

Influence of climate warming and nitrogen deposition on soil phosphorus composition and phosphorus availability in a temperate grassland, China

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Abstract: Climate warming and nitrogen (N) deposition change ecosystem processes, structure, and functioning whereas the phosphorus (P) composition and availability directly influence the ecosystem structure under conditions of N deposition. In our study, four treatments were designed, including a control, diurnal warming (DW), N deposition (ND), and combined warming and N deposition (WN). The effects of DW, ND, and WN on P composition were studied by ³¹P nuclear magnetic resonance (³¹P NMR) spectroscopy in a temperate grassland region of China. The results showed that the N deposition decreased the soil pH and total N (TN) concentration but increased the soil Olsen-P concentration. The solution-state ³¹P NMR analysis showed that the DW, ND and WN treatments slightly decreased the proportion of orthophosphate and increased that of the monoesters. An absence of *myo*-inositol phosphate in the DW, ND and WN treatments was observed compared with the control. Furthermore, the DW, ND and WN treatments significantly decreased the recovery of soil P in the NaOH–EDTA solution by 17%–20%. The principal component analysis found that the soil pH was positively correlated with the P recovery in the NaOH–EDTA solution. Therefore, the decreased soil P recovery in the DW and ND treatments might be caused by an indirect influence on the soil pH. Additionally, the soil moisture content was the key factor limiting the available P. The positive correlation of total carbon (TC) and TN with the soil P composition indicated the influence of climate warming and N deposition on the biological processes in the soil P cycling.

Keywords: climate warming; nitrogen deposition; temperate grassland; ³¹P nuclear magnetic resonance spectroscopy

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Soil phosphorous (P) composition provides important information for grassland ecosystems, especially because P is often the growth-limiting nutrient when nitrogen (N) is not limited (Jouany et al., 2011). The soil organic phosphorus (P_o) can constitute 20% to 80% of the total phosphorus (TP) in the soil (Dalal, 1977; Dai et al., 1996). Although large concentrations of P_o persist in soils (Hawkes et al., 1984; Turner et al.,

2003a, b), its potential availability is determined by the type of soil P compounds (Turner et al., 2004), which suggests that a greater soil concentration of diesters could provide readily available P whereas a greater concentration of monoesters only provides with potentially available P. For example, orthophosphate diesters can be degraded in soil in a short time, whereas phytate is more stable and is difficult to min-

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eralize due to the low phytase activity in soil (Bowman and Cole, 1978). The soil P bioavailability in grassland ecosystems could be influenced by climate warming and N deposition because the P cycle is controlled by biochemical and biological processes (McGill and Cole, 1981).

Climate warming has caused an average global temperature increase of 0.74°C in the past 100 years (IPCC, 2007), and some studies have reported that this has led to an increase in the decomposition rate of soil organic carbon (Kirschbaum, 2000; Davidson and Janssens, 2006). Consequently, these biological processes could release more available inorganic P (P_i) in soils. In addition to the effect on P mineralization, elevated temperatures were reported to affect the soil P composition by decreasing the amount of monoester-P (Sumann et al., 1998). N deposition could also affect both the biological and biochemical mineralization of soil P. For example, N deposition was reported to increase the labile carbon mineralization via biological processes (Currey et al., 2010) and increase the biological P limitation via biochemical processes (Turner et al., 2003a; Hogan et al., 2010), which might influence the composition and availability of P. In addition, the availability of P was reported to play an important role in regulating species diversity under conditions of N deposition (Limpens et al., 2004). The single factor of either warming or N deposition might influence the soil P composition and availability, thus the combination of both was also considered as a manipulated factor.

To investigate the influence of climate warming and N deposition on ecosystem processes, structure, and functioning, Niu and Wan (2008) established a field trial in Inner Mongolia of China in 2006 and found that warming significantly increased the soil temperature by 1.9°C and suppressed the competitive ability of a C_4 grass while increasing that of a C_3 forb and a C_3 grass. The effect of warming on carbon (C) sequestration was insignificant whereas the N deposition stimulated the exchange of C in the ecosystem by increasing the C assimilation process during the wet growing season (Xia et al., 2009).

The effects of warming and N deposition on N use efficiency, C sequestration, and species diversity were

extensively studied (An et al., 2005; Niu and Wan, 2008; Xia et al., 2009), but little information can be found about the soil P composition and P availability in temperate grassland soils under the condition of warming, N deposition and their combination. We hypothesize that climate warming, N deposition or their combination would influence soil P availability (Olsen-P) and soil P composition by changing the concentrations of different P fractions such as phosphonates, orthophosphates, orthophosphate monoesters, orthophosphate diesters, pyrophosphates and polyphosphates.

1 Materials and methods

1.1 Study area

The study area is located in Duolun county, Inner Mongolia, China (42°20'N, 116°17'E; 1,324 m asl). The mean annual precipitation is 385 mm, with 86% of the rainfall occurring from May to September. The annual mean temperature is 2.1°C, with monthly averages ranging from -17.5°C in January to 18.9°C in July. The soil is classified as Haplic Calcisol according to the Food and Agricultural Organization of the United Nations (FAO classification), with 63% sand, 20% silt, and 17% clay (Niu and Wan, 2008). The flora was consistent throughout the area and was dominated by *Stipa krylovii*, *Artemisia frigida*, *Potentilla acaulis*, *Cleistogenes squarrosa*, *Allium bidentatum*, and *Agropyron cristatum*. The study area has been overgrazed since the 1980s and has experienced severe soil degradation and nutrient loss. The field was fenced in 2001 to prohibit grazing for ecological restoration.

1.2 Experimental design

Four treatments were conducted in this study: a control, diurnal (24-h) warming (DW), N deposition (ND), and diurnal warming with N deposition (WN). Each treatment was replicated in six 3 m×4 m plots according to a complete random block design. Three-meter buffer zones separated each plot. The plots were warmed using MSR-2420 infrared radiators (Kalglo Electronics, Bethlehem, Pennsylvania, USA) hung at a height of 2.25 m and set to 1,600 W. Inactivated “dummy” heaters (i.e. non-functional heaters identical to the one used

to treat the 'warming' plots) were installed over the control plots to duplicate the shading effect. The experimental plots were established in September 2005 and the simulated warming experiment was initiated on 23 April 2006. The heaters were kept off from 15 November 2007 to 14 March 2008 and from 1 December 2008 to 1 March 2009 because the station was closed during deep winters. For the ND treatment, urea (10 g N/m²) was applied for the first year in September 2006, and NH₄NO₃ (10 g N/m²) was applied instead of urea on 19 July during the following years.

1.3 Soil properties and sampling

In August 2009, soil cores were sampled at a depth of 15 cm; each sample was composed of three cores per plot. The fresh samples were sieved (<2 mm) and preserved in a refrigerator at 4°C. The subsamples were air-dried at ambient temperature for at least one week and were stored prior to the chemical analysis.

The soil water content was measured gravimetrically after drying at 105°C for 24 h. The soil pH was measured using a glass electrode (soil/water ratio of 1.0:2.5). The total carbon (TC) and total nitrogen (TN) were determined using an automatic element analyzer (Vario EL, Elementar, Germany). The Olsen P was extracted using 0.5 M NaHCO₃ at a pH of 8.5 (Olsen et al., 1954); the P in the filtrate was then determined colorimetrically by the molybdate method (Murphy and Riley, 1962). TP was digested from 2.0 g of the air-dried soil (<0.15 mm) in 30 mL of 70% perchloric acid, and the P was measured colorimetrically (Kuo, 1996).

1.4 Soil P composition with solution-state ³¹P NMR spectroscopy

The soil P composition was determined using solution-state ³¹P NMR spectroscopy. Briefly, the air-dried soil (<2 mm, 2.5 g) was shaken in 50 mL of a 0.25 M NaOH–0.05 M Na₂EDTA solution at 20°C for 16 h. The extracts were centrifuged at 10,000g for 30 min, and 20 mL aliquots from the six replicates were combined into a composite extract for each treatment, and the composite extracts were then frozen and lyophilized. The total P in solution was determined using inductively coupled plasma-optical emission spectroscopy (ICP–OES; PE OPTIMA 3000). The P re-

covery was calculated by comparing the TP in the NaOH–EDTA extracts with the soil TP.

Approximately 200 mg of the freeze-dried extracts were redissolved in 0.1 mL of 10 M NaOH and 0.5 mL D₂O and then decanted into 5-mm NMR tubes. The solution-state ³¹P NMR spectra were acquired at 24°C using a JEOL ECA 600 spectrometer operating at 243 MHz with a 45° pulse width (5.2 μs pulse), an acquisition time of 0.77 s (50×10⁻⁶ window centered at 0×10⁻⁶), a delay time of 1.0 s, broadband proton decoupling, and 26,000 scans. The chemical shifts were determined in relation to external 85% H₃PO₄ (at δ=0). All of the spectral results were processed with the NMR Utility Transform Software (NUTS) for Windows (Acorn NMR, Livermore, CA), and the peaks were assigned by referring to the literature (Turner et al., 2003c; Turner, 2007; Zhang et al., 2012). The amounts of the different forms of P were calculated by multiplying the percentage of the integrated area of each assigned peak to the total integration area by the TP extracted with NaOH–EDTA.

1.5 Statistical analyses

The results were presented as the mean±SE (standard error, *n*=6). The differences among the treatments were tested using the one-way ANOVA in SPSS 11.5 for Windows (SPSS Inc., Chicago, IL). After determining that the within-group variance was equal across the groups associated with each mean, Tukey's method was used to perform a mean separation test and was considered significant at a 5% level (*P*<0.05). A detrended correspondence analysis was applied to determine the lengths of the P composition gradients and the soil properties, which showed that the lengths of the gradients were less than 3. A principal components analysis (PCA) was then applied to identify the effects of climate warming and N deposition on the soil properties, P recovery and P composition using Canoco Software 4.5 (Microcomputer Power, USA).

2 Results

2.1 Soil properties

The soil pH values ranged from 6.7 to 7.3 and decreased significantly under the treatments with N deposition (ND and WN) after 5 years of N applica-

tion (Table 1). The TC concentrations were not affected by warming and N deposition. The ND treatment significantly decreased the soil TN compared with the control, and no differences in soil TP were found among the treatments. The Olsen-P concentration ranged from 14.4 to 23.6 mg/kg soil, with the highest value found for the ND treatment. The tested soils had low soil moisture contents, with even the fresh samples containing only 5.7%–6.5% water, and no significant differences among treatments were found (Table 1).

2.2 Soil P composition

The solution-state ^{31}P NMR spectra with 10 Hz line broadening are shown in Fig. 1. The strong signals at approximately 6.7×10^{-6} were assigned to inorganic orthophosphate, with concentrations ranging from 32 to 50 mg P/kg soil (Table 2). Pyrophosphate was identified at approximately -3.1×10^{-6} , ranging from 2.8 to 5.2 mg P/kg soil (Table 2).

The orthophosphate monoesters were dominant in every treatment, ranging from 104 to 139 mg P/kg soil (72%–75% of extracted P). Only the control showed the *myo*-inositol hexakisphosphate signal with a distinct C-2 position at 6.0×10^{-6} at a concentration of 11 mg P/kg soil (Table 2). The signal for *scyllo*-inositol hexakisphosphate can be identified at approximately 4.1×10^{-6} , with concentrations ranging from 5.5 to 7.5 mg P/kg soil. No orthophosphate diesters, such as DNA or phospholipids, were found in this study (Table 2).

Warming and N deposition markedly decreased the P recovery, slightly decreased the content of soil orthophosphate and increased that of monoesters.

2.3 The PCA of soil properties and P composition

The results of the PCA indicated that Factors 1 and 2 accounted for 82.8% and 14.3% of the total variance, respectively (Fig. 2). Factor 1 was related to the soil moisture content and Factor 2 was related to the soil

Table 1 pH, TC, TN, TP, Olsen-P and moisture content for soils treated for 4 years with four different treatments

Treatment	pH	TC	TN	TP	Olsen-P	Moisture content (%)
		g/kg				
Control	7.2±0.03a	24.9±1.15a	2.01±0.09a	335±19a	16.3±1.8b	6.5 ± 0.59a
DW	7.3±0.05a	24.0±1.12a	1.82±0.07a	335±7.2a	17.4±1.2b	5.7 ± 0.43a
ND	6.7±0.07b	22.6±1.04a	1.70±0.09b	373±23a	23.6±3.2a	5.7 ± 0.23a
WN	6.7±0.05b	22.8±0.74a	1.77±0.04b	310±23a	14.4±1.1b	5.8 ± 0.59a

Note: DW, diurnal warming; ND, N deposition; WN, diurnal warming with N deposition. Results were presented as mean±standard error. Values within one column with the same letter are not significant at 5% level.

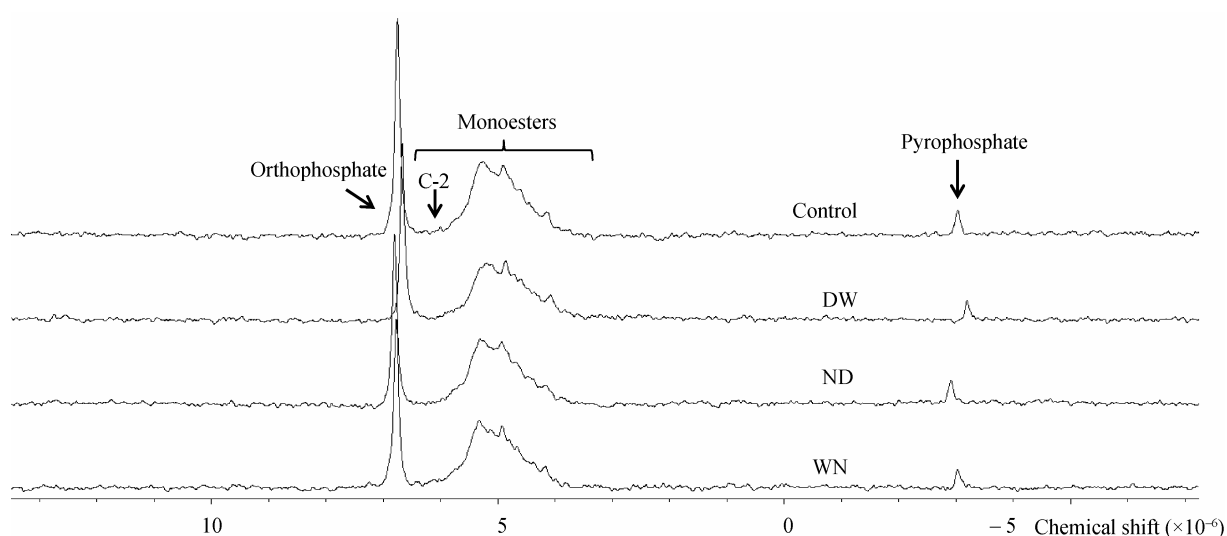


Fig. 1 Solution ^{31}P NMR spectra of NaOH-EDTA of soil extracts in a temperate grassland, China

Table 2 Soil phosphorus concentration (mg P/kg soil) in NaOH–EDTA extracts determined by solution ^{31}P NMR spectroscopy

Treatment	Total P*	Inorganic P		Organic P		
		Orthophosphate	Pyrophosphate	Orthophosphate monoesters		
				Total mono- esters	<i>myo</i> -inositol hexakisphos- phate	<i>scyllo</i> -inositol hexakisphos- phate
Control	194 (58)	50 (26%)	5.2 (2.7%)	139 (72%)	11 (5.9%)	6.9 (3.6%)
DW	140 (41)	32 (23%)	2.8 (2.0%)	104 (75%)	nd	7.5 (5.3%)
ND	141 (38)	32 (23%)	4.5 (3.2%)	104 (74%)	nd	5.5 (3.9%)
WN	143 (47)	33 (23%)	3.0 (2.1%)	106 (74%)	nd	6.0 (4.2%)

Note: DW, diurnal warming; ND, N deposition; WN, diurnal warming with N deposition. nd, not detected; values in parentheses indicate the percentage of the total extracted P; * indicates the values in parentheses stand for recovery of extracted TP from soil TP.

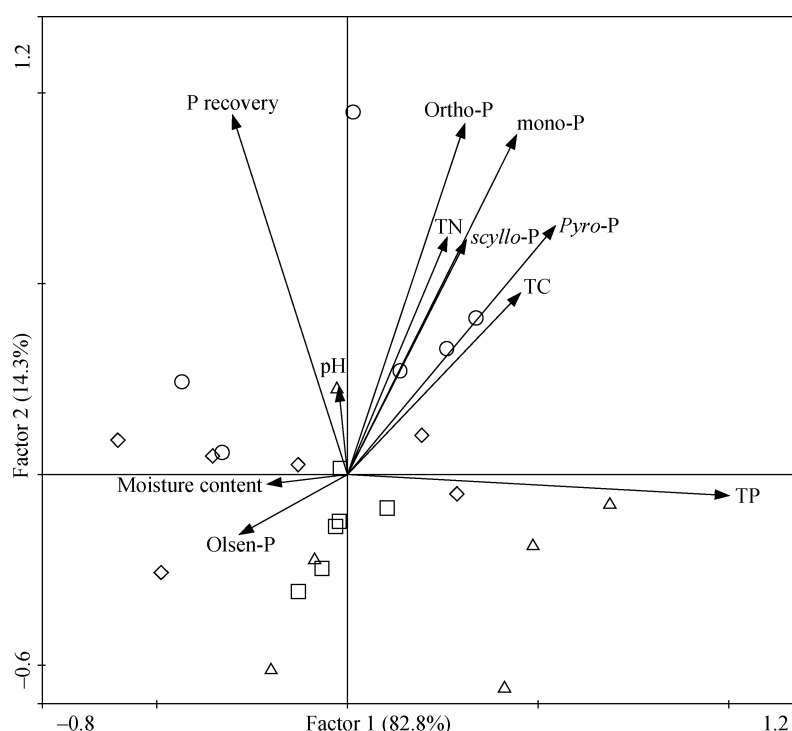


Fig. 2 Principal components analysis (PCA) of soil moisture content, pH, total carbon (TC), total nitrogen (TN), total phosphorus (TP), Olsen-P, orthophosphate (Ortho-P), monoesters (mono-P), *scyllo*-inositol hexaphosphate (*scyllo*-P), and pyrophosphate (*pyro*-P) for soils treated for 4 years with 1) control (○); 2) diurnal (24-h) warming (DW, □); 3) nitrogen deposition (ND, △); 4) diurnal (24-h) warming with N deposition (WN, ◇).

pH. Specifically, the soil P composition and the P recovery in the NaOH–EDTA extract increased with the soil pH, which could be influenced by the N deposition. The soil Olsen-P concentration was positively correlated with the soil moisture content. The results also showed that the soil TC and TN were positively correlated with the soil P composition, which included orthophosphate, monoesters, pyrophosphates and *scyllo*-inositol hexaphosphate.

3 Discussion

3.1 Soil properties

The results of the one-way ANOVA showed that soil pH (sampled to a 15-cm depth) decreased due to the acidification resulting from N fertilization ($P < 0.05$), which has been extensively reported (Guo et al., 2010; Li et al., 2010; Malhi et al., 2011) and regarded to be

connected with the release of H^+ ions during the nitrification process (Bolan et al., 1991). This study determined that N addition had no effect on TC, which was similar to the findings of another report regarding the TC change in a semi-arid grassland (Li et al., 2010). In comparison with our study, many researchers have reported an increase in the TC concentration with soil fertilization. For example, Nyborg et al. (1999) showed that 13 years of N and sulfur fertilization increased the soil TC. Tiemann and Billings (2011) found that after 7 years of N fertilization, the TC concentrations were higher than those in unfertilized plots. The reason for this discrepancy could be that, in this study, the decomposition rate of TC was nearly the same as the accumulation rate of TC in the semi-arid grassland after the addition of N. The soil TN decreased under the ND and WN treatments ($P < 0.05$), which could be attributed to the enhanced plant uptake of N from the soil by the stimulation from N fertilization (Xia et al., 2009). The higher Olsen-P concentration under the ND treatment could be caused by higher P concentration from dead microbes in the air-dried samples (Turner and Haygarth, 2001), as the soil microbial biomass could increase with the addition of N (Johnson et al., 1998). It is also possible that the decreased soil pH under N addition resulted in fewer exchangeable cations and thus decreased the sorption of soil P to cations (Zhang et al., 2013). The PCA showed that the soil moisture content was positively correlated with the Olsen-P concentration, which indicated that the exchangeable P (Olsen-P) was more influenced whereas the adsorbed P (other P fractions) was less influenced by the soil moisture.

3.2 Soil P composition

The orthophosphate monoesters were the dominant P fraction in this study (72%–75% of the total extracted P), which is in agreement with previous studies on grassland soils (McDowell et al., 2005; Murphy et al., 2009). Under the warming and N deposition treatments, the proportion of monoesters increased while the proportion of orthophosphate decreased. In contrast with our study, Sumann (1998) reported that the proportion of monoesters decreased due to higher microbial activities at cooler temperatures. In addition,

Turner et al. (2003a) reported an increase in the orthophosphate proportion under N deposition conditions. These different results could be attributed to the soil moisture content, which was reported to influence microbial activities and phosphatase activities (Donnelly et al., 1990; Turner and Romero, 2010). A greater percentage of orthophosphate monoesters suggests lower environmental risks under warming and N deposition treatments, as the monoesters are easily adsorbed by soil cations and thus decrease the mobility in soil (Celi and Barberis, 2007). No orthophosphate diesters were found in this study, which could be due to the neutral pH and the drier condition of the soils. It has been shown that soil diesters such as DNA accumulate in acidic soils with soil pH less than 5 (Turner and Engelbrecht, 2011). Furthermore, moist soils contain higher concentrations of diesters than drier soils (Guggenberger et al., 1996; Sumann et al., 1998). The positive relationship of TC and TN with soil P fractions indicated that the biological process primarily influenced soil P composition under warming and N deposition treatments. Furthermore, the acid phosphatase, alkaline phosphatase and phosphodiesterase activities in the soil were not affected by warming and N deposition (data not shown), ruling out the biochemical influences of warming and N deposition. The positive effect of the ND treatment on P availability showed no biological P limitations in this area.

Generally, the control contained more orthophosphate and monoesters than the other three treatments, and this result was due to the difference in the total P recovery rate in the NaOH–EDTA solution. There are few reports about the differences in P recovery caused by management methods such as N deposition and environmental warming. Turner et al. (2003b) showed that the P recovery in the NaOH–EDTA solution could increase in a warm and moist climate; however, it could be reduced in semi-arid or arid areas (Turner et al., 2003d). This study indicates that warming, N deposition and their interactions decreased soil P recovery by 17%–20%. The PCA indicated that this may result from the indirect effects of the DW, ND and WN treatments on soil pH.

4 Conclusion

Warming and N addition could indirectly influence the soil P recovery in NaOH–EDTA solution and the soil P composition by changing the soil pH and soil moisture content. The recovery of soil P markedly decreased (17%–20%) under the DW, ND and WN treatments compared with the control. The solution-state ^{31}P NMR spectroscopy analysis showed that the P composition in the NaOH–EDTA extracts was dominated by phosphomonoesters (72%–75%), followed by orthophosphate (23%–26%), and contained small amounts of pyrophosphate. *Scyllo*-inositol hexakisphosphate was found in all treatments, whereas only the control treatment contained *myo*-inositol hexakisphosphate. Apart from soil pH, the effect of warming and N deposition on soil moisture had an important influence on soil P composition. Therefore, further research should be conducted regarding the long-term effect of warming, N deposition and other environmental factors such as water stress on soil P availability and *myo*-inositol hexakisphosphate.

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