THE ROYAL SOCIETY

Received 2 November 2000
Accepted 25 January 2001
Published online 22 April 2002

Inositol phosphates in the environment

Benjamin L. Turner[1*], Michael J. Papházy[2], Philip M. Haygarth[1] and Ian D. McKelvie[2]

[1]*Soil Science Group, Institute of Grassland and Environmental Research, North Wyke, Okehampton, Devon EX20 2SB, UK*
[2]*Water Studies Centre and School of Chemistry, Monash University, Clayton 3168, Victoria, Australia*

The inositol phosphates are a group of organic phosphorus compounds found widely in the natural environment, but that represent the greatest gap in our understanding of the global phosphorus cycle. They exist as inositols in various states of phosphorylation (bound to between one and six phosphate groups) and isomeric forms (e.g. *myo*, D-*chiro*, *scyllo*, *neo*), although *myo*-inositol hexakisphosphate is by far the most prevalent form in nature. In terrestrial environments, inositol phosphates are principally derived from plants and accumulate in soils to become the dominant class of organic phosphorus compounds. Inositol phosphates are also present in large amounts in aquatic environments, where they may contribute to eutrophication. Despite the prevalence of inositol phosphates in the environment, their cycling, mobility and bioavailability are poorly understood. This is largely related to analytical difficulties associated with the extraction, separation and detection of inositol phosphates in environmental samples. This review summarizes the current knowledge of inositol phosphates in the environment and the analytical techniques currently available for their detection in environmental samples. Recent advances in technology, such as the development of suitable chromatographic and capillary electrophoresis separation techniques, should help to elucidate some of the more pertinent questions regarding inositol phosphates in the natural environment.

Keywords: inositol phosphates; phytic acid; organic phosphorus; soils; sediments; bioavailability

1. INTRODUCTION

Inositol phosphates (IP_x, where $x = 1, 2,\ldots6$) are a class of organic compounds found widely in the natural environment. They are synthesized in terrestrial ecosystems by plants and are strongly complexed in soils, where they accumulate to form the dominant class of organic phosphorus (P). As such, they are of central interest in terms of their availability to plants (Hayes *et al.* 2000*b*) and potential leaching to waterbodies (Turner & Haygarth 2000). Large amounts of inositol phosphates are also present in aquatic environments, where they may contribute to the growth of toxin-producing cyanobacteria (Whitton *et al.* 1991), which currently pose the most pervasive threat to global water quality (Kotak *et al.* 1993; Turner & Rabalais 1994; Bowling & Baker 1996).

Despite their widespread occurrence in terrestrial and aquatic systems, and improved techniques for their separation and detection in environmental samples, the role of inositol phosphates in the global P cycle remains poorly understood. Inorganic phosphate is liberated from the parent inositol by the enzyme phytase, but evidence for the hydrolysis and biological utilization of inositol phosphates in both terrestrial and aquatic systems is ambiguous. The continued accumulation of inositol phosphates in soils under cultivation has implications for their transfer to water bodies, but difficulties associated with detecting the relatively low concentrations of organic P species in natural waters means that there is little information on the movement of inositol phosphates from terrestrial to aquatic systems.

The roles of inositol phosphates in biomedical and food chemistry are well documented and understood (Stevenson 1982; Oberleas & Harland 1986; Dean & Beaven 1989; Palmer & Wakelam 1989; Xu *et al.* 1992), but large gaps exist in our understanding of the origins, chemical forms, bioavailability and mobility of these compounds in the environment. This review summarizes current information on inositol phosphates in the environment and the techniques available for their extraction, separation and detection in environmental samples.

2. STRUCTURE, NOMENCLATURE AND CHEMISTRY OF INOSITOL PHOSPHATES

Inositol phosphates are a family of phosphoric esters of hexahydroxycyclohexane (inositol). A number of inositol phosphate isomers exist, based on the stereoisomers of the unsubstituted inositols (figure 1). The *myo* stereoisomer is the most common in nature, although *neo*-, *scyllo*- and D-*chiro*-inositol phosphates are also present in terrestrial and aquatic environments (Cosgrove 1980). The D- and L- prefixes of the *chiro*-inositol isomers indicate that the compounds are optically either dextrorotatory (D-*chiro*-) or laevorotatory (L-*chiro*-), although only D-*chiro*-inositol phosphates appear to exist in the environment. The origins of the various isomers are discussed below (§ 3(a)(ii)).

*Author for correspondence (current address): United States Department of Agriculture—Agricultural Research Service, Northwest Irrigation and Soils Research Laboratory, 3793 N. 3600 E. Kimberly, Idaho 83341, USA (bturner@nwisrl.ars.usda.gov).

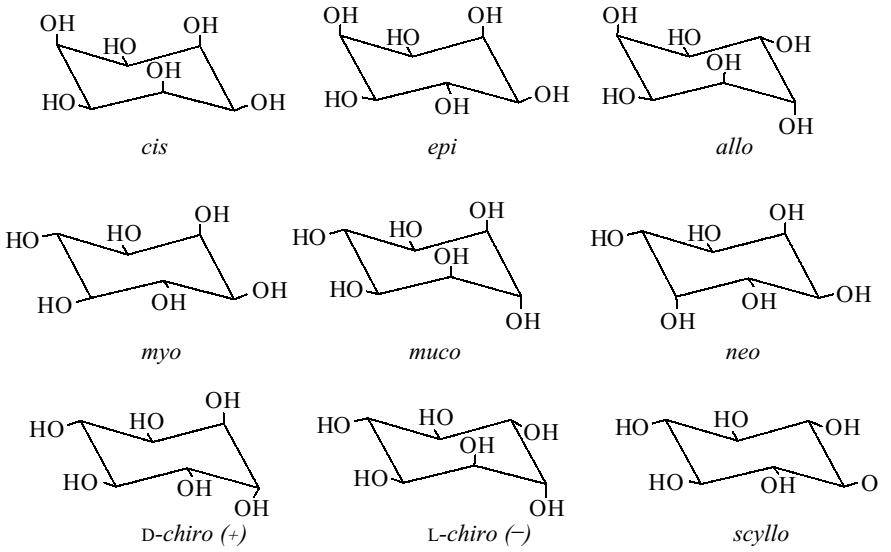

Figure 1. Preferred conformations of unsubstituted inositols (after Cosgrove 1980).

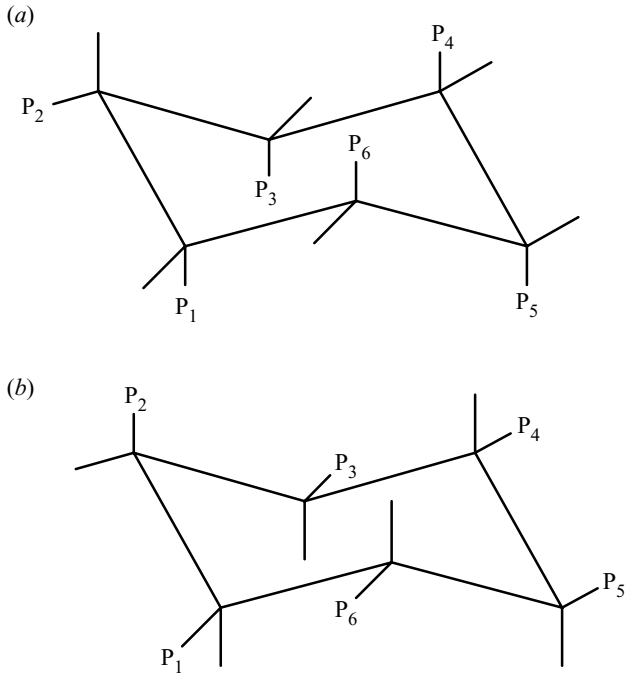

Figure 2. (*a*) Axial form of phytic acid (pH 5–12); (*b*) equatorial form of phytic acid (pH < 5 and pH > 12) (Martin & Evans 1986). $P_i = -OPO_3^{2-}$, $i = 1, 2...6$.

The number of substituted phosphate groups on the inositol ring may vary between one and six, which is indicated by the prefixes mono, bis, tris, tetrakis, pentakis and hexakis (preferred over di, tri, tetra, penta and hexa) (IUPAC 1971). The positions of the phosphate groups are denoted by the position number of the carbon in the inositol ring to which they are attached (figure 2). For a comprehensive description of the numbering system, the reader is referred to the monograph by Cosgrove (1980, pp. 6–9).

The *myo*-inositol hexakisphosphate isomer is referred to by a number of different names: 'phytic acid' is the alternative name for the free acid form, whilst 'phytate' refers to the salt of phytic acid. The term 'phytin' is occasionally used, especially in older texts, and refers specifically to the Ca–Mg salt of phytic acid, which forms the dominant inositol phosphate in cereal grains (Wheeler & Ferrel 1971).

Phytic acid possesses an axial structure between pH 5 and 12 (figure 2*a*) and an equatorial structure outside this range (figure 2*b*). Phytic acid has 12 ionizable protons: pK_a values estimated by measuring the change in ^{31}P nuclear magnetic resonance (^{31}P NMR) chemical shift with change in pH are thought to be more reliable than those obtained by potentiometric methods (table 1).

Inositol phosphates, especially IP_6, act as strong ligands because of their high anionic charge (figure 3). Their propensity for complexing polyvalent cations is well known in the medical and biological fields (Martin & Evans 1986; Luttrell 1992; Martin 1995) and applies similarly to soils, sediments, and colloidal and particulate matter in aquatic systems (Anderson *et al.* 1974; De Groot & Golterman 1993). The reactivity of IP_6 has recently found application in the reclamation of sediments and aquifers contaminated with uranic actinides (Nash *et al.* 1998). The compounds formed between the actinides and IP_6 reduce the contaminant mobility and decompose *in situ* to form compounds that are stable in the environment.

There have been several investigations of the interaction between phytic acid and polyvalent cations at different pH values (Martin & Evans 1986, 1987; Evans & Martin 1991; Luttrell 1992). At low pH, the combining affinity of soluble phytate complexes for metal ions is:

$$Cu(II) \geqslant Zn(II) \sim Cd(II) > Mn(II) > Mg(II) > Co(II) > Ni(II),$$

whilst at mid-range pH values, insoluble metal ion : phytate complexes of 6 : 1 are formed (Martin & Evans 1987).

Phytic acid is strongly adsorbed to the surfaces of ferric oxides (De Groot & Golterman 1993) and the adsorption of IP_6 to non-crystalline aluminium or iron precipitates in

Table 1. pK_a data for *myo*-inositol hexakisphosphate (from the data of Costello *et al.* 1976).

pK_1	pK_2	pK_3	pK_4	pK_5	pK_6	pK_7	pK_8	pK_9	pK_{10}	pK_{11}	pK_{12}
1.1	1.5	1.5	1.7	2.1	2.1	5.7	6.9	7.6	10.0	10.0	12.0

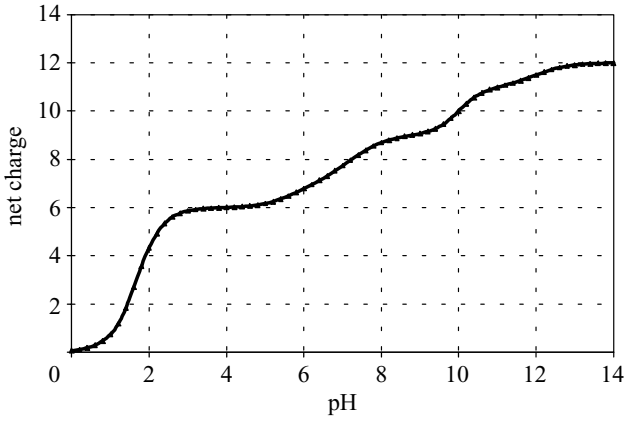

Figure 3. Estimated charge on *myo*-inositol hexakisphosphate over a range of pH values. The calculated charge is based on the pK_a data shown in table 1 (Costello *et al.* 1976).

model solutions is greatly influenced by pH; at pH 4.5, the uptake of IP_6 is more than double that at pH 6.5 (Shang *et al.* 1992). In a comparative study of the adsorptive effects of orthophosphate and phytate, De Groot & Golterman (1993) found that the addition of phytate to a suspension of orthophosphate adsorbed onto Fe(OOH) not only released orthophosphate, but also inhibited orthophosphate adsorption. Significantly, Ca^{2+} enhanced the adsorption of phytate onto Fe(OOH) by a factor of two. Furthermore, the chemical reduction of Fe(OOH)-phytate formed insoluble Fe_4(phytate), rather than releasing soluble phytate, suggesting an irreversible binding of IP_6 under these conditions.

The displacement of orthophosphate into solution upon the addition of IP_6 has also been observed in soils (Bowman *et al.* 1967; Anderson *et al.* 1974). The likely mechanism is IP_6 competing successfully for the same binding sites as orthophosphate (Anderson *et al.* 1974). Indeed, soil IP_{5+6} contents have been strongly correlated with phosphate sorption capacity (McKercher & Anderson 1968*b*). However, Helal & Dressler (1989) noted that the addition of Na-phytate to soil displaced Fe as well as orthophosphate, indicating that the reaction was not a simple phytate/orthophosphate exchange, but involved the modification of soil properties related to sorption. For example, changes in electrochemical properties almost certainly contribute (Celi *et al.* 1999).

Whilst the interaction of phytic acid with iron oxide surfaces has been studied, less is known about its interaction with soluble organic matter, such as humic and fulvic acids (Thurman 1985; Nanny & Minear 1994). However, inositol phosphates clearly form strong associations with these types of compounds. In soils, for example, significant amounts of inositol phosphates are associated with both the humic and fulvic acid fractions (Borie *et al.* 1989) and a similar situation appears to exist in lake waters (Herbes *et al.* 1975; Nanny & Minear 1994).

(a) *Chemical hydrolysis of inositol phosphates*

The phosphate ester linkages in phytic acid are quite stable, especially under basic conditions. Chemical hydrolysis in acidic media is slow, with maximum hydrolysis of phytic acid occurring at pH 4.5 and minimum rates at pH 0–1 (Anderson 1980). Even under conditions of extended reaction (6 h) with concentrated hydrochloric or sulphuric acid at high temperature (100 °C), complete hydrolysis of IP_6 is not achieved (Cosgrove 1980). This results in incomplete recovery of inositol phosphates in waters and soil extracts by conventional digestion procedures for the determination of total P, which commonly involve nitric–sulphuric acid digestion for 40–60 min (Benson *et al.* 1996).

(b) *Enzymatic hydrolysis of inositol phosphates*

Two enzymes are known to catalyse the dephosphorylation of IP_6. These are 3-phytase (EC 3.1.3.8), which is of microbial origin, and 6-phytase (EC 3.1.3.26) from wheat. The enzymes' names derive from their action on the 3 and 6 phosphate bonds of *myo*-IP_6, respectively (Nomenclature Committee of the International Union of Biochemistry 1984). Despite the reported specificity of purified phytase for phytic acid (Cosgrove 1980; Nayini & Markakis 1986), most commercially available sources contain a variety of other enzymes as impurities, notably acid phosphatases. This results in activity towards a wide range of organic P compounds including other orthophosphate monoesters (e.g. sugar phosphates and mononucleotides), inorganic and organic polyphosphates and even nucleic acids (McKelvie *et al.* 1995; Shand & Smith 1997; Turner *et al.* 2002). Dephosphorylation of IP_{2-3} by phytase is considerably slower than of IP_{4-6} and purified phytase does not appear to release orthophosphate from IP_1 (Kemme *et al.* 1999). The final dephosphorylation is achieved by phosphomonoesterase enzymes (acid or alkaline phosphatase, EC 3.1.3.1 and EC 3.1.3.2, respectively), which occur widely in the natural environment and can dephosphorylate all inositol phosphate esters except IP_6 (Meek & Nicoletti 1986; Marko-Varga & Gorton 1990; Shan *et al.* 1993).

There has been little research on phytase in soils and waters because no artificial substrates are presently available that can be used for convenient measurement of activity. Phytase is present in soil and soil solution (Jackman & Black 1952; Shand & Smith 1997) and several plants (including cyanobacteria) and microorganisms excrete extracellular phytase (Greaves & Webley 1965; Whitton *et al.* 1991; Hayes *et al.* 2000*b*; Leake 2002). However, activity appears to be negligible in natural waters (Klotz 1991), possibly due to the low pH optima (pH 2.5 and 5.0) of purified phytase activity towards phytic acid, with activity declining to almost zero at pH 7 (Ullah & Gibson 1987; McKelvie *et al.* 1995). There is also evidence that the activity of wheat phytase is com-

pletely inhibited in the presence of montmorillonite (Leprince & Quiquampoix 1996).

3. THE INOSITOL PHOSPHATE CYCLE

(a) *The terrestrial inositol phosphate cycle*

A conceptual model of the terrestrial inositol phosphate cycle is illustrated in figure 4*a*. The cycle is necessarily qualitative because of the paucity of information available on the magnitude of the compartments and flow paths involved.

(i) *Origins of inositol phosphates in soils*

In the terrestrial environment, inositol phosphates are synthesized in plants, almost exclusively as the *myo* stereoisomer. For example, *myo*-IP_6 often comprises 100% of the total inositol phosphates in most nutritionally important legumes, cereals and oil seeds (Kasim & Edwards 1998). The highest concentrations are found in the seeds, where IP_6 acts as a P storage compound and constitutes up to 90% of the total organic P (Cosgrove 1980). Lower phosphate esters of *myo*-inositol do not appear to exist in nature other than as transient intermediaries in biochemical reactions, although $IP_{1–3}$ are important constituents of the phosphoinositides, a form of phospholipid constituting *ca.* 2–9% of the total phospholipids in plant and animal tissues (Angyal 1963; Cosgrove 1980). Inositol phosphates have also been reported in animal wastes (2–60% total organic P) (Caldwell & Black 1958*a*; Peperzak *et al.* 1959), hay grass (3–7% total organic P) (Becker 1950) and in small amounts in bacteria, fungi and the roots of higher plants (Cosgrove 1980).

Sewage sludge is a potentially important, but unquantified, source of inositol phosphates to soil. These are increasingly being applied to agricultural land as a nutrient source, especially in the UK, where sea dumping was banned in 1998. Sludges can contain large amounts of organic P in the form of orthophosphate monoesters (Condron *et al.* 1997) and, therefore, have the potential to provide large amounts of inositol phosphates to the soil system.

(ii) *Amounts and forms of inositol phosphates in soils*

A large proportion of the total soil P is present in organic forms, typically between 29 and 65%, but up to 90% in some organic soils (Harrison 1987). The majority of the identifiable soil organic P exists as orthophosphate monoesters, which constitute between 60 and 90% of the total organic P in alkaline soil extracts (Condron *et al.* 1985, 1990; Cade-Menun & Preston 1996). Inositol phosphates frequently comprise over 60% of the soil orthophosphate monoesters, because only small amounts of other monoesters (e.g. sugar phosphates) are detected (Dalal 1977; Harrison 1987). Therefore, IP_6 is the major component of the organic P in most soils.

The amounts of IP_6 and its proportional contribution to the total organic P vary widely between soils (table 2). For example, Williams & Anderson (1968) reported that IP_{5+6} in 47 Australian soils varied between 1 and 356 μg P g^{-1} dry soil and constituted between 0.4 and 38% of the total organic P. Similarly, Anderson (1964) reported concentrations in Scottish soils of between 56 and 460 μg P g^{-1} dry soil and between 24 and 58% of the total organic P. However, many of these results must be considered with caution, because the analytical techniques used by some early authors may have either overestimated or underestimated the soil inositol phosphate content. Underestimations were probably caused by incomplete recovery of inositol phosphate from anion exchange columns (Anderson 1964; Martin 1970), whilst overestimations resulted from the inclusion of organic P compounds other than inositol phosphates in the purified extracts (Irving & Cosgrove 1981). Furthermore, large amounts of soil organic P are not extracted using common extracting solutions such as 0.5 M NaOH (Cade-Menun & Preston 1996); the residual fraction probably contains strongly bound inositol phosphates.

Inputs of inositol phosphates to the soil from plant material are principally in the form of *myo*-IP_6 (Cosgrove 1980), but all possible phosphate esters of *myo*-inositol have been detected in the soil, including IP_1 (Martin & Wicken 1966; Wild & Oke 1966; Minear *et al.* 1988), IP_2 (Martin & Wicken 1966; Halstead & Anderson 1970), IP_3 (Halstead & Anderson 1970; Omotoso & Wild 1970), IP_4 (Cosgrove 1963; Omotoso & Wild 1970; Minear *et al.* 1988), IP_5 (Cosgrove 1963; Martin & Wicken 1966; McKercher & Anderson 1968*b*; Minear *et al.* 1988) and IP_6 (table 2). *myo*-IP_6 is usually the dominant form, although *myo*-IP_5 exists in substantial quantities. Typical proportions of the inositol phosphate esters are IP_6 = 83%, IP_5 = 12%, IP_4 = 4%, IP_3 = 1%, IP_2 = trace (Anderson & Malcolm 1974). Inositol monophosphate, which is a well-known microbial metabolite, was not detected in this example, although Wild & Oke (1966) claimed that IP_1, identified by paper chromatography, was the major form of organic P in $CaCl_2$ extracts of two soils from southern England. Despite these reports, it is unclear to what extent esters other than hexakisphosphate may represent the degradation of IP_6 during chemical extraction.

One of the most interesting aspects of soil inositol phosphates is the existence of stereoisomers other than *myo*-inositol phosphates that rarely exist elsewhere in nature. The presence of D-*chiro*-, *scyllo*- and *neo*-IP_6 has been demonstrated, apparently at similar ratios, in a range of soil types from around the world (Cosgrove 1962, 1966; Cosgrove & Tate 1963; McKercher & Anderson 1968*a*; Halstead & Anderson 1970; Omotoso & Wild 1970; Anderson & Malcolm 1974; Baker 1974). These are generally in the order of *myo* > *scyllo* > D-*chiro* > *neo*, with *myo*-IP_6 representing up to 90% of the total IP_6, *scyllo*-IP_6 representing 20–50%, D-*chiro*-IP_6 representing *ca.* 10% and *neo*-IP_6 representing *ca.* 1% (McKercher & Anderson 1968*a*). However, a more recent study reported that *neo*-inositol phosphates represented *ca.* 7% of the total IP_{5+6} in uncultivated soils of southeastern Australia (Irving & Cosgrove 1982). In the lower inositol phosphate esters, Anderson & Malcolm (1974) detected *myo*, *scyllo*, D-*chiro* and *neo* inositol isomers of $IP_{3–6}$ in Scottish soils, whilst Omotoso & Wild (1970) found that only the *myo* stereoisomer existed in the lower esters ($IP_{1–4}$) in a range of English and Nigerian soils.

The origins of the *scyllo*, D-*chiro* and *neo* isomers of IP_6 in soils remain unresolved. Free *scyllo*- and D-*chiro*-inositols occur in various plant and animal sources, including palm trees, fish and urine (Angyal 1963), but never in

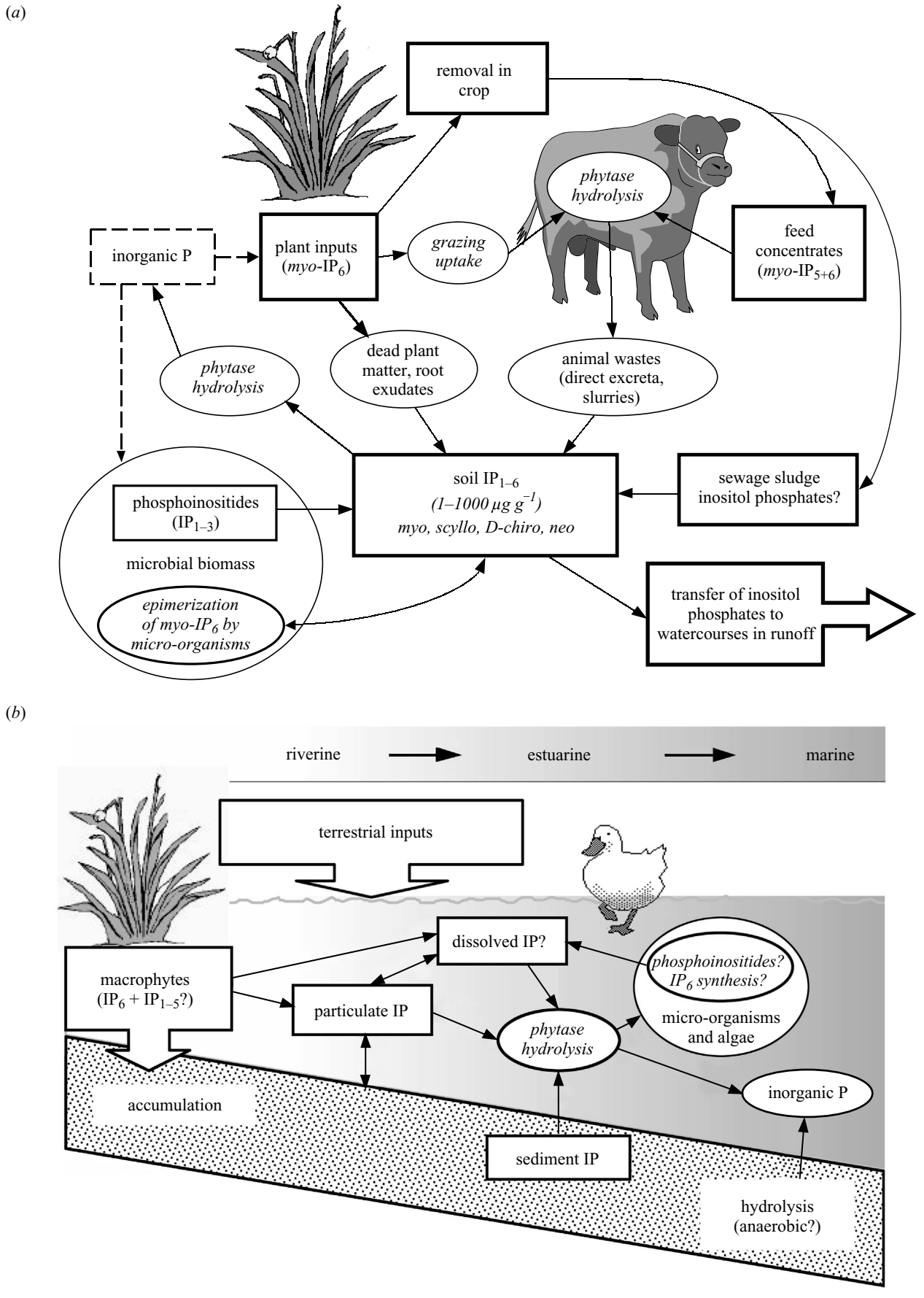

Figure 4. Conceptual biogeochemical cycle of inositol phosphates in (*a*) terrestrial systems, and (*b*) aquatic systems.

Table 2. Reported inositol phosphate concentrations in a range of soil types.

				inositol phosphate content (IP_{5+6})			
reference	location	soil description	number of soils	$\mu g\ g^{-1}$ soil	% of total organic P	extraction technique	isomers detected other than *myo*
Anderson (1964)	Scotland	range of soil types and parent materials	17	56–460	24–58	hot 3M NaOH[c]	—
Anderson & Malcolm (1974)	Scotland	arable soils	1	338.5	53.4	—	*neo*, *scyllo*, D-*chiro* (IP_{3-6})
Appiah & Thomas (1982)	Canada, Ghana	various soil types	5	15.5–30.6	5.1–23	hot 3M NaOH[c]	—
Baker (1974)	New Zealand	two chronosequences	—	0.3–59	2.2–32	hot 3M NaOH[c]	—
Borie *et al.* (1989)	Chile	volcanic soils	9	415–987	42–67	method of Anderson (1964) with hypobromide oxidation[d]	—
Caldwell & Black (1958*b*)	USA	range of soil types and textures	49	4.0–98.5	3.0–52	hot concentrated HCl; 0.5 M NaOH[a]	—
Cosgrove (1963)	Australia	alpine humus	3	92.4–334	12–16	1 M NaOH, 60 °C[b]	D-*chiro*, *scyllo* (IP_6)
Cosgrove (1966)	Scotland, USA	surface soils; range of parent materials and P contents	12	—	—	1 M NaOH, 60 °C[b]	*scyllo* (IP_5); *neo*, *scyllo*, D-*chiro* (IP_6)
Dormar (1967)	Canada	Chernozems	5	14.3–41.9	8.0–12	1 M NaOH, 60 °C[b]; hot 3M NaOH[c]	—
Halstead & Anderson (1970)	Scotland and Canada	surface soils	7	—	8.0–34	hot 3M NaOH[c]	*neo*, D-*chiro*, *scyllo*(IP_{2-6})
Irving & Cosgrove (1982)	southeastern Australia	—	5	12.2–167.4	—	method of Anderson (1964) with hypobromide oxidation[d]	D-*chiro*, *scyllo*, *neo* (IP_{5-6})
Islam & Mandal (1977)	Bangladesh	range of soil types and textures	—	3.0–183.1	2.2–82	—	—
Jencks *et al.* (1964)	USA	calcareous soils	—	25–500	30–100	—	—
Martin (1970)	New Zealand	surface soils	4	—	28–62	0.3 M LiOH	—
Martin & Wicken (1966)	New Zealand	range of surface soil types	5	94–558	4.0–42	0.3 M KOH	D-*chiro* ($IP_{1,2+6}$)
McKercher & Anderson (1968*a*)	Canada and Scotland	surface soils	8	31–460	12.5–50.2	hot 3 M NaOH[c]	D-*chiro*, *scyllo* (IP_5); D-*chiro*, *scyllo*, *neo* (IP_6)
McKercher & Anderson (1968*b*)	Canada	range of surface soil types	18	20–71	10.7–23.4	hot 3 M NaOH[c]	—
		subsurface soils, mostly under grassland	12	18–43	11.2–18.5	hot 3 M NaOH[c]	—
Omotoso & Wild (1970)	England and Nigeria	grassland and forest soils	7	38–513	11.2–30	1 M NaOH, 60 °C[b]	D-*chiro*, *scyllo*, *neo* (IP_6) (*neo* not detected in Nigerian soils)
Oniani *et al.* (1973)	England, USSR, Ceylon	grasslands and tea plantations	4	2.0–90	0.7–45	hot 3M NaOH[c]	—
Steward & Tate (1971)	Australia	—	8	10–290	2.0–31	0.5 M NaOH and ultrasonication	D-*chiro*, *scyllo* (IP_{4-6})
Thomas & Lynch (1960)	Canada	range of soil types, Ah horizons	—	7.3–155.8[e]	2.2–21.9	hot concentrated HCl; 0.5 M NaOH[a]	—
Williams & Anderson (1968)	Australia	wide range of soil types	47	1–365	0.4–38	hot 3M NaOH[c]	—

[a] Caldwell & Black (1958*a*). [b] Cosgrove & Tate (1963). [c] Anderson (1964). [d] Irving & Cosgrove (1981). [e] Total inositol phosphate.

combination with phosphate (Cosgrove 1980). D-*chiro*-IP_6 was reported in pine needles (L'Annunziata & Fuller 1971), but this was subsequently questioned on analytical grounds (Cosgrove 1980). Recently, *neo*-inositol phosphates have been identified in trophozoites of the parasitic amoebae *Entamoeba histolytica* and *Phreatamoeba balamuthi* using two-dimensional nuclear magnetic resonance (NMR) (Martin *et al.* 2000). However, the fact that (i) terrestrial plants only contain the *myo* isomer of IP_6 and (ii) chemical epimerization of *myo*-IP_6 has been ruled out, suggest that microorganisms must play a key role in the synthesis of IP_6 isomers other than *myo*-IP_6 in the soil (Cosgrove 1969*b*). The likely mechanisms of biological production are either the direct synthesis of D-*chiro*-, *scyllo*- and *neo*-inositol phosphates by microorganisms, or the transformation of the *myo* isomer through epimerization reactions. Epimers are stereoisomers that differ only in the arrangement of atoms around a single carbon atom and epimerization is the transformation from one epimer to another. Most evidence points to epimerization rather than direct microbial synthesis as the likely mechanism producing the isomers other than *myo* in soils (Cosgrove 1969*b*; L'Annunziata 1975). This is supported by the fact that stereoisomers present in the soil (D-*chiro*, *scyllo* and *neo*) differ from the *myo* isomer only by the orientation of a single phosphate group, or one carbon atom, whereas other isomers that have not been detected in soils differ by two (e.g. *cis*, *allo*, *muco*). Indeed, the conversion of unphosphorylated *myo*-inositol to D-*chiro*-inositol has been shown in the clover *Trifolium incarnatum* and in the alga *Chlorella fusca* (L'Annunziata 1975). Furthermore, L'Annunziata *et al.* (1977) detected D-*chiro*-inositol after the incubation of ^{14}C-labelled *myo*-inositol with soil; no such transformation took place in sterile soil, strongly suggesting that microbial synthesis or epimerization reactions were responsible.

(iii) *Behaviour of inositol phosphates in soils*

The dominance of inositol phosphates over other forms of soil organic P is not reflected in the composition of organic P inputs to the soil (Magid *et al.* 1996). For example, orthophosphate monoesters constitute only 10–25% of the organic P inputs to the soil from microbial and plant sources (Webley & Jones 1971; Bieleski 1973; Magid *et al.* 1996) of which inositol phosphates are a minor fraction. By contrast, orthophosphate diesters (e.g. nucleic acids and phospholipids) constitute the majority of the organic P in inputs of fresh microbial and plant matter to the soil. Nucleic acids dominate microbial inputs of organic P, constituting *ca.* 60% of the intracellular P in fungi, bacteria and mycorrhizal mycelium (Webley & Jones 1971; Gianfrancesco & Leake 2002). Phospholipids account for 5–30% of the organic P in microorganisms (Webley & Jones 1971; Magid *et al.* 1996) and an even greater proportion in fresh plant tissue (Bieleski 1973).

The disparity between the composition of organic P compounds measured in the soil (dominated by orthophosphate monoesters) and those constituting the majority of the organic P in fresh inputs (dominated by orthophosphate diesters) arises through a differential stabilization of organic P compounds in the soil. Adsorption of organic P in soil is usually determined by the P groups, which represent the dominant charge on most organic P compounds (Celi *et al.* 1999). As a result, organic P compounds are more strongly sorbed as the number of P groups increases. This means that sugar phosphates and orthophosphate diesters are only weakly adsorbed and remain vulnerable to microbial degradation (Greaves & Wilson 1970). However, with six phosphate groups, IP_6 has a high charge density and undergoes strong interaction with the soil, either being adsorbed to clays (Anderson & Arlidge 1962; Celi *et al.* 1999), or precipitated as insoluble salts such as sesquioxides of Fe and Al in acid soils and insoluble calcium salts in alkaline soils (Jackman & Black 1951). The strong reaction compared with other organic P compounds means that IP_6 undergoes preferential stabilization, which prevents interaction with hydrolytic enzymes and results in inositol phosphates accumulating to form the dominant fraction of organic P in most soils (Harrison 1987). Since the number of P groups associated with inositol decreases from hexakisphosphate to monophosphate, the charge density decreases and sorption is reduced. This is mirrored by decreases in the concentrations of these forms in the soil, from hexakisphosphate to monophosphate (Anderson & Arlidge 1962; Anderson & Malcolm 1974), although this also reflects the much smaller inputs of the lower inositol phosphate esters.

(iv) *Factors controlling the amounts of inositol phosphate in soils*

The extent to which inositol phosphates accumulate in the soil depends to a large extent on a range of climatic, soil type and cultivation factors. Using two soil chronosequences in New Zealand, Baker (1974) found that the IP_6 content increased with age up to 3000 years, after which the amounts remained constant for 10 000 years. The ratio of *myo*- to *scyllo*-IP_6 also varied with age, decreasing from 10 : 1 to 2 : 1 between 50 and 10 000 years. It is known that the nature of the soil is critical in determining whether inositol phosphates occur in significant quantities and whether they are available to plants and microbes. Some of the factors that influence the stability of inositol phosphates are soil texture (clay content), pH, organic matter content, climate and soil management/land use.

Soil properties

The most important soil properties controlling the accumulation of inositol phosphates are likely to be similar to those controlling the accumulation of organic matter in general, including soil texture (clay content) and pH (see also § 2). Highly significant differences in organic P contents have been detected with variations in texture, with peat soils containing the greatest proportions and sandy soils the lowest (Harrison 1987). The type of clay also influences the strength of IP_6 sorption, being more strongly sorbed to illite than kaolinite due to differences in the numbers of P groups involved in the stabilization process (Celi *et al.* 1999). Concentrations of IP_6 in soils from around the world relate strongly to organic P, total P, pH, total organic carbon and total nitrogen. For example, Harrison (1987) found that 90% of the variation in concentrations of IP_6 reported in the literature was explained by the total organic P, pH and total carbon. Concentrations of inositol phosphates have also been reported to correlate strongly with the phosphate adsorption capacity

and amorphous (oxalate-extractable) Fe and Al (McKercher & Anderson 1968*b*; Anderson 1980). However, other studies found no significant relationships with soil properties (Williams & Anderson 1968; Appiah & Thomas 1982), which may reflect differences in the extractability of inositol phosphates between soil types, combined with variations in sampling techniques (e.g. sample depth). For example, inositol phosphates accumulate in the surface zone of undisturbed soils (Williams & Anderson 1968).

The soil pH exerts considerable control over the stabilization of inositol phosphates. Acid soils are known to accumulate more organic P than alkaline soils (Harrison 1987), mainly as orthophosphate monoesters (Hawkes *et al.* 1984). Inositol phosphates react strongly with Fe and Al under acidic conditions and become insoluble. Complexes of inositol phosphates with Fe, Al, Ca and clays show pH-dependent variations in solubility, being most stable at pH < 5 and > 7.5 (Goring 1951; Jackman & Black 1951; Emsley & Niazi 1981), which is further regulated by the type of cations in solution (Celi *et al.* 2001). Depressed microbial activity under acidic conditions may also contribute to the stability of inositol phosphates, although microbial phytase activity is optimal at low pH (Ullah & Gibson 1987). Cosgrove (1966) noted that IP_6 was not present in two calcareous soils from the USA, but this is not necessarily representative of all calcareous soils because McKercher & Anderson (1968*b*) found that 31% of the organic P in a Scottish calcareous soil was present as IP_6.

Climate

The effects of climate are difficult to distinguish, but have been attributed to differences in inositol phosphate concentrations between soils (McKercher & Anderson 1968*b*). Soil organic P contents decrease with increasing temperature (Westin & Buntley 1967), probably because temperatures of 30–40 °C are optimal for the breakdown of organic P by the microbial biomass and phosphatase (Eid *et al.* 1951; Spier & Ross 1978). The extent of soil drying can also exert a considerable control on the solubility of inositol phosphates because significantly more are released to water from air-dried soils than from moist soils (Wild & Oke 1966; Turner *et al.* 2002). This is probably due to physical disruption mechanisms involving soil structural changes (Simonsson *et al.* 1999) and may contribute to the substantial increases in organic P released to water following soil drying (Turner & Haygarth 2001). Waterlogging of soils causes chemical reduction of Fe compounds and releases orthophosphate into solution but, in the case of IP_6, probably results in the formation of insoluble Fe_4(phytate) (De Groot & Golterman 1993).

Land use and management

Differences in land use give rise to marked variations in the organic P content. For example, forest soils typically contain greater concentrations and proportions of organic P than arable or cultivated soils (Harrison 1987), although this may, in part, be indirectly related to climatic or soil-type controls on land use. However, the amount and composition of the soil organic P is profoundly influenced by conversion between land-use types. For example, conversion of New Zealand grassland to coniferous forest substantially reduces the concentration of orthophosphate monoesters (Condron *et al.* 1996), possibly through ectomycorrhizal associations with coniferous tree roots (Leake 2002).

Soil organic P is rapidly degraded under cultivation (Tiessen *et al.* 1983), probably through increased aeration and mineralization (Dalal 1977). Williams & Anderson (1968) found that soil inositol phosphate concentrations increased when organic matter accumulated, but decreased under cultivation when organic matter was depleted. However, in the latter case, the inositol phosphate component decreased at a slower rate than total organic P, confirming the stability of IP_6 in soil.

In recent years, the effects of land use and management on soil organic P have been investigated using solution-state ^{31}P NMR. This technique is limited for investigation of inositol phosphates because it does not discriminate between inositol phosphates and other orthophosphate monoesters (see § 4), but the dominance of IP_6 in the orthophosphate monoester pool of most soils suggests that ^{31}P NMR can be used to indicate differences in IP_6 contents amongst soils (Bishop *et al.* 1994). For example, soils under cultivation for 70–80 years contained only orthophosphate monoesters, probably due to the preferential degradation of other less stable forms of organic P (Condron *et al.* 1990). Early studies using ^{31}P NMR were conducted on grasslands, which were dominated by orthophosphate monoesters (Tate & Newman 1982; Hawkes *et al.* 1984; Condron *et al.* 1985). More recently, the technique has been applied to forest and upland organic peat soils, where there are much smaller differences in the proportions of orthophosphate monoesters and diesters (Bedrock *et al.* 1994; Cade-Menun & Preston 1996; Dai *et al.* 1996).

Most studies indicate that the addition of mineral P fertilizer increases the organic P content of surface soils, mainly in the form of orthophosphate monoesters (Condron *et al.* 1985; Borie *et al.* 1989; Bedrock *et al.* 1994). This may result from either greater inputs of plant material through increased growth, or to a reduction in organic P mineralization through decreased requirement for P and suppression of phosphatase enzyme activity. Changes in pH induced by adding lime to pasture soils increases the solubility and mineralization of alkali-extractable organic P, including IP_6 (Pearson *et al.* 1941; Condron & Goh 1989).

(v) *Biological availability of inositol phosphates in soil*

The size of the inositol phosphate pool in most soils suggests that it has great potential to provide P for plant uptake. Several plants grow well with phytate as a sole P source, in some circumstances as efficiently as with orthophosphate and other organic P compounds (Tarafdar & Claassen 1988; Helal & Sauerbeck 1991). However, it is far from clear whether this applies to plants growing in soil. In P-limited environments there is evidence of P uptake from IP_6 in a *Carex* species (Corona *et al.* 1996) and in the dominant arctic tundra plant *Eriophorum vaginatum* (Kroehler & Linkins 1991), in addition to a range of other plant species (Li *et al.* 1997). However, the stabilization and accumulation of IP_6 in most soils suggests that it is relatively unavailable for biological uptake. For example, Greenwood & Lewis (1977) observed that

although soil yeasts of the genus *Cryptococcus* could use soluble Na-IP_6 as a sole P source, they could not use insoluble salts of IP_6, perhaps indicating that soil IP_6 would be inaccessible. This is consistent with the findings of Martin & Cartwright (1971), who showed that IP_6 added to soils was more available to plants in low P-fixing soils than in high P-fixing soils.

Other studies have suggested that the utilization of IP_6 by plants is limited by the presence of phytase, rather than the inaccessible nature of the compound in the soil matrix. For example, Findenegg & Nelemans (1993) concluded that the rate of enzyme-catalysed cleavage limited the availability of IP_6 to maize plants growing in phytate-amended soil, whilst Hayes *et al.* (2000*b*) showed that a range of pasture plants (grasses and legumes) grew poorly on IP_6 due to insufficient production of phytase. Furthermore, Bishop *et al.* (1994) showed that adding commercial phytase to Chilean volcanic soils reduced ^{31}P NMR signals from alkali-extractable orthophosphate monoesters compared with extracts of control soils and soils amended with other phosphatase enzymes.

Several soil microorganisms can access IP_6, which may be an important link in the soil inositol phosphate cycle and provide the mechanism by which plants obtain P from soil phytate (Richardson *et al.* 2001*b*). Greaves & Webley (1965) reported that 30–50% of soil bacterial isolates produced phytase activity, although the ability of soil microorganisms to access IP_6 in the presence of soil was subsequently found to be extremely limited (Greaves & Webley 1969). Ectomycorrhizal fungi may be particularly important because phytase activity has been detected in all 20 species so far tested (Leake 2002). For example, Antibus *et al.* (1992) showed that mycelium isolated from four basidiomycete species could access ^{32}P from labelled IP_6. Many ectomycorrhizal species can access insoluble salts of IP_6 as effectively as orthophosphate, including those associated with coniferous (Sitka spruce) roots. This is consistent with the findings of Condron *et al.* (1996), who discovered that orthophosphate monoesters were substantially depleted when coniferous agroforestry plantations were grown on New Zealand pasture soils. The changes were determined by ^{31}P NMR, so were not directly related to the depletion of IP_6. However, IP_6 is the dominant orthophosphate monoester in most soils, suggesting that ectomycorrhizal associations may be an important mechanism by which plants access recalcitrant soil IP_6.

A potentially important mechanism of plant acquisition of P from soil-bound inositol phosphate complexes may be root-induced changes in rhizosphere pH and/or the excretion of organic acid anions (e.g. citrate, maleate). These processes have been extensively investigated in terms of the solubilization of inorganic P minerals and phosphate rock fertilizers (e.g. Hedley *et al.* 1982; Hinsinger & Gilkes 1996), yet have been relatively ignored in terms of the organic P compounds. However, the dominance of IP_6 in the soil organic P pool suggests that plants in natural environments may rely heavily on their ability to access recalcitrant IP_6 and that root-induced changes in pH or organic acid excretion play a major role in this process. Indeed, inositol phosphates constitute a large proportion of the organic P extracted from soils with citric acid at low pH (Hayes *et al.* 2000*a*).

Understanding the extent to which inositol phosphates are used by plants and how this could be improved is fundamental to the development of sustainable, low-input management systems for agricultural P. Genetic modification to produce plants able to secrete phytase is one approach. For example, the modification of *Arabidopsis thaliana* with the phytase gene from *Aspergillus niger* (plus a signal peptide sequence) has recently been shown to render the plant capable of growth rates equivalent to control plants grown on inorganic orthophosphate (Richardson *et al.* 2001*a*). This approach assumes that inositol phosphate uptake from soil is limited by phytase activity, raising the interesting question of why plants evolving in often P-limited soils containing an abundance of inositol phosphates do not secrete phytase.

From the preceding discussion, it is clear that the extent to which soil inositol phosphates are biologically available remains unresolved. The ample evidence of IP_6 utilization by a range of plant species in culture suggests that inositol phosphates cannot be disregarded as a bioavailable P source, but there is a clear requirement for similar studies in real soil conditions.

(vi) *Mobility of inositol phosphates in the soil and potential transfer to watercourses*

Information on the transfer of inositol phosphates from terrestrial to aquatic systems is fundamental to understanding the potential impact of diffuse agricultural P pollution on water quality. However, the difficulties in detecting low concentrations of trace organic P compounds in natural waters mean that there is little direct evidence of inositol phosphate transfer from the soil in runoff.

The strong sorption of IP_6 to clays and organic matter suggests that desorption of IP_6 to soil solution is unlikely. However, Espinosa *et al.* (1999) reported the first evidence of the presence of IP_6 in soil leachate water, where it constituted around one-third of the identifiable soluble organic P. Furthermore, studies using enzymes on soil-water extracts and soil solutions have revealed that a considerable proportion of the organic P is hydrolysable by phytase (Pant *et al.* 1994*a*; Shand & Smith 1997), which is increased following soil drying (Turner *et al.* 2002). Much of the unhydrolysed organic P may be strongly bound inositol phosphates in high molecular weight complexes with organic matter.

The large amounts of inositol phosphates accumulated in the soil suggest that there is a great potential for transfer to surface waters to occur by erosion mechanisms. The majority of P forms over 0.45 μm in size have been identified as organic P bound to soil particles, although the composition of this fraction is largely unknown (Stevens & Stewart 1982; Heathwaite *et al.* 1990). Thus, it is reasonable to assume that large amounts of inositol phosphates are transferred in runoff adsorbed to soil particles. This is supported by the work of Suzumura & Kamatani (1995*b*), who detected various IP_6 isomers in river-water suspended solids, although these only amounted to *ca.* 5% of the total organic P. Furthermore, a substantial amount of P in runoff can be associated with colloidal-sized (1 nm–1 μm) material (Haygarth *et al.* 1997). These soil-derived colloids consist of clays, organic matter, oxides and oxyhy-

droxides of Fe, Al and Mn, which have a strong affinity for inositol phosphates (Kretzschmar *et al.* 1999).

(vii) *Inositol phosphates in animal manures*

Inositol hexakisphosphate excreted by monogastrics (e.g. poultry and pigs) is a potentially important link in the plant–soil transfer pathway. A large proportion of the P in animal feed is IP_6, but this is unavailable to monogastrics because of low levels of intestinal phytase (Morse *et al.* 1992). To combat this, inorganic P supplements are used to provide sufficient P for optimal animal growth and development, but inefficient uptake means that typically about 70% of the total P intake is lost in excreta. This is ultimately applied to agricultural land, often in addition to mineral P fertilizers, increasing the risk of P pollution in runoff water (Haygarth *et al.* 1998). As a result, there has been considerable recent interest in the use of phytase enzymes as feed supplements for monogastrics and ruminants to increase the hydrolysis of IP_6 in the gut and increase P uptake efficiency (e.g. Poulsen 2000). Phytase supplements also increase the bioavailability of various minerals and trace elements (e.g. Lei *et al.* 1993), but there is evidence that IP_6 has various protective functions, including the reduction of free radicals and the carry-over of heavy metals (Pallauf & Rimbach 1997).

Phytase supplements can reduce the concentrations of IP_6 in pig faeces by between 35 and 47% (Simons *et al.* 1990; Skoglund *et al.* 1998). This has had a tremendous impact in The Netherlands, where phytase use on a large scale has halved P excretion from pig production in 20 years (Lenis & Jongbloed 1999). When used with broiler chickens, phytase allows inorganic P in feed to be reduced by up to 50%, with no effects on feed conversion, growth rate, bodyweight or bird performance (Simons *et al.* 1990; Huff *et al.* 1998). Similarly, the use of phytase in a low-P diet for pigs produces equivalent growth and performance as a diet with normal P contents (Simons *et al.* 1990; Adeola *et al.* 1998; Liu *et al.* 1998). When used in duck diets, it brings additional benefits in terms of improved nutrient yield and bird performance (Farrell & Martin 1998).

Despite the many agronomic advantages of phytase supplements in animal diets, the environmental benefits are less conclusive. Phytase supplements can clearly reduce the concentrations of total P in manures, but the corresponding changes in manure-P composition may have implications for P transfer in runoff. Manure-derived inositol phosphates are strongly fixed in the soil and unlikely to be lost in drainage waters. However, by converting IP_6 to inorganic orthophosphate in the animal, phytase supplements may increase the potential risk of manure-derived P loss in drainage waters. Therefore, the potential fate of manure-derived P must be considered when assessing the value of phytase supplements alongside the perceived benefits of a reduction in the total P. The development of techniques for the determination of inositol phosphates in water and manures will improve our understanding of the impacts of these types of effects on the environment.

(b) *The aquatic inositol phosphate cycle*

(i) *Origins of inositol phosphates in aquatic systems*

A conceptual aquatic inositol phosphate cycle is presented in figure 4*b*. The origins of inositol phosphates in aquatic systems are largely unidentified. It has been speculated that substantial amounts of inositol phosphates are derived from terrestrial environments, but there is little direct evidence for this. Weimer & Armstrong (1979) found that, in aquatic plants, lower inositol phosphate esters ($IP_{1–4}$) were more prevalent than higher esters ($IP_{5–6}$). From this finding, and the inositol phosphate composition of lake sediments, they deduced that inositol phosphate inputs to lake sediments were derived in equal proportions from catchment runoff and autochthonous production by algae and macrophytes. However, this assumption was subsequently questioned, because the extraction process may have degraded higher inositol phosphate esters (Potman & Lijklema 1983). Suzumura & Kamatani (1995*b*) determined IP_6 in samples from various stages of the cycle, including soils, riverine, estuarine and coastal marine sediments. By tracing the isomeric composition of the IP_6 through the system, they deduced that the dominant source of phytate to the catchment was terrestrial.

Some aquatic organisms synthesize inositol phosphates. The phosphorylation of *myo*-inositol to *myo*-IP_6 has been described in *Spirodela polyrhiza* L., a species of floating duckweed that, *in vivo*, can synthesize *myo*-IP_6 from inositol via the intermediates $I(3)P_1$ $I(1,4)P_2$, $I(3,4,6)P_3$, $I(3,4,5,6)P_4$, $I(1,3,4,5,6)P_5$ and $I(1,2,4,5,6)P_5$ (Brearley & Hanke 1996). A similar series of intermediates has been identified in the slime mould *Dictyostelium*, in which inositol phosphates may be involved in cell-signalling processes (Stephens & Irvine 1990). Phosphatidyl inositol has been detected in phytoplankton (Oku & Kamatani 1995), suggesting that inositol phospholipids and lower inositol phosphates play an important role in phytoplankton metabolism and structure. However, Suzumura & Kamatani (1995*b*) did not detect IP_6 in zoo- or phytoplankton, discounting them as a source of aquatic phytate.

(ii) *Amounts and forms of inositol phosphates in aquatic systems*

Inositol phosphates have been detected in substantial quantities in aquatic sediments and potentially constitute an important fraction of the total organic P in the water column. Alkaline bromination followed by gel filtration of dissolved organic P in lake waters has been used to show that $IP_{1–3}$ were present in lake waters at concentrations between 3 and 15 $\mu g\ P\ l^{-1}$, mostly in the form of high molecular weight complexes (Eisenreich & Armstrong 1977). Herbes *et al.* (1975) determined that the majority of the dissolved organic P in lake water was amenable to hydrolysis by phytase. These compounds were suggested to consist of low molecular weight orthophosphate monoesters such as soluble inositol phosphates, plus high molecular weight compounds consisting of inositol phosphates bound to lipids, proteins and fulvic acids. Similarly, McKelvie *et al.* (1995) detected phytase-hydrolysable P compounds in natural waters using phytase in an immobilized, although phytase also hydrolysed a range of other organic P compounds.

Inositol phosphates comprise a substantial proportion of the total P in most lake sediments (Sommers *et al.* 1972;

Weimer & Armstrong 1977; De Groot & Golterman 1993). For example, Sommers *et al.* (1972) detected concentrations between 15 and 34 μg P g^{-1}, although this represented less than 10% of the total organic P. De Groot & Golterman (1993) showed the presence of considerable levels of phytase-hydrolysable P in shallow sediments from a freshwater marsh and a brackish/saltwater lake in southern France. In contrast, Feuillade & Dorioz (1992) detected only small concentrations of phytase-hydrolysable P in lake sediments, although this may have been an artefact of the high pH (7.8) used in these experiments (phytase activity decreases to almost zero at pH 7). In the marine environment, White & Miller (1976) demonstrated the presence of small amounts of IP_6 in coastal sediments, whilst Suzumura & Kamatani (1993) detected *myo*-, *chiro*- and *scyllo*-IP_6 in shallow marine sediments from Tokyo Bay.

(iii) *Availability of inositol phosphates to aquatic organisms*

The bioavailability of inositol phosphates in aquatic systems is an important issue in terms of water quality. The large concentrations of inositol phosphates in aquatic sediments means that they are a potentially important source of P for algal growth, which has implications for water quality and attempts to control eutrophication. However, evidence for the bioavailability of inositol phosphates in aquatic systems is contradictory. Their accumulation in sediments suggests that they are relatively refractory and, hence, biologically unavailable, but there is evidence that 35 different cyanobacterial strains (blue-green algae) can use IP_6 as their sole P source, although not as effectively as orthophosphate or other organic P compounds (Whitton *et al.* 1991). This indicates the synthesis of phytase by these organisms. Other phosphatases produced by algae (e.g. alkaline phosphatase) are not able to hydrolyse IP_6, although they may show activity for $IP_{1–5}$.

The strongest evidence for the potential bioavailability of IP_6 comes from analysis of estuarine and coastal environments, where inositol phosphates appear to be rapidly degraded. For example, Suzumura & Kamatani (1995*a*) found that although IP_6 constituted the majority of the total organic P in estuarine sediments, this was not mirrored in coastal marine sediments, where there was evidence of substantial hydrolysis. Inositol hexakisphosphate concentrations in coastal marine surface sediments were 0.01–0.1 μmol P g^{-1}, representing *ca.* 0.1–1.2% of the total organic P, but were many times lower than in estuarine sediments (0.07–0.66 μmol P g^{-1}) and riverine suspended solids (0.46 μmol P g^{-1}) and two orders of magnitude lower than in soils (0.25–5.65 μmol P g^{-1}). In addition, IP_6 was completely decomposed in subsurface sediments, indicating its ephemeral nature in the marine environment. Further evidence of *in situ* hydrolysis of inositol phosphates in marine sediments was provided by White & Miller (1976) who detected residual *myo-, scyllo-* and *neo*-inositol isomers in marine sediments, but only small amounts of the hexakisphosphate esters.

The reasons for the rapid degradation of IP_6 in marine environments are unclear, but may relate to changes in the solubility of sediment-bound inositol phosphates in more saline environments, or to anaerobic processes in marine sediments. The latter explanation seems the most likely, because in laboratory studies sediment IP_6 was almost completely hydrolysed within 40 days under anaerobic conditions, whereas under aerobic conditions only partial hydrolysis was noted after 60 days (Suzumura & Kamatani 1995*a*). Clearly, many important questions remain to be answered concerning the forms, origins, transformations and bioavailability of inositol phosphates in aquatic systems.

4. ANALYTICAL TECHNIQUES FOR THE DETERMINATION OF INOSITOL PHOSPHATES IN ENVIRONMENTAL SAMPLES

The single greatest limitation on the investigation of inositol phosphates in the environment has been the availability of suitable analytical techniques for their determination in the complex matrices presented by environmental samples. Determination of inositol phosphates in waters, sediments and soils involves three distinct operations: extraction, separation and detection. Successful determination of inositol phosphates can be hindered in each or all of these stages. For example, the efficiency of inositol phosphate extraction from soils is highly dependent on the extraction conditions used and may involve some hydrolysis of the inositol phosphate congeners present. Chromatographic separation of inositol phosphates may be incomplete because of the elution conditions chosen or specific interactions with the stationary phase, or could even entail some on-column hydrolysis. Detection of inositol phosphates after separation usually involves colorimetric determination of molybdate-reactive P following a digestion step, which may or may not be 100% efficient depending on the conditions selected. For example, use of conventional thermal digestion with nitric–sulphuric acid for determination of total P is unlikely to allow complete conversion of phytic acid to orthophosphate for subsequent detection and, consequently, the phytic acid contribution to total P is underestimated or even undetected. For this reason, the use of a digestion reagent that has both oxidative and hydrolytic capability, such as peroxydisulphate–sulphuric acid or peroxydisulphate–perchloric acid, is recommended (Benson *et al.* 1996).

It may also be difficult to achieve the sensitivity in the detection stage that is necessary to determine the low concentrations of inositol phosphates in natural waters. Consequently, there is a need to refine existing techniques and to develop new techniques that have the selectivity and sensitivity required to advance our understanding of the behaviour of inositol phosphates in the environment. A critical part of the validation of new techniques will be a systematic investigation of the transformations between different esters ($IP_{1–6}$) and isomers (*myo*, *scyllo*, D-*chiro*, *neo*) that may occur during extraction, separation and detection.

The following section details the existing procedures and techniques for the determination of inositol phosphates in environmental samples. Limits of detection have been reported where possible (table 3), but many publications do not provide sufficient information to allow this. Similarly, there are few fully validated techniques. In general, validation should be performed against chromatographically purified standards, but there are problems in obtaining suitable reference materials. Many of the

Table 3. Detection limits for inositol phosphates and inositol for a range of analytical techniques.
(IMER, immobilized enzyme reactor; HPLC, high performance liquid chromatography; I(2)P, inositol 2-monophosphate; LC–MS, liquid chromatography–mass spectrometry; MRP, molybdate-reactive phosphorus; NMR, nuclear magnetic resonance; P_i, inorganic phosphate as orthophosphate.)

technique	species detected	detection limit	sample type	reference
anion-exchange chromatography/colorimetric	P_i	0.5 μg P g^{-1}	soil	McKercher & Anderson (1968*b*)
gel filtration/colorimetric	MRP	10 μg P l^{-1}	lake sediments	Eisenreich & Armstrong (1977)
^{31}P NMR	IP_6	< 24 mg P g^{-1}	food	O'Neill *et al.* (1980)
HPLC/colorimetric	MRP	30 μg P g^{-1}	plants	Graf & Dintzis (1982)
ion-exchange column/colorimetric	P_i	10 μg P g^{-1}	soil	Appiah & Thomas (1982)
HPLC–IMER/fluorimetric	P_i	15.5 ng l^{-1}	enzymatic products	Meek & Nicoletti (1986)
HPLC/colorimetric	MRP	25 μg P l^{-1}	model compounds	Minear *et al.* (1988)
HPLC/flow injection analysis	MRP	10 μg P l^{-1}	aqueous	Clarkin *et al.* (1992)
HPLC/colorimetric	$FeCl_3$	2.5 μg P g^{-1}	food	Rounds & Nielsen (1993)
flow injection analysis/colorimetric	MRP	27 μg P l^{-1}	aqueous	McKelvie *et al.* (1993)
flow injection analysis/colorimetric	MRP	4 μg P l^{-1}	aqueous	McKelvie *et al.* (1995)
enzymatic/colorimetric	MRP	90 μg P g^{-1}	food and pharmaceutical	March *et al.* (1995)
HPLC/metal-dye detection	inositol phosphates	0.93 μg P l^{-1}	cells and tissues	Guse *et al.* (1995)
HPLC/colorimetric	MRP	0.3 μg P g^{-1}	coastal sediments	Suzumura & Kamatani (1995*b*)
ion-exchange/thermospray LC–MS	inositol	19 mg P l^{-1}	biomedical	Hsu *et al.* (1990)
capillary electrophoresis	I(2)P	200 μg l^{-1}	physiological	Henshall *et al.* (1992)
HPLC-enzymatic/bioluminescence	inositol	31 ng l^{1}	physiological	Prestwich & Bolton (1991)

reported analytical methods do not list detection limits or test for selectivity, so it is not always possible to compare the relative merits of the different techniques. However, techniques based on chromatographic or capillary electrophoresis separation will generally be more selective than those based on selective or sequential extraction procedures.

(a) *Extraction and clean-up of inositol phosphates from soils and sediments*

The extraction of inositol phosphates from soils and sediments is complicated by their propensity to form complexes with polyvalent cations or to associate with other organic matter in the sample, such as humic acids (McKercher & Anderson 1968*b*; Omotoso & Wild 1970; Appiah & Thomas 1982). The conventional and long-standing approach to extraction of the chemically refractory higher inositol phosphates (IP_{5+6}) from soils has involved the use of a strong alkali solution, typically 3 M NaOH (Irving & Cosgrove 1981). From the late 1960s, the main extraction method used was that described by Anderson (1964) and McKercher & Anderson (1968*b*). The soil sample is first treated with a dilute acid to remove carbonates; this acid is then discarded. The soil is subsequently extracted with hot 3 M NaOH to extract the strongly bound inositol phosphates from the soil. The sesquioxides are removed as precipitates from the alkali extract and the inositol phosphates are then precipitated as barium salts in the presence of ethanol. The inositol phosphates have conventionally been separated by ion-exchange chromatography (McKercher & Anderson 1968*b*).

Despite the widespread use of the McKercher & Anderson method, it can overestimate the concentrations of inositol phosphates in soil extracts because other organic P compounds are present in the ion-exchange fractions that were thought to contain only inositol phosphates (Irving & Cosgrove 1981). This can be overcome by using alkaline bromination, or hypobromite oxidation, which oxidizes soil organic matter without degrading IP_{5+6} (Irving & Cosgrove 1981, 1982). The remaining inositol phosphates can then be analysed without interference from other organic P compounds.

Eisenreich & Armstrong (1977) tested the effect of alkaline bromination on standard solutions of IP_1 and IP_6. Using gel filtration chromatography to separate the two inositol phosphates, they found an average post-alkaline bromination recovery of 87% for IP_6 and 45% for IP_1. The lower recovery for IP_1 was attributed to its greater susceptibility to hydrolysis. Other organic phosphates, including glucose 6-phosphate, DNA and adenosine monophosphate, were completely oxidized by the same treatment. However, it should be noted that it has subsequently been found that DNA is not oxidized by the alkaline bromination procedure (Nanny & Minear 1994).

Weimer & Armstrong (1977) used three methods for extracting inositol phosphates from lake sediments. The first was a basic extraction using NaOH to liberate inositol phosphates from the sediment and, as with the McKercher & Anderson (1968*b*) method, inositol phosphates were precipitated as barium salts after the sesquioxides had been discarded. The second method coupled milder extraction conditions with alkaline bromination, but no significant advantages were observed from this clean-up step. The third method involved a combination of the two previous methods and yielded the best recovery of inositol phosphates from the sediments (12.7% total organic P). Radiolabelling inositol phosphates with ^{32}P showed that an average recovery of 91% was obtained following the extraction and clean-up procedures.

Hong & Yamane (1980) compared NaOH extraction procedures for inositol phosphates in soil humic acid. More inositol phosphates were recovered by increasing the extraction temperature from 60 to 100 °C and allowing a fourfold increase in the time allowed for hypobromite oxidation. Other extraction techniques have involved the initial removal of inorganic orthophosphate with Ca-nitrilotriacetate/dithionite and ethylenediamine tetraacetic acid (EDTA), followed by the removal of an acid-soluble organic P fraction, leaving a residual organic phosphate fraction containing inositol phosphates (De Groot & Golterman 1993). A phytase-hydrolysable fraction was determined once humic acids were removed as a precipitate to prevent interference with the enzyme. This phytase-hydrolysable fraction was used to infer the presence of phytate. No measure of the extraction efficiency was made, although it was demonstrated that phytate formed insoluble complexes with Mn^{2+}, Mg^{2+}, Fe^{2+}, Ca^{2+}, Al^{3+} and Fe^{3+}.

Recently, the recovery of organic P from soil has been improved using an extracting solution containing 0.25 M NaOH plus 0.05 M EDTA (Cade-Menun & Preston 1996). First proposed by Bowman & Moir (1993), extraction with NaOH–EDTA has become widely used for analysis of soil P composition by ^{31}P NMR (e.g. Cade-Menun & Preston 1996; Dai *et al.* 1996), but has yet to be applied to the extraction of inositol phosphates.

(b) *Pre-concentration of inositol phosphates in environmental samples*

The low concentrations of organic P compounds in natural waters has precluded most analytical techniques for their determination, with the result that there is little information on the forms and occurrence of inositol phosphates in water. The requirement for the analysis of inositol phosphates in waters is, therefore, some form of pre-concentration. Soil extracts for analysis by ^{31}P NMR also require some form of pre-concentration. The time and treatment conditions involved in sample pre-concentration introduces the potential for changes in P forms to occur, but are necessary if aquatic samples are to be analysed effectively using current methodologies.

Several methods of pre-concentration have been developed, including rotary evaporation at low temperatures (Newman & Tate 1980), ultrafiltration through low molecular weight filters, reverse osmosis membranes (Clarkin *et al.* 1992; Nanny *et al.* 1994) and precipitation of P compounds with lanthanum (Stevens & Stewart 1982). Clarkin *et al.* (1992) proposed a concentration procedure whereby samples were concentrated and size-fractionated using a series of ultrafiltration and reverse osmosis membranes, although only *ca.* 25% of the initial dissolved P in field samples was retrieved by this technique. This method was used to analyse soluble organic P in lake and stream water samples (Nanny & Minear 1994; Nanny *et al.* 1995). Espinosa *et al.* (1999) described a

method that employs strong anion exchange (SAX) resins for the concentration and speciation of trace amounts of P compounds in soil waters. Pre-concentration was achieved by passing filtered samples through SAX cartridges and eluting the retained negatively charged species with a solution of 0.75 M NaCl and 0.5 mM EDTA. Recoveries of over 75% were achieved for a range of inorganic and organic model P compounds. The eluate was then separated on a strong anion exchange column. Recently, a method has been proposed for pre-concentrating IP_6 onto Sephadex gel (used in gel filtration), which selectively retains IP_6 whilst excluding other organic and inorganic phosphates. The gel-bound inositol phosphates are then quantified directly using solution-state ^{31}P NMR (Turner & McKelvie 2002).

(c) *Techniques for the separation of inositol phosphates*

(i) *Liquid chromatography*

Several methods have been used to separate inositol phosphates, including ion-exchange (high performance liquid chromatography (HPLC) or conventional large-column ion-exchange) and size exclusion/gel filtration (table 3), although most have involved ion-exchange chromatography to isolate the individual congeners (Tangendjaja *et al.* 1980; Camire & Clydesdale 1982; Graf & Dintzis 1982; Batty *et al.* 1985; Wilson *et al.* 1985; Cilliers & Van Niekerk 1986; Meek & Nicoletti 1986; Portilla & Morrison 1986; Horstman *et al.* 1988; Matthews *et al.* 1988; Mayr 1988; Minear *et al.* 1988; Phillipy & Bland 1988; Heathers *et al.* 1989; Taylor *et al.* 1990; Bos *et al.* 1991; Prestwich & Bolton 1991; Clarkin *et al.* 1992; Rounds & Nielsen 1993; Wolters *et al.* 1993; Nanny *et al.* 1994, 1995; Guse 1995; Suzumura & Kamatani 1995*b*). The ability of anion-exchange HPLC to separate the various inositol phosphate congeners effectively and efficiently has led to its widespread use. However, in addition to an often lengthy separation time, there is a need for sensitive detection methods for inositol phosphates following separation. As inositol phosphates cannot be detected directly by either UV or visible spectrophotometry, several studies have used post-column conversion and/or derivatization reactions with subsequent spectrophotometric detection (Cilliers & Van Niekerk 1986; Meek & Nicoletti 1986; Mayr 1988; Haizeng *et al.* 1991; Clarkin *et al.* 1992; McKelvie *et al.* 1993; Rounds & Nielsen 1993; Wolters *et al.* 1993).

Use of low pressure anion-exchange chromatography has been superseded by HPLC because of the inability of the former to resolve inositol phosphates adequately within an acceptable time (Cosgrove 1963, 1969*a*; Cosgrove & Tate 1963; McKercher & Anderson 1968*a*,*b*; Sommers *et al.* 1972; Harland & Oberleas 1977; Ellis & Morris 1982). Further advantages of HPLC over more traditional column or paper chromatographic techniques include better resolution of the various congeners of the inositol phosphate family, faster sample throughput, smaller sample size and improved detection limits. Recently, a simple and inexpensive high performance thin-layer chromatography technique has been developed for the separation of IP_{1-6} in grain extracts (Hatzack & Rasmussen 1999). Anion-exchange HPLC has been the preferred mode for the separation of inositol phosphates, but reversed-phase HPLC has also been applied (Tangendjaja *et al.* 1980), mainly for the separation and quantification of the various inositol phosphate esters in food and biological materials, but only at much higher concentrations than those that are presumed to occur in lake and river sediments (Brando *et al.* 1990; Patthy *et al.* 1990; Lehrfeld & Morris 1992; Tao & Li 1992).

(ii) *Gas chromatography*

Gas chromatography (GC) has been used to detect derivatized volatile inositol phosphate forms, but only after separation by ion-exchange chromatography (Rittenhouse & Sasson 1985; Heathers *et al.* 1989). One method for determining the isomers of IP_{5+6} in soils involved initial isolation of the inositol phosphates with low-pressure anion-exchange chromatography, followed by GC using flame ionization detection (FID) to detect the various isomers as hexakis-*O*-acetyl derivatives (Irving & Cosgrove 1982). An even more involved method used anion-exchange HPLC to collect IP_{1-4}, which were subsequently dephosphorylated to *myo*-inositol before being derivatized to a hexatrimethylsilyl moiety and analysed by GC–FID (Heathers *et al.* 1989). Neither method was used to quantify inositol phosphates.

(iii) *Capillary electrophoresis*

Capillary electrophoresis has been used for the separation of inositol phosphates in physiological samples and in a fermentation broth (Henshall *et al.* 1992; Buscher *et al.* 1994). Typical separation time for the resolution of four of the inositol phosphate congeners was 10 min, and the species of interest were detected via indirect photometric means (Henshall *et al.* 1992). The application of capillary electrophoresis coupled with mass spectrometry detection has the potential to provide detailed and comprehensive information on inositol phosphates in environmental samples, but has not, as yet, to our knowledge, been accomplished.

(d) *Spectroscopic detection techniques*

(i) *Nuclear magnetic resonance spectroscopy*

Although ^{31}P NMR spectroscopy is relatively insensitive compared with HPLC techniques, it has the advantage of being able to distinguish between similar compounds in complex matrices (Nanny & Minear 1994), thereby obviating the need for lengthy chromatographic separation and clean-up procedures (Costello *et al.* 1976; O'Neill *et al.* 1980; Tate & Newman 1982; Crans *et al.* 1993; Johnson *et al.* 1995; Kemme *et al.* 1999). For this reason, the technique has great potential for the investigation of inositol phosphates in environmental samples. The various P compounds are differentiated according to the different environments around the P nuclei, although the chemical shift of the phytate peaks varies with the pH and ionic strength of the solution (Costello *et al.* 1976; O'Neill *et al.* 1980; Nanny & Minear 1994).

Phytate has been quantified in foods by measuring the area of the peaks that occur at the C2 position for phytate (δ = 24 to 28 ppm downfield of tetrametaphosphate), as this peak was well resolved from low-field orthophosphate and other phytate signals (O'Neill *et al.* 1980). Recently, ^{31}P NMR has been used to quantify IP_{1-6} in a range of samples related to animal nutrition (Kemme *et al.* 1999).

The samples were extracted in HCl and purified by freeze-drying and filtration, followed by analysis at alkaline pH (> 12) to ensure the stability of the samples. The use of two-dimensional ^{31}P–^{1}H NMR is a novel application for determining the isomeric composition of inositol phosphates (Johnson *et al.* 1995) and has recently been used to identify *neo*-IP_{4-6} in two species of amoebae following the separation and purification of the compounds by HPLC (Martin *et al.* 2000). Solution-state ^{31}P NMR has also been used to detect IP_6 separated from other organic and inorganic phosphates by selective extraction onto Sephadex gel (Turner & McKelvie 2002).

The application of ^{31}P NMR to the investigation of inositol phosphates in soils appears attractive and has been used to identify IP_6 in alkaline soil extracts using spiked samples (e.g. Newman & Tate 1980). However, the identification and quantification of inositol phosphates is hindered by poor resolution in the orthophosphate monoester region of the NMR spectra ($\delta = +3.5$ to 5.9 ppm), resulting in an inability to differentiate between inositol phosphate esters, other orthophosphate monoesters and often orthophosphate (Hawkes *et al.* 1984; Condron *et al.* 1985, 1990, 1997). Similar problems encountered in the investigation of lake waters were partially overcome by using hypobromite oxidation, leaving only inorganic orthophosphate, inositol phosphates and DNA (Nanny & Minear 1994). In this study, the required ^{31}P NMR detection limits of 10–20 mg P l^{-1} were achieved using ultrafiltration and reverse osmosis techniques (Nanny & Minear 1994), but are more commonly and easily achieved by freeze-drying samples and redissolving in NaOH. Despite this, long run times (often over 16 h) are necessary to achieve acceptable signal-to-noise ratios.

In recent years, high-resolution solid-state ^{31}P NMR has also been applied to soils (Lookman *et al.* 1996, 1997). This has obvious applications for investigating soil processes *in situ* without the need for extraction procedures that can alter the P compounds under investigation. However, the broad peaks that result from the low concentrations of P and high concentrations of paramagnetic impurities (Fe^{3+}, Mn^{2+}), coupled with the inability to distinguish between orthophosphate monoesters and orthophosphate, means that solid-state ^{31}P NMR is of limited value at present (Condron *et al.* 1997).

Therefore, whilst the use of ^{31}P NMR is attractive because various P compounds can be determined directly in complex matrices without the need for separation, the technique remains relatively insensitive and does not permit the easy differentiation between different orthophosphate monoesters. Despite this, it remains a convenient method of quantification and identification of inositol phosphate esters and isomers following chromatographic separation or alkaline bromination.

(ii) *Mass spectrometry*

Chemical ionization gas chromatography–mass spectrometry (GC–MS) has been used to quantify inositol trisphosphate in kidney cells after HPLC separation and acid hydrolysis (Wilson *et al.* 1985). Fast atom bombardment–mass spectrometry (FAB–MS) (Hsu & Sherman 1989) and thermospray liquid chromatography–mass spectrometry (LC–MS) (Wilson *et al.* 1985; Phillipy *et al.* 1987) have also been applied to the analysis of inositol phosphates in biological matter, but not environmental samples *per se*, possibly due to the high detection limit of *ca.* 190 μg P l^{-1} for the LC–MS technique (Hsu *et al.* 1990; Shang *et al.* 1992). This situation seems unlikely to change while there is a preference for cheaper, more versatile instrumentation and, potentially, on-line monitoring of inositol phosphates.

(iii) *Inductively coupled plasma atomic-emission spectroscopy*

Phytic acid has been detected in food and urine using inductively coupled plasma atomic-emission spectroscopy (ICP-AES) by pre-concentrating the phytic acid on an anion-exchange resin (e.g. AGI-X4, Bio-Rad), followed by quantitative determination as P by ICP-AES (Plaami & Kumplainen 1991; March *et al.* 1995; Grases & Llobera 1996). Detection limits for phytate of 0.5 mg P l^{-1} in cereals (Plaami & Kumplainen 1991) and 36 μg P l^{-1} in urine (Grases & Llobera 1996) were reported. Current detection limits for P in the μg l^{-1} range mean that this method has great potential for determining of total P in aqueous samples.

(iv) *Molecular luminescence*

Techniques using either bioluminescence or fluorescence offer a potentially sensitive means for detecting inositol phosphates. When used in combination with the highly selective enzymes, luminescence techniques can offer detection limits that are three orders of magnitude lower than spectrophotometric techniques. One example of a highly sensitive bioluminescence detection of inositol phosphates involved the HPLC separation of IP_{2-4} congeners (Prestwich & Bolton 1991). Collected fractions were desalted, dephosphorylated using alkaline phosphatase and the liberated inositols determined by an inositol dehydrogenase-NADH-linked bioluminescence reaction.

(e) *Radiolabelling techniques*

Radiolabelling with ^{3}H or $^{32}P/^{33}P$ has been used to determine inositol phosphates in lake sediments (Weimer & Armstrong 1977) and biological samples (Batty *et al.* 1985; Rittenhouse & Sasson 1985; Horstman *et al.* 1988; Taylor *et al.* 1990). Radiolabelling with ^{32}P has also been used to identify specific inositol phosphates after HPLC separation (Brando *et al.* 1990; Patthy *et al.* 1990; Taylor *et al.* 1990; Lehrfeld & Morris 1992; Tao & Li 1992), whilst inositols labelled with ^{14}C have been used to investigate the origins of IP_6 stereoisomers in the soil (L'Annunziata *et al.* 1977). Dual-labelled ^{32}P–^{14}C IP_6 would represent a powerful tool for investigating many fundamental questions regarding the origins and transformations of inositol phosphates in soils.

(f) *Enzyme-hydrolysis techniques*

Soluble phytase has been used to infer the presence of IP_6 in lake waters (Herbes *et al.* 1975), aquatic sediments (Feuillade & Dorioz 1992; De Groot & Golterman 1993), soil solutions (Shand & Smith 1997), soil water extracts (Pant *et al.* 1994*a*; Turner *et al.* 2002) and root exudates (Pant *et al.* 1994*b*). The method involves the addition of phytase to an aqueous sample, which hydrolyses any inositol phosphates present. The liberated orthophosphate is then determined by molybdate reaction. More advanced methods have used immobilized phytase columns coupled

with flow injection analysis to determine IP_6 in natural and wastewaters (McKelvie *et al.* 1995).

Enzyme techniques are sensitive, having limits of detection comparable with those for orthophosphate in waters (*ca.* 10–20 $\mu g\ l^{-1}$ using colorimetric detection), especially when coupled with flow injection analysis (Shan *et al.* 1993, 1994; McKelvie *et al.* 1995). The major drawback is that commercial phytase preparations are non-specific and release orthophosphate from a range of orthophosphate monoester, diester and condensed P compounds, in addition to IP_6 (Shand & Smith 1997; Turner *et al.* 2002). This can be overcome by using purified phytase preparations specific to phytic acid (Ullah & Gibson 1987; Hayes *et al.* 2000*a*). Enzyme techniques are readily applicable to the routine analysis of a large number of environmental samples, but must be validated using techniques that have greater sensitivity and specificity for the various inositol phosphate esters.

5. CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE RESEARCH

Inositol phosphates are a fundamental component of the global P cycle because of their relative abundance in soils and sediments; yet the origins, bioavailability and mobility of inositol phosphates in the environment are poorly understood. The similar nature of the six congeners of inositol phosphate has caused difficulty in separating and quantifying the amounts of each species. Thus, it is not surprising that the first complete separation of all six inositol phosphate esters has only been reported in the last decade. Detection of the isomeric forms of inositol phosphate places further demands on analytical sensitivity and the origins of the various isomers in the environment remain enigmatic.

Before significant advances can be made in understanding the mechanisms controlling inositol phosphate dynamics in the environment, it will be necessary to develop inexpensive, robust and reproducible techniques for the extraction, separation and detection of inositol phosphates in environmental samples. Methods for the quantitative extraction of inositol phosphates from soil and sediments remain elusive, although the one-step NaOH–EDTA extraction technique currently used in ^{31}P NMR studies combines high P recoveries with mild extraction conditions. The continued development of techniques employing HPLC separations with anion exchange columns should provide an accurate separation technique that will be available to most laboratories. Thus, chromatographic techniques offer the greatest potential for allowing inositol phosphate determinations to become routine. However, for the analysis of large numbers of environmental samples at low cost, such as in catchment water quality monitoring, the development of enzymatic techniques coupled with flow injection analysis may be preferable. Sensitive techniques for the separation of the isomeric forms of inositol phosphate in environmental samples would be a significant advance, possibly providing key insights into the dynamics and bioavailability of inositol phosphates in the soil. Such techniques may involve capillary electrophoresis mass spectrometry systems, although detection limits will, as ever, present a major limitation. Before any new techniques become accepted, a comprehensive assessment of the transformations that occur during the entire procedure is a fundamental requirement.

Once basic methodologies for inositol phosphate determination are established, it will be possible to begin comprehensive investigation into the dynamics of inositol phosphates in the environment. Aspects requiring immediate investigation include the processes controlling inositol phosphate cycling and bioavailability in soils and waters and the transport of inositol phosphates between the major environmental compartments. This will reveal valuable information about the availability of inositol phosphates to crops, the potential risks associated with their transfer from soil to water and the subsequent impact on water quality through the availability of inositol phosphates to aquatic microorganisms.

An important area of current research into soil P is the development of sustainable management strategies for P in agricultural systems, minimizing the use of mineral fertilizers and, therefore, potential P loss in runoff to watercourses. An obvious route to achieving sustainability is the strategic exploitation of inositol phosphates, which represent a substantial untapped reservoir of soil P. This can only be achieved through a concerted effort towards a comprehensive understanding of inositol phosphate dynamics in the soil system, but will lead to a sustainable future for P from both an agronomic and an environmental perspective.

REFERENCES

Adeola, O., Orban, J. I., Ragland, D., Cline, T. R. & Sutton, A. L. 1998 Phytase and cholecalciferol supplementation of low-calcium and low-phosphorus diets for pigs. *Can. J. Anim. Sci.* **78**, 307–313.

Anderson, G. 1964 Investigations on the analysis of inositol hexaphosphate in soil. *Trans. Int. Congress Soil Sci.* **4**, 563–571.

Anderson, G. 1980 Assessing organic phosphorus in soil. In *The role of phosphorus in agriculture* (ed. F. E. Khasawneh, E. C. Sample & E. J. Kamprath), pp. 411–431. Madison, USA: American Society of Agronomy.

Anderson, G. & Arlidge, E. Z. 1962 The adsorption of inositol phosphates and glycerophosphate by soil clays, clay minerals, and hydrated sesquioxides in acid media. *J. Soil Sci.* **13**, 216–224.

Anderson, G. & Malcolm, R. E. 1974 The nature of alkali-soluble soil organic phosphates. *J. Soil Sci.* **25**, 282–297.

Anderson, G., Williams, E. G. & Moir, J. O. 1974 A comparison of the sorption of inorganic orthophosphate and inositol hexaphosphate by six acid soils. *J. Soil Sci.* **25**, 51–62.

Angyal, S. J. 1963 Cyclitols. In *Carbohydrates, volume V* (ed. M. Florkin & E. H. Stotz), pp. 297–303. Amsterdam: Elsevier.

Antibus, R. K., Sinsabaugh, R. L. & Linkins, A. E. 1992 Phosphatase-activities and phosphorus uptake from inositol phosphate by ectomycorrhizal fungi. *Can. J. Bot.* **70**, 794–801.

Appiah, M. R. & Thomas, R. L. 1982 Inositol phosphate and organic phosphorus contents and phosphatase activity of some Canadian and Ghanaian soils. *Can. J. Soil Sci.* **62**, 31–38.

Baker, R. T. 1974 Chemical nature and distribution of soil organic phosphate in two chronosequences of soils. PhD thesis, Department of Soil Science, University of Canterbury, New Zealand.

Batty, I. R., Nahorski, S. R. & Irvine, R. F. 1985 Rapid forma-

tion of inositol 1,3,4,5-tetrakisphosphate following muscarinic receptor stimulation of rat cerebral cortical slices. *Biochem. J.* **232**, 211–215.
Becker, M. 1950 Phytin in der tierernahrung. *Landw. Forsch.* **2**, 64–74.
Bedrock, C. N., Cheshire, M. V., Chudek, J. A., Goodman, B. A. & Shand, C. A. 1994 Use of ^{31}P-NMR to study the forms of phosphorus in peat soils. *Sci. Total Env.* **152**, 1–8.
Benson, R. L., McKelvie, I. D., Hart, B. T., Truong, Y. B. & Hamilton, I. C. 1996 Determination of total phosphorus in waters and wastewaters by on-line UV/thermal induced digestion and flow injection analysis. *Anal. Chim. Acta* **326**, 29–39.
Bieleski, R. I. 1973 Phosphate pools, phosphate transport and phosphate availability. *A. Rev. Plant Physiol. Plant Mol. Biol.* **24**, 225–252.
Bishop, M. L., Chang, A. C. & Lee, R. W. 1994 Enzymatic mineralization of organic phosphorus in a volcanic soil in Chile. *Soil Sci.* **157**, 238–243.
Borie, F., Zunino, H. & Martinez, L. 1989 Macromolecule-P associations and inositol phosphates in some Chilean volcanic soils of temperate regions. *Commun. Soil Sci. Plant Anal.* **20**, 1881–1894.
Bos, K. D., Verbeek, C., Van Eeden, C. H., Slump, P. & Wolters, M. G. 1991 Improved determination of phytate by ion-exchange chromatography. *J. Agric. Food Chem.* **39**, 1770–1772.
Bowling, L. C. & Baker, P. D. 1996 Major cyanobacterial bloom in the Barwon-Darling River, Australia, in 1991, and underlying limnological conditions. *Marine Freshwater Res.* **47**, 643–657.
Bowman, R. A. & Moir, J. O. 1993 Basic EDTA as an extractant for soil organic phosphorus. *Soil Sci. Soc. Am. Proc.* **57**, 1516–1518.
Bowman, B. T., Thomas, R. L. & Elrick, D. E. 1967 The movement of phytic acid in soil cores. *Soil Sci. Soc. Am. Proc.* **31**, 477–481.
Brando, C., Hoffman, T. & Bonvini, E. 1990 High-performance liquid chromatographic separation of inositol phosphate isomers employing a reversed-phase column and a micellar mobile phase. *J. Chromatogr.* **529**, 65–80.
Brearley, C. A. & Hanke, D. E. 1996 Inositol phosphates in the duckweed *Spirodela polyrhiza* L. *Biochem. J.* **314**, 215–225.
Buscher, B. A., Irth, H., Andersson, E., Tjaden, U. R. & Van der Greef, J. 1994 Determination of inositol phosphates in fermentation broth using capillary zone electrophoresis with indirect UV detection. *J. Chromatogr.* A **678**, 145–150.
Cade-Menun, B. J. & Preston, C. M. 1996 A comparison of soil extraction procedures for ^{31}P NMR spectroscopy. *Soil Sci.* **161**, 770–785.
Caldwell, A. G. & Black, C. A. 1958*a* Inositol hexaphosphate: I. Quantitative determination in extracts of soils and manures. *Soil Sci. Soc. Am. Proc.* **22**, 290–293.
Caldwell, A. G. & Black, C. A. 1958*b* Inositol hexaphosphate: III. Content in soils. *Soil Sci. Soc. Am. Proc.* **22**, 296–298.
Camire, A. L. & Clydesdale, F. M. 1982 Analysis of phytic acid in foods by HPLC. *J. Food Sci.* **47**, 575–578.
Celi, L., Lamacchia, S., Marsan, F. A. & Barberis, E. 1999 Interaction of inositol hexaphosphate on clays: adsorption and charging phenomena. *Soil Sci.* **164**, 574–585.
Celi, L., Presta, M., Ajmore-Marsan, F. & Barberis, E. 2001 Effects of soil pH and electrolytes on inositol hexaphosphate interaction with goethite. *Soil Sc. Soc. Am. J.* **65**, 753–760.
Cilliers, J. J. L. & Van Niekerk, P. J. 1986 LC determination of phytic acid in food by postcolumn colorimetric detection. *J. Agric. Food Chem.* **34**, 680–683.
Clarkin, C. M., Minear, R. A., Kim, S. & Elwood, J. W. 1992 An HPLC postcolumn reaction system for phosphorus-specific detection in the complete separation of inositol phosphate congeners in aqueous samples. *Environ. Sci. Technol.* **26**, 199–204.
Condron, L. M. & Goh, K. M. 1989 Effects of long-term phosphatic fertilizer applications on amounts and forms of phosphorus in soils under irrigated pasture in New Zealand. *J. Soil Sci.* **40**, 4383–4395.
Condron, L. M., Goh, K. M. & Newman, R. H. 1985 Nature and distribution of soil-phosphorus as revealed by a sequential extraction method followed by P-31 nuclear magnetic-resonance analysis. *J. Soil Sci.* **36**, 199–207.
Condron, L. M., Frossard, E., Tiessen, H., Newman, R. H. & Stewart, J. W. B. 1990 Chemical nature of organic phosphorus in cultivated and uncultivated soils under different environmental-conditions. *J. Soil Sci.* **41**, 41–50.
Condron, L. M., Davis, M. R., Newman, R. H. & Cornforth, I. S. 1996 Influence of conifers on the forms of phosphorus in selected New Zealand grassland soils. *Biol. Fertil. Soils* **21**, 37–42.
Condron, L. M., Frossard, E., Newman, R. H., Tekely, P. & Morel, J.-L. 1997 Use of ^{31}P NMR in the study of soils and the environment. In *Nuclear magnetic resonance spectroscopy in environmental chemistry* (ed. M. A. Nanny, R. A. Minear & J. A. Leenheer), pp. 247–271. New York: Oxford University Press.
Corona, M. E., Van der Klundert, I. & Verhoeven, J. T. 1996 Availability of organic and inorganic phosphorus compounds as phosphorus sources for *Carex* species. *New Phytol.* **133**, 225–231.
Cosgrove, D. J. 1962 Forms of inositol hexaphosphate in soils. *Nature* **194**, 1265–1266.
Cosgrove, D. J. 1963 The chemical nature of soil organic phosphorus. I. Inositol phosphates. *Aust. J. Soil Res.* **1**, 203–214.
Cosgrove, D. J. 1966 Detection of isomers of phytic acid in some Scottish and Californian soils. *Soil Sci.* **102**, 42–43.
Cosgrove, D. J. 1969*a* Ion-exchange chromatography of inositol polyphosphates. *Ann. NY Acad. Sci.* **165**, 677–686.
Cosgrove, D. J. 1969*b* The chemical nature of soil organic phosphorus–II: characterization of the supposed DL-chiro-inositol hexaphosphate component of soil phytate as D-chiro-inositol hexaphosphate. *Soil Biol. Biochem.* **1**, 325–327.
Cosgrove, D. J. 1980 *Inositol phosphates—their chemistry, biochemistry and physiology*. Amsterdam: Elsevier Scientific.
Cosgrove, D. J. & Tate, M. E. 1963 Occurrence of *neo*-inositol hexaphosphate in soils. *Nature* **200**, 568–569.
Costello, A. J., Glonek, T. & Myers, T. C. 1976 ^{31}P nuclear magnetic resonance-pH titrations of myo-inositol hexaphosphate. *Carbohydr. Res.* **46**, 159–171.
Crans, D. C., Mikus, M. & Marshman, R. W. 1993 ^{31}P NMR examination of phosphorus metabolites in the aqueous, acidic, and organic extracts of *Phaselous vulgaris* seeds. *Anal. Biochem.* **209**, 85–94.
Dai, K. O., David, M. B., Vance, G. F. & Krzyszowska, A. J. 1996 Characterization of phosphorus in a spruce-fir spodosol by phosphorus-31 nuclear magnetic resonance spectroscopy. *Soil Sci. Soc. Am. J.* **60**, 1943–1950.
Dalal, R. C. 1977 Soil organic phosphorus. *Adv. Agron.* **29**, 85–117.
Dean, N. M. & Beaven, M. A. 1989 Methods for the analysis of inositol phosphates. *Anal. Biochem.* **183**, 199–209.
De Groot, C. J. & Golterman, H. L. 1993 On the presence of organic phosphate in some Camargue sediments: evidence for the importance of phytate. *Hydrobiologia* **252**, 117–126.
Dormar, J. F. 1967 Distribution of inositol phosphates in some chernozemic soils of southern Alberta. *Soil Sci.* **104**, 17–24.
Eid, M. T., Black, C. A. & Kempthorne, O. 1951 Importance of soil organic phosphorus to plant growth at low and high soil temperatures. *Soil Sci.* **71**, 361–370.
Eisenreich, S. J. & Armstrong, D. E. 1977 Chromatographic

investigations of inositol phosphate esters in lake waters. *Environ. Sci. Technol.* **11**, 497–501.

Ellis, R. & Morris, E. R. 1982 Comparison of ion-exchange and iron precipitation methods for analysis of phytate. *Cereal Chem.* **59**, 232–233.

Emsley, J. & Niazi, S. 1981 The structure of *myo*-inositol hexaphosphate in solution: P-31 NMR investigation. *Phosphorus Sulfur* **10**, 401–408.

Espinosa, M., Turner, B. L. & Haygarth, P. M. 1999 Preconcentration and separation of trace phosphorus compounds in soil leachate. *J. Environ. Qual.* **28**, 1497–1504.

Evans, W. J. & Martin, C. J. 1991 The interactions of inositol hexaphosphate with Fe(III) and Cr(III). A calorimetric investigation. XV. *J. Inorg. Biochem.* **41**, 245–252.

Farrell, D. J. & Martin, E. A. 1998 Strategies to improve the nutritive value of rice bran in poultry diets. III. The addition of inorganic phosphorus and a phytase in duck diets. *Br. Poultry Sci.* **39**, 601–611.

Feuillade, M. & Dorioz, J. M. 1992 Enzymatic release of phosphate in sediments of various origins. *Water Res.* **26**, 1195–1201.

Findenegg, G. R. & Nelemans, J. A. 1993 The effect of phytase on the availability of P from myo-inositol hexaphosphate (phytate) for maize roots. *Plant Soil* **154**, 189–196.

Gianfrancesco, R. U. & Leake, J. R. 2002 Utilisation of phosphodiester-P by ectomycorrhizal fungi: use of DNA as a source of phosphorus and preliminary characterisation of phosphatases in *Amanita rubescens* and *Suillus bovinus*. In *Phosphatases in the environment* (ed. B. A. Whitton & I. Hernández). Dordrecht, The Netherlands: Kluwer Academic. (In the press.)

Goring, C. A. I. 1951 Reactions of biological organic phosphorus compounds with clays. *Iowa State Coll. J. Sci.* **25**, 230–232.

Graf, R. & Dintzis, F. R. 1982 High-performance liquid chromatographic method for the determination of phytate. *Anal. Biochem.* **119**, 413–417.

Grases, F. & Llobera, A. 1996 Determination of phytic acid in urine by ICP atomic emission spectrometry. *Anal. Lett.* **29**, 1193–1199.

Greaves, M. P. & Webley, D. M. 1965 A study of the breakdown of organic phosphates by micro-organisms from the root region of certain pasture grasses. *J. Appl. Bacteriol.* **28**, 454–465.

Greaves, M. P. & Webley, D. M. 1969 The hydrolysis of *myo*inositol hexaphosphate by soil microorganisms. *Soil Biol. Biochem.* **1**, 37–43.

Greaves, M. P. & Wilson, M. J. 1970 The degradation of nucleic acids and montmorillonite-nucleic acid complexes by soil microorganisms. *Soil Biol. Biochem.* **2**, 257–268.

Greenwood, A. J. & Lewis, D. H. 1977 Phosphatases and the utilisation of inositol hexaphosphate by soil yeasts of the genus *Cryptococcus*. *Soil Biol. Biochem.* **9**, 161–166.

Guse, A. H., Goldwich, A., Weber, K. & Mayr, G. W. 1995 Non-radioactive, isomer-specific inositol phosphate mass determinations: HPLC micro-metal dye detection strongly improves speed and sensitivity of analyses from cells and micro-enzyme assays. *J. Chromatogr.* B: *Biomed. Sci. Appl.* **672**, 189–198.

Haizeng, C., Li, Y. & Song, S. 1991 Determination of the phytic acid content in soybean by ion-chromatography with post-column detection. *Shipin Yu Faijiao Gongye* **6**, 33–39.

Halstead, R. L. & Anderson, G. 1970 Chromatographic fractionation of organic phosphates from alkali, acid, and aqueous acetylacetone extracts of soils. *Can. J. Soil Sci.* **50**, 111–119.

Harland, B. F. & Oberleas, D. 1977 A modified method for phytate analysis using an ion-exchange procedure: application to textured vegetable proteins. *Cereal Chem.* **54**, 827–832.

Harrison, A. F. 1987 *Soil organic phosphorus. A review of world literature*. Oxford: CAB International.

Hatzack, F. & Rasmussen, S. K. 1999 High-performance thin-layer chromatography method for inositol phosphate analysis. *J. Chromatogr.* B: *Biomed. Sci. Appl.* **736**, 221–229.

Hawkes, G. E., Powlson, D. S., Randall, E. W. & Tate, K. R. 1984 A ^{31}P nuclear magnetic resonance study of the phosphorus species in alkali extracts from soils from long-term field experiments. *J. Soil Sci.* **35**, 35–45.

Hayes, J. E., Richardson, A. E. & Simpson, R. J. 2000*a* Components of organic phosphorus in soil extracts that are hydrolysed by phytase and acid phosphatase. *Biol. Fertil. Soils* **32**, 279–286.

Hayes, J. E., Simpson, R. J. & Richardson, A. E. 2000*b* The growth and phosphorus utilisation of plants in sterile media when supplied with inositol hexaphosphate, glucose 1-phosphate or inorganic phosphate. *Plant Soil* **220**, 165–174.

Haygarth, P. M., Warwick, M. S. & House, W. A. 1997 Size distribution of colloidal molybdate reactive phosphorus in river waters and soil solution. *Water Res.* **31**, 439–442.

Haygarth, P. M., Jarvis, S. C., Chapman, P. & Smith, R. V. 1998 Phosphorus budgets for two contrasting grassland farming systems in the UK. *Soil Use Manag.* **14**, 160–167.

Heathers, G. P., Juehne, T., Rubin, L. J., Corr, P. B. & Evers, A. S. 1989 Anion exchange chromatographic separation of inositol phosphates and their quantification by gas chromatography. *Anal. Biochem.* **176**, 109–116.

Heathwaite, A. L., Burt, T. P. & Trudgill, S. T. 1990 The effect of land use on nitrogen, phosphorus and suspended sediment delivery to streams in a small catchment in southwest England. In *Soil erosion on agricultural land* (ed. J. Boardman, L. D. L. Foster & J. A. Dearing), pp. 161–177. Chichester, UK: Wiley.

Hedley, M. J., Nye, P. H. & White, R. E. 1982 Plant-induced changes in the rhizosphere of rape (*Brassica napus* var. Emerald) seedlings: II. Origin of the pH change. *New Phytol.* **91**, 31–44.

Helal, H. M. & Dressler, A. 1989 Mobilisation and turnover of phosphorus in the rhizosphere. *Zeitschrift für Pflanzenernahrung und Bodenkunde* **152**, 175–180.

Helal, H. M. & Sauerbeck, D. 1991 Soil and root phosphatase activity and the utilization of inositol phosphates as dependent on phosphorus supply. In *Plant roots and their environment*, Proceedings of an ISSR-Symposium. London: Elsevier Science.

Henshall, A., Harrold, M. P. & Tso, J. M. 1992 Separation of inositol phosphates by capillary electrophoresis. *J. Chromatogr.* **608**, 413–419.

Herbes, S. E., Allen, H. E. & Mancy, K. H. 1975 Enzymatic characterization of soluble organic phosphorus in lake water. *Science* **187**, 432–434.

Hinsinger, P. & Gilkes, R. J. 1996 Mobilization of phosphate from phosphate rock and alumina-sorbed phosphate by the roots of ryegrass and clover as related to rhizosphere pH. *Eur. J. Soil Sci.* **47**, 533–544.

Hong, J. K. & Yamane, I. 1980 Examination of the conventional method and a proposal for a new method of determining inositol phosphate in the humic acid fraction. *Soil Sci. Plant Nutr.* **26**, 497–505.

Horstman, D. A., Takemura, H. & Putney, J. W. 1988 Formation and metabolism of [^{3}H] inositol platelets in AR42J pancreatoma cells. *J. Biol. Chem.* **263**, 15 297–15 303.

Hsu, F. F. & Sherman, W. R. 1989 Performing high salt concentration gradient elution ion-exchange separations using thermospray mass spectrometry. *J. Chromatogr.* **479**, 437–440.

Hsu, F. F., Goldman, H. D. & Sherman, W. R. 1990 Thermo-

spray liquid-chromatographic mass-spectrometric studies with inositol phosphates. *Biomed. Environ. Mass Spectr.* **19**, 597–600.

Huff, W. E., Moore, P. A., Waldroup, P. W., Waldroup, A. L., Balog, J. M., Huff, G. R., Rath, N. C., Daniel, T. C. & Raboy, V. 1998 Effect of dietary phytase and high available phosphorus corn on broiler chicken performance. *Poultry Sci.* **77**, 1899–1904.

Irving, G. C. & Cosgrove, D. J. 1981 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.* **12**, 495–509.

Irving, G. C. & Cosgrove, D. J. 1982 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.* **13**, 957–967.

Islam, A. & Mandal, R. 1977 Amounts and mineralization of organic phosphorus compounds and derivatives in some surface soils of Bangladesh. *Geoderma* **17**, 57–68.

IUPAC 1971 *Nomenclature of inorganic chemistry, definitive rules 1970*. London: Butterworths.

Jackman, R. H. & Black, C. A. 1951 Solubility of iron, aluminium, calcium and magnesium inositol phosphates at different pH values. *Soil Sci.* **72**, 179–186.

Jackman, R. H. & Black, C. A. 1952 Phytase activity in soils. *Soil Sci.* **73**, 117–125.

Jencks, E. M., Raese, J. T. & Reese, C. D. 1964 Organic phosphorus content of some West Virginia soils. *Bulletin of the West Virginia University Agricultural Experimental Station* **489**, 3–16.

Johnson, K., Barrientos, L. G., Long, L. & Murthy, P. P. 1995 Application of two-dimensional total correlation spectroscopy for structure determination of individual inositol phosphates in a mixture. *Anal. Biochem.* **231**, 421–431.

Kasim, A. B. & Edwards, H. M. 1998 The analysis for inositol phosphate forms in feed ingredients. *J. Sci. Food Agric.* **76**, 1–9.

Kemme, P. A., Lommen, A., De Jonge, L. H., Van der Klis, J. D., Jongbloed, A. W., Mroz, Z. & Beynen, A. C. 1999 Quantification of inositol phosphates using ^{31}P nuclear magnetic resonance spectroscopy in animal nutrition. *J. Agric. Food Chem.* **47**, 5116–5121.

Klotz, R. L. 1991 Cycling of phosphatase hydrolyzable phosphorus in streams. *Can. J. Fish. Aquat. Sci.* **48**, 1460–1467.

Kotak, B. G., Kenefick, S. L., Fritz, D. L., Rousseaux, C. G., Prepas, E. E. & Hrudey, S. E. 1993 Occurrence and toxicological evaluation of cyanobacterial toxins in Alberta lakes and farm dugouts. *Water Res.* **27**, 495–506.

Kretzschmar, R., Borkovec, M., Grolimund, D. & Elimelech, M. 1999 Mobile subsurface colloids and their role in contaminant transport. *Adv. Agron.* **66**, 121–193.

Kroehler, C. J. & Linkins, A. E. 1991 The absorption of inorganic-phosphate from ^{32}P-labeled inositol hexaphosphate by *Eriophorum vaginatum*. *Oecologia* **85**, 424–428.

L'Annunziata, M. F. 1975 The origin and transformations of the soil inositol phosphate isomers. *Soil Sci. Soc. Am. Proc.* **39**, 377–379.

L'Annunziata, M. F. & Fuller, W. H. 1971 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. America Proc.* **35**, 587–595.

L'Annunziata, M. F., Gonzalez, J. & Olivarez, L. A. 1977 Microbial epimerization of myo-inositol to chiro-inositol in soil. *Soil Sci. Soc. Am. J.* **41**, 733–736.

Leake, J. R. 2002 Organic phosphorus utilisation by mycorrhizal plants and fungi: how much do we really know?. In *Phosphatases in the environment* (ed. B. A. Whitton & I. Hernández). Dordrecht, The Netherlands: Kluwer Academic. (In the press.)

Lehrfeld, J. & Morris, E. G. 1992 Overestimation of phytic acid in foods by the AOAC anion-exchange method. *J. Agric. Food Chem.* **40**, 2208–2210.

Lei, X., Pao, K. K., Miller, E. R., Ullrey, D. E. & Yokoyama, M. T. 1993 Supplemental microbial phytase improves bioavailability of dietary zinc to weanling pigs. *J. Nutr.* **123**, 1117–1123.

Lenis, N. P. & Jongbloed, A. W. 1999 New technologies in low pollution swine diets: diet manipulation and use of synthetic amino acids, phytase and phase feeding for reduction of nitrogen and phosphorus excretion and ammonia emission. *Asian–Australian J. Anim. Sci.* **12**, 305–327.

Leprince, F. & Quiquampoix, H. 1996 Extracellular enzyme activity in soil: effect of pH and ionic strength on the interaction with montmorillonite of two acid phosphatases secreted by the ectomycorrhizal fungus *Hebeloma cylindrosporum*. *Eur. J. Soil Sci.* **47**, 511–522.

Li, M. G., Osaki, M., Rao, I. M. & Tadano, T. 1997 Secretion of phytase from the roots of several plant species under phosphorus-deficient conditions. *Plant Soil* **195**, 161–169.

Liu, J., Bollinger, D. W., Ledoux, D. R. & Veum, T. L. 1998 Lowering the dietary calcium to total phosphorus ratio increases phosphorus utilization in low-phosphorus corn-soybean meal diets supplemented with microbial phytase for growing-finishing pigs. *J. Anim. Sci.* **76**, 808–813.

Lookman, R., Geerts, H., Grobet, P., Merckx, R. & Vlassak, K. 1996 Phosphate speciation in excessively fertilized soil: a ^{31}P and ^{27}Al MAS NMR spectroscopic study. *Eur. J. Soil Sci.* **47**, 125–130.

Lookman, R., Grobert, P., Merckx, R. & Van Riemsdijk, W. H. 1997 Application of ^{31}P and ^{27}Al MAS NMR for phosphate speciation studies in soil and aluminum hydroxides: promises and constraints. *Geoderma* **80**, 369–388.

Luttrell, B. M. 1992 The biological relevance of the binding of calcium ions by inositol phosphates. *J. Biol. Chem.* **268**, 1521–1524.

McKelvie, I. D., Hart, B. T., Cardwell, T. J. & Cattrall, R. W. 1993 Speciation of dissolved phosphorus in environmental samples by gel filtration and flow-injection analysis. *Talanta* **40**, 1981–1993.

McKelvie, I. D., Hart, B. T., Cardwell, T. J. & Cattrall, R. W. 1995 Use of immobilized 3-phytase and flow injection for the determination of dissolved phosphorus species in natural waters. *Anal. Chim. Acta* **316**, 277–289.

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

McKercher, R. B. & Anderson, G. 1968*b* Content of inositol penta- and hexaphosphates in some Canadian soils. *J. Soil Sci.* **19**, 47–55.

Magid, J., Tiessen, H. & Condron, L. M. 1996 Dynamics of organic phosphorus in soils under natural and agricultural ecosystems. In *Humic substances in terrestrial ecosystems* (ed. A. Piccolo), pp. 429–466. Oxford: Elsevier Science.

March, J. G., Villacampa, I. A. & Graces, F. 1995 Enzymatic-spectrophotometric determination of phytic acid with phytase from *Aspergillus ficuum*. *Anal. Chim. Acta* **300**, 269–272.

Marko-Varga, G. & Gorton, L. 1990 Post-column derivatization in liquid chromatography using immobilized enzyme reactors and amperometric detection. *Anal. Chim. Acta* **234**, 13–29.

Martin, J. K. 1970 The measurement of inositol polyphosphates in soil extracts by isotope dilution analysis. *New Zealand J. Agri. Res.* **13**, 930–939.

Martin, C. J. 1995 Reaction of the co-ordinate complexes of inositol hexaphosphate with first row transition series cations

and Cd(II) with calf intestinal alkaline phosphatase. *J. Inorg. Biochem.* **58**, 89–107.

Martin, J. K. & Cartwright, B. 1971 The comparative plant availability of ^{32}P *myo*-inositol hexakisphosphate and $KH_2{}^{32}PO_4$ added to soils. *Commun. Soil Sci. Plant Anal.* **2**, 375–381.

Martin, C. J. & Evans, W. J. 1986 Phytic acid-metal ion interactions. The effect of pH on Ca(II) binding. *J. Inorg. Biochem.* **27**, 17–30.

Martin, C. J. & Evans, W. J. 1987 Phytic acid: divalent cation interactions. V. Titrimetric, calorimetric, and binding studies with cobalt(II), nickel(II) and their comparison with other metal ions. *J. Inorg. Biochem.* **30**, 101–119.

Martin, J. K. & Wicken, A. J. 1966 Soil organic phosphorus: IV. Fractionation of organic phosphorus in alkaline soil extracts and the identification of inositol phosphates. *New Zealand J. Agri. Res.* **9**, 529–535.

Martin, J.-B., Laussmann, T., Bakker-Grunwald, T., Vogel, G. & Klein, G. 2000 *neo*-Inositol polyphosphates in the amoeba *Entamoeba histolytica*. *J. Biol. Chem.* **275**, 10 134–10 140.

Matthews, W. R., Guido, D. M. & Huff, R. M. 1988 Anion-exchange high performance liquid chromatographic analysis of inositol phosphates. *Anal. Biochem.* **168**, 63–70.

Mayr, G. W. 1988 A novel metal-dye detection system permits picomolar-range HPLC analysis of inositol polyphosphates from non-radioactively labelled cell or tissue specimens. *Biochem. J.* **254**, 585–591.

Meek, J. L. & Nicoletti, F. 1986 Detection of inositol triphosphate and other organic phosphates by high-performance liquid chromatography using an enzyme-loaded postcolumn reactor. *J. Chromatogr.* **351**, 303–311.

Minear, R. A., Segars, J. E., Elwood, J. W. & Mulholland, P. J. 1988 Separation of inositol phosphates by high performance ion exchange chromatography. *Analyst* **113**, 645–649.

Morse, D., Head, H. H. & Wilcox, C. J. 1992 Disappearance of phosphorus in phytate from concentrates *in vitro* and from rations fed to lactating dairy cows. *J. Dairy Sci.* **75**, 1979–1986.

Nanny, M. A. & Minear, R. A. 1994 Organic phosphorus in the hydrosphere. Characterization via ^{31}P Fourier transform nuclear magnetic resonance spectroscopy. In *Environmental chemistry of lakes and reservoirs* (ed. L. A. Baker), pp. 161–191. Washington, DC: American Chemical Society.

Nanny, M. A., Kim, S., Gadomski, J. E. & Minear, R. A. 1994 Aquatic soluble unreactive phosphorus: concentration by ultrafiltration and reverse osmosis membranes. *Water Res.* **28**, 1355–1365.

Nanny, M. A., Kim, S. & Minear, R. A. 1995 Aquatic soluble unreactive phosphorus: high-performance liquid chromatography studies on concentrated water samples. *Water Res.* **29**, 2138–2148.

Nash, K. L., Jensen, M. P. & Schmidt, M. A. 1998 Actinide immobilization in the subsurface environment by in-situ treatment with a hydrologically unstable organophosphorus complexant: uranyl uptake by calcium phytate. *J. Alloys Compounds* **271**, 257–261.

Nayini, N. R. & Markakis, P. 1986 Phytases. In *Phytic acid* (ed. E. Graf), pp. 101–118. Minneapolis, USA: Pilatus Press.

Newman, R. H. & Tate, K. R. 1980 Soil phosphorus characterisation by ^{31}P nuclear magnetic resonance. *Commun. Soil Plant Anal.* **11**, 835–842.

Nomenclature Committee of the International Union of Biochemistry 1984 *Enzyme nomenclature*. Orlando, USA: Academic.

Oberleas, D. & Harland, B. F. 1986 Analytical methods for phytate. In *Phytic acid* (ed. E. Graf), pp. 101–118. Minneapolis, USA: Pilatus Press.

Oku, O. & Kamatani, A. 1995 Phospholipid in plankton samples from Tokyo Bay and Sagami Bay. *Nippon Suisan Gakkaishi* **61**, 588–595.

Omotoso, T. I. & Wild, A. 1970 Content of inositol phosphates in some English and Nigerian Soils. *J. Soil Sci.* **21**, 216–223.

O'Neill, I. K., Sargent, M. & Trimble, M. L. 1980 Determination of phytate in foods by phosphorus-31 FT-NMR spectrometry. *Anal. Chem.* **52**, 1288–1291.

Oniani, O. G., Chater, M. & Mattingley, G. E. G. 1973 Some effects of fertilizers and farmyard manure on the organic phosphorus in soils. *J. Soil Sci.* **24**, 1–9.

Pallauf, J. & Rimbach, G. 1997 Nutritional significance of phytic acid and phytase. *Arch. Anim. Nutr.* **50**, 301–319.

Palmer, S. & Wakelam, M. J. 1989 Mass measurement of inositol phosphates. *Biochim. Biophys. Acta* **1014**, 239–246.

Pant, H. K., Edwards, A. C. & Vaughan, D. 1994*a* Extraction, molecular fractionation and enzyme degradation of organically associated phosphorus in soil solutions. *Biol. Fertility Soils* **17**, 192–200.

Pant, H. K., Vaughan, D. & Edwards, A. C. 1994*b* Molecular size distribution and enzymatic degradation of organic phosphorus in root exudates of spring barley. *Biol. Fertility Soils* **18**, 285–290.

Patthy, M., Balla, T. & Aranyi, P. 1990 High-performance reversed-phase ion-pair chromatography study of *myo*-inositol phosphates. *J. Chromatogr.* **523**, 201–216.

Pearson, R. W., Norman, A. G. & Ho, C. 1941 The mineralization of the organic phosphorus of various compounds in soil. *Soil Sci. Soc. Am. Proc.* **13**, 168–175.

Peperzak, P., Caldwell, A. G., Hunziker, R. R. & Black, C. A. 1959 Phosphorus fractions in manures. *Soil Sci.* **87**, 293–302.

Phillipy, B. Q. & Bland, J. M. 1988 Gradient ion chromatography of inositol phosphates. *Anal. Biochem.* **175**, 162–166.

Phillipy, B. Q., White, K. D., Johnston, M. R., Tao, S. & Fox, M. R. 1987 Preparation of inositol phosphates from sodium phytate by enzymatic and nonenzymatic hydrolysis. *Anal. Biochem.* **162**, 115–121.

Plaami, S. & Kumpulainen, J. 1991 Determination of phytic acid in cereals using ICP-AES to determine phosphorus. *J. Assoc. Official Anal. Chem.* **74**, 32–36.

Portilla, D. & Morrison, A. R. 1986 Bradykin-induced changes in inositol trisphosphate in mock cells. *Biochem. Biophys. Res. Commun.* **140**, 644–649.

Potman, W. & Lijklema, L. 1983 Inositol phosphates: hydrolysis, stability and analysis. *Water Res.* **17**, 411–414.

Poulsen, H. D. 2000 Phosphorus utilization and excretion in pig production. *J. Environ. Qual.* **29**, 24–27.

Prestwich, S. A. & Bolton, T. B. 1991 Measurement of picomole amounts of any inositol phosphate isomer separable by HPLC by means of a bioluminescence assay. *Biochem. J.* **274**, 663–672.

Richardson, A. E., Hadobas, P. A. & Hayes, J. E. 2001*a* Extracellular secretion of *Aspergillus* phytase from *Arabidopsis* roots enables plants to obtain phosphorus from phytate. *Plant J.* **25**, 641–649.

Richardson, A. E., Hadobas, P. A., Hayes, J. E., O'Hara, C. P. & Simpson, R. J. 2001*b* Utilization of phosphorus by pasture plants supplied with myo-inositol hexaphosphate is enhanced by the presence of soil micro-organisms. *Plant Soil* **229**, 47–56.

Rittenhouse, S. E. & Sasson, J. P. 1985 Mass changes in *myo*-inositoltrisphosphate in human platelets stimulated by thrombin. *Microbiol. Ecol.* **260**, 8657–8660.

Rounds, M. A. & Nielsen, S. S. 1993 Anion-exchange high performance liquid chromatography with post-column detection for the analysis of phytic acid and other inositol phosphates. *J. Chromatogr.* **653**, 148–152.

Shan, Y., McKelvie, I. D. & Hart, B. T. 1993 Characterization

of immobilized *Escherichia coli* alkaline phosphatase reactors in flow injection analysis. *Anal. Chem.* **65**, 3053–3060.

Shan, Y., McKelvie, I. D. & Hart, B. T. 1994 Determination of alkaline phosphatase-hydrolyzable phosphorus in natural water systems by enzymatic flow injection. *Limnol. Oceanogr.* **39**, 1993–2000.

Shand, C. A. & Smith, S. 1997 Enzymatic release of phosphate from model substrates and P compounds in soil solution from a peaty podzol. *Biol. Fertil. Soils* **24**, 183–187.

Shang, C., Stewart, J. W. B. & Huang, P. M. 1992 pH effects on kinetics of adsorption of organic and inorganic phosphates by short-range ordered aluminium and iron precipitates. *Geoderma* **53**, 1–14.

Simons, P. C. M., Versteegh, H. A. J., Jongbloed, A. W., Kemme, P. A., Slump, P., Bos, K. D., Wolters, M. G. E., Beudeker, R. F. & Verschoor, G. J. 1990 Improvement of phosphorus availability by microbial phytase in broilers and pigs. *Br. J. Nutr.* **64**, 525–540.

Simonsson, M., Berggren, D. & Gustafsson, J. P. 1999 Solubility of aluminium and silica in spodic horizons as affected by drying and freezing. *Soil Sci. Soc. Am. J.* **63**, 1116–1123.

Skoglund, E., Nasi, M. & Sandberg, A. S. 1998 Phytate hydrolysis in pigs fed a barley-rapeseed meal diet treated with *Aspergillus niger* phytase or steeped with whey. *Can. J. Anim. Sci.* **78**, 175–180.

Sommers, L. E., Harris, R. F., Williams, J. D. H., Armstrong, D. E. & Syers, J. K. 1972 Fractionation of organic phosphorus in lake sediments. *Soil Sci. Soc. Am. Proc.* **36**, 51–54.

Spier, T. W. & Ross, D. J. 1978 Soil phosphatase and sulphatase. In *Soil enzymes* (ed. R. G. Burns), pp. 197–250. London: Academic.

Stephens, L. R. & Irvine, R. F. 1990 Stepwise phosphorylation of *myo*-inositol to *myo*-inositol hexakisphosphate in *Dictostelium*. *Nature* **346**, 580–583.

Stevens, R. J. & Stewart, B. M. 1982 Some components of particulate phosphorus in river water entering Lough Neagh. *Water Res.* **16**, 1591–1596.

Stevenson, F. J. 1982 *Humus chemistry. Genesis, composition, reactions*. New York: Wiley.

Steward, J. H. & Tate, M. E. 1971 Gel chromatography of soil organic phosphorus. *J. Chromatogr.* **60**, 75–82.

Suzumura, M. & Kamatani, A. 1993 Isolation and determination of inositol hexaphosphate in sediments from Tokyo Bay. *Geochim. Cosmochim. Acta* **57**, 2197–2202.

Suzumura, M. & Kamatani, A. 1995*a* Mineralization of inositol hexaphosphate in aerobic and anaerobic marine sediments: implications for the phosphorus cycle. *Geochim. Cosmochim. Acta* **59**, 1021–1026.

Suzumura, M. & Kamatani, A. 1995*b* Origin and distribution of inositol hexaphosphate in estuarine and coastal sediments. *Limnol. Oceanogr* **40**, 1254–1261.

Tangendjaja, B., Buckle, K. A. & Wootton, M. 1980 Analysis of phytic acid by high performance liquid chromatography. *J. Chromatogr.* **197**, 274–277.

Tao, L. & Li, W. 1992 Rapid and sensitive anion-exchange high-performance liquid chromatographic determination of radiolabeled inositol phosphates and inositol trisphosphate isomers in cellular systems. *J. Chromatogr.* **607**, 19–24.

Tarafdar, J. C. & Claassen, N. 1988 Organic phosphorus compounds as a phosphorus source for higher plants through the activity of phosphatases produced by plant roots and microorganisms. *Biol. Fertil. Soils* **5**, 308–312.

Tate, K. R. & Newman, R. H. 1982 Phosphorus fractions of a climosequence of soils in New Zealand tussock grassland. *Soil Biol. Biochem.* **14**, 191–196.

Taylor, G. S., Garcia, J. G., Dukes, R. & English, D. 1990 High-performance liquid chromatography analysis of radiolabeled inositol phosphates. *Anal. Biochem.* **188**, 118–122.

Thomas, R. L. & Lynch, D. L. 1960 Quantitative fractionation of organic phosphorus compounds in some Alberta soils. *Can. J. Soil Sci.* **40**, 113–120.

Thurman, E. M. 1985 *Organic geochemistry of natural waters*. Dordrecht, The Netherlands: Kluwer Academic.

Tiessen, H., Stewart, J. W. B. & Moir, J. O. 1983 Changes in organic and inorganic phosphorus composition of two grassland soils and their particle size fractions during 60–90 years of cultivation. *J. Soil Sci.* **34**, 815–823.

Turner, B. L. & Haygarth, P. M. 2000 Phosphorus forms and concentrations in leachate under four grassland soil types. *Soil Sci. Soc. Am. J.* **64**, 1090–1097.

Turner, B. L. & Haygarth, P. M. 2001 Phosphorus solubilisation in rewetted soils. *Nature* **411**, 258.

Turner, B. L. & McKelvie, I. D. 2002 A novel technique for the pre-concentration and separation of inositol hexakisphosphate in soil extracts with determination by phosphorus-31 nuclear magnetic resonance *J. Environ. Qual.* **31**, 466–470.

Turner, R. E. & Rabalais, N. N. 1994 Coastal eutrophication near the Mississippi river delta. *Nature* **368**, 619–621.

Turner, B. L., McKelvie, I. D. & Haygarth, P. M. 2002 Characterisation of water-extractable soil organic phosphorus by phosphatase hydrolysis. *Soil Biol. Biochem.* **34**, 27–35.

Ullah, A. H. J. & Gibson, D. M. 1987 Extracellular phytase (E.C. 3.1.3.8) from *Aspergillus ficuum* NRRL 3135: purification and characterization. *Preparative Biochem.* **17**, 63–91.

Webley, D. M. & Jones, D. 1971 Biological transformation of microbial residues in soil. In *Soil biochemistry, volume II* (ed. A. J. McLaren & J. Skujins), pp. 446–485. New York: Marcel Dekker.

Weimer, W. C. & Armstrong, D. E. 1977 Determination of inositol phosphate esters in lake sediments. *Anal. Chim. Acta* **94**, 35–47.

Weimer, W. C. & Armstrong, D. E. 1979 Naturally occurring organic phosphorus compounds in aquatic plants. *Environ. Sci. Technol.* **13**, 826–829.

Westin, F. C. & Buntley, G. J. 1967 Soil phosphorus in South Dakota. III. Phosphorus fractions of some borolls and ustolls. *Soil Sci. Soc. Am. Proc.* **31**, 521–528.

Wheeler, E. L. & Ferrel, R. E. 1971 A method for phytic acid determination in wheat and wheat fraction. *Cereal Chem.* **48**, 312–320.

White, R. H. & Miller, S. J. 1976 Inositol isomers: occurrence in marine sediments. *Science* **193**, 885–886.

Whitton, B. A., Grainger, S. L. J., Hawley, G. R. W. & Simon, J. W. 1991 Cell bound and extracellular phosphatase activities of cyanobacterial isolates. *Microbiol. Ecol.* **21**, 85–98.

Wild, A. & Oke, O. L. 1966 Organic phosphate compounds in calcium chloride extracts of soils: identification and availability to plants. *J. Soil Sci.* **17**, 356–371.

Williams, C. H. & Anderson, G. 1968 Inositol phosphates in some Australian soils. *Aust. J. Soil Res.* **6**, 121–130.

Wilson, D. B., Connolly, T. M., Bross, T. E., Majerus, P. W., Sherman, W. R., Tyler, A. N., Rubin, L. J. & Brown, J. E. 1985 Isolation and characterization of the inositol phosphate products of polyphosphoinositide cleavage by phospholipase C. *J. Biol. Chem.* **260**, 13 496–13 501.

Wolters, M. G. E., Schreuder, H. A. W., Van Den Heuvel, G., Vanlonkhuijsen, H. J., Hermus, R. J. J. & Voragen, A. G. J. 1993 A continuous *in vitro* method for estimation of the bioavailability of minerals and trace elements in foods: application to breads varying in phytic acid content. *Br. J. Nutr.* **69**, 849–861.

Xu, P., Price, J. & Aggett, P. J. 1992 Recent advances in methodology for analysis of phytate and inositol phosphates in foods. *Prog. Food Nutr. Sci.* **16**, 245–262.