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Impact of poultry manure application, alone or combined with phosphate rock on biogeochemical cycling of C and P in grassland soils.

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“Impact of poultry manure application, alone or combined with phosphate rock, on ryegrass biogeochemical cycling of C and P in grassland soils”

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Abbreviations

P	<i>phosphorus</i>
Pi	<i>inorganic phosphorus</i>
Po	<i>organic phosphorus</i>
Pt	<i>total phosphorus</i>
C	<i>carbon</i>
N	<i>nitrogen</i>
SOC	<i>soil organic carbon</i>
SOM	<i>soil organic matter</i>
Al	<i>aluminum</i>
Fe	<i>iron</i>
PUE	<i>phosphorus use efficiency</i>
FYM	<i>farm yard manure</i>
PM	<i>poultry manure</i>
RP	<i>phosphate rock</i>
PMRP	<i>poultry manure and phosphate rock mixture</i>
FLf	<i>free light fraction from soil organic matter density fractionation</i>
OLf	<i>occluded light fraction from soil organic matter density fractionation</i>
Hf	<i>heavy fraction from soil organic matter density fractionation</i>
PSB	<i>phosphate solubilizing bacteria</i>

Thesis summary and outline

In the light of the need of increasing the sustainability of agricultural production, it is necessary to reduce use of limited resources and to employ negative emission technologies to combat and adapt to climate change (IPCC, 2014; UNEP, 2017). Phosphorus (P) is the most expensive fertilizer applied in agricultural systems, due to its high production cost through phosphate rock (RP) mining. The price of RP has been increasing over the years, and in the future depletion of this non-renewable resource located in only a few countries is expected (Cordell et al., 2009; Reijnders, 2014). Accordingly, it is important to identify management options to reduce RP utilization as mineral fertilizer without sacrificing productivity due to the need of feeding the growing world population (Menezes-Blackburn et al., 2018). The first chapter of this thesis focuses on the importance of grasslands for food, and the principal challenges in terms of soil P fertilization. In addition, we reviewed managements options to enhance the soil P availability and plant use efficiency (PUE) to pasture development and production. According to the literature, poultry manure (PM), resulted to be an interesting option to improve the delivery of phosphate by RP enhancing its solubilization and plant PUE. PM is widely used as soil amendment, nonetheless the mechanisms by which it increases P availability and controls soil organic carbon (SOC) sequestration remain unclear. In the second chapter we assess the impact of a long-term application of PM. In order to investigate the impact of long-term poultry manure application on carbon (C) storage and P forms, sites receiving poultry manure application during five and ten years in different farms of southern Chile were sampled.

Although the improvement of available P and plant yield by using PM it has been demonstrated, no study has been carried out to investigate quantitatively the synergetic or antagonistic effects of the combined application of both materials. Accordingly, we

carried out a short-term study in controlled conditions. We show in the third chapter, results from plant production parameters and soil P availability as response of using PM alone or combined with RP. Moreover, we present the quantification of synergistic and antagonistic effects of using their combination.

On the other hand, as PM is a rich source of organic C and may be enhancing SOC sequestration. The impact of this material on C flow within plant-soil system is poorly known. We hypothesized that RP and PM influence differently SOC storage and distribution. The fourth chapter assesses the importance of plant-derived organic C for soil organic matter (SOM) and P pools in grassland soil.

Finally, in the fifth chapter we present a general discussion of the results obtained in this study and concluding remarks with some future directions.

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CHAPTER I

General Introduction

Chapter 1: General Introduction

1.1.Introduction

Approximately 60% of the world's grasslands is classified as grazing land, which occupies 25% terrestrial surface or about 70% world agricultural area (Snow et al., 2014). Pastures support the livelihoods of ~1 billion people occupying ~40% of the world's land area (excluding Antarctica and Greenland) (Kemp et al., 2013). Permanent pastures are the most common, where a temperate climate predominates (Demagnet et al., 2015). Temperate grazing system, based on natural permanent pastures, account for 13% world's pasture lands, mainly China, northern United States of America and South America. Pastures due to their permanent soil cover and high belowground biomass (Conant et al., 2011) are favorable systems in terms of soil carbon sequestration and soil quality (Rumpel et al., 2015). They have few environmental externalities and their introduction into cropping cycles has been suggested to be beneficial in terms of soil quality (Lemaire et al., 2015). Pastures play an important role in many mixed agricultural systems, providing a break in rotational cropping systems and feed to livestock in systems in which diet comprises both crop residues or by-products and grazed or conserved grass (Orr et al., 2019).

With the increasing meat demand of the global population the proportion of grazing lands is likely to increase (Alexandratos and Bruinsma, 2012). Although pasture-based farming systems are therefore often considered to have few externalities compared with many other farming types, they have been following the general tendency of agricultural activities with high external inputs in forms of nutrients. The most limiting nutrients for crops and their yield are nitrogen (N) and phosphorus (P). The relatively high soil organic matter (SOM) concentrations in the surface of some pasture soils are associated with SOM turnover and with high organic P and N contents in grasslands. While fertilizer N

is taken up rapidly by plants and the microbial biomass, P is immobilized and unavailable for uptake through sorption onto soil minerals (Condrón et al., 2005). In some countries, grasslands are the main base of economy, and agricultural production is performed primarily on soils with a high P sorption capacity. Such situation, requiring high fertilizer inputs, occur for example in countries with volcanic soils, such as Chile and Japan, or tropical countries with highly weathered, such as many African and Asian countries.

The P fertilizers are manufactured from phosphate rock (RP), a finite resource, controlled by only a handful of countries. Recent estimates suggest that the global commercial RP reserves will be depleted in 50 to maximum 400 years (Cordell et al., 2009; Manschadi et al., 2014; Reijnders, 2014). This RP depletion in addition to increasing world's demography levels represent a massive challenge for current agricultural systems, especially in countries with P deficient soils. Management practices for improving P use efficiency (PUE) by plants are needed to decrease P fertilizer doses applied through mobilization of P fixed on the mineral phase. In this review, we will analyze the P status of pasture ecosystems and discuss P dynamics through the plant-soil system. We will present different P sources and their effect in soil and finally propose management strategies to enhance soil P availability in pasture systems.

1.2. Phosphorus status of pasture ecosystems

Although pasture-based farming systems are often considered to be relatively environmentally benign compared with many other farming types, they require high nutrient inputs (Snow et al., 2014). Their productivity is influenced by many parameters depending on agroecological and management factors such as precipitation and temperature variability, soil water holding capacity, soil fertility, as well as aspects such as stocking rate and defoliation frequency (Castellaro et al., 2012).

In intensified systems elemental cycles may be decoupled leading to losses of reactive N and P (Rumpel et al., 2015). High mineral fertilizer N input in pastures may be replaced by the use of leguminous species (Crème et al., 2016), which require P for N fixation. Therefore, P is, after N, the most limiting nutrient for pastures and their yield is limited by P availability in about 40% world's arable land (Divito and Sadras, 2014). In addition, P is often the limiting nutrient in terrestrial primary production (Reijnders, 2014). Soils showing high fixation capacity, are characterized by low pH, and high content of short-range order minerals and/or metal oxides meaning that most of the inorganic P (Pi) added to soil remains unavailable for crop uptake (Mejías et al., 2013).

Volcanic ash derived soils, mainly Andisols and Ultisols, are very commonly used for pasture development (Demanet et al., 2015). These soils have unique properties and contribute significantly to agriculture and forest production (Takahashi and Shoji, 2002). Total P concentration in pasture soils varies depending of the soil type and fertilization management (Table 1). Volcanic soils have a high P adsorption capacity (85-90%) (Borie and Rubio, 2003; Escudey et al., 2001; Mejías et al., 2013) and contain high amounts of extractable and exchangeable aluminum (Al) (Mora et al., 2004). High P retention on volcanic ash soils is attributed to active Al and iron (Fe) associated with organic (mainly Al-humus complexes) and mineral fractions (allophanes and ferrihydrite), which form during soil development (Satti et al., 2007). Most agricultural soils have large capacity to sequester this P via adsorption onto soil mineral surfaces and sparingly soluble P mineral formation, which eventually accumulate in the soil as unavailable P forms having a high concentrations of total P (Pt); with high accumulation of organic P (Po) (more than 50% total P) (Borie and Rubio, 2003; Redel et al., 2016; Turner et al., 2003; Velásquez et al., 2016).

In natural pastures, P fluxes are low, and are directly controlled by plant and animal residues, which are decomposed on soil surface. According to Ramaekers et al., (2010) there are two different types of P deficient soils: i) soils with an overall low total P content and ii) soils with high total P, but with low P availability for plant uptake due to strong P retention. The organic P forms in pasture soils (Table 1) are affected or, at least, correlated with a wide range of soil geochemical, physical and climatic factors, including precipitation and temperature. They have major impacts on biochemical processes, such as plant species and soil mineralogy affecting Po composition in soil (Nash et al., 2014). Commonly soils under intensive grassland, Po and Pi concentrations are equivalent (Redel et al., 2016; Velásquez et al., 2016). Species and concentrations of P in soils will vary according to management and soil property interactions, for example, Stutter et al. (2015) found that ortho-Pi had significantly smaller concentrations and relative contribution to total P in extensive/semi-natural soils than intensive grassland and arable soils. Furthermore, in soil, microbes and plants take up ortho-Pi during growth, returning Po to soils as lysed cell contents, plant detritus, and excreta in grazed systems (Fuentes et al., 2012). Amongst the soil factors studied the reactive properties of soil organic C (SOC) and analytically-defined properties oxalate extractable Al and Fe seem to be particularly important (Matus et al., 2014, 2006; Neculman et al., 2013; Redel et al., 2016) . Moreover, in pasture growing in volcanic soils of southern Chile, P availability was found mainly regulated by the formation of amorphous Al-Po complexes, being Al_{ox} the main factor that governs Po storage (Redel et al., 2016). Furthermore, Velásquez et al. (2016a) showed that Chilean Andisols had a high content of residual P, which was formed by monoester P, including myo- and scyllo -inositol hexakisphosphate (IP6). IP6 an important component of the organic P in temperate pasture soils is considered

inaccessible to plant uptake due to its resistance to hydrolytic attack by phytase, however under certain conditions IP6 could be bioavailable (Turner et al., 2005).

1.3. Phosphorus cycling in grasslands soils

Most P in terrestrial ecosystems is present in soil. Total P in soils ranges from 200 to 800 mg kg⁻¹ in older/highly weathered and younger/less developed soils.(Khaledian et al., 2018). Grazing systems have impacts on nutrient cycling promoting nutrient flux modifications, where animals are catalysts, and soil is the central compartment, in which nutrient cycling processes occur (Costa et al., 2014). Organic matter inputs result in very uneven nutrient availability, consequently, in vegetation dynamics. In fertilized and natural pastures ecosystems the preferential localization of roots in upper soil layers is due to nutrient accumulation in surface horizons as a consequence of the absence of ploughing and low P ion mobility (Jobbágy and Jackson, 2001). Fig. 1 is showed the P cycling which is controlled simultaneously by geochemical and biological processes being determined in extent by the complex nature of interactions between inorganic, organic and microbial forms of P (Condrón and Newman, 2011). Thus, soil P is derived from different organic and inorganic sources added to soil by human activity or derived from soil inherent processes.

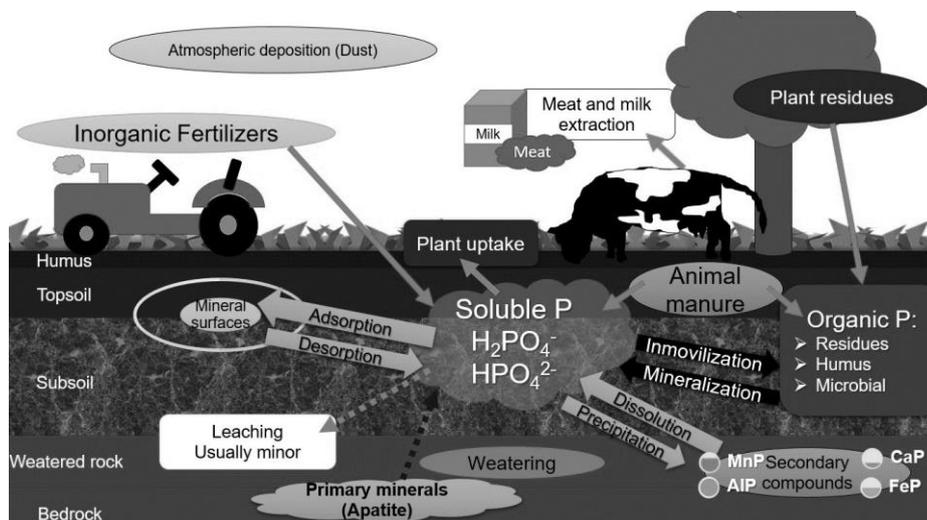


Figure 1. Soil phosphorus forms and dynamics in grassland system–plant system.

Table 1 shows concentration of inorganic, organic and microbial biomass phosphorus in a range of soils under pasture development with different fertilizer management. The total organic P concentration in grassland soils (Table 1) is not related to total stock but is a direct function of total organic C and N concentration (Rumpel et al., 2015). In most soils, it accounts for between 30% and 65% total P, although some soils contain up to 90% organic P (Borie and Rubio, 2003; Murphy et al., 2009; Nash et al., 2014). Soil organic P turnover is governed by a range of abiotic and biotic factors; and determined by immobilization (biological conversion of inorganic P into organic P) and mineralization (process whereby inorganic P is released from organic P) rates (Frossard et al., 2000; Spohn et al., 2013; Stewart and Tiessen, 1987). According to literature, there is only a limited understanding of the mechanisms associated with organic P transformation to inorganic P at process level, and that further work is needed using soil-based studies, especially under field conditions (Nash et al., 2014). There are many different soil P sources (pools), which are constantly interacting: i) plant-available inorganic P (i.e water soluble P), ii) organic P (P associated with living and dead organic matter) and iii) less available inorganic P (poorly soluble mineral P or tightly bound to clay particles) (Condrón et al., 2005) (Fig. 1). In the following, we will present the different P sources present in grassland soils and inputs and outputs in pasture systems.

Table 1. Soil phosphorus properties in grassland ecosystems. * Pt: Total P; Po: Organic P; MBP: Microbial P.

Site location	Soil Type	Fertilizer input (kg ha ⁻¹)	Depth (mm)	Pt (mg kg ⁻¹)	Po (mg kg ⁻¹)	MBP (mg kg ⁻¹)	Reference
Canada	Brown Chernozem (Aridic Haploboroll) and Humic Luvisol (Argiaquoll)	-	0-150	721-1318	122-719	-	Condron et al., 1990
North America	Typic Cryoborolls, Typic Haploborolls, Pachic Udic Haploborolls, Aridic Argiborolls, Argic Cryoborolls, Ustic Haplargids, Aridic Paleustolls	-	0-100	337-1838	-	-	Sumann et al., 1998
England and Wales	Haplaquepts, Dystrochrepts, Hapludalfs, Udipsamments, Paleudalfs, Fluvaquents	-	0-100	376–1,981	257 - 1,083	31-239	Turner et al., 2003
England and Wales	-	-	0-100	-	882	-	Turner and Haygarth, 2005
New Zealand	Allophanic, Oxidic, Pumice, Brown, Gley, Melanic, Pallic, Podzol, Recent, Ultic	<20 kg P	0-75	225-2770	74-991	-	McDowell et al., 2006
New Zealand	Podzol, Allophanic, and Pallic	-	0-75	460-2910	-	-	McDowell and Stewart, 2005
New Zealand	Dystric Regosol, Humic Acrisol, Orthic Acrisol, Gleyic Acrisol and Rendzina	40 kg P, 20 kg N	0-150	938-2405	190-1126	-	McDowell and Stewart, 2006
New Zealand	Humult, Vitrand, Udand, Ustochrept, Dystrochrept, Orthod, and Aquept	-	0 - 75	375 - 2607	-	-	Chen et al., 2003

Australia	Haploxeralf and Rhodoxeralf		0-100	545-1299	-	-	Dougherty et al., 2007
Ireland	Haplic Cambisols, Haplic Gleysols Endoaquept, Haplustert, Hapludalf, Haplorthod, Haplorthod, Hapludox, Paleustalf, and Fragiudult	-	0-20	616-2580	-	-	Murphy et al., 2009
Australia	Cambisol, Histosol, Gleysol, Podzol	-	0-70	886-1865	596-1524	0.1-23.3	Stutter et al., 2015
Southern Chile	Typic Melanoxerands, Typic Hapludands, Typic Durudands	-	0 – 200	1982 – 3560	835 – 1552	-	Borie and Barea, 1983
Southern Chile	Typic Hapludands, Acrudoxic Hydric Melanudands, Eutric Pachic Fulvudands and Acrudoxic Hapludands	50 to 100 kg P, 100 kg N	0 - 200	1,668- 3,079	918 - 1,275	28 - 158	Redel et al., 2016
Southern Chile	Acrodoxic Hydric Melanudands, Pachic Melanudands and Eutric Pachic Fulvudands	0 kg P; 90 to 150 kg P	0-200	982 – 2473	337 – 739	-	Velasquez et al., 2016a

1.3.1 Soil phosphorus forms

To maintain highly productive pasture species, legumes (e.g. clover) in sward regular fertilizer inputs (especially P and sulphur) are required and superphosphate (9% P and 11% S) applications result in P deficiency correction through increasing soil inorganic P content (Williams and Haynes, 1990). Continuous applications of inorganic P fertilizers such as superphosphate (industrially produced from fossil phosphorites and crushed bone mixture, treated with sulphuric acid) (Reijnders, 2014), exceeding plant acquisition will, inevitably, result in P accumulation in soils (Redel et al., 2016; Velásquez et al., 2016a). Phosphate, predominant soil inorganic P form, can be derived from dissolution of primary P containing minerals such as apatite, through the application of mineral P fertilizers or by organic P form mineralization of litter or other organic material by microorganisms. Therefore, soil P concentration increase over the years and extent of such accumulation will depend on fertilizer application rate, years of application, soil matrix surface adsorptive capacity as well as on microbial activity, especially microorganisms involved in P cycling (Spohn et al., 2013). In agricultural soils, almost all P forms are potentially available to plants. The mechanisms involved in phosphate adsorption are: i) exchange of phosphate ions (H_2PO_4^- or HPO_4^{2-}) with surface Fe-OH, Al-OH, Fe-OH $_2^+$ and Al-OH $_2^+$ groups through ligand exchange reactions, ii) adsorption by phyllosilicate clay minerals, and iii) adsorption by calcium carbonate (in calcareous soils) (McLaren and Cameron, 2012). Fig. 2 showed the soil P pools related with its availability for plant uptake, where P can be separated into labile-P (H_2O -, and 0.5 M NaHCO_3 -Pi and Po), moderately labile-P (0.1 M NaOH-Pi and Po), and stable-P (HCl-P and residual) fractions. P fractionation schemes involve sequential extraction with a series of reagents designed to selectively dissolve various forms of P based mainly on the nature and strength of interactions between P moieties and other mineral and organic

components (Condrón and Newman, 2011) (Fig. 2). However, their availability degree will depend on solubility and structure of chemical forms, on susceptibility to microorganism attack, as well as on soil-root environment (Borie and Rubio, 2003).

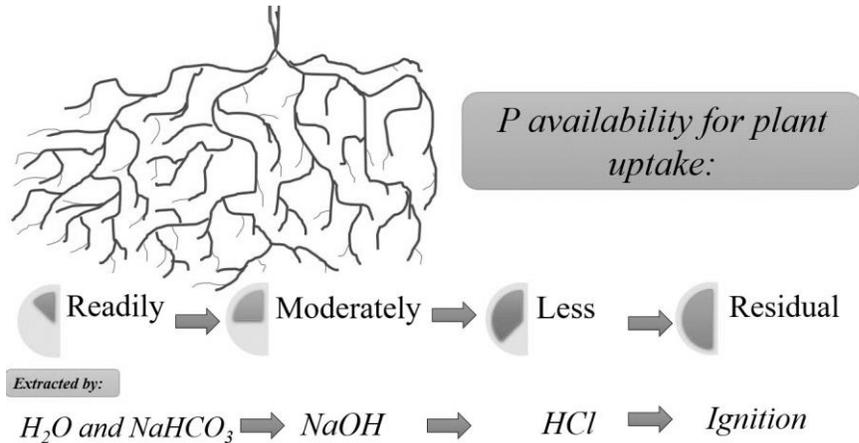


Figure 2. Conceptual soil phosphorus pools characterized in terms of availability to plant roots.

On the other hand, organic P bonded in some way with SOC, is derived mainly from biological processes involving phosphate assimilation and subsequent release as mature and decomposed microbial, animal and plant materials (Condrón et al., 2005; Stewart and Tiessen, 1987). Organic P is classified into phosphate esters (e.g. monoesters: sugar phosphates, phosphoproteins, mononucleotides, and inositol phosphates; diesters: nucleic acids, phospholipids, teichoic acid, and aromatic compounds), phosphonates (e.g. 2-aminoethyl phosphonic acid (AEP)) and anhydrides (the most important are adenosine diphosphate (ADP) and adenosine triphosphate (ATP)) (Condrón et al., 2005; Turner et al., 2003; Turner et al., 2005).

Organic P compounds are composed of inositol phosphate (80% total soil organic P) (Menezes-Blackburn et al., 2013; Richardson et al., 2001; Turner et al., 2005). Their stability is closely linked to phosphate group number, rendering more recalcitrant esters of higher

order to biodegradation, consequently, more abundant. Myo-inositol hexakisphosphate (also known as phytic acid) is the most common stereoisomer in soil (Menezes-Blackburn et al., 2013). Organic P necessarily must be previously mineralized before plant absorption, being a potential source for plant nutrition in grassland ecosystems. Promoting soil organic P mineralization could be used as a tool to improve their availability for plant uptake and decreasing high mineral fertilizer application doses. However, the relative importance of organic P in phosphate contribution for plant uptake compared with direct P mobilization from soil mineral constituents remains relatively poorly understood (Richardson et al., 2009).

1.3.2 Phosphorus inputs and outputs

In pasture system inputs are governing mainly by inorganic P fertilizers, plant litter and animal feces. Manure and dung are constantly produced in grassland ecosystem. In grazed pastures, up to 85% P taken up by animal is returned to soil as dung (Fuentes et al., 2006; Williams and Haynes, 1990). Such deposits can represent P inputs of 35 to 280 kg P ha⁻¹ annually for individual sheep and cattle, respectively (Nash et al., 2014). Making more effective use of organic manures and biosolids might allow a decrease in P fertilizer use, but not an improvement in P fertilizer efficiency (Syers et al., 2008). When inorganic fertilizers are added to soil, phosphate is either sequestered into forms that are not immediately available to plants or extracted from soil water and incorporated into plant and microbial biomass. In grazing systems (Fig. 1), P is further transferred into animal biomass and may be exported from farms as animal (or plant) product (27% milk, 60% feces and 13% animal maintenance) (Hart et al., 1997). Otherwise, P in biomass is returned to soil when plant and animal biomass, and their wastes are recycled and decomposed (Nash et al., 2014).

Soil P outputs are governed principally by leaching and runoff. In cultivated grasslands, the P flux leaving the system is a direct function of N management regime (Stroia et al., 2007;

Watson and Matthews, 2008). According to defoliation (cutting for hay or grazing) methods and fertilization, P balances on field scale can differ considerably, ranging from a negative balance, where large P exports are not counterbalanced by fertilization, to a large surplus in over-fertilized grasslands (Stroia et al., 2007), which can represent important P sources to surface runoff waters. Recently, unexpected incidental P losses between 3 to 14 g dissolved P ha⁻¹ year⁻¹ occurred through surface runoff in Southern Chile volcanic soils (Alfaro and Salazar, 2007). These findings are consequential, because P concentrations in surface runoff were found above 0.01 mg P L⁻¹, which can trigger accelerated growth of algae and aquatic plants in freshwater systems (Mejías et al., 2013). To retain these fluxes, vegetated buffer zones in riparian wetlands are often installed (Haygarth et al., 2006). However, these wetlands may be sinks as well as sources of organic as well as inorganic P depending on the complex interaction between hydroclimatic variability, topography and soil properties (Gu et al., 2017).

1.4. Plant available phosphorus in grassland soils and its uptake by plants

P uptake by plant roots occurs mainly as phosphate ions (H₂PO₄⁻ and HPO₄²⁻) from the soil solution, which depends on release of mineral phosphate through solubilization and release of organic P through mineralization (Hinsinger, 2001). During phosphate uptake, P-depleted area is formed close to roots, since phosphate uptake is faster than its diffusion in soil (Bhat and Nye, 1974). Plants mediate positive and negative interactions via root exudations by rhizosphere (Fig. 3), which is defined as the soil volume around living roots influenced by root activity and their ability to secrete a wide range of compounds into rhizosphere, being one of the remarkable metabolic features of plant roots. The release of inorganic P through

solubilization or mineralization may be influenced by the plants through rhizosphere processes (Richardson et al., 2009).

The P uptake by grassland plants may vary between fertilizer formulation, composition and application, soil characteristics, plant species, and planting-harvesting schemes. Chemical reactions and microbial activity affect P availability for plant uptake. Under acid conditions, P is held tightly by Al and Fe in soil minerals (Borie and Rubio, 2003; Escudey et al., 2001; Hinsinger, 2001; Redel et al., 2016; Velásquez et al., 2016a). Under alkaline conditions, P is held tightly by soil calcium (Abbasi et al., 2015; Divito and Sadras, 2014; Waldrip et al., 2015). The P is strongly adsorbed onto soil colloids and does not form volatile compounds, so its cycle takes place exclusively in the biosphere and losses of P by leaching are generally small. Roots must proliferate throughout the soil to acquire sufficient P for plant nutrition. Figure 3 shows the processes occurring in the rhizosphere to acquire P from the soil for plant uptake. Most plants foster symbiotic relationship with mycorrhizal fungi to increase their ability to explore the soil volume and mobilize P from remote inorganic or organic sources.

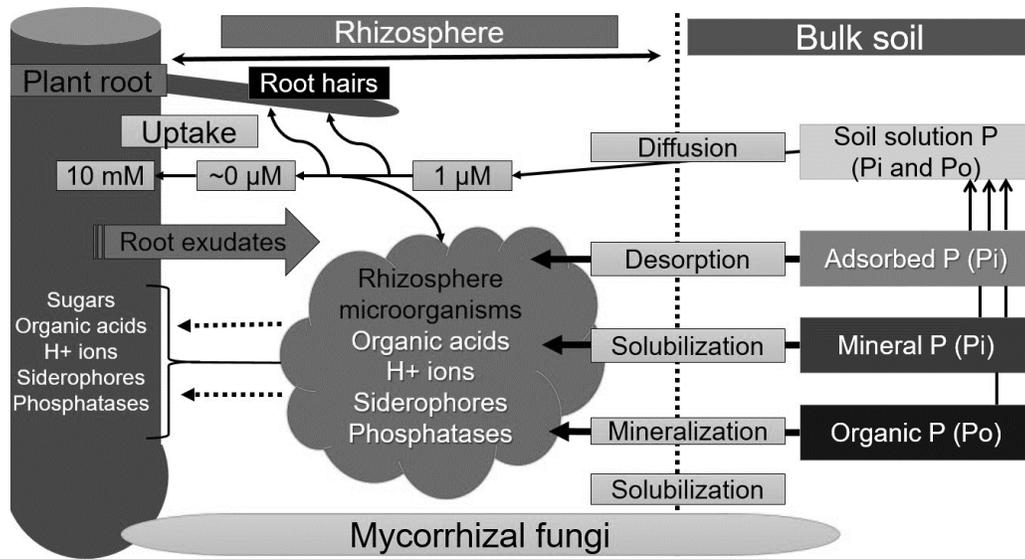


Figure 3. Rhizosphere processes to plant phosphorus uptake from soil

The P-utilization efficiency (the ability of plants to convert absorbed P into yield) is an important parameter because it allows us to have a reference for measuring the sustainability of agricultural systems (Sandaña and Pinochet, 2014). Low concentrations of phosphate in soil solution, slow diffusion P rates in soil and limited capacity for replenishment of soil-solution P are major factors that contribute to P deficiency in plants (Richardson et al., 2009). Figure 4 shows the strategies developed by plants to increase the acquisition and utilization of P. Plant root capacity for P acquisition is one of the key genetic characteristics determining nutrient accumulation (plant capacity for P acquisition and storage in shoot tissues). It is widely recognized that improving P-acquisition efficiency will result in substantial gains in P-use efficiency (PUE) and P-utilization efficiency, in relation to the fact that only 15–30% P fertilizer applied is taken up by plants in the first year of its application (McLaughlin et al., 2011).

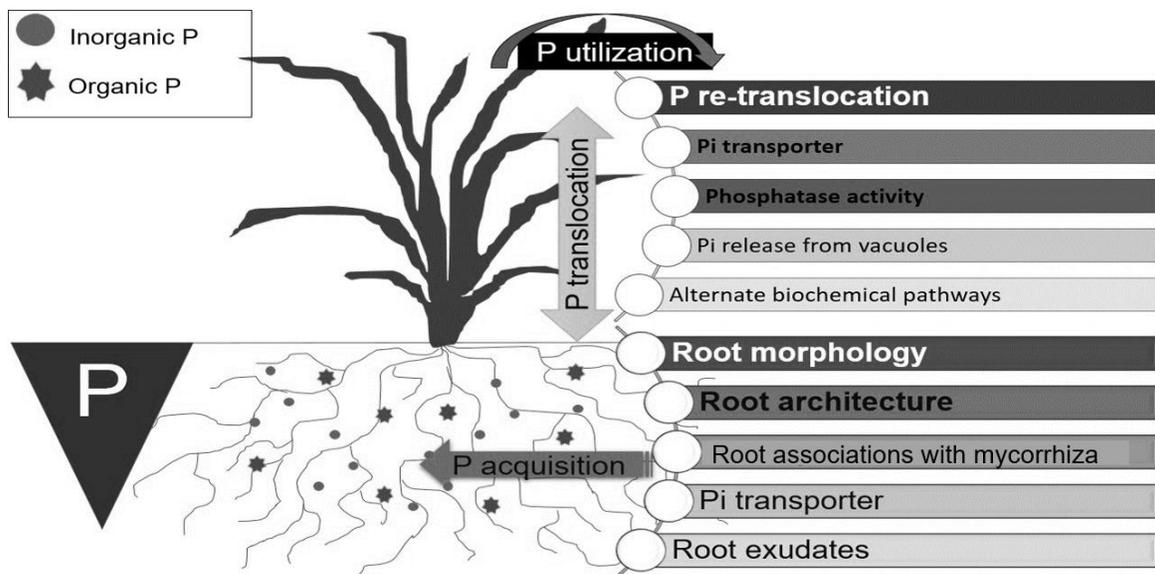


Figure 4. Schematic representation of acquisition mechanisms and utilization of phosphorus by modern crops growing in intensive cropping systems.

To be available to plants, organic P must be mineralized first to release phosphate with phosphate ester (C–O–P), phosphoanhydride (P–O–P) or phosphonate (C–P) bond hydrolysis, being predominantly mediated by phosphatase enzyme action (Bishop et al., 1994). However, plants need first to stimulate microbial degradation of SOM by the roots and mycorrhizae exudation of organic acids through the rhizosphere priming effect (Kuzyakov and Domanski, 2000). In addition, plant can exudate organic acids (Clarholm et al., 2015), which may influence P solubility and consequently its release and availability in the rhizosphere (Hinsinger, 2001). In general, around 5 – 21% total photosynthetically fixed C is transferred into rhizosphere through root exudates. In grassland ecosystem, plant pasture transfers 30 – 50% total assimilated C into soil, released from roots epidermal and cortical cells leading to microorganism proliferation (Haichar et al., 2014). The availability of these compounds to support microorganisms, plant and animal growth is partly dependent on their degradation rate to generate free phosphate (Fig. 3). In absence of readily-available P through inorganic fertilizers input, plants must utilize numerous strategies (Fig. 4) to acquire soil inorganic P and organic P quickly and efficiently to ensure an adequate P supply during growing season (Owen et al., 2015).

Clarholm et al. (2015) proposed that active P acquisition from SOM is achieved by a three-step process and performed by plant-microbe consortia. In step 1, SOM is destabilized by organic anions acids exuded by roots and fungi, which form particularly stable chemical complexes with the polyvalent SOM-bridging metals calcium, Al and Fe. In step 2, exuded hydrolytic enzymes degrade destabilized and newly exposed organic compounds, and release N and P in bioavailable forms. In step 3, there is a local P uptake by roots and fungi. Soil microorganism biomass management (related to plant-root type), the amount of organic anions acids released, and enhanced enzyme behavior could be innovative tools for

improving soil P fertility (Spohn et al., 2013; Zederer et al., 2017), hence, sustainability of low input farming systems, in terms of the agroecological transition of agricultural systems. Soil microorganisms represent an important dynamic nutrient reservoir potentially available to plants, and they play a fundamental role in soil organic P transformation (Lupwayi et al., 2005). Through phosphatase excretion, moderately labile inorganic P forms solubilization, organic P release and synthesis, they also work preventing phosphate sorption by soil inorganic colloids through immobilization in their tissues (Bishop et al., 1994). Then with the microorganism death and cell lysis, P can be released more synchronized with plant demand (Tiecher et al., 2012). Accordingly, soil microbial biomass management could be an affordable tool for improving P soil fertility (Koutika et al., 2013), but their capacity to provide P to plants is increased considerably within rhizosphere, where there is a large metabolizable C supply derived from roots (Richardson et al., 2009). SOC has various roles in producing crops and improving their environment. For improving SOC, organic amendments application is a common practice (see 5.3).

1.5. Management practices to improve soil phosphorus availability

The P bioavailability in productive grasslands is affected by several factors of managements including soils nutrients interactions (Paredes et al., 2011). Due to high phosphate fertilizer prices following phosphate rock (RP) future scarcity (Cordell et al., 2009; Reijnders, 2014), there is a research need for strategies by which P fertilizers can be used more effectively and improve their uptake efficiency by plants. Possible options to do this include the management of: pasture plant species (Cougnon et al., 2018; Klabi et al., 2018; Maltais-Landry, 2015), grazing intensity (Baron et al., 2001; Chaneton et al., 1996; Mundy et al., 2003; Neff et al., 2005; Simpson et al., 2012), cutting intensity, stocking density (Alfaro et al., 2009; Gao et al., 2016; Simpson et al., 2015), and animal manure inputs (Abdala et al., 2015; Adeli et al.,

2003; Costa et al., 2014; Duan et al., 2011; Geisseler et al., 2011; McLaughlin et al., 2004; Takeda et al., 2009; Vanden Nest et al., 2016). In grazed pasture, improving the recycling of P may be achieved through grazing management that reaches more uniform animal excreta distribution, hence, P returns to the pasture (Syers et al., 2008; Williams and Haynes, 1990).

1.5.1. Mowing, grazing and stocking density

Mowing trials are used to measure pasture responses to nutrients because they are much cheaper to operate than grazing trials, but in mowing trials the influence of the grazing animal through excreta return, treading and defoliation is absent (Morton and Roberts, 2001). Long-term biomass removal contribute to a less labile organic P relative to SOC attributed to enhanced mineralization of labile organic P in response to continued depletion of soil inorganic P (Boitt et al., 2017). Simpson et al. (2012) found that inorganic and organic P in the readily available fraction and the residual P were higher in soil from clippings left treatments compared with the no mowing and clippings removed treatments. Moreover, the P uptake for the clipping left was 51-54% higher than no mowing treatment.

Grazing can improve soil quality over time, maintaining higher moderate and labile P pools and contributing increases on pasture yield (Costa et al., 2014). Increasing animal densities reduces the selection for palatable vegetation patches within a grazing camp, and that this can reduce the spatial heterogeneity in vegetation vigor over time (Venter et al., 2019). Also, differences in the stage of the plant before start the grazing is important to consider as nutritive value. Lawson et al. (2017), demonstrated that 3-leaf stage in tall fescue appears to be the most productive of the grazing-management plant persistence and the ability of lactating dairy cows to consume the dry matter (DM) grown efficiently.

Livestock overgrazing is one of the most important factors that results in pasture degradation (Cavagnaro et al., 2018). Livestock grazing alters the cycles of soil nutrients in pastures

ecosystems by the interactions between plants and the soil, maintaining species diversity by light or moderate grazing, while opposite using heavily grazing (Costa et al., 2014; Li et al., 2011). By ingesting herbage, grazing animals encourage pasture plants to grow and therefore take up more nutrients from the soil (Williams and Haynes, 1990). Simpson et al. (2015) found that the stock carrying capacity of pasture growing on P deficient soil is increased by increasing the total soluble P concentration of the soil, which could be attributed to grazing promotes root exudation feeding the microorganisms on the rhizosphere, increasing their activity and consequently the P cycle efficiency (Costa et al., 2014). However, Da Silva et al. (2014) didn't found an interaction of grazing intensity and cattle dung input in a field experiment with Italian ryegrass under four different grazing intensities (0.10, 0.20, 0.30 or 0.40 cm), indicating that high soil P were associated with high grazing intensities.

Alfaro et al. (2009) establish that in grazed areas, depending on the stocking rate and the grazing efficiency, the amounts of P recycled can reach levels equivalent to the amounts applied as mineral fertilizers, especially in low input systems. However, Gao et al. (2016) found that P content was unaffected by stocking rate (2.4 and 4.8 animal unit months ha⁻¹), grazing treatment (continuous vs. discontinued) and their interactions in a cattle grazing study that started in 1949 until 2013. Grazing intensity could promote root proliferation depending of plant species, for example for smooth brome grass (*Bromus inermis* Leys) greater at medium grazing than either heavy or light grazing pressure whereas for meadow brome grass (*Bromus biebersteinii* Roem. & Schult.) was greatest under light grazing pressure (Mapfumo et al., 2002).

1.5.2. Pasture plant species

Pasture are used to graze cattle, sheep, goats or other animals and in this way contribute to the production of food. Among plants digestibility is an important factor in terms of grazing,

for example tall fescue possess on average 8% points lower than ryegrass affecting voluntary intake of animals in 7% lower than ryegrass (Cougnon et al., 2018). Sward composition and the type of grazing (see 5.1) on intake choices revealed differences between animal species, being sheep more selective than cattle (Cuchillo-Hilario et al., 2018). Sheep are able to differentiate between tall fescue genotypes when grown in small plots by digestibility of the organic material, the water-soluble carbohydrate concentration and the acid detergent fiber concentration (Cougnon et al., 2018). Plants may affect their rhizosphere directly or stimulate P mobilization via changes in microbial activity and species composition (Maltais-Landry, 2015). For example, the inclusion of some legume plants (i.e *Medicago sativa*) improve the nutritive value of native grass mixtures by increasing the concentration of nutrients in their tissues (N and P) (Klabi et al., 2018).

Among pasture plant species there are differences according to P requirements to produce biomass (Sandral et al., 2018). For example, the P uptake of Coastal bermudagrass needed to reach 90% of maximum yield was reported as 48 kg ha⁻¹, respectively whereas annual ryegrass needs 34 kg ha⁻¹ (Evers, 2002). Annual ryegrass is considered as the most effective temperate grass to remove soil P, because it is very productive and has a high P concentration (Brink et al., 2002; Evers, 2002; Waldrip et al., 2011). Moreover, Martinefsky et al. (2010) observed that comparing two cultivars of tall fescue species, the temperate cultivar accumulated a higher amount of total biomass as compared with Mediterranean cultivar under non-limiting and moderate limiting P availability conditions, indicating an effect less negative of P deficiency in the former. Simpson et al. (2014) reviewed the P balance of temperate pastures concluding that productive forage plants with lower critical P requirements are necessary for the critical P concentration of the soil–plant system to be lower; as an obvious goal to pursue. P use efficiency (PUE) differs among different pastures

species and their mixtures, being higher on mixtures compared to pure cultivated crops (Ilieva and Vasileva, 2016). According to some studies under pasture system the P plant use efficiency could be increased by using organic manure fertilization management (Abbasi et al., 2015; Khan et al., 2018; Poblete-Grant et al., 2019; Syers et al., 2008).

1.5.3. Animal manure input as fertilization managements

Nowadays, as a consequence of the growing global population, the animal production has been increasing heavily with greatly effects on animal disposal. According to FAO (2018b) beef production in developing countries by 2027 was projected to be 21% higher, also the sheep meat consumption worldwide on a per capita basis will reach 1.8 kg retail weight equivalent (r.w.e.) by 2027, as a consequence its production will experience a higher rate of growth than that of the previous decade. Moreover, sheep and goats increased 60% from 1961-2016, while swine increased 140% and poultry had a remarkable five-fold increase.

Manure applications during grazing periods have a different nutrient content which can vary widely depending on manure type (Table 2), animal physiology, species and age, composition of diets, and moisture content (Fuentes et al., 2006). The inorganic P pool of manures is large and has been reported to vary between 60 – 90%, being quite soluble and readily available to plants (Bolan et al., 2010; He and Honeycutt, 2001; Pagliari and Laboski, 2012; Sato et al., 2005). Options to increase plant-available P pool and optimize plant contribution to modify soil P cycle are the strategic use of organic-anion secreting plants or organic manures (Richardson et al., 2009). During grazing, plants received different manure inputs depending of the grazing animal type (Table 2).

On the other hand, the external manure application after composting may be a way to transform organic waste into organic amendments, which may be used to increase the soil fertility and C sequestration potential (Barthod et al., 2018; Chabbi et al., 2017). Manure

addition may enhance P nutrition of pastures via several mechanisms (Calabi-floody et al., 2012; Dikinya and Mufwanzala, 2010; Fuentes et al., 2012; Giles et al., 2017; Menezes-blackburn et al., 2014; Scotti et al., 2015).

Manure applications stimulate biological activity in soil (Takeda et al., 2009) delivering significant amounts of organic matter and other secondary nutrients, such as calcium, which can be important in stabilizing solution P into calcium-P secondary minerals as soil pH is raised because of the liming effect that manures generally have on soils (Abdala et al., 2015; Azeez and van Averbek, 2012). Manure also, affects P availability by (a) reducing P adsorption through competition for fixation sites by organic acids, (b) favoring the formation of metal-humates-phosphates complexes, and (c) decreasing the rate of precipitation of non-soluble calcium phosphates (Pizzeghello et al., 2014). Characterization of manure by animal type had been reviewed by He et al. (2016). In this review we will focus on changes in the response of pasture plants under manure fertilization management. Thus, understanding such processes within integrated crop-livestock systems is needed to evaluate the long-term manure impacts of soil management on P forms and P transformations.

The available P contents in the soil differed among areas with or without dung input, being higher under the presence of dung input (Da Silva et al., 2014). As manure input significantly increased soil pH and soil organic C levels (Vanden Nest et al., 2016), thus soil type is an important consideration when developing guidelines on manure input (Shepherd and Newell-Price, 2016).

Table 2. Nutrient content from different animal species manures applied into soil with/without crops grown.

Manure type	pH (H ₂ O)	C (g kg ⁻¹)	N (g kg ⁻¹)	P (g kg ⁻¹)	K (g kg ⁻¹)	Soil Taxonomy	Crop	Reference
Cattle	-	260	8	2.3	-	Vertisol	-	Reddy et al., 1996
	-	209	14	1.2	-			Maerere et al., 2001
	-	259	7	1.8	7	Typic Haplustert	Sorghum and soybean	Ghosh et al., 2004
	-	435-472	24-18	4	-	-	-	Leytem and Westermann, 2005
	-	-	3.2	1.8	11	-	-	Mohanty et al., 2006
	-	-	12	6	19	-	-	Antil and Singh, 2007
	-	333	12	8	-	Typic Ustochrepts and Typic Ustifluvents	Maize	Garg and Bahl, 2008
	-	-	7	4.2	7	Typic Haplustert (Vertisol)	Sorghum and soybean	Ghosh et al., 2009
	-	202-243	13-19	4-7	-	Xeric Haplocalcids (fine sandy loam)	-	Tarkalson and Leytem, 2009
	6.6	329	46	10	-	Andosol	African millet	Wickramatilake et al., 2010
	-	395	21	5	6	Durinodic Xeric Haplocalcids	Maize	Leytem et al., 2011
	6.6	329	46	10	-	Regosol	African millet	Wickramatilake et al., 2011
	-	402	17	5	-	Typic Haplorthod	Sorghum	Waldrup et al., 2012
	6.7	21	2.4	4	-	-	-	Lyimo et al., 2012
	-	-	11	18	6	-	-	Dunjana et al., 2012
	-	-	32	7	13	-	pea and cabbage	Korzeniowska et al., 2013
	6.8	112	9	4	8	-	-	Malik et al., 2013
	-	-	19	19	17	-	-	Qureshi et al., 2014
	-	-	5	1.1	6	Aquic Ustipsamment, Vertic Endoaquol, Typic Sulphisaprists Euic (Sandy, Calcareous and Mesic)	wheat, tomato, sugarbeet, maize and sunflower	Pizzeghello et al., 2014
	8.3	39	2	7	-	-	-	Li et al., 2014
7.3	527	20	7	-	-	-	Annaheim et al., 2015	
8.0	-	16	4	6	Old Brahmaputra Floodplain	-	Hafiz et al., 2016	

	-	-	5	3	6	Typic Kandiuustalf (alfisol)	finger mille and groundnut	Sathish et al., 2016
	-	261	12	2.7	7	Arenosols	Brachiaria	McRoberts et al., 2017
Sheep	8.3	279	-	8	-	-	-	Mcdowell and Stewart, 2005
	6.9	506	18	14	14	-	-	Jalali and Ranjbar, 2009
	8.5	364	24	9	24	-	-	Pagliari and Laboski, 2012
	7.9	-	20	27	8	-	-	Elouear et al., 2016
Swine	-	29	34	70	-	-	-	Tunney et al., 1987
	6.9-7.3	44-46	14.-24	47-103	-	Typic Hapludolls and Typic Argiudolls	-	Gollany et al., 2003
	-	233-360	39-56	17-30	-	-	Barley	Leytem and Westermann, 2005
	-	2.3-2.7	1.5	1.0	-	Vertic Ustochrept	Lucerne	Ceotto and Spallacci, 2006
	-	-	15	6.5	8	-	-	Bolan et al., 2010
	6.9-7.2	-	4-6*	0.7-1.5*	2*	-	Poa pratensis L., Phleum pretense L, Medicago sativa L.	Wilson et al., 2011
	-	-	2.6	4	4	Red-Yellow Latossol	Brachiaria brizantha	Costa et al., 2014
	-	150	16	2	24	-	-	Qiao et al., 2017, 2014
	7.1	91	25	15	36	-	-	Barbosa et al., 2015
	-	376.1	33	24	-	-	-	Zhang et al., 2015
	8.0	-	430*	15*	-	Ultic Palexeralf	Ryegrass	Schoebitz and Vidal, 2016
	7.2	205	36	15	16	Typic Hapludox	-	da Silva Oliveira et al., 2017
Poultry	6.5	461	50	6	-	Andie Dystrochrepts	-	Mahimairaja et al., 1993
	8.9	-	29	19	-	Typic Dystrochrept	-	Mahimairaja et al., 1995
	-	150	18	5	-	-	-	Maerere et al., 2001
	-	313	21	11	12	Typic Haplustert	Sorghum and soybean	Ghosh et al., 2004
	8.0	337	29	10	17	-	Maize	Hirzel et al., 2007
	-	25	18	11	-	-	-	Antil and Singh, 2007

-	5.9	19	13	-	Typic Ustochrepts and Typic Ustifluvents	Maize	Garg and Bahl, 2008
6.8	295	43	38	22	-	-	Agbede and Ojeniyi, 2009
7.6	418	36	20	40	Alfisol	Sorghum	Jalali et al 2009
-	413	12	8	10	Entisols and Inceptisols	-	Toor, 2009
-	-	25	10	12	Typic Haplustert (Vertisol)	Sorghum and soybean	Ghosh et al., 2009
-	-	26	7	10	-	-	Bolan et al., 2010
9.7	193	27	31	-	Andosol	African millet	Wickramatilake et al., 2010
9.7	193	27	31	-	Regosol	African millet	Wickramatilake et al., 2011
7.6	341	16	28	12.	-	-	Khan and Sharif, 2012
6.5	336	49	21	29	-	-	Pagliari and Laboski, 2012
-	29.1	4	4	-	-	-	Lyimo et al., 2012
-	446	33	16	-	Oxisol	-	de Souza et al., 2012
7.5	321	21	10	18	Inceptosol	Wheat	Abbasi et al., 2013
7.3	143	9	5	11	-	-	Malik et al., 2013
6.4	36	5	14	-	-	-	Li et al., 2014
-	-	19	13	20	-	-	Qureshi et al., 2014
8.6	231	24	8	10	-	-	Barbosa et al., 2015
-	-	22	11	14	Aquic Hapludoll, Typic Hapludoll, Typic Calciaquoll	Maize	Hoover et al., 2015
-	-	25	16	-	Inceptosol	Capsicum annum	Abbasi et al., 2015
6.8	28	91	37	34	-	Maize	Ahmed et al., 2016
7.7	-	2.2	2	3	Old Brahmaputra Floodplain	-	Hafiz et al., 2016
6.8	121	50	5	-	Alfisols	Sorghum	Ibrahim et al., 2017
7.4	341	16	10	12	Inceptosol	Maize	Abbasi and Manzoor, 2018

*mg L⁻¹

Animal species also influence differences on effect. The long-term application of farmyard manure as P fertilizer affects the pool sizes and dynamics of soil P, while Geisseler et al. (2011) found that the proportion of P in the organic form was only slightly increased with the application of farm yard manure compared to the plots receiving mineral fertilizer. Sheep and cattle have different grazing habits and forage requirements and, consequently, can have different impacts on pasture development (Barthram et al., 2005). Sheep like farmyard manure treatment increased soil soluble P from 1.08 to 8.6 mg L⁻¹ (Jalali and Ranjbar, 2009) and increased activities of alkaline phosphatase and β -glucosidase compared with no manure treatments (Liu and Zhou, 2017). The digestive system among animal species could differ also, being swine not as efficient as that of cattle in absorbing P in feed, and their excrete about 50–60% of their P intake in feces and urine, being necessary additions of enzyme phytase to pig feed to improve P uptake (Hjorth et al., 2010).

According to the review performed by Choudhary et al. (1996) the effectiveness of swine manure has not been studied on temperate pastures, thus they encouraged to increase knowledge of swine manure handling and utilization for crop production in temperate regions. Nowadays, using swine manure had showed to increase crops yields (Adeli et al., 2003; Costa et al., 2014; Duan et al., 2011; McLaughlin et al., 2004) and soil extractable nutrients, mainly soil Olsen P and soil extractable K, with minimal soil NO₃-N accumulation (Wilson et al., 2011). Moreover, swine manure incorporation into the soil (5 and 10 cm) increased the plant PUE in pastures (Adeli et al., 2003), supporting that the use of swine manure could be a source of P in agricultural areas. He et al. (2003) determined that the proportion of bioavailable P was the same for swine (72.8%) and cattle (71.6%) manures, although less recalcitrant P was present in the cattle manure (7.8%) than in the swine manure (21.3%).

Among manures poultry is considered as the most efficient in terms of N and P (He et al., 2016). To enhance the P status of soils, poultry manure (PM) may be a suitable amendment, because it is a type of organic amendment applied to soils as a source of N and P (Table 2). PM is relatively a cheap source of both macronutrients (N, P, K, Ca, Mg, S) (Evers, 2002, 1998; Ghosh et al., 2009; Hirzel et al., 2007; Pederson et al., 2002; Toor, 2002; Waldrip et al., 2011) and micronutrients (Cu, Fe, Mn, B), increases SOC (Li et al., 2018a), soil aggregate size fraction (Ranatunga et al., 2013), and enhance the soil microbial activity (Ghosh et al., 2004; Li et al., 2018a; Malik et al., 2013). In Table 3 is showed the effects of PM on different parameters on soil and plant. In a study performed by Bahl and Toor (2002), PM addition reduced the effective P adsorption capacity, standard phosphate requirement and had a high effect on mobilizing native P forms on soils.

Moreover, Waldrip et al. (2011) demonstrated that PM temporarily increase inorganic P in NaOH and HCl soil fractions attributing this to: (1) stimulation of root phosphatase production, (2) the enrichment of the surrounding soil for microbial phosphatase production or activity, and (3) increases of Al- and Fe-associated phosphates solubility. In addition to P contained in PM, solubilize the native phosphate compounds present in soil by organic acids produced as the organic amendment decomposes, which may have favorable effect on native P solubilization (Toor and Bahl, 1997).

Table 3. Poultry manure effect on soil physical, chemical and biological parameters and plant production.

Effect	Conditions	Rate	Soil type	Soil pH	Plant species	Observations	Reference
Soil physical properties	Field	1.9-3.8 Mg ha ⁻¹	Ferralsol	4.97	Corn, oat and wheat	Promotion of clay flocculation and soil aggregation, increasing the weighted mean diameter.	de Cesare Barbosa et al., 2015
	Field	2.3 Mg ha ⁻¹	Typic Hapludults	5.2	Pastures	Changes regarding aggregates distribution.	Ranatunga et al., 2013
	Field	13 Mg ha ⁻¹	Haplic Yermosols	7.6	Maize	PM alone showed substantial reduction in soil bulk density.	Mahmood et al., 2017
	Field	10-30 Mg ha ⁻¹	Oxic Tropudalf	5.8	Carrot	Increasing amounts of PM, soil moisture and total porosity increased, while soil bulk density and temperature decreased attributed to the increased soil organic matter from the organic amendment.	Agbede et al., 2017
	Field	21 Mg ha ⁻¹	Typic Gleyi-Stagnic Anthrosols		Rice, wheat	Decreasing of the soil mesopore volume and increases the soil micropore and macropore volumes in plow pan. The tensile strength of soil aggregate in the were less than 20 MPa, While the soil aggregate wet stability index was greater than inorganic fertilizers.	Li et al., 2011
Soil chemical properties	Field	5-10 Mg ha ⁻¹	Orthic Humo-Ferric Podzol and Gleyed Regosol	6.2-6.5	Ryegrass, orchardgrass, white clover, red clover.	After 3 years of PM amendment equivalent yield and forage quality as inorganic fertilizers can be obtained.	Warman and Copper, 2000
	Field	1-4 Mg ha ⁻¹	Aquic Arenic Haplustalf	6.1	Maize and cowpea	Efficient in facilitating the release of P from RP was showed as significantly higher available soil P.	Akande et al., 2005
	Greenhouse	30-120 mg P kg ⁻¹	Ferric Acrisol and typic Haplustox	4.2- 6.1	Maize	Enhancing of P availability by its combination with RP decrease with the increasing of water-soluble P content of the P source.	Agyin-Birikorang et al., 2007
	Greenhouse	42.6 Mg ha ⁻¹	Typic Haplorthod	5.2	Ryegrass	Temporary increasing of NaOH and HCl-Pi, being readily transformed into plant-available P, labile P and therefore does not necessarily accumulate as stable HCl-P.	Waldrip et al., 2011

Greenhouse	90 mg P kg ⁻¹	Humic Lithic Eutrudepts	7.57	Chilli	RP when combined with PM released significantly higher amount of P compared to the RP alone (80%).	Abbasi et al., 2015	
Field	10 Mg ha ⁻¹	Humic Lithic Eutrudepts	7.1	Winter wheat	Decreases on pH of soil amended with PM combined with RP.	Abbasi et al., 2013	
Greenhouse	20 Mg ha ⁻¹	Haplargids and Ustorthents	8.1-8.2	Wheat	Decreases of soil available P over time probably due to increases in plant grow and larger root system which could depleted available P more strongly over time.	Malik et al., 2013	
Field	9 Mg ha ⁻¹	silty clay loam	8.15	Winter wheat and summer maize	SOC was increased by 143%. Being labile C markedly increased.	Li et al., 2018	
Field	5.6-22.4 Mg ha ⁻¹	thermic Glossic Fragiudult	4.2-5.1	Bermudagrass	Highest levels of NO ₃ -N in the upper 5 cm showing no differences below 10 cm, so its application does not promoted build-up of excessive NO ₃ -N that could leach into groundwater.	Wood et al., 1993	
Greenhouse	150 ug P g ⁻¹	Humic Lithic Eutrudepts	7.1	Maize	The pH of the soil amended with RP+PM tended to decline.	Abbasi and Manzoor, 2018	
Greenhouse	4.5 Mg ha ⁻¹	Typic Fluvaquents, Ustochrept, Hapludalf	6.0-7.9	Soybean	Combined application of P fertilizers and PM showed greater increases in Olsen P at all fertilizer rates. Mechanisms: i) conversion of insoluble Al and Fe-P into soluble forms; ii) organic acid and humate release; iii) production of CO ₂ increasing solubility of Ca and Mg-P; and iv) humate may form a protective surface over colloidal sesquioxides reducing the P fixation.	Toor, 2009	
Greenhouse	4 Mg ha ⁻¹	Typic Ustochreps, Typic Ustifluvents	7.5-7.9	Maize	PM released more P due to its high P and humic substances and low C:P ratio	Garg and Bahl, 2008	
Field	18 Mg ha ⁻¹	Typic Paleudults	7.1-7.7	Bermudagrass	Residual build-up of C and P, Cu and Zn	Sistani et al., 2008	
Soil biological properties	Greenhouse	42.6 Mg ha ⁻¹	Typic Haplorthod	5.2	Ryegrass	Higher activity of acid compared to alkaline phosphatase, suggesting that the activity of the first one may be involved in maintaining the balance of soil solution P in the rhizosphere.	Waldrip et al., 2011

	Greenhouse	20 Mg ha ⁻¹	Haplargids and Ustorthents	8.1-8.2	Wheat	Microbial activity and biomass C, N and P was greatly increased explained by its higher TOC concentration and also due to its input of enough nutrients to satisfy microbial and plant demand.	Malik et al., 2013
	Field	9 Mg ha ⁻¹	silty clay loam	8.1	Winter wheat and summer maize	Microbial community composition was dominated by bacteria with a higher +G/-G ratio which represent a favorable SOC accumulation. Microbial biomass C increased was probably due by PM input of a readily-available source of C substrate and improving soil environment.	Li et al., 2018
	Greenhouse	5 mg P kg ⁻¹	Regosol	4.0	African millet	PM increased microbial biomass P and population density of PSB. PM treatment was closely related with genus <i>Paenibacillus</i> and <i>Buskholderia</i> .	Wickramatilake et al., 2011
	Greenhouse	13.3 mg P kg ⁻¹	Andosol	5.1	African millet	Phosphate rock-P was efficiently immobilized by microorganisms in the PM. Bioavailability of P depends on the ability of soil microbial biomass to immobilize non-labile forms of soil P.	Wickramatilake et al., 2010
	Lab	9-10 Mg ha ⁻¹	Typic Haploxeralf Calciorthid	8.3	Wheat, rape, vetch	Reduction of earthworms in control soil 48% compared to 22% in PM treatments. Enzymatic activities and soil basal respiration were highly increased by PM.	Delgado et al., 2012
	Greenhouse	4 Mg ha ⁻¹	Typic Ustochreps, Typic Ustifluvents	7.5-7.9	Maize	PM showed the higher alkaline phosphatase activity compared with other manures by its low lignin content and high C, N and cellulose content	Garg and Bahl., 2008
	Field	26 kg P ha ⁻¹	Typic Haplustert	7.8	Soybean, sorghum, wheat	PM inhibited nodulation in soybean due to its high total N in a relatively easily available mineral form (ammoniacal plus Uric N)	Ghosh et al., 2004b
	Field	56-168 kg P ha ⁻¹	Typic Fragiudult	6.2	Soybean	Enzymatic activities of phosphatase were high immediately	Blair et al., 2014
Plant yield	Field	9 Mg ha ⁻¹	thermic Glossaquic Paleudalfs	5.9	Ryegrass, Coastal bermudagrass	PM combined with commercial N fertilizer increased yield and N, P, K uptake.	Evers, 2002

Field	5-10 Mg ha ⁻¹	Orthic Humo-Ferric Podzol and Gleyed Regosol	6.2-6.5	Ryegrass, orchardgrass, white clover, red clover	After 3 years of PM amendment equivalent yield and forage quality as inorganic fertilizers can be arised.	Warman and Copper, 2000
Greenhouse	0-40 g kg ⁻¹	Clay loam	7.9	Tomato	Zn and Rb concentrations in fruit were increased while Br was decreased. PM levels had no significant effects on K, S, Fe, Sr or Ba concentrations in tomato plants.	Demir et al., 2010
Field	1-4 Mg ha ⁻¹	Aquic Arenic Haplustalf	6.1	Maize and cowpea	RP combined with PM improved plant height and yield.	Akande et al., 2005
Greenhouse	30-120 mg P kg ⁻¹	Ferric Acrisol and typic Haplustox	4.2-6.1	Maize	Low rate of PM increased from ~3.6 to 7.6 g pot ⁻¹ while, total P uptake increased from ~15 to 39 mg pot ⁻¹ .	Agyin-Birikorang et al., 2007
Greenhouse	42.6 Mg ha ⁻¹	Typic Haplorthod	5.2	Ryegrass	PM promoted higher root and shoot P uptake and increased root P concentrations.	Waldrip et al., 2011
Greenhouse	90 mg P kg ⁻¹	Humic Lithic Eutrudepts	7.57	Chilli	RP combined with PM displayed a remarkable improvement in the growth and yield probably due to the additional beneficial effect on soil physicochemical properties and root proliferation.	Abbasi et al., 2015
Field	10 Mg ha ⁻¹	Humic Lithic Eutrudepts	7.1	Winter wheat	RP combined with PM increased PUE more than two-fold, moreover yield increased by 52% over RP alone.	Abbasi et al., 2013
Greenhouse	20 Mg ha ⁻¹	Haplargids and Ustorthents	8.1-8.2	Wheat	PM obtained the highest increases on the N, P and K uptake. PM also stimulated root dry weight and number of tillers per plant.	Malik et al., 2013
Field	6-22 Mg ha ⁻¹	thermic Glossic Fragiudult	4.2-5.1	Bermudagrass	Residual effect of PM may result in both increased yields and hay quality with long-term application.	Wood et al., 1993
Field	5.6-11.2 Mg ha ⁻¹	mesic Typic Fragiudult	6.2	Tall fescue	Dry matter was similar between the two PM rates. There was a significant interaction between sample date with the concentration of Cu, Mg, Se and Zn, but not for Mn or Al in herbage. As, Cd, and Cr herbage concentrations were below the limits of detection of the instrument.	Brye and Pirani., 2006

Greenhouse	150 ug P g ⁻¹	Humic Lithic Eutrudepts	7.1	Maize	Combined application of RP with PM increased P contents, P-uptake and PUE compared to their separate application.	Abbasi and Manzoor., 2018
Field	90 kg P ha ⁻¹	mesic Typic Hapludolls	6.6	Maize	Extremely high increments in yield in integrated treatments pf PM with both inorganic P fertilizers when inoculated with PSB.	Zafar et al., 2011
Greenhouse	4.5 Mg ha ⁻¹	Typic Fluvaquents, Ustochrept, Hapludalf	6.0-7.9	Soybean	Combined P fertilizers with PM had a more favorable effect on dry matter yield and P uptake attributed to: i) through enlarged proliferation of roots; ii) reduction of Al and Fe activity by root organic acids release; and iii) reduction of Ca activity in soil solution by bonding Ca on plant root exchanging sites.	Toor, 2009
Field	9 Mg ha ⁻¹	Fine sandy loam	5.1-5.7	Ryegrass, oat, rye, wheat, red clover, white clover.	Ryegrass as effective as other forages in N, P, K, Cu, and Zn uptake.	Pederson et al., 2002
Greenhouse	4 Mg ha ⁻¹	Typic Ustochreps, Typic Ustifluvents	7.5-7.9	Maize	PM treated soil showed the highest P uptake due to its higher mobilization of P as well as higher amount of P added from its own constituent.	Garg and Bahl., 2008
Field	26 kg P ha ⁻¹	Typic Haplustert	7.8	Soybean, sorghum, wheat	Sorghum highly responded to PM because the fact that approximately 74% of total P and 40% of total N were in available form	Ghosh et al., 2004a
Field	26 kg P ha ⁻¹	Typic Haplustert	7.8	Soybean, sorghum, wheat	The total chlorophyll content in sole sorghum was higher in PM treated plots	Ghosh et al., 2004b
Field	18 Mg ha ⁻¹	Typic Paleudults	7.1-7.7	Bermudagrass	No impact on P uptake possibly because initial soil P were high or because bermudagrass requirements for P were relatively low	Sistani et al., 2008
Field	10 - 20 Mg ha ⁻¹	Typic Melanoxerands	6.5	Maize	Agronomical use efficiency of N was higher using PM than those obtained with the mineral fertilizers	Hirzel et al., 2007

Nowadays, the improvement to enhance the manure as a P source for plants by the enrichment with inorganic fertilizers (Dunjana et al., 2012), phosphate solubilizing bacteria (PSB), and nanoclays materials had been performed (Calabi-floody et al., 2012; Fuentes et al., 2009; Mora et al., 2017). The addition of PM plus inorganic fertilizers has beneficial soil effects (Ghosh et al., 2004) increasing Olsen extractable P content. The combined use of manure and phosphate rock (RP) has been assessed as a strategy to decrease fertilization prices through increasing the efficiency of RP (Agyin-Birikorang et al., 2007; Khan and Sharif, 2012; Mahimairaja et al., 1995). The mechanism behind could be organic material mineralization produced following release of organic acid resulting in pH decreases as result of proton reaction, ion chelation, and exchange reactions. Therefore, mineralization of PM provides a favorable environment for RP dissolution (Khan and Sharif, 2012; Mahimairaja et al., 1995).

The use at large scale of recycling of organic P from animal wastes is great option considering the imminent RP scarcity (Abbasi et al., 2015; Menezes-Blackburn et al., 2013). Hirzel et al. (2009), performed a field experiment to assess the effect of applying PM as a nutrient source on silage maize yield and on the properties of volcanic soil (11 mg kg⁻¹, Olsen P). They found that that the different rates of PM (10 to 20 Mg ha⁻¹) applied have no adverse effect on maize yield and that the nutrients supplied by this manure are adequate for a good silage maize harvest obtaining high yields (30 to 37 Mg ha⁻¹). Moreover, Hirzel et al. (2013) carried out a field experiment in a high soil P (22 mg ha⁻¹, Olsen P) concluding that combination of PM at rates of 7.5 to 15.0 Mg ha⁻¹ and conventional N (100 to 300 kg ha⁻¹) improves biomass production in maize. Waldrip et al. (2011) performed a pot experiment to investigate PM effects on soil P availability, using ryegrass (*Lolium perenne*) plants growing in low P soil (5 mg kg⁻¹, Morgan P). They found a higher root P concentration, total P uptake, and an increase of phosphatase

activity by PM application. The fertilization value of PM may also be enhanced through microbial inoculation or addition of enzymes to increase P release (Abbasi et al., 2015; Toor, 2002). Applying commercial N fertilizer in combination with PM to pasture systems increased yield and N, P and K uptake (Evers, 2002; Pederson et al., 2002; Waldrip et al., 2011). This could give promising results, which could be translated in the reduction of the use of high-cost P fertilizers.

1.6. Conclusion and Perspectives

With the currently world's population increase and resource scarcity, strategies to enhance crop yields by agricultural management practices are needed. Soil P importance is massive for plant nutrition, but grasslands managements for improving P fertilizer efficiency uses are still poorly understood.

Animal production generates high sub-product amounts that can be returned to soils avoiding nutrient extraction of these agricultural systems. Integrated fertilizing management (organic-inorganic sources) could be a possible strategy to mobilize native soil P accumulates by intensive fertilization history in agroecosystems. Where pastures are the base of the economy and agricultural production is carried out primarily in soils with a high P content (volcanic soils) but sparingly available to plant uptake being sorbed by soil clays.

Phosphate rock depletion in addition to the increasing of world's demography levels represent a massive challenge for the current agricultural systems. Management practices to improve P efficiency uptake by plants are needed to decrease the P fertilizer doses to be applied and enhance P bioavailability in soils with a high organic P accumulation and make it available to plants. According to the information above mentioned, organic manure could be an interesting option to improve the delivery of P by phosphate rock enhancing its solubilization and plant P use efficiency.

According to literature, there is only a limited understanding of mechanisms associated with organic P transformation into inorganic P, at process level, and further work is needed using soil-based studies, especially under field conditions. Moreover, there are few studies investigating the effect of organic amendments on the solubility of RP. However, no study has been carried out to investigate quantitatively the synergistic or antagonistic effects of the combined application of both materials considering soil type. Moreover, the quantification of C transferred from plant/manure to soil and its fate allocated in different soil organic matter pools is poorly known.

Hypotheses

We hypothesized that

i) Long-term amended soil with composted poultry manure applied alone increases P availability and SOC storage; and ii) the joined application of phosphate rock and poultry manure in short-term induces synergistic effects by enhancing C transfer from plant to soil as well as SOC storage, P availability and biomass production depending of soil properties.

General objective:

To assess the impact of poultry manure application, alone or combined with phosphate rock, on soil quality, carbon sequestration, P availability and ryegrass production.

Specific objectives:

- To investigate the relationship between soil available P forms, carbon concentration, soil particle size distribution, and Al and Fe oxides in Southern Chilean pastures amended with composted PM for several years.
- To determine the effect of PM application, alone or combined with RP, on ryegrass biomass production, P use efficiency, and soil P availability in a moderately acid and an alkaline soil.
- To assess the effect of PM and RP amendments on (1) soil carbon storage and (2) plant-derived carbon allocation in different fractions.

CHAPTER II

Soil available P on Southern Chilean pastures under composted poultry manure is regulated by soil organic carbon, and iron and aluminum complexes

Submitted: Geoderma Regional

Chapter II: Soil available P and particles size distribution on Southern Chilean pastures under composted poultry manure is regulated by soil organic carbon, and iron and aluminum complexes

Abstract

Andisols, rich in minerals like allophane, imogolite and iron- (Fe) or aluminum- (Al) oxides have high phosphorus (P) sorption capacity and require annual P additions to ensure plant productivity. It is known that the use of composted poultry manure (PM) increases soil labile P and carbon concentration, although the mechanisms controlling P availability and soil carbon (C) sequestration remain unclear. The aim of this study was to investigate the relationship between soil available P forms, soil organic C (SOC), aggregation, and Al and Fe forms in Southern Chilean pastures amended with composted poultry manure. Soil samples, were taken from 4 pastoral farms where PM had been applied annually at 3 Mg ha⁻¹ and analyzed for elemental concentrations, P forms through Hedley fractionation, aggregation through particle size analyses and scanning electron microscopy and oxalate and pyrophosphate extractable Fe and Al. Andisols receiving PM application resulted in increases of 22 – 65% of SOC likely due to improved aggregation capacity as indicated by greater soil particle size of amended pasture soils, as compared to the control. Moreover, the readily available inorganic P increased by 56 to 286%. Andisols amended with PM showed decreases in oxalate extractable Al and Fe while, pyrophosphate extractable forms increased and was correlated to C and P concentrations and particle size distribution. We conclude that increases of P availability in Andisols under pasture amended with PM is highly regulated by changing SOC stabilization mechanisms.

Keywords: Poultry manure; Andisols; Phosphorus Availability; Particle size distribution; Al-, Fe-complexes.

2.1. Introduction

Dairy and meat production in Southern Chile is based on the use of permanent pastures on Andisols (Demanet et al., 2015). Andisols have specific andic properties such as high content of short-range order minerals, favoring phosphorus (P) immobilization through sorption and/or precipitation with cations such as aluminum (Al) and iron (Fe) (Borie and Rubio, 2003; Mora et al., 2006; Redel et al., 2016). In these soils, organic matter (SOM) and inorganic and organic forms of P accumulate under pasture (Redel et al., 2016; Velásquez et al., 2016). Consequently, they require high levels of P fertilizer inputs to enhance and maintain plant development and animal productivity. In the current context, the challenge is to implement sustainable intensification with new strategies to increase yields using fewer resources (limited agricultural area and fertilizers) and waste recycling (Calabi-Floody et al., 2018)

Enhancement of plant available P may be achieved by using P-rich organic waste, such as poultry manure (PM) with a low N:P ratio (Shepherd and Withers, 1999). PM has high P availability (He et al., 2008; Pagliari and Laboski, 2012; Waldrip et al., 2011) and is abundant globally due to broiler meat production (FAO, 2018b). Composted PM may be a suitable P fertilizer for soils with high P fixation capacity, such as Andisols. Although PM is widely used as soil amendment, mechanisms by which it increases P availability and controls SOC sequestration remain unclear. In this study, we investigated Andisols under pasture at four farms in Southern Chile. The aim of the study was to determine effects of long-term PM amendment on C sequestration and soil P availability. We

hypothesized that increased P availability in Andisols receiving regular PM amendment is controlled by soil Al and Fe complexes and their interactions with SOC. In order to test this hypothesis, we investigated the relationship between soil available P forms, C concentration, soil particle size distribution, and Al and Fe forms in Southern Chilean pastures amended with composted PM for several years. The specific objectives of this study were to: i) characterize P forms in Andisols under pasture amended with PM for several years, ii) investigate the effect of long-term PM amendment on Al and Fe forms, and iii) determine the effect of long-term PM amendment on soil particle size.

2.2. Material and methods

2.2.1. Study farms and soil sampling

Soil samples were collected in the summer of 2015-2016 from 4 grazing farms located in southern Chile: Copihual (39°13'45"S, 72°12'27"W), Carilafquen (39°01'57"S, 72°03'57"W), Huifquenco (39°17'17"S, 72°14'18"W), and Santa Teresa (39°54'60"S, 72°41'30"W) (Fig. 5). All farms were located on Andisols with loamy texture formed from volcanic parent material and belong to 3 different soil series (Villarica, Cunco and Los Lagos) (CIREN, 2003). The climate in this region is temperate with rainfall ranging between 200 and 2000 mm year⁻¹. Although land management at the farms was similar, at Copihual and Carilafquen the soils received 3 Mg ha⁻¹ of composted PM annually for 5 years and at Huifquenco and Santa Teresa 3 Mg ha⁻¹ of composted PM were added to soils annually for 10 years.

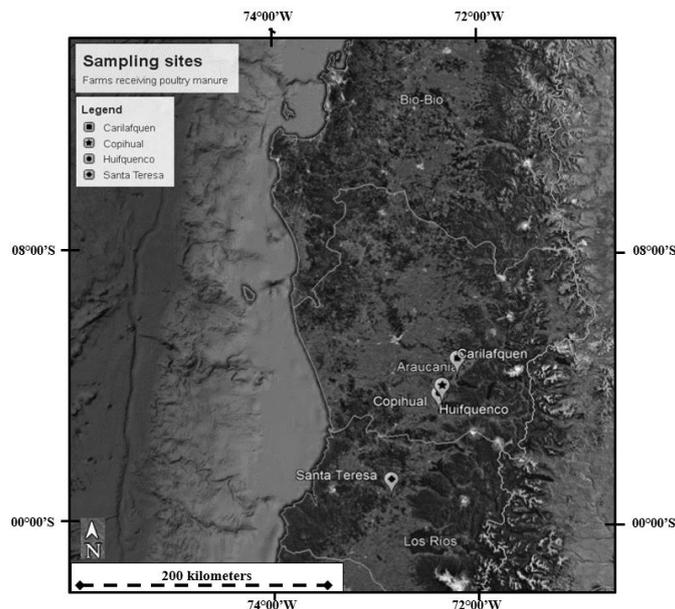


Figure 5. Localization of the sampling sites in the Southern Chilean pastures amended several years with composted poultry manure.

In all farms, the application of PM was carried out between February and April. Animal production and approximate biomass production are shown in Tab. 4. Generally, the PM material applied originated from the central zone of Chile. Poultry manure was stabilized through the composting process after the successive action of populations of beneficial microorganisms developed under controlled conditions of air, temperature and humidity. On each farm, we collected three samples from paddocks amended with PM and three soil samples from reference paddocks without fertilization (Control). Soils under both treatments (PM and Control) were under pasture management (Table 4). Soils were sampled at depth of 0-20 cm. After sampling, the soils samples were air-dried and sieved <2 mm. For this study, PM was sampled from the commercial product distributed by Pucalán, homogenized, freeze-dried, ground to pass 2 mm, and stored at -20°C prior to analyses.

Table 4. Land use, classification and management history in the Southern Chilean pastures amended several years with composted poultry manure.

	Carilafquen	Copihual	Huifquenco	Santa Teresa
Soil series	Cunco	Villarrica	Villarrica	Los Lagos
Soil family	Acrudoxic Hapludand	Acrudoxic Fulvudands	Acrudoxic Fulvudands	Typic Durudands
Soil density (g cm⁻³)	1.05	0.65	0.65	0.84
Organic carbon (%)	3.29	13.03	13.03	8.27
pH H₂O	6.0	5.2	5.2	6.1
Soil texture	Loamy loam	Sandy loam	Sandy loam	Loamy silty clay
Pluviometric (mm per year)	2,000-2,500	750	750	3
Pasture species	<i>Lolium perenne</i> + <i>Dactylis glomerata</i> + <i>Fertuca arundinacea</i> + <i>Trifolium repens</i>	Natural	Natural	<i>Lolium perenne</i> + <i>Trifolium repens</i>
Poultry manure application (years)	5	5	10	10
Fertilization per ha	3 tons Mg + 50 kg Urea	3 Mg PM + 0 kg Urea	3 tons PM + 50 kg Urea	3 tons PM + 100 kg Urea
Cattle	Wagyu	Wagyu	Black Angus	Holstein Freezer
Milk and Meat production	336 kg LW (live weight)	275 kg LW	312 kg LW	9,750 L
Stoking rate (cows per ha)	1.4	1.1	1.2	1.5
Grazing system		Rotating with electrical fences		
Pasture production before PM (kg DM ha⁻¹)	6.8	5.5	5.8	9.5
Pasture production after PM (kg DM ha⁻¹)	10.9	7.3	10.8	11.7

2.2.2. Chemical characterization of poultry manure compost and soil

Chemical analyses of PM and soil samples were carried out according to the methodology described by Sadzawka et al. (2006). pH was determined in H₂O with a 1:2.5 PM sample: water solution ratio. Total C and nitrogen (N) were determined by dry combustion using a CHN auto-analyzer (CHN NA 1500, Carlo Erba). No carbonate was present in the soil; therefore, soil C is considered to be exclusively organic. Basic exchangeable cations (Ca, Mg, Na and K) were extracted with 1 M ammonium acetate (pH 7) and determined by atomic absorption spectrophotometry (AAS). Determination of Al and Fe present in the -humus complex (Al_{pyro} and Fe_{pyro}) were determined by extraction with 0.1 M sodium pyrophosphate diphosphate (pH 10) and amorphous Al and Fe (Al_{ox} and Fe_{ox}) were determined by extraction with 0.2 M ammonium oxalate pH 3.0 (van Reeuwijk, 2002). Al and Fe in pyrophosphate and oxalate extracts were measured by atomic absorption (UNICAM, 969 AA spectrometer) at 309 and 248 nm, respectively.

2.2.3. Phosphorus concentration and fractionation

Total P of PM and soil was determined in extracts by alkaline digestion with sodium hypobromite (NaBrO) (Dick and Tabatabai, 1977). Plant available P was extracted with sodium bicarbonate (0.5 M NaHCO₃ at pH 8.50) (Olsen and Sommers, 1982).

The nature of P in PM and soil was determined by sequential extraction using a scheme based on that proposed by Hedley et al. (1982). Briefly, to quantify readily available P, 1 g of sample was extracted with 25 mL of deionized H₂O. The samples were shaken during

16 h and then centrifuged at 5000g for 20 min. The soil solution was filtered and stored at 4 °C. The remaining sample was extracted, as described above, with sodium bicarbonate (0.5 M NaHCO₃ at pH 8.5), followed by sodium hydroxide (0.1 M NaOH) and hydrochloric acid (1 M HCl) to extract P of decreasing lability. In all extracts, inorganic P was measured directly by colorimetry (Murphy and Riley, 1962), total P was measured by NaBrO digestion (Dick and Tabatabai, 1977), and organic P was calculated as the difference between total and inorganic P.

2.2.4. Soil particle size distribution

Aggregates of soil samples were metered out directly into a beaker with distilled water and the particle-size distribution was measured by the laser diffraction technique. Additionally, aggregate size of PM and soil samples was analyzed by scanning electron microscope (Variable Pressure Scanning electron microscope VP-SEM), with transmission module STEM SU-3500 (Hitachi-Japan). The details regarding applied voltage, magnification used and the scale of the images were implanted on the photographs.

2.2.5 Statistical analysis

Normality and homogeneity of variance were determined before analyses. Statistical differences of means (95% significance level) were analyzed using two ways analyses of variance (two-way ANOVA) with the *aov* function followed by Tukey test using p-value of 0.05. We identified significant differences among treatments in each soil and differences among soils in same treatment. The magnitude of correlations among soil

available P, total C and the particle size distribution with Al and Fe forms parameters were tested by Pearson correlation coefficient. No statistical test was made with the percent of soil P proportion because these results were calculated with values already processed in ANOVAs. Principal component analysis (PCA) was performed using the package Factoextra; we consider the soil P fractions, total carbon concentration, particle size distribution and Al, Fe forms. Statistical testing was done using the statistical program R Foundation for Statistical Computing Version 1.1.456 (R Development Core Team 2009-2018 RStudio, Inc); effects were deemed significant at $P < 0.05$.

2.3. Results

2.3.1. Chemical characterization of PM added to pasture soils

Table 5 shows the PM properties. The pH value was 8.7. Macronutrient concentration was highest for P and N, (25.01 g kg⁻¹ and 37.06 g kg⁻¹, respectively). Composted PM showed also a high total C (267.79 g kg⁻¹) and macronutrient concentration (18.5 g Ca kg⁻¹ and 6.2 g Mg kg⁻¹).

Table 5. Chemical characteristics and phosphorus fractionation of composted poultry manure material. Data expressed in dry weight at 70°C. The values are means of 3 replicates ± standard error (SE).

<i>Chemical characterization</i>		
Total P (g kg⁻¹)		25.01 ±0.11
Total C (g kg⁻¹)		267.79 ±0.06
Total N (g kg⁻¹)		37.06 ±0.08
Calcium (g kg⁻¹)		18.5 ±1.20
Magnesium (g kg⁻¹)		6.2 ±0.93
C:N		7.22 ±0.34
pH(H₂O)		8.77 ±0.15
Moisture (%)		56.07 ±1.99
<i>Phosphorus fractionation</i>		
H₂O	Pi (g kg ⁻¹)	1.27 ±0.32
	Po (g kg ⁻¹)	3.26 ±0.98
NaHCO₃	Pi (g kg ⁻¹)	2.79 ±0.39
	Po (g kg ⁻¹)	3.65 ±1.29
NaOH	Pi (g kg ⁻¹)	5.56 ±0.24
	Po (g kg ⁻¹)	2.25 ±1.72
HCl	Pi (g kg ⁻¹)	1.12 ±0.55
	Po (g kg ⁻¹)	3.29 ±2.29
Residual	Pt (g kg ⁻¹)	1.20 ±0.42

Sequential fractionation showed that composted PM was constituted similarly of organic P and inorganic P forms, representing 51 and 44% of total P, containing a 4.9% as residual P form. The P in PM was distributed as follows 18.5% in H₂O, 26.4% in NaHCO₃, 32.0%

in NaOH, and 18.0% in the HCl extractable fraction (table 5). Additionally, PM had a high contribution of NaHCO₃ extractable organic P.

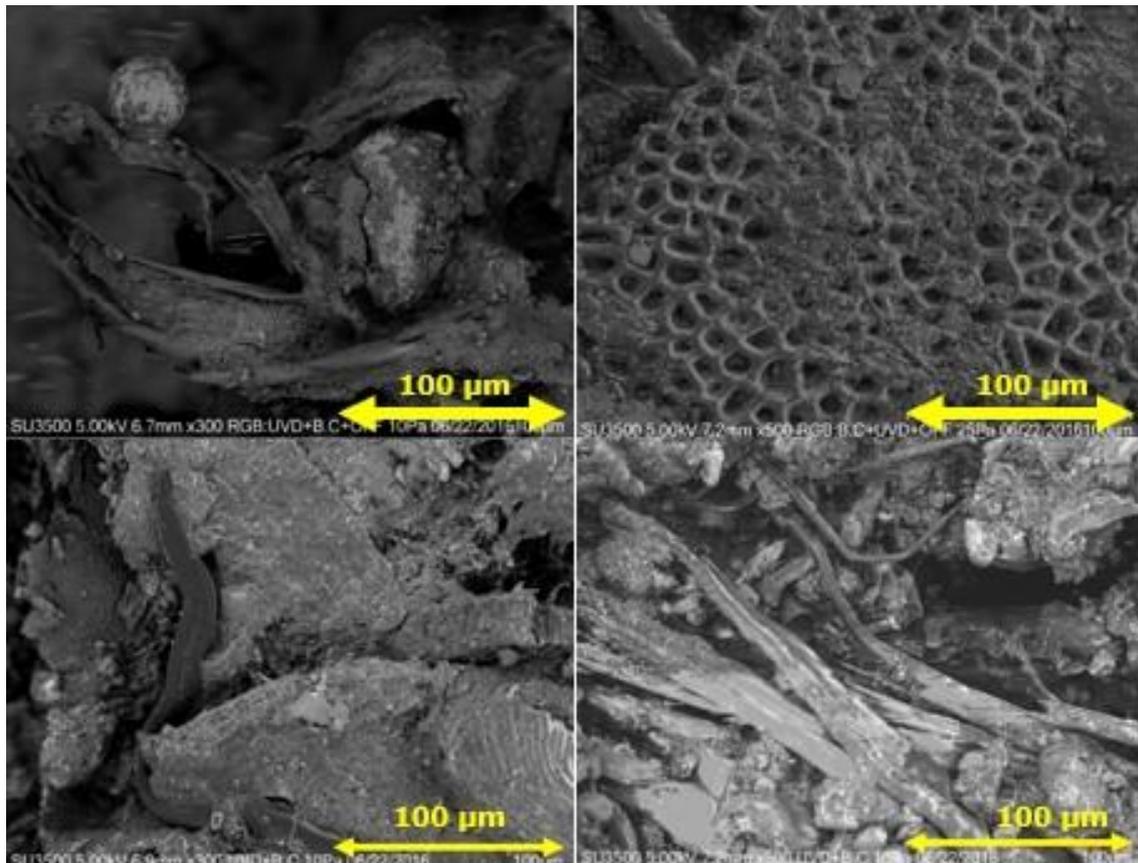


Figure 6. Images scanning electron microscope (SEM) in composted poultry manure amendment showing its morphological structure. Magnification of 300x (left) and 500x (right).

The morphology of PM was heterogenous, being composed of different compounds (animal waste and straw) (Fig. 6). The scanning electron microscopy images showed the porous structure of this amendment and straw residues.

2.3.2. Soil chemical characterization and aggregation of Andisols under pasture amended with PM

The chemical characterization of the soil varied between the control, and the PM application group are shown in Table 6. Soil pH, and total C increased significantly in Huifquenco ($p < 0.001$; $p = 0.003$) and Santa Teresa ($p = 0.002$; $p < 0.001$) for PM amended soil

as compared to the control. SOM ($p < 0.001$) and Total N ($p < 0.001$) content was significantly higher only for the Santa Teresa PM amended soil (9.8 g kg^{-1}), as compared to the control (5.3 g kg^{-1}).

Olsen P concentration was increased significantly ($p < 0.001$) for all soils amended with PM, as compared with the controls. The highest increase was obtained for Santa Teresa Farm being 12-fold higher than the control. The pyrophosphate and oxalate-extractable Al concentrations were higher than both extractable Fe concentrations (Table 6). Pyrophosphate- extractable Al ranged between 6.2 and 20.5 g kg^{-1} and pyrophosphate extractable Fe between 1.7 and 7.0 g kg^{-1} . Oxalate-extractable Al ranged between 35.2 and 48.1 g kg^{-1} and between oxalate-extractable Fe ranged between 9.6 and 17.1 g kg^{-1} . Santa Teresa showed a decrease of oxalate-extractable Al and Fe after the several years of PM application (Table 6). Copihual, Huifquenco and Santa Teresa increased significantly the pyrophosphate extractable Al by the use of PM. moreover, the concentrations of pyrophosphate extractable Fe_{pyro} were also increased for Carilafquen and Santa Teresa.

Table 6. Soil chemical properties determined in the Southern Chilean pastures amended several years with composted poultry manure.

Soils	Carilafquen		Copihual		Huifquenco		Santa Teresa		
	Control	PM	Control	PM	Control	PM	Control	PM	
pH	5.3±0.0 ^{Ab}	5.6±0.0 ^{Ab}	5.7±0.3 ^{Aa}	5.6±0.0 ^{Ab}	5.6±0.0 ^{Bab}	6.1±0.1 ^{Aa}	5.5±0.0 ^{Bab}	6.0±0.0 ^{Aa}	
SOM	(%)	17.4±0.1 ^{Ab}	17.0±0.1 ^{Ab}	14.6±3.0 ^{Abc}	16.1±1.5 ^{Ab}	20.4±0.1 ^{Aa}	19.5±1.2 ^{Aab}	11.0±0.0 ^{Bc}	23.1±0.7 ^{Aa}
Olsen P	mg kg ⁻¹	11.0±1.3 ^{Ba}	16.0±0.5 ^{Ab}	6.1±0.9 ^{Bb}	14.5±1.4 ^{Ab}	6.9±0.1 ^{Bb}	12.1±0.2 ^{Ab}	2.8±0.7 ^{Bc}	32.8±3.6 ^{Aa}
C:N		12.8	11.0	13.0	11.0	21.3	33.8	16.5	14.6
Total C		105.0±0.8 ^{Ab}	109.6±2.4 ^{Ac}	99.4±20.1 ^{Ab}	106.0±3.2 ^{Ac}	141.0±0.2 ^{Ba}	172.2±4.4 ^{Aa}	86.6±7.0 ^{Bb}	142.7±0.6 ^{Ab}
Total N		8.2±0.0 ^{Ba}	9.9±0.3 ^{Aa}	7.7±1.6 ^{Bab}	9.6±0.3 ^{Aa}	6.6±0.2 ^{Aab}	5.1±2.2 ^{Bb}	5.3±0.1 ^{Bb}	9.8±0.2 ^{Aa}
Total P		2.1±0.0 ^{Ba}	2.7±0.1 ^{Aa}	1.3±0.3 ^{Bb}	2.3±0.4 ^{Aab}	1.7±0.0 ^{Aab}	1.9±0.0 ^{Ab}	1.3±0.1 ^{Bb}	2.2±0.2 ^{Ab}
Al_{pyro}	g kg ⁻¹	12.3 ±0.4 ^{Aa}	11.6 ±0.3 ^{Ac}	8.4 ±0.3 ^{Bb}	10.5 ±0.3 ^{Ac}	12.1 ±0.8 ^{Ba}	20.5 ±1.4 ^{Aa}	6.2 ±0.2 ^{Bc}	15.0 ±0.8 ^{Ab}
Fe_{pyro}		3.2 ±0.0 ^{Ba}	4.4 ±0.1 ^{Ab}	2.7 ±0.1 ^{Ab}	2.6 ±0.1 ^{Ac}	4.4 ±0.0 ^{Aa}	4.6 ±0.7 ^{Ab}	1.7 ±0.1 ^{Bc}	7.0 ±0.5 ^{Aa}
Al_{ox}		48.1 ±2.0 ^{Aa}	44.1 ±1.5 ^{Aa}	47.6 ±2.4 ^{Ab}	37.4 ±3.3 ^{Ba}	41.0 ±2.2 ^{Abc}	43.2 ±3.8 ^{Aa}	48.1 ±1.8 ^{Aa}	35.2 ±0.9 ^{Bb}
Fe_{ox}		17.1 ±0.3 ^{Aa}	16.2 ±0.3 ^{Aa}	14.1 ±0.5 ^{Ab}	14.6 ±0.2 ^{Aa}	15.8 ±1.9 ^{Ab}	14.9 ±0.7 ^{Aa}	14.7 ±0.9 ^{Ab}	9.6 ±0.6 ^{Bb}

PM: composted poultry manure amended soils; Control: Unfertilized soils. ±SD. Upper case letters denotes significant differences between treatments for one soil. Lower case letters denote significance differences between soils for one treatment (Tukey's test at p≤0.05).

The control and the Carilafquen and Copihual PM amended soils had a similar distribution of aggregate sizes. In control approximately 25% of particles were larger than 36.3 – 42.8 μm , 50% of particles were larger than 62.6 – 99.3 μm and 75% of particles were larger than 100.2 – 162.4 μm (Table 7). The distribution of aggregate sizes following PM manure application for Huifquenco and Santa Teresa soils showed averages in 25% of particles with a size of 72.2 μm , 50% of all particles were larger than 140.15 μm and 75% of all particles exceeded 251.0 μm .

Table 7. Particle size distribution determined in the Southern Chilean pastures amended several years with composted poultry manure. PM: composted poultry manure amended soils; Control: Unfertilized soils. \pm SD. Upper case letters denote significant differences ($p \leq 0.05$). between treatments for one soil. Lower case letters denote significance differences ($p \leq 0.05$) between soils for one treatment.

Soils		Size particle distribution (μm)		
		25%	50%	75%
Carilafquen	Control	36.3 \pm 3.0 ^{Aa}	62.6 \pm 0.4 ^{Ab}	100.2 \pm 4.7 ^{Aa}
	PM	44.9 \pm 4.1 ^{Ab}	89.0 \pm 3.1 ^{Ab}	148.3 \pm 17.1 ^{Ab}
Copihual	Control	48.5 \pm 7.4 ^{Aa}	99.3 \pm 12.7 ^{Aa}	148.1 \pm 19.9 ^{Aa}
	PM	43.3 \pm 7.0 ^{Ab}	86.8 \pm 14.6 ^{Ab}	132.2 \pm 7.5 ^{Ab}
Huifquenco	Control	48.5 \pm 18.6 ^{Ba}	89.9 \pm 15.0 ^{Bab}	162.4 \pm 24.7 ^{Ba}
	PM	70.7 \pm 5.7 ^{Aa}	139.5 \pm 17.5 ^{Aa}	248.8 \pm 13.0 ^{Aa}
Santa Teresa	Control	42.8 \pm 8.7 ^{Ba}	80.4 \pm 7.5 ^{Bab}	136.6 \pm 10.9 ^{Ba}
	PM	73.8 \pm 5.5 ^{Aa}	140.8 \pm 10.8 ^{Aa}	253.3 \pm 37.1 ^{Aa}

Scanning electron microscope micrographs showed the surface structure in Control and soils after PM application at magnification of 300x (Fig. 7). The SEM micrograph data revealed that PM application increased soil aggregate size, supported by the laser diffraction measurements (Table 7; $p \leq 0.001$).

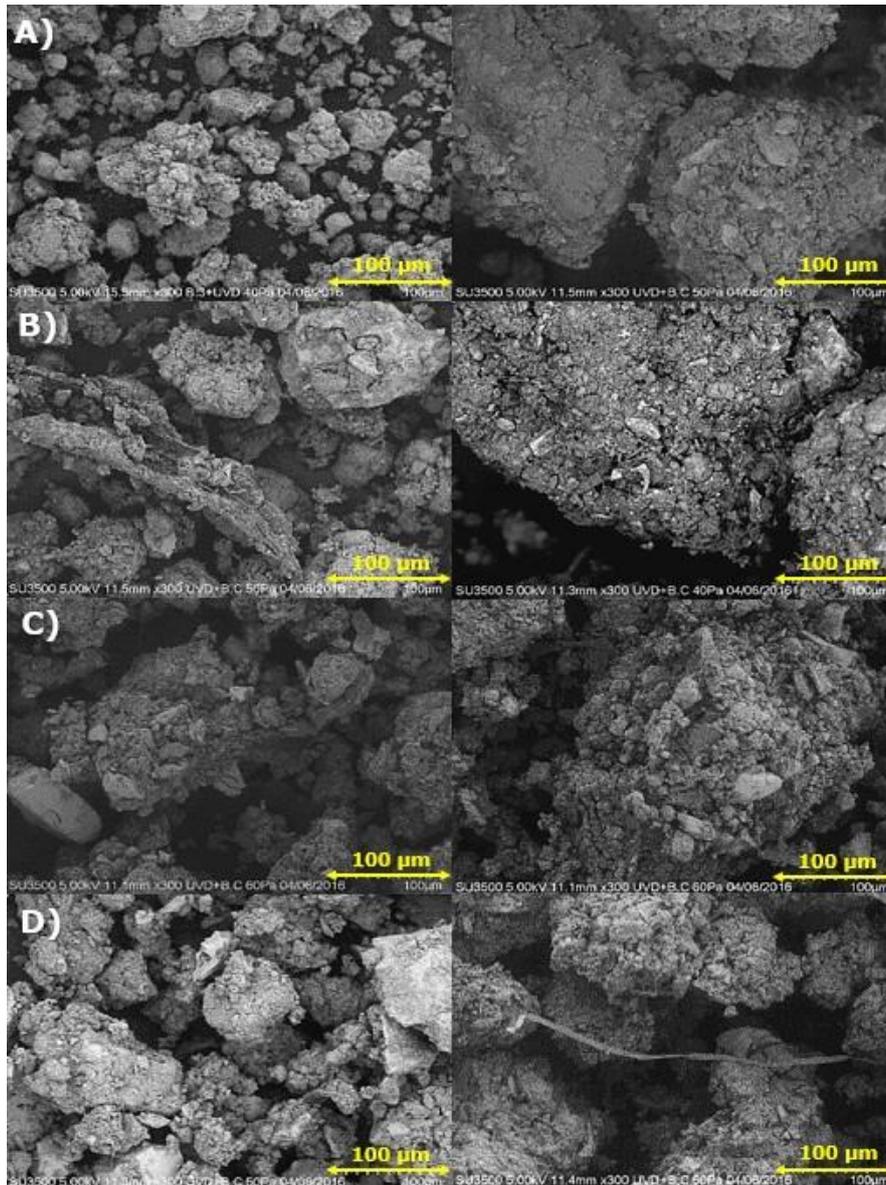


Figure 7. Images scanning electron microscope (SEM) in soils sampled in a) Carilafquen b) Copihual, c) Santa Teresa and d) Huifquenco amended several years with composted poultry manure (right) and their respective control (left).

2.3.3. Soil phosphorus distribution of Andisols under pasture amended with PM

Total P distribution (%) between the 4 fractions for the different treatments are shown in Fig. 8. Relative quantities of P in the readily, moderately and less available soil fractions were similar for all treatments. Less than 3 % of total P was found in the readily available fraction ($H_2O+NaHCO_3$ -P), 29-52% were comprised in the moderately available fraction (NaOH-P) and 6-29% were present in the less labile fraction (HCl-P). The readily

available fraction showed the lowest values among fractions, while the moderately available fraction showed the highest contribution to total P. The residual fraction comprised around 26-44% to total P (Fig. 8).

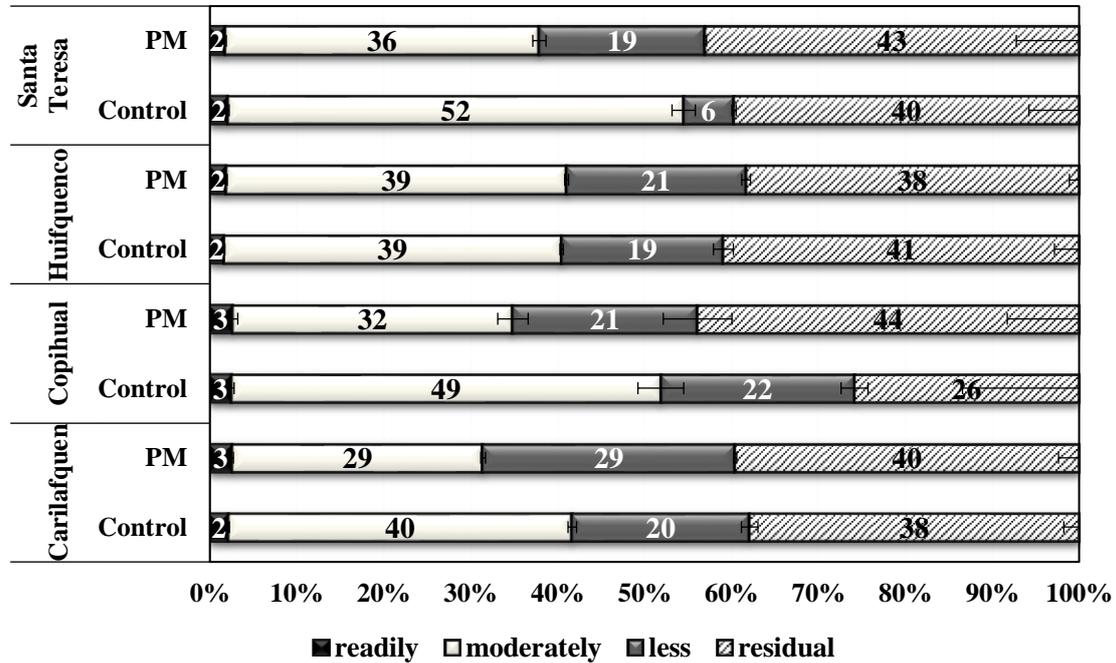


Figure 8. Phosphorus distribution in a) readily available ($H_2O+NaHCO_3$), b) moderately available ($NaOH$) and c) less-available (HCl) fractions in the Southern Chilean pastures amended several years with composted poultry manure. PM: composted poultry manure amended soils; Control: Unfertilized soils. The bars on the columns stand for standard deviation ($\pm SD$).

Inorganic P concentration increased significantly ($p < 0.001$) in the readily and moderately soil fractions amended with PM as compared with their respective control (Fig. 9). Among farms, Santa Teresa showed the strongest increases in inorganic P concentration in the readily available soil fraction amounting to 286% followed by Carilafquen with 126%, Huifquenco with 88% and finally Copihual with 56% (Fig. 9A). PM application increased significantly ($p < 0.001$) the inorganic P for the moderately available fraction by 63% in Santa Teresa and ~30% for Carilafquen and Copihual as compared with the control (Fig. 9B).

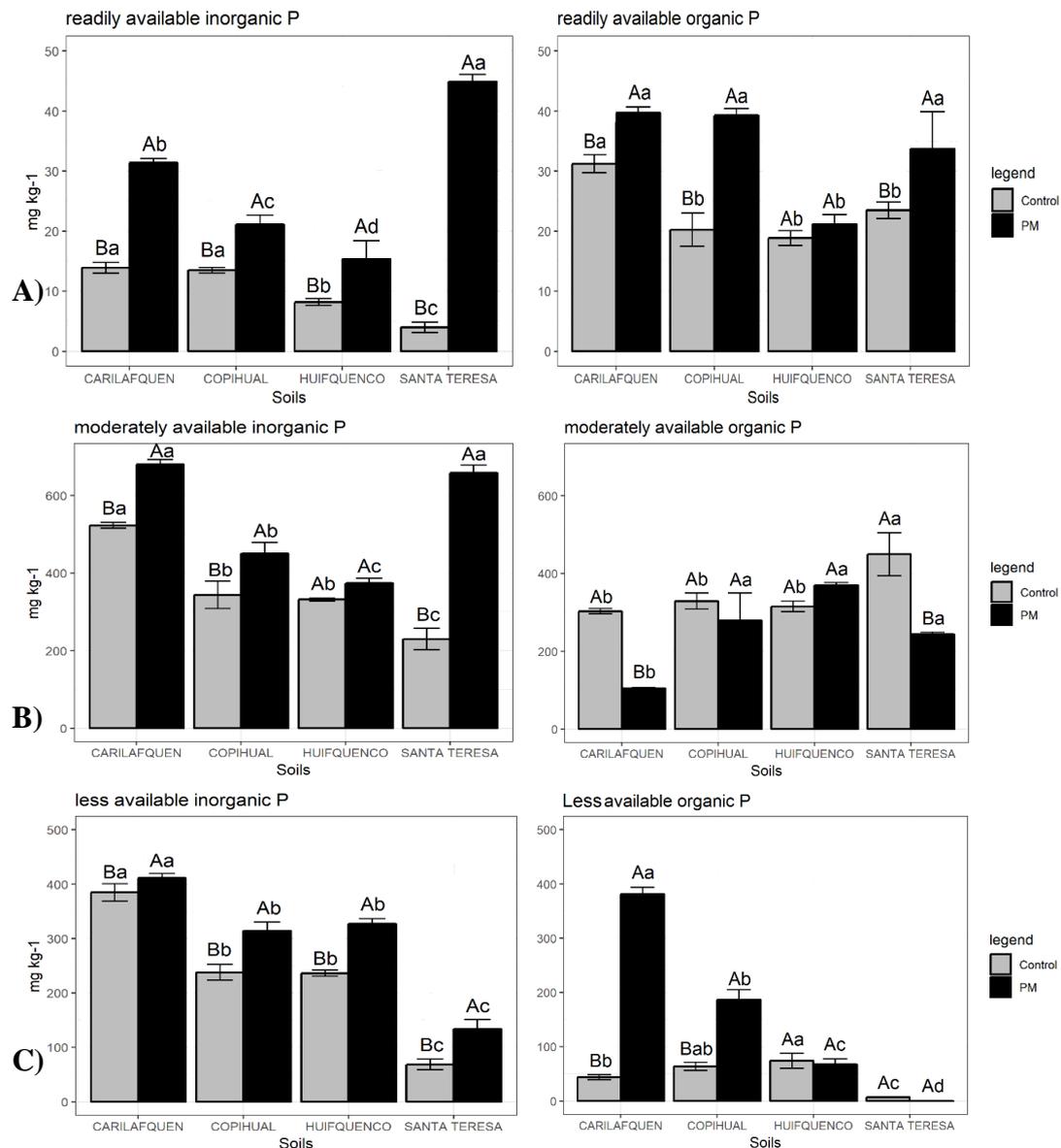


Figure 9. Soil inorganic and organic phosphorus concentration in a) readily available ($H_2O+NaHCO_3$), b) moderately available ($NaOH$) and c) less available (HCl) fraction in the Southern Chilean pastures amended several years with composted poultry manure. PM: composted poultry manure amended soils; Control: unfertilized soils. The bars on the columns stand for standard deviation ($\pm SD$). Upper case letters denote significant differences ($p \leq 0.05$) between treatments for one soil. Lower case letters denote significance differences ($p \leq 0.05$) between soils for one treatment.

Organic P concentration in the readily available soil fraction was significantly higher in PM amended soils ($p < 0.001$) by 94% and 27% for Copihual and Carilafquen, as compared with control. In PM amended soil of Santa Teresa, organic P concentration was 10% lower than control. Organic P concentration decreased significantly in moderately available soil fraction of PM amended soils in Carilafquen, and Santa Teresa, as compared

with controls (Fig. 9B). Inorganic P in the less available fractions increased significantly in all farms ($p < 0.01$). Soil organic P concentration was increased significantly only for Carilafquen and Copihual PM amended soils ($p < 0.001$), showing no significant differences for Huifquenco and Santa Teresa (Fig. 9C).

2.3.4. Relationship between soil parameters and soil particle size:

The eigenvalues of PCA performed with the soil P fractions, indicated that the first two components of PCA explained 73.1% of the total variance (Fig. 10). The first dimension explained 40.7% of the variability and the second dimension explained 32.4% of the variability.

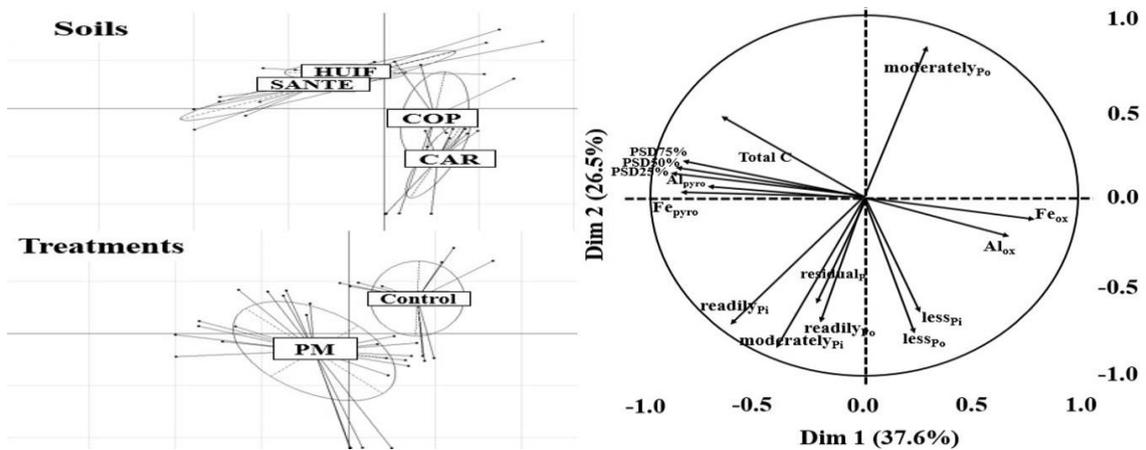


Figure 10. Principal component analysis for the soil variables studied in the Southern Chilean pastures amended for 5 (Carilafquen and Copihual) and 10 (Huifquenco and Santa Teresa) years with composted poultry manure.

The individual representation of treatments on the PCA factor map (Fig. 10) showed spatial separation of treatments in all studied soils. Both groups were separated along the first axis. PM was related to the total soil carbon, particle size distribution, pyrophosphate extractable Al and Fe, readily available P, moderately Pi and residual P. Additionally, soils could be separated into two groups according to the years (5 and 10 years) of PM application according along the 2nd axis. Carilafquen (CAR) and Copihual (COP) were thus associated with less-P, and oxalate extractable Al and Fe, while Santa Teresa

(SANTE) and Huifquenco (HUIF) were associated with soil particle size distribution, total carbon content and pyrophosphate extractable Al and Fe (Fig. 10).

Table 8. Relationship between nitrogen fertilization, total soil organic carbon (SOC), soil phosphorus forms, the size of soil particles distributed in 25, and 75%, and the concentration of Al- and Fe-extractable in oxalate (Al-ox; Fe-ox) and pyrophosphate (Al-pyro; Fe-pyro) in the Southern Chilean pastures amended several years with composted poultry manure.

	PSD (25%)	PSD (50%)	PSD (75%)	SOC	Al-ox	Fe-ox	Al-pyro	Fe-pyro
SOC	0.59*	0.56*	0.59*	-	-0.53*	-0.60*	0.67*	0.86*
Al-ox	-0.53*	-0.52*	-0.57*	-0.53*	-	0.71*	-0.26	-0.61*
Fe-ox	-0.68*	-0.65*	-0.66*	-0.60*	0.71*	-	-0.23	-0.71*
Al-pyro	0.67*	0.65*	0.69*	0.67*	-0.26	-0.23	-	0.63*
Fe-pyro	0.66*	0.61*	0.66*	0.86*	-0.61*	-0.71*	0.63*	-
Readily-Pi	0.34*	0.34*	0.36*	0.54*	-0.41*	-0.53*	0.67*	0.51*
Readily-Po	0.07	0.08	0.03	0.11	0.26	-0.08	0.06	0.04
Mod-Pi	0.09	0.07	0.11	0.44*	-0.18	-0.22	0.19	0.41*
Mod-Po	-0.01	0.00	-0.03	-0.34*	0.21	0.22	0.01	-0.27
Less-Pi	-0.32	-0.31	-0.31	-0.03	0.45*	0.61*	0.20	-0.26
Less-Po	-0.37*	-0.36*	-0.33	-0.23	0.24	0.35*	-0.16	-0.35*
Residual P	0.02	0.04	0.04	0.38*	0.19	-0.15	0.10	0.17

Pearson correlation in Table 8 showed that the readily available soil inorganic P and total carbon concentration were positively and significantly correlated with the pyrophosphate extractable Al and Fe, and the size of soil particles. Additionally, both soil parameters were negatively and significantly correlated with the oxalate extractable Al and Fe.

The relationship between the particle size distribution was significantly affected by Al and Fe forms, showing a positive correlation with pyrophosphate while for oxalate negative relationship was observed. Moreover, we found that the concentration of pyrophosphate extractable Fe was negatively correlated with the oxalate extractable Fe.

2.4. Discussion

As PM is an organic amendment, its application lead to an increase in SOC content (Antil and Singh, 2007; Ewulo and Ojeniyi, 2008). This is supported by our results showing increased in SOC of 22% in Huifquenco and 65% in Santa Teresa soils amended with poultry manure (PM), as compared with control (Table 6). This is very high as compared to Cambisol amended with organic waste materials (Paetsch et al., 2016), and considering the relatively low amount of PM applied (3 Mg ha^{-1}).

The high total C increase may result from a direct effect of PM-C input or may be resulting from better plant growth with higher belowground SOC inputs (indirect effect). N content remained similar in three out of four farms studied, which indicates that the C/N ratio of SOM increases after prolonged PM application as was shown in Huifquenco (Table 6). Increasing C/N ratio may be related to the preservation of fresh plant material in soil larger size particles (Bronick and Lal, 2005). This hypothesis is supported by microscopic investigation and particle size analyses, which showed that composted PM application caused a greater size of soil particles (Table 7; Fig. 7). When organic components increased, the percentage of the smaller particles is reduced, indicating the smaller particles were combining to form larger size particles (Zhao et al., 2017). Moreover, we found a strong correlation between SOC and the size of soil particles ($r=0.56 - 0.59$; $p<0.001$) (Table 8). Increasing the proportion of larger size particles after long-term PM application was observed by other studies (Ewulo and Ojeniyi, 2008). Larger soil particles protects SOC from degrading processes, thus increases the SOC concentration (Holeplass et al., 2004). Thus, PM application may be a suitable soil management practice to increase soil carbon sequestration. We suggest that the SOC sequestration potential through aggregation is especially high in Andisols with particular mineral properties due to their high content of short-range order minerals (Matus et al.,

2014). Enhanced soil size particles distribution induced by PM application may play a crucial role in soil physicochemical and biological processes and may affect the forms and availability of soil P (Li et al., 2016).

This is supported by our results on Andisols amended with PM showed changes in the distribution of the soil P forms. The inorganic P concentration of the readily and moderately soil fraction was greatly increased (Figure 9A-B). The studied pastures managed with PM fertilization showed an average of 50.9 mg kg⁻¹ of total P belonging to readily available fraction, which is similar to pastures on Andisols receiving mineral fertilization (Redel et al., 2016; Velásquez et al., 2016a). Our results thus indicate that the use of PM results in similar P availability as a conventional management. The increase of available P by application of PM agrees with the studies performed by Adeli et al. (2005) and Ewulo et al. (2008). Concomitant decrease of the organic P in moderately available fractions suggest its mineralization from composted PM (He et al., 2008) (Figure 9B). Our results indicated values of soil residual P constituting on average 41.25% of total P in amended PM soils (Fig. 8), which is similar to values recorded for Chilean Andisols under conventionally fertilized pasture (Redel et al., 2016; Velásquez et al., 2016). This may indicate that (1) few chemically stable P is added by PM compared to the standing soil stock and/or (2) chemically stable P present in PM does not accumulate in the residual soil P fraction (He et al., 2008; Pagliari and Laboski, 2014, 2012; Waldrip et al., 2015).

We found that the increase of the readily available inorganic P by using PM amendment over years was positively correlated with total soil carbon ($r=0.54$; $p<0.001$) and the size of soil particles (Table 8). Our study also showed a clear separation in soils amended with PM during 5 (Copihual and Carilafquen) and 10 (Huifquenco and Santa Teresa) years (Fig. 10). Stronger relationship between SOC and readily available inorganic P were

found in some studies demonstrating that SOC is an important factor which can directly affect the adsorption and desorption of P, through various mechanisms, (Yang et al., 2019). In Andisols, the amount of adsorbed P increased when soil organic matter (SOM) was removed, indicating that SOM occupied sites that could adsorb P, by inhibition of P adsorption through competitive adsorption (Hiradate and Uchida, 2004).

On the other hand, the adsorption of P involves a sequence of adsorption processes (such as to clay minerals and Fe/Al oxides) (Gérard, 2016), where increasing of SOC could compete with P for adsorption sites on the surface of minerals, by complexes Al- and Fe-OM thus are decreasing P adsorption by these minerals (Guppy et al., 2005; Mora et al., 2002; Redel et al., 2016). However, there is no consensus on this issue, and some studies have failed to verify the ability of increased levels of organic C to reduce P adsorption (Fink et al., 2016). In pasture growing in volcanic soils of southern Chile managed as conventional fertilizers, the P availability is mainly regulated by the formation of amorphous Al-P_o complexes, being Al-ox the main factor that governs P_o storage (Redel et al., 2016). In contrast, our results showed decreases in oxalate extractable Al and Fe after prolonged PM amendment of Andisols while pyrophosphate forms increase (Table 6; Table 8). This could indicate a shift of C stabilization processes from adsorption onto Al and Fe oxides to organic matter-metal complex formation. Moreover, we found that available P behavior was somewhat related to Al and Fe, as Al-pyrophosphate ($r=0.67$; $p<0.001$) and Fe-pyrophosphate ($r=0.51$; $p=0.001$) was significantly correlated with the inorganic P from readily available fractions (H₂O and NaHCO₃) as shown in Table 8. The bigger size of soil particles found in Andisols receiving several PM applications was also positively correlated with pyrophosphate forms of Al and Fe while negatively related with the oxalate ones. Increased aggregation given by larger soil particles observed in Andisols could result in P protection within soil aggregates (Li et al., 2016). However, Wang et al.

(2001) indicated that freshly applied P may penetrate only a thin layer around soil aggregates surface and that larger soil particles with relatively lower surface as compared to small soil particles could reduce P fixation and result in an increase P availability.

The improvement of the size of soil particles, SOC and P availability in Andisols amended with PM may lead to higher yields, as was shown by other studies on the effect of long-term composted PM application on forage yield in pastures (Evers, 2002; Pederson et al., 2002). The use of PM amendment on pastures changed distribution of soil constituents' and the carbon concentration which demonstrated be the main mechanisms regulating soil P availability. Our study showed that the improvement of carbon concentration of Andisols after the use of PM through Al- and Fe- complex formation and the enhancement of soil particle size, led to increases of available P forms.

2.5. Conclusions

Despite differences in soil type between sampling sites, results showed that long-term inputs of poultry manure increased plant available P in soil. Moreover, poultry manure application showed a significant increase in soil total carbon content and soil aggregation. The larger particle size induced by poultry manure application led to an increase in soil organic C and available P content. This may be explained by decreased adsorption onto Fe and Al oxides and increased organic matter metal complex formation. Further studies are needed to assess the impact of poultry manure input on soil C and P dynamics and the quantification on yield increase under controlled field conditions. Moreover, the consequences of the changed soil parameters on microbial functioning remains to be investigated.

CHAPTER III

*Synergistic and Antagonistic Effects of Poultry Manure and Phosphate
Rock on Soil P Availability, Ryegrass Production, and P Uptake*

Agronomy 9, 191 (2019)

Chapter III: Synergistic and Antagonistic Effects of Poultry Manure and Phosphate Rock on Soil P Availability, Ryegrass Production, and P Uptake

Abstract

To maintain grassland productivity and limit resource depletion, scarce mineral P (phosphorus) fertilizers must be replaced by alternative P sources. The effect of these amendments on plant growth may depend on physicochemical soil parameters, in particular pH. The objective of this study was to investigate the effect of soil pH on biomass production, P use efficiency, and soil P forms after P amendment application (100 mg kg⁻¹ P) using poultry manure compost (PM), rock phosphate (RP), and their combination (PMRP). We performed a growth chamber experiment with ryegrass plants (*Lolium perenne*) grown on two soil types with contrasting pH under controlled conditions for 7 weeks. Chemical P fractions, biomass production, and P concentrations were measured to calculate plant uptake and P use efficiency. We found a strong synergistic effect on the available soil P, while antagonistic effects were observed for ryegrass production and P uptake. We conclude that although the combination of PM and RP has positive effects in terms of soil P availability, the combined effects of the mixture must be taken into account and further evaluated for different soil types and grassland plants to maximize synergistic effects and to minimize antagonistic ones.

Keywords: Poultry manure; phosphate rock; ryegrass; plant biomass; phosphorus uptake; phosphorus availability.

3.1. Introduction

Fertilization of grasslands with mineral phosphorus (P) fertilizer is a common practice in many regions of the world to maintain productivity, especially on soils with high P retention (Redel et al., 2016; Rumpel et al., 2015; Velásquez et al., 2016a). In order to reduce the use of scarce phosphate rock (RP), alternative P fertilizers need to be found (Cordell et al., 2009; Reijnders, 2014). In this context, poultry manure, an abundant organic waste material from the growing broiler industry (FAO, 2018a), is known for its high P content (Pagliari and Laboski, 2012). Its transformation through composting into organic amendments, and their subsequent application in grassland systems may be a promising strategy (Calabi-Floody et al., 2018; Redding et al., 2016) to reduce the use of mineral fertilizers. Several studies showed that plant nutrient uptake and the biomass of several plants could be significantly increased using poultry manure compost (PM) (Evers, 2002; Pederson et al., 2002). The application of PM led to changes in soil P forms and phosphatase activity (Waldrip et al., 2011). However, despite its positive effects on plant nutrient availability and biomass production, the application of PM may lead to the simultaneous introduction of contaminants (Foust et al., 2018) and could also lead to a loss of P to waterways following long term application.

Therefore, nowadays, the use of PM in combination with RP has been considered as good practice to limit the use of both materials without compromising plant requirements (Song et al., 2017). However, the fertilizer value of both substrates may depend on the soil reaction. For example, RP efficiency may be limited in soils with high pH due to its low dissolution rate (Zapata and Roy, 2004), while in soils with acid pH, RP may lead to further acidification (Rajan et al., 1991). The efficiency of PMRP mixture for increasing wheat and chili yield and P uptake has already been demonstrated for acid and alkaline soils (Abbasi et al., 2015, 2013). However, no study has been carried out to investigate

quantitatively the synergetic or antagonistic effects of the combined application of both materials.

In this study, we carried out a growth chamber experiment to investigate the effect of the combined use of PM and RP as compared to their application as a single amendment in soils with similar properties, but contrasting in pH. The objective of the study was to determine the effect of PM application, alone or combined with RP, on ryegrass biomass production, P use efficiency, and soil P availability in an acid and an alkaline soil. We hypothesized that the soils' and plants' response to the combined use of PM and RP in terms of biomass production and P use efficiency may depend on the soil reaction and that the mixture will have additional effects as compared to the use of PM and RP as a single amendment. Moreover, we hypothesized that the combined use of PM and RP will ameliorate P availability and biomass production as compared to their use as a single amendment.

3.2. Materials and Methods

3.2.1. Materials

We used two silty soils (50–60% silt): A Neoluvisol with a pH of 6.1 (moderately acid soil) and a carbonated Luvisol, with a pH of 8.5 (alkaline soil) (Table 9) according to the French Référentiel Pédologique 2008 (Baize and Girard, 2008). Both soils showed similar texture, organic matter content, and soil forming processes (lixiviation). They were differentiated by pH and also their initial Olsen P concentration. They are part of the French observatory SOERE PRO (<https://www6.inra.fr/valor-pro/SOERE-PRO-les-sites>). The Neoluvisol is located in Eastern France at Colmar, and the Luvisol is located in northwestern France in Brittany at Le Rheu. We sampled the first 0 to 30 cm. of the control plots without fertilization at the two sites. After sampling, the soils were transported to the laboratory, air dried, and sieved at 2 mm. The plant species used was ryegrass (*Lolium perenne*), a typical pasture plant used for grazing systems.

Table 9. Soil physical and chemical characterization.

Soil Type	pH	C _{org} g kg ⁻¹	C/N	P olsen mg kg ⁻¹	K ₂ O Cmol + kg ⁻¹	Clay %	Silt	Sand
Moderately acid	6.1	11.9	10	60	0.32	14.6	68.3	16.1
Alkaline	8.5	12.1 *	10	11	0.26	20.7	59.8	6.8

* CaCO₃ = 128 g kg⁻¹.

PM compost was provided by KOMECO B.V in pellet form with a dry matter content of 880 g kg⁻¹ with an organic matter content of 600 g kg⁻¹. Phosphorus content was 13.2 g kg⁻¹ d.w. The material contained 42% organic P and 58% of inorganic P (Table 10). RP was bought from ‘Les comptoirs de Jardin’ and was derived from bones with 30% P and 50% calcium. It was provided in powder form with 90% of the particles smaller than 0.16 mm.

Table 10. Inorganic (Pi) and organic P (Po) in fractions sequentially extracted from poultry manure compost PM.

H ₂ O		NaHCO ₃		NaOH		HCl		Residual
Pi	Po	Pi	Po	Pi	Po	Pi	Po	Pt
mg kg ⁻¹								
187 ± 8	122 ± 43	202 ± 3	149 ± 30	47 ± 4	72 ± 13	181 ± 9	97 ± 8	145 ± 7

3.2.2. Growth Chamber Experiment

The experiment was carried out in pots in the RUBIC V biogeochemical reactor—Servathin, Carrières-sous-Poissy France—for 7 weeks. To account for the contrasting bulk densities, we amended 490 g of the moderately acid soil and 550 g of the alkaline soil per treatment, with four replicates with poultry manure compost (PM), phosphate rock (RP), or their mixture consisting of 70% PM and 30% RP (PMRP) (Appendix 2). In total, 100 mg P per kg⁻¹ soil d.w. were added to each treatment. The amendments were supplied in the form of a dry powder. We added 13.30 g of PM and 0.80 g RP to the pots with a single amendment and 9.31 g of PM and 0.23 g of RP to the pots with amendment mixtures. To account for N and K supplied by PM (262 mg N and 221 mg K per kg d.w. soil), we added the corresponding amounts of K and N in the form of KCl and NH₄NO₃ to all other treatments, including the control. The PM application was equivalent to 9.8 Mg ha⁻¹ when applied in the mixture with RP and 14 Mg ha⁻¹ when applied as a single amendment. The RP application was equivalent to 0.25 Mg ha⁻¹ when applied in a mixture with PM and 0.8 Mg ha⁻¹ when applied as a single amendment.

After addition of the amendments, the soils were thoroughly mixed in plastic bags, added to each pot, and brought to field capacity with tap water. After one day, a total of 97 ryegrass seeds were added to each pot. Seeds were sown on the surface and covered superficially with soil material. Plants were grown at 24 °C (day temperature) and at 17

°C (night temperature) with a day length (light intensity of $650 \mu\text{mol m}^{-2} \text{s}^{-1}$) of 8 h for the first 13 days, and 11 h until the end of the experiment. Soil moisture was maintained at 40% of the available field capacity by watering regularly. Air humidity was 75% to 65, % respectively, for day and night conditions.

After 7 weeks, shoots and roots were separated from soil and their fresh biomass was weighed. Thereafter, biomass was dried at 65 °C for 48 hours. Oven-dried plant material was ground to pass through 20-mesh (0.84 mm) sieves. Microbial biomass was determined using 5 g of fresh samples. The remaining soil masses were oven-dried at 40°C and sieved at 2 mm. An aliquot was ground for further analyses. All data is expressed on a dry weight basis.

3.2.3. Soil Analysis

Phosphorus forms based on P solubility were measured using a modified Hedley fractionation scheme (Hedley et al., 1982) with successive chemical P extraction from soluble to residual fractions. Briefly, 1 g of dry and sieved soil was extracted sequentially by shaking for 16 h with 30 mL of (1) distilled water, (2) 0.5 M NaHCO_3 at pH 8.5, (3) 0.1 M NaOH, and (4) 1 M HCl. Each suspension was centrifuged at 10,000 rpm for 10 min and the supernatants were recovered and analyzed for total P (Pt) and inorganic P (Pi). Organic P (Po) was determined by difference. The residues were dried at 60 °C and used for subsequent extractions. Residual P remaining after the last step was extracted with 1 M sulphuric acid (H_2SO_4) during 24 h, after calcination of the residue for 1 h at 550 °C. Inorganic P was determined in the solutions by the ammonium molybdate-ascorbic acid method (Murphy and Riley, 1962). Total P was determined by taking aliquots from supernatants for digestion using potassium persulfate ($\text{K}_2\text{S}_2\text{O}_8$) and 2.5 M sulphuric acid (H_2SO_4). Fraction one and two represent readily available P. Moderately available P is found in fraction 3, less available P in fraction 4, whereas residual P

represents unavailable P. Pt, Po and Pi of bulk soil were calculated as sum of Hedley fractions.

Total organic C and N concentrations of soil samples were determined using an elemental analyzer (Variopyrocube, Elementar, Langensebold, Hesse, Germany). The acid soil was carbonate free and total soil C therefore corresponded to organic C. For the alkaline soil (carbonated soil), HCl-fumigation was performed before elemental analyses to remove inorganic C (Harris et al., 2001). Microbial biomass P was determined by the chloroform fumigation-extraction method (Brookes et al., 1982). Briefly, 5 g of fresh soil were extracted with 0.5 M NaHCO₃ before and after fumigation with CHCl₃. Total P was determined in the solutions by the ammonium molybdate-ascorbic acid method (Murphy and Riley, 1962). Microbial biomass P was calculated as the difference between fumigated and non-fumigated soil and multiplied by a factor of 0.40 (Brookes et al., 1982).

3.2.4. Biomass Analysis

Total N and C concentrations were measured using an elemental analyzer (Variopyrocube, Elementar, Langensebold, Hesse, Germany). Total shoot P contents were analyzed by calcination followed by acid recovery using inductive coupled plasma mass spectrometry (iCAPTM Q ICP-MS, Thermo ScientificTM, Waltham, MA, USA). The P uptake (mg) was calculated as a product of the shoots' or roots' nutrient concentrations (mg g⁻¹) and shoot or root biomass (g). P use efficiency (PUE) was also calculated according to Baligar et al. (2001) as follows:

$$PUE = \frac{P \text{ uptake in treatment (mg)} - P \text{ uptake in control (mg)}}{\text{total P applied}} \times 100 \quad (1)$$

3.2.5. Synergistic and Antagonistic Effect of Mixture

Based on the quantities of PM and RP applied as single amendments or in mixture (see 2.2), we calculated the additional effect of the PMRP mixture on the soil available P, biomass production, and P uptake resulting from the combined application of PM and RP as compared to their use as a single amendment. This calculation is justified by the observation made by many others that PM would lead to enhanced mineralization of RP due to the release of organic acids. However, this additional effect was never quantified. We used Equation (2) to calculate the additional effect, i.e., change of PMRP as compared to the sum of single amendments:

$$\% \text{ change} = \frac{\textit{observed result} - \textit{expected result}}{\textit{expected result}} \times 100 \quad (2)$$

The observed result was corrected for the control in order to obtain the effect of the amendments. The expected result for PMRP was obtained as a sum of the PM and RM after multiplication of the observed results in the two treatments with a coefficient accounting for the different proportions of the two amendments used in the mixture, i.e., 70% and 30%.

3.2.6. Statistical Analysis

The experiment was arranged in a completely randomized design with four replicates. Data were checked for normality (Shapiro-Wilk test) and homogeneity of variance (Levene test) were determined before analyses. Statistical differences of means (95% significance level) were analyzed using two-way analyses of variance (two-way ANOVA). Post hoc tests with the function Tukey-test were made for the explanatory variables independently when the ANOVA detected significant differences. The relationships between soil available P and plant parameters were tested by Pearson

correlation analyses. Statistical testing was done using the statistical program R Foundation for Statistical Computing Version 1.1.456 (R Development Core Team 2009–2018); effects were deemed significant at $p \leq 0.05$. Principal component analysis (PCA) was performed using the package, Factoextra; we consider one for the soil P fractions and a second one for the plant P uptake and biomass.

3.3. Results

3.3.1. Total Soil C, N, and P Concentrations

After the end of the experiment, PM treatment led to significantly increased total soil C concentrations in the moderately acid and alkaline soil by 57 and 29%, respectively (Table 11). The RP treatment showed no differences as compared to control, while its combination with PM increased the total C concentrations in both soils, with significant effects only in the moderately acid soil. Total N concentration was increased by 29% using PM in the alkaline soil. In the moderately acid soil, PM treatment increased total soil N concentration by 57%, whereas a lower increase was noted in the PMRP treatment (30%). Total P increased in both soils with PM amendment.

The C:N decreased in both soils amended with RP as compared with PM, PMRP, and the control (Table 11). In the moderately acid soil, PMRP treatments showed the highest N:Po and C:Po, while for the alkaline soil, the use of the mixture led to an increase of N:Pi, while C:Po and N:Po decreased. C:Pi was increased by both PM and PMRP treatments in the moderately acid soil only. N:Pi increased in all treatments in the moderately acid soil, while in the alkaline soil, only PMRP and RP increased this ratio significantly as compared with the control (Table 11).

Table 11. Soil parameters in control and soil amended with poultry manure compost (PM), phosphate rock (RP), and their combination (PMRP) after 7 weeks of ryegrass growth.

Soil Type		C	N	C/N	Pt	C:Po	C:Pi	N:Pi	N:Po
		g kg ⁻¹			mg kg ⁻¹				
Moderately acid	Control	10.5 ^{Ca}	1.2 ^{Da}	8.9 ^{Aa}	100.2 ^{Ca}	344.9 ^{Ba}	192.2 ^{Bb}	21.6 ^{Cb}	38.6 ^{Ba}
	RP	11.0 ^{Ca}	1.4 ^{Ca}	7.7 ^{Ba}	97.3 ^{Ca}	314.4 ^{Cb}	194.4 ^{Bb}	25.3 ^{Bb}	40.9 ^{Bb}
	PM	16.5 ^{Aa}	2.0 ^{Aa}	8.5 ^{Aa}	132.1 ^{Aa}	356.4 ^{Ba}	255.9 ^{Aa}	30.2 ^{Aa}	42.1 ^{Ba}
	PMRP	13.7 ^{Ba}	1.7 ^{Ba}	8.3 ^{Aa}	118.0 ^{Ba}	414.9 ^{Aa}	210.5 ^{Aa}	25.3 ^{Bb}	49.8 ^{Aa}
Alkaline	Control	11.5 ^{Ba}	1.3 ^{Ba}	9.2 ^{Aa}	103.2 ^{Ba}	365.3 ^{Ba}	241.9 ^{Aa}	26.3 ^{Ba}	39.8 ^{Ba}
	RP	12.0 ^{Ba}	1.7 ^{Ab}	7.2 ^{Aa}	103.5 ^{Ba}	399.7 ^{Aa}	239.2 ^{Aa}	33.3 ^{Aa}	55.6 ^{Aa}
	PM	14.8 ^{Aa}	1.7 ^{Ab}	8.8 ^{Ba}	127.2 ^{Aa}	383.2 ^{Ba}	244.1 ^{Aa}	28.1 ^{Ba}	44.3 ^{Ba}
	PMRP	13.4 ^{ABa}	1.6 ^{Aa}	8.7 ^{Aa}	120.8 ^{Aa}	275.0 ^{Cb}	290.1 ^{Aa}	33.5 ^{Aa}	31.8 ^{Bb}

* Upper case letters denote significant differences ($p \leq 0.05$) between treatments for one soil.

Lower case letters denote significant differences ($p \leq 0.05$) between soils for one treatment.

3.3.2. Soil Phosphorus Forms

Concentrations of readily available fractions (extractable with water and NaHCO_3) ranged between 5.20 to 14.94 mg P kg⁻¹ soil for inorganic and 6.07 to 27.58 mg P kg⁻¹ soil for organic P (Fig. 11). The moderately available fraction (NaOH extractable) ranged between 6.07 and 27.58 mg P kg⁻¹ soil. Soils treated with PM and PMRP increased significantly inorganic and organic P concentrations in the readily available fraction as compared with the control and RP treatment. In the alkaline soil, RP amendment increased readily available inorganic and organic P on average 1.2-fold as compared with the control.

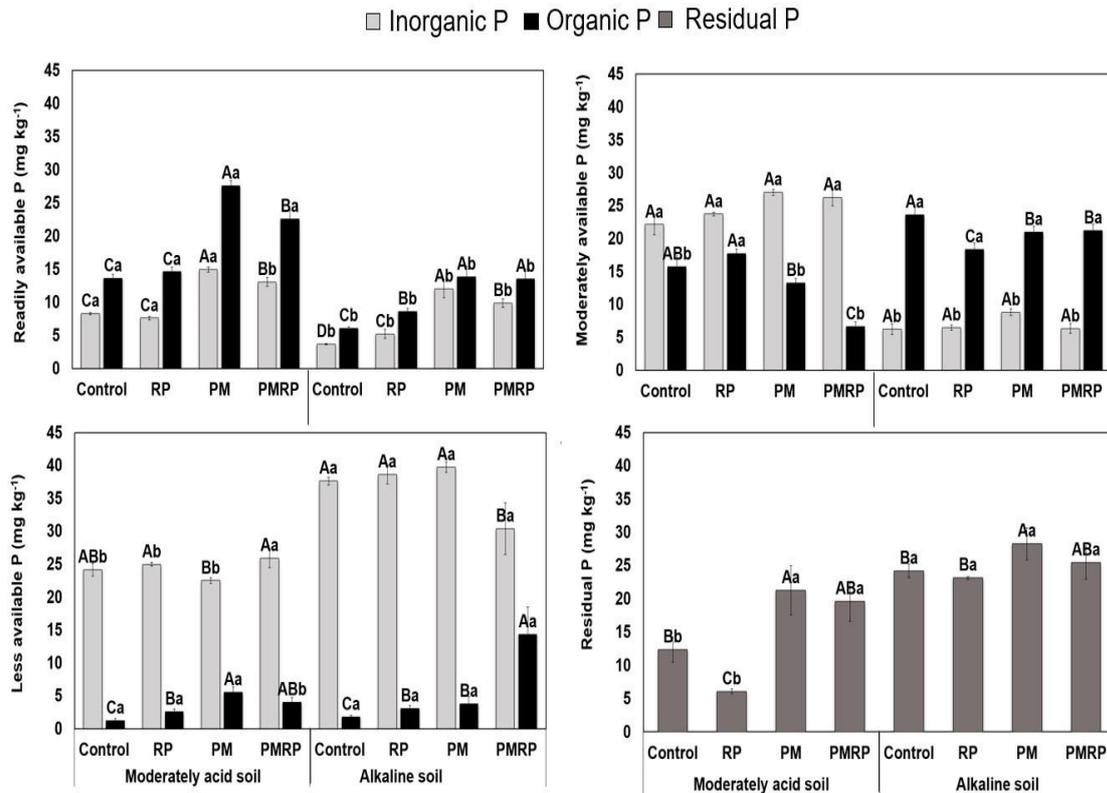


Figure 11. Inorganic and organic P concentration of fractions extracted from control and soil amended with poultry manure compost (PM), phosphate rock (RP), and their combination (PMRP) after 7 weeks of ryegrass growth. Upper case letters denote significant differences ($p \leq 0.05$) between treatments for one soil. Lower case letters denote significant differences ($p \leq 0.05$) between soils for one treatment.

No differences were found for the moderately acid soil (Fig. 11). In the other fractions, the P concentrations ranged between 22.52 to 39.73 and 1.20 to 14.30 mg P kg⁻¹ soil as the inorganic and organic form, respectively. All amendments increased organic P in the less available P fraction in both soils, whereas residual P was increased with regards to the control only by PM amendments.

3.3.3. Microbial Biomass P

Microbial biomass P ranged between 7.55 to 35.10 mg P kg⁻¹ soil, and it was significantly increased with all P amendments as compared to the control (Fig. 12). With PM amendment, microbial biomass P concentrations increased greatly as compared to RP

amendment by 81% to 93% in moderately acid and alkaline soil, respectively (Fig. 12). In the moderately acid soil, PM and its combination with RP induced the greatest increases of microbial biomass P (81–100%) as compared with RP while for the alkaline soil, only PM as a single amendment increased MBP with regards to RP.

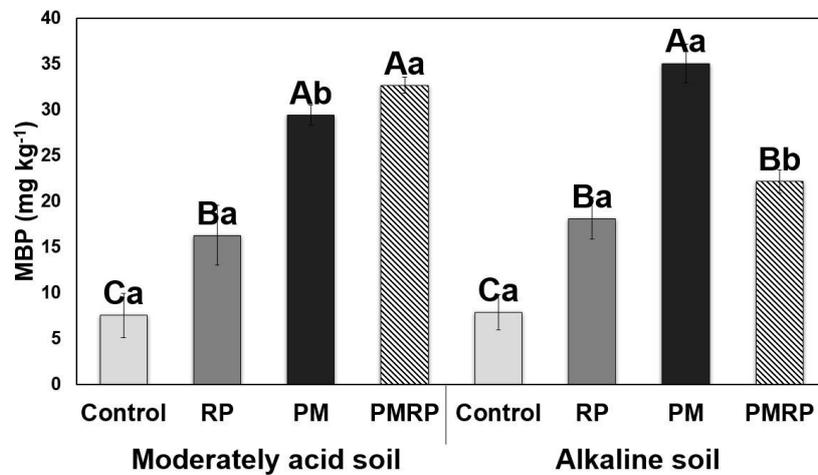


Figure 12. Microbial biomass phosphorus (MBP) in in control and soil amended with poultry manure compost (PM), phosphate rock (RP), and their combination (PMRP) after 7 weeks of ryegrass growth. Upper case letters denote significant differences ($p \leq 0.05$) between treatments for one soil. Lower case letters denote significant differences ($p \leq 0.05$) between soils for one treatment.

3.3.4. Shoot and Root Biomass Production

Plants grown in the moderately acid soil showed greater root biomass in all treatments compared to those grown in the alkaline soil, whereas shoot biomass was similar for both soils, except for the RP treatment (Fig. 13).

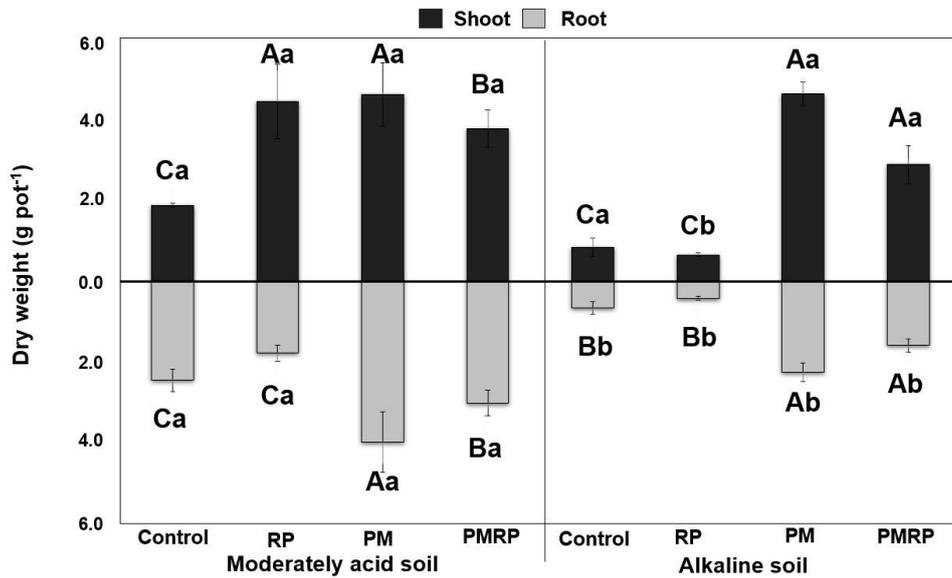


Figure 13. Dry weight of shoots and roots in control and soil amended with poultry manure compost (PM), phosphate rock (RP), and their combination (PMRP) after 7 weeks of ryegrass growth. Upper case letters denote significant differences ($p \leq 0.05$) between treatments for one soil. Lower case letters denote significant differences ($p \leq 0.05$) between soils for one treatment.

The dry weight of plants cultivated in moderately acid soil treated with different P amendments and their combination was significantly higher ($p \leq 0.05$) compared to the control (Fig. 13). The PM and RP increased shoot biomass similarly (~2.5 fold) as compared with the control. Root biomass increased by 1.2 to 3.3-fold in both soils treated with either PM alone or combined with RP.

In the alkaline soil, no difference in dry matter production was observed for the RP treatment with respect to the control. In contrast, PM alone and its combination with RP significantly increased shoot biomass (5.6 and 3.5-fold) and root biomass (3.3 and 2.3-fold) as compared to the control (Fig. 13).

3.3.5. Shoot and Root P Concentrations and Uptake

Shoot and root P concentrations and root P uptake increased significantly with all amendments as compared to the control (Table 12). Shoot P uptake showed no change compared to the control for both soils. The highest increase was noted for PM and PMRP treatments, which showed on average 37% to 48% higher root and shoot P concentrations than plants of the control treatment (Table 12). This is in line with a higher P uptake (Table 12). Shoot P concentration and uptake was higher in all treatments except RP in the moderately acid soil as compared to the alkaline one. Despite differences in uptake, root P concentrations were similar for both soils.

Table 12. P concentration and uptake of roots and shoots and P use efficiency (PUE) after 7 weeks of ryegrass growth in control soil and soil amended with poultry manure compost (PM), phosphate rock (RP), and their combination (PMRP).

Soil Type	Treatment	P conc.		P uptake		PUE % of input
		Shoot g kg ⁻¹	Root g kg ⁻¹	Shoot mg	Root mg	
Moderately acid	Control	7.2 ^{Da}	1.4 ^{Da}	3.6 ^{Ba}	3.6 ^{Ca}	-
	RP	12.5 ^{Ca}	2.2 ^{Ca}	3.5 ^{Ba}	4.6 ^{Ca}	6 ^{Ca}
	PM	24.2 ^{Aa}	3.8 ^{Aa}	4.9 ^{Aa}	14.8 ^{Aa}	28 ^{Aa}
	PMRP	16.6 ^{Ba}	2.8 ^{Ba}	5.0 ^{Aa}	8.7 ^{Ba}	14 ^{Ba}
Alkaline	Control	1.4 ^{Db}	1.7 ^{Ba}	2.6 ^{Bb}	1.1 ^{Cb}	-
	RP	4.9 ^{Cb}	2.1 ^{Ba}	3.0 ^{Ba}	2.8 ^{Ba}	5 ^{Ca}
	PM	17.7 ^{Ab}	3.3 ^{Aa}	3.8 ^{Ab}	9.7 ^{Ab}	25 ^{Aa}
	PMRP	11.3 ^{Bb}	3.0 ^{Aa}	3.8 ^{Ab}	6.9 ^{Aa}	16 ^{Ba}

Upper case letters denote significant differences ($p \leq 0.05$) between treatments for one soil. Lower case letters denote significant differences ($p \leq 0.05$) between soils for one treatment.

Plant P use efficiency of the added P sources and their combinations ranged from 5% to 6% for RP to a maximum of ~28% for PM alone (Table 12). Plant use efficiency of the mixture, PMRP, was in between these values. Few differences were noted between soils.

3.3.6. Relationship between Soil and Plant Parameters

In the alkaline soil, the readily available inorganic and organic P fractions, moderately available Po, residual P, and microbial biomass P were strongly correlated with shoot and root biomass and nutrient uptake (Table 13). In the acid soil, inorganic and organic P in the readily available fraction and moderately available-Pi showed a positive correlation with root biomass and root P uptake (Table 13). Residual P was correlated with root biomass and less available organic P was somewhat correlated with shoot biomass and root P uptake. In this soil, the strongest correlations were noted for P uptake in the root and shoot with readily available P, moderately available Pi, less available Po, microbial P, total C, and total N.

Table 13. Relationship between soil and plant parameters after 7 weeks of ryegrass growth in control and soil amended with poultry manure compost (PM), phosphate rock (RP), and their combination (PMRP). *denotes significant correlation coefficients ($p \leq 0.05$), $n = 16$.

Soil	Parameters	Biomass		P Uptake	
		Shoot	Root	Shoot	Root
Moderately acid	readily-Pi	0.41	0.80*	0.87*	0.92*
	readily-Po	0.51*	0.78*	0.95*	0.96*
	Mod-Pi	0.61*	0.59*	0.83*	0.83*
	Mod-Po	-0.07	-0.43	-0.37	-0.39
	Less-Pi	-0.10	-0.44	-0.40	-0.45
	Less-Po	0.71*	0.61*	0.93*	0.89*
	Residual-P	0.19	0.70*	0.63*	0.74*
	Microbial P	0.62*	0.61*	0.83*	0.78*
	Total N	0.65*	0.69*	0.94*	0.91*
	Total C	0.50*	0.71*	0.89*	0.88*
Alkaline	readily-Pi	0.94*	0.93*	0.96*	0.91*
	readily-Po	0.84*	0.82*	0.89*	0.84*
	Mod-Pi	0.75*	0.67*	0.75*	0.67*
	Mod-Po	0.09	0.16	-0.18	-0.13
	Less-Pi	-0.03	-0.14	-0.03	-0.09
	Less-Po	0.30	0.38	0.33	0.39
	Residual-P	0.81*	0.80*	0.70*	0.66*
	Microbial P	0.88*	0.84*	0.95*	0.88*
	Total N	0.45	0.31	0.60*	0.51
	Total C	0.81*	0.80*	0.82*	0.77*

To investigate the effect of the different types of amendments on soil and plant parameters, we performed PCA analyses. The first two PCA components explained 82% of the total variance of soil P fractions (Fig. 14A). The individual representation of treatments on the factor map showed spatial separation of treatments in both soils, with a tendency to the formation of two groups, one for the PM and PMRP treatments and a second one for the control and RP (Fig. 14A). Both groups were separated along the 2nd axis related to the contribution of less available organic P and residual P. Additionally, acid and alkaline soils could be separated according to soil P forms along the first axis. Alkaline soils were thus associated with less and moderately available Po and less available Pi, while acid soils were associated with readily available Pi and Po and moderately available Pi (Fig. 14A).

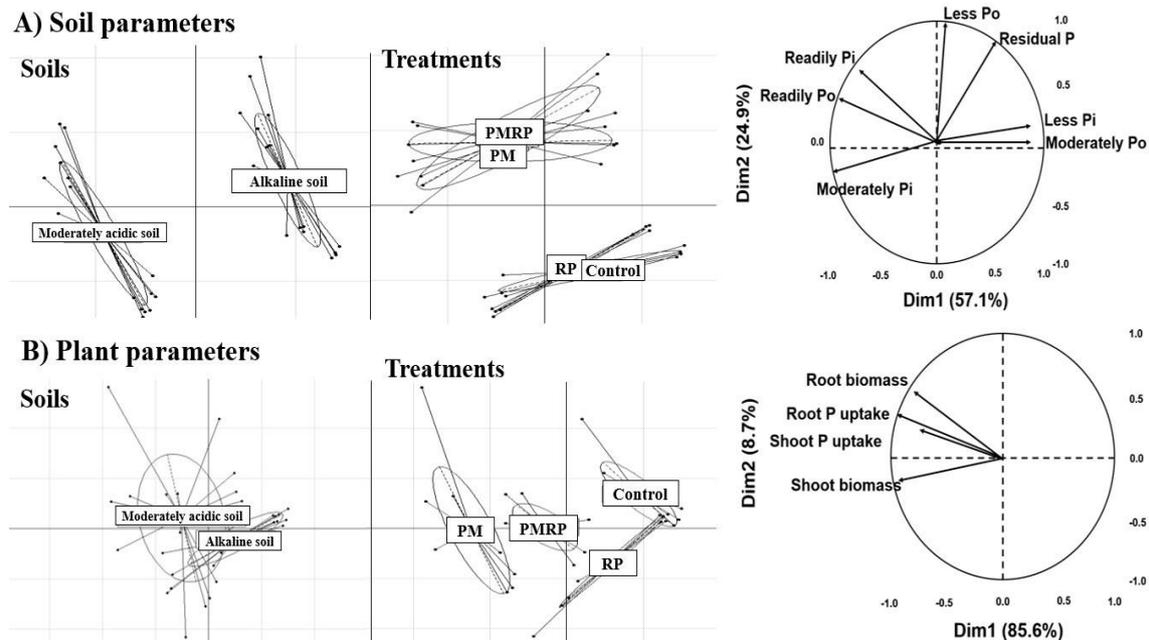


Figure 14. Principal component analysis of soil P forms (A) and plant (B) variables studied as the response to different soil types and treatments.

For the PCA performed with plant parameters, the first two components explained 94% of the total variability. The individual representation of treatments on the factor map (Fig. 14B) showed spatial separation of all the treatments of both soils. Treatments with poultry

manure were associated with P uptake and aboveground and belowground biomass in the positive direction. Plant parameters did not differentiate acid and alkaline soils.

3.3.7. Synergistic and Antagonistic Effects between PM and RP on Soil and Plant Parameters

To elucidate the effects of the amendment mixture, PMRP, on plant and soil parameters, we compared the expected values as the sum of the single use of PM and RP to the observed value for the mixture.

Table 14. Synergistic and antagonistic effect on soil and plant parameters as the response of the combined application of poultry manure compost and phosphate rock applied at a P rate of 100 mg kg⁻¹ soil after 7 weeks of ryegrass growth. \pm SD.

			Observed	Expected	Mixture Effect (%)*
Moderately acid	Soil	P availability (mg kg ⁻¹)	137.1	132.5	9.9
	Shoot	Biomass (g)	1.9	1.6	25.5
		P uptake (mg)	9.4	12.3	-23.6
	Root	Biomass (g)	1.16	0.65	91.3
		P uptake (mg)	1.5	3.97	-59.6
Alkaline	Soil	P availability (mg kg ⁻¹)	133.5	114.4	17.5
	Shoot	Biomass (g)	2.1	2.38	-13.1
		P uptake (mg)	9.97	11.6	-13.6
	Root	Biomass (g)	0.78	0.81	-3.3
		P uptake (mg)	5.5	5.82	1.8

*negative values indicate antagonistic effect and positive values indicate synergistic effect.

Differences were interpreted in terms of synergistic and antagonistic effects. While the mixture had a similar synergistic effect on P availability in both soils, their effect on plant parameters was strongly dependent on the soil type (Table 14). For the alkaline soil, the mixture had no or a slightly antagonistic effect on plant biomass and P uptake, while in

the moderately acid soil, the mixture had an antagonistic effect on P uptake, and a synergistic effect on root and shoot biomass.

3.4. Discussion

3.4.1. Impact of Organic and Inorganic P Amendments on C, N, and P Stoichiometry and Microbial Biomass P

Treatments with poultry manure compost (PM and PMRP) showed increased soil C, N, and P concentrations, most probably related to organic matter input through the amendment as well as higher biomass production (Fig. 13). All amendments changed to some extent soil organic matter stoichiometric ratios. These ratios determine the interlinkage between biochemical cycles of C, N, and P, providing information about nutrient availability following SOM decomposition and stabilization processes. The effect of the amendments on stoichiometric ratios was dependent on the type of amendment and also the soil type. Differences in stoichiometric ratios in the two soil types suggest that the amendment effect could be soil pH dependent.

Amendments increased P incorporation into the microbial biomass. Soil type had an effect on the microbial response to the application of the mixture (PMRP). It was interesting to note that while in the moderately acid soil, microbial biomass P was similar in PM and PMRP, in the alkaline soil, microbial biomass P was lower in PMRP compared to PM (Fig. 12). Similar negative effects of inorganic fertilizers on soil microorganisms have been reported before (Lupwayi et al., 2005). The results of this study show an antagonistic effect of both materials when applied in combination to alkaline soil. In view of the importance of soil microorganisms for the maintenance of soil fertility and the sustainability of grassland ecosystems and their role for P immobilization, especially in soils with low C:P ratios, such as the ones of the present study (Zhang et al., 2018), we

suggest that the soil reaction may be an important criterion to consider when organic P fertilizers are applied in combination with inorganic ones.

3.4.2. Impact of Organic and Inorganic P Amendments on Nutrient Uptake and Biomass Production and Soil P Forms

Our results, indicated that shoot biomass increased with all P sources, except for RP in alkaline soil (Fig. 13). A contrasting effect of RP on plant growth depending on the soil reaction has been observed before and the higher efficiency of RP under acid soil conditions is well documented (e.g., Abbasi et al., 2015). As many studies have shown (Waldrip et al., 2011), biomass production was strongly correlated to readily available inorganic P ($r = 0.94$ for shoot biomass in the alkaline soil and for root biomass in both soils) (Table 14). Readily available soil P may be already present in amendments (Giles et al., 2015) or may have been mineralized from organic forms after amendment addition (Singh et al., 2009). Our data indicate that while large amounts of readily available P were added with PM (Table 10), in both soils, P uptake was correlated to microbial biomass P, less available organic P, and/or residual P, C, and N (Table 11). This suggests that the mineralization of organic matter is an important process for biomass production after amendment addition. Soil reaction may influence the importance of the latter process, as much stronger correlations between those parameters were noted in the acid soil as compared to the alkaline one. The importance of the soil reaction for soil P forms is further illustrated by the PCA analyses of soil parameters (Fig. 14A).

However, this is different for plant parameters, which were differentiated by treatments, but not soil types (Fig. 14B). Our data show that using 14 Mg ha⁻¹ PM as a single amendment supplied sufficient nutrients to meet plant requirements well above the critical N, P, and K concentration in shoots for producing 90% of the maximum ryegrass yield, which are 18, 3.4, and 28 mg g⁻¹ (Evers, 2002). Since PM is a very rich animal

manure compost, it may help to build up soil productivity better than other amendments due to additional effects (Agbede and Ojeniyi, 2009). This is especially true for the moderately acid soil, which was not deficient in plant available P as indicated by the plant P concentrations of the control treatment. However, we found that in both soils, PM addition increased the shoot and root P uptake as compared to the RP when used as single amendments (Table 12). Moreover, PM led to higher plant P use efficiency than RP (Table 12). This could be an indication that there are nutrient limitations other than N, P, and K occurring, which were counterbalanced with PM. Moreover, it is also possible that PM led to the stimulation of microbial activity by promoting P uptake (Abbasi et al., 2015).

3.4.3. Synergistic and Antagonistic Effects of the Combined Application of PM and RP

Our results showed that combining RP with PM may be highly efficient in increasing the soil available P concentrations, thereby enhancing biomass production. While, in general, RP has low concentrations of available P (Kaleeswari and Subramanian, 2001), various studies (Akande et al., 2005; Antil and Singh, 2007; Arcand and Schneider, 2006; Ghosh et al., 2009) have demonstrated that P availability from RP may be increased by co-application of organic amendments. For example, Qureshi et al. (2014) showed that combining RP with compost increases soil P availability, and Abbasi and co-workers (Abbasi et al., 2015) demonstrated that the release of P to the soil increased by 80% compared with when RP was combined with PM.

In this study, our data revealed strong synergistic effects in terms of P availability independent of the soils' pH (Table 14). In contrast, biomass production showed a strong synergistic response in the moderately acid soil and an antagonistic response in the alkaline soil. Soil type dependent antagonistic effects were also observed for P uptake. Root P uptake was lower than expected in the moderately acid soil, while it was similar

to the expected value in the alkaline soil (Table 14). This might be explained by a higher immobilization of P in the microbial biomass in moderately acid soil as compared to the alkaline one (Fig. 13). Microbial P may become available and thus microbial immobilization as well as the antagonistic effect might be transitory. Another explanation of these contrasting effects may be related to the initial P status of the soil. As the moderately acid soil was not P deficient, the increased availability of P following PM and RP did not foster uptake. We suggest that synergistic and antagonistic effects in different soil types should be evaluated and taken into consideration, when elaborating new fertilizer strategies through the combination of organic and inorganic fertilizers.

3.5. Conclusions

Our study showed that the P distributed between inorganic and organic P fractions was greatly affected by the type of amendment and soil type. Poultry manure compost increased highly soil P availability, consequently improving above and belowground plant biomass production on both soil types, while phosphate rock amendment had limited effects on soil P fractions and positive effects on aboveground plant biomass production only in moderately acidic soil. In general, the influence of amendment type on soil parameters was limited and mainly related to the organic matter input. In contrast, the soil amendment type had a strong effect on plant parameters. We found synergistic effects of the combined use of PM and RP for soil available P in both soils. For plant parameters, synergistic and antagonistic effects were soil type dependent. We therefore suggest that fertilizer strategies through the combination of organic and inorganic fertilizers must be tested in different soil types by quantifying their synergistic and antagonistic effects. Moreover, the use of poultry manure compost, alone or combined with phosphate rock, could be a strategy to replace inorganic fertilizers and should be tested in long-term field experiments.

CHAPTER IV

Impact of poultry manure and phosphate rock amendment on C allocation in the rhizosphere of ryegrass plants

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Chapter IV: Impact of poultry manure and phosphate rock amendment on C allocation in the rhizosphere of ryegrass plants

Abstract

Soil carbon (C) sequestration has been identified as a suitable negative emission technology but may have a high nutrient cost. Transfer of C from plant to soil may be influenced by soil physicochemical conditions and type of amendment. The aim of this study was to investigate the effect of different types of phosphorus (P) fertilizer on C flow from plant to soil. In particular we investigated how poultry manure (PM) and phosphate rock (RP) or their mixture affected C allocation in microbial biomass, and (2) we assess the contributions of plant- and poultry manure derived C on rhizosphere soil and physical soil organic matter (SOM) fractions. We performed a growth chamber experiment with ryegrass plants using ^{13}C labelling to investigate the impact of organic (PM) and inorganic phosphate rock (RP) P soil amendment on C dynamics and we fractionated SOM to different soil fractions. This study clearly indicated that both poultry manure and its mixture with phosphate rock applied in two different soil types increased total SOC contents and labile organic C (FLf, OLf and microbial biomass C). Moreover, the greatest contribution to stored soil organic C were observed as poultry manure derived C. Our study showed that in soils amended with PM the plant_{derived} C was allocated mainly in the FLf, while PMRP favored its allocation in the Hf, highlighting that PMRP could represent an interesting alternative to store SOC.

Keywords: Poultry manure; Phosphate rock; C isotopes; SOM density fractionation

4.1. Introduction

Negative emission technologies are now required to effectively reduce high atmospheric CO₂ concentrations and to combat climate change (IPCC, 2014; UNEP, 2017). Soil organic carbon (SOC) sequestration has been identified as a suitable negative emission technology, which also improves soil quality (Smith, 2016). However, SOC sequestration but may have a high nutrient cost (Richardson et al., 2014). In fact, substantial amounts of nitrogen (N) and phosphorus (P) are necessary to increase SOC (Soussana et al., 2017; Van Groenigen et al., 2017). This is problematic especially for P, as P fertilizers manufactured from phosphate rocks may be of limited availability in the future due to resource distribution and depletion (Cordell et al., 2009; Reijnders, 2014). Therefore, alternative P sources have to be found.

One possible option may be poultry manure composts (PM), which has been used as phosphorus fertilizer to improve plant nutrition and crop productivity by increasing soil nutrient status (Azeez and van Averbek, 2012; Dikinya and Mufwanzala, 2010; Singh et al., 2017; Toor, 2009; Waldrip et al., 2011). PM availability may increase in the future due to the high amounts of manure of a growing broiler industry from intensive meat and egg production (FAO, 2018b). These waste materials may be recycled into soil amendments by composting to prevent environmental concerns like greenhouse gas emissions (Bogner et al., 2008; Sánchez et al., 2015), odor (Keener et al., 2014) and pollution of waterways (Szogi et al., 2015) following direct field application or uncontrolled disposal. Therefore, soil application of PM may be a win-win strategy. To optimize resource use and to prevent adverse effects (e.g. heavy metal contamination) it has been suggested to combine PM use and rock phosphate (RP). Although the benefits of the combined utilization of both materials for enhancing plant growth has been demonstrated (Ren et al., 2014), the impact of these material on carbon (C) flow within

plant-soil system is poorly known. This information is however necessary to assess the impact of fertilization management involving PM and RP on soil carbon storage and to understand carbon and nutrient dynamics at the root-soil interphase and to link soil microbial biomass to organic C pools, which are critical for quantifying C fluxes (Eze et al., 2018).

In the present study, we used ^{13}C labelling of ryegrass plants grown in two contrasting soils differentiated by their P status and soil reaction to investigate the impact of organic (PM) and inorganic (RP) P soil amendment on C transfer from plants to soil. Our objective was to assess the effect of PM and RP amendments on plant-derived SOC allocation to different pools. We also studied the fate of C derived from plant- and poultry manure. We hypothesized that RP and PM influence differently plant-derived C distribution depending on soil type. Moreover, we hypothesized that the joined application of RP and PM induces synergistic effects by enhancing C transfer from plant to soil.

4.2. Materials and Methods:

4.2.1. Materials:

Soils utilized were part of the French observatory SOERE PRO (<https://www6.inra.fr/valor-pro/SOERE-PRO-les-sites>). Both soils showed similar texture, organic matter content and soil forming processes (lixiviation). Soil samples were taken from Colmar (Eastern France) and Brittany at Le Rheu (northwestern of France). According to the French Référentiel Pédologique (Baize and Girard, 2008) the soil from Colmar was Neoluvisol with a pH of 6.1 (moderately acid soil) and the soil from Brittany was a carbonated Luvisol, with a pH of 8.5 (Alkaline soil) (Table 15). They were differentiated by pH, initial Olsen P concentration and also by the physical properties. Control plots without fertilization at the two sites were sampled at the first 0-30 cms. After sampling the soils were transported to the laboratory, air dried and sieved at 2 mm. The plant species used was ryegrass (*Lolium perenne*).

Table 15. Soil physical and chemical characterization.

Soil Type	pH	C _{org} g kg ⁻¹	C:N	P olsen mg kg ⁻¹	K ₂ O Cmol + kg ⁻¹	Clay %	Silt	Sand
Moderately acid	6.1	11.9	10	60	0.32	14.6	68.3	16.1
Alkaline	8.5	12.1 *	10	11	0.26	20.7	59.8	6.8

* CaCO₃ = 128 g kg⁻¹.

We used poultry manure material composted in pellet form with a dry matter content of 88% with an organic matter content of 600 g kg⁻¹. The input of organic C was 443 and 310 g C kg⁻¹ from PM and the mixture (PMRP). Phosphorus content was 30 g kg⁻¹ in P₂O₅. RP was derived from bones with 30% P and 50% calcium. It was provided in powder form with 90% of the particles smaller than 0.16 mm.

4.2.2. Growth chamber experiment:

We established a pot experiment in optimal conditions in a growth chamber for 7 weeks. We amended 490 g soil of the moderately acid soil and 550 g of the alkaline soil per

treatment (to account the contrasting bulk densities) with four replicates. Treatments consisted in poultry manure compost (PM), phosphate rock (RP) or their mixture consisting of 70% of PM and 30% of RP (PMRP) (Appendix 2). Treatments were added to supply 100 mg P per kg⁻¹ soil d.w. N and K was also supplied to account the nutrient input of PM (262 mg N and 221 mg K per kg soil) in the form of KCl and NH₄NO₃ to all other treatments including the control. The PM application was equivalent to 9.8 Mg ha⁻¹ when applied in mixture with RP and 14 Mg ha⁻¹ when applied as single amendment. The RP application was equivalent to 0.25 Mg ha⁻¹ when applied in mixture with PM and 0.8 Mg ha⁻¹ when applied as single amendment. Soils were thoroughly mixed in plastic bags after addition of the amendments, transferred to each pot and brought to field capacity with tap water. A total of 97 of ryegrass seeds were added to each pot. Seeds were sown on the surface and cover superficially with soil material. The growth chamber conditions were 24 °C (day temperature) and at 17 °C (night temperature) with a day length (light intensity of 650 μmol m⁻² s⁻¹) of 8 h for the first 13 days and afterwards 11 h until the end of the experiment. Soil moisture was maintained at 40% of the available field capacity by watering regularly. Air humidity (75-65 % respectively for day and night conditions). Air CO₂ concentration was regulated at 400 ppm for daytime.

After 7 weeks, shoots and roots were separated from soil and their fresh weight was recorded. The remaining soil masses were oven-dried at 40°C and sieved at 2 mm. An aliquot was ground for further analyses.

4.2.3. Microbial biomass C

Microbial biomass was determined on a fresh soil samples by the chloroform fumigation-extraction method for C (Vance et al., 1987). The organic C concentration of K₂SO₄-extracted solutions were measured using TOC analyzer (TOC-VCSH by Shimadzu with a sampler ASI-V by Shimadzu). Microbial biomass C (MBC) concentrations were

calculated as the difference between fumigated and non-fumigated soil multiplied by a factor of 2.64 for C.

4.2.4. Soil organic matter density fractionation:

We applied a physicochemical density fractionation and separated into free light fraction (FLf), occluded light fraction (OLf), which together constitute the active pool of SOM; and the heavy fraction (Hf) contributing to the intermediate and passive SOM pool (Von Lützow et al., 2007). Briefly, 10 g of soil were suspended in 25 ml sodium polytungstate with a density of 1.6 g cm³ and centrifuged at 5500 rpm for 30 minutes. Thereafter the supernatant was recovered by filtration. The remaining material was again suspended in sodium polytungstate (1.6 g cm³) and treated by ultrasonication (300 J mL⁻¹) in order to disperse macroaggregates. After recovery of the supernatant corresponding to the occluded light fraction (OLf) the remaining material (Hf) was washed with distilled water. FLf and OLf were overdried at 40°C and the Hf fraction was freeze-dried before elemental and isotopic analyses. C and N content of bulk soil and physical fractions were measured using an elemental analyser Variopyrocube (Elementar). The moderately acid soil was non-carbonate; soil C were considered entirely organic. For the alkaline soil (carbonated soil), a pre-treatment of decarbonation with HCl-fumigation was performed (Harris et al., 2001). The $\delta^{13}\text{C}$ values were measured for all soil treatments with an Isotope Ratio Mass Spectrometer (Micromass Isoprime).

4.2.5. Sources of soil organic carbon:

At the end of the growing cycle, samples of roots and shoots were oven-dried at 50°C, finely ground with a ball mill, and a subsample was analyzed for %C, %N, and $\delta^{13}\text{C}$ with an isotope Ratio Mass Spectrometry (EA-IRMS) Variopyrocube (Elementar) – Micromass Isoprime. Natural abundance of ^{13}C in soil and plant fractions was determined.

The $\delta^{13}\text{C}$ values were calculated from the measured isotope ratios of the sample and standard as follow:

$$\delta^{13}\text{C}(\text{‰}) = (R_{\text{sample}}/R_{\text{standard}}) \times 10^3 \quad (3)$$

where $R = {}^{13}\text{C}/{}^{12}\text{C}$ (molar ratio).

Plant_{derived C} to SOC in rhizosphere soil and SOM fractions were calculated from natural $\delta^{13}\text{C}\text{‰}$ abundance by using the following equation:

$$\text{Plant} - \text{derived C} = \left(\frac{\delta^{13}\text{C sample} - \delta^{13}\text{initial}}{\delta^{13}\text{enriched root} - \delta^{13}\text{initial}} \right) \quad (4)$$

where “ $\delta^{13}\text{C}$ sample” refers to soils amended with P sources at the end of experiment, “ $\delta^{13}\text{C}$ initial” is the soil of the control pots and “ $\delta^{13}\text{C}$ Enriched roots” is the mean $\delta^{13}\text{C}$ value of ryegrass roots of each treatment. The plant_{derived C} without poultry manure (Control, RP) the plant_{derived C} was calculated as follows:

$$\text{SOC}_{\text{plant}} = \text{SOC} \times \text{Plant}_{\text{derived C}} \quad (5)$$

$$\text{SOC}_{\text{Native}} = \text{SOC} \times (1 - \text{Plant}_{\text{derived C}}) \quad (6)$$

where SOC is total soil organic carbon. Then, for the PM and PMRP treatment, manure-derived soil organic carbon was estimated as the difference in total soil organic C and the sum of original and plant_{derived C} in soil (Zhang et al., 2015).

Finally, each source input was calculated as follows:

$$\text{Source input} = \text{Source derived C} (\text{mg g}^{-1}) \times \text{weight of source} (\text{g}) \quad (7)$$

4.2.6. Statistical analysis:

The experiment was arranged in a completely randomized design with four replicates. Data were checked for normality (Shapiro-Wilk test) and homogeneity of variance (Levene test) were determined before analyses. Statistical differences of means (95% significance level) were analyzed using two-way analyses of variance (two-way ANOVA). Post hoc tests with the function Tukey-test were made for the explanatory variables independently when the ANOVAs detected significant differences. We

identified significant differences among treatments in each soil and differences among soils of the same treatment. The relationship between parameters were tested by Pearson correlation analyses. Statistical testing was done using the statistical program R Foundation for Statistical Computing Version 1.1.456 (R Development Core Team 2009-2018 RStudio, Inc); effects were deemed significant at $p \leq 0.05$.

4.3. Results

4.3.1. Plant biomass production and P uptake:

Shoot biomass production was highly increased by all treatments in the moderately acid soil, while in the alkaline soil was by RP being only increasing with PM and PMRP. Root biomass production was increased in both soils amended with both PM and PMRP as compared with RP and control (Table 16). Additionally, P uptake in shoots and roots were greatly increased in both soils amended with PM and PMRP treatments.

Table 16. Dry weight and P uptake of shoots and roots in control and soil amended with poultry manure compost (PM), phosphate rock (RP), and their combination (PMRP) after 7 weeks of ryegrass growth. Upper case letters denote significant differences ($p \leq 0.05$) between treatments for one soil ($p \leq 0.05$). Lower case letters denote significant differences ($p \leq 0.05$) between soils for one treatment.

Soil Type	Treatment	Biomass production		P uptake	
		Shoot g pot ⁻¹	Root	Shoot mg pot ⁻¹	Root
Moderately acid	Control	1.9 ^{Ca}	2.5 ^{Ca}	3.6 ^{Ba}	3.6 ^{Ca}
	RP	4.4 ^{Aa}	1.8 ^{Ca}	3.5 ^{Ba}	4.6 ^{Ca}
	PM	4.6 ^{Aa}	4.0 ^{Aa}	4.9 ^{Aa}	14.8 ^{Aa}
	PMRP	3.8 ^{Ba}	3.0 ^{Ba}	5.0 ^{Aa}	8.7 ^{Ba}
Alkaline	Control	0.8 ^{Ca}	0.7 ^{Bb}	2.6 ^{Bb}	1.1 ^{Cb}
	RP	0.6 ^{Cb}	0.4 ^{Bb}	3.0 ^{Ba}	2.8 ^{Ba}
	PM	4.6 ^{Aa}	2.3 ^{Ab}	3.8 ^{Ab}	9.7 ^{Ab}
	PMRP	2.9 ^{Aa}	1.6 ^{Ab}	3.8 ^{Ab}	6.9 ^{Aa}

4.3.2. Microbial biomass C:

Application of PM significantly increased by 28-125% the microbial biomass C concentrations in the moderately acid and alkaline soil, respectively (Fig. 15). RP had a negative effect on microbial biomass C leading to a decrease of 14–38% in both soils as compared with control, while its combination with PM induced the greatest microbial biomass C increases (107%) for the moderately acid soil. For alkaline soil, microbial biomass showed similar concentrations as the control.

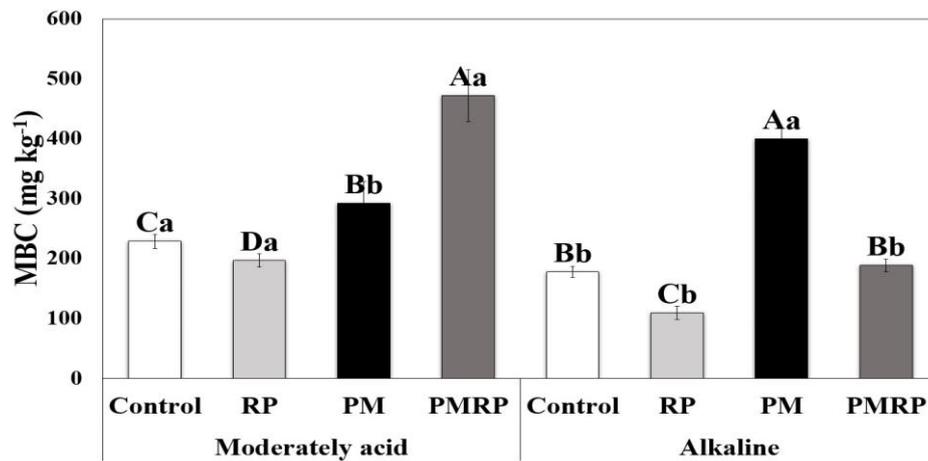


Figure 15. Microbial biomass carbon in control and amended soil with poultry manure, phosphate rock and their combination applied at a P rate of 100 mg kg⁻¹ soil after 7 weeks of ryegrass growth. Control, unamended soil; PM, poultry manure compost; RP, phosphate rock; PMRP, phosphate rock combined with poultry manure compost. \pm SD. Upper case letters denotes significant differences ($p \leq 0.05$) between treatments for one soil. Lower case letters denote significance differences ($p \leq 0.05$) between soils for one treatment.

4.3.3. Carbon derived from plant, poultry manure compost and native C in the rhizosphere soil

At the end of the experiment 0.16 to 0.50 g of plant_{derived} C was present in the alkaline and moderately acid soil (Fig. 16A). In the latter soil, the plant_{derived} C was significantly increased by 64 to 94% with both PMRP and PM treatments as compared to control and RP (Fig. 16A), while for the alkaline soil RP amendment led to less plant_{derived} C (45%) as compared with PM, PMRP and control. The PM_{derived} C with PM accounted 3.69 – 3.81 g, being higher than PMRP treatment compromising to 2.34 – 3.05 g (Fig. 16A).

Changes in plant- and PM_{derived} C in soil are presented in figure 16B. At the end of the experiment, about between 4 and 7% of native SOC had been replaced with plant_{derived} C in all treatments (Fig. 16B). The PM_{derived} C comprised about 36 and 41% of total SOC, and native SOC accounted for 53 and 58% in soils amended with PMRP. PM_{derived} C in the soils amended with PM compromised about 47% of total SOC, and native SOC accounted for 49% in the alkaline and moderately acid soil (Fig. 16B).

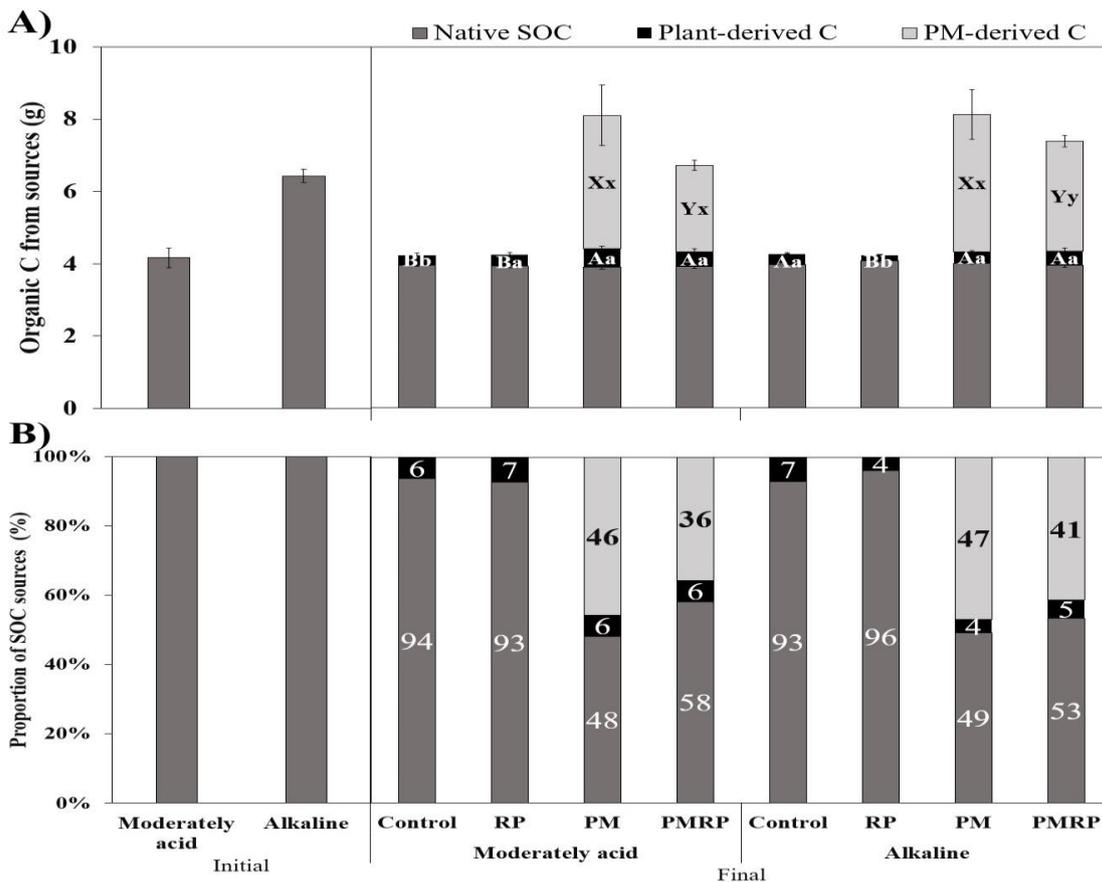


Figure 16. Organic carbon from sources (A) and Proportion of SOC sources (B) in the rhizosphere soil for the moderately acid and alkaline soil, after 7 weeks of ryegrass growth amended with poultry manure, phosphate rock and their combination applied at a P rate of 100 mg kg⁻¹ soil. Control, unamended soil; PM, poultry manure compost; RP, phosphate rock; PMRP, phosphate rock combined with poultry manure compost. ±SD. Upper case letters denotes significant differences ($p \leq 0.05$) between treatments for one soil. Lower case letters denote significance differences ($p \leq 0.05$) between soils for one treatment.

4.3.4. Total C and N, and C derived from plant and poultry manure compost input and their distribution in SOC fractions:

Ryegrass growing on PM, and PMRP amended soils significantly increased by 1.4 to 2.9-fold the proportion of the free light fraction (FLf) (Table 17). Both PM treatments increased by 35 to 43% the occluded light fraction (OLf) in the moderately acid soil. RP led to much lower proportions of the free light fraction in the alkaline soil, being 60% lower than control (Table 17).

Table 17. Portion of each SOM fraction, and total N and C of free (FLf), occluded (OLf) light fraction and heavy fraction (Hf) of SOM separated from soils (0–20 cm) in ryegrass plants after 7 weeks growing in PM with/without phosphate rock amended soil. Upper case letters denote significant differences ($p \leq 0.05$) between treatments for one soil. Lower case letters denote significance differences ($p \leq 0.05$) between soils for one treatment. Legend: RP, phosphate rock; PM, poultry manure compost; PMRP, phosphate rock combined with poultry manure compost.

			Moderately acid soil				Alkaline soil			
			Control	RP	PM	PMRP	Control	RP	PM	PMRP
FLf			4.3 ^{Ca}	5.0 ^{Ca}	12.6 ^{Aa}	9.3 ^{Ba}	3.3 ^{Cb}	1.3 ^{Db}	6.9 ^{Ab}	4.7 ^{Bb}
OLf	Weight	g pot⁻¹	1.7 ^{Bb}	1.6 ^{Bb}	2.2 ^{Aa}	2.4 ^{Aa}	2.3 ^{Aa}	2.4 ^{Aa}	2.6 ^{Aa}	2.2 ^{Aa}
Hf			484.1 ^{Aa}	483.4 ^{Aa}	475.2 ^{Ba}	478.3 ^{Ba}	484.4 ^{Aa}	486.3 ^{Aa}	481.8 ^{Aa}	483.1 ^{Aa}
Free light fraction	N	mg pot⁻¹	48.3 ^{Cb}	55.1 ^{Ca}	313.2 ^{Aa}	208.5 ^{Ba}	53.1 ^{Ca}	21.9 ^{Db}	148.1 ^{Ab}	127.5 ^{Bb}
	C		934.4 ^{Ca}	936.3 ^{Ca}	3890.1 ^{Aa}	2540.5 ^{Ba}	701.2 ^{Cb}	323.0 ^{Db}	1655.9 ^{Ab}	1198.2 ^{Ba}
	C:N		19.3 ^{Aa}	17.2 ^{Aa}	12.7 ^{Ba}	12.4 ^{Ba}	13.3 ^{Ab}	14.7 ^{Ab}	11.8 ^{Aa}	9.6 ^{Bb}
Occluded light fraction	N	mg pot⁻¹	24.9 ^{Cb}	23.2 ^{Cb}	70.2 ^{Ab}	57.2 ^{Bb}	58.7 ^{Ba}	54.8 ^{Ba}	79.9 ^{Aa}	71.6 ^{Aa}
	C		408.0 ^{Bb}	404.1 ^{Bb}	823.0 ^{Ab}	696.8 ^{Ab}	758.4 ^{Ca}	752.9 ^{Ca}	1022.4 ^{Aa}	896.6 ^{Ba}
	C:N		16.3 ^{Aa}	17.8 ^{Aa}	11.7 ^{Ba}	12.0 ^{Ba}	13.0 ^{Ab}	13.7 ^{Ab}	12.8 ^{Aa}	12.5 ^{Aa}
Heavy fraction	N	mg pot⁻¹	657.8 ^{Cb}	617.7 ^{Cb}	935.2 ^{Aa}	750.7 ^{Bb}	771.7 ^{Ba}	756.3 ^{Ba}	787.1 ^{ABb}	802.6 ^{Aa}
	C		5201.5 ^{Cb}	4663.5 ^{Cb}	7496.7 ^{Aa}	6035.9 ^{Bb}	6019.2 ^{Ca}	6327.9 ^{Ca}	7037.8 ^{Aa}	6667.4 ^{Ba}
	C:N		7.9 ^{Aa}	7.6 ^{Ab}	8.2 ^{Aa}	8.1 ^{Aa}	7.8 ^{Ba}	8.4 ^{Ba}	8.9 ^{Aa}	8.2 ^{Ba}

Total N and C in all SOM fractions were significantly higher in both soils amended with PM and PMRP, as compared with RP and Control (Table 17). RP showed no-differences in total N and C for all SOM fractions in the moderately acid as compared to control, whereas C and N were even lower than the control for FLf in the alkaline soil (Table 17). In the alkaline soil, highest amount of plant_{derived} C was allocated to the Hf fraction regardless the treatment (decreasing only with PM), while in the moderately acid soil, the treatment strongly impacted plant_{derived} C input into soil fractions (Fig. 17). In the latter soil, highest amounts of plant_{derived} C were found in the FLf fraction of the RP followed by PM treatment, whereas in the PMRP treatment highest amount of plant_{derived} C were allocated to the Hf fraction (Fig. 17).

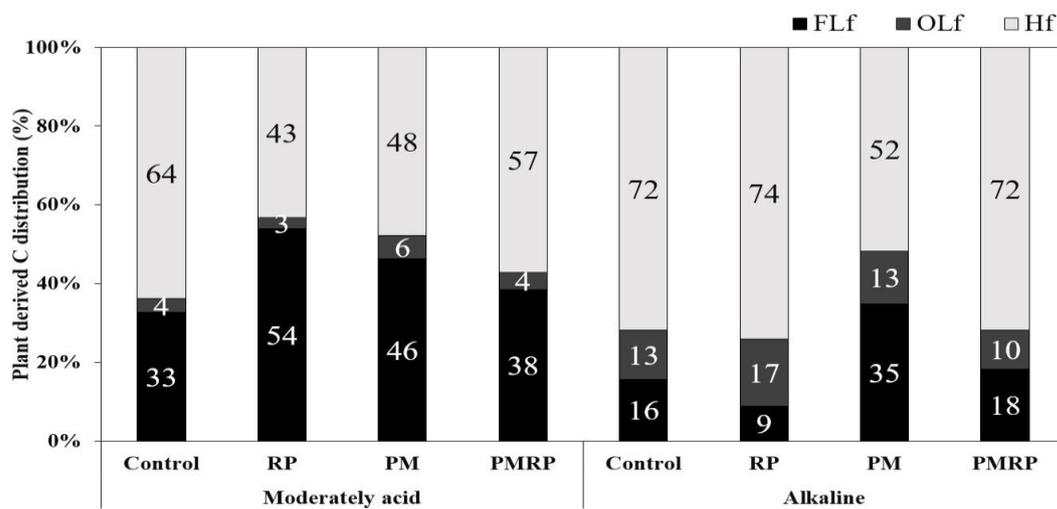


Figure 17. Plant_{derived} C distribution from free light (FLf), occluded light (OLf) and heavy fraction (Hf) of SOM separated from soils (0–20 cm) in ryegrass plants after 7 weeks growing in poultry manure with/without phosphate rock amended soil. Legend: RP, phosphate rock; PM, poultry manure compost; PMRP, phosphate rock combined with poultry manure compost.

The plant_{derived} C inputs, corresponding to FLf, OLf, and Hf were significantly increased compared to the control in both soils amended with PM and PMRP (Fig. 18). For the FLf, plant_{derived} C increased by 2.1 to 4.6-fold in the PM treatment as compared to the control. RP decreased by 50% the plant_{derived} C from the FLf, as compared with the control in the

alkaline soil, while plant_{derived} C was increased by 30% in the moderately acid soil (Fig. 19A). PMRP increased by 50 to 62% the plant_{derived} C in the FLf of moderately acid and alkaline soil, respectively (Fig. 18A).

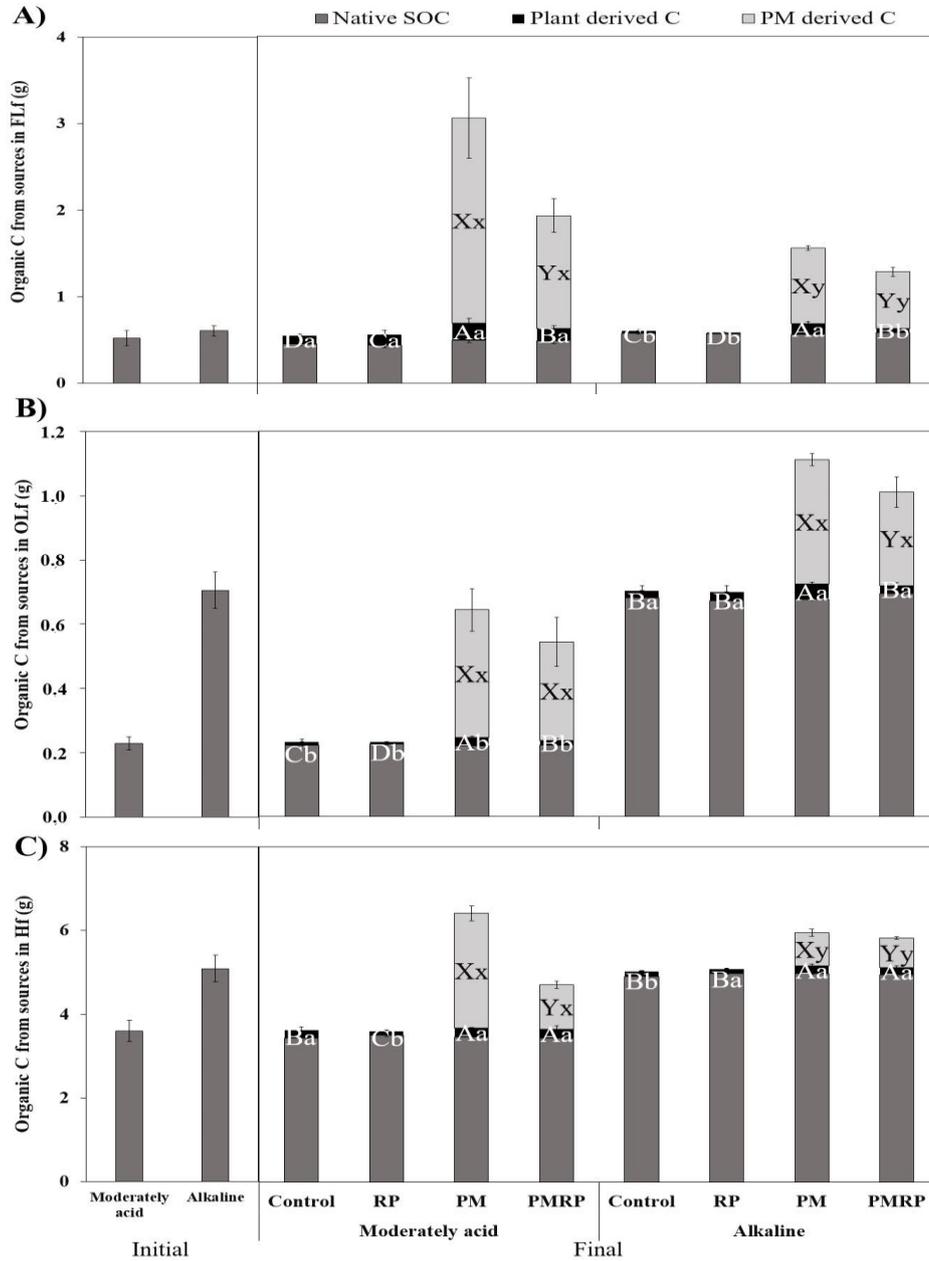


Figure 18. Organic carbon from sources in free light (FLf) (A), occluded light (OLf) (B) and heavy fraction (Hf) (C) of SOM separated from soils (0–20 cm) in ryegrass plants after 7 weeks growing in poultry manure with/without phosphate rock amended soil. \pm SD. Values followed by a different letter over the bars indicate statistically significant differences at $p \leq 0.05$ within soil among fertilization treatments. Legend: RP, phosphate rock; PM, poultry manure compost; PMRP, phosphate rock combined with poultry manure compost.

Plant_{derived} C from the Olf remained similar as control by using RP, and PMRP for the alkaline soil, while using PM its increased by 2.2-fold as compared with control. In the moderately acid soil amended with PM and PMRP, the plant_{derived} C was increased by 2.5 and 1.5-fold, respectively (Fig. 18B).

RP decreased the plant_{derived} C in the Hf by 47% and 7% as compared to the control in the moderately acid and alkaline soil, respectively. The moderately acid soil amended with PM alone and combined with RP increased by 11 and 14% the plant_{derived} C as compared to the control, while in the alkaline soil both treatments showed the highest increases being 48% and 39% respectively compared to the control (Fig. 18C).

4.3.5. Relationship between parameters

Microbial biomass C was highly and positively correlated to plant and poultry manure derived-C from rhizosphere and from both free light and heavy fractions (Fig. 19). In addition, microbial biomass C was correlated with N and C from the free light SOM fraction.

Total N and C from all SOM fractions were highly and significantly correlated with PM_{derived} C from rhizosphere soil. Moreover, we found that PM_{derived} C from all SOM fractions were highly and significantly correlated with total SOC allocated in the free light and heavy fractions (Fig. 20).

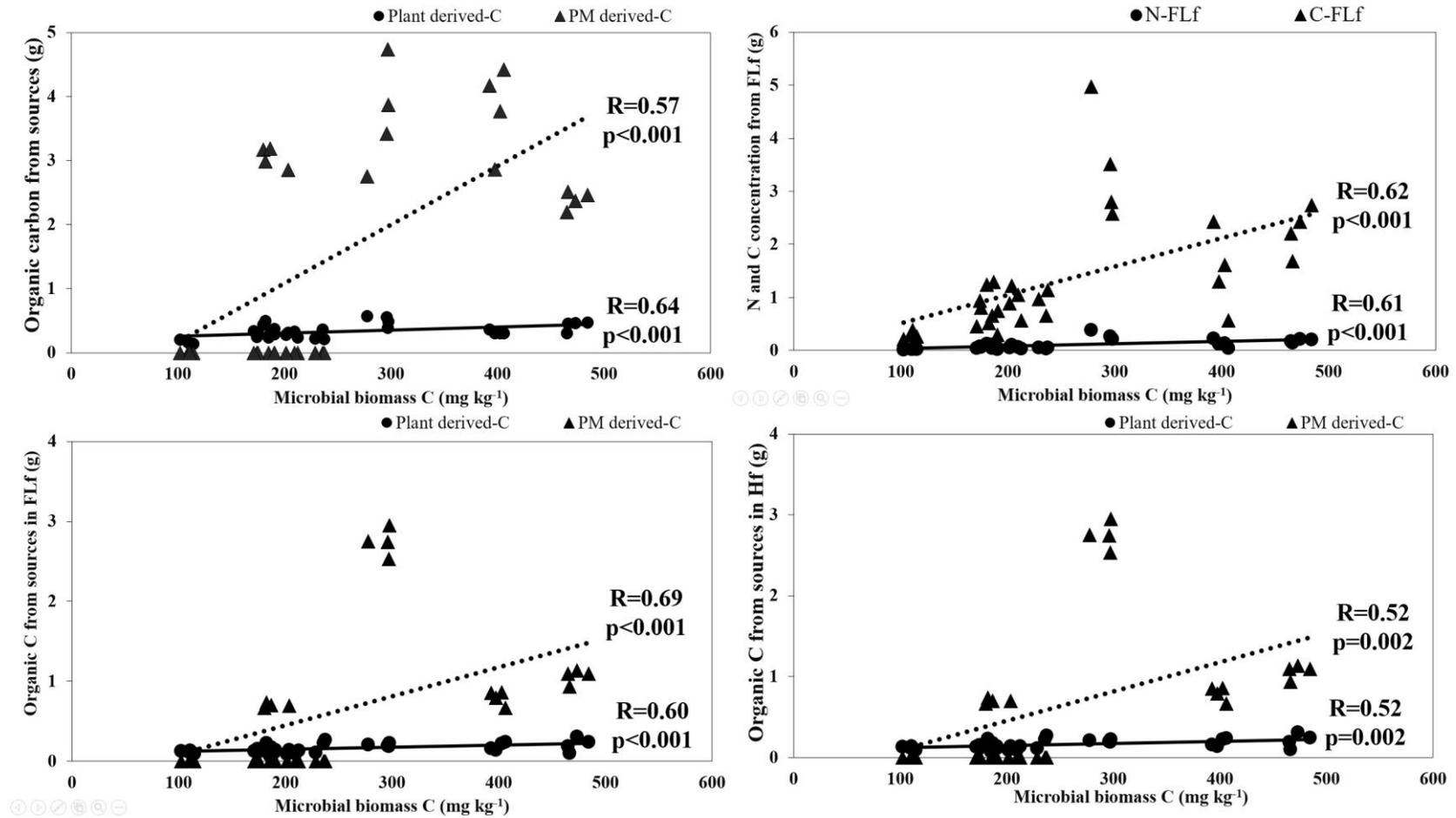


Figure 19. Relationship between soil microbial biomass C with organic C from plant and poultry manure sources from control and amended soil with poultry manure, phosphate rock and their combination applied at a P rate of 100 mg kg⁻¹ soil after 7 weeks of ryegrass growth. n=16.

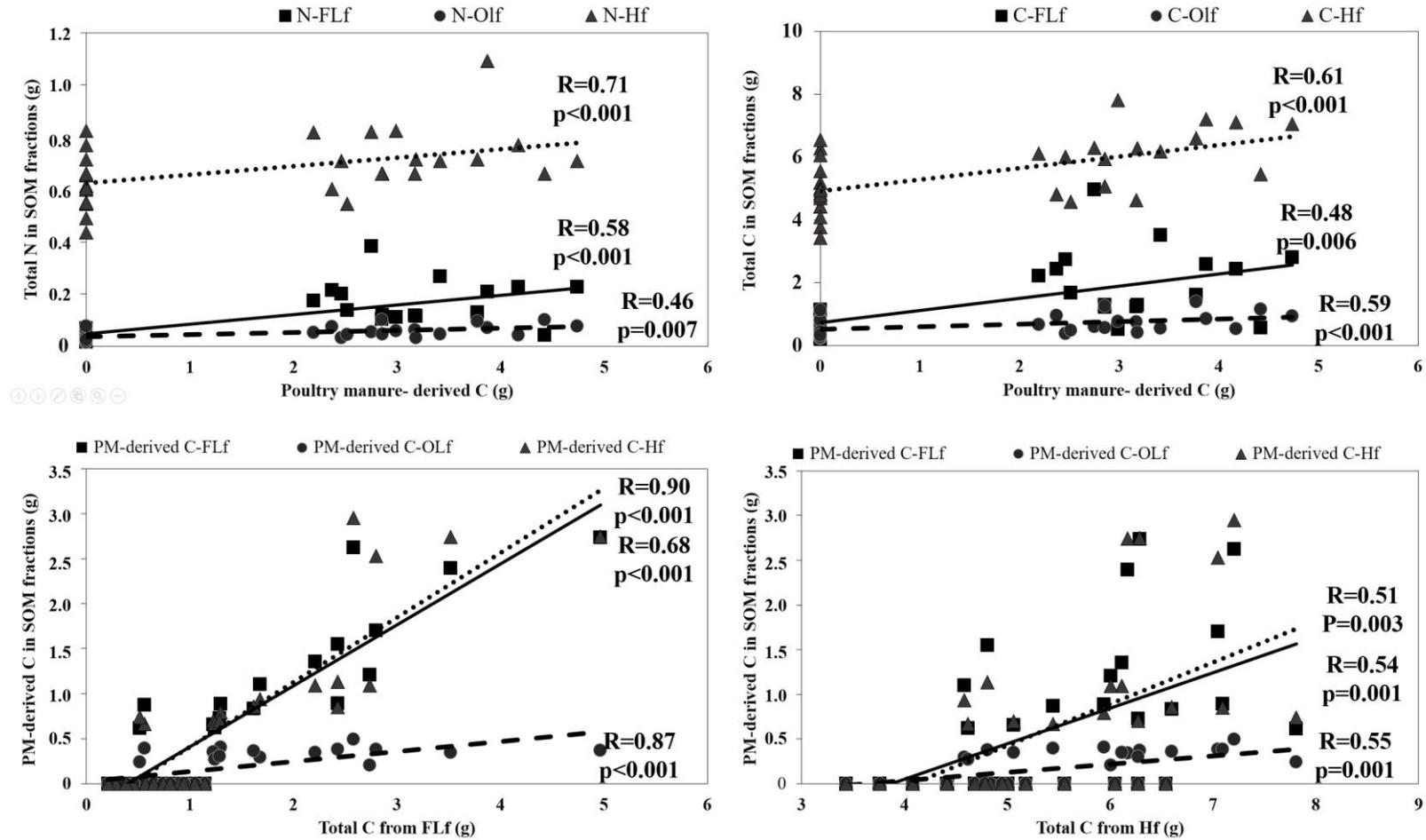


Figure 20. Relationship between poultry manure-derived C with total C in soil organic matter fractions from control and amended soil with poultry manure, phosphate rock and their combination applied at a P rate of 100 mg kg^{-1} soil after 7 weeks of ryegrass growth. $n=16$.

4.4. Discussion:

4.4.1. Effect of amendments on soil C and N status

Our study showed that in both soils amended with RP, the microbial biomass C was much lower than in the control (Fig. 15). Similar negative effects considering fertilizer source on soil microorganisms have been reported before (Lupwayi et al., 2005). He et al. (1997) stated that the principal mechanism responsible for the reduced soil microbial biomass may be increase in soil pH due to liming materials containing CaO (4.7%) which was in agreement of the RP containing 16% CaO used in this study. We found that PM and PMRP highly enhanced microbial biomass C (Fig. 15), moreover, microbial biomass C was highly and significantly correlated to plant- and $PM_{\text{derived C}}$ in the rhizosphere soil and allocated in the F_l and H_f from SOM (Fig. 19). Malik et al. (2013) also found significantly increases in microbial biomass C by applying poultry litter at a rate of 20 Mg dw ha⁻¹. Soil NPK nutrient availability can be critically important for microbial activity (Poeplau et al., 2018), thus P availability highly increased by both PM and PMRP treatments (Poblete-Grant et al., 2019) could be related with increasing microbial biomass C (Spohn et al., 2013).

We found differences between soil type amended with PM and PMRP. For example, the alkaline soil amended with PM showed the highest increases in microbial biomass C (Fig. 15), whereas in the moderately acidic soil, the microbial biomass C concentration for the PM treatment was lower than in the PMRP. Microbial biomass decomposition kinetics and its community composition differs from acid soils to those with higher pH (Paul, 2016). Therefore both, organic material quality and soil type may have affected soil microbial characteristics (Li et al., 2018b). As microorganisms have a positive role on improving soil fertility by producing more aggregates after organic amendment (Lehmann et al., 2011; Rahman et al., 2017), increases in microbial biomass C with PM

treatments is important due to the fact that soil microorganisms mineralize nutrients from organic soil amendments (Luo et al., 2015; Lupwayi et al., 2005). Variations of litter inputs in quality and quantity affect the activity and composition of soil microbial communities, which can also control the stoichiometry of assimilable resources in their local environment by altering the allocation of resources (i.e. rich substrates of C, N and P degraded by enzymes microbial origin) (Bell et al., 2014; Griffiths et al., 2012).

Data obtained for total N and C from all SOM fractions in soils amended with PM and its combination with RP, were significantly higher than the control and RP treatments of both soils. The RP treatment alone did not significantly increase total N and C from all SOM fractions indicating that additional C inputs derived from PM were needed (Table 16). These is supported by significant correlations found between $PM_{\text{derived C}}$ in the rhizosphere soil, and $PM_{\text{derived C}}$ from FLf and Hf with the total C and N (Fig. 20). Similar increasing C allocation to the FLf fraction following organic manure input were reported by Han et al. (2006). The light fraction is readily influenced by recent land use and management practices, and it is more sensitive than the total SOM content to those effects (Bremer et al., 1994; Soon et al., 2009). The results of our study indicate that the organic (PM) and inorganic (RP) fertilization management practices affected total C and N concentration of the soil FLf fractions; however, for some of the variables, those effects were also influenced by soil type (Table 17). For example, in the heavy fraction (Hf) total N and C concentration were increased significantly only by the PM treatment in the moderately acid soil (Table 17). It is worth to mention that the addition of PM alone or combined with RP improved the amount of stored soil C.

4.4.2. Effect of amendments on C transfer from plant to soil

We found differences between soil types for the $plant_{\text{derived C}}$ input and allocation. For example, in the moderately acid soil the $plant_{\text{derived C}}$ was highly increased by both PM

and PMRP as compared with control and RP. Although, both PM treatments showed non differences for plant_{derived} C compared to the control in alkaline soil, the use of RP decreased it by 45% (Fig. 17). These observations could be explained by the higher soil nutrient availability given by the application of PM (Poblete-Grant et al., 2019), which may have led to more allocation of photo-assimilated C to the roots, and then to the soil. In addition, organic amendments applied alone or combined with chemical fertilizers further enhanced C rhizodeposition, probably through impacts on microbial activity/function, and aggregate protection (Qiao et al., 2017). Changes in labile organic C corresponding to FLf can respond more quickly than total SOC content (Li et al., 2018b). In both soils amended with PM, the plant_{derived} C was allocated mainly in the FLf, while PMRP favored its allocation in the Hf (Fig. 18). As the organic matter stability increases from FLf (active pool) to Hf (mineral-associated SOM) (Von Lützow et al., 2007), PMRP could represent an interesting alternative to store SOC.

At the end of the experiment the PM_{derived} C converted into soil organic C ranged from 36 to 47% (Fig. 17). Similar results were obtained by Bol et al. (2004) where after 4 weeks of the applied slurry the 28–36% was sequestered into the soil. In the current study PM_{derived} C was higher as compared with the study of Ma et al. (2013) and Zhang et al. (2015) where only 2.8–3.5% of sheep dung and about 5.1% of pig manure_{derived} C were converted into soil organic carbon after long-term manure input (5 months and 26 years, respectively). According to Zhang et al. (2015), organic manure applied alone or combined with RP not only improved plant biomass production, but also increased soil carbon sequestration. Bol et al. (2003) found relationships between sequestered manure derived-C and microbial biomass C. We also found that microbial biomass C was positive correlated with C from sources, being greater for plant- than PM_{derived} C. In our results reported in Poblete-Grant et al. (2019), we found that microbial biomass P followed the

same trend regarding PM treatments as reported here for microbial biomass C. Considering that the turnover of soil C could strongly be coupled to the turnover of P in microbial biomass, as the C input from PM related to the energy resource and P as a nutrient resource (Chen et al., 2019). While microbial biomass C showed high correlation with plant_{derived} C from the rhizosphere soil, microbial biomass P was highly correlated with PM_{derived} C (Appendix 3). This indicated that soil microbial biomass mediates accumulation and dynamics of SOC (Richardson et al., 2014). Also, we found differences between soil type in PM_{derived} C from FLf and Hf (Fig. 20). The moderately acid soil showed the highest PM_{derived} C compared to alkaline soil, which could may be due to differences in soil pH.

4.5. Conclusion

This study clearly indicated that both poultry manure compost and its mixture with phosphate rock applied in two different soil types increased total SOC contents and labile organic C (FLf, OLf and microbial biomass C). Moreover, the greatest contribution to stored soil organic C were observed as PM_{derived} C. Furthermore, we found high relationships between PM_{derived} C in the rhizosphere soil and from the FLf and Hf SOM fractions. Our study showed that in soils amended with PM the plant_{derived} C was allocated mainly in the FLf, while PMRP favored its allocation in the Hf, highlighting that PMRP could represent an interesting alternative to store SOC. It is worth to note that PM and PMRP strongly increased root biomass which may explain the enhancement of soil microbial biomass C and P, supported by the significantly correlations between both parameters. Another important aspect to consider are the differences on soil physicochemical properties, which highly influenced the soil reaction with the amendment showing contrasting effect on soil microbial biomass C, plant_{derived} C, soil-plant C stock, and C loss (Appendix 5). The current study provided valuable information

by quantifying organic carbon derived from plant and poultry manure allocated in SOM pools, which were highly increased by using organic amendments. Further field experiments are needed to account realistic and effective effect of poultry manure alone and its mixture with phosphate rock to assess long-term soil C stabilization.

CHAPTER V

General discussion and concluding remarks

Chapter 5: General discussion and concluding remarks

5.1 General discussion

Nowadays, increasing prices of P fertilizers have been driven by estimations that in a near future phosphate rock, as non-renewable resource, could be depleted (Cordell et al., 2009; Reijnders, 2014). Therefore, reducing the use of inorganic fertilizer by management strategies to maintain agricultural yields is encouraged. Soil phosphorus (P) importance is massive for plant nutrition, but P fertilizer use efficiency are still poorly understood. This thesis assessed the impact of poultry manure compost (PM) application, alone or combined with phosphate rock (RP), on soil quality, carbon sequestration, P availability and ryegrass production.

According to results from Chapter II, after the study of Andisols with high phosphate sorption capacity amended with PM (3 Mg ha^{-1}), the amendment could supply plant requirements in order to achieve high yields and maintaining suitable levels of soil available P (50.9 mg kg^{-1} of total P belonging to the readily fraction) being comparative to pastures receiving inorganic fertilizers (Redel et al., 2016; Velásquez et al., 2016b). In addition, our study highlighted that applications of more than 10 years were necessary to achieved significant increases in SOC (22-65%) under field conditions. As increases in soil carbon concentration it have been widely reported (Antil and Singh, 2007; Ewulo and Ojeniyi, 2008; Paetsch et al., 2016) and as well in available P (Adeli et al., 2005; Ewulo and Ojeniyi, 2008; Waldrip et al., 2011), Chapter II was focused to elucidate the mechanisms driving those changes.

We found that organic matter complexes with aluminum (Al) and iron (Fe) were favored while the amorphous complexes were decreased. The changes in the soil constituents were highly correlated to our results of increasing SOC and phosphorus availability, and may partly be explained by SOC association with larger soil particles resulting in P

protection within soil aggregates (Li et al., 2016). Accordingly, we found that PM as an organic amendment enhanced the distribution of larger soil particles mainly after 10 years, being positively correlated with increases in soil carbon concentration, P availability and organic matter-Al and -Fe complexes. These findings supported our hypothesis that long-term application of poultry manure to soils increases P availability and soil organic carbon storage.

As the use of PM was suggested by many authors (Abbasi et al., 2015, 2013; Mahimairaja et al., 1995) as an strategy for the enhancement of phosphate rock solubilization, we found that using PM in short-term increased significantly inorganic and organic P concentrations in the readily available fraction, plant response was highly dependent of soil type. Soil P dynamic is influenced by soil properties, such as soil pH (Alt et al., 2011) and clay content (Pagliari and Laboski, 2014). Considering that differences of soil quality and plant parameters were dependent of soil type, we highlighted that soil reaction may be an important criterion to consider when organic P fertilizers are applied in combination with inorganic ones. This supported our hypothesis that soils' and plants' response to the combined use of PM and RP in terms of biomass production and P use efficiency may depend on soil reaction. We also found that both PM and PMRP enhanced P uptake which was attributed to increases in soil available P, probably due to i) the role of PM in increasing net negative charges in the soil that decreases the adsorption of applied P and also, ii) the formation of organic anions, which compete with inorganic P for the same adsorption sites on soil constituents (Abbasi et al 2015; Alloush 2003).

On the other hand, regarding C dynamics this current study showed applying easily available C by both, PM and PMRP treatments as demonstrated increasing microbial biomass C and P. The positive effect of C input by applying manure on soil microbial biomass has been well reported (Li et al., 2018b; Lupwayi et al., 2019; Ma et al., 2016;

Malik et al., 2013). Exogenous C input is related to the energy resource and P is a nutrient resource, however, the turnover of soil C could strongly be coupled to the turnover of P in microbial biomass (Chen et al., 2019). While microbial biomass C showed high correlation with plant_{derived} C from the rhizosphere soil, microbial biomass P was highly correlated with PM_{derived} C (Appendix 3). This indicated that soil microbial biomass mediates accumulation and dynamics of SOC (Richardson et al., 2014). The relationship between plant_{derived} C, microbial biomass and SOC allocation plays a key role understanding C cycling within the rhizosphere. Accordingly, we found that PM and PMRP strongly increased root biomass which may explain the enhancement of soil microbial biomass supported by the significantly correlations between both parameters ($r=0.73$; $r=0.56$, $p<0.001$, Appendix 3). In addition, we found that both microbial biomass C and P were highly correlated with shoot and root P uptake (Appendix 3). Even though carbon input were different by using entirely PM and its mixture with RP, contributions to rhizosphere SOC from PM_{derived} C didn't showed differences between both treatments ranging 36 to 47% among soils. The addition of carbon-rich residues can lead to a net decrease in SOC (Kirkby et al., 2014, 2011), however PM compost as high nutrient availability manure (Poblete-Grant et al., 2019) minimizes the promoting effect and promoted the sequestration of carbon into new SOM.

Data obtained from several studies quantifying C derived from manure differs by type and quality of the organic amendment as well as soil characteristics (Bol et al., 2004; Ma et al., 2013; Zhang et al., 2015). Considering density fractionation as a powerful step for quantitatively isolating SOM pools with relevance to soil function (Adams et al., 2018; Crow et al., 2007; Stahr et al., 2018) it has been reported that light fraction more sensitive to changes in management (Bremer et al., 1994). Our study showed that C:N decreased in the order FLf > OLf > HF. This correspond to mostly studies which reported low C:N

ratio within the heavy fraction whereas the free light fraction has a high C:N ratio (Adams et al., 2018; Stahr et al., 2018). Both poultry manure treatments contributed highly by increasing the FLf proportion, being ~1.8-fold higher for the moderately acid as compared to alkaline soil. In addition, PM alone or combined with RP improved the amount of stored soil C, being highly correlated with $PM_{\text{derived C}}$ from the FLf and Hf. This supports our third hypothesis that RP and PM influence differently SOC storage and distribution depending on soil type. Nonetheless, soils amended with PM, allocated the $plant_{\text{derived C}}$ mainly in the FLf, while PMRP favored its allocation in the Hf. Considering that the organic matter stability increases from FLf (active pool) to Hf (mineral-associated SOM) (Von Lützow et al., 2007), thus PMRP could represent an interesting alternative to store SOC. Finally, the mentioned before partially supported our fourth hypothesis that the joined application of RP and PM induces synergistic effects by enhancing C transfer from plant to soil, P availability and biomass production as compared to their use as single amendment, while the mixture didn't show synergistic effects on biomass production.

The understanding of C and P dynamics at the root-soil interphase is essential for management of C sequestration and agricultural sustainability without sacrificing productivity. This understanding needed to be linked to soil microbial biomass and organic C (SOC) pools, which are critical for quantifying C fluxes (Eze et al., 2018).

Our study assessed the effect of two types of P amendments, phosphate rock and poultry manure in solitary and mixed, on Andisol (long-term) and Luvisol (short-term) soil types. We observed that application of PM and RP amendments showed differences increasing soil P availability and SOC given by differences on soil parental material origins, initial soil pH, P status, and SOM content. Soil pH highly influenced soil reaction with the amendment type used, for example we found that RP amendment was positively effective in moderately acid soils while in alkaline soils its efficacy was almost negligible. Organic

matter content also plays an important role on soil reaction through acidifying and chelation mechanisms, we observed that Luvisols contained 2% of SOM compared to Andisols where SOM ranged from 11 to 20%. Moreover, physical soil properties could influence soil reaction, Andisols are known as high P retention soils given mainly by its allophane content, Luvisols are characterized by its low P retention. Those differences on soil particle constituents are also important in terms of C storage where Andisols stored more C due to its higher specific surface area of short-range-order minerals content compared to Luvisols (Cambisol) (Calaby-Floody et al., 2015). Even though we observed that in all soil types available P and SOC was increased, it is important to consider that the intensity of those changes given by the application of PM in solitary or mixed with RP could be differ regarding differences on soil physicochemical properties (Appendix 5).

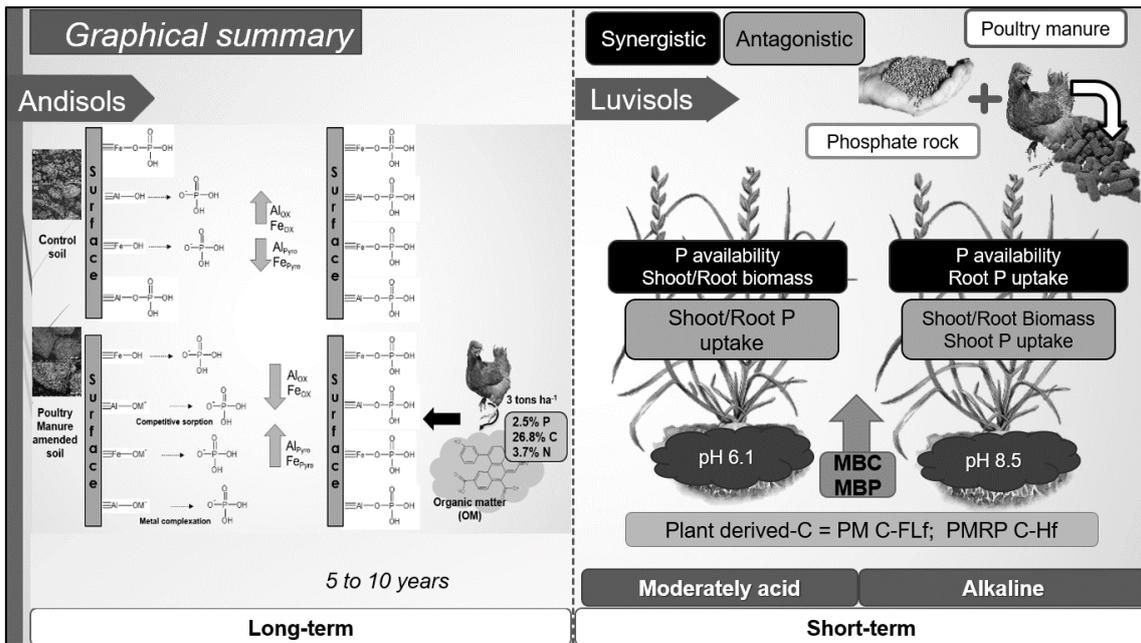
5.2 Concluding remarks and future directions

Poultry meat industry generates high sub-product amounts that can be returned to soils, as a possible strategy to mobilize native soil P accumulates by intensive fertilization history in agroecosystems. Phosphate rock depletion in addition to the increasing of world's demography levels represents a massive challenge for the current agricultural systems. Our research showed that long-term poultry manure compost applications in field conditions increased significantly soil total carbon content and soil aggregation. Promoting larger particle size by poultry manure application led to an increase in soil organic C and available P content. We attributed increases in P availability by decreased adsorption onto Fe and Al oxides and increased organic matter metal complex formation. In addition, we found that short-term inputs (7 weeks) of poultry manure compost increased highly soil P availability, consequently improving above and belowground plant biomass production on both soil types, while phosphate rock amendment had

limited effects on soil P fractions and positive effects on aboveground plant biomass production only in moderately acid soil. Moreover, this research reported for first time the synergistic and antagonistic effects of the combined use of PM and RP, being positive for soil available P in both soils, while for plant parameters, synergistic and antagonistic effects were soil type dependent. On the other hand, our study clearly indicated that both poultry manure compost and its mixture with phosphate rock applied in two different soil types increased total SOC contents and labile organic C (FLf, OLf and microbial biomass C). Moreover, the greatest contribution to stored soil organic C were observed as poultry manure compost-derived C. Furthermore, applying PM favored FLf allocation of the plant-derived C, while PMRP favored its allocation in the Hf, highlighting that PMRP could represent an interesting alternative to store SOC. Application of poultry manure compost alone or combined with phosphate rock is a good way of building the soil organic matter content, which not only enhances soil microbial biomass, it's application also improves soil available P and plant biomass and P use efficiency.

We therefore suggest that fertilizer strategies through the combination of organic and inorganic fertilizers must be tested in different soil types by quantifying their synergistic and antagonistic effects. The current study provided valuable information assessing phosphorus and carbon dynamics, as well as distinguish soil organic carbon derived from plant and poultry manure in the rhizosphere and allocated in SOM pools. Further field experiments considering different soil with different properties are needed to account realistic and effective effect of poultry manure alone and its mixture with phosphate rock to assess long-term soil C stabilization and P availability allocated in SOM pools, including plant productive and quality parameters.

5.3. Graphical summary



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<https://doi.org/10.1016/j.still.2016.11.007>

Appendix

Appendix 1. Publications and presentations

Publications:

1. Patricia Poblete-Grant, Philippe Biron, Thierry Bariac, Paula Cartes, María de La Luz Mora, and Cornelia Rumpel. Synergistic and Antagonistic Effects of Poultry Manure and Phosphate Rock on Soil P Availability, Ryegrass Production, and P Uptake. *Agronomy* 2019, 9, 191; doi:10.3390/agronomy9040191

2. Patricia Poblete-Grant, Jonathan Suazo, Leo Condron, Cornelia Rumpel, Rolando Demanet and María de La Luz Mora. 2019. Soil available P on southern Chilean pastures under composted poultry manure is regulated by soil organic carbon and, iron and aluminum complexes. *Geoderma regional*. Submitted.

3. George, TS, Giles, CD, Menezes-Blackburn, D, Condron, LM, Poblete-Grant, P, et al., & Haygarth, PM. Organic phosphorus in the terrestrial environment: A perspective on the state of the art and future priorities. *Plant and Soil*, doi:10.1007/s11104-017-3391-x, 2017.

4. Velásquez, G., Calabi-Floody, M., Poblete-Grant, P., Rumpel, C., Demanet R., Condron, L., Mora, M.L. 2016. Fertilizer effects on phosphorus fractions and organic matter in Andisols. *Journal of Soil Science and Plant Nutrition*, 16, 294 – 304.

5. Redel, Y., Cartes, P., Demanet, R., Velásquez, G., Poblete-Grant, P., Bol, R., Mora, M.L. 2016. Assessment of phosphorus status influenced by Al and Fe compounds in volcanic grassland soils. *Journal of Soil Science and Plant Nutrition*, 16, 490 – 506.

International and national presentations

1. Patricia Poblete-Grant, Philippe Biron, Thierry Bariac, Paula Cartes, María de La Luz Mora, and Cornelia Rumpel. How does combined use of poultry manure and phosphate rock amendments affect soil p dynamics, plant biomass production? 8th

International Symposium of Interactions of Soil Minerals with Organic Components and Microorganisms. Seville, Spain, June 23-28 2019.

2. Rolando Demanet, Ana Luengo Escobar, Cecilia Paredes, Marcela Calabi, **Patricia Poblete-Grant**, Cornelia Rumpel, María de la Luz Mora. Effect of different sources of phosphorous on the production of a permanent grassland of *Lolium perenne* in an Andisol from Southern Chile. 6th Symposium on Phosphorus in Soils and Plants (PSP6). Leuven, Belgium. 10-13th September 2018.

3. Patricia Poblete-Grant, Philippe Biron, Patricia Richard, Thierry Bariac, María de La Luz Mora, and Cornelia Rumpel. How does the combined use of poultry manure and rock phosphate affect P dynamics in planted soil? European Geosciences Union General Assembly. Vienna, Austria. 8– 13 April 2018.

4. Poblete-Grant, Patricia; Cornelia Rumpel, Leo Condrón, Rolando Demanet and María de La Luz Mora. Soil organic matter and dynamics of phosphorus in pastures soils after several years of composted poultry manure application. 6th International Symposium on Soil Organic Matter. 3 - 7 September, 2017. Rothamsted Research, Harpenden, England.

5. Poblete-Grant, Patricia; Bobadilla, Katterine; Condrón, Leo; Rumpel, Cornelia; Demanet, Rolando and Mora, María de la Luz. P development status of grassland Andisol after contrasting years of poultry manure application. 3er Taller Latinoamericano de PGPR y 2do Workshop en Biotecnología y Medioambiente. Pucón. 28th November – 02nd December 2016.

6. Poblete-Grant, Patricia; Condron, Leo; Demanet, Rolando and Mora, Maria de la Luz. Organic phosphorus fate in grassland Andisol with a poultry manure application history. Workshop of Organic Phosphorus. 5-9 September, 2016. Windermere, England.

7. Poblete-Grant, Patricia; Montalbán-Torres, Nicole and Mora, María de la Luz. Phosphate adsorption dynamics on two contrasting soil management. 5th International workshop advances in science and technology of bioresources, Chile, Pucón, 2015.

8. Poblete-Grant, Patricia, Montalbán-Torres, Nicole; Redel, Yonathan; Demanet, Rolando and Mora, María de la Luz. Changes in phosphorus-fractions and phosphatase-activity in ryegrass with two different phosphorus sources. Poster presentation. Soil Interfaces for Sustainable Development Conference. 5-10 July, 2015. Canada.

Honours and Awards:

International Doctoral Scholarship (Conicyt). Internship in AgroParis Tech (10th may 2017 – 17th july 2018).

SOM2017 Award for “**Best student oral presentation from an eligible nation**”. 6th International Symposium on Soil Organic Matter.

Lincoln University, New Zealand, for three months (March-June 2015) aiming to improve english skills and research. Becas en el Extranjero para Doctorandos FRO1204.

Conicyt National PhD scholarship n°21150715, since 2015 to 2018.

Appendix 2. Laboratory incubation assay:

Materials: Soil was collected from “Santa Elena” farm located at 50 km in Teodoro Schmith state, in Araucanía region, Chile. Soil sample belongs to Barros Arana series, member of the medial Family on skeletal Sandy, Mesic of Typic Hapludand (Andisol).

Table A1. Soil chemical characterization

Soil type	pH	Total N	P olsen	Total K	SOM
	H ₂ O	mg kg ⁻¹			
Andisol	5.72	21	10	125	150

Soils were sieved (2mm), air dried, and stored before experiment initiation. Selected soil properties are listed in Table A1. The poultry manure (PM) properties were described in table A2.

Table A2. Chemical properties and inorganic (Pi) and organic P (Po) in fractions sequentially extracted from poultry manure (PM) compost PM

pH	Total N	Total C	Moisture	H ₂ O		NaHCO ₃				NaOH		HCl		Residual
				Pi	Po	P g kg ⁻¹				Pi	Po	Pi	Po	
H ₂ O	g kg ⁻¹		%			Pi	Po	Pi	Po	Pi	Po	Pi	Po	Pt
8.77	25.01	267.79	56.07	1.27	3.26	2.79	3.65	5.56	2.25	1.12	3.29	1.20		

Methods: 1000 g of bulk soil was moistened to field capacity in polyethylene cups. PM was then thoroughly mixed with remainder of the bulk soils to provide 0, 2.5, 5.0 and 7.5 g P kg⁻¹ dry soil: treatments will be referred to as Control, PM100, PM200 and PM300, respectively. Subsamples were taken at 1, 3, 5, 7, 10 days after incorporation. One soil, four manure concentration, five sampled dates and three replications resulted in a total of 60 experimental units. The prepared soil/PM samples were incubated at 25°C. At each sampling date, samples were stored at -20°C until analysis. P available concentration was measured according to the technique of Olsen based on an extraction with a solution of

0.5 M sodium bicarbonate at pH 8.5, using molybdate colorimetry of Murphy and Riley (1962). In addition, an incubation of PM and phosphate rock (RP) (BIFOX) was performed to measure PM effect in P release of RP. Ratio was 50%PM + 50%RP and 75%PM + 25%RP. Samples were moistened in polyethylene bags and were incubated during 30 days at 25°C. Phosphorus content was measured after the extraction with a solution of 0.5 M sodium bicarbonate at pH 8.5 during 16 hours and determined using molybdate colorimetry.

Results: Available P release from PR was increased during PM decomposition incubated at 30 days (Figure A1). This increase was calculated with the difference between initial values of PM and RP and P content in mixture in each sampled day. Initial content of PM showed values of 2440.13 and RP 156.23 of available P (mg kg^{-1}) respectively. After 1 day, the P release effect of PM under RP showed a significantly increase in P available content. The differences calculated showed that with a mixture of 75% PM and 25% RP was the best alternative to increase the release of available P. This could be due by organic acids released during PM decomposition and microorganisms present in this amendment. This is in agreement with the study performed by Khan and Sharif, (2012) where they conclude that the addition of fresh PM with RP enhance the available P content attributing that to the solubilization of inorganic P involving the secretion of organic acids, lowering of pH as a result of acid production, ion chelation and exchange reaction. Abbasi et al. (2015), indicate that the different types of organic manure increase the microorganisms content, and they found an important increase of P release from RP when they applied in combination with PM (80%) compare to RP alone.

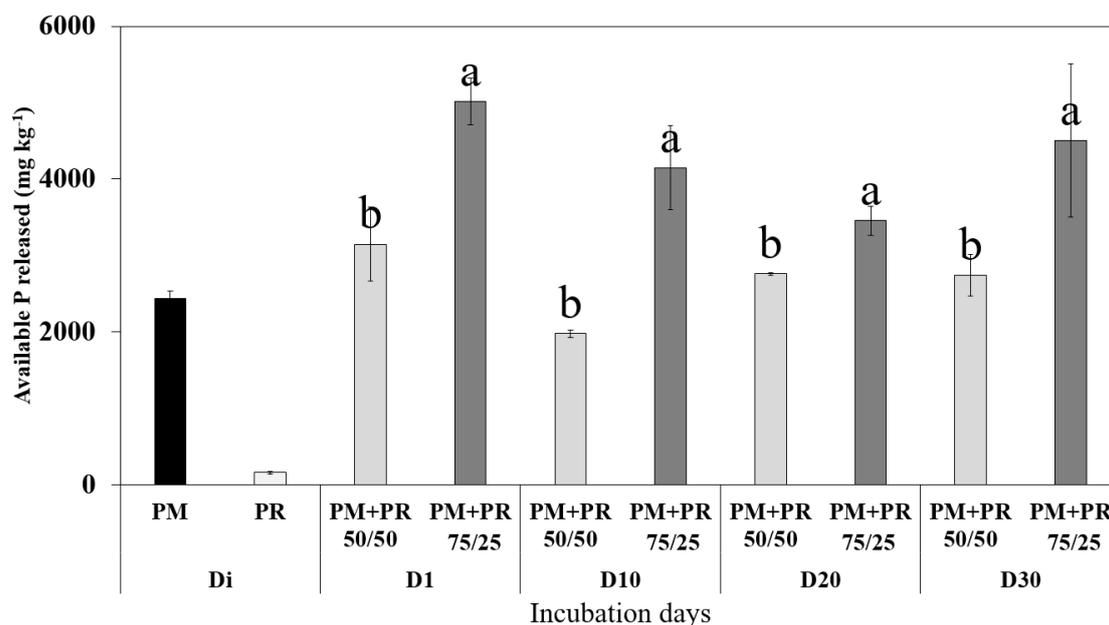


Figure A1. Release of available P derived from phosphate rock during decomposition of poultry manure in 10 days of incubation at 25°C. Letters a and b indicate significant differences ($p < 0.05$) between the means between different treatments (ANOVA followed by Tukey test).

The incubation of soil with different rates of PM resulted in an important increase of soil available P concentration from the first day after establishment (Figure A2). P release capacity of soil amended with PM ranged between 10.41 to 31.83 for PM100, 36.65–65.97 for PM200 and 44.58–131.97 for PM300. The highest values of P concentration were due to the high PM rates used in this study. In practical terms treatments represented 170, 340 and 510 Mg ha⁻¹ of PM, which are very high P input (4250, 8500, 12750 kg P ha⁻¹). Olsen P was increased with the increase of PM rate. Showing the highest P concentration with PM300 at the 3rd day of sampling. Then, available P decreased for 200PM and 300PM treatments, while maintaining higher than the control. In the study performed by Khan and Sharif (2012), the available P increased significantly with PM plus RP. Dikinya and Mufwanzala (2010), also found a significant increase of available P with increasing application rate in three different soils.

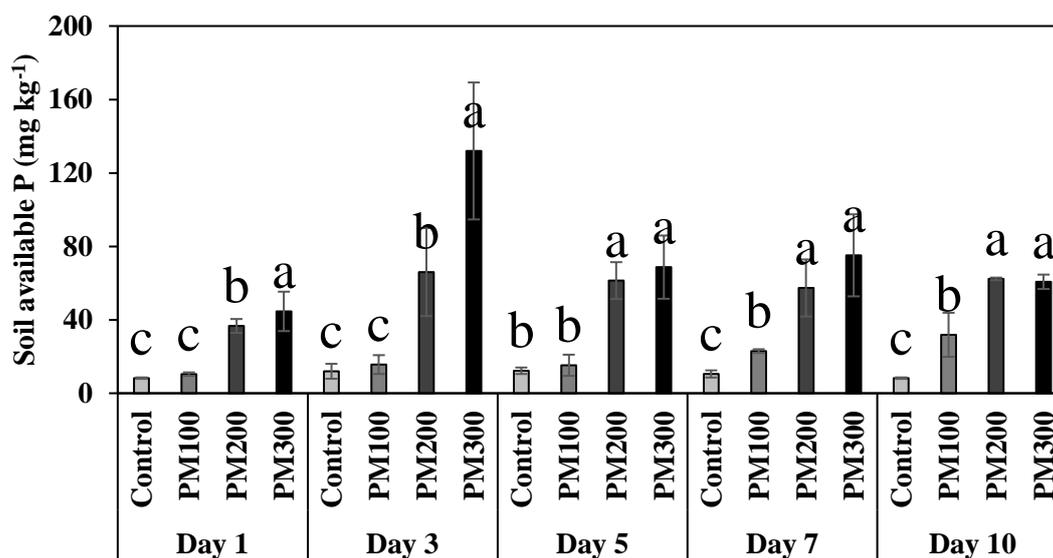


Figure A2. P release capacity of different PM rates applied to a soil incubated (average over three replicates) under controlled laboratory conditions at 25°C. The legend on x axis refers to the different rates: Control, 100PM (2.5 g P per soil kg), 200PM (5g P per soil kg) and 300PM (7.5 g P per soil kg) in different sampling days (1, 3, 5, 7 and 10). Letters a, b and c indicate significant differences ($p < 0.05$) between the means between soils with different rates of poultry manure application and control (ANOVA followed by Tukey test).

Appendix 3. Relationship between soil and plant parameters from control and soil amended with poultry manure compost (PM), phosphate rock (RP), and their combination (PMRP). Legend: MBP, microbial biomass P; MBC, microbial biomass C; readily Pi, readily available inorganic P; readily Po, readily available organic P; mod-Pi, moderately inorganic P; mod-Po, moderately organic P; Less-Pi, less available inorganic P; Less-Po, less available organic P; shoot (g), shoot biomass production; root (g), root biomass production; shoot-P, shoot P concentration; root-P, root P concentration; Shootup-P, shoot P uptake; Rootup-P, root P uptake. * represent significant correlations ($p < 0.05$), $n=16$.

	MBP	Readily-Pi	Readily-Po	mod-Pi	mod-Po	Less-Pi	Less-Po	Residual P	shoot (g)	root (g)	shoot-P	root-P	Shootup P	Rootup P
MBP	1.00	0.72*	0.53*	0.22	-0.25	0.13	0.56*	0.45	0.54*	0.46*	0.59*	0.79*	0.73*	0.71*
MBC	0.60*	0.78*	0.68*	0.53*	-0.42	-0.22	0.15	0.10	0.62*	0.73*	0.70*	0.48*	0.70*	0.63*
Plant-derived C	0.39	0.67*	0.66*	0.47*	-0.32	-0.55*	0.47*	-0.02	0.47*	0.70*	0.72*	0.58*	0.68*	0.64*
PM-derived C	0.78*	0.84*	0.61*	0.24	-0.14	-0.06	0.72*	0.59*	0.54*	0.57*	0.65*	0.86*	0.79*	0.80*
N-FLf	0.50*	0.80*	0.75*	0.48*	-0.39	-0.46*	0.46*	0.09	0.56*	0.78*	0.80*	0.70*	0.80*	0.78*
N-OLf	0.49*	0.32	0.03	-0.15	0.24	0.31	0.50*	0.71*	0.03	0.12	0.15	0.50*	0.26	0.28
N-Hf	0.33	0.34	0.19	0.08	0.09	0.05	0.41	0.45	-0.06	0.24	0.25	0.47*	0.32	0.37
C-FLf	0.44	0.80*	0.79*	0.59*	-0.50*	-0.52*	0.33	-0.01	0.58*	0.82*	0.80*	0.64*	0.81*	0.78*
C-OLf	0.41	0.25	-0.04	-0.19	0.26	0.37	0.45*	0.67*	-0.02	0.04	0.09	0.41	0.20	0.22
C-Hf	0.40	0.41	0.19	0.05	0.06	0.14	0.42	0.51*	0.00	0.22	0.27	0.51*	0.37	0.43
Plant-derived C-FLf	0.44	0.77*	0.80*	0.69*	-0.59*	-0.54*	0.13	-0.17	0.59*	0.79*	0.69*	0.48	0.79*	0.70*
PM-derived C-FLf	0.62*	0.87*	0.81*	0.59*	-0.48*	-0.46*	0.53*	0.18	0.50*	0.79*	0.83*	0.83*	0.87*	0.87*
Plant-derived C-OLf	0.45	0.22	-0.07	-0.26	0.30	0.48*	0.39	0.73*	-0.02	0.04	0.02	0.49*	0.22	0.31

PM-derived C-OLf	0.80*	0.86*	0.67*	0.34	-0.23	-0.11	0.70*	0.50*	0.54*	0.64*	0.72*	0.88*	0.83*	0.85*
Plant-derived C-Hf	0.30	0.61*	0.44	0.32	-0.31	-0.13	0.28	0.21	0.20	0.56*	0.55*	0.33	0.41	0.48*
PM-derived C-Hf	0.55*	0.84*	0.78*	0.58*	-0.43	-0.49*	0.53*	0.18	0.49*	0.77*	0.78*	0.81*	0.86*	0.85*

Appendix 4. Nutrient concentration of roots and shoots after 7 weeks of ryegrass growth in control soil and soil amended with poultry manure compost (PM), phosphate rock (RP), and their combination (PMRP).

Soil	Treatments	Shoots										Root									
		Ca		K		Mg		Na		As		Ca		K		Mg		Na		As	
		g kg ⁻¹										g kg ⁻¹									
		mg kg ⁻¹										mg kg ⁻¹									
Moderately acid	Control	8.1	±1.4	31.7	±1.3	2.4	±0.3	1.4	±0.1	NQ	NQ	3.5	±0.6	5.8	±2.7	1.5	±0.3	0.9	±0.2	4.2	±0.8
	RP	6.9	±1.1	46.1	±5.2	2.3	±0.2	1.9	±0.4	NQ	NQ	4.8	±0.4	3.9	±0.8	1.1	±0.1	0.7	±0.2	2.9	±0.5
	PM	5.3	±0.5	42.6	±2.9	2.7	±0.1	4.2	±1.3	NQ	NQ	9.7	±1.5	5.5	±1.7	1.9	±0.1	0.5	±0.0	4.8	±0.9
	PMRP	6.0	±0.2	36.9	±0.5	2.3	±0.1	1.4	±0.1	NQ	NQ	8.6	±1.2	6.2	±2.0	1.7	±0.1	1.0	±0.5	4.3	±0.9
Alkaline	Control	11.3	±1.0	37.5	±4.3	2.2	±0.3	1.8	±0.3	NQ	NQ	34.7	±7.6	3.8	±0.2	3.9	±1.7	0.6	±0.2	5.3	±1.7
	RP	22.5	±8.0	50.4	±3.7	2.7	±0.2	1.4	±0.1	NQ	NQ	29.4	±4.8	9.8	±2.0	4.1	±0.8	1.1	±0.4	5.7	±0.2
	PM	6.1	±0.8	46.1	±7.4	2.6	±0.6	2.9	±0.8	NQ	NQ	25.0	±6.4	4.5	±0.9	2.3	±0.3	0.9	±0.2	4.5	±0.9
	PMRP	6.9	±1.3	47.5	±3.6	2.3	±0.3	2.6	±1.2	NQ	NQ	30.4	±3.3	3.8	±1.0	2.3	±0.3	0.8	±0.3	3.9	±0.6

Appendix 5. Carbon stock in different compartments (soil-plant) and C loss from soils amended with composted poultry manure (PM), phosphate rock (RP) and their combination (PMRP) after 7 weeks of ryegrass growth.

