REVIEWER REPORT 2

Comment 1
The objective of this manuscript was to characterize and quantify inositol phosphates(IP) in soil extracts following hypobromite oxidation using ^{31}P 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 extra-terrestrial 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 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.

Response 1
Inositol phosphates are a very important component of the P cycle in both agricultural and environmental contexts. Indeed, several recent reviews have highlighted a stagnation of advancing our knowledge of the P cycle to address global challenges due to a lack of knowledge on organic P (George et al., 2018; Haygarth et al., 2018; McLaren et al., 2020). Our study provides new information on the chemical nature of a multitude of organic P species, which is essential to understand processes relating to their flux in nature and their function in the soil system. Furthermore, the production/accumulation as well as the hydrolysis of IP to lower order IP, involves the cycling of P and C in soil.

We used a novel approach of combining chemical extraction, hypobromite oxidation, and multiple NMR techniques to better understand the chemical composition of soil organic P. Furthermore, we strongly believe that our publication will be of great interest to a broad audience, including scientists working in agriculture, environment, sediments and waters. Lastly, we highlight that our study is the first to report the existence of 11 inositol phosphate species using direct spectroscopic evidence, and also provide new insight on the chemical and structural composition of 'complex' phosphomonoesters. We also thank the reviewer for their positive comment of the paper later in their review (see Comment 28).

Comment 2
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.

Response 2
In addition to Response 1 of Reviewer 2, we highlight the discussion in the body text on the importance and implications of our results (lines 24-26 in the Abstract, lines 355-359, 379-380, 388-402, 429-434, 462-468 in the Discussion section and lines 492-497 in the Conclusion section).

Comment 3
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 Ferrosol(Columbia), a Cambisol and a Gleysol froom Switzerland, and a Cambisol from Germany”.

Response 3
Agreed, we have reworded the sentence:

Changed from (lines 14-15): Soil samples analysed include the A horizon of a Ferralsol from Colombia, of a Cambisol from Switzerland, of a Gleysol from Switzerland and of a Cambisol from Germany.

Changed to (lines 14-15): Soil samples analysed include A horizons from a Ferralsol (Colombia), a Cambisol and a Gleysol from Switzerland, and a Cambisol from Germany.

Comment 4
And why is the phrase “(using solution 31P NMR spectroscopy)” included inline 19, given that the method was given in line 13?

Response 4
We have deleted “(using solution 31P NMR spectroscopy)”.

Comment 5
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 characterize 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?

Response 5
Please see Response 1 of Reviewer 2. In addition, the majority of NMR studies have identified a small selection of compounds in the phosphomonoester region of NMR spectra on soil extracts (McLaren et al 2020). These are typically four IP_6 compounds, α- and β-glycerophosphate, and some RNA mononucleotides. Consequently, most studies have focused on the cycling of IP_6, which is considered relatively stable in soil. In the current study, we report up to 70 sharp signals in the phosphomonoester region of NMR spectra on soil extracts following hypobromite oxidation, which is considerably more than that typically reported in the literature. We could identify on average 48% of peaks in this region as arising from inositol phosphates, however, it is likely a much greater proportion of these sharp peaks will be due to inositol phosphates due to their resistance to hypobromite oxidation.

The majority of organic P studies have focused on the cycling of IP_6, particularly of *myo*-IP_6 (McLaren et al. 2020). We show that there is a much greater diversity of organic P compounds than previously thought, and second that they appear to be predominately lower-order inositol phosphates. This has major consequences to our understanding of P cycling, given the different mechanisms and compounds involved than previously thought and their unknown function in the soil system.

Insert Lines 95-98: We hypothesise 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 a wide variety of IP. This would have major

consequences to our understanding of P cycling in terrestrial (and aquatic) ecosystems, as much more organic P compounds and mechanisms would be involved than previously thought.

Comment 6
And what information would be expected from analyzing them in different soils?

Response 6
A diverse set of soils provides the opportunity to identify a greater array of organic P species than what might be present in only one soil. The diversity of soil properties may also reveal different relative contributions of organic P species than that present in a particular soil type.

Comment 7
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.

Response 7
Please see Response 5 of Reviewer 2. In addition, in a recent paper we obtained high-resolution NMR spectra that exhibited a plethora of sharp peaks and an underlying broad peak in the phosphomonoester region on soil extracts (Reusser et al 2020). This suggested a much greater diversity of organic P species than previously thought. The identity of these sharp peaks was largely unknown and could not be attributed to the limited number of RNA mononucleotides and two glycerophosphates often reported in the literature. Furthermore, a review of the literature from the 1950s to 1970s indicated some studies report the presence of lower-order inositol phosphates in soil extracts using chromatographic approaches. Consequently, we hypothesised 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 inositol phosphates. If the majority of sharp peaks disappeared following hypobromite oxidation, then this would indicate that the sharp signals were due to non-inositol phosphate compounds. We combined previously published methods to test this hypothesis, but did not seek to advance or test the efficacy of these methods as is typically done in a 'methods' paper.

Comment 8
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.

Response 8
Agreed, we changed the reference accordingly. This error occurred because of a formatting issue in the EndNote library.

Reference entry changed to (lines 682-685): Riley, A. M., Trusselle, M., Kuad, P., Borkovec, M., Cho, J., Choi, J. H., Qian, X., Shears, S. B., Spiess, B., and Potter, B. V. L.: *scyllo*-Inositol pentakisphosphate as an analogue of *myo*-inositol 1,3,4,5,6-pentakisphosphate: Chemical synthesis, physicochemistry and biological applications, ChemBioChem, 7, 1114-1122, 10.1002/cbic.200600037, 2006.

Comment 9
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.

Response 9
We have updated the reference list.

Comment 10
l. 87: “was resistant” should be “were resistant”, because it modified “signals”, which is plural.

Response 10
Corrected.

Comment 11
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.

Response 11
We are happy to reduce this if requested by the Editor. However, the approach is not well known outside of the NMR and organic P communities, and the additional information may be useful for understanding and for reproducibility in future experiments.

Comment 12
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.

Response 12
We carried out the hypobromite oxidation procedure based on the method of Turner et al. (2012), and prior to the publication of Turner (2020). Briefly, Turner et al (2020) suggest taking a 10 mL aliquot of soil extract, adding 2 g of NaOH, and then adding 0.5 mL of bromine. This is slightly different to that reported in Turner et al (2012). In the current study, we similarly take a 10 mL aliquot of soil extract, but add 1 mL of 10 M NaOH, and add 0.6 mL of bromine. Please see Response 3 of Reviewer 1.

Comment 13
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%?

Response 13
We used 25% of the total filtrate for the hypobromite oxidation. We have made this clearer in the manuscript:

Inserted (lines 121-123): Concentrations of organic P for NMR analysis were carried out using the NaOH-EDTA extraction technique of Cade-Menun et al. (2002) at a soil to solution ratio of 1:10, i.e. extracting 4 g of soil with 40 mL of extractant.

Changed from (lines 121-123): Briefly, 10 mL of the filtrate was placed in a three necked round bottom flask equipped with a septum, a condenser, a magnetic stir bar and thermometer (through a claisen adapter with N_2 adapter).

Changed to (lines 127-129): Briefly, 10 mL of the NaOH-EDTA filtrate (section 2.2) was placed in a three necked round bottom flask equipped with a septum, a condenser, a magnetic stir bar and thermometer (through a claisen adapter with N_2 adapter).

Comment 14
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"

Response 14
Agreed.

Changed from (lines 144-145): The difference in concentrations of total P and MRP in solution is that of molybdate unreactive P (MUP), which is considered to be largely that of organic P.

Changed to (lines 150-151): The difference in concentrations of total P and MRP in solution is molybdate unreactive P (MUP), which is predominantly organic P for these samples.

Comment 15
l. 146-147: "a duplicate sample of the Cambisol and the Gleysol was spiked" should be "duplicate samples of the Cambisol and Gleysol were spiked"

Response 15
Corrected.

Comment 16
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.

Response 16
Vestergren et al. (2012) report in their body text: "Extraction of soils with NaOH/EDTA is known to hydrolyze several forms of phosphodiesters. This is considered an unavoidable drawback of the method, but it has been pointed out that it does not exclude deriving the original P composition when hydrolysis products can be traced back.[21] Therefore, when a hydrolysis product is observed, it must be determined what fraction of the compound was originally present in the soil, versus formed during extraction.[19] Whereas the longer sample preparation time for sulfide treatment increases hydrolysis (Figure S3 of the Supporting Information), the 2D methodology is very well suited to trace observed compounds back to their precursors". The citation of Vestergren et al. (2012) in our manuscript refers to their findings in the Supporting Information (Figure 3). The authors present NMR spectra and report that more hydrolysis of phosphodiesters are due to the "longer exposure to high pH", and that the 'resting' time of the extracts in the study was 18-20 hours at room temperature. The authors note in their study the mechanism of alkaline hydrolysis of organic P compounds to their hydrolysis products and the necessity of a reaction period lasting several hours for sufficient hydrolysis.

Comment 17
l. 193-195: Something seems to be missing here for the measurement of N observability. Using Ptot ICP-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.

Response 17
P_{tot} NMR and P_{tot} ICP-OES refer to the P concentrations in mg P per kg soil measured in the extracts. Hence, the analysed P contents in the extracts were back-calculated to the original concentrations in the soil, including any partitioning in the extraction, freeze-drying and re-dissolving processes. We made this clearer in the text by inserting the units of the two parameters.

Insert (lines 206-208): ,where P_{tot} NMR refers to the total P content in mg P/kg_{soil} detected in the soil extracts using solution ^{31}P NMR spectroscopy and P_{tot} ICP-OES refers to the total P concentration in mg P/kg_{soil} measured in the soil extracts prior to freeze-drying using ICP-OES.

Comment 18
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 thing this much detail is needed, it could be included in the SI.

Response 18
Please see Response 11.

Comment 19
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?

Response 19
We report in our studies average values as well as standard deviations. Furthermore, we carried out the one-way ANOVA with subsequent multi comparison of mean values using the Tukey's significance honestly significant difference procedure to determine whether the T_2 of the broad peak was significantly different from the IP peaks. The result of this statistical analysis is reported in the text, lines 330-332: The average (n=4) T_2 times of the broad peak was significantly different than that of *scyllo*- and *myo*-IP_6 ($p < 0.05$).

Comment 20
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.

Response 20
The main reaction was oxidation, not bromination of the samples. The aim of our study was the identification of IP, whose peaks appear in the phosphomonoester region. Hence, our spectra focus on the phosphomonoester region, which is also where the majority (> 99%) of NMR signals are located. We are unsure why the inclusion of the whole spectrum would add to the information already provided in Table 4. Nevertheless, we are willing to add the spectra of the Gleysol and Cambisol (Figure 2), where considerable amounts of phosphodiesters were measured before hypobromite oxidation, to the supporting information.

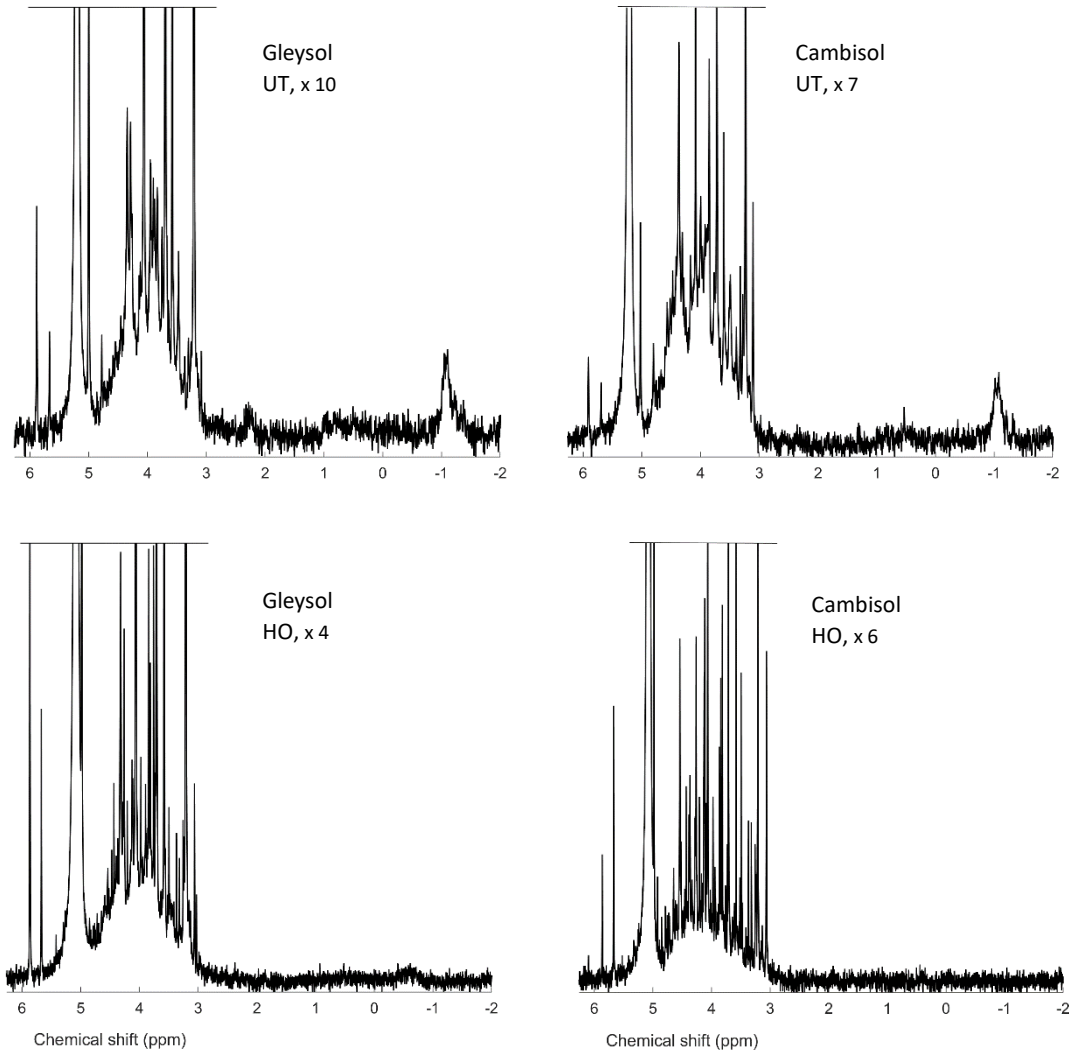

Figure 2. Solution ^{31}P nuclear magnetic resonance (NMR) spectra (500 MHz) of the orthophosphate, phosphomonoester and phosphodiester region on untreated (UT, on top) and hypobromite oxidised (HO, below) 0.25 M NaOH + 0.05 M EDTA soil extracts of the Gleysol (right) and Cambisol (left). Signal intensities were normalised to the MDP peak intensity. The vertical axes were increased for improved visibility of spectral features, as indicated by a factor.

Comment 21

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.

Response 21

The aim of the transverse relaxation (T_2) experiments was to determine if the underlying broad signal itself is caused by sharp peaks of IP or if another compound of larger structure than IP resisted hypobromite oxidation (Please also see Response 28, Reviewer 1). The red line of Figure 3 does not show a normal NMR soil spectrum but the result of the T_2 experiment with the longest spin-echo delay for each hypobromite oxidised soil sample. The spectra in black with the shortest spin-echo delay can be interpreted as a “normal” NMR soil spectra. We applied increasing spin-echo delays and acquired the resulting spectra for each step. However, due to visibility reasons, we only show the results of the shortest (black) and longest spin-echo delay (red). This presentation is normal for T_2 experiments (Claridge, 2016; Li et al., 2018a).

Figure 3 shows that the sharp peaks of IP after a spin-echo delay of 80*τ are still present (red line). In contrast, the broad peak partially disappears along with the orthophosphate peak, showing nothing else than noise. This highlights that the broad peak and orthophosphate peak are not of the same chemical composition as the rest of the sharp peaks, as it would not be visible only in the black spectra. As the T_2 are inversely related to a compound's molecular size, our results support the findings of Jarosch et al. (2015) and McLaren et al. (2015b); McLaren et al. (2019) that the compounds causing the broad signal are of larger molecular size than IP.

Comment 22
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. 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. IP_6) to lower IPs (IP_5 and IP_4)? The recovery of the added *myo*-IP_6 was only 20 and 47%, which suggests it may have been degraded.

Response 22
According to the method, inositol hexakisphosphates and pentakisphosphates are stable to hypobromite oxidation, please see Response 2 of Reviewer 1. We tested the oxidation efficacy in a pilot study (Response 3, Reviewer 1). Furthermore, bromine was added in excess. If not all organic P species have been oxidised, this suggests that they are stable to hypobromite oxidation, highlighting their chemical stability. The losses occurred most certainly during the precipitation and re-dissolving procedure and not because of degradation. Please also see Response 24 of Reviewer 1. Furthermore, we identified inositol pentakisphosphates in untreated extracts, lines 312-320.

Comment 23
l. 255: change "Although," to "However,"

Response 23
Corrected.

Comment 24
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"

Response 24
Agreed.

Changed from (lines 273-274): A detailed view of the phosphomonoester region of spiked extracts is shown in Fig. SI1 to SI5 of the Supporting Information.

Changed to (lines 286-287): Detailed views of the phosphomonoester regions of spiked samples are shown in Fig. SI1 to SI5 of the Supporting Information.

Comment 25
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.

Response 25
The 'spin-echo' analysis was carried out to provide evidence that there were other compounds different to IP resistant to hypobromite oxidation. Without these results, one

could assume that the broad signal itself could be comprised of sharp peaks caused by IP. Please also see Response 27, Review 1.

Comment 26
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.

Response 26
We are unsure what the reviewer means by their comment regarding citations. Citations are primarily used to support the claims of the authors made in the body text. If the reviewer believes we have incorrectly used a citation when supporting a claim, then we are happy to make corrections. Unfortunately, the reviewer has not provided any evidence to support her or his claim.

We are unsure what the reviewer means by this comment regarding NMR sample preparation. A comparison of methods for preparing NMR samples by Dr Ronald Smernik (e.g. Smernik and Dougherty (2007)) and that reported in the current study, clearly shows a large difference in sample preparation. Both of these methods also slightly differ to other groups using NMR approaches (Cade-Menun and Liu, 2014). Indeed, our approach is based on the studies of Vincent et al. (2013) and Spain et al. (2018), which is optimised to the high-resolution NMR spectrometers we have access to.

Lastly, we note that McLaren et al. (2019) is the only study reporting transverse-relaxation (T_2) experiments for organic P compounds in soil mineral samples. In addition, studies by Smernik et al. have also done much work on identifying lower-order IP in plant samples using solution ^{31}P NMR spectroscopy.

Comment 27
In addition, it shows an unfamiliarity with the broader P-NMR literature, which is of concern.

Response 27
Please see Response 26. In addition, we are unsure why the reviewer has made this assertion given the recent review paper on the chemical nature of soil organic P by two of the co-authors (McLaren et al. (2020)). Of course, it is possible that we may have made an error and have missed a relevant study. In this case, we would be happy to make corrections and strengthen the claims already made in the text. Unfortunately, the reviewer has not provided any details where a publication might have been missed or incorrectly cited.

Comment 28
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.

Response 28
We thank the reviewer for the positive comment.

For example, water extractable inorganic P can be very small in terms of concentration but rather important in terms of function. In addition, we note that total IP comprised up to 18% of total P_{org} in hypobromite oxidised extracts and compounds causing the broad signal on average 23% of total P_{org} in untreated extracts. In our opinion, these organic P pool should not be neglected. Furthermore, ratios of IP_6 to IP_5 could provide a tool for assessing stability

of IP in soil systems, please see Response 2. water extractable inorganic P can be very small in terms of concentration but rather important functionally.

Comment 29
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.

Response 29
This section refers to the structural composition and possible stability of compounds causing the broad signal in soil, which has implications to our understanding of soil organic matter and 'legacy' P in agroecosystems. Lines 462-468 in the manuscript: Since a portion of the broad signal is resistant to hypobromite oxidation, this suggests the organic P is complex and in the form of polymeric structures. The chemical resistance of the broad signal to hypobromite oxidation may also indicate a high stability in soil (Jarosch et al., 2015). Annaheim et al. (2015) found that concentrations of the broad signal remained unchanged between three different organic fertiliser strategies after 62 years of cropping. In contrast, the organic P compounds annually added with the fertilisers were completely transformed or lost in the slightly acidic topsoil of the field trial. Nebbioso and Piccolo (2011) reported that high molecular weight material of organic matter in soil is an association of smaller organic molecules. These associations however would still cause a broad signal in the phosphomonoester region of soil extracts and could be a reason that some organic molecules containing P are protected from hypobromite oxidation. The large proportion of the broad signal in the total organic P pool demonstrates its importance in the soil P cycle.

Comment 30
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 McLaren et al., 2015a

Response 30
It is unclear which particular study by He et al the reviewer is referring to. McLaren et al. (2015a) determined the total concentrations of soil P using X-ray fluorescence spectroscopy, which was similarly the case here. The authors then compared these measures with that of aqua regia digestion, the ignition-H_2SO_4 and NaOH-EDTA extraction techniques, and also the summation of P fractions from a sequential chemical fractionation procedure based on Hedley et al. (1982). The authors report that the native soil of their study contained a fraction of strongly-held mineral P that was neither acid nor alkali extractable. They also considered the XRF method to be the most reliable for quantifying concentrations of total P in soil, which was similar to the summation of P fractions by sequential chemical fractionation. Furthermore, the authors provide supporting evidence that a relatively small portion of alkaline soluble organic P was not extracted by NaOH-EDTA.

We report in our study, Lines 337-340: On average, 44 % of total P (as measured with XRF) was extracted by NaOH-EDTA, which is consistent with previous studies (Turner, 2008; Li et al., 2018b; McLaren et al., 2019). The non-extractable pool of P is likely to comprise of inorganic P as part of insoluble mineral phases, but could also contain some organic P (McLaren et al., 2015a). Hence, we refer to the pool of P not extracted by NaOH-EDTA but measured by XRF. Therefore, we consider the publication of McLaren et al. (2015a) as the most suiable in this context.

The reviewer could be referring to He et al. (2007). Here the authors reported that P recoveries in NaOH-EDTA extracts of poultry manure were lower compared to extracts of dairy manure. The authors attributed this lower recovery to the higher Ca content in the poultry manure. Increased Ca in the poultry manure may have resulted in less soluble forms of P that were not extracted with NaOH-EDTA. By using an additional extraction step (1 M

HCl) following the NaOH-EDTA step, the authors were able to recover the remaining P from the poultry manure. Furthermore, solution ^{31}P NMR spectra of the HCl extract revealed that the majority of P was present as orthophosphate and to a lesser extent phytate. However, the study of He et al. (2007) was carried out on manure samples and are not relevant to soil samples.

Comment 31
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?

Response 31
It relates to what happens to the organic molecules containing phosphate as functional group. It gives more detail on what actually happens to the organic molecules during the hypobromite oxidation procedure. We reworded the sentence to make this clearer.

Changed from (lines 333-334): This will result in the production of carbon dioxide and simple organic acids.

Changed to (lines 349-351): The products of hypobromite oxidation are most probably carbon dioxide, simple organic acids from the oxidative cleavage of the phosphoesters and orthophosphate.

Comment 32
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.

Response 32
Please see Responses 3 and 5 of Reviewer 1.

Comment 33
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.

Response 33
It appears the reviewer has misread the sentence. We provide two different citation groups for studies involving chromatography and NMR spectroscopy (see below).

Lines 365-367: The detection of *myo*-, *scyllo*-, *chiro*, and *neo*-IP_6 in untreated and hypobromite oxidised soil extracts is consistent with previous studies using chromatography (Irving and Cosgrove, 1982; Almeida et al., 2018) and NMR (Turner and Richardson, 2004; McLaren et al., 2015b; Jarosch et al., 2015; Vincent et al., 2013; Doolette et al., 2011a).

Comment 34
l. 356-363: As noted above, the authors did not have compete 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.

Response 34
We can confirm that bromine was present in excess and that soil extracts were kept at reflux following bromine addition. Furthermore, the volume of bromine added relative to the aliquot of soil extract was similar or greater in our study compared to that in previous studies (Turner et al 2012; Turner & Richardson 2004). Please see Responses 3 and 5 of Reviewer 1.

Unfortunately, the reviewer has not provided the reference to support his or her claim. We are not aware of any study that has identified another organic P species at the chemical shift at or near that of α-glycerophosphate. Nevertheless, in the current study, the assignment of α-glycerophosphate was based on spiking experiments in untreated soil extracts. Following hypobromite oxidation, this peak disappeared, revealing two peaks belonging to IP. This then provided strong evidence that the peak originally assigned to α-glycerophosphate was in fact due to an IP.

The assignment of one of the aforementioned peaks in hypobromite extracts was confirmed by spiking experiments with *neo*-IP_6 in the 2-equatorial/4-axial conformation. This resulted in the increased peak intensity at 4.37 ppm (C2,5) and its corresponding peak at 4.11 ppm (C1,3,4,6), which occurred at the known peak ratio of 4:2 for *neo*-IP_6 in the 2-equatorial/4-axial conformation, see Figure SI4 with the spiking results. Consequently, our results highlight the need for caution when assigning the α-glycerophosphate peak based on spiking experiments alone with α-glycerophosphate in untreated soil extracts. We would recommend that spiking with *neo*-IP_6 would also occur. We have revised the text, lines 376-380: Whilst a peak at δ 4.36 ppm would be assigned to α-glycerophosphate based on spiking experiments in the untreated extracts of the Cambisol and the Gleysol, hypobromite oxidation revealed the presence of the 2-equatorial/4-axial C2,5 peak of *neo*-IP_6 at δ 4.37 ppm, and also an unidentified peak at δ 4.36 ppm in the Cambisol. Therefore, the assignment and concentration of α-glycerophosphate may be unreliable in some soils of previous studies.

Comment 35
l. 370: change “extracts, which the” to “extracts, of which the”

Response 35
Corrected.

Comment 36
l. 383: add spaces between the numbers and words here: “1axial” should be “1 axial” or “1-axial”, etc.

Response 36
Agreed.

Changed from (line 383): the 1axial/5equatorial and 5axial/1 equatorial forms of *myo*-(1,2,3,4,6)-IP_5 are in a dynamic equilibrium,

Changed to (lines 403-405): the 1-axial/5-equatorial and 5-axial/1-equatorial forms of *myo*-(1,2,3,4,6)-IP_5 are in a dynamic equilibrium,

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