

# Dissolved Organic Phosphorus: A Mini-Review of Composition, Transformations and Loss

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## ABSTRACT

Despite comprising of 10-80% of total phosphorus (P) in soil solution, little is known of the composition, transformations and loss of dissolved organic P (DOP). Once dissolved in solution, organic P can either be mineralized into phosphate or lost in flowing waters. However, the potential for mineralization and loss depends on the chemical form of DOP. For instance, orthophosphate diesters such as RNA are known to be much more labile than compounds such as phosphonates or inositol phosphates (a subset of orthophosphate monoesters). This review highlights recent developments in characterising DOP, the mineralization and utilization of DOP by microbes and plants (largely via extracellular enzymes or organic acids), and the potential for loss in overland or subsurface flow under different landuses.

**Keywords:** bioavailability, enzyme, microbes, nuclear magnetic resonance, phosphatase

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## INTRODUCTION

Phosphorus (P) is vital for primary production. On land, the importance of P is emphasized in agricultural production systems, which regularly apply P in the form of inorganic fertilisers or in a combination of inorganic and organic forms in animal faeces (e.g., effluent, slurry, manure, dung, composts). In water, P is commonly the most limiting nutrient for eutrophication and associated cyanobacterial blooms (Schindler 1977). The occurrence of eutrophication has become a major issue facing industrialized nations that have increased agricultural production systems and in-turn the loss of P from land to water. However, while much research over the past 30-40 years has focused on the role of inorganic P in productivity, recent research has highlighted the role of organic P.

The percentage of organic P in soils varies from 20-80% (Harrison 1987), and a similar percentage has been found for organic P in solution (Turner *et al.* 2005). The cycling of P in both terrestrial and aquatic systems inevitably involves P coming into solution. For the sake of this mini-review this is termed dissolved organic P (DOP). Once in solution, DOP can be either lost in flow (e.g., overland flow or tile-drainage to waterways) or it becomes biologically active and utilized by biota. A number of mechanisms exist by which DOP can be utilized. These include: the synthesis of phosphatase enzymes that are either excreted from within cells or resident on the cell surface, exudation of organic acids that hydrolyze DOP, and, as in the case of some aquatic bacteria, DOP may simply be taken within a cell for later processing (Heath *et al.* 2005).

Although it is clear that DOP plays an important role in productivity, little is known of its composition, transforma-

tions and loss within terrestrial and aquatic systems. This mini-review aims to outline information on DOP forms, how they change or are utilized, and how potentially mobile they are.

## COMPOSITION, CONCENTRATION AND MOVEMENT OF DOP

Studies examining water and calcium chloride extracts of soil have indicated the presence of at least 30 distinct DOP compounds (Espinosa *et al.* 1993; McDowell *et al.* 1998; McDowell and Stewart 2005a; Nanny and Minear 1993). Initially, only inositol monophosphates could be distinguished (Wild and Oke 1966), whereas techniques such as high performance liquid chromatography and nuclear magnetic resonance spectroscopy now regularly identify phosphonates (direct C-P bond), sugar phosphates and inositol hexakisphosphate (Espinosa *et al.* 1999; McDowell and Stewart 2005a).

Examination of organic P in soils has become more common via analysis of total organic P extracts (e.g., NaOH-EDTA, Bowman and Moir 1989) with solution <sup>31</sup>P-nuclear magnetic resonance (<sup>31</sup>P-NMR). This technique initially classified organic P into distinct classes of phosphonates, pyrophosphates, polyphosphates, orthophosphate diesters and orthophosphate monoesters (Newman and Tat 1980). However, classification has improved with the advent of peak assignments for specific P compounds in NaOH-EDTA solutions, and in water extracts, lake water or soil solutions between pH 5 and 13 (Turner *et al.* 2003; McDowell and Stewart 2005a). Orthophosphate diesters are commonly broken into phospholipids, DNA, and teichoic acids (McDowell and Stewart 2005a). Stereo-isomers of

inositol phosphates, a sub-class of orthophosphate monoesters, have also been split into *myo*-inositol hexakisphosphate, largely from plants, and *scyllo*-inositol hexakisphosphate, largely from microbes (Turner and Richardson 2004).

Generally, the proportion of different P species within the soil is dominated by orthophosphate or orthophosphate monoesters followed by orthophosphate diesters, pyrophosphates or polyphosphates, and phosphonates. However, apart from orthophosphate monoesters the identification of sub-species in other classes has been hindered by low concentrations and/or uncertainty over peak assignments due to variation with pH and line broadening. While equations exist to calculate the position of peaks with pH (McDowell *et al.* 2006), the problem of peak broadening is sample specific and depends on the concentration of paramagnetics (e.g., Mn, Fe, Cu). Paramagnetics can be removed from samples (McDowell and Stewart, 2005b), but they are also required so that spectra are quantitative, i.e. so that the spin lattice relaxation time,  $T_1$ , is reached. Equations for A) orthophosphate and B) orthophosphate monoesters, orthophosphate diesters, and pyrophosphate are (McDowell *et al.* 2006):

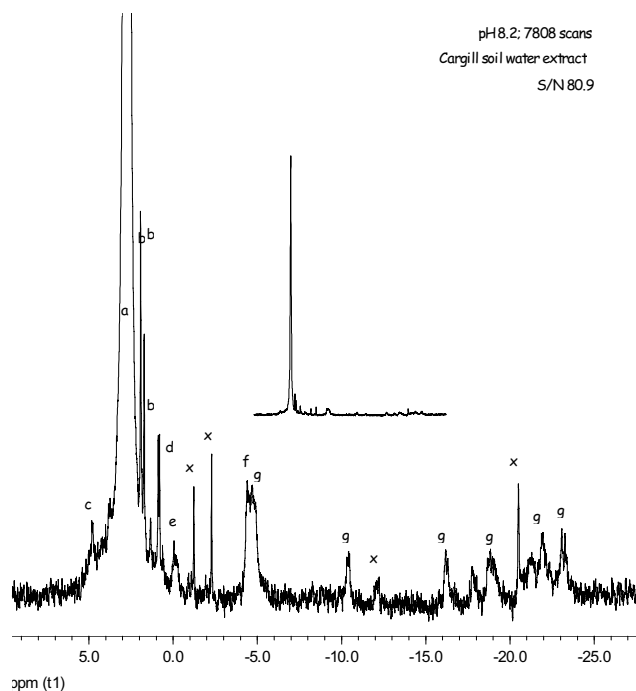
$$A) \quad T_1 = 0.286 \left( \frac{P}{Fe + Mn} \right) + 0.313$$

$$B) \quad T_1 = 0.062 \left( \frac{P}{Fe + Mn} \right) + 0.690$$

where P, Fe and Mn are concentrations in w/v within the NMR sample tube.

Studies that have directly examined DOP in soil solutions, leachates, overland flow or surface waters with  $^{31}\text{P}$ -NMR are far fewer than those looking at organic P in soil. However, McDowell *et al.* (1998) analysed dilute calcium chloride extracts of soils that were designed to estimate P in soil solution, Nanny and Minear (1993) examined concentrated lake water samples, Toor *et al.* (2003) examined soil leachates, and McDowell and Stewart (2005a) analyzed lake water samples, soil leachate, overland flow samples and water extracts of soil and dung. An example of a spectra and species identified in a soil water extract is given in **Fig. 1**. The sample pH was unaltered, and analyzed at pH 8.2, which contrasts to almost all other studies of soil organic P and the study of Toor *et al.* (2003) which altered pH to >12. While altering pH to >12 yields a constant pH that makes peak assignments simpler, it also results in hydrolysis of some organic P species such as some orthophosphates diesters (Leinweber *et al.* 1997; Turner *et al.* 2003).

Having isolated and quantified the relative forms of DOP, many authors have shown that their relative mobility varies (e.g., Leytem *et al.* 2002). Diesters have a lower charge density than monoesters since phosphate is bound to C by two ester linkages - meaning there is less ionic interaction with the soil. In contrast, alkali extractable monoesters include inositol phosphates such as phytic acid (Turner and Richardson 2004), which bind strongly to soil particles - even more so than orthophosphate (Leytem *et al.* 2002). Depending on the flow route taken and soil management this means that in general phytate is less mobile than many other DOP species and orthophosphate. For instance, McDowell *et al.* (2005) found that in a pasture receiving dairy shed effluent there was a significant increase in phytate concentration in the topsoil due to sorption, but also an increase in DOP in tile drainage water over a three year period. This was attributed to a combination of enhanced mobility, due to poor sorption to soil of DOP over orthophosphate, and occupation of P sorption sites by phytate and organic matter from the effluent. The rapid increase in DOP leaching was attributed to movement in macropores created by earthworms. Goehring *et al.* (2001) stated that soluble P loss in macropores is due to negligible P sorption to pore walls, while McDowell *et al.* (2005) further postulated that this was due to repeated exposure of macropores to effluent



**Fig. 1**  $^{31}\text{P}$ -NMR spectra of a soil water extract of a Cargill pasture soil treated with chelating resin. Miniature spectra show full height of orthophosphate peak vis-à-vis other peaks. S/N is the signal to noise ratio. Peak assignment are a = orthophosphate, b = phytate (4<sup>th</sup> peak obscured by orthophosphate), c = orthophosphate monoesters, d = phospholipids, e = DNA, f = pyrophosphate, g = polyphosphates, h = phosphonates, and x = unknown but most likely either monoester or polyphosphate. Adapted from McDowell RW, Stewart I (2005a).

resulting in P sorption saturation of pore walls. In soils with little macropore flow, the loss of P is generally minimal via sub-surface routes unless the soil is very free draining or has little capacity to sorb P. For example, Chardon *et al.* (1997) found that the application of pig slurry to a sandy free-draining soil in the Netherlands caused DOP, not dissolved inorganic P, to be the dominant fraction at depth.

Another route for DOP to be lost to waterways is via overland flow (**Fig. 2**). Fewer studies have examined DOP in overland flow compared to sub-surface routes. However, these losses can be of more concern since the time taken for DOP to travel to waterways via overland flow is commonly less than via sub-surface routes, unless large macropores are connected to tile drains (McDowell *et al.* 2004). This also means that overland flow can interact less with the soil than sub-surface flow. Intuitively, a shorter contact time should result in less time for P desorption into flow. However, since P is highly sorbed by the soil, the topsoil tends to be enriched with P due to fertiliser applications or dung returns. This means that the concentration of P in overland flow can and is usually greater than in subsurface flow (**Table 1**). However, it is well known that in P enriched soils most P accumulates in inorganic P forms (Stewart and Tiessen 1987), consequently the percentage of total P as DOP is less than expected. Furthermore, McDowell *et al.* (2002) showed that the percentage of total P as DOP in overland flow was inversely related to soil Olsen P (bicarbonate extractable inorganic P) concentration.

Concentrations of DOP in soil solution, drainage waters, overland flow and streams/rivers were summarized in a recent review on the loss of DOP from land to water by Turner (2005). Of dissolved P (i.e. P that passes through filters ranging in pore size from 0.8 to 0.22  $\mu\text{m}$  depending on the study), DOP comprised 0-96% relating to concentrations of 0 to 1.087  $\text{mg L}^{-1}$ . Compared to other fractions, DOP is a small component of total P lost from cropped land due to the dominance of particulate P losses, or from heavily fertilized or manured soils since most of the P applied is in inor-

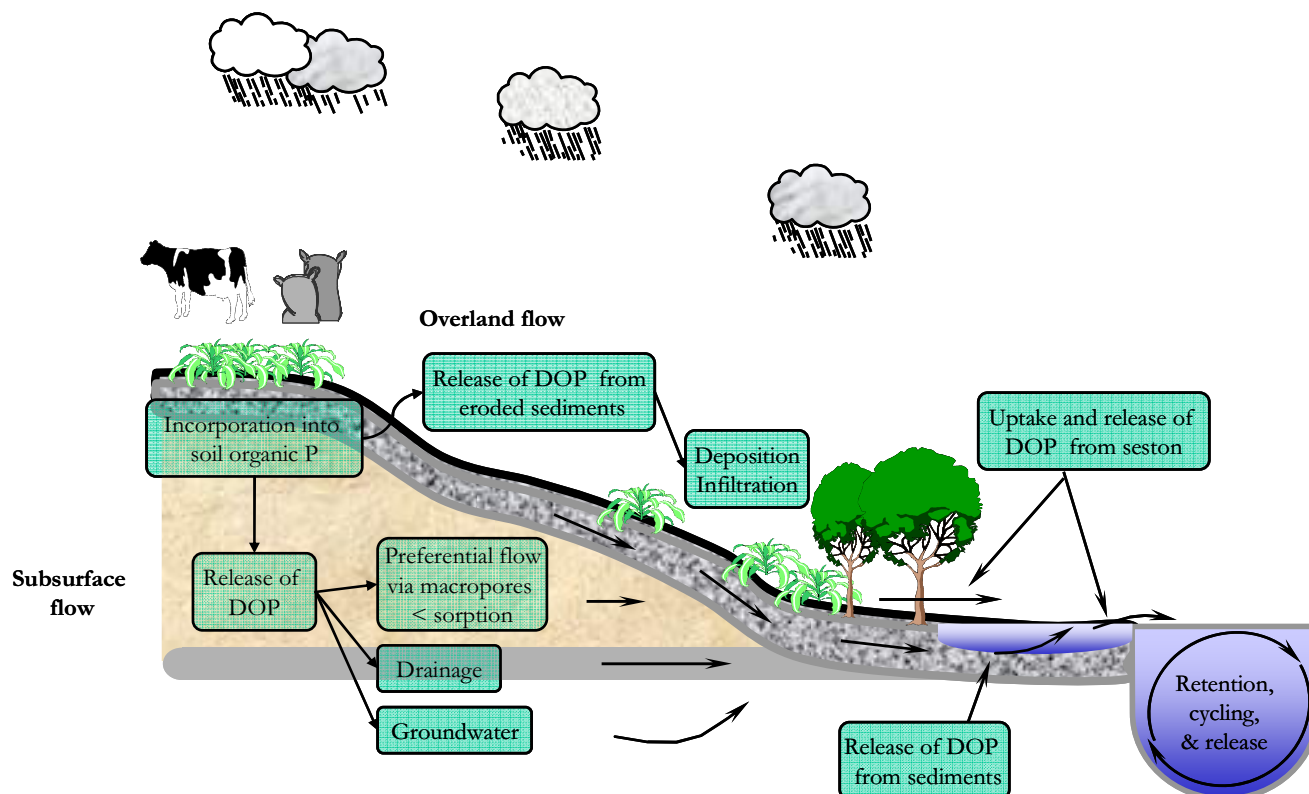


Fig. 2 Transformations and flow of DOP within terrestrial and aquatic environments.

ganic form. Many factors influence DOP concentrations, but chief among these is the concentration of organic P in the soil. Soils that tend to have more P in organic than inorganic forms tend to be cool, moist, and receive little in the way of P fertilizers, which forces productivity to be cycled through the organic pool. For instance, in studying the fate of 50 years of superphosphate application to a trial under pasture in New Zealand, Metherell *et al.* (2001) found that inorganic P increased at a much greater rate than organic P, and consequently, organic P occupied a greater proportion of total P in low- than high-fertility (e.g., Olsen P) soils.

Notwithstanding climatic and soil forming factors, land use and management have a profound influence on organic P and DOP concentrations. In general, pastures increase the concentration of organic matter in the soil with age. Hence the proportion of DOP is generally greater in soils under pasture than cropland (Table 1). For instance, it is common that the proportion of DOP in drainage waters from arable sites in the UK and USA is <10% (Culley *et al.* 1983; Heckrath *et al.* 1995), whereas in drainage from pastures in New Zealand the proportion was 50–80% (McDowell and Monaghan 2002). Like land use, changing soil management, such as increased intensification can alter the concentration of DOP. For instance, concentrations of DOP commonly increase along with other P fractions as stream orders increase i.e. from headwaters (1<sup>st</sup> order streams) influenced

by extensive land use (e.g., high country sheep grazing) to large rivers that receive more inputs from intensive agricultural land uses such as dairying (Wilcock *et al.* 1999).

## TRANSFORMATIONS AND UTILIZATION OF DOP

The utilization of DOP can occur in terrestrial and aquatic ecosystems. In non-agricultural ecosystems and in agricultural systems low in available inorganic P, mineralisation of DOP can be the major mechanism for generating plant available P (Fox and Comerford 1992). In order for plants to maximise the uptake of mineralised P, roots have adapted by increasing root growth and length, the degree of branching, depth of rooting, and the number and length of root hairs. Other adaptations include symbiotic relationships with mycorrhizal fungi that enable larger volumes of soil and soil solution to be explored (Gahoonia and Nielsen 1997), and changing rhizosphere dynamics and the activity of microorganisms by releasing organic acids or phosphatase enzymes (Richardson 1994). For example, the exudation of organic acids by white lupin (*Lupinus albus* L.) and radiata pine (*Pinus radiata* L.) has been well documented as a measure to liberate P from the rhizosphere (Gardner *et al.* 1983; Davis 1995; Chen *et al.* 2004). Tarafdar and Jungk (1987) noted a 65 and 87% decrease in total soil organic P from the rhizosphere of Berseem clover (*Trifolium alexandrinum* L.) and wheat crops, respectively.

Many studies have shown that under low-P conditions the activity of extracellular phosphatase enzymes associated with plant roots increases (McLachlan 1980; Tarafdar and Jungk 1987). These enzymes are usually alkaline phosphatases broadly grouped by substrate into phosphomonoesterases and phosphodiesterases. They can exist either as associated with plant cell walls or exuded into the rhizosphere as exozymes (McLachlan 1980; Gaume *et al.* 2001). However, the response of plant-mediated enzymes to soil-P can often be clouded in soil by phosphatase enzymes associated with microbial activity (Richardson *et al.* 2005). For instance, Adams and Pate (1992) showed that while enzyme activity was able to meet P nutrition requirements when ribonucleic acid and  $\beta$ -glycerophosphate were added to lupins

**Table 1** Range of DOP concentrations and the median percentage of dissolved organic P (DOP) as a proportion of total P for different flow routes in grassland, arable and forested land uses. Data from Turner (2005) and references therein.

Landuse	Number of studies	Flow route	DOP range (mg L <sup>-1</sup> )	Median percentage of TP
Grassland	4	Soil solution	0.055–0.464	54
	5	Sub-surface	0.001–0.300	27
	4	Overland	0.010–0.447	14
Arable	5	Sub-surface	0.010–0.600	11
Forest	2	Soil solution	0.226–1.087	31

grown in soil or sand, the authors were not able to isolate the effect of plant roots from microorganisms. In an effort to isolate plant effects, several authors have shown secretion of extracellular phosphatases occurs under sterile conditions. For instance, Richardson *et al.* (2000) was able to demonstrate that plants grown on sterile agar obtained P from a range of monoester and diester substrates. However, conditions must be completely sterile as phosphatase remains active even after  $\gamma$ -irradiation (Ohel *et al.* 2001).

Increasingly, work is focused on utilisation of phytate, a major component of soil organic P (about 30-60%). Although additional studies examining the availability of *myo*-inositol hexakisphosphate, a major stereoisomer of phytate, showed little of the substrate was utilised (Hayes *et al.* 2000; Richardson *et al.* 2000). Chen *et al.* (2004) showed that *myo*-inositol hexakisphosphate was depleted in soils growing ryegrass (*Lolium perenne* L.) and radiata pine (*Pinus radiata* L.) for one year compared control soils supporting no plants. Further studies have also shown the extracellular secretion of phytase occurs in maize (*Zea mays* L.), and potato (*Solanum tuberosum* L.). Additional work is now looking at transgenic plants such as subterranean clover (*Trifolium subterraneum* L.) that include genes from *Aspergillus niger* for phytase generation and secretion (George *et al.* 2004).

Similar to plants that maintain low-P concentrations in the rhizosphere, microbial phosphorus uptake can be equal to, if not greater than, plant mediated organic P mineralisation. This means that DOP cycling can be controlled by microbes in the soil (Oberson and Joner 2005). Net mineralisation, defined as the difference between mineralisation and immobilisation is affected by factors such as flushes from drying and wetting, basal mineralisation of organic matter and re-mineralisation of microbial P after the principal decomposition of organic matter. The quantity of soil microbial P tends to be greater in grassland than forest or arable soils. However, Chen *et al.* (2003) estimated that the annual release of P from soil microbial biomass was 30 mg P kg<sup>-1</sup> (14 kg P ha<sup>-1</sup>) under grassland and 35 mg P kg<sup>-1</sup> (15 kg P ha<sup>-1</sup>) under forest, resulting in a turnover time of 1.3 and 0.8 years, respectively. While much of this P may be recycled within the soil plant system, actions such as re-wetting very dry soils can liberate a lot of DOP into the soil solution from lysed microbial cells, which can then be transported in overland or sub-surface flow (Turner 2005).

Once in a waterbody, DOP can be either utilised via mineralisation or by cyanobacteria, phytoplankton and other seston. Alternatively, DOP can condense into higher molecular weight compounds and become colloidal P. Phytoplankton contain many P esters, which can be released to water upon death or actively released or consumed (mineralisation and direct uptake) by microorganisms (Berman 1970). Like P-poor soils, the activity of alkaline phosphatase enzymes - soluble or bound to algal membranes, increases when P becomes limiting (Newman *et al.* 1994). For example, the concentration of monoesters in lake and marine systems has been negatively correlated with phosphomonoesterase activity (Heath and Cooke 1975; Rivkin and Swift 1980). However, again like soils, the activity of this enzyme class is clouded by microbes that do not require P limitation to enzymatically sequester P (Jansson *et al.* 1988). Furthermore, higher organisms such as zooplankton also have the ability to utilise these enzymes for P nutrition (Wynne and Gophen 1981). Nevertheless, studies using filtration to partly isolate enzymes from phytoplankton (1-25  $\mu$ m) to those from bacteria, have shown that the activity of soluble alkaline phosphatase, mostly from bacteria, varies with season and depth in the water column (Jones 1972). For instance, epilimnetic alkaline phosphatase activity is greatest during periods of high growth potential in spring and summer (Wetzel 1991). There are now also thoughts that greater phosphomonoesterase to phosphodiesterase activity could be used as an indicator of growing phototrophs (Whitton *et al.* 2005).

An exception to the negative feedback loop exhibited

by many alkaline phosphatases is bacterial 5'-nucleotidase, which catalyses the cleavage of phosphoryl groups from 5'-nucleotides. Activity of 5'-nucleotidase has been found to be prevalent in microbiota (<1  $\mu$ m) especially in lakes with little DOP. However, activity is maintained in high-P environments even though the P is not utilised. Consequently, Cotner and Wetzel (1991) concluded that this enzyme was important in P generation in oligotrophic habitats.

Although lytic processes have been the focus of many studies of DOP utilisation, heterotrophic bacteria can directly uptake DOP without hydrolysis (Cotner and Wetzel 1992; Heath 2005). For example, *E. coli* is known to exhibit two mechanisms to uptake glycerol 3-phosphate, and Winkler (1973) notes that mechanisms for the uptake of glucose-6-phosphate, which also supplies a C source, is broadly distributed among bacterial taxa. However, the importance of this uptake mechanism compared to lytic processes, especially where possible C limitation is concerned, is unclear.

## CONCLUDING REMARKS

Dissolved organic P is a mediator of biological nutrition in terrestrial and aquatic systems. As such, our challenge for the future is to maximise the utilisation of DOP to provide for plant production without promoting the loss of DOP to aquatic ecosystems where increases in productivity are undesirable. In order to do this we need to understand the forms of DOP that are most active, not only for potential uptake, but also with respect to mobility in terrestrial and aquatic ecosystems. At present, our understanding of DOP transformations in relation to plant nutrition is restricted to a few classes of DOP, some individual DOP species, and a few crops of economic importance (e.g., pine or clover). Studies have begun to use molecular techniques to enhance the organic P utilisation (e.g., phytate) of some crops, which will be an important step in decreasing our reliance on P fertilisers and provide a method for improved yield to those who cannot afford to supplement inorganic P. However, this objective needs to be put into an ecosystems perspective and will only be realised once our understanding of different land managements is more complete. This requires knowledge not only DOP interactions within the soil-plant continuum, but also how DOP is transported spatially and temporally within a catchment and the downstream effects on surface water quality.

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