



Impacts of Climate Warming on Soil Phosphorus Forms and Transformation in a Tibetan Alpine Meadow

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Abstract

The alpine meadow ecosystem in Tibetan Plateau is highly sensitive and vulnerable to climate change. The impact of climate warming on soil phosphorus (P) forms and transformation remains poorly understood. Here, a 5-year field experiment investigated the influences of warming on soil inorganic P (Pi) and organic P (Po) forms in an alpine meadow of the Tibetan Plateau. The field experiment included three treatments: unwarmed control (CK), winter warming (WW), and year-round warming (YW). Open top chambers were used to impose warming and increased average soil temperature at 5 cm depth by 1.3 °C compared with CK. Chemical fractionation and solution ³¹P nuclear magnetic resonance (NMR) spectroscopy were used to speciate P forms. Compared with CK, total P concentration decreased by 11.7% ($P < 0.05$) and 2.46% ($P > 0.05$) while available P concentration increased by 19.1% and 12.7% ($P < 0.05$) in WW and YW, respectively. Chemical fractionation showed that water (H₂O)- and bicarbonate (NaHCO₃)-extractable Pi concentrations were higher, while sodium hydroxide (NaOH)- and hydrochloric acid (HCl)-extractable Pi and H₂O-, NaHCO₃-, NaOH-, and HCl-extractable Po concentrations were lower in WW and YW than in CK. ³¹P NMR indicated that orthophosphate concentration was 12.4% and 5.54% ($P > 0.05$) higher, while pyrophosphate, orthophosphate monoesters, and orthophosphate diester concentrations were lower in WW and YW than in CK. Acid phosphomonoesterase was a significant factor in shaping soil P forms and transformation. Short-term warming can mitigate P limitation by promoting the transformation of moderately labile and stable Pi fractions and various forms of Po to bioavailable P. This study provides novel insights into understanding terrestrial ecosystem P cycling in response to future climate change.

Keywords Experimental warming · Soil phosphorus availability · Chemical fractionation · Solution ³¹P NMR · Inceptisol · Alpine meadow

1 Introduction

According to the Synthesis Report from the Intergovernmental Panel on Climate Change (IPCC), average global surface temperature (GST) has risen approximately 0.72 °C from 1951 to 2012. It was forecasted that GST may increase by another 0.3 °C to 0.7 °C by 2035 (Pachauri et al. 2014). Rising temperature has profound impact on nutrient cycling in terrestrial ecosystems (Rolph 2003; Ren et al. 2018; Gong et al. 2020). The behaviors of nutrients (e.g., nitrogen, phosphorus, and sulfur) depend on the organic and inorganic forms that exist in terrestrial ecosystems. Therefore, understanding the responses of nutrient forms to soil temperatures is necessary to project nutrient cycling processes in the context of future climate change.

Phosphorus (P) is an essential and crucial limiting nutrient for plant growth in terrestrial and aquatic ecosystems

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(Hou et al. 2018a; Zhang 2021). In general, P exists in two pools in soil, i.e., inorganic P (Pi) and organic P (Po). By means of chemical fractionation method, Pi and Po can be sequentially divided into H_2O -, NaHCO_3 -, NaOH -, and HCl -extractable fractions. But, chemical fractionation assigns P fractions based on their solubility in different extracting agents and consequently cannot specify P forms in these fractions (Hashimoto and Watanabe 2014). By contrast, solution ^{13}P NMR spectroscopy on alkaline extracts is a powerful technique to identify and quantify P forms at the molecular level (Zhang et al. 2019a, b). But, solution ^{31}P NMR technique is limited by the requirement of an alkaline extraction procedure that may chemically alter P forms in soil samples (Schmieder et al. 2018). Therefore, the combined use of chemical sequential fractionation and solution ^{31}P NMR spectroscopy can provide a better understanding on soil P forms and transformation.

The Tibetan Plateau, located in northwest China and referred to as the third pole of the earth, is considered to be an ecologically fragile area and sensitive to global climate change (Yao et al. 2012; Wang 2016). Observations and simulation results demonstrated that the Plateau has undergone significant warming over recent decades, with rates of warming ranging between 0.16 and 0.67 °C decade⁻¹ since the 1950s (Kuang and Jiao 2016). The rate of warming on the Plateau exceeds that of the northern hemisphere and the global average because of the high altitude (average exceeding 4000 m above sea level) in the region (Bai et al. 2013). Furthermore, warming was stronger in winter than the other seasons on the Plateau (Zhang et al. 2019b). Alpine grassland on the Tibetan Plateau, which covers more than 60% of the entire Plateau, is one of the largest regions of alpine grassland globally (Yan and Lu 2020). Several previous studies have focused on the influences of climate warming on soil carbon (C) and nitrogen (N) cycling in the Tibetan alpine grassland ecosystems (Chen et al. 2020). However, little attempt has been made to explore soil P forms and transformation under warming in alpine grassland ecosystems, which has limited our understanding of how P cycling may respond to future climate change in this area.

Phosphorus (P) is an essential and crucial limiting nutrient for plant growth in terrestrial and aquatic ecosystems (Hou et al. 2018a; Zhang 2021). In general, P exists in two pools in soil, namely, inorganic P (Pi) and organic P (Po). By means of chemical fractionation, Pi and Po can be sequentially divided into H_2O -, NaHCO_3 -, NaOH -, and HCl -extractable fractions. However, chemical fractionation assigns P fractions based on their solubility in different extracting agents and consequently cannot specify what form of P is present in these fractions (Hashimoto and Watanabe 2014). By contrast, solution ^{13}P NMR spectroscopy on alkaline extracts is a powerful technique to identify and quantify P forms at the molecular level (Zhang et al. 2019a). The

solution ^{31}P NMR technique is limited by the requirement of an alkaline extraction procedure that may chemically alter P forms in soil samples (Schmieder et al. 2018). Therefore, the combined use of chemical sequential fractionation and solution ^{31}P NMR spectroscopy may provide a better understanding of soil P forms and transformations.

Alpine meadow, covering approximately 35% of the Plateau area (Yang and Sun 2021), is especially sensitive to global climate change (Jiang et al. 2016). The main aims of this study were to evaluate the effects of winter and year-round warming on soil P forms and transformations in an alpine meadow of the Tibetan Plateau by using a combination of chemical fractionation and solution ^{31}P NMR spectroscopy. The key factors driving soil P forms and transformations were also explored. We hypothesized that warming will affect the forms of P present in soil and their transformations by altering P input via plant litterfall, due to a decline in plant aboveground biomass (AGB) under warming conditions (Guan et al. 2018). Furthermore, we hypothesized that warming will promote soil Po mineralization by increasing soil phosphatase activity.

2 Materials and Methods

2.1 Study Area, Experimental Design, and Soil Sampling

The passive warming experiment was performed in a fenced *Kobresia*-dominated alpine meadow in the Tibetan Plateau, at the Damxung Grassland Station, located at latitude 30.85°N, longitude 91.08°E, and an altitude of 4333 m. Average annual temperature and precipitation are 1.3 °C and 477 mm, respectively. The growing season is between May and September, and the remainder of the year is referred to as the freezing season (winter). The soil type is Typical Matti-Gelic Cambosols according to the Chinese Soil Taxonomy (Cooperative Research Group on Chinese Soil Taxonomy (CRGCST) 2001). At the beginning of this experiment in July 2010, some basic soil properties at 0–15 cm depth were as follows: pH, 6.95; soil organic C (SOC), 11.3 g kg⁻¹; total N, 1.20 g kg⁻¹; sand, 67.0%; silt, 18.2%; and clay, 14.7%.

The field experiment was installed in a randomized complete block design with five replications. Three treatments were included in this study: (1) unwarmed control (CK), (2) winter warming (WW), and (3) year-round warming (YW). Open top chambers (OTCs) chosen by the International Tundra Experiment were used as warming devices. The OTCs in YW plot were installed in the field throughout the duration of this experiment, while those in WW were only installed in the field during the freezing season (from late September of each year to early May of the following year). Soil

temperature was synchronously recorded at 30 min intervals using a HOBO weather station (Onset, Bourne, MA, USA). The monitoring data during the period from June 2012 to May 2015 demonstrated that average soil temperature at a depth of 5 cm increased by 1.3 °C in WW and YW compared with CK.

After 5 years of warming, soil samples at 0–20 cm depth were collected as five 5-cm-diameter cores from each plot and later homogenized into one composite sample on 26 June 2015. The samples were sieved through a 2-mm screen. More details about the field experiment and soil sampling have been reported elsewhere (Guan et al. 2018). Soil moisture, SOC, total N, and phosphatase activity were determined according to the procedures recommended (Lu 2000), and the results are listed in Table S1.

2.2 Soil Analysis

Soil total P and available P were extracted using concentrated H₂SO₄-HClO₄ digestion and 0.5 M NaHCO₃, respectively (Olsen and Sommers 1982). Before and after ignition (550 °C, 1 h) of soil samples, Po was extracted using 1.0 M H₂SO₄ and calculated as difference of P concentration in acid extracts between ignited and non-ignited samples (Walker and Adams 1958). All P concentrations in these extracts were measured colorimetrically by ammonium molybdate and ascorbic acid (Murphy and Riley 1962).

Soil chemical P fractionation was conducted following a modified Hedley (Hedley et al. 1982) sequential extraction method (Teng et al. 2020). Specifically, soil samples (1.0 g) were sequentially extracted with 30 mL of H₂O, 0.5 M NaHCO₃ (pH 8.5), 0.1 M NaOH, and 1.0 M HCl at room temperature for 16 h. Total P concentrations in the sequentially extracted fractions were measured through digesting in an autoclave (121 °C) with H₂SO₄ and (NH₄)₂S₂O₈. Pi concentration was measured colorimetrically (Murphy and Riley 1962), and Po concentration was calculated as the difference between total P and Pi concentration (Tiessen and Moir 1993).

Solution ³¹P NMR analysis was performed following the procedures of Cade-Menun and Preston (1996). Briefly, air-dried soil samples (4 g) were extracted by 0.25 M NaOH-0.05 M EDTA solution (40 ml) at 20 °C for 16 h. Total P concentrations in aliquots of extracts were tested with an iCAP6300 inductively coupled plasma-optical emission spectrometer (Thermo Fisher Scientific, Waltham, MA, USA). The residual extracts were lyophilized, and the freeze-dried samples (approximate 300 mg) were then re-dissolved in 0.25 M NaOH (1 ml) and mixed with D₂O (0.05 ml) for NMR measurement. The spectra were collected with AVANCE III HD 500 spectrometer (Bruker Biospin, Fällanden, Switzerland) equipped with a 5-mm broadband probe and operated at 202.5 MHz, using 13.0 μs pulse width,

3.0 s delay time, 0.4 s acquisition time, and 15,000 scans. The obtained NMR spectra were analyzed with MestReNova 5.3.1 software (Mestrelab Research, Santiago de Compostela, Spain). Chemical shifts were assigned according to spiking experiments with *myo*-inositol hexakisphosphate (IHP), α-glycerophosphate, β-glycerophosphate, adenosine 5′ monophosphate, phosphocholine, α-D-glucose 1-phosphate, and D-glucose 6-phosphate (Sigma, St. Louis, MO, USA) and previously published literatures (McDowell et al. 2007; Doolette et al. 2009; Cade-Menun 2015). The concentration of each identified compound was acquired through multiplying the percentage of the integrated area of the corresponding compound to total integration area by NaOH-EDTA extracted total P concentration. The orthophosphate monoesters and diesters were corrected by moving α- and β-glycerophosphate and mononucleotides from monoesters into diesters (He et al. 2011).

2.3 Statistical Analysis

To detect whether warming had statistically significant impacts on soil total P and P forms after 5 years of field experiment, one-way analysis of variance (ANOVA) followed by Fisher's least significant difference (LSD) test ($P < 0.05$) was applied when data followed normal distribution (Shapiro-Wilks test, $P > 0.05$) and variances were homogenous (Levene test, $P > 0.05$). When necessary, data were square root- or log-transformed and retested. The relationships between P forms from sequential chemical fractionation and from solution ³¹P NMR spectroscopy, as well as relationships between P forms and environmental factors (AGB, soil moisture, SOC, total N, C/N ratio, C/P ratio, N/P ratio, and phosphatase activity), were assessed by Pearson correlation at $P < 0.05$ and $P < 0.01$. Statistical analyses were performed using SPSS 16.0 for Windows (SPSS Inc., Chicago, Illinois, USA).

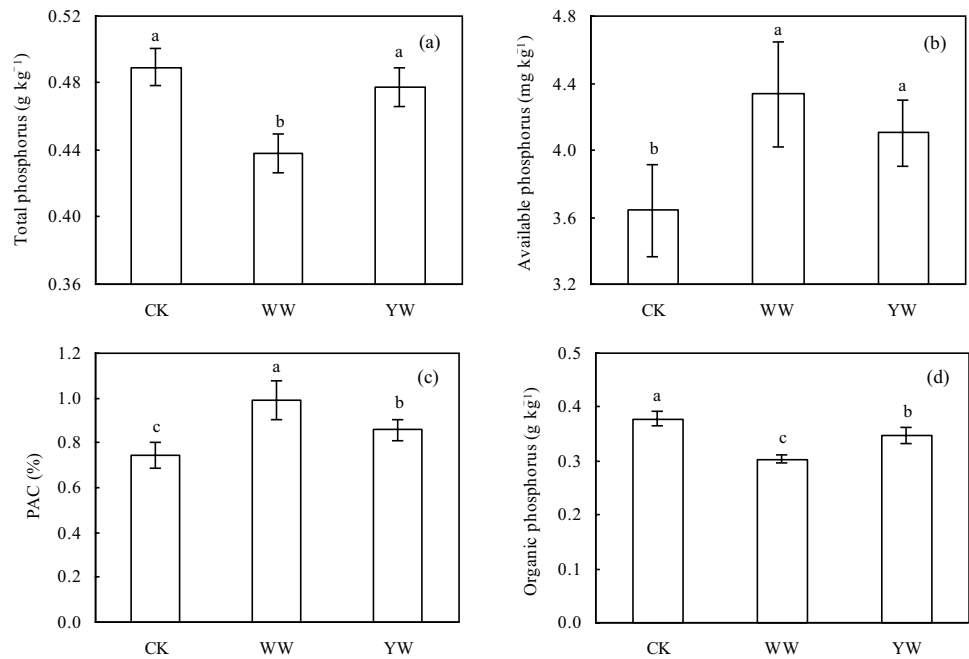
Redundancy analysis (RDA) was conducted to detect the main environmental factors affecting soil total P and P forms with Canoco 5.0 for Windows (Microcomputer Power, Ithaca, New York, USA). The significance level of explanatory environmental factors was evaluated by Monte Carlo permutation test (number of permutations: 499) at $P < 0.05$.

3 Results

3.1 Soil Total Phosphorus, Available Phosphorus, and Organic Phosphorus Concentrations

After 5 years of warming, total P concentration decreased by 11.7% ($P < 0.05$) in WW and 2.46% ($P > 0.05$) in YW compared with CK. Furthermore, total P concentration was 9.02% ($P < 0.05$) lower in WW than in YW (Fig. 1a). In

Fig. 1 Total phosphorus (a), available phosphorus (b), phosphorus activity coefficient (PAC) (c), and organic phosphorus (d) in soil under unwarmed control (CK), winter warming (WW), and year-round warming (YW)



contrast, available P concentration was 19.1% and 12.7% ($P < 0.05$) higher in WW and YW compared to CK, respectively, and available P concentration was 5.65% ($P > 0.05$) higher in WW than in YW (Fig. 1b). The phosphorus activity coefficient (PAC, i.e., percentage of available P to total P) ranged from 0.74 to 0.99% and significantly increased in the order of CK < YW < WW (Fig. 1c). The Po concentration, accounting for 69.3–77.2% of total P, significantly decreased in the order of CK > YW > WW (Fig. 1d).

3.2 Soil Phosphorus Forms by Chemical Sequential Fractionation

Among the sequentially extracted fractions, total P concentration in the NaOH fraction was the largest (197–208 mg kg⁻¹), followed by HCl (34.5–40.5 mg kg⁻¹) and NaHCO₃ (13.2–13.7 mg kg⁻¹) fractions, and the lowest total P concentration was in the H₂O fraction (3.00–3.85 mg kg⁻¹). After 5 years of warming, total P concentrations of all sequentially extracted fractions were lower in WW and YW compared to CK, with significant differences between CK with WW for H₂O-P and NaOH-P and between CK with WW and YW for HCl-P. Moreover, total P concentrations of these sequentially extracted fractions were lower in WW than in YW, but the differences were not significant (Fig. 2a and b).

The concentrations of Pi fractions were in the order HCl-Pi > NaOH-Pi > NaHCO₃-Pi > H₂O-Pi. Compared with CK, H₂O-Pi concentration increased by 41.2% ($P < 0.05$) and 9.16% ($P > 0.05$), and NaHCO₃-Pi concentration increased by 8.99% ($P < 0.05$) and 3.75% ($P > 0.05$) in WW and YW,

respectively. By contrast, NaOH-Pi concentration was 0.70% and 3.95% ($P > 0.05$) lower and HCl-Pi concentration was 16.6% and 12.5% ($P < 0.05$) lower in WW and YW compared to CK, respectively. Although there were no significant differences between WW and YW for all Pi fractions, H₂O-Pi, NaHCO₃-Pi, and NaOH-Pi concentrations were 29.4%, 5.05%, and 3.23% higher and HCl-Pi concentration was 3.62% lower in WW than in YW (Fig. 2c and d).

The concentrations of Po fractions were in the order NaOH-Po > NaHCO₃-Po > HCl-Po > H₂O-Po. Relative to CK, H₂O-Po concentration decreased by 57.5% and 17.1% ($P < 0.05$), NaHCO₃-Po concentration decreased by 13.9% ($P < 0.05$) and 7.17% ($P > 0.05$), NaOH-Po concentration decreased by 6.34% ($P < 0.05$) and 2.53% ($P > 0.05$), and HCl-Po concentration decreased by 24.7% and 6.48% ($P > 0.05$) in WW and YW, respectively. Furthermore, H₂O-, NaHCO₃-, NaOH-, and HCl-Po concentrations were 34.5% ($P < 0.05$), 6.26% ($P > 0.05$), 3.72% ($P > 0.05$), and 17.1% ($P > 0.05$) lower in WW than in YW, respectively (Fig. 2e and f).

3.3 Soil Phosphorus Forms by Solution ³¹P NMR Spectroscopy

The NMR spectra were composed of Pi and Po compounds. The Pi compounds included orthophosphate and pyrophosphate, and Po compounds comprised orthophosphate monoesters and diesters (Fig. 3). In the orthophosphate monoesters, *myo*-IHP, *scyllo*-IHP, α -glycerophosphate, β -glycerophosphate, mononucleotides, and phosphocholine were identified, and the remaining unidentified monoesters

Fig. 2 Phosphorus forms including total phosphorus (P) (a, b), inorganic phosphorus (Pi) (c, d), and organic phosphorus (Po) (e, f) by chemical sequential fractionation with H₂O, NaHCO₃, NaOH, and HCl in soil under unwarmed control (CK), winter warming (WW), and year-round warming (YW)

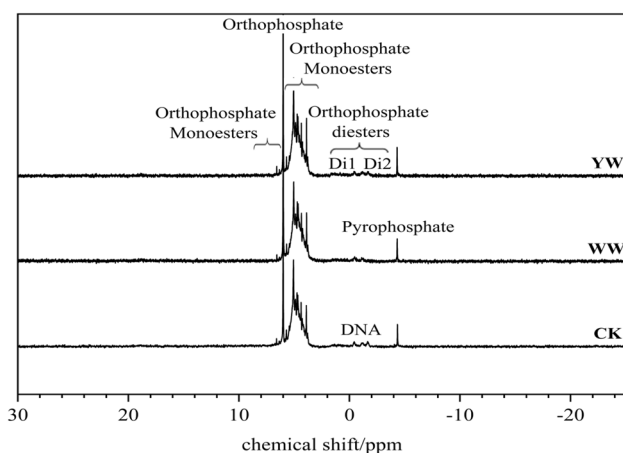
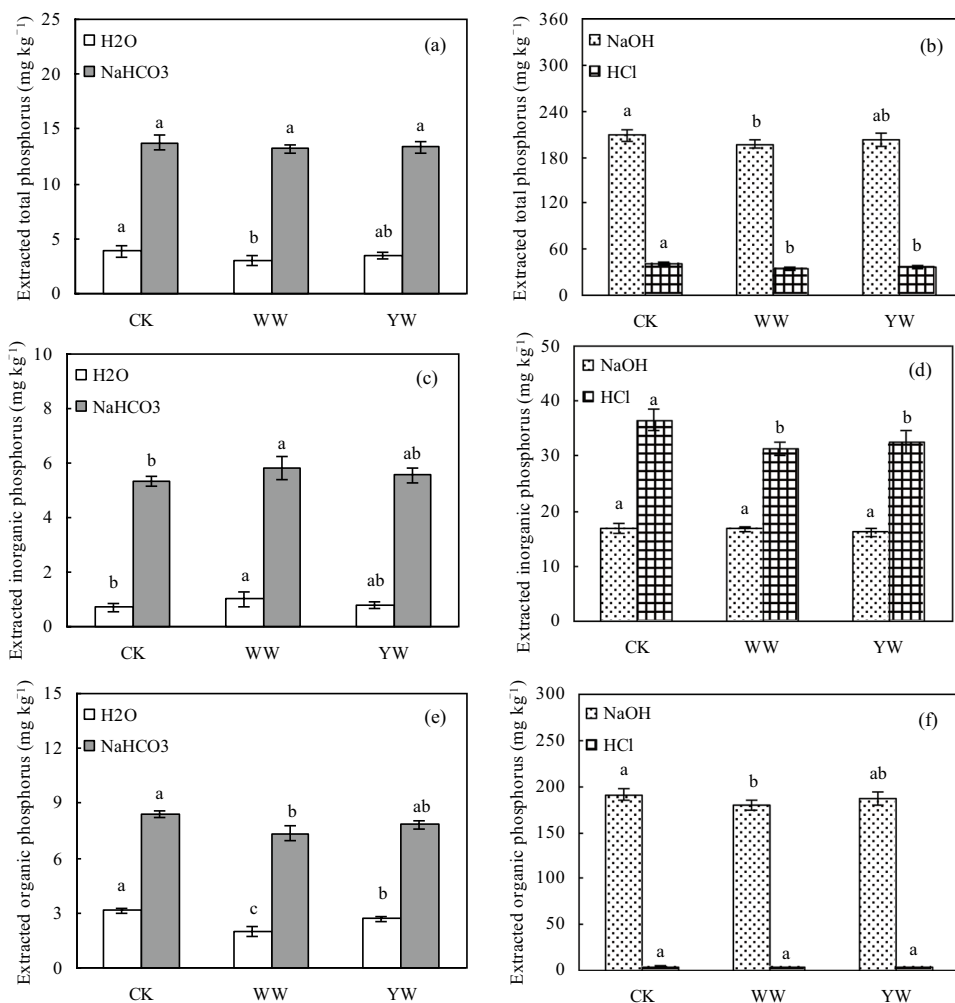


Fig. 3 Solution phosphorus-31 nuclear magnetic resonance (³¹P NMR) spectra of NaOH-EDTA extracts in soil under unwarmed control (CK), winter warming (WW), and year-round warming (YW). Di1 and Di2 represent unidentified orthophosphate diesters from regions 1 and 2, respectively

were divided into monoester 1, monoester 2, and monoester 3 groups. In the orthophosphate diesters, deoxyribonucleic acid (DNA) was identified, and the other unidentified diesters were assigned to diester 1 and diester 2 groups (Fig. 4, Fig. S1).

After 5 years of warming, the NaOH-EDTA extracted total P (Ept), ranging between 318.1 and 341.8 mg kg⁻¹ and accounting for 69.0 to 72.6% of soil total P, was 7.45% ($P < 0.05$) and 3.73% ($P > 0.05$) lower in WW and YW compared to CK, respectively, and 3.58% ($P > 0.05$) lower in WW than in YW. In the Pi compounds, the proportion of orthophosphate (7.49–9.03% of Ept) was larger than that of pyrophosphate (0.86–0.99% of Ept). In the Po compounds, the proportion of unidentified orthophosphate monoesters (61.0–63.4% of Ept) was the largest, followed by glycerophosphates (sum of α - and β -glycerophosphate, 12.5–13.4% of Ept), IHP (sum of *myo*- and *scyllo*-IHP, 8.86–10.6% of Ept), unidentified orthophosphate diesters (2.72–3.68% of Ept), mononucleotides (0.88–1.07% of Ept), choline phosphate (0.80–0.93% of Ept), and DNA (0.82–0.86% of Ept). Furthermore, *myo*-IHP showed a larger

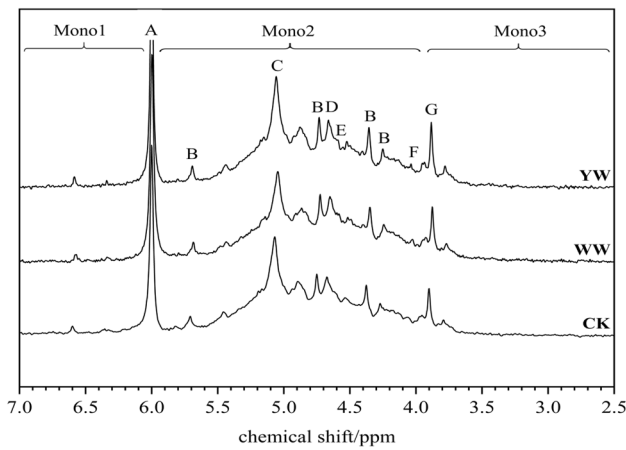


Fig. 4 Solution phosphorus-31 nuclear magnetic resonance (^{31}P NMR) spectra of NaOH-EDTA extracts in soil under unwarmed control (CK), winter warming (WW), and year-round warming (YW) showing the assignments of peaks in the orthophosphate monoester region. A, B, C, D, E, F, and G represent orthophosphate, *myo*-inositol hexakisphosphate, α -glycerophosphate, β -glycerophosphate, mononucleotide, choline phosphate, and *scyllo*-inositol hexakisphosphate, respectively; Mono1, Mono2, and Mono3 represent unidentified orthophosphate monoesters from regions 1, 2, and 3, respectively

proportion (6.86–8.22% of EPt) in the two stereoisomers of IHP, and α -glycerophosphate exhibited a larger proportion (9.13–9.71% of EPt) in the two glycerophosphates. Compared with CK, orthophosphate concentration was 12.4% and 5.54% ($P > 0.05$) higher in WW and YW, respectively. In contrast, other P compounds exhibited lower concentrations in WW and YW than in CK, with significant differences between CK with WW and YW for *myo*-IHP and α -glycerophosphate. Although there were no significant differences between WW and YW, the extent of increases or decreases of all P forms in WW was larger than those in YW relative to CK (Table 1).

Across the three treatments, the proportion of total Pi (i.e., sum of orthophosphate and pyrophosphate, 8.43–10.0% of EPt) was lower than that of total Po (i.e., sum of orthophosphate monoesters and orthophosphate diesters, 89.1–90.5% of EPt). Furthermore, orthophosphate monoesters exhibited larger proportion (86.4–87.0%) than orthophosphate diesters (3.55–4.54%) in all the three treatments, even after correction for degradation products (72.5–73.1% versus 16.9–19.1%). Compared with CK, the concentration of total Pi was 10.8% and 3.47% ($P > 0.05$) higher in WW and YW, respectively. In contrast, the concentrations of total Po and uncorrected/corrected orthophosphate monoesters and diesters all decreased in WW and YW than in CK, with significant differences between CK and WW. There were no significant differences between WW and YW, while the extents of increase or decrease were greater for WW than for YW relative to

Table 1 Proportions (%) and concentrations (mg kg^{-1}) of phosphorus (P) compounds in soil NaOH-EDTA extracts under unwarmed control (CK), winter warming (WW), and year-round warming (YW) in a Tibetan alpine meadow by solution ^{31}P NMR spectroscopy

Treatment	EPT		Pi		Po									
	Ortho	Pyro	Ortho	Pyro	Monoesters	Diesters		Nucl		Pchol		Others		
	myo-IHP	scyllo-IHP	α -Glyc	β -Glyc	DNA	Others	DNA	Others						
Proportion (%)														
CK	69.8 ± 1.44a	7.49 ± 1.21b	0.94 ± 0.16a	8.22 ± 0.32a	2.36 ± 0.37a	9.71 ± 0.56a	3.74 ± 0.16a	1.07 ± 0.30a	0.93 ± 0.16a	61.0 ± 1.73b	0.86 ± 0.18a	3.68 ± 0.89a		
WW	72.6 ± 3.09a	9.03 ± 0.43a	0.99 ± 0.12a	6.86 ± 0.41b	2.00 ± 0.11b	9.15 ± 0.43a	3.33 ± 0.46a	0.88 ± 0.09a	0.80 ± 0.04a	63.4 ± 1.52a	0.83 ± 0.21a	2.72 ± 1.08a		
YW	69.0 ± 4.12a	8.19 ± 0.59ab	0.86 ± 0.09a	7.08 ± 0.25b	2.06 ± 0.07ab	9.13 ± 0.41a	3.45 ± 0.13a	0.91 ± 0.11a	0.80 ± 0.05a	63.1 ± 0.42a	0.82 ± 0.04a	3.57 ± 0.62a		
Concentrations (mg kg^{-1})														
CK	341.8 ± 8.62a	25.6 ± 4.06a	3.22 ± 0.51a	28.1 ± 1.57a	8.10 ± 1.39a	33.2 ± 2.24a	12.8 ± 0.71a	3.64 ± 1.00a	3.17 ± 0.57a	208.5 ± 7.32a	2.93 ± 0.60a	12.6 ± 3.05a		
WW	318.1 ± 15.2b	28.8 ± 2.36a	3.15 ± 0.41a	21.8 ± 1.01b	6.35 ± 0.07b	29.1 ± 2.06b	10.6 ± 1.41b	2.80 ± 0.26a	2.53 ± 0.03b	201.9 ± 13.9a	2.62 ± 0.59a	8.54 ± 3.17a		
YW	329.5 ± 13.8ab	27.0 ± 2.60a	2.82 ± 0.34a	23.3 ± 1.38b	6.80 ± 0.34ab	30.0 ± 0.58b	11.4 ± 0.77ab	3.00 ± 0.20ab	2.70 ± 0.18a	208.0 ± 9.16a	2.70 ± 0.18a	11.8 ± 2.09a		

Different lowercase letters within the same column indicate significant differences at $P < 0.05$ (mean ± standard deviation, $n = 5$). *EPt*, NaOH-EDTA extractable total P; *Ortho*, orthophosphate; *Pyro*, pyrophosphate; *myo-IHP*, *myo*-inositol hexakisphosphate; *scyllo-IHP*, *scyllo*-inositol hexakisphosphate; α -Glyc, α -glycerophosphate; β -Glyc, β -glycerophosphate; *Nucl*, mononucleotides; *Pchol*, choline phosphate; *DNA*, deoxyribonucleic acid

CK. Both the uncorrected and corrected orthophosphate diesters to monoester (diesters/monoesters) ratios exhibited no significant differences among the three treatments (Table 2).

3.4 Relationships between Soil Phosphorus Forms and Environmental Factors

Soil total P was positively correlated but available P was negatively correlated with *myo*-IHP, *scyllo*-IHP, α -glycerophosphate, orthophosphate monoesters, and corrected orthophosphate diesters. The H₂O- and NaHCO₃-Pi were positively correlated with orthophosphate, NaOH-Pi was positively correlated with mononucleotides and DNA, and HCl-Pi was positively correlated with *myo*-IHP, *scyllo*-IHP, α -glycerophosphate, β -glycerophosphate, mononucleotides, choline phosphate, and corrected diesters. The H₂O-, NaHCO₃-, NaOH-, and HCl-Po were all positively correlated with *scyllo*-IHP (Table S2).

Soil available P, H₂O-Pi, and NaHCO₃-Pi were positively correlated while total P, HCl-Pi, and most Po forms were negatively correlated with acid phosphomonoesterase activity. Furthermore, H₂O-Po, *myo*-IHP, and phosphocholine were negatively correlated with neutral and alkaline phosphomonoesterase activities. Except for pyrophosphate, no significant correlations were observed between soil P forms and AGB (Table S3).

RDA results showed that biotic and abiotic factors explained 87.6% of the variations in total P and P forms. RDA1 and RDA2 explained 69.4% and 11.9% in total P and P form variations, respectively. The acid phosphomonoesterase was a significant factor in controlling total P and the distribution of P forms and explained 28.7% of the observed total variances in total P and P forms (Fig. 5).

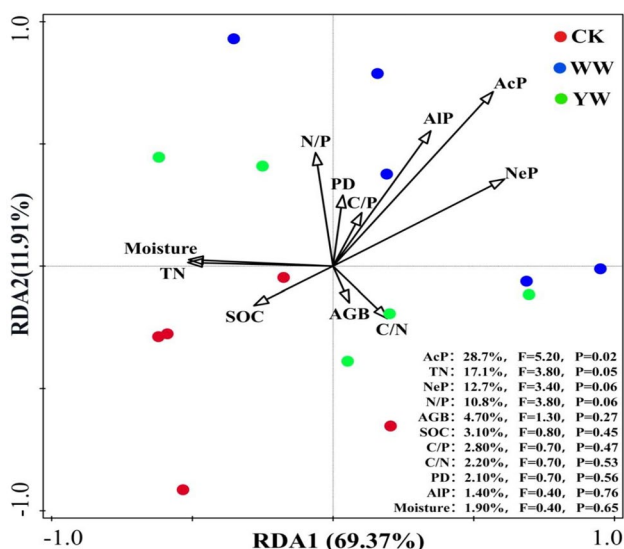


Fig. 5 Redundancy analysis (RDA) plot illustrating relationships between soil total phosphorus (P), available P, and inorganic P (Pi) and organic P (Po) forms with soil properties under unwarmed control (CK), winter warming (WW), and year-round warming (YW). SOC: soil organic carbon; TN: total nitrogen; AcP: acid phosphomonoesterase; NeP: neutral phosphomonoesterase; AIP: alkaline phosphomonoesterase; PD: phosphodiesterase; AGB: aboveground biomass

4 Discussion

4.1 Warming Impacts on Soil Total P Concentration

The results from the present study indicated that short-term warming had a negative effect on soil total P concentration (Fig. 1a). In a 3-year field warming experiment, Rui et al. (2012) observed that soil total P concentration was significantly reduced at a depth of 0–10 cm in a Tibetan

Table 2 Proportions (%) and concentrations (mg kg⁻¹) of inorganic P (Pi), organic P (Po), orthophosphate monoesters (Mono), orthophosphate diesters (Di), corrected orthophosphate monoesters (Cmono), corrected orthophosphate diesters (Cdi), Di to Mono (Di/Mono) ratio,

Treatment	Total Pi	Total Po	Mono	Di	Cmono	Cdi	Di/Mono	Cdi/Cmono
Proportion (%) ^a								
CK	8.43 ± 1.35 b	91.6 ± 1.35 a	87.0 ± 1.40 a	4.54 ± 1.02 a	72.5 ± 1.52 a	19.1 ± 1.20 a	0.05 ± 0.01 a	0.26 ± 0.02 a
WW	10.0 ± 0.40 a	90.0 ± 0.40 b	86.4 ± 0.99 a	3.55 ± 1.27 a	73.1 ± 1.45 a	16.9 ± 1.72 b	0.04 ± 0.02 a	0.23 ± 0.03 a
YW	9.04 ± 0.52 ab	91.0 ± 0.52 ab	86.6 ± 0.28 a	4.39 ± 0.65 a	73.1 ± 0.35 a	17.9 ± 0.80 ab	0.05 ± 0.01 a	0.24 ± 0.01 a
Concentration (mg kg ⁻¹)								
CK	28.8 ± 4.51 a	313.0 ± 9.81 a	297.5 ± 9.70 a	15.5 ± 3.50 a	247.9 ± 8.57 a	65.1 ± 4.52 a		
WW	31.9 ± 2.41 a	286.2 ± 13.2 b	275.0 ± 15.7 b	11.2 ± 3.69 a	232.6 ± 14.3 b	53.6 ± 4.40 b		
YW	29.8 ± 2.55 a	299.7 ± 11.9 ab	285.2 ± 11.5 ab	14.5 ± 2.21 a	240.8 ± 10.6 ab	58.9 ± 2.77 ab		

Different lowercase letters within the same column indicate significant differences at $P < 0.05$ (mean ± standard deviation, $n = 5$). Total Pi = orthophosphate + pyrophosphate, Total Po = Mono + Di. ^aThe proportion to total P in soil NaOH-EDTA extracts

alpine meadow ecosystem, consistent with our results. A decrease in soil total P concentration under warming was also observed or projected in shrubland (Sardans et al. 2006), heath (Rinnan et al. 2008), and everglades ecosystems (Orem et al. 2015), as well as at global scale (He et al. 2021; Zhang 2021). In contrast, some other researchers observed that warming had no significant effects on soil total P concentration in alpine (Wang et al. 2014) and temperate grassland ecosystems (Zhang et al. 2013, 2014; Fang et al. 2020).

First, weathering of P-containing minerals is an important process controlling P transformation in natural grassland ecosystems (Ippolito et al. 2010). Climate warming can accelerate the weathering of P-containing minerals (Flicoteaux and Lucas 1984) and subsequently lead to the depletion of P through biomass removal, erosion, runoff, and leaching (Zhou et al. 2017; Gao et al. 2019). However, we believe that mineral weathering only contributed a small proportion to soil P loss due to the short duration of warming and low warming intensity in this field experiment. Second, plant biomass input was a major source of P in grassland soil (Nash et al. 2014). The decline of ABG under warming in the present study (Table S1) led to a smaller return of P to soil via plant litterfall, which may have contributed to the reduction of soil total P concentration. There was no significant correlation between soil total P concentration and AGB in the current study (Table S3), implying that plant biomass was not a crucial factor contributing to the depletion of soil P in this study. In a previous study, Sardans et al. (2006) suggested that warming-induced declines in soil total P could result from the increasing accumulation of P in plant biomass coupled with a decreasing P concentration in litterfall. This requires verification in our future studies. On the other hand, the non-significant correlation between soil total P concentration and AGB (Table S3) also suggested that P was not a limiting factor for grassland plant productivity in the Tibetan alpine meadow ecosystem. Third, total P is composed of Pi and Po pools in soil. The significant and positive correlations between total P and some Pi and Po forms (Table S2) suggested that the loss of specific P forms due to plant uptake, leaching, and surface runoff under warming was mainly responsible for the depletion of soil total P. Detailed discussions on soil Pi and Po forms under warming are described in the following sections.

4.2 Warming Impacts on Soil Inorganic Phosphorus Forms

Among sequentially extracted Pi fractions, H₂O-Pi and NaHCO₃-Pi (i.e., Pi adsorbed on crystalline surfaces) are considered as labile Pi, NaOH-Pi (i.e., Pi associated with aluminum and iron oxides or carbonates) as moderately labile Pi, and HCl-Pi (i.e., Pi bound to calcium) as stable Pi fraction (He et al. 2008; Negassa and Leinweber 2009; Hou

et al. 2018b). In our present study, HCl-Pi was the dominant Pi fraction among all Pi fractions (Fig. 2b), which supports previous findings showing that calcium-bound Pi was the dominant Pi fraction in alpine meadow soil (Li et al. 2020). The results of sequential fractionation (Fig. 2) indicated that short-term warming caused an increase in labile Pi fractions but led to a decrease in moderately labile and stable Pi fractions.

The available P (i.e., Olsen P), which accounted for 60.1–64.9% of total labile Pi (i.e., sum of H₂O-Pi and NaHCO₃-Pi) in the tested soil, is also commonly considered as labile Pi that can be directly absorbed by plants (Gao et al. 2016; Menezes-Blackburn et al. 2019). Our present study demonstrated that short-term warming exerted a positive effect on soil available P concentration (Fig. 1b). The increment in available P concentration was identical with increases in the concentrations of labile Pi fractions (Fig. 2b). However, some previous studies found that warming exerted positive (Zi et al. 2018), negative (Gong et al. 2015), or no effects (Zhang et al. 2013, 2014; Wang et al. 2014; Fang et al. 2020) on available P concentration in other grassland ecosystems.

We hypothesize three possible reasons to explain the increase in soil labile Pi forms under warming. First, the enhancements of soil phosphatase activities, as observed in this study (Table S1), were beneficial to Po mineralization into labile Pi. The enhancement of phosphatase activity under warming has also been reported in other grassland ecosystems (Rui et al. 2012; Zi et al. 2018; Gong et al. 2020). The positive correlation between soil available P, H₂O-Pi and NaHCO₃-Pi with acid phosphomonoesterase activity (Table S3) further indicated that acid phosphomonoesterase contributed to greater Pi availability. The mineralization of Po has been considered as the main process controlling the availability of soil P due to low rates of weathering from P-containing primary minerals in alpine ecosystems (Rui et al. 2012). Second, plant roots tend to exude more organic acids under elevated temperature (Jia et al. 2015), which could promote the transformation from moderately labile and stable Pi fractions to labile Pi fractions by chelating with metal cations (Taghipour and Jalali 2013; Wang et al. 2015) or facilitated the desorption of Pi by competing with phosphate for adsorption sites on soil particle surfaces (Hinsinger 2001; Almeida et al. 2020). The PAC value has been used as an indicator to evaluate the degree of the difficulty of the transformation between total P and available P, with a larger value showing that the transformation from total P to available P was easier (Xu et al. 2019). The significant increase in the PAC in our present study (Fig. 1c) suggested that warming facilitated conversion of total P into available P and consequently improved the availability of P in soil. The significant and negative correlation between available P and HCl-Pi ($r = -0.66$, $P < 0.01$) further demonstrated

that the stable Pi fraction was the major source of available P. Third, soil available P level for optimum grassland production was within the range of 16–24 mg kg⁻¹ (Tunney 2002). In the present study, soil available P concentrations (3.64–4.33 mg kg⁻¹) across all treatments were much lower than these optimum threshold levels. The P-limited condition under warming could accelerate the production of bioavailable P at the expense of Po. In addition, the change in plant biomass may partly contribute to the improvement of soil P availability. Gong et al. (2015) demonstrated that AGB increased under warming, which promoted plant P uptake and reduced soil available P content. By contrast, the warming-induced decrease of plant AGB observed in our present study could cause more available P to remain in soil. Similar to soil total P, the non-significant correlation between soil Pi forms and AGB (Table S3) also suggested that P was not a limiting factor for grassland plant productivity in this Tibetan alpine meadow ecosystem. Orthophosphate represented labile Pi (Table S2) and the non-significant increase of orthophosphate concentration might be attributed to the slightly greater orthophosphate input by Po mineralization than its output via plant uptake, surface runoff, and leaching under warming.

4.3 Warming Impacts on Soil Organic Phosphorus Forms

The results from the present study indicated that short-term warming had a significantly negative effect on soil Po concentration (Fig. 1d). In a 3-year field warming experiment, Rui et al. (2012) observed that soil Po concentration significantly decreased at 0–10 cm depth in a Tibetan alpine meadow ecosystem, consistent with our present findings. A previous study by Guan et al. (2018) found that warming had no significant impact on SOC content, implying that soil Po was a more sensitive indicator to climate warming than SOC. As indicated above, the enhancements of soil phosphatase activities under warming could accelerate the mineralization of soil Po. Similar to available P and labile Pi fractions, the significant and negative correlation between acid phosphomonoesterase activity and Po concentration (Table S3) suggested that acid phosphomonoesterase was crucial in driving soil Po mineralization.

Similar to Pi fractions, H₂O-Po and NaHCO₃-Po represent labile Po, NaOH-Po represents moderately labile Po, and HCl-Po represents stable Po among sequentially extracted Po fractions (He et al. 2008; Negassa and Leinweber 2009). In the present study, the results of sequential fractionation (Fig. 2) indicated that warming decreased the concentrations of all Po fractions. By using a modified Hedley and Stewart fractionation scheme, Rui et al. (2012) observed that warming significantly decreased the concentration of NaOH-Po (N(I)Po) but not that of other Po fractions at 0–10 cm depth

under short-term (3 years) warming in a Tibetan alpine meadow ecosystem. The negative correlations between available P with NaHCO₃-Po ($r = -0.63$, $P < 0.05$) and NaOH-Po ($r = -0.73$, $P < 0.01$) suggested that labile and moderately labile Po fractions contributed more to soil P availability under warming. Moreover, acid phosphomonoesterase activity was significantly and negatively correlated with H₂O-, NaHCO₃-, NaOH-, and HCl-Po concentrations (Table S3), which further suggests that acid phosphomonoesterase was crucial in driving soil Po mineralization, and labile H₂O-Po was more sensitive to acid phosphomonoesterase activity, as evidenced by the larger correlation coefficient between acid phosphomonoesterase and H₂O-Po.

The concentrations of NaOH-EDTA extractable Po compounds (Tables 1 and 2) followed a pattern similar to those of sequentially extracted Po fractions (Fig. 2). Although inositol hexakisphosphates are strongly stabilized in soil via interactions with mineral matrices and organic matter (Celi and Barbaris 2005), significant reductions in *myo*- and *scyllo*-IHP concentrations under warming were observed (Table 1). The bioavailability of IHP can be related to nutrient status of soil. Turner et al. (2005) showed that inositol hexakisphosphates were prone to mineralize under P-limited conditions, while they tended to accumulate under P-sufficient conditions. In the present study, Po concentration was lower than the Po threshold value (i.e., 0.5 g kg⁻¹) reported for P-limited conditions (Turner et al. 2005). The P limitation could accelerate the mineralization of recalcitrant inositol phosphates under warming. On the other hand, the increased exudation of organic acids from plant roots under elevated temperature (Jia et al. 2015) could also solubilize recalcitrant IHP and promote the hydrolysis of IHP into Pi through phytase (Almeida et al. 2018). In addition, it has been well-established that *myo*-IHP derives from plants (especially seeds) and *scyllo*-IHP derives from microbial synthesis (Xin et al. 2019). The declines in AGB (Table S1) and microbial P immobilization could also partly contribute to the decreasing concentrations of the two inositol hexakisphosphates. The α - and β -glycerophosphates originate from phospholipids that are present in plant residuals and microbial cells (Liu et al. 2019). Thus, the significant decreases in the two glycerophosphates likely reflected the reduced input of plant litterfall and microbe sources. In addition, the non-significant correlation between soil Po forms and AGB (Table S3) further confirmed that P was not a limiting factor for grassland plant productivity in this Tibetan alpine meadow ecosystem.

The mineralization of Po in soil follows the process in which orthophosphate diesters are first hydrolyzed into orthophosphate monoesters and then orthophosphate monoesters are hydrolyzed into inorganic orthophosphate (Turner and Haygarth 2005). Because orthophosphate diesters are more susceptible to mineralization than

orthophosphate monoesters (Zhang et al. 2019a), the diester/monoester ratio has been used as indicator to describe the lability of soil Po (Baumann et al. 2017; Aleixo et al. 2020). Therefore, our present study (Table 2) suggested that short-term warming did not affect soil Po lability. However, we also observed a decline of the corrected diester/monoester ratio under both WW and YW. Presumably, long-term warming would cause a marked decline of the ratio and thus exacerbate P limitations.

5 Conclusions

This study demonstrated that soil warming of 1.3 °C achieved in OTCs experiment decreased soil total P, NaOH-Pi, HCl-Pi, H₂O-Po, NaHCO₃-Po, NaOH-Po, and HCl-Po and pyrophosphate, orthophosphate monoesters, and orthophosphate diester concentrations. In contrast, warming increased available P, H₂O-Pi, NaHCO₃-Pi, and orthophosphate concentrations. The extent of increases or decreases for total P and P forms was generally larger in winter warming than in year-round warming. Our results suggest that warming can mitigate P limitation during the short-term by promoting the transformations of moderately labile and stable Pi fractions and various forms of Po into bioavailable P. Winter warming exerted a stronger impact on soil P forms and transformations, implying that summer warming can weaken the impacts of winter warming. Soil Po was a more sensitive indicator to climate warming than SOC. However, acid phosphomonoesterase rather than AGB was a significant factor in shaping soil P forms and transformation, contrary to our initial hypothesis. Furthermore, P was also not a limiting factor for grassland plant productivity in the Tibetan alpine meadow ecosystem.

Although warming can mitigate P limitation during the short-term, the depletions of total P and Po concentrations may aggravate P limitation in the long-term. Therefore, an optimum P fertilization strategy, especially the inclusion of manure, should be exploited to sustain grassland productivity in this alpine meadow region to cope with future climate change. However, further study is required to evaluate long-term fates of soil P pools under warming for accurate predictions of the responses of P cycling in terrestrial ecosystems to global climate change.

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Declarations

Conflict of Interest The authors declare no competing interests.

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