

Interactive comment on “Identification of lower-order inositol phosphates (IP₅ and IP₄) in soil extracts as determined by hypobromite oxidation and solution ³¹P NMR spectroscopy” by Jolanda E. Reusser et al.

Anonymous Referee #2

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The objective of this manuscript was to characterize and quantify inositol phosphates (IP) in soil extracts following hypobromite oxidation using ³¹P nuclear magnetic resonance (P NMR) spectroscopy. This is a very technical paper with respect to the chemical methods utilized. Given that the mandate of this journal is: “interactions between the biological, chemical, and physical processes in terrestrial or extraterrestrial life with the geosphere, hydrosphere, and atmosphere. The objective of the journal is to cut across the boundaries of established sciences and achieve an interdisciplinary view of these interactions” (from the journal website)”, this paper does not seem like a good

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fit for the journal. While the authors identified a wide range of different P compounds in their four soil samples, no attempt was made to relate these compounds back to broader biological, chemical or physical processes within these soils. As such, it will not be of interest to the majority of Biogeosciences readers, as currently written, and will likely be overlooked by the scientists who would be interested in such a technical paper. In my opinion, this would be a better fit in either an environmental chemistry journal or in the chemistry section of a soil science journal. Thus, in my opinion the authors should withdraw this paper from this journal and submit it to another journal that better fits the paper's focus. If the authors choose not to do this, then they must significantly revise the manuscript to keep it within the journal's scope, to clearly demonstrate the significance of these identified P compounds to P cycling in these soils, and to P cycling more broadly.

Abstract: As written, the abstract make it clear that this is chemistry methods paper, not a biogeochemical study, because the results and conclusions highlighted in the abstract indication only that the authors were able to identify these peaks, but make no reference to their relative importance in the studied soils and to P cycling in these and other soils. This supports my point above that this is not an appropriate journal for this paper as currently written. In addition the abstract needs to be more carefully edited, as it is awkwardly written in places. For example, lines 14-15: "include the A horizon of a Ferrasol from Columbia, of a Cambisol from Switzerland, of a Gleysol from Switzerland and of a Cambisol from Germany" should be "include A horizons from a Ferrasol (Columbia), a Cambisol and a Gleysol from Switzerland, and a Cambisol from Germany". And why is the phrase "(using solution ^{31}P NMR spectroscopy)" included in line 19, given that the method was given in line 13?

Introduction: The introduction provides a good overview of the chemical methodology for extracting and characterizing IP in soil, as would be expected for a chemical methods paper. It gives a very brief overview of the factors generally controlling IP in soils, but doesn't give much information about why there is a need to specifically character-

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ize all of these different IP forms. What insights into soil P cycling would we gain from identifying these compounds that we don't already have by from the IP compounds we can already identify? And what information would be expected from analyzing them in different soils? And the hypothesis seems to be something that was tacked on at the end, and doesn't make a lot of sense: "We hypothesize that a large portion of sharp peaks in the phosphomonoester region of untreated soil extracts would be resistant to hypobromite oxidation, which would indicate the presence of IP". This again emphasizes that this is a chemical methods paper only. Other points in the Introduction: l. 35: "Riley Andrew et al., 2006" why is the authors first name included (Andrew M. Riley is the first author of the paper)? This should be "Riley et al., 2006". And the listing in the References (l. 641-644) contains the first names of other authors of this paper. "Shears Stephen, B" should be "Shears, SB", and "Potter Barry VL" should be "Potter BVL". The correct names are very obvious when reading the manuscript, so I'm not sure why they are incorrect here. l. 39 and elsewhere in the text: when citing a list of references, it is conventional to list them in order from oldest to most recent. l. 87: "was resistant" should be "were resistant", because it modified "signals", which is plural.

Methods: As written, there is far too much technical information (e.g. about the transverse relaxation experiments), which will not be of any interest to the majority of readers of this journal. And other important information seems to be missing. See specific points listed below. Also, I believe that Turner has published a new paper of the hypobromite oxidation method. How does the method used compare to that method. l. 117: Please provide information on the total volume of extractant used and the total volume of filtrate produced, to help the reader put the hypobromite oxidation experiments into context. In line 121, it indicates that "10 mL of the filtrate was used". What proportion of the total filtrate is this – 10% or 100%? l. 144-145: This sentence is awkwardly written. Change "...in solution is that of molybdate unreactive P (MUP), which is considered to be largely that of organic P" to "in solution is molybdate unreactive P (MUP), which is predominantly organic P for these samples" l. 146-147: "a duplicate sample of the Cambisol and the Gleysol was spiked" should be "duplicate samples of the Cambisol

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and Gleysol were spiked” l. 161-162: The inclusion of the Vestergren et al. 2012 paper here confused me. This group left their samples to sit overnight because they used a sulfide treatment to remove paramagnetic ions. Was this also done for the current study? If so, then please describe the sulfide treatment more clearly. If not, then it would be better to replace this reference with one that is more appropriate. l. 193-195: Something seems to be missing here for the measurement of N observability. Using PtotICP-OES only makes sense if the entire sample after freeze-drying was used for the NMR analysis. However, that does not seem to be the case for this study. While it appears that the total mass of lyophilized material was used for the brominated samples (l. 167-168), a set mass (120 g) of the non-brominated lyophilized material was used, with no indication of how much of the total lyophilized material this represents. The proportion of total mass used must be factored into the equation to correctly determine NMR observability. This would also explain the differences in observability reported in the supplementary information (SI) for the brominated and unbrominated samples. l. 206-225: There is no need to include this much detail about the transverse relaxation papers. As noted above, the majority of readers of this paper in this journal will not be interested in these details. In addition, this appears to be a repeat of what was done for the McLaren et al. 2019 study. As such, all that is needed is to cite the previous publication. If the authors really think this much detail is needed, it could be included in the SI. L. 226-233: Why are methods for statistical analyses reported here, when no results of statistical analysis are included in the Results, Discussion or SI?

Results: 1. Please provide spectra showing the entire spectrum for each brominated and unbrominated sample, scaled to allow the reader to see the full height of orthophosphate and the relative heights of other peaks compared to orthophosphate. All of the spectra currently in the manuscript show the monoester region only, with the orthophosphate peak truncated. This is needed to get a full sense of all the peaks for each sample, especially for the brominated samples. 2. The usefulness of the spectra shown in Fig. 3 are not clear. I am used to looking at NMR spectra, and I found these confusing, as with the exception of the Gleysol the red lines show little but noise. Again,

this would be appropriate for a chemical methods paper, emphasizing that this is not the best journal for this study. 3. I am concerned that the authors report signals for non-IP compounds in their brominated spectra. In my experience with this technique, if there are any peaks for non-IP compounds, that suggests that the oxidation was incomplete. And that in turn raises questions about the authors' assignment of peaks in the brominated samples. 4. How confident are the authors that all of the peaks were present in their soils prior to extraction and hypobromite oxidation? Isn't it possible that bromination degraded some high IPs (e.g. IP6) to lower IPs (IP5 and IP4)? The recovery of the added myo-IP6 was only 20 and 47%, which suggests it may have been degraded. l. 255: change "Although," to "However," l. 273: "A detailed view of the phosphomonoester region of spiked extracts is shown" should be "Detailed views of the phosphomonoester regions of spiked samples are shown" l. 306-316: I do not see the need to include any of this information about spin-echo analysis of selected P compounds in the current paper, as it will not be of any interest to the majority of readers of this paper in this journal.

Discussion: The P-NMR literature cited in this section seems biased to papers by the Smernik group. I have concerns about this because that group prepared their samples for NMR differently from most other groups, and from what was done for the current study. As such, results from that group may not be directly comparable here. In addition, it shows an unfamiliarity with the broader P-NMR literature, which is of concern. In general, however, I think the authors have done a reasonable job of trying to relate these P compounds to the literature and to the soils, which would be suitable to this journal. However, they should note the overall small proportion of total P that some of these compounds comprise. Are compounds in such low concentrations really an integral component of P cycling. And in my opinion, section 4.3 is not appropriate for this journal and would not be of interest to the majority of readers, and so should be cut. l. 322-324: Other studies have looked at what was not extracted by NaOH-EDTA, including with acid extraction after NaOH-EDTA or with solid-state P-NMR. See for example studies by He et al. These would be more appropriate to cite here than

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McLaren et al., 2015a l. 333-334: “This will result in the production of carbon dioxide and simple organic acids” This sentence does not seem to be relevant here. How is this related to P? l. 340-342: If the authors had not shown peaks other than monoesters and orthophosphate, I might agree with them that the peaks in the monoester region are all IP. However, it is clear from the results they have shown that they did not have complete oxidation of all P compounds. So how can they be confident that they only have IP in the monoester region? This must be addressed. l. 348-350: I’m confused by the some of the papers cited here. Why are studies that did not use chromatography cited here to make a point about chromatography. Please rephrase, or remove the non-chromatography references. l. 356-363: As noted above, the authors did not have complete oxidation of all non-IP compounds in their extracts. So how can they be certain that this peak at 4.36 is an IP compound and not α -glycerol. In addition, other groups have reported a peak that sits very close to α -glycerol, and have urged caution about identifying this peak without spiking. This emphasizes a need for a broader review of the literature than just papers from the Smernik group.

l. 370: change “extracts, which the” to “extracts, of which the” l. 383: add spaces between the numbers and words here: “1axial” should be “1 axial” or “1-axial”, etc.

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