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Interactive comment on "Soil phosphorus dynamics on terrestrial natural ecosystems" *by* Leonardo Deiss et al.

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We sincerely thank the editor and reviewers for evaluating our manuscript. We have responded to each comment. As requested by the editorial board, we are providing in this document responses to comments only, and not the revised manuscript, even though we already made several changes on it following the reviewers' suggestions, and specific details are presented on this document. Comments made by the reviewer are identified by "R1", and responses from authors are identified with "Response to R1".

R1 General comment.

Organic phosphorus (P) cycling in soils is a topic that has received attention in recent

years. As more papers are published, meta-analyses that link the data from these papers together to identify trends in organic P cycling become possible, at least in theory, and a paper presenting novel findings could be of interest to readers. However, deriving meaningful interpretations from a meta-analysis of soil P-NMR studies requires a clear understanding of the P-NMR method and its limitations, in order to correct for known artifacts of analysis. This was not done for this manuscript. As such, it cannot be published in its present form, and will require a major revision, including reanalysis of data, to make it publishable.

Response to R1 General comment.

We understand the point the reviewer is making about using the correction for potentially degraded peaks (of diesters converted to monoesters). Just to clarify, we did not use the correction previously because 39% of inositol phosphate (comprehending all tropical results and other locates) and 12% of DNA results were absent from the compiled data. We knew that correction was possible through adding to the total diesters concentration, the α- and β-glycerophosphate concentrations (potentialy degraded peaks), but the reviewer also provided additional details that could improve our analysis. To address the issue, we will follow the reviewer suggestion. Using the available data, we will focus on specific organic P compounds (i.e. DNA and IHP) instead of its respective functional groups (diester and monoester). Given the huge proportions of potentially degraded peaks (non-inositol monoesters), and the uncertain about which compounds were present in this potentially degraded fraction, we choose to not to work with the corrected di-to-mono ratio, focusing on DNA and IHP compounds instead.

R1 Comment 1.

Writing quality: a) The quality of English in the manuscript is poor in many places. If the authors revise this manuscript, I suggest they have it read by someone more familiar with English, who also understands the research field. b) Please check that you are using the correct spelling of the names of authors whose papers are cited.

For example, “Vincent” is repeatedly cited as “Vicent”, including in the supplemental files. c) Be specific with terminology. The term “P” is an abbreviation for the element for phosphorus. However, the authors use it interchangeably for phosphate, which is incorrect.

Response to R1 Comment 1.

a) In the new manuscript version, a native language specialist will revise the English.

b) “Vicent” will be replaced by “Vincent”, and we will check all the names of the other authors whose papers are cited.

c) In the new manuscript version, the terminology will be revised regarding the proper use of abbreviations. “P” will be used as an abbreviation for the element phosphorus, Po and Pi will be used for the respective organic and inorganic pools, and the other P compounds will be described by their proper names.

R1 Comment 2.

As P-NMR has become more widely used to characterize soil P forms, enough data has become available to indicate the possibility of using these data in meta-analyses to look at soil factors controlling P forms, especially organic P. However, those of us who use this technique the most also recognize its limitations. Although the use of P-NMR has advanced our understanding of soil organic P cycling more than almost any other method to date, the technique is not perfect. It is important to understand the artifacts of the method. It is also important to separate P-NMR results on a soil extract from the P forms that would have been present in the original soil sample prior to extraction. After all, isn’t that the objective of a soil science study? Unfortunately, it isn’t clear to me that the authors of this manuscript are familiar enough with the soil P-NMR technique to understand its limitations and address them. This has produced a study that clearly involved a lot of work by the authors, but which ultimately has not produced any new insights with respect to soil P.

Response to R1 Comment 2.

We recognize that P-NMR can have limitations, and we have addressed them in specific parts of the manuscript. We will emphasize those limitations according to the suggested comments. Regarding the separations of P-NMR results from other P forms present in the original soil sample, we worked with P-NMR results obtained from NaOH-EDTA extracts only (Y axis on figures 2, 3, 4, 5, and 7, which do not include the residual P, i.e. difference between soil total P and NaOH-EDTA P). The total P of NaOH-EDTA extracts could be obtained by adding Organic P (e.g. figure 3A) to Inorganic P (e.g. figure 2A), but it does not correspond to the soil total P. The total P (obtained with other method – not P-NMR, e.g. digestion) was also presented in the manuscript, but acknowledging that it was obtained by a different method. In the new manuscript version, we will add more information in the figure captions to avoid misunderstandings, i.e., results in the Y axis are from NaOH-EDTA P-NMR results.

R1 Comment 2a.

a) Concentration: It is not possible to determine absolute concentrations of P forms or compound classes using NMR; only relative percentages can be determined, because it is a compositional analysis in which the total must be 100%. Concentrations of P forms are then determined by multiplying by the total extracted P concentration by the percentage of each P form, which is still based on the compositional analysis. This is why the proportions and concentrations of total organic P and total inorganic P (Figs. 2 and 3) show inverse relationships to one another – together they have to add to 100%. This is exactly what would be expected, so it is strange to me that the authors would comment on this (p. 6, lines 13-16). The authors also do not seem to understand the relationship between total P in the soils and P extraction in NaOH-EDTA. In natural (non-tilled) samples, P is stratified, such that concentrations are higher at the soil surface and lower with depth. There will also be an increase in organic P at the soil surface from inputs of plant material, which will decrease with depth – especially in forests with limited mixing and with greater fungal activity in mats in the

forest floor (as is typical for temperate forests, where the majority of these studies were conducted). This needs to be accounted for somehow.

Response to R1 Comment 2a.

Our total organic and inorganic P results, on mg kg-1 basis, are from NaOH–EDTA extracts only (do not include residual P, i.e. difference between soil total P and total P of NaOH-EDTA extracts). Based on our understanding, the results on mg kg-1 basis were determined from the proportion (%) of each P compound or functional group on spectra (determined by integration of peaks area or deconvolution) multiplied by the total P extracted with NaOH–EDTA. Most authors have presented their P-NMR results (forms and compounds) on both % and mg kg-1 basis (from P-NMR results of NaOH–EDTA extracts), including most of the ones we compiled data from. In the new manuscript version, we will add more information on figure captions to state that results on Y axis are from NaOH–EDTA extracts only. Usually, P-NMR results from NaOH-EDTA soil extracts are presented in both ways: (a) on mg $kg-1$ basis (non-including residual P), and (b) relative distribution of P (%). We followed the same criteria used by those papers to present our results. We do understand that results are based on a compositional analysis (i.e. P forms are determined by multiplying the total P extracted with NaOH-EDTA by the percentage of each P form), but the description of the inverse relation (obviously a inverse relation) between organic and inorganic concentration (% of total NaOH EDTA P) meant to explore the phenomena of pH or other variable impacting these forms. It was the way we found to describe our results. In the new manuscript version, we will reformulate the text avoiding the obviousness on describing results from percentages. In the specific case, the sentence containing "they showed a contrasting behavior" will be excluded. We do understand that soil total P is different than soil P extracted with NaOH- EDTA. We have mentioned that on Page 7 lines 10-13 "It's important to note that the reported total P is the one obtained by digestion and usually comprise the residual P non-recovered by the NaOH EDTA extractant. The recovery of total P by NaOH EDTA extraction is variable depending on soil characteristics and

laboratory procedures (Cade-Menun and Liu, 2014)." Moreover, knowing that there is a potential effect of soil conditions and laboratory procedures, we used the P recovery (percentage of P extracted with NaOH EDTA from soil total P) as a random factor in all bivariate regression models. We agree that natural (non-tilled) samples have stratified nutrient distributions. Our supplemental Figure S2 presented the results obtained regarding this effect. But contrary to what was expected, we found no effect of sampling depth over organic P concentration in mg $kg-1$, neither for both organic and inorganic on % basis (even though functional groups of organic and inorganic P responded dynamically to soil depth, even having contrasting responses for organic and mineral soil layers). We did find a sampling depth effect for inorganic P concentration in mg $kg-1$. Therefore, knowing that there is a potential effect of sampling depth, we used it as a random factor in all bivariate regression models.

R1 Comment 2b.

b) Extraction efficiency and soil pH: It has been very well established that the recovery of total P from soil samples with NaOH-EDTA extraction is never 100%, and is higher from samples with lower pH. The extraction seems to favor samples high in iron and aluminum, with generally poor P recovery from samples high in calcium; the reasons for this are unclear. As such, any meta-analysis comparing across a range of sample must take into account differences in P recovery among studies, and even among depths within the same soil profile or at different points along a soil chronosequence. For example, the recovery of total P in the samples for the Turner et al. (2003) paper ranged from 14-45%, in the Turner et al. (2007) paper 63-91%, and in the McDowell et al. (2007) paper 11-75%. If the purpose of this meta-analysis is to look at factors controlling soil P, then these differences in recovery must be factored in. Is it even possible to compare the results for a soil where only 11% of the total P was extracted to one with 91% extraction? What about the 89% of total P that wasn't extracted? The authors of this manuscript don't even mention this as a factor, let alone correct for it. And that, unfortunately, undermines their results.

Response to R1 Comment 2b.

We do understand that soil chemical characteristics can impact the recovery of P with a NaOH-EDTA extraction. We also agree that an "analysis comparing across a range of sample must take into account differences in P recovery among studies, and even among depths within the same soil profile". We have already addressed that using the 1) P recovery, and 2) sampling depths as random factors (and also latitude for other purpose not directly associated with the comment) in the analysis (which are described in the methods section Page 5 lines 7-15). An example of the impact of a random factor is described in the Page 6 lines 18-20: "There was no pH effect over this inorganic compounds in the organic layer (even though there is an apparent trend, these relationships became non-significant after including sampling depth as random effect on models; Supplementary Appendix S2 shows the sampling depth effect over soil P composition)."

R1 Comment 2c.

c) Degradation: As noted, it is important for any soil study to ensure that the forms discussed, or the ratios of compound classes such as orthophosphate monoesters and diesters, are based on what was in the original soil sample, and not what was produced during extraction and analysis. It is well established that some orthophosphate diesters such as RNA and phospholipids can degrade to the orthophosphate monoesters α- and β-glycerophosphates (phospholipids) and various monophosphates (RNA) when analyzed at the high pH required for good peak separation in P-NMR spectra [e.g. Turner et al. 2003; Doolette et al. 2009; He et al. 2011, Vincent et al. 2013; Schneider et al. 2016. The degree of degradation will vary depending on the length of NMR experiment and other factors [see Cade-Menun and Liu (2014) and Cade-Menun (2015) for more details]. It is essential that these degradation peaks are identified and quantified in order to determine the correct concentrations of orthophosphate monoesters and diesters that were in the original soil sample; doing so improves any comparison of these P forms to other soil properties (e.g. Young et al., 2013; Liu et al. 2013

J. Environ. Qual. 42:1763-1770). Unfortunately, most studies before 2010 did not identify these compounds and correct for degradation. The authors of this manuscript acknowledge that degradation can occur (p. 4), but for some reason have chosen to ignore it, which is a major problem. The issue of degradation MUST be addressed for any study of edaphic and climatic characteristics to have any meaning. If the concentrations of orthophosphate monoesters and diesters were not corrected in the original study, then the authors of this manuscript could have applied some correction factor to compensate. For example, Vincent et al. (2013) note that most non-inositol phosphate monoesters were diester breakdown products (p. 160). The studies used by the authors here all included some measurement of inositol phosphates (at least myo-IHP and scyllo-IHP). As such, the authors could have assumed that those were the only true monoesters, and corrected the remaining proportion of monoesters to diesters. It would have at least been more meaningful that what they did, which was to ignore degradation but then reach the conclusion that the ratio of diesters to monoesters was a significant factor in the study.

Response to R1 Comment 2c.

We understand and agree with the reviewer's comment. But, as described in the methods section Page 4 lines 14-16: "We know that it is possible to correct degraded peaks of diesters converted to monoesters (e.g., Young et al., 2013 and Cade-Menun et al., 2010), but since some papers only showed functional groups like monoesters and diesters, and not species (specific P compounds) inside these functional groups, this correction was not done." Not all studies used in this manuscript included some measurement of inositol phosphates (at least myo-IHP and scyllo-IHP). Specifically, the following papers did not present P species (including myo-IHP and scyllo-IHP) inside these functional groups (monoesters and diesters) are: Celi et al., 2013, n=4; Vincent et al. 2010, n=1; Turner, 2008b, n=1; Turner et al 2003 (native soil sample), n=1; Turner and Engelbrecht, 2011, n=19; Turner et al 2014, n=10; and therefore correction was not possible to be addressed properly based on our previous knowledge. Some

of these authors acknowledge that there is a small contribution of inositol phosphates (most tropical soils) while others have provided no explanation about with they did not present specific P compounds results. Therefore, we thought it will still be biased to assume something that we were not certain of (i.e. amount of inositol phosphates). As described earlier, to address the issue, we will follow the reviewer suggestion. Using the available data, we will focus on specific organic P compounds (i.e. DNA and IHP) instead of its respective functional groups (diester and monoester). According to the gathered data, non-inositol monoesters (potentially degraded peaks, as suggested by the reviewer) corresponded to 66.76 % in average of the total amount of non-corrected monoesters (ranging from 7.8 to 100%), previously reported as total monoesters content, from papers that presented IHP results (n=61). The same non-inositol monoesters (potentially degraded peaks) corresponded to 53.94 % in average of the total NaOH EDTA organic P amount (ranging from 6.47 to 100%) from papers that presented IHP results (n=61). Based of the results presented by the authors we could not calculate how much of the potentially degraded peaks were: α- and β-glycerophosphate (Doolette et al., 2009), nor RNA and phospholipid (which includes glycerophosphates) (Vincent et al., 2013); which were determined as degraded peaks by those authors. Therefore, given the proportions, correcting for potentially degraded peaks has a huge impact on the results, and it is a not completely unbiased calculation, since we don't know if all potentially degraded peaks were α- and β-glycerophosphate (Doolette et al., 2009), or RNA and phospholipid (Vincent et al., 2013), so we choose to not work with the di-to-mono ratio. Inositol plus DNA represented 59.20% in average of total NaOH EDTA organic P (n=51) from papers that presented both DNA and IHP results. Therefore, it is also a huge proportion and could be an unbiased approach for those results. The reported proportions are not closing exactly due to the different datasets (n = 51 and n=61). To re-analyze data, IHP will not be considered for tropical soil results because they have non-detected concentrations of this compound (but tropical results will be maintained for the other variables). The following two paragraphs were written just to clarify why we have done the analysis in the previous way. We tried to be as

clear as possible about this issue, as it is written in the Page 4 lines 18-20: "We expect for future researches to provide results of as much soil P species they can find rather than functional groups only, even when species concentrations are low (and describe when species are not detected), what may enable future analysis to avoid possible confounding effects of organic P species inside functional groups (e.g., inositol and monoesters)." So, we believe that some questions will still remain to be addressed regarding soil P composition in terrestrial natural ecosystems, but our manuscript will provide significant and robust information using currently available results from literature. We understand the importance of what the reviewer is asking for, and recognize that in the manuscript, but as described we could not reach that level of detail due to absence of data (all specific P compounds). We have used an approach used by other authors. The same approach of not correcting for potentially degraded peaks was used in another recent paper, for example, that combined results from pasture soils using P-NMR results of NaOH–EDTA extracts (Nash et al., 2014). Essentially, they did not corrected for any degraded peak to determine the diester-to-monoester ratio, and described that this was out of their scope, but we agree that their approach is also not optimal.

R1 Comment 3.

Selection of studies: The authors indicate in the methods that they were careful in their selection of papers to include in their meta-analysis, such as native vegetation. As such, I am puzzled as to why the Turner et al. 2003 paper was included as the only study from the USA, because it used agricultural soils. And while the abstract and elsewhere in the text indicate a "dataset including 88 sites", these are overwhelmingly biased to sites in New Zealand (59) and Panama (21), which does not cover a range of "temporal, edaphic and climatic characteristics". The sites selected are also mainly from chronosequence studies, which may also have affected the P forms and their relationship to soil properties.

Response to R1 Comment 3.

The Turner et al. 2003 paper included most soils under arable cropping, although there was a native site, and this was the one we included in our analysis. We understand that we were not able to cover a vast representative sample, at global level, but we included as much as we could, given the data availability on the literature. This compilation made this study to have the wider geographical coverage on the topic (terrestrial environments with native vegetation - P-NMR results of NaOH–EDTA extracts).

R1 Comment 4.

Introduction: a) Please include references for all statements of fact, and make sure those facts are correct. For example, p. 1, lines 24-25: "Once P has been dissolved as free orthophosphate" It isn't possible for free orthophosphate to exist in the soil solution; it will still be associated with cations, although as more soluble forms. b) Be careful with terminology. Page 2, line 1: "inorganic and organic P pools are each composed by fractions or functional groups". No, they are composed of specific P compounds. The term "functional group" is used elsewhere in the introduction. Please indicate what is meant by this term, which isn't one used for soil P chemistry. And note that fractionation measures operationally-defined P pools, rather than specific P forms. c) Page 2, line 10: Turner 2007 is not cited in the references.

Response to R1 Comment 4.

a) In the new manuscript version, all statements of fact will be referenced, and it was make sure that those facts were correct. Specifically, "as free orthophosphate" will be excluded from the sentence. In other occurrence we will use "available" instead of "free" when referring to P that could be potentially taken up by plants.

b) In the new manuscript version, the statement will be reviewed clarifying that inorganic and organic P pools are composed of specific P compounds. "Functional groups" were changed to compounds in the whole manuscript when describing P compounds.

c) It will be corrected in the new manuscript version. The correction is "Turner et al.,

2007", which was previously cited in other parts of the manuscript.

R1 Comment 5.

Methods: a) See comments above about site selection. b) Page 4, lines 14-23: This discussion about degradation belongs in the Discussion section, not the methods section. c) The authors have made a lot of assumptions here, particularly for soil classification. Please justify these assumptions in the Discussion section of the manuscript.

Response to R1 Comment 5.

a) It was answered on Authors' response to comment 3.

b) In the new manuscript version, we will move the part about degradation to the Discussion section.

c) In the new manuscript version, the assumptions about soil classification will be justified in the discussion section. The assumptions include: The soil total P content depends on both weathering stages and parent material, but generally decreases with increasingly weathered soil orders (Yang and Post, 2011). The soil weathering stages classification also takes into account changes in soil P composition, and generally follows the Walker and Syers (1976) conceptual model: there is a gradual decrease and eventual depletion of primary mineral P (mainly apatite P), a decrease of total P, an increase and then decrease of total organic P, and a increase and eventual dominance of occluded P during soil development (Yang and Post, 2011). In highly weathered soils, occluded P increases at the expense of organic P through by encapsulation of mineralized P inside of Fe and Al minerals (Crews et al., 1995).

R1 Comment 6.

Results: a) I am puzzled by the phrase "concentration (% of total NaOH EDTA P)", page 6 line 30. Do you mean % or concentration in mg/kg? They are not the same thing, although they are derived from the same data (% of P forms multiplied by extract concentration). b) As noted above, any results related to total concentrations

or percentages of orthophosphate monoesters, orthophosphate diesters and the diester:monoester ratio are meaningless if not corrected for degradation. The authors must remove all reference to uncorrected concentrations and ratios. They could correct them as suggested above, or they could focus on specific P forms (e.g. DNA or IHP).

Response to R1 Comment 6.

a) We meant % of NaOH EDTA P in %.

b) We consider to be the same response of "Authors' response to comment 2c)". We did not correct them for degradation in the previous manuscript version. As described earlier, to address the issue, we will follow the reviewer suggestion. Using the available data, we will focus on specific organic P compounds (i.e. DNA and IHP) instead of its respective functional groups (diester and monoester).

R1 Comment 7.

Discussion: Given the issues noted above, I am not sure there is anything meaningful in the discussion section, which as written is a review of the temporal, edaphic and climatic characteristics affecting P forms in NaOH-EDTA extracts, rather than in the original soils themselves. This is really unfortunate given the amount of work the authors put into this study. I hope the authors will address these issues. When they do, I expect much of the discussion section to change.

Response to R1 Comment 7.

As described above, we will focus on specific organic P compounds (i.e. DNA and IHP). Specifically, we deleted discussion about the mechanisms that prompted the inverse response of monoesters and diesters as P limitation increased (since those functional groups results were excluded from the manuscript). Discussion was added about why DNA concentration increased as both P limitation and soil acidity increased in older, more weathered soil systems. Discussion was also added about the increase in inositol

phosphates concentrations at more acidic soil environments.

R1 Comment 8.

Figures: a) The two figures used for Figure 1 were both published elsewhere, and thus are covered by copyright. However, the authors do not indicate anywhere that they have permission to use these figures in their manuscript, which must be obtained from the publishers of the original papers. b) All figures containing references to total orthophosphate monoesters and diesters, and the diester:monoester ratio (e.g. 3, 5, 7, 8, 9, S4.1, S4.2, S4.3, S4.4) must be corrected for degradation. And all figures will likely change when the authors have normalized the data used in this study for P recovery.

Response to R1 Comment 8.

a) In the new manuscript version, we will provide the coverage by copyright. License Numbers: 4210920836823 (Elsevier) and 4210930550479âĂĺ(John Wiley and Sons).

b) The response about the correction for degradation is on "Authors' response to comment 2c)", and regarding the normalization for P recovery is addressed on the "Authors' response to comment 2a)".

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