# Variations in the nitrogen saturation threshold of soil respiration in grassland ecosystems

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**Abstract** Over the last century, anthropogenic activities have increased nitrogen (N) deposition considerably, which significantly affects ecosystem processes and has the potential to induce N saturation in the future. The continuous increase in N deposition may cause a non-linear response in soil respiration (Rs), an important component of carbon (C) cycling. However, little is known about N saturation threshold of soil respiration. In this study, we conducted coordinated experiments in four grassland types across northern China with four N addition levels to explore patterns in the Rs saturation threshold. Our results showed that an Rs saturation threshold generally exists in grassland ecosystems in response to N addition gradients. The N saturation threshold of Rs occurred at an average rate of 50 kg N ha$^{-1}$ yr$^{-1}$, but varied widely with grassland type; the N saturation threshold occurred at rates of 100, 50, 50, and 25 kg N ha$^{-1}$ yr$^{-1}$ in the alpine meadow, meadow steppe, typical steppe, and desert steppe, respectively. Autotrophic respiration (Ra) and heterotrophic respiration (Rh) responded to N addition gradients differently. Ra increased initially and became saturated at a rate of 50 kg N ha$^{-1}$ yr$^{-1}$ and declined thereafter. In

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contrast, Rh decreased monotonically after N addition. Structural equation models further confirmed that the effects of N addition gradients on Rs were primarily determined by the non-linear response of belowground biomass. Interestingly, the compiled global dataset showed that the N saturation threshold of Rs increased with precipitation and soil moisture. These findings indicate that the stimulating effect of N deposition on Rs and Ra might diminish with increasing N deposition in the future, especially in dry grassland ecosystems.

## Introduction

Nitrogen (N) is an important nutrient in terrestrial ecosystems, and the continuous increase in reactive N deposition substantially alters carbon (C) cycling (Janssens et al. 2010; Zhou et al. 2014; Greaver et al. 2016). Soil respiration (Rs), an important component of ecosystem C cycling (Schlesinger and Andrews 2000), is influenced considerably by N deposition (Zhou et al. 2014; Greaver et al. 2016). Generally, the effects of N addition on Rs largely result from altering plant production and species composition (Raich and Tufekciogul 2000; Xu et al. 2015), transforming microbial communities (Janssens et al. 2010), and changing environmental conditions (e.g. pH; Raich and Schlesinger 1992; Bond-Lamberty and Thomson 2010; Chen et al. 2015). Significant increases in Rs have been reported with N addition (Zhou et al. 2014), but the magnitude of the increase has varied with N levels (Liu and Greaver 2010). Larger increases in Rs have been found at a medium N addition, and relatively smaller increases at low or high levels of N addition (Liu and Greaver 2010; Peng et al. 2011; Zhang et al. 2014; Zhai et al. 2016). Thus, there is likely a threshold for Rs in response to N addition gradients. Previous studies have mostly focused on the direction and magnitude of the Rs response to N addition, such as Rs decreases in forests and increases in grasslands following N addition (Janssens et al. 2010; Zhou et al. 2014; Greaver et al. 2016). However, little is known about the Rs saturation threshold along N addition gradients, especially

from field experiments (Brook et al. 2013). Therefore, a better understanding of the N saturation threshold of Rs is important for predicting ecosystem C balances in the future.

Previous studies have shown that in response to N addition Rs increases at first, reaches a plateau, and then declines at high levels of N addition (Peng et al. 2011; Zhang et al. 2014; Zhai et al. 2016). During the initial stage of N addition, autotrophic respiration (Ra) increases rapidly following N addition (Zhou et al. 2014) due to the quick increase in aboveground net primary production (ANPP) and belowground biomass (BGB) (LeBauer and Treseder 2008; Liu and Greaver 2010). Meanwhile, a reduction in heterotrophic respiration (Rh) following N addition (Zhou et al. 2014) is caused by the depressed soil microbial activity (Treseder 2004; Wessén et al. 2010; Janssens et al. 2010; Ramirez et al. 2012; Leff et al. 2015) and a decrease in soil labile carbon (Riggs et al. 2015). N-induced increases in Ra are larger than decreases in Rh, leading to increases in Rs and a positive nitrogen response efficiency (NRE, Rs response per unit N addition) (Zhou et al. 2014, 2016; Shcherbak et al. 2014; Tian et al. 2016a; Fig. 1b). However, with continuous N addition, the ecosystem becomes saturated with nitrogen. The increase in net primary production (NPP) gradually levels off (Aber et al. 1998), and the enrichment in aluminum ions in the soil depresses root growth (Tian et al. 2016b; Horswill et al. 2008), leading to a decrease in BGB (Aber et al. 1998) and a subsequent decline in Ra. As a result, Rs decreases at high levels of N addition, leading to a saturation threshold for Rs (van Nes et al. 2016; Fig. 1a). Therefore, exploring Ra and Rh responses, and their relative contributions to total soil respiration among different ecosystems is key to understanding Rs saturation threshold patterns in response to N addition gradients.

The N saturation threshold of Rs is potentially influenced by precipitation and soil moisture. It has been reported that in grassland ecosystems, precipitation affects the Rs response to N addition by altering the Ra response (Yan et al. 2010). In croplands, the Rs response was reported to peak at 550 mm of precipitation after which little response was demonstrated under extremely low or high mean annual precipitation (Chen et al. 2017). In addition, recent studies have indicated that precipitation influences the N saturation threshold of ANPP at a global scale (Tian et al. 2016a).
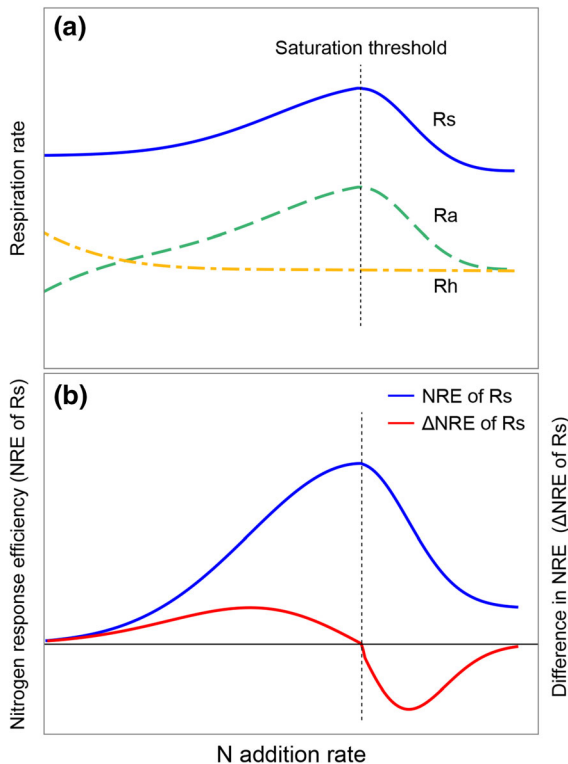
**Fig. 1** Conceptual models of the response ratio of soil respiration and its components along the N-addition gradients (RR), **a** the solid line represents soil respiration (Rs), the long-dash line represents autotrophic respiration (Ra), and the dashed line represents heterotrophic respiration (Rh). The nitrogen response efficiency of Rs (NRE of Rs, soil respiration response per unit N addition) and the difference in NRE between two adjacent N addition levels (ΔNRE of Rs) along the N-addition gradients. **b** The blue solid line represents NRE of Rs, and the red solid line represents ΔNRE of Rs. The N saturation threshold of Rs occurred when the ΔNRE was lower than zero. (Color figure online)

Precipitation can regulate the saturation threshold of NPP and Rh in response to temperature change, besause resource availability increases with increasing precipitation, which then alleviates the adverse effect of high temperature on NPP and Rh (Zhou et al. 2008). Similarly, an increase in precipitation or soil moisture potentially alters the N saturation threshold of plants by increasing water availability, and subsequently influences the N saturation threshold of Rs.

Grasslands are among the most widespread vegetation types in the world, and play a critical role in the global C cycle (Suttie et al. 2005; Ni 2002; Kang et al. 2007; Fang et al. 2010). As BGB in grassland ecosystems ranges from 45 to 2785 g m$^{-2}$ (Yang et al. 2010), the contribution of Ra to Rs varies widely

from 15 to 90% (Norman et al. 1992; Dugas et al. 1999; Wang et al. 2007). Hence, with precipitation varying from 200 to 1600 mm (Christensen et al. 2004; Gill et al. 2002), grasslands are ideal ecosystems for the study of variations in the saturation threshold of soil respiration along N addition gradients. To investigate the N saturation threshold of Rs, we conducted coordinated experiments involving four N addition levels in four different grassland types: alpine meadow, meadow steppe, typical steppe, and desert steppe (Fig. 2). We hypothesized the following: (1) increasing N inputs will lead to a saturation threshold for Rs in grasslands, and (2) saturated plant production resulting from N addition may cause a saturation threshold for Ra/Rh, subsequently leading to a saturation threshold for Rs. As the limiting resource of ecosystems shifts from nitrogen to water under continuous N addition (Aber et al. 1998), we also hypothesize that (3) precipitation and/or soil moisture increases the N saturation threshold of Rs across grassland types.

## Materials and methods

### Sites and experimental design

The coordinated study was conducted since 2011 along environmental and biological gradients in an alpine meadow, a meadow steppe, a typical steppe, and a desert steppe across the northern grasslands of China. Respectively, these sites are located at the Haibei National Field Research Station of the Alpine Grassland Ecosystem, Chinese Academy of Sciences (3200 m above sea level); the Hailar National Field Research Station of Grassland Ecosystems, Chinese Academy of Agriculture Sciences (684 m above sea level); the Xilinhot Field Research Station of Grassland Ecosystems, Inner Mongolia University (1100 m above sea level); and the Xilamuren desert grassland ecosystem (1700 m above sea level) (Fig. 2). The dominant plant species were *Kobresia humilis* and *Stipa aliena* in the alpine meadow, *Stipa baicalensis* in the meadow steppe, *Leymus chinensis* in the typical steppe, and *Stipa breviflora* in the desert steppe. From 1986 to 2015, the mean annual precipitation was 486, 352, 336, and 288 mm, and the mean annual temperature (MAT) was –1.2, –1.3, 0.8, and 2.6 °C in the alpine meadow, meadow steppe, typical steppe, and
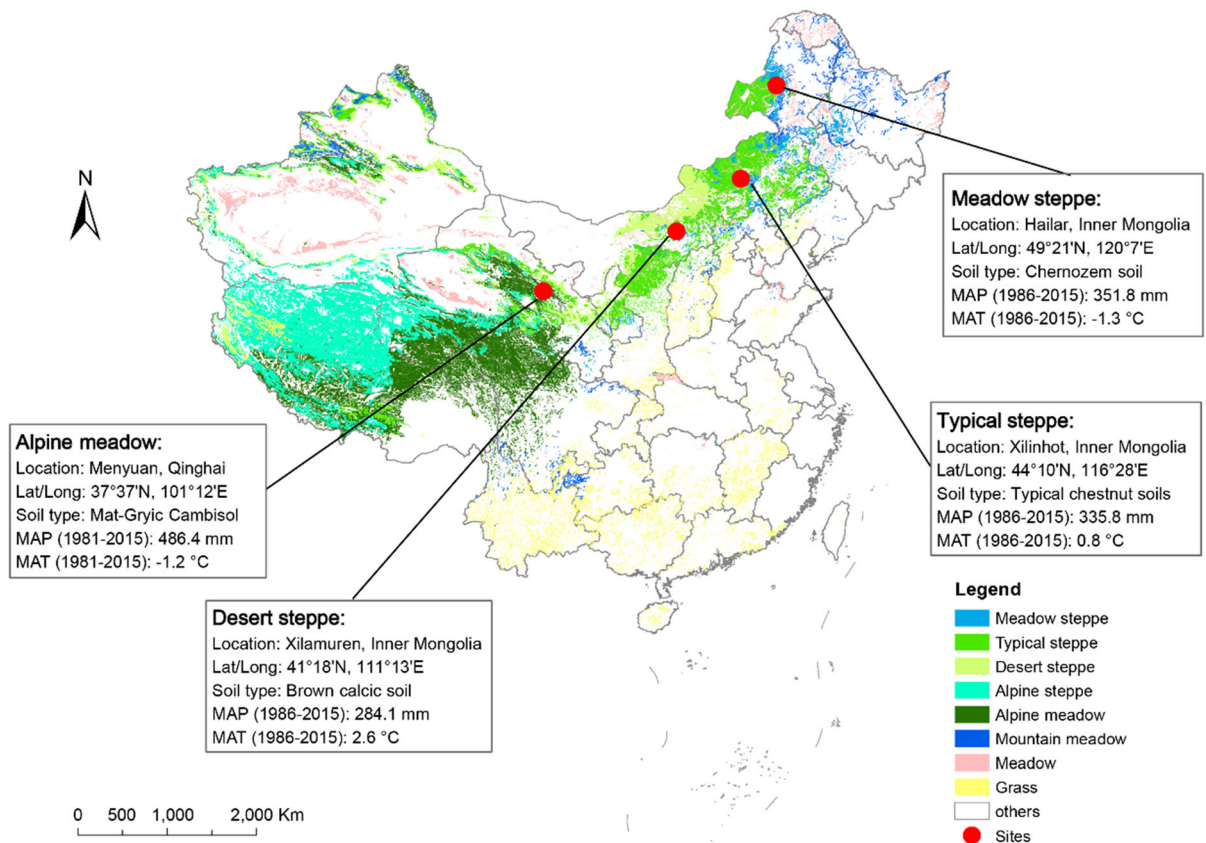
**Fig. 2** Spatial distribution and basic conditions of the study sites in grasslands across northern China

**Table 1** Environmental parameters measured during the experimental period in the study sites of the four different grassland types

| Parameters | Alpine meadow | Meadow steppe | Typical steppe | Desert steppe |
|---|---|---|---|---|
| ANPP (g m$^{-2}$) | 361 ± 23.7c | 354 ± 17.82c | 271 ± 12.04b | 131 ± 13.06a |
| BGB (g m$^{-2}$) | 2136 ± 213bc | 2403 ± 234c | 1961 ± 247b | 1237 ± 89a |
| Standing litter biomass (g m$^{-2}$) | 126 ± 13.1b | 387 ± 22.6c | 136 ± 31.2b | 27.9 ± 1.89a |
| SOC (g kg$^{-1}$) | 72.41 ± 3.16d | 38.29 ± 2.20c | 13.46 ± 0.65b | 10.73 ± 0.85a |
| TN (g kg$^{-1}$) | 8.80 ± 0.26c | 3.81 ± 0.21b | 1.55 ± 0.08a | 1.68 ± 0.09a |
| Soil temperature (°C) | 10.99 ± 0.33a | 16.25 ± 0.48b | 15.60 ± 0.25b | 17.10 ± 1.01b |
| Soil moisture (%) | 25.93 ± 0.51d | 20.91 ± 1.09c | 13.92 ± 1.21b | 4.79 ± 0.50a |
| Precipitation (mm) | 454.7 | 417.4 | 322.6 | 321.1 |
| MAT (°C) | − 1.14 | − 1.27 | 3.37 | 3.29 |

Different letters (a, b, c, and d) within the same row indicate significant differences among the four grassland types based on a linear mixed-model and Tukey's HSD test

Data shown are means ± se

*ANPP* aboveground net primary production, *SOC* soil organic carbon, *TN* total soil nitrogen, *BGB* belowground biomass soil temperature is measured at a soil depth of 5 cm; soil moisture is measured at a soil depth of 10 cm, *MAT* mean annual temperature

desert steppe, respectively (Fig. 2). Other basic environmental conditions during the experimental period are shown in Table 1.

A four level N addition experiment with a randomized block design consisting 5–6 blocks (six replicates for the alpine meadow and five replicates for the meadow steppe, typical steppe, and desert steppe) was conducted at each study site since 2011. The four N addition levels were no nitrogen addition (control), low N addition (LN, 25 kg N ha$^{-1}$ yr$^{-1}$ as urea), medium N addition (MN, 50 kg N ha$^{-1}$ yr$^{-1}$ as urea), and high N addition (HN, 100 kg N ha$^{-1}$ yr$^{-1}$ as urea). At each study site, the 5–6 replicate blocks were separated by 2-m-wide walkways. Within each block, four plots (6 × 6 m) were selected and randomly assigned to the four N addition treatments. Plots within blocks were separated by 1-m-wide walkways to minimize disturbance from neighboring treatments. Fertilizer was applied at the beginning of May, June, and July. Additional information regarding the experimental design is provided in He et al. (2015) and Ren et al. (2016).

## Measurements of soil respiration, soil temperature and moisture

Soil respiration (Rs) and heterotrophic respiration (Rh) were measured manually between 9:00 and 11:00 am at an interval of 10–15 days using a closed chamber connected to a portable infrared gas analyzer (LI-8100; Li-Cor Inc., Lincoln, NE, USA) from May to September throughout the study from 2011 to 2015. Two different types of polyvinyl chloride (PVC) collars were installed in the field. The shallow collars (20 cm in interior diameter and 10 cm in height) were installed in 2011 at a soil depth of 5 cm and then used to measure Rs. The deep collars (20 cm in interior diameter and 70 cm in height), which were used to measure Rh, were installed permanently at a soil depth of 65 cm to exclude plant roots and belowground organic matter input (> 95% of belowground biomass was distributed in the first 60 cm of soil). The Rh was measured at least one year after the deep collars were installed (i.e., deep collars were inserted in the alpine meadow in 2012, and Rh data were collected beginning in 2013) because the influence of decomposing dead roots on Rh disappears within one year (Wang et al. 2014). Autotrophic respiration (Ra) was calculated as the difference between Rs and Rh.

Soil temperature at a depth of 5 cm (ST) and soil moisture at a depth of 10 cm (SM) were measured at the same time that Rs measurements was taken. Soil temperature was recorded using a soil temperature probe (LI-8100–102, Li-Cor Inc., Lincoln, NE, USA) connected to the portable soil respiration system, and soil moisture was measured with a TDR probe (IMKO, Ettlingen, Germany). We also collected daily air temperature and precipitation data from weather stations located within 100 m of our study sites.

## Plant biomass and SOC analysis

Aboveground net primary production (ANPP), standing litter biomass (LB), belowground biomass (BGB), and soil organic carbon (SOC) were measured in each grassland type during the study period. Plot plant biomass was measured at the time of peak standing biomass in August of each year. In each plot, aboveground plant biomass was harvested in a randomly selected 0.2 × 0.5 m quadrat (0.5 × 0.5 m in the alpine meadow) and care was taken not to resample areas that were previously clipped. Harvested biomass was identified to the species level, sorted into functional groups (i.e., grasses, sedges, legumes, and non-leguminous forbs) and plant litter, and later oven-dried at 65 °C for 48 h to calculate the ANPP and litter stocks. After clipping, BGB was determined by obtaining three replicate soil cores (7 cm in diameter) within the same quadrat. Soil samples were divided into three Sects. (0–10, 10–20 and 20–40 cm), and roots were extracted by sieving and washing the sampled soil cores through a 0.5-mm mesh sieve. The BGB dry weight was measured after drying at 65 °C for 48 h. In addition, when the plant biomass was harvested, five additional soil cores were taken from the top soil layer (0–10 cm) of each plot to measure the SOC (He et al. 2016).

## Data analysis

The response ratio (RR), nitrogen response efficiency (NRE, Rs response per unit N addition), and the difference in NRE between two adjacent N addition levels (ΔNRE) were used to determine the saturation threshold of Rs (Shcherbak et al. 2014; Tian et al. 2016a):

$$RR = \frac{\overline{x}_t - \overline{x}_c}{\overline{x}_c} \tag{1}$$

$$NRE = \frac{RR}{NR} \tag{2}$$

$$\Delta NRE1 = \frac{NRE2 - NRE1}{NR2 - NR1} \tag{3}$$

where $\overline{x}_t$ and $\overline{x}_c$ are mean values of the variables of interest, such as Rs and Rh, in the treatment and control plots, respectively, and NR is the N addition rate. The saturation threshold of Rs was defined as the N addition rate where the $\Delta$NRE was lower than zero. For example, when the $\Delta$NRE was lower than zero at 50 kg N ha$^{-1}$ (the difference in NRE between 50 and 100 kg N ha$^{-1}$), this meant that Rs became saturated with an N addition rate of 50 kg N ha$^{-1}$.

The relationship between environmental factors and soil respiration was investigated by ordinary linear regression (OLR) analysis, and the relationship between saturation threshold of soil respiration and precipitation/soil moisture was investigated by piecewise linear regression (pls) analysis (R-function "segmented"). Furthermore, we quantified the contributions of climate (precipitation, air temperature), soil factors (SOC, soil moisture, and soil temperature), and abiotic factors (ANPP, BGB, standing litter biomass) to the soil respiration variation using a variation partitioning analysis (R-function "varpart"). The impact of N addition on soil respiration was examined using linear mixed-effects models (R-function "lme"), in which, grassland type, N addition level and year were used as fixed effects, and the date, block and plot were used as random effects. A structural equation model (SEM) was employed to evaluate the hypothesized underlying causal relationships among variables following N addition (Wang et al. 2017) using the "piecewise-SEM" package in R. The SEM model was developed to relate the Rs to Rh, Ra, ANPP, BGB, LB, and SOC based on a priori hypotheses at the ecosystem level (Luo et al. 2006). We normalized all the variables across grassland types using Eq. (2), and the response ratio of each variable was used in the SEM. The model was assessed using Fisher's $C$ statistic, AIC values, and $P$-values. A model with lower AIC and higher $P$-values was chosen for use in the study.

All statistical analyses and graphs were prepared in R 3.5.1 (R Core Team 2018). Differences were considered to be statistically significant at $P \leq 0.05$.

## Results

### N-induced changes in the Rs saturation threshold and its components

Across all grassland types, soil respiration (Rs) had a distinct seasonal pattern with the highest value occurring in the summer (Fig. 3). Rs was higher in the alpine meadow (3.31 µmol $CO_2$ m$^{-2}$ s$^{-1}$) and meadow steppe (3.83 µmol $CO_2$ m$^{-2}$ s$^{-1}$) compared to the typical steppe (1.91 µmol $CO_2$ m$^{-2}$ s$^{-1}$) and desert steppe (1.18 µmol $CO_2$ m$^{-2}$ s$^{-1}$), even under N addition (Table 2 and Fig. 3). Along the N addition gradients in our coordinated experiments, Rs reached a maximum with the intermediate level N addition and declined with the highest N addition level (Fig. 4). During the initial stage, both the response ratio (RR) and nitrogen response efficiency (NRE) of the Rs significantly increased with N addition (Table 3). The $\Delta$NRE of the Rs was significantly lower than zero at the N addition rate of 50 kg N ha$^{-1}$ yr$^{-1}$ (Fig. 4g); thus Rs became saturated at an N addition rate of 50 kg N ha$^{-1}$ yr$^{-1}$ (Fig. 4a and d). Further analysis showed that the N saturation threshold of Rs differed among the four grassland types, with Rs becoming saturated at rates of 50, 50, 25, and 100 kg N ha$^{-1}$ yr$^{-1}$ in the meadow steppe, typical steppe, desert steppe, and alpine meadow, respectively (Fig. 4a). The NRE of the Rs peaked at a rate of 50, 50, 25, and 100 kg N ha$^{-1}$ yr$^{-1}$ in the meadow steppe, typical steppe, desert steppe, and alpine meadow, respectively. (Fig. 4d). Similarly, the $\Delta$NRE of the Rs was lower than zero at the N addition rate of 50 kg N ha$^{-1}$ yr$^{-1}$ in the meadow steppe, typical steppe, and alpine meadow, and a rate of 25 kg N ha$^{-1}$ yr$^{-1}$ in the desert steppe (Fig. 4g).

Heterotrophic respiration (Rh) significantly decreased after N addition with no saturation threshold, and the NRE of the Rh continuously increased along the N addition gradients across all grassland types (Fig. 4b and e). The autotrophic respiration (Ra) was saturated by N addition at the same N addition level as the Rs saturation threshold (on average at 50 kg N ha$^{-1}$ yr$^{-1}$; Fig. 4c). Again, similar to Rs, Ra
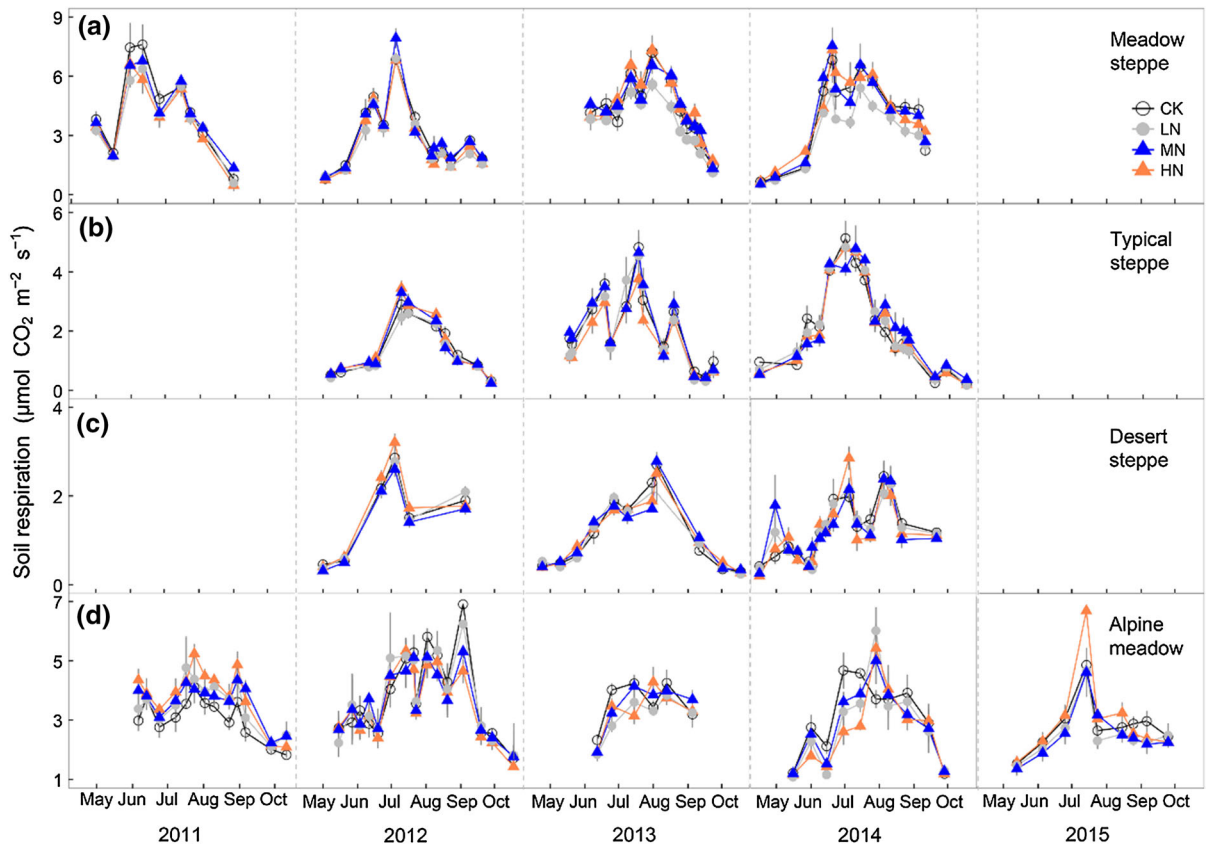
**Fig. 3** Seasonal variability of soil respiration in four grassland types. The four rows represent four different grassland types: Meadow steppe **a**, typical steppe **b**, desert steppe **c**, and alpine meadow **d**. The five columns represent years 2011 to 2015. The different symbols represent different nitrogen addition levels: CK, control; LN, MN, and HN: nitrogen levels at 25, 50, and 100 kg N ha$^{-1}$ year$^{-1}$, respectively

**Table 2** Effect of N addition on soil respiration across four grassland types based on a linear mixed-model analysis

| Fixed-terms | numDF | denDF | F-value | P-value |
|---|---|---|---|---|
| N | 3 | 1889 | 14.55 | < 0.001 |
| Grassland type | 3 | 77 | 285.8 | < 0.001 |
| Year | 3 | 166 | 0.29 | 0.59 |
| N*Grassland type | 9 | 77 | 9.55 | < 0.001 |
| N*Year | 9 | 1889 | 5.62 | < 0.001 |
| Grassland type*Year | 9 | 77 | 41.05 | < 0.001 |
| N*Grassland type*Year | 27 | 77 | 6.18 | < 0.001 |

Fixed terms were N addition, grassland type, and year, and random terms were date, block, and plot

peaked at the N addition rate of 50 kg N ha$^{-1}$ yr$^{-1}$ (Fig. 4f), and the ΔNRE of the Ra was significantly lower than zero at the N addition rate of 50 kg N ha$^{-1}$ yr$^{-1}$ (Fig. 4i). Thus, the N saturation threshold of Rs was consequent to having a Ra saturation threshold along the N addition gradients.

Effects of biotic and abiotic factors on Rs and its components

Aboveground net primary production, standing litter biomass (LB), belowground biomass (BGB), and soil organic carbon (SOC) were higher in the alpine meadow and meadow steppe than in the typical steppe
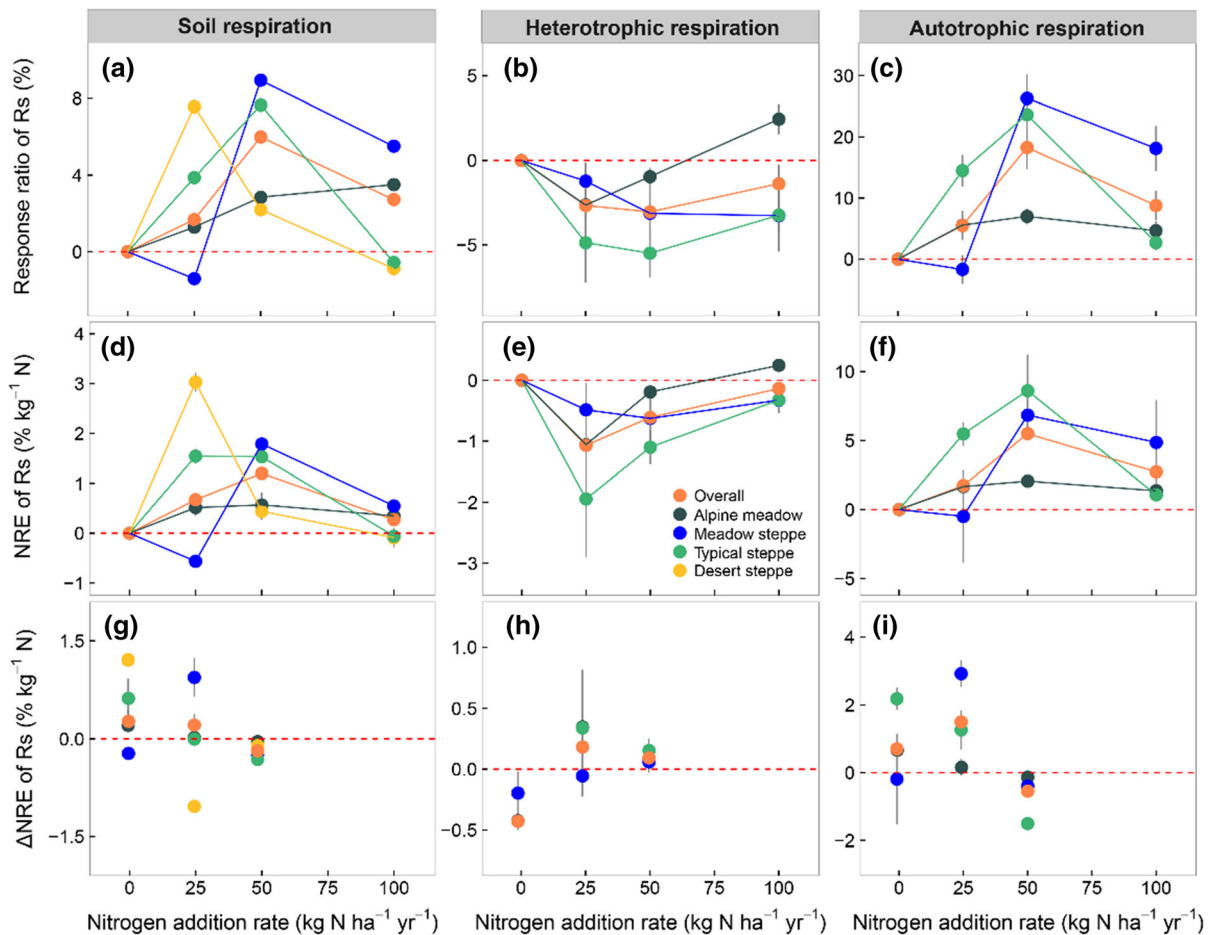
**Fig. 4** Changes in RR, NRE, and ΔNRE of Rs, Rh, and Ra along N addition gradients in each field experiment. The columns represent different respiration types, and the rows represent different indexes. For ΔNRE, if the difference in NRE was between 25 and 0 kg N ha$^{-1}$, the value was plot at 0 kg N ha$^{-1}$ along the x-axis and so on for the other addition levels. Each color represents a grassland type. Abbreviations: Overall, the average of respiration across four grassland types; Rs, soil respiration; Ra, autotrophic respiration; Rh, heterotrophic respiration; RR, response ratio; NRE, respiration response per unit N addition; ΔNRE, the difference in NRE between two adjacent N addition levels

and desert steppe (Table 2), and both of BGB and SOC showed positive correlations with precipitation and soil moisture (Fig. 5b). The results of the variation partitioning analysis showed that the amount of variation captured by all selected variables was 78% for the overall Rs (Fig. 5a). The effects of soil factors, abiotic factors, and climate accounted for 40%, 50%, and 76%, respectively. In addition, the joint effects of the three dominant factors accounted for 25% (Fig. 5a). Therefore, the variation in Rs across the four grassland types is primarily explained by the differences in climate.

The structural equation model revealed that the non-linear impact of N addition on Rs, with a saturation threshold at intermediate addition levels, was due to the different responses of its components along the N addition gradients. At the low level, N addition increased Rs through two pathways: (1) directly by increasing Ra, and (2) indirectly through N-induced changes in LB, which decreased the Rh (Fig. 6a). At the medium level, N addition increased Rs directly by increasing Ra (Fig. 6b). However, at the high level, N addition increased Rs indirectly by increasing BGB, which increased Ra, and subsequently increased Rs (Fig. 6c).
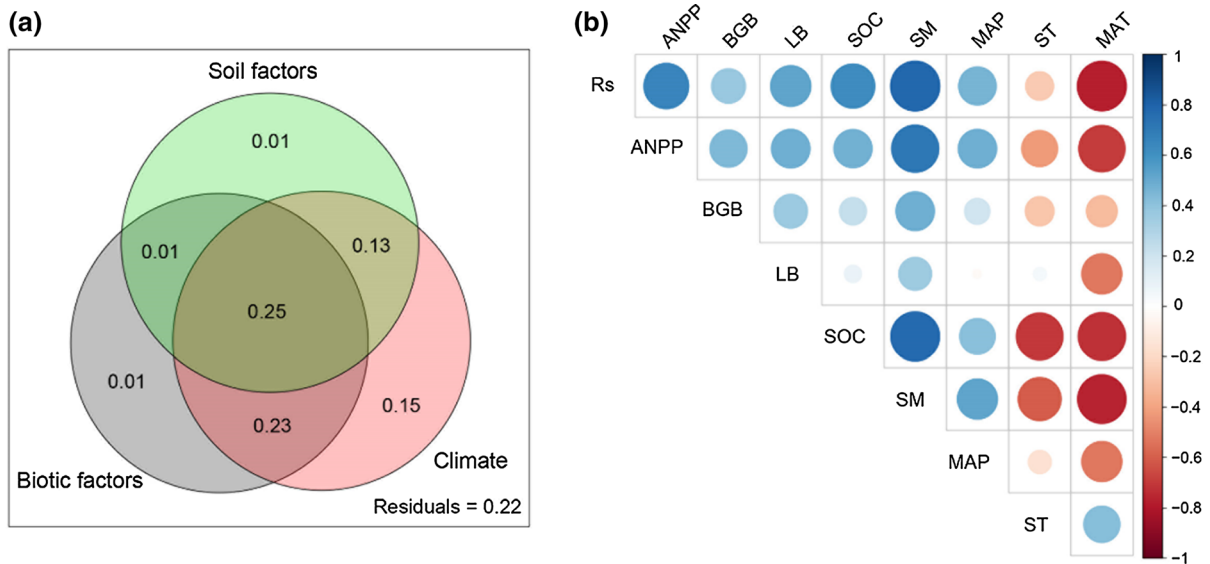
**(a)**



**(b)**



**Fig. 5** Results of variation partitioning for the dominant factors (e.g. soil factors, abiotic factors, and climate) regulating soil respiration across four grassland types **a**. Relationship between soil respiration and environmental factors across four grassland types **b**. The size of each circle represents the correlation coefficient of two parameters. Rs, soil respiration; ANPP, aboveground net primary production; BGB, belowground biomass; LB, standing litter biomass; SOC, soil organic carbon; ST, soil temperature at a in depth of 5 cm; SM, soil moisture at a depth of 10 cm; MAP, mean annual precipitation; MAT, mean annual temperature

### Environmental factors affecting the Rs saturation threshold

Precipitation and soil moisture were found to be important factors affecting the N saturation threshold of Rs among grassland types (Fig. 7). The N saturation threshold of Rs increased linearly with increasing precipitation up to a precipitation level of 465 mm. This was observed in all grassland types at the regional scale (Fig. 7a). A positive relationship was also found between the N saturation threshold of Rs and the soil moisture in grasslands (Fig. 7b).

### Discussion

Our study is among the first to provide strong evidence regarding the nitrogen saturation of soil respiration (Rs) along N addition gradients across grassland types. The Rs saturation threshold was observed at the N addition rate of 50 kg N ha$^{-1}$ year$^{-1}$, and was found to be primarily determined by the response of below-ground biomass (BGB).

### Nitrogen saturation threshold of Rs

The Rs saturation threshold under N deposition is difficult to examine across ecosystems due to its heterogeneous responses in space (Brook et al. 2013). In this study, using coordinated experiments in grasslands across northern China, we observed that Rs saturation in response to N addition gradients exists across the four grassland types studied, although the value varied between grasslands. Previous studies have found the N-induced increase in Rs to be highest at N addition rates of 40 and 80 kg N ha$^{-1}$ year$^{-1}$ in the typical and alpine steppes of China (Li et al. 2015; Peng et al. 2017), which is similar to the N saturation threshold of Rs determined in our study (50 kg N ha$^{-1}$ year$^{-1}$). Results from a meta-analysis also showed the response of Rs to N addition gradients to be highest at the addition rate of 50–100 kg N ha$^{-1}$ year$^{-1}$ across all grassland ecosystems (Zhou et al. 2014). This suggests showed that the saturation of Rs in response to N addition gradients is a general phenomenon in grassland ecosystems from a local scale to a global scale. A similar phenomenon has also been documented in croplands (Li et al. 2015; Zhai et al. 2016). However,
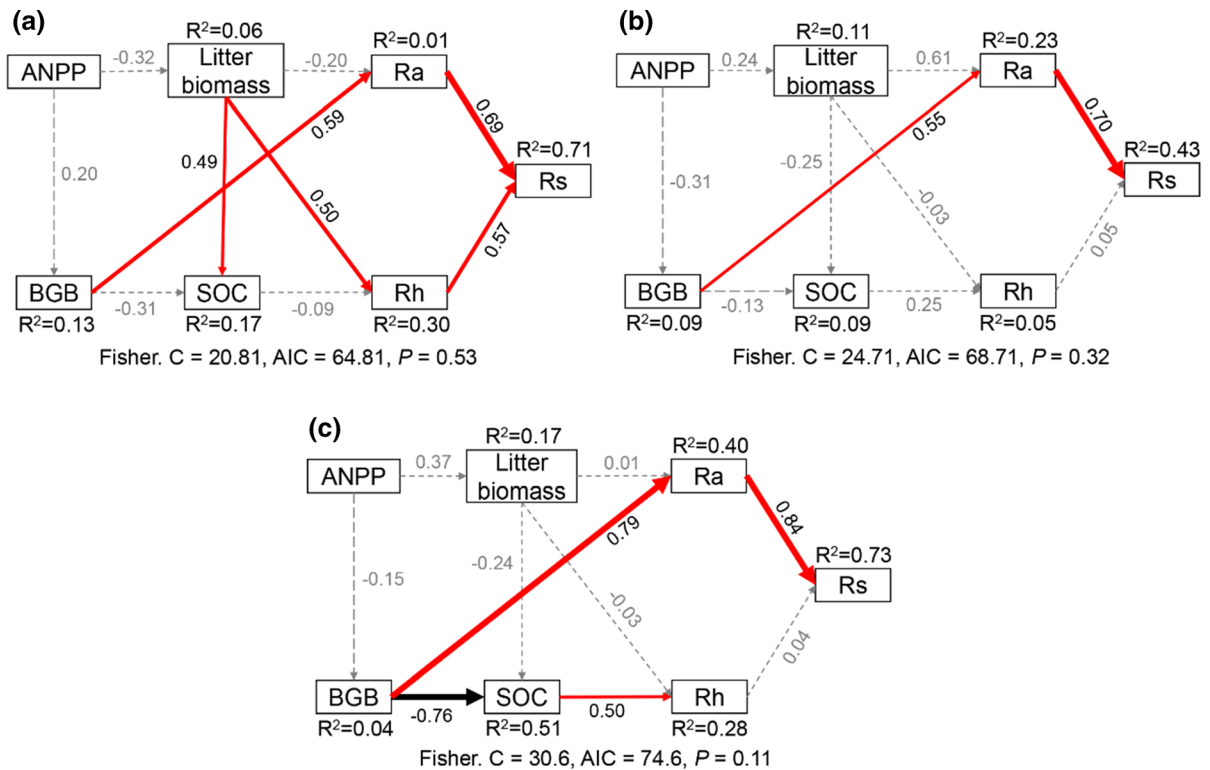
**Fig. 6** Final structural equation model (SEM) for the response ratio of Rs under low **a**, medium **b**, and high **c** N addition levels for the coordinated experiments. Measured exogenous and endogenous variables are represented by boxes. The numbers beside arrows are standardized path coefficients. The width of each arrow indicates the strength of the relationship with thicker arrows representing stronger relationships. Solid red arrows represent positive paths ($P < 0.05$), solid black arrows represent negative paths ($P < 0.05$), and dashed gray arrows represent non-significant paths ($P > 0.05$). Abbreviations: Rs, soil respiration; Rh, heterotrophic respiration; Ra, autotrophic respiration; ANPP, aboveground net primary production; BGB, belowground biomass; SOC, soil organic carbon

Rs often decreases following N addition in forests (Janssens et al. 2010), resulting in no Rs saturation threshold along N addition gradients.

Our results showed the Rs saturation threshold to coincide with the N saturation threshold of aboveground net primary production (ANPP) and BGB across grassland types, and the increase in ANPP and BGB gradually leveled off, even diminished, when the N addition rate was higher than 50 kg N ha$^{-1}$ yr$^{-1}$ (Fig. S3a and b). The increase in ANPP following N addition has been reported to plateau at rates of 175, 52.5, and 80 kg N ha$^{-1}$ yr$^{-1}$ in tall fescue grassland, alpine meadow, and typical steppe, respectively (Bai et al. 2010; Tian et al. 2015, 2016b). These data show that grassland ecosystems will become saturated with nitrogen in the future under continuous N deposition. The limiting resource for ecosystems may shift from nitrogen to water or phosphorus (Aber et al. 1998), and

the N-uptake and N-use efficiency of the ecosystem will decrease quickly (Liu et al. 2013). Therefore, a reduction in fossil fuels usage is urgently needed to prevent the N saturation of grassland ecosystems through N deposition.

**N saturation threshold of Rs determined by the non-linear response of BGB**

Autotrophic respiration (Ra) plays an important role in the Rs response to N addition gradients in grasslands. In our experiments N addition increased Rs by increasing Ra throughout the N addition gradients, which led to a consistent N saturation threshold for Ra along with Rs (Fig. 6). At the initial stage of N addition, ANPP increased quickly (Fig. S3a), which required more BGB to capture nutrient resources and water from the soil (Chapin 1980) and led to an
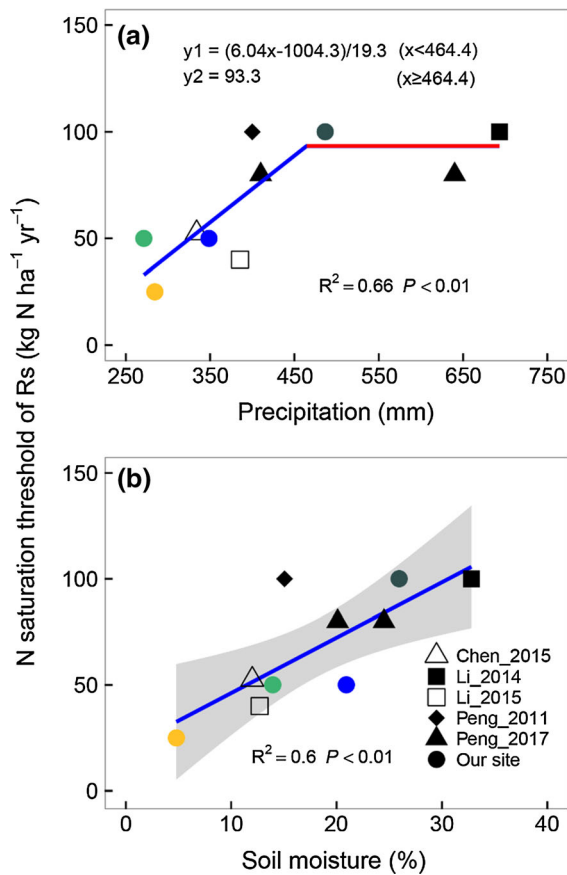
Fig. 7 Relationship of the N saturation threshold of Rs with precipitation (a) and soil moisture (b). Different shapes represent individual studies, colored circles represent the experimental field data of the coordinated study, and the gray point represents data from the meta-analysis. Abbreviations: Rs, soil respiration

increase in Ra. With continuous N addition, ecosystem production leveled off at the specific N addition rate, where the limiting resource for plants shifted from nitrogen to water or phosphorus (Aber et al. 1998). This resulted in no further increases, or decreases, in ANPP and BGB (Fig. S3a and b), but lead to a subsequent decrease in Ra. Moreover, when the N addition rate was higher than the N saturation threshold of plants, the enrichment of aluminum ions in the soil depressed the activity and biomass of roots (Tian et al. 2016b), which caused a significant decline in unit belowground biomass autotrophic respiration (Fig. S4). Thus, the response of Ra to N addition showed three distinct stages along N addition gradients, which led to a Ra saturation threshold. However, heterotrophic respiration (Rh) decreased along N addition gradients, which was different from the Ra response to N addition gradients. The N-induced decrease in standing litter biomass decreased the input of labile carbon from aboveground sources into the soil along N addition gradients (Fig. S3c; Knorr et al. 2005). Meanwhile, a previous study showed that N addition promoted the formation of large macro-aggregates in soil (Luo et al. 2018), thereby protecting soil organic carbon from microbial decomposition along N addition gradients (Riggs et al. 2015). In addition, the reduction of Rh was not caused by the soil acidification following N addition (Fig. S3d). Hence, Rh decreased due to a decrease in labile carbon along N addition gradients, leading to no saturation threshold for Rh. Consequently, the difference in the N

**Table 3** $F$-values of the effect of N addition on soil respiration in four grassland types based on a linear mixed-model analysis

| Year | Alpine meadow | Meadow steppe | Typical steppe | Desert steppe |
|---|---|---|---|---|
| 2011 | 12.3** | 4.68** | – | – |
| 2012 | 2.22[†] | 5.01** | 9.31** | 2.78* |
| 2013 | 2.57[†] | 12.19** | 5.81** | 1.29 |
| 2014 | 0.58 | 12.29** | 0.86 | 2.87* |
| 2015 | 6.41** | – | – | – |
| Overall | 4.33** | 23.11** | 2.59* | 0.61 |

The fixed term was N addition (numDF = 3) Random terms were date, block, and plot. Table entries are $F$-values with asterisks indicating significance levels (**$P < 0.01$; * $0.01 < P < 0.05$; [†] $0.05 < P < 0.1$)

The dash means that data are not available

saturation threshold of BGB caused the variation in the Ra, as well as the Rs, saturation threshold across grassland types.

## N saturation threshold of Rs modified by precipitation

Across grassland ecosystems, climate may affect the Rs saturation threshold resulting from N addition gradients (Yan et al. 2010; Tian et al. 2016a). Our results showed that the N saturation threshold of Rs was positively correlated with precipitation and soil moisture (Fig. 7), indicating that precipitation and soil moisture may be the crucial environmental factors affecting the N saturation threshold of Rs across grassland types. Precipitation or soil moisture is the main driver of BGB in grasslands at a regional scale (Ma et al. 2008; Yang et al. 2010; Tang et al. 2017). Increases in BGB have been reportedly followed by increases in the N uptake capacity of ecosystems (Aber et al. 1998), resulting in a higher N saturation threshold for BGB (Bai et al. 2010; Del Grosso et al. 2008; Tian et al. 2016a). In addition, the variation in the N saturation threshold of Rs among different grassland types was caused by differences in the N saturation threshold of BGB (Fig. 4 and Fig. S3b). Hence, precipitation/soil moisture influence the saturation threshold of Rs by altering the N saturation threshold of BGB. Moreover, in high precipitation grasslands, a considerable amount of nitrogen leaks from ecosystems (Hogberg et al. 2006; Harpole et al. 2007; Ren et al. 2017), which decreases the N-use efficiency, and thus the addition of more nitrogen is potentially needed to achieve the N saturation threshold of the ecosystems. Consequently, the positive correlation between the N saturation threshold of BGB and precipitation led to a positive relationship between the N saturation threshold of Rs and precipitation and soil moisture across grassland types.

The N saturation threshold of Rs increased linearly with increasing precipitation and soil moisture (Fig. 7), but it no longer increased when precipitation was higher than 465 mm (Fig. 7a). One reason potentially explaining the different influences of precipitation and soil moisture on the N saturation threshold of Rs is the positive relationship that exists between soil moisture and precipitation and the fact that surface runoff and soil infiltration increase exponentially with increasing precipitation (Bell

et al. 2010; Miao et al. 2017), which causes soil moisture to not increase monotonously with increasing precipitation. Hence, the N saturation threshold increased linearly with an increase in soil moisture, but only increased to some extent with an increase in precipitation.

## Conclusions

Our results from coordinated experiments in four grassland types across northern China help to elucidate the N saturation threshold of soil respiration and its components along N addition gradients. An average saturation threshold of soil respiration (Rs) in grasslands was determined at the N addition rate of $50 \text{ kg N ha}^{-1} \text{ yr}^{-1}$, at which point the Rs response shifted from being linear to non-linear with increasing N input, and the stimulation effects of N deposition on Rs were observed to diminish. This information may be useful for simulating and predicting the Rs response to different N deposition levels in the future. The N saturation threshold of Rs was determined by the non-linear response of belowground biomass, suggesting that the response of plants to N addition is an important indicator for the Rs saturation threshold. Moreover, the N saturation threshold of Rs increased with precipitation and soil moisture, which demonstrated that grassland ecosystems with higher precipitation may be able to bear more N deposition in the future.

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