

Dear Professor Nobuhito Ohte,

We thank you very much for considering our manuscript (bg-2019-173), “Exogenous phosphorus compounds interact with nitrogen availability to regulate dynamics of soil inorganic phosphorus fractions in a meadow steppe”. We appreciate the reviewers for their positive and constructive comments and suggestions on our manuscript. Here, we provided the point-to-point responses to reviewers’ comments. And a marked-up manuscript with red color indicating the changes was attached after the point-to-point responses.

10 **Reviewer 1**

Comment 1: I wonder whether authors could extract Ca₂-P and Olsen-P properly. I agree that the potential limitation of the sequential extraction methods as described in p. 8. Line 18-20 but I think that authors have to refer to the difference between Ca₂-P and Olsen-P in Introduction or Materials and methods. Although both of them are extracted with NaHCO₃, Ca₂-P is classified into moderate-cycling IP and Olsen-P into labile-P. Moreover, the concentration of Ca₂-P and Olsen-P in each treatment are really similar (Fig.3 a,b and Fig. 5 a,b) and they are significantly positively correlated each other (r= 0.63 and 0.53 in Table 3). I wonder if authors extracted almost same chemical properties. Refer difference between Ca₂-P and Olsen-P in terms of chemical compounds. If they are chemically similar compounds, Ca₂-P should be eliminated from Moderate-cycling IP when SEM were built.

Response: Thanks for the comment. And we agree with the reviewer’s comment that Ca₂-P and Olsen P are chemically similar. The description about the difference between Ca₂-P and Olsen-P was added in the Introduction section as “Soil Olsen P could be directly absorbed and utilized by plants, which includes all water-soluble P, some of the absorbed and soluble IP, and mineralizable organic P (Tang et al., 2009; Cao et al., 2012). Ca₂-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)” (P. 4 Lines 23-24). Therefore, we defined Ca₂-P as labile IP and Olsen-P as available P (P. 4 Lines 16-19). And the SEM has been reanalyzed by eliminating Ca₂-P from moderate-cycling IP, where it was defined as the labile IP in the SEM modeling.

Reference cited:

Shen, J., Li, R., Zhang, F., Fan, J., Tang, C., Renfenl, Z.: *Crop yields, soil fertility and phosphorus fractions in response to long-term fertilization under the rice monoculture system on a calcareous soil*, *Filed Crop Res.* 86, 225-238.

5 Tang, X., Ma, Y., Hao, X., Li, X., Li, J., Huang, S., Yang, X.: *Determining critical values of soil Olsen-P for maize and winter wheat from long-term experiments in China*, *Plant Soil*, 323:143-151.

Cao, N., Chen, X., Cui, Z., Zhang, F.: *Change in soil available phosphorus in relation to the phosphorus budget in China*, *Nutr. Cycl. Agroecosyst*, 94: 161-170, 10.1007/s10705-012-9530-0, 2012.

10 Zhao, F., Zhang, Y., Dijkstra, F. A., Li, Z., Zhang, Y., Zhang, T., Lu, Y., Shi, J., and Yang, L.: *Effect of amendments on phosphorous status in soils with different phosphorous levels*, *Catena* 172, 97-103.

Comment 2: I have some concern about SEM. At first, how do the authors handle N addition effects when making SEM? There is no description about that. Second, the description “Moderate-cycling IP was mainly regulated by plant biomass” (e.g. p.2 line 14-15) sounds strange. In this study, plant biomass is also affected by soil IP. Finally, not only IP but Organic P also affects TP because the percentage of each IP fraction to total P (TP) is less than 50%. Organic P (TP-TIP) fraction accounts large part of TP and must have great effects on TP in this study. Author should explain these three points and reanalyze them.

Response: Thanks for the constructive suggestions. We revised the manuscript according to all the suggestions as mentioned above. First, N and P addition effects have been incorporated into the SEM (please see Fig. 6), which was described in the Statistical analysis section as “Structural equation models (SEM) were built to clarify direct and indirect N and P addition effects on soil P fractions through the changes in plant P uptake and soil pH” (P. 11 Lines 7-8). Second, we replaced the parameter of plant biomass with plant P uptake in the SEM, because the plant P uptake intensity (amount) instead of plant biomass can directly affect soil P dynamics. Third, we fully agree with the point that soil organic P has great effects on TP and we tried to add the soil organic P into the SEM. However, there are multiple collinearities (variance proportions = 0.97) between soil organic P and soil TP resulting in the failure of the SEM. Thus, we added the correlation between organic P and TP to illustrate the significant contribution of organic P to TP in Table 3.

35 **Comment 3:** I have serious concern about description in Results section of this study. This paper has several mistakes in the Results section. Some description in Results do not correspond to the relevant Table and Figure. Some specific comments are written

below.

Response: We have addressed these concerns in the following responses to specific comments.

Specific comments

5 p.4 line 16-18: It may be appropriate to show which fraction of inorganic P increased in the previous study.

Response: Thanks for the comment. We have added the information of which inorganic P fractions increased in the previous study as “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, 10 Ca_2 -P, Ca_8 -P and O-P) and total P (TP) concentrations in a calcareous soil (Wang et al. 2010)” (P. 5 Lines 22-24).

p. 6 line 4-7 (2nd hypothesis): I cannot understand how hypothesis 2 was derived. I particularly do not understand why authors hypothesized that recalcitrant P would be higher with KH_2PO_4 addition than with $\text{Ca}(\text{H}_2\text{PO}_4)_2$ addition. I think that it takes 15 much time to form recalcitrant P from labile-P. Authors should add explanation about chemical properties of recalcitrant IP with introducing previous studies.

Response: Thanks for the comment. We hypothesized that recalcitrant P would be higher with KH_2PO_4 addition than with $\text{Ca}(\text{H}_2\text{PO}_4)_2$ addition, because more soluble KH_2PO_4 can convert into labile and moderate-cycling IP fractions faster and 20 consequentially promoting the formation of recalcitrant IP fractions. We agree that it takes much time to form recalcitrant P from labile P, but the time should be shorter for KH_2PO_4 addition than $\text{Ca}(\text{H}_2\text{PO}_4)_2$. Here, hypothesis 2 has been clarified as “addition of soluble KH_2PO_4 would be more efficient in increasing soil labile IP, moderate-cycling IP fractions, recalcitrant P fractions and total P (TP) than 25 less-soluble $\text{Ca}(\text{H}_2\text{PO}_4)_2$, because of faster conversion of KH_2PO_4 into labile and moderate-cycling IP fractions and consequentially promoting the formation of recalcitrant fractions” (P. 7 Lines 9-14). We have added chemical properties of soil recalcitrant IP in the introduction section as “Soil recalcitrant IP is relatively stable and unavailable for plants, which is mainly converted from the fixation of labile and 30 moderate-cycling IP (Shen et al., 2004; Zhao et al., 2019)” (P. 4 Line 25-P. 5 Line 1). We hope this information would help to support the 2nd hypothesis.

Reference cited:

Shen, J., Li, R., Zhang, F., Fan, J., Tang, C., Renfenl, Z.: Crop yields, soil fertility and phosphorus fractions in response to long-term fertilization under the rice monoculture

system on a calcareous soil, *Field Crop Res.* 86, 225-238, [10.1016/j.fcr.2003.08.013](https://doi.org/10.1016/j.fcr.2003.08.013), 2004.

5 Zhao, F., Zhang, Y., Dijkstra, F. A., Li, Z., Zhang, Y., Zhang, T., Lu, Y., Shi, J., and Yang, L.: *Effect of amendments on phosphorous atatus in soils with different phosphorous levels*, *Catena*, 172, 97-103, <https://doi.org/10.1016/j.catena.2018.08.016>, 2019.

p.4 line 16-20: These two sentences introduce the previous studies which results were opposite. The connection of them is not good. I think that these two sentences should change to “Some previous study showed that~~~ and other one showed that ~~~.”

10 Response: Thanks for the helpful suggestion. This sentence has been corrected accordingly (P. 5 Line 22- P. 6 Line 1).

It may be appropriate to explain how large three species of plants (*Stipa baicalensis*, *Leymus chinensis* and *Carex duriuscula*) are occupied per area if authors have some data. Is there difference of dominant ratio among treatments?

15 Response: As suggested, we calculated and analyzed the relative biomass proportion of the three species (shown in P. 8 Lines 2-6) which showed no difference among P addition rates but significantly increased with N addition. The data for relative proportion of the three dominant species has been listed in the following table.

20 **Table** Sum of the relative biomass (%) of three dominant plant species (*Stipa baicalensis*, *Leymus chinensis* and *Carex duriuscula*) as affected by P addition type and rate without and with N addition, respectively.

P rate (kg P ha ⁻¹ yr ⁻¹)	KH ₂ PO ₄		Ca(H ₂ PO ₄) ₂	
	Without N	With N	Without N	With N
0	61.07±6.75	82.16±4.31	61.07±6.75	82.16±4.31
20	65.35±8.34	80.73±5.62	54.56±4.77	81.21±4.62
40	63.94±6.83	83.15±7.56	67.16±6.61	85.06±3.96
60	73.61±2.77	83.37±6.00	50.29±7.12	89.05±3.25
80	67.39±6.89	89.23±2.30	59.60±6.28	91.92±2.22
100	74.75±5.86	72.43±9.92	65.07±3.30	83.11±5.30

25 p.9 line 3-5 and p.10 line 11 and other: “P uptake” should be replaced by another word (i.e. the biomass-weighted P concentration). In this study, authors just determined the concentration of P in plant body not the P uptake.

Response: We thank the reviewer for the suggestion. But here we calculated the

amount of P uptake by the three dominant species via multiplying P concentration by plant biomass (P. 10 Lines 13-15). It's not biomass-weighted P concentration which is determined via multiplying P concentration by plant biomass proportion.

5 Explain about dataset authors have. According to Materials and methods, authors used 110 plots in total including replication, which means that 0 kg P ha⁻¹ yr⁻¹ treatments are overlapped between KH₂PO₄ and Ca(H₂O₄)₂ fertilization. Although most of their data looks same among KH₂PO₄ and Ca(H₂O₄)₂ fertilization when 0 kg P were added but some are different. For example, soil pH is almost same between N treatment when 0 kg KH₂PO₄ were added (Fig. 1e) but there is difference between N treatment
10 when 0 kg Ca(H₂O₄)₂ were added. Explain why some data are difference when 0 kg P were added as seen in soil pH.

Response: Sorry for the confusion. In the field experiment, we have 5 replicate control plots for both KH₂PO₄ and Ca(H₂O₄)₂ fertilization. In our previous version, we forgot to overlap the control plots between KH₂PO₄ and Ca(H₂O₄)₂ fertilization for soil pH.
15 But it is correct for all the other parameters. Now, we have corrected and reanalyzed the data of soil pH (Fig. 1e, f).

p.10 line 21-23: Unclear, explain more in detail

Response: Thanks for the comments. This sentence mainly explained the interactive P_r×N and P_t×P_r×N effects on soil Al-P. It has been clarified into “For instance, Al-P concentration was higher with KH₂PO₄ addition than Ca(H₂PO₄)₂ at P addition level
20 of 60 kg P ha⁻¹ yr⁻¹ when N was not added, but it was higher for P addition levels of 60, 80 and 100 kg P ha⁻¹ yr⁻¹ when N was added” (P. 12 Lines 5-8).

Results 3.3 and 3.4: There are many faults. Relevant Figure and Table does not show the result which are written in manuscript. First of all, it is strange that there are two
25 “3.3” section. Specific comments are below.

Response: Sorry for the confusion. We have changed the second 3.3 to 3.4 and changed 3.4 to 3.5. Responses for all the specific comments are listed below.

p.11 line 10-13: Fig 4a, b and Table S2 does not show the results as described in manuscript. I think authors should replace “0, 40, 100” by “20, 60, 80” according to
30 Table S2. Moreover, Table S2 just shows whether there is difference of soil IP fraction between types of added P compounds (KH₂PO₄ and Ca(H₂O₄)₂) or not but it does not show which is higher or lower between them. However, Table S2 does not coincide

with Fig.4a and b. For example, Table S2 shows that soil Ca₁₀-P are significantly different (Student t-test, p<0.01) between KH₂PO₄ and Ca(H₂PO₄)₂ addition when 0 kg P were added but they look no different in Fig 4a and b. I think something is wrong and authors should reconfirm the dataset and reanalyze them.

5 Response: We thank the reviewer for pointing this out. We checked all the data and presented Table S2 with means ± standard error in order to show which P type was higher. According to Table S2, we replaced 0, 40 ,100 by 40, 60, 100. We feel really sorry that we mistakenly did not present the data from the same control of KH₂PO₄ addition as Ca(H₂PO₄)₂ in Table S2 in our previous version (but the data of 0 kg P presented in Figure 4 were correct) (same correction mentioned for soil pH data). This has been corrected in the current version where we now used the data from the same control plots.

p.11 line 14-16: There is no asterisk in Fig. 4d. I cannot judge whether there is significant difference between Without N and N addition at 60 and 80 kg P ha⁻¹ yr⁻¹.

15 Response: Sorry for the confusion. We added the asterisks in Fig. 4d.

p.11 line18-20: Unclear, explain more in detail. What about O-P for 0 kg P ha⁻¹ yr⁻¹ treatment with N addition? Table S2 shows the significant difference between KH₂PO₄ and Ca(H₂PO₄)₂ addition (p<0.05).

20 Response: We thank the reviewer for pointing this out. We feel sorry for our mistake in presenting the data from control plots. The O-P concentration should be the same in the control plots for KH₂PO₄ and Ca(H₂PO₄)₂ addition, as we used the same control plots for the two P types as described in the Material and Method section. This has been corrected.

p.12 line 3-4: Fig. 5d does not show that results. Soil TP with Ca(H₂PO₄)₂ does not increase with increasing P addition when N was added.

Response: Thanks for the observation. This sentence has been corrected into “KH₂PO₄ addition increased soil TP irrespective of N addition, while Ca(H₂PO₄)₂ addition only increased soil TP without N addition”.

p.12 line 7-8: There are no results which indicate correlation between TP and moderate-inorganic IP (Fe-P, Al-P, Ca₂-P, Ca₈-P) in Table 3.

30 Response: Thanks so much for the observation. We added the correlation between soil TP and moderate-cycling IP (Al-P, Fe-P and Ca₈-P) in Table 3.

p.13 line 1: “applied P was immobilized mainly into inorganic forms” sounds strange. “Immobilization” is the conversion of inorganic materials to organic ones.

Response: As per suggestion, we replaced the word “immobilized” with “fixed” (P. 14 Line 16).

5 Add “soil” before each P fraction. Some are already added but I think that authors should unify the expression about soil IP fraction.

Response: We agree with the reviewer and have added “soil” before each P fractions in the manuscript.

Is the term “recalcitrant inorganic phosphorus” used commonly?

10 Response: Yes, we found some references about recalcitrant IP. In this study, we defined Ca₁₀-P and O-P as the recalcitrant IP which is insoluble and unavailable for plants. Miller et al (2001) introduced that most soil P exists in recalcitrant minerals and forms soil recalcitrant phosphate with the depletion of primary minerals. In addition, Lawrence et al (2001) also proposed the conversion of recalcitrant inorganic
15 phosphorus into other P forms.

Reference cited:

Miller, A., Schuur, E., Chadwick, O.: Redox control of phosphorus pools in Hawaiian montane forest soils, Geoderma, 102: 0-237, 10.1016/s0016-7061(01)00016-7, 2001.

20 *Lawrence, D and Schlesinger, W.: Changes in soil phosphorus during 200 years of shifting cultivation in Indonesia, Ecology, 82: 2769-2780, 10.2307/2679959, 2001.*

p.15 line 13: not “P demand” but “concentration of P in plant” as pointed out above.

Response: It’s not “concentration of P in plant”, because plant P uptake (g P per m²)
25 was calculated via multiplying plant P concentration (g P kg⁻¹ biomass) by plant biomass (g m⁻²).

p. 15 line 3-6: The sentence is wordy.

Response: Thanks for the comments. We reanalyzed the SEM and clarified the description into “The decrease in soil pH contributed to the increase in labile P with
30 Ca(H₂PO₄)₂ addition (Fig. 6b)” (P. 16 Lines 20-21) according to the SEM model.

Conclusion: I think that authors should suggest how to fertilize P and N appropriately on grassland ecosystems to maintain plant productivity in Conclusion based on the

results of this study.

Response: Thanks for the constructive suggestion. This information has been added in the Conclusion section as “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” (P. 19 Lines 23-25).

Technical corrections

p.3 line 17 and other: Olsen P => Olsen-P

Response: As per suggestion, we replaced “Olsen P” with “Olsen-P”.

10 p.4 line17: inorganic P => IP

Response: As per suggestion, we replaced “inorganic P” with “IP”.

p.7 line 1: phosphorus => P

Response: As per suggestion, we replaced “phosphorus” with “P”.

Caption of Fig. 7: moderate-cycling P => moderate-cycling IP

15 Response: As per suggestion, we replaced “moderate-cycling P” with “moderate-cycling IP” in Fig. 7 caption.

Fig. 4c: Replace lowercase letters (i.e. a, b) by capital letters (i.e. A, B).

Response: As suggested, we replaced lowercase letters (i.e. a, b) by capital letters (i.e. A, B) in Fig 4c.

20 Fig. 5a: The vertical axis labels of Fig. 3a and c and the horizontal axis labels of Fig. 5a are written in bold type and they should be changed.

Response: Thanks for the observation. We have corrected the vertical axis labels of Fig. 3a and c and the horizontal axis labels of Fig. 5a.

Fig. 6: Explain what the width of arrows means.

25 Response: We thank the reviewer for pointing this out. The width of arrows is proportional to the strength of the relationship, which has now been explained in the figure caption.

Fig. S1 and 2: Replace “N0” and “N10” by “Without N” and “N addition.” The terms “N0” and “N10” are not used in the main manuscript.

30 Response: Thanks, we have replaced “N0” and “N10” with “Without N” and “N addition” in Fig. S1 and Fig. S2.

I recommend that authors use consistent color for “Without N” and “N addition” in figures for easy understand. In current manuscript, white bar represents “Without N” in the bar graph, whereas white dot represents “N addition” in the scatter plots. Red fitted line represents “Without N” in Fig. 1c, d and “N addition” in Fig. 2, 3.

5 Response: Thanks for pointing this out. We have corrected the dot figures for consistency using white and black colors to represent “Without N” and “N addition”, respectively. Moreover, black and red lines were fitted for “Without N” and “N addition” treatments, respectively.

10 **Reviewer 2**

P2 Line 14-15: logic leaps exist in the statement ‘Moderate-cycling IP was mainly regulated by aboveground plant biomass with KH_2PO_4 addition, while by soil pH and plant biomass with addition of $\text{Ca}(\text{H}_2\text{PO}_4)_2$ ’. Please change ‘plant biomass’ into ‘plant P uptake’.

15 Response: As suggested, we have reworded “plant biomass” into “plant P uptake” and changed the parameter when re-running the SEM model (P. 2 Line 14).

P2 Line 24: Please change the keyword ‘soil phosphorus’ into ‘nitrogen enrichment’.

Response: As suggested, we have changed the keyword “soil phosphorus” into “nitrogen enrichment” (P. 3 Line 1).

20 P3 Line 3: change ‘50-90%’ into ‘50%-90%’.

Response: As suggested, we have changed “50-90%” into “50%-90%” (P. 4 Line 3).

P3 Line 17-18: ‘labile/ available P (Olsen P), moderate-cycling P fractions (Al-P, Fe-P, $\text{Ca}_2\text{-P}$ and $\text{Ca}_8\text{-P}$)’. according to the Methods introduced by the authors, ‘Olsen-P was extracted from air-dried soil with 0.5 M NaHCO_3 (pH 8.5)(Olsen et al., 1954)’, while ‘ $\text{Ca}_2\text{-P}$ was determined by shaking 0.5 g soil with 25 ml 0.25 M NaHCO_3 (pH 7.5)’, and in contrast to Fig. 3a,b and Fig5.a,b, $\text{Ca}_2\text{-P}$ is more suitable to classified into ‘labile’ fraction. The statement should be ‘labile P ($\text{Ca}_2\text{-P}$)/ available P (Olsen P), moderate-cycling P fractions (Al-P, Fe-P, and $\text{Ca}_8\text{-P}$)’.

25 Response: Thanks for the comments. We fully agree with the comment and define $\text{Ca}_2\text{-P}$ as labile P in the study. The statement has been changed into “labile P ($\text{Ca}_2\text{-P}$) / available P (Olsen-P), moderate-cycling P fractions (Al-P, Fe-P and $\text{Ca}_8\text{-P}$)” (P. 4 Line 30

17).

P3 Line 21: delete 'understanding'.

Response: Yes, we deleted "understanding".

P4 Line 1: reword 'content' into 'concentration'.

5 Response: We have replaced 'content' into 'concentration' (P. 5 Line 7)

P5 Line 6: suggest to rephrase 'release of P from soil organic P' into 'mineralization of soil organic P'.

Response: As suggested, we have replaced 'release of P from soil organic P' into 'mineralization of soil organic P' (P. 6 Line 12).

10 P5 Line 25: 'soil labile IP (Olsen-P)': 'Soil labile IP (Ca₂-P) or available P (Olsen-P)'.

Response: As suggested, we have replaced 'soil labile IP (Olsen-P)' into 'soil labile IP (Ca₂-P)' (P. 7 Line 6).

P6 Line 1: remove 'Ca₂-P'.

Response: We have removed Ca₂-P in the sentence (P. 7 Line 7).

15 P7 Line 12-13: maybe, this sentence should be written as 'The plants were sorted to species and oven-dried at 65 for 48 h, then weighed and ground'? Because the way you did these should be to determined aboveground net primary productivity and get plant samples ground to measure plant P concentration.

20 Response: As suggested, we have changed the description into 'The plants were sorted to species and oven-dried at 65 °C for 48 h, then weighed and ground' (P. 8 Lines 22-23).

P7 Line 24: change 0.5g into 0.5 g.

Response: As suggested, 0.5g were changed into 0.5 g.

25 P8 Line 16-18: change 'Total IP (TIP) concentration was defined as the sum of moderate-cycling IP (Al-P, Fe-P, Ca₂-P and Ca₈-P) and recalcitrant IP (Ca₁₀-P and O-P).' into 'Total IP (TIP) concentration was defined as the sum of labile IP (Ca₂-P), moderate-cycling IP (Al-P, Fe-P and Ca₈-P) and recalcitrant IP (Ca₁₀-P and O-P).'

Response: Thanks for the suggestion. We have changed 'Total IP (TIP) concentration was defined as the sum of moderate-cycling IP (Al-P, Fe-P, Ca₂-P and Ca₈-P) and

recalcitrant IP (Ca₁₀-P and O-P).’ into ‘Soil total IP (TIP) concentration was defined as the sum of soil labile IP (Ca₂-P), moderate-cycling IP (Al-P, Fe-P and Ca₈-P) and recalcitrant IP (Ca₁₀-P and O-P)’ (P. 9 Line 24-P. 10 Line 1).

5 P9 Line 24: plant P uptake should be incorporated in the SEM model instead of plant biomass. If plant biomass is included, it should be explained by P fractions/ P availability. But do not use plant biomass to explain P fractions.

Response: As per suggestion, we used P uptake instead of plant biomass in the SEM and reanalyzed the SEM.

10 P 12 Line 12: Even though plant biomass production consume soil P, it would be better to directly use the parameter of plant P uptake as you calculated in P 9 Line 5.

Response: As per suggestion, we changed “plant biomass” into “plant P uptake” (P. 14 Line 2).

15 P15 Line 16-17: In this study, you were unable to determine the relative transformation rates of the two compounds. But the results of higher O-P and Ca₁₀-P suggested that a higher proportion of Ca(H₂PO₄)₂ was converted into stable inorganic P forms. So, please correct this point.

Response: Thanks for the comment. We have corrected the sentence into “This suggests that a higher proportion of Ca(H₂PO₄)₂ was converted into stable inorganic P forms than the more soluble KH₂PO₄ (P. 17 Lines 7-9).

20 P16 Line 7 & 22: Be aware that the mechanistic description might change if you replace the parameter of plant biomass with plant P uptake.

Response: Thanks for the reviewer’s observation. We have updated the description in the Result and Discussion section.

25 P18 Line 19-20 change ‘Phosphate Release Kinetics in Calcareous Grassland and Forest Soils in Response to H⁺ Addition’ into ‘Phosphate release kinetics in calcareous grassland and forest soils in response to H⁺ addition’

Response: Yes, we have corrected ‘Phosphate Release Kinetics in Calcareous Grassland and Forest Soils in Response to H⁺ Addition’ into ‘Phosphate release kinetics in calcareous grassland and forest soils in response to H⁺ addition’

30 P19 Line 9: *Lolium perenne* and *Pinus radiata*, italic.

Response: Yes, we changed *Lolium perenne* and *Pinus radiata* into *Lolium perenne* and *Pinus radiata*.

P20 Line 1-2: change ‘Do Nutrient Limitation Patterns Shift from Nitrogen Toward Phosphorus with Increasing Nitrogen Deposition Across the Northeastern United States?’ into ‘Do nutrient limitation patterns shift from nitrogen toward phosphorus with increasing nitrogen deposition across the Northeastern United States?’

Response: This has been corrected.

P20 Line 24-25: change ‘Soil fertility and fertilizers an introduction to nutrient management’ into ‘Soil Fertility and Fertilizers: An Introduction to Nutrient Management’

Response: Yes, we have changed ‘Soil fertility and fertilizers an introduction to nutrient management’ into ‘Soil Fertility and Fertilizers: An Introduction to Nutrient Management’.

P24 Line 20-21: change ‘Nitrogen Fertilization Effects on Grassland Soil Acidification: Consequences on Diffusive Phosphorus Ions’ into ‘Nitrogen fertilization effects on grassland soil acidification: consequences on diffusive phosphorus ions’

Response: Yes, we have changed ‘Nitrogen Fertilization Effects on Grassland Soil Acidification: Consequences on Diffusive Phosphorus Ions’ into ‘Nitrogen fertilization effects on grassland soil acidification: consequences on diffusive phosphorus ions’.

P26 Line 1: ‘*Larix gmelinii*’, italic?

Response: Yes, we changed ‘*Larix gmelinii*’ into ‘*Larix gmelinii*’.

P30 Line 8: ‘P’, italic.

Response: Yes, we changed “P” into “*P*”.

P33 Line 8: ‘P’, italic.

Response: Yes, we changed “P” into “*P*”.

P35 Fig. 6: reshape the ‘Structural equation model’ and rewrite the Results and Discussion sections related to the SEM.

Response: As suggested, we have reshaped the SEM and rewrote the Results and Discussion sections related to the SEM (P. 13 Line 23-P. 14 Line 3, P. 16 Lines 19-20, P. 17 Lines 20-24).

P36 Fig. 7: The content in the figure is hard to read because of the background color.

- 5 Remove Ca₂-P from the ‘moderate-cycling P’, and instead of using Olsen as labile P, I suggest the authors use Ca₂-P as labile P.

Response: As suggested, we have changed the background color in Fig. 7. We removed Ca₂-P from the ‘moderate-cycling IP’ and use Ca₂-P as labile IP.

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With above corrections, the manuscript is hereby resubmitted to the journal. We are thankful for the reviewers’ work and glad to respond any further questions that you have. We look forward a positive response from you.

Thanking you,

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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 $\text{Ca}(\text{H}_2\text{PO}_4)_2$) 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 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 KH_2PO_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 KH_2PO_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 KH_2PO_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 chemical form of P fertilizer should be considered during fertilization management for maintaining soil available P.

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Key words semi-arid steppe; nitrogen enrichment; fertilization management;
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 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 (Ca₂-P), octacalcium phosphate (Ca₈-P), hydroxylapatite (Ca₁₀-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 Ca₂-P > Ca₈-P > Ca₁₀-P (Jiang and Gu, 1989). Although these element-bound P fractions are not readily available to plants, soil Al-P, Fe-P, Ca₂-P and Ca₈-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 (Ca₂-P) / available P (Olsen-P), moderate-cycling P fractions (Al-P, Fe-P and Ca₈-P) and recalcitrant P fractions (Ca₁₀-P and O-P). But the category is not universal which 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). Ca₂-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). 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 KH_2PO_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 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 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 found that long-term application of $\text{Ca}(\text{H}_2\text{PO}_4)_2$ increased soil IP fractions (Al-P, Fe-P, Ca_2 -P, Ca_8 -P and O-P) and total P (TP) concentrations in a calcareous soil (Wang et al. 2010); and other one 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). 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 (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 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 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 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 $\text{Ca}(\text{H}_2\text{PO}_4)_2$ 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 KH_2PO_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 KH_2PO_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 KH_2PO_4 into labile and moderate-cycling IP fractions and consequentially promoting the formation of recalcitrant fractions; 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.

2. Materials and methods

2.1. Study sites and experimental design

The study site (119° 22' E, 50° 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 to a temperate continental monsoon climate. The mean annual precipitation and mean annual temperature is 375 mm and -3 °C, 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 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 was 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.*, KH_2PO_4 and $\text{Ca}(\text{H}_2\text{PO}_4)_2$, and were applied at six levels: 0, 20, 40, 60, 80 and 100 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 . 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 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 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 $\text{Ca}(\text{H}_2\text{PO}_4)_2$ treatment, so that in total, there were 110 plots of 8 m × 8 m, separated by 1-m wide buffer zones. The initial pH of KH_2PO_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 m × 1 m quadrat placed randomly within each plot. The plants were sorted to species and oven-dried at 65 °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

5 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, soil Ca₂-P was determined by shaking 0.5 g soil with 25 ml 0.25 M NaHCO₃ (pH 7.5), and then
10 centrifuged at 3500 rpm for 8 min to measure soil Ca₂-P. The remaining soil was washed two times with 25 ml 95% C₂H₅OH and extracted with 25 ml 0.5 M NH₄Ac (pH = 4.2) to determine soil Ca₈-P (Jiang and Gu, 1989). After this, the soil was shaken with 25 ml 1 M NH₄Cl and centrifuged at 3500 rpm to discard the supernatant. The remaining soil was then shaken with 25 ml 0.5 M NH₄F (pH 8.2) and centrifuged
15 at 3500 rpm for 8 min to analyze the soil 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 Na₂CO₃ for 2 h at 25 °C, and then centrifuged at 4500 rpm for 10 min to measure soil Fe-P in the supernatant. The remaining samples were washed as above and extracted with 25 ml mixture of 0.3
20 M Na₃(citrate)-Na₂S₂O₄ and 0.5 M NaOH to measure soil O-P. Finally, the remaining samples were shaken with 0.25 M H₂SO₄ for 1 h at 25 °C, and centrifuged at 3800 rpm for 10 min to determine soil Ca₁₀-P. The P concentration in all the extractants was determined by the molybdenum blue colorimetric method at wavelength of 700 nm with a UV-VIS spectrometer (UV-1700, Shimadzu) (Murphy and Riley, 1962). Soil
25 total IP (TIP) concentration was defined as the sum of soil labile IP (Ca₂-P),

moderate-cycling IP (Al-P, Fe-P and Ca₈-P) and recalcitrant IP (Ca₁₀-P and 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 phosphate dissolved in the extractants (Jiang and Gu, 1989).

Soil TP concentration was determined after digestion with 8 ml 85% HNO₃ +4 ml 72% HClO₄ +1 ml 40% HF (Sommers and Nelson, 1972), and Olsen-P was extracted from air-dried soil with 0.5 M NaHCO₃ (pH 8.5) (Olsen et al., 1954). Plant TP concentration was determined by acid digestion using H₂SO₄-H₂O₂ (Thomas et al., 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:

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

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 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_i) 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. 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 between the two P compounds (KH_2PO_4 vs. $\text{Ca}(\text{H}_2\text{PO}_4)_2$) 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 N and P addition effects on soil IP fractions through the changes in plant P uptake and soil pH.

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 $P < 0.05$.

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 levels of P addition, either as KH_2PO_4 or as $\text{Ca}(\text{H}_2\text{PO}_4)_2$ (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 rates with significantly higher overall KH_2PO_4 effect than $\text{Ca}(\text{H}_2\text{PO}_4)_2$. KH_2PO_4 addition showed no impact on soil pH (Fig. 1e), while $\text{Ca}(\text{H}_2\text{PO}_4)_2$ decreased soil pH without N addition (Fig. 1f).

3.2. Soil labile and moderate-cycling inorganic phosphorus fractions

For both KH_2PO_4 and $\text{Ca}(\text{H}_2\text{PO}_4)_2$ 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_2PO_4 than with $\text{Ca}(\text{H}_2\text{PO}_4)_2$ addition, especially at higher P rates (Fig. 2; Table S1). 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_2PO_4 addition than $\text{Ca}(\text{H}_2\text{PO}_4)_2$ at P addition level of $60 \text{ kg P ha}^{-1} \text{ yr}^{-1}$ when N was not added, but it was higher for P addition levels of 60, 80 and $100 \text{ kg P ha}^{-1} \text{ yr}^{-1}$ when N was added. Nitrogen addition decreased both soil Al-P and Fe-P concentrations, particularly at higher levels of P addition (Fig. 2; Table 2 and S1).

Addition of both P compounds significantly increased soil $\text{Ca}_2\text{-P}$ and $\text{Ca}_8\text{-P}$ concentrations with and without N addition (Fig. 3a, b, c, d). With KH_2PO_4 addition, soil $\text{Ca}_2\text{-P}$ and $\text{Ca}_8\text{-P}$ concentrations were higher than with $\text{Ca}(\text{H}_2\text{PO}_4)_2$ addition (Fig. 3; Table S1). Nitrogen addition significantly decreased soil $\text{Ca}_2\text{-P}$ and $\text{Ca}_8\text{-P}$ 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 $\text{Ca}_2\text{-P}$ and $\text{Ca}_8\text{-P}$ and $P_r \times N$ interactive effect on soil $\text{Ca}_2\text{-P}$ (Table 2).

3.3. Soil recalcitrant inorganic phosphorus fractions

$\text{Ca}(\text{H}_2\text{PO}_4)_2$ addition significantly increased soil $\text{Ca}_{10}\text{-P}$ concentration, while KH_2PO_4 addition showed no impact (Fig. 4a, b). Soil $\text{Ca}_{10}\text{-P}$ concentration was higher with $\text{Ca}(\text{H}_2\text{PO}_4)_2$ addition than with KH_2PO_4 addition for all levels except for $60 \text{ kg P ha}^{-1} \text{ yr}^{-1}$ without N addition and for 40, 60 and $100 \text{ kg P ha}^{-1} \text{ yr}^{-1}$ with N addition (Fig. 4a, b; Table S2). There was no main N addition and $N \times P_r$ interactive effect on soil $\text{Ca}_{10}\text{-P}$ for both P compounds (Table 2). Nitrogen addition had also no significant effect on soil O-P with KH_2PO_4 addition but significantly decreased it with $\text{Ca}(\text{H}_2\text{PO}_4)_2$

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 KH₂PO₄ when added with N, and with Ca(H₂PO₄)₂ independent of N addition. Soil O-P concentration was lower with Ca(H₂PO₄)₂ addition than with KH₂PO₄ addition for 60 and 80 kg P ha⁻¹ yr⁻¹ with N addition (Fig. 4c, d; Table S2). The relative proportions of soil O-P and Ca₁₀-P to soil TIP decreased while the proportions of soil Al-P, Ca₂-P, and Ca₈-P increased with increasing P addition rates for both P compounds (Fig. S1).

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 KH₂PO₄ addition than with Ca(H₂PO₄)₂ addition, resulting in significantly higher soil Olsen-P with KH₂PO₄ addition in the 100 kg P ha⁻¹ yr⁻¹ treatment with and without N addition (Fig. 5a, b). KH₂PO₄ addition increased soil TP irrespective of N addition, while Ca(H₂PO₄)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, Ca₂-P and Ca₈-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, Ca₂-P, and Ca₈-P ($P < 0.01$) (Table 3). Soil Ca₁₀-P had significantly positive correlations with soil Fe-P and Ca₈-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 KH₂PO₄ (Fig. 6a) and with Ca(H₂PO₄)₂ addition

(Fig. 6b). N addition had a positive impact on plant P uptake under both P compounds addition (Fig. 6a ,b). **Plant P uptake** had a negative impact on soil moderate-cycling IP both with KH_2PO_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, with a higher contribution for KH_2PO_4 addition (Fig. 6a,b). Soil moderate-cycling IP contributed to the increase of 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/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 then 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, Ca₂-P and Ca₈-P concentrations (Fig. 7).

5 Inconsistent with our first hypothesis, we found significant changes in soil recalcitrant P fractions (Ca₁₀-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 of soil Ca₁₀-P with Ca(H₂PO₄)₂ addition was not expected; and it was inconsistent with a previous study from a
10 calcareous soil showing unchanged soil Ca₁₀-P after 21-years of superphosphate application (Wang et al., 2010). In this calcareous soil, Ca(H₂PO₄)₂ addition enhanced the transformation of soil Ca₂-P and Ca₈-P into more stable soil Ca₁₀-P fractions for reasons that are not clear. In return, soil Ca₁₀-P can be a potential P sink of soil moderate-cycling IP pools because of its significant correlation with soil Fe-P under
15 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 KH₂PO₄ without N addition (Fig. 4c, d). This is in contrast to a 21-year long study where increased levels of Ca(H₂PO₄)₂ significantly increased soil O-P (Wang et al., 2010). The discrepancy might be due to 1) differences in soil type affecting soil P
20 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
25 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 compounds 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, Ca₂-P and Ca₈-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 Ca₈-P tended to be higher with soluble KH₂PO₄ addition than with less-soluble Ca(H₂PO₄)₂ addition with significant increases in some P addition levels (Fig. 2 & Fig. 3). Compared to Ca(H₂PO₄)₂, KH₂PO₄ 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 CaCO₃ (Havlin et al., 2005). **The decrease in soil pH contributed to the increase in labile IP with Ca(H₂PO₄)₂ addition (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 Ca(H₂PO₄)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 KH_2PO_4 addition (Fig. 6a). Under both P compounds, soil moderate-cycling IP fractions contributed to the increase of 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/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 KH_2PO_4 addition (Fig. 4 & Fig. 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 KH_2PO_4 . Additionally, lower soil O-P and $\text{Ca}_{10}\text{-P}$ concentrations could be caused by enhanced plant P uptake intensity under KH_2PO_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 KH_2PO_4 in maintaining soil recalcitrant IP fractions in the 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 KH_2PO_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 at a shorter time and generates more free PO_4^{3-} (Chien et al., 2011). Soil moderate-cycling IP fractions also contributed to elevated soil Olsen-P concentrations with stronger correlations under KH_2PO_4 addition (Table 3). Soil moderate-cycling IP fractions showed a stronger direct and positive effect on soil labile IP with KH_2PO_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 Ca₂-P and Al-P were the dominant soil IP fractions to improve soil P availability in the calcareous soil. Our results suggest that KH₂PO₄ was better than Ca(H₂PO₄)₂ in alleviating P limitations of soil microorganisms and plants by promoting the formation of soil labile IP and moderate-cycling IP fractions in the semi-arid 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 Ca₁₀-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 (Fig. 1a,b & Fig. 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 non-significant 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 PO₄³⁻ 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 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 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).

10

5. Conclusions

Addition of P compounds significantly increased soil moderate-cycling IP fractions of Al-P, Fe-P, Ca₂-P and Ca₈-P, which may have contributed to higher available P (Olsen P) in the soil. Soil moderate-cycling IP fractions were higher with soluble KH₂PO₄ addition, but in contrast, soil recalcitrant fractions of Ca₁₀-P and O-P were higher with Ca(H₂PO₄)₂ 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 promoted 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.**

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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 \pm 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

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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), 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, Olsen-P, total phosphorus (TP) and organic P (TOP) with Ca(H₂PO₄)₂ and KH₂PO₄ addition in the meadow steppe.

		Fe-P	Ca ₂ -P	Ca ₈ -P	Ca ₁₀ -P	O-P	Olsen-P	TP	TOP
KH ₂ PO ₄ 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 ₂ -P			0.92**	0.23	0.12	0.63**	0.41**	-0.28*
	Ca ₈ -P				0.29*	0.10	0.57**	0.37**	-0.29*
	Ca ₁₀ -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 ₂ PO ₄) ₂ 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 ₂ -P			0.84**	0.42	-0.03	0.53**	0.32*	-0.06
	Ca ₈ -P				0.50*	0.05	0.34**	0.26**	0.02
	Ca ₁₀ -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 and 0.01, respectively

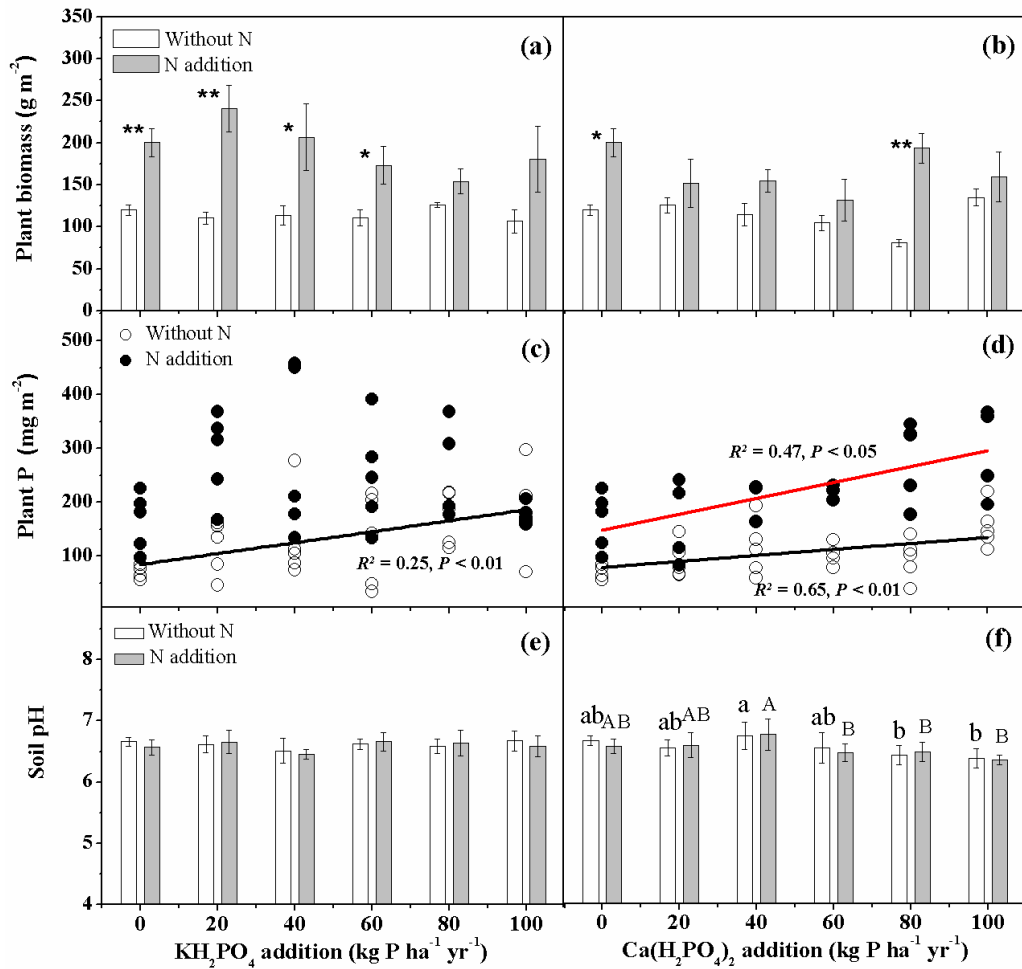


Fig. 1 Aboveground plant biomass (a, b), biomass-weighted phosphorus (P) uptake of three dominant plant species (c, d) and soil pH (e, f) as affected by KH_2PO_4 and $\text{Ca}(\text{H}_2\text{PO}_4)_2$ additions with and without N inputs. Data are represented as means \pm SE for panels a, b, e and f. Letters indicate significant differences between P rates of KH_2PO_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).

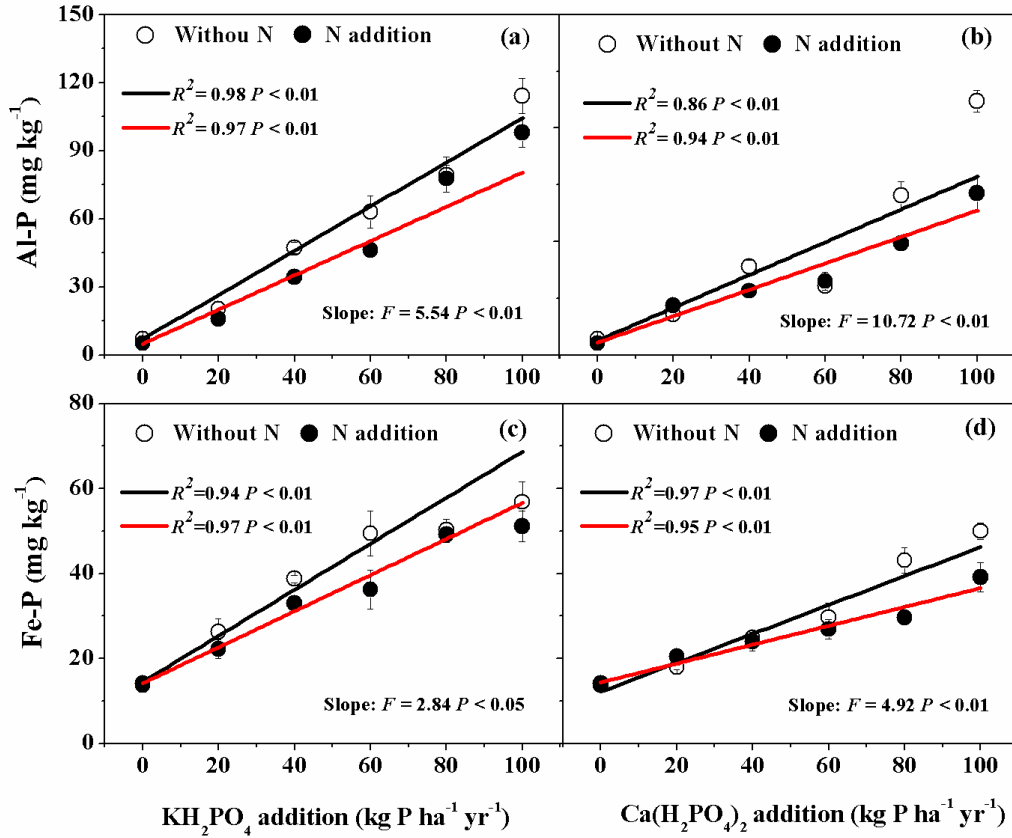


Fig. 2 Effects of phosphorus (P) and nitrogen (N) additions on soil variscite (Al-P) and strengite (Fe-P). Data are represented as means \pm standard error. Phosphorus types include KH₂PO₄ and Ca(H₂PO₄)₂ at rates of 0, 20, 40, 60, 80 and 100 kg P ha⁻¹ yr⁻¹. Fitted lines are based on linear regression models. The black and red line represent without N and N addition, respectively. Significance was labeled for slopes of the black and red lines.

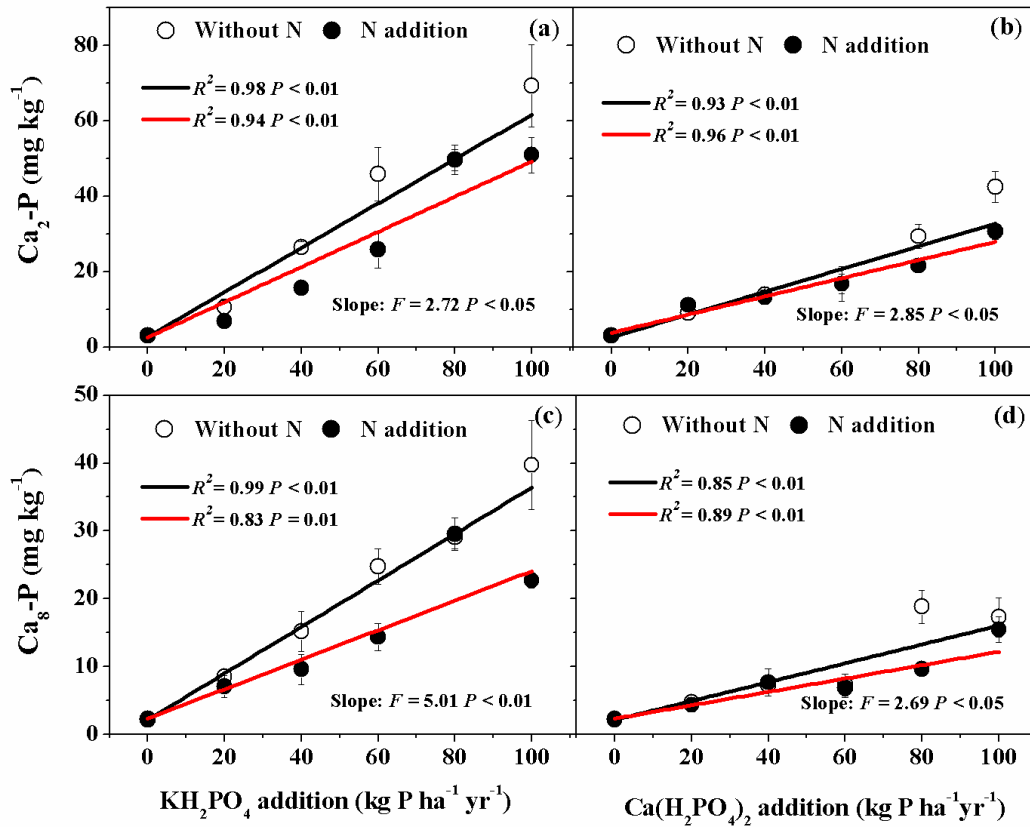


Fig. 3 Effects of phosphorus (P) and nitrogen (N) additions on soil dicalcium phosphate (Ca₂-P) and octacalcium phosphate (Ca₈-P). Phosphorus types include KH₂PO₄ and Ca(H₂PO₄)₂ at rates of 0, 20, 40, 60, 80 and 100 kg P ha⁻¹ yr⁻¹. Data are represented as means ± standard error. Fitted lines are based on nonlinear regression models. The black and red line represent without N and N addition, respectively. Significance was labeled for slopes of the black and red lines.

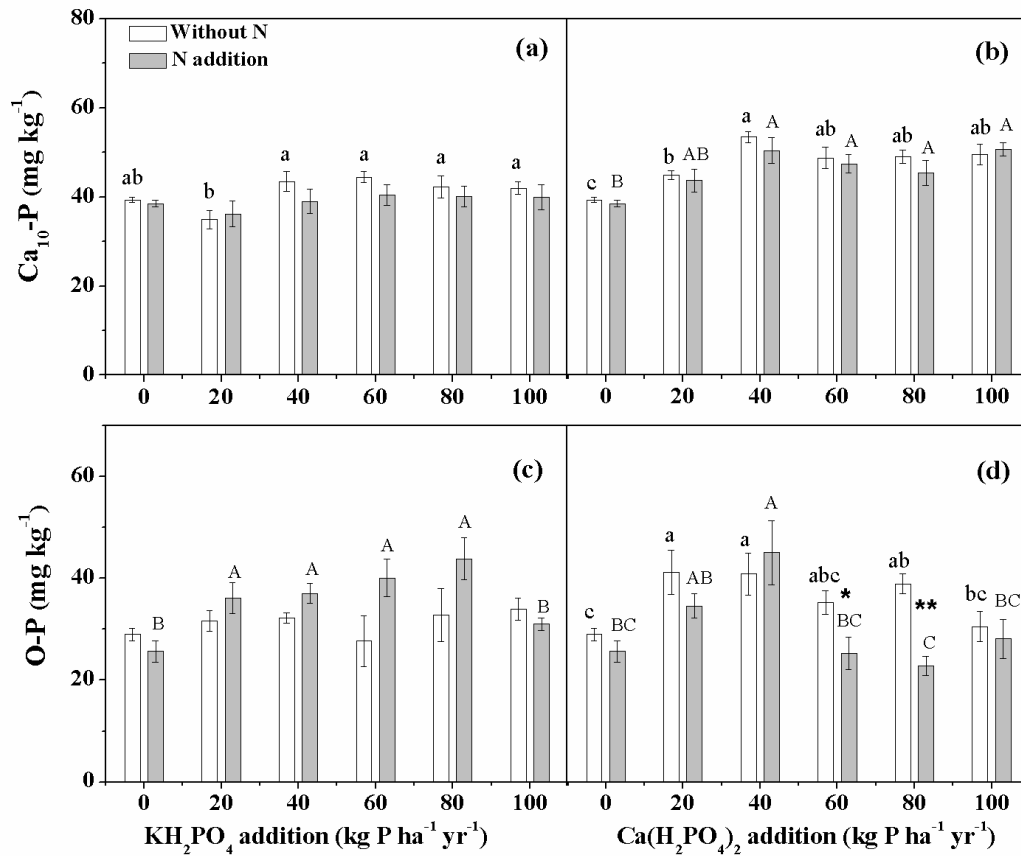


Fig. 4 Effects of phosphorus (P) and nitrogen (N) additions on soil hydroxylapatite (Ca₁₀-P) and occluded P (O-P). Phosphorus types include KH₂PO₄ and Ca(H₂PO₄)₂ at rates of 0, 20, 40, 60, 80 and 100 kg P ha⁻¹ yr⁻¹. Data are represented as means ± standard error. Letters indicate significant differences between P rates of KH₂PO₄ or Ca(H₂PO₄)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).

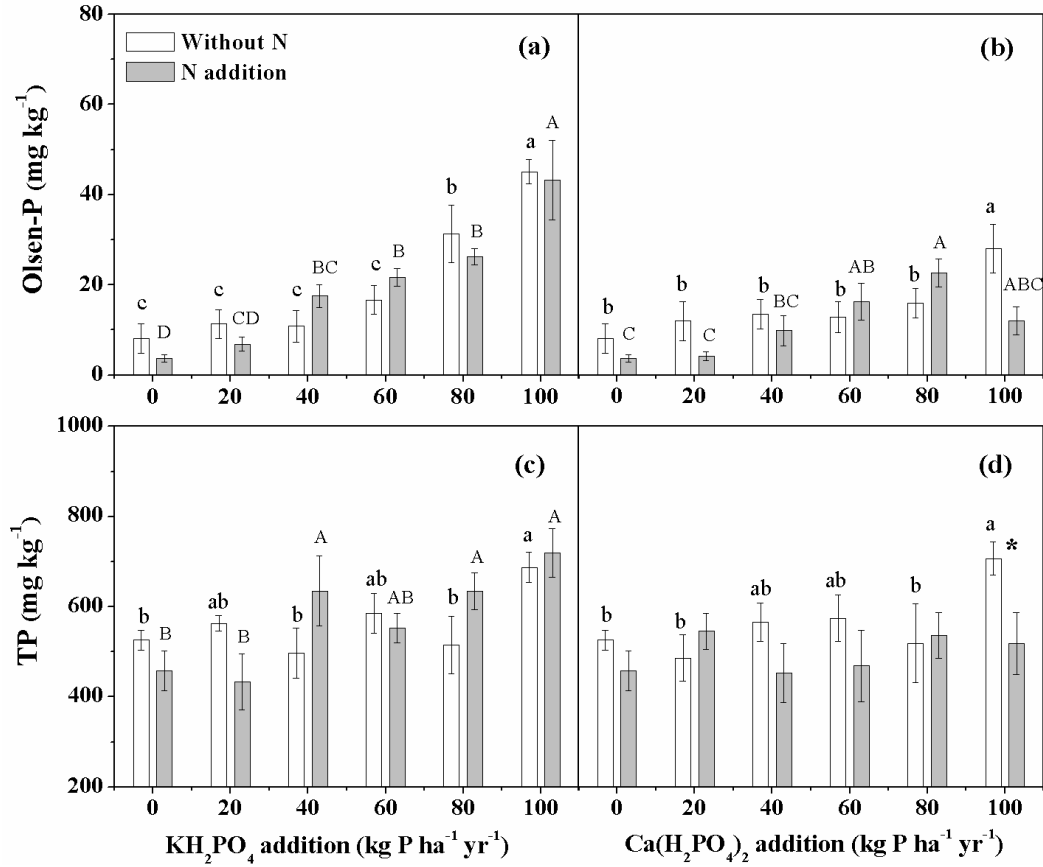


Fig. 5 Effects of phosphorus (P) and nitrogen (N) additions on soil Olsen-P and total P (TP) in the meadow steppe. Phosphorus types include KH₂PO₄ and Ca(H₂PO₄)₂ at rates of 0, 20, 40, 60, 80 and 100 kg P ha⁻¹ yr⁻¹. Data are represented as means ± standard error. Letters indicate significant differences between P rates of KH₂PO₄ or Ca(H₂PO₄)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).

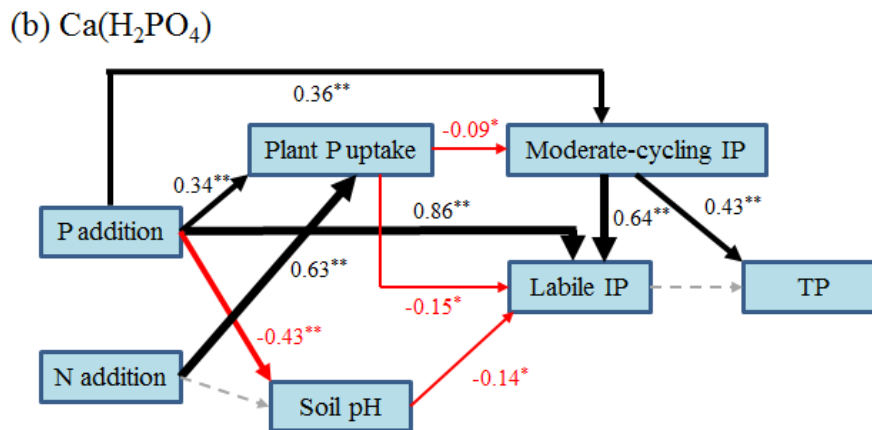
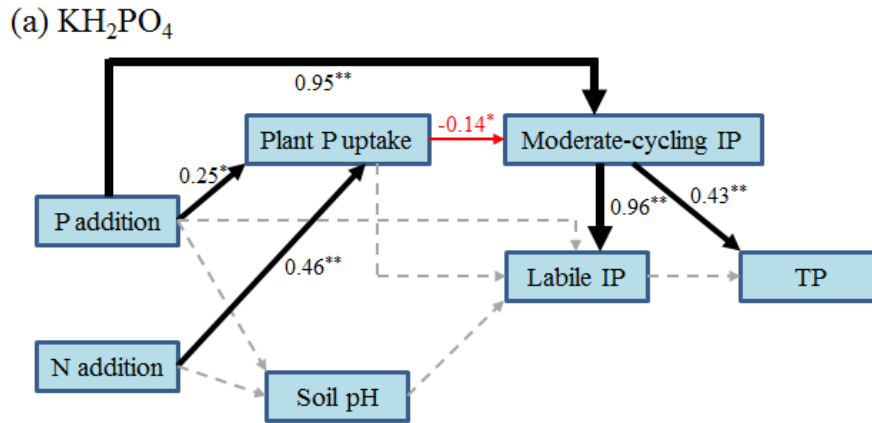


Fig. 6 Structural equation model of the relationships between soil pH, plant P uptake, soil moderate-cycling inorganic phosphorus (IP), labile P and TP under KH_2PO_4 (a, $\chi^2 = 23.44$, $P = 0.38$, RMSEA = 0.03, AIC = 67.44) and $\text{Ca}(\text{H}_2\text{PO}_4)_2$ additions (b, $\chi^2 = 27.01$, $P = 0.08$, RMSEA = 0.09, AIC = 79.01) across N treatments. Arrows indicate positive (black), negative (red) and neutral (grey-dotted) 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).

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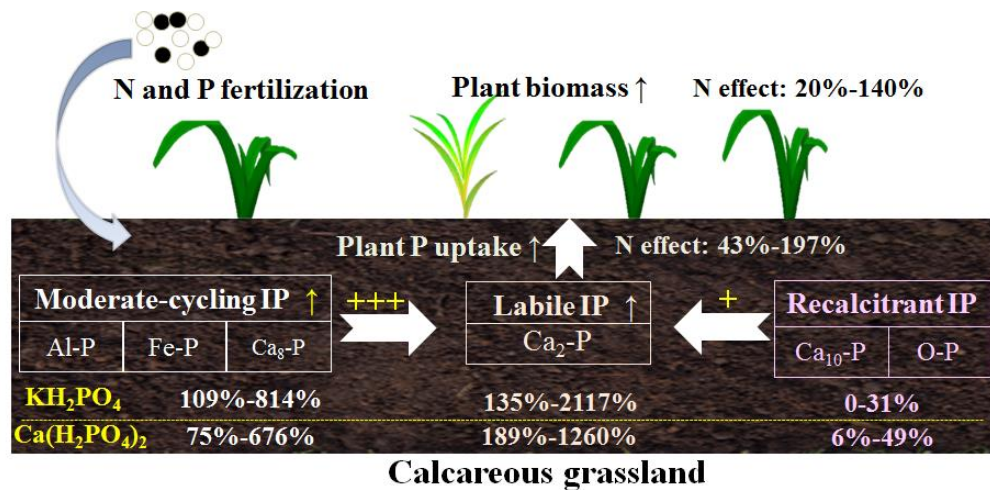


Fig. 7 Schematic diagram shows the effects of phosphorus (P) compound addition on soil inorganic P transformations in the calcareous grassland of Inner Mongolia. The percentages represent increase (“↑” in the diagram) of soil inorganic P fractions, plant P uptake and plant biomass as affected by fertilization across P addition rates.

5 KH₂PO₄ addition had a larger impact on moderate-cycling IP (sum of Al-P, Fe-P and Ca₈-P) as compared to Ca(H₂PO₄)₂ (109%-814% vs. 75%-676%). However, recalcitrant P (sum of Ca₁₀-P and O-P) increased more with Ca(H₂PO₄)₂ addition relative to KH₂PO₄ (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.