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Title page

Title: Response of soil respiration to nitrogen addition along a degradation gradient in a temperate steppe of northern China

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Abstract

Although numerous studies have been conducted on the responses of soil respiration (*Rs*) to nitrogen (N) addition in grassland ecosystems, it remains unclear whether a nonlinear relationship between *Rs* and N addition exists and whether there is a uniform response across grasslands with different degradation status. We established a field experiment with six N treatments (0, 10, 20, 30, 40, and 50 g N m^{-2} y^{-1}) on four grassland sites, each with a varied degradation states in the Inner Mongolia steppe of northern China during the growing seasons of 2012 and 2013. *Rs* and its major influential factors, including aboveground biomass, root biomass, plant tissues carbon (C) and N concentrations, soil organic carbon (SOC) and soil total nitrogen (STN), microbial biomass and soil pH, were measured. Results show that N fertilization did not change the seasonal patterns of *Rs* but it changed the magnitude of *Rs* in grasslands with a different degradation status and only degradation had signification effects on *Rs*. This shows that variations of *Rs* in degraded grasslands were due to the difference in SOC content. The response of *Rs* to N addition differed with the severity of degradation. Furthermore, the response of *Rs* to N addition slowed down over time. The dominant factor controlling *Rs* changed across different degradation grasslands. The leading factors for *Rs* were SOC and STN in non-degraded and moderately degraded grassland; soil pH in severely degraded grassland; and aboveground biomass and root biomass in extremely degraded grassland. Our results highlight the importance of considering the degradation level of grassland to identify soil carbon emissions in grassland ecosystems, and N addition may alter the difference of soil carbon emissions in different degraded grasslands and change its soil carbon emissions pattern.

Keywords: nitrogen addition; soil respiration; soil organic carbon; degraded grassland

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1 Introduction

Soil respiration (*Rs)* consists mainly of microbial respiration and root respiration. As an important part of the underground carbon (C) cycle, *Rs* is a major process of C exchange between the atmosphere and soil, as well as a vital source of atmospheric carbon dioxide (CO_2) (Fang et al., 2001; Shao et al., 2014). Approximately 10% of the global annual atmospheric CO_2 release is derived from *Rs*, and the carbon emission from *Rs* is more than 10-fold that released from fossil fuel combustion (Bond-Lamberty and Thomson, 2010; IPCC, 2007; Silver, 2014). Consequently, a minor variation in the rate of *Rs* can result in a large change in the turnover rate of soil organic carbon (SOC), greatly altering atmospheric CO_2 concentrations (Riley et al., 2005). This minor variation, therefore, may have implications for the future global climate (Knops and Reinhart, 2000).

Grassland is the second largest area of green vegetation on land after forest. Unlike other ecosystem types, grassland has a large root system (Soussana et al., 2004), and approximately 90% of C is stored in the soil (Soussana et al., 2004). The major process of C cycling is completed in the soil (Sharrow and Ismail, 2004; Soussana et al., 2004). Hence, regulations and mechanisms of grassland *Rs* are crucial for evaluating the response of C release to global changes, which has significant effects on the assessment and prediction of global change, as well as the pattern of C cycling (Asner et al., 2004; Jia et al., 2013).

In the coming decades, an increasing amount of nitrogen (N) is predicted to enter grassland ecosystems due to the increase of atmospheric N deposition (Galloway et al., 2004; Galloway et al., 2008) and anthropogenic N fertilization (Field et al., 2014; Law, 2013). N addition will change soil nutrient conditions (Lu et al., 2013; Zhang et al., 2014), affecting plant growth (Nadelhoffer et al., 1999; Zong et al., 2013), plant tissue N content (Iversen et al., 2010; Li et al., 2015), microbial biomass (Compton et al., 2004; Frey et al., 2004), soil extracellular enzyme activity (Esch et al., 2013; Wang et al., 2014), soil

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physical and chemical properties such as soil pH (Janssens et al., 2010), soil organic carbon (SOC) and soil total nitrogen (STN) (He et al., 2013; Mueller et al., 2013). All of these factors will affect the magnitude of *Rs* by influencing microbial respiration (Ramirez et al., 2012) and root respiration (Vose and Ryan, 2002). Numerous studies have investigated the responses of *Rs* to N addition in forests (Fan et al., 2014; Hogberg, 2007; Li et al., 2014; Thomas et al., 2010). However, there are fewer studies on the grassland ecosystem, and these have commonly focused on Europe and North America (Jones et al., 2006; Li et al., 2013). Moreover, previous research has focused on the effects of hydrothermal factors (Jia et al., 2006; Luo et al., 2001), grazing (Cao et al., 2004), land-use change (Qi et al., 2007), and fire (Xu and Wan, 2008) on *Rs*, while reporting fewer details on the effect of N addition on *Rs*. Specifically, the effect of *Rs* to N addition in different degraded grasslands has been rarely reported (Peng et al., 2011).

The response of *Rs* to N addition may differ in grasslands with a different degradation status. On the one hand, degradation causes the death of aboveground biomass and root biomass (Cheng et al., 2007; Yan et al., 2006), which may reduce photosynthetic products from above- to below-ground and the substrate of *Rs*. With N addition, the increase of plant growth and photosynthetic products from above- to below-ground (Du et al., 2014) is inevitably influenced by the increase in the availability of N in the soil (Keuter et al., 2013; Ladwig et al., 2012), enhancing the substrate of *Rs*. Thus, the differences between non-degraded grassland (NDG) and degraded grasslands are likely to reduce following N addition and promote *Rs* rate by increasing the growth of aboveground plants. On the other hand, with the increase of N, the excess N can cause soil acidification (Yao et al., 2014), the inhibition of microbial respiration (Janssens and Luyssaert, 2009; Phillips and Fahey, 2007), plant root growth (Liu et al., 2013) and root respiration (Högberg et al., 2010) in non-degraded grassland. Therefore, *Rs* may have a nonlinear response to N addition, increasing at first and then declining in non-degraded grassland. *Rs* in severely degraded grassland may increase linearly with N addition. However, the responses of *Rs* to N addition in grasslands with a different degradation status are rarely studied.

In China, grassland is one of the most widespread vegetation types, occupying approximately 40% of the national total land area (Kang et al., 2007). Approximately 78% of the grasslands are in the northern temperate and semiarid areas (Chen and Wang, 2000). Severe climate conditions together with human activities cause most of the areas to suffer from desertification or degradation, and maintain N-deficient status (Cao et al., 2004; Hooper and Johnson, 1999; Zhang and Han, 2008). At present, 61.49% of northern grasslands in China have different degradation gradients (Zhou et al., 2014). However, it is unclear how increasing N availability affects the process of soil carbon emissions of grasslands with a different degradation status.

In this study, we conducted a controlled experiment on the Ulan Buton steppe at the southeastern edge of the Inner Mongolian Plateau, China, by selecting four experimental sites in different stages of degradation. Each site had six N-treatments to determine the response of *Rs* to N addition and the mechanisms involved. Specifically, we aimed to determine (1) how degradation affects grassland *Rs*; and (2) if the effects of N addition on the grassland *Rs* differ with degradation status. Our hypotheses were: (1) *Rs* would reduce with the degradation because of decreased plant biomass and photosynthetic product transport; and (2) *Rs* in grassland with a lower degradation level would increase at first and then decrease, while *Rs* with a higher degradation level would increase linearly as N is added; and finally, N addition would affect *Rs* mainly via the change in plant growth.

2. Materials and methods

2.1 Site description

The study was conducted on the Ulan Buton steppe, Inner Mongolian Plateau, China (Fig. 1).

Annual mean air temperature and precipitation are −1.4°C and 400 mm, respectively. The soil was classified as Chernozems, with sand and silt dominating its surface layer (Liu et al., 2008). Four 100 m × 100 m experimental fields were fenced on the flat land surface in 2011 after communication with local people about history of human disturbances at each site. The details about our study site can be found in Xu et al. (2015). The distances between these fields were no more than 10 km, which ensured that they shared similar climatic conditions (e.g., temperature and precipitation) and original vegetation types. In fact, among all vegetation and soil features, plant species composition and community structure can indicate the status of grassland degradation well. Liu et al. (2008) found that in this region, the herb species of grassland could be categorized into three groups: annuals (mainly appearing in the seriously degraded steppe), moderate grazing degradation indicators, and climax species in mature steppe. we followed the method in the study of Xu et.al. (2015) to quantify the grassland degradation level. Specifically, extremely degraded grassland had the highest proportion of annuals among the four fields and non-degraded grassland had the highest proportion of climax species, while the proportion of moderately grazing degradation indicators was high in the other two fields. The relative covers (ranging from 0 to 1) of climax species were: 0.34 in extremely degraded grassland (EDG), 0.40 in severely degraded grassland (SDG), 0.54 in moderately degraded grassland (MDG), and 0.74 in non-degraded grassland (NDG) (Xu et al., 2015).

The plant species composition is shown in Table 1. The EDG was open to local grazing and resulted in low species richness. The SDG was a high-pasture two decades ago, while it became degraded with overgrazing until 2011. The MDG was a pasture under managed grazing along with relatively low biomass. The NDG has been fenced for preventing grazing since 2000 and the species richness was high.

2.2 Experimental design

We divided each of the fields into three blocks, separated by a 2 m buffer zone. In each block, we selected 12 plots of 6 m × 6 m, separated by a 1 m buffer zone for different treatments. Each plot was further divided into four parts with observation, plant sampling, soil sampling, and *Rs* measurement areas (Fig. 2).

N addition began in May 2011, and urea was added as the fertilizer. There were six N addition amounts: 0 (CK, control check), 10, 20, 30, 40, and 50 g N m^{-2} y^{-1}. We followed the N-treatment design of Xu et al. (2015). N was applied four times in the first 10 days of May, June, July, and August using a quarter of the annual amount each time.

2.3 Soil respiration

Rs was measured using a Li-8100 soil CO_2 flux system (LI-COR Inc. Lincoln, NE, USA). Measurements were conducted at least once per month during the growing season (July–September) in 2012 and 2013. Every field had three experimental replications and there were two polyvinyl chloride (PVC) collars in each plot. The PVC collar (20 cm inner diameter, 6 cm height) was inserted 3 cm into the soil to measure *Rs*.

We used the single measured value of *Rs* as the average of the day. However, *Rs* obviously changes dynamically and the *Rs* measured at a different time of the day may result in a large bias. Based on previous studies on the *Rs* of grassland (Eler et al., 2013; Plestenjak et al., 2012) and field conditions, we selected the fine sunny days and measured *Rs* between 9:00 and 14:00 in the daytime to minimize the influence of the dynamic changes to *Rs*.

Rs in the growing season was obtained from the field data using linear extrapolation methods with following equation:

$$R = \sum(R_i \cdot \Delta t) \quad (1)$$

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where, R is the soil respiration during the growing season; R_i is the Rs at the measurement time in the growing season; and Δt is the measurement time interval (Gomez-Casanovas et al., 2013).

2.4 Sampling and measurements

2.4.1 Soil sampling

Soils were sampled from all plots in mid-August 2012 to a soil depth of 10 cm using a 5.8 cm diameter soil corer. The root, litter, and small stones were removed from the samples by hand and sieved with a 2 mm mesh sieve. The samplings were divided into two parts: fresh, 2 mm sieved soil was used to measure microbial biomass; and air-dried, 2 mm sieved soil was used to measure SOC, STN, and soil pH. All measurements were repeated independently in triplicate.

Microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) were measured using the chloroform fumigation extraction technique (Brookes et al., 1985; Vance et al., 1987). Briefly, two replicate samples were taken; one was fumigated with alcohol-free $CHCl_3$ for 24 h, while the other remained unfumigated. Fumigated and unfumigated samples were extracted using 0.5 mol L^{-1} K_2SO_4 (1:2.5 w/v) with agitation for 30 min. The extracts were analyzed for total dissolved C and N using a total C analyzer (TOC-500; Shimadzu, Kyoto, Japan). The microbial biomass was calculated as the difference in extractable C and N between the fumigated and unfumigated soils.

The soil pH value was determined using air-dried soil by a 1:5 soil:water ratio with a pH meter (Model PHS-2; INESA Instrument, Shanghai, China). The SOC and STN were measured by an element analyzer (Vario EL III, Elementar, Hanau, Germany).

2.4.2 Plant sampling

Aboveground and root biomass were sampled in the middle of August. Aboveground biomass was collected by clipping with a 50 cm × 50 cm sampling frame, dried, and weighed in each replicate plot.

Root biomass was collected from a soil depth of 30 cm using a 5.8 cm diameter soil corer with three repetitions. The roots were separated from the soil by washing, and then dried at 60°C for 48 h, and weighed. Root samples were ground and analyzed for total C and N using an element analyzer (Vario EL III, Elementar, Hanau, Germany).

2.5 Data analysis

All statistical analyses were performed using SPSS statistical software (SPSS 17.0 for Windows; SPSS Inc., Chicago, IL, USA). One-way analysis of variance (ANOVA) was performed to compare the differences of abiotic and biotic variables among different N addition treatments and degradation levels. Factorial ANOVA with Duncan's test was applied to identify independent and their interaction effects of degradation and N addition treatments on *Rs* and abiotic and biotic variables. Piecewise linear regression analysis was used to determine the relationship between *Rs* and pH. Simple linear regression was performed to determine the relationship between *Rs* and SOC, STN, MBC, MBN, root C and N concentrations, aboveground biomass, and root biomass. Significant effects were determined at $P < 0.05$, unless otherwise stated. Data were expressed as mean values ± S.E. (standard error).

3. Results

3.1 Seasonal dynamics of *Rs*

There was no significant difference in seasonal dynamics of *Rs* between non-degraded grassland (NDG) and grasslands with a varied degradation status, with the highest rates of *Rs* in June and the lowest in autumn for all treatments in both 2012 and 2013. Compared with degraded grassland, non-degraded grassland had a greater variation of *Rs* rate in the growing season (Fig. 3).

3.2 Response of *Rs* to different N addition gradients

Response of *Rs* to N addition differed with the severity of degradation, and the intensity of response

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slowed down with increasing time (Fig. 4). In 2012, the *Rs* of NDG, MDG, and SDG reached its maximum with an N addition amount of 20 or 30 g N m^{-2} y^{-1}, and then decreased. In EDG, *Rs* maintained an increasing trend although no significant difference was observed ($P > 0.05$). However, no significant effect of N addition on *Rs* was found in all treatments in 2013 ($P > 0.05$).

3.3 Difference of *Rs* in different degraded grasslands

The intensity of N addition changed the relative magnitude of *Rs* in grasslands with a varied degradation status (Fig. 5). Usually, *Rs* decreased with degradation without fertilization. With an increasing amount of N addition, the *Rs* of EDG increased to a similar magnitude as NDG. Moreover, the *Rs* of EDG was significantly higher than NDG in 2013 ($P < 0.05$). In addition, factorial ANOVA showed that degradation had significant effects on *Rs*, while N addition did not significantly affect *Rs*. Finally, no significant interaction between N addition and degradation was observed for *Rs* (Table 2).

3.4 Biotic and abiotic variables

Soil pH decreased significantly with the N addition treatment ($P < 0.05$, Table 3). No significant effect on SOC and root C concentration was found in all the N fertilization treatments ($P > 0.05$, Table 3). N addition did not significantly alter STN and root biomass, but changed those in SDG ($P > 0.05$, Table 3). The response of soil microbial biomass to N fertilization differed with the severity of degradation (Table 3). Except for MDG, the variation of root N concentration did not reach a significant level under N addition ($P > 0.05$, Table 3). Furthermore, the effects of N fertilization on aboveground biomass were greater than its effect on *Rs*, and there was no significant difference with degradation levels (Fig. 6).

Factorial ANOVA (Table S1) showed expect root N concentrations and belowground biomass, degradation significantly affected nearly all abiotic and biotic factors. N addition did not significantly affect SOC, STN, root C concentrations and belowground biomass, but significantly affect MBC, MBN, root N concentrations, aboveground biomass and soil pH value. In addition, there was a significantly interaction effect between N fertilization and degradation on MBC, MBN and aboveground biomass.

Correlations between *Rs* and abiotic and biotic factors varied in grasslands with different degradation statuses. Specifically, there was a significant linear relationship between *Rs* and SOC, STN in NDG and MDG (Figs. S1 and S2, $P < 0.05$); while *Rs* and soil pH were significantly correlated in SDG (Fig. S3, $P < 0.05$). Piecewise linear regression showed that *Rs* reached its maximum at a pH value of 6.28 and then decreased with the increase of pH. In EDG, a significant linear correlation was found between *Rs* and vegetation factors, including aboveground biomass and root biomass (Fig. S4, $P < 0.05$).

4 Discussion

Previous studies have reported that short-term N addition increased soil CO_2 fluxes (Bowden et al., 2004; Fang et al., 2012). Our results showed that *Rs* responded non-linearly to short-term N fertilization. *Rs* reached its maximum with an N addition amount of 20 or 30 g N m^{-2} y^{-1} from NDG to SDG and then decreased, and *Rs* was inhibited at the higher-N treatments. The initial increase at lower-N treatments may be due to the reduced soil C:N ratio from increased N availability, which therefore accelerated the decomposition of SOM (Gundersen, 1998). However, we did not find that the soil C:N ratio decreased significantly due to N addition (Fig. S5). In addition, the increased plant biomass with fertilization may account for the initial increase of *Rs*. Previous research has demonstrated that our study area is an N-limited ecosystem (Xu et al., 2015) and degradation deteriorates N deficiency. N addition would increase the availability of N in soil, promoting plant growth, and resulting in increased photosynthetic products transported from above- to below-ground (Du et al., 2014). Consequently, plant C may prime the growth and activity of mycorrhizal fungi (Craine et al., 2007) and rhizospheric microbes (Högberg et al., 2010), thus increasing *Rs*.

Rs reduced from NDG to SDG at high N amounts, potentially due to the saturation phenomenon in these three fields. When N addition surpassed its saturation point, the increase of plant growth slowed, and photosynthetic products from aboveground decreased. As a result, the deficiency of carbon for microbial decomposition will affect microbial growth (Table 3). Thus, higher N addition will instead reduce *Rs*. Furthermore, we found that the response of plant growth to N fertilization was greater than the impact of N fertilization on *Rs* (Fig. 6). With an increasing amount of N, the proportion of N fertilization to promote plant growth slowed down (Fig. S6). In other words, under high N treatment, aboveground biomass tends to have a lower increase than that under low N treatment. We therefore conclude that the effect of *Rs* on N addition is mainly due to the variation of plant growth by N fertilization.

We also found that the dominant factor influencing *Rs* changed with the severity of degradation. In NDG and MDG, SOC and STN were the dominant factors influencing *Rs*, which was consistent with the result of Bazzaz and Williams (1991). However, other studies have reported that no significant relationship was found between soil organic matter (SOM) and *Rs* (Zhang et al., 2009). In SDG, soil pH became the dominant factor. Numerous studies have shown that there is a significant positive correlation between *Rs* and soil pH (Bowden et al., 2004; Phillips and Fahey, 2007; Vanhala, 2002). In our study, there was a threshold of 6.28 in the soil pH value. Specifically, a positive correlation occurred before and a negative correlation after the threshold was reached. This suggests that *Rs* requires a suitable soil pH. Xie et al. (2009) also found that higher soil pH inhibits *Rs*. In EDG, the dominant factor changed to aboveground and root biomass, which was consistent with the results of Wardle et al. (2004). The reason for the change of the dominant factor in different degraded grasslands may be because SOC and STN are the substrates of *Rs* at the low degradation level, and they mainly determine the magnitude of *Rs* (Bazzaz and Williams, 1991). With N addition, microorganisms accelerate decomposition due to the increased availability of substrate N. When a grassland is severely degraded, due to the lack of soil nutrients, *Rs* may be mainly dependent on the plant growth, including above- and belowground biomass, to supply the substrate for rhizospheric and microbial respiration (Wardle et al., 2004).

In addition, we found that the response of *Rs* to N fertilization slowed down with time. In 2012, *Rs* increased, and then decreased with N addition in NDG, MDG, and SDG. However, in EDG, *Rs* was not significantly elevated by the N addition. Variations of *Rs* responding to N addition did not reach significant levels in all fields following the second year. Firstly, this is partly because after the first year of fertilization, much of the available soil C had been consumed, and thus large amounts of CO_2 were emitted from the soil. Meanwhile, due to low precipitation, high evaporation, and wind erosion in our study area, the accumulation of SOC was relatively slow. Thus, if the external C input could not replenish the consumable soil C, the previous N-limited status may have become a limitation of C resources. Former study observed that microbial respiration was rarely affected by N addition in the C-limited status (Micks et al., 2004). Borton et al. (2004) also found that N addition did not affect root biomass and root respiration, and *Rs* was influenced by the limitation of other nutrient resources. With changes to limiting nutrient resources, the response of *Rs* to N addition would become weaker (Peng et al., 2011). Consequently, the response of *Rs* to N addition is a result of multiple factors other than N availability alone. Secondly, due to the disturbance history of grazing in our degraded grasslands, the experiment itself (for which plot were fenced) would be interfere with the disturbance, causing confounding influences of short-term recovery and N addition. The decreased temporal response to N addition may also partly arise from the reduced/remove disturbance element in degraded grasslands. Lastly, it is

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noteworthy that our study only measured total *Rs*, however, N addition have had a different effect on its heterotrophic and autotrophic components. Therefore, we need to separate soil autotrophic and heterotrophic components in future research to better understand *Rs* variation with degradation and N fertilization.

5. Conclusions

The response of *Rs* to N addition differed in grasslands with different degradation levels, which was related to soil nutrient status before N fertilization. With N addition, *Rs* increased at first and then decreased from NDG to SDG; whereas there was a linear increase in EDG. The response of *Rs* to N addition in degraded grassland was consistent with aboveground biomass, emphasizing the close association of above- and belowground C processes. Furthermore, effect of N fertilization on *Rs* slowed down with time. This suggests the importance of substrate quantity for *Rs*. Finally, the response of *Rs* to N addition differed with the severity of degradation, emphasizing that the degree of degradation is a key factor to consider when assessing grassland ecosystem soil carbon emissions.

Author contributions

W.W. designed the experiment, J.C. conducted the experiment and wrote the main manuscript text as well as prepared the figures. X.X., H.L. and W.W. revised the first drafts. All authors reviewed the manuscript.

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Zong, N., Shi, P. L., Jiang, J., Song, M. H., Xiong, D. P., Ma, W. L., Fu, G., Zhang, X. Z., and Shen, Z. X.: Responses of ecosystem CO_2 fluxes to short-term experimental warming and nitrogen enrichment in an alpine meadow, Northern Tibet Plateau, Sci World J, 2013.

483 Table 1. Species composition in different degraded grasslands

Degradation degree	Species composition	Dominant species
Extremely degraded grassland	*Carex regescens*、*Leymus chinensis*、*Setaria viridis*	*L. chinensis*
Moderately degraded grassland	*C. regescens*、*L. chinensis*、*Potentilla longifolia*、*Poa sphondylodes* 、*Stipa baicalensis*	*C regescens*、*L. chinensis*、
Non-degraded grassland	*Bromus japonicas*、*Bromus inermis*、*Bupleurum chinense*、*C. regescens*、*Cleistogenes squarrosa*、*L. chinensis*、*P. sphondylodes*、*Sanguisorba officinalis*、*Vicia sepium*	*S officinalis*、*V sepium*
Severely degraded grassland	*Artemisia capillaris*、*Artemisia frigida*、*B. japonicas*、*C. regescens*、*C. squarrosa*、*L. chinensis Potentilla acaulis*、*P. longifolia*、*P. sphondylodes*、*S. baicalensis*、	*C. regescens*、*C. squarrosa*、*L. chinensis*

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Table 2. ANOVA of the effects of nitrogen fertilization and degradation degree on soil respiration

Term	Df	F- Value	P -Value
N-treatment (N)	5	0.711	0.618
Degradation status (D)	3	9.123	< 0.001
N×D	15	0.44	0.956

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Table 3. Effects of nitrogen (N) addition on soil respiration, microbial biomass carbon, microbial biomass nitrogen, and soil organic carbon (SOC), soil total nitrogen (STN), root C and N concentration, soil pH, and above- and belowground biomass. Data are expressed as mean value ± S.E. (Standard Error). Different letters in the same row indicate significant differences among N treatments, while different capital letters in the same column indicate significant differences among degradation levels at 0.05 level of P-value. DG = desertification grassland, SDG = severely degraded grassland, MDG = moderately degraded grassland, NDG = non-degraded grassland.

Item	Degradation	Nitrogen fertilization (g N m^{-2} y^{-1})					
	degree	0	10	20	30	40	50
Soil microbial biomass carbon (mg kg^{-1})	NDG	33.36±2.32$^{a\,A}$	32.81±10.01$^{a\,A}$	97.63±33.53$^{b\,A}$	27.66±5.23$^{a\,A}$	61.14±1.11$^{a\,A}$	39.49±0.21$^{a\,A}$
	MDG	14.24±6.33$^{a\,B}$	38.22±17.59$^{a\,A}$	19.14±1.36$^{a\,B}$	13.20±2.60$^{a\,BC}$	16.51±8.14$^{a\,B}$	22.99±8.44$^{a\,AB}$
	SDG	9.03±0.97$^{a\,B}$	7.49±1.38$^{a\,A}$	8.15±3.29$^{a\,B}$	3.73±2.21$^{a\,C}$	7.37±4.52$^{a\,B}$	12.44±2.81$^{a\,B}$
	EDG	13.80±2.19$^{a\,B}$	27.71±3.37$^{a\,A}$	18.74±1.81$^{a\,B}$	20.16±3.67$^{a\,AB}$	56.42±20.29$^{b\,A}$	28.83±4.16$^{a\,AB}$
Soil microbial biomass nitrogen (mg kg^{-1})	NDG	3.84±0.32$^{a\,A}$	7.73±2.63$^{a\,A}$	10.30±2.75$^{a\,A}$	34.27±13.52$^{a\,A}$	15.27±4.11$^{a\,AB}$	21.50±2.43$^{a\,A}$
	MDG	2.16±0.24$^{a\,A}$	2.40±1.71$^{a\,A}$	8.81±2.42$^{ab\,A}$	16.98±3.76$^{ab\,AB}$	13.74±4.01$^{ab\,AB}$	21.15±8.45$^{b\,A}$
	SDG	5.02±1.04$^{a\,A}$	2.65±0.41$^{a\,A}$	3.38±0.26$^{a\,A}$	4.21±0.44$^{a\,B}$	3.61±2.08$^{a\,B}$	2.76±0.97$^{a\,A}$
	EDG	3.00±1.29$^{a\,A}$	6.10±2.81$^{a\,A}$	11.51±5.62$^{a\,A}$	4.98±1.09$^{a\,B}$	63.82±31.99$^{b\,A}$	22.61±12.60$^{ab\,A}$
Soil organic carbon content (%)	NDG	4.49±0.11$^{a\,A}$	5.36±1.66$^{a\,A}$	5.17±0.38$^{a\,A}$	5.43±0.63$^{a\,A}$	4.44±1.23$^{a\,A}$	4.79±0.75$^{a\,A}$
	MDG	0.87±0.18$^{a\,C}$	0.86±0.15$^{a\,B}$	1.06±0.08$^{a\,B}$	0.75±0.12$^{a\,B}$	0.62±0.26$^{a\,B}$	0.89±0.15$^{a\,B}$
	SDG	1.37±0.21$^{a\,B}$	1.75±0.30$^{a\,B}$	1.26±0.21$^{a\,B}$	1.02±0.13$^{a\,B}$	1.13±0.08$^{a\,B}$	0.88±0.06$^{a\,B}$
	EDG	0.18±0.05$^{a\,D}$	0.27±0.17$^{a\,B}$	0.13±0.03$^{a\,B}$	0.12±0.05$^{a\,B}$	0.24±0.04$^{a\,B}$	0.14±0.03$^{a\,B}$
Soil total nitrogen content (%)	NDG	0.37±0.01$^{a\,A}$	0.43±0.14$^{a\,A}$	0.41±0.04$^{a\,A}$	0.44±0.05$^{a\,A}$	0.35±0.10$^{a\,A}$	0.38± 0.06$^{a\,A}$
	MDG	0.07±0.02$^{a\,B}$	0.08±0.02$^{a\,B}$	0.09±0.01$^{a\,B}$	0.08±0.01$^{a\,B}$	0.06±0.03$^{a\,B}$	0.09±0.01$^{a\,B}$
	SDG	0.11±0.01$^{ab\,B}$	0.14±0.02$^{b\,B}$	0.10±0.01$^{a\,B}$	0.10±0.01$^{a\,B}$	0.10±0.01$^{a\,B}$	0.07±0.01$^{a\,B}$
	EDG	0.02±0.01$^{a\,C}$	0.03±0.01$^{a\,B}$	0.02±0.01$^{a\,B}$	0.02±0.01$^{a\,B}$	0.02±0.01$^{a\,B}$	0.02±0.01$^{a\,B}$
root C concentration (%)	NDG	21.89±4.81$^{a\,A}$	29.39±5.18$^{a\,A}$	20.87±4.88$^{a\,A}$	18.10±1.67$^{a\,A}$	21.46±3.63$^{a\,A}$	17.65±1.35$^{a\,A}$
	MDG	19.88±2.99$^{a\,A}$	22.98±2.30$^{a\,AB}$	18.44±2.16$^{a\,A}$	18.82±4.49$^{a\,A}$	18.20±1.79$^{a\,A}$	24.82±4.00$^{a\,A}$
	SDG	20.90±3.85$^{a\,A}$	22.91±3.29$^{a\,AB}$	18.70±0.63$^{a\,A}$	27.81±3.27$^{a\,A}$	25.48±0.45$^{a\,A}$	25.53±4.75$^{a\,A}$
	EDG	17.25±3.66$^{a\,A}$	12.35±3.04$^{a\,B}$	16.95±4.81$^{a\,A}$	20.53±5.82$^{a\,A}$	21.43±6.76$^{a\,A}$	14.24±2.23$^{a\,A}$
root N concentration (%)	NDG	0.58±0.14$^{a\,A}$	0.82±0.17$^{a\,A}$	0.81±0.19$^{a\,A}$	0.78±0.23$^{a\,A}$	1.05±0.32$^{a\,A}$	0.76±0.13$^{a\,A}$
	MDG	0.45±0.04$^{a\,A}$	0.80±0.06$^{b\,A}$	0.63±0.08$^{ab\,A}$	0.62±0.13$^{ab\,A}$	0.63±0.07$^{ab\,A}$	0.84±0.05$^{b\,A}$
	SDG	0.55±0.09$^{a\,A}$	0.67±0.08$^{a\,A}$	0.53±0.03$^{a\,A}$	0.73±0.07$^{a\,A}$	0.75±0.02$^{a\,A}$	0.79±0.16$^{a\,A}$

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	EDG	$0.38\pm0.06^{a\ A}$	$0.49\pm0.17^{a\ A}$	$0.58\pm0.12^{a\ A}$	$0.77\pm0.19^{a\ A}$	$1.09\pm0.28^{a\ A}$	$0.74\pm0.20^{a\ A}$
pH	NDG	$6.13\pm0.21^{ab\ A}$	$6.19\pm0.06^{b\ A}$	$5.78\pm0.20^{ab\ A}$	$5.82\pm0.19^{ab\ A}$	$5.58\pm0.15^{a\ A}$	$5.72\pm0.13^{ab\ A}$
	MDG	$6.55\pm0.05^{c\ AB}$	$6.30\pm0.07^{bc\ AB}$	$6.40\pm0.13^{bc\ B}$	$6.33\pm0.12^{bc\ A}$	$6.18\pm0.15^{b\ A}$	$5.81\pm0.11^{a\ A}$
	SDG	$6.61\pm0.10^{c\ B}$	$6.53\pm0.06^{c\ B}$	$6.35\pm0.06^{bc\ B}$	$6.16\pm0.19^{ab\ A}$	$5.99\pm0.11^{a\ A}$	$5.93\pm0.02^{a\ A}$
	EDG	$6.58\pm0.09^{c\ B}$	$6.38\pm0.15^{c\ AB}$	$6.17\pm0.10^{bc\ AB}$	$6.17\pm0.02^{bc\ A}$	$5.70\pm0.34^{ab\ A}$	$5.32\pm0.17^{a\ B}$
Belowground biomass ($g\ m^{-2}$)	NDG	$1146.14\pm386.9^{a\ A}$	$1022.44\pm567.9^{a\ A}$	$802.81\pm291.07^{a\ A}$	$525.11\pm178.91^{a\ A}$	$405.19\pm15.30^{a\ A}$	$355.96\pm57.97^{a\ A}$
	MDG	$720.76\pm222.86^{a\ A}$	$974.47\pm566.11^{a\ A}$	$578.12\pm156.22^{a\ A}$	$724.54\pm107.32^{a\ A}$	$922.72\pm254.09^{a\ A}$	$1317.81\pm174.04^{a\ B}$
	SDG	$1048.95\pm200.8^{ab\ A}$	$850.77\pm430.53^{a\ A}$	$592.01\pm89.78^{a\ A}$	$830.58\pm176.14^{a\ A}$	$1664.94\pm154.9^{b\ B}$	$600.84\pm172.83^{a\ A}$
	EDG	$424.12\pm184.33^{a\ A}$	$419.07\pm203.18^{a\ A}$	$661.43\pm255.91^{a\ A}$	$521.32\pm185.60^{a\ A}$	$859.61\pm272.55^{a\ A}$	$977.0\pm324.5^{a\ AB}$
Aboveground biomass($g\ m^{-2}$)	NDG	$498.13\pm32.47^{a\ A}$	$713.33\pm151.80^{a\ A}$	$768.00\pm90.19^{a\ A}$	$819.87\pm126.07^{a\ A}$	$552.80\pm72.05^{a\ A}$	$549.73\pm93.07^{a\ A}$
	MDG	$236.93\pm13.69^{a\ B}$	$248.26\pm21.82^{a\ B}$	$241.60\pm28.11^{a\ B}$	$272.40\pm32.31^{a\ B}$	$287.60\pm34.11^{a\ A}$	$258.40\pm15.37^{a\ A}$
	SDG	$212.00\pm62.01^{a\ B}$	$357.07\pm19.54^{b\ B}$	$363.20\pm30.32^{b\ B}$	$416.53\pm27.72^{bc\ B}$	$391.47\pm45.13^{bc\ A}$	$499.20\pm34.58^{c\ A}$
	EDG	$295.87\pm59.04^{a\ B}$	$233.60\pm84.20^{a\ B}$	$414.27\pm28.54^{a\ B}$	$354.40\pm23.63^{a\ B}$	$505.33\pm36.46^{a\ A}$	$391.87\pm94.77^{a\ A}$

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Figure legends

Fig. 1. Locations of the study area and grassland.

Fig. 2. Experimental setup of control and fertilized treatments in different degraded grasslands. 10 - 50 represents 10, 20, 30, 40, and 50 g N m^{-2} y^{-1}, respectively. A, B, and C indicate the three replicates.

Fig. 3. Soil respiration rate of degraded grasslands in control (red circle), 10 g N m^{-2} y^{-1} (green circle), 20 g N m^{-2} y^{-1} (yellow triangle), 30 g N m^{-2} y^{-1} (white triangle), 40 g N m^{-2} y^{-1} (black square), and 50 g N m^{-2} y^{-1} (blue square) fertilized plots during the growing seasons of 2012 and 2013.

Fig. 4. Comparison of growing season soil respiration between the control and fertilized treatments in 2012 and 2013. Significant differences between N treatments are indicated by different letters.

Fig. 5. Comparison of growing-season soil respiration between different degraded grasslands in 2012 (yellow) and 2013 (green). Significant differences between degradation levels are indicated by different letters. EDG = extremely degraded grassland, SDG = severely degraded grassland, MDG = moderately degraded grassland, NDG = non-degraded grassland.

Fig. 6. Relationship between the proportion of change of total aboveground biomass and the proportion of change of growing season soil respiration. EDG = extremely degraded grassland, SDG = severely degraded grassland, MDG = moderately degraded grassland, NDG = non-degraded grassland.

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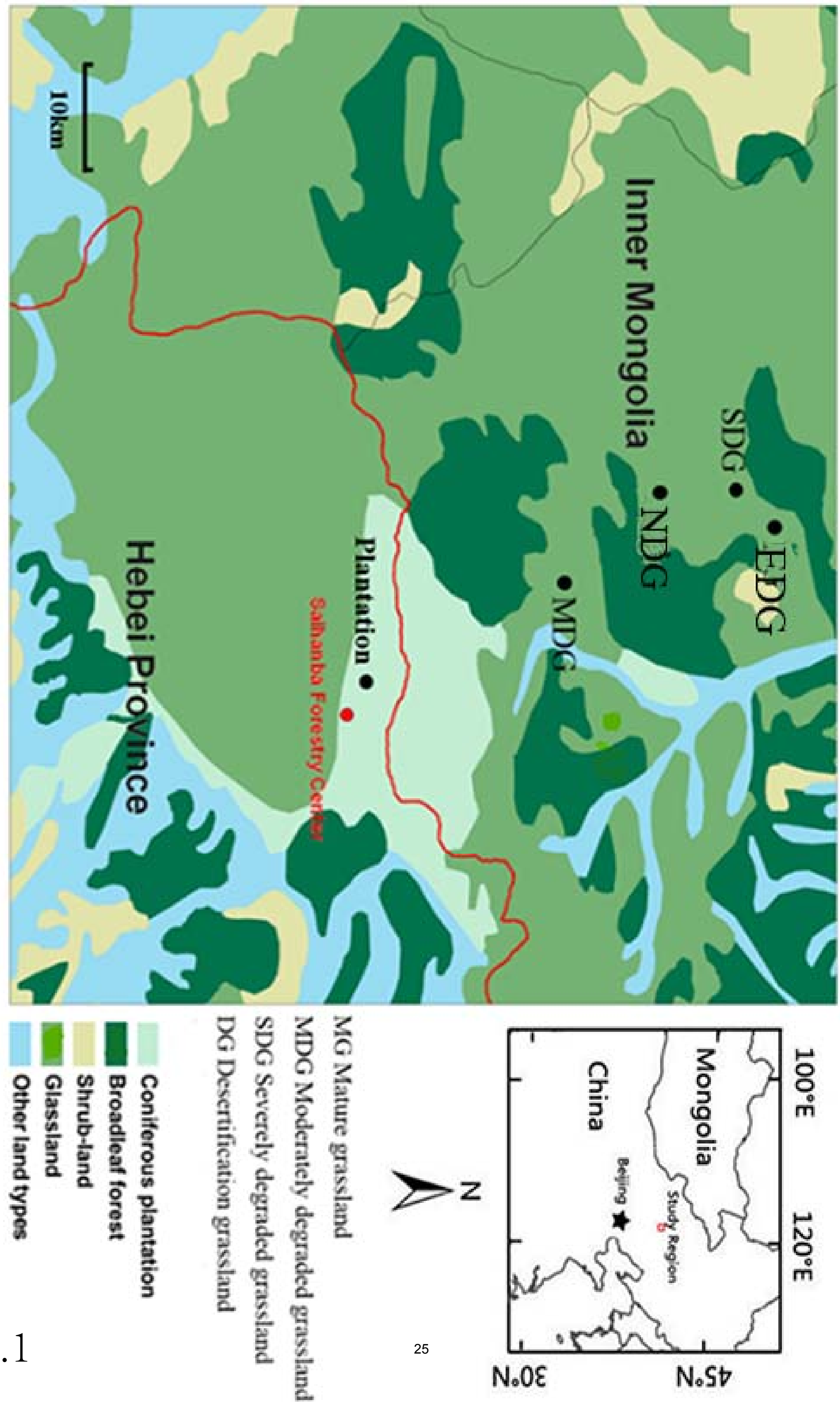

Fig.1

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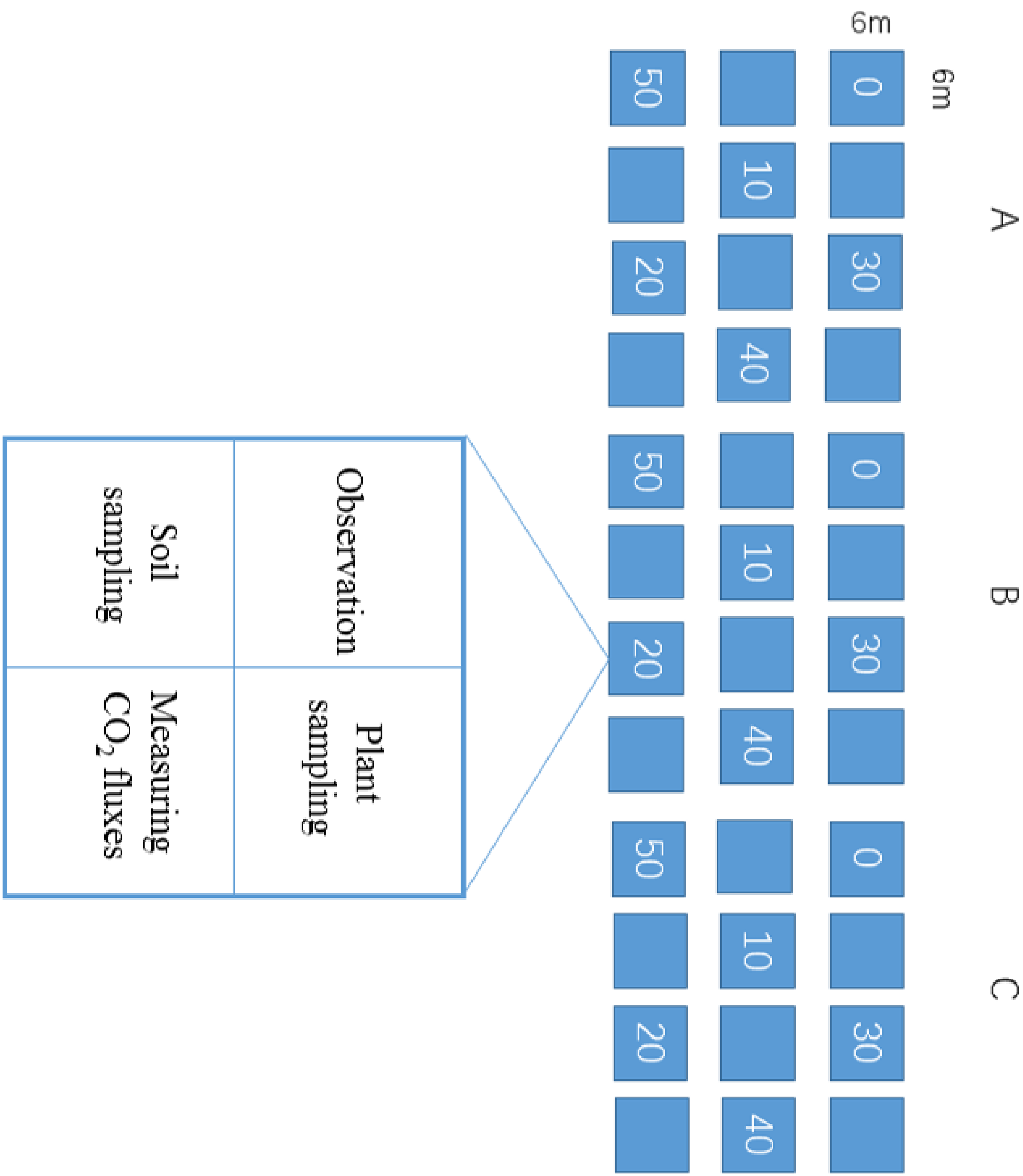

Fig.2

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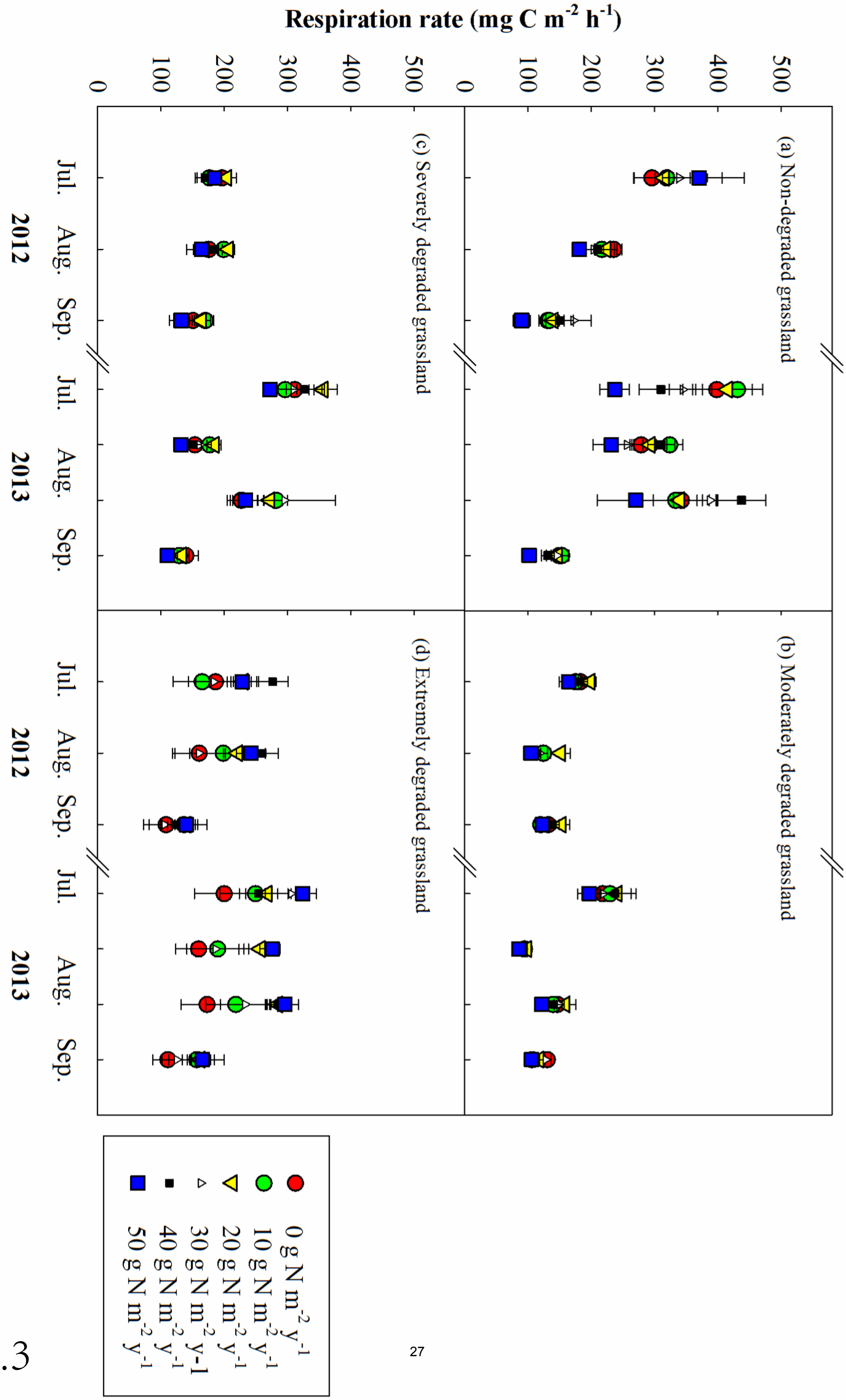

Fig.3

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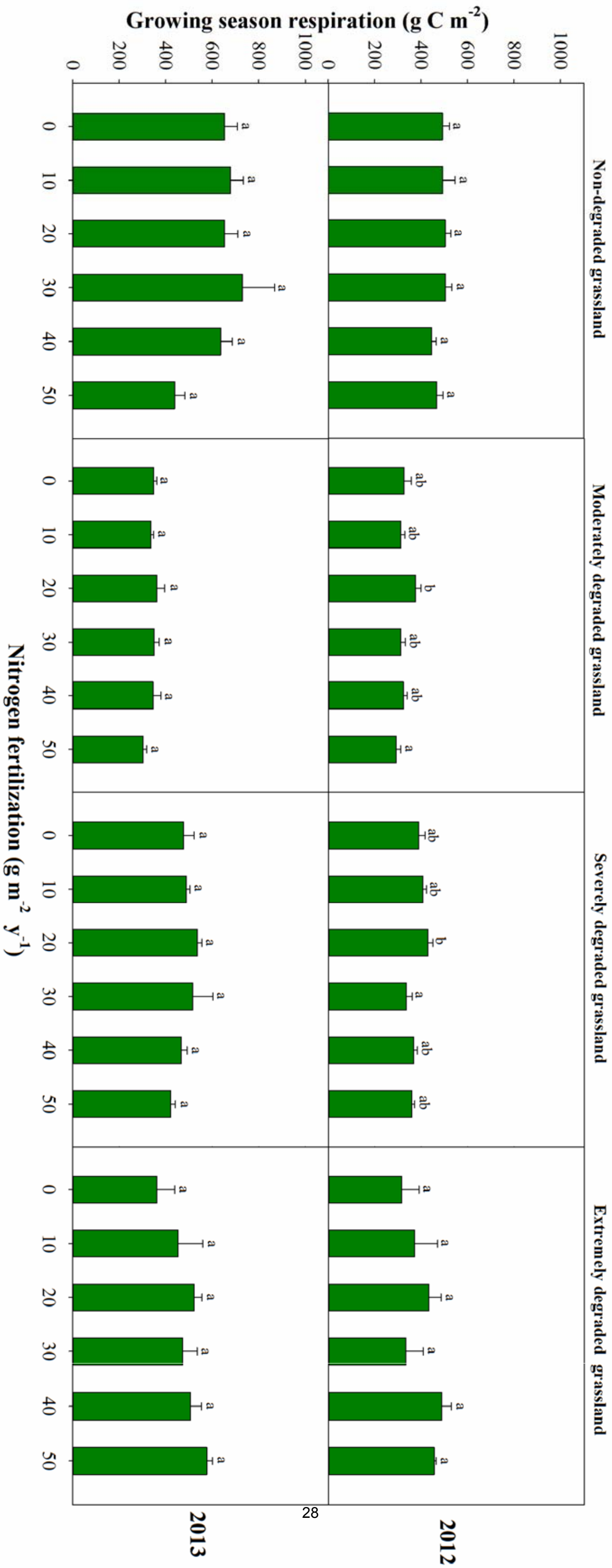

Fig.4

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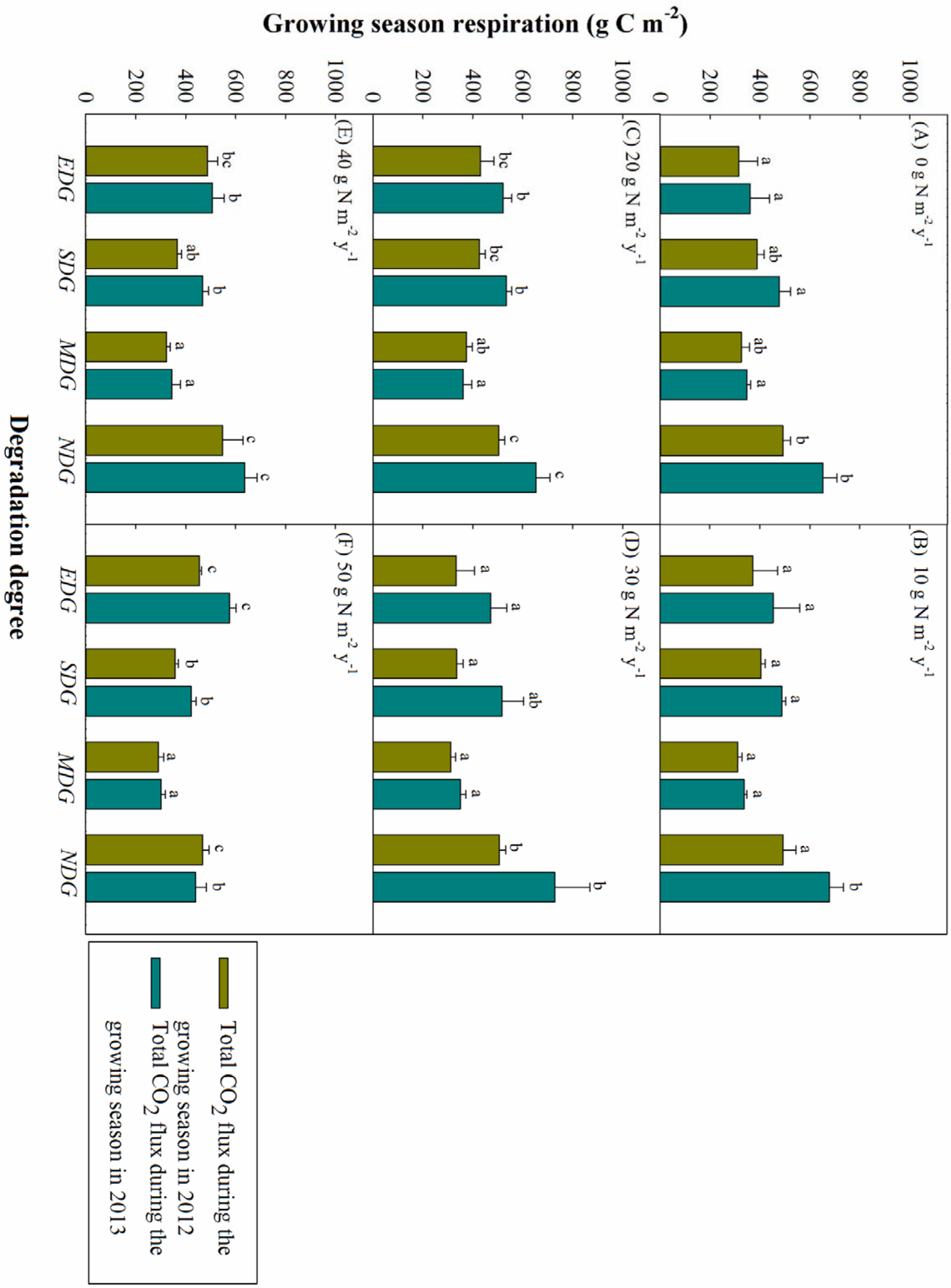

Fig.5

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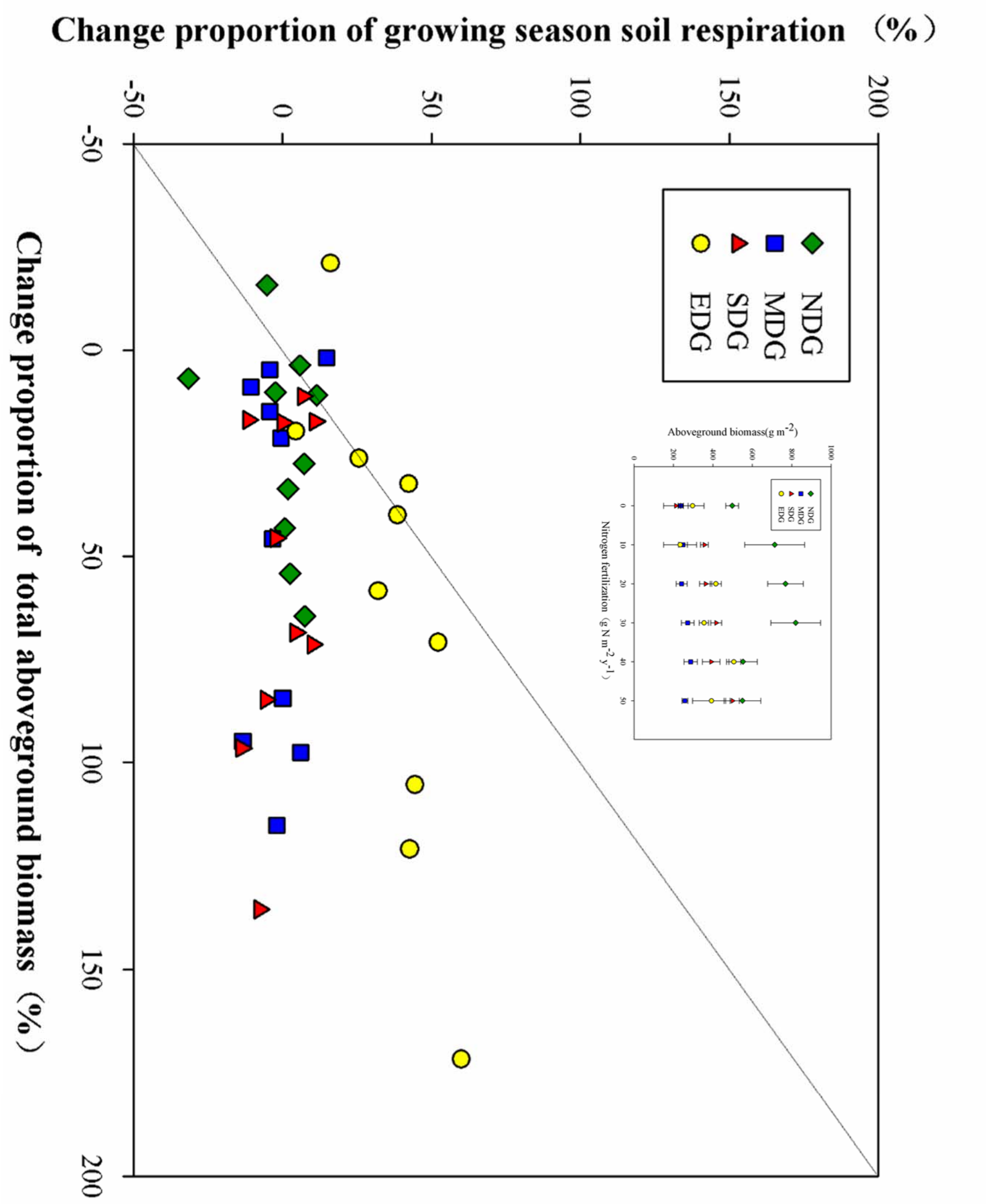

Fig.6