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 the quantification of lower-order IP 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 A horizons from a Ferralsol (Colombia), a Cambisol and a Gleysol from Switzerland, and 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$_4$. 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.

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 (Angyal, 1963; Cosgrove and Irving, 1980). 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 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 (Strickland, 1973; Cosgrove and Irving, 1980).

Inositol phosphates have been reported to comprise more than 50 % of total organic phosphorus (P_{org}) in some soils (Cosgrove and Irving, 1980; McDowell and Stewart, 2006; Turner, 2007). 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 %) (Cosgrove and Irving, 1980; Turner et al., 2012). 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 mostly incapable of digesting myo-IP_6 without the addition of phytases to their diets (Leytem et al., 2004; Leytem and Maguire, 2007; Turner et al., 2007b). An exception to this are pigs, which were found to at least partially digest phytate (Leytem et al., 2004), and transgenic pigs expressing salivary phytase (Golovan et al., 2001; Zhang et al., 2018). 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 (Anderson et al., 1974; Ognalaga et al., 1994), 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; Anderson and Malcolm, 1974; Cosgrove and Irving, 1980; Irving and Cosgrove, 1982). 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, 1968b). 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 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 (Anderson and Malcolm, 1974; Cosgrove and Irving, 1980; Turner et al., 2002; 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 (Newman and Tate, 1980; Cade-Menun and Liu, 2014). 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 and a chelating agent (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 (Smith and Clark, 1951; Anderson, 1955; Cosgrove, 1963). 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 hypobromite oxidised extracts. Interestingly, the authors also reported the presence of NMR signals in the phosphomonoester region that could not be assigned to IP_6 and were 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 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. Furthermore, a better understanding of these organic P compounds in soil would also help improve strategies to increase their biological utilisation, which may reduce the amount of fertiliser needed in agricultural systems and thus influence the transfer of P to aquatic/marine ecosystems.

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). The four soil samples were chosen from a larger collection based on their diverse concentration of P_{org} and composition of the phosphomonoester region in NMR spectra (Reusser et al., 2020). 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 (Bünemann et al., 2016; Lang et al., 2017). 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 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, i.e. extracting 4 g of soil with 40 mL of extractant.

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). The hypobromite oxidation procedure is similar to that reported in Turner (2020). 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). 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 (Figure SI9). 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 molybdate unreactive P (MUP), which is predominantly organic P for these samples. To assess the of effect hypobromite oxidation on the stability of an IP_6, duplicate samples of the Cambisol and the Gleysol were 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):

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

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)

185   (Bruker Corporation; Billerica, MA). The ^{31}P frequency for this NMR spectrometer was 202.5 MHz and gated

186   broadband proton decoupling with a 90° pulse of 12 μs was applied. Spectral resolution under these conditions for

187   ^{31}P was < 1 Hz. Longitudinal relaxation (T_1) times were determined for each sample with an inversion recovery

188   experiment (Vold et al., 1968). This resulted in recycle delays ranging from 8.7 to 30.0 sec for the untreated

189   extracts and 7.8 to 38.0 sec for the hypobromite oxidised soil extracts. The number of scans for the untreated

190   extracts was set to 1024 or 4096, depending on the signal to noise ratio of the obtained spectrum. All hypobromite

191   oxidised spectra were acquired with 3700 to 4096 scans.

192   **2.6   Processing of NMR spectra**

193   All NMR spectra were processed with Fourier transformation, phase correction, and baseline adjustment within

194   the TopSpin® software environment (Version 3.5 pl 7, Bruker Corporation; Billerica, MA). Line broadening was

195   set to 0.6 Hz. Quantification of NMR signals involved obtaining the integrals of the following regions: 1) up to

196   four phosphonates (δ 19.8 to 16.4 ppm); 2) the added MDP (δ 17.0 to 15.8ppm) including its two carbon satellite

197   peaks; 3) the combined orthophosphate and phosphomonoester region (δ 6.0 to 3.0 ppm); 4) up to four

198   phosphodiesters (δ 2.5 to -3.0 ppm), and 5) pyrophosphate (δ -4.8 to -5.4 ppm). Due to overlapping peaks in the

199   orthophosphate and phosphomonoester region, spectral deconvolution fitting (SDF) was applied as described in

200   Reusser et al. (2020). In brief, the SDF procedure involved the fitting of an underlying broad signal, based on the

201   approach of Bünemann et al. (2008) and McLaren et al. (2019). We carried out the SDF with a non-linear

202   optimisation algorithm in MATLAB® R2017a (The MathWorks, Inc.) and fitted visually identifiable peaks by

203   constraining their line-widths at half height as well as the lower and upper boundary of the peak positions along

204   with an underlying broad signal in the phosphomonoester region. The sharp signals of high intensity (e.g.

205   orthophosphate) and the broad peak were fitted using Lorentzian lineshapes, whereas sharp signals of low intensity

206   were fitted using Gaussian lineshapes. The NMR observability of total P (P_{tot}) in NaOH-EDTA extracts was

207   calculated using Eq. (2) (Dougherty et al., 2005; Doolette et al., 2011b):

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

209   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

210   spectroscopy and P_{tot} ICP-OES refers to the total P concentration in mg P/kg$_{soil}$ measured in the soil extracts prior

211   to freeze-drying using ICP-OES.

212   **2.7   Spiking experiments**

213   To identify the presence of IP in hypobromite oxidised extracts, samples were spiked with a range of standards

214   and then analysed again using NMR spectroscopy. This involved the addition of 5 to 20 μL aliquots of an IP

215   standard solution directly into the NMR tube, which was then sealed with parafilm, manually shaken, and then

216   allowed to settle prior to NMR analysis. Each sample extract was consecutively spiked with no more than four IP

217   standards. The NMR spectra of soil extracts after spiking were overlaid with the NMR spectra of unspiked soil

218   extracts to identify the presence of IP across all soil samples. This comparison of NMR spectra was possible due

219   to negligible changes in the chemical shifts of peaks among soil samples. The IP standards used in this study are

220   listed in Table 2.

221   [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 McLaren et al. (2019). The T$_2$ times of the partitioned NMR signals were calculated using Eq. (3) within RStudio© (version 1.1.442):

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

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.

260 [Suggested location Table 3]

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

262 The most prominent signal in the NMR spectra of untreated NaOH-EDTA soil extracts was that of orthophosphate
263 at δ 5.25 (±0.25) ppm, followed by the phosphomonoester region ranging from δ 6.0 to 3.0 ppm (Fig. 1). There
264 were also some minor signals due to pyrophosphate δ -5.06 (±0.19) ppm (all soils), phosphodiesters ranging from
265 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
266 and 18.3 ppm (not detected in the Gleysol). However, these compounds comprised less than 8 % of the total NMR
267 signal.

268 Following hypobromite oxidation of NaOH-EDTA extracts, the most prominent NMR signals were found in the
269 orthophosphate (65 % of total NMR signal) and phosphomonoester (35 % of total NMR signal) region across all
270 soils (Fig. 1). Phosphodiesters and pyrophosphates were removed following hypobromite oxidation in the
271 Ferralsol, the Vertisol and the Cambisol (DE). However, some signal remained in the Gleysol at low concentrations
272 (0.4 % of the total NMR signal). Phosphonates were removed following hypobromite oxidation in the Ferralsol
273 and the Vertisol, but a total of five sharp peaks in the phosphonate region were detected (δ 19.59, 18.58, 17.27 and
274 9.25 ppm) in the Cambisol. These peaks comprised 0.6 % of the total NMR signal.

275 The phosphomonoester region of NMR spectra on untreated NaOH-EDTA extracts exhibited two main features:
276 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
277 256.12 Hz; and 2) the presence of between 19 and 34 sharp signals. This was similarly the case on hypobromite
278 oxidised extracts, except there was a decrease in the intensity of the broad signal and a change in the distribution
279 and intensity of sharp signals. For the Cambisol and Gleysol, the number of sharp signals in the phosphomonoester
280 region approximately doubled (to 40 and 70 sharp signals, respectively) following hypobromite oxidation. In
281 contrast, less than half of the sharp signals remained in the Ferralsol following hypobromite oxidation (i.e. 14 of
282 the 30 peaks originally present in the untreated extract), whereas one peak was removed following hypobromite
283 oxidation in the Vertisol. There was little change (0.23 ppm) in the chemical shifts of peaks between the untreated
284 and hypobromite oxidised extracts.

285
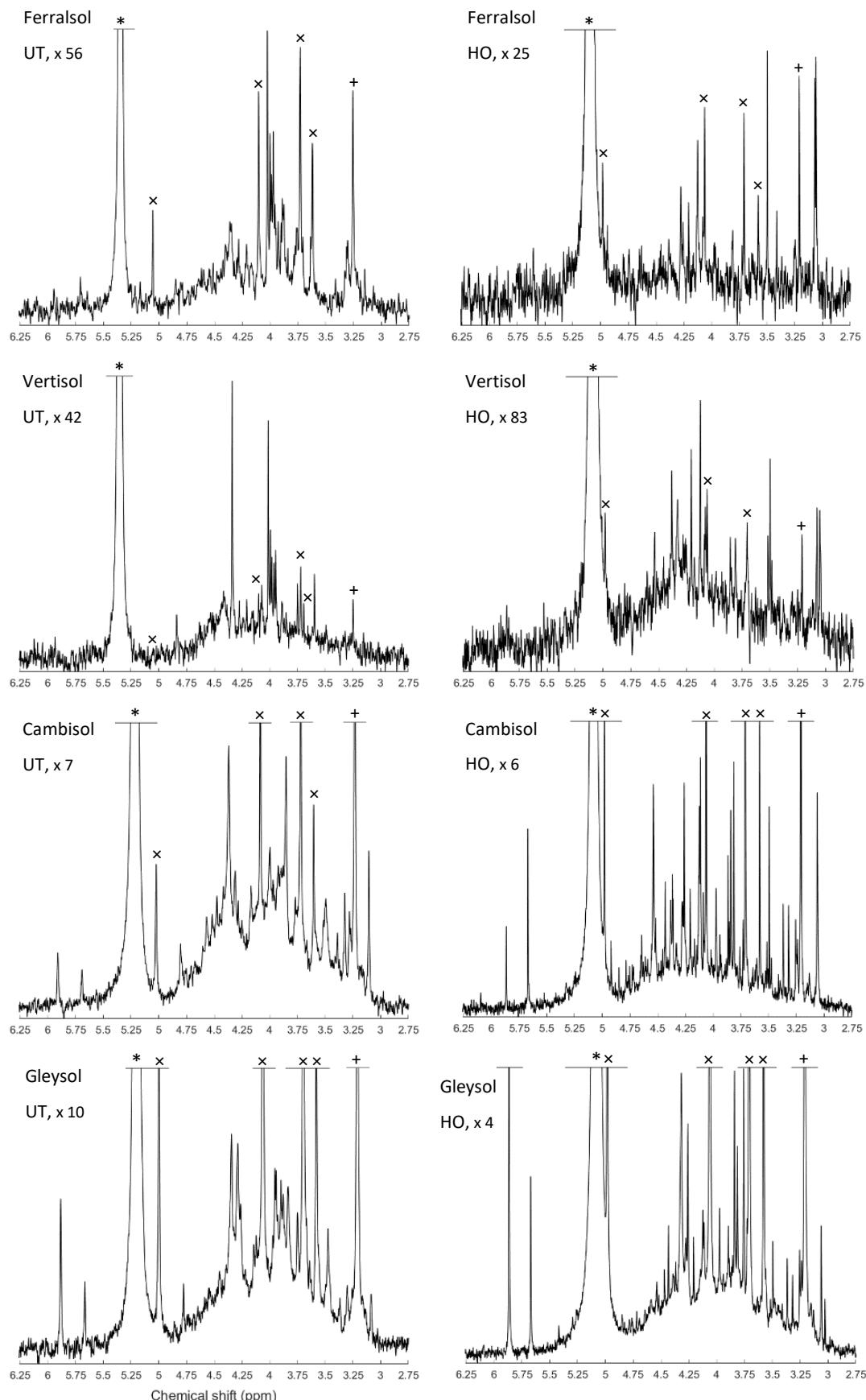
Figure 1. Solution ^{31}P nuclear magnetic resonance (NMR) spectra (500 MHz) of the orthophosphate and phosphomonoester region on untreated (UT) and hypobromite oxidised (HO) 0.25 M NaOH + 0.05 M EDTA soil extracts (Ferralsol, Vertisol, Cambisol and Gleysol). Signal intensities were normalised to the 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$_6$.

287   [Suggested location Table 4]

3.3   Identification and quantification of inositol phosphates (IP$_6$, IP$_5$ and IP$_4$) in soil extracts

289   Detailed views of the phosphomonoester regions of spiked samples are shown in Fig. SI1 to SI5 of the Supporting
290   Information. The number of identified sharp peaks in the phosphomonoester region ranged from 7 (Vertisol) to 33
291   (Gleysol). *myo*- and *scyllo*-IP$_6$ were identified in the hypobromite oxidised extracts of all soils (Table 5). On
292   average, 72 % of *myo*-IP$_6$ and 56 % of *scyllo*-IP$_6$ present in the untreated extracts remained in the hypobromite
293   oxidised extracts (Table SI1 in the Supporting Information). *neo*-IP$_6$ was identified in the the 2-equatorial/4-axial
294   and 4-equatorial/2-axial conformations, and c*hiro*-IP$_6$ in the 2-equatorial/4-axial confirmation, of the oxidised
295   extracts in the Cambisol and Gleysol, but were absent in the Ferralsol and the Vertisol (Fig. SI4 and SI5 in the
296   Supporting Information).

297   The *myo*, *scyllo*, *chiro* and *neo* stereoisomers of IP$_5$ were identified in various hypobromite oxidised extracts (Table
298   5). Two isomers of *myo*-IP$_5$ were identified in some extracts, which included *myo*-(1,2,4,5,6)-IP$_5$ and *myo*-
299   (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
300   evidence for the presence of *myo*-IP$_4$ in these soil samples, as only one of the two peaks of this compound was
301   present in the NMR spectra of untreated extracts. This could possibly be due to the partial dephosphorylation of
302   *myo*-IP$_4$ during the hypobromite oxidation procedure. The reason of the reduced resistance of lower order IP to
303   hypobromite oxidation compared to IP$_{5+6}$ might be due to their reduced steric hindrance and charge density, as less
304   phosphate groups are bound to the inositol ring.

305   Concentrations of total IP ranged from 1.4 to 159.3 mg P/kg$_{soil}$ across all soils, which comprised between 1 %
306   (Vertisol) and 18 % (Gleysol) of the organic P in untreated NaOH-EDTA extracts (Table 3). Pools of IP$_6$ were the
307   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
308   IP$_6$ stereoisomers across all soils were in the order of *myo* (61 %, SD=12), *scyllo* (29 %, SD=3), *chiro* (6 %, SD=8)
309   and *neo* (4 %, SD=5). Similarly, the *myo* and *scyllo* stereoisomer were also the most predominant forms of IP$_5$,
310   but comprised between 83 % (Cambisol) and 100 % (Ferralsol and Vertisol) of total IP$_5$ (Table 5). Trace amounts
311   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.
312   2).

313   [Suggested location Table 5]
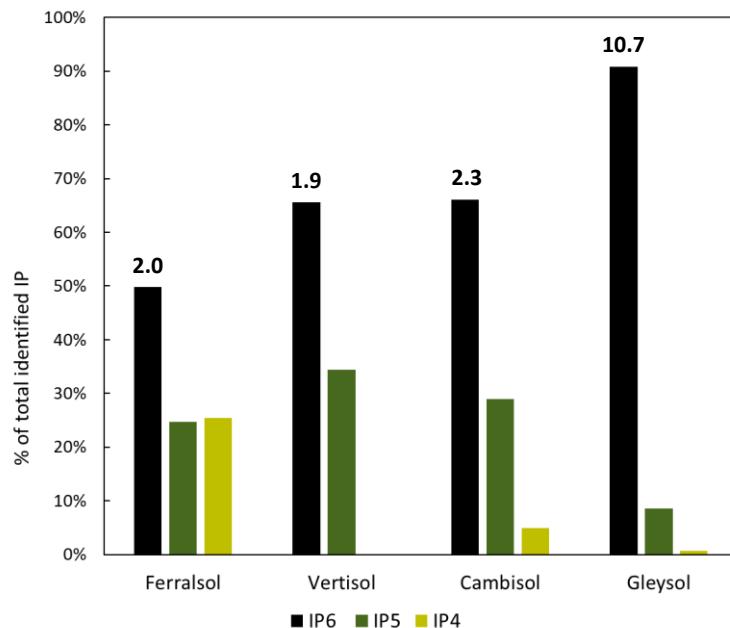
Figure 2. The proportion of total identifiable pools of inositol hexakisphosphates (IP₆), -pentakisphosphates (IP₅) or – tetrakisphosphates (IP₄) to that of the total pool of identifiable IP, as determined by solution ³¹P NMR spectroscopy on four soil extracts (Ferralsol, Vertisol, Cambisol and Gleysol) following hypobromite oxidation. Values located above the IP₆ bar are the ratio of total identifiable IP₆ to that of IP₅ in each soil sample.

314

315   If sharp peaks arising from IP were identified in the NMR spectra on hypobromite oxidised extracts, a comparison

316   was made with that of their corresponding untreated extracts. The sharp peaks of all stereoisomers of IP₆ were

317   present in the untreated extracts. The five peaks of *myo*-(1,2,4,5,6)-IP₅ and the three peaks of *scyllo*-IP₅ were also

318   identified. However, it was not possible to clearly identify other IP₅ compounds in untreated extracts due to

319   overlapping signals. In the Gleysol, all three peaks of *scyllo*-IP₅ were detected, but only two of the possible five

320   peaks could be clearly assigned to *myo*-(1,2,4,5,6)-IP₅. In the Ferralsol, both peaks of *scyllo*-IP₄ were present in

321   the untreated extract, but only two of the three possible peaks could be assigned to *scyllo*-IP₅. In the Vertisol, no

322   IP₅ was identified. Concentrations of IP in untreated extracts assessed by spectral deconvolution fitting were

323   generally double than that measured in hypobromite oxidised extracts. Recoveries of added *myo*-IP₆ in the Gleysol

324   and Cambisol following hypobromite oxidation were 47 % and 20 %, respectively.

325   **3.4   Spin-echo analysis of selected P compounds**

326   Due to the presence of sharp and broad signals in hypobromite oxidised soil extracts, the structural composition

327   of phosphomonoesters was probed. A comparison of the NMR spectra at the lowest (1*τ) and highest (80*τ) pulse

328   delays revealed a fast decaying broad signal for all hypobromite oxidised soil extracts, which was particularly

329   evident in the Gleysol (Fig. 3). Calculated T_2 times of all IP₆ stereoisomers were longer than that of the broad

330   signal (Table 6). The T_2 times of *scyllo*-IP₆ (on average 175.8 ms, SD=49.7) were generally the longest of all

331   stereoisomers of IP₆. The T_2 time of the orthophosphate peak was the shortest, which was on average 11.5 ms

332   (SD=4.9).

333   The average (n=4) T_2 times of the broad peak was significantly different than that of *scyllo*- and *myo*-IP₆ ($p <$

334   0.05). Significant differences in the T_2-times of *neo*- and D-*chiro*-IP₆ were not tested, as these compounds were

335   not detected in the Ferralsol and the Vertisol.
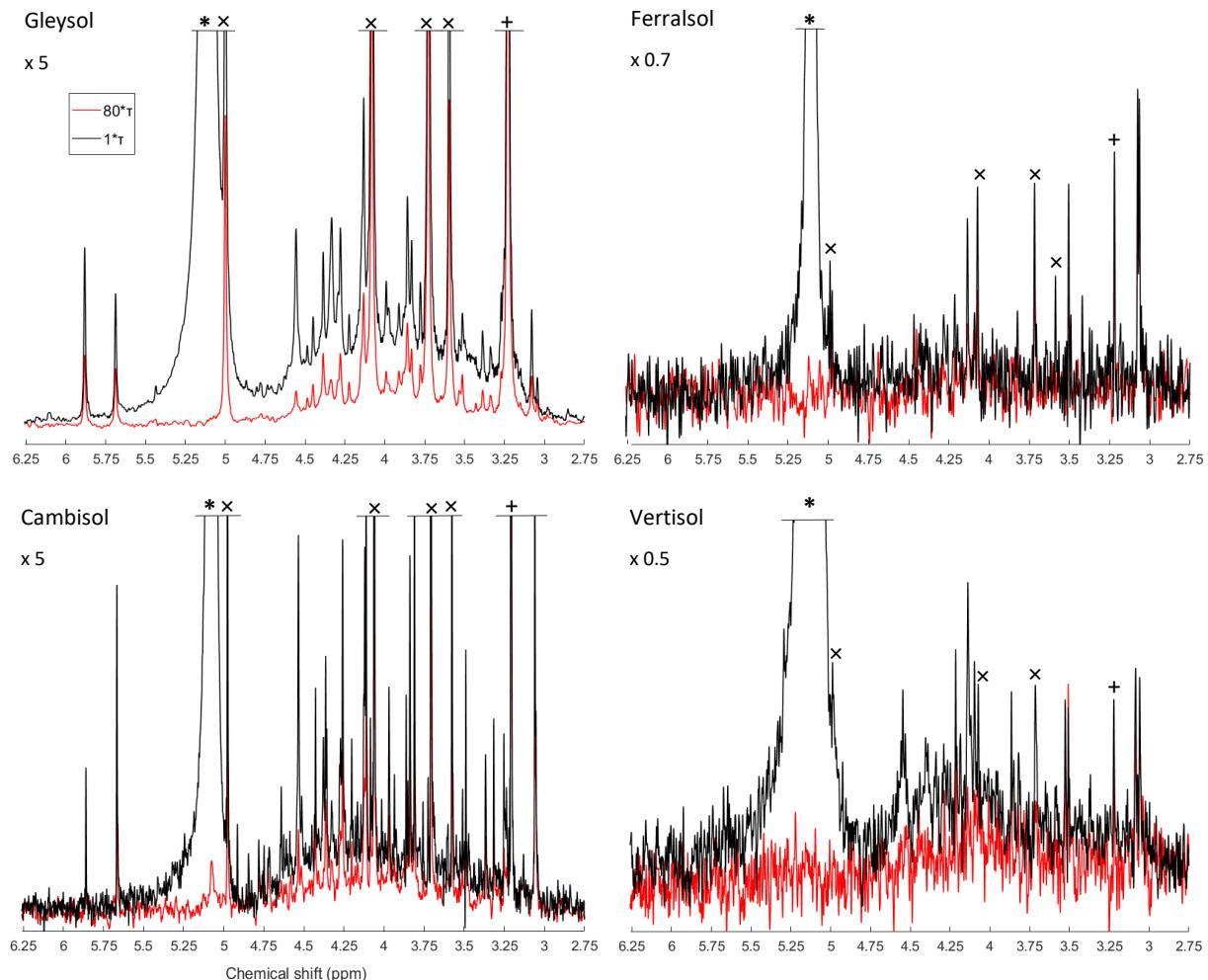
Figure 3. Solution ^{31}P NMR spectra of hypobromite oxidised soil extracts acquired with a CPMG pulse sequence with 1*τ (black) and 80*τ (red) spin-echo delays. The orthophosphate (*), *scyllo*-IP$_6$ (+) and *myo*-IP$_6$ peaks (×) are marked accordingly. Spectra were normalised to the maximum *scyllo*-IP$_6$ peak intensity in the 1*τ spectrum for each soil. The vertical axes were increased/decreased for better visualisation by an indicated factor.

4   Discussion

4.1   Pools of phosphorus in untreated and hypobromite oxidised soil extracts

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., 2018; 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). Nevertheless, the NaOH-EDTA extraction technique is considered to be a measure of total organic P in soil, which can be subsequently characterised by solution ^{31}P NMR spectroscopy (Cade-Menun and Preston, 1996).

Hypobromite oxidation resulted in a decrease in the concentration of inorganic and organic P in NaOH-EDTA extracts across all soils. The decrease of organic P is consistent with previous studies (Turner and Richardson, 2004; Turner et al., 2012; Almeida et al., 2018). However, Almeida et al. (2018) reported an overall increase in the concentration of inorganic P following hypobromite oxidation, which the authors proposed to be caused by the

350   degradation of organic P forms not resistant to hypobromite oxidation. A decrease in the concentration of organic
351   P in NaOH-EDTA extracts following hypobromite oxidation was expected based on the oxidation of organic
352   molecules containing P. The products of hypobromite oxidation are most probably carbon dioxide, simple organic
353   acids from the oxidative cleavage of the phosphoesters and orthophosphate (Irving and Cosgrove, 1981; Sharma,
354   2013).

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

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

367   ## 4.2 Phosphorus assignments of sharp peaks in hypobromite oxidised extracts

368   The detection of *myo-, scyllo-, chiro*, and *neo*-IP_6 in untreated and hypobromite oxidised soil extracts is consistent
369   with previous studies using chromatography (Irving and Cosgrove, 1982; Almeida et al., 2018) and NMR (Turner
370   and Richardson, 2004; Doolette et al., 2011a; Vincent et al., 2013; Jarosch et al., 2015; McLaren et al., 2015b).
371   Turner et al. (2012) suggested that hypobromite oxidised extracts only contained *neo*-IP_6 in the 4-equatorial/2-
372   axial conformation due to the absence of signals from the 2-equatorial/4-axial conformation. In the current study,
373   both conformations could be identified in two of the four soil extracts, which is likely due to improved spectral
374   resolution and sensitivity. The relative abundances of the four identified stereoisomers of IP_6 in soil extracts were
375   similar to previous studies (Irving and Cosgrove, 1982; Turner et al., 2012).

376   Several studies have shown overlap of peaks relating to RNA mononucleotides and that of α-and β-
377   glycerophosphate, which are the alkaline hydrolysis products of RNA and phospholipids, respectively. However,
378   in the current study, several sharp peaks were present in hypobromite oxidised extracts which are in the chemical
379   shift range of RNA mononucleotides and α-and β-glycerophosphate. Whilst a peak at δ 4.36 ppm would be
380   assigned to α-glycerophosphate based on spiking experiments in the untreated extracts of the Cambisol and the
381   Gleysol, hypobromite oxidation revealed the presence of the 2-equatorial/4-axial C2,5 peak of *neo*-IP_6 at δ
382   4.37 ppm, and also an unidentified peak at δ 4.36 ppm in the Cambisol. Therefore, the assignment and
383   concentration of α-glycerophosphate may be unreliable in some soils of previous studies.

384   For the first time, we identified lower-order IP (IP_5 and IP_4) in soil extracts using solution ^{31}P NMR spectroscopy.
385   Smith and Clark (1951) were the first to suggest the presence of IP_5 in soil extracts using anion-exchange
386   chromatography, which was later confirmed (Anderson, 1955; Cosgrove, 1963; McKercher and Anderson, 1968b).
387   Halstead and Anderson (1970) reported the presence of all four stereoisomers (*myo, scyllo, neo* and *chiro*) in the
388   lower ester fractions (IP_2-IP_4) as well as the higher ester fractions (IP_5, IP_6) isolated from soil, with the *myo*
389   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, of which the *myo* and *scyllo* stereoisomers were the most abundant. 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). It is possible that an abiotic transformation of *myo*-IP_6 to *myo*-(1,3,4,5,6)-IP_5 occurs, which could then be adsorbed by soil constituents. Stephens and Irvine (1990) reported *myo*-(1,3,4,5,6)-IP_5 as an intermediate in the synthesis of IP_6 from *myo*-IP in the cellular slime mould *Dictyostelium*. Therefore, *myo*-(1,3,4,5,6)-IP_5 could have been biologically added to the soil. Furthermore, *myo*-(1,3,4,5,6)-IP_5 was present in different animal feeds and manures (Sun and Jaisi, 2018). Sun et al. (2017) reported *myo*-(1,3,4,5,6)-IP_5 and *myo*-(1,2,4,5,6)-IP_5 as intermediates in the minor, resp. 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 1-axial/5-equatorial and 5-axial/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. Turner and Richardson (2004) reported NMR-signals for two other *scyllo*-IP_4 isomers, which could not be tested for in this study due to the lack of available standards.

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 $\gg$ Cambisol > Vertisol > Ferralsol. McKercher and Anderson (1968a) 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 (1968b) 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 (McDowell et al., 2007; Turner et al., 2007a; Turner et al., 2014). Similarly,

431   Baker (1976) found that the $IP_6 + IP_5$ concentrations in the Franz Josef chronosequence increased until 1000 years,

432   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

433   SOM content, suggesting a possible stabilization of IP_6 due to association with SOM (Borie et al., 1989; Makarov

434   et al., 1997). In contrast, the Ferralsol sample containing high amounts of Fe and Al showed the smallest IP_6 to IP_5

435   ratio, even though IP_6 is known to strongly adsorb to sesquioxides (Anderson and Arlidge, 1962; Anderson et al.,

436   1974). However, the production, input and mineralisation rates of IP_6 and IP_5 are not known for our soil samples.

437   Further research is needed to understand the mechanisms controlling the flux of lower-order IP in soil.

438   In the Ferralsol and the Cambisol, there was an overall decrease in the concentration of IP_6 and IP_5 following

439   hypobromite oxidation compared to the untreated extracts. Since the main cause of resistance of IP to hypobromite

440   oxidation is that of steric hindrance, which generally decreases with decreasing phosphorylation state and

441   conformation of the phosphate groups (axial vs. equatorial), we assume that low recoveries of added myo-IP_6 is

442   due to losses of precipitated P_{org} compounds during the precipitation and dissolution steps. This is supported by

443   the decrease in the concentration of orthophosphate following hypobromite oxidation compared to untreated

444   extracts. Therefore, quantities of IP as reported in the current study should be considered as conservative.

445   **4.3   Structural composition of phosphomonoesters in hypobromite oxidised soil extracts**

446   The NMR spectra on hypobromite oxidised soil extracts revealed the presence of sharp and broad signals in the

447   phosphomonoester region. Transverse relaxation experiments revealed a rapid decay of the broad signal compared

448   to the sharp peaks of IP_6, which support the hypothesis that the compounds causing the broad signal arise from P

449   compounds other than IP. These findings are consistent with that of McLaren et al. (2019), who probed the

450   structural composition of phosphomonoesters in untreated soil extracts. Overall, measured T_2 times in the current

451   study on hypobromite oxidised extracts were markedly longer compared to that on untreated extracts reported in

452   McLaren et al. (2019). This could be due to removal of other organic compounds by hypobromite oxidation in the

453   matrix and therefore a decrease in the viscosity of the sample. This would result in an overall faster tumbling of

454   the molecules and hence an increased T_2 relaxation time. As reported by McLaren et al. (2019), calculations of the

455   broad signal's linewidth based on the T_2 times were considerably lower compared to that of the standard

456   deconvolution fitting (SDF). When applying the same calculations to our samples, the linewidth of the broad signal

457   at half height is on average 5.2 Hz based on the T_2 times. In contrast, the linewidths acquired from the SDF average

458   to 256.1 Hz. McLaren et al. (2019) suggested that the broad signal is itself comprised of more than one compound.

459   Our results are consistent with this view and therefore it is likely that the main cause of the broad signal is a

460   diversity of P molecules of differing chemical environments within this region, rather than the slow tumbling of

461   just one macromolecule. Nebbioso and Piccolo (2011) reported that high molecular weight material of organic

462   matter in soil is an association of smaller organic molecules. We suggest that these associations would still cause

463   a broad signal in the phosphomonoester region of soil extracts and could be a reason that some organic molecules

464   containing P are protected from hypobromite oxidation.

465   Since a portion of the broad signal is resistant to hypobromite oxidation, this suggests the organic P is complex

466   and in the form of polymeric structures. The chemical resistance of the broad signal to hypobromite oxidation may

467   also indicate a high stability in soil (Jarosch et al., 2015). Annaheim et al. (2015) found that concentrations of the

468   broad signal remained unchanged between three different organic fertiliser strategies after 62 years of cropping.

469   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 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 great diversity and abundance of IP in soils and therefore their importance in terrestrial P cycles. Further research on the mechanisms and processes involved in the cycling of this wide variety of IP in soil will have implications on our understanding of organic P turnover as well as plant availability, and possibly help improve fertiliser strategies in agricultural systems.

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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References

Almeida, D. S., Menezes-Blackburn, D., Turner, B. L., Wearing, C., Haygarth, P. M., and Rosolem, C. A.: Urochloa ruziziensis cover crop increases the cycling of soil inositol phosphates, Biology and Fertility of Soils, 54, 935-947, 10.1007/s00374-018-1316-3, 2018.

Anderson, G.: Paper chromatography of inositol phosphates, Nature, 175, 863-864, 10.1038/175863b0, 1955.

Anderson, G., and Arlidge, E. Z.: The adsorption of inositol phosphates and glycerophosphate by soil clays, clay minerals, and hydrated sesquioxides in acid media., Journal of Soil Science, 13, 216-224, 10.1111/j.1365-2389.1962.tb00699.x, 1962.

Anderson, G., and Malcolm, R. E.: The nature of alkali-soluble soil organic phosphates., Journal of Soil Science, 25, 282-297, 10.1111/j.1365-2389.1974.tb01124.x, 1974.

Anderson, G., Williams, E. G., and Moir, J. O.: A comparison of the sorption of inorganic orthophosphate and inositol hexaphosphate by six acid soils, Journal of Soil Science, 25, 51-62, 10.1111/j.1365-2389.1974.tb01102.x, 1974.

Angyal, S. J.: Chapter VIII - Cyclitols, in: Comprehensive Biochemistry, edited by: Florkin, M., and Stotz, E. H., Elsevier, 297-303, 1963.

Annaheim, K. E., Doolette, A. L., Smernik, R. J., Mayer, J., Oberson, A., Frossard, E., and Bünemann, E. K.: Long-term addition of organic fertilizers has little effect on soil organic phosphorus as characterized by ^{31}P NMR spectroscopy and enzyme additions, Geoderma, 257-258, 67-77, https://doi.org/10.1016/j.geoderma.2015.01.014, 2015.

Baker, R. T.: Changes in the chemical nature of soil organic phosphate during pedogenesis., Journal of Soil Science, 27, 504-512, 10.1111/j.1365-2389.1976.tb02020.x, 1976.

Borie, F., Zunino, H., and Martínez, L.: Macromolecule‐P associations and inositol phosphates in some chilean volcanic soils of temperate regions, Communications in Soil Science and Plant Analysis, 20, 1881-1894, 10.1080/00103628909368190, 1989.

Bühler, S., Oberson, A., Sinaj, S., Friesen, D. K., and Frossard, E.: Isotope methods for assessing plant available phosphorus in acid tropical soils, European Journal of Soil Science, 54, 605-616, 10.1046/j.1365-2389.2003.00542.x, 2003.

Bünemann, E. K., Smernik, R. J., Marschner, P., and McNeill, A. M.: Microbial synthesis of organic and condensed forms of phosphorus in acid and calcareous soils, Soil Biology and Biochemistry, 40, 932-946, https://doi.org/10.1016/j.soilbio.2007.11.012, 2008.

Bünemann, E. K., Augstburger, S., and Frossard, E.: Dominance of either physicochemical or biological phosphorus cycling processes in temperate forest soils of contrasting phosphate availability, Soil Biology and Biochemistry, 101, 85-95, https://doi.org/10.1016/j.soilbio.2016.07.005, 2016.

Cade-Menun, B., and Liu, C. W.: Solution phosphorus-31 nuclear magnetic resonance spectroscopy of soils from 2005 to 2013: a review of sample preparation and experimental parameters, Soil Science Society of America Journal, 78, 19-37, 10.2136/sssaj2013.05.0187dgs, 2014.

Cade-Menun, B. J., and Preston, C. M.: A comparison of soil extraction procedures for ^{31}P NMR spectroscopy, Soil Science, 161, 1996.

Cade-Menun, B. J., Liu, C. W., Nunlist, R., and McColl, J. G.: Soil and litter phosphorus-31 nuclear magnetic resonance spectroscopy, Journal of Environmental Quality, 31, 457-465, 10.2134/jeq2002.4570, 2002.

Cade-Menun, B. J.: Improved peak identification in ^{31}P-NMR spectra of environmental samples with a standardized method and peak library, Geoderma, 257-258, 102-114, https://doi.org/10.1016/j.geoderma.2014.12.016, 2015.

Celi, L., and Barberis, E.: Abiotic reactions of inositol phosphates in soil, in: Inositol phosphates: linking agriculture and the environment, edited by: Turner, B. L., Richardson, A. E., and Mullaney, E. J., CABI, Wallingford, 207-220, 2007.

Chung, S.-K., Kwon, Y.-U., Chang, Y.-T., Sohn, K.-H., Shin, J.-H., Park, K.-H., Hong, B.-J., and Chung, I.-H.: Synthesis of all possible regioisomers of *scyllo*-Inositol phosphate, Bioorganic & Medicinal Chemistry, 7, 2577-2589, https://doi.org/10.1016/S0968-0896(99)00183-2, 1999.

573 Claridge, T. D. W.: Chapter 2 - Introducing High-Resolution NMR, in: High-Resolution NMR techniques
574 in organic chemistry, 3 ed., edited by: Claridge, T. D. W., Elsevier, Boston, 11-59, 2016.
575 Cosgrove, D.: The chemical nature of soil organic phosphorus. I. Inositol phosphates, Soil Research, 1,
576 203-214, https://doi.org/10.1071/SR9630203, 1963.
577 Cosgrove, D. J., and Irving, G. C. J.: Inositol phosphates : their chemistry, biochemistry and physiology,
578 Studies in organic chemistr, Amsterdam : Elsevier, 1980.
579 Doolette, A. L., Smernik, R. J., and Dougherty, W. J.: Spiking improved solution phosphorus-31 nuclear
580 magnetic resonance identification of soil phosphorus compounds, Soil Science Society of America
581 Journal, 73, 919-927, 10.2136/sssaj2008.0192, 2009.
582 Doolette, A. L., Smernik, R. J., and Dougherty, W. J.: A quantitative assessment of phosphorus forms in
583 some Australian soils, Soil Research, 49, 152-165, https://doi.org/10.1071/SR10092, 2011a.
584 Doolette, A. L., Smernik, R. J., and Dougherty, W. J.: Overestimation of the importance of phytate in
585 NaOH–EDTA soil extracts as assessed by 31P NMR analyses, Organic Geochemistry, 42, 955-964,
586 https://doi.org/10.1016/j.orggeochem.2011.04.004, 2011b.
587 Doolette, A. L., and Smernik, R. J.: Phosphorus speciation of dormant grapevine (Vitis vinifera L.) canes
588 in the Barossa Valley, South Australia, Australian Journal of Grape and Wine Research, 22, 462-468,
589 10.1111/ajgw.12234, 2016.
590 Doolette, A. L., and Smernik, R. J.: Facile decomposition of phytate in the solid-state: kinetics and
591 decomposition pathways, Phosphorus, Sulfur, and Silicon and the Related Elements, 193, 192-199,
592 10.1080/10426507.2017.1416614, 2018.
593 Dougherty, W. J., Smernik, R. J., and Chittleborough, D. J.: Application of spin counting to the solid-
594 state ^{31}P NMR analysis of pasture soils with varying phosphorus content, Soil Science Society of
595 America Journal, 69, 2058-2070, 10.2136/sssaj2005.0017, 2005.
596 Dougherty, W. J., Smernik, R. J., Bünemann, E. K., and Chittleborough, D. J.: On the use of hydrofluoric
597 acid pretreatment of soils for phosphorus-31 nuclear magnetic resonance analyses, Soil Science
598 Society of America Journal, 71, 1111-1118, 10.2136/sssaj2006.0300, 2007.
599 Dyer, W. J., and Wrenshall, C. L.: Organic phosphorus in soils: III. The decomposition of some organic
600 phosphorus compounds in soil cultures, Soil Sci., 51, 323, 1941.
601 FAO, and Group, I. W.: World reference base for soil resources 2014, World soil resources reports,
602 Food and Agriculture Organization of the United Nations FAO, Rome, 2014.
603 Fatiadi, A. J.: Bromine oxidation of inositols for preparation of inosose phenylhydrazones and
604 phenylosazones, Carbohydrate Research, 8, 135-147, https://doi.org/10.1016/S0008-6215(00)80149-
605 4, 1968.
606 Goring, C. A. I., and Bartholomew, W. V.: Microbial products and soil organic matter: III. Adsorption of
607 carbohydrate phosphates by clays, Soil Science Society of America Journal, 15, 189-194,
608 10.2136/sssaj1951.036159950015000C0043x, 1951.
609 Halstead, R. L., and Anderson, G.: Chromatographic fractionation of organic phosphates from alkali,
610 acid, and aqueous acetylacetone extracts of soils, Canadian Journal of Soil Science, 50, 111-119,
611 10.4141/cjss70-018, 1970.
612 Hochberg, Y., and Tamhane, A. C.: Multiple comparison procedures, Wiley series in probability and
613 mathematical statistics. Applied probability and statistics, Wiley New York, 1987.
614 Irvine, R. F., and Schell, M. J.: Back in the water: the return of the inositol phosphates, Nature Reviews
615 Molecular Cell Biology, 2, 327, 10.1038/35073015, 2001.
616 Irving, G. C. J., and Cosgrove, D. J.: The use of hypobromite oxidation to evaluate two current methods
617 for the estimation of inositol polyphosphates in alkaline extracts of soils, Communications in Soil
618 Science and Plant Analysis, 12, 495-509, 10.1080/00103628109367169, 1981.
619 Irving, G. C. J., and Cosgrove, D. J.: The use of gas‐liquid chromatography to determine the
620 proportions of inositol isomers present as pentakis‐ and hexakisphosphates in alkaline extracts of
621 soils, Communications in Soil Science and Plant Analysis, 13, 957-967, 10.1080/00103628209367324,
622 1982.
623 Jarosch, K. A., Doolette, A. L., Smernik, R. J., Tamburini, F., Frossard, E., and Bünemann, E. K.:
624 Characterisation of soil organic phosphorus in NaOH-EDTA extracts: a comparison of ^{31}P NMR

spectroscopy and enzyme addition assays, Soil Biology and Biochemistry, 91, 298-309, https://doi.org/10.1016/j.soilbio.2015.09.010, 2015.

L'Annunziata, M. F.: The origin and transformations of the soil inositol phosphate isomers, Soil Science Society of America Journal, 39, 377-379, 10.2136/sssaj1975.03615995003900020041x, 1975.

Lang, F., Krüger, J., Amelung, W., Willbold, S., Frossard, E., Bünemann, E. K., Bauhus, J., Nitschke, R., Kandeler, E., Marhan, S., Schulz, S., Bergkemper, F., Schloter, M., Luster, J., Guggisberg, F., Kaiser, K., Mikutta, R., Guggenberger, G., Polle, A., Pena, R., Prietzel, J., Rodionov, A., Talkner, U., Meesenburg, H., von Wilpert, K., Hölscher, A., Dietrich, H. P., and Chmara, I.: Soil phosphorus supply controls P nutrition strategies of beech forest ecosystems in Central Europe, Biogeochemistry, 136, 5-29, 10.1007/s10533-017-0375-0, 2017.

Leytem, A. B., Turner, B. L., and Thacker, P. A.: Phosphorus composition of manure from swine fed low-phytate grains, Journal of Environmental Quality, 33, 2380-2383, 10.2134/jeq2004.2380, 2004.

Leytem, A. B., and Maguire, R. O.: Environmental implications of inositol phosphates in animal manures, in: Inositol phosphates: linking agriculture and the environment, edited by: Turner, B. L., Richardson, A. E., and Mullaney, E. J., CABI, Wallingford, 150-168, 2007.

Li, M., Cozzolino, V., Mazzei, P., Drosos, M., Monda, H., Hu, Z., and Piccolo, A.: Effects of microbial bioeffectors and P amendments on P forms in a maize cropped soil as evaluated by ^{31}P–NMR spectroscopy, Plant and Soil, 427, 87-104, 10.1007/s11104-017-3405-8, 2018.

Makarov, M. I., Malysheva, T. I., Haumaier, L., Alt, H. G., and Zech, W.: The forms of phosphorus in humic and fulvic acids of a toposequence of alpine soils in the northern Caucasus, Geoderma, 80, 61-73, https://doi.org/10.1016/S0016-7061(97)00049-9, 1997.

McDowell, R. W., and Stewart, I.: The phosphorus composition of contrasting soils in pastoral, native and forest management in Otago, New Zealand: Sequential extraction and ^{31}P NMR, Geoderma, 130, 176-189, https://doi.org/10.1016/j.geoderma.2005.01.020, 2006.

McDowell, R. W., Cade-Menun, B., and Stewart, I.: Organic phosphorus speciation and pedogenesis: analysis by solution ^{31}P nuclear magnetic resonance spectroscopy, European Journal of Soil Science, 58, 1348-1357, 10.1111/j.1365-2389.2007.00933.x, 2007.

McKercher, R. B., and Anderson, G.: Characterization of the inositol penta- and hexaphosphate fractions of a number of Canadian and Scottish soils, Journal of Soil Science, 19, 302-310, 10.1111/j.1365-2389.1968.tb01542.x, 1968a.

McKercher, R. B., and Anderson, G.: Content of inositol penta- and hexaphosphates in some Canadian soils, Journal of Soil Science, 19, 47-55, 10.1111/j.1365-2389.1968.tb01519.x, 1968b.

McKercher, R. B., and Anderson, G.: Organic phosphate sorption by neutral and basic soils, Communications in Soil Science and Plant Analysis, 20, 723-732, 10.1080/00103628909368112, 1989.

McLaren, T. I., Smernik, R. J., Guppy, C. N., Bell, M. J., and Tighe, M. K.: The organic P composition of vertisols as determined by ^{31}P NMR spectroscopy, Soil Science Society of America Journal, 78, 1893-1902, 10.2136/sssaj2014.04.0139, 2014.

McLaren, T. I., Simpson, R. J., McLaughlin, M. J., Smernik, R. J., McBeath, T. M., Guppy, C. N., and Richardson, A. E.: An assessment of various measures of soil phosphorus and the net accumulation of phosphorus in fertilized soils under pasture, Journal of Plant Nutrition and Soil Science, 178, 543-554, 10.1002/jpln.201400657, 2015a.

McLaren, T. I., Smernik, R. J., McLaughlin, M. J., McBeath, T. M., Kirby, J. K., Simpson, R. J., Guppy, C. N., Doolette, A. L., and Richardson, A. E.: Complex forms of soil organic phosphorus–A major component of soil phosphorus, Environmental Science & Technology, 49, 13238-13245, 10.1021/acs.est.5b02948, 2015b.

McLaren, T. I., Verel, R., and Frossard, E.: The structural composition of soil phosphomonoesters as determined by solution ^{31}P NMR spectroscopy and transverse relaxation (T$_2$) experiments, Geoderma, 345, 31-37, https://doi.org/10.1016/j.geoderma.2019.03.015, 2019.

Meiboom, S., and Gill, D.: Modified spin‐echo method for measuring nuclear relaxation times, Review of Scientific Instruments, 29, 688-691, 10.1063/1.1716296, 1958.

Milliken, G. A., and Johnson, D. E.: Analysis of messy data. Volume 1: designed experiments, 2nd ed. ed., Boca Raton, Fla : CRC Press, 2009.

Newman, R. H., and Tate, K. R.: Soil phosphorus characterisation by ^{31}P nuclear magnetic resonance, Communications in Soil Science and Plant Analysis, 11, 835-842, 10.1080/00103628009367083, 1980.

Ognalaga, M., Frossard, E., and Thomas, F.: Glucose-1-phosphate and *myo*-inositol hexaphosphate adsorption mechanisms on goethite, Soil Science Society of America Journal, 58, 332-337, 10.2136/sssaj1994.03615995005800020011x, 1994.

Ohno, T., and Zibilske, L. M.: Determination of low concentrations of phosphorus in soil extracts using malachite green, Soil Science Society of America Journal, 55, 892-895, 10.2136/sssaj1991.03615995005500030046x, 1991.

Reusser, J. E., Verel, R., Frossard, E., and McLaren, T. I.: Quantitative measures of *myo*-IP$_6$ in soil using solution ^{31}P NMR spectroscopy and spectral deconvolution fitting including a broad signal, Environmental Science: Processes & Impacts, 10.1039/C9EM00485H, 2020.

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.

Sharma, V. K.: Oxidation of amino acids, peptides, and proteins : kinetics and mechanism, Wiley series of reactive intermediates in chemistry and biology, Hoboken : Wiley, 2013.

Smith, D. H., and Clark, F. E.: Anion-exchange chromatography of inositol phosphates from soil, Soil Science, 72, 353-360, 1951.

Spain, A. V., Tibbett, M., Ridd, M., and McLaren, T. I.: Phosphorus dynamics in a tropical forest soil restored after strip mining, Plant and Soil, 427, 105-123, 10.1007/s11104-018-3668-8, 2018.

Stephens, L. R., and Irvine, R. F.: Stepwise phosphorylation of *myo*-inositol leading to *myo*-inositol hexakisphosphate in Dictyostelium, Nature, 346, 580-583, 10.1038/346580a0, 1990.

Strickland, K. P.: The chemistry of phospholipids, Second, completely revised and enlarged edition. ed., Form and Function of Phospholipids, edited by: Ansell, G. B., Hawthorne, J. N., and Dawson, R. M. C., Elsevier Scientific Publ. Company, 1973.

Sun, M., Alikhani, J., Massoudieh, A., Greiner, R., and Jaisi, D. P.: Phytate degradation by different phosphohydrolase enzymes: contrasting kinetics, decay rates, pathways, and isotope effects, Soil Science Society of America Journal, 81, 61-75, 10.2136/sssaj2016.07.0219, 2017.

Sun, M., and Jaisi, D. P.: Distribution of inositol phosphates in animal feed grains and excreta: distinctions among isomers and phosphate oxygen isotope compositions, Plant and Soil, 430, 291-305, 10.1007/s11104-018-3723-5, 2018.

Suzumura, M., and Kamatani, A.: Isolation and determination of inositol hexaphosphate in sediments from Tokyo Bay, Geochimica et Cosmochimica Acta, 57, 2197-2202, https://doi.org/10.1016/0016-7037(93)90561-A, 1993.

Turner, B. L., Papházy, M. J., Haygarth, P. M., and McKelvie, I. D.: Inositol phosphates in the environment, Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences, 357, 449, 2002.

Turner, B. L., and Richardson, A. E.: Identification of *scyllo*-inositol phosphates in soil by solution phosphorus-31 nuclear magnetic resonance spectroscopy, Soil Science Society of America Journal, 68, 802-808, 10.2136/sssaj2004.8020, 2004.

Turner, B. L.: Inositol phosphates in soil: Amounts, forms and significance of the phosphorylated inositol stereoisomers., in: Inositol phosphates: Linking agriculture and the environment., edited by: Turner, B. L., Richardson, A. E., and Mullaney, E. J., CAB International, Wallingford, Oxfordshire, UK, 186-206, 2007.

Turner, B. L., Condron, L. M., Richardson, S. J., Peltzer, D. A., and Allison, V. J.: Soil organic phosphorus transformations during pedogenesis, Ecosystems, 10, 1166-1181, 10.1007/s10021-007-9086-z, 2007a.

Turner, B. L., Richardson, A. E., and Mullaney, E. J.: Inositol phosphates: linking agriculture and the environment, CABI, Wallingford, xi + 288 pp. pp., 2007b.

Turner, B. L.: Soil organic phosphorus in tropical forests: an assessment of the NaOH–EDTA extraction procedure for quantitative analysis by solution ^{31}P NMR spectroscopy, European Journal of Soil Science, 59, 453-466, 10.1111/j.1365-2389.2007.00994.x, 2008.

729 Turner, B. L., Cheesman, A. W., Godage, H. Y., Riley, A. M., and Potter, B. V.: Determination of *neo-* and
730 D-*chiro*-inositol hexakisphosphate in soils by solution ^{31}P NMR spectroscopy, Environ Sci Technol, 46,
731 4994-5002, 10.1021/es204446z, 2012.

732 Turner, B. L., Wells, A., and Condron, L. M.: Soil organic phosphorus transformations along a coastal
733 dune chronosequence under New Zealand temperate rain forest, Biogeochemistry, 121, 595-611,
734 10.1007/s10533-014-0025-8, 2014.

735 Turner, B. L.: Isolation of inositol hexakisphosphate from soils by alkaline extraction and hypobromite
736 oxidation, in: Inositol Phosphates: Methods and Protocols, edited by: Miller, G. J., Springer US, New
737 York, NY, 39-46, 2020.

738 Vestergren, J., Vincent, A. G., Jansson, M., Persson, P., Ilstedt, U., Gröbner, G., Giesler, R., and
739 Schleucher, J.: High-resolution characterization of organic phosphorus in soil extracts using 2D ^{1}H–^{31}P
740 NMR correlation spectroscopy, Environmental Science & Technology, 46, 3950-3956,
741 10.1021/es204016h, 2012.

742 Vincent, A. G., Vestergren, J., Gröbner, G., Persson, P., Schleucher, J., and Giesler, R.: Soil organic
743 phosphorus transformations in a boreal forest chronosequence, Plant and Soil, 367, 149-162,
744 10.1007/s11104-013-1731-z, 2013.

745 Vold, R. L., Waugh, J. S., Klein, M. P., and Phelps, D. E.: Measurement of spin relaxation in complex
746 systems, The Journal of Chemical Physics, 48, 3831-3832, 10.1063/1.1669699, 1968.

747 Volkmann, C. J., Chateauneuf, G. M., Pradhan, J., Bauman, A. T., Brown, R. E., and Murthy, P. P. N.:
748 Conformational flexibility of inositol phosphates: influence of structural characteristics, Tetrahedron
749 Letters, 43, 4853-4856, https://doi.org/10.1016/S0040-4039(02)00875-4, 2002.

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752 **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

753

754 **Table 2. Standard solutions used for the spiking experiment of the hypobromite oxidised soil extracts. All standards**
755 **were dissolved in 0.25 M NaOH and 0.05 M Na₂EDTA.**

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

756

757 **Table 3. Concentrations of total P as measured by XRF and 0.25 M NaOH + 0.05 M EDTA extractable P before and**
758 **after hypobromite oxidation of soil extracts. Concentrations of total P in NaOH-EDTA extracts were determined by**
759 **ICP-OES, whereas that of molybdate reactive P (MRP) was determined by the malachite green method of Ohno and**
760 **Zibilske (1991). Concentrations of molybdate unreactive P (MUP) were calculated as the difference between total P and**
761 **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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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 soil extracts (Ferralsol, Vertisol, Cambisol and Gleysol) before and after hypobromite oxidation (HO). Quantification was based on spectral integration and deconvolution fitting. The proportion of P detected in hypobromite oxidised 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 in phosphomonoester region	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	-	-	-	-

768 **Table 5. Concentrations of identified inositol phosphates (IP) in hypobromite oxidised 0.25 M NaOH + 0.05 M EDTA**
769 **soil extracts (Ferralsol, Vertisol, Cambisol and Gleysol). Concentrations were calculated from solution ^{31}P NMR spectra**
770 **using spectral deconvolution fitting including an underlying broad signal. When no concentration is given, the IP**
771 **compound was not detected in the respective soil extract. Chemical shift positions are based on the NMR spectrum of**
772 **the Cambisol extract (Fig. SI8 in the Supporting Information). Peak positions varied up to +0.018 ppm (Gleysol).**
773 **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
D-*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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Table 6. Transversal relaxation times (T$_2$) of various P species in the orthophosphate and phosphomonoester regions as determined by solution ^{31}P nuclear magnetic resonance (NMR) spectroscopy and a Carr-Purcell- Meiboom-Gill (CPMG) pulse sequence on hypobromite oxidised soil extracts.

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