Interactive comment on “Identification of lower-order inositol phosphates (IP$_5$ and IP$_4$) in soil extracts as determined by hypobromite oxidation and solution $^{31}$P NMR spectroscopy” by Jolanda E. Reusser et al.

Anonymous Referee #1

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I am happy to see this study on the inositol phosphate stereoisomers in soils, particularly the lower-order esters. The inositol phosphates are a quantitatively important and ecologically interesting group of phosphorus compounds in soils, but much remains unknown. This study uses hypobromite oxidation and solution $^{31}$P NMR spectroscopy to identify inositol phosphate stereoisomers in four soils. The spectroscopic work is of high quality. The presence of the higher-order stereoisomers is well-established, but this work identifies several lower-order esters in various stereoisomeric forms. Although these have been reported previously by chromatography, and inferred in NMR studies based on resistance to bromination, this is the first direct identification by solution $^{31}$P NMR. I recommend publication, but ask the authors to consider the following comments in their revision.

1. Hypobromite oxidation destroys organic matter except the inositol phosphates, but this statement seems true only for the higher-order esters. The hexaphosphates definitely resist bromination (e.g. Turner et al. 2012). However, it seems that earlier papers on the method suggested at least partial decomposition of the pentakisphosphates and complete decomposition of other esters. If these compounds persisted here, particularly the tetrakisphosphates, this suggests the possibility that oxidation was incomplete (see below). Did the authors test the resistance of the target compounds to bromination? If not, it might be worth adding a statement about the extent to which the lower esters are expected to resist bromination.

2. There appears to be a couple of problems with the bromination procedure here. First, it appears that there was incomplete oxidation, with persistence of some diesters, phosphonates, inositol tetrakisphosphates, and the broad signal (assuming it represents high molecular weight organic matter). Second, and as discussed by the authors, there appears to have been considerable loss of phosphorus during bromination, perhaps through precipitation, as indicated by a loss of orthophosphate, pyrophosphate, and the inositol hexakisphosphates. Inorganic phosphate should increase markedly following bromination, as organic phosphates are destroyed and converted to inorganic orthophosphate. This isn’t a problem for identification, but represents a problem for the quantification of compounds in the brominated extracts, at least if these values are to represent concentrations of the identified forms in the original soil. Given the precipitation issue, the concentrations in brominated extracts should probably be considered unreliable, and it’d be better to give quantitative values only from those signals identified in the unbrominated extracts. Data from the brominated extracts are of course still useful as qualitative identifications.

3. It has been claimed that inositol phosphates account for a negligible amount of soil
organic phosphorus and that their importance in the soil has been over-emphasized in the literature. This argument was made sufficiently strongly by one group that a prominent mycorrhizal ecologist, now sadly deceased, rewrote the section on inositol phosphate utilization by ectomycorrhizal fungi in her influential textbook. The authors might consider mentioning this in the discussion section, given the relatively large concentrations of inositol phosphates they detected in their soils.

4. The ‘broad signal’ is supposed to consist of high molecular weight organic compounds. These should be destroyed by hypobromite oxidation. If not, this suggests that either (1) the oxidation was incomplete, or (2) the broad signal is caused by something else other than high molecular weight compounds. The authors might comment on this.

5. Related to the broad signal, I think it would be worth explaining a little more about the deconvolution procedure used here. Some recent studies appear to have deconvoluted from the baseline to the top of the peaks in the monoester region, which is certain to overestimate the proportion of each signal. This might in turn exaggerate differences between signals in brominated unbrominated extracts, given that the ‘broad signal’ appears to be reduced by bromination.

Line-by-line comments:

Line 12: most studies have identified inositol phosphates by NMR in recent decades, not chromatography. Perhaps you refer specifically to lower esters, in which case perhaps state this at the start of the sentence.

Line 17: shouldn’t the ‘broad signal’ be destroyed by hypobromite oxidation?

Line 20: I understood that one of the myo-IP5 forms (myo-inositol-1,3,4,5,6) is supposed to be rare in nature and therefore unlikely to occur in soils. This is because phytases cleave phosphates other than the C-2 phosphate, often leaving myo-inositol-2-phosphate as the final product. It’s therefore a surprise to see this compound detected in two of the soils here. Could the authors comment on this?


Line 76: perhaps add ‘and a chelating agent’ – the EDTA is important in the single-step extraction.

Line 80: this was presumably the case in Turner and Richardson 2004, who presented chemical shifts of lower scyllo-IP esters, but did not detect the corresponding signals in NMR spectra of soil extracts.

Line 97: it’s not clear why these four soils were chosen for study – perhaps add a brief explanation.

Line 118: This sentence seems redundant if the method was the same. Delete?


Line 190 and 221: Please provide more information on the deconvolution procedure. Some recent studies appear to have deconvoluted from the baseline to the top of the peaks in the monoester region, which is certain to overestimate the proportion of each signal. This might in turn lead to differences between signals in brominated unbromi-
nated extracts.

Line 262: What could the broad signal possibly be, in brominated extracts?

Line 225: comma instead of period. The persistence of some phosphodiesters sug-

Line 276: this depends on how spectra were deconvoluted – see point above.

Line 278: It's interesting to see evidence for the two conformers of neo-IP6. The pro-

Line 283: Aren't lower-order esters destroyed by bromination?

Line 292: Turner and Richardson 2004 reported signals for two different scyllo-IP4

Line 311: 6 in subscript.

Line 327: orthophosphate should increase following bromination, as organic phos-

Line 404: also along the Haast chronosequence: Turner, B. L., A. Wells, and L. M. Con-

Table 3 – you could combine this table with Table 1 to streamline display items.

Table 4 – indicate that the broad peak also represents phosphomonoesters.

Table 5 – I think it's fairly safe to assume that the chiro-IP6 is the D form, given that

Table S1 – this indicates a considerable proportion of the phosphorus has been lost
during the bromination procedure.