Reply to Handling topic editor:

Dear Editor,
Thank you very much for providing us an opportunity to revise the previously submitted manuscript entitled “Intensive agricultural management-induced subsurface accumulation of water extractable colloidal P in lime concretion black soil” (No. egusphere-2023-709). We are grateful to you for the constructive suggestions on the manuscript. The manuscript has been carefully revised according to the comments with highlighted changes in yellow mark. We have also revised and re-edited the whole manuscript for English. Enclosed below are our point-by-point responses to the comments.

We would like also to thank you for allowing us to submit a revised copy of the manuscript.
and hope the revised manuscript is acceptable for publication in the journal.

(1) Please, make a more detailed classification of the selected soils according to the WRB system, as required by the two referees. The Vertisol is not sufficient to define the type of soil and, please, substitute the WRB name throughout the text, including in the title.

Reply: Thanks a lot for this suggestion. We checked the references related to this type of soil and many references (Rahman et al., 2017; Chen et al., 2022; Wang et al., 2022a and b) defined it as Vertisol. For example:

Shajiang black soil (Vertisol) has a high shrinkage capacity and contains many calcareous concretions (Chen et al., 2022).
The soil, locally known as Shajiang black soil, is classified as a Vertisol according to USDA soil Taxonomy (Rahman et al., 2017; Soil Taxonomy, USDA, 2010).

Based on it, we classified it as vertisol and gave more detailed information on this type of soil in Line 48-52: Vertisol (locally known as Shajiang black soil) covers approximately 3.13 million hectares in the Huang-Huai-Hai Plain of China (Wei et al., 2018). It is characterized by low soil organic matter (SOM), low water-air permeability, poor fertility, and strong swelling-shrinkage properties, thus contributing to low and medium crop yields.
Line 99-100: The soil is classified as a Vertisol according to USDA soil Taxonomy (Soil Survey Staff, 2010).

References:

Chen, Y., Zhang, Z., Guo, Z., et al., Impact of calcareous concretions on soil shrinkage of a Vertisol and their relation model development, Geoderma, 420, 115892. https://doi.org/10.1016/j.geoderma.2022.115892, 2022.

Wang, Y., Zhang, Z., Tian, Z., et al., Determination of soil bulk density dynamic in a Vertisol during wetting and drying cycles using combined soil water content and thermal property sensors, Geoderma, 428, 116149, https://doi.org/10.1016/j.geoderma.2022.116149, 2022.

Wang, Y., Zhang, Z., Guo, Z., et al., The dynamic changes of soil air-filled porosity associated with soil shrinkage in a Vertisol, Eur J Soil Sci. https://doi.org/10.1111/ejss.13313, 2022.

Rahman, M., Zhu, Q., Zhang, Z., et al., The roles of organic amendments and microbial community in the improvement of soil structure of a Vertisol, Appl. Soil Ecol. 111, 84-93. http://dx.doi.org/10.1016/j.apsoil.2016.11.018, 2017.

Soil Survey Staff, 2010. Keys to Soil Taxonomy, 11th ed. USDA-Natural Resources Conservation Service, Washington, DC.

(2) The hypothesis and the objectives of the work remain weak, even after your reply, thus a more robust revision of them should be done.

Reply: Thanks a lot for the constructive suggestion and we revised it as follows:
Line 85-92: The aim of this research was to explore (1) the physicochemical properties and speciation of colloidal P in the soil profile and (2) whether the colloidal organic and inorganic P exist in the subsoil and play important roles for the transport of P in intensively managed Vertisol. We assumed that the transport of colloidal P with inorganic and organic forms from the topsoil to subsoil of Vertisol is certainly possible as a result of long-term intensive agricultural management, which could bring an unavoidable risk on transport and loss of subsurface P in agricultural soil.

(3) The processes that could lead to the presence of (organically-bound) colloidal P in subsoils should be further revised since your reply is not so convincing.

Reply: Thanks a lot for the comment and we revised it as follows:
Line 337-355: In principle, there are four processes that could lead to the presence of organically-bound colloidal P in subsoils: i) the mobilization and subsequent transport of colloids from topsoils into subsoils, ii) the leaching of dissolved organic P and their subsequent sorption to surfaces of WDC in subsoils, iii) direct input of organic P into subsoils due to root exudation or root decay, and their subsequent sorption to surfaces of WDC in subsoils, and iv) the leaching of P from subsoils and its subsequent assimilation by microorganisms colonizing mineral surfaces in subsoils. As discussed above, the first process was probably dominated because more negative values of zeta potential at surface soil colloids are beneficial for the transport of colloids from

topsoil to subsoil by increasing repulsion force with soil clay particles. Additionally, the presence of abundant swelling-shrinkage clays increases preferential flow which also facilitates the downward transport of colloids. The process ii) and iii) were also possible but their contributions were not clear. The process iv) seems not important because the OC/OP in all soil samples ranged from 4.9 to 49.6, which did not support the immobilization of P. Additionally, microbial phosphorus is mainly phosphate diesters (Turner et al., 2005). The relatively small fraction of orthophosphate diester indicated by ^{31}P-NMR measurements in subsoil suggested that the process iv) was probably not the most relevant process explaining the accumulation of WDC containing organically-bound P in subsoils.

Turner, B.L., Cade-Menun, B.J., Condron, L.M., Newman, S., Extraction of soil organic phosphorus, Talanta, 66, 294-306. doi:10.1016/j.talanta.2004.11.012, 2005.

(4) The explanation of zeta potential changes along the profile should be also reconsidered, giving a more complete explanation of the complex processes that can affect this parameter. In addition to pH changes and P desorption/sorption processes, cation eluviation/illuviation along the profile could affect cation-particle interactions that can explain the more positive values, as shown by the increase of hydroxyapatite.

Reply: Thanks a lot for your useful suggestion and we revised it as follows:
Line 275-279: The cation eluviation such as Ca^{2+} from the topsoil to subsoil and the subsequence illuviation in the subsoil probably affect cation-particle interactions and contribute to the more positive zeta potential in subsoil, as shown by the increase of hydroxyapatite with depth detected by P K-edge XANES results (see section 3.4).

(5) Finally, I am wondering whether montmorillonite and other 2:1 phyllosilicates can retain significant amounts of P in the colloidal fraction. In general, 2:1 phyllosilicates have a net negative charge and their retention capacity may be related to edges, becoming significant in soils where these phyllosilicate strongly dominate over Fe and Al (hydr)oxides.

Reply: montmorillonite and other 2:1 phyllosilicates could retain significant amounts of P in the colloidal fraction and we added some discussion in Line 320-324:

The minerals in all colloids included montmorillonite, nontronite, and illite, indicating the existence of swelling-shrinkage clays. These secondary minerals all have a significant adsorption capacity for P due to great surface area and the structural binding sites of clay mineral edges (Gérard, 2016; Chen et al., 2020).

Line 289-291 : The soil colloids enriched in secondary clays such as montmorillonite, nontronite, and illite as shown in XRD results could readily immobilize P.

Response to reviewer 1：

General comments

The manuscript describes the characterization of depth distributions of P forms in “Lime concretion black soils” in China with a focus on water dispersible colloids. Water dispersible colloids were extracted from the soils using an extraction with water plus sedimentation and centrifugation. The extracted and isolated colloids were subsequently characterized with wet-chemical methods as well as 31P-NMR, P K-edge XANES and XRD measurements. The presence of organically-bound P in the water dispersible colloid fraction, negative surface charges of the extracted colloids and larger WDC-P concentrations compared to dissolved P concentrations in subsoils are interpreted as indicators for a prominent role of colloid transport for the migration of P from topsoils to subsoils in the investigated soils. Although much work has been devoted to the characterization of the P forms in the soil profiles, several points prevent a publication of the manuscript in my opinion.

Reply: We thank the reviewer 1 think that “much work has been devoted to the characterization of the P forms in the soil profiles”.

First, the World Reference Base of soil resources (WRB, 2014 upated 2015) does not know “lime concretion black soils”. From the soil data presented and the soil description the investigated soils could be Chernozems, perhaps Vertic Chernozems. Please classify the soils properly according to the WRB system.

Reply: Thanks a lot for the suggestion. The soil belongs to lime concretion black soil in Chinese Soil Taxonomy (Wei et al., 2018) and Vertisol according to World Reference Base (WRB, 2014). We will add this information in Materials and Methods.

Wei C., Gao W., William R., Li B., 2018. Shrinkage characteristics of lime concretion black soil as affected by biochar amendment. Pedosphere, 28(5),713-725.

WRB, 2014. World Reference Base for soil resources 2014: international soil classification system for naming soils and creating legends for soil maps. Rome, Italy.

Second, the chosen sedimentation and centrifugation scheme fails to separate colloidal matter from particulate matter and dissolved compounds. The common definition of colloids comprises a size range from 10 nm (sometimes 1 nm) to 1000 nm, while the authors chose a size range from 350 nm to 2000 nm. This means that their water dispersible colloid (WDC) fraction includes particles larger than 1000 nm, which are commonly excluded from the colloid fraction and attributed to the particulate fraction and excludes the smallest colloids, which have the highest specific surface area and reactivity as well as mobility. This hampers comparisons of the results with other recent studies on colloidal and nanoparticulate P.

It is difficult to understand why ultracentrifugation was not used to separate the dissolved components of the extracts from the colloidal ones (e.g., Ilg et al. J. Environ Qual. 34:926–935, 2005; Missong et al. J. Plant Nutr. Soil Sci. 179:159-167, 2016; Missong et al. Plant and Soil 427:71–86, 2018).

In fact, Missong et al. used the supernatant of the Sequaris and Lewandowski (2003) method to extract and isolate the colloids and nanoparticles and NOT to separate the dissolved fraction.

Hence the manuscript should discuss the implications of their choice of size range for the extracted and isolated particles for their results.

It is possible that the study underestimated the relevance of P binding to collloids and colloid-facilitated P transport by neglecting the smallest colloid fractions.

Reply: The defined sizes of water dispersible colloids (WDC) are not consistent in the current literatures. For example, in the literature by Séquaris et al. (2013), the size of WDC is defined from 0.05 to 2 µm. Jiang et al. (2012, 2017) defined the WDC as particles < 2 µm of clay fraction. The study from Li et al. (2022) defined the size of WDC as 0.22-2 µm. In the study from Wang et al. (2020), WDC were chosen as the size below 300 nm. In this study, we used the sedimentation and centrifugation scheme based on the study of Séquaris et al. (2013). That is why we defined the water dispersible colloids below 2 µm.

On the other side, it is difficult to collect all the smallest colloids using ultracentrifugation. In the paper from Missong et al. (Plant and Soil 427:71–86, 2018), the WDC was separated by ultra-centrifugation at 14,000×g for 60 min. However, she did not calculate or measure the smallest size. In the study from Ilg et al., he also did not calculate the smallest size based on the ultracentrifugation. There is no information in their studies if all the smallest colloids were collected. In the study from Séquaris et al. (2013), The WDC size fraction from 0.05 to 2 µm was obtained by high-speed centrifugation (10,000 g; 90 min) of the soil colloidal phase. In our previous study, after centrifugation at 2525 × *g* for 68 min (Biofuge, Heraeus) of colloid suspension, the final supernatant still contained the remaining fine colloids with an average hydrodynamic diameter about 180 nm according to DLS result. In this study, although <0.35 µm was defined as dissolved fraction, we furthermore distinguished total P concentrations (DTP) and reactive P concentrations (DRP) of dissolved fractions. Actually the difference of DTP and

DRP belongs to the smallest colloidal P fraction with size <0.35 µm and we already considered it as colloidal fraction and discussed it in the line 253-258.

Line 279-282: It is important to note that DRP accounted for only 29-51% of DTP (Table 2). Furthermore, the sum of colloidal P concentration for <0.35 µm (i.e. the difference between DTP and DRP) and 0.35-2 µm was about 38-93 mg/kg soil, which is 6-37 folds of DRP. It suggested that the contribution of CP to transport P in both surface and subsurface soil environment is important and should not be overlooked

References

Séquaris J., Klumpp E., Vereecken H., 2013. Colloidal properties and potential release of water-dispersible colloids in an agricultural soil depth profile. Geoderma, 193-194, 94-101.

Jiang C., Séquaris J., Vereecken H., Klumpp E., 2012. Effects of inorganic and organic anions on the stability of illite and quartz soil colloids in Na-, Ca- and mixed Na–Ca systems. Colloids and Surfaces A: Physicochemical and Engineering Aspects. 415, 134-141.

Jiang C., Séquaris J., Vereecken H., Klumpp E., 2017. Effects of temperature and associated organic carbon on the fractionation of water-dispersible colloids from three silt loam topsoils under different land use. Geoderma, 299, 43-53.

Li Y., Livi K., Arenberg M., Xu S., Arai Y., 2022. Depth sequence distribution of water extractable colloidal phosphorus and its phosphorus speciation in intensively managed agricultural soils. Chemosphere, 286, 131665.

Wang L., Missong A., Amelung W., Willbold S., Prietzel J., Klumpp E., 2020 Dissolved and colloidal phosphorus affect P cycling in calcareous forest soils. Geoderma, 375, 114507.

Jiang X., Bol R., Nischwitz V., Siebers N., Willbold S., Vereecken H., Amelung W., Klumpp E., 2015. Phosphorus containing water dispersible nanoparticles in arable soil. Journal of Environmental Quality, 44, 1772-1781.

The manuscript does not specify which assumptions were made regarding the density of the water extractable colloids and particles. However, the calculated centrifugation speeds and times for the separation of colloids and particles critically depend on particle densities, which may vary due to the different fraction of organic material in the particles. Did the authors check the diameter of the extracted and isolated particles with dynamic light scattering (DLS)?

Reply: Thanks a lot for this useful suggestion. The density of particles is assumed as 2.65 g cm^{-3}. We have measured the final supernatant by DLS and the measured size was 351.3±6.6 nm. We have added this information in Line 143-145 of the manuscript.

Line 144-145: The final supernatant only contained the remaining fine colloids with an average hydrodynamic diameter of 351.3±6.6 nm (according to dynamic light scattering [DLS] result) as well as the electrolyte phase.

In principle, there are four processes that could lead to the presence of (organically-bound) colloidal P in subsoils: i) the mobilization and subsequent transport of colloids from topsoils into subsoils, ii) the leaching of dissolved inorganic or organic P and their subsequent sorption to surfaces of water-dispersible colloids in subsoils, iii) the leaching of P from subsoils and its subsequent assimilation by microorganisms colonizing mineral surfaces in subsoils, and iv) direct input of organic P into subsoils due to root exsudation or root decay. While the manuscript focuses on process i), it doesn´t consider processes ii)-iv).

Reply: Thanks a lot for this useful suggestion. We have discussed all these processes in the discussion as follows:

Line 337-355: In principle, there are four processes that could lead to the presence of organically-bound colloidal P in subsoils: i) the mobilization and subsequent transport of colloids from topsoils into subsoils, ii) the leaching of dissolved inorganic or organic P and their subsequent sorption to surfaces of WDC in subsoils, iii) direct input of organic P into subsoils due to root exsudation or root decay, and their subsequent sorption to surfaces of WDC in subsoils, and iv) the leaching of P from subsoils and its subsequent assimilation by microorganisms colonizing mineral surfaces in subsoils. As discussed above, the first process was probably dominated due to more negative values of zeta potential at surface soil colloids and the presence of abundant swelling-shrinkage clays which facilitates preferential flow. The process ii) and iii) were also possible but their contributions were not much. As discussed before, no colloids existed in the depth >60 cm of sample B which supported the limited generation of colloids in subsoils of lime concretion black soil. The process iv) seems not important because the OC/OP in all soil samples ranged from 4.9 to 49.6, which did not support the immobilization of P. Additionally, the relatively small fraction of orthophosphate diester indicated by ^{31}P-NMR measurements suggested that the process iv) was probably not the most relevant process explaining the accumulation of WDC containing organically-bound P in subsoils.

The finding that the WDC had zeta potentials smaller than -15 mV equivalent to the critical zeta potential for the mobilization of goethite particles by P sorption (Ilg et al., Eur J Soil Sci 59:233–246, 2008) supports the hypothesis of the transport of P containing colloids from topsoils to subsoils. However, the finding that the zeta potential shifts to more positive values with increasing depth suggests that this process is perhaps not the most relevant one. If colloid transport from topsoils to subsoils would be the dominant process, then one would expect a shift to more negative zeta potentials due to the increase in pH with increasing depth.

Reply: Thanks a lot for this comment and we will add the literature of Ilg et al. (2008) to support the hypothesis of the transport of P containing colloids from topsoils to subsoils. There is a possibility that the transported colloids could desorb P from the surface due to the increasing pH in the subsoil by the competition adsorption of OH^-. That is probably the reason why more positive zeta potentials occurred with increasing depth.

The relatively small fraction of diester P indicated by 31P-NMR measurements suggests that also process iii), the P assimilation by microorganisms is probably not the most relevant process explaining the accumulation of WDC containing organically-bound P in subsoils. However, the fraction of diester P might have been underestimated due to its hydrolysis during the extraction procedure (see Missong et al., 2016 and papers cited therein).

Reply: Thanks a lot for this suggestion. Previous reports have identified that the degradation of orthophosphate diesters resulted in α- and β- glycerophosphates and mononucleotides (Glyc+nucl) during ^{31}P NMR analysis (Doolette et al., 2009), which were detected in the orthophosphate monoester region, then these compounds were categorized as orthophosphate diesters rather than to monoesters in our study.

We have mentioned it in the Line 165 to 167 in the manuscript: The α-, β-glycerophosphates and mononucleotides (Glyc+nucl) were classified as orthophosphate diesters rather than to monoesters (Young et al., 2013; Liu et al., 147 2018).) However, we forgot to explain it in the Table 3. We added this information in the Table 3 too.

Inositolhexaphosphate (IHP) is an important storage form of P in plants. Although IHP is mainly stored in seeds, the significant levels of IHP in the WDC fraction might point to the relevance of direct P inputs via decaying roots for P translocation into subsoils.

Reply: This is useful information and we have discussed it in Line 332-334:

Line 332-334: The significant level of IHP showed by P K-edge XANES spectra suggested the direct P inputs via decaying roots for P translocation into subsoils.

The dominance of hydroxyapatite in the subsoils might point to the relevance of leaching of dissolved ortho-P from topsoils into subsoils with subsequnqt precipitation of apatite due to the increase in pH and Ca^{2+} concentrations. On the other hand, the dominance of hydroxyapatite P in the WDC of subsoils may also reflect the presence of apatite in the unweathered parent material of soil formation.

Reply: This is useful information and we have added this point in the manuscript Line 325-330.

Overall, the informative value of the manuscript is moderate, also because the results can hardly be linked to results of other studies on colloidal P due to the exclusion of medium to small colloids and the inclusion of non-colloidal particles in the WDC fraction.

Reply: As mentioned above, the definitions of colloid size are not consistent, and there are still considerable amounts of literatures that define colloids as less than 2 µm. On the other side, we considered the small colloids by discussing the difference of DTP and DRP in the "dissolved" fraction with soil particle size below 350 nm.

Line 279-282: It is important to note that DRP accounted for only 29-51% of DTP (Table 2). Furthermore, the sum of colloidal P concentration for <0.35 µm (i.e. the difference between DTP and DRP) and 0.35-2 µm was about 38-93 mg/kg soil, which is 6-37 folds of DRP. It suggested that the contribution of CP to transport P in both surface and subsurface soil environment is important and should not be overlooked

Furthermore, a clear hypothesis is lacking leading to a rather explorative nature of the study, for which the number of investigated soils profiles is quite small since only two profiles were analyzed intensively.

Reply: Thanks a lot for the suggestion and we gave a clear hypothesis in the manuscript as follows:

Line 88-92: We assumed that the subsurface accumulation of colloidal P in lime concretion black soil as a result of long-term intensive agricultural management contributes to subsurface P loss, which brought an unavoidable potential on the transport of P in agricultural soil.

Additionally, all four soil profiles showed similar physicochemical properties and P distribution, suggesting that the colloids in the four soil profiles were similar. That is why we investigated the colloids of two soil profiles as representatives.

Specific comments

Lines 67-68: Ditches can increase the transport of P from soils to surface waters. Why should they increase the leaching of P from topsoils to subsoils? Because the volume of drainage is increased by the lowering of groundwater levels? Is there shallow groundwater at the investigated sites?

Reply: We changed this sentence to Line 81-84: "Additionally, the artificial ditches could also facilitate the transport of dissolved and colloidal P from both surface and subsurface soil to surface waters, which is an important non-point source of eutrophication."

Line 70: I don´t understand the unit hm2. Does this mean hecto square meters, i.e. 100 m2? If yes, then the application of fertilizer would be excessive (60000 kg per hectare, or 6 tons per hectare). Please use the unit m^2, or hectare (ha) if the Journal allows the use of non SI units.

Reply: Thanks a lot for the useful suggestion. We changed “600 kg/hm^2” to “600 kg per hectare”.

Line 72: Is this accepted or rather expected?

Reply: Thanks a lot for the useful suggestion. We changed “accepted” to “expected”.

Line 120: Do you mean Duran (Trademark for borosilicate glass)?

Reply: Yes, we changed it as suggested.

Lines 124-128: A similar procedure was used by Missong et al. (2016, 2018) to isolate the colloidal fraction in the supernatant. The supernatant of the centrifugation at 3500 g will certainly contain many colloids and nanoparticles, therefore it is misleading to denominate this fraction as "dissolved" fraction. Ultracentrifugation or ultrafiltration would have been necessary to remove most colloids and nanoparticles from the supernatant...

Reply: Thanks a lot for the useful suggestion. As mentioned before, we considered the “small colloids and nanoparticle” part by discussing the difference of DTP and DRP in the “dissolved” fraction with soil particle size below 350 nm.

Line 275-278: It is important to note that DRP accounted for only 29-51% of DTP (Table 2). Furthermore, the sum of colloidal P concentration for <0.35 µm (i.e. the difference between DTP and DRP) and 0.35-2 µm was about 38-93 mg/kg soil, which is 6-37 folds of DRP. It suggested that the contribution of CP to transport P in both surface and subsurface soil environment is important and should not be overlooked

Line 130: Did you freeze-dry the colloids?

Reply: Yes and we have added this information in the manuscript.

Line 132: Why wasn´t this ultracentrifugation used to separate the dissolved fraction from the colloidal fraction?

Reply: I have already explained it in the 3rd question.

Line 205: It would be good to calculate the degree of P saturation of the soil samples as DPS=Pox/[0.5*(Feox+Alox)], e.g. van der Zee and van Riemsdijk

(Transport in Porous Media 1: 339-359, 1986), Ilg et al. (J. Environ. Qual. 34:926–935, 2005).

Reply: Thanks a lot for this useful suggestion and we calculated the degree of P saturation as suggested.

Table 1 Physicochemical characteristics of bulk soils.

Sample	Depth(cm)	pH	%TC	%TN	OC/OP (%)	ACP (μg/(g*h))	ALP (μg/(g*h))	OA-P (mg/kg)	DCB-P (mg/kg)	DPS (%)
A	0-20	5.13±0.04e	0.91±0.00a	0.10±0.00a	40.9±3.4ab	647.8±13.5a	465.5±10.2a	142.9±0.8a	178.3±12.3a	4.6±0.0c
	20-40	6.43±0.10d	0.54±0.01d	0.07±0.01b	26.3±2.4b	280.5±4.1b	305.3±14.1d	121.4±5.5b	121.4±5.5bc	4.4±0.0c
	40-60	7.23±0.02c	0.52±0.00e	0.06±0.01bc	30.2±15.8ab	213.3±6.7c	358.6±4.9c	137.6±6.5b	137.6±6.2b	6.8±0.0b
	60-80	7.52±0.02b	0.67±0.01c	0.06±0.01bc	33.9±3.8ab	127.8±9.8d	390.3±8.1b	31.9±3.5c	98.8±22.1c	6.3±0.0b
	80-100	7.52±0.02b	0.75±0.01b	0.07±0.00b	49.6±5.3a	122.6±11.7d	414.0±11.1b	67.0±6.2b	103.1±16.7bc	10.0±0.0a
	100-120	7.70±0.02a	0.50±0.01f	0.06±0.01c	43.9±6.7ab	96.2±8.2e	346.4±16.6c	75.8±15.3b	100.6±5.5c	7.2±0.0b
B	0-20	4.88±0.07e	1.17±0.01a	0.14±0.00a	40.2±4.6a	997.2±14.6a	701.4±14.7a	141.70±9.5a	185.9±2.8a	6.5±0.0c
	20-40	6.28±0.02d	0.66±0.01b	0.08±0.00b	37.2±5.1a	413.0±4.7b	514.7±5.8b	84.7±1.3b	121.4±7.8b	17.4±0.0a
	40-60	7.41±0.01c	0.48±0.00d	0.06±0.00c	26.2±3.9b	186.6±3.8c	509.3±17.9c	59.7±12.7c	66.6±11.4d	16.1±0.0a
	60-80	8.03±0.01b	0.35±0.01e	0.05±0.00d	10.4±1.8c	147.2±14.3d	392.5±16.6d	73.7±3.2bc	99.8±2.8c	11.0±0.0b
	80-120	8.37±0.01a	0.52±0.01c	0.04±0.00e	17.5±1.4c	100.1±9.3e	326.2±14.3e	60.7±1.9c	79.1±0.9d	5.2±0.0c
C	0-20	5.01±0.02e	0.94±0.00a	0.11±0.00a	19.7±2.2a	1177.6±31.8a	510.6±15.2b	161.7±5.6a	182.3±6.0a	5.3±0.0c
	20-40	6.23±0.03d	0.54±0.00b	0.07±0.00b	15.9±3.0a	332.4±12.8b	377.3±15.3d	33.7±0.7b	70.4±3.90b	15.7±0.0b
	40-60	7.32±0.04c	0.48±0.00c	0.06±0.00c	9.3±1.0b	190.0±4.7c	578.1±7.6a	29.7±13.4b	45.6±8.8c	19.2±0.0a
	60-80	7.60±0.03b	0.39±0.01d	0.04±0.01d	5.4±0.9b	176.6±3.2c	489.5±4.2b	41.7±6.2b	43.8±5.3c	14.3±0.0b
	80-120	7.74±0.03a	0.27±0.00e	0.04±0.00d	4.9±1.0b	126.8±17.9d	448.1±7.4c	48.7±4.81b	50.1±4.3c	4.8±0.0c
D	0-20	5.17±0.04e	0.88±0.01a	0.10±0.01a	28.7±2.3a	749.6±9.6a	502.1±12.9bc	126.7±6.2a	129.2±6.30a	6.1±0.0d
	20-40	6.97±0.06d	0.57±0.02b	0.07±0.00b	33.1±3.1a	435.4±3.2b	557.2±14.6a	33.7±2.7b	51.4±2.07c	19.4±0.0ab
	40-60	7.46±0.03c	0.50±0.00c	0.06±0.00c	32.7±3.8a	136.7±5.8c	526.0±16.3b	34.7±7.0b	65.6±2.28b	21.0±0.0a
	60-80	7.78±0.03b	0.36±0.01d	0.04±0.01cd	21.1±1.0b	175.9±6.5d	497.1±1.7c	42.7±5.7b	49.8±49.1c	13.7±0.0c
	80-120	8.04±0.01a	0.25±0.00e	0.03±0.01d	13.1±1.7c	97.9±2.3e	349.4±10.3d	37.7±1.7b	40.1±1.93c	17.2±0.0b

TC: total carbon, TN: total nitrogen, ACP: acid phosphatase activity, ALP: alkaline phosphatase activity, OA-P: oxalic acid extractable P, DCB-P: dithionite–citrate–bicarbonate extracted P. DPS: degrees of phosphorus saturation (DPS=[OA-P]/0.5([OA-Fe]+[OA-Al])). OA-Fe: oxalic acid extractable Fe. OA-Al: oxalic acid extractable Al.

Line 220: I think the authors mean the OC/OP ratio, not the TC/TP ratio…

Reply: Thanks a lot for the suggestion. We changed it as follows:

Line 242: The OC/OP ratio of > 200:1 is favorable for P immobilization.

Line 238: “existed” instead of “exited”?

Reply: Thanks a lot for the suggestion and we changed it as suggested.

Line 251: Yes, but if the colloids found below 20 cm depth came from topsoils, why should the zeta potential increase? Increasing pH should even cause a further shift to more negative zeta potentials?

Reply: There is a possibility that the transported colloids could desorb P from the surface due to the increasing pH in the subsoil by the competition adsorption of OH^-. That is probably the reason why more positive zeta potentials occurred with increasing depth.

Line 258: Perhaps this suggests a POTENTIAL CONTRIBUTION of colloidal P to P leaching, but it could also point to transport of dissolved P with subsequent sorption to the colloidal fraction of subsoils…

Reply: Thanks a lot for your constructive suggestion and we revised the manuscript as suggested:

Line 278-281: It suggested that the potential contribution of CP to transport P in both surface and subsurface soil environment is important and should not be overlooked. However, it is not excluded the transport of dissolved P with subsequent sorption to the colloidal fraction of subsoils.

Line 265-266: “Most colloidal P at the surface soil was from fertilizers and plant residues”? How was this proven?

Reply: Thanks a lot for pointing it. This sentence is not entirely correct and we deleted this sentence.

Lines 285-286: How did you know that these colloids were mobile?

Reply: Thanks a lot for pointing it and we deleted the word “mobile”.

Line 308: Do you mean “dispersed and transported down to subsoils”?

Reply: Yes, we revised the sentence as suggested: “The P-rich WECs was dispersed and transported from surface soils to subsoils”.

Lines 313ff: “colloids with a greater negative charge resulting from P adsorption may be repulsed by negatively charged soil mineral surfaces,…” Refer to Ilg et al. (Eur J Soil Sci 59:233–246, 2008) to underpin this statement?

Reply: Revised it as suggested.

Lines 310-320: Why should the ditches promote the leaching from topsoils to subsoils? They rather facilitate the transport from soils to surface waters?

Reply: Thanks a lot for pointing it and we deleted this sentence.

Lines 427-430: This paper is listed twice in the references.

Reply: Thanks and we revised it as suggested.

Figure 4: Does this figure really show the linear combination fitting or rather the raw XANES spectra?

Reply: Thanks a lot for pointing it and we changed the figure as follows:

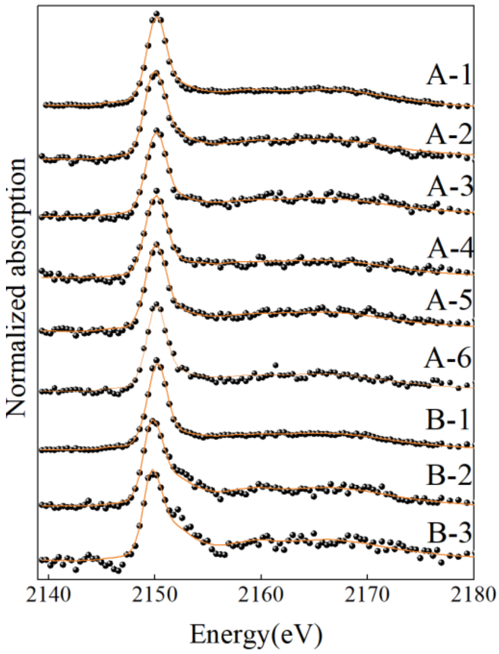

Fig. 4 presents the results of the linear combination fitting (LCF) of the P K- edge XANES spectra for soil colloids of samples A and B. The raw data is represented by points and the fitting results are shown by solid lines. The LCF fitting results can be found in Table 4.

Sample	Depth (cm)	Soil texture	Soil sand fractions (>20 μm, %)	Soil silt fractions (2-20 μm, %)	Colloidal fraction (0.35-2 μm, %)	Zeta potential (mV)	Colloidal P (0.35-2 μm) (mg/kg soil)	DTP (<0.35μm, mg/kg soil)	DRP (<0.35μm, mg/kg soil)	CP/TP (%)
	0-20	sandy loam	57.0±4.0ab	34.8±3.8ab	6.2±0.3c	-19.1±2.7	80.7±3.6a	22.1±1.0a	10.0±1.3a	15.6±1.0ab
	20-40	sandy loam	55.7±1.4ab	34.2±1.2ab	6.3±0.3bc	-18.5±1.1	51.0±1.7b	15.3±0.9b	7.4±0.4b	13.1±0.7b
A	40-60	sandy loam	57.3±4.5 ab	33.6±4.4ab	6.9±0.2bc	-17.9±0.6	52.4±1.9b	12.3±1.2c	4.6±1.1c	14.4±1.5b
	60-80	sandy loam	56.8±2.7ab	33.0±2.5ab	7.3±0.6ab	-17.5±2.9	52.9±4.4b	6.7±1.4d	2.6±0.5cd	14.1±1.9b

	80-100	sandy loam	59.7±2.9 a	30.4±3.3b	7.0±0.5bc	-15.7±2.7	37.3±1.3c	6.0±0.3d	2.0±0.7d	12.0±0.8b
	100-120	loam	50.3±1.6 b	38.8±0.9a	8.3±0.5a	-15.8±0.8	41.2±1.4c	4.1±0.8d	1.2±0.3d	19.2±1.7b
	0-20	sandy loam	56.3±3.4a	35.9±3.6a	5.3±0.3b	-22.0±1.7	61.3±2.7a	19.5±1.1a	10.0±1.3a	10.6±1.1a
B	20-40	sandy loam	56.5±3.2a	34.8±4.1a	5.3±0.4b	-18.9±1.0	31.6±1.5b	14.4±1.2b	6.3±0.4b	10.1±0.8a
	40-60	sandy loam	57.0±1.9a	33.1±1.2a	6.5±0.3a	-9.0±0.6	31.1±1.1b	11.2±0.9c	4.6±0.3b	11.4±0.7a

Table 1: C/P ratios in the range of 15-22 make no sense. Please calculate OC/OP ratios instead of TC/TP ratios.

Reply: Thanks and we revised it as suggested and showed the OC/OP data in the Table 1 which have showed above.

Table 2: Include the percentage of colloidal P in relation to TP?

Reply: Revise as suggested.

Table 2 Soil fractionation, physicochemical characteristics and P levels of water extracted colloids.

DTP: total P concentrations of dissolved fractions (<0.35 µm); DRP: reactive P concentrations of dissolved fractions (<0.35 µm); CP: colloidal P; TP: total P.

Table 3: NMR typically doesn't measure a concentration but rather a relative fraction of the signal of different P forms in relation to the total signal. How did you derive the concentrations of the different P forms in mg/kg soil. What does the * after Diesters mean? Was the diester contribution corrected for the hydrolysis of diesters during the extraction?

Reply: It is correct that NMR results showed a relative fraction of the signal of different P forms in relation to the total signal. The concentrations of individual P species were calculated by multiplying ^{31}P-NMR proportions by the total NaOH-Na_2EDTA extractable P concentration. We have added this sentence in the materials and methods.

* after diesters means calculation by including diester degradation products (i.e. Glyc+nucl: α/β- glycerophosphate, and mononucleotides) with orthophosphate diesters (Diesters) rather than orthophosphate monoesters (Monoesters). We have added this explanation in the Table 3.

Response to reviewer 2：

In this study, soil samples for different soil profiles were collected form four agricultural field sites. The physicochemical character and P distribution were investigated. At the same time, the colloidal phosphorus fractions in different soil profiles were also determined using 31P-NMR, P K-edge XANES and XRD measurements. The authors have done a lot of work in the determination of soil parameters. However, the distribution and composition of colloidal phosphorus in the lime concretion black soil in this study are similar to other types of soil in the existing references, which hinders the innovation of this study. Therefore, the author should focus on refining the innovation of this work. The specific comments as follow:

Abstract is very important, but there are a lot of details missing and many mistakes in this text, and the first sentence of the abstract needs to point out the existing problems and innovation. Language must be improved.

Reply: We changed it in the abstract:

Long-term excessive application of mineral fertilizer leads to accumulation of phosphorus (P) in lime concretion black soil, which increases the risk of P migration and loss from soil profile. The colloids in the soil profile are important carriers for P migration due to high P adsorption and transport capacity. It is not clearly understood how colloidal P is distributed in subsoils (<1.2m) of lime concretion black soil, contributing to subsurface P loss. Understanding the depth sequence distribution of colloidal P and its speciation in the soil profile is critical in assessing P loss comprehensively.

Line 16, “~” should be “-”.

Reply: Revised as suggested.

Line 16, what kind of soil?

Reply: We added the soil type in the sentence as follows:

Water extractable colloids (WECs) of lime concretion black soils were obtained from 0-120 cm soil profile by soil fractionation method, and their physicochemical properties were analyzed.

Lines 19, colloidal P should be colloidal P (CP)?

Reply: Thanks and we revised as suggested.

Line 24, change colloidal P (CP) to CP.

Reply: Revised as suggested.

I can’t find any experiments or treatments information in the abstract section, the author must clear this in lines 17-20.

Reply: We added the experiments and treatments information as follows:

Line18-24: Water extractable colloids (WECs) of lime concretion black soils with size of 0.35-2 µm were obtained from 0-120 cm soil profile by sedimentation and centrifugation scheme. The dissolved reactive P (DRP) and dissolved total P (DTP) in soil supernatant with particle sizes <0.35 µm were measured by molybdate blue colorimetry. Solution ^{31}P nuclear magnetic resonance (NMR) and P K-edge XANES were used to characterize the species and distribution of colloidal P in fertilized farmland soil profile.

Line 27, Usually CP is defined in 1-1000 µm, why did the author limit the size to 2µm?

Reply: The defined sizes of water dispersible colloids (WDC) are not consistent in the current literatures. For example, in the literature by Séquaris et al. (2013), the size of WDC is defined from 0.05 to 2 µm. Jiang et al. (2012, 2017) defined the WDC as particles < 2 µm of clay fraction. The study from Li et al. (2022) defined the size of WDC as 0.22-2 µm. In the study from Wang et al. (2020), WDC were chosen as the size below 300 nm. In this study, we used the sedimentation and centrifugation scheme based on the study of Séquaris et al. (2013). That is why we defined the water dispersible colloids below 2 µm.

References

Séquaris J., Klumpp E., Vereecken H., 2013. Colloidal properties and potential release of water-dispersible colloids in an agricultural soil depth profile. Geoderma, 193-194, 94-101.

Jiang C., Séquaris J., Vereecken H., Klumpp E., 2012. Effects of inorganic and organic anions on the stability of illite and quartz soil colloids in Na-, Ca- and mixed Na–Ca systems. Colloids and Surfaces A: Physicochemical and Engineering Aspects. 415, 134-141.

Jiang C., Séquaris J., Vereecken H., Klumpp E., 2017. Effects of temperature and associated organic carbon on the fractionation of water-dispersible colloids from three silt loam topsoils under different land use. Geoderma, 299, 43-53.

Li Y., Livi K., Arenberg M., Xu S., Arai Y., 2022. Depth sequence distribution of water extractable colloidal phosphorus and its phosphorus speciation in intensively managed agricultural soils. Chemosphere, 286, 131665.

Wang L., Missong A., Amelung W., Willbold S., Prietzel J., Klumpp E., 2020 Dissolved and colloidal phosphorus affect P cycling in calcareous forest soils. Geoderma, 375, 114507.

NMR and P K-edge XANES are important methods for this study, but the authors didn't provide any important results or data related to the above methods.

Reply: We added the related results in the abstract and discussion part as follows:

Line 34-41: The relatively small fraction of orthophosphate diesters indicated by ^{31}P-NMR measurements suggests that the P assimilation by microorganisms for the accumulation of WDC containing organically-bound P was limited in subsoils. The XANES results of WECs showed that the proportions of Al-P, Fe-P, and inositol hexakisphosphate (IHP) of WECs decreased but hydroxyapatite (HAP) increased with soil depth.

The authors should provide more information about the lime concretion black soil.

Reply: we added the related information as follows:

Line 48-55: Lime concretion black soil is classified by Chinese Soil Taxonomy and covers approximately 3.13 million hectares in China (Wei et al., 2018). It is one of the major soil types in the Huang-Huai-Hai Plain of China (Wei et al., 2018) and characterized by low soil organic matter (SOM), low water-air permeability, poor fertility, and strong swelling-shrinkage properties, thus contributing to low crop field (Chen et al., 2020).

References

Wei, C., Gao, W., William, R., Li, B.: Shrinkage characteristics of lime concretion black soil as affected by biochar amendment, Pedosphere, 28(5), 713-725, https://doi.org/10.1016/S1002-0160(18)60041-4, 2018.

Chen, L., Li, F., Li, W., Ning, Q., Li, J., Zhang, J., Ma, D., and Zhang, C.: Organic amendment mitigates the negative impacts of mineral fertilization on bacterial communities in Shajiang black soil, Appl. Soil Ecol., 150, 103457, https://doi.org/10.1016/j.apsoil.2019.103457, 2020.

Line 67, why and how the artificial ditches facilitate the transport of dissolved and colloidal P from both surface and subsurface soil?

Reply: The artificial ditches are normally 1-1.5 m deep and thus facilitate the transport of dissolved and colloidal P from both surface and subsurface soil to surface water system.

Lines 69-70, Liang et al., 2016 did not use any lime concretion black soil in his study.

Reply: Liang et al. (2016) found that the intensive input of fertilizer in soil facilitates the release of colloidal P.

Line 85, lime concretion black soil is not a typical soil type according to the international classification, the author should provide the correct soil classification naming and references.

Reply: Thanks a lot for the suggestion. The soil belongs to lime concretion black soils in Chinese Soil Taxonomy and Vertisol according to US Soil Taxonomy (Wei et al., 2018) and World Reference Base (WRB, 2014). We will add this information in Materials and Methods.

References:

Wei C., Gao W., William R., Li B., 2018. Shrinkage characteristics of lime concretion black soil as affected by biochar amendment. Pedosphere, 28(5),713-725.

WRB, 2014. World Reference Base for soil resources 2014: international soil classification system for naming soils and creating legends for soil maps. Rome, Italy.

Line 86, why the four sites were selected?

Reply: The soils in four sites belong to the lime concretion black soil but with different soil subtypes: loamy thinly covered lime concretion black soil, clay covered lime concretion black soil, clay thinly covered lime concretion black soil and green black soil.

Line 88, In what season was the soil taken?

Reply: The season is Autumn 2021 and we have added this information in the manuscript.

Line 97, remove “by” before “using”

Reply: Revised as suggested.

Line 124 the colloids with size of 0.35-2 μm should be verified by dynamic light scattering method.

Reply: Thanks a lot for this useful suggestion. We have measured the final supernatant by DLS and the measured size was 351.3±6.6 nm. We will add this information in the manuscript Line 143-145.

Line 171 please provide the full name of ANOVA, actually one way ANOVA is single factor analysis of variance.

Reply: Thanks a lot for the suggestion and we revised as follows:

Line 193-194: One way analysis of variance (ANOVA) as a single factor analysis of variance was used to test significant differences of soil indicators with different soil profiles.

Line 193-194 please check the format of the unit

Reply: we have checked the unit.

Lines 229-230, surface soils (0-20 cm) contained 20 to 40 mg kg^{-1} P doesn't mean that this meets the growth requirement of the winter wheat and corn.

Reply: The mean critical values for maize ranged from 12.1 to 17.3 mg P kg^{-1} (average 15.3 mg P kg^{-1}) and for winter wheat from 12.5 to 19.0 mg P kg^{-1} (average 16.3 mg P kg^{-1}) in China according to the study of Tang et al. (2009). We added this literature in the manuscript.

Tang X., Ma Y., Hao X., et al., 2009. Determining critical values of soil Olsen-P for maize
and winter wheat from long-term experiments in China

Response to reviewer 3：

The lime concretion black soil is typic soil in the Huang-huai-hai plain of China but the information related to its colloidal P and P distribution in soil profile was rare which limited our understanding of nutrient managements in this type of soil. In this study, soil samples from four agricultural field sites were investigated. A lot of work related to the physicochemical properties especially for P in bulk soil and colloidal samples in soil profile were investigated. ^{31}P-NMR, P K-edge XANES and XRD measurements were used to measure the colloidal P species and distribution in different soil profiles, which is important to understand the colloidal P distribution and their migration in lime concretion black soil. Generally, the manuscript is well-written and organized. However, there are some concerns should be addressed by authors, as follows.

(1) The lime concretion black soil is classified based on Chinese soil taxonomy, please also provide the soil classification according to the international classification.

Reply: Thanks a lot for the suggestion and we revised it as follows:

The soil belongs to lime concretion black soil in Chinese Soil Taxonomy (Wei et al., 2018) and Vertisol according to World Reference Base (WRB, 2014). We will add this information in Materials and Methods.

References:

Wei C., Gao W., William R., Li B., 2018. Shrinkage characteristics of lime concretion black soil as affected by biochar amendment. Pedosphere, 28(5),713-725.

WRB, 2014. World Reference Base for soil resources 2014: international soil classification system for naming soils and creating legends for soil maps. Rome, Italy.

(2) It is better to put the map of the soil sampling area in SI to show their representativeness in the local region.

Reply: Thanks for your suggestion. The distribution of lime concretion black soil in China and the location of the study site was shown in Fig.S1 of Bai et al. (2023). We cited this literature in the manuscript.

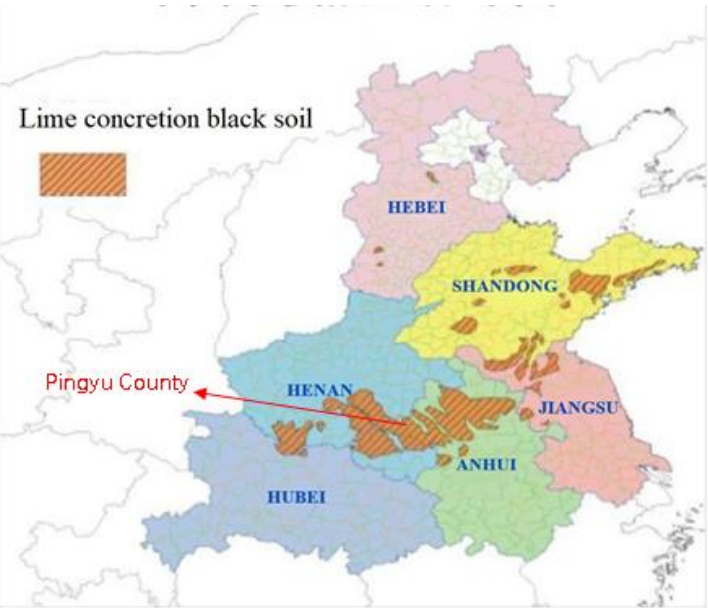

Fig. S1 Distribution of lime concretion black soil in China and the location of the study site (Bai et al., 2003)

Bai, S., Tan, J., Zhang, Z., Wei, M., Zhang, H., and Jiang, X.: Phosphorus speciation and colloidal phosphorus response to the cessation of fertilization in lime concretion black soil, Pedosphere, https://doi.org/10.1016/j.pedsph.2023.01.004, 2023.

(3) Keywords are not appropriate as some keywords already appear in the title.

Reply: Thanks for your suggestion. We changed it as follows:

Keywords: subsurface soil; colloidal P, organic P, solution 31P-NMR, P K-edge XANES

(4) More information about lime concretion black soil needs to be provided in the introduction.

Reply: we added the related information as follows:

Line 48-53: Lime concretion black soil is classified by Chinese Soil Taxonomy and covers approximately 3.13 million hectares in China (Wei et al., 2018). It is one of the major soil types in the Huang-Huai-Hai Plain of China (Wei et al., 2018) and characterized by low soil organic matter (SOM), low water-air permeability, poor fertility, and strong swelling-shrinkage properties, thus contributing to low crop field (Chen et al., 2020).

References

Wei, C., Gao, W., William, R., Li, B.: Shrinkage characteristics of lime concretion black soil as affected by biochar amendment, Pedosphere, 28(5), 713-725, https://doi.org/10.1016/S1002-0160(18)60041-4, 2018.

Chen, L., Li, F., Li, W., Ning, Q., Li, J., Zhang, J., Ma, D., and Zhang, C.: Organic amendment mitigates the negative impacts of mineral fertilization on bacterial communities in Shajiang black soil, Appl. Soil Ecol., 150, 103457, https://doi.org/10.1016/j.apsoil.2019.103457, 2020.

(5) Further clarification is needed on current research limitations on colloidal P in lime concretion black soil.

Reply: We changed it in the abstract:

Line 12-19： Long-term excessive application of mineral fertilizer leads to accumulation of phosphorus (P) in lime concretion black soil, which increases the risk of P migration and loss from soil profile. The colloids in the soil profile are important carriers for P migration due to high P adsorption and transport capacity. It is not clearly understood how colloidal P is distributed in subsoils (<1.2m) of lime concretion black soil, contributing to subsurface P loss. Understanding the depth sequence distribution of colloidal P and its speciation in the soil profile is critical in assessing P loss comprehensively.

(6) Colloids with size of 0.35-2 μm should be further measured by dynamic light scattering.

Reply: Thanks a lot for this useful suggestion. We have measured the final supernatant by DLS and the measured size was 351.3±6.6 nm. We will add this information in the manuscript Line 143-145.

(7) More discussion and evidence need to provide to support the transport of colloidal P from surface to subsurface soil.

Reply: Thanks a lot for this useful suggestion. We have discussed it as follows:

Line 337-355: In principle, there are four processes that could lead to the presence of organically-bound colloidal P in subsoils: i) the mobilization and subsequent transport of colloids from topsoils into subsoils, ii) the leaching of dissolved inorganic or organic P and their subsequent sorption to surfaces of WDC in subsoils, iii) direct input of organic P into subsoils due to root exsudation or root decay, and their subsequent sorption to surfaces of WDC in subsoils, and iv) the leaching of P from subsoils and its subsequent assimilation by microorganisms colonizing mineral surfaces in subsoils. As discussed above, the first process was probably dominated due to more negative values of zeta potential at surface soil colloids and the presence of abundant swelling-shrinkage clays which facilitates preferential flow. The process ii) and iii) were also possible but their contributions were not much. As discussed before, no colloids existed in the depth >60 cm of sample B which supported the limited generation of colloids in subsoils of lime concretion black soil. The process iv) seems not important because the OC/OP in all soil samples ranged from 4.9 to 49.6, which did not support the immobilization of P. Additionally, the relatively small fraction of orthophosphate diester indicated by ^{31}P-NMR measurements suggested that the process iv) was probably not the most relevant process explaining the accumulation of WDC containing organically-bound P in subsoils.

(8) The format of references is inconsistent.

Reply: Revised as suggested.