Determination of *neo*- and D-*chiro*-Inositol Hexakisphosphate in Soils by Solution ^{31}P NMR Spectroscopy

Benjamin L. Turner,[†,]* Alexander W. Cheesman,[†] H. Yasmin Godage,[‡] Andrew M. Riley,[‡] and Barry V. L. Potter[‡]

[†]Smithsonian Tropical Research Institute, Apartado 0843-03092, Balboa, Ancón, Republic of Panama
[‡]Wolfson Laboratory of Medicinal Chemistry, Department of Pharmacy and Pharmacology, University of Bath, Bath BA2 7AY, United Kingdom

ⓢ *Supporting Information*

ABSTRACT: The inositol phosphates are an abundant but poorly understood group of organic phosphorus compounds found widely in the environment. Four stereoisomers of inositol hexakisphosphate (IP_6) occur, although for three of these (*scyllo*, *neo*, and D-*chiro*) the origins, dynamics, and biological function remain unknown, due in large part to analytical limitations in their measurement in environmental samples. We synthesized authentic *neo*- and D-*chiro*-IP_6 and used them to identify signals from these compounds in three soils from the Falkland Islands. Both compounds resisted hypobromite oxidation and gave quantifiable ^{31}P NMR signals at $\delta = 6.67$ ppm (equatorial phosphate groups of the 4-equatorial/2-axial conformer of *neo*-IP_6) and $\delta = 6.48$ ppm (equatorial phosphate groups of the 2-equatorial/4-axial conformer of D-*chiro*-IP_6) in soil extracts. Inositol hexakisphosphate accounted for 46−54% of the

soil organic phosphorus, of which the four stereoisomers constituted, on average, 55.9% (*myo*), 32.8% (*scyllo*), 6.1% (*neo*), and 5.2% (D-*chiro*). Reappraisal of the literature based on the new signal assignments revealed that *neo*- and D-*chiro*-IP_6 occur widely in both terrestrial and aquatic ecosystems. These results confirm that the inositol phosphates can constitute a considerable fraction of the organic phosphorus in soils and reveal the prevalence of *neo*- and D-*chiro*-IP_6 in the environment. The hypobromite oxidation and solution ^{31}P NMR spectroscopy procedure allows the simultaneous quantification of all four IP_6 stereoisomers in environmental samples and provides a platform for research into the origins and ecological significance of these enigmatic compounds.
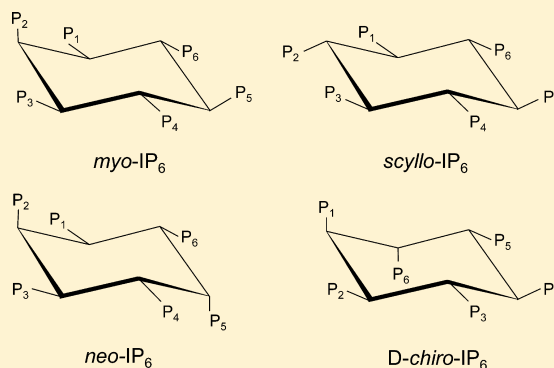
INTRODUCTION

A considerable proportion of the phosphorus in many soils occurs as stereoisomers of inositol hexakisphosphate (IP_6).[1,2] The most abundant of these is *myo*-IP_6, which occurs as a phosphorus storage compound in seeds,[3] although there is evidence that three additional stereoisomers also occur in the environment.[4] In a remarkable series of papers published in the 1960s, Dennis Cosgrove identified *scyllo*-, *neo*-, and D-*chiro*-IP_6 in soil extracts using a combination of alkaline extraction, column chromatography, dephosphorylation, and paper chromatography of the free inositols.[5−7] Subsequent studies have detected the stereoisomers of IP_6 in a number of soils and sediments,[8−12] yet they are extremely rare elsewhere in nature: *scyllo*-IP_6 has never been identified in an organism,[4] while *neo*- and D-*chiro*-IP_6 have been identified only in human intestinal amoebae[13] and velvet mesquite leaves,[14] respectively. A report of *muco*-IP_6 [14] was criticized on analytical grounds,[1] and this compound does not appear to exist in nature. The remaining four inositol stereoisomers (*allo*, L-*chiro*, *cis*, *epi*) do not appear to occur naturally in phosphorylated forms.

Given that the origins of the stereoisomeric forms of IP_6 remain unknown, it is perhaps not surprising that the pathways

to their synthesis, biological function, and behavior in the environment are also unknown. Each of the three stereoisomers differs from the *myo* form in the orientation of a single phosphate group, so it is possible that they are synthesized by epimerization of *myo*-IP_6 as well as by phosphorylation of the base isomer.[15] It is well-known that *myo*-IP_6 reacts strongly with metal ions to form insoluble complexes,[1] and the other stereoisomeric forms presumably react in a similar manner. The inositol phosphates are therefore often considered to be relatively recalcitrant in the environment.[2] Despite this, a number of organisms can synthesize phytase enzymes that allow them to acquire phosphorus from *myo*-IP_6,[16,17] although *scyllo*-, *neo*-, and D-*chiro*-IP_6 are all more resistant to phytase hydrolysis than the *myo* isomer.[18] Taken together, these properties suggest that possible functions of the stereoisomers might include protection against metal toxicity, conservation
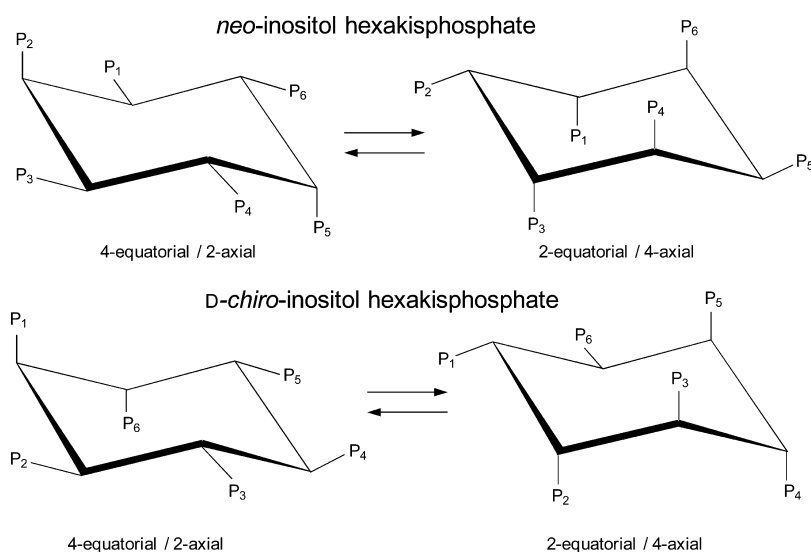
Figure 1. Conformational changes in *neo*- and D-*chiro*-inositol hexakisphosphate (IP$_6$) depending on solution pH. Phosphate groups are indicated by 'P', with the number of the associated carbon group indicated by the subscript numeral. At low pH both *neo*- and D-*chiro*-IP$_6$ adopt the 4-equatorial/2-axial conformation. At high pH, the 2-equatorial/4-axial and 4-equatorial/2-axial forms exist in equilibrium, and the relative proportions of the two conformers might be influenced by factors other than pH (e.g., metal counterions). In ^{31}P NMR spectra of alkaline soil extracts, *neo*-IP$_6$ was observed (within the limits of detection) to be exclusively in the 4-equatorial/2-axial conformation, while D-*chiro*-IP$_6$ occupied the 2-equatorial/4-axial conformation.

and protection of phosphorus from competitors, or an abiotic role in soil structure.[4]

Despite the widespread occurrence of inositol phosphates in the environment, the stereoisomers of IP$_6$ other than *myo* have been virtually unstudied for the previous 40 years in any field (but see refs 19 and 20), including biomedicine, where the inositol phosphates are of particular interest for their role in cell signaling.[21] This is unsatisfactory given the importance of the inositol phosphates in the phosphorus cycle in both terrestrial and aquatic ecosystems but is due in large part to analytical limitations inherent in the measurement of inositol phosphates in environmental samples.[2] The study of organic phosphorus in such samples has been improved dramatically by the application of solution ^{31}P NMR spectroscopy.[22] This technique has been adopted widely because it provides information on phosphorus compounds in alkaline extracts of soils or sediments without the need for the complex extraction and sample clean up procedures involved in conventional chromatography.[23] However, solution ^{31}P NMR spectroscopy has been applied only recently to the quantification of IP$_6$ in environmental samples: a technique using spectral deconvolution was used to quantify *myo*-IP$_6$,[24] while a procedure involving hypobromite oxidation, which destroys all organic matter except the inositol phosphates, and spiking with authentic compounds was used to identify *scyllo*-IP$_6$.[12]

Despite these advances, it has not been possible to identify *neo*- and D-*chiro*-IP$_6$ by solution ^{31}P NMR spectroscopy, due principally to the difficulty in obtaining authentic samples of these compounds. Here we report the chemical synthesis of *neo*- and D-*chiro*-IP$_6$, the first efficient and high-yielding route to the latter, and their identification in soil extracts by hypobromite oxidation and solution ^{31}P NMR spectroscopy. Our results confirm the presence of all four stereoisomers of IP$_6$ in soils (i.e., *myo*, *scyllo*, *neo*, and D-*chiro*) and, by enabling a re-evaluation of the literature on solution ^{31}P NMR spectroscopy of soils and sediments, reveal the widespread occurrence of *neo*- and D-*chiro*-IP$_6$ in both terrestrial and aquatic ecosystems.

■ MATERIALS AND METHODS

Synthesis of D-*chiro*-Inositol 1,2,3,4,5,6-Hexakisphosphate. Bis(cyanoethyl)(*N*,*N*-diisopropylamino)phosphine (2.40 g, 9.00 mmol) was added to a stirred suspension of D-*chiro*-inositol (180.2 mg, 1.00 mmol) and 5-phenyltetrazole (1.75 g, 12.00 mmol) in anhydrous dichloromethane (10 mL) under an argon atmosphere. Stirring continued for 20 h at room temperature. The reaction mixture was cooled to −40 °C, and *tert*-butyl hydroperoxide (70%, 1.72 mL, 12.00 mmol) was added portion-wise while stirring. The mixture was allowed to warm to room temperature and stirred for a further 1 h. After removal of solvent under reduced pressure, the residue was redissolved in dichloromethane (100 mL) and washed with a 10% sodium sulfite solution (2 × 100 mL), the organic phase was dried (MgSO$_4$), and the solvent was concentrated under reduced pressure to afford the crude 1,2,3,4,5,6-hexakis-*O*-[bis(cyanoethyloxy)phosphoryl] D-*chiro*-inositol. Without further purification, the crude material was dissolved in concentrated aqueous ammonia solution (30 mL), and the mixture was heated at 60 °C overnight in a Pyrex pressure tube. After evaporation of the solution under vacuum, the residue was purified by ion exchange chromatography to afford the pure triethyl ammonium salt of D-*chiro*-inositol 1,2,3,4,5,6-hexakisphosphate (1.06 g, 91% from D-*chiro*-inositol) as a hygroscopic white solid[25] (analytical information presented in the Supporting Information).

Synthesis of *neo*-Inositol 1,2,3,4,5,6-Hexakisphosphate. Authentic *neo*-IP$_6$ was synthesized by a similar procedure to that described above for D-*chiro*-IP$_6$ (see the Supporting Information).

Preparation of Authentic Compounds for Solution ^{31}P NMR Spectroscopy. To determine chemical shifts of *neo*- and D-*chiro*-IP$_6$ in NaOH−EDTA solution by solution ^{31}P NMR spectroscopy, compounds (5 mg) were dissolved in deionized water (4 mL) to yield solutions containing ∼200 µg P mL$^{−1}$. Of this, 3 mL was adjusted to pH >13 by addition of 10 M NaOH, while 1 mL was pretreated by hypobromite oxidation (see
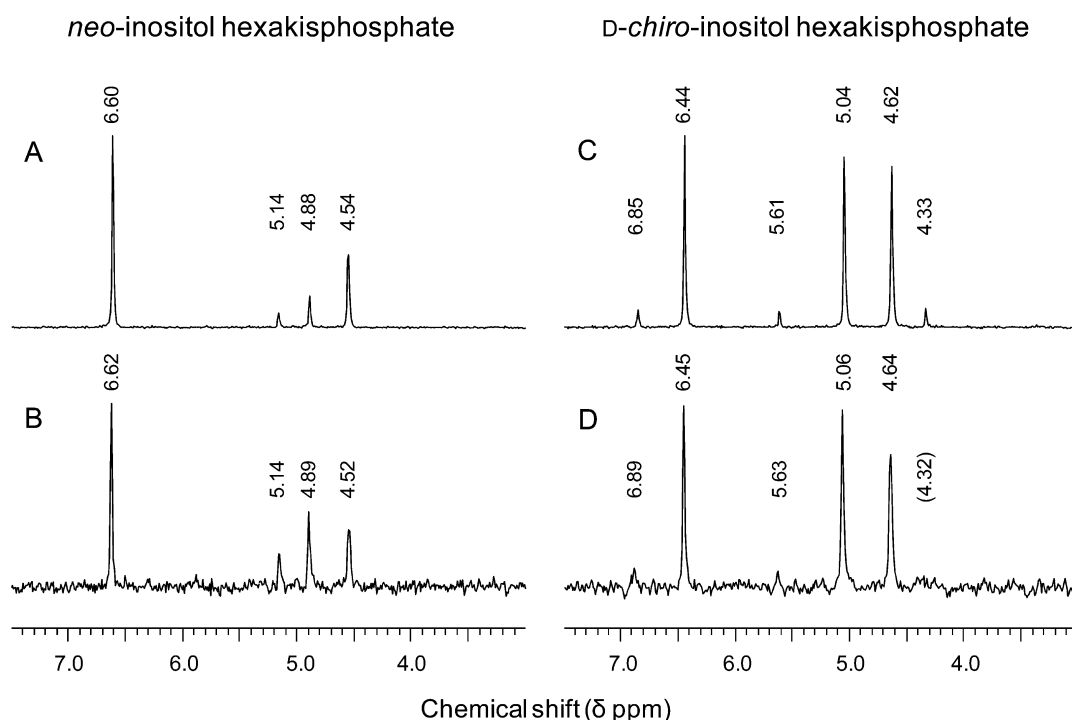
Figure 2. Solution ^{31}P NMR spectra of authentic *neo-* and D-*chiro*-inositol hexakisphosphate dissolved in NaOH−EDTA. Spectra show samples before (A, C) and after (B, D) pretreatment by hypobromite oxidation. Spectra are plotted with 1 Hz (A−C) or 2 Hz (D) line broadening.

below). Both samples were then analyzed by solution ^{31}P NMR spectroscopy, and the unbrominated samples were used to prepare a spike solution (see below).

Soils and Phosphorus Extraction. We studied three soils from the Falkland Islands known to contain inositol phosphates (B. L. Turner, unpublished data). The soils were from three distinct locations and were all under pasture consisting mainly of white grass (*Cortaderia pilosa*) with some Christmas bush (*Baccharis magellanica*, an evergreen shrub). The soils were rich in organic matter (11−23% total C), moderately acidic (pH in water 5.2−5.4), and contained relatively high phosphorus concentrations (753−1107 mg P kg^{-1}) (see Table S1 in the Supporting Information). Soils were extracted in a solution containing 0.25 M NaOH and 50 mM disodium EDTA (ethylenediaminetetraacetate) for 16 h in a 1:20 solid/solution ratio at 22 °C, centrifuged (8000 *g*, 30 min), lyophilized, and ground to a fine powder. An aliquot of each extract was taken prior to lyophilization for determination of total phosphorus by inductively coupled plasma optical-emission spectrometry (ICP−OES).

Hypobromite Oxidation. Solutions containing authentic inositol phosphates and soil NaOH−EDTA extracts were analyzed by solution ^{31}P NMR spectroscopy before and after treatment by hypobromite oxidation (also known as alkaline bromination). The method was adapted from previous studies[12,26,27] and is reported in full in the Supporting Information. Briefly, samples were made strongly alkaline and treated with ice-cold pure bromine. After 1 h at room temperature the mixture was boiled (5 min), acidified to pH <3 (accompanied by a color change from yellow to orange), and reheated to dispel excess bromine (color change from orange to yellow), and the pH was adjusted to 8.5 (color change from yellow to colorless). Phosphates were precipitated with barium acetate, resuspended using cation exchange resin (Amberlite IR120, hydrogen form; Sigma-Aldrich, St Louis,

MI), and the pH adjusted to >13 with 1 M NaOH. Solutions containing authentic compounds were analyzed directly, while soil extracts were frozen and lyophilized for later analysis.

Addition of Authentic Inositol Phosphates to Soil Extracts. Two solutions were prepared containing authentic inositol phosphates. These were added as spikes to soil extracts to identify individual signals. The first spike solution contained *myo-* and *scyllo*-IP$_6$, while a second spike solution contained *neo-* and D-*chiro*-IP$_6$. Spike solutions were prepared by combining 0.25 mL of each of the authentic compound solutions with 0.5 mL of a solution containing 1.0 M NaOH and 100 mM EDTA. For analysis, 0.25 mL of spike solution was added to 0.75 mL of reconstituted soil extract (see below) and 1 mL of 1.0 M NaOH and 100 mM EDTA solution. The spike was therefore added at relatively similar concentrations of both *neo-* and D-*chiro*-IP$_6$ compared to soil extracts.

Solution ^{31}P NMR Spectroscopy. Samples were analyzed by solution ^{31}P NMR spectroscopy using a procedure similar to that described previously,[12,28] with details reported in the Supporting Information. Briefly, compounds were redissolved in D$_2$O and the 1 M NaOH−100 mM EDTA solution, vortexed (1 min), and transferred to a 10 mm NMR tube. Soil extracts were filtered (1 μm GF-B filter, Whatman) prior to analysis. Spectra were acquired on a Bruker Avance 500 using a 30° pulse, 0.58 s acquisition time, and a 2 s T$_1$ delay. These parameters yield quantitative spectra based on literature reports (e.g., ref 29). The number of scans varied from ∼1000 for authentic compounds to ∼30,000 for soil extracts to ensure acceptable signal-to-noise ratios. Chemical shifts of signals were determined in parts per million (ppm) relative to an external orthophosphoric acid standard (85%) set to δ = 0 ppm. Signals were subsequently adjusted using the chemical shift of *scyllo*-IP$_6$ (δ = 4.03 ppm) to facilitate signal identification among spectra and assigned based on literature reports of model compounds spiked into NaOH−EDTA soil extracts.[30] Signal areas were

4996

dx.doi.org/10.1021/es204446z | *Environ. Sci. Technol.* 2012, 46, 4994−5002

Table 1. Phosphorus Composition of NaOH−EDTA Extracts of Three Soils Determined by Solution ^{31}P NMR Spectroscopy with or without Pretreatment by Hypobromite Oxidation[a]

		% of total spectra area					
		inorganic phosphorus		organic phosphorus			
soil	pretreatment	orthophosphate	pyrophosphate	phosphomonoester	phospholipid	DNA	phosphonate
East 50	none	20.3	2.4	67.1	1.8	5.9	2.4
	bromination	42.3	0.7	57.0	0	0	0
West 18	none	18.8	1.4	66.4	4.4	7.5	1.5
	bromination	43.4	0.7	55.7	0	0	0
West 22	none	17.3	2.3	70.2	3.9	5.2	1.2
	bromination	47.2	2.6	50.2	0	0	0

[a]Values are the proportion (%) of the total spectral area assigned to functional groups of phosphorus compounds.

calculated by integration, and deconvolution was performed on the region between δ = 3.0 and 7.5 ppm to separate orthophosphate from phosphomonoesters and to quantify signals from inositol phosphates (see below). Finally, spectra were plotted with 1 Hz line broadening to show fine resolution in the phosphomonoester region.

Quantification of Inositol Hexakisphosphate Stereoisomers. The proportion of the total spectral area was determined for the four IP$_6$ stereoisomers (*myo*, *scyllo*, *neo*, D-*chiro*). The proportion of *scyllo*-IP$_6$ was determined from the strong signal at δ = 4.03 ppm. The proportion of *myo*-IP$_6$ was determined by summing the four signals in a 1:2:2:1 pattern (see below for chemical shifts). The proportion of *neo*-IP$_6$ was determined by multiplying the signal at δ = 6.67 ppm by 1.5 (i.e., the signal represents four of the six phosphates on the molecule). The proportion of D-*chiro*-IP$_6$ was determined by multiplying the signal at δ = 6.48 ppm by three (i.e., it represents two of the six phosphates on the molecule). We assumed that all of the *neo*- and D-*chiro*-IP$_6$ molecules were present as a single conformer in soil extracts, given the absence of detectable signals from the alternate conformers in spectra of brominated samples (see below).

RESULTS

Signals from *neo*- and D-*chiro*-Inositol Hexakisphosphates in NaOH−EDTA. Both *neo*- and D-*chiro*-IP$_6$ can occur in two conformational forms in solution (Figure 1), and both forms were present in preparations of the authentic samples in alkaline solution (Figure 2). The authentic sample of *neo*-IP$_6$ in NaOH−EDTA gave four signals in solution ^{31}P NMR spectroscopy (Figure 2A). Two signals at δ = 6.61 and δ = 4.54 ppm in a 4:2 ratio was assigned to the 4-equatorial/2-axial conformation, based on ^{1}H, ^{13}C, and 2-dimensional NMR experiments (see the Supporting Information). Two smaller signals at δ = 4.88 and δ = 5.14 ppm, also in a 4:2 ratio, were assigned to the 2-equatorial/4-axial conformation (Figure 2A). The ratio of the two conformers was 6:1. The chemical shifts of the signals were essentially unchanged by hypobromite oxidation (Figure 2B), but the ratio of the two conformers was reduced to 2:1.

The authentic sample of D-*chiro*-IP$_6$ gave six signals in solution ^{31}P NMR spectroscopy (Figure 2C). Three large signals at δ = 4.62, 5.04, and 6.44 ppm in a 2:2:2 pattern were assigned to the 2-equatorial/4-axial conformation, based on ^{1}H, ^{13}C, and two-dimensional NMR experiments (see the Supporting Information). A second set of smaller signals at δ = 4.33, 5.61, and 6.85 ppm, also in a 2:2:2 pattern, were assigned to the 4-equatorial/2-axial conformation. The ratio of

the two sets of signals was 11:1. The chemical shifts of signals from D-*chiro*-IP$_6$ were essentially unchanged following hypobromite oxidation (Figure 2D), although poor resolution in the brominated sample due to the low phosphorus concentration prevented clear quantification of signals from the 4-equatorial/2-axial conformer.

In both cases, no inorganic phosphate was detected in brominated samples, confirming the resistance of *neo*- and D-*chiro*-IP$_6$ to hypobromite oxidation.

Phosphorus Composition of Soil NaOH−EDTA Extracts and the Effect of Hypobromite Oxidation. Untreated NaOH−EDTA extracts of the three soils were similar in terms of their broad phosphorus composition (Table 1, Figure 3A). The NaOH−EDTA solution recovered ≥90% of the total phosphorus from the three soils (see Table S1 in the Supporting Information), consistent with the efficacy of this extraction solution for high latitude soils rich in carbon and phosphorus.[31−33] Most of the extracted phosphorus was in organic forms, including phosphomonoesters (66−70% extracted phosphorus), DNA (DNA; 5.2−7.5%), alkali-stable phospholipids (1.8−4.4%), and phosphonates (1.2−2.4%). Inorganic phosphorus included orthophosphate (17−20% extracted phosphorus) and pyrophosphate (1.4−2.4%).

Hypobromite oxidation destroyed phospholipids, DNA, phosphonates, and some phosphomonoesters (Table 1, Figure 3B). Pyrophosphate also declined partially in two of the three soils. Inorganic orthophosphate increased to 42−47% of the total phosphorus following bromination, while phosphomonoesters declined to 50−57% (Table 1). The phosphomonoesters persisting after hypobromite oxidation presumably represent only inositol phosphates.[12,26]

Identification of Signals from Inositol Hexakisphosphate Stereoisomers in Soil Extracts. All four stereoisomers of IP$_6$ were identified in extracts of the three soils by spiking solutions with the respective stereoisomers (Table 2, Figure 4). The presence of *myo*- and *scyllo*-IP$_6$, their chemical shifts, and their resistance to hypobromite oxidation[12,26] was confirmed by spiking with authentic compounds (data not shown). The large stable signal from *scyllo*-IP$_6$ was adjusted to δ = 4.03 ppm (the chemical shift of this compound in the unbrominated East 50 soil) in all spectra, to facilitate comparison among spectra with small differences in pH and salt content. Based on this, signals from *myo*-IP$_6$ occurred at δ = 4.38, 4.53, 4.89, and 5.79 ppm in a 1:2:2:1 pattern (Figure 4, Figure S1 in the Supporting Information). The chemical shift of inorganic orthophosphate varied slightly among samples, presumably due to variation in salt content,[34] but was typically within 0.05 ppm of δ = 5.95 ppm (Figure 4). Chemical shifts of
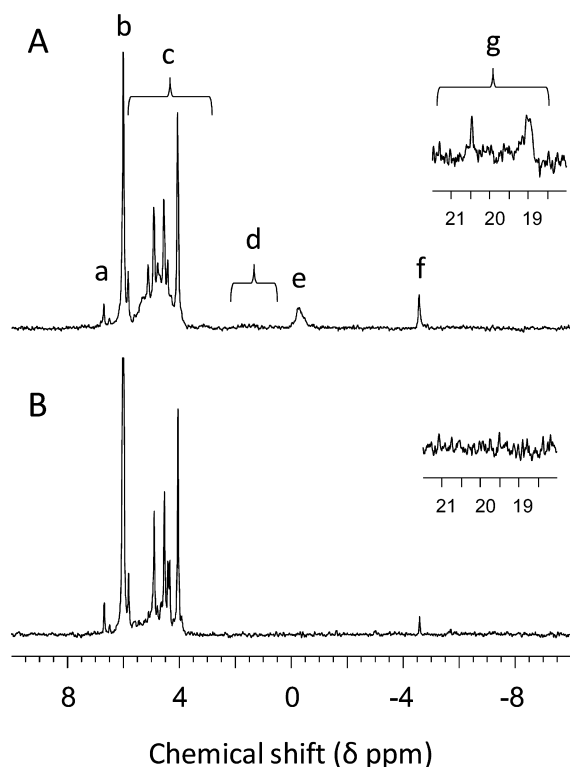
Figure 3. Solution ³¹P NMR spectra of NaOH−EDTA extracts of a soil (East 50) from the Falkland Islands before (A) and after (B) pretreatment with hypobromite oxidation to destroy all organic phosphorus other than the higher-order inositol phosphates. The zoomed insets show the phosphonate signals between δ = 19 and 21 ppm. Spectra are plotted with 5 Hz line broadening. In (B) the spectrum is truncated vertically to show the *scyllo*-inositol hexakisphosphate signal at the same height in both spectra. Signal assignments are as follows: a, *neo*- and D-*chiro*-inositol hexakisphosphate; b, inorganic orthophosphate; c, phosphomonoesters other than the signals from *neo*- and D-*chiro*-inositol hexakisphosphate between δ = 6.4 and 6.9 ppm; d, alkali-stable phospholipids; e, DNA; f, pyrophosphate; g, phosphonates.

the remaining signals in the phosphomonoester region were remarkably consistent across soils and treatments.

The second spike solution contained *neo*-IP₆ conformers in a 1:4 ratio (spectrum not shown), which did not appear to change after addition of the spike to the soils extracts. The ratio was different in the soil extracts themselves, because the largest *neo*-IP₆ signal was at δ = 6.67 ppm (i.e., from the four equatorial phosphates of the 4-equatorial/2-axial conformer), with no similar signal detected at δ = 4.93 ppm (i.e., from the four axial phosphates of the 2-equatorial/4-axial conformer) in any of the brominated soil extracts. For quantification, we therefore assumed that all *neo*-IP₆ in soil extracts was in the 4-equatorial/2-axial conformation.

Signals at δ = 6.48 and 6.67 ppm downfield of orthophosphate in all soils were identified as being from D-*chiro*- and *neo*-IP₆, respectively. These signals were present in both brominated and untreated soil extracts. A signal at δ = 6.90 ppm from the 4-equatorial/2-axial conformer of D-*chiro*-IP₆ present in the spike was not detected in any soil extract (untreated or brominated). Other signals from D-*chiro*- and *neo*-IP₆ were not identified clearly in soil extracts either with or without pretreatment by hypobromite oxidation. A signal at δ = 5.08 ppm in untreated extracts coincided with one of the three

Table 2. Solution ³¹P NMR Spectroscopy Chemical Shifts (δ ppm) of Phosphates in Inositol Hexakisphosphate (IP₆) Stereoisomers in Alkaline Soil Extracts (pH > 13)[a]

stereoisomer	configuration	contributing P groups	orientation (ax/eq)	chemical shift (δ ppm)
scyllo-IP₆	6 ax	C1, C2, C3, C4, C5, C6	ax	4.03
myo-IP₆	1-eq/5-ax	C5	ax	4.38
		C1, C3	ax	4.53
		C4, C6	ax	4.89
		C2	eq	5.79
neo-IP₆	4-eq/2-ax	C2, C5	ax	4.58
		C1, C3, C4, C6	eq	6.67
	2-eq/4-ax	C1, C3, C4, C6	ax	4.93
		C2, C5	eq	5.17
D-*chiro*-IP₆	4-eq/2-ax	C1, C6	ax	4.33
		C2, C5	eq	5.66
		C3, C4	eq	6.90
	2-eq/4-ax	C3, C4	ax	4.66
		C2, C5	ax	5.08
		C1, C6	eq	6.48

[a]Signals are scaled relative to the large stable signal from *scyllo*-IP₆ at δ = 4.03 ppm. Values varied by ± 0.01 ppm or less among samples and treatments (unbrominated, brominated, spiked). Eq, equatorial; ax, axial.

signals from the 2-equatorial/4-axial conformer of D-*chiro*-IP₆, as shown by spiking, but was considerably reduced by hypobromite oxidation. This indicates that the signal was from an organic phosphorus compound other than an inositol phosphate, probably α-glycerophosphate.[35] It should be noted that the signals assigned to D-*chiro*-IP₆ could also arise from its enantiomer L-*chiro*-IP₆, although we consider this unlikely given that the latter has never been identified in nature (see Discussion). A small signal at δ = 4.32 ppm increased in extracts of all soils after hypobromite oxidation. This remains unidentified but appears commonly following pretreatment by this procedure (B. L. Turner, unpublished observations).

Quantification of Inositol Hexakisphosphate Stereoisomers. Total IP₆ in the three soils was between 36.7 and 41.4% of the extracted phosphorus (mean 38.6 ± 2.6%) with little variation between values calculated from untreated and brominated extracts (Table 3). Inositol hexakisphosphate therefore accounted for an average of 48.9 ± 4.2% of the soil organic phosphorus and 57.0 ± 4.7% of the phosphomonoesters (Table S2 in Supporting Information). These values are representative of the total soil phosphorus given that NaOH−EDTA extracted ≥90% of the total soil phosphorus. Thus, about half of the organic phosphorus in the three soils was IP₆. The remaining phosphomonoesters resisting hypobromite oxidation presumably represented lower-order inositol phosphates and constituted an additional 18−21% of the soil organic phosphorus (see Table S2 in the Supporting Information).

Most of the IP₆ was in the form of *myo*-IP₆ (55.9 ± 1.3% of the total IP₆) and *scyllo*-IP₆ (32.8 ± 3.3%), with smaller proportions of the *neo* (6.1 ± 0.9%) and D-*chiro* (5.2 ± 1.7%) stereoisomers (Table 3). Values were remarkably consistent across the three soils, and there was little variation between untreated and brominated extracts for all four stereoisomers in almost all cases. Overall, the stereoisomers were present in the following order: *myo* > *scyllo* > *neo* > D-*chiro*. The ratio of the four stereoisomers was 10.78:6.32:1.17:1.
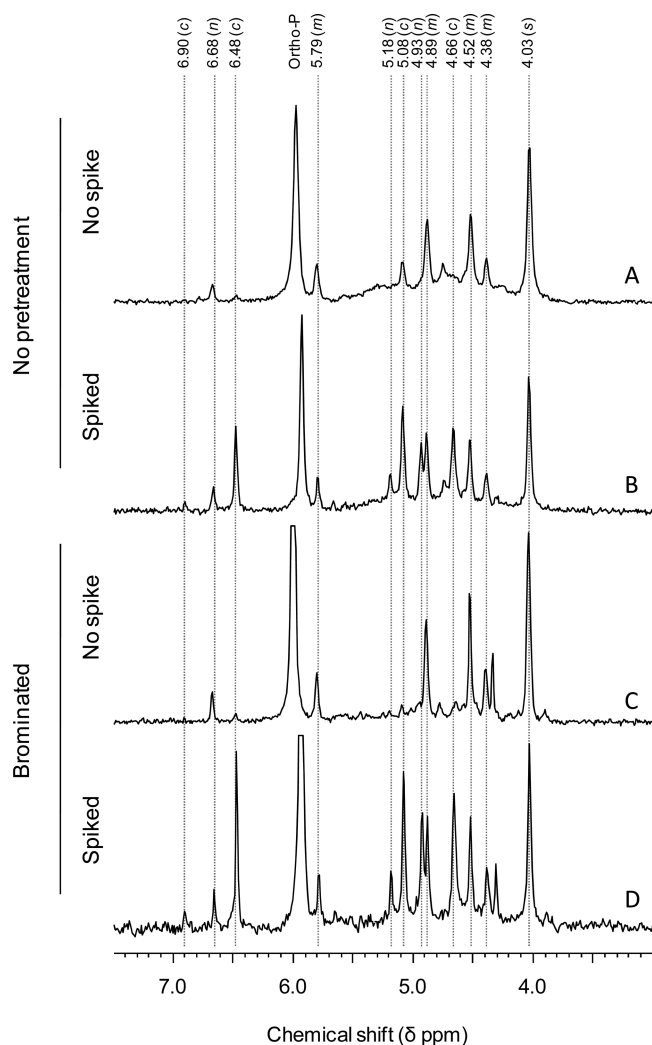
Figure 4. Solution ^{31}P NMR spectra of NaOH−EDTA extracts of a soil from the Falkland Islands (East 50) showing the phosphomonoester region in detail ($\delta = 3.0$ to 7.5 ppm). The following treatments are shown: untreated (A), untreated and spiked with a mixture of *neo*- and D-*chiro*-inositol hexakisphosphate (IP$_6$) (B), pretreated by hypobromite oxidation (C), pretreated with hypobromite oxidation and spiked with a mixture of *neo*- and D-*chiro*-IP$_6$ (D). Signal assignments are from the untreated spiked sample and assigned to *myo* (*m*), *scyllo* (*s*), D-*chiro* (*c*), or *neo* (*n*) IP$_6$ stereoisomers. Inorganic orthophosphate (Ortho-P) is the large signal close to $\delta = 6.00$ ppm. Spectra are plotted with 1 Hz line broadening and referenced to the chemical shift of *scyllo*-IP$_6$ in spectrum A ($\delta = 4.03$ ppm). The signal at $\delta = 4.58$ ppm from the two axial phosphates of the 4-equatorial/2-axial conformer of *neo*-IP$_6$ were small in the spike solution and were not detectable in soil extracts. Spectra for two additional soils showing the same treatments are in the Supporting Information (Figure S1).

■ DISCUSSION

Solution ^{31}P NMR spectroscopy is the most widely used procedure for determining the chemical nature of organic phosphorus in soils and sediments.[23] Our results extend the scope of this technique to include identification of all four stereoisomeric forms of IP$_6$ present in nature. Both *neo*- and D-*chiro*-IP$_6$ can be identified readily by signals downfield of orthophosphate between $\delta = 6.40$ and 6.70 ppm (relative to *scyllo*-IP$_6$ at $\delta = 4.03$ ppm) and are detectable at relatively low concentrations given that the signals are well-resolved from the large orthophosphate signal close to $\delta = 6.00$ ppm.

Importantly, both *neo*- and D-*chiro*-IP$_6$ resist hypobromite oxidation. Our results indicate that it is possible to quantify the IP$_6$ in well-resolved spectra containing high concentrations of inositol phosphates without brominating samples. However, bromination is clearly a useful pretreatment for estimation of the IP$_6$ in environmental samples by solution ^{31}P NMR spectroscopy, as indicated by its ability to distinguish between α-glycerophosphate and one of the three signals from the 2-equatorial/4-axial conformer of D-*chiro*-IP$_6$ at $\delta = 5.08$ ppm. The impact of this procedure on lower-order esters of all four stereoisomers remains unknown.

Both *myo*- and *scyllo*-IP$_6$ can occur in two conformations depending on solution pH,[20,36,37] although both appear in a single conformation at the high pH of the extracts used in solution ^{31}P NMR spectroscopy of soils. This yields consistent and stable signals and facilitates their identification and quantification.[12,24] In contrast, both conformers of *neo*- and D-*chiro*-IP$_6$ can be present in strongly alkaline extracts, as indicated by spectra of authentic compounds shown here. In soil extracts, however, only a single conformer of each stereoisomer was detected. Specifically, no signals were detected at $\delta = 4.93$ or 5.18 ppm from the 2-equatorial/4-axial conformer of *neo*-IP$_6$, nor at $\delta = 6.90$ ppm from two equatorial phosphates of the 4-equatorial/2-axial conformer of D-*chiro*-IP$_6$. This simplifies quantification of these compounds in soil extracts, which can be achieved using the two signals at $\delta = 6.67$ and 6.48 ppm for *neo*- and D-*chiro*-IP$_6$, respectively. The possible presence of other conformers is indicated by signals at $\delta = 6.90$ ppm (D-*chiro*-IP$_6$) or $\delta = 5.18$ ppm (*neo*-IP$_6$; the signal at $\delta = 4.92$ ppm from this conformer may be obscured by a signal from *myo*-IP$_6$). It remains unclear why both conformers were present in the authentic samples at high pH, especially as signals from these compounds in the spike solution were unchanged following addition to soil extracts. Solution pH was strongly alkaline in all cases, so it seems possible that conformers might be stabilized by interaction with metals.[38] Further experiments are required to understand why the ratio of the two *neo*-IP$_6$ conformers varies at strongly alkaline pH.

A caveat to the identification of D-*chiro*-IP$_6$ is that identical signals in solution ^{31}P NMR spectroscopy would be expected from its enantiomer L-*chiro*-IP$_6$, so the possibility remains that the form detected in soil extracts is the L-, rather than the D-, enantiomer. We assume that the signals are from D-*chiro*-IP$_6$, however, given that *chiro*-IP$_6$ in soils was previously identified as the D-enantiomer[39,40] and that the L-enantiomer has never been detected in nature in phosphorylated form.[1,4]

Very few studies have quantified *neo*- or D-*chiro*-IP$_6$ in soils or sediments since the early work of Cosgrove.[5,6] For the soils studied here, both compounds were present in much lower concentrations than either the *myo* or *scyllo* stereoisomers, which is in agreement with the older literature.[4] However, the concentration of the *neo* isomer appears to be greater than or similar to the D-*chiro* isomer in all three soils studied here. This contrasts with early reports that the D-*chiro* isomer occurred at greater concentration[8,9] but is consistent with the suggestion that the *neo* isomer might have been underestimated in studies that did not include pretreatment by hypobromite oxidation.[26]

The identification of *neo*- and D-*chiro*-IP$_6$ reported here allows a reappraisal of publications that have applied solution ^{31}P NMR spectroscopy to soils and aquatic sediments. A number of studies reported signals downfield of orthophosphate in solution ^{31}P NMR spectra of soil and sediment extracts but did not identify them as *neo*- or D-*chiro*-IP$_6$. It was initially

Table 3. Inositol Hexakisphosphate (IP$_6$) Stereoisomers in NaOH−EDTA Extracts of Three Soils from the Falkland Islands Determined by Solution ^{31}P NMR Spectroscopy with or without Pretreatment by Hypobromite Oxidation (Bromination)[a]

| | inositol hexakisphosphate stereoisomer | | | | | | | | |
| | % of spectra area | | | | | % of total IP$_6$ | | | |
soil/treatment	*myo*	*scyllo*	*neo*	D-*chiro*	total IP$_6$	*myo*	*scyllo*	*neo*	D-*chiro*
East 50									
none	22.4	15.1	2.50	1.69	41.7	53.8	36.2	6.0	4.1
bromination	22.7	14.7	2.37	1.37	41.1	55.1	35.8	5.8	3.3
average	*22.5*	*14.9*	*2.44*	*1.53*	*41.4*	*54.5*	*36.0*	*5.9*	*3.7*
West 18									
none	21.4	12.4	1.83	0.81	36.4	58.7	34.1	5.0	2.2
bromination	21.6	12.6	2.19	2.88	39.3	54.9	32.1	5.6	7.3
average	*21.5*	*12.5*	*2.01*	*1.84*	*37.8*	*56.8*	*33.1*	*5.3*	*4.8*
West 22									
none	21.0	10.9	2.63	2.84	37.4	56.3	29.1	7.0	7.6
bromination	20.4	10.6	2.55	2.38	36.0	56.8	29.5	7.1	6.6
average	*20.7*	*10.7*	*2.59*	*2.61*	*36.7*	*56.5*	*29.3*	*7.1*	*7.1*
overall average	21.6 ± 0.9	12.7 ± 2.1	2.35 ± 0.30	1.99 ± 0.56	38.6 ± 2.5	55.9 ± 1.3	32.8 ± 3.3	6.1 ± 0.9	5.2 ± 1.7

[a]Values are expressed as both the proportion (%) of total spectral area and the proportion (%) of the total IP$_6$. The overall average is the mean ± standard deviation of the three averages for each soil.

speculated that the signals represented aromatic phosphodiesters based on their similarity to compounds such as binaphthyl diylhydrogen phosphate,[41,42] although the resistance of the signals to hypobromite oxidation subsequently indicated the likelihood that they represented inositol phosphates.[12] We can now assign these signals to *neo*- and D-*chiro*-IP$_6$.

A series of temperate pasture soils yielded signals that we can now assign to *neo*- and D-*chiro*-IP$_6$ at concentrations of 8−51 mg P kg^{-1}, which represented 2−5% of the extracted phosphorus.[42] The same signals were also reported in Madagascan rice soils at concentrations of 0.8−4.9 mg P kg^{-1} (0.5−1.3% of extracted phosphorus)[43] (note that these values are 6-fold lower than the erroneous values in the original article). Similar signals were also reported in extracts of Russian grassland soils,[44] Scottish mineral soil,[41] sewage sludge,[45] and humic acids extracted from an agricultural soil.[46] However, only traces were detected in subarctic tundra and lowland temperate and tropical rain forests.[32,47,48] The humic acid study also detected *scyllo*-IP$_6$ but not the more widespread *myo* isomer, suggesting a difference in the mechanisms by which the various stereoisomeric forms of IP$_6$ become stabilized in soils.[46]

Importantly, we can now extend the identification of *neo*- and D-*chiro*-IP$_6$ to aquatic systems. Previously, the only report of either of these stereoisomeric forms was the measurement of D-*chiro*-IP$_6$ by gas chromatography in riverine, estuarine, and marine sediments of Tokyo Bay and the surrounding catchment.[11] In that study, most of the IP$_6$ was in the *myo* form, with 10−15% as *scyllo* and 1−5% as D-*chiro* (*neo*-IP$_6$ was not detected). Elsewhere, signals that can now be assigned to *neo*- and D-*chiro*-IP$_6$ were reported in sediments from a wide range of Danish lakes,[49] settling seston and sediments from a eutrophic lake in China,[50,51] and sediments from a brackish embayment in Helsinki.[52] In the latter study, the signal corresponding to *neo*-IP$_6$ constituted 0.3−3.0% of the organic phosphate and was greatest in sediment layers dated to periods of high pollutant inputs into the embayment. No signals corresponding to *neo*- and D-*chiro*-IP$_6$ were reported in NMR studies of marine sediments,[53,54] although D-*chiro*-IP$_6$ was detected in sediments from Toyko Bay.[27]

Although it has been known for some time that inositol phosphate concentrations vary widely among soils (from none to all of the organic phosphorus, reviewed in ref 4), it has been suggested recently that the amount of inositol phosphate in soil has been overestimated and is quantitatively unimportant.[34,35] Here, IP$_6$ accounted for half the soil organic phosphorus extractable in NaOH−EDTA, which in turn represented ≥90% of total soil phosphorus. These results demonstrate unequivocally that IP$_6$ can constitute a considerable proportion of the organic phosphorus in some soils.

Despite the apparent abundance of IP$_6$ stereoisomers in terrestrial and aquatic ecosystems, their origins, dynamics, and biological function remain unknown. Given the importance of phosphorus as both a nutrient and a pollutant, it is remarkable that so little is known about one of the most widespread groups of organic phosphates in the environment. Results presented here confirm the prevalence of the IP$_6$ stereoisomers in soils and lake sediments and support previous reports that the relative abundance of the four stereoisomeric forms of IP$_6$ follows the pattern *myo* >*scyllo* >*neo* >D-*chiro* (although the *scyllo* isomer can be the dominant form in some environments; e.g. ref 47). The investment in energy and phosphorus involved in the synthesis of the stereoisomeric forms of IP$_6$ suggests that they have some important yet currently unknown biological function. Research on this topic has been restricted by the difficulty in identifying the inositol phosphate stereoisomers in environmental samples, but the methodology presented here allows the simultaneous determination of all four stereoisomeric forms of IP$_6$ in a single analysis. This opens up new possibilities for studying the origins and ecological significance of these enigmatic compounds.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Detailed information on analytical procedures, a table of soil properties, and a figure of NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Phone: +507-212-8171. Fax: +507-212-8148. E-mail: TurnerBL@si.edu.

Notes

The authors declare no competing financial interest.

■ REFERENCES

(1) Cosgrove, D. J. *Inositol Phosphates: Their Chemistry, Biochemistry and Physiology*; Elsevier Scientific: Amsterdam, 1980; p 197.

(2) Turner, B. L.; Papházy, M. J.; Haygarth, P. M.; McKelvie, I. D. Inositol phosphates in the environment. *Philos. Trans. R. Soc. London, Ser. B* **2002**, *357*, 449−469.

(3) Raboy, V. *myo*-Inositol-1,2,3,4,5,6-hexa*kis*phosphate. *Phytochemistry* **2003**, *64*, 1033−1043.

(4) Turner, B. L. Inositol phosphates in soil: amounts, forms and significance of the phosphorlyated inositol stereoisomers. In *Inositol Phosphates: Linking Agriculture and the Environment*; Turner, B. L., Richardson, A. E., Mullaney, E. J., Eds.; CAB International: Wallingford, UK, 2007; pp 186−207.

(5) Cosgrove, D. J. Forms of inositol hexaphosphate in soils. *Nature* **1962**, *194*, 1265−1266.

(6) Cosgrove, D. J.; Tate, M. E. Occurrence of *neo*-inositol hexaphosphate in soil. *Nature* **1963**, *200*, 568−569.

(7) Cosgrove, D. J. The chemical nature of soil organic phosphorus—II. Characterization of the supposed DL-*chiro*-inositol hexaphosphate component of soil phytae as D-*chiro*-inositol hexaphosphate. *Soil Biol. Biochem.* **1969**, *1*, 325−327.

(8) McKercher, R. B.; Anderson, G. Characterization of inositol penta- and hexaphosphate fractions of a number of Canadian and Scottish soils. *J. Soil Sci.* **1968**, *19*, 302−310.

(9) Omotoso, T. I.; Wild, A. Content of inositol phosphates in some English and Nigerian soils. *J. Soil Sci.* **1970**, *21*, 216−223.

(10) Irving, G. C. J.; Cosgrove, D. J. The use of gas-liquid chromatography to determine the proportions of inositol isomers present as pentakis- and hexakisphosphates in alkaline extracts of soils. *Commun. Soil Sci. Plant Anal.* **1982**, *13*, 957−967.

(11) Suzumura, M.; Kamatani, A. Origin and distribution of inositol hexaphosphate in estuarine and coastal sediments. *Limnol. Oceanogr.* **1995**, *40*, 1254−1261.

(12) Turner, B. L.; Richardson, A. E. Identification of *scyllo*-inositol phosphates in soils by solution phosphorus-31 nuclear magnetic resonance spectroscopy. *Soil Sci. Soc. Am. J.* **2004**, *68*, 802−808.

(13) Martin, J.-B.; Laussmann, T.; Bakker-Grunwald, T.; Vogel, G.; Klein, G. *neo*-Inositol polyphosphates in the amoeba *Entamoeba histolytica*. *J. Biol. Chem.* **2000**, *275*, 10134−10140.

(14) L'Annunziata, M. F.; Fuller, W. H. Soil and plant relationships of inositol phosphate stereoisomers: the identification of D-chiro- and muco-inositol phosphates in a desert soil and plant system. *Soil Sci. Soc. Am. Proc.* **1971**, *35*, 587−595.

(15) L'Annunziata, M. F.; Gonzalez, I., Soil metabolic transformations of carbon-14-*myo*-inositol, carbon-14-phytic acid and carbon-14-iron(III) phytate. In *Soil Organic Matter Studies*; Interna-tional Atomic Energy Agency: Vienna, Austria, 1977; Vol. *1*, pp 239−253.

(16) Hill, J. E.; Richardson, A. E. Isolation and assessment of microorganisms that utilize phytate. In *Inositol Phosphates: Linking Agriculture and the Environment*; Turner, B. L., Richardson, A. E., Mullaney, E. J., Eds.; CAB International: Wallingford, UK, 2007; pp 61−77.

(17) Cheng, C.; Lim, B. L. Beta-propeller phytases in the aquatic environment. *Arch. Microbiol.* **2006**, *185*, 1−13.

(18) Cosgrove, D. J. Inositol phosphate phosphatases of microbiological origin. Inositol phosphate intermediates in the dephosphorylation of the hexaphosphates of *myo*-inositol, *scyllo*-inositol, and D-*chiro*-inositol by a bacterial (*Pseudomonas* sp.) phytase. *Aust. J. Biol. Sci.* **1970**, *23*, 1207−1220.

(19) Adelt, S.; Podeschwa, M.; Dallmann, G.; Altenbach, H.-J.; Vogel, G. Stereo- and regiospecificity of yeast phytases-chemical synthesis and enzymatic conversion of the substrate analogues *neo*- and L-*chiro*-inositol hexakisphosphate. *Bioorg. Chem.* **2003**, *31*, 44−67.

(20) Volkmann, C. J.; Chateauneuf, G. M.; Pradhan, J.; Bauman, A. T.; Brown, R. E.; Murthy, P. P. N. Conformational flexibility of inositol phosphates: influence of structural characteristics. *Tetrahedron Lett.* **2002**, *43*, 4853−4856.

(21) Michell, R. H.; Heath, V. L.; Lemmon, M. A.; Dove, S. K. Phosphatidylinositol 3,5-bisphosphate: metabolism and cellular functions. *Trends Biochem. Sci.* **2006**, *31*, 52−63.

(22) Newman, R. H.; Tate, K. R. Soil phosphorus characterisation by ^{31}P nuclear magnetic resonance. *Commun. Soil Sci. Plant Anal.* **1980**, *11*, 835−842.

(23) Cade-Menun, B. J., Using phosphorus-31 nuclear magnetic resonance spectroscopy to characterize organic phosphorus in environmental samples. In *Organic Phosphorus in the Environment*; Turner, B. L., Frossard, E., Baldwin, D. S., Eds.; CAB International: Wallingford, UK, 2005; pp 21−44.

(24) Turner, B. L.; Mahieu, N.; Condron, L. M. Quantification of *myo*-inositol hexakisphosphate in alkaline soil extracts by solution ^{31}P NMR spectroscopy and spectral deconvolution. *Soil Sci.* **2003**, *168*, 469−478.

(25) Cosgrove, D. J. Synthesis of the hexaphosphates of *myo*-, *scyllo*-, *neo*-, and D-inositol. *J. Sci. Food Agric.* **1966**, *17*, 550−554.

(26) Irving, G. C. J.; Cosgrove, D. J. The use of hypobromite oxidation to evaluate two current methods for the estimation of inositol polyphosphates in alkaline extracts of soils. *Commun. Soil Sci. Plant Anal.* **1981**, *12*, 495−509.

(27) Suzumura, M.; Kamatani, A. Isolation and determination of inositol hexaphosphate in sediments from Tokyo Bay. *Geochim. Cosmochim. Acta* **1993**, *57*, 2197−2202.

(28) Cheesman, A. W.; Turner, B. L.; Inglett, P. W.; Reddy, K. R. Phosphorus transformations during decomposition of wetland macrophytes. *Environ. Sci. Technol.* **2010**, *44*, 9265−9271.

(29) Cade-Menun, B. J.; Liu, C. W.; Nunlist, R.; McColl, J. G. Soil and litter phosphorus-31 nuclear magnetic resonance spectroscopy: extractants, metals, and phosphorus relaxation times. *J. Environ. Qual.* **2002**, *31*, 457−465.

(30) Turner, B. L.; Mahieu, N.; Condron, L. M. Phosphorus-31 nuclear magnetic resonance spectral assignments of phosphorus compounds in soil NaOH−EDTA extracts. *Soil Sci. Soc. Am. J.* **2003**, *67*, 497−510.

(31) Turner, B. L.; Chudek, J. A.; Whitton, B. A.; Baxter, R. Phosphorus composition of upland soils polluted by long-term atmospheric nitrogen deposition. *Biogeochemistry* **2003**, *65*, 259−274.

(32) Turner, B. L.; Baxter, R.; Mahieu, N.; Sjogersten, S.; Whitton, B. A. Phosphorus compounds in subarctic Fennoscandian soils at the mountain birch (*Betula pubescens*)−tundra ecotone. *Soil Biol. Biochem.* **2004**, *36*, 815−823.

(33) Bowman, R. A.; Moir, J. O. Basic EDTA as an extractant for soil organic phosphorus. *Soil Sci. Soc. Am. J.* **1993**, *57*, 1516−1518.

(34) Smernik, R. J.; Dougherty, W. J. Identification of phytate in phosphorus-31 nuclear magnetic resonance spectra: the need for spiking. *Soil Sci. Soc. Am. J.* **2007**, *71*, 1045−1050.

(35) Doolette, A. L.; Smernik, R. J.; Dougherty, W. J. Spiking improved solution phosphorus-31 nuclear magnetic resonance identification of soil phosphorus compounds. *Soil Sci. Soc. Am. J.* **2009**, *73*, 919−927.

(36) Emsley, J.; Niazi, S. The structure of *myo*-inositol hexaphosphate in solution: ^{31}P N.M.R. investigation. *Phosphorus Sulfur Relat. Elem.* **1981**, *10*, 401−408.

(37) Barrientos, L. G.; Murthy, P. P. N. Conformational studies of *myo*-inositol phosphates. *Carbohydr. Res.* **1996**, *296*, 39−54.

(38) Veiga, N.; Torres, J.; González, G.; Gómez, K.; Mansell, D.; Freeman, S.; Domínguez, S.; Kremer, C. Insight into the protonation and K(I)-interaction of the inositol 1,2,3-trisphosphate as provided by ^{31}P NMR and theoretical calculations. *J. Mol. Struct.* **2011**, *986*, 75−85.

(39) Cosgrove, D. J. The chemical nature of soil organic phosphorus I. Inositol phosphates. *Aust. J. Soil Res.* **1963**, *1*, 203−214.

(40) L'Annunziata, M. F.; Fuller, W. H.; Brantley, D. S. D-*chiro*-inositol phosphate in a forest soil. *Soil Sci. Soc. Am. Proc.* **1972**, *36*, 183−184.

(41) Bedrock, C. N.; Cheshire, M. V.; Chudek, J. A.; Goodman, B. A.; Shand, C. A. Use of ^{31}P-NMR to study the forms of phosphorus in peat soils. *Sci. Total Environ.* **1994**, *152*, 1−8.

(42) Turner, B. L.; Mahieu, N.; Condron, L. M. The phosphorus composition of temperate pasture soils determined by NaOH−EDTA extraction and solution ^{31}P NMR spectroscopy. *Org. Geochem.* **2003**, *34*, 1199−1210.

(43) Turner, B. L. Organic phosphorus in Madagascan rice soils. *Geoderma* **2006**, *136*, 279−288.

(44) Amelung, W.; Rodionov, A.; Urusevskaja, I. S.; Haumaier, L.; Zech, W. Forms of organic phosphorus in zonal steppe soils of Russia assessed by ^{31}P NMR. *Geoderma* **2001**, *103*, 335−350.

(45) Smith, M. T. E.; Cade-Menun, B. J.; Tibbett, M. Soil phosphorus dynamics and phytoavailability from sewage sludge at different stages in a treatment stream. *Biol. Fertil. Soils* **2006**, *42*, 186−197.

(46) He, Z.; Olk, D. C.; Cade-Menun, B. J. Forms and lability of phosphorus in humic acid fractions of Hord Silt Loam soil. *Soil Sci. Soc. Am. J.* **2011**, *75*, 1712−1722.

(47) Turner, B. L.; Condron, L. M.; Richardson, S. J.; Peltzer, D. A.; Allison, V. J. Soil organic phosphorus transformations during pedogenesis. *Ecosystems* **2007**, *10*, 1166−1181.

(48) Turner, B. L.; Engelbrecht, B. M. J. Soil organic phosphorus in lowland tropical rain forests. *Biogeochemistry* **2011**, *103*, 297−315.

(49) Jørgensen, C.; Jensen, H. S.; Andersen, F. Ø.; Egemose, S.; Reitzel, K. Occurrence of orthophosphate monoesters in lake sediments: significance of *myo*- and *scyllo*-inositol hexakisphosphate. *J. Environ. Monit.* **2011**, *13*, 2328−2334.

(50) Bai, X.; Ding, S.; Fan, C.; Liu, T.; Shi, D.; Zhang, L. Organic phosphorus species in surface sediments of a large, shallow, eutrophic lake, Lake Taihu, China. *Environ. Pollut.* **2009**, *157*, 2507−2513.

(51) Ding, S.; Xu, D.; Li, B.; Fan, C.; Zhang, C. Improvement of ^{31}P NMR spectral resolution by 8-hydroxyquinoline precipitation of paramagnetic Fe and Mn in environmental samples. *Environ. Sci. Technol.* **2010**, *44*, 2555−2561.

(52) Turner, B. L.; Weckström, K. Phytate as a novel phosphorus-specific paleo-indicator in aquatic sediments. *J. Paleolimnol.* **2009**, *42*, 391−400.

(53) Cade-Menun, B. J.; Benitez-Nelson, C. R.; Pellechia, P.; Paytan, A. Refining ^{31}P nuclear magnetic resonance spectroscopy for marine particulate samples: Storage conditions and extraction recovery. *Mar. Chem.* **2005**, *97*, 293−306.

(54) Ahlgren, J.; Reitzel, K.; Tranvik, L.; Gogoll, A.; Rydin, E. Degradation of organic phosphorus compounds in anoxic Baltic Sea sediments: A ^{31}P nuclear magnetic resonance study. *Limnol. Oceanogr.* **2006**, *51*, 2341−2348.