

Biogeosciences

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Soil phosphorus dynamics on terrestrial natural ecosystems

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This is a second revision of a previously submitted manuscript. I also reviewed the previous submissions.

For the most part, the manuscript has been significantly improved with each revision. However, there are still a few problems to be addressed, as noted below.

1. Writing quality: for the most part, the quality of English has improved. However, there are still some problems in some places, which are noted below.
2. I am puzzled about some of the literature cited in the introduction and discussion, and still think there is a bias to citations of specific authors rather than a good overview of the literature.
 - a) Please cite original papers where possible, rather than recent review papers. For example, why cite Nash et al. (2014), rather than Newman and Tate 1980, *Commun. Soil Sci. Plant Anal* 11:835-842) or Tate and Newman (1982, *Soil Biol. Biochem* 14:191-196) for the description of P forms on p. 2, line 17? Why cite Turner 2008a for phosphatase and orthophosphate uptake (p. 2, line 20) – this was well-known prior to that paper. Why cite Huang et al. 2017 for vegetation and organisms (p. 10, l. 24) or mycorrhizae (p. 13, l. 16), when it presents no new information on either of these topics? The same is also true for Yu et al. 2013. There has been a lot of good research into many aspects of P cycling by a lot of good scientists; citing the review papers instead of the original research is unfair to the original researchers, and runs the risk of introducing errors into the literature if the original work was cited incorrectly in the review. Would the authors of this manuscript not prefer to have it cited directly?
 - b) Please be careful citing studies using fractionation methods (e.g. Hedley) in study discussing specific chemical forms. Fractions from fractionation methods are operationally defined, not chemically defined, especially the Hedley fractionation method.
3. Abstract:

Lines 11-12: “nuclear magnetic resonance of NaOH-EDTA extracts” should be “nuclear magnetic resonance spectroscopy of NaOH-EDTA extracts” (something I missed in my previous review.

Lines 16-17: “organic and inorganic P compounds variations” should be “variations in organic and inorganic P compounds”

Lines 19-20: “and after altogether with plant and microbe” I still do not understand what the authors are trying to say here; something seems to be missing. Do they mean “and together with plant and microbe”?
4. Introduction:

p. 2, line 6 and elsewhere: “phosphorus” should be “P” after the first use of the abbreviation, except at the start of a sentence.

p. 2, line 14: “composed by specific” should be “composed of specific”

p. 2, lines 17 and 18: please replace “Nash et al. 2014” and “Cade-Menun and Preston 1996” with more appropriate references, because these compound classes were recognized prior to the publication of these papers

p. 2, line 20: “As most enzymes, the activity of soil P cycling enzymes” should be “As with most enzymes, the activities of soil phosphatases”

p. 2, line 21: “specific enzyme optimum” should be “specific enzyme optima”

p. 2, lines 24-25: DNA adsorption is only below 5 (the isoelectric point of DNA); also, why is a review paper (Yu et al. 2013) cited here, rather than one of the original references about pH? Please see the comments for page 12, below.

p. 2, lines 27-31: studies using P fractionation (e.g. Walker and Syers 1976, Yang and Post 2011) do not give any information about P_o and P_i forms and compounds, because fractionation by definition can only give information about operationally-defined pools. Please rewrite this paragraph, and cite better references that actually describe changes in P_i and P_o compounds

p. 3, line 6: “While this study” should be “While the Feng et al. (2016) study”

p. 3, line 11: “Variation in” should be “Variations in”

p. 3, lines 27-28: “the NaOH-EDTA extraction does not separate Fe-, Al-and Ca-phosphate compounds (Kizewski et al. 2011)” although this might be what Kizewski et al. said, it isn't very accurate. A better way to say it is: “the high pH of the NaOH-EDTA extraction separates P species from the cations (e.g. Al, Fe, Ca) with which they were associated in soil”.

- p. 3, lines 28-30: Yes, there are other methods to study P dynamics and soil P composition, but none of these methods is perfect individually. For example, while XANES is a solid-state technique that does not require extraction, P concentrations are often below the detection limit, so it can only detect broad P species groups (e.g. Fe-P, Ca-P), but can't for example say if DNA is sorbed to Fe or Al. The most thorough studies of soil P use a combination of techniques together, and not any single technique (e.g. Liu et al. 2013 J. Environ. Qual 42:1763-1770; Liu et al. 2015 Environ Sci Technol 49:168-176).
- p. 3, lines 30-32: "This does not mean that the results on pyrophosphate, polyphosphate and total orthophosphate concentrations are not useful, however, there are other inorganic P compounds of importance in soils". This sentence makes no sense to me. No, there are no other inorganic P compound in soil other than orthophosphate, pyrophosphate and polyphosphate. However, as noted above, extraction with NaOH-EDTA removes inorganic (and organic) P compound from the cations with which they are associated in soils. Thus, as noted below, a combination of techniques will give the most complete picture of soil P speciation and dynamics. Please rewrite this sentence.
5. Methods: These are now clear and well-written.
6. Results:
- p. 7, line 1: "there was no pH effect on both pools" should be "there was no pH effect on either pool"
- p. 7, line 6: "additional Appendix S3" delete "additional"
- p. 7, line 8: "additional Appendix S6" delete "additional"
- p. 9, lines 5-6: "organic and inorganic P" should be " P_o and P_i " to be consistent with the rest of the manuscript
7. Discussion: In general, this part of the manuscript is improved compared to previous versions.
- p. 10, line 5: "P organic and inorganic compounds" should be " P_o and P_i compounds"
- p. 10, line 8: "the decaying degree of C element is lower than the P" I do not understand what you are trying to say here. Is it "organic matter degrades more slowly than P_o compounds"? Please rewrite.
- p. 10, line 9: "Turner and Condron 2013" is an opening paper introducing a special issue, and does not contain data to support this statement of fact. Please cite another reference that actually contains data.
- p. 10, lines 16-17: It is not possible to determine specific P compounds such as apatite with Hedley fractionation, and the long extraction times likely also degrade organic P. Please cite a better reference, with actual soil chemical data to support this point, and not a review article of Hedley fractionation (Yang and Post 2011).
- p. 10, lines 23-25: please cite an original study that contains data, and not a review paper (Huang et al. 2017) to support this statement of fact
- p. 11, line 6: "proportion" should be "proportions".
- p. 11, lines 26-28: These are also conditions under which ectomycorrhizal fungi are found. These fungi produce hyphal mats in the forest floor, so an increase in polyphosphates could reflect an increase in ectomycorrhizal hyphal mats.
- p. 12, line 5: "pH affect" should be "pH affects"
- p. 12, line 5: "organic and inorganic P compounds" should be " P_o and P_i compounds"
- p. 12, line 14: "on the clay minerals", delete "the"
- p. 12, lines 15-18: I am pleased to see the authors citing original studies about DNA sorption at the start of this section. However, I do not understand why they cited Yu et al. 2013 at the end of the section (line 18), because this is a review paper. Please replace this citation with a paper containing original research to support this statement of fact.
- p. 12, line 22: "for the P acquisition" delete "the"
- p. 12, line 25: "organic P" should be " P_o "
- p. 12, line 28: "George et al. 2017" is a broad review paper; please cite a more specific reference to support this statement of fact.
- p. 12, line 28: "Plant and microorganism" should be "Plants and microorganisms"; "diesters" should be "orthophosphate diesters" or "phosphodiester".
- p. 12, line 29: "monoesters" should be "orthophosphate monoesters", or "phosphomonoesters"; monoesters and diesters are general bond descriptions; the "orthophosphate" or "phospho" is more specific
- p. 12, line 30: Why is Turner 2008a cited to support this statement of fact about phosphatases? This was known years before the Turner paper was published (e.g. Halsted 1964, Skujins 1967, Tabatabai and Bremner 1969).
- p. 12, line 33: "both solubilization and hydrolysis by the phytase" As written, this implies that phytase both solubilizes and hydrolyzes inositol hexaphosphates, which is incorrect. Organic acids are required to desorb inositol phosphates, so that phytases can hydrolyze them. Please rewrite these lines, with a more appropriate reference than Turner 2008a.
- p. 13, line 7: "effect in" should be "effect on"
- p. 13, line 8: "did not had" should be "had no"
- p. 13, line 16: Huang et al. 2017 provides no direct evidence of phytate mineralization by ectomycorrhizal fungi. Please cite a better reference to support this statement of fact.

- p. 14, lines 12-13: Turner et al. 2002 is a review paper about inositol phosphates, so why is it being cited to support a general statement about temperature and phosphatase activity? Please replace this with a more appropriate reference.
- p. 14, lines 24-29: Why are Walker and Syers 1976 and Feng et al. 2016 being cited to support discussion about decreased orthophosphate measured in NaOH-EDTA extracts by NMR? Neither of the cited references used NaOH-EDTA extraction or NMR, so they are irrelevant to the discussion here. Please replace these with better references. The authors should also mention here that caution needs to be used when discussing changes in orthophosphate extracted by NaOH-EDTA for NMR, because it will preferentially extract organic P rather than orthophosphate. As such, studies of the residuals after NaOH-EDTA show that it is mainly orthophosphate, especially for soils with low total P recovery by NaOH-EDTA.
- p. 15, line 9, "Even though" should be "However"
- p. 15, lines 13 and 17: "Vitousek et al. 1995" is cited in the text but is not listed in the References
- p. 15, lines 23-25: "phosphorus" should be abbreviated to be consistent with the rest of the manuscript.
- p. 15, line 29: "why inositol hexakisphosphates have not been found" should be "why are inositol hexakisphosphates not found"
- p. 16, line 15: "functional groups only (i.e. diesters and monoesters)" should be "broad compound classes only (i.e. orthophosphate diesters and monoesters)"
- p. 16, line 15: "when compounds concentrations" should be "when compound concentrations"

8. Conclusions:

p. 15, line 27: "as pedogenesis evolve" should be "as pedogenesis evolves"

9. Acknowledgements: "NSERC-Discover" should be "NSERC Discovery"; "from which studies" should be "from whose studies"

10. References: in general, this section is greatly improved compared to the previous version of this manuscript. However, there are still differences in formatting (e.g. Cloy et al. 2014) and some spelling mistakes (e.g. "Biophysical" in Wang et al. 2014". Please proofread this section carefully.