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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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Abstract. Inositol phosphates (IP) are a major pool of identifiable organic phosphorus (P) in soil. However, insight on their distribution and cycling in soil remains limited, particularly of lower-order IP (IP_5 and IP_4). This is because their quantification typically requires a series of chemical extractions, including hypobromite oxidation to isolate IP, followed by chromatographic separation. Here, for the first time, we identify the chemical nature of organic P in four soil extracts following hypobromite oxidation using solution ^{31}P NMR spectroscopy and transverse relaxation (T_2) experiments. 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. Solution ^{31}P NMR spectra of the phosphomonoester region on soil extracts following hypobromite oxidation revealed an increase in the number of sharp signals (up to 70), and an on average 2-fold decrease in the concentration of the broad signal compared to the untreated soil extracts. We identified the presence of four stereoisomers of IP_6, four stereoisomers of IP_5, and *scyllo*-IP_4 (using solution ^{31}P NMR spectroscopy). We also identified for the first time two isomers of *myo*-IP_5 in soil extracts: *myo*-(1,2,4,5,6)-IP_5 and *myo*-(1,3,4,5,6)-IP_5. Concentrations of total IP ranged from 1.4 to 159.3 mg P/kg_{soil} across all soils, of which between 9 % and 50 % were comprised of lower-order IP. Furthermore, we found that the T_2 times, which are considered to be inversely related to the tumbling of a molecule in solution and hence its molecular size, were significantly shorter for the underlying broad signal compared to the sharp signals (IP_6) in soil extracts following hypobromite oxidation. In summary, we demonstrate the presence of a plethora of organic P compounds in soil extracts, largely attributed to IP of various order, and provide new insight on the chemical stability of complex forms of organic P associated with soil organic matter.

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1 Introduction

Inositol phosphates (IP) are found widely in nature and are important for cellular function in living organisms. They are found in eukaryotic cells where they operate in ion-regulation processes, as signalling or P storage compounds (Irvine and Schell, 2001). The basic structure of IP consists of a carbon ring (cyclohexanehexol) with one to six phosphorylated centers (IP_{1-6}) and up to nine stereoisomers (Cosgrove and Irving, 1980; Angyal, 1963). An important IP found in nature is *myo*-IP_6, which is used as a P storage compound in plant seeds. Another important species of IP is that of *myo*-(1,3,4,5,6)-IP_5, which is present in most eukaryotic cells at concentrations ranging from 15 to 50 µM (Riley Andrew et al., 2006). Species of IP_{1-3} are present in phospholipids such as phosphatidylinositol diphosphates and are an essential structural component of the cell membrane system (Cosgrove and Irving, 1980; Strickland, 1973).

Inositol phosphates have been reported to comprise more than 50 % of total organic phosphorus (P_{org}) in some soils (Cosgrove and Irving, 1980; Turner, 2007; McDowell and Stewart, 2006). Four stereoisomers of IP have been detected in soils, with the *myo* stereoisomer being the most abundant (56 %), followed by *scyllo* (33 %), *neo* and D-*chiro* (11 %) (Turner et al., 2012; Cosgrove and Irving, 1980). The largest input of *myo*-IP_6 to the soil occurs via the addition of plant seeds (Turner et al., 2002). However, the addition of *myo*-IP_6 to soil can also occur via manure input because monogastric animals are incapable of digesting *myo*-IP_6 without the addition of phytases to their diets (Turner et al., 2007b; Leytem and Maguire, 2007). The accumulation of *myo*-IP_6 in soil occurs due to the negative charge of the deprotonated phosphate groups, which can coordinate to the charged surfaces of Fe- and Al-(hydro)-oxides (Ognalaga et al., 1994; Anderson et al., 1974), clay minerals (Goring and Bartholomew, 1951) and soil organic matter (SOM) (McKercher and Anderson, 1989), or form insoluble precipitates with cations (Celi and Barberis, 2007). These processes lead to the stabilisation of IP in soil resulting in its accumulation and reduced bioavailability (Turner et al., 2002). In contrast, the sources and mechanisms controlling the flux of *scyllo*-, *neo*- and D-*chiro*-IP_6 in soil remain unknown but are thought to involve epimerization of the *myo* stereoisomer (L'Annunziata, 1975).

Chromatographic separation of alkaline soil extracts revealed the presence of four stereoisomers of IP_6 and lower-order IP_{1-5} (Halstead and Anderson, 1970; Irving and Cosgrove, 1982; Cosgrove and Irving, 1980; Anderson and Malcolm, 1974). Irving and Cosgrove (1981) used hypobromite oxidation prior to chromatography to isolate the IP fraction in alkaline soils. The basis of this approach is that IP are considered to be highly resistant to hypobromite oxidation, whereas other organic compounds (e.g. phospholipids and nucleic acids) will undergo oxidation (Dyer and Wrenshall, 1941; Turner and Richardson, 2004). The resistance of IP to hypobromite oxidation is thought to be due to the high charge density and steric hindrance, which is caused by the chair conformation of the molecule and the bound phosphate groups, with the P in its highest oxidation state. Hypobromite oxidation of inositol (without phosphate groups) mainly results in the formation of inososes, which have an intact carbon ring (Fatiadi, 1968). Fatiadi (1968) considered that the oxidation of bromine with inositol is stereospecific and comparable to catalytic or bacterial oxidants.

A limitation of chromatographic separation of alkaline extracts is that there is a mixture of unknown organic compounds that can co-elute with IP, and result in an overestimation of IP concentrations (Irving and Cosgrove, 1981). However, this can also occur for IP, and historically, studies often reported the combined concentration of IP_6 and IP_5 due to a lack of differentiation in their elution times (McKercher and Anderson, 1968a). More recently, Almeida et al. (2018) investigated how cover crops might mobilize soil IP using hypobromite oxidation on NaOH-EDTA extracts followed by chromatographic separation. The authors found that pools of *myo*-IP_6 and 'unidentified

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IP' accounted for 30 % of the total extractable pool of P and hypothesised that the 'unidentified IP' pool consists solely of lower-order *myo*-IP. Pools of lower order IP_{1-5} comprise on average 17 % of the total pool of IP in soil and account for an important pool of soil organic P in terrestrial ecosystems (Turner et al., 2002; Anderson and Malcolm, 1974; Cosgrove and Irving, 1980; Turner, 2007).

Since the 1980s, solution ^{31}P nuclear magnetic resonance spectroscopy (NMR) has been the most commonly used technique to characterise the chemical nature of organic P in soil extracts (Cade-Menun and Liu, 2014; Newman and Tate, 1980). An advantage of this technique is the simultaneous detection of all forms of organic P that come into solution, which is brought about by a single step extraction with alkali (Cade-Menun and Preston, 1996). However, a limitation of the technique has been the loss of information on the diversity and amount of soil IP compared to that typically obtained prior to 1980 (Cosgrove, 1963; Smith and Clark, 1951; Anderson, 1955). To date, solution ^{31}P NMR spectroscopy on soil extracts has only reported concentrations of *myo*-, *scyllo*-, *chiro*- and *neo*-IP_6. The fact that lower-order IP were not reported in studies using NMR spectroscopy might be due to overlap of peaks in the phosphomonoester region, which makes peak assignment of specific compounds difficult (Doolette et al., 2009).

Turner et al. (2012) carried out hypobromite oxidation prior to solution ^{31}P NMR analysis of alkaline soil extracts to isolate the IP fraction. This had the advantage of reducing the number of NMR signal in the phosphomonoester region and consequently the overlap of peaks. The authors demonstrated the presence of *neo*- and *chiro*-IP_6 in NMR spectra via spiking of brominated extracts. Interestingly, the authors also reported the presence of NMR signals in the phosphomonoester region that could not be assigned to IP_6 and was resistant to hypobromite oxidation. They were not able to attribute the NMR signals to any specific P compounds, but hypothesised based on their resistance to hypobromite oxidation that they were due to lower-order IP.

The aim of this study was to identify and quantify IP in soil extracts following hypobromite oxidation using solution ^{31}P NMR spectroscopy. In addition, the structural composition of phosphomonoesters in soil extracts following hypobromite oxidation was probed using solution ^{31}P NMR spectroscopy and transverse relaxation experiments. 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 IP.

2 Experimental section

2.1 Soil collection and preparation

Soil samples were collected from the upper horizon of the profile at four diverse sites. These include a Ferralsol from Colombia, a Vertisol from Australia, a Cambisol from Germany, and a Gleysol from Switzerland (FAO, 2014). Background information and some chemical properties of the soils are reported in Table 1. Briefly, the Ferralsol was collected from an improved grassland in 1997 at the Carimagua Research Station's long-term Culticore field experiment in Columbia (Bühler et al., 2003). The Vertisol was collected from an arable field in 2018 located in southern Queensland. The site had been under native shrubland prior to 1992. The Cambisol was collected from a beech forest in 2014, and is part of the "SPP 1685 – Ecosystem Nutrition" project (Lang et al., 2017; Bünemann et al., 2016). The Gleysol was collected from the peaty top soil layer of a drained marshland in 2017, which has been under grassland for at least 20 years.

Soil samples were passed through a 5 mm sieve and dried at 60°C for 5 days, except for the Ferralsol (sieved <2 mm) and the Vertisol (ground <2 mm), which were received dried. Total concentrations of C and N in soils were

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obtained using combustion of 50 mg ground soil (to powder) weighed into tin foil capsules (vario PYRO cube®, Elementar Analysesysteme GmbH). Soil pH was measured in H_2O with a soil to solution ratio of 1:2.5 (w/w) using a glass electrode.

[Suggested location Table 1]

2.2 Soil phosphorus analyses

Total concentrations of soil P were carried out by X-ray fluorescence spectroscopy (SPECTRO XEPOS ED-XRF, AMETEK®) using 4.0 g of ground to powder soil sample mixed with 0.9 g of wax (CEREOX Licowax, FLUXANA®). The XRF instrument was calibrated using commercially available reference soils. 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. This extraction procedure is the same as described in McLaren et al. (2019).

2.3 Hypobromite oxidation

Hypobromite oxidation of NaOH-EDTA soil filtrates was carried out based on a modified version of the method described in Suzumura and Kamatani (1993) and Turner et al. (2012). 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). After the addition of 1 mL 10 M aqueous NaOH and vigorous stirring, an aliquot of 0.6 mL Br_2 (which was cooled prior to use) was added, resulting in an exothermic reaction where some of the soil extracts nearly boiled. The optimal volume of Br_2 for oxidation was assessed in a previous pilot study using 0.2, 0.4, 0.6 and 0.8 mL Br_2 volumes, and then observing differences in their NMR spectral features (data not shown). The reaction was heated to 100 °C within 10 min and kept at reflux for an additional 5 min. After cooling to room temperature, the solution was acidified with 2 mL of 6 M aqueous HCl solution in order to obtain a pH < 3, which was confirmed with a pH test strip. The acidified solution was reheated to 100 °C for 5 min under a stream of nitrogen to vaporise any excess bromine. The pH of the solution was gradually increased to 8.5 using 10 M aqueous NaOH solution. After dilution with 10 mL of H_2O, 5 mL 50 % (w/w) ethanol and 10 mL 10 % (w/w) barium acetate solution was added to the solution in order to precipitate any IP (Turner et al., 2012). The solution was then heated and boiled for 10 min and allowed to cool down overnight. The solution was subsequently transferred to a 50 mL centrifuge tube and a 10 mL aliquot of 50 % (w/w) ethanol was added, manually shaken, and centrifuged at 1500 g for 15 min. The supernatant was removed and a 15 mL aliquot of 50 % (w/w) ethanol was added to the precipitate, shaken, and then centrifuged again as before. The supernatant was removed and the process repeated once more to further purify the pool of IP. Afterwards, the precipitate was transferred with 20 mL of H_2O into a 100 mL beaker that contained a 20 mL volume (equating to a mass of 15 g) of Amberlite® IR-120 cation exchange resin beads in the H^+ form (Sigma-Aldrich, product no. 06428). The suspension was stirred for 15 min and then passed through a Whatman no. 42 filter paper. A 9 mL aliquot of the filtrate was frozen at – 80 °C and then lyophilised prior to NMR analysis. This resulted in 18 - 26 mg of lyophilised material across all soils. Concentrations of total P in solutions were obtained using inductively coupled plasma-optical emission spectrometry (ICP-OES). Concentrations of molybdate reactive P (MRP) were obtained using the malachite green method of Ohno and Zibilske (1991). 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.

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To assess the of effect hypobromite oxidation on the stability of an IP_6, a duplicate sample of the Cambisol and the Gleysol was spiked with 0.1 mL of a 11 mM *myo*-IP_6 standard. The recovery of the added *myo*-IP_6 following hypobromite oxidation was calculated using Eq. (1):

$$Spike\ recovery\ (\%) = \frac{C_{spiked}\left(\frac{mg}{L}\right) - C_{unspiked}\left(\frac{mg}{L}\right)}{C_{standard\ added}\left(\frac{mg}{L}\right)}, \quad (1)$$

where C_{spiked} and $C_{unspiked}$ are the concentrations of *myo*-IP_6 in NaOH-EDTA extracts following hypobromite oxidation of the spiked and unspiked samples, respectively. $C_{standard\ added}$ is the concentration of the added *myo*-IP_6 within the standard. As ^{31}P NMR spectroscopy of the standard revealed impurities, the concentration of *myo*-IP_6 in the standard was calculated based on the ^{31}P NMR spectrum.

2.4 Sample preparation for solution ^{31}P NMR spectroscopy

The lyophilised material of the untreated soil extracts was prepared for solution ^{31}P NMR spectroscopy based on a modification of the methods of Vincent et al. (2013) and Spain et al. (2018). Briefly, 120 mg of lyophilised material was taken and dissolved in 600 µL of 0.25 M NaOH-0.05 M Na_2EDTA solution (ratio of 1:5). However, for the Cambisol sample, this ratio resulted in a NMR spectrum that exhibited significant line broadening. Therefore, this was repeated on a duplicate sample but at a smaller lyophilised material to solution ratio (ratio of 1:7.5), as suggested in Cade-Menun and Liu (2014), which resolved the issue of poor spectral quality. The suspension was stored overnight to allow for complete hydrolysis of phospholipids and RNA (Doolette et al., 2009; Vestergren et al., 2012), which was then centrifuged at 10621 g for 15 min. A 500 µL aliquot of the supernatant was taken, which was subsequently spiked with a 25 µL aliquot of a 0.03 M methylenediphosphonic acid standard made in D_2O (Sigma-Aldrich, product no. M9508) and a 25 µL aliquot of sodium deuteroxide at 40 % (w/w) in D_2O (Sigma-Aldrich, product no. 372072). The solution was then mixed and transferred to a 5 mm diameter NMR tube.

A similar procedure was used for the soil extracts that had undergone hypobromite oxidation, except the total mass of lyophilised material (18 - 26 mg) was dissolved with 600 µL of a 0.25 M NaOH-0.05 M Na_2EDTA solution. However, for the Cambisol sample, the NMR spectrum exhibited considerable line-broadening, and an additional 400 µL aliquot of NaOH-EDTA solution was added to the NMR tube, mixed, and then returned to the NMR spectrometer. This resolved the issue of poor spectral quality.

2.5 Solution ^{31}P NMR spectroscopy

Solution ^{31}P NMR analyses were carried out on all untreated and hypobromite oxidised soil extracts at the NMR facility of the Laboratory of Inorganic Chemistry (Hönggerberg, ETH Zürich). All spectra were obtained with a Bruker AVANCE III MD 500 MHz NMR spectrometer equipped with a cryogenic probe (CryoProbe™ Prodigy) (Bruker Corporation; Billerica, MA). The ^{31}P frequency for this NMR spectrometer was 202.5 MHz and gated broadband proton decoupling with a 90° pulse of 12 µs was applied. Spectral resolution under these conditions for ^{31}P was < 1 Hz. Longitudinal relaxation (T_1) times were determined for each sample with an inversion recovery experiment (Vold et al., 1968). This resulted in recycle delays ranging from 8.7 to 30.0 sec for the untreated extracts and 7.8 to 38.0 sec for the hypobromite oxidised soil extracts. The number of scans for the untreated extracts was set to 1024 or 4096, depending on the signal to noise ratio of the obtained spectrum. All hypobromite oxidised spectra were acquired with 3700 to 4096 scans.

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2.6 Processing of NMR spectra

All NMR spectra were processed with Fourier transformation, phase correction, and baseline adjustment within the TopSpin® software environment (Version 3.5 pl 7, Bruker Corporation; Billerica, MA). Line broadening was set to 0.6 Hz. Quantification of NMR signals involved obtaining the integrals of the following regions: 1) up to four phosphonates (δ 19.8 to 16.4 ppm); 2) the added MDP (δ 17.0 to 15.8ppm) including its two carbon satellite peaks; 3) the combined orthophosphate and phosphomonoester region (δ 6.0 to 3.0 ppm); 4) up to four phosphodiesters (δ 2.5 to -3.0 ppm), and 5) pyrophosphate (δ -4.8 to -5.4 ppm). Due to overlapping peaks in the orthophosphate and phosphomonoester region, spectral deconvolution fitting was applied as described in McLaren et al. (2019). The NMR observability of total P (P_{tot}) in NaOH-EDTA extracts was calculated using Eq. (2) (Doolette et al., 2011b; Dougherty et al., 2005):

$$NMR\ observability\ (\%) = \frac{P_{tot}\ NMR}{P_{tot}\ ICP-OES} * 100\ \%\ , \quad (2)$$

where P_{tot} NMR refers to the total P content detected in the soil extracts using solution ^{31}P NMR spectroscopy and P_{tot} ICP-OES refers to the total P concentration measured in the soil extracts prior to freeze-drying using ICP-OES.

2.7 Spiking experiments

To identify the presence of IP in hypobromite oxidised extracts, samples were spiked with a range of standards and then analysed again using NMR spectroscopy. This involved the addition of 5 to 20 µL aliquots of an IP standard solution directly into the NMR tube, which was then sealed with parafilm, manually shaken, and then allowed to settle prior to NMR analysis. Each sample extract was consecutively spiked with no more than four IP standards. The NMR spectra of soil extracts after spiking were overlaid with the NMR spectra of unspiked soil extracts to identify the presence of IP across all soil samples. This comparison of NMR spectra was possible due to negligible changes in the chemical shifts of peaks among soil samples. The IP standards used in this study are listed in Table 2.

[Suggested location Table 2]

2.8 Transverse relaxation (T_2) experiments

Due to the presence of sharp and broad signals in the phosphomonoester region of NMR spectra on hypobromite oxidised soil extracts, transverse relaxation (T_2) experiments were carried out to probe their structural composition. The transverse relaxation (originally spin-spin relaxation) describes the loss of magnetisation in the x-y plane. This loss occurs due to magnetic field differences in the sample, arising either by instrumentally caused magnetic field inhomogeneities or by local magnetic fields in the sample caused by intramolecular and intermolecular interactions (Claridge, 2016). Generally, small, rapidly tumbling molecules exhibit longer T_2 relaxation times compared to large, slowly tumbling molecules (McLaren et al., 2019).

Briefly, solution ^{31}P NMR spectroscopy with a Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence (Meiboom and Gill, 1958) was carried out on all hypobromite oxidised soil extracts, as described in McLaren et al. (2019). This involved a constant spin-echo delay (τ) of 5 ms, which was repeated for a total of eight iterations (spin-echo periods of 5, 50, 100, 150, 200, 250, 300, and 400 ms). A total of 4096 scans and a recycle delay of 4.75 sec was used for all iterations. Transverse relaxation times for the aforementioned integral ranges were calculated using Eq. (3) within the TopSpin® software environment. Due to overlapping peaks in the orthophosphate and phosphomonoester region, spectral deconvolution was carried out to partition the NMR signal, as described in

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McLaren et al. (2019). The T_2 times of the partitioned NMR signals were calculated using Eq. (3) within RStudio© (version 1.1.442):

$$M(t) = M_0 * e^{(-t*T_2{}^{-1})}, \quad (3)$$

where M refers to the net magnetisation derived from the average angular momentum in the x–y plane, τ refers to the spin-echo delay in milliseconds (ms), and T_2 refers to the transverse relaxation time (ms).

2.9 Statistical analyses and graphics

Statistical analyses were carried out using Microsoft® Excel 2016 and MATLAB R2017a (©The MathWorks, Inc.). Graphics were created with Microsoft® Excel 2016 and MATLAB R2017a (©The MathWorks, Inc.). Solution (1D) ^{31}P NMR spectra were normalised to the peak intensity of MDP (δ 16.46 ppm). Spectra from the T_2 experiments were normalised to the peak intensity of *scyllo*-IP_6 (δ 3.22 ppm).

A one-way ANOVA was carried out in MATLAB R2017a (©The MathWorks, Inc.) with a subsequent multi comparison of mean values using the Tukey's honestly significant difference procedure based on the studentised range distribution (Hochberg and Tamhane, 1987; Milliken and Johnson, 2009).

3 Results

3.1 Phosphorus concentrations in soil extracts

Concentrations of total soil P as determined by XRF ranged from 320 to 3841 mg P/kg_{soil} across all soils (Table 3). Concentrations of total P as estimated by the NaOH-EDTA extraction technique ranged from 160 to 1850 mg P/kg_{soil}, which comprised 28 to 51 % of the total soil P as determined by XRF. Pools of organic P comprised 28 to 72 % of the total P in NaOH-EDTA untreated soil extracts.

Concentrations of total P in NaOH-EDTA soil extracts following hypobromite oxidation ranged from 77 to 578 mg P/kg_{soil} (Table 3), which accounted for 31 to 48 % (on average 38 %) of the total P originally present in the extracts. Similarly, pools of organic P in NaOH-EDTA extracts following hypobromite oxidation were lower, comprising 22 to 48 % (on average 36 %) of that originally present in untreated NaOH-EDTA extracts across all soils.

[Suggested location Table 3]

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

The most prominent signal in the NMR spectra of untreated NaOH-EDTA soil extracts was that of orthophosphate at δ 5.25 (±0.25) ppm, followed by the phosphomonoester region ranging from δ 6.0 to 3.0 ppm (Fig. 1). There were also some minor signals due to pyrophosphate δ -5.06 (±0.19) ppm (all soils), phosphodiesters ranging from δ 2.5 to -2.4 ppm (not detected in the Vertisol), and phosphonates (not including the added MDP) at δ 19.8, 19.2 and 18.3 ppm (not detected in the Gleysol). However, these compounds comprised less than 8 % of the total NMR signal.

Following hypobromite oxidation of NaOH-EDTA extracts, the most prominent NMR signals were found in the orthophosphate (65 % of total NMR signal) and phosphomonoester (35 % of total NMR signal) region across all soils (Fig. 1). Phosphodiesters and pyrophosphates were removed following hypobromite oxidation in the Ferralsol, the Vertisol and the Cambisol (DE). Although, some signal remained in the Gleysol at low concentrations (0.4 % of the total NMR signal). Phosphonates were removed following hypobromite oxidation in

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the Ferralsol and the Vertisol, but a total of five sharp peaks in the phosphonate region were detected (δ 19.59, 18.58, 17.27 and 9.25 ppm) in the Cambisol. These peaks comprised 0.6 % of the total NMR signal.

The phosphomonoester region of NMR spectra on untreated NaOH-EDTA extracts exhibited two main features: 1) the presence of a broad signal centered at around δ 4.1 (±0.1) ppm with an average line-width at half height of 256.12 Hz; and 2) the presence of between 19 and 34 sharp signals. This was similarly the case on hypobromite oxidised extracts, except there was a decrease in the intensity of the broad signal and a change in the distribution and intensity of sharp signals. For the Cambisol and Gleysol, the number of sharp signals in the phosphomonoester region approximately doubled (to 40 and 70 sharp signals, respectively) following hypobromite oxidation. In contrast, less than half of the sharp signals remained in the Ferralsol following hypobromite oxidation (i.e. 14 of the 30 peaks originally present in the untreated extract), whereas one peak was removed following hypobromite oxidation in the Vertisol. There was little change (0.23 ppm) in the chemical shifts of peaks between the untreated and hypobromite oxidised extracts.

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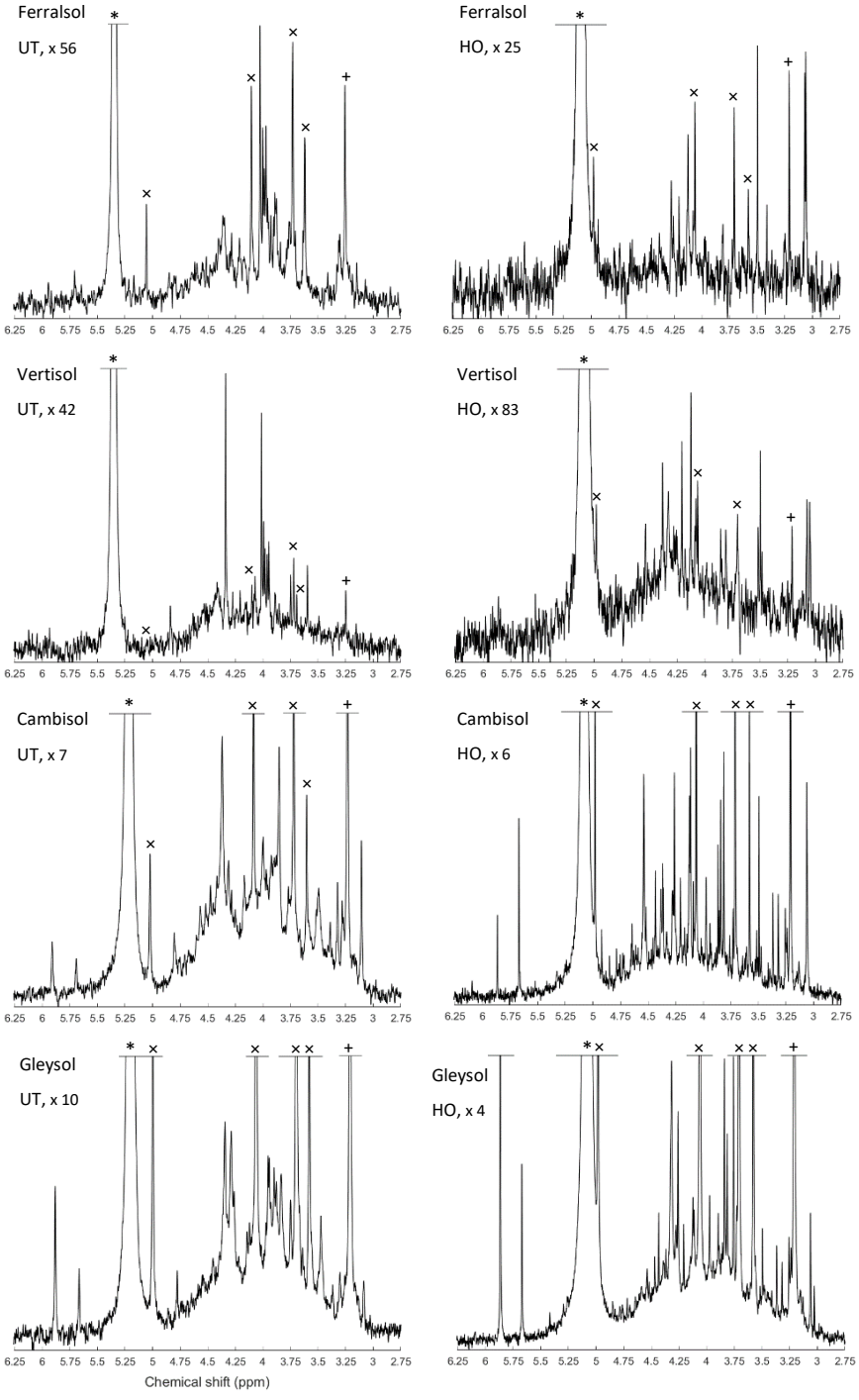

Figure 1. Solution ^{31}P nuclear magnetic resonance (NMR) spectra (500 MHz) of the orthophosphate and phosphomonoester region on untreated (UT) and hypobromite oxidised (HO) 0.25 M NaOH + 0.05 M EDTA soil extracts (Ferralsol, Vertisol, Cambisol and Gleysol). Signal intensities were normalised to the MDP peak intensity. The vertical axes were increased for improved visibility of spectral features, as indicated by a factor. The orthophosphate peak is marked with an asterisk. The symbol '×' marks the four individual peaks of *myo*-IP$_6$ and '+' the peak of *scyllo*-IP$_6$.

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271 [Suggested location Table 4]

272 **3.3 Identification and quantification of inositol phosphates (IP_6, IP_5 and IP_4) in soil extracts**

273 A detailed view of the phosphomonoester region of spiked extracts is shown in Fig. SI1 to SI5 of the Supporting
274 Information. The number of identified sharp peaks in the phosphomonoester region ranged from 7 (Vertisol) to 33
275 (Gleysol). *myo*- and *scyllo*-IP_6 were identified in the hypobromite oxidised extracts of all soils (Table 5). On
276 average, 72 % of *myo*-IP_6 and 56 % of *scyllo*-IP_6 present in the untreated extracts remained in the hypobromite
277 oxidised extracts (Table SI1 in the Supporting Information). *neo*-IP_6 was identified in the the 2-equatorial/4-axial
278 and 4-equatorial/2-axial conformations, and c*hiro*-IP_6 in the 2-equatorial/4-axial confirmation, of the oxidised
279 extracts in the Cambisol and Gleysol, but were absent in the Ferralsol and the Vertisol (Fig. SI4 and SI5 in the
280 Supporting Information).
281 The *myo*, *scyllo*, *chiro* and *neo* stereoisomers of IP_5 were identified in various hypobromite oxidised extracts (Table
282 5). Two isomers of *myo*-IP_5 were identified in some extracts, which included *myo*-(1,2,4,5,6)-IP_5 and *myo*-
283 (1,3,4,5,6)-IP_5. In addition, *scyllo*-IP_4 was detected in all soils except that of the Vertisol. There was insufficient
284 evidence for the presence of *myo*-IP_4 in these soil samples, as only one of the two peaks of this compound was
285 present in the NMR spectra of untreated extracts.
286 Concentrations of total IP ranged from 1.4 to 159.3 mg P/kg_{soil} across all soils, which comprised between 1 %
287 (Vertisol) and 18 % (Gleysol) of the organic P in untreated NaOH-EDTA extracts (Table 3). Pools of IP_6 were the
288 most abundant form of IP, which ranged from 0.9 to 144.8 mg P/kg_{soil} across all soils (Table 5). The proportion of
289 IP_6 stereoisomers across all soils were in the order of *myo* (61 %, SD=12), *scyllo* (29 %, SD=3), *chiro* (6 %, SD=8)
290 and *neo* (4 %, SD=5). Similarly, the *myo* and *scyllo* stereoisomer were also the most predominant forms of IP_5,
291 but comprised between 83 % (Cambisol) and 100 % (Ferralsol and Vertisol) of total IP_5 (Table 5). Trace amounts
292 of *scyllo*-IP_4 were also detected in three of the four soils. The ratio of total IP_6 to IP_5 differed across all soils (Fig.
293 2).
294 [Suggested location Table 5]

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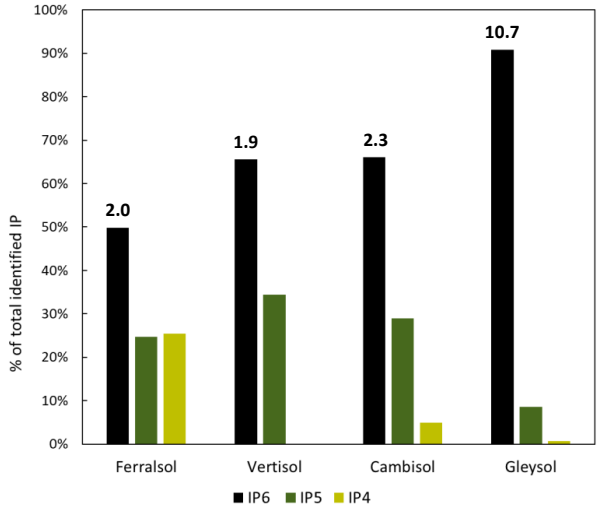

Figure 2. The proportion of total identifiable pools of inositol hexakisphosphates (IP_6), -pentakisphosphates (IP_5) or –tetrakisphosphates (IP_4) to that of the total pool of identifiable IP, as determined by solution ^{31}P NMR spectroscopy on four soil extracts (Ferralsol, Vertisol, Cambisol and Gleysol) following hypobromite oxidation. Values located above the IP_6 bar are the ratio of total identifiable IP_6 to that of IP_5 in each soil sample.

295

296 If sharp peaks arising from IP were identified in the NMR spectra on hypobromite oxidised extracts, a comparison
297 was made with that of their corresponding untreated extracts. The sharp peaks of all stereoisomers of IP_6 were
298 present in the untreated extracts. The five peaks of *myo*-(1,2,4,5,6)-IP_5 and the three peaks of *scyllo*-IP_5 were also
299 identified. However, it was not possible to clearly identify other IP_5 compounds in untreated extracts due to
300 overlapping signals. In the Gleysol, all three peaks of *scyllo*-IP_5 were detected, but only two of the possible five
301 peaks could be clearly assigned to *myo*-(1,2,4,5,6)-IP_5. In the Ferralsol, both peaks of *scyllo*-IP_4 were present in
302 the untreated extract, but only two of the three possible peaks could be assigned to *scyllo*-IP_5. In the Vertisol, no
303 IP_5 was identified. Concentrations of IP in untreated extracts assessed by spectral deconvolution fitting were
304 generally double than that measured in hypobromite oxidised extracts. Recoveries of added *myo*-IP_6 in the Gleysol
305 and Cambisol following hypobromite oxidation were 47 % and 20 %, respectively.

306 **3.4 Spin-echo analysis of selected P compounds**

307 Due to the presence of sharp and broad signals in hypobromite oxidised soil extracts, the structural composition
308 of phosphomonoesters was probed. A comparison of the NMR spectra at the lowest (1*τ) and highest (80*τ) pulse
309 delays revealed a fast decaying broad signal for all hypobromite oxidised soil extracts, which was particularly
310 evident in the Gleysol (Fig. 3). Calculated T_2 times of all IP_6 stereoisomers were longer than that of the broad
311 signal (Table 6). The T_2 times of *scyllo*-IP6 (on average 175.8 ms, SD=49.7) were generally the longest of all
312 stereoisomers of IP_6. The T_2 time of the orthophosphate peak was the shortest, which was on average 11.5 ms
313 (SD=4.9).
314 The average (n=4) T_2 times of the broad peak was significantly different than that of *scyllo*- and *myo*-IP_6 ($p <$
315 0.05). Significant differences in the T_2-times of *neo*- and D-*chiro*-IP_6 were not tested, as these compounds were
316 not detected in the Ferralsol and the Vertisol.

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317 [Suggested location Table 6]

318

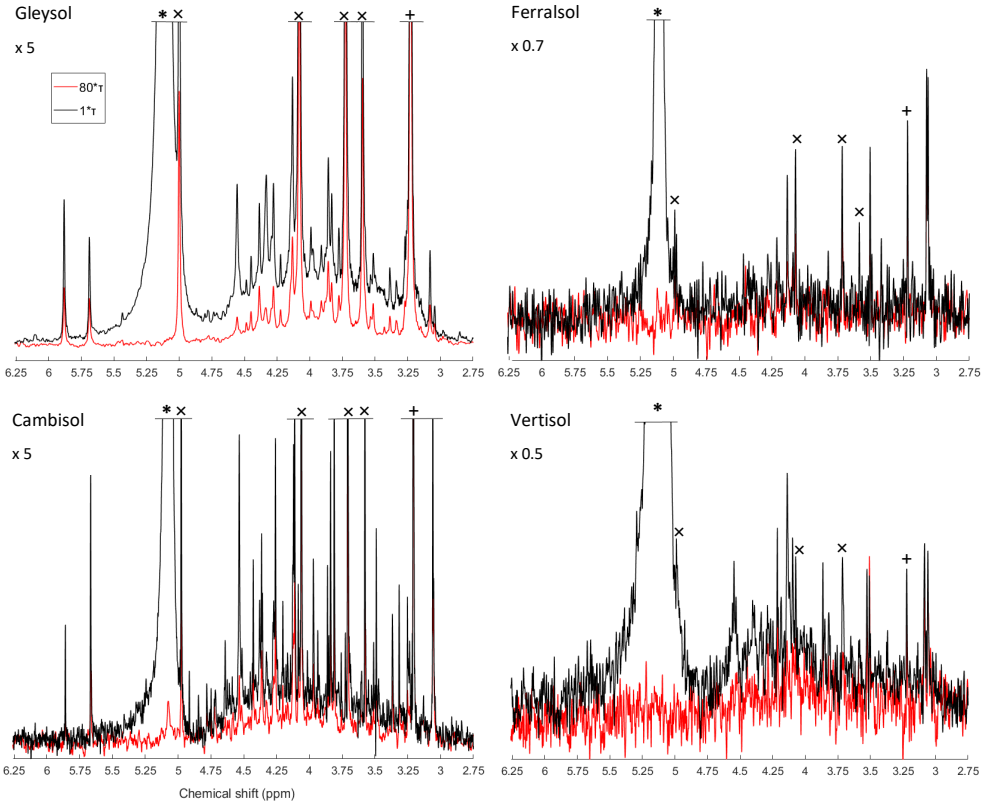

Figure 3. Solution ^{31}P NMR spectra of hypobromite oxidised soil extracts acquired with a CPMG pulse sequence with 1*τ (black) and 80*τ (red) spin-echo delays. The orthophosphate (*), *scyllo*-IP$_6$ (+) and *myo*-IP$_6$ peaks (×) are marked accordingly. Spectra were normalised to the maximum *scyllo*-IP$_6$ peak intensity in the 1*τ spectrum for each soil. The vertical axes were increased/decreased for better visualisation by an indicated factor.

319 **4 Discussion**

320 **4.1 Pools of phosphorus in untreated and hypobromite oxidised soil extracts**

321 On average, 44 % of total P (as measured with XRF) was extracted by NaOH-EDTA, which is consistent with
322 previous studies (McLaren et al., 2019; Li et al., 2018; Turner, 2008). The non-extractable pool of P is likely to
323 comprise of inorganic P as part of insoluble mineral phases, but could also contain some organic P (McLaren et
324 al., 2015a). Nevertheless, the NaOH-EDTA extraction technique is considered to be a measure of total organic P
325 in soil, which can be subsequently characterised by solution ^{31}P NMR spectroscopy (Cade-Menun and Preston,
326 1996).
327 Hypobromite oxidation resulted in a decrease in the concentration of inorganic and organic P in NaOH-EDTA
328 extracts across all soils. The decrease of organic P is consistent with previous studies (Turner et al., 2012; Almeida
329 et al., 2018; Turner and Richardson, 2004). However, Almeida et al. (2018) reported an overall increase in the
330 concentration of inorganic P following hypobromite oxidation, which the authors proposed to be caused by the

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degradation of organic P forms not resistant to hypobromite oxidation. A decrease in the concentration of organic P in NaOH-EDTA extracts following hypobromite oxidation was expected based on the oxidation of organic molecules containing P. This will result in the production of carbon dioxide and simple organic acids (Irving and Cosgrove, 1981; Sharma, 2013).

Overall, hypobromite oxidation of NaOH-EDTA soil extracts resulted in a considerable increase in the number of sharp peaks and a decrease in the broad underlying peak in the phosphomonoester region compared to that of untreated soil extracts. This was particularly the case for the Cambisol and the Gleysol, which had high concentrations of extractable organic P. Since the broad peak is thought to be closely associated with the SOM (Bünemann et al., 2008; Dougherty et al., 2007; McLaren et al., 2015b), its decrease in soil extracts following hypobromite oxidation is consistent with that observed for other organic compounds (Turner et al., 2012). Our results indicate that the majority of sharp peaks present in the phosphomonoester region of untreated soil extracts are stable to hypobromite oxidation, and are therefore likely to be IP.

Across all soils, 5 to 15 peaks in the phosphomonoester region were removed following hypobromite oxidation compared to those in untreated extracts, which are likely due to the oxidation of: α-and β-glycerophosphate (Doolette et al., 2009; McLaren et al., 2015b), RNA mononucleotides (8 peaks) (Vincent et al., 2013), glucose 6-phosphate, phosphocholine, glucose 1-phosphate, or phosphorylethanolamine (Cade-Menun, 2015).

4.2 Phosphorus assignments of sharp peaks in hypobromite oxidised extracts

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). Turner et al. (2012) suggested that hypobromite oxidised extracts only contained *neo*-IP_6 in the 4-equatorial/2-axial conformation due to the absence of signals from the 2-equatorial/4-axial conformation. In the current study, both conformations could be identified, which is likely due to improved spectral resolution and sensitivity. The relative abundances of the four identified stereoisomers of IP_6 in soil extracts were similar to previous studies (Turner et al., 2012; Irving and Cosgrove, 1982).

Several studies have shown overlap of peaks relating to RNA mononucleotides and that of α-and β-glycerophosphate, which are the alkaline hydrolysis products of RNA and phospholipids, respectively. However, in the current study, several sharp peaks were present in hypobromite oxidised extracts which are in the chemical shift range of RNA mononucleotides and α-and β-glycerophosphate. 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 (Doolette et al., 2009), 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.

For the first time, we identified lower-order IP (IP_5 and IP_4) in soil extracts using solution ^{31}P NMR spectroscopy. Smith and Clark (1951) were the first to suggest the presence of IP_5 in soil extracts using anion-exchange chromatography, which was later confirmed (Cosgrove, 1963; McKercher and Anderson, 1968a; Anderson, 1955). Halstead and Anderson (1970) reported the presence of all four stereoisomers (*myo*, *scyllo*, *neo* and *chiro*) in the lower ester fractions (IP_2-IP_4) as well as the higher ester fractions (IP_5, IP_6) isolated from soil, with the *myo* stereoisomer being the main form in all fractions. In the current study, all four stereoisomers of IP_5 could be detected in the hypobromite oxidised soil extracts, which the *myo* and *scyllo* stereoisomers were the most abundant.

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The relative abundances of IP_5 stereoisomers are consistent with the findings of Irving and Cosgrove (1982) using gas-liquid chromatography on the combined IP_6 + IP_5 fraction. The detection of all four stereoisomers of IP_5 in NMR spectra provides direct spectroscopic evidence for their existence in soil extracts.

In addition to the four stereoisomers of IP_5, we were able to identify the presence of two isomers of *myo*-IP_5 in the Cambisol and Gleysol, i.e. *myo*-(1,2,4,5,6)-IP_5 and *myo*-(1,3,4,5,6)-IP_5. These two isomers have not yet been detected in soil extracts. A distinction of different *myo*-IP_5 isomers was not reported in earlier studies using chromatographic separation. In non-soil extracts, *myo*-(1,2,4,5,6)-IP_5 was detected by Doolette and Smernik (2016) in grapevine canes, and *myo*-(1,3,4,5,6)-IP_5 as the thermal decomposition product of a phytate standard (Doolette and Smernik, 2018). Sun et al. (2017) reported *myo*-(1,2,4,5,6)-IP_5 as an intermediate in the major pathways of Aspergillus niger phytase and acid phosphatase (potato) phytate degradation. The presence of *myo*-(1,2,3,4,6)-IP_5 could not be confirmed as NMR analyses on the compound itself exhibited a broad NMR signal (Fig. SI7 in the Supporting Information). This is because in solutions with a pH of 9.5 or above, the 1axial/5equatorial and 5axial/1 equatorial forms of *myo*-(1,2,3,4,6)-IP_5 are in a dynamic equilibrium, which can cause broadening (Volkmann et al., 2002). According to Turner and Richardson (2004) and Chung et al. (1999), the two identified *scyllo*-IP_4 peaks (signal pattern 2:2) can be attributed to the *scyllo*-(1,2,3,4)-IP_4 isomer. However, the two peaks of *scyllo*-IP_4 overlapped in the Cambisol and Gleysol with the peak at the furthest upfield chemical shift of *myo*-(1,2,4,5,6)-IP_5 at δ 3.25 ppm, and with the peak at the furthest downfield chemical shift of *myo*-(1,3,4,5,6)-IP_5 at δ 4.12 ppm.

Whilst on average 48 % of the sharp peaks in the phosphomonoester region of soil extracts following hypobromite oxidation could be attributed to IP_6, IP_5 and *scyllo*-IP_4, the identity of many sharp peaks remain unknown. An unidentified peak at δ 4.33 ppm is present in all soil samples except in the Ferralsol, with concentrations of up to 10 mg P/kg_{soil} (Cambisol). Other unidentified peaks at δ 3.49, 3.86, 4.20 and 3.91 ppm were detected in all soils, with concentrations ranging from 1 to 2 mg P/kg_{soil}. Interestingly, two peaks upfield of *scyllo*-IP_6 became more prominent (at δ 3.08, 3.05 ppm) following hypobromite oxidation, which was particularly the case in the Vertisol soil. The diversity of organic P species in the Vertisol soil appears to be much greater than previously reported (McLaren et al., 2014). We hypothesise that many of these unidentified peaks arise from other isomers of *myo*- and *scyllo*-IP_5, based on the higher abundance of their IP_6 counterparts.

The ratio of IP_6 to lower-order IP varied across soils, which ranged in decreasing order: Gleysol ≫ Cambisol > Vertisol > Ferralsol. McKercher and Anderson (1968b) found a higher ratio of IP_6 to IP_5 in some Scottish soils (ratio 1.8 to 4.6) compared to some Canadian soils (0.9 to 2.4). The authors attributed this difference to the greater stabilization of IP_6 relative to lower esters in the Scottish soils, possible due to climatic reasons or effects of different soil properties. In a subsequent study, McKercher and Anderson (1968a) observed increased IP contents with increasing total organic P content. Studies of organic P speciation along chronosequences found that *myo*-IP_6 concentrations declined in older soils (Turner et al., 2007a; McDowell et al., 2007). Similarly, Baker (1976) found that the IP_6 + IP_5 concentrations in the Franz Josef chronosequence increased until 1000 years, followed by a rapid decline. In our soil samples, the highest IP_6 to IP_5 ratio was found in the soil with the highest SOM content, suggesting a possible stabilization of IP_6 due to association with SOM (Makarov et al., 1997; Borie et al., 1989). In contrast, the Ferralsol sample containing high amounts of Fe and Al showed the smallest IP_6 to IP_5 ratio, even though IP_6 is known to strongly adsorb to sesquioxides (Anderson et al., 1974; Anderson and Arlidge, 1962). However, the production, input and mineralisation rates of IP_6 and IP_5 are not known for our soil samples. Further research is needed to understand the mechanisms controlling the flux of lower-order IP in soil.

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In the Ferralsol and the Cambisol, there was an overall decrease in the concentration of IP_6 and IP_5 following hypobromite oxidation compared to the untreated extracts. Since the main cause of resistance of IP to hypobromite oxidation is that of steric hindrance, which generally decreases with decreasing phosphorylation state and conformation of the phosphate groups (axial vs. equatorial), we assume that low recoveries of added *myo*-IP_6 is due to losses of precipitated P_{org} compounds during the precipitation and dissolution steps. This is supported by the decrease in the concentration of orthophosphate following hypobromite oxidation compared to untreated extracts. Therefore, quantities of IP as reported in the current study should be considered as conservative.

4.3 Structural composition of phosphomonoesters in hypobromite oxidised soil extracts

The NMR spectra on hypobromite oxidised soil extracts revealed the presence of sharp and broad signals in the phosphomonoester region. Transverse relaxation experiments revealed a rapid decay of the broad signal compared to the sharp peaks of IP_6, which support the hypothesis that the compounds causing the broad signal arise from P compounds other than IP. These findings are consistent with that of McLaren et al. (2019), who probed the structural composition of phosphomonoesters in untreated soil extracts. Overall, measured T_2 times in the current study on hypobromite oxidised extracts were markedly longer compared to that on untreated extracts reported in McLaren et al. (2019). This could be due to removal of other organic compounds by hypobromite oxidation in the matrix and therefore a decrease in the viscosity of the sample. This would result in an overall faster tumbling of the molecules and hence an increased T_2 relaxation time. As reported by McLaren et al. (2019), calculations of the broad signal's linewidth based on the T_2 times were considerably lower compared to that of the standard deconvolution fitting (SDF). When applying the same calculations to our samples, the linewidth of the broad signal at half height is on average 5.2 Hz based on the T_2 times. In contrast, the linewidths acquired from the SDF average to 256.1 Hz. McLaren et al. (2019) suggested that the broad signal is itself comprised of more than one compound. Our results are consistent with this view and therefore it is likely that the main cause of the broad signal is a diversity of P molecules of differing chemical environments within this region, rather than the slow tumbling of just one macromolecule.

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. The large proportion of the broad signal in the total organic P pool demonstrates its importance in the soil P cycle.

Unexpectedly, the transverse relaxation times of orthophosphate were shorter than that of the broad signal. This was similarly the case in an untreated NaOH-EDTA extract of a forest soil with the same origin as the Cambisol as reported in McLaren et al. (2019). The authors hypothesised that this might be due to the sample matrix (i.e. high concentration of metals and organic matter). Whilst these factors are likely to affect T_2 times, they do not appear to be the main cause as the hypobromite oxidised extracts in the current study contained low concentrations of organic matter and metals as a consequence of the isolation procedure. The fast decay of orthophosphate was found across all four soil extracts with a diverse array of organic P concentrations and compositions of organic P in the phosphomonoester region. Therefore, another possible explanation could be a matrix effect or an association with large organic P compounds causing the broad signal (McLaren et al., 2019). It is known that dynamic

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intramolecular processes as ring inversion and intermolecular processes such as binding of small-molecule ligands to macromolecules can cause a broadening or a doubling of resonances (Claridge, 2016). When the smaller molecule is bound to the larger molecule, it experiences slower tumbling in the solution and hence a shorter T_2 time. It is possible that a chemical exchange of the orthophosphate with a compound in the matrix or an organic P molecule could result in the short T_2 time of the orthophosphate peak. We carried out a T_2 experiment on a pure solution of monopotassium phosphate (described in the Supporting Information), in which the matrix effects should be considerably reduced compared to the soil extracts. We found that the T_2 time of orthophosphate (203 ms) in the pure solution was considerably longer than that reported in soil extracts following hypobromite oxidation.

5 Conclusion

Inositol phosphates are an important pool of organic P in soil, but information on the mechanisms controlling their flux in soil remain limited due in part to an inability to detect them using solution ^{31}P NMR spectroscopy. For the first time, we identified six different lower-order IP in the solution ^{31}P NMR spectra on soil extracts. Solution ^{31}P NMR spectra on hypobromite oxidised extracts revealed the presence of up to 70 sharp peaks, which about 50 % could be identified. Our results indicate that the majority of the sharp peaks in solution ^{31}P NMR soil spectra were resistant to hypobromite oxidation, and therefore suggest the presence of diverse IP. Our study highlights the abundance of IP in soils and therefore their importance in terrestrial P cycles. Furthermore, we provide new insight on the large pool of phosphomonoesters represented by the broad signal, of which a considerably portion was resistant to hypobromite oxidation. Further research is needed to understand the chemical composition of the broad signal, and the mechanisms controlling its flux in terrestrial ecosystems.

Data availability

All data presented in this study and the Supplement is also available by request from the corresponding author.

Author contribution

The experimental design was planned by JR, TM, DZ, RV and EF. The experiments were carried out by JR under supervision of TM, DZ and RV. RV provided the MATLAB code for the standard deconvolution fitting of the NMR spectra. The data was processed, analysed and interpreted by JR with support from TM, DZ and RV. JR prepared the manuscript with contributions from all co-authors.

Conflicts of interest

The authors declare that they have no conflict of interest.

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690 **Table 1. General characteristics of soil samples used in this study.**

Soil type	-	Ferralsol	Vertisol	Cambisol	Gleysol
Country	-	Colombia	Australia	Germany	Switzerland
Coordinates sampling site	-	4°30' N / 71°19' W	27°52' S / 151°37' E	50°21' N / 9°55' E	47°05' N / 8°06' E
Elevation	m ASL	150	402	800	612
Sampling depth	cm	0-20	0-15	0-7	0-10
Year of sampling	year	1997	2017	2014	2017
Land use	-	Pasture	Arable field	Forest	Pasture
C_{tot}	g C/kg_{soil}	26.7	23.9	90.3	148.3
N_{tot}	g N/kg_{soil}	1.7	1.9	6.6	10.9
pH in H_2O	-	3.6	6.1	3.6	5.0

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692 **Table 2. Standard solutions used for the spiking experiment of the hypobromite oxidised soil extracts. All standards**
693 **were dissolved in 0.25 M NaOH and 0.05 M Na_2EDTA.**

Standard	Product number	Company/origin	Concentration of standard in NaOH-EDTA (mg/mL)
myo-IP_6	P5681	Merck (Sigma-Aldrich)	8.10
L-*chiro*-IP_6	Collection of Dr Max Tate		2.39
D-*chiro*-IP_6	CAY-9002341	Cayman Chemical	2.00
neo-IP_6	Collection of Dr Dennis Cosgrove, made up in 15 mM HCl		4.62
D-*myo*-(1,2,4,5,6)-IP_5	CAY-10008452-1	Cayman Chemical	2.00
myo-(1,2,3,4,6)-IP_5	93987	Merck (Sigma-Aldrich)	2.00
D-*myo*-(1,3,4,5,6)-IP_5	CAY-10009851-1	Cayman Chemical	2.00
D-*myo*-(1,2,3,5,6)-IP_5	CAY-10008453-1	Cayman Chemical	2.00
scyllo-IP_5	Collection of Dr Dennis Cosgrove		2.64
L-*chiro*-IP_5	Collection of Dr Dennis Cosgrove		2.24
neo-IP_5	Collection of Dr Dennis Cosgrove		2.45
myo-IP_4	Collection of Dr Dennis Cosgrove		2.76
scyllo-IP_4	Collection of Dr Dennis Cosgrove		2.41
neo-IP_4	Collection of Dr Dennis Cosgrove		2.33

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695 **Table 3. Concentrations of total P as measured by XRF and 0.25 M NaOH + 0.05 M EDTA extractable P before and**
696 **after hypobromite oxidation of soil extracts. Concentrations of total P in NaOH-EDTA extracts were determined by**
697 **ICP-OES, whereas that of molybdate reactive P (MRP) was determined by the malachite green method of Ohno and**
698 **Zibilske (1991). Concentrations of molybdate unreactive P (MUP) were calculated as the difference between total P and**
699 **MRP.**

Measure		Ferralsol	Vertisol	Cambisol	Gleysol
XRF	P_{tot} (mg P/kg_{soil})	320	1726	3841	2913
NaOH-EDTA extractable P (untreated)	P_{tot} (mg P/kg_{soil})	160	484	1850	1490
	MRP (mg P/kg_{soil})	67	351	525	610
	MUP (P_{org}) (mg P/kg_{soil})	93	133	1326	880
NaOH-EDTA extractable P (hypobromite oxidised)	P_{tot} (mg P/kg_{soil})	77	158	580	578
	MRP (mg P/kg_{soil})	32	111	283	231
	MUP (P_{org}) (mg P/kg_{soil})	45	47	297	348

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701 **Table 4. Concentrations (mg P/kg$_{soil}$) of P compounds in solution ^{31}P NMR spectra of 0.25 M NaOH + 0.05 M EDTA**
702 **soil extracts (Ferralsol, Vertisol, Cambisol and Gleysol) before and after hypobromite oxidation (HO). Quantification**
703 **was based on spectral integration and deconvolution fitting. The proportion of P detected in hypobromite oxidised**
704 **extracts compared to that in untreated extracts is provided in brackets.**

Phosphorus class		Ferralsol	Vertisol	Cambisol	Gleysol
Phosphonates	before HO	1.0	2.6	14.5	-
	after HO	-	-	3.0 (21)	0.2
Orthophosphate	before HO	54.8	221.4	434.3	368.3
	after HO	32.0 (58)	116.6 (53)	329.3 (76)	243.4 (66)
Phosphomonoester	before HO	36.3	39.1	501.1	399.2
	after HO	12.7 (35)	24.2 (62)	210.3 (42)	292.1 (73)
Broad peak	before HO	21.6	30.9	305.8	216.7
	after HO	8.3 (39)	19.3 (63)	99.2 (32)	108.4 (50)
Phosphodiester	before HO	5.1	-	28.2	26.9
	after HO	-	-	-	2.0 (8)
Pyrophosphate	before HO	1.9	1.8	12.9	23.9
	after HO	-	-	-	-

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706 **Table 5. Concentrations of identified inositol phosphates (IP) in hypobromite oxidised 0.25 M NaOH + 0.05 M EDTA**
707 **soil extracts (Ferralsol, Vertisol, Cambisol and Gleysol). Concentrations were calculated from solution ^{31}P NMR spectra**
708 **using spectral deconvolution fitting including an underlying broad signal. When no concentration is given, the IP**
709 **compound was not detected in the respective soil extract. Chemical shift positions are based on the NMR spectrum of**
710 **the Cambisol extract (Fig. SI8 in the Supporting Information). Peak positions varied up to +0.018 ppm (Gleysol).**
711 **Conformation equatorial (eq) and axial (ax) according to Turner et al. (2012).**

Phosphorus compound	Chemical shift δ ppm	Concentrations (mg P/kg$_{soil}$)			
		Ferralsol	Vertisol	Cambisol	Gleysol
***myo*-IP$_6$**	4.97, 4.06, 3.70, 3.57	1.1	0.6	26.3	85.0
***scyllo*-IP$_6$**	3.20	0.4	0.3	15.6	41.1
***neo*-IP$_6$ 4-eq/2-ax**	5.86, 3.75	-	-	1.4	8.8
***neo*-IP$_6$ 2-eq/4-ax**	4.36, 4.11	-	-	4.0	1.3
***chiro*-IP$_6$ 2-eq/4-ax**	5.66, 4.25, 3.83	-	-	9.4	8.6
***myo*-(1,2,4,5,6)-IP$_5$**	4.42, 3.97, 3.72, 3.36, 3.25	-	-	7.0	4.1
***myo*-(1,3,4,5,6)-IP$_5$**	4.12, 3.60, 3.23	-	-	2.8	1.3
***scyllo*-IP$_5$**	3.81, 3.31, 3.05	0.7	0.5	10.8	6.1
***neo*-IP$_5$**	4.64, 4.27, 4.01, 3.87, 3.13	-	-	3.3	2.1
***chiro*-IP$_5$**	4.61, 3.39	-	-	0.9	-
***scyllo*-(1,2,3,4)-IP$_4$**	4.12, 3.25	0.8	-	4.3	1.0
Total IP		3.0	1.4	85.9	159.3

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713 **Table 6. Transversal relaxation times (T_2) of various P species in the orthophosphate and phosphomonoester regions as**
714 **determined by solution ^{31}P nuclear magnetic resonance (NMR) spectroscopy and a Carr-Purcell- Meiboom-Gill**
715 **(CPMG) pulse sequence on hypobromite oxidised soil extracts.**

716

Phosphorus compound	T_2 [ms]			
	Ferralsol	Vertisol	Cambisol	Gleysol
***myo*-IP_6**	163	140	139	121
***scyllo*-IP_6**	250	155	154	144
***neo*-IP_6**	-	-	203	102
***D-chiro*-IP_6**	-	-	108	132
orthophosphate	14	9	17	6
broad peak	44	69	89	62