



# Exogenous phosphorus compounds interact with nitrogen availability to regulate dynamics of soil inorganic phosphorus fractions in a meadow steppe

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Received: 6 May 2019 – Discussion started: 11 June 2019

Revised: 15 October 2019 – Accepted: 15 October 2019 – Published: 12 November 2019

**Abstract.** Here we investigated the effects of P compounds ( $\text{KH}_2\text{PO}_4$  and  $\text{Ca}(\text{H}_2\text{PO}_4)_2$ ) with different addition rates of 0, 20, 40, 60, 80, and 100 kg P ha<sup>-1</sup> yr<sup>-1</sup> and  $\text{NH}_4\text{NO}_3$  addition (0 and 100 kg N ha<sup>-1</sup> yr<sup>-1</sup>) on soil labile inorganic phosphorus (IP) (dicalcium phosphate,  $\text{Ca}_2\text{-P}$ ), moderate-cycling IP, and recalcitrant IP fractions in a calcareous grassland of northeastern China. Soil moderate-cycling IP fractions, not readily available to plants but transforming into soil-available P quickly, include variscite (Al-P), strengite (Fe-P) and octacalcium phosphate ( $\text{Ca}_8\text{-P}$ ); recalcitrant IP fractions include hydroxylapatite ( $\text{Ca}_{10}\text{-P}$ ) and occluded P (O-P). Soil labile and moderate-cycling IP fractions and total P significantly increased with increasing P addition rates, with higher concentrations detected for  $\text{KH}_2\text{PO}_4$  than for  $\text{Ca}(\text{H}_2\text{PO}_4)_2$  addition. Combined N and P treatments showed lower soil labile IP and moderate-cycling IP fractions compared to ambient N conditions, due to enhanced plant productivity. Moderate-cycling IP was mainly regulated by P addition and plant P uptake to further enhance labile IP and total P concentrations with  $\text{KH}_2\text{PO}_4$  and  $\text{Ca}(\text{H}_2\text{PO}_4)_2$  addition. Soil labile IP was also directly and negatively affected by soil pH and plant P uptake with  $\text{Ca}(\text{H}_2\text{PO}_4)_2$  addition.  $\text{Ca}(\text{H}_2\text{PO}_4)_2$  addition significantly increased the soil recalcitrant IP ( $\text{Ca}_{10}\text{-P}$ ) fraction, while  $\text{KH}_2\text{PO}_4$  addition showed no impact on it. A significant positive correlation was detected between soil labile IP,

moderate-cycling IP fractions and soil Olsen-P which illustrated that labile IP and moderate-cycling IP fractions were important sources for soil-available P. Our results suggest that moderate-cycling IP fractions are essential for grassland P biogeochemical cycling and the chemical form of P fertilizer should be considered during fertilization management for maintaining soil-available P.

## 1 Introduction

Phosphorus (P) is an essential nutrient affecting terrestrial plant productivity and ecosystem functions (Luo et al., 2015). Soil inorganic P (IP) could occupy 50%–90% of soil total P (Jiang and Gu, 1989; Feng et al., 2016) and mainly includes adsorbed and mineral forms of phosphate (Hinsinger, 2001). Soil mineral-bound phosphate falls into two groups: those containing calcium (Ca-P) and those containing iron and aluminum (Chen et al., 2002), which are the leading sources of plant-available P (Meena et al., 2018). The detailed classification of these two groups includes soil variscite (Al-P), strengite (Fe-P), dicalcium phosphate ( $\text{Ca}_2\text{-P}$ ), octacalcium phosphate ( $\text{Ca}_8\text{-P}$ ), hydroxylapatite ( $\text{Ca}_{10}\text{-P}$ ) and occluded P (O-P) (Zhao et al., 2019). Soil IP is dom-

inated by Al-P and Fe-P in acidic soils and by Ca-P fractions in calcareous soils (Baumann et al., 2017). The solubility of soil Ca-P fractions decreases in the order  $\text{Ca}_2\text{-P} > \text{Ca}_8\text{-P} > \text{Ca}_{10}\text{-P}$  (Jiang and Gu, 1989). Although these element-bound P fractions are not readily available to plants, soil Al-P, Fe-P,  $\text{Ca}_2\text{-P}$  and  $\text{Ca}_8\text{-P}$  can be converted to free phosphates, serving as important buffering pools for available P (Herlihy and McGrath, 2006; Zhao et al., 2019). Based on P transforming dynamics, soil IP could be fractionated into soil labile ( $\text{Ca}_2\text{-P}$ ) or available P (Olsen-P), moderate-cycling P fractions (Al-P, Fe-P and  $\text{Ca}_8\text{-P}$ ) and recalcitrant P fractions ( $\text{Ca}_{10}\text{-P}$  and O-P). But the category is not universal and mainly depends on the sensitivity of soil IP fractions in different studies (Schrijver et al., 2012 vs. Zhao et al., 2019). Soil Olsen-P could be directly absorbed and utilized by plants, which includes all water-soluble P, some of the adsorbed and soluble IP, and mineralizable organic P (Tang et al., 2009; Cao et al., 2012).  $\text{Ca}_2\text{-P}$ , chemically similar to Olsen-P, includes water-soluble P, citrate-soluble P and partially surface-adsorbed P (Shen et al., 2004; Zhao et al., 2019). Soil recalcitrant IP is relatively stable and unavailable for plants, which is mainly converted from the fixation of labile and moderate-cycling IP (Shen et al., 2004; Zhao et al., 2019). Therefore, soil IP transformation is crucial for nutrient cycling in terrestrial ecosystems.

Adding P to soil is an effective way to enhance soil-available P and sustain plant productivity (Marklein and Houlton, 2012). However, P addition changes ecosystem P cycling (Herlihy and McGrath, 2006) and the reactions between free phosphate and cations in soil, thereby influencing the concentration and transformation of soil IP fractions (Walker and Syers, 1976). As such, chronic P input can result in insoluble phosphate accumulation and decrease P use efficiency without providing additional benefits for plant growth (Maistry et al., 2015; Halajnia et al., 2009; Maistry, 2015). This could then increase the risk of soil P leaching losses (Shen et al., 2004). Therefore, exploring the effect of P addition on soil IP fractions is important for understanding soil-available P supply and P dynamics in terrestrial ecosystems (Sigua et al., 2017).

Biogeochemical P dynamics can also be influenced by the chemical form of applied P (Margenot et al., 2017). Frequently used P compounds include  $\text{Ca}(\text{H}_2\text{PO}_4)_2$  (slow-release P fertilizer) and  $\text{KH}_2\text{PO}_4$  (fast-release P fertilizer) (Mortvedt et al., 1999), which have played critical roles in agricultural ecosystems (Nguyen et al., 2012). Both P fertilizers can convert into various inorganic P fractions including soil Fe-P (especially in paddy soils with high  $\text{Fe}^{2+}$ ) (Sah et al., 1989) and soil Al-P, which can account for 33 % of the total soil P in weathered soils (Margenot et al., 2017). Soil  $\text{Ca}_2\text{-P}$  and  $\text{Ca}_8\text{-P}$  could be also formed with P addition, especially in calcareous soils with pH higher than 7 (Al-Khateeb et al., 1986). In a previous study it was found that long-term application of  $\text{Ca}(\text{H}_2\text{PO}_4)_2$  increased soil IP fractions (Al-P, Fe-P,  $\text{Ca}_2\text{-P}$ ,  $\text{Ca}_8\text{-P}$  and O-P) and total P (TP) concentra-

tions in a calcareous soil (Wang et al., 2010), and another study showed that long-term  $\text{Ca}(\text{H}_2\text{PO}_4)_2$  addition decreased soil P availability due to fixation of P to the soil constituents (Xiong et al., 2018).  $\text{KH}_2\text{PO}_4$  addition was suggested to enhance the reaction of phosphate with Fe and Al oxides and with hydroxyl ions under lower soil pH conditions (Shuman, 1988). While these studies provided insight into the relationships between P addition and IP fractions (Condrón and Goh, 1989), little is known about the effects of different P compound additions on soil IP fractions and P availability.

Nitrogen enrichment can influence soil chemical properties, thereby affecting soil P transformations and consequently aboveground biomass and foliar P concentrations (Crowley et al., 2012). For instance, N addition often decreases soil pH and promotes the release of P from Fe and Al oxides (Gustafsson et al., 2012; Yang et al., 2014) and can result in redistribution of soil IP fractions (Zhao et al., 2019). Nitrogen addition could also promote the mineralization of soil organic P by increasing phosphatase activities (Olander and Vitousek, 2000). The effect of combined N and P addition on ecosystem P dynamics may further depend on their relative amounts added to the soil. For instance, inorganic P solubility and availability were particularly large when P was applied with N (Ross et al., 1995), while N and P applied at higher N : P ratios increased plant P use efficiency and leaf phosphatase activity (Hogan et al., 2010). It is well known that variation in N : P input ratios can also alter plant litter N : P ratios (Vitousek et al., 2010; Güsewell, 2004; Sun et al., 2018), thereby further affecting N and P availability through litter decomposition. However, it remains unclear how applications of N and P added at different ratios will affect soil IP fractions.

The meadow steppe in northern China is an important part of the Eurasian grassland ecosystem (Kang et al., 2007). With the intensive environmental stresses of overgrazing and enhanced outputs of animal products from a sharply rising population, Inner Mongolian grassland ecosystems have potentially become more nutrient-limited, predominantly in regards to N and P (Kang et al., 2007; Gong et al., 2011). Hence, N and P additions are necessary to enhance ecosystem productivity in the meadow steppe. The purpose of our study was to monitor the effects of various levels of  $\text{KH}_2\text{PO}_4$  and  $\text{Ca}(\text{H}_2\text{PO}_4)_2$  with and without N addition on concentrations of soil IP fractions, available P and TP. We hypothesized that (1) soil labile IP ( $\text{Ca}_2\text{-P}$ ) and moderate-cycling IP fractions (Al-P, Fe-P and  $\text{Ca}_8\text{-P}$ ) would significantly increase with  $\text{KH}_2\text{PO}_4$  and  $\text{Ca}(\text{H}_2\text{PO}_4)_2$  addition, while soil recalcitrant IP fractions ( $\text{Ca}_{10}\text{-P}$  and O-P) would not be affected because of different solubility in IP fractions; (2) addition of soluble  $\text{KH}_2\text{PO}_4$  would be more efficient in increasing soil labile IP, moderate-cycling IP fractions, recalcitrant P fractions, and total P (TP) than less soluble  $\text{Ca}(\text{H}_2\text{PO}_4)_2$  because of faster conversion of  $\text{KH}_2\text{PO}_4$  into labile and moderate-cycling IP fractions, consequentially promoting the formation of recalcitrant fractions; and (3) the concentrations of

soil IP fractions would be lower under combined P and N additions than those under P addition alone due to facilitation of plant P uptake with N addition.

## 2 Materials and methods

### 2.1 Study sites and experimental design

The study site (50°10' N, 119°22' E, elevation 523 m a.s.l.) is located at the Erguna Forest-Steppe Ecotone Research Station of Inner Mongolia, China. This area belongs to a temperate continental monsoon climate. The mean annual precipitation and mean annual temperature are 375 mm and  $-3^{\circ}\text{C}$ , respectively. Rainfall at the site is mainly concentrated during the period from June to August and the average growing season is about 150 d. The soil is a chernozem according to the Food and Agricultural Organization of the United Nations classification (IUSS Working Group WRB, 2014), and the soil chemical characteristics of the site are reported in Table 1. The dominant plant species include *Stipa baicalensis*, *Leymus chinensis* and *Carex duriuscula*. The relative biomass proportion of the three dominant species combined to the total aboveground biomass were 50.29%–74.75% and 72.43%–91.92% in the plots without and with N addition, respectively.

The experiment, established in 2014, was arranged in a randomized block design with 24 treatments and five replicates. Phosphorus addition included two compounds, i.e.,  $\text{KH}_2\text{PO}_4$  and  $\text{Ca}(\text{H}_2\text{PO}_4)_2$ , and were applied at six levels: 0, 20, 40, 60, 80 and  $100\text{ kg P ha}^{-1}\text{ yr}^{-1}$ . Half of the plots were applied with  $100\text{ kg N ha}^{-1}\text{ yr}^{-1}$  in the form of  $\text{NH}_4\text{NO}_3$ . P and N were added in the middle of May. All treatment plots were balanced for K using potassium chloride (KCl) to maintain the same amount of K input as in the treatment with  $100\text{ kg P ha}^{-1}\text{ yr}^{-1}$  of  $\text{KH}_2\text{PO}_4$  addition ( $132\text{ kg K ha}^{-1}$ ). To balance for Cl along with KCl addition,  $\text{CaCl}_2$  was applied to maintain equal Cl inputs for all plots ( $121\text{ kg Cl ha}^{-1}$ ). Calcium was not balanced in this calcareous soil, where Ca is already abundant. In this study we chose the same control plots for the  $\text{KH}_2\text{PO}_4$  and  $\text{Ca}(\text{H}_2\text{PO}_4)_2$  treatment, so that, in total, there were 110 plots of  $8\text{ m} \times 8\text{ m}$ , separated by 1 m wide buffer zones. The initial pH of  $\text{KH}_2\text{PO}_4$  and  $\text{Ca}(\text{H}_2\text{PO}_4)_2$  solutions were 4.91 and 4.50, respectively.

### 2.2 Sample collection

In August 2016, aboveground biomass was harvested by clipping all living tissues using a  $1\text{ m} \times 1\text{ m}$  quadrat placed randomly within each plot. The plants were sorted to species and oven-dried at  $65^{\circ}\text{C}$  for 48 h, then weighed and ground. Soil samples were taken from the surface (10 cm depth) using a 5 cm diameter soil auger. Five soil cores were collected from each plot and mixed into one composite sample. Each sample was air-dried and passed through a 2 mm sieve to remove litter and detritus. A subsample of the air-dried soil was ground

using a ball mill to pass through a 0.15 mm sieve for further analyses of IP fractions and TP.

### 2.3 Soil chemical analyses

Soil pH was measured in a 1 : 5 soil-to-water slurry with a pH meter (S210 SevenCompact™, Mettler, Germany). Soil IP fractions were extracted according to the sequential fractionation scheme proposed by Chang and Jackson (1958) which is modified to suit for calcareous soils (Jiang and Gu, 1989). Briefly, soil  $\text{Ca}_2\text{-P}$  was determined by shaking 0.5 g soil with 25 mL 0.25 M  $\text{NaHCO}_3$  (pH 7.5) and then centrifuged at 3500 rpm for 8 min to measure soil  $\text{Ca}_2\text{-P}$ . The remaining soil was washed two times with 25 mL 95 %  $\text{C}_2\text{H}_5\text{OH}$  and extracted with 25 mL 0.5 M  $\text{NH}_4\text{Ac}$  (pH = 4.2) to determine soil  $\text{Ca}_8\text{-P}$  (Jiang and Gu, 1989). After this, the soil was shaken with 25 mL 1 M  $\text{NH}_4\text{Cl}$  and centrifuged at 3500 rpm to discard the supernatant. The remaining soil was then shaken with 25 mL 0.5 M  $\text{NH}_4\text{F}$  (pH 8.2) and centrifuged at 3500 rpm for 8 min to analyze the soil  $\text{Al-P}$  fraction in the supernatant. The remaining soil was washed two times with 25 mL saturated NaCl and then sequentially shaken with 25 mL mixture of 0.1 M NaOH and  $\text{Na}_2\text{CO}_3$  for 2 h at  $25^{\circ}\text{C}$  and then centrifuged at 4500 rpm for 10 min to measure soil  $\text{Fe-P}$  in the supernatant. The remaining samples were washed as above and extracted with 25 mL mixture of 0.3 M  $\text{Na}_3(\text{citrate})\text{-Na}_2\text{S}_2\text{O}_4$  and 0.5 M NaOH to measure soil  $\text{O-P}$ . Finally, the remaining samples were shaken with 0.25 M  $\text{H}_2\text{SO}_4$  for 1 h at  $25^{\circ}\text{C}$  and centrifuged at 3800 rpm for 10 min to determine soil  $\text{Ca}_{10}\text{-P}$ . The P concentration in all the extractants was determined by the molybdenum blue colorimetric method at a wavelength of 700 nm with a UV-VIS spectrometer (UV-1700, Shimadzu) (Murphy and Riley, 1962). Soil total IP (TIP) concentration was defined as the sum of soil labile IP ( $\text{Ca}_2\text{-P}$ ), moderate-cycling IP ( $\text{Al-P}$ ,  $\text{Fe-P}$  and  $\text{Ca}_8\text{-P}$ ) and recalcitrant IP ( $\text{Ca}_{10}\text{-P}$  and  $\text{O-P}$ ). Total organic P (TOP) was calculated as the difference between TP and TIP concentrations. The potential limitation of the extraction methods is that they may not be very specific in separating the different forms of P minerals, as a small amount of other phosphates dissolved in the extractants (Jiang and Gu, 1989).

Soil TP concentration was determined after digestion with 8 mL 85 %  $\text{HNO}_3$  + 4 mL 72 %  $\text{HClO}_4$  + 1 mL 40 % HF (Sommer and Nelson, 1972), and Olsen-P was extracted from air-dried soil with 0.5 M  $\text{NaHCO}_3$  (pH 8.5) (Olsen et al., 1954). Plant TP concentration was determined by acid digestion using  $\text{H}_2\text{SO}_4\text{-H}_2\text{O}_2$  (Thomas et al., 1967). Soil TP, Olsen-P and plant TP were analyzed by the molybdenum blue colorimetric method at 700 nm.

**Table 1.** General characteristics of the soil from the experimental site before nitrogen and phosphorus manipulation. Selected parameters include sand, silt, and clay fractions; soil pH; soil organic matter (SOM); total nitrogen (TN); total phosphorus (TP); total inorganic phosphorus (TIP); organic P; exchangeable Ca and Al; and available Fe and Mn. Data are mean  $\pm$  standard error ( $n = 5$ ).

Sand (%)	Silt (%)	Clay (%)	Soil pH	SOM (g kg <sup>-1</sup> )	TN (g kg <sup>-1</sup> )	TP (mg kg <sup>-1</sup> )	TIP (mg kg <sup>-1</sup> )	Organic P (mg kg <sup>-1</sup> )	Ca (cmol kg <sup>-1</sup> )	Al (cmol kg <sup>-1</sup> )	Fe (mg kg <sup>-1</sup> )	Mn (mg kg <sup>-1</sup> )
36.75	39.61	23.64	6.68	43.89	1.83	508.23	105.05	403.17	13.41	0.05	36.76	30.70
$\pm 0.93$	$\pm 0.96$	$\pm 0.78$	$\pm 0.06$	$\pm 1.14$	$\pm 0.06$	$\pm 21.30$	$\pm 2.87$	$\pm 22.95$	$\pm 0.43$	$\pm 0.01$	$\pm 1.77$	$\pm 0.93$

## 2.4 Statistical analyses

Plant P uptake of three dominant species was calculated using the following equation:

$$P \text{ uptake} = \sum_i^n P_i \times B_i,$$

where  $P_i$  is TP concentration of species  $i$  and  $B_i$  is the biomass of species  $i$ . All the data were shown as mean  $\pm$  standard error. The Kolmogorov–Smirnov test was performed to determine whether the data had a normal distribution. Three-way ANOVAs were conducted to determine the effects of N addition ( $N$ ), P addition rate ( $P_r$ ), P compounds ( $P_t$ ) and their interactions on soil IP fractions and Olsen-P concentration. For each P compound and N treatment, the effect of P addition rates on moderate-cycling IP fractions and plant P were determined using polynomial contrasts, the effect of P addition rates on plant biomass, soil pH, soil recalcitrant IP fractions, Olsen-P and TP were analyzed using Duncan's multiple-range tests. A Student's  $t$  test was used to determine the difference between two P compounds within each P addition rate and N treatment (without and with N) and between N treatments within each P compound and addition rate. For moderate-cycling IP fractions, one-way analysis of covariance (ANCOVA) was employed to distinguish the slopes between the two N treatments (without N vs. with N) for each P compound and between the two P compounds (KH<sub>2</sub>PO<sub>4</sub> vs. Ca(H<sub>2</sub>PO<sub>4</sub>)<sub>2</sub>) for each N treatment. Pearson correlations were used to test the relationships between soil variables. All of the above statistics were carried out using SPSS 16.0 (SPSS Inc., Chicago, USA).

Structural equation models (SEMs) were built to clarify direct and indirect N and P addition effects on soil IP fractions through the changes in plant P uptake and soil pH. A chi-square test, Akaike information criteria (AIC) and root-mean-square error of approximation (RMSEA) were used to evaluate the fit of the model. The SEM analyses were performed using AMOS 7.0 (Amos Development Co., Greene, Maine, USA). Statistical significance was accepted at  $P < 0.05$ .

## 3 Results

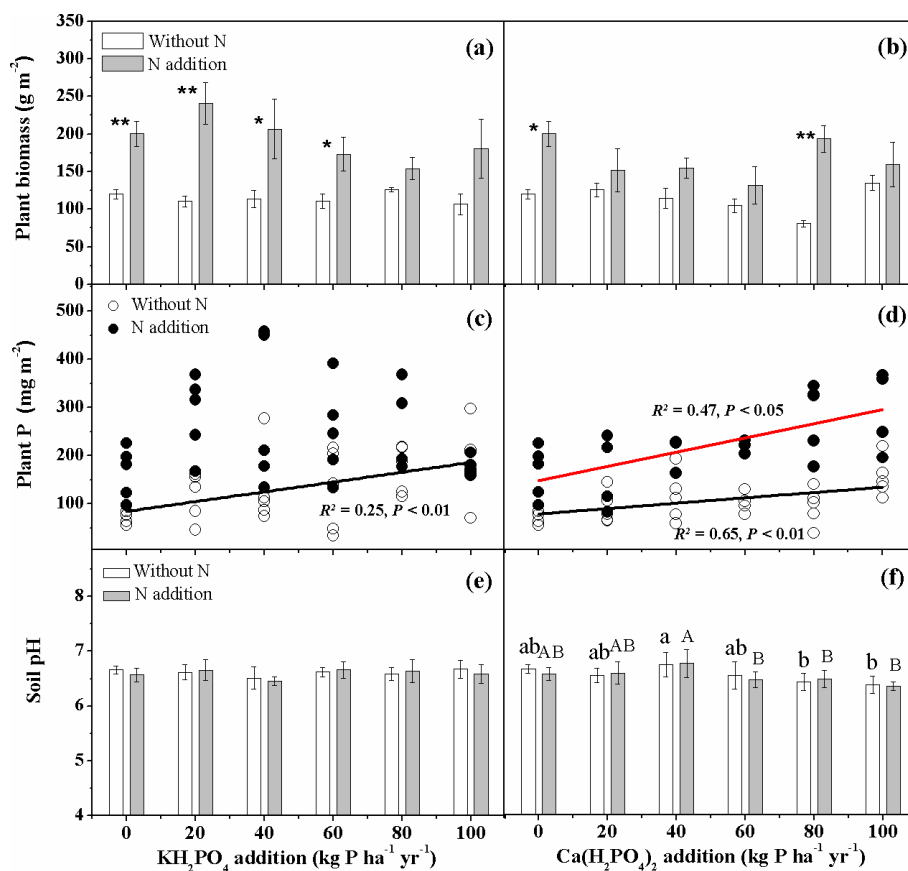
### 3.1 Aboveground plant biomass and soil pH

Nitrogen addition increased aboveground biomass production (Fig. 1a, b). Aboveground biomass production did not show a clear trend in response to different levels of P addition, either as KH<sub>2</sub>PO<sub>4</sub> or as Ca(H<sub>2</sub>PO<sub>4</sub>)<sub>2</sub> (Fig. 1a, b). Nitrogen addition significantly increased plant P uptake of the three dominant species *Stipa baicalensis*, *Leymus chinensis* and *Carex duriuscula* for both P compounds (Fig. 1c, d). Plant P uptake increased with increasing P addition rates with significantly higher overall KH<sub>2</sub>PO<sub>4</sub> effect than Ca(H<sub>2</sub>PO<sub>4</sub>)<sub>2</sub>. KH<sub>2</sub>PO<sub>4</sub> addition showed no impact on soil pH (Fig. 1e), while Ca(H<sub>2</sub>PO<sub>4</sub>)<sub>2</sub> tended to decrease soil pH at 80 and 100 kg P ha<sup>-1</sup> yr<sup>-1</sup> without N addition and at 60, 80 and 100 kg P ha<sup>-1</sup> yr<sup>-1</sup> with N addition (Fig. 1f).

### 3.2 Soil labile and moderate-cycling inorganic phosphorus fractions

For both KH<sub>2</sub>PO<sub>4</sub> and Ca(H<sub>2</sub>PO<sub>4</sub>)<sub>2</sub> additions, soil Al-P and Fe-P concentrations significantly increased with increased P addition rates (Fig. 2a, b, c, d). Soil Al-P and Fe-P concentrations were higher with KH<sub>2</sub>PO<sub>4</sub> than with Ca(H<sub>2</sub>PO<sub>4</sub>)<sub>2</sub> addition, especially at higher P rates (Fig. 2; Table S1 in the Supplement). This resulted in significant interactive  $P_t \times P_r$  effects on soil Al-P and Fe-P concentrations (Table 2). Significant interactive  $P_r \times N$  and  $P_t \times P_r \times N$  effects were also found on soil Al-P. For instance, Al-P concentration was higher with KH<sub>2</sub>PO<sub>4</sub> addition than Ca(H<sub>2</sub>PO<sub>4</sub>)<sub>2</sub> at P addition level of 60 kg P ha<sup>-1</sup> yr<sup>-1</sup> when N was not added, but it was higher for P addition levels of 60, 80 and 100 kg P ha<sup>-1</sup> yr<sup>-1</sup> when N was added. Nitrogen addition decreased both soil Al-P and Fe-P concentrations, particularly at higher levels of P addition (Fig. 2; Tables 2 and S1).

Addition of both P compounds significantly increased soil Ca<sub>2</sub>-P and Ca<sub>8</sub>-P concentrations with and without N addition (Fig. 3a, b, c, d). With KH<sub>2</sub>PO<sub>4</sub> addition, soil Ca<sub>2</sub>-P and Ca<sub>8</sub>-P concentrations were higher than with Ca(H<sub>2</sub>PO<sub>4</sub>)<sub>2</sub> addition (Fig. 3; Table S1). Nitrogen addition significantly decreased soil Ca<sub>2</sub>-P and Ca<sub>8</sub>-P concentrations for both P compounds in some P addition levels (Fig. 3; Table S1). Therefore, significant  $P_t \times P_r$  interactive effects were detected on soil Ca<sub>2</sub>-P and Ca<sub>8</sub>-P and  $P_r \times N$  interactive effect on soil Ca<sub>2</sub>-P (Table 2).



**Figure 1.** Aboveground plant biomass (a, b), biomass-weighted phosphorus (P) uptake of three dominant plant species (c, d) and soil pH (e, f) as affected by  $\text{KH}_2\text{PO}_4$  and  $\text{Ca}(\text{H}_2\text{PO}_4)_2$  additions with and without N inputs. Data are represented as mean  $\pm$  standard error for panels (a), (b), (e) and (f). Letters indicate significant differences between P rates of  $\text{KH}_2\text{PO}_4$  or  $\text{Ca}(\text{H}_2\text{PO}_4)_2$  addition without N (lowercase letters) and with N addition (capital letters). Asterisks represent significance between N treatments within each P type and rate (\* and \*\* for  $P < 0.05$  and 0.01, respectively).

**Table 2.** Results ( $F$  values) of three-way ANOVAs on the effect of phosphorus (P) fertilizer type ( $P_t$ ), P addition rate ( $P_r$ ), and nitrogen (N) addition and their interactions on soil inorganic phosphorus fractions of variscite (Al-P), strengite (Fe-P), dicalcium phosphate ( $\text{Ca}_2$ -P), octacalcium phosphate ( $\text{Ca}_8$ -P), hydroxylapatite ( $\text{Ca}_{10}$ -P), occluded phosphate (O-P), Olsen-P, total inorganic phosphorus (TIP), and soil total P (TP) with  $\text{KH}_2\text{PO}_4$  and  $\text{Ca}(\text{H}_2\text{PO}_4)_2$  addition.

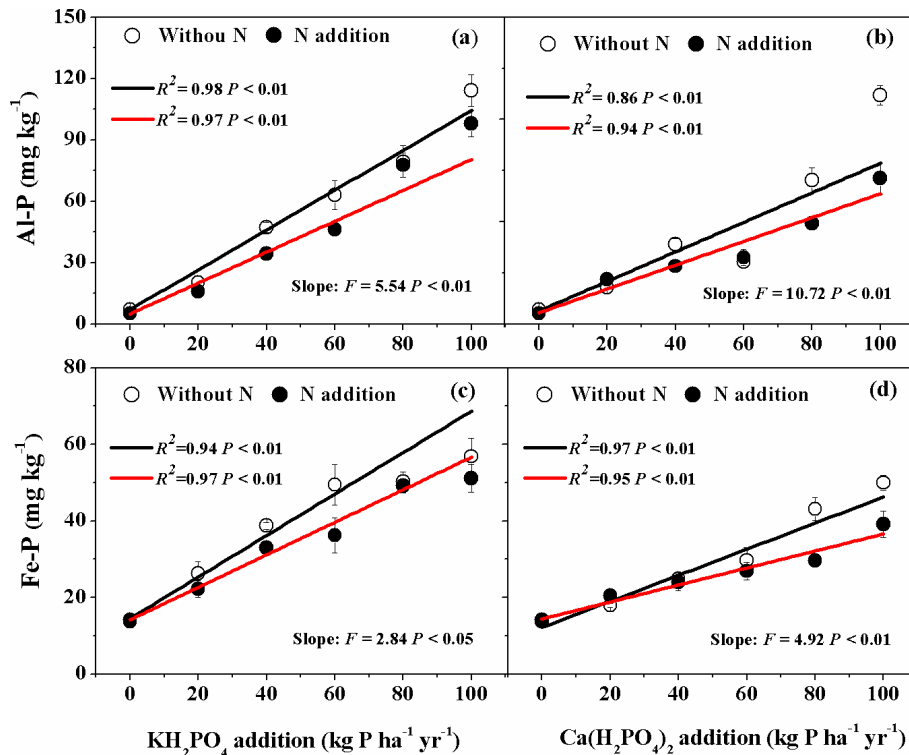
	Al-P	Fe-P	$\text{Ca}_2$ -P	$\text{Ca}_8$ -P	$\text{Ca}_{10}$ -P	O-P	Olsen-P	TIP	TP
$P_t$	32.4**	76.7**	59.9**	101**	98.1**	0.60	21.5**	60.4**	2.90
$P_r$	268**	97.3**	73.8**	62.6**	6.30**	3.88*	25.0**	192**	7.70**
$N$	37.1**	18.2**	11.1**	21.9**	5.20*	0.00	1.90	36.8**	3.80
$P_t \times P_r$	7.90**	3.40*	11.6**	8.40**	1.00	3.21*	5.90**	13.6**	0.90
$P_t \times N$	0.40	0.10	5.30	6.20	0.10	19.7**	1.80	0.00	6.00*
$P_r \times N$	6.30**	2.10	2.70*	2.20	0.60	0.70	2.00	4.90**	0.80
$P_t \times P_r \times N$	4.20**	2.60*	0.90	4.00**	0.40	2.81*	1.30	3.40**	3.90**

\* Significance level at 0.05. \*\* Significance level at 0.01.

### 3.3 Soil recalcitrant inorganic phosphorus fractions

$\text{Ca}(\text{H}_2\text{PO}_4)_2$  addition significantly increased soil  $\text{Ca}_{10}$ -P concentration, while  $\text{KH}_2\text{PO}_4$  addition showed no impact (Fig. 4a, b). Soil  $\text{Ca}_{10}$ -P concentration was higher with

$\text{Ca}(\text{H}_2\text{PO}_4)_2$  addition than with  $\text{KH}_2\text{PO}_4$  addition for all levels except for 60  $\text{kg P ha}^{-1} \text{ yr}^{-1}$  without N addition and for 20, 60 and 80  $\text{kg P ha}^{-1} \text{ yr}^{-1}$  with N addition (Fig. 4a, b; Table S2). There was no main N addition and  $N \times P_r$  interactive effect on soil  $\text{Ca}_{10}$ -P for both P compounds (Table 2).



**Figure 2.** Effects of phosphorus (P) and nitrogen (N) additions on soil variscite (Al-P) and strengite (Fe-P). Data are represented as mean  $\pm$  standard error. Phosphorus types include  $\text{KH}_2\text{PO}_4$  and  $\text{Ca}(\text{H}_2\text{PO}_4)_2$  at rates of 0, 20, 40, 60, 80 and  $100 \text{ kg P ha}^{-1} \text{ yr}^{-1}$ . Fitted lines are based on linear regression models. The black and red lines represent without N and N addition, respectively. Significance is labeled for slopes of the black and red lines.

Nitrogen addition also had no significant effect on soil O-P with  $\text{KH}_2\text{PO}_4$  addition but significantly decreased it with  $\text{Ca}(\text{H}_2\text{PO}_4)_2$  addition at 60 and  $80 \text{ kg P ha}^{-1} \text{ yr}^{-1}$  (Fig. 4c, d). Soil O-P showed a hump-shaped relationship along the P addition gradient with  $\text{KH}_2\text{PO}_4$  when added with N and with  $\text{Ca}(\text{H}_2\text{PO}_4)_2$  independent of N addition. Soil O-P concentration was lower with  $\text{Ca}(\text{H}_2\text{PO}_4)_2$  addition than with  $\text{KH}_2\text{PO}_4$  addition for 60 and  $80 \text{ kg P ha}^{-1} \text{ yr}^{-1}$  with N addition (Fig. 4c, d; Table S2). The relative proportions of soil O-P and  $\text{Ca}_{10}\text{-P}$  to soil TIP decreased while the proportions of soil Al-P,  $\text{Ca}_2\text{-P}$ , and  $\text{Ca}_8\text{-P}$  increased with increasing P addition rates for both P compounds (Fig. S1 in the Supplement).

### 3.4 Soil Olsen-P and total P

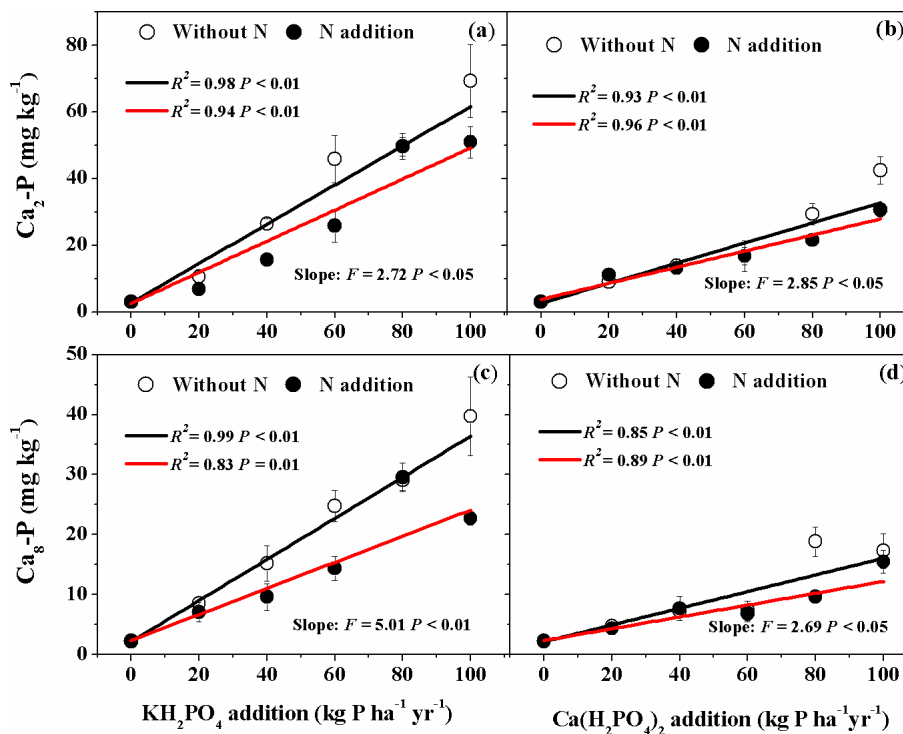
For both P compounds, P addition significantly increased soil Olsen-P concentration regardless of N addition (Fig. 5a, b). Soil Olsen-P concentration increased more strongly with increased levels of  $\text{KH}_2\text{PO}_4$  addition than with  $\text{Ca}(\text{H}_2\text{PO}_4)_2$  addition, resulting in significantly higher soil Olsen-P with  $\text{KH}_2\text{PO}_4$  addition in the  $100 \text{ kg P ha}^{-1} \text{ yr}^{-1}$  treatment with and without N addition (Fig. 5a, b).  $\text{KH}_2\text{PO}_4$  addition increased soil TP irrespective of N addition, while

$\text{Ca}(\text{H}_2\text{PO}_4)_2$  addition only increased soil TP without N addition (Fig. 5c, d).

### 3.5 Correlation between soil inorganic fractions with soil characteristics

For both P compounds, soil Al-P, Fe-P,  $\text{Ca}_2\text{-P}$  and  $\text{Ca}_8\text{-P}$  had significantly positive correlations with each other. In addition, soil TP and Olsen-P were all positively correlated with soil Al-P, Fe-P,  $\text{Ca}_2\text{-P}$  and  $\text{Ca}_8\text{-P}$  ( $P < 0.01$ ) (Table 3). Soil  $\text{Ca}_{10}\text{-P}$  had significantly positive correlations with soil Fe-P and  $\text{Ca}_8\text{-P}$  for both P compounds (Table 3). Soil TOP had significantly positive correlations with soil TP (Table 3). Furthermore, soil TIP concentration was positively correlated with the level of P addition for both P compounds (Fig. S2).

The SEM suggested that P addition had a positive impact on plant P uptake and soil moderate-cycling IP both with  $\text{KH}_2\text{PO}_4$  (Fig. 6a) and with  $\text{Ca}(\text{H}_2\text{PO}_4)_2$  addition (Fig. 6b). N addition had a positive impact on plant P uptake under both P compound additions (Fig. 6a, b). Plant P uptake had a negative impact on soil moderate-cycling IP both with  $\text{KH}_2\text{PO}_4$  (Fig. 6a) and with  $\text{Ca}(\text{H}_2\text{PO}_4)_2$  addition (Fig. 6b), while soil pH also negatively influenced soil labile IP with  $\text{Ca}(\text{H}_2\text{PO}_4)_2$ . Soil moderate-cycling IP fractions drove the increase in soil labile IP concentration for both P compounds,

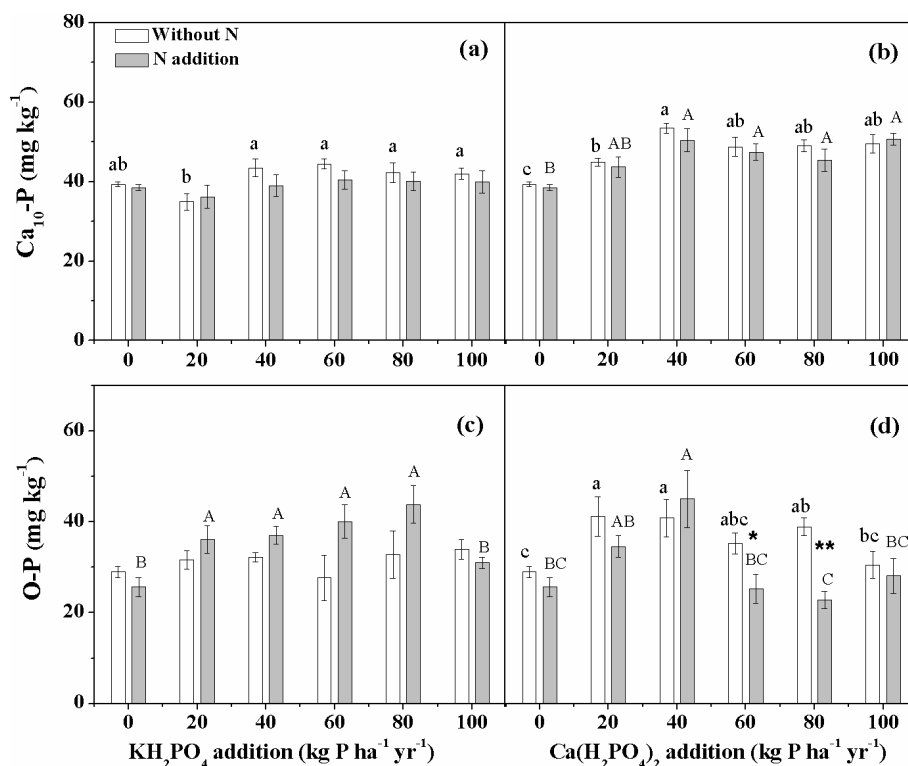


**Figure 3.** Effects of phosphorus (P) and nitrogen (N) additions on soil dicalcium phosphate (Ca<sub>2</sub>-P) and octacalcium phosphate (Ca<sub>8</sub>-P). Phosphorus types include KH<sub>2</sub>PO<sub>4</sub> and Ca(H<sub>2</sub>PO<sub>4</sub>)<sub>2</sub> at rates of 0, 20, 40, 60, 80 and 100 kg P ha<sup>-1</sup> yr<sup>-1</sup>. Data are represented as mean ± standard error. Fitted lines are based on linear regression models. The black and red lines represent without N and N addition, respectively. Significance was labeled for slopes of the black and red lines.

**Table 3.** Correlation analyses (*R* values) among soil inorganic fractions, Olsen-P, total phosphorus (TP) and organic P (TOP) with Ca(H<sub>2</sub>PO<sub>4</sub>)<sub>2</sub> and KH<sub>2</sub>PO<sub>4</sub> addition in the meadow steppe.

		Fe-P	Ca <sub>2</sub> -P	Ca <sub>8</sub> -P	Ca <sub>10</sub> -P	O-P	Olsen-P	TP	TOP
KH <sub>2</sub> PO <sub>4</sub> addition	Al-P	0.92**	0.93**	0.87**	0.25	0.15	0.70**	0.44**	-0.26*
	Fe-P		0.95**	0.86**	0.27*	0.19	0.59**	0.41**	-0.28*
	Ca <sub>2</sub> -P			0.92**	0.23	0.12	0.63**	0.41**	-0.28*
	Ca <sub>8</sub> -P				0.29*	0.10	0.57**	0.37**	-0.29*
	Ca <sub>10</sub> -P					0.00	0.17	0.06	-0.17
	O-P						0.07	0.15	0.01
	Olsen-P							0.52**	0.04
TP								0.74**	
Ca(H <sub>2</sub> PO <sub>4</sub> ) <sub>2</sub> addition	Al-P	0.91**	0.92**	0.80**	0.47*	-0.04	0.48**	0.29*	-0.08
	Fe-P		0.94**	0.87**	0.49*	-0.03	0.46**	0.31*	0.01
	Ca <sub>2</sub> -P			0.84**	0.42	-0.03	0.53**	0.32*	-0.06
	Ca <sub>8</sub> -P				0.50*	0.05	0.34**	0.26**	0.02
	Ca <sub>10</sub> -P					0.26*	0.11	0.17	-0.12
	O-P						-0.29*	-0.03	-0.03
	Olsen-P							0.34*	0.14
TP								0.88**	

\* Significance level at 0.05. \*\* Significance level at 0.01.



**Figure 4.** Effects of phosphorus (P) and nitrogen (N) additions on soil hydroxylapatite (Ca<sub>10</sub>-P) and occluded P (O-P). Phosphorus types include KH<sub>2</sub>PO<sub>4</sub> and Ca(H<sub>2</sub>PO<sub>4</sub>)<sub>2</sub> at rates of 0, 20, 40, 60, 80 and 100 kg P ha<sup>-1</sup> yr<sup>-1</sup>. Data are represented as mean ± standard error. Letters indicate significant differences between P rates of KH<sub>2</sub>PO<sub>4</sub> or Ca(H<sub>2</sub>PO<sub>4</sub>)<sub>2</sub> addition without N (lowercase letters) and with N addition (capital letters). Asterisks represent significance between N treatments within each P type and rate (\* and \*\* for  $P < 0.05$  and  $0.01$ , respectively).

with a higher contribution for KH<sub>2</sub>PO<sub>4</sub> addition (Fig. 6a, b). Soil moderate-cycling IP contributed to an increase in soil TP under both P compounds (Fig. 6a, b).

## 4 Discussion

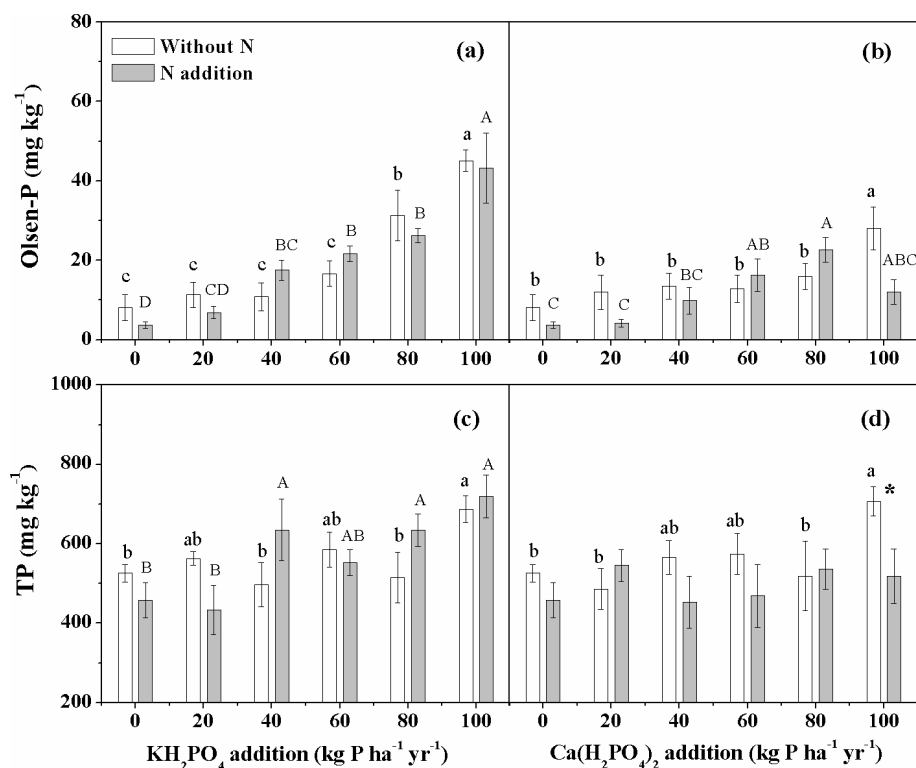
### 4.1 Effect of P additions rates on soil IP fractions

With increasing P addition levels, the increase in soil labile IP and moderate-cycling IP fractions was consistent with our first hypothesis and the findings from previous studies (Wang et al., 2010; Zhao et al., 2019). Soil IP fractions can be affected by nutrient addition, soil type and soil chemical properties (Daly et al., 2001; Stroia et al., 2011). The positive linear correlation of soil TIP concentration and P addition rates (Fig. S2) indicates that applied P was fixed mainly into inorganic forms (Chauhan et al., 1981) and converted to various soil P fractions (Piegholdt et al., 2013). Most of the applied P transformed into soil moderate-cycling IP fractions as seen from the decrease in the relative proportion of soil recalcitrant IP fractions (Fig. S1). Indeed, applied P fertilizer can be quickly bound by P-fixing constituents, e.g., Fe and Al oxides and clay minerals (Devau et al., 2011). In calcareous

soils, precipitation is the main process retaining applied P in soils, especially precipitation with Ca at relatively high soil pH (Wang et al., 2010). Likely, the amount of P from input processes (exogenous P and weathering) was higher than the amount from output processes (plant uptake, erosion and leaching losses), which can eventually cause P accumulation in the soil as insoluble soil P fractions (Song et al., 2017). Therefore, both the monopotassium phosphate and monocalcium phosphate fertilizers transformed into soil labile IP and moderate-cycling IP fractions and contributed to the increase in soil Al-P, Fe-P, Ca<sub>2</sub>-P and Ca<sub>8</sub>-P concentrations (Fig. 7).

Inconsistent with our first hypothesis, we found significant changes in soil recalcitrant P fractions (Ca<sub>10</sub>-P and O-P), which accounted for 21 %–73 % of soil TIP (Fig. S1) and potentially played an important role in supplying soil-available P in this meadow steppe (Fig. 7). The significant increase in soil Ca<sub>10</sub>-P with Ca(H<sub>2</sub>PO<sub>4</sub>)<sub>2</sub> addition was not expected; and it was inconsistent with a previous study from a calcareous soil showing unchanged soil Ca<sub>10</sub>-P after 21 years of superphosphate application (Wang et al., 2010). In this calcareous soil, Ca(H<sub>2</sub>PO<sub>4</sub>)<sub>2</sub> addition enhanced the transformation of soil Ca<sub>2</sub>-P and Ca<sub>8</sub>-P into more stable soil Ca<sub>10</sub>-P fractions for reasons that are not clear. In return, soil Ca<sub>10</sub>-P can be





**Figure 5.** Effects of phosphorus (P) and nitrogen (N) additions on soil Olsen-P and total P (TP) in the meadow steppe. Phosphorus types include  $\text{KH}_2\text{PO}_4$  and  $\text{Ca}(\text{H}_2\text{PO}_4)_2$  at rates of 0, 20, 40, 60, 80 and 100  $\text{kg P ha}^{-1} \text{yr}^{-1}$ . Data are represented as mean  $\pm$  standard error. Letters indicate significant differences between P rates of  $\text{KH}_2\text{PO}_4$  or  $\text{Ca}(\text{H}_2\text{PO}_4)_2$  addition without N (lowercase letters) and with N addition (capital letters). Asterisks represent significance between N treatments within each P type and rate (\* and \*\* for  $P < 0.05$  and 0.01, respectively).

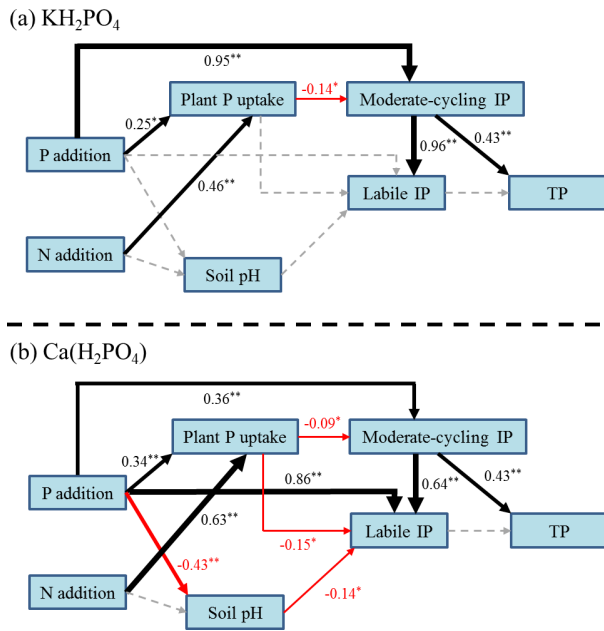
a potential P sink of soil moderate-cycling IP pools because of its significant correlation with soil Fe-P under both chemical P forms (Table 3). Soil O-P showed a hump-shaped relationship with P addition for both P compounds when N was also supplied, while no relationship was found for  $\text{KH}_2\text{PO}_4$  without N addition (Fig. 4c, d). This is in contrast to a 21 year long study where increased levels of  $\text{Ca}(\text{H}_2\text{PO}_4)_2$  significantly increased soil O-P (Wang et al., 2010). The discrepancy might be due to (1) differences in soil type affecting soil P dynamics differently (a Calcarid Regosol in the Wang et al., 2010, study and a Chernozem in this study); (2) different P compounds having different effects on soil O-P cycling; (3) N addition interacting with P to affect plant P uptake and soil O-P transformations (Marklein and Houlton, 2012; Zhang et al., 2004). Thus, P addition effects on soil O-P and other soil recalcitrant P fractions were complex, particularly when N was also added.

The significant increase in soil Olsen-P with P addition levels was consistent with previous studies (Karaca et al., 2002; Zhou et al., 2018). Significant increase in soil Olsen-P most likely was a consequence of inputs of P going into this pool surpassing output processes of mineral fixation, leaching and plant uptake. Dissolution of exogenous P com-

pounds could be the primary input process, which then directly contributed to the increase in soil Olsen-P concentration. Moreover, the soil IP pools, especially soil Fe-P, Al-P,  $\text{Ca}_2\text{-P}$  and  $\text{Ca}_8\text{-P}$ , can also be an important source of bioavailable P (Zhang et al., 2012). Significantly positive correlations between soil moderate-cycling IP fractions and soil labile IP (Table 3; Fig. 6) suggest that soil moderate-cycling IP fractions contributed strongly to enhance soil P availability (Fig. 7). Indeed, soil moderate-cycling IP fractions could release soil-available P more easily than recalcitrant IP fractions (Zhang et al., 2012).

#### 4.2 Effects of compound-specific P additions

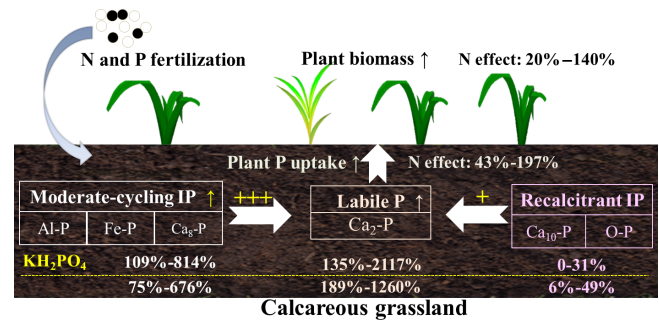
Consistent with our second hypothesis, soil labile IP and moderate-cycling IP fractions of Al-P, Fe-P and  $\text{Ca}_8\text{-P}$  tended to be higher with soluble  $\text{KH}_2\text{PO}_4$  addition than with less soluble  $\text{Ca}(\text{H}_2\text{PO}_4)_2$  addition with significant increases in some P addition levels (Figs. 2 and 3). Compared to  $\text{Ca}(\text{H}_2\text{PO}_4)_2$ ,  $\text{KH}_2\text{PO}_4$  is more effective in elevating soil phosphate levels to form soil labile IP and moderate-cycling IP fractions by rapidly interacting with Fe- and Al-oxides and  $\text{CaCO}_3$  (Haylin et al., 2005). The decrease in soil pH contributed to the increase in labile IP with  $\text{Ca}(\text{H}_2\text{PO}_4)_2$  addi-



**Figure 6.** Structural equation model of the relationships between soil pH, plant P uptake, soil moderate-cycling inorganic phosphorus (IP), labile P, and TP under  $\text{KH}_2\text{PO}_4$  (a,  $\chi^2 = 23.44$ ,  $P = 0.38$ , RMSEA = 0.03, AIC = 67.44) and  $\text{Ca}(\text{H}_2\text{PO}_4)_2$  additions (b,  $\chi^2 = 27.01$ ,  $P = 0.08$ , RMSEA = 0.09, AIC = 79.01) across N treatments. Arrows indicate positive (black), negative (red) and neutral (dotted grey) effects. Arrow width is proportional to the strength of the relationship. The number adjacent to each arrow is the standardized path coefficient with corresponding significance (\*, \*\* for  $P < 0.05$  and 0.01, respectively).

tion (Fig. 6b). Lower soil pH could accelerate soil recalcitrant IP fractions converting into soil labile IP, moderate-cycling IP and Olsen-P (Alt et al., 2013). Under the  $\text{Ca}(\text{H}_2\text{PO}_4)_2$  treatment, significant negative relationships of soil O-P and Olsen-P further support this argument (Table 3). On the other hand, plant P uptake instead of soil pH was more responsible for variations in soil moderate-cycling IP fractions with  $\text{KH}_2\text{PO}_4$  addition (Fig. 6a). Under both P compounds, soil moderate-cycling IP fractions contributed to an increase in soil labile IP across N treatments (Fig. 6), which suggested that higher plant growth and P demand (Fig. 1c, d) could enhance the conversion of soil moderate-cycling IP fraction into labile or available P.

In contrast to our expectation, soil recalcitrant IP fractions of O-P and  $\text{Ca}_{10}\text{-P}$  were higher with  $\text{Ca}(\text{H}_2\text{PO}_4)_2$  than with  $\text{KH}_2\text{PO}_4$  addition (Figs. 4 and 7). This suggests that a higher proportion of  $\text{Ca}(\text{H}_2\text{PO}_4)_2$  was converted into stable inorganic P forms than the more soluble  $\text{KH}_2\text{PO}_4$ . Additionally, lower soil O-P and  $\text{Ca}_{10}\text{-P}$  concentrations could be caused by enhanced plant P uptake intensity under  $\text{KH}_2\text{PO}_4$  addition, which facilitated more soil recalcitrant IP fractions transforming into moderate-cycling IP and labile IP. Thus,  $\text{Ca}(\text{H}_2\text{PO}_4)_2$  appears to be better than the  $\text{KH}_2\text{PO}_4$  at main-



**Figure 7.** Schematic diagram shows the effects of phosphorus (P) compound addition on soil inorganic P transformations in the calcareous grassland of Inner Mongolia. The percentages represent increase (“↑” in the diagram) of soil inorganic P fractions, plant P uptake and plant biomass as affected by fertilization across P addition rates.  $\text{KH}_2\text{PO}_4$  addition had a larger impact on moderate-cycling IP (sum of Al-P, Fe-P and  $\text{Ca}_8\text{-P}$ ) as compared to  $\text{Ca}(\text{H}_2\text{PO}_4)_2$  (109%–814% vs. 75%–676%). However, recalcitrant P (sum of  $\text{Ca}_{10}\text{-P}$  and O-P) increased more with  $\text{Ca}(\text{H}_2\text{PO}_4)_2$  addition relative to  $\text{KH}_2\text{PO}_4$  (6%–49% vs. 0%–31%). Nitrogen addition decreased moderate-cycling P by enhancing plant biomass and plant P uptake for both P compound additions across P addition rates. Therefore, moderate-cycling P showed a higher contribution to soil labile P than recalcitrant P, as represented by “+++” and “+”, respectively.

taining soil recalcitrant IP fractions in the grassland soil. Although soil recalcitrant IP fractions (O-P and  $\text{Ca}_{10}\text{-P}$ ) are relatively stable, they play essential roles in buffering the depletion of soil moderate-cycling P and maintaining soil-available P levels (Seeling and Jungk, 1996; Vu et al., 2008).

As expected, more soluble  $\text{KH}_2\text{PO}_4$  could result in higher labile IP (Fig. 7) in soil solution through dissolution. It is generally observed that this more soluble P fertilizer type dissolves in soil water over a shorter time period and generates more free  $\text{PO}_4^{3-}$  (Chien et al., 2011). Soil moderate-cycling IP fractions also contributed to elevated soil Olsen-P concentrations with stronger correlations under  $\text{KH}_2\text{PO}_4$  addition (Table 3). Soil moderate-cycling IP fractions showed a stronger direct and positive effect on soil labile IP with  $\text{KH}_2\text{PO}_4$  addition than  $\text{Ca}(\text{H}_2\text{PO}_4)_2$  addition, while plant P uptake showed both indirect negative (by negatively affecting soil moderate-cycling IP) and direct negative effects on labile IP with  $\text{Ca}(\text{H}_2\text{PO}_4)_2$  addition (Fig. 6). Based on the correlation analyses between soil IP fractions and Olsen-P, we found that soil  $\text{Ca}_2\text{-P}$  and Al-P were the dominant soil IP fractions to improve soil P availability in the calcareous soil. Our results suggest that  $\text{KH}_2\text{PO}_4$  was better than  $\text{Ca}(\text{H}_2\text{PO}_4)_2$  at alleviating P limitations of soil microorganisms and plants by promoting the formation of soil labile IP and moderate-cycling IP fractions in the meadow steppe.

### 4.3 Nitrogen-addition-regulated P effects on soil inorganic P fractions

We predicted that the concentrations of soil IP fractions would be lower under combined P and N additions than under P addition alone because of increased plant P uptake with N addition (third hypothesis). We found some support for this, where combined P and N additions decreased most soil IP fractions compared to P addition alone, except for the soil Ca<sub>10</sub>-P fraction. We also found a significant increase in plant biomass with combined P and N addition (Fig. 1a, b) and a negative correlation between plant P uptake and moderate-cycling IP fractions (Fig. 6a, b). Therefore, the decrease in soil moderate-cycling IP fractions with N addition could have been due to enhanced plant P uptake (Fig. 1c, d) as a result of increased plant biomass (Figs. 1a, b and 7). Under N addition, simultaneous increases in soil Olsen-P output (plant uptake) and input pathways (transformation from soil moderate-cycling IP fractions) may have resulted in mostly nonsignificant difference in soil Olsen-P concentrations between combined P and N addition and P addition alone (Fig. 5a, b). Nitrogen addition can potentially increase soil P availability by promoting solubilization of soil IP fractions in the short term (Wang et al., 2016). However, long-term N deposition resulted in soil IP-exhaustion, thereby constraining the growth of plants (Olander and Vitousek, 2000; Yang et al., 2014). Previous research has also found that decades of N addition could accelerate PO<sub>4</sub><sup>3-</sup> release (Malik et al., 2012; Stroia et al., 2011) and enhance conversion of soil recalcitrant IP fractions to soil labile IP and moderate-cycling IP fractions as a result of soil acidification (Alt et al., 2013). Additionally, N addition was found to suppress acid and alkaline phosphatase enzymes, resulting in a decrease in soil organic P mineralization in a similar semiarid grassland (Tian et al., 2016). Therefore, combined N and P addition might decrease soil IP fractions by reducing the conversion of soil organic P to IP as compared to P addition alone. Our results clearly illustrate that N effects on soil IP fractions depended on P inputs, where combined N and P additions could accelerate conversion of soil moderate-cycling IP fractions into soil-available P and enhance plant P uptake and biomass (Fig. 7).

## 5 Conclusions

Addition of P compounds significantly increased soil moderate-cycling IP fractions of Al-P, Fe-P, Ca<sub>2</sub>-P and Ca<sub>8</sub>-P, which may have contributed to higher available P (Olsen-P) in the soil. Soil moderate-cycling IP fractions were higher with soluble KH<sub>2</sub>PO<sub>4</sub> addition, but, in contrast, soil recalcitrant fractions of Ca<sub>10</sub>-P and O-P were higher with Ca(H<sub>2</sub>PO<sub>4</sub>)<sub>2</sub> addition. Combined N and P addition decreased soil IP fractions due to enhanced plant P uptake compared to P addition alone for both P compounds. Thus, N addition pro-

moted the transformation of soil moderate and recalcitrant IP fractions into available forms. Soil moderate-cycling IP fractions had a greater contribution to soil P availability than soil recalcitrant P fractions. Our findings elucidated the interactive effects of N and P addition on soil IP dynamics and presented the first evidence for the relative roles of exogenous P compounds in regulating P availability in the meadow steppe grassland. Overall, P fertilization is necessary for promoting productivity and sustainable management of grasslands by maintaining soil P availability and pools under scenarios of ecosystem N enrichment.

*Data availability.* The datasets used and/or analyzed in the present study are available from the corresponding author on reasonable request.

*Supplement.* The supplement related to this article is available online at: <https://doi.org/10.5194/bg-16-4293-2019-supplement>.

*Author contributions.* ZW and YJ designed the study. HL, HW and ZS performed samplings of soils and plants. HL completed laboratory analyses. RW and FAD contributed to the interpretation and discussion of the results. HL prepared the manuscript with suggestions from all the co-authors.

*Competing interests.* The authors declare that they have no conflict of interest.

*Acknowledgements.* We acknowledge the Erguna Forest-Steppe Ecotone Ecosystem Research Station, Institute of Applied Ecology, Chinese Academy of Sciences, for logistical support.

*Financial support.* This research has been supported by the National Natural Science Foundation of China (grant nos. 31770525 and 31870441), the National Key Research and Development Program of China (grant no. 2016YFC0500707), and the Youth Innovation Promotion Association CAS (grant no. Y9QCH121YY).

*Review statement.* This paper was edited by Nobuhito Ohte and reviewed by two anonymous referees.

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