2011 (middle panels) and 2012 (right panels). Vertical bars represent the standard error of the mean (n=3). C, control; N, nitrogen addition; W, water addition; NW, the concurrent addition of water and nitrogen.

Figure 3. Growing season above ground net primary productivity (A: ANPP, g m⁻²), CO₂ (B: g C m⁻²), CH₄ (C: g C m⁻²) and N₂O (D: g N m⁻²) under four treatments in 2010, 2011 and 2012. Values represent the mean ±SE (n=3). * on the pillar indicates significant difference compared with control if P<0.05. Different letters in a column mean significant difference among years in the same treatment if P<0.05. Four bars from left to right are control plots, nitrogen addition, water addition, and combined addition of water and nitrogen.

Figure 4. N-induced changes in CO₂ (A), CH₄ (C), and N₂O (E) in the un-watered and watered plots from 2010 to 2012, and the water-induced changes in CO₂ (B), CH₄ (D), and N₂O (F) in the unfertilized and fertilized plots (Asterisks in the figures represent the difference between treatments; *, p<0.05; **, p<0.01)

Figure 5. Summarized interactive impacts of N addition and water amendment on NEE, CH₄, and N₂O fluxes over the study period (solid lines indicate positive impact while dash lines indicate negative impacts)

Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

Fig. 5

Figure 5. Summarized impacts of N addition and water amendment on CO₂ flux, CH₄, and N₂O fluxes over the study period (solid lines indicate significance at P < 0.05 level, dash lines indicate insignificance at P < 0.05 level)