Can the soil phosphorus bank be unlocked by plants ?

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Abstract: The regular application of phosphorus (P)-based fertilizers to Australian pasture soils has resulted in an increase in total soil P content. This accumulated soil P can be likened to a 'bank account' to which farmers regularly make 'deposits', but from which it is more difficult to make 'withdrawals'. In many soils the 'bank' of soil P is now an important resource which represents considerable 'capital value'. Our research is concerned with developing ways to increase the ability of plants to draw upon the soil P bank.

Most Australian soils are deficient in the form of phosphorus (P) that is readily available to plants. Application of P-based fertilizers is therefore necessary to achieve and maintain high levels of productivity in agricultural systems. In combination with the introduction of productive legumes such as subterranean clover (*Trifolium subterraneum*), the use of superphosphate has been an important means for pasture improvement for some decades. Primary producers in Australia currently spend an estimated $600 million per annum on phosphate-based fertilizer. Approximately half of this P is applied to pastures. However, P usage on pastures is variable and in recent years has declined. For instance, in Victoria the average rate of annual application of P fertilizer to pastures has decreased; from some 14 kg P/ha to approximately 10 kg P/ha, and the percentage of pastures fertilized annually has declined; from almost 100% in the 1950's to approximately 20 to 40% throughout the early 1990's (McLaughlin, 1992). For the NSW tablelands, it is estimated that less than 30% of farmers have maintained adequate P fertilizer management programs over the past decade. This decline in P usage on pastures has occurred even though many of the soils concerned remain deficient in forms of P that are available to plants.

Despite the widespread deficiency of plant-available P, most soils do however contain large amounts of total P. A proportion of this P occurs naturally ('native' soil P), a significant amount may also have 'accumulated' as a consequence of previous applications of P fertilizers. Our research aims to understand the cycling of P within grazed pasture systems and to identify the relative importance of various soil P fractions for plant nutrition. Importantly, we have recently identified key processes by which plants are able to potentially access P from the poorly available forms of P that accumulate in soils. An increase in the capacity of plants to acquire P from poorly available sources in soil would increase the overall efficiency of P-fertilizer use on pastures.

Accumulation of soil phosphorus

Phosphorus in soil is associated with either soil organic matter (organic P; Po), or occurs in inorganic forms (Pi) The inorganic forms of P are either predominantly bound to charged soil particles ('adsorbed' P), or occur as poorly soluble precipitates (Fig. 1). For a range of soils throughout south-eastern Australia, McLaughlin *et al.*, (1990) have shown a mean total soil P content of 250 µg P/g soil (15 soils; 0 to 10 cm depth) and that this ranged between 100 and 460 µg P/g soil. These amounts of total soil P equate to a mean of 320 kg P/ha and a range of 130 to 580 kg P/ha (assuming an average soil bulk density of 1.25 g/cm^3). Importantly, the soils examined in the study were obtained from pastures that either had no fertilizer history (lower values), or had regularly recieved 'normal' rates of P fertilizer (higher values). In the case of the fertilized pastures, it is clear that the soils now contain a large amount of total soil P (in excess of 300 kg P/ha) relative to their annual input requirement of around 10 to 20 kg P/ha per year.

Application of P-based fertilizer therefore generally leads to a net accumulation of total soil P (Fig. 1). For pasture soils, P accumulates into both the Pi and Po fractions with rates of accumulation of around 5 to 10 kg P/ha/yr being typical for moderately fertilized pasture soils (McLaughlin *et al.*, 1991). As an example, we have observed the net accumulation of over 200 kg P/ha in the top 10 cm of a Rutherglen soil (A. Richardson, unpublished) that recieved some 4.5 tonnes of superphosphate/ha between 1914 and 1986 (*ie.* equivalent to approximately 5.5 kg P/ha/yr; Ridley *et al.*, 1990). The accumulation of P in pasture systems is further illustrated by a comparison of P inputs (as fertilizer) relative to P exports (as pastoral commodities). For

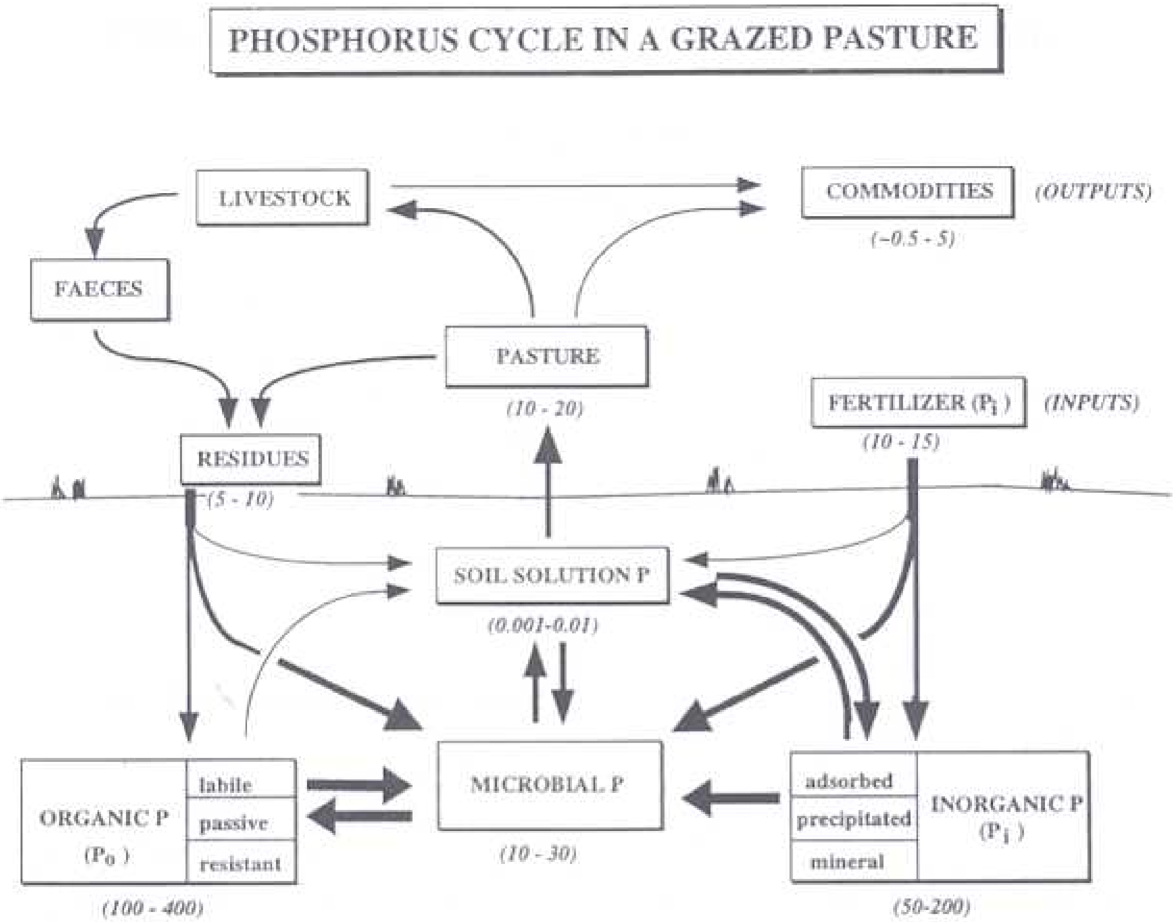

Figure 1: Schematic representation of the major components of the soil-plant phosphorus (P) cycle under a grazed and fertilized pasture in south-eastern Australia. Major P fractions, and the amount of P (kg/ha; 0-10 cm) typically associated within each fraction are shown in parenthesis. Major P transformation processes and the passage of P between the various fractions are indicated by arrows.

pastures in general, a ratio of around 5:1 is reported (McLaughlin *et al.*, 1991), thereby indicating a net accumulation of approximately 80% of the P inputs. This level of P accumulation in soil is consistent with various studies that have shown that the immediate recovery of fertilizer P by plants is only 10 to 20% of that applied. High levels of P input are therefore required to overcome this sequestration of P into soils. In the long term and with sustained P fertilization, whereby the total P content of soils is increased, it is conceivable that pasture systems may eventually approach a higher degree of sustainability, such that the cycling of P within the system is adequate to maintain the annual needs of a pasture, provided that sufficient maintenance requirements are met (*ie.* to replace exported P). However, given the very low P status of Australian soils, combined with their high capacity for adsorption and precipitation of P, it is unlikely that such an equilibrium will be achieved in the short term by economically feasible rates of P-fertilizer, as may have occurred on highly P-fertile soils elsewhere in the world (Harrison, 1985). For Australian soils it is essential to use P-fertilizers at rates which give profitable returns, but in addition, it would be desirable to improve the efficiency by which plants utilize fertilizer P. One such possibility could be achieved by increasing the capacity of pasture plants to obtain their P from accumulated sources of soil P.

Forms of accumulated soil phosphorus

Organic forms of P in soil generally account for at least 50% and, for pastures, up to 85% of the total P present (McLaughlin *et al.*, 1990). Although a large proportion of the Po in soil is poorly characterized, inositol phosphates and their various metal ion derivatives (*ie.* soil phytate) have been shown to represent a major component. In Australian soils, inositol P may account for up to 38% (mean of 16%) of the total Po content (Williams and Anderson, 1968). Lesser amounts of other phosphate esters (*eg.* phospholipids, nucleic acids and sugar phosphates) have also been identified. Organic P in soil originates from the deposition of organic materials (*ie.* plant and animal residues), or accumulates as a consequence of microbial activity. Rapid 'immobilisation' of P, applied as either fertilizer or as plant residues, into microbial biomass and subsequent deposition into organic P fractions has been reported (Richardson, 1994).

Inorganic forms of P in soil occur as either phosphate anions that are adsorbed to various soil constituents including iron (Fe) and aluminium (Al) oxides, Al silicates and calcium (Ca) carbonates; or as sparingly soluble precipitates of Fe and Al (particularly in acidic soils) or Ca (in alkaline soils) (Sanyal and DeDatta, 1991). The formation and presence of the various forms of Pi in soil is both complex and subject to a wide range of physical and chemical factors, but importantly it accumulates directly as a consequence of the application of P-based fertilizers.

Availability of phosphorus to plants

Despite the large amount of total P in soil, plants almost exclusively derive their P requirement from phosphate anions (predominantly HPO_4^{2-}) dissolved in the soil solution (Wild, 1988). This pool of 'immediately available' P is extremely small (around 0.01 kg P/ha) and must therefore be replenished regularly to meet plant requirements (*ie*. some 10 to 20 kg of P/ha per annum; Fig. 1). Whilst many factors may affect the concentration of phosphate in the soil solution, the capacity of phosphate anions to desorb and diffuse within the soil, the degree of solubility of the various P precipitates and the susceptibility of Po substrates to mineralization (*ie*. the release of phosphate from Po substrates by enzymes) are of major importance. However, in most soils these processes alone do not supply P at sufficient rates to support maximum plant growth. Consequently, root morphological characteristics; such as rate of root growth and total root length, the kinetics of P uptake at the root surface and the abundance and distribution of root hairs also play an important role in plant P nutrition (Ozanne, 1980). Phosphorus acquisition by plants is therefore largely governed by the capacity of roots to 'explore' the soil. The effective volume of soil that is explored by plants is further enhanced significantly by the formation of associations between the roots and mycorrhizal fungi (Robson *et al*., 1993).

The availability of soil P to plants is also influenced markedly by biological processes that occur at the root surface. In particular, the production of phosphatase enzymes and the release of specific root exudates that either directly acidify the rhizosphere (*eg*. H^+ ions and organic acids), or support the general growth of soil microorganisms (*ie*. sugars and other energy-rich carbon compounds) can influence the availability of soil P (Richardson, 1994).

The presence of organic acids in the rhizosphere, whether of plant or microbial origin, are effective in releasing soil P through either the solubilization and dissociation of precipitated P (ie., by reduced pH), or by the chelation of metal ions that are associated with the P (eg., Al, Fe and Ca), thus resulting in mobilization of phosphate. For some plant species, such as white lupin (*Lupinus albus*), it is well established that large amounts of citric acid (as citrate) is released from specialized (proteoid) roots (Dinkelaker *et al*., 1995), and that this citrate can mobilize plant-available phosphate from highly P-fixing soils (Hocking *et al*., 1997).

The availability of phosphate from soil organic P is mediated by the action of phosphatase enzymes. A wide range of phosphatases that release phosphate from various Po substrates, including soil phytate, have been described from both plants and soil microorganisms. High levels of phosphatase activity and significant zones of Po depletion have also been demonstrated within plant rhizospheres (Tarafdar and Jungk, 1987). However, distinction of the relative importance of phosphatases of either plant or microbial origin to the P nutrition of plants is unclear (Richardson, 1994).

Can the efficiency of phosphorus-use by plants be improved ?

A number of possibilities exist for improving the P-use efficiency of plants. Various attributes of plants that are amenable to manipulation have been identified and, in some cases, used successfully in breeding programs to develop plants that show improved P-use efficiency (Caradus, 1995). However, these attributes are most often associated with differences in root morphology and, in most cases, selection has only resulted in marginal benefits. By comparison, the selection of P-efficient variants on the basis of altered root function, although having received less attention, may offer greater benefits in terms of plant P nutrition. For instance, an increase in the capacity of plant roots to access the large reserves of poorly available P that have accumulated in soils may provide an effective means for improving plant P-use efficiency. We are currently investigating the possibility of using genes cloned from soil microorganisms as a novel approach to improve the capacity of plants to acquire P from accumulated sources.

Manipulating the secretion of organic acids by roots.

Plants are capable of synthesizing and, in some cases, exuding from their roots a range of organic acids which have the potential to solubilize precipitated forms of soil P. Most notable is the release of citrate by the proteoid roots of white lupin (Dinkelaker *et al*., 1995). Currently, we are investigating factors which regulate the synthesis and release of citrate by both lupin roots (Keerthisinghe *et al*. 1998) and by a range of other plant species (E. Delhaize, unpublished). Of particular interest is (i) the protein citrate synthase, which is a key enzyme involved in the biosynthesis of citrate, and (ii) specific 'transport channels' which may be required for the release of organic acids (P. Ryan, unpublished). Citrate synthase genes have been cloned from bacteria and these will be 'over-expressed' in the roots of various plant species, including the pasture legumes subterranean clover and lucerne (*Medicago sativa*). In tobacco (*Nicotiana tabacum*), expression of a bacterial citrate synthase gene has been shown to increase significantly both the internal concentration and the efflux of citrate from roots by up to 5 and 3-fold relative to control plants, respectively (de la Fuente *et al.*, 1997). We consider that an enhancement of citrate exudation by roots, or its introduction into plant species which otherwise do not release citrate, could significantly increase the ability of plants to solubilize, and thus access accumulated forms of soil P. In laboratory studies, we have shown that citric acid is very effective at releasing both inorganic and organic forms of soil P (P. Hocking, J. Hayes, unpublished).

Manipulating the phosphatase (phytase) activity of roots.

We have shown that pasture plants grown under controlled conditions have only limited capacity to directly obtain P from inositol phosphates (soil phytate). For instance, in sterile sand-vermiculate culture, subterranean clover plants supplied with inositol hexaphosphate (IHP) contained only 8% of the P content of plants supplied with an equivalent amount of P as phosphate (Richardson *et al.*, 1998). However, if the plants were inoculated with a population of culturable soil microorganisms, or with a specific strain of Pseudomonas bacteria that was selected for its ability to release P from IHP (Richardson and Hadobas, 1997), subterranean clover could grow equally as well as those plants supplied with P as phosphate. The release of phosphate from IHP requires the action of a specific phosphatase enzyme (*ie.* phytase) and we have recently characterized phytase activity in roots from a wide range of pasture species (Hayes *et al*, 1998). For subterranean clover, phytase constituted less than 2% of the total root acid phosphatase activity and, importantly, only some 2 to 3% of the total phytase activity appeared to be extracellular to the root. A phytase gene has therefore been cloned from the fungus *Aspergillus* and we are currently investigating the possibility of expressing this gene directly in roots of pasture legumes such as subterranean clover (A. Richardson, unpublished). We consider that an increase in the extracellular phytase activity in roots of subterranean clover may enhance the ability of plants to access P from soil phytate.

Conclusions

The majority of Australian pastoral soils contain significant amounts of total soil P, which to a large extent have accumulated as a consequence of previous applications of P fertilizer. However, total soil P and accumulated forms of soil P are only poorly available to plants. Our research is aimed at understanding mechanisms by which the availability of these sources of P in soils could be increased. Currently, we are using genes obtained from soil microorganisms that encode for citrate synthase and phytase, to genetically engineer pasture plants to increase their capacity to acquire P from the poorly-available forms of P that accumulate in soils. An improvement in the ability of plants to acquire P from accumulated sources would decrease the annual requirement of pastures for P fertilization and thus provide significant benefits to the pastoral industries by reducing fertilizer costs and for the development of more sustainable agricultural systems with reduced dependence on applied fertilizer.

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