

Review

Using organic phosphorus to sustain pasture productivity: A perspective [☆]



David M. Nash ^{a,*}, Philip M. Haygarth ^b, Benjamin L. Turner ^c, Leo M. Condron ^d, Richard W. McDowell ^e, Alan E. Richardson ^f, Mark Watkins ^a, Michael W. Heaven ^a

^a Victorian Department of Environment and Primary Industries – Ellinbank, RMB 2460 Hazeldean Road, Ellinbank, Victoria 3821, Australia

^b Centre for Sustainable Water Management, Lancaster Environment Centre, Lancaster University, Bailrigg, Lancaster LA14YW, United Kingdom

^c Smithsonian Tropical Research Institute, Apartado 0843-03092, Balboa, Ancon, Panama

^d Faculty of Agriculture and Life Sciences, Lincoln University, Lincoln, PO Box 85084, 7647 Christchurch, New Zealand

^e AgResearch – Invermay Agricultural Centre, Puddle Alley, Private Bag 50034, Mosgiel 9053, New Zealand

^f CSIRO Plant Industry – PO Box 1600, Canberra, ACT 2601, Australia

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ABSTRACT

Organic phosphorus (P) in grazed pastures/grasslands could sustain production systems that historically relied on inorganic P fertiliser. Interactions between inorganic P, plants and soils have been studied extensively. However, less is known about the transformation of organic P to inorganic orthophosphate. This paper investigates what is known about organic P in pasture/grassland soils used for agriculture, as well as the research needed to utilise organic P for sustainable plant production.

Organic P comprises > 50% of total soil P in agricultural systems depending on location, soil type and land use. Organic P hydrolysis and release of orthophosphate by phosphatase enzymatic activity is affected by a range of factors including: (a) the chemical nature of the organic P and its ability to interact with the soil matrix; (b) microorganisms that facilitate mineralisation; (c) soil mineralogy; (d) soil water electrolytes; and (e) soil physicochemical properties.

Current biogeochemical knowledge of organic P processing in soil limits our ability to develop management strategies that promote the use of organic P in plant production. Information is particularly needed on the types and sources of organic P in grassland systems and the factors affecting the activity of enzymes that mineralise organic P. Integrated approaches analysing the soil matrix, soil water and soil biology are suggested to address this knowledge gap.

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1. Introduction

Phosphorus (P) is an essential input for many agricultural production systems. For example, the productivity of most Australian soils in their native state was limited by P and nitrogen (N) availability (Sale, 1992). Through the use of P fertilisers, productive pasture-based grazing systems have been developed in which legumes, predominantly introduced clovers, fix atmospheric N, helping to rectify both P and N

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* Corresponding author. Tel.: +61 3 5624 2253.

E-mail address: david.nash@depi.vic.gov.au (D.M. Nash).

limitations (Magid et al., 1996). While some recent studies suggest that the need for P fertilisers has diminished, especially in the more intensive systems (Condon, 2004; Gourley et al., 2012), ongoing P additions are often required to sustain optimal production (Frossard et al., 2000). In most instances the use of P remains inefficient and there is clear need to increase P-use efficiency (Burkitt et al., 2002; Haygarth et al., 2013; Richardson et al., 2011; Simpson et al., 2011).

Inorganic P in the form of orthophosphate is a primary constituent of most P fertilisers. When added to soil, orthophosphate is either sequestered into forms that are not immediately available to plants (Engelstad and Terman, 1980; McLaughlin et al., 2011) or extracted from soil water and incorporated into plant and microbial biomass. In grazing systems, P is further transferred into animal biomass and may be exported from farms as animal (or plant) product. Phosphorus in biomass is otherwise returned to the soil when plant and animal biomass, and their wastes, are recycled and decomposed.

Decomposing plant and animal products, along with the soil microflora and fauna that undertake such decomposition, provide a significant store and a source of organic P, which commonly comprises >50% of total soil P in agricultural systems (Stutter et al., 2012), and inorganic P (Tisdale et al., 1985). In grazed pastures, up to 85% of the P taken up by plants is returned to the soil in dung. Such deposits can represent P inputs of 35 and 280 kg P ha⁻¹ annually for individual sheep and cattle, respectively (Haynes and Williams, 1993). Importantly, P is often returned to soil at a location far removed from where it was consumed, so stock transfer represents a loss in pastoral systems (Kemp et al., 2000) that can account for approximately 5% of P fertiliser inputs (Simpson et al., 2011).

Despite predictions of future P shortages (Cordell et al., 2009), there appears to be no immediate concerns regarding the supply of P fertilisers (Heffer and Prud'homme, 2011). However, the offsite impacts of P derived from farmland (Schröder et al., 2010) and the susceptibility of the supply chain to short-term disruptions in nations supplying raw materials (i.e. Morocco) and associated market volatility, has increased scientific interest in accessing 'stored' organic P for plant production, thereby lowering the short to medium term (i.e. <10 year) need for continued P additions (Haygarth et al., 2013; Stutter et al., 2012).

An important consideration for more sustainable use of world P reserves and agriculture production is whether the productivity of pasture-based grazing systems can be sustained by greater utilisation of soil organic P if fertiliser applications are curtailed? In this paper we consider the forms of organic P in pasture soils and factors that regulate their bio-availability, with an emphasis on phosphomonoesters, the singly most abundant form of organic P. In this context we use the term "pasture" to refer to native or introduced grass species that are grazed as part of an agricultural production system established through the use of P amendments. Our aim is to identify gaps in knowledge that need to be addressed in order to optimise the use of organic P for pasture production, particularly in Australia and New Zealand.

2. Forms of organic phosphorus in pasture soils

Orthophosphate, the predominant form of inorganic P in soil, can be derived from dissolution of primary P containing minerals such as apatite, through the application of mineral P fertilisers or by mineralisation of organic forms of P by microorganisms (Frossard et al., 2000; Stewart and Tiessen, 1987). Other forms of inorganic P commonly found in soils include pyrophosphate and polyphosphates which may be associated with high levels of fungal activity (Bunemann et al., 2008; Makarov et al., 2005). Organic P on the other hand is derived mainly from biological processes involving assimilation of orthophosphate and subsequent release as microbial, animal and plant materials mature and decompose (Condon et al., 2005).

Typical organic P compounds (Figs. 1 and 2) include: (i) phosphomonoesters (compounds with a single ester linkage to orthophosphate) such as the inositol phosphates; (ii) phosphodiester

(compounds with two ester linkages to orthophosphate) such as ribonucleic acid (RNA), deoxyribonucleic acid (DNA), lipoteichoic acid, phospholipid fatty acids (e.g. lecithin); and (iii) organic polyphosphates such as adenosine triphosphate. Phosphomonoesters and diesters, including specific compounds such as *myo*-inositol hexakisphosphate (IP₆) and DNA are routinely determined by extraction with a solution containing 0.25 M NaOH and 50 mM Na₂EDTA and detection by solution ³¹P nuclear magnetic resonance (NMR) spectroscopy (Cade-Menun, 2005; Condon et al., 2005; Murphy et al., 2009). Lower extractant concentrations may enhance phosphodiester recovery compared to phosphomonoester recovery (Turner, 2008), while hydrolysis of phosphodiester during extraction and analysis has been shown to occur with manures and may bias quantitative assessments (Cade-Menun, 2011; Turner et al., 2003d).

Some characteristics of P in NaOH-EDTA extracts of selected pasture/grassland soils analysed using ³¹P NMR are presented in Table 1. Collectively, phosphomonoesters and diesters predominate and comprise ~25% of the total P in pasture soils. The most prevalent phosphomonoester is *myo*-IP₆ (Fig. 2) (Cosgrove, 1980; Turner et al., 2002a), which contains six phosphate moieties and exists as nine possible stereoisomers, although only four of these appear to occur in the environment (Turner, 2007). The *myo*-IP₆ stereoisomer (also termed phytic acid (Shears and Turner, 2007)) is the most common form identified in soil, followed by the *scyllo*-, *neo*-, and *D-chiro*- forms (Turner, 2007; Turner et al., 2012). Phytic acid is synthesised by plants and is stored primarily in seeds where it can represent 60% to 80% of total plant P. Seeds of major cereal crops typically contain between 3.0 and 4.0 mg P/g dry weight (Raboy, 1997), so *myo*-IP₆ enters the soil through direct deposition of plant material (including seeds) (Noack et al., 2012), and from animal faeces, especially non-ruminant animals that consume feed containing IP₆ (Maguire et al., 2004). Non-ruminant animals do not produce gut phytase (i.e. phosphatase enzyme required for specific hydrolysis of IP₆) so a large proportion of IP₆ in the diet may be excreted in the faeces (Lei and Porres, 2007; Leytem et al., 2004; Turner, 2004; Turner and Leytem, 2004). Nonetheless the origins of phytate in soil remain to be more fully elucidated. For example, the presence of hexakisphosphates of *scyllo*-, *neo*-, and *D-chiro*-inositol stereoisomers in soil, which do not occur in higher plants, suggests that direct synthesis by microorganisms may be a significant source of inositol phosphates in some soils (Anderson, 1980; Turner, 2007).

From a production standpoint the considerable variability in organic P forms and their concentration in different soils and across farming systems (see Table 1), are both an opportunity and a concern. Moreover, the significance of organic P in plant nutrition is not reflected in agronomic soil tests which commonly involve dissolution and extraction of inorganic P and thus do not take adequate account of biological processes (Condon and Newman, 2011; Coventry et al., 2001; Murphy et al., 2009). Therefore, accessing the potential value of organic P as a 'fertiliser' resource requires a more comprehensive understanding of organic P transformations in soil and factors that affect its mineralisation, along with possibly new methods of assessing its role in plant nutrition in different pastoral systems. The variability in organic P between otherwise similarly managed sites also suggests that rates of organic P mineralisation vary widely and could be manipulated to release inorganic P for plant production.

3. Factors affecting organic phosphorus species and concentrations in pasture soils

Forms of organic P in soil are affected, or at least correlated with a wide range of soil geochemical, physical and climatic factors, including precipitation and temperature, which have major impacts on biological processes (Harrison, 1987). The proportion of phosphodiester extracted from bulk soil and the clay fraction increased with mean annual precipitation and temperature in a study of pasture/grassland soils of western USA (Sumann et al., 1998). Phosphomonoester proportions in

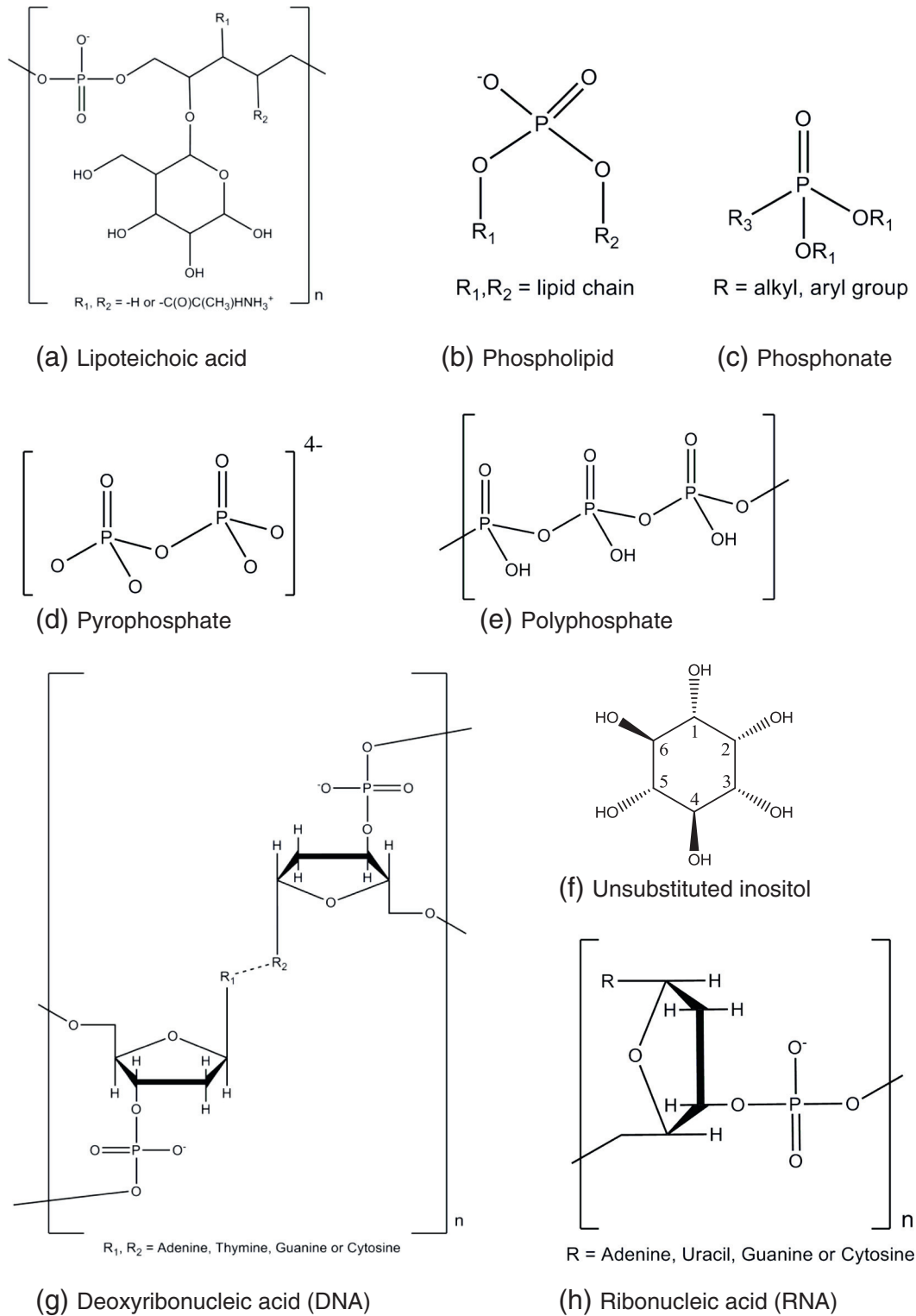


Fig. 1. Chemical structures of selected phosphorus containing molecules found in soil. (a) Lipoteichoic acid, (b) phospholipid, (c) phosphonate, (d) pyrophosphate, (e) polyphosphate, (f) unsubstituted inositol, (g) deoxyribonucleic acid (DNA), and (h) ribonucleic acid (RNA).

bulk soil, on the other hand, decreased in the clay fraction with increased mean annual temperature and with increased mean annual precipitation. In another study investigating primarily irrigated, arable soils from the western USA, temperature was negatively correlated with both the concentrations and proportions of phosphomonoesters and diesters (Turner et al., 2003a). The comparative lack of relationships with precipitation may in this case in part reflect irrigation.

The phosphodiester to monoester ratio is another soil metric that may be affected by climatic factors. In one study, phosphodiester to monoester ratios appeared to increase with mean annual temperature (Sumann et al., 1998). In a climosequence of New Zealand tussock grassland soils correlation coefficients suggested that the phosphodiester to monoester ratio tended to decrease with temperature (Tate and Newman, 1982). Some of the variability (i.e. <25%) observed in

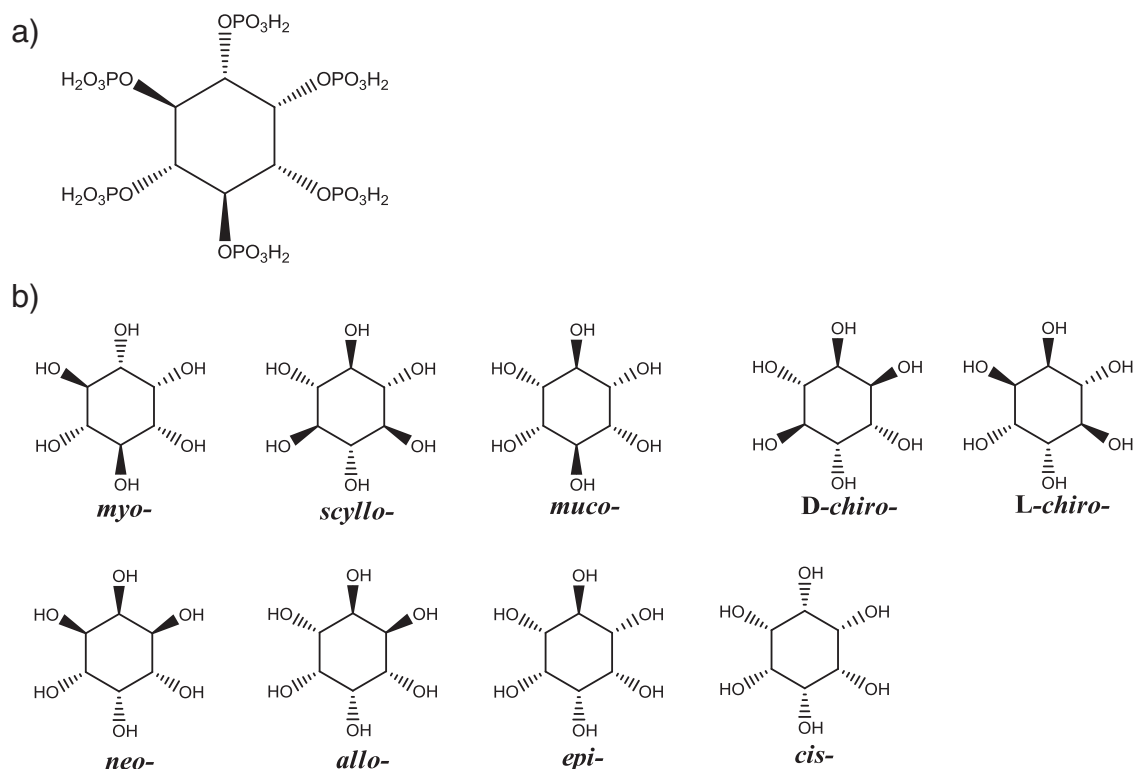


Fig. 2. The structure of a) phytic acid (*myo*-inositol hexakisphosphate) and b) conformations of unsubstituted inositols.

phosphodiester to monoester ratios between experiments may be accounted for by the different extraction procedures (Cade-Menun and Liu, 2013), between-year variation and the time of sampling as seasonal trends in ratios have been observed in pasture/grassland soils (Turner et al., 2003b).

There are limited data on the effects of climatic factors on the composition of different organic P classes. In an Irish study (Murphy et al., 2009) using four non-basaltic soils, the concentration of *myo*-IP₆ ranged from 97 to 185 mg P/kg of soil (representing 20 to 52% of the total organic P) while *scyllo*-IP₆ ranged between 23 and 99 mg P/kg (representing 12 to 17%). The ratio of *scyllo*- to *myo*-isomers varied from 0.23 to 0.85 (Murphy et al., 2009). Similar results were found in twenty nine temperate pasture soils from England and Wales (Turner et al., 2005), where the ratio of *scyllo*- to *myo*-isomers ranged from 0.31 to 0.79 (mean 0.45). In these samples *scyllo*-IP₆ concentrations ranged from 11 to 130 mg P/kg of soil (representing 4 to 15% of the organic P extracted) and *myo*-IP₆ concentrations ranged between 26 and 189 mg P/kg (representing 11 to 35% of the extracted organic P). In the future, the forms of inositol P may prove to be important if the enzymes responsible for dephosphorylation show specificity for particular structural isomers.

Plant species and soil mineralogy also affects organic P composition in soil. In a Brazilian study, tropical pasture/grassland soils had a low phosphodiester to monoester ratio (0.001) which was significantly higher where legumes were planted, (i.e. between 0.17 and 0.34, depending on species) (Canellas et al., 2004). In Brazilian Oxisols (high in Al and Fe oxides), the phosphodiester to monoester ratios on a range of pastures varied from 0.20 to 0.28 (Chapuis-Lardy et al., 2001), with the predominance of phosphomonoesters being attributed largely to the soil mineralogy (i.e. >50% gibbsite, 13–20% goethite, 10–20% kaolinite and 6–8% haematite). In this case, the phosphodiester to monoester ratio showed no clear trend as a result of land-use changing from savannah to pasture (Chapuis-Lardy et al., 2001).

Based on the available data, it is difficult to draw any firm conclusions regarding the effects of climate or plant species on organic P. This is not surprising given the large range of reported data (see Table 1), the likelihood that the primary effects of climate, soil and

plant species along with soil formation are strongly interrelated, and the lack of consistent analytical protocols between studies (Cade-Menun and Liu, 2013). Clearly, further work measuring organic P species in soils using standardised procedures, along with meta-analysis of existing data sets, is required to identify factors that regulate organic P composition across different environments.

4. Stabilisation and mineralisation of organic phosphorus in soil

Mineralisation of organic P in soil is governed by a range of both abiotic and biotic factors (George et al., 2007b). Access to the substrate is a primary requirement for organic P hydrolysis, with Al and Fe oxides being particularly important. Inositol phosphates have greater affinity than orthophosphate for Fe oxides (Martin et al., 2004) as the high anionic charge of inositol phosphates, particularly IP₆, facilitates the formation of strong electrostatic bonds (Menezes-Blackburn et al., 2012; Turner et al., 2002a). Originally it had been thought that adsorption of IP₆ onto goethite (a common soil Fe oxide mineral) is through ligand exchange at the surface, releasing OH⁻ and H₂O to the solution and formation of an inner sphere complex (i.e. ions bind directly to the surface with no intervening water molecules) (Ognalaga et al., 1994). However, a recent study suggests that some of the phosphate groups may form an outer sphere complex and that hydrogen bonding plays an important role in binding of IP₆ to goethite (Johnson et al., 2012). It would seem likely that bonds between IP₆ and soil constituents like goethite have features of both inner and outer sphere complexes, with the balance dependant on soil pH. This would explain why phosphomonoester concentrations are commonly related to the Fe and Al content of forestry (Vincent et al., 2012) and pasture (Murphy et al., 2009; Turner et al., 2003c) soils.

Model (i.e. laboratory) studies have shed further light on the reactions of *myo*-IP₆ with other soil constituents (Celi et al., 1999). Goethite adsorption of *myo*-IP₆ (0.64 μmol P/m²) was greater than for phyllosilicate minerals (illite 0.38 μmol P/m²; kaolinite, 0.27 μmol P/m²). The stronger adsorption of *myo*-IP₆ to Fe oxides in that study was attributed to the electrostatic association of four of the six orthophosphate groups, rather than two in the cases of illite and kaolinite. The study suggested that IP₆

Table 1
Selected characteristics of phosphorus in NaOH-EDTA extracts of selected grassland.

Site location	Number of sites	Vegetation classification	Sampling depth (mm)	Total P (mg/kg)	P extracted (mg/kg)	Phosphomonoester P (mg/kg)	Phosphodiester P (mg/kg)	Phosphodiester/monoester (%)	Reference
Canada	3	Grassland (native) ^a	0–150	956 (721–1318) ^b	696 (410–1136)	307 (202–474)	43 (12–94)	14.0	Condron et al. (1990)
Australia	12	Pasture	0–100	1262 (257–2526)	856 (252–1903)	272 (149–496)	18 (4–39)	6.6	Doolette et al. (2011)
Australia	3	Pasture	0–100	984 (545–1299)	695 (360–948)	209 (154–294)	15 (11–19)	7.2	Dougherty et al. (2007)
New Zealand	5	Pasture	0–150	1533 (938–2405)	1085 (411–2243)	442 (209–848)	18 (7–43)	4.1	McDowell and Stewart (2006)
New Zealand	24	Pasture	0–75	1268 (225–2770)	870 (128–2292)	349 (55–613)	19 (5–62)	5.4	McDowell et al. (2005)
New Zealand	3	Pasture	0–75	1433 (460–2910)	1207 (417–2290)	423 (204–613)	27 (6–62)	6.5	McDowell and Stewart (2005)
Ireland	25	Grassland (deforested)	0–20	1428 (616–2580)	718 (355–2353)	339 (30–653)	16 (0–53)	4.7	Murphy et al. (2009)
North America	18	Grassland	0–100	1292 (337–1838)	670 (393–1043)	350 (196–653)	19.6 (0.5–53)	5.6	Sumann et al. (1998)
England and Wales	29	Pasture	0–100	939 (376–1981)	686 (330–1538)	343 (154–751)	39 (11–109)	11.4	Turner et al. (2003c)
Weighted Mean				1216	776	345	27	7.8	

^a Additional descriptor.

^b Range.

changed the surface properties of the mineral. Net negative charge on the surface increased and dispersion of the particles was enhanced, presumably as a result of the unattached phosphate groups in IP₆ modifying the electrochemical properties. This suggests that IP₆ may, in some soils, increase colloidal P concentrations in soil water and leachate. Calcite (CaCO₃) is another common soil mineral that has been shown to adsorb and retain IP₆ (17.8 μmol P/m²) (Celi et al., 2000).

Similar model studies suggest that the electrolyte solution surrounding goethite also affects *myo*-IP₆ and orthophosphate adsorption. IP₆ and orthophosphate adsorbed onto goethite decreased with increasing pH in the presence of KCl, but increased with a CaCl₂ solution (Celi et al., 2001). Compared to *myo*-IP₆, a less pronounced decrease in adsorption of orthophosphate with increased pH in a KCl solution was attributed to the higher negative charge on *myo*-IP₆ and the tendency for phosphate groups on *myo*-IP₆ to be less effective in neutralising the hydroxyl groups released during adsorption. It was suggested that precipitation of Ca salts may partly contribute to “apparent” adsorption of both *myo*-IP₆ and orthophosphate above pH 5 (Celi et al., 2001). As both Ca and K salts in this study were used at similar concentrations (0.01 M), the effects of an interaction between the charge cloud surrounding the solid phase (i.e. electrical double layer) (van Olphen, 1977) may also warrant consideration.

Compared to adsorption, desorption of inositol phosphates from soil minerals has received less attention. Desorption of *myo*-IP₆ from goethite has been shown to increase with pH and the number of desorption cycles (Martin et al., 2004). The amount of *myo*-IP₆ desorbed from goethite was less than 20% of the desorption of orthophosphate, which was attributed to the strength of the bonds and the high negative charge of the complex, hindering the influence of other cations and/or ligands. The same study examined the effects of organic anions, which may be released by plant roots and are commonly assumed to influence the desorption of orthophosphate in soil (Ryan et al., 2001). After five extraction cycles, 2.6% of the initially adsorbed IP₆ was extracted with citrate and 4.5% with a KCl solution. The equivalent data for orthophosphate were 32.7% and 20.6%, respectively. It was concluded that the effects of the organic anions were negligible.

Solid phase adsorption of inositol phosphates is commonly reported to hinder their degradation in soils (Madrid and Diaz-Barrientos, 1998; Turner et al., 2002b). For example, in model studies, IP₆ adsorbed to iron oxides (goethite and haematite) and montmorillonite appeared to be inaccessible for enzyme hydrolysis and subsequent release of orthophosphate (Giaveno et al., 2010). However, in the same study, enzymes

were able to hydrolyse some of the IP₆ adsorbed to two soil clays. The latter results were attributed to the heterogeneous surface of the clays and organic matter that may hinder the perfect arrangement of IP₆ on those surfaces (Rao et al., 1996, 2000). The relatively high organic matter concentrations in the surface of some pasture soils, and associated soil organic matter turnover, would therefore be expected to enhance the hydrolysis of IP₆ in field studies (Nash et al., 2007a; Nash et al., 2007b).

When in solution, enzyme hydrolysis of inositol phosphates can be influenced by complexation reactions. For example, reaction of *myo*-IP₆ with Fe²⁺ occurs more quickly in solution and provides greater protection from enzyme hydrolysis than Fe³⁺-*myo*-IP₆ complexes (Heighton et al., 2008). The formation of such complexes has received little study, but is likely to depend strongly on soil pH and soil redox potential, which affect the concentrations and relative proportion of Fe²⁺ and Fe³⁺ in soil water, and P adsorption (Bohn et al., 1985). It follows that periodic change in soil oxic/anoxic conditions, such as would occur in irrigated or occasionally waterlogged soils and subsoils, could affect the formation of Fe²⁺-*myo*-IP₆ complexes and stabilisation of organic P in soils, intermittently facilitating the release of plant available P from *myo*-IP₆.

Like inositol phosphates, phosphodiester such as DNA can be protected from dephosphorylation by adsorption to the soil matrix. Factors such as mineralogy of the solid phase, pH, ionic strength and composition, and molecular size of the DNA affect that process (Levy-Booth et al., 2007; Ogram et al., 1988). For example, at pH > 5 DNA is negatively charged and its adsorption to minerals such as montmorillonite is facilitated by cation bridging that is not necessary at lower pH when DNA is positively charged (Greaves and Wilson, 1969). Notably, pH 5 is within the range encountered in pasture soils (Crawford et al., 1994). The location of DNA and RNA adsorption on montmorillonite, and therefore susceptibility to biodegradation, has also been shown to depend on solution pH (Greaves and Wilson, 1969, 1970). In general DNA adsorption to 2:1 phyllosilicates (e.g. montmorillonite) appears stronger than to 1:1 phyllosilicates (e.g. kaolinite) (Cai et al., 2006). A detailed discussion of DNA and RNA adsorption is outside the scope of this review but readers are referred to reviews which comprehensively examine this complex topic (Pietramellara et al., 2009; Trevors, 1996; Yu et al., 2013).

5. Reaction of phosphatases and their efficacy in soil

While availability of the substrate is critical for the transformation of organic P to orthophosphate, so too are the phosphatase enzymes in soil

which catalyse dephosphorylation reactions. In P deficient environments, selection pressure commonly results in the proliferation of free-living microorganisms and symbiotic associations with mycorrhizal fungi that have potential to mobilise and mineralise otherwise unavailable organic P (and solubilise inorganic P) (Mander et al., 2012; Richardson and Simpson, 2011). For example, in pot trials ryegrass (*Lolium perenne* L.) was grown using orthophosphate and *myo*-IP₆ adsorbed to goethite as sources of P (Martin et al., 2004). Compared to plants receiving no P, P concentrations in the shoots increased by factors of approximately 3 and 6 where *myo*-IP₆ and inorganic P adsorbed to goethite were supplied, respectively. These results suggest that some *myo*-IP₆ was converted to orthophosphate probably through microbial processes.

Dephosphorylation of IP₆ is generally attributed to microorganisms, but plant roots also appear to possess limited phytase activity (Richardson, 1994). In model studies, pasture grasses (*Danthonia richardsonii*, *Phalaris squatica*) and legumes (*Medicago polymorpha*, *M. sativa*, *Trifolium repens*, *T. subterraneum*) were grown in sterile agar or sterile sand-vermiculite media (Richardson et al., 2001). Under these conditions plants utilising *myo*-IP₆ contained only 20–34% of the total P content, and had restricted growth, when compared to plants supplied with an equivalent amount of orthophosphate. This effect was even more pronounced for the sand-vermiculite treatments where total P uptake from plants utilising IP₆ was only 5 to 10% of those receiving orthophosphate. Phosphorus uptake from the *myo*-IP₆ treatments increased by 3.9 to 6.8-fold when plants were inoculated with soil microorganisms, although the response depended on the growth medium used and the source of the inoculant. Impaired *myo*-IP₆ availability was suggested as the major reason why the sand-vermiculite medium was the least responsive to inoculation. A similar mechanism would explain the lack of a response to P additions where a Fe-IP₆ complex was added to the soil under exotic pasture in Australia (Taranto et al., 2000). By comparison, in this study P supplied as RNA was mineralised within two months, which is consistent with RNA being less tightly held on adsorption sites and thus more labile than *myo*-IP₆.

Mineralisation of different inositol phosphate isomers was studied in a 10-month pot trial using perennial ryegrass (*Lolium perenne*) growing in seven pasture/grassland soils from New Zealand (Chen et al., 2004). The inositol phosphate concentrations in soil were 142 to 598 mg P/kg and under ryegrass, *myo*-IP₆ decreased in four soils (3 to 20% of inositol phosphates) and increased in three soils (6 to 12%) whereas *scyllo*-IP₆ decreased in three soils (1 to 5%) and increased in three soils (2 to 8%) (Chen et al., 2004; Turner et al., 2005). Importantly, the changes in concentrations of the two isomers were not consistent across soil types. For example, in the Stratford soil, *myo*-IP₆ decreased by 18% while the *scyllo*-IP₆ increased by 8%. These results may be attributed to: (a) specific enzyme activities (i.e., compared to *myo*-IP₆, *scyllo*-IP₆ has been shown to be more resistant to enzyme hydrolysis by phytase (Cosgrove, 1966; He et al., 2011)); and (b) an interaction between root exudates, pH in the root zone, and soil properties, which may affect the abiotic stabilisation of the isomers differently. These factors could similarly contribute to the varying *scyllo*-IP₆/*myo*-IP₆ ratios (0.29–0.69) measured in 29 lowland permanent pasture soils from England and Wales (Turner et al., 2005).

Soil pH appears to be a particularly important factor affecting efficacy and biochemical availability of dephosphorylating enzymes in soil. For example, phytases from *Aspergillus niger* and *Peniophora lycii*, with differing biochemical properties including isoelectric point, were added to suspensions of three contrasting soils (Spodosol, Oxisol, Alfisol) and their activities for dephosphorylation of IP₆ monitored (George et al., 2007a). At pH 7.5 both phytases remained in solution, but at pH 5.5 *A. niger* phytase was rapidly adsorbed to the soil solid phase. The result was that *P. lycii* phytase, which had the more acidic isoelectric point, stayed in the solution and was more effective at hydrolysing IP₆ added to the soil and endogenous organic P. In a companion experiment, the activity of *A. niger* phytase in solution declined rapidly (i.e. <10 min.) when added to a soil suspension. The activity lost from

solution phase was largely recovered (57 to 86%) on the soil solid phase, although this recovery varied with soil type (Alfisol > Spodosol > Oxisol) (George et al., 2005). Two other observations from this study were also notable. First, that soil taken from the rhizosphere slowed the rate at which phytase activity declined by 2–4 times, suggesting that rhizosphere soils (which in this study lowered soil pH by ~0.5 units) may modify phytase adsorption and thus the release of orthophosphate from organic P. Second, by increasing the soil suspension pH from 5.5 to 7.5, 5 to 50% of the activity of phytase that had been adsorbed to the soil solid phase for up to 28 days could be desorbed. This suggests that adsorption of phytase to the solid matrix may be an important mechanism for the protection and longer-term persistence of activity in field soils. However, in that context, it would also be important to consider the effects of pH on the structure of the phytase (i.e. charge density) and the concentrations of Fe and Al in solution.

The efficacy of phosphatases is also influenced by companion cations and other possible soil water constituents. For example in model studies representatives of three classes of phytase, some of which may be present in soil (Lim et al., 2007) (histidine acid phosphatases (HAPs), β -propeller phytases (BPPs), purple acid phosphatases (PAPs)), were unable to hydrolyse Al³⁺, Fe²⁺, Fe³⁺, Cu²⁺ or Zn²⁺ salts of IP₆, but were able to hydrolyse Ca²⁺, Mg²⁺ and Mn²⁺ salts (Tang et al., 2006). Additionally, hydrolysis of Ca-IP₆ was prevented when Al³⁺, Fe²⁺, Fe³⁺, Cu²⁺ or Zn²⁺ ions were present. In a second part of the study, IP₆ was adsorbed to Al precipitates. When organic anions were added to desorb IP₆, hydrolysis occurred with effectiveness in the order citrate > oxalate > malate. It was notable that excessive concentrations of organic acids inhibited enzyme activity with PAP being more resistant to this inhibitory effect than HAP.

There is need to develop a consistent procedure for quantitatively comparing the potential of different microorganisms, especially those in the rhizosphere of different plants, to release orthophosphate from different sources. Orthophosphate release, especially from mineral sources is routinely assessed under laboratory conditions by incubating microbial isolates with various forms of organic P or poorly soluble inorganic P sources (Nautiyal, 1999; Schneider et al., 2010). By contrast, a soil-based procedure to quantitatively assess P mobilisation potential directly in soils has recently been proposed (Wang et al., 2012). The technique involves incubation of rewetted soils with poorly available P sources for up to 30 days with adequate addition of C and N to ensure that P was limiting microbial growth. In one application, the technique suggested that P mobilisation potential (measured as the sum of microbial and resin P) of rhizosphere soil from different crops decreased in the order faba bean (*Vicia faba* L.) > chickpea (*Cicer arietinum* L.) and lupin (*Lupinus albus* L.) > wheat (*Triticum aestivum* L.). Interestingly, the use of specific inorganic P and organic P substrates provided some indication of the mechanisms responsible for P mobilisation, although the effects of high C and N concentrations on soil redox status and associated properties were not investigated.

It is clear from the literature that there is only a limited understanding of the mechanisms associated with transformation of organic P to inorganic P at the process level, and that further work is needed using soil-based studies, especially under field conditions. In most studies, the experimental conditions, by necessity, removed interactions between system components and it follows that while these studies indicate what is possible, they do not necessarily indicate what is probable. Such knowledge of organic P transformations is a prerequisite for understanding the potential contribution organic P mineralisation could make to pasture productivity.

6. Implications for the P nutrition of pastures

The underlying purpose of this paper was to address whether the production of intensive pasture systems can be sustained by greater access to soil organic P when fertiliser applications are reduced. For example, in a Mediterranean climate like south-eastern Australia, organic P is

likely to accumulate as pastures mature, and remain high over summer. Decomposition of detrital material is likely to be initiated by the following seasonal (autumn) rains. This incidentally is when most inorganic P fertilisers are applied (Nash and Hannah, 2011) and when organic N is also mineralised. In most years the commencement of seasonal rain (autumn break) is relatively gentle (i.e. little runoff) and occurs before low soil temperatures (i.e. <8 °C) suppress microbial activity. It follows that orthophosphate would be released for plant growth. As seasons progress, some of the organic P accumulated over winter would be converted to orthophosphate when temperatures rise in early spring. This may coincide with waterlogging, anoxia and the conversion of Fe³⁺ to Fe²⁺, releasing orthophosphate derived from inorganic sources into solution. Waterlogging and anoxia are also likely to affect soil pH and the efficacy of phosphorylating enzymes. As the soil dries in late spring/early summer, conversions of organic P to orthophosphate and plant growth are both likely to decline due to a lack of soil water. Whether or not these processes are in synchrony with plant demands and can thus sustain pasture production, especially in improved pasture systems, and for how long, is however unclear (refer <http://www.dairyaustralia.com.au/>).

There seems little doubt that organic P can be a substantial reserve of P that is plant available (Stutter et al., 2012). Phosphomonoesters and diesters comprise at least 25% of total P (Table 1). Assuming an A horizon depth of 150 mm and bulk density of 1.25 kg/dm³, soil phosphomonoester and diester concentrations of 345 and 27 mg P/kg, respectively, equate to >600 and >50 kg P/ha, more than or comparable with fertiliser application rates (Gourley et al., 2012). However, these concentrations reflect the balance between rates of decomposition and production in the period leading up to sampling. While such analyses can be useful for investigating paedogenesis, what is lacking is quantitative data relating to the release of orthophosphate from different organic P species under field conditions and in a timeframe consistent with the needs of pasture plants.

In forest systems ³²P has been used to examine rates of organic P mineralisation *in vitro* and to compare organic P mineralisation rates to soil properties (Harrison, 1982a; Harrison, 1982b). Enzyme activity has also been used to infer the potential contributions of organic P to forest production (Achat et al., 2012). However, isotopic techniques are difficult to apply *in situ* and for pasture production systems, which are potentially susceptible to short-term (i.e. monthly or even weekly) changes in orthophosphate supply, using enzyme techniques the “apparent” capacity of organic P to sustain pasture production may well be different from its actual ability. There is anecdotal evidence that organic P mineralisation rates may be quite high (i.e. >100% p.a. for phosphodiester) in pasture systems but there appear few techniques that can provide direct, quantitative measures of organic P mineralisation rates *in situ*.

A study in Pennsylvania (USA) investigated changes over several years in cropping soils receiving dairy, swine and spent mushroom compost as compared to adjacent pasture/grasslands (Dou et al., 2009). Inorganic orthophosphate comprised 79 to 93% of NaOH-EDTA extracted P while the equivalent range for unmanured soils was 33 to 71%, suggesting that addition of organic amendments does not necessarily increase the proportions of organic P in soil. Phosphomonoesters comprised 7 to 58% of extracted P whereas phosphodiester were present in only a small (<40%) number of samples and at low concentrations. The latter results are in keeping with other studies (Hansen et al., 2004; Koopmans et al., 2003) and are interesting given that such amendments commonly have >4% and up to 10% phosphodiester (He et al., 2007; McDowell et al., 2008; Turner, 2004). Also of note, IP₆ concentrations were similar in manured (52 to 116 mg P/kg) and unmanured soils (53 to 137 kg P/kg), even when poultry manures, that are typically high (i.e. >50%) in IP₆ (Leytem et al., 2006; Maguire et al., 2004) were applied, which suggests that manure-derived phosphodiester and IP₆ were mineralised.

There is a pressing need to quantify phosphodiester and monoester turnover rates on a more frequent basis in pasture soils. A hysteresis in

inorganic P reactions in soil (Barrow, 1980, 1983; Holford, 1989, 1991) is almost certainly mirrored in the organic P pools, especially given the reliance on enzymes for dephosphorylation. If organic P turnover rates cannot be directly measured, they could be quantified using observational data and a mass balance approach. That would require longitudinal studies across the year rather than snapshot studies at a single point in time. Simultaneous monitoring of other soil properties such as soil pH, redox potential, Fe and Al concentrations, and microbial activity (including community composition) would provide additional insights into the processes that affect organic P mineralisation. A consistent analytical protocol for extraction and analyses of soils (0.25 M NaOH and 50 mM EDTA extraction followed by ³¹P NMR spectroscopy) which has become the norm in recent years will help with the interpretation of, and comparisons between, such longitudinal studies.

At a process level the most significant gaps in our knowledge would appear to relate to: (a) the transition of organic P substrates and phosphatase enzymes from their point of origin, be that the soil matrix or an organism, to the site where decomposition and mineralisation actually occurs; and (b) the role of the microbial biomass and specific microorganisms in the organic P mineralisation process. There are few studies investigating the role of soil water in facilitating organic P mineralisation. Similarly there has been little study of organic compounds that may complex organic P and inorganic P in that transition phase, and affect adsorption/desorption processes (Guppy et al., 2005a, 2005b; Singh and Jones, 1976). This knowledge will be fundamental to provide land managers with the confidence to lower fertiliser P application rates and to ensure that accessing organic P can adequately contribute to pasture nutrition without unexpected side effects associated with P loss.

Combining new technologies such as those used in metabolomics (i.e. the study of metabolites) to analyse temporal changes in soil water constituents and quantitative PCR (i.e. quantification of microbial community structures), with established soil extraction/³¹P NMR and enzymatic techniques, offers the possibility of studies simultaneously addressing both the chemistry and biology of the decomposition and mineralisation of organic P in soil. Further, where these techniques are applied to the same system, data mining techniques (i.e. PCA, PLS-DA) can be more easily used. While not demonstrating “cause and effect”, these data mining techniques demonstrate covariance that can be used for generating hypotheses that are subsequently tested in model systems. They are particularly useful in structured experiments where through, for example, cultivation and the application of organic amendments, a range of soil conditions have been established at one site, minimising between plot variation that is attributable to climate, animal management and soil type. Given the importance of P to agriculture and receiving waters in agricultural catchments, we believe there is a compelling argument for a more innovative, integrated and international approach to investigating organic P transformations in pasture systems.

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