

Soil phosphorus dynamics on terrestrial natural ecosystems

Leonardo Deiss, B. Anibal de Moraes and Vincent Maire

This is a revision of a previously submitted manuscript.

For the most part, the manuscript has been significantly improved compared to the original submission. In particular, the authors have constrained the studies used to avoid methodological differences, and have focused on specific P compounds to minimize differences among research groups (e.g. with respect to correcting for degradation), which could hamper the type of global comparisons they are trying to make. I still think the studies they have included are too heavily skewed to studies by a single author (Turner) and a single location (New Zealand). However, that is partially due to issues with analyses by some studies by other groups or from other locations. Hopefully, future studies will avoid these problems, allowing this type of analysis to be expanded in time. But for now, this is an interesting first attempt at this type of analysis.

There are still some problems that will need to be addressed before this is publishable. The majority of these fall more into minor or moderate revisions, as indicated below. However, the discussion will need some substantial revision, because many parts of it, as indicated below, are based merely on speculation, and are not grounded in fact or even in the authors' own results.

1. Writing quality: for the most part, the quality of English has improved. However, there are still some problems with some sections. These are specifically addressed below, but I still recommend that the authors have the revised version read by a native English speaker who is familiar with the research topic. There are still problems with the writing in that the authors make sweeping statements of fact, without citing any references to support these statements. This is particularly true in the introduction, and specific instances are noted below.
2. Title: The current title isn't very informative or very well written (e.g. "soil" by definition is "terrestrial", so including both is redundant). I suggest: "A meta-analysis of ecosystem properties influencing soil phosphorus dynamics in natural ecosystems".
3. Abstract: Although the main body of the paper appears to have been revised with the assistance of a native English speaker, the abstract appears to have been overlooked.
Line 7: "phosphorus (P) compounds can be modified by distinctive ecosystem properties" Using the term "modifies" implies that the ecosystem properties directly change P forms. However, most of the changes are indirect. For example, pH can change the soil environment to influence sorption of P forms, or it can affect the organisms that produce P forms, or that produce enzymes to mineralize P forms. But pH itself isn't necessarily changing P forms, except potentially alter the charge of the P form. As such, I think it would be best to change this to "phosphorus (P) compounds can be influenced by distinctive ecosystem properties".
Line 8: "soil P dynamics on terrestrial natural ecosystems, relating its organic" should be "soil P dynamics in natural ecosystems, relating organic"
Line 11: "determined the soil P composition" should be "determined soil P composition"
Lines 11-12: "nuclear magnetic resonance of soils extracted with NaOH EDTA" should be "nuclear magnetic resonance of NaOH-EDTA extracts of soils"
Line 12: "models were used to better understand the soil P" should be "models were used to better understand the factors influencing soil P"
Line 13: "relationships, soil P compounds had similar overall behaviors on mineral and organic layers but with different slopes". This analysis does not give any information about the behavior of soil P compounds; instead, it shows the relationship of soil P compounds with various factors. As such, this should be "relationships, trends for soil P compounds were similar for mineral and organic layers but with different slopes".
Line 16: "Soil, particularly" should be "Soil factors, particularly"; "ratio" should be "ratios"
Line 18: "soil P composition on terrestrial natural ecosystems" should be "soil P composition in natural ecosystems"
Lines 19-20: "and after altogether with plant and microbe coexistence" I do not understand what the authors are trying to say here; something seems to be missing.
4. Introduction:
p. 2, line 4: "derives essentially" should be "derives predominantly"; "as an eolian deposit" should be "as eolian deposits". However, I disagree with this statement, because it is only relevant for younger soils (e.g. Chadwick et al. 1999 Nature 397:491-497). And because this is a statement of fact, a reference needs to be cited here.

- p. 2, lines 5-6: "Phosphorus goes back to soil as organic materials (Noack et al. 2012" but not necessarily as organic P; Noack et al. showed that much of the P in plant residues was orthophosphate. I think the authors need to explicitly state that here, that "organic materials" are not primarily composed of organic P forms.
- p. 2, lines 7-9: "Each new cycle...primary minerals" Please cite a reference to support this statement of fact, because I am not sure that it is in fact true.
- p. 2, line 10: Jenny (1941) described these as the five factors of soil formation, not ecosystem functioning. Please cite another reference to support using this term in this way.
- p. 2, line 14:"main Po compounds are" should be "main Po compound categories are", because monoesters, diesters, etc. are compound categories, not forms.
- p. 2, lines 15-16: "orthophosphate monoester" should be "orthophosphate monoesters", "orthophosphate diester" should be "orthophosphate diesters" and "phosphonate" should be "phosphonates", because these broad compound classes contain a number of different P compounds.
- p. 2, line 18: "used by" should be "directly taken up by", because all P compounds can be used by plants and microbes after transformation, just not directly taken up by them.
- p. 2, lines 17-19: "Specific phosphatase...and microbes" and "Obviously...optimum" Please cite references to support these statements of fact.
- p. 2, lines 19-20: "Phosphomonoesterase is more active in acidic soils while phosphodiesterase is optimized in basic soils (Turner and Haygarth 2005)" NO! It simply is not possible to make such a broad, sweeping statement based on a single study of pasture soils from England. Please rewrite.
- p. 2, lines 28-29: "As soil ages...colimitation intermediate stage" Please cite a reference to support this statement of fact.
- p. 3, line 4: "precipitations" should be "precipitation".
- p. 3, lines 9-14: I think "parent rock" should be changed to "parent material", because soils can form on materials other than a specific type of rock (e.g. sand, glacial till).
- p. 3, line 16: "Soil P composition has been studied in soils from ecosystems worldwide" should be "Soil P composition has been studied in ecosystems worldwide"
- p. 3, line 17: "(NMR) was a widely used method" should be "(NMR) is a widely-used method", because to the best of my knowledge it is still being used.
- p. 3, line 19: "NaOH and chelating agent EDTA" should be "NaOH combined with the chelating agent EDTA"
- p. 3, line 20 (and elsewhere throughout the manuscript): "NaOH EDTA" should be "NaOH-EDTA".
- p. 3, lines 22-24: "The NaOH EDTA...(Turner and Blackwell 2013)" This sentence does not make any sense as written. How does this demonstrate that NaOH-EDTA is quantitative? Please rewrite and include more references.
- p. 3, lines 26-27: "state factors of ecosystem functioning" see previous comment and rewrite.
- p. 3, line 27: "because known responses were obtained from case-specific conditions". I do not understand what the authors are trying to say here. Please rewrite.

5. Methods:

- p. 4, line 5: "that namely estimated an adequate delay time" should be "that used an adequate time"
- p. 4, line 15: "only control (unchanged) was used" should be "only control (unchanged) samples were used"
- p. 4, lines 23-23: "organic P and its compound" should be "organic P and the compounds"
- p. 4, line 24: "NaOH EDTA inorganic P, and its compounds" should be "and NaOH-EDTA inorganic P and the compounds"
- p. 4, line 27: "climate characteristic" should be "climate characteristics", because it refers to both precipitation and temperature.
- p. 5, lines 29-30: "how the inorganic and organic P compounds variation" should be "how variations in inorganic and organic P compounds"
- p. 6, line 4: "Differently" should be "In contrast"

6. Results:

- p. 6, line 13: "McDowell and Stewart" should be "McDowell and Stewart"; the name is spelled correctly in the References section.
- p. 6, line 20: "And temperate rain forest" Don't some of the New Zealand sites fall into temperate rain forest? The authors should check to confirm.
- p. 6, line 25: "were absented" should be "were absent"
- p. 6, lines 26-27: "All compiled results...polyphosphate" This sentence repeats information already given (p. 4, lines 22-24), and should be deleted in one of these locations in the text.
- p. 7, line 3: "The compounds proportions" should be "The proportioning of compounds"
- p. 7, line 6: "Into the Pi pool" should be "In the Pi pool"; "layers, the orthophosphate" should be "layers, orthophosphate"
- p. 7, lines 12-13: "There was no clay effect on both Pi and Po and their compounds proportions" should be "Clay had no effect on either Pi or Po, or on the proportions of compounds in these categories"

- p. 7, line 18: “effect on both Pi and Po” should be “effect on either Pi or Po”
- p. 7, line 24: “in both mineral and organic “ should be “in either mineral or organic”
- p. 7, lines 30-31: “In is important to note...usually comprises the residual P not recovered by the NaOH EDTA extractant”. This sentence doesn’t make sense to me. Firstly, the x axes in figures 2 and 3 vary depending on the factor (e.g. %, % organic P, log scale mg/kg). Secondly, total P doesn’t just include residual P (which is what “comprises the residual P” means”), it includes both the extracted P and the residual P. This sentence needs to be rewritten.
- p. 8, line 2: “As the percentage” should be “As a percentage”
- p. 8, line 12: “on the soil P composition on terrestrial natural ecosystems” should be “ in the soil P composition in natural ecosystems”
- p. 8, line 13: “over total “ should be “on total”
- p. 8, line 18 and line 19: “affected the soil age and CP ratio” should be “affected soil age and CP ratios”
- p. 8, line 29: “of the ecosystem’s properties” should be “of ecosystem properties”
- p. 8, line 31: “and its compound” should be “and its compounds”
- p. 9, line 2: “poorly defined” should be “poorly-defined”
- p. 9, line 3: “of the ecosystem’s properties” should be “of ecosystem properties”
- p. 9, lines 7-8: “phosphonate had most of its variation explained by” should be “most of the variation in phosphonates was explained by”
- p. 9, line 19: “by precipitation into the “ should be “by precipitation in the”
- p. 9, line 21: “Into the” should be “in the”
- p. 9, line 26: “Pyrophosphate had a positive influence of” should be “Pyrophosphate was positively influenced by”
- p. 9, line 27: “inositol was negatively” should be “inositol phosphates were negatively”
- p. 9, line 29: “Phosphonate was” should be “Phosphonates were”
7. Discussion: Much of this is very poorly done, and relies on speculation rather than data. Major revisions are needed in this part of the manuscript.
- p. 10, line 2: “compounds respond to” should be “compounds responded to”
- p. 10, line 3: “at a wide” should be “on a wide”
- p. 10, line 4: “terrestrial nature ecosystems” should be “natural ecosystems”
- p. 10, line 7: “persistence on ecosystems” should be “persistence in ecosystems”
- p. 10, lines 7-11. This is a very long, confusing sentence; as written, the point that the authors are trying to make with this sentence is not clear. It should be rewritten.
- p. 10, line 14: “the decaying degree of C element is lower than the P” I do not understand what the authors are trying to say here.
- p. 10, line 15: “resulting in the slowed decomposition of the older soil system” Again, I do not understand what the authors are trying to say here. As currently written, it indicates that the soil system itself is decomposing more slowly. However, soils do not decompose. Do they mean that decomposition is slower in older soil systems? If so, then decomposition of what? Plant material? Or do they mean mineralization of specific P forms? This needs to be rewritten for clarity.
- p. 10, lines 16-19: “more weathered soils are remote from the parent material”. Again, this is confusing as written. Do the authors mean physically remote? If so, wouldn’t that depend on the nature of the soil weathering processes, and even on the soils themselves? Or do they mean “substantially changed”? Please rewrite.
- p. 10, line 25: “occluded P increases at the expense of organic P”. This sentence implies that organic P compounds cannot be occluded, which isn’t the case – one of the reasons that inositol phosphates increase with weathering is that they become occluded in the same way that inorganic P compounds become occluded. The inaccuracy of this statement is due to citing a dated reference (Crews et al. 1995) that relied on Hedley fractionation. Please update this statement and cite something that uses P-NMR or other modern techniques.
- p. 10, line 31 and p. 11, line 2: “phosphorus” should be abbreviated as P.
- p. 11, line 2: “with species maximum” should be “with species’ maximum”
- p. 11, line 5: “shapes” should be “shaped”, because it is discussing a student that has been published.
- p. 11, line 9: “As soil aged” should be “As soils aged”
- p. 11, line 11: “weathering stages had a major role” should be “weathering stage had a major influence”
- p. 11, line 11: “dynamic” should be “dynamics”
- p. 11, lines 16-23: It is well-established that ectomycorrhizal fungi convert the orthophosphate that they take up from the soil into polyphosphates, and translocate the polyphosphate along fungal hyphae, sometimes a great distance from where the orthophosphate is taken up (e.g. Bücking and Heyser 1999 Mycol. Res. 103:31-39; Plassard and Dell 2010 Tree Physiol. 30:1129-1139). This needs to be mentioned, as well as the need to define plant and microbial communities in studies of P forms.
- p. 11, line 28 to p.12, line 2: Polyphosphates can potentially degrade to pyrophosphates during extraction and analysis by P-NMR (e.g. Cade-Menun et al. 2006, Environ. Sci. Technol. 40:7874-7880), so pyrophosphate and polyphosphate shouldn’t be considered as fully distinct P forms, and the authors should use care when discussing hydrolysis of pyrophosphate separately from polyphosphate.

- p. 12, lines 5-6: "Plant and microorganism breakdown diesters need" should be "Plant and microorganism breakdown of diesters needs"
- p. 12, lines 8-9: "P limitation increased phosphoesterases synthesis as a way to increase the organic P breakdown to the bioavailable P". As written, it doesn't make sense. Do the authors want to say: "P limitation may stimulate increased phosphoesterase synthesis as a way to increase bioavailable P by the mineralization of organic P"?
- p. 12, lines 12-16: "Therefore we hypothesize that as P got scarcer, plant and soil microorganisms may have been stimulated to produce phosphomonoesterases in greater amounts compared to phosphodiesterase because of the lower investment required for the organic P acquisition. Even though acid phosphatases require greater activation energy than alkaline phosphatases (Hui et al. 2013), breaking down diesters would require both enzymes; therefore a greater investment in energy". These two sentences are baseless speculation, and should be deleted from the paper. The authors did not include any measures of phosphatase activities of any kind from any of the published studies, and thus are unqualified to say anything about how enzyme activity changes with soil factors. Their results in Fig. 3 show that the proportion of total organic P, as well as the proportions of IHP and DNA decrease with increasing pH. This is related to increased charge of these compounds with decreasing pH, and thus increased sorption. This is discussed in detail in Condrón et al. 2005, among other papers.
- p. 12, lines 18-20: As noted for the introduction, it isn't possible to take the results of one study into pasture soils from one country and extrapolate to a global model about enzyme activity, especially when the current study did not include any measures of enzyme activity. This needs to be deleted.
- p. 12, lines 23-26: As noted above, the influence of pH on DNA sorption is well-established (Condrón et al. 2005). The authors need to revise these lines of the discussion.
- p. 12, lines 28 to p. 13, line 12: The authors need to be very precise in their terminology. In this section, they discuss "inositol phosphates". However, the data they include from various studies is for inositol hexaphosphates, which very specifically are inositols with 6 phosphate groups. These will behave very differently in soils from inositol phosphates with fewer phosphate groups. Please rewrite this section to be more precise. In addition, all of the processes discussed here to govern the behavior of "inositol phosphates" in soil will also apply to other P forms, especially DNA.
- p. 13, lines 9-10: This sentence discusses changes in the concentration of "inositol phosphate" with soil weathering. However, the authors only present changes in "inositol phosphates" as proportions of organic P, not as concentrations. As such, this is all speculation and should be revised or deleted.
- p. 13, line 14: "there were no inositol phosphates on tropical, more weathered soils". Could this not also relate to either inputs from plants or production of phytases by plants and microbes? The authors did not include any factors that might influence the cycling of IHP in these types of soils. As such, this is merely speculation and should be deleted.
- p. 13, lines 20-23: "Recent investigations have contradicted the often-cited literature" please cite references here to support this statement of fact, both for the "often cited literature", and the "recent investigations".
- p. 14, lines 10-30: One thing missing from this section is the role of vegetation with respect to inputs of different P forms. It should also be noted that a greater soil wetness is not necessarily associated with leaching, if there is impeded drainage of the soils in some ways (e.g. from the formation of placic horizons). This section of the manuscript is really vague, and doesn't add anything to our knowledge of P cycling in soils. The authors must do a far more thorough literature review to discuss their results properly.
- p. 14, line 32: "As the orthophosphate percentage decreased following precipitation, the pyrophosphate percentage increased". This does not necessarily mean that the concentration of orthophosphate decreased, or that of pyrophosphate increased, or imply a cause-and-effect relationship. This line suggests to me that the authors do not understand compositional analysis: as the percent of one thing decreases the percentage of another thing will increase because the total must add to 100%. In my opinion, reporting and discussing these as percentages is misleading, for this reason. It would be far better to use concentrations.
- p. 15, lines 6, 7: "phosphorus" should be "P"
- p. 15, lines 7-8: "greater organic P concentrations were associated with increasing biomass production". Studies of biomass (e.g. Noack et al. 2012) show that the majority of P in plant biomass is as orthophosphate, not organic P compounds. As such, this sentence doesn't make sense to me, and isn't supported by the literature. Please revise.

8. Conclusions:

p. 15, lines 22-23: "after altogether with plant and microbe coexistence" as noted for the abstract, I do not understand what the authors are trying to say here. Something seems to be missing, or mistranslated.

9. Figures: In all figures, "phosphonate" should be "phosphonates", because this is a general compound category containing a number of specific P compounds. Also, "inositol" should be IHP or inositol hexakisphosphate.

10. References: there are many problems with this section of the manuscript. The authors need to carefully proof-read and correct this section.

- a) Many of the references are out of order alphabetically, including the entire "Y" section (which should come after W), Deiss et al. 2017 should come after Damon et al. 2014; Laliberté et al. 2017 should come before Legendre and Legendre 2102 and Li et al. 2015; Whittaker 1975 should come before Wilson et al. 2013.
- b) There are formatting differences among references. Cade-Menun et al. 2000 has the volume and page number listed as 30:1714-1725, while other references use a comma (e.g. Celi et al., 2013, 367, 121-134). Chen et al. 2004 does not include periods after abbreviations in the journal title (*Aust J Soil Res*); Cade-Menun and Preston 1996, Kizewski et al. 2011, Turner et al. 2013 have all the words capitalized in the manuscript title.
- c) The journal volume number and/or page numbers are missing from Deiss et al. 2017, Doolette et al. 2016, Li et al. 2015, while George et al. 2017 is missing the name of the journal as well as the volume number.
- d) Rumpel et al. is 2015 in the References, but 2016 in the text; Bünemann is "Bunemann" in the text (p. 11, line 16)

Condon, L.M., B.L. Turner and B.J. Cade-Menun. 2005. Chemistry and dynamics of soil organic phosphorus. pp. 87-121. In: J.T. Sims and A.N. Sharpley, eds. *Phosphorus, Agriculture and the Environment*. Monograph no 46. Soil Science Society of America. Madison, WI.