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Comment on soil-2021-127

Anonymous Referee #1

Referee 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-RC1, 2021

Phosphorus (P) is an essential crop nutrient but its cycling is still poorly understood. Globally, there are concerns about the long-term sustainability of the rock phosphate reserves that are used to make chemical P fertilizers. The use of recycled wastes, including animal manures, could be used to replace some or all of P fertilizers, but studies are needed to understand the availability of these wastes to crops. The objective of this study was to investigate P availability over time in incubated soils after amendment with recycled wastes, including poultry and cattle manure and maize straw. In general, the topic of the study is suitable for this journal, and could be of interest to readers. However, there are a number of problems with the manuscript that must be addressed before the manuscript can be accepted for publication.

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.

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 to just the soils used in this study.

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 including 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-Menun 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.

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 adsorption near edge structure) spectroscopy (e.g. Saunders 1959. Nature 4704:2037; Condron 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).

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?

4b. I am pleased to see that the authors have not labelled the fractions with any specific chemical terms. However, I am concerned that the authors have interpreted changes in these soil fraction 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 for the soil samples immediately after the fertilizers were added, in addition to data after 70 days of incubations.

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.

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 very pool 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, labelled "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 labelled as "C" can contain a number of 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 in order for these P-NMR results to be publishable in any scientific journal.

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 modelling (SEM) to draw conclusions about 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).

7. References: there are a number of 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.

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 reference. The author's name is Frederic Gérard, which the authors somehow split into to 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.

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 greatly" should be "The relative contents of inorganic and organic P in soil are greatly", because the verb modifies "contents". The authors need to carefully edit any revised manuscript.