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Phosphorus Legacy: Role of Long-Term Soil Phosphorus Accumulation in the Sustainable Management of Intensive Agroecosystems

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A thesis

submitted in partial fulfilment

of the requirements for the Degree of

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by

Gustavo Boitt

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Abstract of a thesis submitted in partial fulfilment of the requirements for the Degree of Doctor of Philosophy.

Phosphorus Legacy: Role of Long-Term Soil Phosphorus Accumulation in the Sustainable Management of Intensive Agroecosystems

by

Gustavo Boitt

Phosphorus (P) is an essential nutrient for all organisms. Phosphate rock is primarily utilised for the manufacture of P fertilisers, and is a finite resource. Most agricultural lands worldwide present low levels of available soil P, thus requiring P inputs for productive agriculture. However, constant P inputs result in accumulation of soil P (legacy P), increasing risk of eutrophication of waterways. Efficient P use of agriculture require improvements in utilisation of legacy P. The objective of this work was to investigate and quantify the impact of contrasting agricultural land uses and management on the nature and dynamics of P. Three long-term, replicated field trials were selected: i) long-term irrigation trial, Winchmore, New Zealand; ii) long-term pig slurry inputs, Santa Catarina, Brazil; iii) long-term ecology trial, Lincoln, New Zealand. Four experiments were conducted. The first study investigated the impact of 62 years of irrigation on the amounts and distribution of soil profile P to 100 cm under grazed pasture. Despite identical P inputs, total soil profile P accumulation was inversely proportional to water input rates (6423, 5908 and 5054 kg P ha^{-1} for the control, low and high irrigation rates, respectively). Differences were mainly attributed to inorganic P forms. Phosphorus removal and transfer/loss occurred under irrigation. For a 3-fold increase in irrigation frequency, P removal in irrigation outwash increased by 13-fold. Combined, annual removal in animal products, internal transfer, and outwash losses were directly related to irrigation frequency and increased from 8 to 18.6 kg P ha^{-1} for treatments receiving annually, 2.6 or 7.7 100-mm irrigations, respectively. The second experiment quantified the impacts of P inputs in pig slurry to a high P-sorbing Oxisol under cropping in southern Brazil. Fifteen years of slurry additions resulted in P accumulations and vertical movement proportional to application rates. However, changes were confined to the 0-20 cm depth. Phosphorus accumulated mainly in inorganic forms. Slurry input rates of 25, 50, 100 and 200 m^3 ha^{-1} y^{-1} resulted in accumulations of 25, 57, 106 and 159 kg P ha^{-1} y^{-1} (0-40 cm), where 8, 10, 23 and 28 kg P ha^{-1} y^{-1} were organic P forms. Mass balance confirmed that most of

P added to the system accumulated in the soil. The third and fourth experiments assessed, respectively, long-term and short-term impacts of plant biomass retention or removal in soil biogeochemical properties after 20 years, in absence of P inputs. Grassland plants utilised 35% of the P legacy, mainly from inorganic forms. Plant production and P uptake were up to 2-fold higher for the biomass retained comparatively to biomass removed. Mineralisation of soil organic P was limited following P depletion. Contrastingly, despite increased microbial P immobilisation soils under biomass retained, 20% faster turnover rates and 2-fold increase in P fluxes through microbial biomass were observed. The collective findings of this research show that legacy P in soils plays a dominant role in determining P availability as influenced by land management. Further research is necessary to investigate strategies to enhance legacy P mobilisation and utilisation by plants.

Keywords: soil phosphorus, legacy phosphorus, phosphorus accumulation, phosphorus depletion, legacy phosphorus utilisation, soil phosphorus mobilisation, irrigation, grazed pasture, corn responses, biomass management, phosphorus mass balance, soil phosphorus fractionation, ^{31}P nuclear magnetic resonance spectroscopy, microbial phosphorus dynamics, microbial phosphorus flux, turnover rates, phosphatase enzyme activity.

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Chapter 1

Introduction

Phosphorus (P) is an essential element for all life cycles (Elser, 2012). In nature, P occurs predominantly in the form of phosphate (four oxygen [O] atoms surrounding the central P atom; PO_4^{-3}). Phosphate plays an essential role in all living cells in both genetic and the energy transformations processes. Photosynthesis is dependent on P. For example, a nitrogenous base, a ribose sugar and a phosphate group form the key molecules deoxyribonucleic acid (DNA) and ribonucleic acid (RNA), responsible for genetic information in all life forms. Adenosine triphosphate (ATP), essential for biochemical processes of energy storage and transfer, consist of an adenine base attached to sugar ribose and three phosphate groups. Phosphorus is also part of the cell membrane structure. The polar end of the lipid (the hydrophilic head) molecules in cell membranes (constituents of lipid bilayer), contains a phosphate group (Butusov and Jernelöv, 2013).

In terrestrial ecosystems, P is one of seventeen essential nutrients for plant growth, however, it is one of the major limiting elements (Bieleski, 1973). Despite its ubiquitous presence on the earth`s surface, processes of ecosystem development and nutrient dynamics lead towards P depletion (Walker and Syers, 1976; Schulze, 2002; Elser, 2012; Turner et al., 2012; Turner and Condron, 2013). Phosphorus limitation in soils occur as a consequence of soil weathering processes. During the various stages of soil development, P is successively transformed through biological, biochemical and physico-chemical pathways. At young stages of development, P is predominantly present in the crystalline structures of primary minerals which in turn are originated from the parent material (also known as parent rock or bed-rock). Apatite is the primary mineral source of P, with a unitary chemical composition equal to $Ca_5(PO_4)_3(\chi)$, where the ion represented by χ corresponds to OH^-, F^- or Cl^- for hydroxyapatite, fluoroapatite and chloroapatite, respectively. Processes of pedogenesis (driven by climatic factors such as precipitation and temperature) result in transformations of the primary minerals, and therefore, formation of secondary minerals, formation and accumulation of organic matter. Consequently, transformations on the chemical nature of P (originally from primary P minerals) occur through intensive processes of recycling. Phosphorus molecules are adsorbed (adsorption is a physico-chemical phenomenon in which ions, atoms, molecules or dissolved solids [adsorbate] “adhere” to a surface [adsorbent]) to the secondary minerals formed, and become part of organic structures after biological uptake (primarily by microorganisms, algae and plants), return to the soil following cell death (of microorganism, plants and animals), and can accumulate as part of

the soil organic matter (Walker and Syers, 1976; Tiessen et al., 1984; Smeck, 1985; Crews et al., 1995; Cross and Schlesinger, 1995; Novais and Smyth, 1999; Wardle et al., 2004; Lynch, 2007; McDowell et al., 2007; Turner et al., 2007; Yang and Post, 2011; Turner and Condron, 2013; Turner et al., 2013). Organic forms of P are defined as P bound in some way to carbon (C) (Dalal, 1977; McGill and Cole, 1981; Stewart and Tiessen, 1987; Condron et al., 2005). In highly weathered soils, P is accumulated, residually as recalcitrant inorganic and organic forms (Cross and Schlesinger, 1995; Yang and Post, 2011). Therefore, at these stages of the ecosystem development, P becomes a limiting nutrient.

Most of the understanding of long-term P transformation is soils as a function of ecosystem development is derived from studies of chronosequences. Chronosequences are sequences (or gradients) of soil (and ecosystem) development stages with ages raging from decades to millions of years (Stevens and Walker, 1970). One of the most widely recognized models describing process of P transformation in natural environments was proposed by Walker and Syers (1976) based on analyses of various chronosequences in New Zealand (Figure 1.1). Other well studied chronosequences are the Hawaiian Island chronosequence, the Lake Michigan dunes in the USA, the Cooloola sand dune chronosequence in Australia and the Haast dune chronosequence, formed following Alpine fault earthquakes in South Westland, New Zealand (Thompson, 1981; Crews et al., 1995; Lichter, 1998; Wells and Goff, 2007; Turner et al., 2012).

Occlusion of P occurs following adsorption of ions phosphate to mineral structures of secondary mineral silicates or residual aluminium (Al) and iron (Fe) oxides and oxyhydroxides (Smeck, 1985; Parfitt, 1989; Sanyal and De Datta, 1991). Reactions with Fe and Al occur in acid soils whereas co-precipitation with calcium (Ca) is particularly important in alkaline soils Liu et al. (2008). Phosphorus adsorption to soil colloids (such as Fe/Al oxides/oxyhydroxides and silicate clays) is a complex mechanism occurring in various stages and with different degrees of affinity of ions PO_4^{3-} by the adsorption sites. This sorptive characteristic of P by soils occur predominantly at the aluminol and silanol groups, and Lewis acid sites where the OH^- and OH^{2-} (hydroxyls) groups bound to the metals (Fe or Al) are exchanged by the PO_4^{3-} in a reaction of ligands exchange. In this process, the electrical charge of the soil solid components (commonly negative for the majority of soils in the world) is of little influence because it occurs through covalent bond (i.e. with electron sharing between the atoms) (Bigham et al., 1978; McLaughlin et al., 1981; Barrow, 1983; Parfitt, 1989; Sanyal and De Datta, 1991; Torrent et al., 1992; Sparks, 2003; Sposito, 2008).

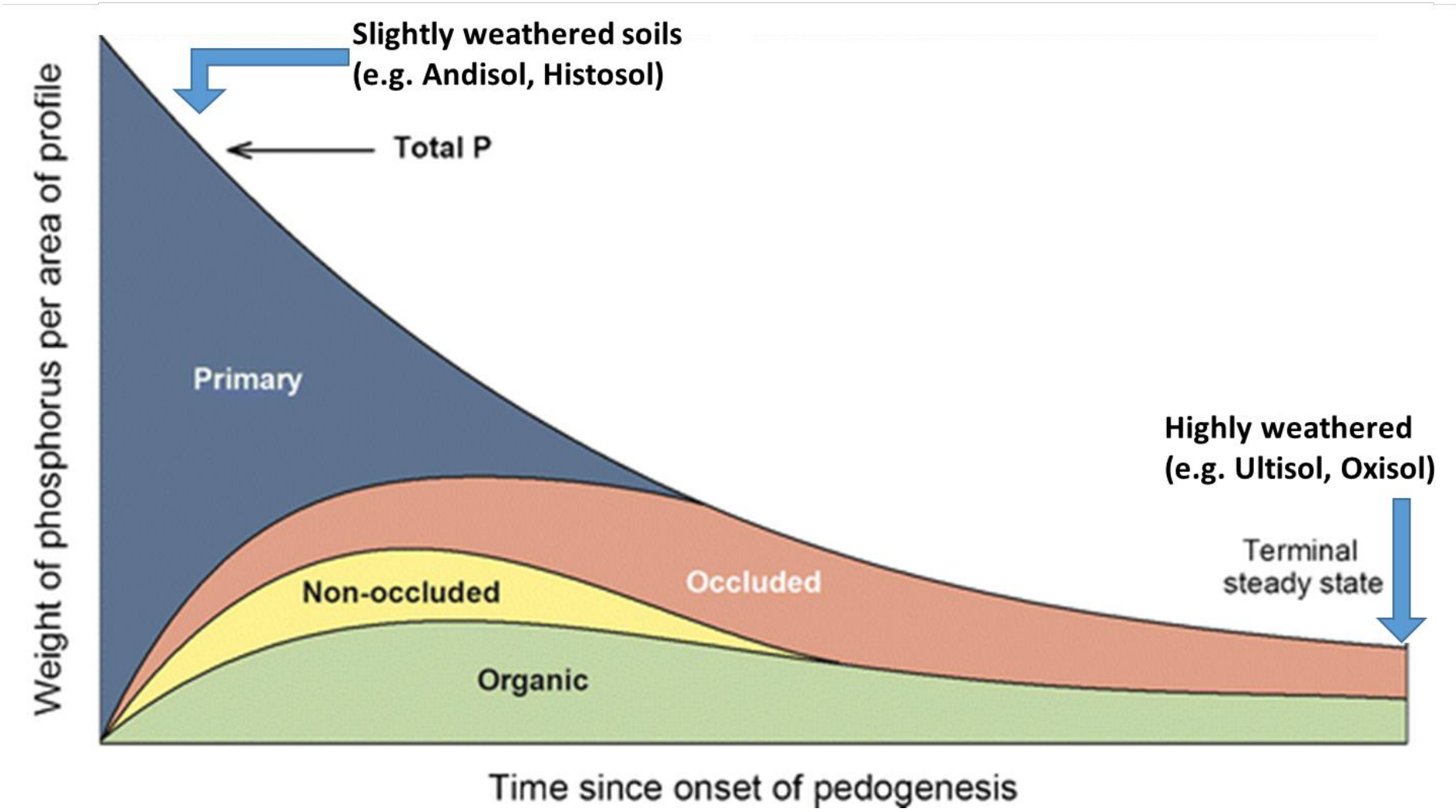


Figure 1.1 The Walker and Syers (1976) conceptual model of phosphorus dynamics during long-term ecosystem development. Figure adapted from Walker and Syers (1976), Cross and Schlesinger (1995), Yang and Post (2011) and Turner and Condron (2013).

This phenomenon is commonly known as inner sphere complexes and/or specific adsorption and is the dominant mechanism governing P availability (to plants and microorganism) from the soil mineral phase (Bigham et al., 1978; Novais and Smyth, 1999; Frossard et al., 2000; Condron, 2004). The inner sphere complex reaction can be represented by the example, where phosphate is adsorbed by an Al/Fe adsorption site (Al- or Fe-); OH and H_2PO_4 are the adsorbed anions; and OH^- and $H_2PO_4^-$ are respectively the anions in the soil solution as follows:

Equation 1 $$\mathbf{Al/Fe\text{-}OH + H_2PO_4^- = Al/Fe\text{-}H_2PO_4 + OH^-}$$

For example, Boitt (2014) presented an example of the naturally occurring residual accumulation of P in a set of highly weathered soils (Oxisols) formed from similar parent material, and differing in mineralogy (kaolinite>hematite/goethite to kaolinite/gibbsite) in a transect south-north in the central-southern Brazil. Highly stable P (occluded P forms) represented between 59-77% of the total soil P (total P between 714-1500 mg P kg^{-1}) and are highly unlikely to have significant participation for plant nutrition due to extremely slow desorption rates. In the same soils, potential P adsorption capacity, defined by the Langmuir model revealed overwhelming values ranging from 2007 to 2260 mg P kg^{-1} in the 0-20 cm soil depth (equivalent to approximately 4300 kg P ha^{-1}).

Soils vary widely with respect to total concentrations of P (100-3000+ mg P kg^{-1} (Novais and Smyth, 1999; Frossard et al., 2000; Condron, 2004; Haygarth et al., 2013). Nonetheless, only a small proportion (<0.1%; or 0.1-10 µM) of the total soil P is present in the soil solution at any time (Novais and Smyth, 1999; Hinsinger, 2001; Condron, 2004; Richardson et al., 2009a; Gatiboni et al., 2013a). Phosphorus take up by plants and microorganism occur almost entirely from the soil solution as inorganic orthophosphate (HPO_4^{2-} and $H_2PO_4^-$, for the common soil pH range 4-8) (Hinsinger, 2001; Condron, 2004).

Following removal from the soil solution, P is replenished by the soil solid phase (inorganic and organic) to maintain equilibrium conditions. Moreover, P is distributed in various forms in soils, with different degrees of stability (commonly referred to according to its lability). In other words, the capacity of a certain form of P to replenish the soil solution (mobilization) with dissolved inorganic P via desorption and/or dissolution (from adsorbed mineral forms), and mineralization (from organic forms) will be given by the concentration in the soil solution, the diffusivity (of P) to the soil solution and the degree of affinity (binding energy) between phosphate ions and the colloidal phase (Figure 1.2) (Sparks, 2003; Sposito, 2008).

The distributions of different forms of soil P can be operationally defined (e.g. P pools) by using several different procedures. The chemical soil P fractionation, for example, is based on multiple

sequential extractions of a soil sample with solution with different chemical strength and therefore extraction capacities. The most widely used protocols were introduced by Chang and Jackson (1957), considering inorganic forms of P bound to Fe, Al and Ca, and by Hedley et al. (1982) that yields a complex and detailed distribution of P forms present in inorganic and organic pools, including in the microbial biomass. The soil P fractionation scheme developed by Hedley et al. (1982) provides a useful index of the relative importance of P cycling by soil biological and geochemical processes at different stages of ecosystem development and as affected by land use changes (Cross and Schlesinger, 1995; Negassa and Leinweber, 2009; Yang and Post, 2011). It is important to note that, the definition of P pools (compartments) in soils is strictly operational. Changes and dynamic interrelationships between the different forms of P occur continuously to maintain equilibrium conditions (Figure 1.2). The original procedure proposed by Hedley et al. (1982) has been modified and adapted by following studies, however the concept has been maintained, where extractant solutions extract P forms of decreasing lability (i.e. increasing stability). For example, a derivate fractionation protocol utilises, sequential extractions with a salt such as 1M ammonium chloride (NH_4Cl) or sodium chloride (NaCl) for extraction of weakly bound P and removal of exchangeable cations; labile forms of inorganic and organic P determined after extraction with 0.5M sodium bicarbonate ($NaHCO_3$); moderately labile inorganic and organic P (bound to Fe/Al oxides and silicate clays) extracted by 0.1M sodium hydroxide (NaOH); inorganic P forms bound to Ca following extraction with 1M hydrochloric acid (HCl); a second extraction with 0.1M NaOH (inorganic and organic P physically protected within the microaggregates) and finally a digestion procedure with concentrated acids (such as concentrated sulfuric acid [H_2SO_4]) at elevated temperatures to extract residual forms of P (inorganic and organic) of high stability (recalcitrant inorganic and organic P physically protected within minerals/micro aggregates/organic matter) (Hedley et al., 1982; Olsen and Sommers, 1982; Smeck, 1985; Condron and Goh, 1989; Cross and Schlesinger, 1995; Condron et al., 1996; Guo and Yost, 1998; Blake et al., 2003; Condron et al., 2005; Gatiboni et al., 2007; Negassa and Leinweber, 2009; Condron and Newman, 2011; Yang and Post, 2011; Gatiboni et al., 2013a).

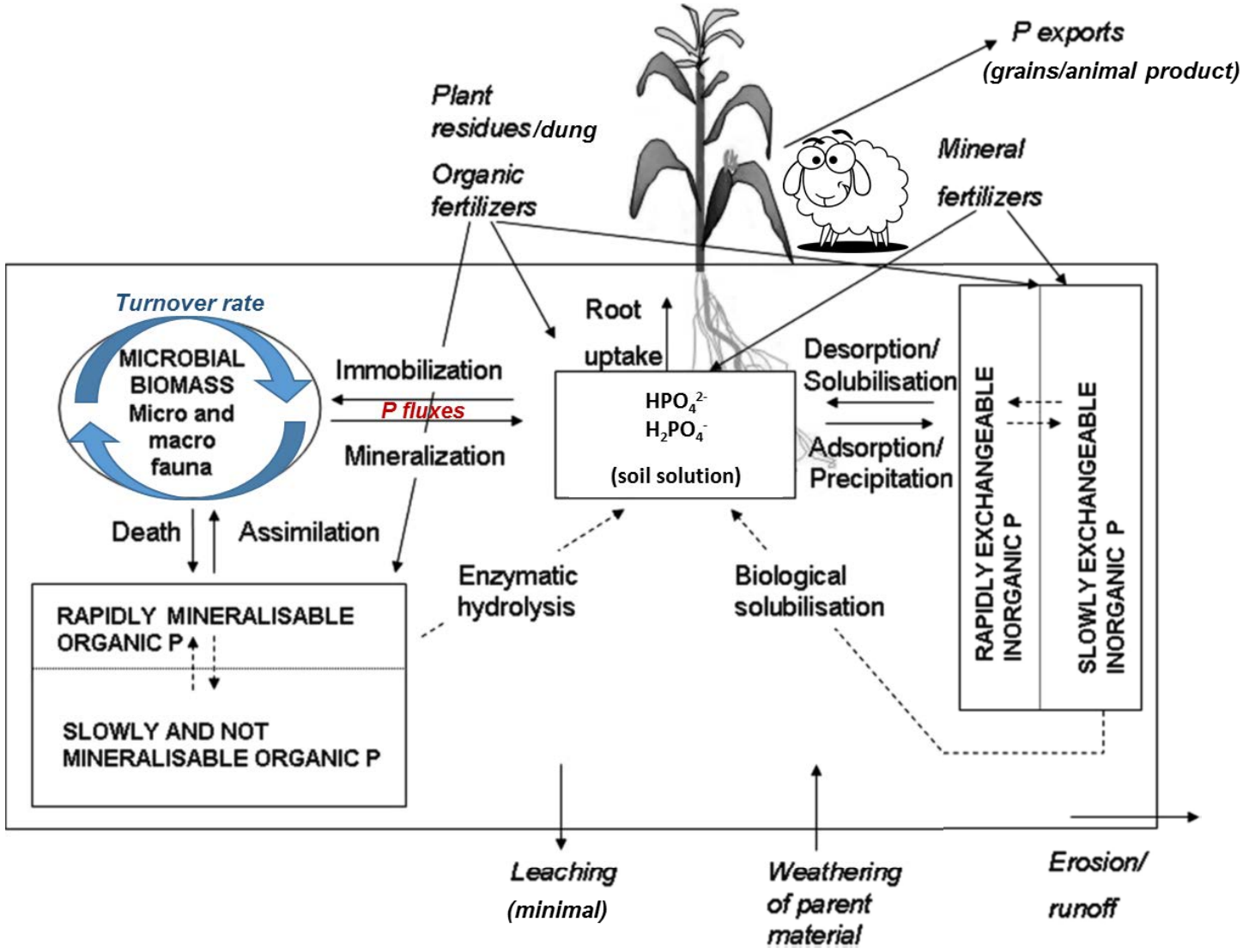


Figure 1.2 The phosphorus cycle in the soil-plant-animal system. The size of represented pools are not at the same scale. Adapted from Frossard et al. (2011).

Organic P can represent between 20 and 80 % of total phosphorus in the majority of soils (Dalal, 1977; Stewart and Tiessen, 1987; Condron et al., 2005). Additionally, accumulation of organic P and chemical nature are associated to ecological processes during ecosystems development (McDowell et al., 2007; Turner et al., 2007; Turner et al., 2013). As previously commented organic P can be defined as P containing at least one covalent bond to a C atom, generally through an ester linkage (i.e. through an oxygen atom) (Doolette and Smernik, 2011). Soil organic P originates following returns of organic matter from biological processes involving assimilation of orthophosphate from the soil solution into the biomass of animals, plants and microorganisms (Dalal, 1977; Stewart and Tiessen, 1987; Condron and Tiessen, 2005; Condron et al., 2005; Gatiboni et al., 2013a). Organic P compounds are mainly classified into orthophosphate esters, phosphonates and anhydrides based on the nature of the P bond (Condron et al., 2005). Orthophosphate esters, are subdivided into orthophosphate monoesters and orthophosphate diesters, according to number of ester groups linked to each orthophosphate. Orthophosphate monoesters are the most common forms of organic P found in soils and can comprise the entire organic P fraction (Condron et al., 2005). Inositol phosphates are the most significant group among the orthophosphate monoesters. Generally, small proportions of orthophosphate diesters have been found in agricultural soils (Rheinheimer et al., 2002; Condron et al., 2005; Stutter et al., 2015).

Orthophosphate diesters compounds can be easily degraded (mineralised), resulting in inorganic P release in soil environments (Hinedi et al., 1988; Condron et al., 1990; Condron et al., 2005; Gatiboni et al., 2005; McDowell et al., 2005; Stutter et al., 2015) and include nucleic acids, phospholipids, teichoic acids and aromatic compounds (Condron et al., 2005). On the other hand, in some cases, DNA can be stabilized and accumulated as part of the soil organic matter (Turner et al., 2007).

Thus, inositol phosphates can comprise more than 80% of the total organic phosphorus and are considered the main compound of the phosphate monoesters in soils (Quiquampoix and Mousain, 2005; Menezes-Blackburn et al., 2012). Gatiboni et al. (2013b) also identified accumulation of inositol phosphates following over nine years of different manure additions to soil.

First used by Newman and Tate (1980), characterization of soil organic P using nuclear magnetic resonance spectroscopy (^{31}P-NMR) has increased successfully the understanding in regard of this important P pool in soils and environmental samples since then. This technique can identify and quantify the organic P compounds in either extracted samples (solution spectroscopy) or solid samples (solid-state spectroscopy). Although, solution ^{31}P-NMR shows a higher spectral resolution than solid-state spectroscopy (Cade-Menun, 2005; Condron et al., 2005) but, are more likely to show hydrolysis of the species extracted (Turner et al., 2003). For P extraction and ^{31}P-NMR determination,

the method employing NaOH plus Ethylenediaminetetraacetic acid (EDTA) (0.25 mol L^{-1} NaOH plus 0.05 mol L^{-1} Na_2EDTA) has been show as suitable due to its high extraction capacity (Cade-Menun and Preston, 1996; Cade-Menun et al., 2002; Turner et al., 2005).

Soil microorganisms play a central role in the P cycle in natural and managed ecosystems (Stewart and Tiessen, 1987; Magid et al., 1996; Frossard et al., 2000; Condron et al., 2005; Richardson and Simpson, 2011; Turner et al., 2013; Nash et al., 2014). Microbial biomass P comprises a small but significant proportion of total soil P (2-5 %) depending on soil type and land use (Hedley and Stewart, 1982; Brookes et al., 1984; Magid et al., 1996; Jakobsen et al., 2005; Oberson and Joner, 2005; Bünemann et al., 2011). However the flow or flux of P through the microbial biomass means that this small pool plays a vital role in determining the dynamics and bioavailability in soil-plant systems (Jakobsen et al., 2005; Oberson and Joner, 2005; Bünemann et al., 2012). Microorganisms and plants exudate phosphatase enzymes in response to low availability and/or demand for inorganic P (McGill and Cole, 1981; Richardson et al., 2011), while the associated role and function of root exudates (including low molecular weight organic anions) in the interface root-soil (known as rhizosphere) remains unclear (Jones et al., 2009; Richardson et al., 2009a; Nannipieri et al., 2011; Ryan et al., 2012; Clarholm et al., 2015).

Thus, due to its complexity and dynamic nature of processes, most of functions in the rhizosphere are still debatable and beyond the current methods of analyses. Biological and biochemical factors such as plant species, quantity and composition of root exudates influencing soil microorganisms and microbial community structure and composition (Shi et al., 2013); presence of highly specialized microbial communities interacting with the host plant by trading carbon and nutrients in mutualistic relationships (Domanski et al., 2001; Jones et al., 2009; Richardson et al., 2009a; Blossfeld et al., 2011; Ryan et al., 2012; Philippot et al., 2013), exudation of extracellular enzymes (Turner and Haygarth, 2005; Richardson et al., 2009a; Nannipieri et al., 2011; Spohn et al., 2013; Spohn and Kuzyakov, 2013, 2014; Giles et al., 2017); and physicochemical processes with large pH shifts occurring as a result of organic acids and H^+ exudation and therefore, chemical alterations on the solubility and availability of nutrients (Chen et al., 2002; George et al., 2006; Richardson et al., 2009a; Blossfeld et al., 2011; Santner et al., 2012; Blossfeld et al., 2013; Giles et al., 2017) are among the main contributors for the complexity of the rhizosphere.

Mycorrhizal associations also have a fundamental role in the P cycling and plant nutrition. These symbioses enhance the P uptake by plants mainly by increasing the root net that explore the bulk-soil, but also have been found to accelerate weathering of P-bearing minerals (Jansa et al., 2011).

Phosphorus uptake by plants and soil microorganisms creates a depletion zone in the rhizosphere (also including mycorrhizal fungi and plant associations, known as mycorrhizosphere (Richardson et al., 2009a); Figure 1.3). The mass flow is not sufficient for adequately supply inorganic P for uptake and thus, diffusion is the dominant mechanism for P providing to the roots (and microbial biomass) which is more pronounced towards the rhizosphere (and mycorrhizosphere) (Richardson et al., 2009a).

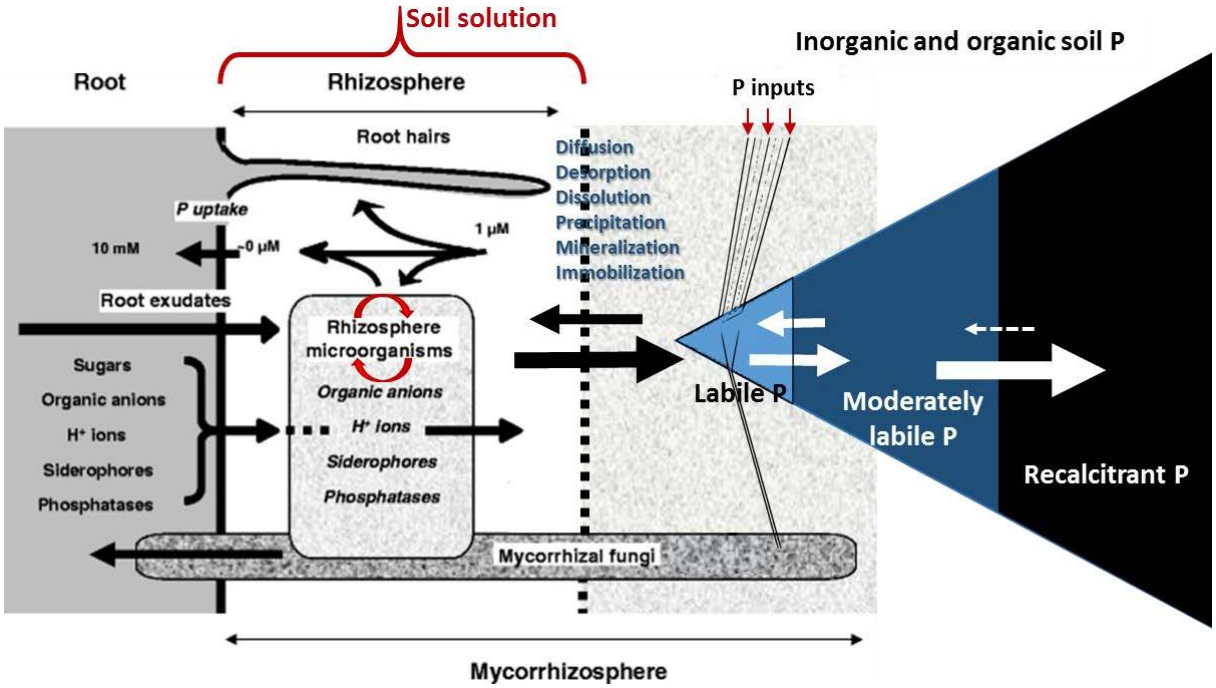


Figure 1.3 Representation of physiological and biochemical processes that influence the availability and transformation of phosphorus in the rhizosphere (and mycorrhizosphere). Adapted from Richardson et al. (2009a).

Utilisation of organic P forms by plants and microorganisms requires the orthophosphate to be cleaved form the organic molecule (mineralisation). This mineralisation is preceded by the production and release of organic anions (or organic acids), extracellular phosphatase enzymes followed by P uptake (Richardson and Simpson, 2011; Clarholm et al., 2015).

The enhanced phosphatase activity is often reported in the rhizosphere environment, and also to be higher in low available P soils (Tarafdar and Jungk, 1987; Chen et al., 2002; George et al., 2002). For example, Chen et al. (2002) observed nearly a 3-fold increase in the microbial biomass in the rhizosphere of ryegrass (*Lolium perenne* L.) and radiata pine (*Pinus radiata* D. Don.), and the effect was extended to up to 5 mm from the root surface. Cover crops can also efficiently mobilise organic P. Phosphatase activity and organic P mineralisation was 30-35% higher under oilseed radish (*Raphanus sativus* L. var. *Oleiferus* Metzg) (non-mycorrhizal species) relative to a control (Kunze et

al., 2011). The effect of the cover crop in the soil enzymatic profile persisted through subsequent periods under the following crop (summer crop).

Inorganic P released into soil solution from microbial cells (i.e. mineralized) can be adsorbed onto soil colloid surfaces, re-immobilized in microbial cells, or taken up by plants (Stewart and Tiessen, 1987; Magid et al., 1996; Jakobsen et al., 2005; Oberson and Joner, 2005). The process and rate of microbial biomass turnover (immobilization, mineralization, re-immobilization and re-mineralization) are key determinants of plant P availability through biological pathways (Condron and Tiessen, 2005; Oberson and Joner, 2005; Bünemann et al., 2012).

Nevertheless, given the general P deficiency in agricultural soils across the world, especially in highly weathered and tropical soils, adequate plant P nutrition and satisfactory yields are highly dependent to external P inputs as fertiliser (Walker and Syers, 1976; Tiessen et al., 1984; Crews et al., 1995; Cross and Schlesinger, 1995; Ker, 1995; Novais and Smyth, 1999; Almeida et al., 2003; Negassa and Leinweber, 2009; Yang and Post, 2011; Elser, 2012; Turner and Condron, 2013). In an ideal situation, the amount of P input (e.g. as fertiliser, manure, etc.) to the system is equal to amount of P exported in products (Simpson et al., 2011; Weaver and Wong, 2011). However, as discussed above, P is efficiently fixed (adsorbed or "P sink") in some degree by soils (Figure 1.4) (Sample et al., 1980; Novais and Smyth, 1999; McLaughlin et al., 2011; Schnug and Haneklaus, 2016). Consequently, low P fertilizer use efficiency is frequently observed in agricultural systems, where P inputs are greater than P removal by crops and livestock. Commonly, 5-20% of the P fertilizer is utilized by crops (Sattari et al., 2012; Haygarth et al., 2013). Moreover, phosphate rock reserves (deposits with economic viability to mine), the primary source for P fertiliser production, is a finite and non-renewable resource (Liu et al., 2008; Cordell et al., 2009; Dawson and Hilton, 2011; Elser, 2012; Scholz et al., 2013; Ulrich et al., 2013; Van Kauwenbergh et al., 2013).

This imbalanced P cycle is generally observed in high productivity agriculture (George et al., 2016). As a result, P surpluses (P inputs exceeding P exports) are observed (Condron, 2004; Bouwman et al., 2009; MacDonald et al., 2011). Accumulation of "residual P" or "legacy P" (the term "residual P" referred here comprise all forms of P accumulated in soils as a result of continued P additions and must not be confused with the residual P fraction determined by soil P fractionation protocols, i.e. non-extracted P fraction) in soils as a result of continued P inputs to maintain satisfactory yields is of global importance (Figure 1.5) (MacDonald et al., 2011; Sattari et al., 2012; Stutter et al., 2012). The term legacy P comprises inorganic and organic P forms of different solubility in soils (Condron, 2004; Stutter et al., 2012; Condron et al., 2013; Nash et al., 2014).

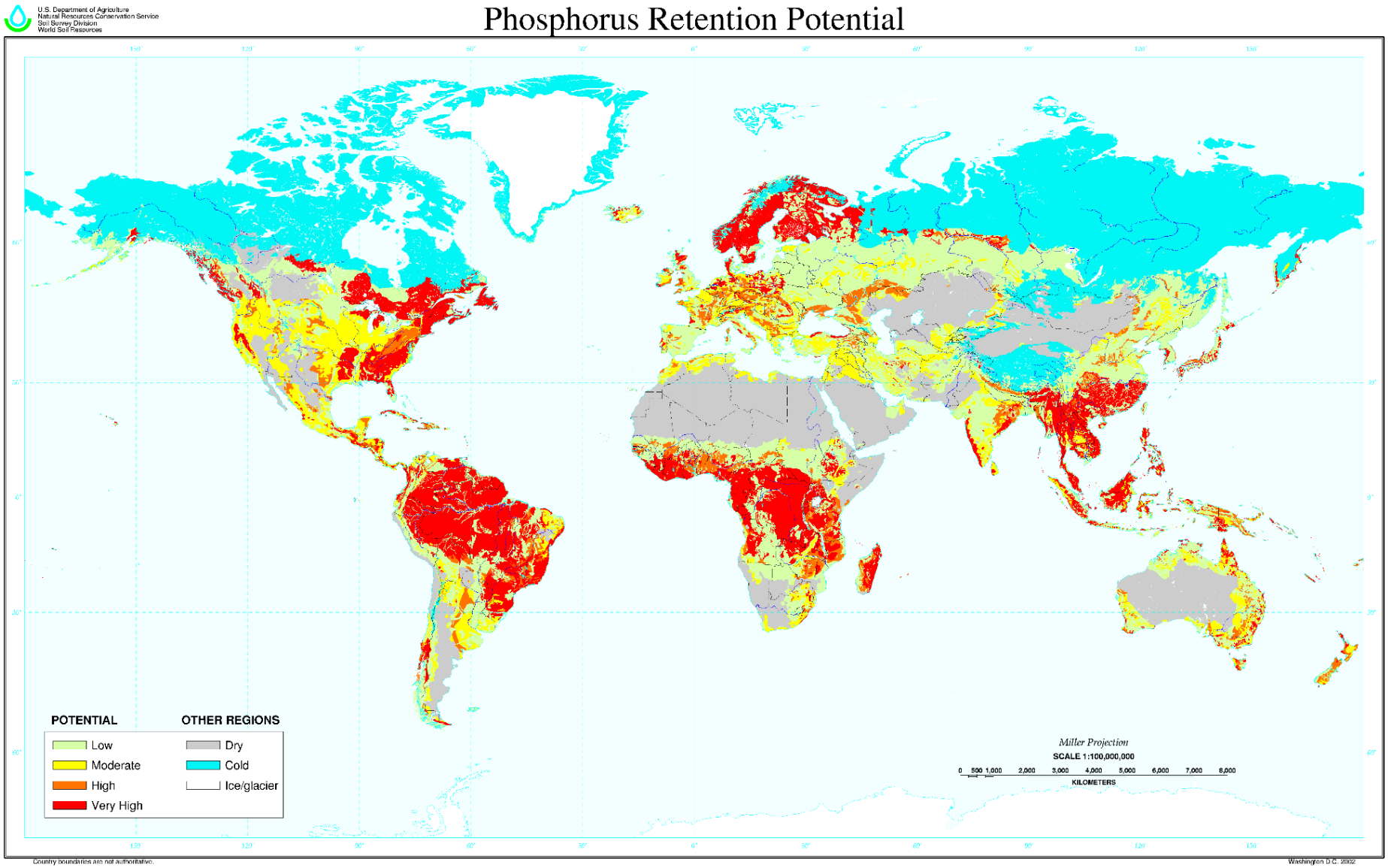


Figure 1.4 Overview of the phosphorus retention (adsorption) capacity in soil across the world (USDA-NRCS, 1998).

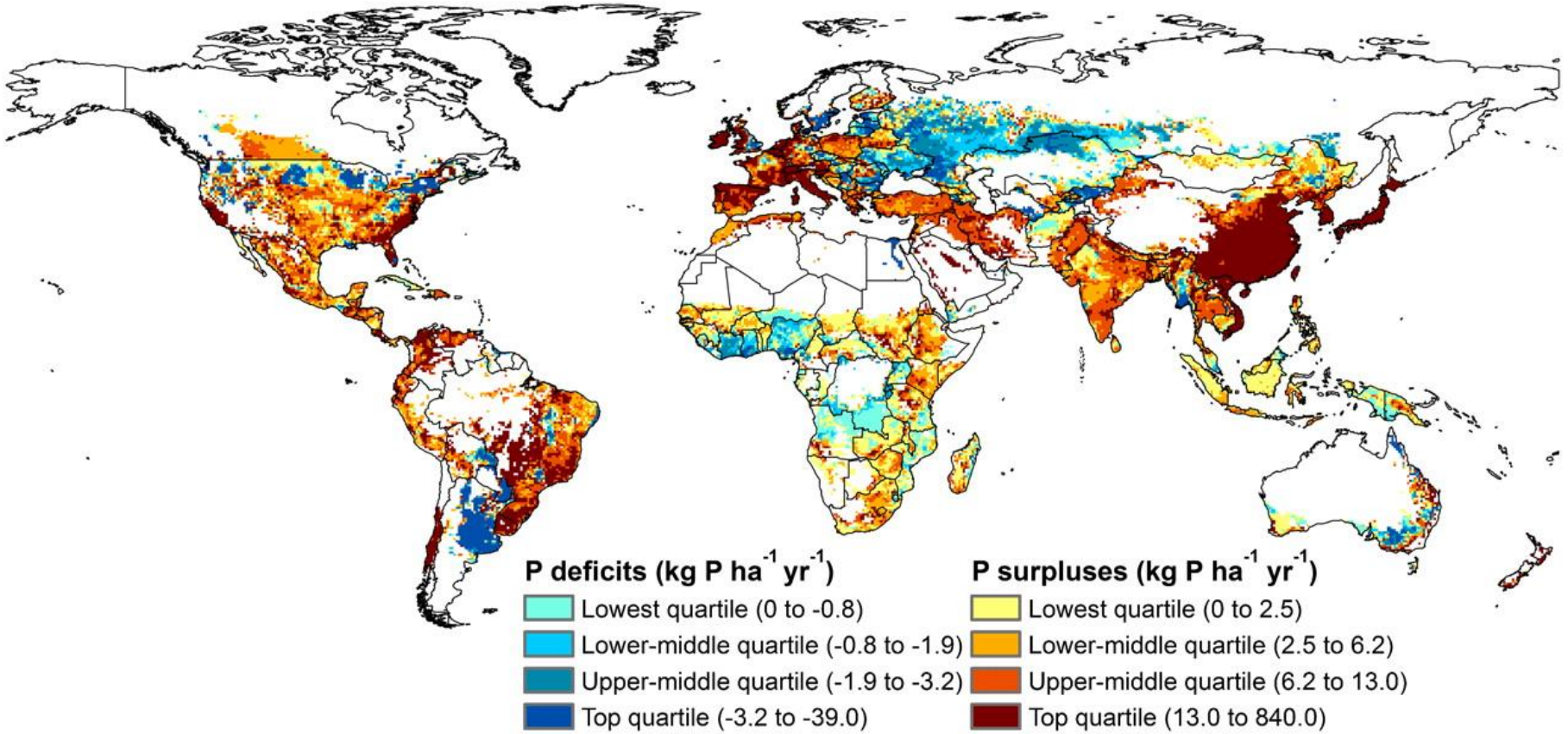


Figure 1.5 Global map of agronomic P imbalances for the year 2000 expressed per unit of cropland area in each 0.5° grid cell. Extracted from MacDonald et al. (2011).

MacDonald et al. (2011) estimated that over 70% of the world's cropland area had overall P surpluses (Figure 1.5). Moreover, the largest P surpluses (median of 26 kg P ha^{-1} y^{-1}) were attributed to 10% of the cropland area (covering mainly East Asia, coastal USA, and southern Brazil), which in turn, was responsible for 45% of the cumulative global P surplus. Additionally, P fertiliser input exceeding crop (and livestock) P removal corresponded to over 85% of the global cropland area that had large P surpluses (i.e. >13 kg P ha^{-1} y^{-1}) (MacDonald et al., 2011). This reflects in an overall decrease in the "P use efficiency" or "P balance efficiency" indicators in agricultural systems (MacDonald et al., 2011; Simpson et al., 2011; Weaver and Wong, 2011; Simpson et al., 2014). The P balance efficiency is a useful index of agronomic effectiveness of fertiliser use, and can be defined as:

Equation 2 $$\textbf{P balance efficiency} = \frac{P_{outputs}}{P_{inputs}} \times 100$$

Where, P_{output} is the P removal in products (grains, animal, etc.) and P_{inputs} comprises external P application (as fertiliser, manure, etc.). High P balance efficiency can only be achieved in very low input, and therefore low productivity agricultural system; in soil conditions with low P adsorption capacity; or under high fertility soils, i.e. high P-buffering capacity systems (MacDonald et al., 2011; McIvor et al., 2011; Simpson et al., 2014). On the other hand, this indicator may be biased in conditions where there is a necessity of constant P inputs well beyond plant P requirements (in productive systems) due to the high reactivity to soil colloid particles (particularly highly weathered soils). During soil P fertility build-up phase; and large livestock density and manure disposal may also lead to low P budget efficiency. Therefore, precaution is required when assessing indices of P balance efficiency in various agricultural systems. Nevertheless, the majority of low P use efficiency agricultural fields, in global assessments, are located in areas with excessive P fertiliser (and manure) inputs and relatively small increases in crop (and animal) productivity (e.g. China, Midwestern USA, and southern/western Europe); and areas with low P inputs and low crop production (e.g. East Africa and north-eastern Brazil), mainly resulting from constrains to agricultural production other than P fertiliser (MacDonald et al., 2011; Schröder et al., 2011). Phosphorus audits in different farming systems and managements are vital for the identifying unsustainable practices and inefficient P use. For example, Haygarth et al. (1998) compared farming systems in the UK, and quantified P accumulation rates of 27 kg P ha^{-1} y^{-1} under dairying in comparison to 0.28 kg P ha^{-1} y^{-1} under sheep. In a long-term fertiliser trial under sheep grazing pasture in New Zealand, treatment plots receiving P fertiliser rates of 18 and 36 kg P ha^{-1} y^{-1} compared to an unfertilised control, net accumulation in soils was shown to be 8 and 20 kg P ha^{-1} y^{-1}, respectively (Tian et al., 2017). The remaining amounts of P were transferred within plots, removed as animal product, remaining in plant residues and were lost

in irrigation water (Nguyen and Goh, 1992; Williams and Haynes, 1992; McDowell and Rowley, 2008; Condron et al., 2014; Tian et al., 2017).

Excessive P inputs to land are directly related to deterioration of water bodies (Jarvie et al., 2015; Powers et al., 2016). Accumulated P in soils and poor farming management, such as the lack of preventive and conservationist soil practices, substantially increase the potential of P transfer and water eutrophication (Carpenter et al., 1998; Sharpley et al., 2013; Rissman and Carpenter, 2015).

The majority (80-90%) of phosphate rock mined annually is used for the manufacture of fertilisers (Cordell et al., 2009; Dawson and Hilton, 2011; Van Kauwenbergh et al., 2013; Walan et al., 2014; Withers et al., 2014). Moreover, 75% of the phosphate rock reserves that can be economically mined with current technology is concentrated in Morocco and Western Sahara (Figure 1.6) (Dawson and Hilton, 2011; Van Kauwenbergh et al., 2013). Concerns regarding phosphate rock resources supply and volatile costs have been given increasing focus in recent years, in special, after the 2007/2008 global crisis when phosphate rock commodities prices spiked by ~700% in a 14-month period (Figure 1.6) (Elser, 2012; Cordell and White, 2014). Growing world population, and therefore the demand for food relies on the maintenance and improvements on crop and livestock yields, with a minimal impact to the environment. Unfortunately, P is one of the key limiting nutrients across the globe`s croplands and is essential for achieving food security, especially in countries such as in Brazil, India, China and African countries (Sanginga et al., 2003; Cordell et al., 2009; MacDonald et al., 2011; Elser, 2012; Condron et al., 2013; Cordell and Neset, 2014; Cordell and White, 2014; Mikkelsen et al., 2014; George et al., 2016; Nziguheba et al., 2016). Identifying the P flows in a global scale is a necessary step towards defining political and economic strategies inherent to the food production chain worldwide and regionally (Liu et al., 2008; Cordell et al., 2009; Cordell et al., 2011; Elser, 2012; Cordell and Neset, 2014; Mikkelsen et al., 2014).

On the other hand, many decades of continuous P applications in European and North American soils, for example, have resulted in stabilisation and/or decrease in P fertiliser demands (Lynch, 2007; Cordell et al., 2009; Sattari et al., 2012; Sharpley et al., 2013). Understanding the role and dynamics of biological processes related to P recycling, chemical form and bioavailability are essential for maintaining the sustainability (i.e. without nutrient depletion from soils and minimal impacts to the environment), and productivity of agroecosystems based on adequate plant P nutrition (Magid et al., 1996; Richardson, 2001; Jakobsen et al., 2005; Richardson et al., 2009a; Richardson et al., 2009b; George et al., 2011; Jones and Oburger, 2011; Richardson and Simpson, 2011; Simpson et al., 2011; Mikkelsen et al., 2014).

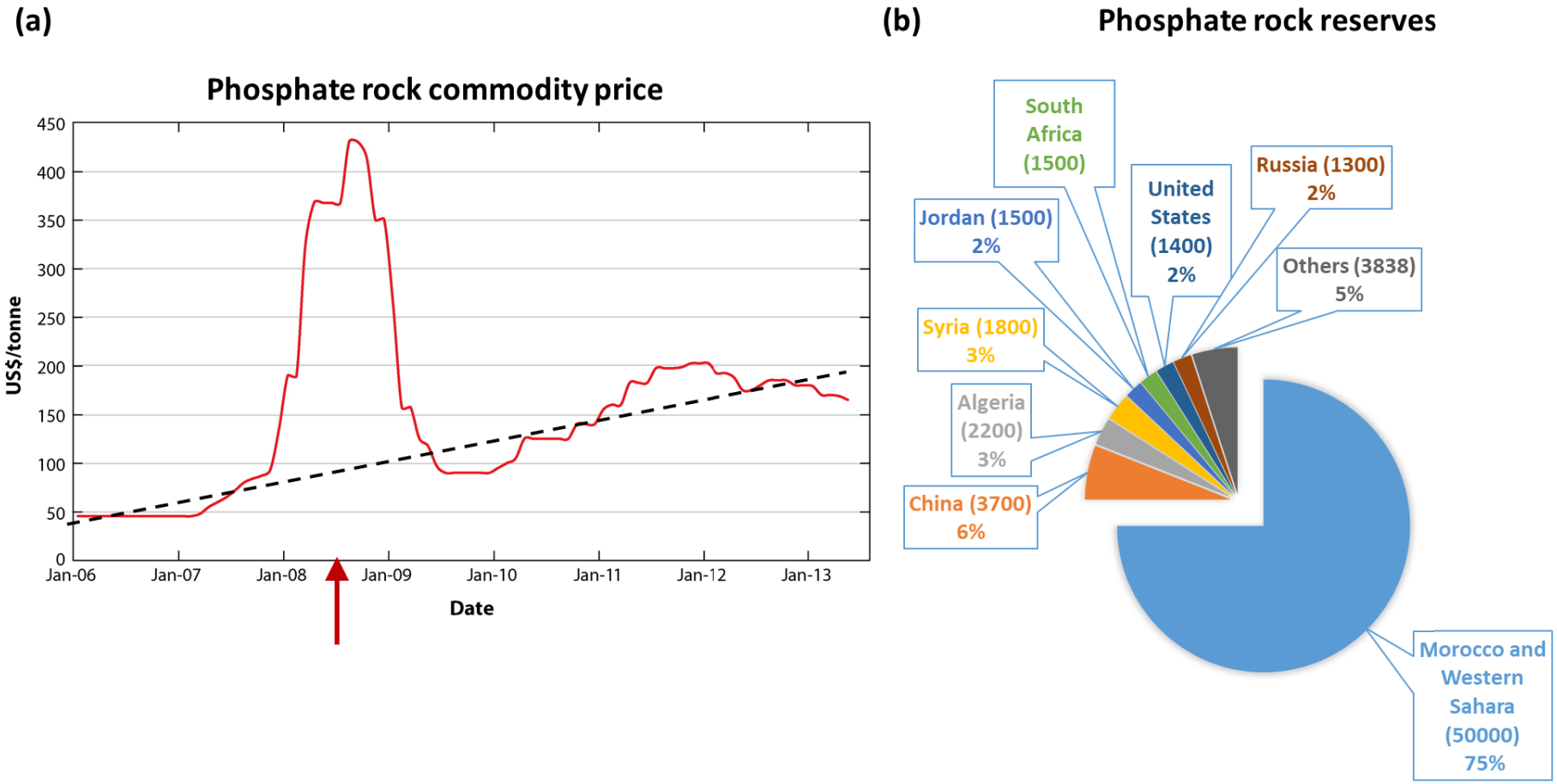


Figure 1.6 (a) Phosphate rock commodity price during 2006-2013. The red arrow indicates the sudden spike experienced in 2007/2008. Figure adapted from Cordell and White (2014). (b) Phosphate rock reserves in 2012. Numbers in parenthesis represent the reserves expressed in million tonnes. Diagram created with data from Van Kauwenbergh et al. (2013).

In this scope, many efforts have been directed towards enhancing P fertiliser efficiency and rational use; enhancing P uptake by crops, for example by association with a range of soil microorganisms (e.g. mycorrhiza fungi and P solubilizing bacteria), including the role of soil microorganism grazers; utilisation of legacy P, including the soil organic and occluded P forms accumulated from previous inputs; management practices to lower P fertiliser inputs; and genetic engineering (Haynes and Williams, 1993; Richardson, 2001; Bonkowski, 2004; Lynch, 2007; Richardson et al., 2009a; Richardson et al., 2009b; Gaxiola et al., 2011; Richardson et al., 2011; Richardson and Simpson, 2011; Simpson et al., 2011; Weaver and Wong, 2011; Hendrix, 2012; Sattari et al., 2012; Stutter et al., 2012; Condron et al., 2013; Haygarth et al., 2013; Nash et al., 2014; Simpson et al., 2014; Withers et al., 2014; George et al., 2016; George et al., 2017).

1.1 Hypothesis and Study Objectives

The literature review revealed that phosphorus legacy in soils under intensive and productive agricultural systems is consistent worldwide. Quantifications of the impacts of long-term P accumulation in soils under different management systems is a common interest, and necessary for establishing more sustainable P-use management strategies in agriculture. Following this scope, the hypothesis of this work is:

Phosphorus inputs and phosphorus accumulation in soils play a dominant role in determining phosphorus availability in agricultural systems as influenced by land use and management.

The general objective is to investigate the impact of different agricultural land management strategies and amendments on the quantities and nature of inorganic and organic phosphorus, together with the effects on the dynamics of microbial biomass phosphorus and related parameters.

The specific objectives are subdivided into two main sections as follows:

a) Long-term management impacts

To assess and quantify the impact of contrasting long-term management regimen in the role of phosphorus depletion/accumulation processes determining/influencing soil P dynamics and bioavailability. Three well established, contrasting long-term field experiments were studied, and are briefly described as follows: a first experiment, the long-term irrigation trial, Winchmore, New Zealand, receiving P inputs for over 60 years and different irrigation management; a second trial receiving long-term pig slurry additions, Santa Catarina, Brazil, receiving P inputs at different rates in pig manure for 15 years under cropping; and a third experiment, the "long-term ecology trial", Lincoln, New Zealand, under contrasting grassland management regimen (grassland biomass retained or removed), non-grazed with a long-term history of P inputs prior to the establishment of the trial, followed by absence of P inputs for over 20 years (i.e. since beginning of experiment).

Importantly, these contrasting long-term management scenarios compose a range of land use, management strategies, forms and quantities of P inputs, soil types and climate conditions. The separate studies of the long-term irrigation trial, long-term pig slurry inputs and long-term ecology trial will compose the chapters 2, 3, and 4, respectively (Figure 1.7).

b) Short-term P dynamics

To assess and quantify the short-term impacts of biological and biochemical processes in soil P dynamics and bioavailability. To achieve this objective, the long-term ecology trial, New Zealand, was

evaluated on a temporal basis for 17 months during 2015-2017. This experiment will be presented on chapter 5 of this thesis (Figure 1.7).

A general discussion and critical analysis will be presented on chapter 6, where the main findings and practical implications of each separate study will be further analysed. This discussion will also discuss some future research priorities and perspectives related to the research topic. The protocols related to soil P analyses utilised here are presented in the appendix section.

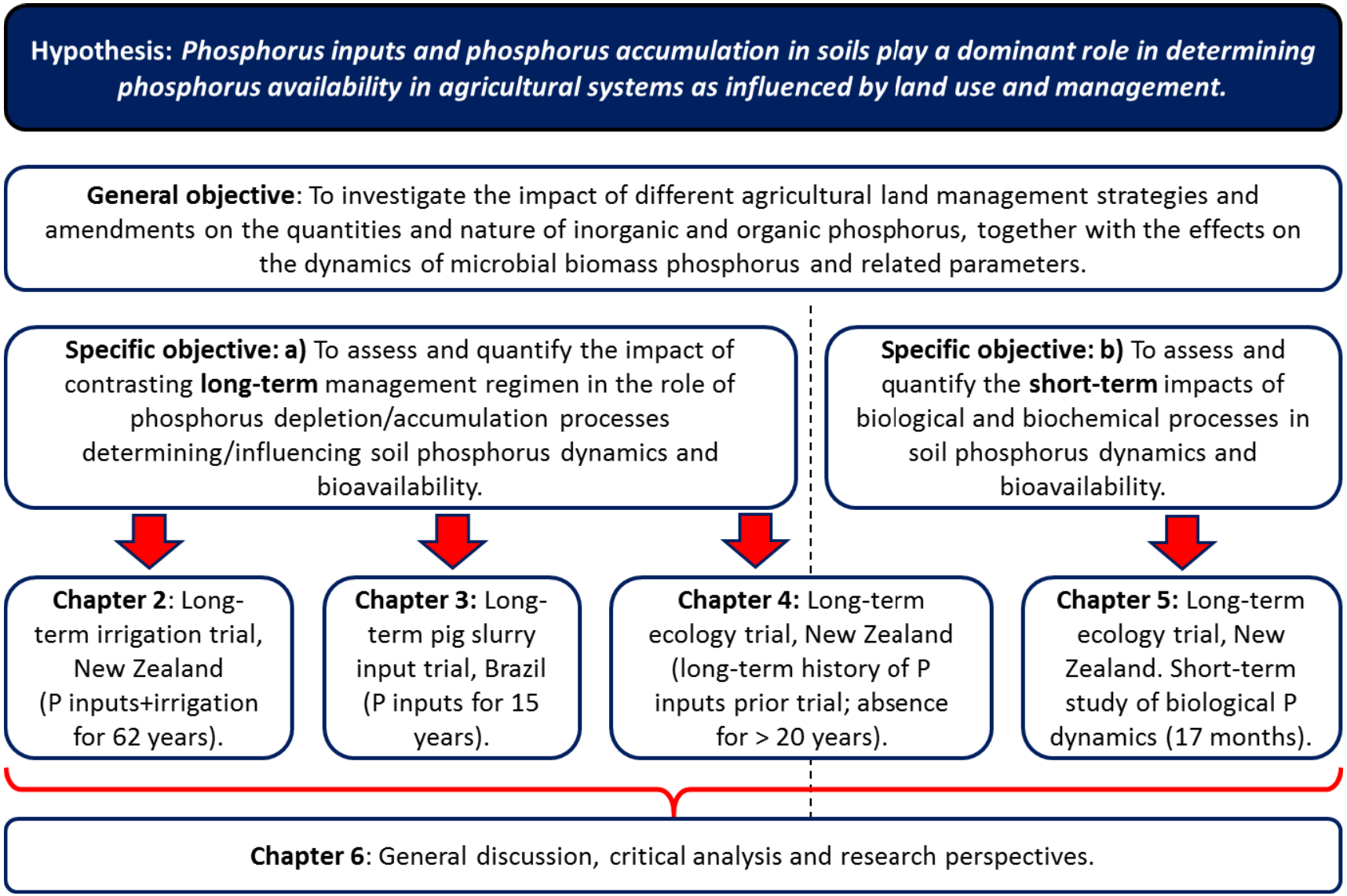


Figure 1.7 Overview of thesis structure.

Chapter 2

Effects of Long-Term Irrigation on Soil Phosphorus under Temperate Grazed Pasture

2.1 Introduction

Phosphorus (P) dynamics and availability in soil–plant systems are controlled by complex biogeochemical interrelations among various abiotic and biotic pools (Frossard et al., 2000). Plant and animal production in agroecosystems can be increased considerably by the addition of P in the form of mineral fertilizers or manures, or both, although chemical adsorption and biological immobilization of P in soil mean that regular inputs of P are required to maintain satisfactory levels of production (Haygarth et al., 2013). In many areas of the world, fertilizer inputs have been combined with irrigation to increase plant and thereby animal production, resulting in increased food supply and security (Lal, 2007; Darko et al., 2016).

In New Zealand, for example, the eastern areas of both main islands receive low rainfall and are prone to summer droughts that severely restrict the growth of pasture, particularly on shallow free draining stony soil (Williams, 2002). The development of large-scale irrigation schemes has enabled substantial expansion in intensive pastoral agriculture (predominantly dairying), which in turn has adversely affected water quality by increased transfer of nitrogen and P into many natural water bodies (Williams, 2004; Wright, 2012; Ministry for the Environment and Statistics New Zealand, 2015). The effect of long-term P and irrigation inputs on the dynamics and distribution of P in soil under intensive management is not well understood, but remains a critical issue for the sustainability and productivity of pastoral farming in temperate regions around the world.

A series of comprehensive field experiments were established in the late 1940s–early 1950s at Winchmore, Canterbury, New Zealand. These pioneering experiments were focussed primarily on assessing and quantifying the effects of P fertilizer and irrigation inputs on the productivity of ryegrass (*Lolium perenne* L.) –clover (*Trifolium repens* L.) pasture grazed by sheep (McDowell and Condron, 2012). These trials were maintained for over 60 years, and included an experiment with variable inputs of irrigation combined with a constant rate of addition of annual P fertilizer (Condron et al., 2006; Condron et al., 2014).

The Winchmore field trial represents a unique opportunity to assess the combined effects of irrigation and P fertilizer inputs on the amounts, forms and distribution of P in the soil profile under grazed pasture in a long-term field-based context. Given the shallow, stony nature of the soil at

Winchmore, it was expected that increased irrigation would result in enhanced depletion of soil P compared with the control, including increased transfer of P in drainage.

2.2 Materials and Methods

2.2.1 Winchmore long-term irrigation field trial

The Winchmore Irrigation Research Station, located in mid-Canterbury, New Zealand (171°48'E, 43°47'S) (Figure 2.1), was established in 1947 to investigate the management of border-dyke flood irrigation for pastures and crops. The soil is classified as a shallow, free-draining Lismore-series stony silt loam (Stony Brown in New Zealand; Typic Dystrustept according to the USDA; Endoskeletic Cambisol; IUSS Working Group, 2015), formed from moderately weathered greywacke loess over gravels. Full details of the long-term irrigation trial are provided by McDowell and Rowley (2008), Rickard and Moss (2012), Kelliher et al. (2012), and Condron et al. (2014). Briefly, the trial was established in 1947 and comprised four replicate plots of different treatments arranged in randomized blocks. Each treatment was grazed by a separate flock of sheep to avoid nutrient transfer between treatments. The stocking rates were adjusted to ensure 80% of pasture utilization across all treatments. For this study, the following three treatments were sampled: control plots, consisting of unirrigated (ambient rainfall) pasture, plots receiving supplemental irrigation when gravimetric moisture (GM; kg water kg^{-1} soil) in the topsoil (0–15 cm) reached 10% ($irrigation_{10\%GM}$; close to permanent wilting point, 12%, for this soil) and plots irrigated when GM reached 20% ($irrigation_{20\%GM}$). All treatments received rainfall at an average of 740 mm $year^{-1}$. A border-dyke (flood) irrigation scheme delivered 100-mm increments between October (spring) and April (autumn) each year. The $irrigation_{10\%GM}$ plots were irrigated at an average of 2.6 events $year^{-1}$ (260 mm $year^{-1}$ of supplemental irrigation). The $irrigation_{20\%GM}$ plots received 7.7 events $year^{-1}$, on average (770 mm $year^{-1}$ of supplemental irrigation). All treatments received 250 kg single superphosphate ha^{-1} $year^{-1}$ (average P content in single superphosphate in New Zealand since 1947 = 9.3%, equivalent to 23.3 kg P ha^{-1} $year^{-1}$). Lime ($CaCO_3$) was applied in 1947 (5 Mg ha^{-1}) and again in 1965 (1.9 Mg ha^{-1}) to maintain soil pH above 6.

2.2.2 Soil sampling

Soil sampling was carried out in 2009, 62 years after the trial was established, as described by Condron et al. (2014). A 100-cm wide × 125-cm deep × 200-cm long pit was excavated in each replicate of the three treatments (12). Material collected from six depths intervals (0–7.5, 7.5–15, 15–25, 25–50, 50–75 and 75–100 cm) was separated by sieving (soil was defined as passing through 2 mm), and the fractions were weighed for each depth increment (Figure 2.2).

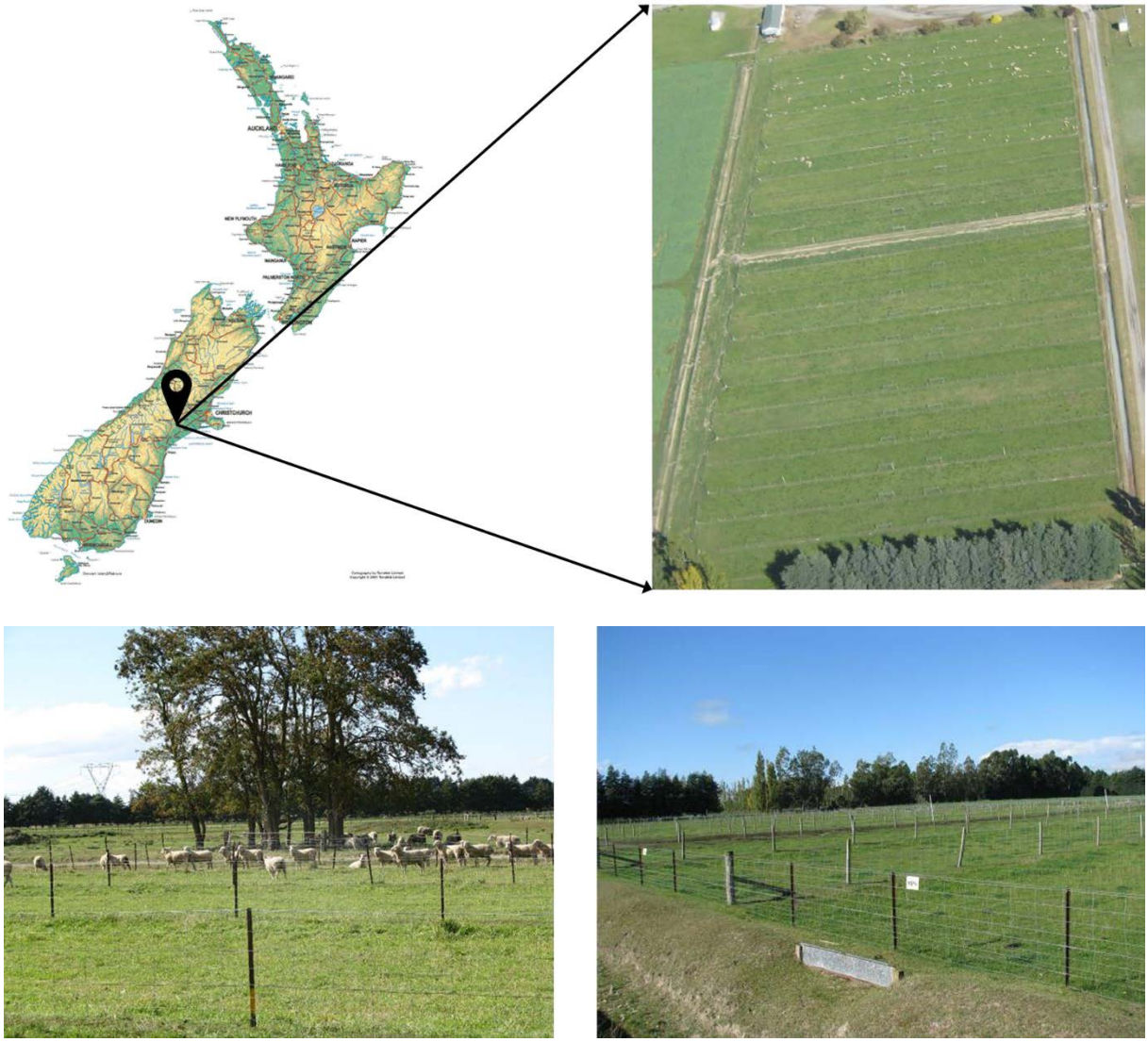


Figure 2.1 Location of the Winchmore Research Station, New Zealand (top left). Aerial image of the long-term irrigation trial (top right). Grazing sheep and fencing separating treatments (bottom left and right).

Figure 2.2 Excavating pits and soil sampling (top). Soil profile under irrigation treatment (bottom; photo from a replicate plot of the Irrigation$_{10\%GM}$ treatment).

2.2.3 Fractionation of P

Soil P fractionation was carried out according to the method described by Condron et al. (1996), and modified by analysing the residual P (i.e., non-extracted P) using H_2SO_4 + H_2O_2 digestion (Olsen and Sommers, 1982). Inorganic (Pi) and organic P (Po) were determined in solutions after sequential extraction of the soil fraction (passing through 2-mm sieve) with ammonium chloride, 1 M NH_4Cl (Pi_{AM}), sodium bicarbonate, 0.5 M $NaHCO_3$ pH 8.5 (Pi_{bic} and Po_{bic}), sodium hydroxide, 0.1 M NaOH (Pi_{OH}_I and Po_{OH}_I), hydrochloric acid, 1 M HCl (Pi_{HCl}), 0.1 M NaOH (Pi_{OH}_II and Po_{OH}_II) and digestion with H_2SO_4 + H_2O_2 (P_{res}). These fractions were grouped into four P pools according to their relative lability as follows: 'labile P' (sum of fractions Pi_{AM}, Pi_{bic} and Po_{bic}), 'moderately labile P' (Pi_{OH}_I + Po_{OH}_I), 'stable P' (Pi_{HCl} + Pi_{OH}_II + Po_{OH}_II) and 'residual P' (P_{res}) (Cross and Schlesinger, 1995). The total inorganic P extracted was obtained by summing the fractions Pi_{AM}, Pi_{bic}, Pi_{OH}_I, Pi_{HCl} and Pi_{OH}_II, whereas the total organic P extracted was given by the sum of Po_{bic}, Po_{OH}_I and Po_{OH}_II. Total soil P was determined separately by sodium carbonate fusion (Kuo, 1996). All soil P data are expressed in kg P ha^{-1}, which was calculated by multiplying the concentration of P (mg P kg^{-1}) by the quantity of soil determined in each depth increment. Importantly, this accounts for the volumetric content of stone and gravel at this site.

2.2.4 Statistical analysis

Differences in the means of soil P fractions across treatments for a given depth were investigated by one-way analysis of variance (ANOVA). The field trial encompasses three treatments arranged randomly in each of four blocks (3 treatments × 4 blocks, total of 12 plots). Accordingly, for the ANOVA summary, 3 degrees of freedom (d.f.) were attributed to the random effect of blocks, while 2 d.f. were attributed to treatments (total d.f. = 11). The normality of the residuals, homogeneity of variances and independence of samples (ANOVA assumptions) were considered during the data analysis. When differences associated with treatments were observed (P<0.05), Fisher's least significant difference (LSD, α = 0.05) was used to evaluate the significance of differences between means. All statistical analyses were done with R statistical software version 3.3.3 (R Core Team, 2017). The 'agricolae' package was used for calculating LSD values (de Mendiburu, 2017).

2.3 Results

Mean amounts of P (kg ha^{-1}) determined in the four pooled soil fractions (labile, moderately labile stable, and residual) for the various treatments at the different soil depth increments are presented in Table 2.1 and Figure 2.3 (P concentrations in mg P kg-1 for all fractions are presented in Appendix A, Table A.1). The moderately labile P fraction was the largest (245–725 kg P ha^{-1},average 441 kg P ha^{-1}) compared with stable P (129–466 kg P ha^{-1}, average 247 kg P ha^{-1}), residual P (60–274 kg P ha^{-1}, average 175 kg P ha^{-1}) and labile P (17–116 kg P ha^{-1}, average 53 kg P ha^{-1}) fractions. Labile P

decreased with soil depth, whereas most other P fractions were either similar or increased with depth to 50 cm and then decreased.

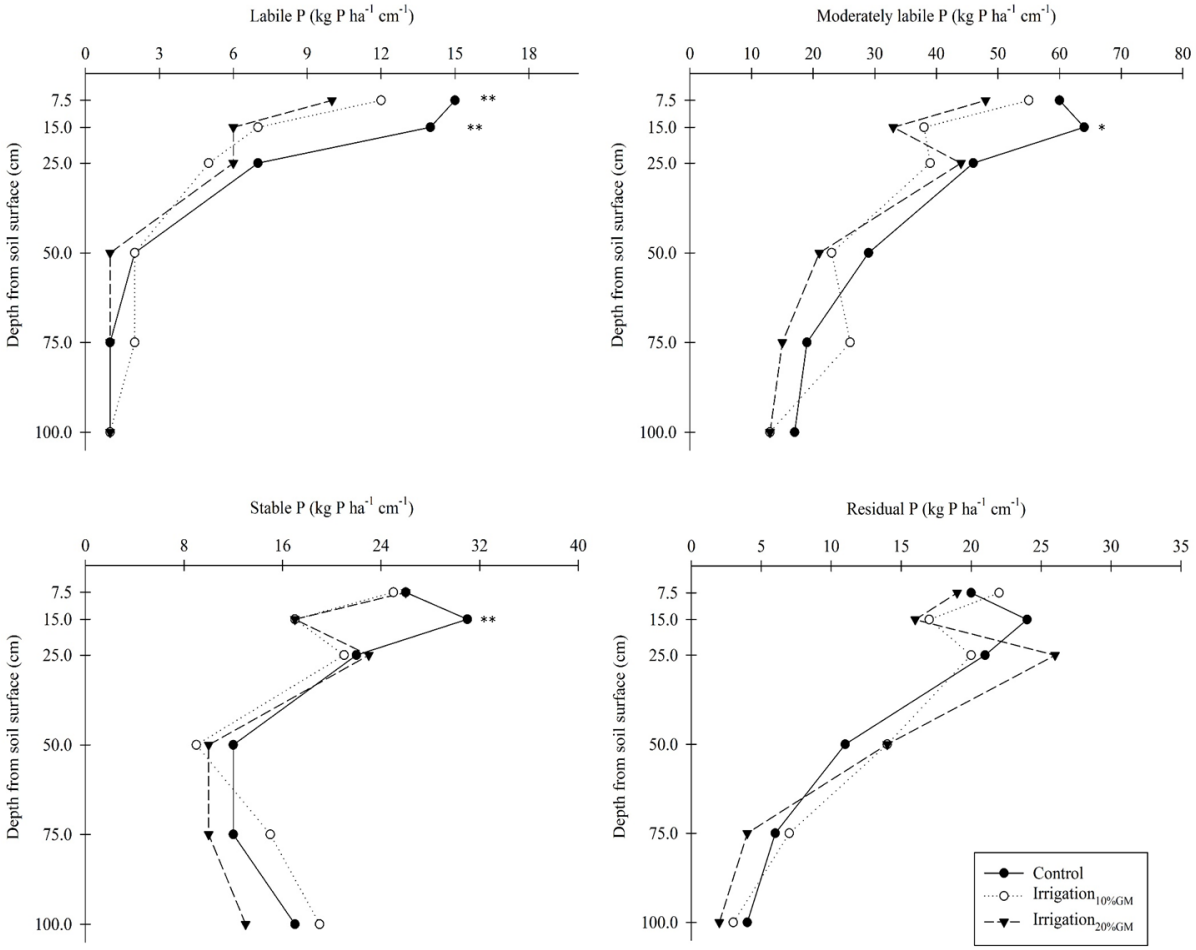


Figure 2.3 Mean amounts of various P fractions normalized to constant depth increments (kg P ha^{-1} cm^{-1}) in soils sampled to 100 cm depth (soil samples were collected from the layers 0-7.5, 7.5-15, 15-25, 25-50, 50-75 and 75-100 cm) under a not irrigated control and irrigated grazed pasture. One or two asterisks indicate significant differences at *P*<0.05 and *P*<0.01, respectively.

Significant differences among treatments were evident within the top 25 cm of soil for the labile, moderately labile and stable P fractions. Labile P was substantially greater in the top 15 cm of soil under the control (221 kg P ha^{-1}) than in the corresponding soil samples under both the irrigation$_{10\%GM}$ (143 kg P ha^{-1}) and irrigation$_{20\%GM}$ (114 kg P ha^{-1}) treatments. Comparable data for moderately labile P were 934, 699 and 608 kg P ha^{-1} for the control, irrigation$_{10\%GM}$ and irrigation$_{20\%GM}$ treatments, respectively. Differences in labile and moderately labile P determined among treatments in soil below 25 cm were not significant, although quantities of P in both fractions were generally smallest in soil under the irrigation$_{20\%GM}$ treatment between 25 and 75 cm.

Table 2.1 Average quantities of P (kg P ha^{-1}) determined in P fractions for soils sampled from different depths after 62 years under control and irrigated grazed pasture: labile P (Pi_{AM} + Pi_{bic} + Po_{bic}), moderately labile P (Pi_{OH}_I + Po_{OH}_I), stable P (Pi_{HCl} + Pi_{OH}_II + Po_{OH}_II), and residual P (P_{res}).

Treatment	Depth / cm					
	0–7.5	7.5–15	15–25	25–50	50–75	75–100
			Labile P / kg P ha^{-1}			
Control	116	105	69	59	32	25
$Irrigation_{10\%GM}$	87	56	48	60	48	17
$Irrigation_{20\%GM}$	71	43	55	23	29	19
LSD (5%)	23.6	30.6	20.0	45.0	40.4	13.6
P value*	0.010	0.006	0.099	0.153	0.495	0.330
			Moderately labile P / kg P ha^{-1}			
Control	454	480	464	725	486	431
$Irrigation_{10\%GM}$	412	287	391	563	654	335
$Irrigation_{20\%GM}$	363	245	439	528	374	316
LSD (5%)	71.1	133.9	108.9	421.2	371.7	206.9
P value	0.056	0.011	0.319	0.512	0.258	0.403
			Stable P / kg P ha^{-1}			
Control	197	230	219	307	292	427
$Irrigation_{10\%GM}$	186	129	205	227	385	466
$Irrigation_{20\%GM}$	194	125	229	242	253	314
LSD (5%)	35.3	50.6	53.2	176.8	302.0	205.2
P value	0.751	0.004	0.581	0.538	0.581	0.250
			Residual P / kg P ha^{-1}			
Control	152	183	207	274	147	90
$Irrigation_{10\%GM}$	164	128	204	340	169	72
$Irrigation_{20\%GM}$	144	118	259	353	94	60
LSD (5%)	22.8	57.4	82.5	191.6	89.5	35.1
P value	0.184	0.066	0.262	0.586	0.187	0.181

LSD (least significant difference) set at 5% probability, *testing the differences associated with treatments in each depth (columns), *P* value of *F* value of treatment effect (degrees of freedom 2 and 6).

.

The only other significant difference observed among treatments was for the stable soil P fraction at 7.5–15 cm, where P was greater under the control (290 kg P ha^{-1}) than the irrigated treatments (125 and 129 kg P ha^{-1} for $irrigation_{10\%GM}$ and $irrigation_{20\%GM}$ treatments, respectively). Similar trends were evident for residual P at this depth.

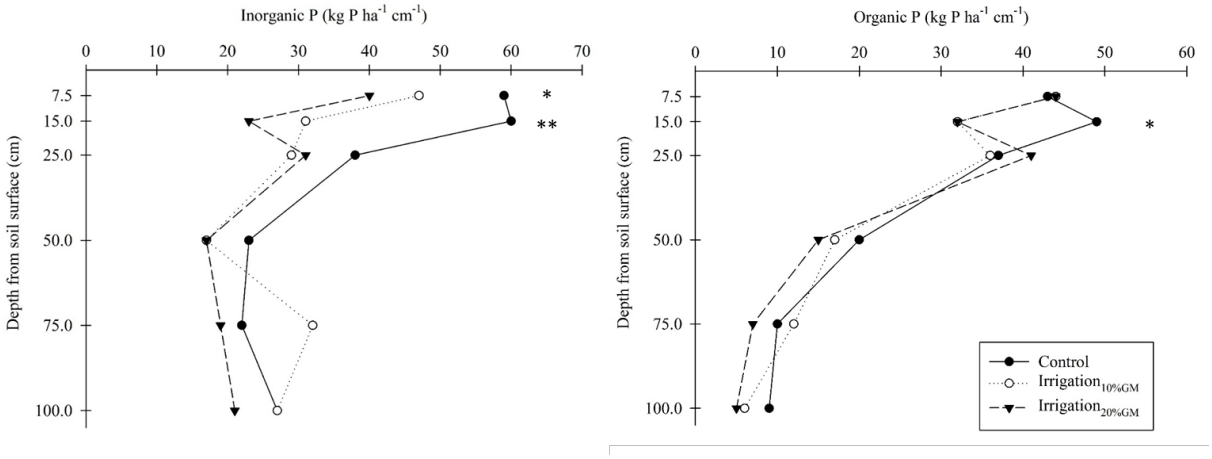


Figure 2.4 Mean amounts of inorganic and organic P pools normalized to constant depth increments (kg P ha^{-1} cm^{-1}) in soils sampled to 100 cm depth (soil samples were collected from the layers 0-7.5, 7.5-15, 15-25, 25-50, 50-75 and 75-100 cm) under a not irrigated control and irrigated grazed pasture. One or two asterisks indicate significant differences at *P*<0.05 and *P*<0.01, respectively.

Table 2.2, Figure 2.4 and Figure 2.5 show the quantities (kg P ha^{-1}) of total extracted inorganic and organic P (i.e. sum of inorganic and organic P determined in labile, moderately labile and stable P fractions), together with total soil P determined for the various treatments at different soil depths. For most soil depths and treatment combinations, the amounts of inorganic P were greater than that of organic P, especially below 50 cm. Significant differences among treatments for inorganic P were confined to the top 15 cm (0–7.5, 7.5–15 cm) whereby amounts were substantially greater under the control (893 kg P ha^{-1}) than for the $irrigation_{10\%GM}$ (586 kg P ha^{-1}) and $irrigation_{20\%GM}$ (472 kg P ha^{-1}) treatments. Similar trends were evident for inorganic P in the 15–50-cm (15–25, 25–50 cm) soil layer. Quantities of organic P were also significantly greater in the 7.5–15-cm and 75–100-cm soil layers under the control than for the irrigated treatments. Similar trends were detected for the 75–100-cm soil depth. No significant differences in soil organic P among treatments were observed at other soil depths, although organic P was consistently less under the $irrigation_{20\%GM}$ treatment than the control and $irrigation_{10\%GM}$ treatments between 25 and 75 cm.

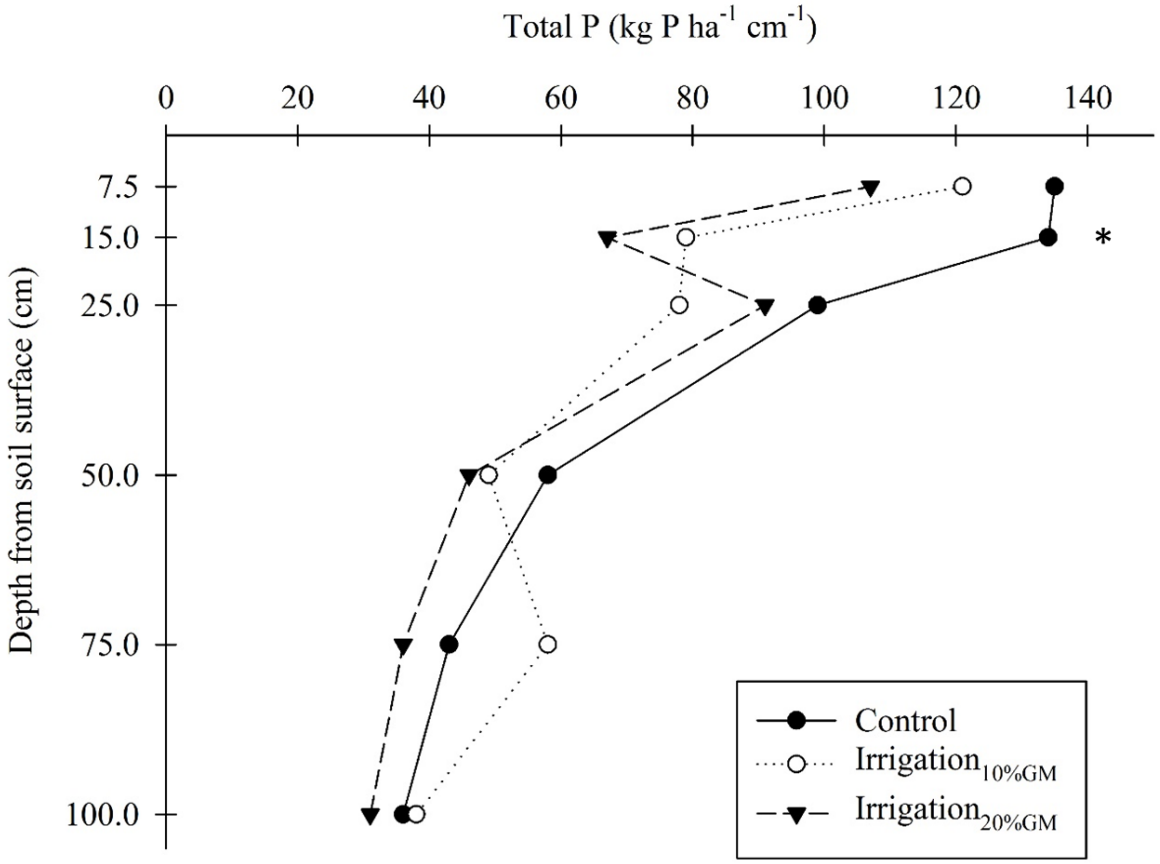


Figure 2.5 Amounts of total P normalized to constant depth increments (kg P ha^{-1} cm^{-1}) in soils sampled to 100 cm depth (soil samples were collected from the layers 0-7.5, 7.5-15, 15-25, 25-50, 50-75 and 75-100 cm) under a not irrigated control and irrigated grazed pasture. The asterisk indicates significant difference at *P*<0.05.

Quantities of total soil P were greater under the control than irrigated treatments for every soil depth except for the 50–100-cm depth, although these differences were significant for the 7.5–15-cm depth only (Table 2.2, Figure 2.5); this was consistent with the data for total extracted P. When total soil P was summed for all depths to 100 cm, however, it amounted to 6423, 5908 and 5054 kg P ha^{-1} for the control, irrigation$_{10\%GM}$ and irrigation$_{20\%GM}$ treatments, respectively (*P* value = 0.047; LSD (5%) = 1038). The difference in P between the control and irrigation$_{20\%GM}$ treatments was significant (*P*<0.05).

Table 2.2 Average quantities (kg P ha^{-1}) of total extracted inorganic P (Pi), total extracted organic P (Po), and total P determined for soils sampled from different depths after 62 years under a not irrigated control and irrigated grazed pasture (total extracted inorganic P = sum of Pi_{AM}, Pi_{bic}, Pi_{OH}_I, Pi_{HCl}, Pi_{OH}_II, total extracted organic P = sum of Po_{bic} + Po_{OH}_I + Po_{OH}_II).

Treatment	Depth / cm					
	0–7.5	7.5–15	15–25	25–50	50–75	75–100
			Total extracted Pi / kg P ha^{-1}			
Dryland	442	451	378	584	552	663
10% Irrigated	355	231	289	431	795	672
20% Irrigated	299	174	313	427	486	534
LSD (5%)	100.8	117.7	79.3	362.4	527.6	346.4
P value*	0.035	0.003	0.076	0.519	0.381	0.583
			Total extracted Po / kg P ha^{-1}			
Dryland	324	364	373	507	258	220
10% Irrigated	329	241	355	419	291	146
20% Irrigated	329	239	411	366	170	115
LSD (5%)	42.4	91.2	112.4	278.6	178.5	69.7
P value	0.947	0.024	0.506	0.497	0.300	0.025
			Total P / kg P ha^{-1}			
Dryland	1007	1000	1005	1439	1063	907
10% Irrigated	908	596	784	1225	1448	947
20% Irrigated	803	499	932	1160	892	768
LSD (5%)	196.2	275.9	372.3	783.0	892.6	402.3
P value	0.110	0.010	0.393	0.677	0.359	0.553

LSD (least significant difference) set at 5% probability, *testing the differences associated with treatments in each depth (columns), *P* value of *F* value of treatment effect (degrees of freedom 2 and 6).

2.4 Discussion

The results of this study clearly demonstrated that quantities of P present in the soil profile to 100 cm, after 62 years of identical P fertilizer inputs, were significantly different depending on the irrigation inputs and consequent increases in plant and animal productivity. The enhanced removal and transfer of P that occurred from the soil P pool under irrigation would probably have been influenced by a combination of factors including differences in pasture production, stocking rate and frequency of irrigation. For example, pasture production and animal stocking rate were 44 and 72% larger for the $irrigation_{10\%GM}$ and $irrigation_{20\%GM}$ treatments, respectively, than the control (Condron et al., 2014). There are five potential mechanisms and pathways that may collectively account for the apparent decline in soil P under irrigated pasture: (i) increased plant P uptake and retention; (ii) P

removal in animal products; (iii) P transfer in excreta; (iv) P losses in outwash associated with flood irrigation events; and (v) downward P transfer by leaching (Nguyen and Goh, 1992; Williams and Haynes, 1992; Haynes and Williams, 1993; Kemp et al., 2000; Sinaj et al., 2002; Toor et al., 2004a; Toor et al., 2004b; Toor et al., 2005; McDowell and Rowley, 2008; McDowell and Condron, 2012). These factors, combined with the long-term history of P fertilizer inputs, can explain the fact that most of the differences observed were between the control and irrigated plots in the 7.5–15-cm soil layer.

Williams and Haynes (1992) collected and examined soil P data (0–20 cm), together with plant and animal P data from the adjacent long-term fertilizer field trial at Winchmore. They calculated total gains and losses of P for an unfertilized adjacent area compared with plots, which received approximately 19 and 38 kg P ha^{-1} per year as single superphosphate for 38 years (1952–1990). Results of this study revealed that 35–37 % of the total fertilizer P applied over the period could not be accounted for in animal products removed or accumulation in residual soil and plant P pools. The authors concluded that most of the unaccounted fertilizer P was transferred in overland flow as suspended soil particles and animal manure during flood irrigation, although some P loss by saturated or preferential flow might have occurred from the soil. It is interesting to note that Nguyen and Goh (1992) used a mass-balance modelling approach to examine the P budget on the same trial. This study included assessment of within-field P transfers by animals (stock camping) and overland flow that occurred within the experimental plots. With this approach, they found that 83–94% of the fertilizer P applied over 35 years could be accounted for by a combination of animal products removed, P transfer and residual P in various soil and plant pools. This, in turn, suggested that net P loss from grazed pasture at Winchmore was substantially less than that indicated by Williams and Haynes (1992). The contrasting findings of these studies, carried out in the same trial reflect differences in the approaches used (e.g. comparison of fertilized treatments with nil P (control) treatment or wilderness site), together with substantial difficulties associated with determining the size of the various pools and associated connectivity and flux. Neither Nguyen and Goh (1992) or Williams and Haynes (1992) were able to include data on likely P transfer in irrigation outwash or by leaching because these data were not available at the time of their studies.

We used a combination of new data from this study, together with information and data from other related studies, to compile and compare P inputs, pools and outputs from the control, $irrigation_{10\%GM}$ and $irrigation_{20\%GM}$ treatments (Table 2.3, Figure 2.6). The sizes of the various P pools, except for the soil P pool, and outputs calculated on an annual and cumulative basis reflected differences in pasture production and grazing capacity determined by irrigation inputs; i.e. $irrigation_{20\%GM}$ > $irrigation_{10\%GM}$ > control. With regard to outputs, quantities of P removed in animal products (meat and wool) were small (0.8–2.4 kg P ha^{-1} yr^{-1}), as were estimated P leaching losses (0.15–0.42 kg P ha^{-1}

yr^{-1}). However, amounts of P transferred to stock camping areas (strip areas along the fence lines of the plots where sheep tended to camp, resulting in increased deposition of faeces (and nutrients)) within the plots by grazing animals in excreta (dung) were greater across all treatments (2.1–9.0 kg P ha^{-1} yr^{-1}). The net transfer of P from the plots in irrigation outwash (mainly dung) was 13 times larger from the $irrigation_{20\%GM}$ treatment (9.2 kg P ha^{-1} yr^{-1}) than the $irrigation_{10\%GM}$ treatment (0.7 kg P ha^{-1} yr^{-1}). Thus, outwash P transfer accounted for 44% of total P outputs from the $irrigation_{20\%GM}$ treatment compared with only 8% for the $irrigation_{10\%GM}$ treatment. It is evident, therefore, that the significant reduction (*P* value = 0.047) in soil profile P to 100 cm determined for the $irrigation_{20\%GM}$ ($irrigation_{20\%GM}$ = 5054 kg P ha^{-1}, control = 6423 kg P ha^{-1}; LSD (5%) = 1038) treatment could be attributed primarily to enhanced transfer and removal of P in irrigation outwash.

Quantities of P determined in the plant pools together with the various outputs and transfers collectively accounted for 75 and 91% of the observed decreases in soil P to 100 cm for the $irrigation_{10\%GM}$ and $irrigation_{20\%GM}$ treatments, respectively (Table 2.3). There are several factors that could account for these differences in recovery, and the failure to account for the decline in soil profile P compared with the control. This could be attributed mainly to the fact that, by necessity, most of the data for the various pools and outputs included in Table 2.3 were calculated rather than measured in the field trial. The soil P pool data to 100 cm was determined for this study, whereas irrigation outwash P was measured and reported by McDowell and Rowley (2008). On the other hand, data for both plant P pools, together with animal P removal, excretal P transfer and P leaching were calculated with information from a variety of sources which, in turn, relied mainly on measurements taken on the adjacent long-term fertilizer field trial at Winchmore. This experiment included variable inputs of annual P fertilizer with a uniform irrigation regime, and many more studies have been conducted and published from this trial compared with the irrigation trial (McDowell & Smith, 2012)

It is possible that estimated loss of P by leaching might have been underestimated, especially for the $irrigation_{20\%GM}$ treatment. Lysimeter studies carried on a similar Lismore soil measured annual P losses in drainage at 70 cm of 0.6–2.5 kg P ha^{-1} under simulated grazing with variable P and nitrogen inputs (Toor et al., 2004a; Toor et al., 2004b; Toor et al., 2005), however P inputs in those studies were considerably higher than in this experiment.

Table 2.3 Major P pools, inputs and outputs determined for a non-irrigated control and irrigated grazed pasture following over 62 years of trial.

P balance in the soil-plant-animal system	Irrigation rates					
	Control		$Irrigation_{10\%GM}$		$Irrigation_{20\%GM}$	
	kg P ha^{-1} $year^{-1}$	kg P ha^{-1}	kg P ha^{-1} $year^{-1}$	kg P ha^{-1}	kg P ha^{-1} $year^{-1}$	kg P ha^{-1}
Inputs						
Applied fertilizer P[a]		1440		1440		1440
Pools						
Soil P pool[b]		6423		5909		5054
Plant P residue[c]	2.5	155	3.5	217	4.6	285
Root P residue + stored root P[d]	0.15	25	0.16	27	0.19	30
Outputs						
Animal P removal[e]	0.8	50	1.9	115	2.4	147
Outwash P removal[f]	0.0	0	0.7	43	9.2	570
Excretal P transfer[g]	2.1	130	5.4	335	9.0	558
P leaching[h]	0.15	9	0.27	17	0.42	26
Total P accounted[i]		369	2.7	753	12.9	1617
Unaccounted or lost P[j]			2.1	130	2.0	122
Recovery (%) of decrease in soil P[k]				75		91

[a]Calculated based on data from Brown (1981) (concentrations of P in single superphosphate in New Zealand from 1947-1981) and personal communication with Alister Metherell (% of P in single superphosphate from 1981-2009).

[b]Total P in soil determined after fusion with Na_2CO_3 (n=24) according to Kuo (1996).

[c]Estimated based on 80% pasture utilization and 53% annual turnover rate (Nguyen and Goh, 1992); and P concentrations in pasture determined in 2003 and; pasture dry matter productivity from Condron et al. (2014).

[d]Estimated based on root P determinations, annual root residue (50%) and root P residue turnover (98%) from Nguyen and Goh (1992).

[e]Calculated from Nguyen et al. (1989) (P concentration in animal tissues) and Condron et al. (2014) (number of ewes ha^{-1} $year^{-1}$).

[f]Data from McDowell and Rowley (2008).

[g]Estimated from Nguyen and Goh (1992) as a direct function of the number of ewes ha^{-1} $year^{-1}$ and indirectly to the pasture dry matter yield (Condron et al., 2014).

[h]Estimated from Toor et al. (2004a).

[i]Sum of P pools and outputs, except soil P pool.

[j]Difference of the soil P pool in the irrigated treatments in relation to the control minus the difference of the accounted P.

[k]Recovery of decrease in soil P (%) = $\frac{\text{(Accounted P in the irrigated treatment - Accounted P in the control)}}{\text{(Soil P pool in the control - Soil P pool in the irrigated treatment)}}$ *100

Results of this study also revealed that long-term irrigation resulted in reduced amounts of soil organic P compared with the control. This is consistent with other data from this trial which showed that quantities of soil organic matter were significantly smaller under irrigated pasture than the control treatment (Condron et al., 2014). This was attributed to a combination of factors including enhanced biological activity (including earthworms) because of the larger soil moisture content, and changes in the amounts and quality of organic matter inputs.

2.5 Conclusions

The findings of this study demonstrated clearly that, after 62 years of identical annual P fertilizer inputs, flood irrigation of grazed pasture had resulted in depletion of soil profile P compared with non-irrigated control treatments. This was attributed to increased pasture production and grazing capacity which increased P removal in animal products and irrigation outwash, together with enhanced P transfer to animal camp sites. These results emphasize the importance of designing and adjusting irrigation inputs to grazed pasture to optimize increases in plant and animal production while limiting P transfer from the soil–plant system in drainage.

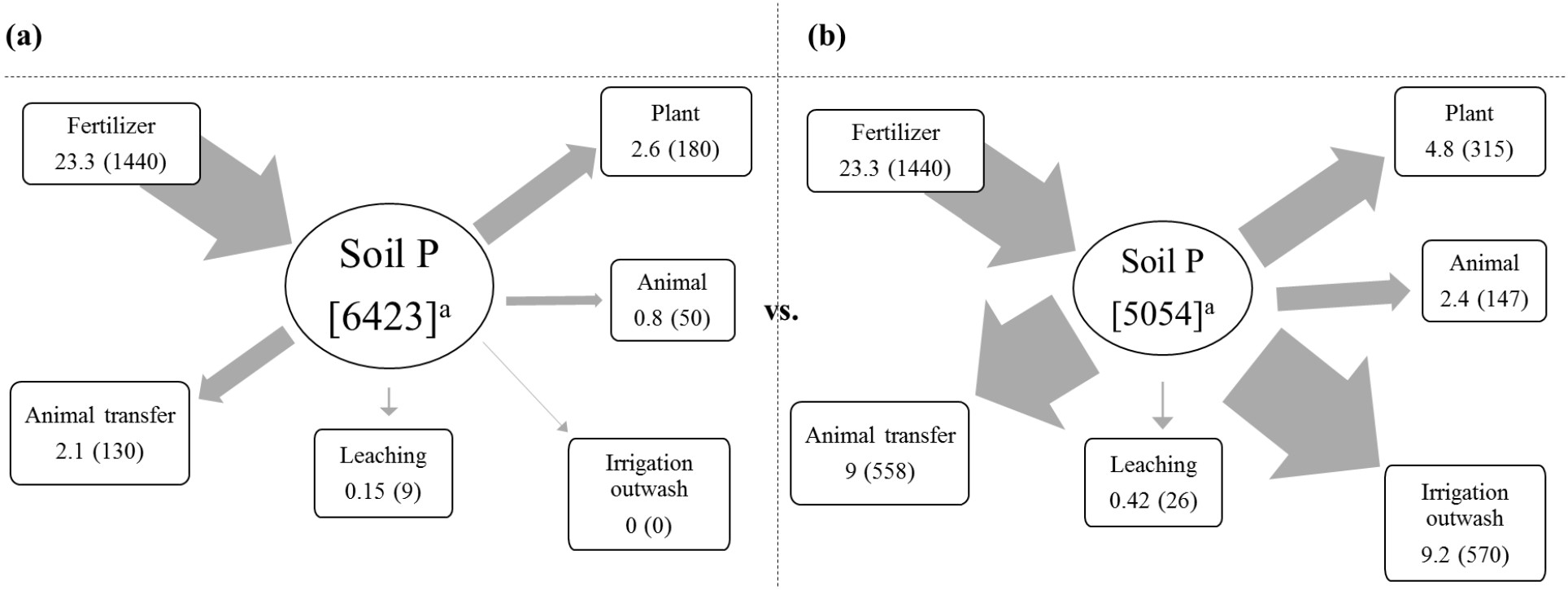


Figure 2.6 Phosphorus inputs, outputs and pools in the soil–plant–animal system compared to the control without irrigation (a) (rainfall, average of 740 mm year^{-1}) versus the contrasting irrigation$_{20\%GM}$ treatment (b) (rainfall plus irrigation applied when soil gravimetric moisture reached 20% [740 + 770 mm year^{-1}]). Numbers without parenthesis represent the annual P fluxes (kg P ha^{-1} year^{-1}). Numbers between parentheses are the accumulated amounts of P (kg P ha^{-1}) after 62 years. [a]Numbers in brackets are the total amounts of P (kg P ha^{-1}) in the soil profile to 100 cm after 62 years. Arrows representing fertilizer inputs are not at the same scale as others. Annual P inputs as fertilizer were calculated from the P concentration in single superphosphate (average = 9.3%) in New Zealand since 1947. Arrows indicating the P outputs are represented as an approximate relative scale. Phosphorus pools represented by plant (plant residue), animal and animal transfer (animal transfer within plots) were calculated from available literature, whereas leaching was estimated from Toor *et al*. (2004a). McDowell & Rowley (2008) measured phosphorus in the irrigation outwash. Soil P pool was quantified by the present study.

Chapter 3

Fate of Phosphorus Applied to Soil in Pig Slurry under Cropping in Southern Brazil

3.1 Introduction

The state of Santa Catarina is the largest producer and exporter of pork in Brazil. Despite being the 7th smallest state, it is responsible for nearly 28% of total pork production and 35% of total Brazilian pork exports (ABPA, 2016). In Brazil, the vast majority of commercial pig (*Sus scrofa domesticus*) production is conducted in confined (housed) systems and the waste from these generated as a slurry. Given the size of the local pork sector, this waste is of particular significance to Santa Catarina, and can represent either an important fertiliser or a potential environmental hazard depending on how the resource is managed.

To meet dietary requirements, pigs in housed systems are commonly fed corn (*Zea mays*) and soybean (*Glycine max*) based feed formulations. The main form of phosphorus (P) in seeds/grains is *myo*-Inositol-1,2,3,4,5,6-hexa*kis*phosphate, which is also referred to as 'phytic acid' or 'inositol phosphate' (Raboy, 2003). In order to be utilised, the phosphate anion (PO_4^{-3}) needs to be cleaved from the organic complex (inositol), a process catalysed by the enzyme phytase (Shin et al., 2001). Monogastric animals, including pigs, have low efficiency in utilising P present in grains (<15% for swine) due to their insufficient production of extracellular phytase, therefore resulting in P-rich animal waste (Cromwell, 2005; Cunha, 2012).

Pig waste, containing substantial quantities of P (and other nutrients) is commonly applied to agricultural land (Cassol et al., 2001). This practice is regarded as an effective strategy to reduce or eliminate the dependency of mineral P fertiliser inputs to maintain crop/pasture productivity, and results in looped nutrient cycles and sustainable productive systems (Choudhary et al., 1996; Cassol et al., 2001; Pandolfo and Ceretta, 2008). However, the repeated application of pig-waste to agricultural fields may lead to significant accumulation of both inorganic and organic P in soils. This may increase the potential for enhanced P loss in drainage, resulting in contamination of aquatic ecosystems (Berwanger et al., 2008; Gatiboni et al., 2008; Pandolfo et al., 2008; Lourenzi et al., 2014; De Conti et al., 2015; Gatiboni et al., 2015). Moreover, long-term P input and accumulation (commonly referred to

as ‘legacy P’, or ‘residual P’) are of global concern given the increasingly volatile supply and costs of phosphate rock resources (Cordell et al., 2011; Sattari et al., 2012; Haygarth et al., 2013; Ulrich and Frossard, 2014). Understanding the risk of repeated inputs of P-containing waste to soils is therefore important to facilitate sustainable management of animal waste in agroecosystems, and to remain resilient to variation in P-commodity prices and currency values on international markets.

Highly weathered soils dominate in tropical and subtropical parts of the globe (Cross and Schlesinger, 1995; Yang and Post, 2011). Oxisols cover more than 60% Brazil, are widely P limiting, and have an inherently high P adsorption capacity. Therefore, high inputs of P are normally required to support economically viable levels of agricultural production (Ker, 1997; Novais and Smyth, 1999; Motta et al., 2002; Almeida et al., 2003; Embrapa, 2013).

A field trial was established in Santa Catarina, Brazil in 2001 to assess agronomic responses of corn to different rates of pig slurry additions, together with changes in soil properties, and associated potential environmental impacts (Cassol et al., 2012; Mafra et al., 2015; Grohskopf et al., 2016). The objective of this study was to quantify the accumulation, distribution, and indicators of potentially adverse environmental impacts after the long-term pig slurry inputs to soils under cropping. Given the high P adsorption capacity of these soils, we hypothesised that the majority of P added via pig slurry was retained in the soil.

3.2 Materials and methods

3.2.1 Long-term field trial, Santa Catarina, Brazil

Soil properties and the agronomic responses of corn were determined in soil to which different rates of pig slurry additions had been applied. The trial was established in 2001 in Campos Novos, Santa Catarina, Brazil (27°23’ 34” S, 51°21’ 47” W) on an Oxisol formed from basalt rocks (Typic Hapludox [USDA]; Latossolo Vermelho Distroférrico [Embrapa, 2013]) (Figure 3.1). The soil is naturally acidic (pH 4.5) and P limited (Mehlich-1 extractable P < 3 mg kg^{-1}) (Almeida et al., 2003). The A horizon (0-20 cm depth) has 65% clay, 32% silt, and 3% sand. The clay fraction is predominantly kaolinite, 2:1 Al-hydroxy interlayer smectite and iron oxides (hematite and goethite) (Almeida et al., 2003). The climate at the location is classified as being humid mesothermal with mild summers (Cfb according to Köppen). The elevation is 863 m above sea level, the rainfall well distributed throughout the year (average = 1480 mm $year^{-1}$), and mean annual temperature is 16°C (January 21°C; June 12°C) (Epagri/Ciram). Prior to the establishment of the trial, the area had been used for cropping

(rotation of corn, soybean, wheat, and oats) under a no-tillage system for many years. The chemical properties of the soil to 20 cm at the trial establishment were: total carbon 25 g kg^{-1}, available P (Mehlich-1) 6 mg kg^{-1}, and pH (H_2O 1:1 v/v) 6.1. Further details of the site and trial set up are described in Cassol et al. (2012) and Mafra et al. (2015).

The five treatments investigated had received 15 annual additions (2001-2015) of pig slurry at rates of 0, 25, 50, 100, and 200 m^3 ha^{-1} y^{-1} (Figure 3.2). Each treatment had four replicate plots, each 12 m × 6.3 m, randomly arranged in blocks (total of 20 plots). Pig slurry was applied to the soil surface in October of each year after the die-down of the winter cover-crop (generally this was black oat [*Avena strigosa*], but was radish [*Raphanus sativus*, in 2005 and 2008]). Corn (*Zea mays*) was planted annually (except in 2003 when black bean [*Phaseolus vulgaris*] was grown) as the summer (main) crop in rotation with winter crops (utilised as cover crop, i.e. not harvested). The area remained under no-tillage system during the period of experiment.

The pig slurry originated from finishing pig barns that had been collected into open effluent ponds. Physicochemical properties (analysed each year) of the slurry were: pH 7.2 ± 0.3; dry matter 44 ± 17 kg m^{-3}; carbon (C) 22 ± 7 kg m^{-3}; total P 1.37 ± 0.6 kg m^{-3}; total nitrogen (N) 3.31 ± 17 kg m^{-3}; C:N ratio 6.6 ± 1.8; total potassium 1.62 ± 0.6 kg m^{-3}; total calcium 1.88 ± 0.7 kg m^{-3}; and total magnesium 0.77 ± 0.3 kg m^{-3}.

3.2.2 Soil sampling and analyses

In May 2016, soil samples were obtained by randomly collecting 8 subsamples per plot at the depths of 0-2.5, 2.5-5, 5-10, 10-20, and 20-40 cm. The composite soil samples were dried at 65°C to constant weight and sieved (<2 mm) prior to analyses. Soil bulk density was also assessed (Table 3.1) in order to correct the soil attributes and concentrations to units of mass per area in hectares. This involved collecting a known volume of soil in metal rings (5.8 cm diameter) at the respective soil layer, and weighing after drying at 105°C to constant mass. Total soil C and N were analysed by an elemental analyser (LECO 2000 CNS Analyser). The quantities of total C and N (in units of Mg ha^{-1} and kg ha^{-1}, respectively) for each soil layer were normalised to a constant depth increment and are expressed in units of Mg ha^{-1} cm^{-1} and kg ha^{-1} cm^{-1}, respectively, to allow comparison between depths.

Soil P was characterized by chemical fractionation with ammonium chloride (1 M NH_4Cl), sodium bicarbonate (0.5 M $NaHCO_3$, pH 8.5), sodium hydroxide (0.1 M NaOH), hydrochloric

acid (1 M HCl), and a second extraction with 0.1 M NaOH (sonicated for 5 minutes) (Hedley et al., 1982; Condron et al., 1996; Condron and Newman, 2011). Total soil P was determined in a separate soil sample after digestion with concentrated sulphuric acid (H_2SO_4) and hydrogen peroxide (H_2O_2 30% v/v) according to Olsen and Sommers (1982). Total extracted organic P was determined as the sum of organic P extracted with $NaHCO_3$ and first and second extractions with NaOH, whereas total extracted inorganic P was calculated as the sum of inorganic P in all extracted fractions. The difference between total soil P and total extracted P (inorganic + organic) was regarded as non-extractable soil P. These multiple soil P fractions were grouped into four P pools according to their relative lability as follows: 'labile P' (sum of fractions NH_4Cl and $NaHCO_3$ [inorganic and organic]), 'moderately labile P' (first NaOH [inorganic and organic]); 'stable P' (HCl + second NaOH [inorganic and organic]); and 'residual P' ('non-extractable P') (Cross and Schlesinger, 1995). The quantities of total P, total inorganic P, total organic P and inorganic and organic P fractions in the soil to 40 cm were expressed in units of kg ha^{-1}, while for each depth increment these P parameters were expressed in units of kg ha^{-1} cm^{-1} to allow comparison between depths.

The degree of phosphate saturation (DPS) was determined as described in Pierzynski (2000). The DPS index relates the proportion of potential P adsorption sites occupied by P, and is an indicator of potential risks and P movement in the soil profile. In brief, composite samples (combined replicates) of the contrasting treatments receiving pig slurry at rates of 0 and 200 m^3 ha^{-1} $year^{-1}$ were extracted with oxalate solution (0.114 M ammonium oxalate + 0.09 M oxalic acid, pH 3.0). Concentrations of P, iron (Fe) and aluminium (Al) in the extracts were determined by ICP-OES and followed by the calculations:

Equation 3 $$\mathbf{PSI = P_{ox} / (Fe_{ox} + Al_{ox})}$$

Equation 4 $$\mathbf{DPS = 200 \times PSI}$$

Where, PSI: phosphorus sorption index; P_{ox}, Fe_{ox} and Al_{ox} are the molar concentrations (mmol kg^{-1}) of the elements and DPS: degree of phosphate saturation.

Potential for P release in drainage was assessed by plotting values of P concentration (in mg P kg^{-1}) present in the first extraction solution of the P fractionation scheme (NH4Cl-P). Only the topmost soil layers (i.e. 0-2.5 and 2.5-5 cm) were considered following recommendations by McDowell et al. (2001) and Schroeder et al. (2004) for environmental assessment. Easily extractable P in soil is considered as an indicator of potential adverse effects resulting from soil erosion and surface runoff (McDowell and Sharpley, 2001;

Sharpley et al., 2001; McDowell et al., 2002). A ‘change-point’ is reached when the soil can no longer adsorb P, and consequently, large amounts of weakly bound P are present (here represented by concentrations of NH_4Cl-P) so that potential P transfers are increased (McDowell et al., 2001; Schroeder et al., 2004).

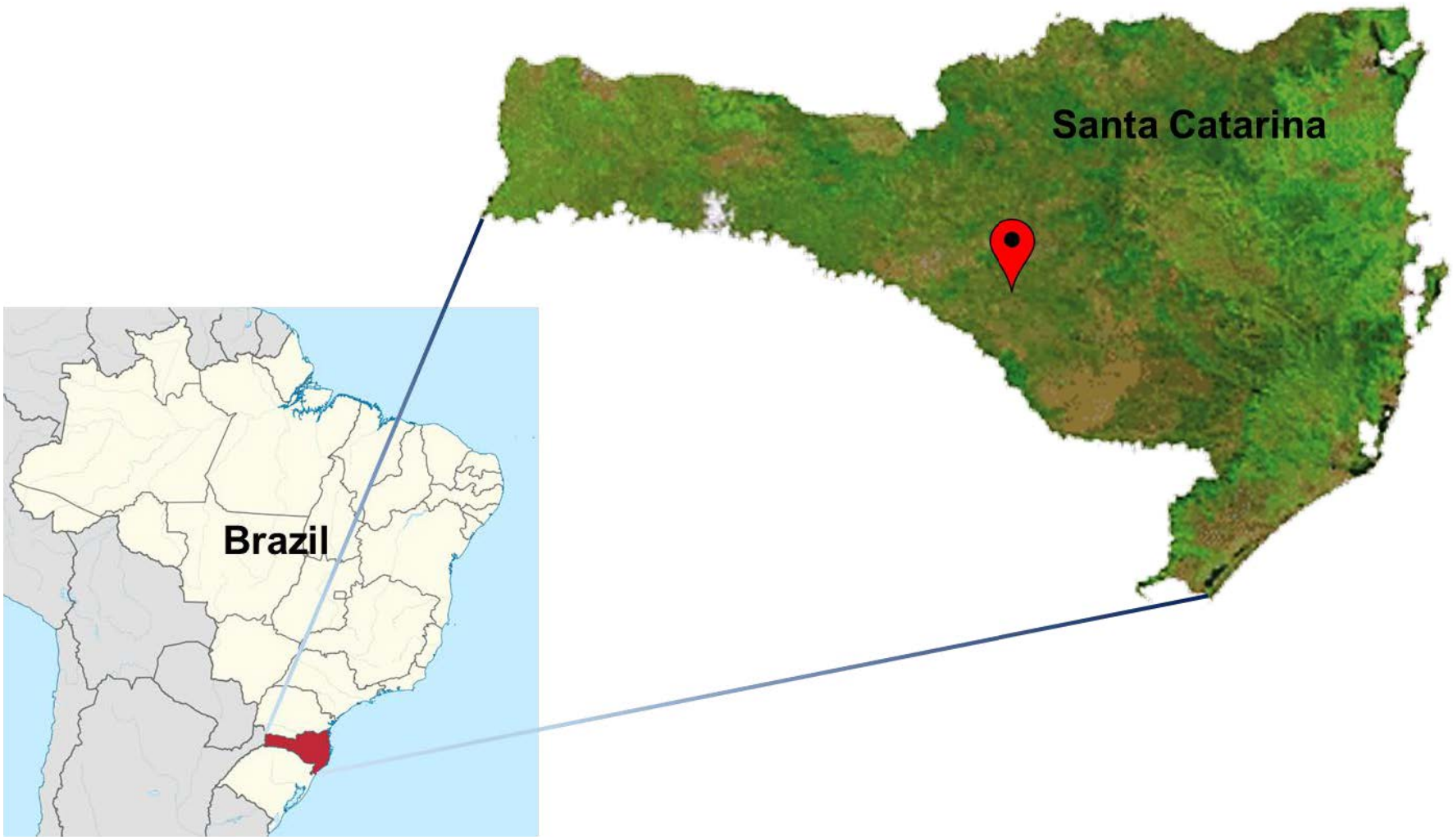


Figure 3.1 Location of the long-term field trial, Santa Catarina, Brazil.

Figure 3.2 Pig slurry addition to the treatment plots (top left), sown of corn (top right), and different stages of corn development (bottom left and right). Images kindly provided by Wagner Sacomori.

3.2.3 Phosphorus mass balance

The productivity of corn and black bean (2003 main crop) was determined as harvestable grain yields. Total amounts of P exported were calculated based on the grain production and concentrations of P in grains (measured for corn and estimated for black beans). To determine the P budget of the system, the following approach was employed (units of kg P ha^{-1}):

Equation 5 $$P_{input} - \Delta P_{soil} - P_{output} - P_{residue} - P_{unaccounted} = 0$$

Where, P_{input} is P added in pig slurry, ΔP_{soil} is the change in soil P storage (soil P_{final} - soil $P_{initial}$), P_{output} is P exported in grain product, $P_{residue}$ is the P content of the crop residues, and $P_{unaccounted}$ is the difference between P_{input} and the sum of P pools accounted for. Therefore, the term $P_{unaccounted}$ reflects the cumulated uncertainties on the P accounting in the mass balance. For the control treatment, P_{input} was zero and $P_{unnacounted}$ was assumed to be negligible given the absence of P inputs and minimal soil losses in erosion and surface runoff (Cogo et al., 2003; Bagatini et al., 2011; Panachuki et al., 2011). Therefore, the control treatment was utilised to solve for 'soil $P_{initial}$', and was assumed to be equal across the experiment area. This allowed ΔP_{soil} in systems receiving pig slurry to be determined. Thus, accounted P in the system (for treatments receiving pig slurry inputs) was determined as:

Equation 6 $$\text{Accounted P (\%)} = \left(1 - \left(\frac{P_{unaccounted}}{P_{input}}\right)\right) \times 100$$

The P balance efficiency of the system was calculated following Simpson et al. (2011):

Equation 7 $$\text{P balance efficiency} = \frac{P_{output}}{(P_{output} + \Delta P_{soil})} \times 100$$

3.2.4 Statistics

Differences in the means of soil attributes and P fractions across treatments for a given depth were determined using one-way ANOVA and least significant difference ($\alpha = 0.05$). Corn yields were modelled as a function of pig slurry input via regression. All statistical analyses were conducted with GenStat 16 (GenStat, 2013).

3.3 Results

3.3.1 Pig slurry effects on soil carbon, nitrogen and phosphorus

Normalised amounts of soil C (Mg ha^{-1} cm^{-1}) and N (kg ha^{-1} cm^{-1}) in the soil profile to 40 cm depth under long-term pig slurry additions are presented in Figure 3.3. The respective quantities for each soil layer (in Mg C ha^{-1} and kg N ha^{-1}), together with the C:N and C:P mass ratios, are presented in Table 3.2. Increasing rates of pig slurry inputs resulted in significant increases in total amounts of soil C and N. The substantial increases in C were mostly confined to the top 0-5 cm soil, but for N these extended down to 10 cm. Concomitantly, C:N ratios significantly decreased in the 0-2.5 and 2.5-5 cm soil layers receiving pig-slurry inputs, although, differences were more evident at the highest input rates (100 and 200 m^3 ha^{-1} y^{-1}). Similar trends were observed for C:P ratios with significant decreases proportional to the rates of pig slurry inputs, however differences were only present in the soil to 20 cm.

Results of the P distribution among different pools in the soil are presented in Figure 3.4, and Figure 3.5. Total quantities of each P pool (kg P ha^{-1}) in the respective layers are presented in Table 3.3 and Table 3.4. Constant additions of pig slurry significantly increased P in all fractions, except for the residual pool (i.e. non-extractable by the chemical fractionation, Figure 3.4). Significant increases in the amounts of labile P as a function of slurry inputs were evident at rates equal or higher to 50 m^3 ha^{-1} y^{-1}. Again, these differences were confined to the top 20 cm soil.

Similar trends were observed for stable P fractions for slurry rates equal or higher to 50 m^3 ha^{-1} y^{-1} to 20 cm depth. Increases in the moderately labile P fraction followed the same trend with significant increases in P concentrations and downward movement as a result of increasing input rates of pig slurry. Moreover, a significant increase in this P pool was observed in the subsoil (20-40 cm) under the treatment receiving 200 m^3 ha^{-1} y^{-1} of slurry.

A similar pattern was observed for the total inorganic P (Figure 3.5). Long-term pig slurry additions resulted in increases in total inorganic P in the 0-40 cm soil layer that were proportional to the amount of slurry applied. Substantial amounts of total organic P accumulated in the upper part of the soil profile (0-10 cm), where significant differences were evident under the highest input rates (100 and 200 m^3 pig slurry ha^{-1} y^{-1}). Increases in organic P were corresponded with increases in soil C and N, and their distribution in the soil profile imposed by the no-tillage system (i.e. no disturbance in the topsoil).

Increases in the amounts of total P and profile distribution were consistent and proportional to input rates (Figure 3.6; Figure 3.7; Figure 3.9). Nonetheless, differences were confined to top 0-20 cm soil layer. The highest rate of pig slurry input (200 m^3 ha^{-1} y^{-1}) led to a 2-fold increase in total P in the 0-20 cm soil layer compared with the control. On average, pig slurry inputs resulted in total P increases of 25, 57, 106, and 159 kg P ha^{-1} y^{-1} in the soil to 40 cm depth under the treatments receiving 25, 50, 100, and 200 m^3 slurry ha^{-1} y^{-1}, respectively (Figure 3.9B). The corresponding annual rate of organic P accumulation under the same treatments were equal to 7.6, 9.9, 23, and 27.5 kg P ha^{-1}, respectively. Extractable inorganic and organic P forms accumulated in soil (0-40 cm) corresponded on average, to 57 ± 10 and 22 ± 5 % of the total soil P, respectively.

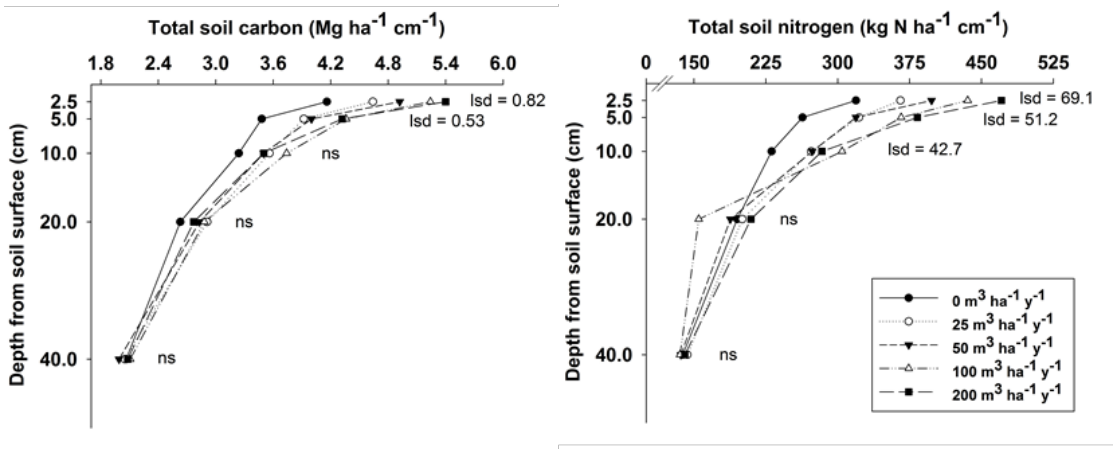


Figure 3.3 Amounts of soil carbon (Mg ha^{-1} cm^{-1}) and nitrogen (kg ha^{-1} cm^{-1}) to 40 cm depth after long-term pig slurry additions at different rates. Significant differences (P<0.05) among treatments for a given depth are represented by the presence of the least significant difference value (lsd). ns = not significant. Note that the scales are different.

Table 3.1 Mean data of soil bulk density in different layers to 40 cm under a no-tillage cropping system, and receiving long-term pig slurry additions.

Pig slurry rate	Depth (cm)					Mean
	0-2.5	2.5-5	5-10	10-20	20-40	
	Soil bulk density (g cm^{-3})					
0 m^{-3} ha^{-1} y^{-1}	1.22	1.27	1.29	1.24	1.21	1.25
25 m^{-3} ha^{-1} y^{-1}	1.20	1.17	1.33	1.34	1.22	1.25
50 m^{-3} ha^{-1} y^{-1}	1.24	1.26	1.30	1.33	1.24	1.28
100 m^{-3} ha^{-1} y^{-1}	1.22	1.12	1.22	1.31	1.23	1.22
200 m^{-3} ha^{-1} y^{-1}	1.10	1.21	1.22	1.28	1.19	1.20
Mean	1.20	1.21	1.27	1.30	1.22	1.24

Table 3.2 Amounts of carbon (Mg C ha^{-1}), nitrogen (kg N ha^{-1}) and ratios C:N and C:P in soils under cropping receiving yearly pig slurry additions at different rates for 15 years. One, two or three asterisks indicate significant differences among treatments respectively at *P*<0.05, *P*<0.01 and *P*<0.001. ns = not significant. LSD = least significant difference.

Pig slurry rate	Depth (cm)					
	0-2.5	2.5-5	5-10	10-20	20-40	0-40
			Carbon (Mg C ha^{-1})			
0 m^{-3} ha^{-1} y^{-1}	10.4	8.7	16.2	26.3	41.3	103
25 m^{-3} ha^{-1} y^{-1}	11.6	9.8	17.8	29.1	41.0	109
50 m^{-3} ha^{-1} y^{-1}	12.3	10.0	17.5	28.3	39.8	108
100 m^{-3} ha^{-1} y^{-1}	13.1	10.9	18.7	28.8	42.1	114
200 m^{-3} ha^{-1} y^{-1}	13.5	10.8	17.5	27.7	41.6	111
LSD	2.05*	1.32*	ns	ns	ns	4.87**
			Nitrogen (kg N ha^{-1})			
0 m^{-3} ha^{-1} y^{-1}	798	658	1155	1951	2767	7330
25 m^{-3} ha^{-1} y^{-1}	914	806	1361	2005	2862	7949
50 m^{-3} ha^{-1} y^{-1}	995	798	1367	1880	2727	7767
100 m^{-3} ha^{-1} y^{-1}	1089	916	1523	1550	2709	7786
200 m^{-3} ha^{-1} y^{-1}	1177	958	1418	2095	2810	8457
LSD	173**	128**	213*	ns	ns	673*
			C:N ratio			
0 m^{-3} ha^{-1} y^{-1}	13.1	13.3	14.0	13.5	14.9	14.0
25 m^{-3} ha^{-1} y^{-1}	12.7	12.1	13.1	14.5	14.4	13.7
50 m^{-3} ha^{-1} y^{-1}	12.3	12.6	12.8	15.1	14.6	13.9
100 m^{-3} ha^{-1} y^{-1}	12.0	11.9	12.3	14.7	15.6	14.6
200 m^{-3} ha^{-1} y^{-1}	11.5	11.3	12.4	13.5	14.9	13.2
LSD	0.58***	0.51***	ns	ns	ns	ns
			C:total P ratio			
0 m^{-3} ha^{-1} y^{-1}	28.8	27.0	28.2	27.4	27.4	27.6
25 m^{-3} ha^{-1} y^{-1}	28.4	25.9	26.1	27.5	27.0	27.0
50 m^{-3} ha^{-1} y^{-1}	25.1	22.0	22.9	25.5	24.9	24.4
100 m^{-3} ha^{-1} y^{-1}	20.9	18.4	18.0	22.9	26.4	22.2
200 m^{-3} ha^{-1} y^{-1}	15.7	13.2	13.6	20.8	27.4	19.1
LSD	2.27***	3.03***	2.81***	3.36**	ns	2.6***

The concentration of total soil P accumulated under the different treatments were reflected in changes in the P saturation measured in the soil profile (Figure 3.6). Substantial differences were mainly present in the 0-20 cm layer. The magnitude of increases in this index in the 200 m^3 ha^{-1} y^{-1} slurry treatment compared to the control were 8-, 12-, 8-, 3-, and 2-fold in the soil layers 0-2.5, 2.5-5, 5-10, 10-20, and 20-40 cm, respectively (Figure 3.6).

Figure 3.7 summarizes the impact of long-term pig slurry additions on the amounts of total P (kg P ha^{-1}) accumulated in the soil to 40 cm, and concentrations of weakly bound P extracted with NH_4Cl salt (NH_4Cl-P, mg P kg^{-1}) in the topmost soil layers (0-2.5 and 2.5-5 cm). A characteristic 'change-point' (threshold after which large P concentrations in NH_4Cl-P were observed) was observed in the top soil layers (0-2.5, and 2.5-5 cm) under slurry rates exceeding 50 m^3 ha^{-1} y^{-1}, where significant concentrations of weakly bound P were present.

3.3.2 Phosphorus mass balance

Inputs of P via pig slurry were: 0, 34, 69, 137, and 275 kg P ha^{-1} y^{-1} on average for the treatments receiving 0, 25, 50, 100, and 200 m^3 slurry ha^{-1} y^{-1} (Table 3.5). Figure 3.8 presents corn yield responses to pig slurry input rates. Estimated maximum corn grain yield (average of 13 years) was 9.2 Mg ha^{-1} with the slurry rate of *c*. 150 m^3 ha^{-1} y^{-1}, whereas 90% of maximum yield was estimated as 8.3 Mg ha^{-1} obtained with the slurry rate input of *c*. 90 m^3 ha^{-1} y^{-1}. That is, for a 10% increase in yield, pig slurry input was required to increase by 70% (corresponding to adding an extra 60 m^3 ha^{-1} y^{-1}).

Table 3.5 and Figure 3.9A present the P mass balance in various pools in the soil-plant system. Phosphorus exported from the system after 15 years were related to grain yields and increased from 7 to 18 kg P ha^{-1} y^{-1} on average, for the treatments receiving 0 and 200 m^3 pig slurry ha^{-1} y^{-1}, respectively (P exported is the sum of total P output in products, including P exported in black bean in 2003). Except for the 200 m^3 ha^{-1} y^{-1} treatment, the majority of added P to the cropping system could be accounted for by either grain exports, soil storage, or crop residues (86 to 94 % for slurry inputs of 25 to 100 m^3 ha^{-1} y^{-1}). Between 58 and 82% of the total P added by slurry was accumulated in the soil to 40 cm (Table 3.5). The highest P balance efficiency was achieved at the lowest pig slurry rate (25 m^3 ha^{-1} y^{-1}).

Table 3.3 Amounts of phosphorus (kg P ha^{-1}) determined in different fractions in soils under cropping receiving yearly pig slurry additions at different rates for 15 years. One, two or three asterisks indicate significant differences among treatments respectively at *P*<0.05, *P*<0.01 and *P*<0.001. ns = not significant. LSD = least significant difference.

Pig slurry rate	Depth (cm)					
	0-2.5	2.5-5	5-10	10-20	20-40	0-40
	Labile P (kg P ha^{-1})					
0 m^{-3} ha^{-1} y^{-1}	8.5	6.8	13	19	25	73
25 m^{-3} ha^{-1} y^{-1}	9.5	8.5	15	23	28	85
50 m^{-3} ha^{-1} y^{-1}	16	10	18	18	25	87
100 m^{-3} ha^{-1} y^{-1}	37	32	35	24	26	154
200 m^{-3} ha^{-1} y^{-1}	64	61	69	25	27	246
LSD	6.2***	7.7***	10***	4.4*	ns	24***
	Moderately labile P (kg P ha^{-1})					
0 m^{-3} ha^{-1} y^{-1}	142	124	229	307	401	1203
25 m^{-3} ha^{-1} y^{-1}	195	172	284	359	401	1410
50 m^{-3} ha^{-1} y^{-1}	240	212	324	388	429	1594
100 m^{-3} ha^{-1} y^{-1}	327	324	505	472	429	2057
200 m^{-3} ha^{-1} y^{-1}	454	440	674	597	530	2695
LSD	57***	47***	76***	109***	34*	284***
	Stable P (kg P ha^{-1})					
0 m^{-3} ha^{-1} y^{-1}	65	61	115	193	213	646
25 m^{-3} ha^{-1} y^{-1}	85	79	134	198	234	730
50 m^{-3} ha^{-1} y^{-1}	106	92	158	211	236	802
100 m^{-3} ha^{-1} y^{-1}	134	127	206	264	238	969
200 m^{-3} ha^{-1} y^{-1}	188	171	292	263	244	1159
LSD	26***	16***	35***	49*	ns	122***
	Residual P "non-extractable" (kg P ha^{-1})					
0 m^{-3} ha^{-1} y^{-1}	190	173	287	560	1055	2265
25 m^{-3} ha^{-1} y^{-1}	171	169	332	611	1053	2335
50 m^{-3} ha^{-1} y^{-1}	193	202	363	657	1140	2554
100 m^{-3} ha^{-1} y^{-1}	208	190	416	670	1122	2605
200 m^{-3} ha^{-1} y^{-1}	201	198	416	634	921	2479
LSD	ns	ns	ns	ns	ns	ns

Table 3.4 Quantities of inorganic, organic and total phosphorus (kg P ha^{-1}) accumulated in soils under cropping receiving yearly pig slurry additions at different rates for 15 years. One, two or three asterisks indicate significant differences among treatments respectively at *P*<0.05, *P*<0.01 and *P*<0.001. ns = not significant. LSD = least significant difference.

Pig slurry rate	Depth (cm)					
	0-2.5	2.5-5	5-10	10-20	20-40	0-40
	Total extracted inorganic P (kg P ha^{-1})					
0 m^{-3} ha^{-1} y^{-1}	118	99	173	229	292	910
25 m^{-3} ha^{-1} y^{-1}	171	137	216	301	274	1100
50 m^{-3} ha^{-1} y^{-1}	247	204	277	310	284	1323
100 m^{-3} ha^{-1} y^{-1}	350	318	460	382	308	1819
200 m^{-3} ha^{-1} y^{-1}	487	474	715	583	416	2675
LSD	58***	48***	71***	109***	74**	265***
	Total extracted organic P (kg P ha^{-1})					
0 m^{-3} ha^{-1} y^{-1}	98	93	184	289	347	1011
25 m^{-3} ha^{-1} y^{-1}	118	122	217	279	389	1125
50 m^{-3} ha^{-1} y^{-1}	115	110	223	307	406	1159
100 m^{-3} ha^{-1} y^{-1}	148	165	287	377	384	1361
200 m^{-3} ha^{-1} y^{-1}	219	198	320	301	385	1424
LSD	44***	43**	59***	ns	ns	171**
	Total P (kg P ha^{-1})					
0 m^{-3} ha^{-1} y^{-1}	406	364	645	1078	1694	4187
25 m^{-3} ha^{-1} y^{-1}	460	428	765	1190	1716	4559
50 m^{-3} ha^{-1} y^{-1}	555	516	863	1273	1829	5036
100 m^{-3} ha^{-1} y^{-1}	706	673	1162	1429	1814	5784
200 m^{-3} ha^{-1} y^{-1}	966	920	1451	1518	1722	6578
LSD	119***	108***	158***	294***	ns	862***

3.4 Discussion

As expected, long-term pig slurry inputs and associated no-tillage increased quantities of P in soil with concomitant vertical movement proportional to application rate. However, increases in total amounts of P were only evident in the upper soil layer (0-20 cm. Phosphorus was preferentially accumulated in inorganic forms (Figure 3.5); while increases in organic P pools were confined to the soil 0-10 cm depth which was consistent with corresponding increases in soil organic C. It is possible that accumulation of organic P was due to the greater biomass production and residue returns to the soil (Rheinheimer et al., 2002; Condron et al., 2005; Mafra et al., 2015). Continuous P inputs via pig slurry at rates exceeding 50 m^3 ha^{-1} y^{-1} resulted in significant increases in concentrations of weakly bound P in the topsoil (0-5 cm), and therefore increasing potential for enhanced P transfer in overland flow (runoff) and associated adverse environmental effects (Carpenter et al., 1998; Ockenden et al., 2017). Increases in P levels were also consistently related to increases in the degree of P saturation index in the soil profile.

There is no evidence in this trial to support P losses via leaching processes (Cassol et al., 2012; Grohskopf et al., 2016). It is also unlikely that movement to deeper soil layers through macropore flow is an important pathway of P transfer to groundwater, especially given the high P sorption capacity of the soil (Almeida et al., 2003; Beven and Germann, 2013). Nevertheless, high doses of pig slurry inputs significantly increased nitrate (NO_3^-) leaching and potential contamination of water bodies with N (Grohskopf et al., 2016; Sacomori et al., 2016). Moreover, soil erosion and surface runoff can constitute significant pathways of P losses in managed systems under no-tillage (McDowell and Sharpley, 2001; Hart et al., 2004; Ceretta et al., 2005; Kleinman et al., 2009; Ceretta et al., 2010; O'Flynn et al., 2012; Wang et al., 2013; Lourenzi et al., 2014). For example, Ceretta et al. (2010), quantified P losses by surface runoff in an experiment in southern Brazil of between 6.4 to 14.4 kg P ha^{-1} y^{-1} from soils receiving pig slurry at rates of 20 to 80 m^3 ha^{-1} y^{-1}, respectively.

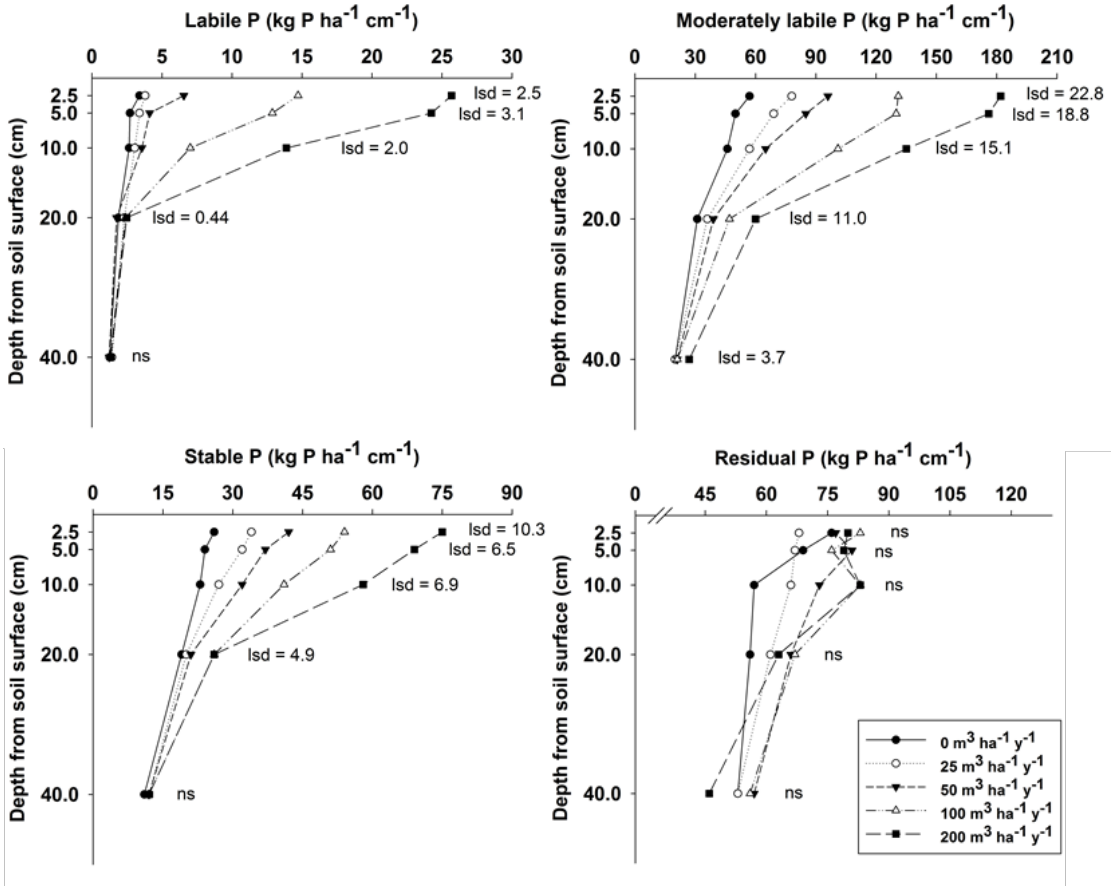


Figure 3.4 Distribution of phosphorus pools in the soil profile after long-term pig slurry additions at different rates. Note that amounts of P (in kg P ha^{-1}) represented by the horizontal axis (x-axis) were normalised to one cm increments in the depth. Significant differences (P<0.05) among treatments for a given depth are represented by the presence of the least significant difference value (lsd). ns = not significant. Note that the scales are different.

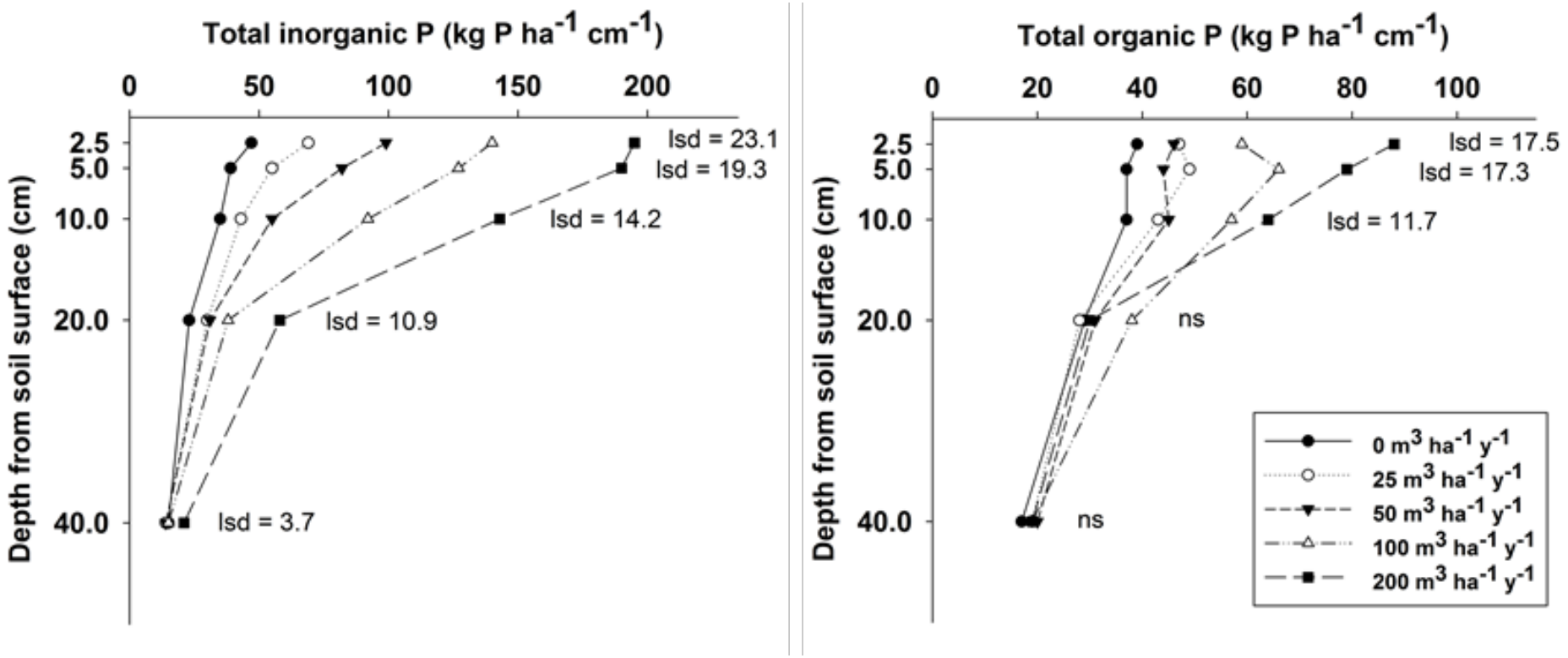


Figure 3.5 Inorganic and organic of phosphorus fractions accumulated in the soil profile after long-term pig slurry additions at different rates. Note that amounts of P represented by the horizontal axis (x-axis) were normalised to constant depth increments (kg P ha^{-1} cm^{-1}). Significant differences (P<0.05) among treatments for a given depth are represented by the presence of the least significant difference value (lsd). ns = not significant. Note that the scales are different.

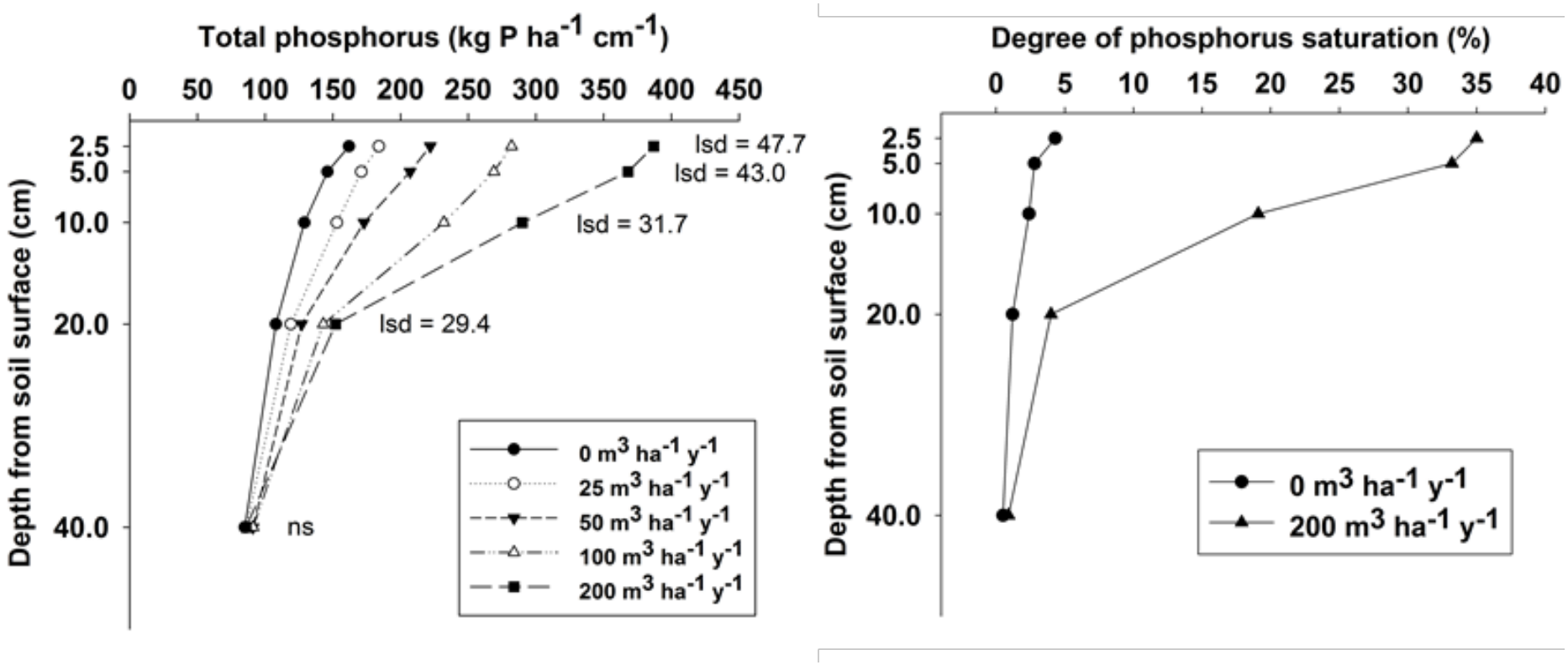


Figure 3.6 Total phosphorus accumulated in the soil profile (left); and degree of phosphorus saturation (right) after 15 years of pig slurry additions at different rates. Note that amounts of P represented by the horizontal axis (x-axis) were normalised to constant depth increments (kg P ha^{-1} cm^{-1}). Significant differences (P<0.05) among treatments for a given depth are represented by the presence of the least significant difference value (lsd). ns = not significant. Note that the scales are different.

Accumulation of soil P in inorganic fractions and movement in the soil profile is consistent with other studies evaluating the impacts of long-term P inputs as fertiliser, manures and organic residues (Leinweber et al., 1997; Gatiboni et al., 2008; Scherer et al., 2010; Guardini et al., 2012; Annaheim et al., 2015; De Conti et al., 2015; Schefe et al., 2015; Tian et al., 2017). Two similar studies in southern Brazil observed P movement to 30 cm depth in soils receiving between 38 to 350 kg P ha^{-1} y^{-1} via pig slurry after 8 years (Guardini et al., 2012; De Conti et al., 2015). However, soils from those studies had a lower P adsorption capacity (< 25% clay) in comparison to soils from this study. Gatiboni et al. (2008) on the other hand, observed downward movement of P to 15 cm in soils after only 2 years pig slurry applications with excessive P input rates up to 2040 kg P ha^{-1} y^{-1} to a sandy soil. In a contrasting environment, Tian et al. (2017) observed significant increases in soil P and degree of P saturation in the soil to 25 cm depth after 57 years of P inputs at rates of 18 and 36 kg P ha^{-1} y^{-1} under grazed pasture in New Zealand.

Mass balance calculations accounted for the majority of P added to the system in pig slurry. However, between 20 and 103 kg P ha^{-1} y^{-1} (corresponding to 15 and 37% of added P) could not be accounted for under the treatments receiving 100 and 200 m^3 slurry ha^{-1} y^{-1}, respectively (Table 3.5, Figure 3.9). This 5-fold increase in the unaccounted P between the treatments is consistent with increases in indicators of potential P losses (weakly bound P level and degree of P saturation shown in Figure 3.6 and Figure 3.7). Nonetheless, P accumulated in soil represented the main P pool in this agricultural system, and clearly demonstrate the great P adsorption capacity by these high clay content Oxisol soils (Novais and Smyth, 1999; Motta et al., 2002; Almeida et al., 2003). As discussed above, P transfer and loss by leaching was not significant in these soils, and so 6-38% of P inputs that could be accounted for by the mass balance may represent P removed in drainage by overland flow.

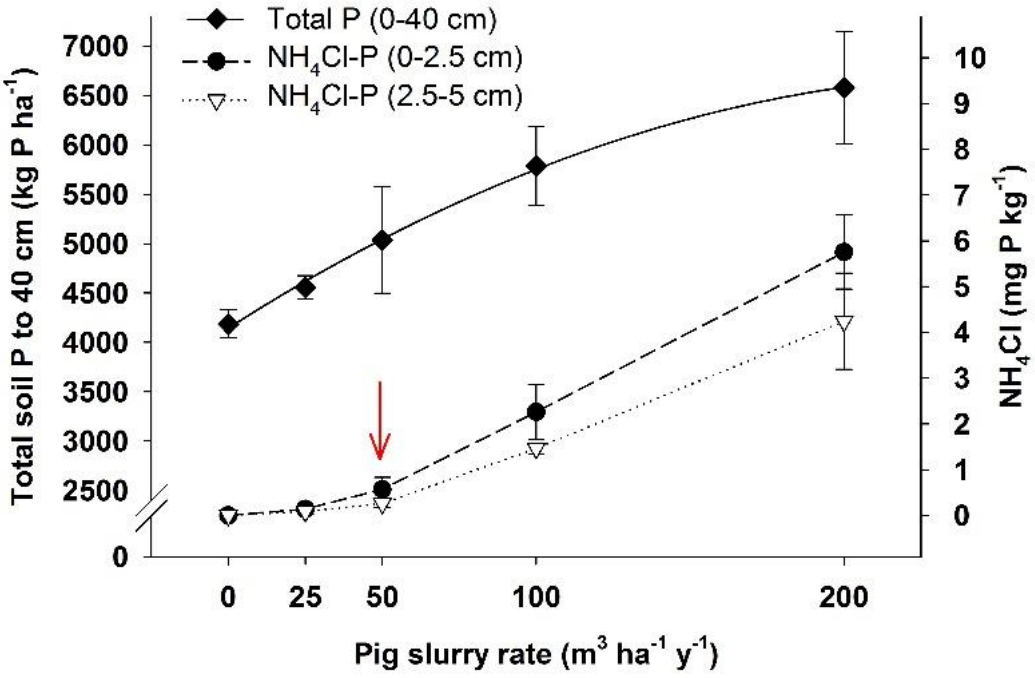


Figure 3.7 Representation of total phosphorus accumulated in the soil profile to 40 cm (left vertical axis, kg P ha^{-1}) after long-term pig slurry additions at different rates (horizontal axis); and respective amounts of weakly bound P extracted with 1M NH_4Cl (right vertical axis, mg P kg^{-1}) in the topmost soil layers (0-5 cm). Substantial increases in this labile fraction impose potential environmental risks mainly following soil erosion by runoff. Vertical bars represent standard deviation of averages. The arrow represents a 'change-point' (at slurry input rate of 50 m^3 ha^{-1} y^{-1}), where large amounts of P are at increased risk of movement by surface runoff (McDowell et al., 2001).

3.4.1 Practical implications

Until 2014, state legislation in Santa Catarina limited the rate of pig slurry application to 50 m^3 ha^{-1} y^{-1} (FATMA, 2003). However, this value was empirical and factors such as P retention capacity were disregarded. After 2014, the legislation was changed and the maximum slurry rate is now based on nutrient exports (P, N, and K) by crops (FATMA, 2014). However, because of excessive application of pig slurry over the years, many soils have high P contents, raising concerns about the continued use of these soils for pig slurry application. An approach to establish environmental safety thresholds for soil P levels in Santa Catarina was developed by Gatiboni et al. (2015). The method is based on the concentrations of bioavailable P (Mehlich-1) in the 0-10 cm soil layer, soil P adsorption capacity and clay contents according to the equation: P threshold $\left(\text{mg P kg}^{-1}\right)$ = 40 + Clay content (%). By

employing the proposed P-threshold prediction to the soil of this study, the critical level is estimated as 126 kg P ha^{-1} (65% clay, soil bulk density = 1.2 g cm^{-3} in 0-10 cm layer). Considering the amounts of labile P (Figure 3.4; Table 3.3) determined by P fractionation and establishing an approximate relationship, it was estimated that the P-threshold could be achieved with the pig slurry input at a rate of 129 m^3 ha^{-1} y^{-1}. However, at this rate, levels of weakly bound P (NH_4Cl-P, Figure 3.7) were estimated as 2-4 mg P kg^{-1} in the topmost soil layers to 5 cm depth, and therefore represent enhanced risk of surface runoff P losses (Carpenter et al., 1998; McDowell et al., 2001; Sharpley et al., 2001; Gatiboni et al., 2015).

With respect to corn productivity, data presented here and by Cassol et al. (2012) showed potential yield responses to pig slurry additions at rates up to 150 m^3 ha^{-1} y^{-1}. Despite that, critical levels of P in soils must be carefully monitored (Sharpley et al., 2015). The P mass balance results presented here clearly demonstrated the unbalanced P inputs needed in this conditions in order to achieve satisfactory yields, thereby leading to P accumulation in the soil pool and low efficiency of P fertilisations (Simpson et al., 2011). Research is urgently needed to find ways to mobilise legacy P and to reduce dependency of constant P inputs to highly weathered soils (Sanginga et al., 2003; Simpson et al., 2011; Sattari et al., 2012; Stutter et al., 2012; Condron et al., 2013; Haygarth et al., 2013; Sharpley et al., 2013).

Results of this study demonstrated that crop yields were substantially increased by pig slurry inputs, and therefore emphasise the value of this waste as fertiliser. However, for the conditions of this study, long-term input rates higher than 50 m^3 ha^{-1} y^{-1} significantly increased soluble and labile P fractions, which are of particular importance in the uppermost soil layers. This in turn raises concerns regarding continued P inputs, and build-up of legacy P in soils, following long periods of pig slurry applications to the same areas. Increased potential for P losses through soil erosion, and surface runoff require constant monitoring and adoption of soil conservationist practices Labrière et al. (2015).

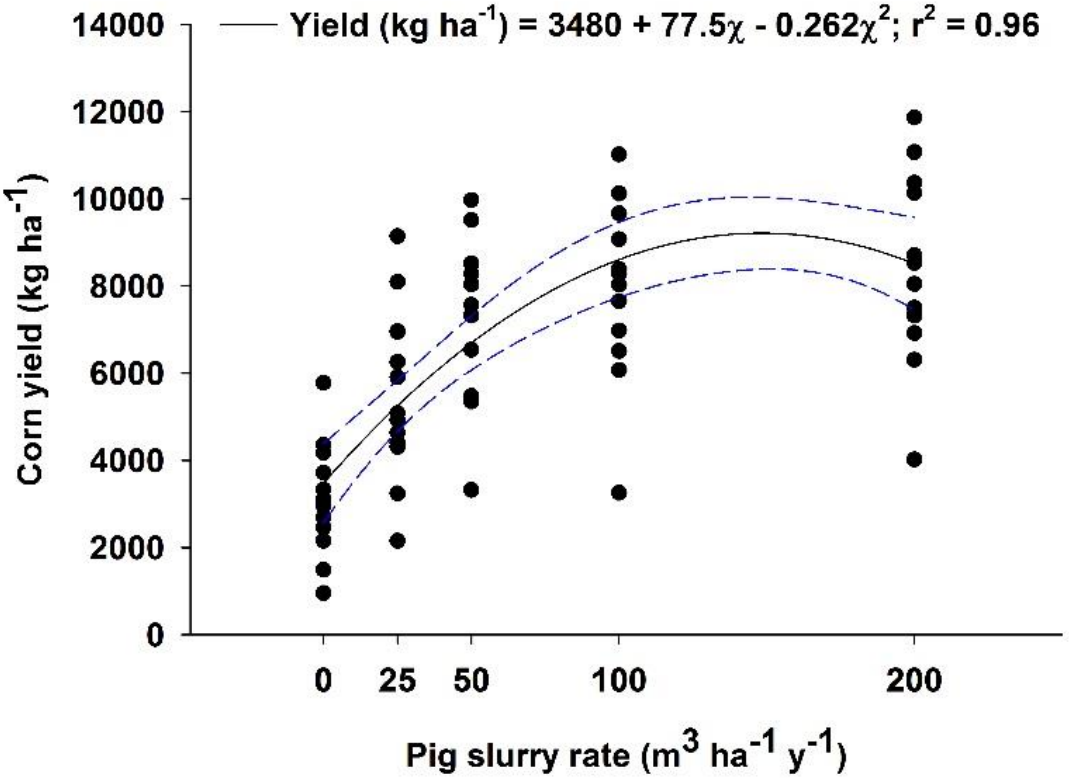


Figure 3.8 Corn yield (grain) response as a function of annual inputs of pig slurry at increasing rates. The adjusted function was computed with corn yield data of 13 years (in 2003 black bean [*Phaseolus vulgaris*] was utilised as main crop; and corn production from 2006 was compromised by a severe drought with yields < 820 kg ha^{-1}). Dashed lines represent the 95% confidence interval.

Improvements on P utilisation in the various stages of the pig production system should also be investigated and adopted. Use of low phytate (inositol phosphate) grains and phytase supplementation in feed formulations are currently some of the more viable options (Ludke et al., 2002; Raboy, 2003; Leytem et al., 2004). Genetically modified pigs able to produce high amounts of phytase in their saliva have already been developed (Golovan et al., 2001; Forsberg et al., 2003). Alternatively, concentration of P (and other nutrients) present in the effluent produced (for example precipitation and recovery as struvite [$MgNH_4PO_4 \cdot 6H_2O$]) may warrant efficient relocation of nutrient rich residues to low fertility areas (Makara and Kowalski, 2015; Fang et al., 2016; Taddeo et al., 2016).

Table 3.5 Major P pools accounted for in the cropping system receiving long-term P additions via pig slurry at different rates.

P balance in the system		Pig slurry application rate (m^3 ha^{-1} $year^{-1}$)				
		0	25	50	100	200
		kg P ha^{-1}				
Inputs	P added via slurry[a]	0	515	1030	2060	4120
Pools	Soil P pool[b]	4187	4559	5036	5784	6578
	Crop P residue[c]	1	3	4	4	5
Outputs	Grain P export[d]	101	180	233	261	276
Total P accounted for[e]		4289	4742	5273	6049	6859
$P_{unaccounted}$[f]		-	62	45	299	1550
Accounted P in the system[g] *(%)*		-	88	94	86	62
Added P accumulated in soil[h] *(%)*		-	72	83	78	58
P balance efficiency[i] *(%)*		-	40	24	15	11

[a]Total P added via pig slurry after 15 years (2001-2015). Additions occurred in October of every year.

[b]Total soil P in the layer 0-40 cm depth.

[c]Amounts of P stored in crop residues are an approximation in function of produced residues (cover crops + crop residues) in the no-tillage system, concentrations of P in biomass and turnover rates from Crusciol et al. (2008) and Giacomini et al. (2003). Biomass production by cover crops varied between 1.6 to 4.6 Mg ha^{-1} (average of 6 evaluations) for control and 200 m^3 ha^{-1} y^{-1}, respectively.

[d]Total amount of P exported in grain (corn and beans [in 2003]) in 15 years as a function of P concentration in grain (% P) and total quantities of produced grain (kg ha^{-1}).

[e]Sum of P accounted for in pools (soil and residues) and P outputs (P exported in grain)

[f]$P_{unaccounted}$ (kg P ha^{-1}) = $P_{input} - (\Delta P_{soil}+P_{output}+P_{residue})$

[g]Accounted P (%) = $\left(1-\left(\frac{P_{unaccounted}}{P_{input}}\right)\right)\times 100$

[h]Accumulated P in soil (%) = $\frac{\Delta P_{soil}}{P_{input}}\times 100$

[i]P balance efficiency (%) = $\frac{P_{output}}{(P_{output}+\Delta P_{soil})}\times 100$

3.5 Conclusions

Long-term P inputs in pig slurry resulted in P accumulations proportional to the application rate, and were confined to the top 20 cm soil layer. This was mainly attributed to the high adsorption capacity of the soil under this experiment. Soil P accumulated primarily as inorganic P forms. Specifically for the soil type of this experiment, after 15 years of pig slurry additions at rates exceeding 50 m^3 ha^{-1} y^{-1}, potential P transfers and associated environmental increased. Therefore, P threshold levels and agronomic recommendations for P inputs established by Gatiboni et al. (2015) and CQFS RS/SC (2016) must be observed. Phosphorus mass balance revealed that between 62 to 94% of the P inputs in slurry could be accounted for in grain exports, soil storage, and crop residues, and that the majority of P added (58-83%) to this cropping system was accumulated in the soil to 40 cm. Unaccounted P represented between 6-38%, which was presumed to have been lost in drainage by overland flow. Adoption of conservation farming practices to avoid surface runoff and soil losses is required.

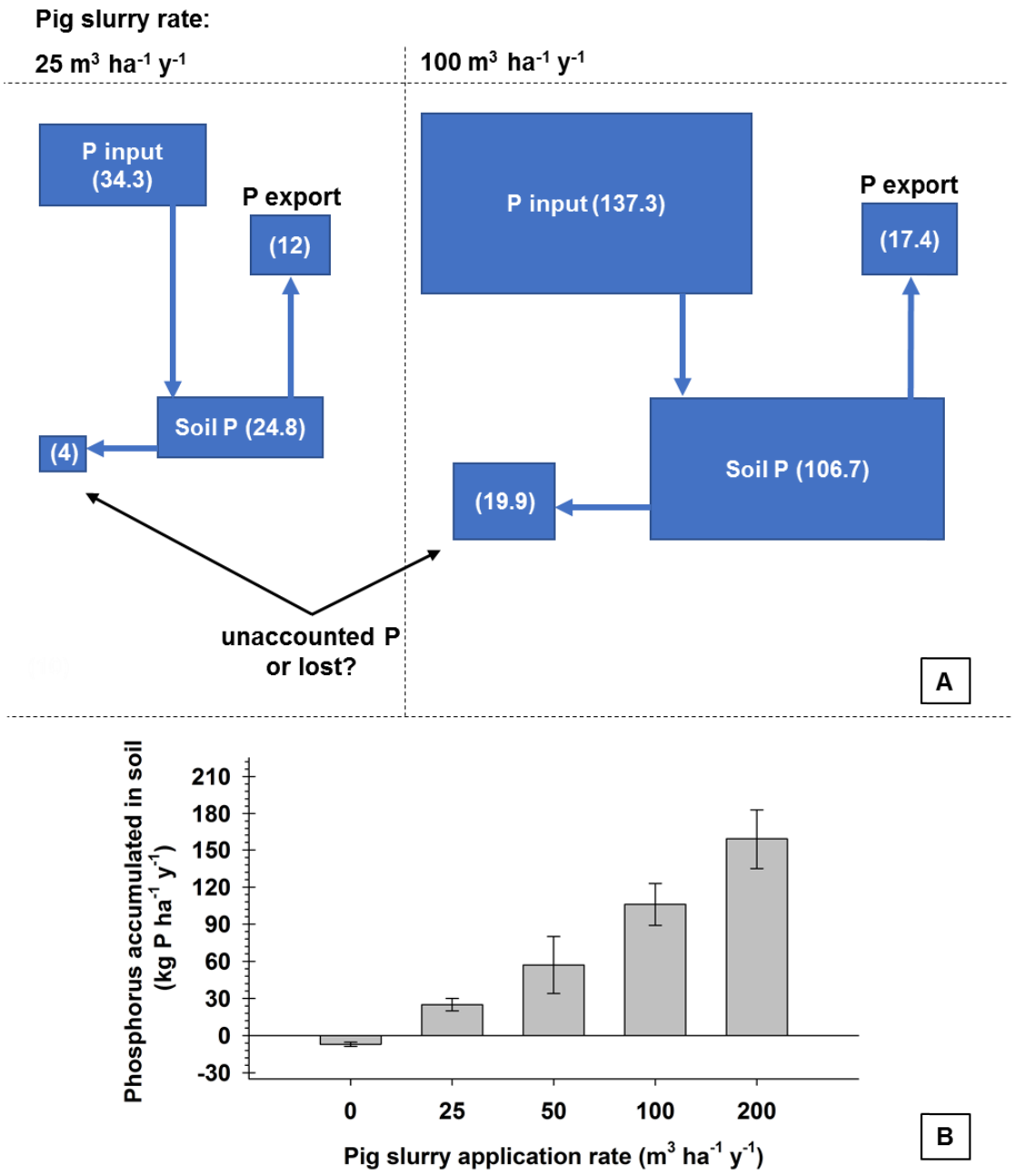


Figure 3.9 [A] Schematic representation to scale, of P flows in the cropping system. In the example, treatments receiving 25 and 100 m^3 ha^{-1} y^{-1} were considered. All numbers within parenthesis are expressed in kg P ha^{-1} y^{-1}. 'Soil P' represents the amount of P accumulated yearly in the soil to 40 cm. [B] Rate of phosphorus accumulation (expressed in kg P ha^{-1} y^{-1}) in the soil to 40 cm receiving annual pig slurry inputs. Rate of soil P depletion in the control treatment was estimated based on amounts of P exported in grains.

Chapter 4

Impacts of Long-Term Plant Biomass Management on Soil Phosphorus under Temperate Grassland

4.1 Introduction

Managed grassland ecosystems account for 25% of all ice-free land (FAOSTAT, 2011), and therefore make a significant contribution to global food supply and ecosystem services (White et al., 2000; Wright, 2005). For example, dairy, meat, and wool products from grazed pasture in New Zealand make up 35-50% of total export income (Hodgson et al., 2005; Statistics New Zealand, 2015), while pastoral agriculture is a significant component of many economies in North America (e.g. USA), South America (e.g. Brazil) and Western Europe (e.g. Ireland).

Inputs of phosphorus (P), mainly in the form of mineral fertilizers derived from phosphate rock, are essential to enhance and maintain the productivity of agroecosystems, including grasslands (Smit et al., 2009). However, most of the P applied in fertiliser is not immediately utilised or retained by plants and grazing animals and therefore accumulates in the soil as various inorganic and organic P forms of different labilities (commonly referred to as “legacy P” or “residual P”) (Haynes and Williams, 1993; Sattari et al., 2012; Stutter et al., 2012; Nash et al., 2014). There are also ongoing concerns and debate around addressing the adverse environmental impacts of high P inputs and the long-term future supply and cost of finite phosphate rock resources (Cordell et al., 2009; Haygarth et al., 2013; Ulrich and Frossard, 2014). These issues have highlighted the need to reassess and improve the overall utilisation efficiency of P inputs in agroecosystems, including the potential to enhance mobilisation of and use of P accumulated in soil from previous inputs (Condron et al., 2013; Haygarth et al., 2013; George et al., 2016).

A field experiment was established at Lincoln University, New Zealand in 1994 to investigate and quantify the long-term impacts of contrasting grassland management regimes on above- and below-ground properties and processes (Simpson et al., 2012; Adair et al., 2013; Farrell et al., 2014; McDowell et al., 2016). The specific objective of this study was to determine the cumulative impact of 20 years of plant biomass and P conservation and depletion on the amounts, forms and associated dynamics of topsoil P. We hypothesised that significant and relatively similar depletion of soil inorganic and organic P would have occurred in response to continued removal of P in biomass, and that changes in soil organic matter and P would be more pronounced at the soil surface (0-2.5 cm) compared with lower depths (2.5-10 cm).

4.2 Materials and methods

4.2.1 Long-term ecology field trial (LTET)

The field trial was established in September 1994 at Lincoln University, New Zealand (S 43°38'51, E 172°28'05) on a Wakanui silt loam soil (Mottled Immature Pallic [NZ]; Udic Ustochrept [USDA]) (Figure 4.1). The trial was primarily designed to investigate and quantify the impacts of a variety of grassland management strategies (no mowing, biomass retention, biomass removal), with and without annual nitrogen (N) fertilizer inputs, on soil properties and processes (Simpson et al., 2012; Farrell et al., 2014; McDowell et al., 2016). The trial site had previously been managed under arable cropping with regular inputs of mineral fertiliser (including P) and the soil P fertility status at establishment was relatively high (Olsen P = 28 mg kg^{-1} for 0-7.5 cm soil) for lowland agricultural soils in New Zealand. The site was cultivated and sown with a mixture of red clover (*Trifolium pratense* L.), white clover (*Trifolium repens* L.), perennial ryegrass (*Lolium perenne* L.), and cocksfoot (*Dactylis glomerata* L). Treatments were established on 5 × 5 m plots arranged in randomised blocks with four replicates each. The trial included a total of 8 treatments, although this study focused on comparison between two treatments, namely mowing with biomass retained (whereby organic matter and nutrients were conserved – hereafter referred to as "biomass retained") and mowing with biomass removed (whereby organic matter and nutrients were depleted - hereafter referred to as "biomass removed") (Figure 4.2). Mowing to 4 cm was carried out when the sward reached a height of approximately 20 cm, which occurred 4-6 times per year between spring (August) and autumn (April). The trial was not grazed and the selected treatments did not receive any fertiliser or irrigation inputs.

4.2.2 Plant sampling and analysis

Plant production and nutrient uptake were not routinely determined on the field trial. Accordingly, assessment of the impact of 20 years of contrasting biomass management on plant growth and P uptake were carried out for the spring-early summer part of the 2013-2014 growing season (August-November). This involved harvesting vegetation at ground-level using randomly placed, 50 × 50 cm quadrats in each replicate plot of the two treatments (8 plots) on three consecutive occasions (mid-August, mid-September, early November). Biomass from the three harvests from each plot were dried at 65°C, bulked and weighed prior to digestion and P analysis (Miller 1998). Aboveground plant biomass dry matter and P uptake were expressed in units of Mg ha^{-1} and kg ha^{-1}, respectively. Root biomass assessment was carried out from soil core samples in November 2014. Duplicate 5.4 cm diameter cores were collected in each replicate plot to 10 cm depth. The cores were divided into 0-2.5, 2.5-5, and 5-10 cm depth intervals. Roots (>1 mm) were separated from soil by washing, dried at 65°C, and weighted. Root biomass dry matter to 10 cm was expressed in units of Mg ha^{-1}, while root

biomass for each soil depth increment was expressed in units of g dm^{-3} to allow comparison between depths.

4.2.3 Soil sampling and analyses

Soil samples were taken from the biomass retained and removed treatments in November 2014 after 20 years of contrasting plant biomass management. Soil samples were randomly taken from the four replicate plots of each treatment by using a spade to collect a 3 cm thick × 20 cm wide slice of soil. These were then partitioned into three different depth increments of 0-2.5, 2.5-5, and 5-10 cm. Duplicate sub-samples from each plot and at each depth were bulked, dried at 65°C, and ground to <2 mm prior to soil analyses.

Soil pH was determined in water (ratio 1:2.5; v/v), and total organic carbon (C; also referred to as total C) and total nitrogen (N) were analysed by an elemental analyser (LECO 2000 CNS Analyser, LECO, Brisbane, Australia). Soil bulk density was determined for each soil depth in order to correct the soil attributes and concentrations to units of mass per hectare. This involved collecting a known volume of soil in metal rings (5.4 cm diameter) at the respective depth, which was then dried to constant mass at 105°C, and weighed. The quantities of total C and N in the top 10 cm were expressed in units of Mg ha^{-1} and kg ha^{-1}, respectively, while for each depth increment total C and N were expressed in units of Mg ha^{-1} cm^{-1} and kg ha^{-1} cm^{-1}, respectively, to allow comparison between depths.

Soil P fractionation involved sequential extraction of replicate samples with ammonium chloride (1 M NH_4Cl), sodium bicarbonate (0.5 M $NaHCO_3$ pH 8.5), sodium hydroxide (0.1 M NaOH), hydrochloric acid (1 M HCl), a second extraction with 0.1 M NaOH (concentrations of inorganic and organic P in this fraction were respectively summed to the first extraction with NaOH), and finally digestion with concentrated sulphuric acid (H_2SO_4) and hydrogen peroxide (H_2O_2) (Condron et al. 1996; Olsen and Sommers 1982). Total organic P was determined as the sum of organic P extracted with $NaHCO_3$ and NaOH, and total soil P was calculated as the sum of total P in all fractions. These multiple soil P fractions were designated as “soil solution P” (NH_4Cl), “labile inorganic and organic P” ($NaHCO_3$), “moderately labile inorganic and organic P” (NaOH), “calcium-bound inorganic P” (HCl), and “highly stable P” (conc. H_2SO_4 + H_2O_2) (Cross and Schlesinger 1995). The quantities of total P, total inorganic P, total organic P and inorganic and organic P fractions in the top 10 cm were expressed in units of kg ha^{-1}, while for each depth increment these P parameters were expressed in units of kg ha^{-1} cm^{-1} to allow comparison between depths.

Equivalent data for total C, total N, total P, total organic P, and labile organic P were used to calculate the following mass ratios: C:N, C:total P, C:total organic P, N:total organic P, C: labile organic P.

In order to assess whether long-term biomass management affected the chemical nature of soil organic P, soils from each depth (0-2.5, 2.5-5, 5-10 cm) were extracted with 0.25 M NaOH + 0.05 M EDTA and P species analysed using ^{31}P nuclear magnetic resonance spectroscopy (^{31}P NMR) (Cade-Menun and Liu 2014). It was not possible to analyse all four replicate soil samples from each depth due to time and cost constraints associated with NMR analysis. Therefore, soil extracts from each replicate were combined using equal volumes of solution. The inorganic phosphorus present was determined in the combined extracts using molybdate blue colorimetry (Dick and Tabatabai 1977), and the total phosphorus content was measured by ICP-OES (Pierzynski 2000). Combined NaOH-EDTA extracts were prepared and analysed by ^{31}P NMR according to the procedures and protocols described in McDowell et al. (2016). The quantities of P species determined by ^{31}P NMR were expressed in units of kg ha^{-1} cm^{-1} to allow comparison between depths.

4.2.4 Statistical analysis

Statistical analyses of the data were carried out using GenStat 16 (GenStat 2013). One-way ANOVA was carried out to test differences in plant and soil properties between treatments (2 treatments, 3 depths and 4 replicates; total n = 24). For soil data (including mass ratios) where significant differences between treatments were observed, an additional two-way ANOVA was carried out to test treatment × depth interactions. If the treatment × depth interaction was not significant, then one-way ANOVA was used to test the significance of differences between treatments within each depth.

Figure 4.1 Aerial image of the long-term ecology trial, Lincoln University, New Zealand (2016).

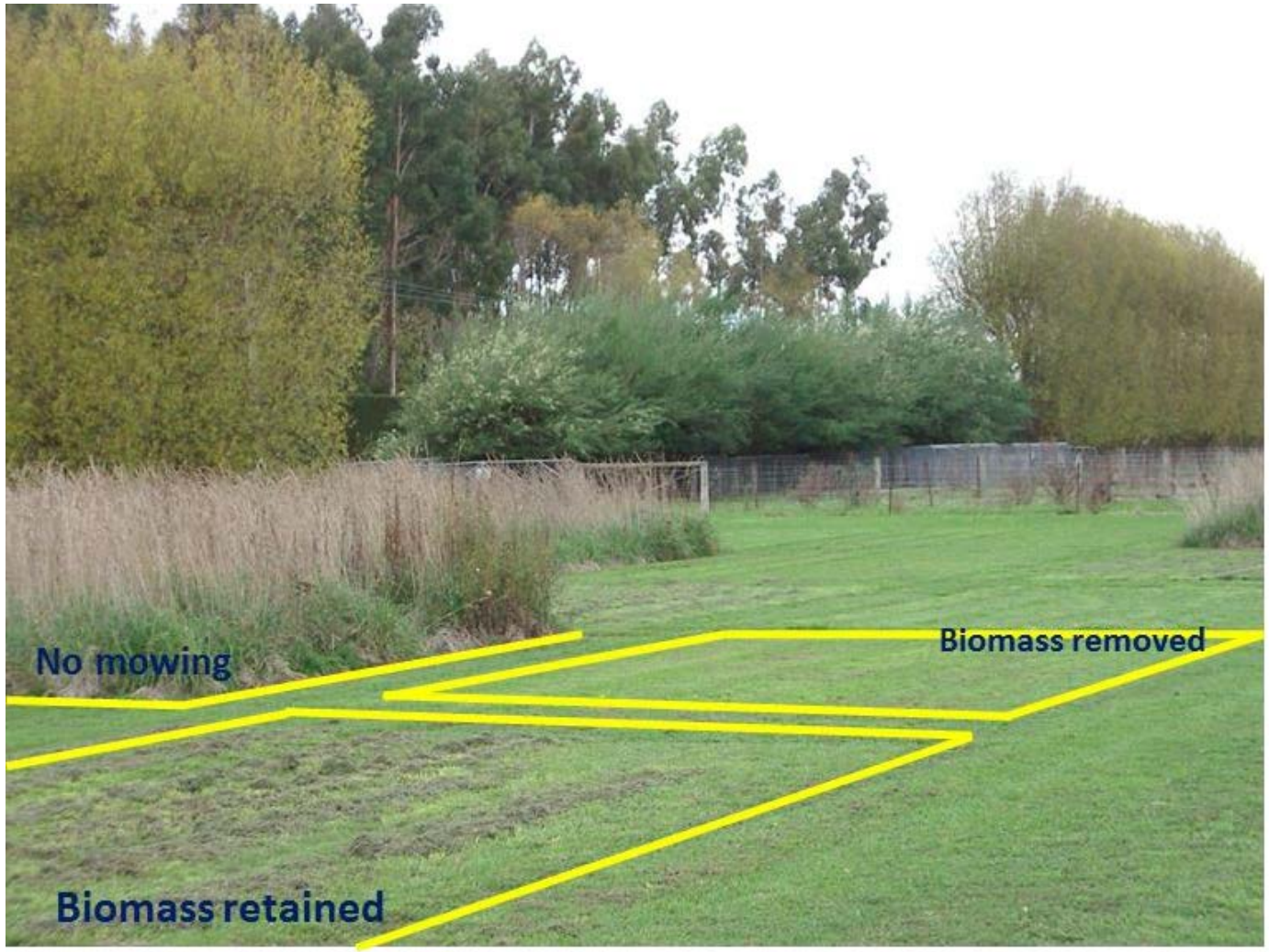


Figure 4.2 The long-term ecology field trial (Lincoln University, New Zealand) showing the three main treatments, namely no mowing, mowing with biomass removed (upper right), and mowing with biomass retained (lower left).

4.3 Results

After 20 years dry matter production was markedly and significantly higher under biomass retention (12.5 Mg ha^{-1}) compared with biomass removal (5.4 Mg ha^{-1}) which was also reflected in the corresponding data for above-ground P uptake (Table 4.1). Similarly, below-ground root biomass was significantly higher in the topmost soil layer (0-2.5 cm) under biomass retention (27.2 g dm^{-3}) compared with removal (15.7 g dm^{-3}), and while a similar trend was evident in the lower soil depths (2.5-10 cm) the differences were not significant.

Table 4.1 Mean data for above-ground dry matter production and P uptake determined between August and November 2014 for the biomass retained and biomass removed treatments, together with below-ground biomass data for 0-10 cm soil determined in November 2014.

Above-ground	Dry matter (Mg ha^{-1})	P uptake (kg P ha^{-1})
Biomass retained	12.5	5.9
Biomass removed	5.4	2.2
	*	*

Below-ground	Dry Matter (g dm^{-3})			0-10 cm (Mg ha^{-1})
	0-2.5 cm	2.5-5 cm	5-10 cm	
Biomass retained	27.2	3.4	1.5	8.4
Biomass removed	15.7	2.5	1.3	5.2
	*	ns	ns	*

One-way ANOVA summary: n = 4; LSD = least significant difference set at 5% probability; Asterisk indicate significant statistical differences associated to treatments in each depth (columns), degrees of freedom (treatments within depth) total = 7; ns = not significant.

Data for selected soil properties and ratios for the different treatments and depths are shown in Figure 4.3 and Table 4.2. Total C to 10 cm was significantly higher under biomass retention than removal, while corresponding differences in total N were not significant (Figure 4.3). The C:N and C:total organic P ratios were significantly higher as a consequence of biomass retention compared with removal, while the opposite trend was observed for C:labile organic P. Differences in the other mass ratios between treatments were not significant. Data presented in Table 4.2 revealed that soil bulk density and pH were not affected by long-term biomass management. However, total C was consistently and significantly higher under biomass retention than removal in all soil depths, although there was no significant interaction between treatment and depth. The magnitude of these differences was similar for each depth, whereby total C was 17-26% higher as a result of biomass retention. Similarly, for the 0-2.5 cm soil all mass ratios except C:labile organic P were significantly

higher under the biomass retention regime, while similar differences were found for C:N and C:organic P in the 5-10 cm soil depth. On the other hand, C:labile organic P was significantly lower under biomass retention in the 2.5-5 cm soil.

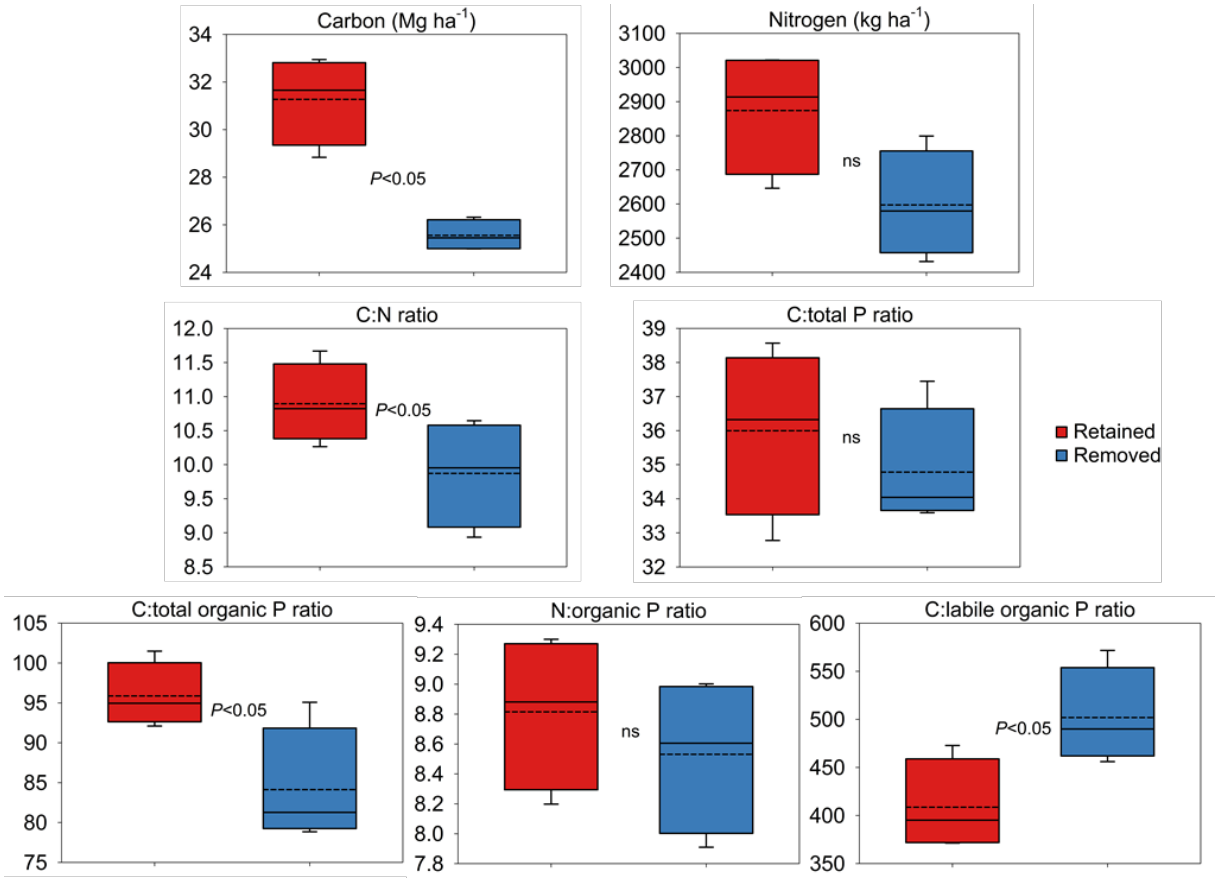


Figure 4.3 Amounts of carbon, nitrogen and various component mass ratios determined for the 0-10 cm soil layer after 20 years of aboveground biomass retention compared to removal, in a long-term field experiment under temperate grassland at Lincoln, New Zealand. Continuous line within the box is the median (50th percentile), whereas the dashed line is the mean. Note that the scales are different. *P*<0.05 indicates significant difference at 5% level, while ns = not significant.

Table 4.2 Mean data (n = 4) for bulk density, pH, total C, total N, and various component mass ratios determined for soils taken after 20 years of biomass retention and removal.

Soil attribute	Plant biomass management	Depth (cm)			Treat. × Depth[§]
		0-2.5	2.5-5.0	5.0-10	
Soil bulk density (g cm^{-3})	Retained	0.7	0.8	1.1	–
	Removed	0.8	0.9	1.1	
		ns	***ns***	***ns***	
pH	Retained	5.6	5.4	5.5	–
	Removed	5.7	5.4	5.6	
		ns	***ns***	***ns***	
Carbon (Mg ha^{-1} cm^{-1})	Retained	3.4	3.5	2.8	***ns***
	Removed	2.7	3.0	2.3	
		*****	*****	*****	
Nitrogen (kg ha^{-1} cm^{-1})	Retained	299	324	264	–
	Removed	276	295	234	
		ns	***ns***	***ns***	
C:N	Retained	11.4	10.8	10.7	–
	Removed	9.9	10.1	9.7	
		*****	***ns***	*****	
C:total P	Retained	49.0	40.3	29.3	–
	Removed	40.8	37.6	30.6	
		*****	***ns***	***ns***	
C:total organic P	Retained	127	103	80.7	–
	Removed	101	95.4	71.6	
		*****	***ns***	*****	
N:organic P	Retained	11.2	9.5	7.6	–
	Removed	10.2	9.5	7.4	
		*****	***ns***	***ns***	
C:labile organic P	Retained	746	436	315	–
	Removed	714	539	413	
		ns	*****	***ns***	

One-way ANOVA was carried out to test differences on the overall treatment effect (2 treatments, 3 depths, 4 replicates; total n = 24). [§]In case of presence of significant differences between treatment means, an additional two-way ANOVA was carried out to test the treatment × depth interaction. Significance was set to 5% probability, where $P<0.05$ = *P*-values are shown; otherwise, ***ns*** = not significant is shown. In the absence of treatment × depth interaction, the statistical analysis was complemented by one-way ANOVA testing differences between treatments within depths separately, where asterisks indicate $P<0.05$.

Soil P data are presented in Figure 4.4, Figure 4.5, and Table 4.3. Figure 4.4 shows the quantities of total and extracted soil P fractions determined for the 0-10 cm soils. Total P and total inorganic P were significantly higher under biomass retention compared with removal, while there was no difference in total organic P. For the P fractions, quantities of soil solution, labile and moderately labile inorganic P were consistently and significant higher as a consequence of biomass retention compared with removal, while labile organic P was also significantly higher under biomass retention. Within the inorganic P fractions, the relative magnitude of the difference between biomass retention and removal was greater for soil solution inorganic P (2.7-fold) compared with labile inorganic P (2-fold), and moderately labile inorganic P (1.4-fold). There were no significant differences between biomass treatments for moderately labile organic P, calcium-bound inorganic P, and highly stable P. Figure 4.5 shows data for the different depths for total P, total inorganic P, total organic P, soil solution inorganic P, labile inorganic and organic P and moderately labile inorganic and organic P. These data confirmed that quantities of total P and total inorganic P were significantly higher under biomass retention, except for total P in the 0-2.5 cm soil. Soil solution inorganic P, labile inorganic and organic P, and moderately labile inorganic P were significantly higher under biomass retention at all soil depths. Significant interactions were determined between treatment and depth for total P, total inorganic P, soil solution inorganic P, labile organic P and moderately labile inorganic P (Table 4.3 and Table 4.5).

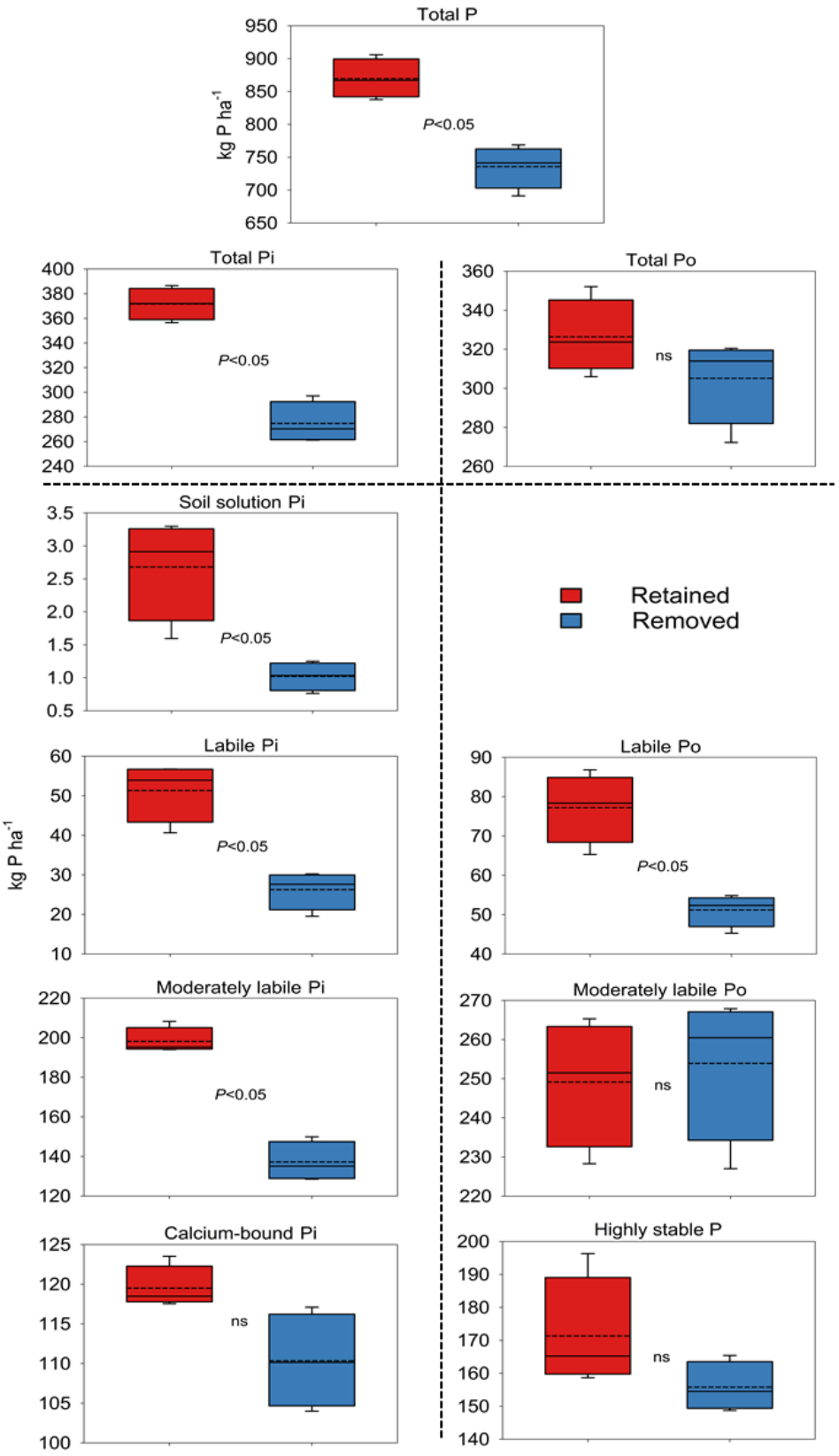


Figure 4.4 Amounts of total P, total inorganic P (Pi), total organic P (Po) and various P pools (kg P ha^{-1}) quantified for the topsoil (0-10 cm depth) after over 20 years of aboveground biomass retention compared to removal, in a long-term field experiment under temperate grassland at Lincoln, New Zealand. Continuous line within the box is the median (50th percentile), whereas the dashed line is the mean. Note that the scales are different. *P*<0.05 indicates significant difference at 5% level, while ns = not significant.

Table 4.3 Mean data (n = 4) for total P, and P fractions (kg ha^{-1} cm^{-1}) determined for soils at different depths taken after 20 years of biomass retention and removal.

Soil phosphorus (P) pool (kg ha^{-1} cm^{-1})	Plant biomass management	Depth (cm) 0-2.5	2.5-5.0	5.0-10	Treat. × Depth§
Total P	Retained	69.1[c]	87.1[b]	95.8[a]	**<0.001**
	Removed	66.6[b]	78.1[a]	74.8[a]	
		ns	*****	*****	
Total inorganic P	Retained	28.9[c]	36.6[b]	41.6[a]	**0.005**
	Removed	24.6[b]	30.6[a]	27.3[b]	
		*****	*****	*****	
Total organic P	Retained	26.7	34.2	34.8	–
	Removed	27.2	31.2	31.9	
		ns	***ns***	***ns***	
Soil solution P (NH_4Cl-Pi)	Retained	0.5[a]	0.3[b]	0.2[c]	**0.035**
	Removed	0.2[a]	0.1[a]	0.1[a]	
		*****	*****	*****	
Labile inorganic P ($NaHCO_3$-Pi)	Retained	5.4	5.8	4.7	***ns***
	Removed	3.2	2.8	2.3	
		*****	*****	*****	
Labile organic P ($NaHCO_3$-Po)	Retained	4.6[c]	8.1[b]	9.1[a]	**0.007**
	Removed	3.9[b]	5.5[a]	5.5[a]	
		*****	*****	*****	
Moderately labile inorganic P (NaOH-Pi)	Retained	13.8[c]	19.0[b]	23.3[a]	**<0.001**
	Removed	11.6[b]	15.7[a]	13.8[a]	
		*****	*****	*****	
Moderately labile organic P (NaOH-Po)	Retained	22.1	26.1	25.7	–
	Removed	23.3	25.7	26.3	
		ns	***ns***	***ns***	
P-calcium bound (HCl-Pi)	Retained	9.2	11.6	13.5	–
	Removed	9.6	12.1	11.2	
		ns	***ns***	***ns***	
Highly stable P (conc. H_2SO_4+H_2O_2 Pi+Po)	Retained	13.4	16.3	19.4	–
	Removed	14.8	16.3	15.6	
		ns	***ns***	***ns***	

One-way ANOVA was carried out to test differences on the overall treatment effect (2 treatments, 3 depths, 4 replicates; total n = 24). §In case of presence of significant differences between treatment means, an additional two-way ANOVA was carried out to test the treatment × depth interaction. Significance was set to 5% probability, where $P<0.05$ = P-values are shown; otherwise, ***ns*** = not significant is shown. Different lower case superscript letters show significant differences (least significant difference set at 5% probability) among depths within treatments. In the absence of treatment × depth interaction, the statistical exploration was complemented by one-way ANOVA testing differences between treatments within depths separately, where asterisks indicate $P<0.05$.

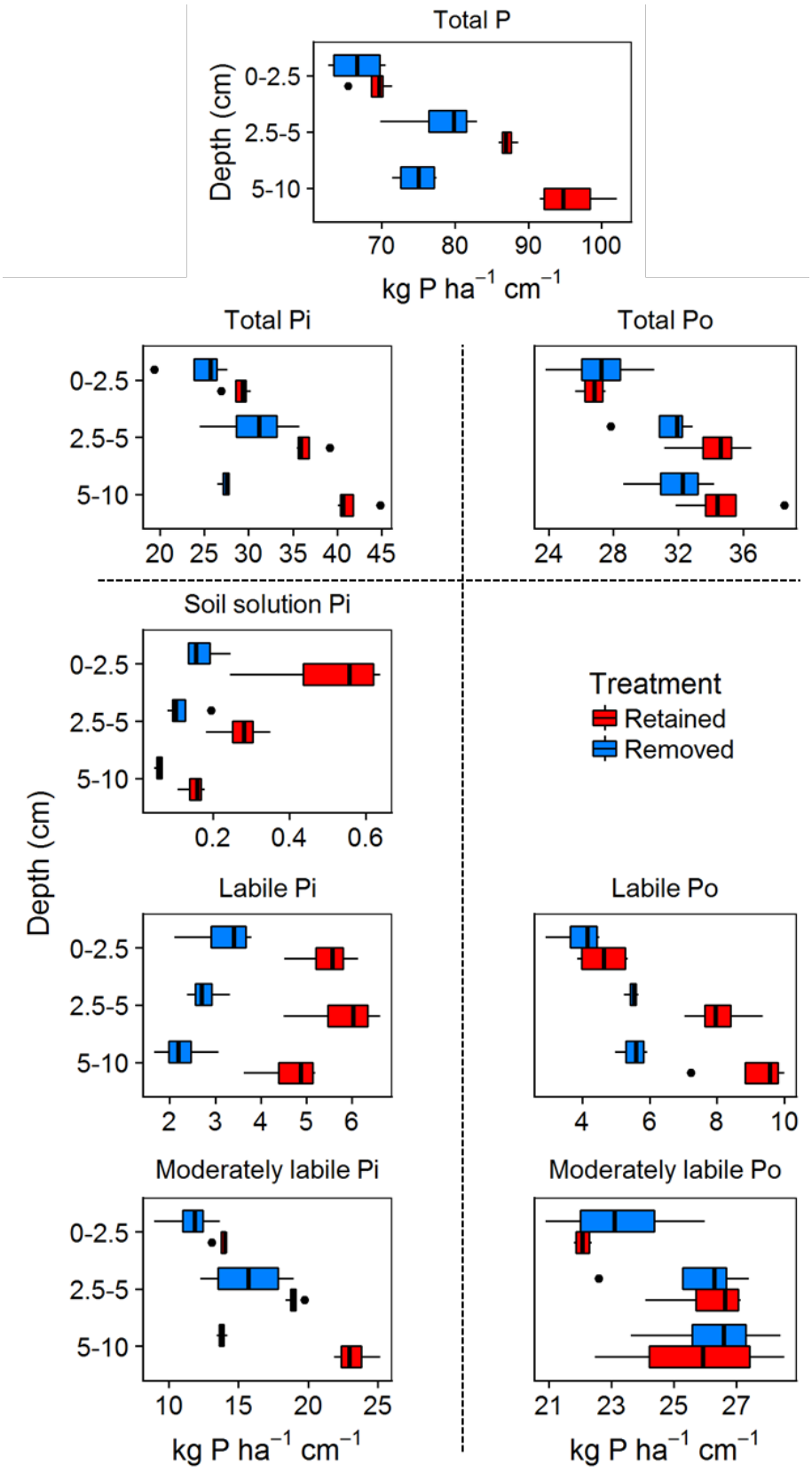


Figure 4.5 Amounts of total P, total inorganic P (Pi), total organic P (Po) and various P pools (normalised to constant depth increment, kg P ha^{-1} cm^{-1}) quantified for different depths (0-2.5, 2.5-5 and 5-10 cm) in soils under contrasting long-term aboveground biomass management (retention versus removal) at Lincoln, New Zealand. Note that the scales are different. Line within the box is the median (50th percentile). Dots indicate data points that fall outside the 1.5 × the interquartile range.

Extractable P forms determined by ^{31}P NMR analysis of soils to 10 cm is shown in Table 4.4. As expected, orthophosphate inorganic P and monoester organic P were the predominant P forms present, with trace quantities of diester organic P and pyrophosphate P detected. Amounts of orthophosphate and monoester P were higher under biomass retention compared with biomass removal, which is consistent with the soil P fractionation results described above. However, in the 0-2.5 cm soil the relative proportion of extracted P present as orthophosphate was higher for biomass removed (78%) than retained (69%), while the opposite trend was evident for monoester P (20% and 29% for the removed and retained treatments, respectively). The corresponding relative proportions of orthophosphate and monoester P were similar between treatments in the 2.5-5 cm and 5-10 cm soils.

Table 4.4 Extractable soil P forms (kg ha^{-1} cm^{-1}) identified by ^{31}P NMR spectroscopy analysis for combined soil replicates at different depths (0-2.5, 2.5-5 and 5-10 cm) sampled after 20 years of biomass retention and removal.

Plant biomass management	NaOH-EDTA-P	Orthophosphate [6.5][b]	Monoesters [3 to 6]	Diesters [2 to -1]	Pyro-phosphate [-3 to -6]
		0-2.5 cm			
Retained	46.8 (44)[a]	34.2 (69)[c]	13.7 (29)	0.1 (0.1)	0.7 (2)
Removed	44.0 (34)	32.4 (78)	8.8 (20)	-	0.7 (1)
		2.5-5 cm			
Retained	61.6 (45)	37.8 (61)	24.2 (39)	-	0.4 (1)
Removed	51.8 (32)	30.9 (60)	20.0 (39)	-	0.9 (2)
		5-10 cm			
Retained	57.7 (45)	35.6 (62)	22.1 (38)	-	0.7 (1)
Removed	43.6 (29)	26.4 (61)	17.2 (39)	-	-

[a]Percentage of the NaOH-EDTA-P extract present as inorganic P determined by colorimetry.

[b]Chemical shift (δ ppm).

[c]Proportion of the NaOH-EDTA-P detected as P species by ^{31}P NMR.

Table 4.5 ANOVA summary for the normalised data.

Variable		One-way ANOVA summary												Two-way ANOVA summary		
		Treatment				Treatment (*P*-value)	Depth (cm)						Depth (*P*-value)	Treatment (*P*-value)	Depth (*P*-value)	Treatment × Depth (*P*-value)
		Retained		Removed			0-2.5		2.5-5.0		5.0-10					
		Mean	SE	Mean	SE		Mean	SE	Mean	SE	Mean	SE				
kg ha^{-1} cm^{-1}	NH_4Cl-Pi	0.3	0.2	0.1	0.1	0.00240	0.3	0.2	0.2	0.1	0.1	0.1	0.01131	<0.001	<0.001	0.03455
	$NaHCO_3$-Pi	5.3	0.9	2.7	0.7	<0.001	4.3	1.4	4.3	1.7	3.5	1.4	0.45575	<0.001	0.04055	0.52907
	$NaHCO_3$-Po	7.3	2.2	5.0	0.9	0.00314	4.3	0.8	6.8	1.5	7.3	2.1	0.00188	<0.001	<0.001	0.00737
	HCl-Pi	11.4	2.0	11.0	1.6	0.55173	9.4	0.9	11.8	1.4	12.4	1.4	<0.001	0.34087	<0.001	0.03320
	NaOH-Pi	18.7	4.1	13.7	2.6	0.00175	12.7	1.8	17.3	2.7	18.5	5.1	0.00795	<0.001	<0.001	<0.001
	NaOH-Po	24.6	2.5	25.1	2.3	0.65874	22.7	1.6	25.9	1.7	26.0	2.2	0.00201	0.58361	0.00383	0.68060
	H_2SO_4conc./H_2O_2-Pi+Po	16.4	3.2	15.6	1.1	0.41741	14.1	1.1	16.3	0.7	17.5	3.1	0.00865	0.24357	0.00218	0.01265
	Total P	84.0	12.0	73.2	6.4	0.01152	67.9	3.4	82.6	6.2	85.3	11.9	<0.001	<0.001	<0.001	<0.001
	Total organic P	31.9	4.3	30.1	3.1	0.24178	27.0	1.9	32.7	2.7	33.3	2.9	<0.001	0.06607	<0.001	0.23580
	Total inorganic P	35.7	5.7	27.5	4.1	<0.001	26.8	3.5	33.6	4.6	34.5	7.8	0.02200	<0.001	<0.001	0.00469
Carbon (Mg ha^{-1} cm^{-1})		3.2	0.4	2.6	0.3	<0.001	3.1	0.4	3.2	0.3	2.5	0.3	0.00241	<0.001	<0.001	0.72948
Nitrogen (kg ha^{-1} cm^{-1})		295	34	268	33	0.06108	288	32	310	23	249	21	<0.001	0.00952	<0.001	0.94734
Mass ratios	C:N	11.0	0.6	9.9	0.9	0.00290	10.7	1.2	10.4	0.7	10.2	0.8	0.62552	0.00499	0.52826	0.70221
	C:organic P	103.4	20.3	89.2	15.4	0.06769	113.7	16.5	99.0	6.8	76.2	7.5	<0.001	<0.001	<0.001	0.03900
	N:organic P	9.4	1.6	9.0	1.4	0.53459	10.7	1.1	9.5	0.4	7.5	0.4	<0.001	0.18118	<0.001	0.34075
	C:P	39.6	8.7	36.4	5.1	0.29748	44.9	4.8	39.2	2.8	29.9	2.5	<0.001	0.00810	<0.001	0.00733
	C:labile organic P	499	201	555	157	0.45189	730	131	488	60	364	71	<0.001	0.13340	<0.001	0.25021
pH		5.5	0.2	5.6	0.1	0.25860	5.6	0.2	5.4	0.1	5.5	0.2	0.08787	0.24685	0.10422	0.86430
Soil bulk density (g cm^{-3})		0.87	0.22	0.95	0.15	0.32189	0.77	0.09	0.86	0.21	1.10	0.04	<0.001	0.15989	<0.001	0.76659

4.4 Discussion

The above- and below-ground plant data clearly demonstrated that current dry matter production and root biomass had been markedly reduced by 38-57% as a consequence of continued biomass removal over 20 years, while seasonal plant P uptake had been reduced by 63%. The latter in particular indicated that the plant availability of soil P had been dramatically lowered by cumulative removal of P in biomass over 20 years. This was confirmed by the corresponding relative differences determined for various soil inorganic P fractions under the contrasting biomass management regimes. Thus quantities of solution, labile, and moderately labile inorganic P were reduced by 63, 49, and 31%, respectively, as a consequence of long-term biomass P removal (Figure 4.6). These data are consistent with changes in soil inorganic P fractions determined over time in archived 0-7.5 cm soils taken from the biomass removed treatment (McDowell et al. 2016). The declining proportional decreases with reducing relative solubility (i.e. solution > labile > moderately labile), together with the absence of any concomitant differences in the calcium bound and highly stable P pools, provides empirical confirmation of the long-term relative bioavailability of these soil P pools, which in turn validates the use of sequential soil P fractionation in biogeochemical studies (Chen et al. 2003; Condron and Newman 2011; Cross and Schlesinger 1995; Negassa and Leinweber 2009; Yang and Post 2011).

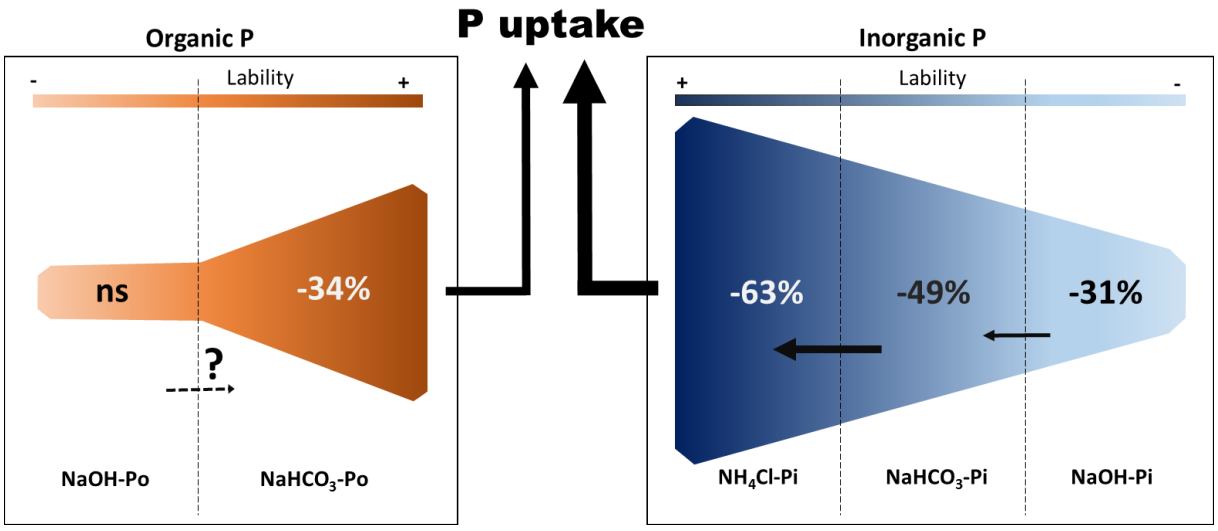


Figure 4.6 Relative difference in selected soil inorganic and organic P fractions (0-10 cm) determined after 20 years of aboveground biomass/P removal versus biomass/P retention.

These findings clearly demonstrate that soluble and readily available inorganic P reserves accumulated from previous fertiliser inputs were at least partly utilised by plants in response to cessation of P inputs and continued removal of P in biomass over 20 years. However, this

mobilisation of soil inorganic P was only sufficient to maintain plant production and P uptake at 37-45% compared with biomass retention, while there was no significant depletion of more stable forms of inorganic P. This in turn may reflect the relative capacity of the plant species present and their associated flora and fauna to mobilise stable/recalcitrant forms of soil inorganic and organic P (Clarholm et al. 2015; Condron and Newman 2011).

In addition to differences in soil inorganic P, the fact that labile soil organic P was 34% lower under biomass removal compared with retention (Figure 4.6) could be attributed to either enhanced immobilisation as a consequence of continued biomass retention or enhanced mineralisation of organic P in this pool in response to depletion of soil P by long-term P removal in biomass.

With regard to the possibility of increased P immobilisation under biomass retention, the regular and sustained cutting and return of grassland biomass C to the soil could have resulted in enhanced net conversion of inorganic to organic P in the soil (Hopkins and Dungait 2010; Magid et al. 1996). This is consistent with the fact that after 20 years total organic C was 17-26% higher in all three soil layers to 10 cm under biomass retention compared with removal. However, the C:total organic P ratios were higher under biomass retention in all 3 depths (significant for the 0-2.5 cm and 5-10 cm soils). This is not consistent with enhanced immobilisation of inorganic P, as the C:total organic P ratios would have been similar if this was the case. This reflects the fact that relative differences in soil C between the biomass treatments were markedly greater than the corresponding non-significant differences in total organic P (-2 to +10%). The absence of any significant differences in the predominant moderately labile soil organic P pool, which accounts for 76-83% of total soil organic P compared with 17-24% for the labile pool, does not indicate that enhanced immobilisation of P occurred in response to continued biomass retention over 20 years. Furthermore, C:N ratios determined in the different soil depths were similarly higher under biomass retention (10.7-11.4) than removal (9.7-10.1) compared with total C. These various differences indicate that there was a shift towards more nutrient depleted soil organic matter in response to increased organic matter inputs under biomass retention (Condron et al. 2010; Horwath 2017; McGill and Cole 1981; Tipping et al. 2016), especially in the top 2.5 cm soil which reflects the continued mowing and return of plant biomass to the soil surface.

With regard to the possibility of increased organic P mineralisation under biomass removal, with particular reference to the significant differences in labile organic P observed between the biomass management treatments. While the C:labile organic P ratio in the 0-2.5 cm soil was lower under biomass removal than retention, the opposite trend was evident in the lower soil depths (significant for the 2.5-5 cm soil), while the corresponding ratio for the combined 0-10 cm soil was significantly higher under biomass removal (502) compared with retention (409). These trends may be attributed

to differences in the relative magnitude of changes in total C and labile organic P between treatments at different depths. Thus differences in total C and labile organic P in the 0-2.5 cm soil were similar (26 and 17%, respectively), whereas differences in labile organic P were substantially higher than total C in the 2.5-5 cm (17 and 47%, respectively) and 5-10 cm (22 and 65%, respectively) soils. This means that there was less labile organic P relative to organic carbon in the soils maintained under biomass removal after 20 years, which in turn could be attributed to enhanced mineralisation of labile organic P in response to continued depletion of soil inorganic P. Other studies conducted in temperate grasslands have demonstrated that labile soil organic P can make a significant contribution to short-term plant P requirements (Perrott et al. 1990; Scott and Condron 2003; Tate et al. 1991). On the other hand, the absence of any significant change in moderately labile organic P in response to soil inorganic P depletion was not consistent with results from P depletion studies related to land-use changes in plant species and associated flora and fauna (Chen et al. 2000; Condron et al. 1996; Hedley et al. 1982; Richter et al. 2006).

The ^{31}P NMR data showed that almost all of the extractable organic P in the LTET soils was present as monoesters with little or no diesters detected. This is consistent with results reported by McDowell et al (2016) for archived 0-7.5 cm soils taken from the LTET, but is not consistent with data from temperate grazed pasture soils (Condron et al. 1985; Magid et al. 1996).

4.5 Conclusions

The findings of this study revealed that plant production and P uptake were 45 and 63% lower, respectively, as a result of 20 years of biomass and P removal in the absence of P inputs. Consequently quantities of soluble and labile inorganic P were 31-63% lower in soil maintained under biomass removal than retention. Corresponding differences in organic C and organic P in the different soil depths to 10 cm indicated that significant mineralisation of labile organic P had occurred in response to continued P removal, while there was no change in the main moderately labile organic P pool. Changes in soil inorganic and organic P that occurred in response to long-term P removal highlight limitations in the mobilisation and reutilisation of legacy or residual P accumulated in soil from previous fertiliser P inputs, especially for organic P and recalcitrant inorganic P. This in turn suggests that enhanced utilisation of legacy P may require changes in plant species/varieties and/or management, including the use of green manure/cover crops and microbial innoculants (Calabi-Floody et al. 2017; Haygarth et al. 2013; Richardson et al., 2011; Simpson et al., 2014).

Chapter 5

Plant Biomass Management Impacts on Short-Term Soil Phosphorus Dynamics in a Temperate Grassland

5.1 Introduction

Biological activity is a key driver of phosphorus (P) dynamics in soil-plant systems (Stewart and Tiessen, 1987; Magid et al., 1996; Richardson and Simpson, 2011). While microbial biomass P commonly accounts for less than 5% of total soil P (Brookes et al., 1984; Oberson and Joner, 2005), functionally it is important as P flux through the microbial biomass (and other biota) has a crucial role in determining turnover and bioavailability of P, especially at the soil-plant interface (rhizosphere) (Jakobsen et al., 2005; Richardson et al., 2009; Bünemann et al., 2012). Furthermore, inorganic P released into soil solution from microbial cells can be taken up by plants or microbes, or adsorbed onto soil colloid surfaces (Stewart and Tiessen, 1987; Jakobsen et al., 2005; Oberson and Joner, 2005). As the size of the microbial biomass is generally greater in the plant rhizosphere (e.g. Chen et al., 2002), these dynamics have a profound effect on primary production.

In agroecosystems, land use and management practices quantitatively and qualitatively influence soil organic matter, and a range of nutrients including P. In turn, these determine the size and activity of the soil microbial biomass. For example, long-term P inputs at similar rates to soils under organic farming practices (bio-dynamic and bio-organic) had higher microbial biomass and organic P mineralization rates comparative to conventional (mineral) fertiliser practices (Oberson et al., 1996; Oehl et al., 2001; Oehl et al., 2004). Another study showed that incubation with green manure led to a 5-fold increase in the daily gross organic P mineralisation, linked to increased biological activity (Randhawa et al., 2005). Soil microbial biomass is also affected by short-term changes in environmental conditions, including soil moisture, temperature and evapotranspiration, particularly in temperate ecosystems (Magid et al., 1996; Frossard et al., 2000). Thus, decomposition of organic matter and consequent mineralisation of P have been shown to be higher in spring when environmental conditions favour microbial activity in the soil (He et al., 1997; Wardle et al., 1999; Chen et al., 2003; Oberson and Joner, 2005). Furthermore, rapid P immobilisation may also occur in response to carbon (C) and nitrogen (N) additions to soil (Oehl et al., 2001; Bünemann et al., 2004; Ehlers et al., 2010).

A field trial was established in 1994 at Lincoln University, New Zealand to assess and quantify the long-term impacts of contrasting temperate grassland management regimes on soil properties and processes (Simpson et al., 2012; Adair et al., 2013; Farrell et al., 2014). This experiment was

maintained for over 22 years and after this time substantial and significant differences in soil organic matter and P occurred as a consequence of aboveground biomass management (retention versus removal) (McDowell et al., 2016; Boitt et al., 2017). Thus, quantities of soil C and N were, respectively, 18% and 10% higher under biomass retention compared with removal, and while significant depletion of soil inorganic P occurred in response to biomass removal, there was limited mobilization of organic P (Boitt et al. 2017). The latter does not mean that contrasting biomass management did not affect biological and biochemical P dynamics in the soil, including P flux through the microbial biomass. Accordingly, the objective of this study was to assess and quantify the combined impact of long-term biomass retention versus removal, and environmental conditions on soil microbial P and phosphatase activity determined on 14 occasions over a 17-month period.

5.2 Materials and methods

5.2.1 Long-term field trial, New Zealand

The 'long-term ecology field trial' was established in September 1994 at Lincoln University, New Zealand. The purpose of the field trial was to assess the impacts of aboveground grassland management practices on soil properties and processes (Simpson et al., 2012; McDowell et al., 2016; Boitt et al., 2017). (172°28'05'' E, 43°38'51'' S). The trial site is situated on a Wakanui silt loam soil (Mottled Immature Pallic [NZ]; Udic Ustochrept [USDA]), site elevation at the area is 10 m above sea level, historic annual average rainfall is 620 mm $year^{-1}$, and mean annual temperature is 12°C (January 17°C; July 6°C) (National Institute of Water and Atmospheric Research, 1981-2010). Prior the establishment of the trial, the area had been used mainly for arable cropping and bioavailable inorganic P was therefore relatively high (Olsen P = 28 mg kg^{-1} for the 0-7.5 cm layer) for lowland agricultural soils in New Zealand.

The research site was cultivated and sown with a mixture of red clover (*Trifolium pratense* L. cv. Pawera), white clover (*Trifolium repens* L. cv. Tahora), perennial ryegrass (*Lolium perenne* L.) and cocksfoot (*Dactylis glomerata* L. cv. Kahu). Treatments were established on 5 × 5 m plots arranged in randomised blocks with four plot-replicates per treatment. The main treatments sampled for this study constituted: (i) aboveground grassland mowed and biomass and associated nutrients maintained on the research plots - restorative management – hereafter referred to as "biomass retained" and; (ii) a contrasting treatment mown with aboveground grassland biomass removed – depletive system – hereafter referred to as "biomass removed". Mowing to 4 cm was carried out when the vegetation reached a height of approximately 20 cm (5-6 times per annum); the trial was not grazed and did not receive any fertiliser or irrigation inputs after establishment.

5.2.2 Meteorological data

Rainfall, soil temperature and potential evapotranspiration (PEVT) were interpolated using the Virtual Climate Station from NIWA (VCS; Tait and Turner, 2005; Chichota et al., 2008). For calculations and comparisons of seasonal trends, cumulated rainfall and averages of soil temperature and PEVT of one week prior to the respective sampling date were considered.

5.2.3 Soil sampling and analyses

The biomass retained treatments and the biomass removed treatments were sampled on 14 occasions during September 2015 to January 2017. Soil samples were obtained by randomly collecting and bulking 7 soil cores (diameter 2.5 cm) from the 0-2.5 and 2.5-5 cm depths from each replicate plot. Fresh soil samples were immediately brought to the laboratory for sieving (<2 mm) and then stored at 4°C until further processing within 24 hours.

Soil physicochemical properties were assessed at the beginning of the current experiment in September 2015. Soil pH was determined in H_2O (ratio 1:2.5 v/v), total carbon (C) and nitrogen (N) were analysed by an elemental analyser (LECO 2000 CNS Analyser). Total soil P was determined after sulfuric digestion (concentrated H_2SO_4 + H_2O_2 30% v/v) according to Olsen and Sommers (1982). Total extractable organic P was measured after extraction with NaOH 0.25M + EDTA 0.05M (Turner et al., 2005). Soil attributes and concentrations were corrected to units of mass per area, in hectares, by using the soil bulk density data for this site as presented by Boitt et al. (2017). Temporal net changes in soil microbial biomass P (microbial P) were analysed after chloroform-fumigation and extraction with 0.5M $NaHCO_3$ The re-adsorption of P during the fumigation process was corrected by adding a P spike (25 mg L^{-1}) to a third set of non-fumigated samples (Morel et al. 1996). The recovery of P from the microbial biomass was further corrected by a Kp factor of 0.4 (Brookes et al., 1982; Hedley and Stewart, 1982). Acid phosphatase enzyme activity (phosphatase activity) was measured by the release of ρ-nitrophenol (ρ-NP) after incubation of a soil sample with ρ-nitrophenyl phosphate (Tabatabai and Bremner, 1969). Bioavailable inorganic P (bioavailable P) was measured after extraction with 0.5M $NaHCO_3$ at pH 8.5 (Olsen, 1954). All soil parameters were additionally corrected to oven-dry basis after determination of gravimetric soil moisture. Phosphorus data are expressed in kg P ha^{-1}, whereas phosphatase activity is presented in µmol ρ-NP g^{-1} h^{-1}.

5.2.4 Estimates of turnover rates and P fluxes

Rates of microbial biomass P turnover were calculated as the ratios of total annual biomass P released (loss) to annual average microbial biomass P according to method described by McGill et al. (1986). Annual biomass P flux (kg P ha^{-1} yr^{-1}) were calculated by method proposed by Brookes et al. (1984) and reviewed by Oberson and Joner (2005) according to:

Equation 8 $$\text{P flux (kg P ha}^{-1}\text{ yr}^{-1}) = \frac{\text{annual biomass P content (kg ha}^{-1})}{\text{turnover time (years)}}$$

5.2.5 Statistical analysis

Ordination by principal component analysis (PCA) was used to determine underlying structure of the seasonal observations together; i.e. similarities, trends and patterns of the dependent variables bioavailable P, microbial P and phosphatase activity.

Differences in soil properties associated with treatments (biomass retained versus biomass removed) were analysed using one-way ANOVA at each depth increment separately. ANOVAs were also used to explore differences among means of microbial biomass P and phosphatase activity at each sampling date at the respective soil depth separately. Two-way ANOVA was performed to test the presence of interaction between treatments and sampling dates across the sampling period at each soil depth. In the absence of interaction, one-way ANOVA was performed across all sampling dates for each treatment and depth separately.

Pearson correlations were used to investigate associations between variables. The temporal observations in bioavailable P, microbial P, and phosphatase activity (dependent variables) between treatments and depths, and their relationships with environmental factors (independent variables), were additionally investigated using regression tree analysis. The tree depth parameter (i.e., how many levels of nodes to use) was tuned via *k*-fold cross-validation (*k*-fold CV) using the "rpart" package (details can be found in Kuhn (2008) and Therneau et al. (2015)). Repeated *k*-fold CV, as suggested by Kohavi (1995), is an adequate measure of predictive error for models such as regression trees, thus 10 repetitions of 10-fold CV were used to fit the tree model and to estimate predictive root-mean square error ($RMSE_{pred}$) and predictive R^2 (R^2_{pred}) as indicators of model performance. Statistical analysis were conducted using GenStat v.16 (GenStat, 2013) for one and two-way ANOVAs and correlation coefficients. PCA and regression tree were performed using R statistical software (R 3.3.3; R Core Team, 2017).

5.3 Results

5.3.1 General soil properties

Table 5.1 presents the changes in general soil parameters as an effect of treatments at the depths studied. Detailed analysis of the distribution of P pools and forms in the topsoil (depths 0-2.5, 2.5-5 and 5-10 cm) can be found in Boitt et al. (2017). Long-term aboveground plant biomass removal led to a 13% reduction on the total soil P in the 0-2.5 and 2.5-5 cm soil layers combined (Table 5.1). However, no significant differences were observed on the amounts of total extractable organic P at the same soil depth. Biomass retention resulted in an 18% increase in soil C to 5 cm compared with

soil maintained under biomass removal. However, concomitant increases on C:P, C:N, C:organic P, and N:organic P were only evident in the 0-2.5 cm soil (Table 5.1).

5.3.2 Climatic parameters

Accumulated rainfall was 646 mm over the 17-month sampling period (monthly rainfall ranged from 7 to 117 mm, Figure 5.1) however, no clear seasonal trend was observed. As expected soil temperature and PEVT were lowest during winter months (Figure 5.1). Soil moisture (Figure 5.2) ranged from 9% to a maximum of 29% and, was significantly correlated with PEVT (r = -0.754, *P*<0.001), soil temperature (r = -0.680, *P*<0.001), and to a lesser extent with rainfall (r = 0.343, *P*<0.001).

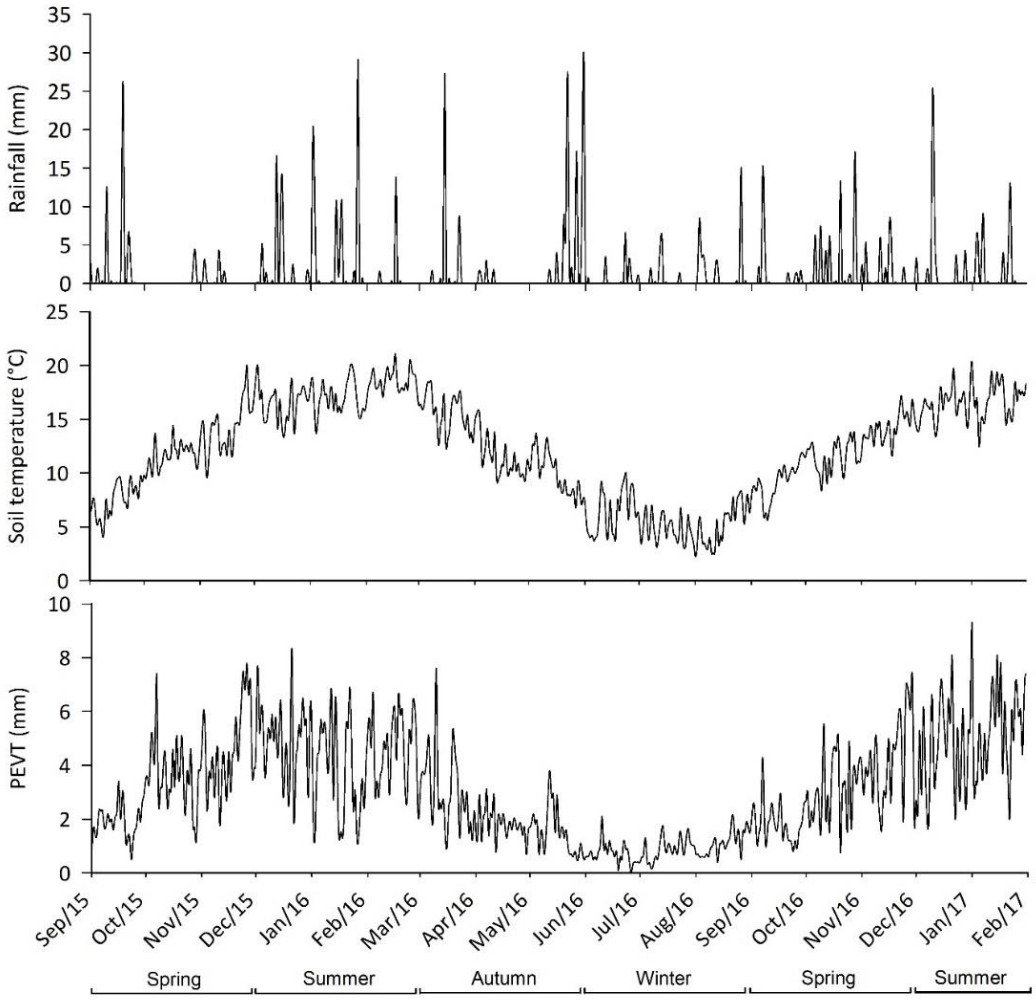


Figure 5.1 Meteorological parameters of daily rainfall, soil temperature and potential evapotranspiration (PVET) during the sampling period (September 2015 to January 2017) in Lincoln, New Zealand.

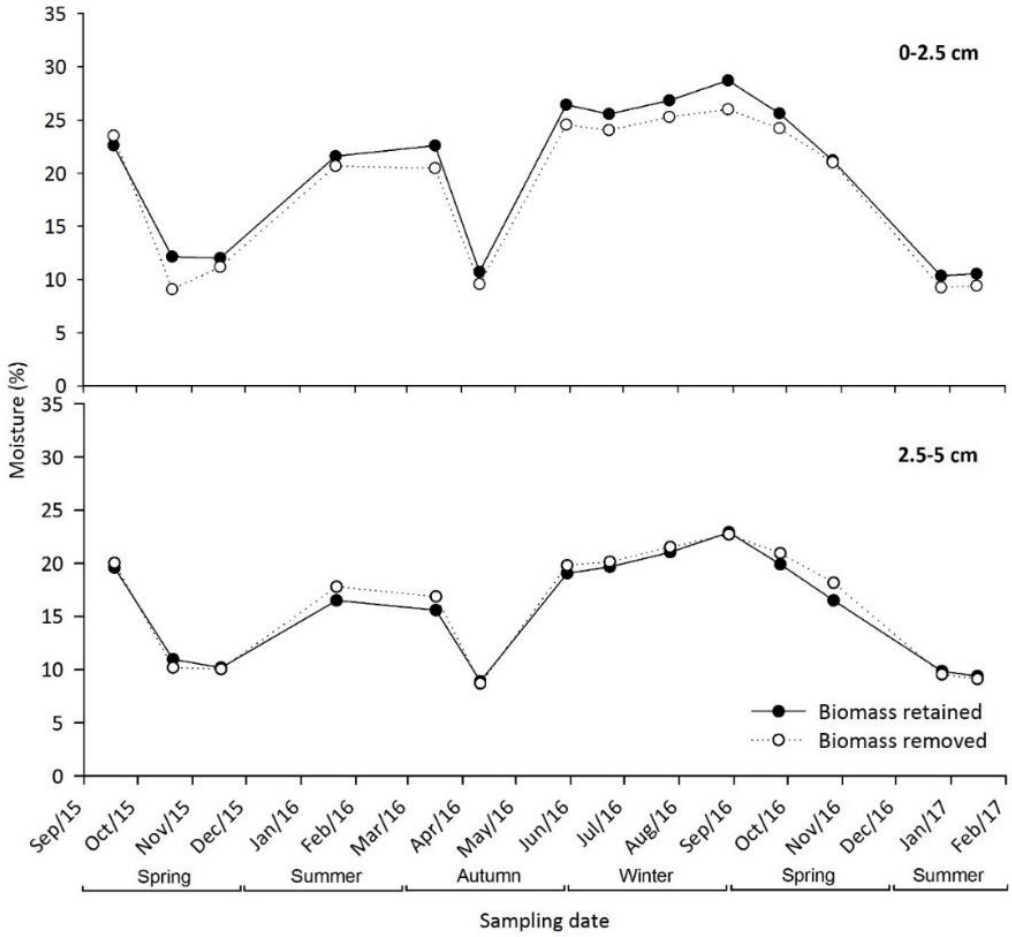


Figure 5.2 Gravimetric soil moisture (%) determined for the soil layers 0-2.5 and 2.5-5 cm over the sampling period September 2015 to January 2017 at the field research trial.

5.3.3 Temporal changes in soil P parameters with respect to treatments

Ordination by PCA is shown in Figure 5.3. The contribution variable to a particular axis (principal component or PC) are estimated by the "loadings" and are represented by the arrows. The horizontal and vertical magnitudes of a loading in reference to the correlation circle correspond to that variable's covariance with the first and second PC's, respectively. Two clusters can be identified which corresponded to the biomass retained and biomass removed treatments (Figure 5.3). The first PC explained 57% of the variability in the data; the three variables associated with this were bioavailable P (loading = -0.56), microbial P (-0.58), and phosphatase activity (-0.59). Principal component 2 explained an additional 23% of the variation, where bioavailable P was the predominant variable (loading = -0.82).

Distribution of the soil P parameters over the sampling period, together with the respective regression tree analysis, are presented in Figures 5.4, 5.5, and 5.6, respectively for bioavailable P,

microbial P, and phosphatase activity. The amount of bioavailable P was significantly affected by grassland management. The treatment effect identified in Figure 5.4a, and the first split performed by the regression tree shown in Figure 5.4b, show that continued removal of biomass resulted in a 70% decrease on the bioavailable P pool in the soil to 5 cm (both soil layers combined). However, the amplitude of temporal changes were small and not significant.

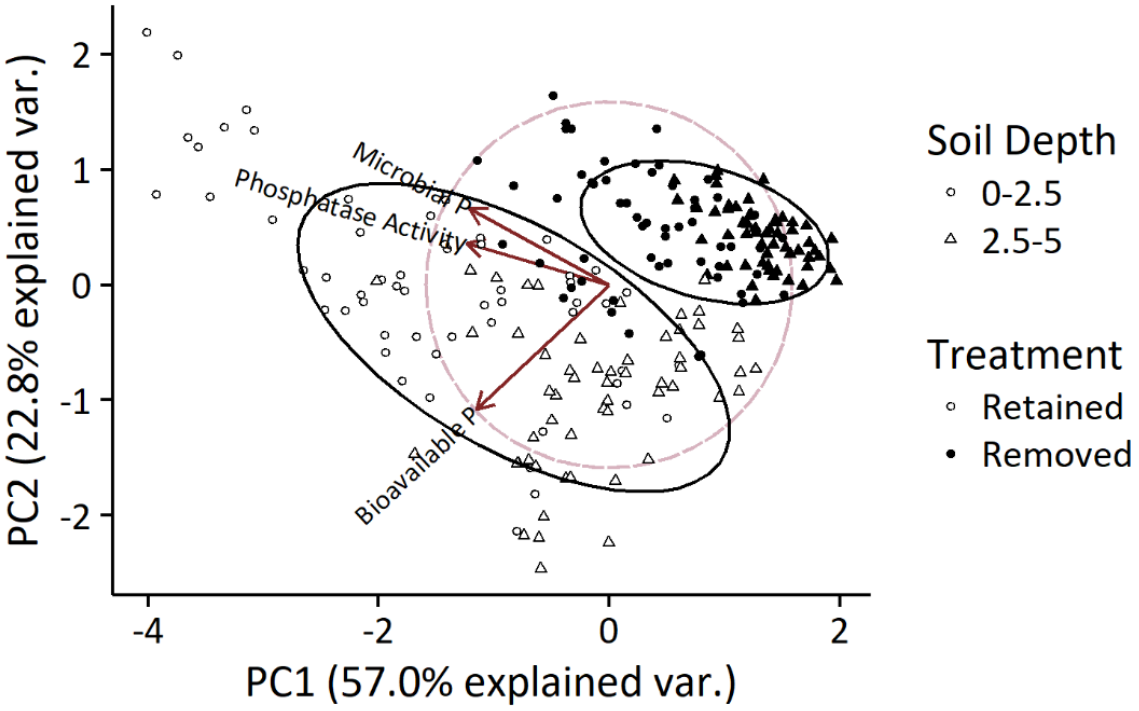


Figure 5.3 Principal component analysis (PCA) investigating the influence of microbial P, phosphatase activity and bioavailable P ($NaHCO_3$-P) in soils from different biomass management and at two different depths. Arrows within the circle (dashed line) represent the correlation of individual variables with the principal components. The length of the arrows indicate the magnitude of increase of that variable; the direction indicate the axis towards which correlation exists.

Temporal fluctuations of microbial P in soils at 0-2.5 and 2.5-5 cm are presented on Figure 5.5a. Microbial biomass P in the soil to 5 cm was 35% higher when biomass was retained. On average, microbial P corresponded to 3.4 and 2.6% of the total soil P in the 0-5 cm soil layer for the biomass retained and biomass removed treatments, respectively. Similar trends were observed for both treatments at the two depths evaluated, with largest amounts of P immobilized in the microbial biomass during spring of 2016. A positive correlation was found (r = 0.675, P<0.001) between microbial P and soil moisture. This was supported by regression tree analysis, where the first branch occurred when the soil moisture was below 21%; no significant differences were apparent between

treatments. However when soil moisture was higher than 21%, clear separation between the grassland management systems was evident as a further branch on the regression tree (Figure 5.5b).

Table 5.1 Selected soil properties determined for soils under long-term (>20 years) aboveground biomass management systems (biomass retained versus biomass removed) at depths 0-2.5 cm and 2.5-5.0 cm.

Soil property	Grassland management					
	Retained	Removed		Retained	Removed	
	0-2.5 cm			2.5-5 cm		
pH	5.6	5.7	ns	5.4	5.4	ns
Carbon (Mg ha^{-1})	8.5	6.8	*	8.8	7.4	*
Nitrogen (kg ha^{-1})	747	691	ns	809	738	ns
Total P (kg ha^{-1})	173	162	ns	217	177	*
Total organic P (kg ha^{-1})	66.8	68.0	ns	85.6	77.9	ns
C:P	49	42	*	41	42	ns
C:N	11.4	9.9	*	10.8	10.1	ns
C:organic P	127	101	*	103	95	ns
N:organic P	11.2	10.2	*	9.5	9.5	ns

Asterisks indicate significant differences between means (n=4) at each soil depth (one-way ANOVA, $P \leq 0.05$). ns = not significant. * = significant at $P<0.05$.

Similar temporal trends among treatments was measured for phosphatase activity (Figure 5.6a). Generally, higher values of phosphatase activity were observed in winter/early spring in both soil depths. Significantly higher phosphatase activity ($P<0.001$) was measured on the 0-2.5 cm soil layer under both treatments, and this was identified by the first branch on the regression tree (Figure 5.6b. Moreover, average phosphatase activity was greater ($P<0.001$) under the biomass retained treatment at both depths increments compared with biomass removed. Enzyme activity was significantly correlated with microbial P, bioavailable P, and soil moisture (Table 5.2).

Table 5.2 Pearson correlation coefficients obtained between the variables evaluated.

	Bioavailable P	Enzyme activity	Soil moisture	Rain	Soil temperature	PEVT
Microbial P	0.324***	0.393***	0.675***	-0.0376^{ns}	-0.373***	-0.391***
Bioavailable P		0.346***	0.053^{ns}	-0.0117^{ns}	0.0343^{ns}	0.035^{ns}
Enzyme activity			0.222***	0.0902^{ns}	-0.155*	-0.167*
Soil moisture				0.343***	-0.68***	-0.754***
Rain					0.0209^{ns}	-0.357***
Soil temperature						0.838***

*P<0.05; ***P<0.001; ns = not significant. PEVT = potential evapotranspiration.

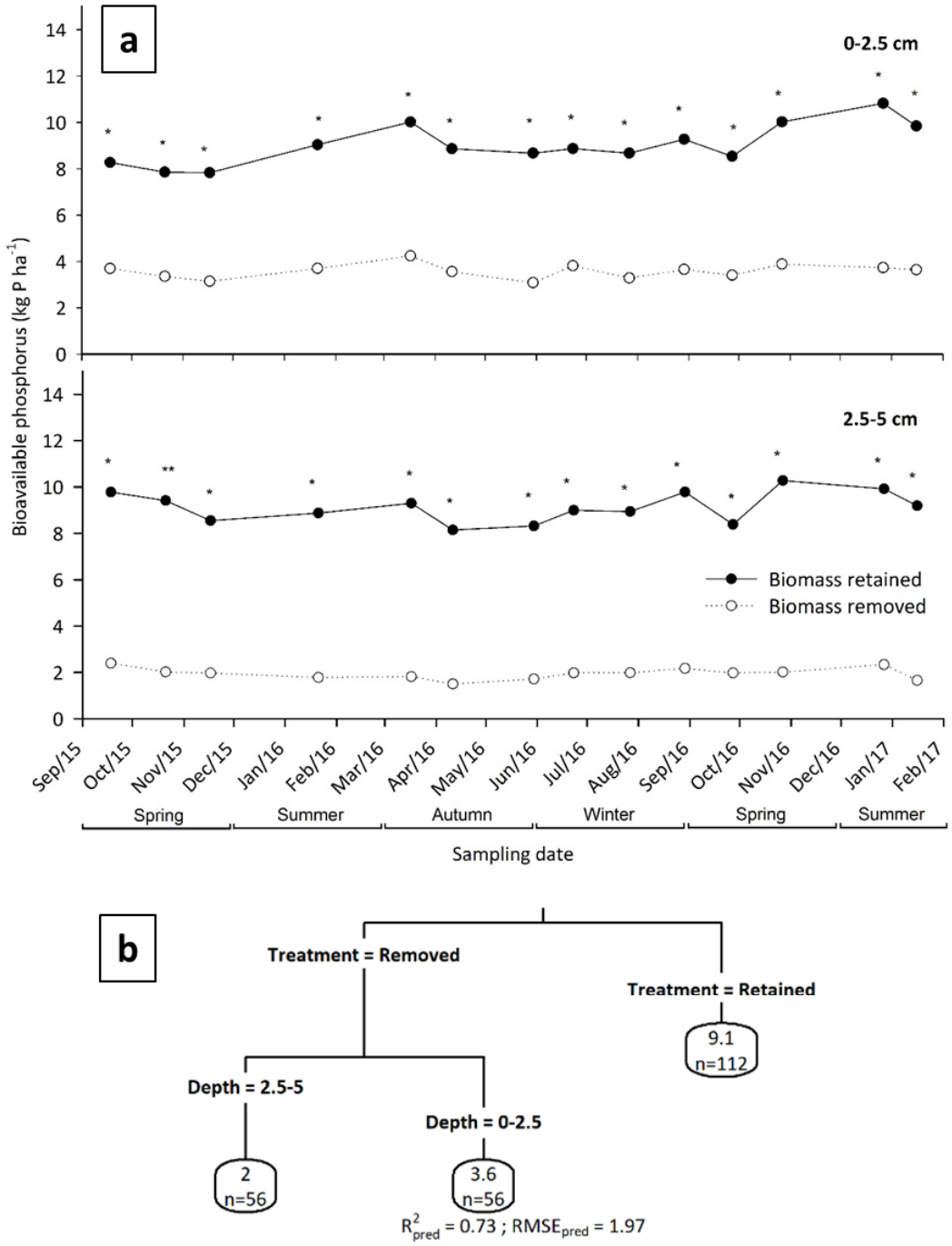
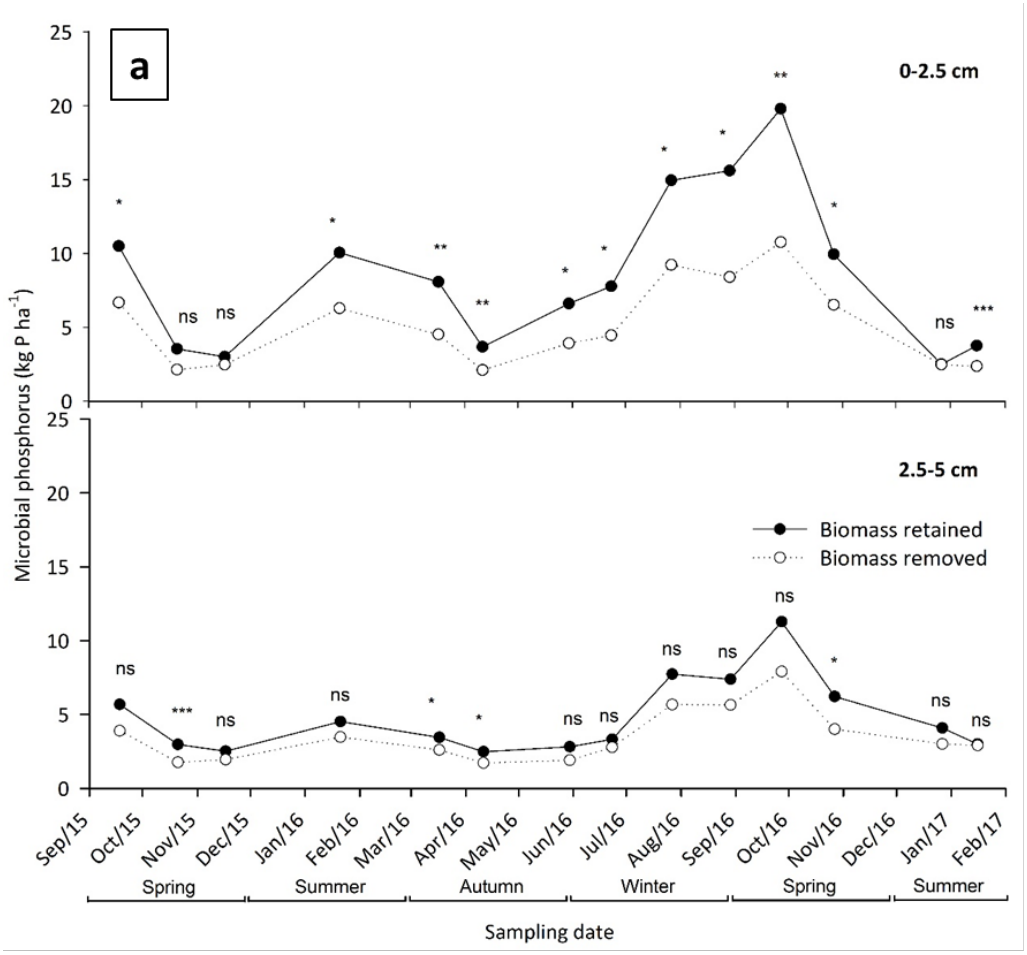


Figure 5.4 (a) Bioavailable phosphorus estimated after extraction with 0.5M $NaHCO_3$ pH 8.5 in non-fumigated samples taken on 14 occasions over the period from September 2015 to January 2017 in soils form 0-2.5 and 2.5-5 cm depths under permanent grassland taken from the long-term ecology trial at Lincoln, New Zealand. Asterisks above the data indicate significant effects (P*<0.05; ***P*<0.01). (b) Regression tree analysis of the variable bioavailable P; modelled values are expressed in kg P ha^{-1}.**



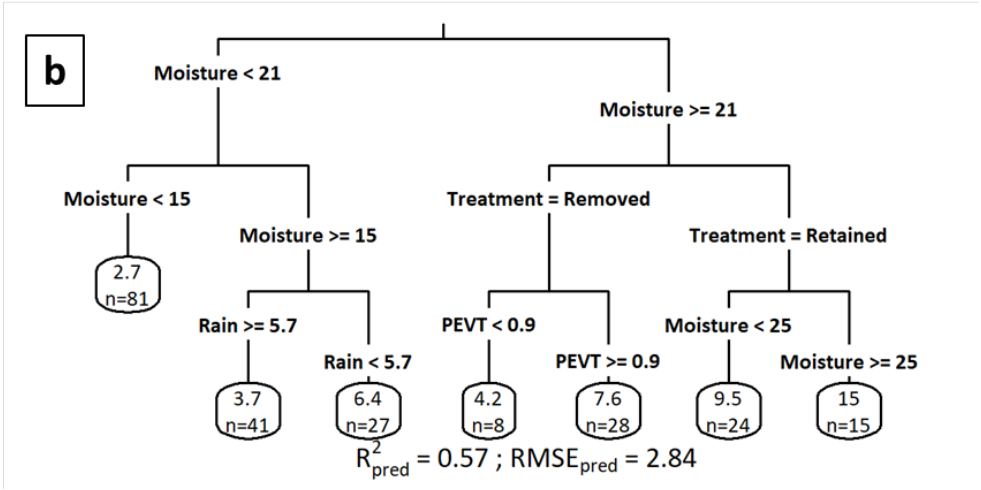
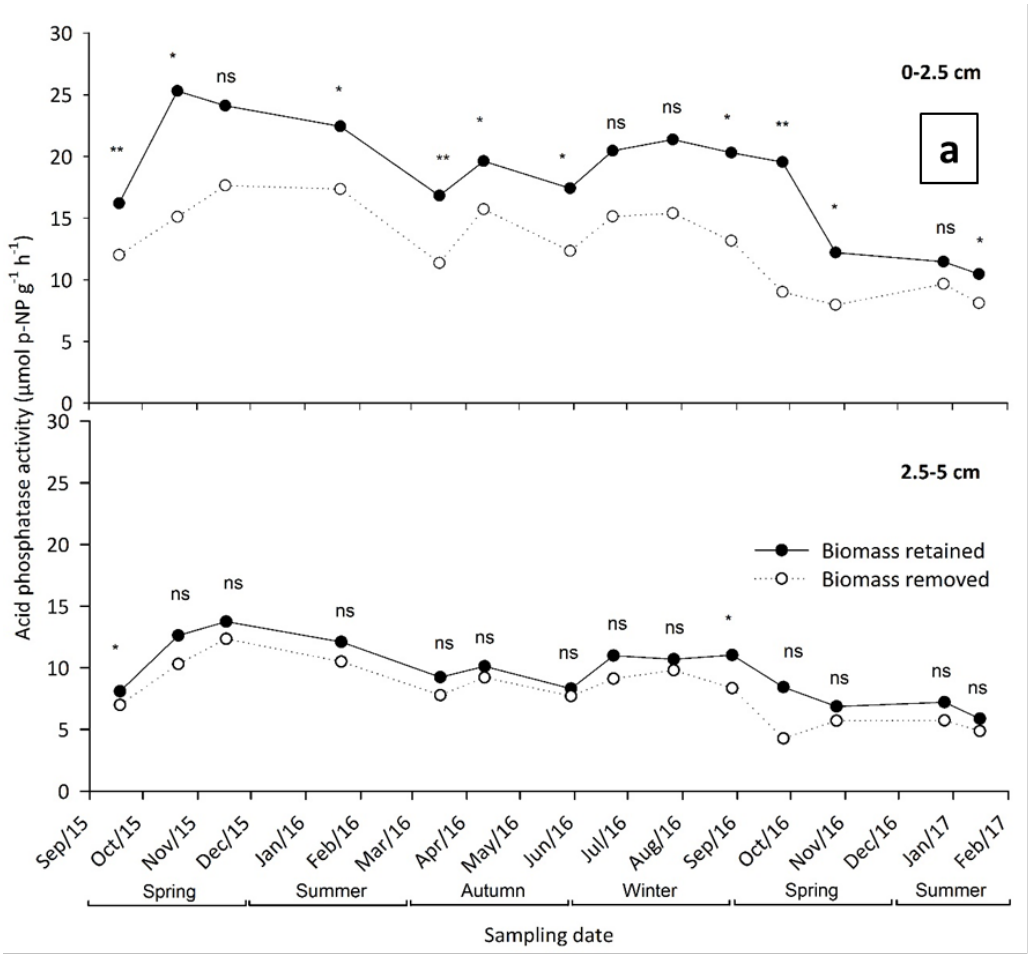


Figure 5.5 (a) Microbial biomass phosphorus measured on 14 occasions over the period from September 2015 to January 2017 in the 0-2.5 and 2.5-5 cm soil layers under permanent grassland taken from the long-term ecology trial at Lincoln, New Zealand. Asterisks above the data indicate significant treatment effects (P*<0.05; ***P*<0.01; ****P*<0.001; ns = not significant). (b) Regression tree analysis of the explanatory variable microbial biomass P; modelled values are expressed in kg P ha^{-1}.**



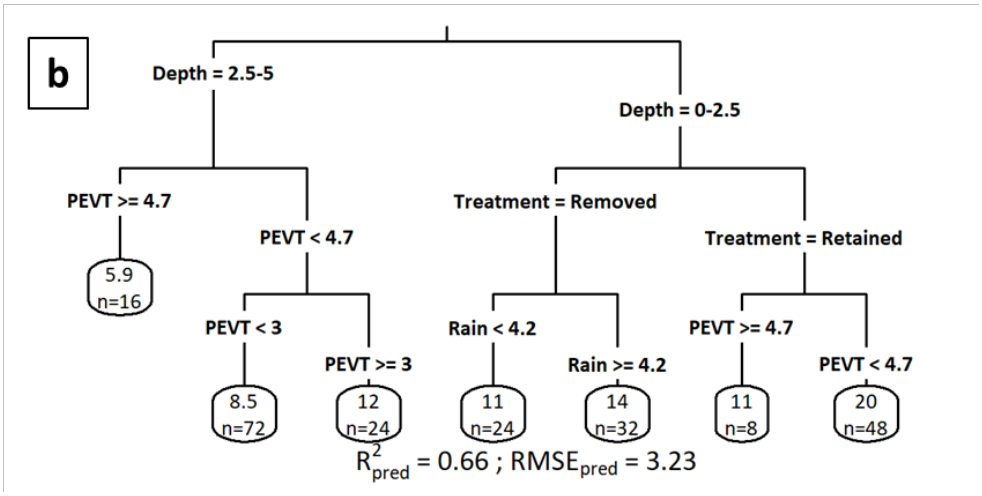


Figure 5.6 (a) Acid phosphatase activity measured on 14 occasions over the period from September 2015 to January 2017 in the 0-2.5 and 2.5-5 cm soil layers under permanent grassland taken from the long-term ecology trial at Lincoln, New Zealand. Asterisks indicate significant treatment effect (*P<0.05; **P<0.01; ns = not significant). (b) Regression tree analysis of the explanatory variable phosphatase activity; modelled values are expressed in µmol ρ-NP g^{-1} h^{-1}.

5.3.4 Microbial P fluxes

Table 5.3 presents estimated turnover rates and P fluxes through microbial biomass. Turnover rates were = 1.24 and 0.97 per year for the 0-5 cm soil under biomass retained and biomass removed, respectively. In this soil layer, P fluxes were 13.4 kg P ha^{-1} $year^{-1}$ for biomass retained and 7.2 kg P ha^{-1} $year^{-1}$ for biomass removed. Microbial P fluxes and turnover rates were more pronounced in the top soil layer (0-2.5 cm) than at 2.5-5 cm. Biomass removal resulted in significantly (P<0.05) lower P fluxes (51% reduction) compared to the biomass retained treatment in the soil to 2.5 cm. Similar trends were evident for the soil layer 2.5-5 cm (29% lower P fluxes under biomass removed than the biomass retained).

Table 5.3 Estimated turnover rates and annual P fluxes though the microbial biomass under contrasting grassland management systems for the period from September 2015 to January 2017.

P released (-) from or stored (+) in microbial biomass over the sampling period (kg P ha^{-1})	Grassland management			
	Retained	Removed	Retained	Removed
	0-2.5 cm		2.5-5 cm	
September 2015	4.4	3.0	2.3	1.4
October 2015	-2.6	-1.6	-0.5	-0.7
November 2015	-3.1	-1.2	-0.9	-0.6
January 2016	4.0	2.6	1.1	0.9
March 2016	2.0	0.8	0.0	0.1
April 2016	-2.4	-1.6	-1.0	-0.8
May 2016	0.5	0.2	-0.6	-0.6
June 2016	1.7	0.8	-0.1	0.3
July 2016	8.9	5.5	4.3	3.2
August 2016	9.5	4.7	4.0	3.1
September 2016	13.7	7.1	7.8	5.4
October 2016	3.8	2.8	2.8	1.5
December 2016	-3.6	-1.2	0.7	0.5
January 2017	-2.3	-1.3	-0.7	0.5
Total stored (annual)	**34.6**	**19.7**	**16.4**	**12.0**
Total released (annual)	**-10.3**	**-5.0**	**-3.1**	**-2.2**
Turnover rate ($year^{-1}$)	**1.60**	**1.31**	**0.90**	**0.89**
Turnover time (years)	**0.68**	**0.80**	**1.14**	**1.15**
Annual biomass P flux[a] (kg P ha^{-1} yr^{-1})	**10.3**	**5.0***	**3.1**	**2.2^{ns}**

[a]Asterisk indicates significant differences between treatment means (n=4) for the respective soil depth (one-way ANOVA, P=0.032, LSD=4.45). ns = not significant. Two-way ANOVA showed significant differences in treatment (P=0.012) and depth (P<0.001) effects and not significant interactions.

5.4 Discussion

Quantities of P in both the bioavailable inorganic and microbial biomass pools were consistently and significantly lower in soil that had been maintained under long-term grassland biomass removal compared with biomass retention. These findings are consistent with substantial reductions in inorganic and total soil P that occurred as a consequence of 20 years of biomass-P removal. In addition retention of the biomass carbon and increased levels of soil organic carbon may have supported enhanced soil biological activity resulting in higher microbial biomass P (McDowell et al., 2016; Boitt et al., 2017). Furthermore, differences in soil P pools between the contrasting biomass management regimes were greater in the top 0-2.5 cm soil compared with 2.5-5 cm soil, which is consistent with the return of plant biomass to the soil surface at each mowing event. These findings highlight the importance of studies linking soil biodiversity, fertility and plant productivity carried out on the uppermost soil layers, also suggesting major negative impacts of surface soil degradation to nutrient cycling and primary productivity (Delgado-Baquerizo et al., 2017).

The size of the bioavailable inorganic P fraction, in all soil depths and under both treatments, remained unchanged over the 17-month duration of the experiment. This was surprising given that plant growth and P uptake would have been expected to change with time over this period (not determined), particularly on a seasonal basis. In the same geographic location (Lincoln, New Zealand), Scott and Condron (2003) found that concentrations of bicarbonate-extractable inorganic P (bioavailable P) in soil (0-20 cm) under agroforestry (grazed pasture - radiata pine (*Pinus radiate* D. Don.)) were significantly higher in November (spring) compared with May (autumn) which was attributed to enhanced organic P mineralisation in spring. On the other hand, quantities of microbial biomass P varied up to 10-fold between sampling dates in the 0-2.5 cm soil, from 2 to 20 kg P ha^{-1}. Thus microbial biomass P was highest in spring (August-September-October 2016) compared with other periods. These findings indicate that the microbial biomass was an important source of P for plants in this grassland environment, which is consistent with recent data from New Zealand forest ecosystems (Turner et al., 2013).

Regression tree analysis revealed that microbial biomass P was most closely correlated with soil moisture over the 14 sampling dates. This is consistent with water availability being an important determinant driver of microbial activity and nutrient acquisition in soil (Oberson and Joner, 2005), especially in dryland environments (McGill et al., 1986; Perrott et al., 1990; Tate et al., 1991; Perrott et al., 1992; He et al., 1997; Chen et al., 2003). Enhanced soil microbial biomass P in spring is also consistent with findings from other short-term studies conducted in temperate grassland environments (Perrott et al., 1990; Tate et al., 1991; He et al., 1997; Chen et al., 2003; Scott and Condron, 2003).

Increased phosphatase enzyme activity in the surface layer (0-2.5 cm) of the biomass retained soil treatments was consistent with changes in soil microbial biomass and soil organic carbon in those samples (discussed above). The absence of clear differences in phosphatase activity between biomass treatments in the 2.5-5 cm soil may reflect the relative magnitude of differences in soil organic carbon and microbial biomass P between treatments in this soil compared with the 0-2.5cm soil. On the other hand, a reduction in soil bioavailable inorganic P as a consequence of long-term biomass removal might have been expected to result in higher acid phosphatase enzyme activity reflecting enhanced mineralisation of soil organic P by microbes and plant roots (McGill and Cole, 1981; Stewart and Tiessen, 1987; Nannipieri et al., 2011). It is possible that long-term bioavailable inorganic P depletion by biomass removal increased acid phosphatase enzyme production by microbes and roots, but this was off-set by enhanced stabilisation and persistence of measured enzyme activity by adsorption onto increased accumulation of soil organic matter under biomass retention (McGill and Cole, 1981). This could be at least partly resolved by assessment of the impact of long-term biomass management on the abundance and expression of microbial phosphatase genes (Fraser et al., 2015). Furthermore, the fact that temporal changes in soil acid phosphatase activity were minor and inconsistent suggests that enzyme activity, as determined by the assay technique, might not adequately reflect short-term changes in enzyme production by microbes and plant roots linked to environment conditions. This may be biased toward measurement of stabilised enzyme activity, which in turn would be influenced by long-term changes in soil properties linked to management (McGill and Cole, 1981; Magid et al., 1996; Oberson and Joner, 2005).

Turnover rates and P flux through microbial biomass affect the wider dynamics and availability of P in agroecosystems (Magid et al., 1996; Frossard et al., 2000; Oberson and Joner, 2005; Bünemann et al., 2012). Results presented here consistently showed considerable seasonal variability, greater amounts of P in the microbial biomass (Figure 5.3, Figure 5.5a and 5.5b), and P fluxes through microbial biomass (Table 5.3) in soils under biomass retained treatment compared to biomass removed management. These parameters were substantially more pronounced in the 0-2.5 cm soil layer.

Augmented organic matter inputs and accumulation as soil organic matter (soil carbon, Table 5.1), increased plant P demand and dry-matter yields (biomass retained), and reduced P availability (Boitt et al., 2017) as a result of long-term plant biomass exports (biomass removed) can explain this enhanced soil microbial activity observed for the treatment with biomass retained. Compared to the P flux data shown in Table 5.2, plant P uptake in the grassland biomass are estimated as 10-12 kg P ha^{-1} $year^{-1}$ for biomass retained and 4-5 kg P ha^{-1} $year^{-1}$ for biomass removed (using data presented in Boitt et al., 2017) and therefore, clearly demonstrates the role and importance of microbial P in redistributing and delivering plant available P (Richardson et al., 2009; Richardson and Simpson,

2011). Brookes et al. (1984) estimated annual P fluxes of 7-40 kg P ha^{-1} for grassland soils, 2-11 kg P ha^{-1} for arable soils and 21.7 kg P ha^{-1} for a woodland soil in the U.K. (assuming turnover time = 2.5 years). In fertilized pastures in the U.K., He et al. (1997) measured expressive amounts of P in the microbial biomass equivalent to 17-290 kg P ha^{-1} in the soil to 15 cm depth. They considered a turnover time of 1.5 years (turnover rate = 0.67 per year) and estimated P fluxes through the microbial pool of 11-190 kg P ha^{-1} $year^{-1}$ which, in terms of comparison, were several times larger than P removed in herbage (2-11 kg P ha^{-1} $year^{-1}$; dry matter yields of 1.13-4.06 t ha^{-1}). Chen et al. (2003) estimated release of P from microbial biomass of 13.8 kg P ha^{-1} $year^{-1}$ for grassland and 16.1 kg P ha^{-1} $year^{-1}$ for forest soils (0-5 cm) in New Zealand high country. The authors found faster turnover rates in forest soils (1.28 per year) than grassland soils (0.80 per year), despite the lower concentrations of microbial P under the forest. Differences were attributed to the role of the forest litter-fall in P recycling processes (Chen et al., 2003; Vincent et al., 2010). However, in soils from the north island of New Zealand, Sparling et al. (1994) estimated microbial P fluxes, assuming a turnover rate of 0.3 (turnover time = 3.33 years), of 36 kg P ha^{-1} $year^{-1}$ for pastures and about 20 kg P ha^{-1} $year^{-1}$ on forest and scrubland sites in the 0-20 cm soil layer. The enhanced microbial activity and P fluxes observed in soils under the biomass retained treatment is supported by a number of studies using isotopic dilution techniques. Collectively these found increased potential organic P mineralisation rates as a result of organic amendments to soils and land use practices (Oberson et al., 1996; Oberson et al., 2001; Oehl et al., 2001; Oehl et al., 2004; Randhawa et al., 2005; Bünemann et al., 2012).

Continued biomass removal resulted in marked reductions in soil P (McDowell et al., 2016; Boitt et al., 2017). Reduced soil P availability ultimately resulted in decreases in plant P uptake as a result of continued biomass removal (Simpson et al., 2012; Boitt et al., 2017). Simpson et al. (2012) measured a higher P uptake by red clover (*Trifolium pratense* L. cv. Pawera) and Italian ryegrass (*Lolium multiflorum* L. cv. Grasslands Moata) grown in soils taken from the biomass retained plots in comparison to biomass removed treatment. They attributed the differences to possible enhancements in microbial activity and returns of organic matter, which was confirmed by the findings of the present study. While the results of this study confirmed the importance of the soil microbial biomass in P cycling, we acknowledge that soil fauna such as protozoa and nematodes play a vital role in the release and subsequent bioavailability of microbial P in soil (Cole et al., 1977; Magid et al., 1996; Bonkowski, 2004; Oberson and Joner, 2005; Richardson and Simpson, 2011).

5.5 Conclusions

Above-ground biomass management and environmental conditions had a significant impact on the short-term biogeochemical soil P cycling in this grassland ecosystem. Microbial biomass, soil phosphatase activity, and bioavailable P were consistently higher in soils under biomass retained compared to removed. Environmental factors were the main drivers of the temporal variations in microbial biomass and phosphatase activity. The important role of the soil microbial biomass in P dynamics was demonstrated by the fact that microbial P turnover in the topsoil (0-2.5 cm) was 22% faster under biomass retained, while P flux through the microbial pool was 2-fold higher under biomass retained compared to removal over a 17-month period.

Chapter 6

General Discussion and Future Research

Long-term phosphorus (P) inputs and P accumulation in highly productive agricultural systems is a predominant condition in soils worldwide. Thus, the major goal of this work was to quantify the impact of long-term contrasting land-use and management strategies on the P dynamics, and implications to the P use and legacy P in soils. The reason for establishing this objective was to highlight the importance on adopting and adjusting intervention practices to maximise P use efficiency of agriculture. In spite of divergences on the current projections of phosphate rock depletion, sustainable agriculture can only be achieved with maximised productivity while minimising impacts to the wide environment.

Accordingly, three comprehensive, adequately established, under consistent long-term management, and adequately replicated field trials were chosen and utilised for this study. These long-term field trials encompassed a contrasting range of land-use and environments. Nonetheless, these different agricultural systems presented one inherent characteristic in common: substantial P inputs and accumulation of residual P or legacy P in soil. Additionally, the first two long-term field trials studied here; i) Winchmore, New Zealand; ii) Santa Catarina, Brazil; were originally designed to evaluate agronomic responses in primary products to quantitative inputs of resources; whereas the third long-term field experiment; iii) Lincoln, New Zealand; was primarily designed to investigate the impacts of biomass management practices and biogeochemical soil properties and processes.

The first study, presented on Chapter 2, demonstrated the impacts of 62 years of constant P inputs and varying levels of supplemental irrigation to a temperate grazed pasture system. Total P accumulation in soil to 100 cm depth was inversely proportional to the water input rates, and is graphically represented in Figure 6.1. Furthermore, P mass balance provided invaluable insight of the P dynamics on the soil-plant-animal agroecosystem. Phosphorus removed in the irrigation outwash was confirmed as the major pathway of P losses following intensification of the irrigation rates. For a 3-fold increase in irrigation inputs, i.e. from 260 to 770 mm $year^{-1}$ of supplemental irrigation (to maintain gravimetric moisture at a minimum of 10 or 20%, respectively), P removal in overland flow increased by a 13-fold in magnitude. Phosphorus losses in irrigation outwash accounted for 8 and 42% of the total P decline under these irrigation rates, respectively. Summed, P losses in outwash and internal P transfers both comprised the majority of the observed soil P depletion under irrigated plots. The remaining amounts of P were accounted for in plant residues and animal product removal. There was no evidence was found to support significant P transfers within the profile or P loss through leaching in the soil below 100 cm under irrigated grazed pasture after the long-term.

Despite advances in technology and efficiency of irrigation systems, surface irrigation (i.e. areas equipped with furrow, border strip and basin irrigation systems) encompasses the majority (86%) of the irrigated area globally (mainly located in India, China and Pakistan). On the other hand, sprinkler irrigation areas correspond to 11% of the irrigated land, mainly located in the USA, China and Brazil (FAO, 2016).

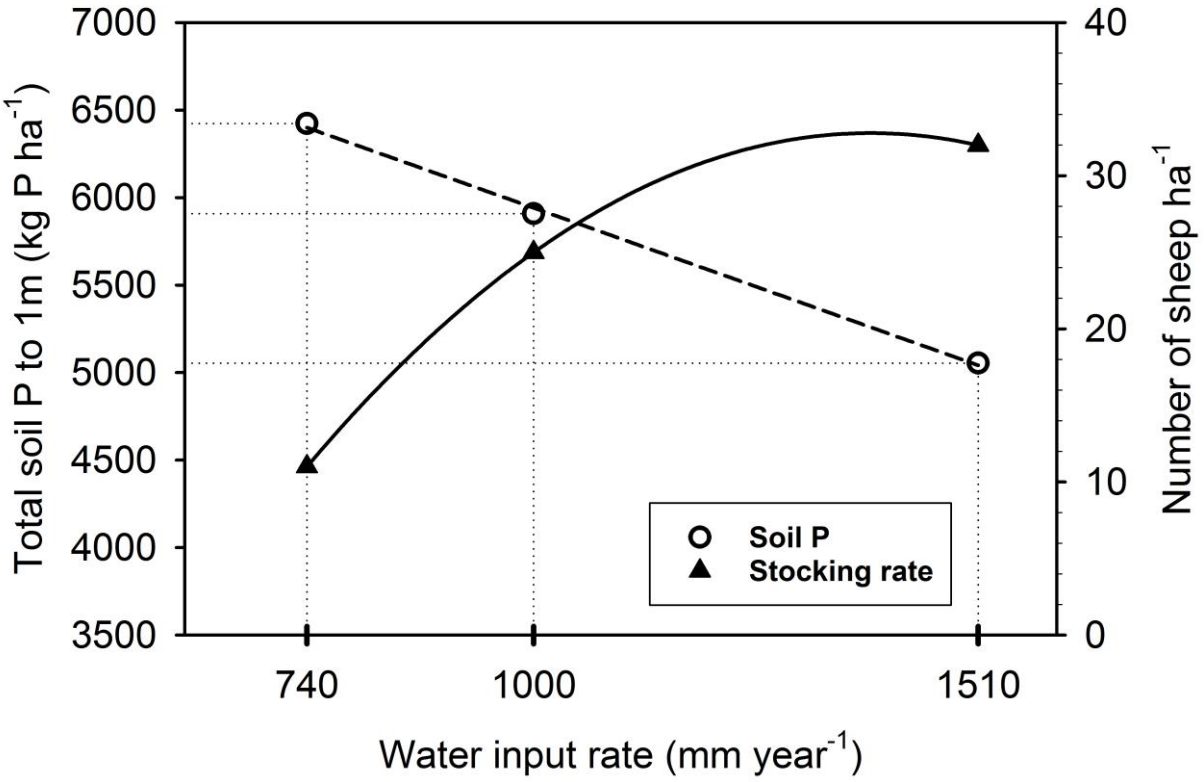


Figure 6.1 Overview of the long-term irrigation impacts on total soil P to 100 cm after 62 years, and sheep stocking rate at the long-term irrigation trial, Winchmore, New Zealand. The water input rates, on the *x*-axis, correspond to the average rainfall (740 mm year^{-1} [control]) and supplemental irrigation (740 + 260 mm year^{-1} [irrigation$_{10\%GM}$]; and 740 + 760 mm year^{-1} [irrigation$_{20\%GM}$]).

Phosphorus fractionation analyses revealed large quantities of inorganic and organic P forms accumulated in soils under no-tillage cropping receiving long-term pig slurry inputs to a high P-sorbing Oxisol. Accumulation of total P and vertical movement were proportional to the input rates, and confined to the top 20 cm soil layer. Phosphorus mass balance confirmed that the majority of P added, at rates up to 275 kg P ha^{-1} year^{-1}, was accumulated in the soil mainly in sparingly available inorganic and organic P forms (Figure 6.2). Phosphorus in sparingly available forms accumulated linearly, at a rate of 6.2 kg P ha^{-1} year^{-1} for every 10 m^3 of pig slurry added (equation in Figure 6.2B).

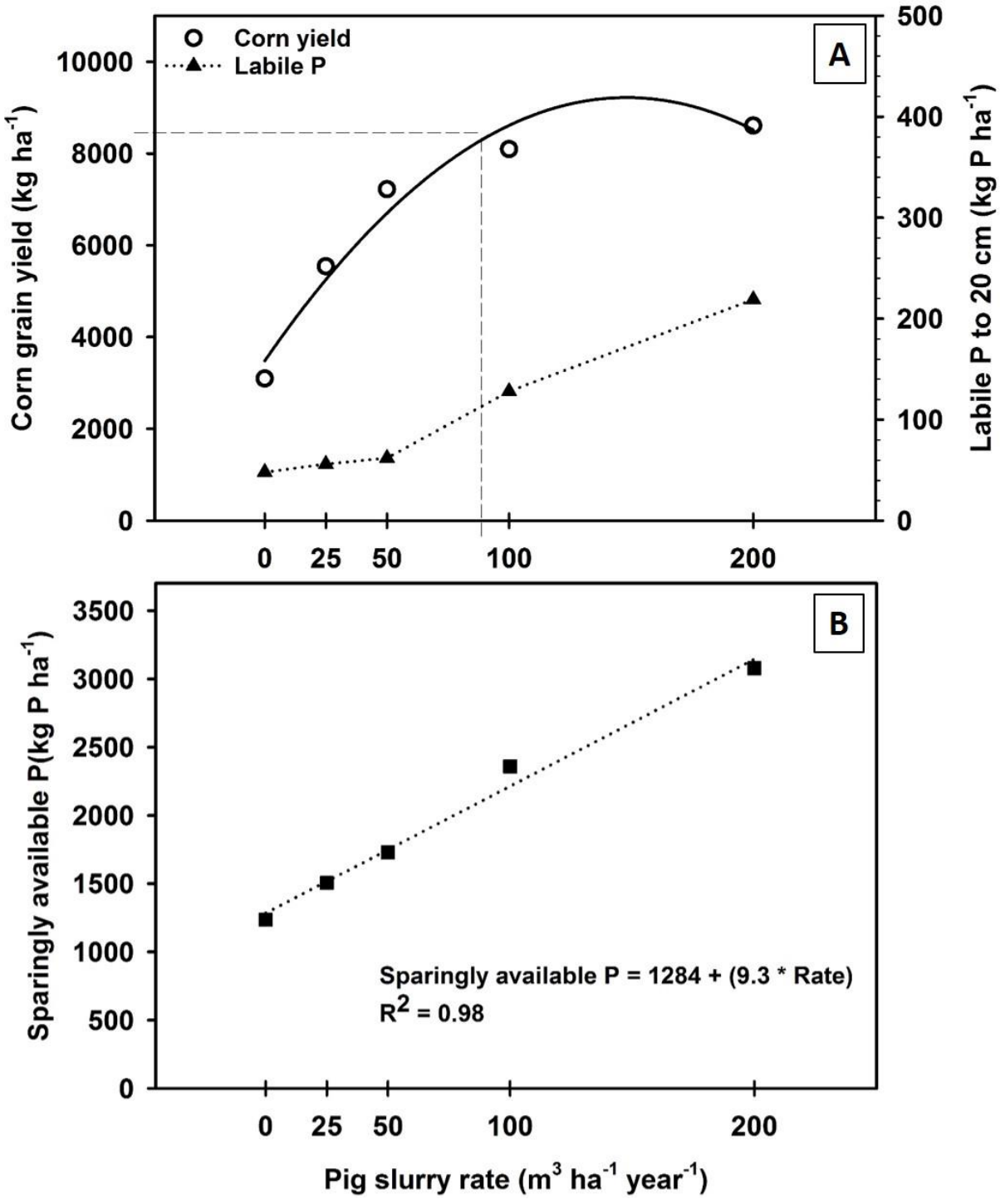


Figure 6.2 Overview of the agronomic responses of corn grain yields (average of 13 years), and amounts of labile P (NH_4Cl-P + $NaHCO_3$-P) in the top 20 cm soil layer [A]; and, amounts of sparingly available P (NaOH-P + HCl-P) [B] accumulated in soils (0-20 cm) after long-term (15 years) annual pig slurry inputs in an Oxisol in Santa Catarina, Brazil. The dashed lines [A] indicate 90% of the maximum potential yield (y-axis), and an interpolation (x-axis) showing the estimated pig slurry input rate to achieve 90% of the maximum yield.

Additionally, the P mass balance demonstrated the large disparities between P inputs and P exports in products on highly P retention soils. These imbalances led to decreased on the P balance efficiency index. For example, P input rates necessary to achieve the agronomic optimum (i.e. 90% of maximum yield) were nearly 8-fold higher than P removed in corn grain (average P input = 124 kg P ha^{-1} y^{-1}; P exports = 17 kg P ha^{-1} y^{-1}). It is also important to note that this cropping system can be considered a medium-high technology. Moreover, a "change-point" was observed in soils receiving pig slurry input rates equivalent to 69 kg P ha^{-1} y^{-1} (50 m^3 slurry ha^{-1} y^{-1}) (Figure 6.2). After 15 years of pig slurry inputs, substantial amounts of organic P were accumulated (between 8 and 28 kg organic P ha^{-1} y^{-1}). Average rates of organic P accumulation were similar or even higher than quantities of P exported in grain (on average for 15 years). Hence, immobilisation of P in the soil organic P fraction constituted an important part of the legacy P in these intensive agroecosystems.

The long-term ecology trial in Lincoln, New Zealand consists of a simple and objective experimental design, invaluable nonetheless. Chapter 4 presents some new research regarding utilisation of legacy P in the event of P fertiliser inputs cessation. Data presented in the Chapter 4 of this thesis demonstrated the role and importance of legacy P, the effects of P depletion on the P bioavailability and responses to plant P nutrition. The ultimate soil test P, i.e. the plant P uptake and responses of the grassland clearly demonstrated the role and bioavailability of the residual P or legacy P accumulated in soils. Drawdown of 35% of inorganic P reserves, as a consequence of biomass removal and withhold on P inputs was followed by significant constrains to grassland biomass production (i.e. plant biomass production was reduced by 50%). On the other hand, similar amounts of total soil organic P were identified, despite long-term P depletion. Nonetheless, enhanced amounts of labile organic P forms were present in soil under biomass retained regime than biomass removed. There are three possible explanations to explain the apparent "stability" of the organic P pool in this system: i) the organic P species present a limited solubility/high stability (as evidenced by the predominance of monoester phosphate); ii) the inorganic P pool is not limiting (evidenced by the relative low C:P and N:P ratios comparing to tropical soil for instance); and iii) organic P is retained with the biomass (evidenced by the concomitant increases in soil organic matter and C:N ratios).

These findings led to the fourth study, presented on Chapter 5. Despite apparent relative stability of the organic P pool, biological P is likely to play a pivotal role on the ecosystem function in soils under long-term management. The microbial and biochemical indicators assessed (microbial P and phosphatase activity) were highly dependent on environmental and management practices. Phosphorus fluxes through the microbial biomass, estimated in the 0-5 cm soil alone, were nearly equal to the P uptakes and exports in grassland biomass (or potentially exported in the case of biomass retained regimen). Moreover, estimated microbial P fluxes and turnover rates in the 0-2.5 cm depth increased by over 100% and 20%, respectively, in soils under biomass retained comparing

to biomass removed in a 17-month period. Figure 6.3 presents a conceptual diagram summarizing the main findings of Chapters 4 and 5.

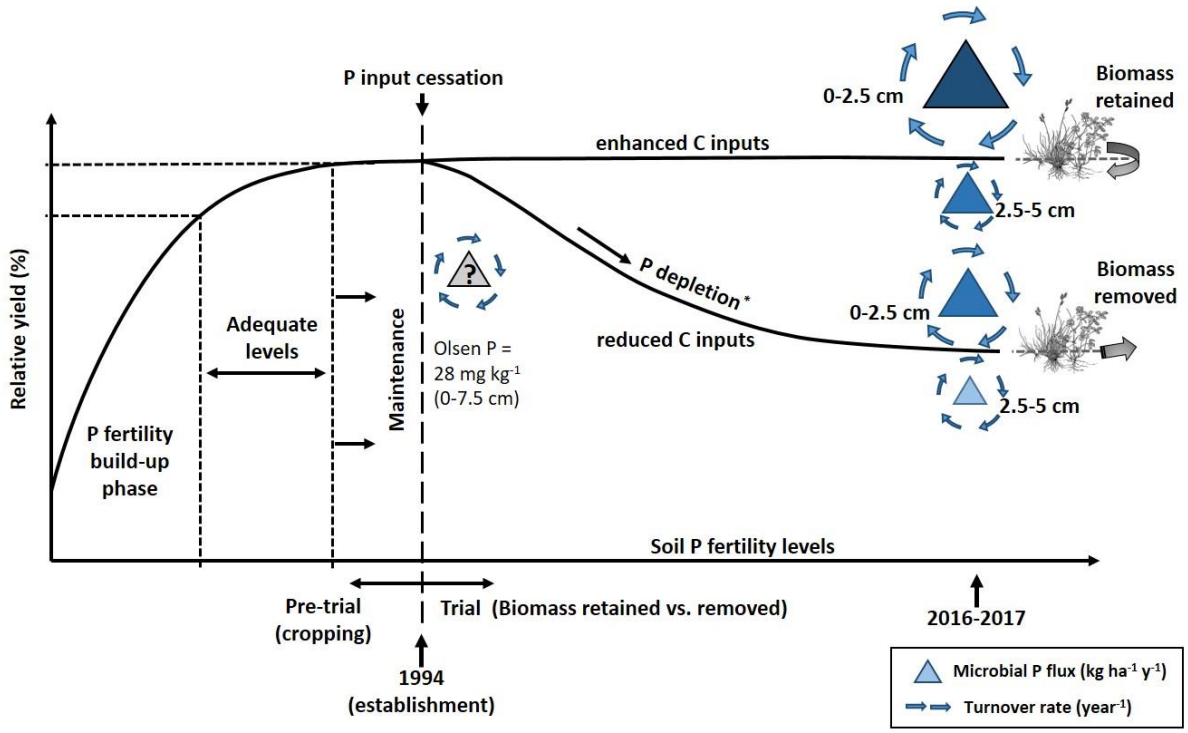


Figure 6.3 Conceptual diagram of the P dynamics in the long-term ecology trial, Lincoln, New Zealand. The *x*-axis hypothetically represents the soil P fertility levels along the time. *The shape of the soil P depletion curve is based on data shown by Dodd et al. (2012) and McDowell et al. (2016). Microbial P fluxes and turnover rates are represented as an approximated relative scale.

Long-term P accumulation in sparingly available inorganic and organic forms, together with P depletion in soils as affected by land management strategies, play a dominant role in determining P availability and plant P nutrition efficiency. Strategies to enhance microbial biomass activity are of crucial importance for improving P-use efficiency of agriculture. Intensively managed agroecosystems should always consider the agronomic optimum recommendations, together with adoption of soil conservationist practices to minimise soil and nutrient losses, and thus support sustainability. Long-term field trials are invaluable resources for studying biogeochemistry and dynamics of nutrients in agricultural systems.

Based on the findings of these studies, more research is necessary to investigate and quantify the role of legacy P under intensive agroecosystems as influenced by land management practices. Quantification of turnover rates of organic and microbial P together with their role in the short-term plant nutrition (e.g. over the duration of a growing season) under different land-uses and

management can potentially yield data to support strategic decisions to improve microbial activity and biodiversity in managed agroecosystems.

The role of legacy P at a field scale together with economic efficiencies in these agricultural systems is largely unknown in highly P-fixing soils in tropical and subtropical environments. Phosphorus use efficiency of agriculture on highly P-sorbing soils can be potentially increased, on the long-term, by adopting strategies to enhance soil organic matter and microbial activity. Significant mobilization of legacy P (inorganic and organic P accumulated) is likely to be achieved by the inclusion of plants species, in the crop rotation scheme, with enhanced capabilities to access sparingly soluble P forms, such as lupins (*Lupinus* spp.) and oilseed radish (*Raphanus sativus*).

Detailed studies of the dynamic and ecology of beneficial associations of soil microorganisms with plants in the rhizosphere of different plants will potentially expand our knowledge of soil processes related to residual P mobilisation, including organic P forms. Recent developments in transcriptomic techniques for determining phosphatase gene expression in the soil-plant interaction, together with recently developed protocols for determining enzyme activity in the rhizosphere with soil zymography will potentially advance our knowledge regarding mobilisation of legacy P in soils, and plant growth promotion. The role and function of root exudates (including low molecular organic anions) in the plant-soil-microorganism association, in the rhizosphere remains unclear.

Appendix A

A.1 Table A.1. Mean concentrations of P (mg P kg^{-1}) determined in P fractions for soils sampled from different depths after 62 years under control and irrigated grazed pasture: total inorganic P (Pi) = sum of Pi_{AM}, Pi_{bic}, Pi_{OH}_I, Pi_{HCl}, Pi_{OH}_II, total organic P (Po) = sum of Po_{bic} + Po_{OH}_I + Po_{OH}_II. Total P concentrations determined after Na_2CO_3 fusion.

Irrigation rate	Depth (cm)	Pi_{NH4Cl}	Pi_{bic}	Po_{bic}	Pi_{OH}_I	Po_{OH}_I	Pi_{HCl}	Pi_{OH}_II	Po_{OH}_II	$P_{residual}$	Total Pi (sum)	Total Po (sum)	P_{total}
Control	0-7.5	4.6	97.7	69.2	345.3	325.1	140.5	65.6	83.9	224.4	878.0	478.1	1489.9
$Irrigation_{10\%GM}$	0-7.5	0.1	12.0	5.7	177.1	124.5	180.3	33.0	22.8	74.0	476.5	153.0	1074.6
$Irrigation_{20\%GM}$	0-7.5	1.1	12.8	18.7	171.0	146.5	74.1	45.0	28.3	128.9	432.8	193.5	836.2
Control	7.5-15	0.3	24.3	11.4	272.2	187.3	126.4	53.2	72.6	122.9	599.2	271.3	1060.2
$Irrigation_{10\%GM}$	7.5-15	0.7	12.2	21.4	128.2	206.7	54.8	51.4	54.6	187.1	434.4	282.7	685.9
$Irrigation_{20\%GM}$	7.5-15	0.8	23.1	51.7	147.1	319.7	88.8	61.7	89.4	228.9	550.5	460.8	887.3
Control	15-25	0.5	30.1	25.5	167.1	223.0	63.6	54.9	56.7	170.8	487.1	305.3	1178.7
$Irrigation_{10\%GM}$	15-25	1.3	42.0	57.9	206.1	308.1	110.3	60.2	65.0	229.2	649.1	431.1	762.1
$Irrigation_{20\%GM}$	15-25	1.6	35.2	43.7	189.5	250.9	161.4	54.4	74.7	167.2	609.3	369.3	899.9
Control	25-50	0.3	24.3	11.4	272.2	187.3	126.4	53.2	72.6	122.9	599.2	271.3	1060.2
$Irrigation_{10\%GM}$	25-50	0.7	12.2	21.4	128.2	206.7	54.8	51.4	54.6	187.1	434.4	282.7	685.9
$Irrigation_{20\%GM}$	25-50	0.8	23.1	51.7	147.1	319.7	88.8	61.7	89.4	228.9	550.5	460.8	887.3
Control	50-75	0.9	29.0	15.4	192.3	173.6	182.2	39.9	36.8	103.8	548.1	225.8	915.6
$Irrigation_{10\%GM}$	50-75	1.1	16.9	7.8	214.3	138.1	140.5	42.4	14.0	93.5	508.8	159.9	674.7
$Irrigation_{20\%GM}$	50-75	0.2	10.0	10.0	136.8	174.4	43.0	52.4	55.3	211.9	454.3	239.7	763.8
Control	75-100	1.2	29.9	40.0	171.8	274.2	91.4	58.2	63.0	208.5	561.0	377.2	765.0
$Irrigation_{10\%GM}$	75-100	1.7	42.9	56.2	202.5	318.4	109.6	63.7	88.6	213.1	633.5	463.2	783.8
$Irrigation_{20\%GM}$	75-100	0.5	13.5	4.0	203.6	97.5	238.0	35.3	12.0	56.0	546.8	113.5	725.9

Appendix B

Methods of Soil Phosphorus Analysis

B.1 Soil phosphorus fractionation scheme

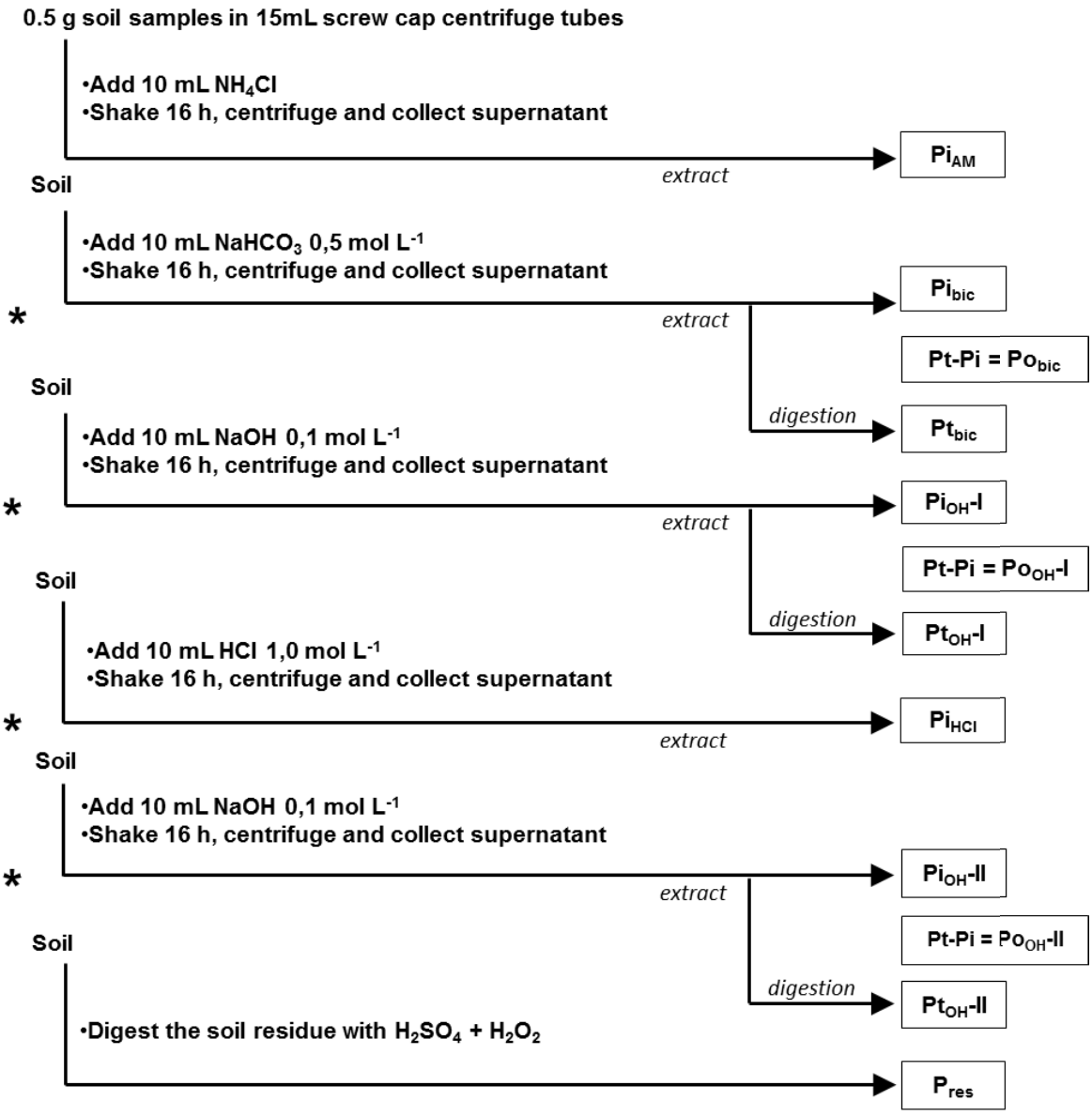


Figure A. 1 Soil phosphorus fractionation scheme (Hedley et al., 1982; Olsen and Sommers, 1982; Condron et al., 1996; Condron and Newman, 2011).

B.2 Soil phosphorus fractionation procedure compiled from Hedley et al. (1982), Olsen and Sommers (1982), Condron et al. (1996) and Condron and Newman (2011)

Reagents and solutions:

NH_4Cl 1 mol L^{-1}: Dissolve 53.5 g of NH_4Cl (ammonium chloride, MW 53.5g) and transfer to a 1000 mL volumetric flask and complete the volume with distilled water (DI H_2O)

$NaHCO_3$ 0.5 mol L^{-1}: Weigh 42.00 g of $NaHCO_3$ (sodium bicarbonate, MW 84g) in a 1000 mL Beaker, add approximately 900 mL of DI H_2O and dissolve the reagent. Adjust the pH to 8.5 with NaOH or HCl. Transfer the solution to a 1000 mL volumetric flask and complete the volume with DI H_2O. Prepare the solution immediately before use.

NaCl 0.5 mol L^{-1}: Dissolve 29.25 g of NaCl (sodium chloride, MW 58.5g) and transfer to a 1000 mL volumetric flask and complete the volume with DI H2O.

NaOH 0.1 mol L^{-1}: Weigh 4.00 g of NaOH (sodium hydroxide, MW 40g) in a 1000 mL Beaker, add approx. 900 mL of DI H_2O and dissolve the reagent. Transfer the solution into a 1000 mL volumetric flask and complete the volume with DI H_2O.

HCl 1 mol L^{-1}: Add (slowly) 84 ml of concentrated HCl (hydrochloric acid, 37% v/v) in a 1000 mL Beaker containing approx. 700 mL of DI H_2O. Transfer to a 1000 ml volumetric flask and make up the volume using DI H_2O.

Procedure:

a) weigh 0.5 grams of soil to 15 mL centrifuge tube with screw cap;

b) add 10 ml of NH_4Cl 1 mol L^{-1};

c) shake for 16 hours in the "end-over-end" (33 rpm);

d) centrifuge at 4000 rpm for 15 min;

e) reserve the supernatant for P analysis;

f) determine inorganic P in the extract (according to Appendix A.3);

g) **add 10 ml of $NaHCO_3$ 0.5 mol L^{-1} pH 8.5;**

h) shake for 16 hours in the "end-over-end" (33 rpm);

i) centrifuge at 4000 rpm for 15 min;

j) reserve the supernatant for P analysis;

k) add 5 mL of NaCl 0.5 mol L^{-1}, carefully to not stir the soil;

l) centrifuge at 4000 rpm for 5 min and add the supernatant to the extract collected previously;

m) determine inorganic P (according to Appendix A.4) and total P in the extract (Appendix A.5);

n) **add 10 ml of NaOH 0.1 mol L^{-1} ;**

o) shake for 16 hours in the "end-over-end" (33 rpm);

p) centrifuge at 4000 rpm for 15 min;

q) reserve the supernatant for P analysis;

r) add 5 mL of NaCl 0.5 mol L^{-1}, carefully to not stir the soil;

s) centrifuge at 4000 rpm for 5 min and add the supernatant to the extract collected previously;

t) determine inorganic P (Appendix A.4) and total P in the extract (Appendix A.5);

u) **add 10 ml of 1.0 mol HCl L^{-1};**

v) shake for 16 hours in the "end-over-end" (33 rpm);

w) centrifuge at 4000 rpm for 15 min;

x) reserve the supernatant for P analysis;

y) add 5 mL of NaCl 0.5 mol L^{-1}, carefully to not stir the soil;

z) centrifuge at 4000 rpm for 5 min and add the supernatant to the extract collected previously;

aa) determine inorganic P (Appendix A.3);

bb) **add 10 ml of NaOH 0.1 mol L^{-1} ;**

cc) shake for 16 hours in the "end-over-end" (33 rpm);

dd) centrifuge at 4000 rpm for 15 min;

ee) reserve the supernatant for P analysis;

ff) add 5 mL of NaCl 0.5 mol L^{-1}, carefully to not stir the soil;

gg) centrifuge at 4000 rpm for 5 min and add the supernatant to the extract collected previously;

hh) determine inorganic P (Appendix A.4) and total P in the extract (Appendix A.5).

ii) **place the samples in a drying oven at 50 °C and digest a subsample of soil according to Appendix A.6.**

***Comments:**

For quality control, standard/reference soils should be included in every fractionation batch. Chosen standard soils should compromise a wide range of properties (e.g. high and low clay content, organic matter, pH, P concentration, etc.) and/or be similar to unknown samples being analyzed. These can be calibrated simply by repetitively analyzing them and calculating averages of P concentrations and standard deviations for each P fraction in each standard soil. Blanks also must be included in each step of the P fractionation.

B.3 Determination of phosphorus in acid soil extracts according to Murphy and Riley (1962)

Reagents and solutions:

Solution A: dissolve 15.35 g $(NH_4)_6Mo_7O_{24}.4H_2O$ (Ammonium molybdate, MW 1235.9 g) with approx. 200 mL of DI H_2O in a 500 mL Beaker. Dissolve 0.2743 g $K(SbO)C_4H_4O_6$ (Potassium antimony, MW 324.9 g) with approx. 100 mL of DI H_2O in a 250 mL Beaker. In a 1000 mL Beaker containing approx. 300 mL of DI H_2O, very slowly add 178 mL of concentrated H_2SO_4 (Sulfuric acid, 95-98%, d=1.84 g/mL [high analytical grade]). After this solution has cooled down, transfer to a 1000 mL volumetric flask, add the solutions of ammonium molybdate and potassium antimony and adjust the volume with DI H_2O.

Solution B: dissolve 1.356 g $C_6H_8O_6$ (Ascorbic acid, MW 176.12 g) with 100 mL of Solution A in a 100 mL volumetric flask. This solution must always be prepared freshly before use.

NaOH 10 mol L^{-1}: dissolve 400 g of NaOH in a 1000 mL Beaker containing approx. 600 ml of DI H_2O. After cooling down, transfer to a 1000 mL volumetric flask and adjust the volume with DI H_2O. Store in a plastic bottle.

ρ-Nitrophenol indicator (yellow in pH 6-7) : weigh 0.25 g of ρ-Nitrophenol (MW 139.11 g) and dissolve to 100 mL using DI H_2O in a volumetric flask. Store in the fridge in a dark bottle.

Procedure:

a) pipette an aliquot of the extract to be analyzed into a 35 mL vial (or similar);
b) add DI H_2O in order to achieve a final volume of 3 mL;
c) add one drop of ρ-Nitrophenol indicator;
d) neutralize the solution with NaOH 10 mol L^{-1} added drop-wise;
e) add 0.5 mL of solution B;
f) read the absorbance using a UV-Vis spectrophotometer set to the wavelength 882 *n*m after 30 minutes. The color is stable for 3-4 hours;
g) the method and spectrophotometer need to be calibrated by preparing a calibration curve with P standard solutions.

Calculations:

The amount of P in each fraction is calculated using the following equation:

P concentration in given fraction (mg kg^{-1}) =

[Conc. of P (mg/L)] x [dilution of extract] x [Volume of extractant (mL) ÷ mass of soil (g)]

B.4 Determination of inorganic phosphorus in alkaline soil extracts according to Dick and Tabatabai (1977) with modification of He and Honeycutt (2005)

Solutions:

Solution A: dissolve 8.80 g $C_6H_8O_6$ (Ascorbic acid, MW 176.12 g) and 41.00 g of Trichloroacetic acid (Cl_3CCOOH, MW 163.39 g [high analytical grade]) in a 500 mL Beaker containing approx. 400 mL of DI H_2O. Transfer to a 500 mL volumetric flask and adjust the volume with DI H_2O. This solution must always be prepared freshly before use.

Solution B: dissolve 6.20 g $(NH_4)_6Mo_7O_{24}.4H_2O$ (Ammonium molybdate, MW 1235.9 g) in a 500 mL volumetric flask and adjust the volume with DI H_2O.

Solution C: in a 1000 mL Beaker containing **at least** 800 mL DI H_2O:

- dissolve 29.40 g (tri-Sodium citrate, MW 294.1 g) while stirring with a magnetic stirrer;
- once fully dissolved, add 26.00 g of (Sodium arsenite, MW 129.91 g), and fully dissolve;
- add 50 ml glacial acetic acid (99%, d=1.05 g/mL). Transfer the content of the Beaker to a 1000 mL volumetric flask and adjust the volume with DI H_2O.

Procedure:

a) pipette 2 mL of the alkaline extract to be analyzed into a 35 mL vial (or similar). The extract here should be previously diluted as needed;

b) add 2.5 ml of solution A;

c) add immediately 0.5 mL of solution B;

d) add 1.25 mL of solution C after a fixed and standardized length of time (e.g. 45 seconds). This step is crucial to assure the reproducibility and sensitivity of the method;

e) the final volume is 12.5 mL;

f) read the absorbance using a UV-Vis spectrophotometer set to the wavelength 850 *n*m (He and Honeycutt, 2005) after 15 minutes. The color is stable for 24 hours;

g) the method and spectrophotometer need to be calibrated by preparing a calibration curve with P standard solutions.

Calculations:

The amount of P in each fraction is calculated using the following equation:

P concentration in given fraction (mg kg^{-1}) =

[Conc. of P (mg/L)] x [dilution of extract] x [Volume of extractant (mL) ÷ mass of soil (g)]

B.5 Auto-clave digestion of alkaline soil extracts (extracted with NaOH and $NaHCO_3$) for total phosphorus analysis according to USEPA (1983) with recommendations by do Nascimento et al. (2015)

Solutions:

Sulfuric acid 1:1: Add, slowly, 500 ml of concentrated H_2SO_4 (Sulfuric acid, 95-98%, d=1.84 g/mL) in approx. 500 ml DI H_2O. Wait for it to cool down, transfer to a 1000 mL volumetric flask and make up to volume with DI H_2O.

Ammonium persulfate 7.5% (w/v): Dissolve 75 g of Ammonium persulfate ($(NH_4)_2S_2O_8$, MW 228.2 g [high analytical grade]) in 800 ml of DI H_2O. Transfer to a 1000 mL volumetric flask and complete the volume with DI H_2O. This solution should be prepared freshly.

Procedure:

a) pipette an aliquot of the alkaline soil extract into a digestion tube with screw cap;

b) add 10 mL of Ammonium persulfate 7.5%;

c) add 1 mL of H_2SO_4 1:1;

d) loosely screw the caps onto the tubes;

e) autoclave at 121 °C and 103 kPa for 120-240 minutes or until complete digestion (depending on the nature and complexity of the extract being analyzed);

f) after the digestion has finished, let them cool down and make the volume up to 20 mL using a volumetric cylinder;

g) determine phosphorus concentration according to Appendix A.3.

   a. (Optional): If using a dilution step with Appendix A.3, use a solution of NaOH where the added OH^- is equivalent to the H^+ associated with the H_2SO_4, thus neutralizing for color development.

***Comments:**

This method can be replaced for the emission spectrometry for determination of the total concentration of P in the extracts, i.e. using ICP-OES (Inductively Coupled Plasma - Optical Emission Spectrometry) if facilities are available (see do Nascimento et al., 2015 for more details). In this case, the high temperature (8000-1000 Kelvin) of the plasma effectively digests all organic P forms and the detection system accurately determines the concentration of total P in the sample. It is important to include blanks, P standards and P peaks in selected samples in every batch. This is a straightforward way to analyze the total P concentration in soil extracts, although the costs can be higher as well and need to be consider previously.

B.6 Block digestion of residual soil for total phosphorus analysis (Olsen and Sommers, 1982)

Equipment:

Aluminum digestion block with adjustable temperature;

Glass tubes diameter: 25 mm and height: 250 mm;

Glass funnels 25-35 mm diameter.

Reagents:

H_2SO_4 concentrated (Sulfuric acid, 95-98%, d=1.84 g/mL [high analytical grade]);

H_2O_2 concentrated (Hydrogen peroxide 30% w/v, [high analytical grade]).

Procedure:

a) weigh approx. 0.1000 g of finely ground soil (42 Mesh sieve) into a glass digestion tube (25 x 250 mm);
b) add 1 ml of H_2SO_4 conc.;
c) place a reflux funnel on the top of the digestion tube;
d) gradually (e.g. 5°C min^{-1}) raise the temperature to 225°C, holding this temperature for 1 hour;
e) remove the samples from the block and let them cool down to room temperature;
f) remove the funnels and add 2 mL of H_2O_2 conc.;
g) place the funnels back on the top of the digestion tube;
h) gradually raise the temperature to 135°C, holding this temperature for 1 hour;
i) if the sample has not clarified (visually checking) due to the presence of organic matter, repeat the steps “e” to “h”, but adding portions of 1 mL of H_2O_2 conc. at this time;
j) to ensure H_2O_2 has completely been degraded, gradually raise the temperature to 150°C and hold for 30 min. [Any leftover H_2O_2 will interfere with colorimetric methods, see Olsen and Sommers, 1982.]
k) remove the samples from the block and let them cool down to room temperature;
l) dilute the samples to 40 mL using a volumetric cylinder;
m) determine phosphorus according to Appendix A.3.

Calculations:

The amount of P in the residual fraction is calculated using the following equation:

Residual P concentration (mg kg^{-1}) =

[Conc. of P (mg/L)] x [Final dilution volume (40 mL) ÷ mass of soil (g)]

B.7 Total soil phosphorus (H_2SO_4+H_2O_2) (Olsen and Sommers, 1982)

Equipment:

Aluminum digestion block with adjustable temperature;

Glass tubes diameter: 25 mm and height: 250 mm;

Glass funnels 25-35 mm diameter.

Reagents:

H_2SO_4 concentrated (Sulfuric acid, 95-98%, d=1.84 g/mL [high analytical grade]);

H_2O_2 concentrated (Hydrogen peroxide 30% w/v, [high analytical grade]).

Procedure:

a) weigh approx. 0.1000 g of finely ground soil (42 Mesh sieve) into a glass digestion tube (25 x 250 mm);
b) add 4 ml of H_2SO_4 conc.;
c) place a reflux funnel on the top of the digestion tube;
d) gradually (e.g. 5°C min^{-1}) raise the temperature to 225°C, holding this temperature for 1.5 hours;
e) remove the samples from the block and let them cool down to room temperature;
f) remove the funnels and add 2 mL of H_2O_2 conc.;
g) place the funnels back on the top of the digestion tube;
h) gradually raise the temperature to 135°C, holding this temperature for 1 hour;
i) if the sample has not clarified (visually checking) due to the presence of organic matter, repeat the steps “e” to “h”, but adding portions of 1 mL of H_2O_2 conc. at this time;
j) to ensure H_2O_2 has completely been degraded, gradually raise the temperature to 150°C and hold for 30 min. [Any leftover H_2O_2 will interfere with colorimetric methods, see Olsen and Sommers, 1982.]
k) remove the samples from the block and let them cool down to room temperature;
l) dilute the samples to 100 mL using a volumetric cylinder or volumetric flask;
m) determine phosphorus according to Appendix A.3.

Calculations:

The amount of total P calculated using the following equation:

$$\text{total P concentration (mg kg}^{-1}\text{)} =$$

$$[\text{Conc. of P (mg/L)}] \times [\text{Final dilution volume (100 mL)} \div \text{mass of soil (g)}]$$

B.8 Total soil phosphorus (fusion with Na_2CO_3) (Kuo, 1996)

Equipment:

Platinum (Pt) crucibles with lid;

Bunsen burner;

Hot plate.

Reagents:

H_2SO_4 concentrated (Sulfuric acid, 95-98%, d=1.84 g/mL [high analytical grade]);

Na_2CO_3 (Sodium carbonate, anhydrous).

Procedure:

a) Mix 0.1000 g of finely ground soil (42 Mesh sieve) into platinum crucible with 1.0 g of Na_2CO_3;
b) Add an additional 1.0 g of Na_2CO_3 on the top, covering the mixture;
c) Cover the crucible with lid and place in blue bunsen flame for 5 minutes until the mass is fused. An example is available at: https://www.youtube.com/watch?v=U_01G1qW-20&t=6s;
d) The liquid melt is additionally heated for 3 min with lid half off and a further 2 minutes without lid;
e) The melted is allowed to cool. Place the crucible and lid in a 250 mL beaker and dissolve with 15 mL of 4.5 M sulphuric acid (H_2SO_4);
f) Add 20 mL of hot distilled water, cover beaker with watchglass and heat on a hot plate at 100 °C for one hour;
g) Adjust the solution to a final volume of 100 mL;
h) Filter with Whatman 41
i) Determine P concentration according to Appendix A.3.

Calculations:

The amount of P in the total P is calculated using the following equation:

$$\text{total P concentration (mg kg}^{-1}) =$$

$$[\text{Conc. of P (mg/L)}] \times [\text{Final dilution volume (100 mL)} \div \text{mass of soil (g)}]$$

B.9 Olsen P (available phosphorus index) (Olsen, 1954)

Reagents and solutions:

$NaHCO_3$ 0.5 mol L^{-1}: Weigh 42.00 g of $NaHCO_3$ (sodium bicarbonate, MW 84g) in a 1000 mL Beaker, add approximately 900 mL of DI H_2O and dissolve the reagent. Adjust the pH to 8.5 with NaOH or HCl. Transfer the solution to a 1000 mL volumetric flask and complete the volume with DI H_2O. Prepare the solution immediately before use.

Procedure:

a) weigh 3.0 g of soil in a 50 mL falcon tube;

b) add 30 mL of $NaHCO_3$ 0.5 mol L^{-1};

c) shake for 30 min in an "end-over-end" (33 rpm);

d) centrifuge at 4000 rpm for 15 min;

e) determine inorganic P (according to Appendix A.4);

Calculations:

Olsen P concentration (mg kg^{-1}) =

[Conc. of P (mg/L)] x [Final dilution volume (30 mL) ÷ mass of soil (g)]

B.10 Extractable organic phosphorus by NaOH-EDTA (Bowman and Moir, 1993; Turner et al., 2005; Cade-Menun and Liu, 2014)

Reagents and solutions:

0.25 mol L^{-1} NaOH + 0.05 mol L^{-1} EDTA : Weigh 10 g of NaOH (sodium hydroxide, MW 40g) in a 1000 mL Beaker, add approximately 900 mL of DI H_2O and dissolve the reagent. Add 18.61 g of EDTA (Ethylenediaminetetraacetic acid), and dissolve. Transfer the solution to a 1000 mL volumetric flask and complete the volume with DI H_2O.

Procedure:

a) weigh 1.5 g of soil in a 50 mL falcon tube;

b) add 30 mL of NaOH+EDTA solution;

c) shake for 16 hours in an "end-over-end" (33 rpm);

d) centrifuge at 4000 rpm for 15 min;

e) filter with Whatman GF/F;

f) dilute accordingly and determine inorganic P (according to Appendix A.4) and total P (Appendix A.5);

g) This extract is also utilised for ^{31}P-NMR analysis after lyophilisation (freeze-drying). See Turner et al. (2003) and Turner et al. (2005).

Calculations:

Inorganic P concentration (mg kg^{-1}) =

[Conc. of P (mg/L)] x [Final dilution volume (30 mL) ÷ mass of soil (g)]

Total extractable organic P = Inorganic P in the NaOH-EDTA extract – Total P

B.11 Degree of phosphorus saturation (DPS) (Pierzynski, 2000)

Solutions:

Oxalate solution (0.114 M ammonium oxalate + 0.09 M oxalic acid, pH 3.0): dissolve 16.2g of $C_2H_8N_2O_4$ (ammonium oxalate, MW 124.1) and 10.8g of $C_2H_2O_4$ (oxalic acid, MW 90.03) in a 1000 mL volumetric flask and complete the volume with DI H_2O.

HCl 0.01 mol L^{-1}: pipette 10 mL of 1 mol L^{-1} HCl (dilute hydrochloric acid) in a 1000 ml volumetric flask and adjust the volume using DI H_2O.

Procedure:

a) Weigh 2 g of soil into 50 mL falcon tube;
b) Add 40 mL of oxalate solution;
c) Shake for 2 hour in horizontal shaker (180rpm) in complete dark;
d) Centrifuge at 4000 rpm and filter with Whatman 41;
e) Pipette 2 mL of filtrate and add 8 mL of HCl 0.01 mol L^{-1};
f) Determine concentrations of P, iron (Fe) and aluminium (Al) in the extracts by ICP-OES.

Calculations:

$$\boldsymbol{PSI = P_{ox}/(Fe_{ox} + Al_{ox})}$$

$$\boldsymbol{DPS = 200 \times PSI;}$$

Where, PSI: phosphorus sorption index; P_{ox}, Fe_{ox} and Al_{ox} are the molar concentrations (mmol kg^{-1}) of the elements and DPS: degree of phosphorus saturation.

B.12 Phosphorus in the microbial biomass – Microbial P (Brookes et al., 1982; Hedley and Stewart, 1982; Morel et al., 1996)

Solutions:

$NaHCO_3$ 0.5 mol L^{-1}: Weigh 42.00 g of $NaHCO_3$ (sodium bicarbonate, MW 84g) in a 1000 mL Beaker, add approximately 900 mL of DI H_2O and dissolve the reagent. Adjust the pH to 8.5 with NaOH or HCl. Transfer the solution to a 1000 mL volumetric flask and complete the volume with DI H_2O. Prepare the solution immediately before use.

$NaHCO_3$ 0.5 mol L^{-1} + 25 mg P L^{-1}: Weigh 42.00 g of $NaHCO_3$ (sodium bicarbonate, MW 84g) in a 1000 mL Beaker, add approximately 900 mL of DI H_2O and dissolve the reagent. Add 25 mL of a 1000 mg P L^{-1} primary stock solution. Adjust the pH to 8.5 with NaOH or HCl. Transfer the solution to a 1000 mL volumetric flask and complete the volume with DI H_2O. Prepare the solution immediately before use.

Concentrated $CHCl_3$ (chloroform).

Procedure:

a) weigh 2 grams of field moist soil, in triplicate (sets A, B and C) into 50 mL centrifuge tubes;
b) place the set of samples “A” in the fumigation chamber together with a beaker containing approx. 50mL of chloroform;
c) evacuate the chamber with a vacuum pump. The objective is to create an ambient saturated with chloroform vapor;
d) fumigate for 24 hours;
e) remove the samples from the chamber and add 30 mL of $NaHCO_3$ 0.5 mol L^{-1} pH 8.5 to extract the fumigated and non-fumigated samples (set ”A” and “B”); and set “C” with $NaHCO_3$ 0.5 mol L^{-1} + 25 mg P L^{-1};
f) shake for 30 minutes in the "end-over-end" (33 rpm);
g) centrifuge at 3000 rpm for 10 min;
h) dilute the aliquots and determine inorganic P in the tree sets of samples (Appendix A.4);

Calculations:

$$\text{Microbial P (mg kg}^{-1}) = 25 * (P_A - P_B)/0.40 * (P_C - P_B)$$

B.13 Acid phosphatase activity in soils (Tabatabai and Bremner, 1969)

Solutions:

Modified Universal Buffer (MUB) pH 6.5: Dissolve 12.1 g of TRIS, 11.6 g of maleic acid, 14 g of citric acid and 6.3 g of boric acid in a 1000 mL beaker with approximately 300mL of DI H_20. Add 500 ml of NaOH 1 mol L^{-1} and adjust the pH to 6.5 with HCl. Transfer to 1000 ml volumetric flask and adjust the volume with distilled water.

Substrate ρ-Nitrophenyl phosphate: Add 1.067g of ρ-Nitrophenyl phosphate disodium salt hexahydrate (the reagent is stored in the freezer at -20 °C) in a 25 mL volumetric flask and dissolve and make up the volume with MUB pH 6.5 (prepare the solution immediately before use).

$CaCl_2$ 0.5 mol L^{-1}: Dissolve 55.5 g of $CaCl_2$ (calcium chloride, MW 58.5g) and transfer to a 1000 mL volumetric flask and complete the volume with DI H2O.

NaOH 0.5 mol L^{-1}: Weigh 20.00 g of NaOH (sodium hydroxide, MW 40g) in a 1000 mL Beaker, add approx. 900 mL of DI H_2O and dissolve the reagent. Transfer the solution into a 1000 mL volumetric flask and complete the volume with DI H_2O.

Toluene.

Procedure:

a) weigh 1 g of field moist soil to 15 mL centrifuge tubes;
b) add 1 mL of the solution ρ -Nitrophenyl phosphate substrate + 4 mL of MUB pH 6.5 + 0.25 mL of Toluene;
c) close the tubes and shake manually; Incubate for 1 hour at 37 °C in the dark;
d) add 1 mL of $CaCl_2$ 0.5 mol L^{-1} + 4 mL of NaOH 0.5 mol L^{-1}, close the tubes and shake them manually again, let them rest for a few minutes;
e) Filter the supernatant with paper filter Whatman 2
f) Dilute accordingly read the absorbance in UV-Vis spectrophotometer set to the wavelength 400 *n*m.
g) Prepare a standard calibration curve with ρ -Nitrophenol

Calculations: ρ-Nitrophenol (μg g^{-1} dry soil h^{-1}) = C * V / DS * MS * t

where: C = concentration of p-nitrophenol (μg ml^{-1} filtered); V = the volume of the suspension (in ml); DS = amount of dry soil incubated; MS = amount of moist soil used (1 g); t = time of incubation (1 hour). (MW of ρ-Nitrophenol = 139.11 g mol^{-1})

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