Soil Biology & Biochemistry 65 (2013) 96–104

Contents lists available at SciVerse ScienceDirect

Soil Biology & Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

Effects of warming and nitrogen deposition on the coupling mechanism between soil nitrogen and phosphorus in Songnen Meadow Steppe, northeastern China

Nanyi Zhang [a], Rui Guo [b], Piao Song [a,c], Jixun Guo [a,*], Yingzhi Gao [a,**]

[a] *Key Laboratory for Vegetation Ecology of the Ministry of Education, Northeast Normal University, Changchun, Jilin 130024, China*
[b] *Institute of Environment and Sustainable Development in Agriculture, Chinese Academy of Agricultural Sciences, Key Laboratory of Dryland Agriculture, Ministry of Agriculture, Beijing 100081, China*
[c] *State Key Laboratory of Vegetation and Environmental Change, Institute of Botany, The Chinese Academy of Sciences, Xiangshan, Beijing 100093, China*

ARTICLE INFO

Article history:
Received 18 December 2012
Received in revised form
13 March 2013
Accepted 16 May 2013
Available online 3 June 2013

Keywords:
Warming
N addition
Soil nitrogen
Soil phosphorus
Global change
Coupling mechanism

ABSTRACT

Songnen Meadow Steppe, which is in northeast China, is increasingly affected by global warming and incremental increases in atmospheric nitrogen deposition. However, the responses of nitrogen (N) and phosphorus (P) in steppe soil, and of the coupling mechanism between them, to the dual effects of global warming and N deposition are still unknown. In this study, the effects of simulated atmospheric warming and N deposition on N and P in Songnen steppe soil, as well as on the coupling between N and P, were examined under in situ conditions. Infrared heaters were used to elevate soil temperature by approximately 1.7 °C since 2006. N additions were treated once a year with aqueous ammonium nitrate at a rate of 10 g m^{-2} a^{-1}. During the four-year study, addition of N increased the amount of total N, and available N, as well as the rate of N mineralization in the soil. Moreover, the amounts of total P and available P in the soil were considerably reduced. Thus, the N:P ratio increased, and the coupling between N and P decreased. Similar values for the N:P ratio were obtained for the addition of N by itself and for the combination of warming and addition of N, which indicates that a small amount of soil warming in Songnen Meadow Steppe would not have a substantial effect on the ratio. With the growth of China's industrialization, N deposition continues to increase. The study area of Songnen Meadow Steppe, and northeast China in general, are characterized by widespread distribution of saline alkali soil. Therefore, the finding of increased P limitation in the soil of Songnen Meadow Steppe has major implications for ecosystems in northeast China. Reasonable regulation and management of meadow soil nutrients will be of great importance in increasing soil productivity and promoting sustainable use of grassland ecosystems.

1. Introduction

Global change, including warming, N deposition, increasing concentration of CO_2, and changes in atmospheric composition are associated with ecological problems that have become important topics of current study (Zhang et al., 2007). According to the Fourth Evaluation Report of the IPCC, the trend in global warming over the past fifty years indicates an increase in temperature by an average of 0.13 °C per decade, almost the twice the rate of increase over the past 100 years; in the next 20 years, the temperature is predicted to increase by an average of 0.2 °C per decade (IPCC, 2007). Meanwhile, the amount of atmospheric N deposition is also increasing steadily (Aber, 1992; Kaiser, 2001). Currently, North America, Europe, and East Asia (especially China) are the regions experiencing the greatest amount of N deposition worldwide (Liu et al., 2013; Vitousek et al., 1997; Galloway et al., 2008). Estimates of the active N generated by human activity have increased from 15 Tg N a^{-1} in 1860 to 165 Tg N a^{-1} in 1995 (Galloway and Cowling, 2002), an increase of a factor of 11; the 1995 estimate represents about 1.6 times the critical load of global N of 100 Tg N a^{-1} (Kaiser, 2001). The continual increase of N deposition will seriously affect the circulation of soil nutrients and the relationships among nutrients, and will affect ecosystem stability (Galloway et al., 1994; Melillo and Cowling, 2002). Therefore, it is essential to explore the

---

[*] Corresponding author. Tel.: +86 431 85098937; fax: +86 431 85695065.
[**] Corresponding author. Tel.: +86 431 85099992; fax: +86 431 85695065.
E-mail addresses: zhangny282@nenu.edu.cn (N. Zhang), gjixun@nenu.edu.cn (J. Guo), gaoyz108@nenu.edu.cn (Y. Gao).

http://dx.doi.org/10.1016/j.soilbio.2013.05.015

effects of global warming and N deposition on soil nutrient stoichiometry of grassland ecosystems, these data will contribute to the understanding of soil nutrient status and may have potential significant implications for the management of grassland restoration.

Temperature is the most important non-biological factor affecting N mineralization. An increase in soil temperature affects N mineralization, decomposition of organic material, amount of soil nutrients present, and migration of soil nutrients, thus altering ecosystem structure and function (Agren et al., 1991; Coughenour and Chen, 1997). Some research indicates that the increase in temperature will influence microbial processes in the soil, increasing the activity of soil enzymes and the bioavailability of N (Zhang et al., 2005; Rustad et al., 2001). The increase of the amount of available nutrients will increase the primary productivity of vegetation and reduce species richness, leading to the redistribution of soil nutrients. However, the specific mechanisms for this process are still unknown, and further exploration and research is required (Freeman et al., 1993; Zak et al., 1999; Maestre et al., 2005). Other research indicates that warming has no noticeable influence on the rate of net mineralization of N and P, or on net primary productivity, and therefore will not facilitate vegetation growth (Rinnan et al., 2007; Menge and Field, 2007). However, there is a lack of information on the response of the coupling mechanism between N and P in soil as temperature increases.

A great deal of research on land plants indicates that the available N:P ratio is sensitive to occurrences of nutrient limitation during vegetation growth and is closely related to the N:P ratio in plant foliage. Therefore, the available N:P ratio can be regarded as an effective index for evaluating ecosystem health (Schipper et al., 2004). Previous study observed that an ecosystem is controlled by N when the N:P ratio is low (<10), by P when the N:P ratio is high (>20), and by both N and P when the N:P ratio is between 10 and 20 (Güsewell, 2004). However, results vary in some ecosystem studies. For example, research conducted in Dutch wetlands indicated that the ecosystem is controlled by N when the N:P ratio is low (<14), by P when the ratio is high (>16), and by both N and P when the ratio is between 14 and 16 (Koerselman and Meuleman, 1996). The critical values of the N:P ratio for barren grassland are considered to be 10 and 14 (Braakhekke and Hooftman, 1999). Different threshold values of the N:P ratio have been suggested for perennial grassland: the system is considered to be controlled by N when the ratio is less than 21, and by P when the ratio is more than 23 (Zhang et al., 2004).

The influence of N deposition on soil nutrients is an issue of great interest among researchers (Adams et al., 2004; Robinson et al., 2004; Xia et al., 2009; Liu et al., 2011). Much of the research indicates that N deposition promotes net N mineralization in soil, increases the bioavailability of N in soil, and increases the net productivity of plants and the output of litter (Aerts et al., 2006; Vourlitis et al., 2007; Sirulnik et al., 2007). Long-term deposition of N will reduce the richness of the plant community and change its composition (Nkana et al., 1999; Prieto et al., 2009). Global change factors such as N, CO_2 and warming frequently limit plant growth, can increase or decrease primary production (Field et al., 1992). The increase in the availability of N will increase primary production, plants need to maintain a stable N:P ratio which encourages the absorption and utilization of soil P (Menge and Field, 2007). Thereby it increasing P demand or limitation and changing N limitation in soil to P limitation.

The influence of the increase of N on soil enzyme activity may be the major reason leading to the change of the limiting factor (Wedin and Tilman, 1993; Rinnan et al., 2007). An increase in N deposition can increase the output of litter, which is an important source of available soil nutrients because it adds available N and P to soil and increases the net P mineralization rate (Bradley et al., 2006). In studies of the influence of N deposition on availability of P, some researchers have noted that the addition of N increases the amount of inorganic N but reduces the amount of P in soil, thereby increasing the N:P ratio (Limpens et al., 2004).

In summary, research on the influence of global change on important soil nutrient factors is mostly centered on a single factor. However, the interaction between warming and N deposition will cause changes in the absolute amount of N and P in soil as well as in their relative proportions. These changes will profoundly influence vegetation growth, species competition, community composition, and ecosystem function. Unfortunately, the current lack of studies on how warming and nitrogen deposition influence the N:P ratio leaves a major gap in our understanding of the effects of alterations in this ratio.

Because about one-third of Earth's land surface is covered by grassland, the effects of global change on grasslands have major implications for the planet (Gao et al., 2006). *Leymus chinensis* Meadow, located in Songnen Meadow Steppe in northeast China, is the most typical and the largest grassland type in China (Zhu, 2004). This is an important area to study the effects of climate change and to determine possible ways to mitigate them. In nearly twenty years, the average temperature of Songnen Meadow Steppe has increased by 2 °C (Wang et al., 2006). The average atmospheric N deposition is approximately 10.5 g m^{-2} a^{-1} (Bai et al., 2010), and it is increasing year by year. Soil N and P are not only important nutrients but also are limiting factors on vegetation growth (Güsewell, 2004). This limiting function is more pronounced in the Songnen Meadow Steppe because of the widespread distribution of saline alkali soil (Su, 1995). Songnen Meadow Steppe was chosen as the research site for this study, which explores the influence of simulated warming and N deposition on soil N, soil P, and the coupling mechanism linking them under in situ conditions. The hypothesis under investigation is that there is coupling mechanism between soil N and P, and that N addition by itself, or a combination of N addition and warming, will increase the rate of net mineralization of N in soil, thus increasing the amount of available N and facilitating vegetation growth. Increased absorption of P by vegetation will lead to a reduction in the amount of soil P. These processes will ultimately reduce the coupling mechanism between soil N and P. This research provides a theoretical basis for understanding the influence of ongoing trends related to global changes on soil nutrients in Songnen Meadow Steppe, and it is relevant to determining methods to facilitate the recovery of grassland ecosystems and protect the environment as global changes occur.

2. Materials and methods

2.1. Study site

The study was conducted at the Songnen Grassland Ecosystem Research Station (44°45′ N, 123°45′ E, 160 m a.s.l.), Jilin Province, northeastern China. This region has a temperate semi-arid monsoon climate. The annual average air temperature is 4.9 °C. Mean annual precipitation (1980–2006) is approximately 410 mm, with more than 70% received from June to September. The total precipitation received during 2007–2010 was 275.9 mm, 384.2 mm, 390 mm, and 352.5 mm, respectively. The type of grassland is meadow steppe, and most of the grassland has a sodic saline meadow soil with a pH value of 8.2. The vegetation at the site is dominated by the perennial grass *Leymus chinensis* (Trin.) Tzvel.; *Phragmites communis*, *Kalimeris integrifolia* Turcz. Ex DC. and *Carex duriuscula* C. A. Mey. are also present at lower density.

2.2. Experiment design and treatments

This study used a completely randomized block factorial experimental design, with warming and N addition as fixed factors. There were four treatments: control (C), warming (W), N addition (N), and a combination of warming and N addition (WN), with six replicates for each treatment. The size of each plot was 3 m × 4 m, and the interval between plots was 3 m. We added a pulse of aqueous ammonium nitrate at a rate of 10 g m^{-2} a^{-1} to fertilized plots on the first day of May of each year. The warmed plots were continuously heated by infrared radiators (Kalglo Electronics Inc. Bethlehem, PA, MSR-2420, USA). Each heater was suspended 2.25 m above the center of a plot and was set at an output of approximately 1700 W, resulting in a 1.7 ± 0.1 °C temperature increase.

2.3. Measurement of soil temperature and soil water content

Soil temperature and water content were measured using an ECH_2O dielectric aquameter (Em50, USA). The apparatus automatically measured soil temperature and soil water content at a depth of 15 cm at 8:00–9:00 a.m. in late May, mid-June, mid-July, mid-August, mid-September, and mid-October from 2007 through 2010.

2.4. Sampling and sample analysis

Soil core samples were collected during the growth season (late May, mid-June, mid-July, mid-August, mid-September, and mid-October) from 2007 through 2010. We collected samples from two random locations in each plot to account for soil heterogeneity. Soil samples were taken with a cylindrical soil sampler (5-cm inner diameter, 15-cm length) for the 0–15 cm layer, and then were preserved in a 4 °C cooler prior to conducting laboratory measurements.

The net N mineralization rate and the amounts of total N, total P, available N, available P, N in ammonium (NH_4^+-N), and N in nitrate (NO_3^--N) were determined in the laboratory. An aerobic procedure (Neve and Hofman, 1996) was used to measure the soil net N mineralization rate. Fresh soil samples were used to minimize disturbance of microbial activity (Marrs et al., 1988; Bao, 1999). Two soil subsamples (about 20 g dry weight each) were transferred to 250-ml triangular bottles, and soil water content was adjusted to 60% water-holding capacity. One subsample was incubated, and one was not. For the incubated samples, the bottles were covered with plastic film (0.01-mm thick) and placed in a constant-temperature cabinet at 25 °C (HPG- 400, China) for 14 days. The bottles were weighed periodically during incubation, and we added deionized water to keep the water content constant.

The available N ($NH_4^+-N + NO_3^--N$) content of each incubated and un-incubated soil sample was determined by the Kjeldahl method. We added 100 ml of 2 M KCl solution to each sample, shook the samples for 60 min using a reciprocal shaker, and then centrifuged them for 10 min. Supernatant was collected with a pipette and then frozen for future analysis. The procedures of Allen et al. (1974) were used in the steam distillation and titration of the supernatant for both the incubated and un-incubated soil subsamples. The NO_3^--N content was determined using UV photometry at 210 nm (Stenger et al., 1995). The NH_4^+-N content was calculated by the difference between available N ($NH_4^+-N + NO_3^--N$) and NO_3^--N. The net N mineralization rate was calculated by the change in available N between the unincubated and incubated subsamples. The total N content was determined by the Kjeldahl method, using digestion by H_2SO_4 (Bremner and Mulvaney, 1982).

Total P content was determined using a $HClO_4-H_2SO_4$ heating digestion method and taking measurements with an inductively coupled plasma emission spectrometer (ICP). Available P content was determined with $NaHCO_3$ extraction and a molybdenum blue colorimetric method using UV photometry at 660 nm.

2.5. Statistical analysis

Seasonal mean values used in this study were calculated from the monthly mean values, which were first averaged from all measurements in the same month. Repeated measures ANOVAs in a general linear model were used to examine temporal (inter- or intra-annual) variations and effects of warming and N addition on soil net N mineralization rate as well as on soil temperature, water content, total N, total P, available N, available P, NH_4^+-N, NO_3^--N, total N:P, available N:P, and $NH_4^+-N:NO_3^--N$. Significant differences among treatments means were analyzed using Tukey's multiple comparison pos hoc test within each year. Pearson's correlation coefficients were used to determine the relationships between soil total N, total P, available N, available P, total N:P, available N:P, net N mineralization and soil temperature as well as soil water content in different treatment plots. Statistical analyses were conducted by SPSS (SPSS 13.0 for windows, USA) and displayed using Sigmaplot 12.0 software.

3. Results

3.1. Effects of warming and N addition on soil temperature and water content

During the four growing seasons during the study, soil temperature displayed a seasonal response to treatments. In an individual growing season, soil temperature (at 0–15 cm) reached a unimodal peak in August (Fig. 1a–d). In general, warming raised the soil temperature ($p < 0.05$) by an average of 1.12 °C, but N addition produced no apparent effect on soil temperature ($p = 0.42$). Combined warming and N addition also markedly increased soil temperature ($p < 0.05$), except in 2008, but the difference between combined warming with N addition and warming by itself is not significant.

Similar to the response of soil temperature, soil water content also displayed a seasonal trend, with water content peaking in July and August (Fig. 1e–h). Warming significantly reduced soil water content ($p < 0.05$), and the addition of N by itself had no apparent effect on the water content ($p = 0.38$). Combined warming and addition of N reduced soil water content ($p < 0.05$), except in 2008, the difference between combined warming with N addition and warming by itself is not significant.

3.2. Effects of warming and N addition on soil N and P

Consistent results were obtained in this study: N addition clearly enhances the amount of total soil nitrogen as well as available N and NH_4^+-N, but these amounts are not significantly affected by warming ($p < 0.001$, Fig. 2a, c, e). The combination of warming and N addition enhanced the amount of total N and available N compared with the control ($p < 0.05$). For available N, the effect of combined warming and N addition was higher than for the control but lower than for N addition by itself. In general, the rate of net mineralization of soil N increased with N addition, warming, and the combination of warming and N addition. The addition of N and warming had apparent effects on the rate of net mineralization of soil N. The amount of soil NO_3^--N decreased with N addition, warming, and the combination of warming and N addition. The addition of N has no apparent effect on the amount of NO_3^--N, but warming clearly decreased the amount of NO_3^--N ($p < 0.01$, Fig. 2f). The addition of N

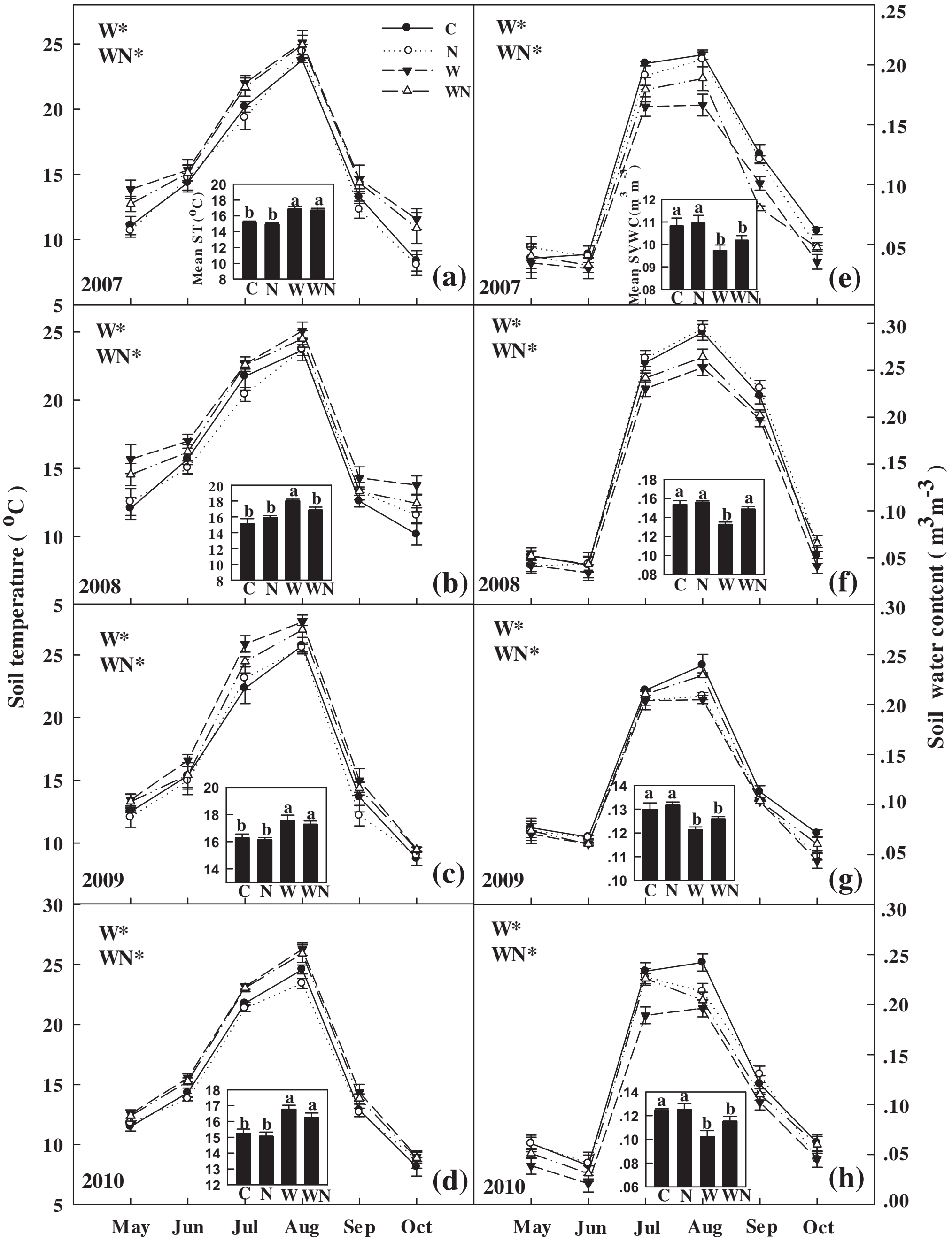

Fig. 1. Effects of warming and nitrogen addition on soil temperature and soil water content. Values show the means of soil temperature (ST) and soil water content (SWC) from May to October for four years. The inset graphs show the seasonal mean ST and SWC values for the four growing seasons. Vertical bars indicate standard errors of the means ($n = 6$). Different lowercase letters indicate statistically significant differences ($p < 0.05$). C = control, N = nitrogen addition, W = warming, and WN = combined warming and nitrogen addition.

significantly reduced the amounts of total P and available P ($p < 0.01$, Fig. 2b, d), but warming produced no apparent effect on them. The interaction between N addition and warming decreased the amount of total P in soil ($p < 0.01$) but had little effect on the amount of available P (Fig. 2b, d).

3.3. *Effects of warming and N addition on the N:P ratio and the NH_4^+–N:NO_3^-–N ratio*

The addition of N increases the N:P and NH_4^+–N:NO_3^-–N ratios (Fig. 3). In 2008, the ratio of available N:P reached 21.12. Although warming tends to increase the available N:P ratio, warming has little effect on the total N:P ratio and the available N:P ratio (Fig. 3a, b). The total and available N:P ratios by combined warming and N addition are higher than that of control, but for the total N:P ratio, there is little difference between N addition by itself and when combined with warming. For the available N:P ratio, the ratio for N addition by itself is higher than that for combined warming and N addition because the amount of NO_3^-–N is significantly reduced when warming and N addition are combined ($p < 0.05$, Fig. 2f; Fig. 3a, b). Surprisingly, given the saline soil in Songnen Meadow Steppe, N or warming by itself did not noticeably increase the NH_4^+–N:NO_3^-–N ratio, but the combination of warming and N addition significantly enhanced it ($p < 0.05$, Fig. 3c). Interestingly, regardless of the

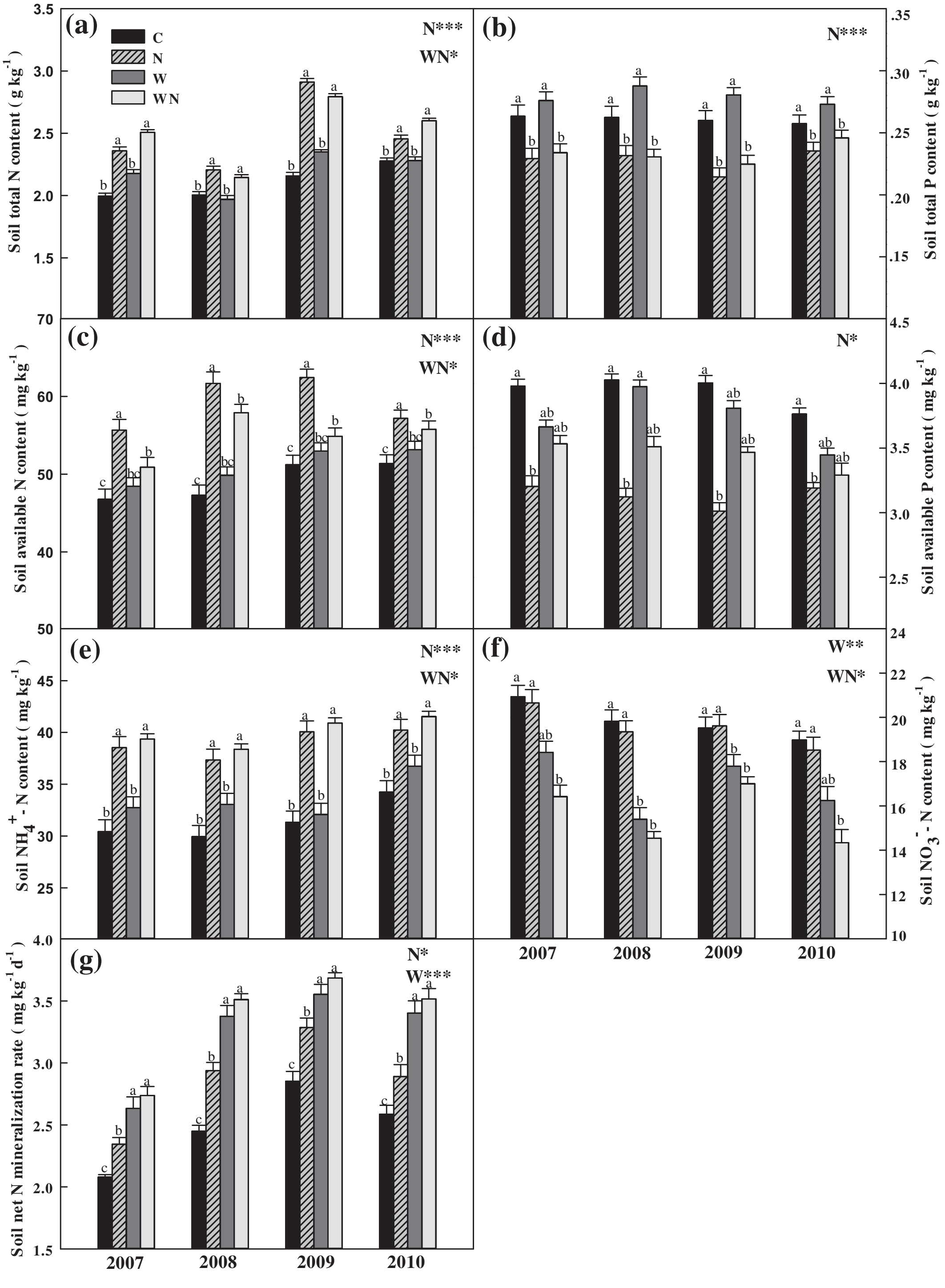

Fig. 2. Effects of warming and nitrogen addition on seasonal changes in soil nutrients. Values show the means of soil total N, total P, available N, available P, NH_4^+–N, NO_3^-–N and the net mineralization rate from May to October for four years. Vertical bars indicate standard errors of the means ($n = 6$). Different lowercase letters indicate statistically significant differences ($p < 0.05$). C = control, N = nitrogen addition, W = warming, and WN = combined warming and nitrogen addition. *, ** and *** indicate a significant difference between treatment at $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively.

treatment, the amount of NH_4^+–N is far greater than that of NO_3^-–N, causing the NH_4^+–N:NO_3^-–N ratio to range between 1.5 and 3.0.

3.4. *Correlation of soil nutrients and nutrient ratios with soil temperature and soil water content*

Correlation analysis showed that soil temperature and water content were positively correlated with the amount of total N, total P, available N, and available P; the total and available N:P ratios; and the net N mineralization rate. Correlation coefficients range from 0.297 to 0.853 (Table 1). The highest correlations between each of these measures and soil temperature or water content occurred in the control plots, except for the net nitrogen mineralization rate. The lowest correlations occurred in the plots treated with the combination of warming and N addition because of the more complex response of soil nutrients to the combined treatment.

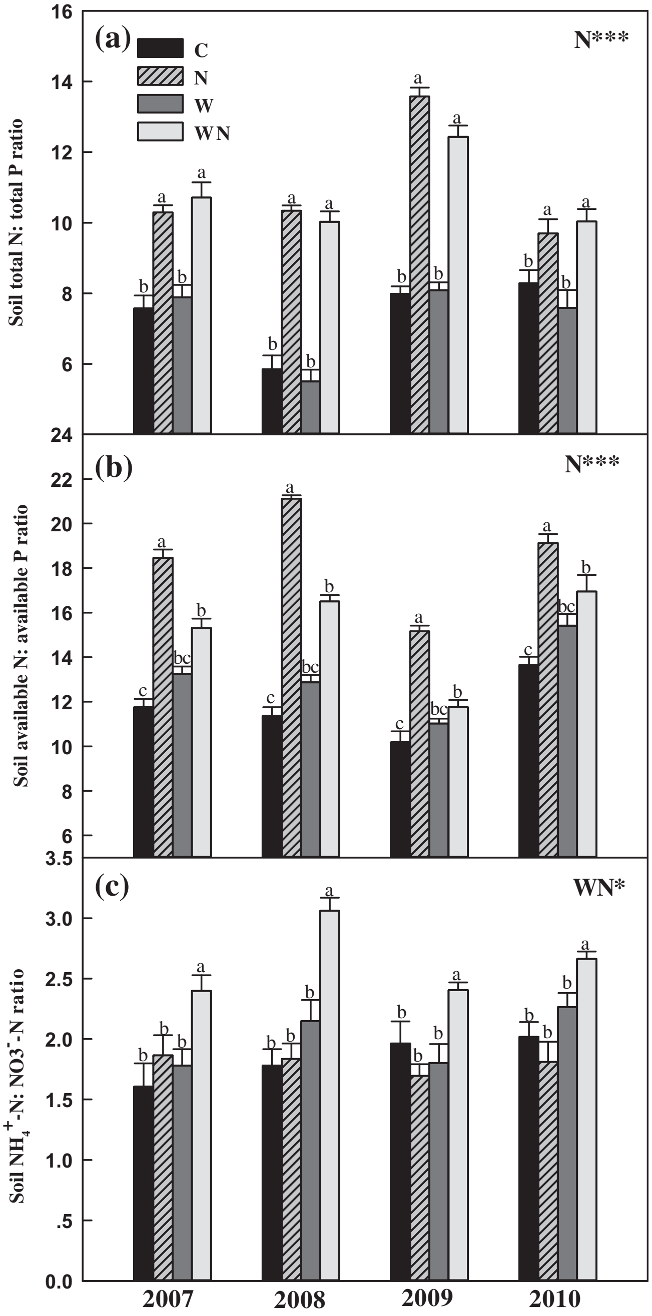

Fig. 3. Effects of warming and nitrogen addition on seasonal changes in soil N:P and NH_4^+-N:NO_3^--N ratios. Values show the means of soil N:P and NH_4^+-N:NO_3^--N ratios from May to October for four years. Vertical bars indicate standard errors of the means ($n = 6$). Different lowercase letters indicate statistically significant differences ($p < 0.05$). C = control, N = nitrogen addition, W = warming, and WN = combined warming and nitrogen addition. *, and *** indicate a significant difference between treatment at $p < 0.05$ and $p < 0.001$, respectively.

Correlations between total and available N and soil temperature or water content were higher than for the correlations of total and available P and soil temperature or water content. The correlations between available N and P and soil temperature or water content were considerably higher than the correlations of total N and P with soil temperature or water content (Table 1). The correlations were generally highest between the net N mineralization rate and soil temperature or water content in the control plots, and were lowest between N addition and soil temperature or water content in N addition plots, but the variation in correlation coefficients among the different treatments was not marked (Table 1).

4. Discussion

4.1. Effects of warming and N deposition on soil N and P

The mineralization, nitration, fixation, and absorption of N, and the internal recycling of nutrients by plants, are the major pathways of N movement in a grassland ecosystem. Mineralization of soil N is an important link in the soil N cycle, whose processes depend on the amount of organic N present, temperature, moisture level, and activity of microorganisms (Puri and Ashman, 1998; Shaw and Harte, 2001a). Currently, whether N deposition affects the net N mineralization rate is a topic of intense debate. Some believe that the net N mineralization rate accelerates with increasing N deposition (Loiseau and Soussana, 2000; Li et al., 2006; Vourlitis and Zorba, 2007). The addition of N enhances the amount of available N in soil, and the amount of available N increases as more N is added, as the duration the addition increases, and as the rate of net mineralization increases (Robinson et al., 2004). However, the results of the study observed that although the mineralization rate for soil N increases with long-term N addition, the rate of net mineralization decreases from an initial peak to a value near or lower than that of the control. Some other previous studies are also in agreement with our results (Tietema et al., 1998; Aber et al., 1998; Throop et al., 2004).

N addition facilitates mineralization of soil N and enhances the amount of available N. The reason is that the addition of N enhances microbial activity and triggers a priming effect that further facilitates the mineralization of soil organic matter (Fig. 2c, g). Moreover, the combination of surplus N and organic matter lowers the soil C:N ratio and accelerates the decomposition rate of organic matter and the release of nutrients (Lovell and Hatch, 1998; Throop et al., 2004). Thus, N deposition facilitates the release of available N in the soil and provides more usable N for the growth of vegetation. However, the mineralization of soil N does not increase continuously when the level of N addition remains the same. In the third growing season (2009), mineralization of soil N declined after reaching a peak, probably because N input changed the chemical characteristics of the organic matter in soil (Aber et al., 1998), or the gradual adaption of aboveground vegetation and soil microbes to the experimental treatments as well as from feedbacks affecting soil nutrient conditions.

Temperature is another important factor controlling the mineralization of N. Higher temperature can facilitate the mineralization of N and accelerate the accumulation of soil N (Piatek and Allen, 1999; Cookson et al., 2002). A positive correlation exists between the mineralization of soil N and temperature (Table 1), which is probably a result of the increase of soil microbial activity and microbial biomass in the warming plots, accelerating the rate of decomposition of soil organic matter and thus facilitating the net mineralization of N (Rustad et al., 2001; Shaw and Harte, 2001b).

In this study, the amounts of total and available N were apparently not affected by warming as had been assumed, mainly because warming is associated with vigorous seasonal vegetation growth that increases the aboveground biomass (unpublished data), and thus the absorption of the nutrients is also enhanced. Therefore, large accumulations of total and available N in soil do not occur, although a large amount of available N remains after mineralization (Fig. 2a, c), and this amount does not change much (Nemani et al., 2003).

Many researchers have attempted to show that NO_3^--N is the main form of available N in arid and semi-arid meadow soil (Ma et al., 1999; Scherer et al., 2003; Turpault et al., 2005). However,

Table 1
Relationships between soil total N, total P, available N, available P, total N:P, available N:P, NNM rate and soil temperature as well as soil water content in different treatment plots.

Soil nutrients	Soil temperature				Soil water content			
	C	N	W	WN	C	N	W	WN
Total N	0.548^{**}	0.475^{*}	0.492^{*}	0.439^{*}	0.596^{**}	0.408^{*}	0.351^{*}	0.430^{*}
Total P	0.531^{**}	0.401^{*}	0.409^{*}	0.297^{*}	0.399^{**}	0.348^{*}	0.326^{*}	0.290^{*}
Available N	0.853^{**}	0.767^{**}	0.742^{**}	0.717^{**}	0.744^{**}	0.519^{**}	0.592^{**}	0.454^{**}
Available P	0.741^{**}	0.659^{**}	0.558^{**}	0.538^{**}	0.784^{**}	0.435^{**}	0.438^{**}	0.391^{**}
Total N:P ratio	0.630^{*}	0.580^{*}	0.457^{*}	0.529^{*}	0.478^{*}	0.471^{*}	0.449^{*}	0.420^{*}
Available N:P ratio	0.812^{**}	0.715^{**}	0.672^{**}	0.625^{**}	0.754^{**}	0.736^{**}	0.660^{**}	0.653^{**}
NNM rate	0.786^{**}	0.770^{**}	0.783^{**}	0.775^{**}	0.807^{**}	0.771^{**}	0.776^{**}	0.780^{**}

Pearson correlation coefficients (R) and their significance (p) are given as: $^{*}p < 0.05$ and $^{**}p < 0.01$, respectively. C = control, N = nitrogen addition, W = warming, WN = combined warming and nitrogen addition, and NNM rate = net N mineralization rate.

the results indicate that NH_4^+–N is the main form of available N in the surficial soil (0–15 cm) of Songnen Meadow Steppe, regardless of the experimental treatment (Fig. 3c). This discrepancy between expectations and results may arise from two characteristics of the soil environment of Songnen Meadow. The first is that NH_4^+–N is likely to be adsorbed and fixed in the soil by clay minerals and organic colloids, causing the amount of NH_4^+–N to tend to rise gradually as the vegetation grows, while the soil NO_3^-–N is used by the steppe plants primarily to form green biomass; therefore, the net surplus of NH_4^+–N in soil is reduced (Gao et al., 2004). The second is that the accumulation of NO_3^-–N is closely related to soil water content. Seasonal waterlogging of the surface is often seen in Songnen Meadow Steppe. Nitrobacterial activity is affected by flooded conditions, which hinder the transformation from NH_4^+–N to NO_3^-–N, causing the amount of NO_3^-–N to tend to decrease (Rückauf et al., 2004). Moreover, leaching, which is the major abiotic channel for NO_3^-–N loss in this special meadow, is mainly influenced by changes in rainfall amounts (Galbally et al., 2008). The reason is that nitrate ions are difficult to be absorbed by soil particles and easily moved with water in soil. As a consequence, the high intensity and frequency of rainfall in summer may facilitate the nitrate ions leaching into deep soil layer and make them plant unavailable. Furthermore, the water table is shallow (about 2 m deep) in Songnen Meadow Steppe, and strong rainfalls in summer facilitate the leaching of NO_3^-–N (Xiao et al., 2005), increasing the loss of NO_3^-–N, and causing the amount of NH_4^+–N to rise to surplus levels. However, some research has found a positive correlation between the amount of NO_3^-–N and conductivity or pH values (Curtin et al., 1998; Zhang and Brian, 2002; Pascual et al., 2007). These correlations indicate that under saline conditions, soil pH and conductivity are higher, which promotes nitrifying processes in the soil and increases the amount of soil NO_3^-–N. This is inconsistent with our results, probably because the influence of pH and conductivity on NO_3^-–N is far less than on the two above processes, but the issue requires further study.

The warming treatment caused soil temperature to rise, and the additional mineralization of N was partially offset by absorption by vegetation, leading to a clear reduction in the amount of NO_3^-–N (Ineson et al., 1998). The addition of N also tended to reduce the amount of NO_3^-–N, so the combined treatment of warming and N addition caused the amount of NO_3^-–N to decline more than for the other two treatments or the control. In 2008, the relatively high amount of rainfall enhanced the leaching of NO_3^-–N, causing the amount of NO_3^-–N in that year to reach a minimum. However, in that same year, the combined treatment of warming and N addition increased the amount of NH_4^+–N, so the NH_4^+–N:NO_3^-–N ratio reached a maximum, with the amount of NH_4^+–N being three times that of NO_3^-–N.

The addition of N considerably reduced the amount of total and available P, mainly because the addition of N facilitates vegetation growth. During growth, plants need to maintain a stable N:P ratio, which encourages the absorption and utilization of soil P (Goodale et al., 2000; Lilleskov et al., 2002; Zhang et al., 2004; Menge and Field, 2007). Therefore, the increasing uptake of N facilitates vegetation growth and leads to the loss of soil P. Further, plant tissue P was difficult to decompose because it existed in the form of phospholipid and phytate, etc. This part of organic P could be decomposed by phosphatase and return back to soil, which would be affected by various factors such as phosphatase activity, temperature and pH (Chen et al., 2004). As a consequence, P absorbed by plants could not soon be released into soil and the P availability was temporarily decreased in soil.

Microbes also play a role in the amount of P present. Microbial functions in soil include immobilization and mineralization (Qin et al., 2006). After some interval of N addition, the relative rate of microbial immobilization of P is higher than the rate of mineralization, so the amount of available P in soil declines (Kouno et al., 2002). However, results of our study show that the amount of soil P was not appreciably reduced by warming (Fig. 2b, d), mainly because of an adaptation process involving microorganisms and enzymatic activity in soil in response to increased temperature; in addition, the activity of phosphatase may be influenced by soil and vegetation characteristics, species biodiversity, and so on (Dick et al., 2000).

Warming increases the activity of soil phosphatase, and this increase has been noted in each year that this study has been extended (unpublished data). Due to the increase in the amount of soil phosphatase, it is clear that available P is somehow being supplied. Therefore, the amount of available P is not noticeably decreased by warming. The productivity of aboveground vegetation is increased by warming, so the need for soil P by plants is also increased. The decrease in soil P content is led by the movement of P to the surface, and soil microbial activity is enhanced by warming, which strengthens the decomposition of the litter. A large amount of P is returned to the soil, and the amount of total P is not much reduced (Chen et al., 2003).

4.2. Effects of warming and N deposition on the coupling mechanism between soil N and P

The N:P ratio increases with N addition, and the increase in the amount of N, as well as the decrease in the amount of P, have different effects on N and P. Therefore, the coupling mechanism between soil N and P is reduced, as reflected by the significant reduction in the liner relationship between total N and available P (Co-variance, $p < 0.01$) as well as between available N and available P (Co-variance, $p < 0.001$) in N addition compare with control. The coupling mechanism is not only influenced by interaction between N and P but also by abiotic factors such as soil temperature, water content, and soil pH. Although results of this study show positive correlations between soil N or P and temperature, the extent of the influence of temperature on P is lower than for N and C (Oberson et al., 1993), and the different responses of N and P to temperature changes alter the coupling relationship. Moreover, N addition can also lead to soil acidification, and the volume and timing of N addition are positively correlated with the extent of decrease of soil pH (Fan et al., 2007). In addition, soil acidification affects phosphatase activity (Gundersen and Rssmussen, 1990), and affects the amount of P in soil, so changes in soil acidification also alter the coupling mechanism between N and P. The N:P ratio is not much changed by warming or by the combination of warming and N addition, so no influence on the coupling mechanism between N and P is apparent (Fig. 3a, b), which indicates that an increase in atmospheric N deposition is likely to weaken the coupling mechanism, but little to no effect can be attributed to warming.

It is surprising that current research on the N:P ratio focuses solely on vegetation, while data on the combined effects of warming and N addition on N:P ratios have not been reported until now. In this study, results show that the addition of N increases the N:P ratio, especially the ratio of available N:P (AN:AP ratio$_{2008}$ = 21.12, Fig. 3b), and that warming also tends to increase the available N:P ratio. The available N:P ratio is lower when a combination of warming and N addition occurs than when N addition occurs by itself (Fig. 3b), mainly due to the low amount of NO_3^-—N (Fig. 2f) associated with the combined treatment. The increase in the N:P ratio shows that the coupling mechanisms between total N and total P, and between available N and available P, are weakened by N addition. In addition, it shows that P becomes the limiting nutrient in the soil, and that P is being lost in Songnen Meadow Steppe, especially when rainfall is plentiful and evenly distributed.

It is clear that atmospheric N deposition will be an important factor affecting the circulation of N and P in across northeast China as global change progresses, and that the coupling mechanism of soil N and P will be reduced by the increase in N deposition. The weakening of the coupling mechanism may negatively affect the grassland ecosystem. The deposition of N is increasing in response to China's increasing industrialization, P may need to be supplied in the future to enhance the availability of soil P and to maintain the dynamic balance between N and P. Because it is of great importance for slowing the negative effects of global change on grassland ecosystems and increasing their productivity.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (31170303, 31270444, 31200243), the Strategic Priority Research Program of the Chinese Academy of Sciences (XDA05050601), the Research Fund for the Doctoral Program of Higher Education of China (20090043110007). Authors would like to thank Linna Ma, Jianqin Yang, Shiwei Gong and Li Jiang for supporting the filed work and laboratory work. We also thank two anonymous reviewers, Qibing Wang and Xiaotao Lü for their constructive comments and suggestions on the early draft of this manuscript.

References

Aber, J.D., 1992. Nitrogen cycling and nitrogen saturation in temperate forest ecosystems. Trends in Ecology and Evolution 7, 220–223.

Aber, J.D., McDowell, W., Nadelhoffer, K.J., Magil, A., Berntson, G., Kamake, M., McNulty, S., Currie, W., Rustad, L., Fernande, I., 1998. Nitrogen saturation in temperate forest ecosystems. Bioscience 48, 921–934.

Adams, M., Ineson, P., Binkley, D., Cadisch, G., Tokuchi, N., Scholes, M., Hicks, K., 2004. Soil functional responses to excess nitrogen inputs at global scale. AMBIO 33 (8), 530–536.

Aerts, R., van Logtestijn, R.S., Karlsson, P.S., 2006. Nitrogen supply differentially affects litter decomposition rates and nitrogen dynamics of sub-arctic bog species. Oecologia 146, 652–658.

Agren, G.I., Mcmurtrie, R.F., Parton, W.J., Pastor, J., Shugart, H.H., 1991. State-of-the-art of models of production decomposition 1inkages in conifer and grassland ecosystems. Ecological Applications 1, 118–138.

Allen, S.E., Grimshaw, H.M., Parkinson, J.A., 1974. In: Chemical Analysis of Ecological Materials. England, Blackwell Scientific Publishers, London.

Bai, Y.F., Wu, J.G., Clark, C.M., Naeem, S., Pan, Q.M., Huang, J.H., Zhang, L.X., Han, X.G., 2010. Tradeoffs and thresholds in the effects of nitrogen addition on biodiversity and ecosystem functioning: evidence from Inner Mongolia grasslands. Global Change Biology 16, 358–372.

Bao, S.D., 1999. In: Agriculture Soil Chemical Analysis. Science Press, Beijing, China, pp. 263–271.

Braakhekke, W.G., Hooftman, D.A.P., 1999. The resource balance hypothesis of plant species diversity in grassland. Journal of Vegetation Science 10, 187–200.

Bradley, K., Drijber, R.A., Knops, J., 2006. Increased N availability in grassland soils modifies their microbial communities and decreases the abundance of arbuscular mycorrhizal fungi. Soil Biology and Biochemistry 38, 1583–1595.

Bremner, J.M., Mulvaney, C.S., 1982. Nitrogen-total. In: Page, A.L., Miller, R.H., Keeney, D.R. (Eds.), Methods of Soil Analysis. American Society of Agronomy, Madison, pp. 595–608.

Chen, C.R., Condron, L.M., Davis, M.R., Sherlock, R.R., 2003. Seasonal changes in soil phosphorus and associated microbial properties under adjacent grassland and forest in New Zealand. Forest Ecology and Management 177, 539–557.

Chen, C.R., Condron, L.M., Davis, M.R., Sherlock, R.R., 2004. Effects of plant species on microbial biomass phosphorus and phosphatase activity in a range of grassland soils. Biology and Fertility of Soils 40, 313–322.

Cookson, W.R., Cornforth, I.S., Rowarth, J.S., 2002. Winter soil temperature (2–5 °C) effects on nitrogen transformations in clover green manure amended or unamended soils: a laboratory and field study. Soil Biology and Biochemistry 34, 1401–1415.

Coughenour, M.B., Chen, D.X., 1997. Assessment of grassland ecosystem response to atmospheric change using 1inked plant-soi1 process models. Ecological Applications 7, 802–827.

Curtin, D.C., Campbell, A., Jail, A., 1998. Effects of acidity on mineralization: pH—dependence of organic matter mineralization in weakly acidic soils. Soil Biology Biochemistry 30, 57–64.

Dick, W.A., Cheng, L., Wang, P., 2000. Soil acid and alkaline phosphatase activity as pH adjustment indicators. Soil Biology and Biochemistry 32 (13), l915–1919.

Fan, H.B., Liu, W.F., Li, Y.Y., Liao, Y.C., Yuan, Y.H., Xu, L., 2007. Tree growth and soil nutrients in response to nitrogen deposition in a subtropical Chinese fir plantation. Acta Ecologica Sinica 27 (11), 4630–4642.

Field, C.B., Chapin III, F.S., Matson, P.A., Mooney, H.A., 1992. Responses of terrestrial ecosystems to the changing atmosphere: a resource-based approach. Annual Review of Ecology and Systematics 23, 201–235.

Freeman, C., Lock, M.A., Reynolds, B., 1993. Climatic change and the release of immobilized nutrients from Welsh riparian wetland soils. Ecological Engineering 2, 367–373.

Galbally, I., Kirstine, W.V., Meyer, C.P., Wang, Y.P., 2008. Soil-atmosphere trace gas exchange in semiarid and arid zones. Journal of Environmental Quality 37, 599–607.

Galloway, J.N., Cowling, E.B., 2002. Relative nitrogen and the world: 200 years of change. AMBIO 31, 64–71.

Galloway, J.N., Levy, I.I.H., Kasibhatla, P.S., 1994. Year 2020: consequences of population growth and development on deposition of oxidized nitrogen. AMBIO 23 (2), 120–123.

Galloway, J.N., Townsend, A.R., Erisman, J.W., Bekunda, M., Cai, Z., Freney, J.R., Martinelli, L.A., Seitzinger, S.P., Sutton, M.A., 2008. Transformation of the nitrogen cycle: recent trends, questions, and potential solutions. Science 320 (5878), 889–892.

Gao, Y.Z., Wang, S.P., Han, X.G., Chen, Q.S., Wang, Y.F., Zhou, Z.Y., Zhang, S.M., Yang, J., 2004. Soil nitrogen regime and the relationship between aboveground green phytobiomass and soil nitrogen fractions at different stocking rates in the Xilin River, Inner Mongolia. Acta Phytoecologica Sinica 28 (3), 285–293.

Gao, X.S., Tian, Z.C., Hao, X.N., Jiang, G.X., 2006. The changes of alpine grassland soil nutrition at different deteriorate degree on high mountain meadow of Three River Source. Journal of Qinghai University 24 (5), 37–40.

Goodale, C.L., Aber, J.D., McDowell, W.H., 2000. The long-term effects of disturbance on organic and inorganic nitrogen export in the White Mountains, New Hampshire. Ecosystems 3, 433–450.

Gundersen, P., Rssmussen, L., 1990. Nitrification in forest soil: effects from nitrogen deposition on soil acidification and aluminium release. Reviews of Environmental Contamination and Toxicology 113, 1–45.

Güsewell, S., 2004. N: P ratios in terrestrial Plants: variation and functional significance. New Phytologist 164, 243–266.

Ineson, P., Benham, D.G., Poskitt, J., Harrison, A.F., Taylor, K., Woods, C., 1998. Effects of climate change on nitrogen dynamics in upland soils. Global Change Biology 4 (1), 153–161.

IPCC, 2007. IPCC WGI Fourth Assessment Report. Climatic Change 2007: The Physical Science Basis. Intergovernmental Panel on C1imate Change, Geneva.

Kaiser, J., 2001. The other global pollutant: nitrogen proves tough to curb. Science 294, 1268–1269.

Koerselman, W., Meuleman, A.F.M., 1996. The vegetation N: P ratio: a new tool to detect the nature of nutrient limitation. The Journal of Applied Ecology 33, 1441–1450.

Kouno, K., Wu, J., Brookes, P.C., 2002. Turnover of biomass C and P in soil following incorporation of glucose or ryegrass. Soil Biology and Biochemistry 34, 617–622.

Li, X.Y., Meixner, T., Sickman, J.O., Miller, A.E., Schimel, J.P., Melack, J.M., 2006. Decadal-scale dynamics of water, carbon and nitrogen in a California chaparral ecosystem: DAYCENT modeling results. Biogeochemistry 77, 217–245.

Lilleskov, E.A., Fahey, T.J., Horton, T.R., Lovett, G.M., 2002. Belowground ectomycorrhizal fungal community change over a nitrogen deposition gradient in Alaska. Ecology 83, 104–115.

Limpens, J., Berendse, F., Klees, H., 2004. How phosphorus availability affects the impact of nitrogen deposition on Sphagnum and Vascular Plants in Bogs. Ecosystems 7 (8), 793–804.

Liu, X.J., Duan, L., Mo, J.M., Du, E.Z., Shen, J.L., Lu, X.K., Zhang, Y., Zhou, X.B., He, C.E., Zhang, F.S., 2011. Nitrogen deposition and its ecological impact in China: an overview. Environmental Pollution 159, 2251–2264.

Liu, X.J., Zhang, Y., Han, W.X., Tang, A.H., Shen, J.L., Cui, Z.L., Vitousek, P., Erisman, J.W., Goulding, K., Christie, P., Fangmeier, A., Zhang, F.S., 2013. Enhanced nitrogen deposition over China. Nature. http://dx.doi.org/10.1038/nature11917.

Loiseau, P., Soussana, J.F., 2000. Effects of elevated CO_2, temperature and N fertilization on fluxes in a grassland ecosystem. Global Change Biology 6, 953–965.

Lovell, R.D., Hatch, D.J., 1998. Stimulation of microbial activity following spring applications of nitrogen. Biology and Fertility of Soils 26, 28–30.

Ma, B.L., Lianne, M.D., Edward, G.G., 1999. Soil nitrogen amendment effects on seasonal nitrogen mineralization and nitrogen cycling in maize production. Agronomy Journal 91, 1003–1009.

Maestre, F.T., Bradford, M.A., Reynold, J.F., 2005. Soil nutrient heterogeneity interacts with elevated CO_2 and nutrient availability to determine species and assemblage responses in a model grassland community. New Phytologist 168 (3), 637–650.

Marrs, R.H., Proctor, J., Heaney, A., Mountford, M.D., 1988. Changes in soil N mineralization and nitrification along an altitudinal transect in tropical rain forest in Costa Rica. The Journal of Ecology 76, 466–482.

Melillo, J.M., Cowling, E.B., 2002. Reactive nitrogen and public policies for environmental protection. AMBIO 31 (2), 150–158.

Menge, D.N.L., Field, C.B., 2007. Simulated global changes alter phosphorus demand annual grassland. Global Change Biology 13, 2582–2591.

Nemani, R.R., Keeling, C.D., Hashimoto, H., Jolly, W.M., Piper, S.C., Tucker, C.J., Myneni, R.B., Running, S.W., 2003. Climate-driven increases in global terrestrial net primary production from 1982 to 1999. Science 300, 1560–1563.

Neve, S.D., Hofman, G.H., 1996. Modeling N mineralization of vegetable crop residues during laboratory incubation. Soil Biology and Biochemistry 28, 1451–1457.

Nkana, V.J.C., Tack, F.M.G., Verloo, M.G., 1999. Dynamics of nutrients in tropical acid soils amended with paper pulp sludge. Waste Management and Research 17, 198–204.

Oberson, A., Fardeau, J.C., Bessob, J.M., Sticher, H., 1993. Soil phosphorus dynamics cropping systems managed according to conventional and biological agricultural methods. Biology and Fertility of Soils 16, 111–117.

Pascual, I., Antolín, M.C., García, C., Polo, A., Sánchez-Díaz, M., 2007. Effect of water deficit on microbial characteristics in soil amended with sewage sludge or inorganic fertilizer under laboratory conditions. Bioresource Technology 98 (l), 29–37.

Piatek, K.B., Allen, H.L., 1999. Nitrogen mineralization in a pine plantation fifteen years after harvesting and site preparation. Soil Science 63, 990–998.

Prieto, P., Penuelas, J., Lloret, F., Llorens, L., Estiarte, M., 2009. Experimental drought and warming decrease diversity and slow down post-fire succession in a Mediterranean shrubland. Ecography 32, 623–636.

Puri, G., Ashman, M.R., 1998. Relationship between soil microbial biomass and gross N mineralization. Soil Biology and Biochemistry 30 (20), 251–256.

Qin, S.J., Liu, J.S., Wang, G.P., 2006. Mechanism of phosphorus availability changing in soil. Chinese Journal of Soil Science 37 (5), 1012–1016.

Rinnan, R., Michelsen, A., Baath, E., Jonasson, S., 2007. Mineralization and carbon turnover in subarctic heath soil as affected by warming and additional litter. Soil Biology and Biochemistry 39, 3014–3023.

Robinson, C.H., Saunders, P.W., Madan, N.J., Pryce-Miller, E.J., Pentecost, A., 2004. Does nitrogen deposition affect soil microfungal diversity and soil N and P dynamics in a high Arctic ecosystem. Global Change Biology 10, 1065–1079.

Rückauf, U., Augustin, J., Russow, R., Merbach, W., 2004. Nitrate removal from drained and reflooded fen soils affected by soil N transformation processes and plant uptake. Soil Biology and Biochemistry 36, 77–90.

Rustad, L.E., Campbell, J.L., Marion, G.M., Norby, R.J., Mitchell, M.J., Hartley, A.E., Cornelissen, J.H.C., Gurevitch, J., 2001. A meta-analysis of the response of soil respiration, net nitrogen mineralization, and aboveground plant growth to experimental ecosystem warming. Oecologia 126, 543–562.

Scherer, L.M., Palmborg, C., Prinz, A., Schulze, E.D., 2003. The role of plant diversity and composition for nitrate leaching in grasslands. Ecology 84, 1539–1552.

Schipper, L.A., Percival, H.J., Sparling, G.P., 2004. An approach for estimating when soils will reach maximum nitrogen storage. Soil Use and Management 20 (3), 281–286.

Shaw, M.R., Harte, J., 2001a. Response of nitrogen cycling to simulated climate change: differential responses along a subalpine ecotone. Global Change Biology 7, 193–210.

Shaw, M.R., Harte, J., 2001b. Control of litter decomposition in a subalpine meadow-sagebrush steppe ecotone under climate change. Ecological Applications 11 (4), 1206–1223.

Sirulnik, A.G., Allen, E.B., Meixner, T., Fenn, M.E., Allen, M.F., 2007. Changes in N cycling and microbial N with elevated N in exotic annual grasslands of southern California. Applied Soil Ecology 36, 1–9.

Stenger, R., Priesack, E., Beese, F., 1995. Rates of net nitrogen mineralization in disturbed and undisturbed soils. Plant Soil 171, 323–332.

Su, D.C., 1995. Study on new methods promoting availability of soil phosphorus and phosphate fertilizer on the basis of soil phosphorus distribution characteristics. Journal of Zhejiang University 3, 74–77.

Throop, H.L., Holland, E.A., Parton, W.J., Ojima, D., Keough, C., 2004. Effects of nitrogen deposition and insect herbivory on patterns of ecosystem level carbon and nitrogen dynamics: results from the century model. Global Change Biology 10, 1092–1105.

Tietema, A., Emmett, B.A., Gundersen, P., Kjonaas, O.J., Koopmans, C.J., 1998. The fate of 15N–labelled nitrogen deposition in coniferous forest ecosystem. Forest Ecology and Management 101, 19–27.

Turpault, M.P., Uterano, C., Boudot, J.P., Ranger, J., 2005. Influence of mature Douglas fir roots on the solid soil phase of the rhizosphere and its solution chemistry. Plant and Soil 275, 327–336.

Vitousek, P.M., Aber, J.D., Howarth, R.W., Likens, G.E., Matson, P.A., Schindler, D.W., Schlesinger, W.H., Tilman, D.G., 1997. Human alteration of the global nitrogen cycle: sources and consequences. Ecological Applications 7 (3), 737–750.

Vourlitis, G.L., Zorba, G., 2007. Nitrogen and carbon mineralization of semi-arid shrubland soil exposed to long-term atmospheric nitrogen deposition. Biology and Fertility of Soils 43 (5), 611–615.

Vourlitis, G.L., Zorba, G., Pasquini, S.C., Mustard, R., 2007. Chronic nitrogen deposition enhances nitrogen mineralization potential of semiarid shrubland soils. Soil Science Society of America Journal 71, 836–842.

Wang, Z.M., Song, K.S., Zhang, B., Liu, D.W., 2006. Analyses of features of agro-climatic changes in Songnen plain in the past 40 years. Chinese Agricultural Science Bulletin 22 (12), 241–246.

Wedin, D., Tilman, D., 1993. Competition among grasses along a nitrogen gradient: initial conditions and mechanisms of competition. Ecological Monographs 63, 199–229.

Xia, J.Y., Niu, S.L., Wan, S.Q., 2009. Response of ecosystem carbon exchange to warming and nitrogen addition during two hydrologically contrasting growing seasons in a temperate steppe. Global Change Biology 15, 1544–1556.

Xiao, C.L., Zhang, L.C., Zheng, J., 2005. Variation of geohydrological condition by prediction of numerical simulation for underground water in south area of Songnen plain. Jilin Water Resources 10, 1–6.

Zak, D.R., Holmes, W.E., MacDonald, N.W., Pregitzer, K.S., 1999. Soil temperature, metric potential, and the kinetics of microbial respiration and nitrogen mineralization. Soil Science Society of American Journal 63, 575–584.

Zhang, R., Brian, J.W., 2002. The effect of soil moisture on mineral nitrogen, soil electrical conductivity and pH. Nutrient Cycling in Agroecosystems 63 (1), 251–254.

Zhang, L.X., Bai, Y.F., Han, X.G., 2004. Differential responses of N: P stoichiometry of *Leymus chinensis* and *Carex korshinskyi* to N addition in a steppe ecosystem in Nei Mongol. Acta Botanica Sinica 46 (3), 259–270.

Zhang, W., Parker, K.M., Luo, Y., Wan, S., Wallace, L.L., Hu, S., 2005. Soil microbial responses to experimental warming and clipping in a tallgrass prairie. Global Change Biology 11, 266–277.

Zhang, N.L., Guo, J.X., Wang, X.Y., Ma, K.P., 2007. Soil microbial feedbacks to climate warming and atmospheric N deposition. Acta Phytoecologica Sinica 31 (2), 252–261.

Zhu, T.C., 2004. In: Yang-cao Biological Ecology. Jilin Science and Technology Press.