



Reply on RC1

Yuan Wang et al.

Author comment on "The distribution of phosphorus from phosphorus derived materials to different soil fractions determines the phosphorus availability in the soil" by Yuan Wang et al., SOIL Discuss., <https://doi.org/10.5194/soil-2021-127-AC1>, 2022

Dear reviewer,

We greatly appreciate your time and expertise in reviewing our manuscript (soil-2021-127). We have carefully modified the manuscript based on your constructive comments, which significantly improve the manuscript. Appended is our point-by-point response to the comments. The detailed information is as follows:

1. This manuscript presents the results of a very simple study: the authors added four fertilizers developed from recycled waste materials, plus a chemical P fertilizer and a check with no fertilizer, to two soils and incubated them for 70 days. They monitored soil test (Olsen) P regularly over the 70-day incubation but did more detailed analyses on samples incubated for the full 70 days only. However, as the authors themselves point out in the Introduction and the Discussion, incubation studies with organic P sources such as manures are very common, and the majority of the conclusions of this study (e.g., "different P sources had different effects on soil P availability", "soil Olsen-P content was mainly affected by the labile P fraction", etc.) have been shown many times before. Therefore, while the results may be useful in the region where this study was conducted, the overall novelty is low as currently written. If this manuscript is revised, the authors must clearly indicate the factors that make this study different from previous studies, including novel results not shown by any previous study.

Thanks for your nice suggestions. Based on the comments, we have conducted a detailed revision in the Introduction. The main objective of this study is to provide a basis for the

closed- P cycle in farming systems by recovering P from agricultural wastes. We attempted to explore promising renewable P-containing materials for achieving a closed cycle of P by understanding the transformation dynamics of different renewable P-containing materials in soil and their P availability. The soil texture and physicochemical properties such as pH and organic matter determined the P sorption reaction (Xiong et al., 2022; Debicka et al., 2016; Bouray et al., 2021). Quantifying the transformations of different phosphorus-containing materials in soils with different soil conditions is necessary to enhance phosphorus utilization and reduce phosphorus resource limitation. We have supplemented this background in the introduction.

To understand the transformation dynamics of different P-containing materials in the soil, we measured the P fractions of the initial soil, four renewable P-containing materials, and two soils with different P-containing materials on days 0, 35, and 70 of incubation. The P fractions were not significantly different on day 70 of incubation compared to day 0 of incubation. Therefore, we analyzed the data from day 70 of incubation in the manuscript. However, we did not realize that these data are indispensable to understanding the mechanisms of transformation of different P-containing materials in two soils. The analysis and discussion of these data have been supplemented in the modified manuscript. We believe that this study is helpful and meaningful for understanding the mechanisms of P-containing material transformation in different soils.

And the conclusions were modified as follows: Compared with other renewable P-containing materials, CM is a superior source for improving soil P availability in fluvo-aquic and red soils. Compared to fluvo-aquic soil, phosphorus from SSP, PM, and CM was more strongly immobilized in red soil. Further analysis of the P fraction of two soils with different P-containing materials at days 0, 35 and 70 of incubation revealed that the distribution of CM to the soil labile P fraction was significantly increased compared to other renewable P-containing materials. And compared with fluvo-aquic soil, the contribution of different P-containing materials to the labile P fraction of red soil was significantly decreased. Changes in P fractions at different incubation periods in soils with different P-containing materials show that most soil P fractions have no significant difference on day 70 of incubation compared to day 0 of incubation. That suggests, in the short term, the difference of potential bioavailability of P from various sources is determined by the distribution to soil labile P fractions rather than its transformation in the soil. In general, there is promising potential to reduce P limitation by recovering cattle manure as an alternative source of P supply. This study provides a basis for closing the P cycle in agricultural systems and for sustainable on-farm P management strategies.

2. One major concern is the lack of detailed information about the soils used in this study. The authors describe them as "calcareous fluvo-aquic soil in Quzhou" and "red soil in Shilin County" (line 108), and include very limited information about these soils in lines 109-113". However, in the discussion, they make statements indicating that they view the

results of this study to be widely applicable (e.g. "suggests that the application of bone meal in red soil", lines 273-273; "adding maize straw and cattle bone meal to fluvo-aquic soil", line 297). The authors need to provide a lot more information about these soils to demonstrate that the results of this study can be more widely applied than just the soils used in this study.

Thanks for pointing this out. The transformation process of P in soil is closely related to soil properties. Therefore, we selected two typical soils with different textures and pH for analysis. We have supplemented the details of these two soils in the materials and methods, to provide a reference for the wider application of this study. The modified as follows: Soil samples were collected from fluvo-aquic soil (calcareous alluvial soil) in Hebei Province and red soil (ultisol) in Yunnan Province. The soil texture of fluvo-aquic soil is silt loam soil with 7.9% of clay (<2 μm), 55.3% of silt (2–20 μm), and 36.8% of sand (20–2,000 μm). The soil texture of red soil is clay with 47.5% of clay (<2 μm), 25.3% of silt (2–20 μm), and 27.2% of sand (20–2,000 μm).

3. Another concern, related to the previous point, is the incomplete descriptions of the recycled P materials used in this study. The authors seem to assume that "poultry manure", "cattle manure", "maize straw" and "cattle bone powder" are adequate descriptions, despite indicating in the introduction that these materials can vary in composition (lines 65-70). based on the literature cited in lines 79-82. However, it is well-established in the literature that this is not true, especially for manure P. Many things will influence P forms and their cycling in manures even within the same species, including diet, animal age and life stage, animal bedding that may be included with animal feces, and storage and treatment of the manures before adding to soils. Diet formulations, including high or low concentrations of dietary P and the addition of phytase, has shown to strongly affect P species and concentrations in manure, including for poultry (Maguire et al. 2004 *J. Environ. Qual.* 33:2306-2316; McGrath et al. 2005 *J. Environ. Qual.* 34:1896-1909; Leytem et al. 2007 *J. Sci. Food Agric.* 87:1495-1501), swine (Yi et al. 1996 *J. Anim. Sci.* 74:1601-1611; Leytem and Thacker 2008 *J. Anim. Vet. Advan.* 7:113-120), sheep (Leytem et al. 2007 *An. Feed. Sci. Technol.* 138:13-28) and dairy (Toor et al., 2005 *J. Environ. Qual.* 34:1380-1391; McDowell et al. 2008 *J. Environ. Qual.* 37:741-752; He et al. 2009 *J. Environ. Qual.* 38:1909-1918). Storage conditions, length of storage, and amendments during storage, including additives such as phytase or alum, will also affect manure P forms and their availability (e.g. Dao et al. 2001 *J. Environ. Qual.* 30:1693-1698; Moore and Edwards 2007 *J. Environ. Qual.* 36:163-174; Warren et al. 2008 *J. Environ. Qual.* 37:469-476; Hill and Cade-Menu 2009 *J. Environ. Qual.* 38:130-138; Casteel et al. 2011 *Poult. Sci.* 90:2689-2696; Peirce et al. 2013 *Plant Soil* 373:359-372; Huang et al. 2018 *J. Environ. Qual.* 47:345-352). The authors have provided very limited information about the manures used in this study, beyond the amount of each fertilizer added (Table 1) and some very bad NMR spectra in the supplemental materials. They have not even included P pools from sequential fractionation (Table 2) for the fertilizers. This is not enough. They must include more detailed descriptions of the sources of these manures, including feed, storage, and treatments of manure (if any) during storage; concentrations of agronomically-relevant nutrients, and N and P pools in these manures (e.g. Olsen P or other soil test P values; nitrate, ammonium or other soil test N values); total organic P; pH, exchangeable cations, etc. Without detailed information about the organic fertilizers, it is difficult to extrapolate the results of

this study to other manures; instead, the results become specific only to these particular fertilizers in these particular soils. And that is not very relevant scientifically, and will not be of interest to other readers of this journal.

Good suggestion. We have analyzed the basic properties of different P-containing materials including P fractions before the experiment, and the specific information about the four renewable P-containing materials has been supplemented in the materials and methods. For specific data, please refer to the materials and methods and supplementary materials of the revised manuscripts.

As suggested by the reviewers, we have modified as follows: The phosphorus fractions in manure are dependent on various factors, including manure type, solid-liquid separation status, manure removal method, handling way and degree of decomposition, etc (Li et al., 2014; Pagliari and Laboski, 2013). Especially for manure type, the differences in the digestive system and feed composition of different animals cause large differences in phosphorus concentration and fractions in different manures (Garcia-Albacete et al., 2012; Freiberg et al., 2020). Meanwhile, straw turnover is usually applied directly to the soil in agricultural practice, and the P availability in straw requires in-depth analysis (Guan et al., 2020). The bone meal which can be recycled and used as a large amount of organic fertilizer in the future remains unclear in terms of P reuse (Ylivainio et al., 2008). Thus, it is necessary to identify and quantify P fractions from different P-containing materials and their distribution in soil P fractions to determine the potential bioavailability and the environmental impact of P from various sources.

4. The main method for soil P pools was a modified version of the Hedley fractionation method, which the authors used for soils only. Sequential P fraction is a common technique that is widely used. However, this is mainly because it is a simple, inexpensive method, rather than because it is chemically precise. All fractionation methods are operationally-defined, meaning that they are defined by the extractants used and the steps in the fractionation method (the order in which each extractant is used). Most extractants used are not specific for any particular P compounds, with the result that the method yields little meaningful data about specific soil P chemistry, as has been discussed for decades, and which has been demonstrated by comparing fractionation results to those from more advanced techniques such as P K-edge XANES (x-ray absorption near edge structure) spectroscopy (e.g. Saunders 1959. *Nature* 4704:2037; Condon and Newman 2011 *J. Soil Sediments* 11: 830-840; Kar et al. 2011 *Soil Sci.* 176:589-595; Klotzbücher et al., 2019. *J. Plant Nutr. Soil Sci.* 182:570-577; Barrow et al., 2021 *Plant Soil* 459:1-11; Gu and Marginot 2021 *Plant Soil* 459:13-17).

We agree with you that the new techniques such as P K-edge XANES is useful to

understanding the soil P transformation progress, while it is difficult for us now due to the crowded monitoring. We will try the advanced techniques to improve our future work.

4a. The authors used a very long extraction procedure, with many of the steps requiring 16 hours of extraction (Fig. S1). However, they do not indicate that they added anti-microbial agents (e.g. toluene, sodium azide). With long extractions such as this, microbial growth can transform P within the samples, either by mineralization of organic P species or by uptake and conversion of phosphate to complex inorganic P forms (e.g. polyphosphates) or organic P forms such as phospholipids or DNA. How certain are the authors that the fractionation results reflect P in the soil samples and not transformations during the fractionation method?

Thanks for pointing out this important point. We strictly follow the improved P sequential fractionation method by Tiessen and Moir. We agree that microorganisms will have an impact on the transformation of P in the long-term extraction process. Therefore, we strictly managed to shake time, environmental temperature, and chromogenic time in the operation of the test. All samples were measured under the same conditions to minimize the effects of P transformation on the results during the extraction process.

4b. I am pleased to see that the authors have not labeled the fractions with any specific chemical terms. However, I am concerned that the authors have interpreted changes in these soil fractions as "transformations" (e.g., lines 28, 236, 346). The authors do not provide any information about the fractions in the fertilizers themselves. In my opinion, all the changes they see after incubations reflect the properties of the fertilizers added. If the authors genuinely want to show transformations of soil during the incubation experiment, then they need to provide data for the fertilizer materials and the soil samples immediately after the fertilizers were added, in addition to data after 70 days of incubations.

Thanks for the helpful suggestions. We have supplemented the data of soil P fractions on days 0 and 35 of incubation in the supplementary materials and supplemented the discussion of these results in the manuscript.

4c. I am concerned about the authors' determination of organic P in their fractions. First,

the methods described in lines 138-142 describe the measurement of phosphate colorimetrically in each fraction before and after digestion. The authors are correct that the measurement after digestion is total P (TP) in each extract. However, the colorimetric measurement before digestion is not total inorganic P but is merely the phosphate that can react with the color reagent (molybdate-reactive P, MRP). Thus, the difference between TP and MRP is not organic P but is molybdate-unreactive P (MUP), which can include complex inorganic P compounds such as pyrophosphate.

Thanks. We followed the improved P sequential fractionation method to classify different P fractions. The P fractions before and after digestion are divided into inorganic P and organic P. We agree with the reviewer's suggestion and annotate this point in the Materials and Methods section of the manuscript.

5. A second main method used to characterize P in these samples was ^{31}P nuclear magnetic resonance spectroscopy (P-NMR). The spectra shown in Figs. 4 and S2 are of very poor quality. There are also problems with the identification of peaks in these samples. For example, the authors indicate "inositol hexaphosphate" for a general region of the spectra, labeled "C", rather than identifying any individual peaks. There are several stereoisomers of inositol hexakisphosphates that can be present in spectra of soil extracts, each with multiple peaks that must be identified to confirm the presence of these compounds in samples. In addition, the broad region of the spectra labeled as "C" can contain several other compounds, products of diester degradation during sample extraction and analysis. Any peak identification requires spiking samples with known P compounds after the initial P-NMR analysis and then reanalyzing the samples by P-NMR to confirm peak identifications. If the authors did this, then they need to show the results of these spiking experiments to confirm their peak identifications. If they did not conduct spiking experiments, then they need to do so for these P-NMR results to be publishable in any scientific journal.

Thank you for pointing out this lack of detailed information. This information has been supplemented with the modified manuscript.

6. I am also concerned by the authors' correlation results in Fig. S4. Only independent variables should be correlated with each other. Methods such as NMR and sequential fractionation produce auto-correlated results, not independent variables: NMR because the results are determined as relative proportions, and P fractionation because each fractionation will depend on what is extracted in the previous fraction. Thus, many of the correlation results in Fig. S4 are meaningless because they are not all for independent variables. I am also concerned about the use of structural equation modeling (SEM) to conclude factors influencing P cycling in these soils, because SEM is merely a fancy

method of correlation, and so is governed by the same rules as for simple correlations (e.g., using independent variables), and because I have concerned about the results in general (see previous points).

Thanks for the helpful suggestions. We modified the graphical representation of the correlation between Olsen-P and P fractions in the supplementary materials. Please refer to supplementary material figure S4 for specific modifications. Structural equation modeling (SEM) can intuitively show the complex path relationship. SEM is also widely used in the study of the soil P cycle (Hou et al., 2016; Costa et al., 2016; Qaswar et al., 2020). In this study, SEM helps us to better understand the process and environmental factors driving soil P transformation. We have redcribed the SEM results in the modified manuscript.

7. References: there are several problems with the references in this manuscript.

7a. The number of references cited is out of proportion to the length of the paper: the total length of the manuscript (including abstract and conclusions) is 326 lines, while the References is 220 lines. The authors should carefully check each reference to see if it is necessary.

We have updated the references accordingly.

7b. There are problems with many of the listings in the References. For example, "Gerard and Frederic" (lines 424-425) and "Gérard" (lines 426-427) are the same references. The author's name is Frederic Gérard, which the authors somehow split into two different authors. Unfortunately, they cite both of these in the text (lines 301-302). There are also problems with other references (e.g. Jiang et al. 2012).

Thank you for pointing this out. We have updated the references accordingly.

8. There are problems with editing and quality of English through the text (e.g., "The relative contents of inorganic and organic P in soil is great" should be "The relative contents of inorganic and organic P in soil are great" because the verb modifies "contents". The authors need to carefully edit any revised manuscript.

We would like to express our heartfelt thanks to the reviewer for the thorough reading of our manuscript and the very useful suggestions. We carefully considered the reviewers' suggestions and made corresponding modifications, which greatly improved our manuscript. During the revision process, we have also done very careful language polishing accordingly.

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