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

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Abstract

Here we investigated the effects of P compounds (KH_2PO_4 and $Ca(H_2PO_4)_2$) with

different addition rates of 0, 20, 40, 60, 80 and 100 kg P ha^{-1} yr^{-1} and NH_4NO_3

addition (0 and 100 kg N ha^{-1} yr^{-1}) on soil labile (Olsen-P), moderate-cycling and

5   recalcitrant inorganic phosphorus (IP) fractions in a calcareous grassland of

northeastern China. Soil moderate-cycling IP, not readily available to plants but

transforming into available P quickly, include variscite (Al-P), strengite (Fe-P),

dicalcium phosphate (Ca_2-P) and octacalcium phosphate (Ca_8-P); recalcitrant fractions

include hydroxylapatite (Ca_{10}-P) and occluded P (O-P). Soil labile and

10   moderate-cycling IP fractions and total P significantly increased with increasing P

addition rates, with higher concentrations detected for KH_2PO_4 than for $Ca(H_2PO_4)_2$

addition. Combined N and P treatments showed lower moderate-cycling IP fractions

compared to ambient N conditions due to enhanced plant productivity.

Moderate-cycling IP was mainly regulated by aboveground plant biomass with

15   KH_2PO_4 addition, while by soil pH and plant biomass with addition of $Ca(H_2PO_4)_2$.

$Ca(H_2PO_4)_2$ addition significantly increased the soil recalcitrant IP (Ca_{10}-P) fraction,

while KH_2PO_4 addition showed no impact on it. A significant positive correlation was

detected between soil moderate-cycling IP fractions and soil Olsen-P which illustrated

that moderate-cycling IP fractions were important sources for available P. Our results

20   suggest that moderate-cycling IP fractions are essential for grassland P

biogeochemical cycling and chemical form of P fertilizer should be considered during

fertilization management for maintaining soil available P.

Key words semi-arid steppe; soil phosphorus; fertilization management;

25   mineral-bound phosphorus; occluded phosphorus

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

5 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 variscite (Al-P), strengite (Fe-P), dicalcium phosphate (Ca_2-P), octacalcium phosphate (Ca_8-P),

10 hydroxylapatite (Ca_{10}-P) and occluded P (O-P) (Zhao et al., 2019). Soil IP is dominated 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 Ca_2-P > Ca_8-P > Ca_{10}-P (Jiang and Gu, 1989). Although these element-bound P fractions are not readily available to plants, Al-P, Fe-P, Ca_2-P and Ca_8-P can be

15 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 labile/ available P (Olsen P), moderate-cycling P fractions (Al-P, Fe-P, Ca_2-P and Ca_8-P) and recalcitrant P fractions (Ca_{10}-P and O-P). But the category is not universal which mainly depends on the sensitivity of P

20 fractions in different studies (Schrijver et al., 2012 *vs.* Zhao et al., 2019). Therefore, understanding 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

25 cycling (Herlihy and McGrath, 2006) and the reactions between free phosphate and

cations in soil thereby influencing the content 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). This could then increase the risk of soil P leaching

5   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 $Ca(H_2PO_4)_2$

10   (slow-release P fertilizer) and KH_2PO_4 (fast-release P fertilizer) (Mortvedt et al.,

1999), which have played crital roles in agricultural ecosystems (Nguyen et al., 2012).

Both P fertilizers can convert into various inorganic P fractions including Fe-P

(especially in paddy soils with high Fe^{2+}) (Sah et al., 1989) and Al-P, which can

account for 33% of the total soil P in weathered soils (Margenot et al., 2017). And

15   Ca_2-P and Ca_8-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 shown that

long-term application of $Ca(H_2PO_4)_2$ increased soil inorganic P and total P (TP)

concentrations in a calcareous soil (Wang et al. 2010). However, long-term

$Ca(H_2PO_4)_2$ addition decreased P availability due to fixation of P to the soil

20   constituents (Xiong et al., 2018). KH_2PO_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 (Condron and Goh, 1989), little is

known about the effects of different P compound additions on soil IP fractions and P

25   availability.

Nitrogen enrichment can influence soil chemical properties thereby affecting soil P transformations, and consequently above-ground 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

5   al., 2014), and can result in redistribution of soil IP fractions (Zhao et al., 2019). Nitrogen addition could also promote the release of P from 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

10   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.

15   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,

20   Inner Mongolia grassland ecosystems have potentially become more nutrient-limited, predominantly by 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 KH_2PO_4 and $Ca(H_2PO_4)_2$ with and without N addition on concentrations of soil IP fractions,

25   available P and TP. We hypothesized that 1) soil labile IP (Olsen-P) and

moderate-cycling IP fractions (Al-P, Fe-P, Ca_2-P and Ca_8-P) would significantly

increase with KH_2PO_4 and $Ca(H_2PO_4)_2$ addition, while soil recalcitrant IP fractions

(Ca_{10}-P and O-P) would not be affected, because of different solubility in IP fractions;

2) addition of soluble KH_2PO_4 would be more beneficial to increasing soil labile IP,

5   moderate-cycling IP fractions, recalcitrant P fractions and total P (TP) than

less-soluble $Ca(H_2PO_4)_2$, because of faster conversion into these fractions with the

more soluble KH_2PO_4; 3) the concentrations of soil IP fractions would be lower under

combined P and N additions than that under P addition alone due to facilitation of

plant P uptake with N addition.

10

2. Materials and methods

2.1. Study sites and experimental design

The study site (119º 22′ E, 50º 10′ N, elevation 523 m a.s.l.) is located in the Erguna

Forest-Steppe Ecotone Research Station of Inner Mongolia, China. This area belongs

15   to a temperate continental monsoon climate. The mean annual precipitation and mean

annual temperature is 375 mm and -3 ℃, respectively. Rainfall of the site is mainly

concentrated during the period from June to August and the average growing season is

about 150 days. The soil is a Chernozem according to the Food and Agricultural

Organization of the United Nations classification (WRB, 2014), and the soil chemical

20   characteristics of the site are reported in Table 1. The dominant plant species include

Stipa baicalensis, *Leymus chinensis* and *Carex duriuscula*.

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., KH_2PO_4 and $Ca(H_2PO_4)_2$, and were applied at six levels: 0, 20, 40, 60, 80 and 100

25   kg P ha^{-1} yr^{-1}. Half of the plots were applied with 100 kg N ha^{-1} yr^{-1} in the form of

NH_4NO_3. Phosphorus 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 kg P ha^{-1} yr^{-1} of KH_2PO_4 addition (132 kg K ha^{-1}). To balance for Cl along with KCl addition, $CaCl_2$ was applied to maintain equal Cl

5   inputs for all plots (121 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 KH_2PO_4 and $Ca(H_2PO_4)_2$ treatment, so that in total, there were 110 plots of 8 m $\times$ 8 m, separated by 1-m wide buffer zones. The initial pH of KH_2PO_4 and $Ca(H_2PO_4)_2$ solutions were 4.91 and 4.50, respectively.

10   **2.2. Sample collection**

In August 2016, aboveground biomass was harvested by clipping all living tissues using a 1 m $\times$ 1 m quadrat placed randomly within each plot. The plants were sorted to species and oven-dried at 65 ℃ for 48 h. Soil samples were taken from the surface (10 cm depth) using a 5-cm diameter soil auger. Five soil cores were collected from

15   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

20   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 (1957) which is modified to suit for calcareous soils (Jiang and Gu, 1989). Briefly, Ca_2-P was determined by shaking 0.5g soil with 25 ml 0.25 M $NaHCO_3$ (pH 7.5), and then

25   centrifuged at 3500 rpm for 8 min to measure soil Ca_2-P. The remaining soil was

washed two times with 25 ml 95% C_2H_5OH and extracted with 25 ml 0.5 M NH_4Ac

(pH = 4.2) to determine soil Ca_8-P (Jiang and Gu, 1989). After this, the soil was

shaken with 25 ml 1 M NH_4Cl and centrifuged at 3500 rpm to discard the supernatant.

The remaining soil was then shaken with 25 ml 0.5 M NH_4F (pH 8.2) and centrifuged

5   at 3500 rpm for 8 min to analyze the 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 Na_2CO_3 for 2 h at 25 ℃, and then centrifuged

at 4500 rpm for 10 min to measure Fe-P in the supernatant. The remaining samples

were washed as above and extracted with 25 ml mixture of 0.3 M

10   Na_3(citrate)-$Na_2S_2O_4$ and 0.5 M NaOH to measure O-P. Finally, the remaining

samples were shaken with 0.25 M H_2SO_4 for 1 h at 25 ℃, and centrifuged at 3800

rpm for 10 min to determine Ca_{10}-P. The Ca_2-P and Ca_8-P fractions were extracted

separately from the Al-P, Fe-P, O-P and Ca_{10}-P frations. The P concentration in all the

extractants was determined by the molybdenum blue colorimetric method at

15   wavelength of 700 nm with a UV-VIS spectrometer (UV-1700, Shimazu) (Murphy

and Riley, 1962). Total IP (TIP) concentration was defined as the sum of

moderate-cycling IP (Al-P, Fe-P, Ca_2-P and Ca_8-P) and recalcitrant IP (Ca_{10}-P and

O-P). 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

20   phosphate dissolved in the extractants (Jiang and Gu, 1989).

Soil TP concentration was determined after digestion with 8 ml 85% HNO_3 +4 ml

72% $HClO_4$ +1 ml 40% HF (Sommers and Nelson, 1972), and Olsen-P was extracted

from air-dried soil with 0.5 M $NaHCO_3$ (pH 8.5) (Olsen et al., 1954). Plant TP

concentration was determined by acid digestion using H_2SO_4-H_2O_2 (Thomas et al.,

25   1967). Soil TP, Olsen P and plant TP were analyzed by the molybdenum blue

colorimetric method at 700 nm.

2.4. Statistical analyses

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

equation:

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$$P \; uptake = \sum_{i}^{n} P_i \times B_i \, ,$$

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 ± standard error. The Kolmogorov-Smirnov test was

performed to determine whether data had a normal distribution. Three-way ANOVAs

were conducted to determine the effects of N addition (N), P addition rate (P_r), P

10   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, recalcitrant IP

fractions, Olsen-P and TP were analyzed using Duncan's multiple range tests.

15   Student 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

20   between the two P compounds (KH_2PO_4 *vs.* $Ca(H_2PO_4)_2$) for each N treatment.

Pearson correlations were used to test the relationships between soil variables. All

the above statistics were carried out using SPSS 16.0 (SPSS Inc., Chicago, USA).

Structural equation models (SEM) were built to clarify direct and indirect

relationships between plant biomass, soil pH, moderate-cycling IP fractions,

25   recalcitrant IP fraction, Olsen-P and TP. Chi-square test, Akaike information criteria

(AIC) and the 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 $P < 0.05$.

5

3. Results

3.1. Aboveground plant biomass and soil pH

Nitrogen addition significantly increased aboveground biomass production (Fig. 1a,b).

Aboveground biomass production did not show a clear trend in response to different

10   levels of P addition, either as KH_2PO_4 or as $Ca(H_2PO_4)_2$ (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 rate with significantly higher overall

KH_2PO_4 effect than $Ca(H_2PO_4)_2$. KH_2PO_4 addition showed no impact on soil pH (Fig.

15   1e), while $Ca(H_2PO_4)_2$ decreased soil pH without N addition (Fig. 1f).

3.2. Soil moderate-cycling inorganic phosphorus fractions

For both KH_2PO_4 and $Ca(H_2PO_4)_2$ additions, Al-P and Fe-P concentrations

significantly increased with increased P addition rates (Fig. 2a, b, c, d). Al-P and Fe-P

concentrations were higher with KH_2PO_4 than with $Ca(H_2PO_4)_2$ addition, especially at

20   higher P rates (Fig. 2; Table S1). This resulted in significant interactive $P_t \times P_r$ effects

on Al-P and Fe-P concentrations (Table 2). For instance, Al-P was higher with

KH_2PO_4 addition than $Ca(H_2PO_4)_2$ at 60 kg P ha^{-1} yr^{-1} without N addition and at 60,

80 and 100 kg P ha^{-1} yr^{-1} levels with N addition. Nitrogen addition decreased both

Al-P and Fe-P concentrations, particularly at higher levels of P addition (Fig. 2; Table

25   2 and S1).

Addition of both P compounds significantly increased Ca_2-P and Ca_8-P

concentrations with and without N addition (Fig. 3a, b, c, d). With KH_2PO_4 addition,

soil Ca_2-P and Ca_8-P concentrations were higher than with $Ca(H_2PO_4)_2$ addition (Fig.

3; Table S1). Nitrogen addition significantly decreased soil Ca_2-P and Ca_8-P

5   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 Ca_2-P and Ca_8-P and

$P_r \times N$ interactive effect on Ca_2-P (Table 2).

3.3. Soil recalcitrant inorganic phosphorus fractions

$Ca(H_2PO_4)_2$ addition significantly increased soil Ca_{10}-P concentration, while KH_2PO_4

10   addition showed no impact (Fig. 4a, b). Ca_{10}-P concentration was higher with

$Ca(H_2PO_4)_2$ addition than with KH_2PO_4 addition for all levels except for 60 kg P ha^{-1}

yr^{-1} without N addition and for 0, 40 and 100 kg P ha^{-1} yr^{-1} with N addition (Fig. 4a, b;

Table S2). There was no main N addition and $N \times P_r$ interactive effect on Ca_{10}-P for

both P compounds (Table 2). Nitrogen addition had also no significant effect on O-P

15   with KH_2PO_4 addition but significantly decreased it with $Ca(H_2PO_4)_2$ addition at 60

and 80 kg P ha^{-1} yr^{-1} (Fig. 4c, d). Soil O-P showed a hump-shaped relationship along

the P addition gradient with KH_2PO_4 when added with N, and with $Ca(H_2PO_4)_2$

independent of N addition. The O-P concentration was lower with $Ca(H_2PO_4)_2$

addition than with KH_2PO_4 addition for 60 and 80 kg P ha^{-1} yr^{-1} with N addition (Fig.

20   4c, d; Table S2). The relative proportions of O-P and Ca_{10}-P to TIP decreased while

the proportions of Al-P, Ca_2-P, and Ca_8-P increased with increasing P addition rate for

both P compounds (Fig. S1).

3.3. Soil Olsen-P and total P

For both P compounds, P addition significantly increased Olsen-P concentration

25   regardless of N addition (Fig.5a, b). Olsen-P concentration increased more strongly

with increased levels of KH_2PO_4 addition than with $Ca(H_2PO_4)_2$ addition, resulting in

significantly higher Olsen-P with KH_2PO_4 addition in the 100 kg P ha^{-1} yr^{-1} treatment

with and without N addition (Fig. 5a, b). Soil TP increased with P addition both with

KH_2PO_4 and $Ca(H_2PO_4)_2$ addition without and with N addition (Fig. 5c, d).

5 **3.4. Correlation between soil inorganic fractions with soil characteristics**

For both P compounds, Al-P, Fe-P, Ca_2-P and Ca_8-P had significantly positive

correlations with each other. In addition, TP and Olsen-P were all positively correlated

with Al-P, Fe-P, Ca_2-P, and Ca_8-P ($P < 0.01$) (Table 3). Ca_{10}-P had significantly

positive correlations with Fe-P and Ca_8-P for both P compounds (Table 3).

10 Furthermore, soil TIP concentration was positively correlated with the level of P

addition for both P compounds (Fig. S2).

The SEM suggested that plant biomass had a negative impact on moderate-cycling

IP both with KH_2PO_4 (Fig. 6a) and with $Ca(H_2PO_4)_2$ addition (Fig. 6b), while soil pH

also negatively influenced moderate-cycling IP with $Ca(H_2PO_4)_2$. Moderate-cycling IP

15 fractions drove the increase in Olsen-P (labile P) concentration for both P compounds,

with a higher contribution for KH_2PO_4 addition (Fig. 6a,b). Olsen-P was negatively

affected by recalcitrant IP only with $Ca(H_2PO_4)_2$ addition (Fig. 6b).

4. Discussion

20 **4.1. Effect of P additions rates on soil IP fractions**

With increasing P addition levels, the increase in soil Olsen-P 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.,

25 2011). The positive linear correlation of TIP concentration and P addition rates (Fig.

S2) indicates that applied P was immobilized mainly into inorganic forms (Chauhan et al., 1981) and converted to various P fractions (Piegholdt et al., 2013). Most of the applied P transformed into moderate-cycling IP fractions as seen from the decrease in the relative proportion of recalcitrant IP fractions (Fig. S1). Indeed, applied P fertilizer

5 can be quickly bound by P-fixing constituents, *e.g.*, Fe/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

10 losses), which can then cause P accumulation in the soil as insoluble P fractions (Song et al., 2017). Therefore, both the monopotassium phosphate and monocalcium phosphate fertilizers transformed into moderate-cycling IP fractions and contributed to the increase in Al-P, Fe-P, Ca_2-P and Ca_8-P concentrations (Fig. 7).

Inconsistent with our first hypothesis, we found significant changes in recalcitrant

15 P fractions (Ca_{10}-P and O-P), which accounted for 21%-73% of TIP (Fig. S1) and potentially played an important role in supplying available P in this meadow steppe (Fig. 6b & Fig. 7). The significant increase of Ca_{10}-P with $Ca(H_2PO_4)_2$ addition was not expected; and it was inconsistent with a previous study from a calcareous soil showing unchanged Ca_{10}-P after 21-years of superphosphate application (Wang et al.,

20 2010). In this calcareous soil, $Ca(H_2PO_4)_2$ addition enhanced the transformation of moderate-cycling IP fractions (Ca_2-P and Ca_8-P) into more stable Ca_{10}-P fractions for reasons that are not clear. In return, Ca_{10}-P can be a potential P sink of moderate-cycling IP pools because of its significant correlation with Fe-P under both chemical P forms (Table 3). Soil O-P showed a hump-shaped relationship with P

25 addition for both P compounds when N was also supplied, while no relationship was

found for KH_2PO_4 without N addition (Fig. 4c, d). This is in contrast to a 21-year long

study where increased levels of $Ca(H_2PO_4)_2$ significantly increased 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

5   Chernozem in this study); 2) different P compounds having different effects on O-P

cycling; 3) N addition interacting with P to affect plant P uptake and O-P

transformations (Marklein and Houlton, 2012; Zhang et al., 2004). Thus, P addition

effects on O-P and other recalcitrant P fractions were complex, particularly when N

was also added.

10   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

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 compounds could be the primary input process, which then directly

15   contributed to the increase in Olsen-P concentration. Moreover, the soil IP pools

associated with moderate-cycling of P, especially Fe-P, Al-P, Ca_2-P and Ca_8-P, can also

be an important source of bioavailable P (Zhang et al., 2012). Significantly positive

correlations between moderate-cycling IP fractions and Olsen-P (Table 3; Fig. 6)

suggest that moderate-cycling P fractions contributed strongly to enhance soil P

20   availability (Fig. 7). Indeed, moderate-cycling IP fractions could release soil available

P more easily than recalcitrant fractions (Zhang et al., 2012).

4.2. Effects of compound-specific P additions

Consistent with our second hypothesis, soil moderate-cycling P fractions of Al-P, Fe-P,

Ca_2-P, Ca_8-P tended to be higher with soluble KH_2PO_4 addition than with less-soluble

25      $Ca(H_2PO_4)_2$ addition with significant increases in some P addition levels (Fig. 2 & Fig.

3). Compared to Ca(H$_2$PO$_4$)$_2$, KH$_2$PO$_4$ is more effective in elevating soil phosphate levels to form moderate-cycling IP fractions by rapidly interacting with Fe- and Al-oxides and CaCO$_3$ (Havlin et al., 2005). The negative correlation between soil pH and moderate-cycling IP fractions with Ca(H$_2$PO$_4$)$_2$ addition further suggests that the

5   decrease in soil pH contributed to the increase in moderate-cycling IP fractions with Ca(H$_2$PO$_4$)$_2$ addition (Fig. 6b). Lower soil pH could accelerate recalcitrant IP fractions converting into moderate-cycling IP and Olsen P (Alt et al., 2013). Under the Ca(H$_2$PO$_4$)$_2$ treatment, significant negative relationships of recalcitrant IP and Olsen P further support this argument (Fig. 6b). On the other hand, plant biomass instead of

10   soil pH was more responsible for variations in moderate-cycling IP fractions with KH$_2$PO$_4$ addition (Fig. 6a). Under both P compounds, moderate-cycling IP fractions contributed to the increase of soil Olsen P across N treatments (Fig. 6), which suggested that higher plant growth and P demand (Fig. 1c,d) could enhance the conversion of moderate-cycling IP fraction into available P.

15   In contrast to our expectation, the recalcitrant IP fractions of O-P and Ca$_{10}$-P were higher with Ca(H$_2$PO$_4$)$_2$ than with KH$_2$PO$_4$ addition (Fig. 4 & Fig. 7). This suggests that Ca(H$_2$PO$_4$)$_2$ could be converted more rapidly into stable inorganic P forms than the more soluble KH$_2$PO$_4$. Additionally, lower O-P and Ca$_{10}$-P concentrations could be caused by enhanced plant P uptake intensity under KH$_2$PO$_4$ addition which

20   facilitated more recalcitrant IP fractions transforming into moderate-cycling IP and Olsen P. Thus, Ca(H$_2$PO$_4$)$_2$ appears to be better than the KH$_2$PO$_4$ in maintaining recalcitrant IP fractions in the soil. Although soil recalcitrant IP fractions (O-P and Ca$_{10}$-P) are relatively stable, they play essential roles in buffering the depletion of moderate-cycling P and maintaining soil available P levels (Seeling and Jungk, 1996;

25   Vu et al., 2008).

As expected, more soluble KH_2PO_4 could result in higher available P (Fig. 7) in soil solution through dissolution. It is generally observed that this more soluble P fertilizer type dissolves in soil water at a shorter time and generates more free PO_4^{3-} (Chien et al., 2011). Moderate-cycling IP fractions also contributed to elevated

5   Olsen-P concentrations with stronger correlations under KH_2PO_4 addition (Table 3). Moderate-cycling IP factions showed a stronger direct and positive effect on Olsen-P with KH_2PO_4 addition than $Ca(H_2PO_4)_2$ addition, while plant biomass showed both indirect negative (by negatively affecting moderate-cycling IP) and positive effects on Olsen-P with KH_2PO_4 addition (Fig. 6). Based on the correlation analyses between

10   soil IP fractions and Olsen-P, we found that Ca_2-P and Al-P were the dominant IP fractions to improve soil P availability in the calcareous soil. Our results suggest that KH_2PO_4 was better than $Ca(H_2PO_4)_2$ in alleviating P limitations of soil microorganisms and plants by promoting the formation of moderate-cycling IP fractions and Olsen-P in the semi-arid steppe.

15   **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

20   alone, except for the Ca_{10}-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 biomass 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 (Fig. 1a,b &

25   Fig. 7). Under N addition, simultaneous increases in Olsen-P output (plant uptake)

and input pathways (transformation from moderate-cycling IP fractions) may have

resulted in mostly non-significant difference in 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 IP fractions in

5   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_4^{3-} release (Malik et al., 2012; Stroia et al., 2011) and enhance

conversion of recalcitrant IP fractions to labile and moderate-cycling IP fractions as a

10   result of soil acidification (Alt et al., 2013). Additionally, N addition was found to

suppress acid and alkaline phosphatase enzymes resulting in the decrease of soil

organic P mineralization in a similar semi-arid grassland (Tian et al. 2016). Therefore,

combined N and P addition might decrease soil IP fractions by reducing the

conversion of organic P to IP as compared to P addition alone. Our results clearly

15   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

available P and enhance plant P uptake and biomass (Fig. 7).

5. Conclusions

20   Addition of P compounds significantly increased soil moderate-cycling IP fractions of

Al-P, Fe-P, Ca_2-P and Ca_8-P, which may have contributed to higher available P (Olsen

P) in the soil. Soil moderate-cycling IP fractions were higher with soluble KH_2PO_4

addition, but in contrast, recalcitrant fractions of Ca_{10}-P and O-P were higher with

$Ca(H_2PO_4)_2$ addition. Combined N and P addition decreased soil IP fractions due to

25   enhanced plant P uptake compared to P addition alone for both P compounds. Thus, N

addition promoted the transformation of moderate and recalcitrant IP fractions into available forms. Moderate-cycling IP fractions had a greater contribution to soil P availability than recalcitrant P fractions. Overall, our findings elucidated the interactive effects of N and P addition on soil IP dynamics and presented the first

5   evidence for the relative roles of exogenous P compounds in regulating P availability in the meadow steppe grassland.

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Tables

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 means ±SE (n = 5).

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

5

Table 2 Results (F values) of three-way ANOVAs on the effect of phosphorus (P)

fertilizer type (P_t), P addition rate (P_r), nitrogen (N) addition and their interactions on

soil inorganic phosphorus fractions of variscite (Al-P), strengite (Fe-P), dicalcium

phosphate (Ca_2-P), octacalcium phosphate (Ca_8-P), hydroxylapatite (Ca_{10}-P),

5   occluded phosphate (O-P), Olsen-P, total inorganic phosphorus(TIP), soil total P (TP)

with KH_2PO_4 and $Ca(H_2PO_4)_2$ addition.

	Al-P	Fe-P	Ca_2-P	Ca_8-P	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 and 0.01, respectively

Table 3 Correlation analyses (R values) among soil inorganic fractions and Olsen-P with $Ca(H_2PO_4)_2$ and KH_2PO_4 addition in the meadow steppe.

		Fe-P	Ca_2-P	Ca_8-P	Ca_{10}-P	O-P	Olsen-P
KH_2PO_4 addition	Al-P	0.92[**]	0.93[**]	0.87[**]	0.25	0.15	0.70[**]
	Fe-P		0.95[**]	0.86[**]	0.27[*]	0.19	0.59[**]
	Ca_2-P			0.92[**]	0.23	0.12	0.63[**]
	Ca_8-P				0.29[*]	0.10	0.57[**]
	Ca_{10}-P					0.00	0.17
	O-P						0.07
$Ca(H_2PO_4)_2$ addition	Al-P	0.91[**]	0.92[**]	0.80[**]	0.47[*]	-0.04	0.48[**]
	Fe-P		0.94[**]	0.87[**]	0.49[*]	-0.03	0.46[**]
	Ca_2-P			0.84[**]	0.42	-0.03	0.53[**]
	Ca_8-P				0.50[*]	0.05	0.34[**]
	Ca_{10}-P					0.26[*]	0.11
	O-P						-0.29[*]

[*], [**] Significance level at 0.05 and 0.01, respectively

5
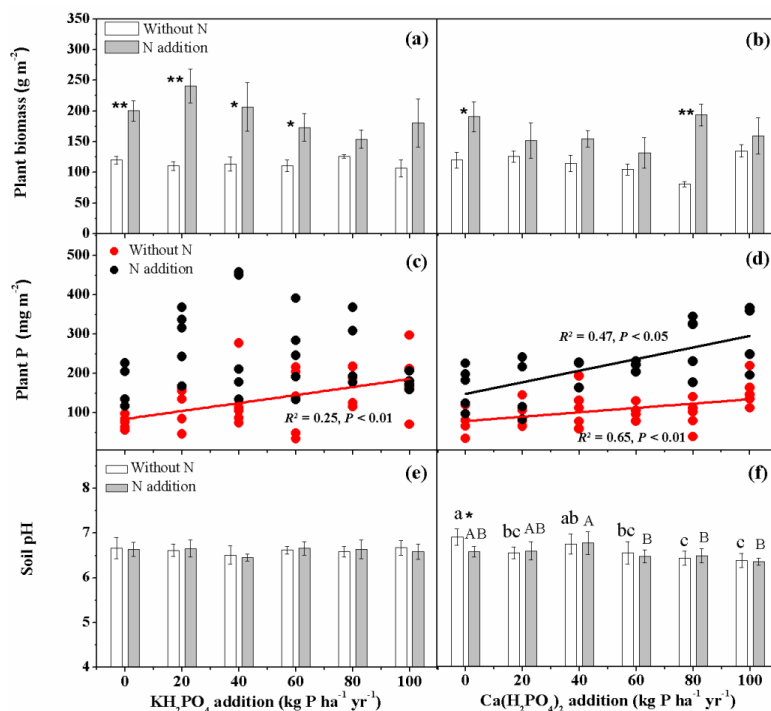
Fig. 1 Aboveground plant biomass (a, b), biomass-weighted phosphorus (P) uptake of three dominant plant species (c, d) and soil pH (e, f) as affected by KH_2PO_4 and $Ca(H_2PO_4)_2$ additions with and without N inputs. Data are represented as means $\pm$ SE

5 for panels a, b, e and f. Letters indicate significant differences between P rates of KH_2PO_4 or $Ca(H_2PO_4)_2$ 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).
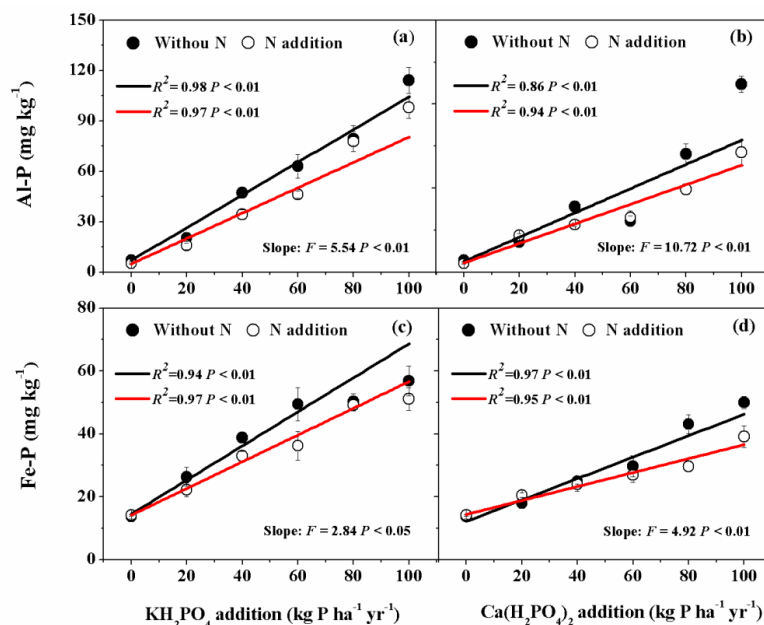
Fig. 2 Effects of phosphorus (P) and nitrogen (N) additions on soil variscite (Al-P) and strengite (Fe-P). Data are represented as means ± standard error. Phosphorus types include KH_2PO_4 and $Ca(H_2PO_4)_2$ at rates of 0, 20, 40, 60, 80 and 100 kg P ha^{-1} yr^{-1}. Fitted lines are based on linear regression models. The black and red line represent without N and N addition, respectively. Significance was labled for slopes of the black and red lines.
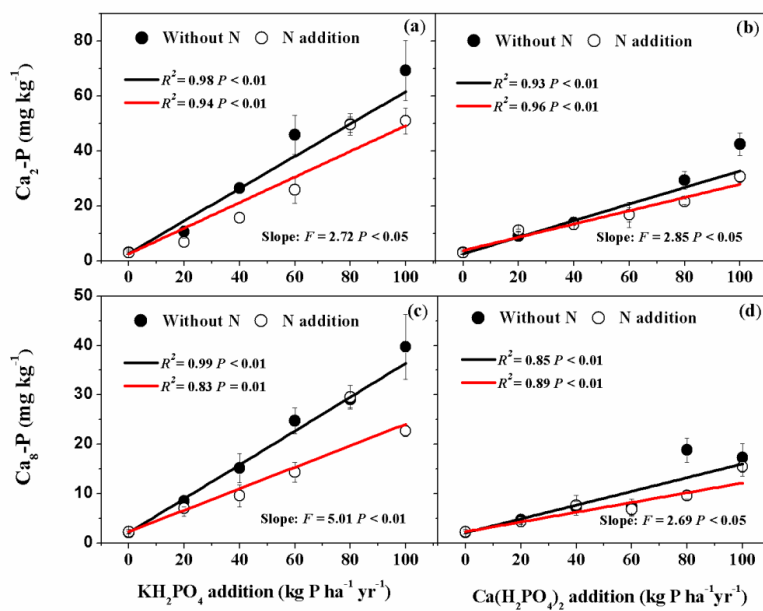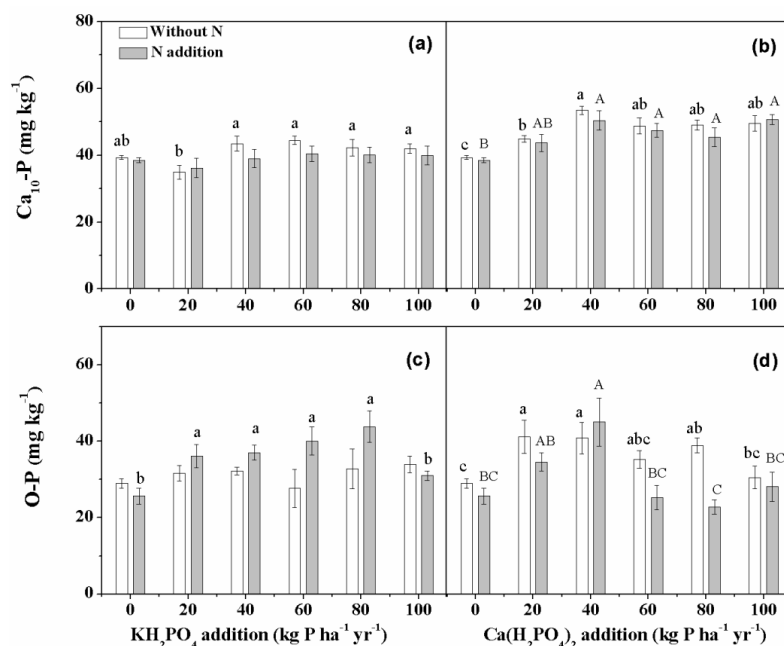
Fig. 3 Effects of phosphorus (P) and nitrogen (N) additions on soil dicalcium

phosphate (Ca_2-P) and octacalcium phosphate (Ca_8-P). Phosphorus types include

KH_2PO_4 and $Ca(H_2PO_4)_2$ at rates of 0, 20, 40, 60, 80 and 100 kg P ha^{-1} yr^{-1}. Data are

5   represented as means ± standard error. Fitted lines are based onlinear regression

models.The black and red line represent without N and N addition, respectively.

Significance was labled for slopes of the black and red lines.

Fig. 4 Effects of phosphorus (P) and nitrogen (N) additions on soil hydroxylapatite (Ca_{10}-P) and occluded P (O-P). Phosphorus types include KH_2PO_4 and $Ca(H_2PO_4)_2$ at rates of 0, 20, 40, 60, 80 and 100 kg P ha^{-1} yr^{-1}. Data are represented as means ±

5   standard error. Letters indicate significant differences between P rates of KH_2PO_4 or $Ca(H_2PO_4)_2$ 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).
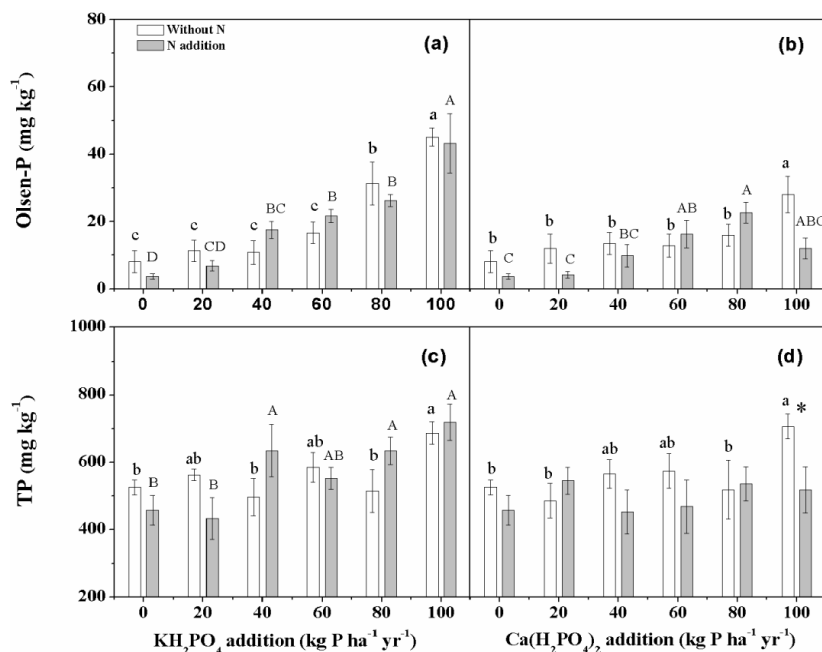
Fig. 5 Effects of phosphorus (P) and nitrogen (N) additions on soil Olsen-P and total P

(TP) in the meadow steppe. Phosphorus types include KH_2PO_4 and $Ca(H_2PO_4)_2$ at

rates of 0, 20, 40, 60, 80 and 100 kg P ha^{-1} yr^{-1}. Data are represented as

5   means±standard error. Letters indicate significant differences between P rates of

KH_2PO_4 or $Ca(H_2PO_4)_2$ addition without N (lowercase letters) and with N addition

(capital letters). Asterisks represent significance between N treatments within each P

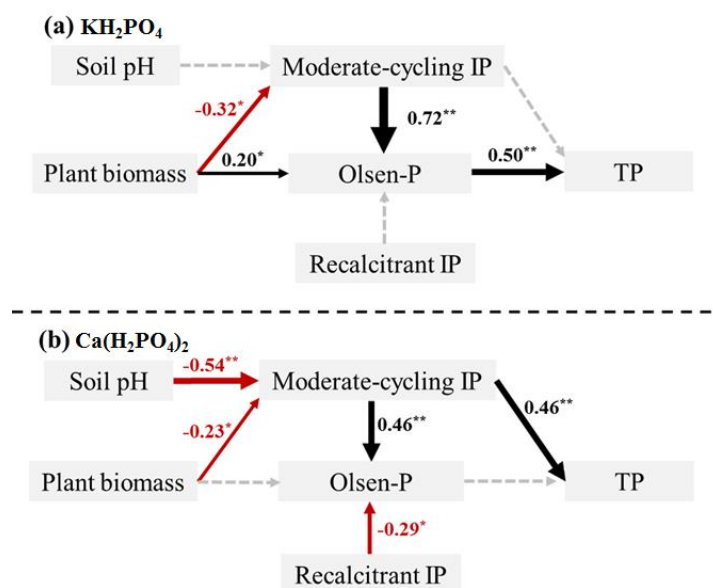type and rate (* and ** for $P < 0.05$ and 0.01, respectively).

Fig. 6 Structural equation model of the relationships between soil pH, aboveground plant biomass, moderate-cycling inorganic phosphorus (IP), recalcitrant IP, Olsen-P and TP under KH_2PO_4 (a, $\chi^2 = 9.37$, $P = 0.59$, RMSEA = 0.00, AIC = 41.37) and

5    $Ca(H_2PO_4)_2$ additions (b, $\chi^2 = 15.14$, $P = 0.13$, RMSEA = 0.09, AIC = 49.14) across N treatments. Arrows indicate positive (black), negative (red) and neutral (grey-dotted) effects. The number adjacent to each arrow is the standardized path coefficient with corresponding significance (*, ** for $P < 0.05$ and 0.01, respectively).
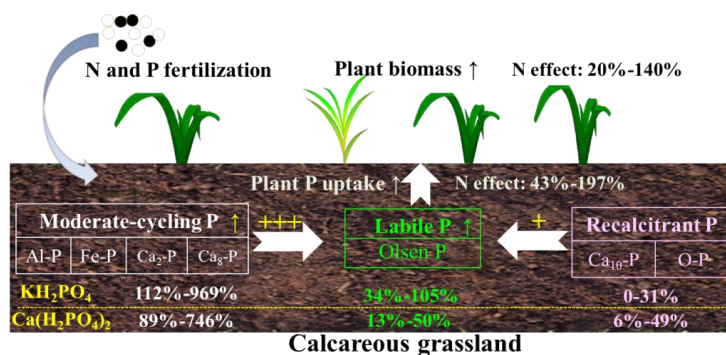
Fig. 7 Schematic diagram shows the effects of phosphorus (P) compound addition on soil inorganic P transformations in the calcareous grassland of Inner Mongolia. The percentages represent increase ("↑" in the diagram) of soil inorganic P fractions, plant

5   P uptake and plant biomass as affected by fertilization across P addition rates. KH_2PO_4 addition had a larger impact on moderate-cycling P (sum of Al-P, Fe-P, Ca_2-P and Ca_8-P) as compared to $Ca(H_2PO_4)_2$ (112%-969% *vs.* 89%-746%). However, recalcitrant P (sum of Ca_{10}-P and O-P) increased more with $Ca(H_2PO_4)_2$ addition relative to KH_2PO_4 (6%-49% *vs.* 0-31%). Nitrogen addition decreased

10   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.