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Supplement of

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

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NMR observability. Measures of NMR observability were calculated for the untreated and the hypobromite oxidised extracts of all soils. Measures of NMR observability refer to the percentage of total P detected using NMR compared to that by ICP-OES. For the untreated soil extracts, measures of NMR observability ranged from 52 % (Gleysol) to 89 % (Ferralsol), with an average NMR observability of 66 %. For the hypobromite oxidised extracts, measures of NMR observability ranged from 58 % (Ferralsol) to 94 % (Cambisol), with an average value of 83 %.

Inositol hexakisphosphate concentrations before and after hypobromite oxidation.

Table SI1. Concentrations of inositol hexakisphosphates in 0.25 M NaOH + 0.05 M EDTA soil extracts before and after hypobromite oxidation (HO). Quantification was based on spectral integration and deconvolution fitting of solution ^{31}P NMR spectra. The proportion of P (%) detected in hypobromite oxidised extracts compared to that in untreated extracts is provided in brackets.

Concentrations (mg P/kg$_{soil}$)		Ferralsol	Vertisol	Cambisol	Gleysol
***myo*-IP$_6$**	before HO	4.4	0.6	46.2	90.4
	after HO	1.1 (25)	0.6 (111)	26.3 (57)	85.0 (94)
***scyllo*-IP$_6$**	before HO	2.5	0.4	34.9	42.6
	after HO	0.4 (14)	0.3 (68)	15.6 (45)	41.1 (97)
***neo*-IP$_6$ 4-eq/2-ax**	before HO	-	-	4.2	7.0
	after HO	-	-	1.4 (33)	8.8 (126)
D-*chiro*-IP$_6$	before HO	-	-	7.2	6.7
	after HO	-	-	9.4 (130)	8.6 (128)

Solution ^{31}P NMR spectra of spiked hypobromite oxidised soil extracts

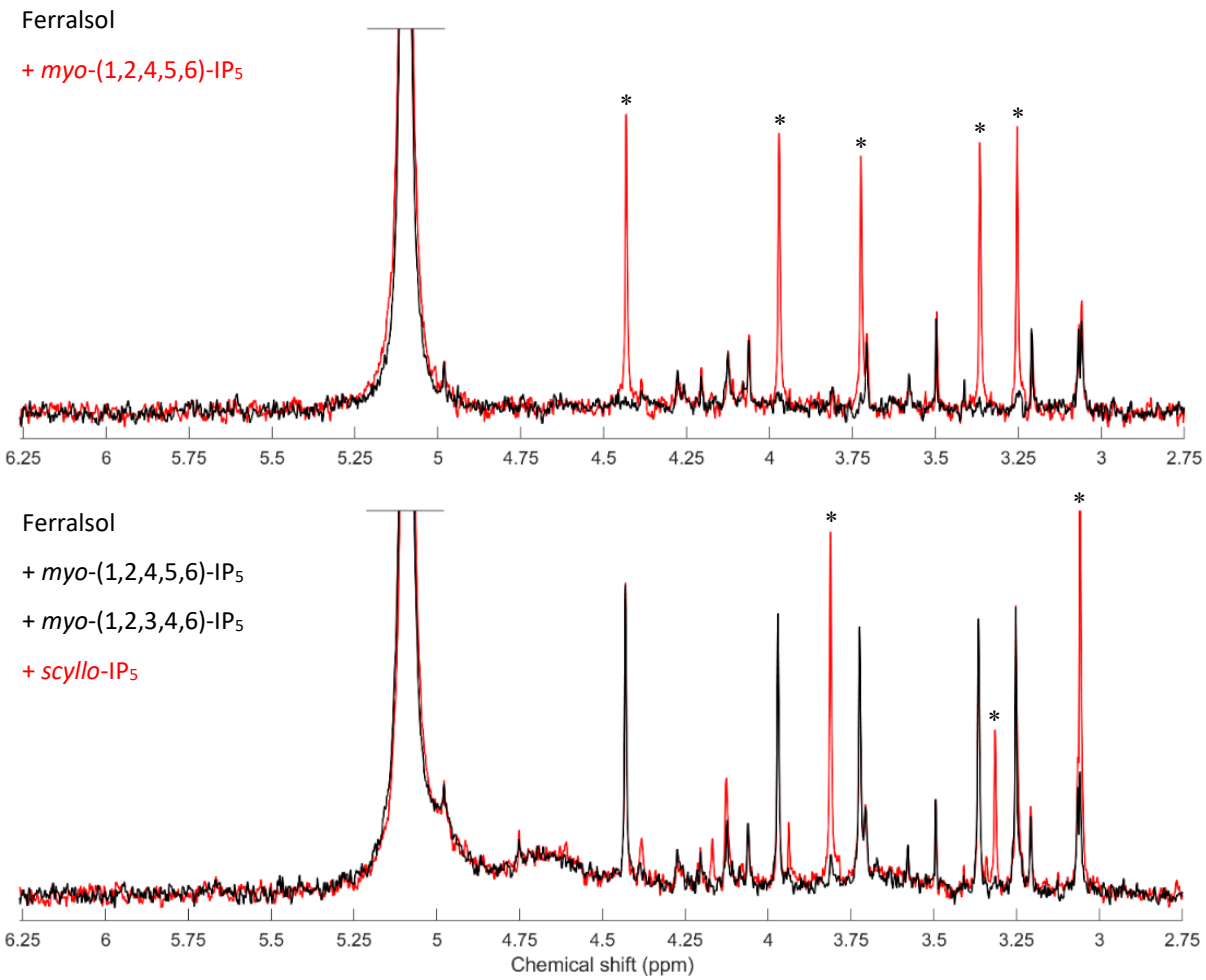

Figure SI1. Solution ^{31}P NMR spectra of the orthophosphate and phosphomonoester region on Ferralsol extract following hypobromite oxidation (black trace), and also that following a spike with an IP standard (red trace). Peaks assigned to the IP standard marked with *.

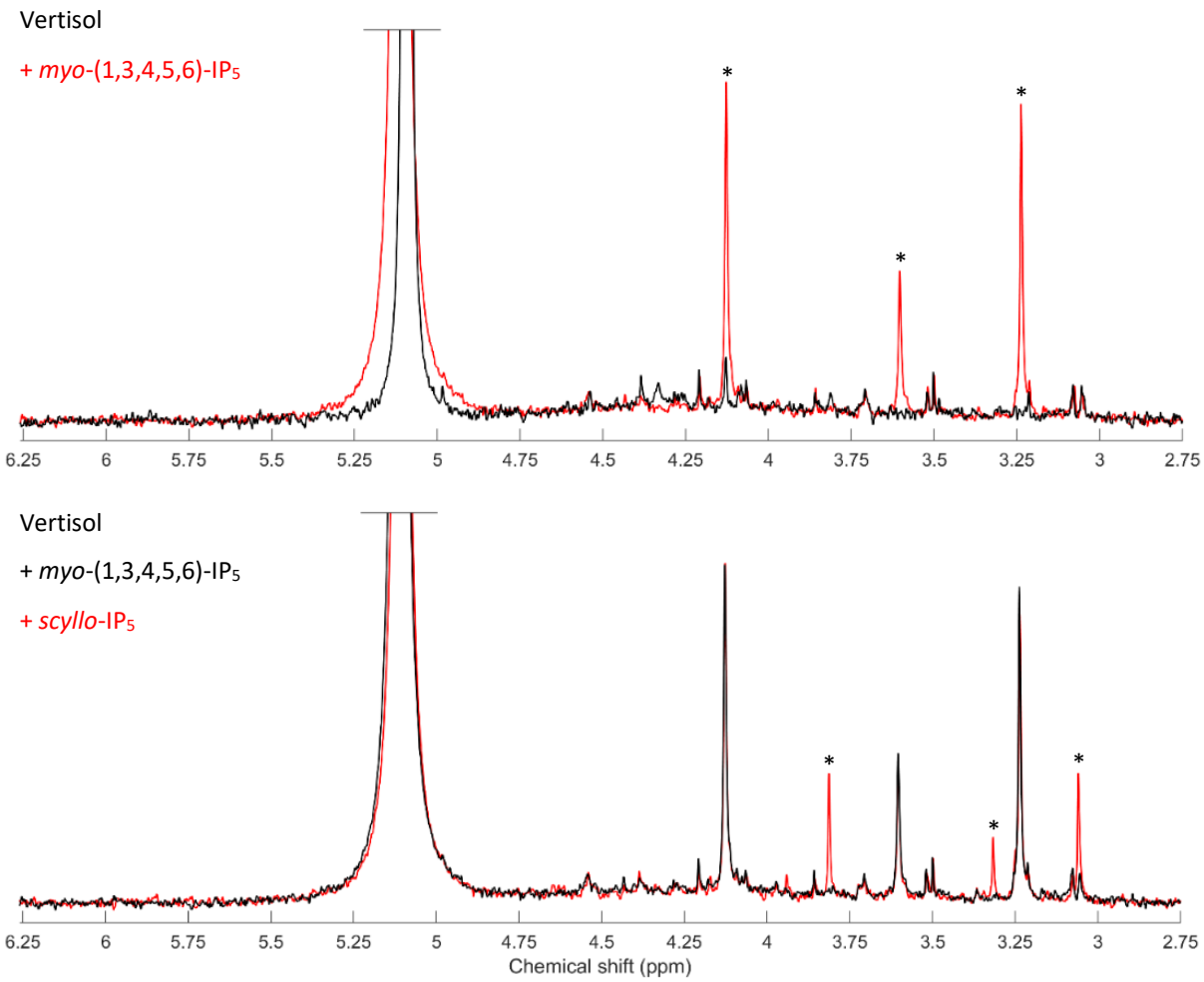

Figure SI2. Solution ^{31}P NMR spectra of phosphomonoester region of hypobromite oxidised 0.25 M NaOH + 0.05 M EDTA Vertisol extract. Spiked spectrum with indicated standard in red. Peaks assigned to standard marked with *.

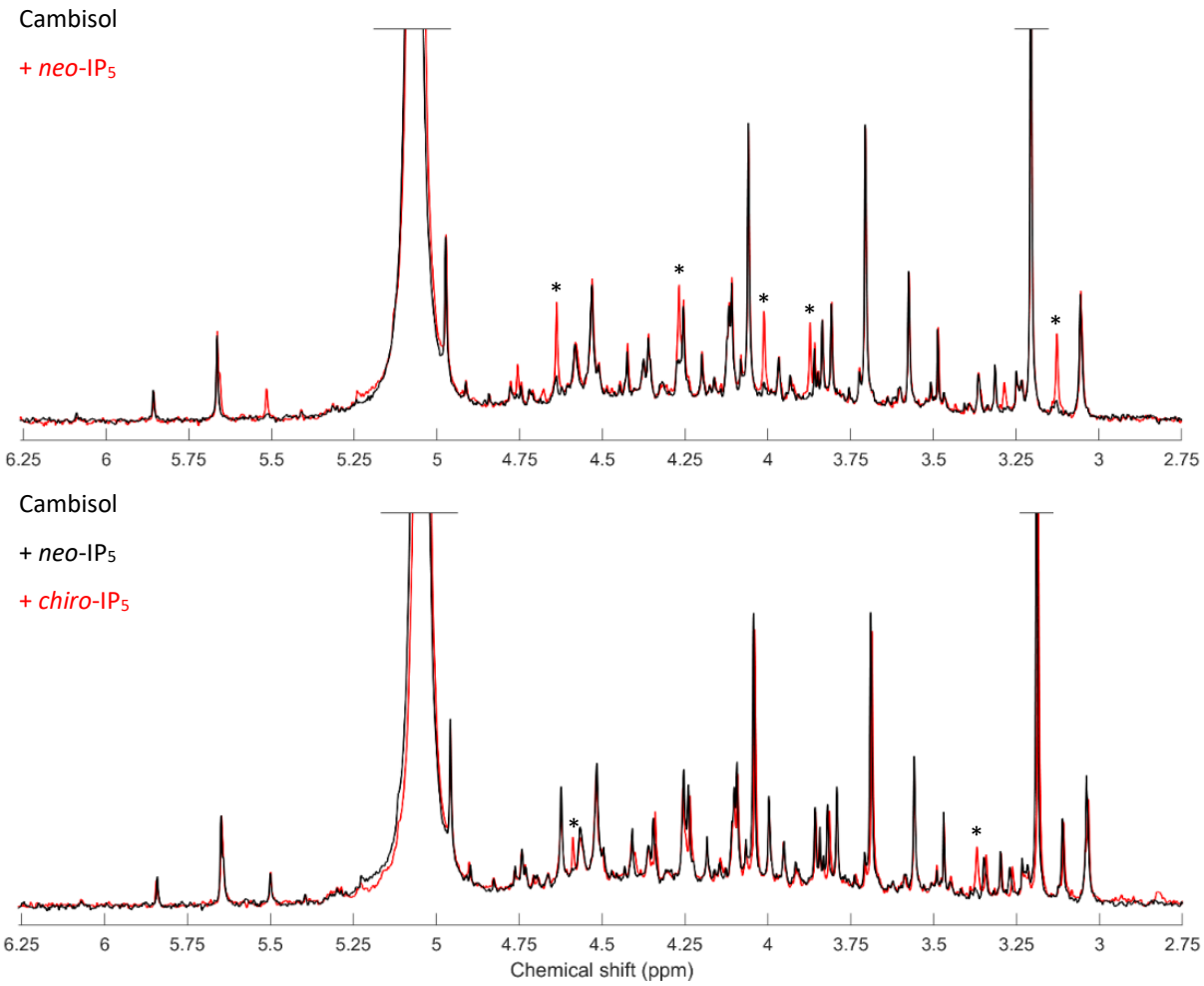

Figure SI3. Solution ^{31}P NMR spectra of phosphomonoester region of hypobromite oxidised 0.25 M NaOH + 0.05 M EDTA Cambisol extract. Spiked spectrum with indicated standard in red. Peaks assigned to standard marked with *.

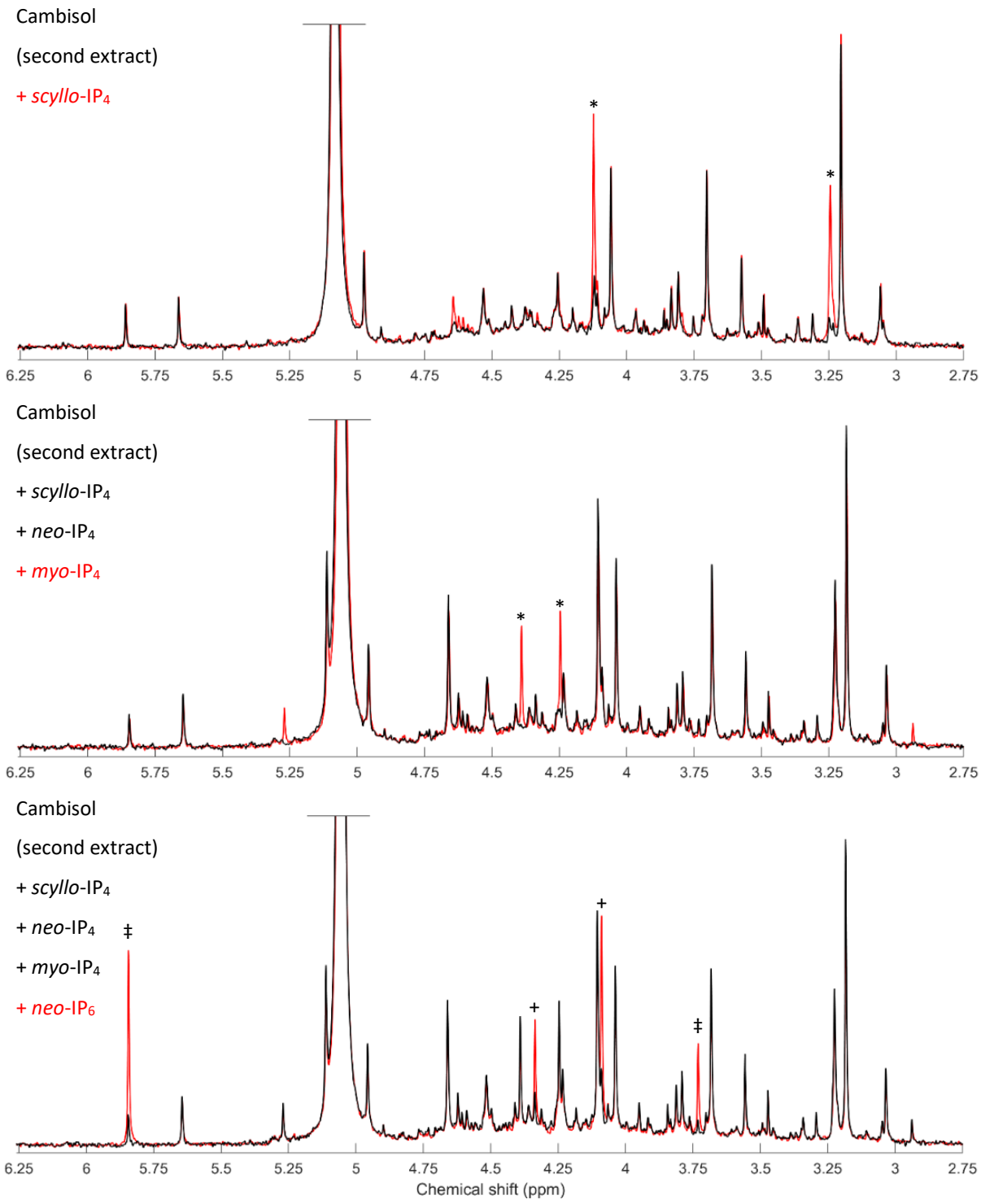

Figure SI4. Solution ^{31}P NMR spectra of phosphomonoester region of hypobromite oxidised 0.25 M NaOH + 0.05 M EDTA Cambisol extract. Spiked spectrum with indicated standard in red. Peaks assigned to 4-equatorial/2-axial conformation marked with ‡, peaks assigned to 2-equatorial/4-axial conformation marked with +.

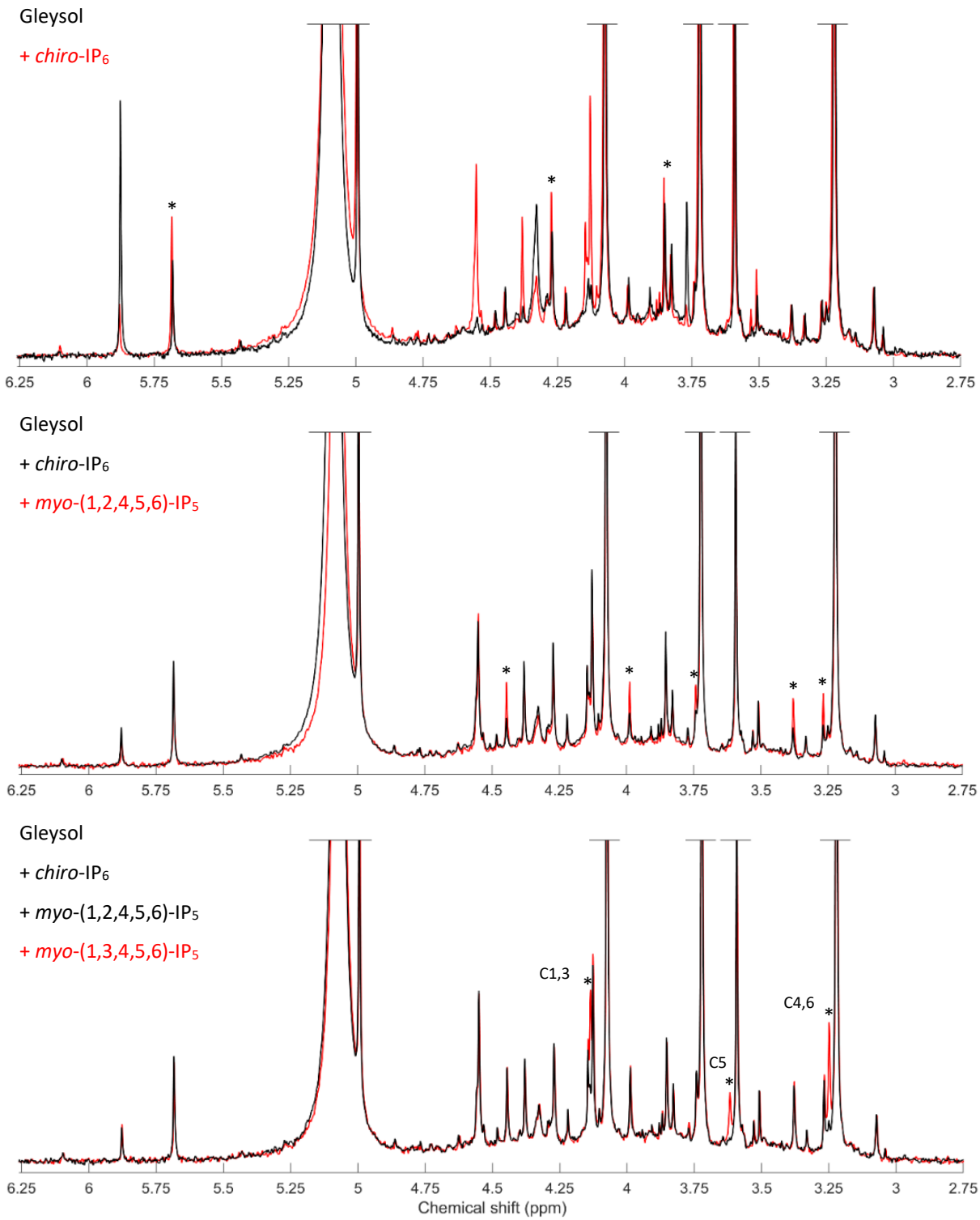

Figure SI5. Solution ^{31}P NMR spectra of phosphomonoester region of hypobromite oxidised 0.25 M NaOH + 0.05 M EDTA Gleysol extract. Spiked spectrum with indicated standard in red. Peaks assigned to standard marked with *. For *myo*-(1,3,4,5,6)-IP$_5$, the respective phosphorylated carbon nuclei of the inositol have been marked based on the ^{31}P NMR spectrum prediction of the program Mnova 11.0.4 (©Mestrelab Research).

Transverse relaxation time of an orthophosphate solution. The analysis of a 0.25 M NaOH + 0.05 M EDTA solution containing 910 mg KH_2PO_4/L resulted in a single orthophosphate peak in the NMR spectrum (δ 5.09 ppm) with a linewidth at peak half height of 0.56 Hz. Transverse relaxation experiments were carried out (similar to that previously described) on the solution, which resulted in a T_2 time of 203 ms for orthophosphate.

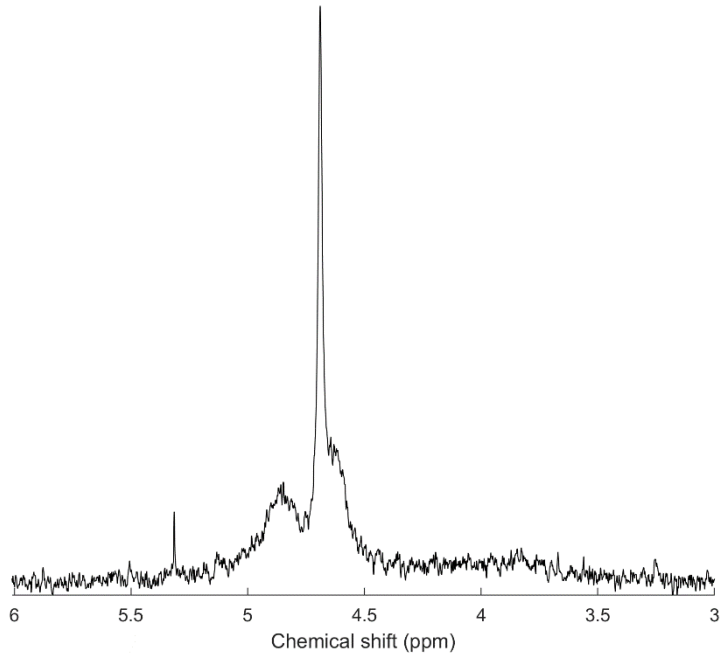

Figure SI7. Solution ^{31}P NMR spectrum of phosphomonoester region of purchased *myo*-(1,2,3,4,6)-IP_5 standard dissolved in 0.25 M NaOH + 0.05 M EDTA.

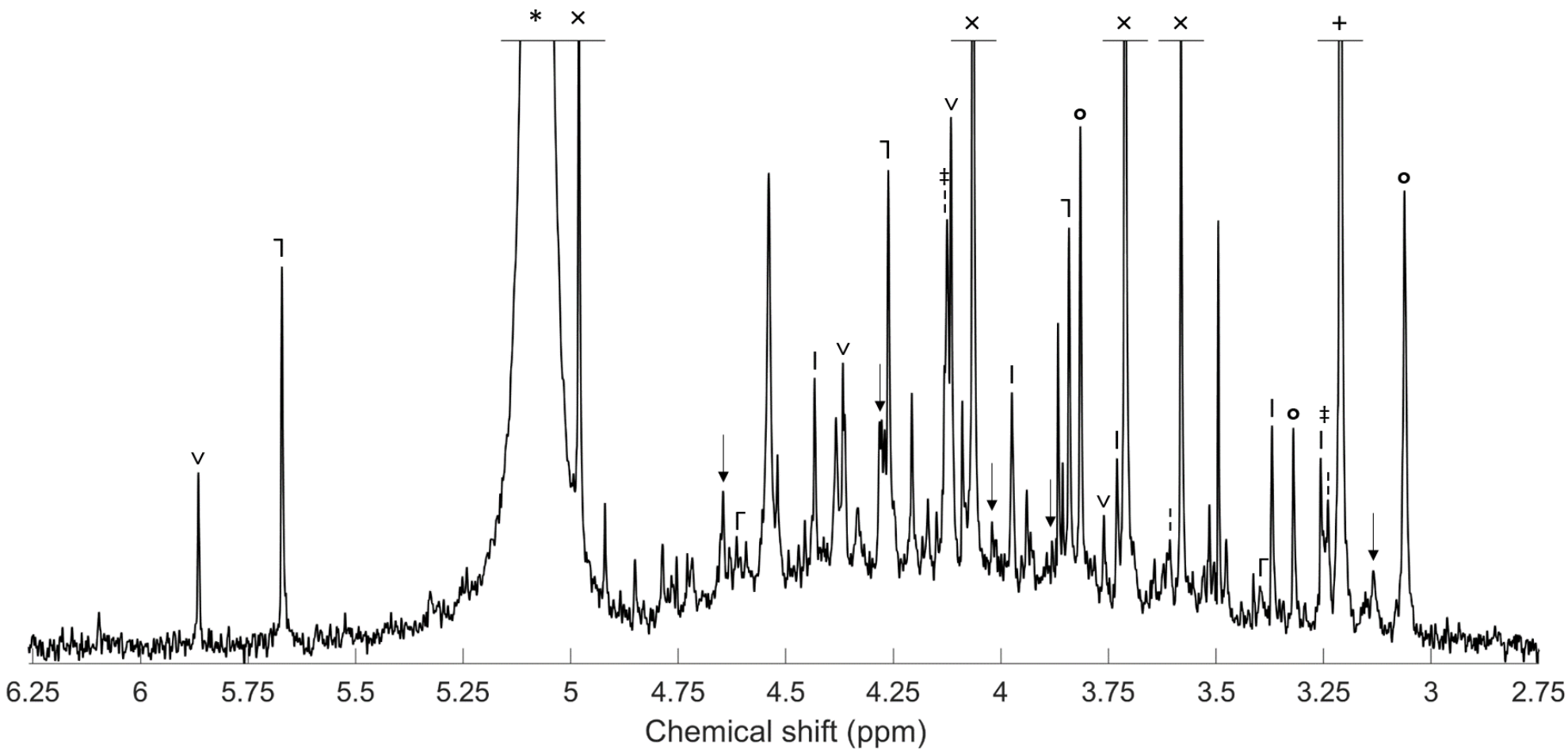

Figure SI8. Solution ^{31}P NMR spectra of phosphomonoester region of hypobromite oxidised 0.25 M NaOH + 0.05 M EDTA Cambisol extract. All identified peaks are marked: orthophosphate (*), *myo*-IP$_6$ (×), *scyllo*-IP$_6$ (+), *neo*-IP$_6$ (ᐯ), *chiro*-IP$_6$ (⅂), *myo*-(1,2,4,5,6)-IP$_5$ (ǀ), *myo*-(1,3,4,5,6)-IP$_5$ (¦), *scyllo*-IP$_5$ (°), *neo*-IP$_5$ (↓), *chiro*-IP$_5$ (Γ), *scyllo*-(1,2,3,4)-IP$_4$ (‡). The chemical shifts in ppm of all identified peaks are listed in Table 5.

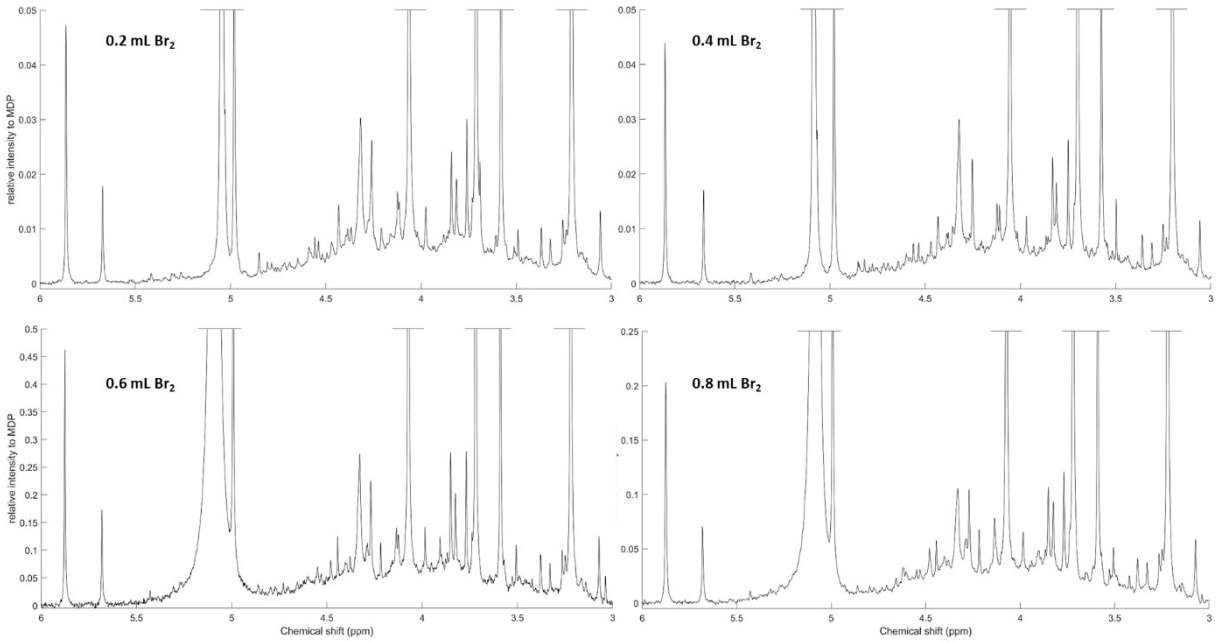

Figure SI9. Solution ^{31}P nuclear magnetic resonance (NMR) spectra (500 MHz) of the orthophosphate and phosphomonoester region of hypobromite oxidised 0.25 M NaOH + 0.05 M EDTA Gleysol extract, using 0.2 mL, 0.4 mL, 0.6 mL and 0.8 mL Br_2 in the hypobromite oxidation procedure. Signal intensities were normalised to the MDP peak (intensity of 1 on y-axes).

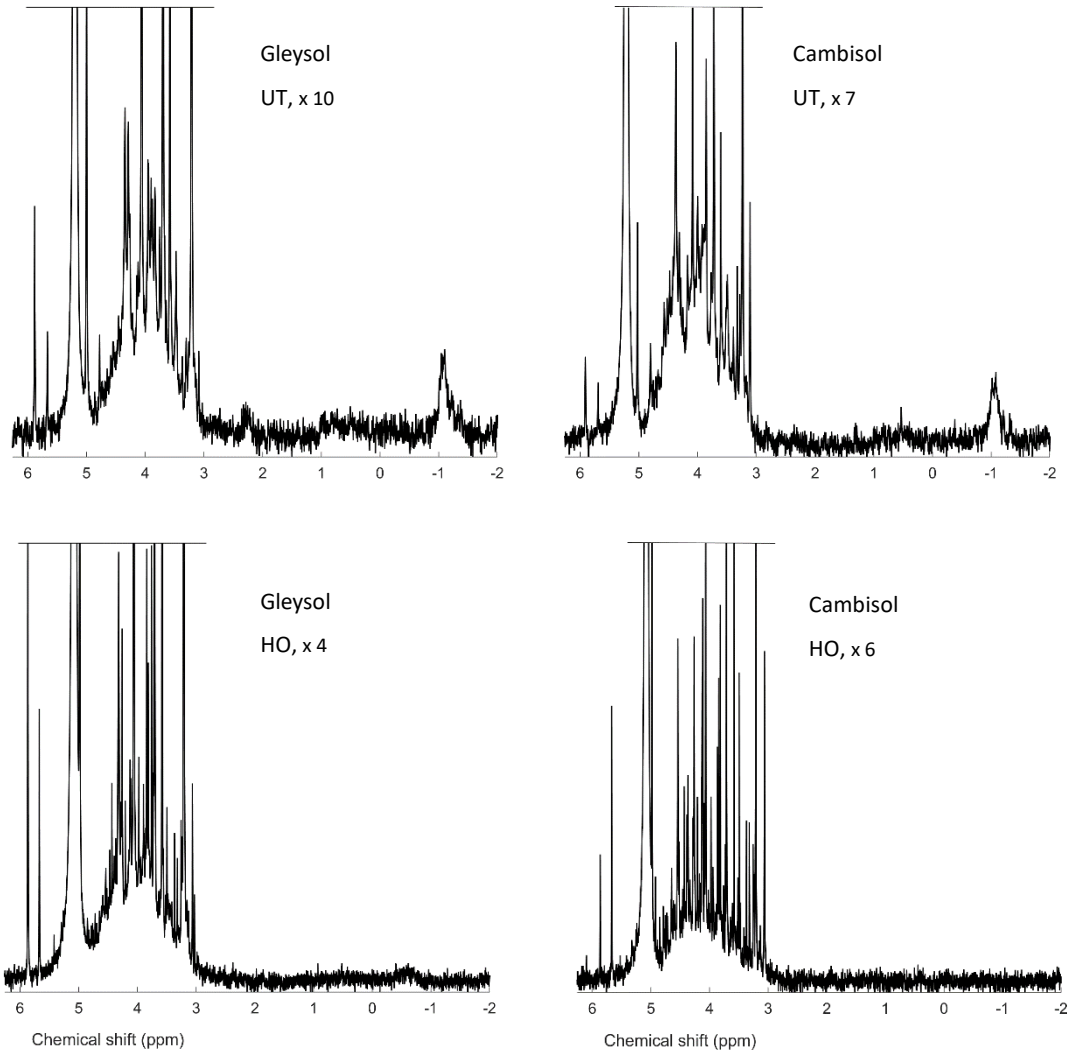

Figure SI10. Solution ^{31}P nuclear magnetic resonance (NMR) spectra (500 MHz) of the orthophosphate, phosphomonoester and phosphodiester region on untreated (UT, on top) and hypobromite oxidised (HO, below) 0.25 M NaOH + 0.05 M EDTA soil extracts of the Gleysol (right) and Cambisol (left). Signal intensities were normalised to the MDP peak intensity. The vertical axes were increased for improved visibility of spectral features, as indicated by a factor.