UNIVERSIDAD DE LA FRONTERA

Facultad de Ingeniería, Ciencias y Administración

Programa de Doctorado en Ciencias de Recursos Naturales

THE EFFECTS OF COMBINED CITRUS WASTES-CATTLE MANURE ADDITION ON PHOSPHORUS AVAILABILITY IN SOIL AND ITS POTENTIAL USE AS A BIOFERTILIZER IN PASTURES

DOCTORAL THESIS IN FULFILLMENT OF THE REQUERIMENTS FOR THE DEGREE DOCTOR OF SCIENCES IN NATURAL RESOURCES

CECILIA DE LOURDES PAREDES NEGRON

TEMUCO – CHILE

2020

“THE EFFECTS OF COMBINED CITRUS WASTES-CATTLE MANURE ADDITION ON PHOSPHORUS AVAILABILITY IN SOIL AND ITS POTENTIAL USE AS A BIOFERTILIZER IN PASTURES”

Esta tesis fue realizada bajo la supervisión del director de Tesis Dra. MARÍA DE LA LUZ MORA GIL, perteneciente al Departamento de Ciencias Químicas y Recursos Naturales de la Universidad de La Frontera y es presentada para su revisión por los miembros de la comisión examinadora.

CECILIA DE LOURDES PAREDES NEGRON

Dr. Andrés Quiroz Cortez
Director del Programa de Doctorado en Ciencias de Recursos Naturales

----
Dra. María de la Luz Mora Gil
(Advisor)

Dra. Siobhan Staunton
(Co-Advisor)

Dr. Mauricio Escudey

Dra. Mónica Rubilar Díaz
Directora Académica de Postgrado
Universidad de La Frontera

Dr. María Cristina Diez Jerez

Dr. Fernando Borie Borie

Dra. Mara Cea

Para mi pequeña Aurora y a mis queridas abuelas

por enseñarme el valor del conocimiento

Acknowledgements/Agradecimientos

Creo que para nadie es fácil comenzar con esta sección porque son tantas las personas que, de alguna u otra forma, a través de una palabra de aliento, compartiendo conocimientos, soporte para el cuidado de los hijos o simplemente un gesto, colaboraron para que yo pueda finalizar éste Doctorado. Sin embargo, no cabe duda que mis primeros agradecimientos son para mi familia, Carlos, Matías y Vicente, sin su apoyo, paciencia y comprensión culminar está etapa hubiese sido muy difícil. Gracias por el tiempo dado en el que me dediqué a ser estudiante y dejé de lado mi rol de madre y compañera. Quiero agradecer especialmente a Carlos, por su constante motivación, respaldo y cariño. Gracias por creer en mí y ayudarme a cumplir con ésta importante etapa de mi vida. Agradezco de manera especial a mi tutora de tesis, Dra. María de la Luz Mora, por haberme dado la posibilidad de realizar éste Doctorado, guiándome con sus conocimientos en cada etapa de esta investigación doctoral. Le agradezco también el haberme dado su apoyo económico y facilitarme los medios para llevar a cabo todas las actividades propuestas durante el desarrollo de esta tesis. Agradeceré infinitamente las oportunidades brindadas para realizar pasantías internacionales y por permitirme vivir experiencias tan importantes que contribuyeron a enriquecer mi formación como investigadora y a nivel personal.

Quiero agradecer infinitamente a la Dra. Siobhan Staunton por su paciencia, disponibilidad y por compartir su amplio conocimiento y experiencia, enriqueciendo el trabajo de esta investigación. A nivel personal, quiero agradecer sus palabras de apoyo y contención ya que me dieron la fuerza para volver a creer en mis capacidades y poder finalizar ésta tesis doctoral. Muchas gracias de todo corazón y espero podamos seguir colaborando en un futuro próximo.

Quiero agradecer, además, a todas las personas que trabajan en el laboratorio de suelo, Olguita, Sra. Carmen, Sra. Brígida, Patricia, Gerson y a Juanita, que, aunque ya no ésta con nosotros, siempre le estaré muy agradecida por su gran ayuda y disposición. A las secretarias del Doctorado, Gladys y Mariela, muchas gracias por escucharnos y siempre darnos palabras de aliento. A mis compañeros de doctorado que ahora son flamantes Doctores, Patty y Francisco. Quiero agradecer además a mis compañeras del laboratorio de Molecular, Anita y Karina, nuestros cafés y conversaciones me alegraron la vida en éstos tiempos de pandemia, muchas gracias a la dos. Además, quiero dar mis más sinceros agradecimientos a mis compañeras Leyla y Sofia, han sido un soporte fundamental todo éste tiempo, siempre dispuestas a ayudar y a compartir sus conocimientos y a darme ánimo cuando más lo necesitaba.

Quiero agradecer a mis suegros, Elizabeth y Carlos y a mi cuñada Isabel, siempre dispuestos a cuidar a mis hijos cuando tenía que realizar mis pasantías, muchas gracias por el cariño y dedicación que le entregaron a ambos. A mi hermana Natalia, que también colaboró con el cuidado de sus sobrinos y cada vez que le solicité su ayuda.

Y, por supuesto, el agradecimiento más profundo es para mis padres, Julio y Patricia, por enseñarme el valor de las cosas, por siempre desear y anhelar lo mejor para mí y mi familia y entregarme las herramientas para desarrollarme como persona. ¡Los quiero mucho!

Summary and thesis outline

Resource scarcity and waste disposal are the challenges addressed by the Circular Economy (Araujo Galvão et al., 2018), therefore, innovative strategies must be implemented to management and use of waste to allow sustainable use of raw materials. The global food production is highly dependent on phosphorus fertilizers produced from phosphate rock (PR). Nevertheless, PR is a finite resource whose price has been increasing over the years, additionally the biggest supplies are mined in politically unstable places, posing risks to many countries that have little or no reserves (Blackwell et al., 2019). In consequence, it is crucial to search for alternatives sources of phosphate from organic residues, such as animal manures or agroindustrial wastes, since they are important sources of nutrients for crops, and improve overall soil quality (Diacono and Montemurro, 2010). This doctoral thesis is structured as follows. Chapter I is a general introduction to this research and presents the hypotheses and aims of this study.

Chapter II provides a brief literature review about the benefits of using cattle animal residues as nutrient source for improving soil quality and nutrient uptake by plants. Additionally, we explore the general background of citrus waste and its potential benefits to be used as an organic amendment.

Chapter III describes the study of effect of citrus waste alone (lemon (L) and mandarin (M) fruit), or in combination with cattle manure, on soil phosphorus availability through controlled laboratory incubations. The results showed that the combined amendment with citrus wastes enhanced the positive effect of cattle manure on soil water-extractable phosphorus and liming effect, with only a small effect on phosphatase activity.

Chapter IV presents the results of the study the combined effects of cattle manure (CM) and lemon peel (LP), on soil biological properties, plant nutrition and antioxidant responses in ryegrass grown under controlled conditions. The addition of both organic wastes improved both soil and plant

biochemical properties and caused a significant increase in P nutrition with a concomitant effect on ryegrass yields.

Finally, in the fifth chapter we present a general discussion of the results obtained in this study along with final remarks on the contributions of this study and further research.

Table of Contents

Figure index

Tables index

CHAPTER I

General introduction

1.1 General Introduction

The reserves of raw material to produce inorganic phosphorus (P) fertilizers are limited since they are mainly produced from mined phosphate rock - a non-renewable, finite and geographically restricted resource (Chowdhury et al., 2017). Furthermore, P is classified as an essential nutrient for plants, required in the large amounts for suitable development and growth (Malhotra et al., 2018). One important characteristic of P is its low availability due to its slow diffusion and high fixation in soils, limiting plant growth. At a local scale, around 60% of agricultural soils in southern Chile are Andisols, which are characterized by low pH and low available P concentrations, despite high total P contents (Mora et al., 2017; Gabriela Velásquez et al., 2016). This is because in Andisols P forms complexes between cations such as aluminium (Al) or iron (Fe) and with soil organic matter (SOM) causing the retention of huge quantities of P, reducing their availability in the soil (Borie and Zunino, 1983; Redel et al., 2016; Velásquez et al., 2016b) and limiting plant growth.

To cope with limited P supplies, plants have evolved various strategies to increase P-use efficiency or help to extract more P from the soil (Richardson et al., 2011; Schachtman et al., 1998; Shen et al., 2011; Simpson et al., 2011). These strategies include changes to cellular metabolism and root development; initiation of mycorrhizal associations (Borie et al., 2010; Gianinazzi et al., 2010); acidification of the rhizosphere which can promote the release of nutrients from soil minerals; the exudation of organic anions to mobilize sparingly available P by chelation and ligand exchange, and release of phosphatases to mobilize organic P by enzyme-catalyzed hydrolysis (Ryan et al., 2001; Wang and Lambers, 2020; Zhang et al., 2010). Organic anions can also stimulate microbial activity in the rhizosphere, which could also influence the availability of other minerals and nutrients (Adeleke et al., 2017; Menezes-Blackburn et al., 2016; Shahzad et al., 2015).

Organic residues such an animal manure, biosolids from human wastes, biochar crop and agrofood residues are used as organic fertilizer alternative to reduce dependency on chemical fertilizer use (Alobwede et al., 2019). Of these, manures, crop and agrofood residues are potentially the most important nutrient sources and could be used as alternative sources of organic fertilizers to provide plants with an optimal combination of macro and micronutrients. In addition, these amendments would improve the physical structure of soil, and so would be hugely beneficial for the agricultural industry. In June 2016, the Chilean Ministry of Environment passed the law N° 20.920, that establishes a framework for waste management and promoting recycling as part of their Circular Economy Model. The same is happening in Europe through its Circular Economy Action Plan (European Commission, 2015), whose aim is to promote resource efficiency with regards to the fertilizer sector in order to create new business opportunities for farmers, as well as help them become more competitive in recycling organic nutrients compared to purchasing inorganic fertilizers (European Economic and Social Committee, 2016).

One of the advantages of the use of organic residues is that they provide crops with nutrients over a long period of time in a slow and extended release process. Among organic residues cattle manure is an important example, where phosphorus (P) is one of the essential nutrients, containing different forms P compounds (organic and inorganic) and total P concentrations ranging from less than 1% to more than 3% (Abdala et al., 2015; Fuentes et al., 2006; Pagliari et al., 2020). In this context, several studies have been carried out to enhance the bioavailability of P from manure using biotechnological strategies such as phosphate solubilizing bacteria (PSB) and phosphatase-nanoclay complexes (Calabi-Floody et al., 2012; Fuentes et al., 2009; D. Menezes-Blackburn et al., 2016; Menezes-Blackburn et al., 2013). Furthermore, cattle manure is a good source of organic matter, improving soil properties such as water-holding capacity, soil structure, and aggregate stability. It also increases soil biota diversity and activity (Parham et al., 2003, 2002) improves soil sustainability and increases productivity of crop (Zhou et al., 2015).

Other types of residues are citrus waste, which produces large quantities of solid pulp and peel wastes. Commonly, the composition of citrus peel includes carbohydrates, nitrogen compounds, volatile compounds, essential oils, enzymes, pigments, vitamins, and short chain organic acids (Sharma et al., 2017). The predominant acids are citric and malic, but tartaric, benzoic, oxalic and succinic acids (Ladaniya, 2008) are also found. Currently this residue is mainly used as an animal foodstuff, thanks to its nutritional capacity. In addition, citrus peel has been used as organic soil conditioner or as substrate for compost production, improving organic matter content of soil (Chaudry et al., 2004; Gelsomino et al., 2010; Tuttobene et al., 2009).

1.2 Hypotheses and research objectives

The continuous use of P fertilizer has resulted in a high accumulation of total P, most of it being sparingly available for plant nutrition. In addition, we know that Andisols exhibit high organic P (Po) content and high inorganic P (phosphate) fixation, and only a small proportion is immediately available to plants. In addition, animal wastes such as cattle manure contain high amounts of unavailable forms of inorganic and organic P that could be solubilized trough biotechnological strategies or agronomic management. On the other hand, the ability of low molecular weight organic anions to release inorganic anions such as phosphate from soil and agricultural waste is well established, but studies involving their influence on the release of P from cattle manure are scarce. Moreover, the citrus waste contains low molecular weight organic acids, and the use of this residue to improve P availability from cattle manure aimed to be used as biofertilizer in pastures have never been reported. With this background the following hypotheses are proposed:

1.2.1 Hypotheses

1. The lability of phosphorus from cattle manure will increase in the presence of citrus waste because of the presence of organic acids (e.g. citric, oxalic and malic acids) from thus creating a possible new biofertilizer.

2. The addition of citrus waste and therefore organic acids will mobilize sparingly soluble soil phosphorus and improve phosphorus acquisition by ryegrass.

1.2.2 General Objective

To evaluate the effect of citrus waste on P availability in soil and cattle manure and its potential use as a biofertilizer for ryegrass growing in volcanic acid soils.

1.2.3 Specific Objectives

* To evaluate the release of available P from soil and cattle manure following application of citrus wastes at different rates under controlled laboratory
* To assess the effect of citrus waste alone or in combination with cattle manure on soil microbial biomass, enzymes activity and changes on soil community.
* To evaluate the efficiency of combined cattle manure+citrus waste for improving P acquisition by ryegrass to assess its possible use as a biofertilizer

CHAPTER II

Improving phosphorus efficiency in soil using cattle manure and citrus residues: a review

Improving phosphorus efficiency in soil using cattle manure and citrus residues: a review

Abstract

Phosphorus (P) is an essential input for many agricultural production systems, indispensable for plant, human and animal life and plays an important role in soil fertility and world food security. The major source of P in current use, phosphate rock, is a non-renewable resource. It is necessary to improve the sustainability of this resource and to increase the use of other P sources. Organic waste can be a valuable and inexpensive fertilizer and a source of plant nutrients. Animal manure is an organic waste that contains a large amount of in different forms with varying proportions of organic and inorganic forms of P which can be assimilated by plants. In addition, that the large organic matter content of manure leads to an increase in soil organic matter and various soil properties are improved including soil water-holding capacity, soil structure and aggregate stability, water infiltration rates, soil biota diversity and activity, and soil fertility and soil erosion. This review focuses on the chemistry of P in soils manures and on the benefits of manure use in soil and how its properties help to improve plant P uptake. The composition and agronomic potential of citrus waste is also reviewed.

2.1 Introduction

The application of phosphate fertilizers in agricultural practices has been used increasingly since the nineteenth century, these fertilizers are made from phosphate rock a non-renewable resource, therefore finite. Phosphorus (P) plays an essential role in agriculture and all forms of life: respiration, photosynthesis in green leaves, microbial turnover, and decomposing litter all require adequate levels of P in specialized forms (Haygarth et al., 2013). One of the main characteristics of phosphorus is its low availability due to slow diffusion and high fixation in soils. All of this means that P can be a major limiting factor for plant growth. Soil P exists under various chemical forms, including inorganic P (Pi) and organic P (Po), which differ widely in their behavior and fate in soils (Hansen et al., 2004; Shen et al., 2011), specifically about bioavailability, as various forms can undergo cycling at different rates, being retained in soils or made available to plants (Chen et al., 2003; Waithaisong et al., 2015).

Organic phosphorus mineralization plays a key role in soil P cycling and may be involved in P availability to plants. This is especially the case in soils of forest and grassland in which the total soil organic P fraction can represent up to 70 to 80% of the total P (Achat et al., 2010; Foster et al., 2002; Redel et al., 2016, 2007). Soil organic P includes P in living soil organisms (microbial biomass) and P in dead soil organic matter (Nziguheba and Bünemann, 2005). Consequently, in addition to physical– chemical processes (e.g. adsorption–desorption), two biological processes (fluxes) may play a role in soil P availability to plants by supplying phosphate ions in solution, either by gross mineralization of microbial biomass or by gross mineralization of P from dead soil organic matter (Liu et al., 2020; Zhang et al., 2010).

In agriculture, there is no known substitute for phosphorus. If the soil is deficient in phosphorus, food production will be limited unless this nutrient contribution is in the form of fertilizer (Balemi and Negisho, 2012). By, therefore, increasing crop yields is essential to an adequate supply of phosphorus. Global crop production depends on fertilizers derived from phosphate rock to maintain high crop yields. Population increase, changing dietary preferences towards more meat and dairy products, and the continuing intensification of global agriculture supporting this expansion will place increasing pressure on an uncertain, but finite supply of high-quality phosphate rock (Cordell et al., 2011).

To maintain soil fertility, it is necessary to add nutrients to agricultural systems; farmers use mineral fertilizers, organic waste, and various management techniques. Nowadays, recovering nutrients by reincorporating organic wastes in farming systems is a sustainable alternative that can contribute to restoring the natural environmental equilibrium (van der Wiel et al., 2019). Manure is used as a source of organic matter (OM) for improving soil quality properties, including water-holding capacity, soil structure, and aggregate stability, water infiltration rates, soil biota diversity, and activity as well as the traditional source of crop nutrients (Hati et al., 2008; Sharpley et al., 2004).

2.2.- Nutrient composition of manure

Throughout the history of agriculture, man has applied all kinds of organic residues to cultivated soils. A study, led by Bogaard et al. (2013), revealed that Europe's first farmers were fertilizing their crops with manure 8000 years ago, thousands of years earlier than previously thought. While in our continent, the Incans introduced natural fertilizers to maintain the fertility of the soil, without the use of fallow crop rotation. These fertilizers included camelid manure, island birds' manure (guano), or some other form of nitrogen-rich organic material (www.stanford.edu).

Animal manure is one of the most widely used organic sources of plant nutrients. It is one of the main land-applied organic sources of plant phosphorus (P) for crop production used globally. The gradual increase in intensive livestock production in the prairie regions has promoted the increased use of animal manures as a P source for crop production (Zhang and Schroder, 2014). Manure is an abundant source of macro and micronutrients for crop and grass production. The most abundant macronutrient found in manure is nitrogen (He and Zhang, 2014; Pagliari et al., 2020). Nitrogen can exist in either mineral or organic form in manure. In fresh manure, nitrogen exists primarily in the organic form, although bacterial processes can convert one form of N to another. Phosphorus is another macronutrient present in manure that exists in both mineral and organic forms. The mineral form is readily available to plants. The organic form must be mineralized before it is available to plants. About 70% of the P in manure is in the organic, unavailable form (Sharpley et al., 2004; Turner, B and Leytem, 2004).

About 90 % of the total Potassium (K) in manure is in a form that is available to the plant in the first year of application. About 75% of K in manure is water soluble. Calcium (Ca), Magnesium (Mg) and Sodium (Na) are nutrients required for plant growth. They mainly exist as dissolved ions and as exchangeable cation adsorbed on clay minerals and organic matter. Sulphur (S) exists as both organic S and inorganic S. The organic S must be mineralized to the sulphate form before it is available to plants (Eghball et al., 2002) .

Many different types of manure are available for crop production. The nutrient content of manures varies with animals, bedding, storage, and processing. The approximate nutrient composition of cattle manure samples is presented in Table 2.1.

Manures supplies many macro- and micro-nutrients for crop production, and they are also valuable sources of organic matter. Increasing soil organic matter improves soil structure, increases the water-holding capacity of coarse-textured sandy soils, improves drainage in fine-textured clay soils, provides a source of slow release nutrients, reduces wind and water erosion, and promotes growth of earthworms and other beneficial soil organisms (Zhang and Schroder, 2014).

On the other hand, commercialized inorganic products can be blended in various proportions to achieve the desired nutrient balance required by plants. However, the nutrients in manure are not balanced for optimal crop growth as they are in commercial inorganic fertilizers. For example, cattle feedlot manure contains about four times more nitrogen (N) than P, while plants require about eight times more N than P (Qian et al., 2004). Therefore, when manure is applied based on plant requirement for one nutrient, other nutrients are usually either over-or under-applied. According to Whalen and Chang (2001), continuous application of manure-based on crop N requirement results in the accumulation of P in the soil increases the risk of P loss from the soil system, through erosion, runoff, and leaching processes.

Table 2.1. Selected chemical properties in cattle manure samples considered in this literature review

Type of manure	Element							References
	N	P	Ca (g kg^{-1})	Mg	K	pH	C:N	
Beef Manure	5.9	0.57	-	-	5.31	-	25-30	Rayne and Aula (2020) US
	3.18	0.79	-	-	2.63	-	-	Sawyer, 2009, Iowa
	29	4.7	11	5	27	8.0	-	Pagliari and Laboski 2012, Wisconsin.
Cattle Manure	14	6	-	-	13	-	25	Lekasi et al., 2003, Kenya
	-	7.6	9.9	-	-	6–7	17.7	Fuentes et al., 2009, Chile
	22.8	5.2	0.56	1.39	8.39	6.8	12	Whalen et al., 2000, US
Dairy Manure	11	0.57	-	-	4.14	-	25-30	Rayne and Aula (2020), US
	7.37	1.97	-	-	7.59	-	13.9	Houlbrooke et al., 2011, New Zealand
	27	4.8	18	5	26	8	13	Pagliari and Laboski 2012, Wisconsin.
	17	5	-	-	-	-	24	Waldrip et al., 2012, US
	4.54	0.59	-	-	2.25	-	-	Sawyer, 2009, Iowa
Farmyard manure	9	4	-	-	8	6.8	12	Malik et al., 2013, Pakistan
	7	1.8	-	-	7	-	37	Ghosh et al., 2004, India
	133	23	-	-	202	-	-	Christensen and Sommer, 2013

2.3.- Manure application and its effects on the physical, chemical and biological soil properties

2.3.1.- Effect on physical soil properties

The incorporation of organic matter in the form of manures affects crop growth and yield either directly by supplying nutrients or indirectly by modifying soil physical properties such as stability of aggregates and porosity that can improve the root environment and stimulate plant growth (Jiang et al., 2018; Williams et al., 2017). Soil organic matter serves as a chelating agent and buffering material, affects the cation exchange capacity of soil, and is an important agent for soil aggregation (El-Nagar and Mohamed, 2019). A study of long-term manure application carried out by Arriaga and Lowery, (2003), showed that manure increased soil-water retention capacity and decreased differences in water retention between different erosion levels, concluding that manure application is a possible management alternative for restoring the physical properties of eroded soil. Hati et al., (2006) showed that application of 10 Mg ha^{-1} manure for three years in conjunction with the recommended rate of inorganic fertilizers to soybean in a soybean–mustard crop rotation improved soil physical conditions through better aggregation, increased saturated hydraulic conductivity, reduced mechanical resistance and bulk density, and enhanced root proliferation of soybean. Dunjana et al., (2012) evaluated the effects of cattle manure and inorganic N-fertilizer application on soil organic carbon, bulk density, macro-aggregate stability and aggregate protected carbon were determined on clay and sandy soils. These researchers that found the addition of cattle manure increased soil organic carbon, macro-aggregate stability and aggregate protected carbon in clay soils, nevertheless on the sandy soils only soil organic carbon increased with the addition of cattle manure, while bulk density, macro-aggregate stability and aggregate protected carbon were not significantly changed. Another study performed on a sandy clay loam soil showed that the utilization of cattle manure significantly decreased soil bulk density and particle density, and in contrast, porosity, organic matter content and saturated hydraulic conductivity increased (Rasoulzadeh and Yaghoubi, 2010). After 71 years of manure application on fine sandy loam soil, Blanco-canqui et al., (2014) showed that manure application reduced compactibility and increased water retention in this semiarid soil. It has been shown that conventional tillage can lead to a reduction of soil organic carbon with consequent degradation in soil physical properties (Büchi et al., 2017). Hati et al., (2008) conducted a study to found the long-term effects of inorganic fertilizer, manure and lime application on organic carbon content and physical properties of an acidic Alfisol under an annual soybean–wheat crop rotation. They found that after 29 crop cycles, the application of balanced fertilizer along with manure or lime improved soil aggregation, soil water retention, microporosity and available water capacity and reduced bulk density. They suggesting that soil management practices in acidic Alfisols should include integrated use of mineral fertilizer and organic manure or lime to maintain soil organic carbon status.

2.3.2.-Effect on chemical soil properties

Animal manure can supply most of the nutrients required by plants for optimal growth, since manure is rich in nitrogen (N), phosphorus (P), and carbon (C) and other secondary nutrients, such as calcium (Ca), magnesium (Mg) and potassium (K) (Pagliari and Laboski, 2012). In a long-term experiment, Liu et al., (2010), showed that manure application caused significant changes in soil chemical properties, increasing significantly nitrogen and total phosphorus, along with the content of phosphorus and available potassium. Zhao et al., (2009) investigated the changes of soil physical, chemical and biological characteristics in calcareous soil under a long-term field experiment. They reported that organic manures significantly influenced soil chemical properties increasing soil organic carbon, available nitrogen and available phosphorus. A liming effect can occur as a result of applied manure to soil because feed rations usually incorporate calcium carbonate (Eghball, 1999). So, Whalen et al., (2000) conducted a study to evaluated the effect of fresh cattle manure on soil acidity and nutrient availability in two acid soils. They reported that soils manure-amended significantly enhance the pH and this raised was attributed to buffering capacity from bicarbonates and organic acids in cattle manure. They also reported an increment on N, P, K, Ca, and Mg available, immediately after manure application and remained significantly high after the 8-week soil incubation. Additionally, others authors such as Tang et al., (2007), Naramabuye and Haynes, (2006) and Wong et al., (1998) have shown that addition of animal manures to acid soils increased pH. A long-term experiment was conducted in a sandy loam soil to study the effects of organic and inorganic sources of nutrients on grain yield (soybean and wheat) and nutrient soil status. The results indicated that combined use of inorganic fertilizer and manure increased soil organic carbon, total N, total P, Olsen P, and exchangeable K compared to the sole application of inorganic source (Kundu et al., 2007). Other study, carried out by Bhattacharyya et al., (2008), showed that the combined use of inorganic and organic fertilizer increased SOC, total N, Olsen P and exchangeable K, compared with data recorded under inorganic fertilizer alone.

2.3.3.- Effect on biological soil properties

Soil microbial biomass and its activity responds to soil management practices such as organic manure and inorganic fertilizers application (Böhme et al., 2005). It has been frequently reported that soil microorganisms and the processes that they control are essential in regulating soil properties because they mediated for example biological nitrogen fixation, residue decomposition, mineralization/immobilization turnover and, nutrient cycling (Schloter et al., 2003).

A field study was conducted by Liu et al., (2017), over two consecutive rice growing seasons to investigate the effect of applying inorganic and organic amendment on the soil nutrient status, enzyme activities and rice yields. The results showed that the combined use of inorganic and organic amendment exhibited the highest levels of microbial biomass carbon (MBC) and the activities of most enzymes. Additionally, they showed through redundancy analysis that MBC and available N were the key determinants affecting the soil enzyme activities and microbial community. Chu et al., (2007) described the effects of balanced versus nutrient-deficiency fertilization on soil microbial biomass, activity, and bacterial community structure in a long-term (16 years) field experiment. They found that, organic manure had a significantly greater impact on the biomass C and its activity, compared with mineral fertilizers. In addition, phylogenetic analysis showed that the change of bacterial community in organic manure-fertilized soil might not be because of the direct influence of the bacteria in the compost, but because of the promoting effect of the compost on the growth of an indigenous Bacillus sp. in the soil. During the evaluation of the long-term cattle manure application in soil, Parham et al., (2003) found that cattle manure application promoted the growth of bacteria, but not fungi, when it was compared to control soil. Moreover, observed that application of chemical fertilizers enriched the K-strategist bacterial community, while application of manure enriched both r- and K-strategists. The richness and evenness of the bacterial community were enhanced by manure treatment and treatments that included N and P, which were positively correlated with soil productivity. Bowles et al., (2014) investigated microbial communities and soil carbon (C) and nitrogen (N) availability on 13 organically-managed fields. Redundancy analysis showed distinct profiles of enzyme activities across the fields, such that C-cycling enzyme potential activities increased with inorganic N availability while those of N-cycling enzymes increased with C availability. In addition, fatty acid methyl esters (FAMEs) suggested that microbial community composition was less variable across fields than enzyme activities. Similarly, Zhang et al. (2012), investigated the effect of organic and inorganic fertilizers on microbial communities in alluvial paddy soil. They conclude that organic manures enhance the bacterial and fungal communities rather than actinomycetes; whereas, impact of chemical fertilizers was vice versa indicating deficiency of organic carbon and nutrients in the soil. Further, actinomycetes and Gram-positive bacteria seem to be the indigenous microbiota of paddy soil, which was dominated by Gram-negative bacteria and fungi after the addition of organic manures. Table 2.2 shows a review with comparative effects between inorganic and organic fertilizers and its response on biological, chemical and physical soil properties.

Table 2.2: Comparative effects of inorganic and organic fertilizers on biological, chemical and physical soil properties

Properties	Treatments	Effects	Author(s)
Biological	Cattle manure addition and rotation with green garlic to improve yield and reduce	The populations of soil bacteria and actinomycetes and the bacteria/fungi ratio increased significantly and soil enzyme activities were generally enhanced under treatments.	Yang et al. (2016)
	Long-term annual application of farmyard manure (FYM)	Manure addition enhanced microbial biomass, xylanase and invertase activity. In addition, FMY induced a shift of the microbial community towards a more bacteria-dominated community in the coarse sand fraction	Poll et al. (2003)
	3 years poultry manure, FYM, sesbania and gliricidia residues, control	Organic manures increased microbial biomass, activity, diversity, and C turnover	Dinesh et al. (2000)
	Long-term FYM, metal contaminated sewage sludge, NPK mineral fertilizer	Microbial Biomass-C and total bacterial numbers greater in the FYM-treated soil than in NPK and sludge-amended soils.	Abaye et al. (2005)

9-year study of traditionally composted FYM, 2 types of biodynamically composted manure	FYM increased microbial biomass, dehydrogenase activity, decomposition (cotton strips), but not saccharase activity, microbial basal respiration, or metabolic quotient. Biodynamic manure preparation decreased soil microbial basal respiration and metabolic quotient compared to non-biodynamic manure.	Zaller and Köpke (2004)
Composts of soymilk residues, cow manure, poultry manure, and sewage sludge	Soil respiration increased rapidly initially, but patterns differed among the composts. Composted soymilk treatment gave higher CO_2-evolution and lower metabolic quotient than the other composts.	Miyittah and Inubushi (2003)
Cattle manure, P, NP, NPK, and NPK plus lime	Effect on soil phosphorus levels, microbial biomass C, and dehydrogenase and phosphatase activities. Long-term application of cattle manure promoted microbiological activities and P cycling in soil.	Parham et al. (2002)

	Cattle manure, P, NP, NPK, and NPK plus lime	Effect on soil microbial populations and community structure. The richness and evenness of the bacterial community were enhanced by manure treatment and treatments that included N and P, which were positively correlated with soil productivity.	Parham et al. (2003)
Chemical	Swine and dairy manure	The consecutive application of animal manures was shown to have an effect on the transformation of crystalline into amorphous Fe- and Al-containing minerals, as evidenced by ammonium oxalate extractions of Fe and Al and confirmed by visual inspection of P K-edge XANES spectra, showing the presence of the diagnostic pre-edge feature of crystalline Fe (III)-minerals in the adjacent area and its absence in manured soils.	Abdala et al. (2015)
	Chemical fertilizer, organic manure and straw	The results demonstrated that continuous 17 year applications of manure significantly increased soil organic carbon and total nitrogen concentrations, microbial biomass carbon, microbial biomass nitrogen.	He et al. (2015)

	Farmyard manure and mineral fertilizer	Treatments affected the pseudo-total Al and Fe with higher contents in manure than mineral, whereas mineral induced higher oxalate/total ratios of both Fe and Al than manure and untreated. Treatment affected the P content with a pattern strongly influenced by soil type and depth.	Pizzeghello et al. (2014)
	Ten years of annual application Chemical fertilizer and organic manure	A positive relationship between C sequestration and organic manure input indicates that the soil has not reached its maximum capacity of C sequestration. Application of organic manure with chemical fertilizer was found to produce greater size of both labile and recalcitrant pools than application of mineral fertilizers alone.	Ding et al. (2012)
Chemical and biological	Cattle farmyard manure and ammonium nitrate	The application of composted FYM with burnt lime added increased the content of available phosphorus. The application of FYM stored under aerobic conditions with burnt lime added resulted in a significant increase in the alkaline phosphatase activity in soil but a significant inhibition of acid activity.	Lemanowicz et al. (2014)

Physical	Inorganic fertilizers and combined application of farmyard manure	The conjunctive use of recommended dose of fertilizer and farmyard manure (NPK + FYM) resulted in significant decrease of bulk density, soil penetration resistance and increase in hydraulic conductivity and mean weight diameter of the water stable aggregates and soil organic carbon content compared to the sole use of fertilizer NPK.	Bandyopadhyay et al. (2010)
	Green manure, farmyard manure, crop residue and urea	The use of green manure, farmyard manure and crop residues restore the damaged soil structure by increasing its organic carbon content, size and stability of aggregates, water retention and infiltration; and decreasing bulk density, dispersion ratio and soil strength.	Singh et al. (2007)
	Long-term manure applications	After 10 years of annual manure applications, bulk density and hydraulic conductivity increased slightly with erosion level. Water retention decreased with increased erosion level at deeper. Manure increased soil-water retention capacity and decreased differences in water retention between erosion levels, especially at low suctions (0 to 20 kPa).	Arriaga and Lowery (2003)

	Long-term beef cattle manure and inorganic N fertilizer application	Manure application improved soil properties at the 0- to 30-cm depth, but inorganic fertilization had no effects. Manure application reduced maximum Proctor bulk density by 6% and increased Proctor soil critical water content by about 17%. Manured plots also retained more soil water, resulting in about 16% more plant available water compared with nonmanured plots.	Blanco-canqui et al. (2014)
	Cattle manure application	After Nine months of cattle manure applications, soil bulk density and particle density decreased significantly. While, porosity, organic matter content and saturated hydraulic conductivity increased with application of 30 and 60 Mg ha^{-1}	Rasoulzadeh and Yaghoubi (2018)
Physical and biological	Chemical and manure fertilization	Manure applications greatly increased the contribution of microbial residues to soil organic carbon in small macroaggregates and large microaggregate. We conclude that higher manure input may promote soil aggregation and higher SOC storage, which is closely related to a greater microbial residues-mediated improvement of soil aggregate stability.	Ding et al. (2015)

2.4.-Phosphorus Compound in manure

The application of manure to soil can increase P fertility. Manure contains organic and inorganic P compounds at total P concentrations ranging from lower than 1 to greater than 3% (Abdala et al., 2015). The total P content in manure is very variable and nearly 70% of total P in manure is labile. The inorganic P pool in manures is large and has been reported to vary between 60% and 90%. It is essentially quite soluble and, therefore, readily available to plants (Sharpley and Moyer, 2000). In the fraction of inorganic P, are found calcium phosphates, phosphate ions in solution and phosphate ions adsorbed on the organomineral surface of the manure (Fuentes et al., 2006; Toor et al., 2006).

Phosphorus in manures can vary widely depending on animal physiology, species and age, composition of diets, duration of manure storage, moisture content and type of bedding material (Mcdowell and Stewart, 2005). Furthermore, the main differences in P quantity and species in manures is the product of P content variation in the diet, where P is added as an orthophosphate supplement or a vegetable ingredient that contains P in ATP, nucleic acids, phospholipids, phosphoproteins and phosphoglucides, where, the phytic acid was the most important phosphoglucides, with percentages of 60-80% of total P in grain (Flachowsky and Hennig 1990).

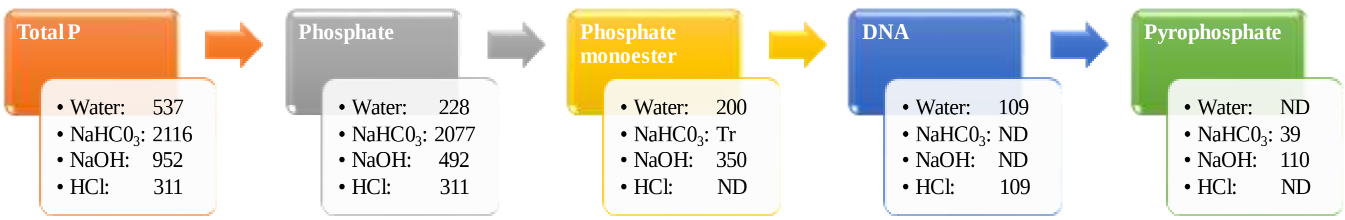
The water soluble P is the most available form, labile P, which is the next most available form, is easily liberated to be taken up by the plant, although some of the water soluble inorganic P is very mobile and thus susceptible to runoff in surface waters. This form is often referred to as the bicarbonate-extractable inorganic and organic P found in manure (Pagliari and Laboski, 2012). The third most available form of P is the sorbed inorganic and organic P that is characterized by its solubility in sodium hydroxide solution. The most recalcitrant form of P, is acid-extractable and generally exists in a stable residual form in the soil

(Sharpley and Moyer, 2000). On the other hand, dairy manure contained the greatest proportion of the inorganic water soluble form of P, relative to the other species of livestock (Pagliari et al., 2020; Pagliari and Laboski, 2012). In poultry manure, the largest portion of P was found to be in the acid-extractable or most stable form, whereas in the swine slurry, the greatest portion of P was found to be in the inorganic hydroxide extracted P form (Table 2.3). Since these forms have different plant availabilities, it could be inferred that dairy manure amended soil would have proportionately higher amounts of the readily available P form compared to swine manure amended soils. Likewise, the labile P, which is relatively more abundant in poultry manure than other manures would be available for plant uptake. The abundance of the readily available forms of P in dairy and poultry manure should be considered when planning applications in areas where water quality may be at risk due to topographical limitations.

Table 2.3: Inorganic and organic P fraction concentration and percentages in dairy, poultry and swine manures (Sharpley and Moyer, 2000)

P form	Extractant	Dairy Manure		Poultry Manure		Swine Slurry	
		mg P kg^{-1}	% of Total P	mg P kg^{-1}	% of Total P	mg P kg^{-1}	% of Total P
Inorganic	Water	2,030	51	7,430	26	6,045	18
	Bicarbonate	360	9	7,180	25	4,168	13
	Hydroxide	70	2	320	1	16,620	50
	Acid	60	1	9,320	32	3,294	10
Organic	Water	470	12	2,360	8	1,526	5
	Bicarbonate	90	2	1,100	4	657	2
	Hydroxide	420	11	470	2	281	1
Total	Inorganic	2,520	63	24,250	84	30,127	91
	Organic	980	25	3,930	14	2,464	8
	Residual	487	12	472	2	361	1
	Total	3,987		28,652		32,952	

Manure also contains large amounts of Po, such as phospholipids and nucleic acids (Turner and Leytem 2004), which can be released to increase soil Pi concentrations by mineralization. The organic P in animal manure it can reach 40% of total P (Hansen et al., 2004). Within the fraction of organic P, inositol phosphate compounds are the most abundant and among them, it has been found mainly inositol hexakisphosphate or phytate, corresponding to the salt of phytic acid (Fuentes et al., 2009; Mcdowell and Stewart, 2005). These compounds are considered recalcitrant due to its affinity for the surface of colloids, which decreases the possibilities of degradation and subsequent release of ions phosphate (Turner and Leytem, 2004). The fate of organic P in manure-amended soils is dependent on the chemical forms of the organic P. Orthophosphate diesters appear to be mineralized more rapidly than orthophosphate monoesters (Condron and Newman, 2011; Turner et al., 2002). Within the orthophosphate monoesters, myo-inositol hexakisphosphate (phytic acid) has a higher charge density than other monoesters such as sugar phosphates or mononucleotides, allowing it to form relatively stable complexes in soil that are protected from microbial degradation (Fuentes et al., 2009, 2008; Gerke, 2015). In order to determine P from manure, Turner and



Leytem (2004) used the Hedley fractionation procedure, which originally was developed for soils, but commonly applied to manures.

Figure 2.1. Concentrations (mg P kg^{-1} dry wt) of phosphorus compounds in sequential extracts of cattle manure from the Hedley fractionation procedure determined by Solution ^{31}PNMR Spectroscopy and ICP-OES Spectrometry. Adapted from Turner and Leytem (2004) (ND: not detected; Tr: trace)

2.5 The plant response to organic manure

It is well known that organic manures are traditionally used for supplying plant nutrients along with improving the soil conditions. In agriculture systems where nutrient availability is a major constraint for agriculture and food production, this management is especially important (Jamraiz Kiani et al., 2005). Recently, in small farmer fields in Northern Rwanda, a field experiments were conducted to evaluate the effect of mineral fertilizers, alone or in combination with farmyard manure on nitrogen fixation and grain yields bean. The results showed that manure and fertilizer application led to greater yields in all fields, and the largest yields were achieved when manure was combined with mineral fertilizer, increasing the grain yield and N and P uptake (Rurangwa et al., 2020). Other recent study of Kandil et al., (2020) carried out to evaluated the response of maize to organic manure and different potassium sources. They showed that organic manure applied at increasing rate, increased plant height, ear length, grains number/row, grains number/ear and 100- grain weight of the maize. Their results coincided with those reported by Agegnehu et al., (2016) who found that organic amendment increased crop productivity in terms of the yield and yield component of maize. Conversely, Hijbeek et al., (2017) using data from 20 field experiments in Europe, reported that crop yields increased by 2.0 Mg ha^{-1} due to synthetic fertilization and had negligible change in response to manure. Nevertheless, Cai et al., (2019) suggest that manure acts as a better fertilizer than synthetic fertilizer because increasing crop yields by improving soil properties. They found that manure alone or combined with synthetic fertilizer significantly increased crop yields, SOC, soil nutrients and soil pH compared with non-fertilizer soil. Overall, the application of manure could provide not only carbon but also different nutrients for crop uptakes, which determine the benefits of agricultural SOC sequestration and nutrient release (Demelash et al., 2014). The residual effect of manure application was visible after many years, leading to higher nutrient availability for crop growth (Cai et al., 2018).

2.6 Value-added products from agroindustrial citrus wastes

Citrus production and consumption worldwide have increased hugely over the past few decades and citrus processing industry plays an important role in the agro-industrial sector. The main producers are Brazil, China, European Union, United States and Mexico. In 2019/2020 more than 91 million Mg of citrus were produced, of which about 76% was consumed as fresh fruit and the remaining 24% was destined to industrial processing (USDA, 2020).

The production and export of citrics of Chile is composed by mandarins, oranges, lemons and grapefruits. The citrus industry as export sector shows a clear trend towards consolidation, this is reflected in export value, since, the season 2014, Chile exported a value close to 157,000 tons.

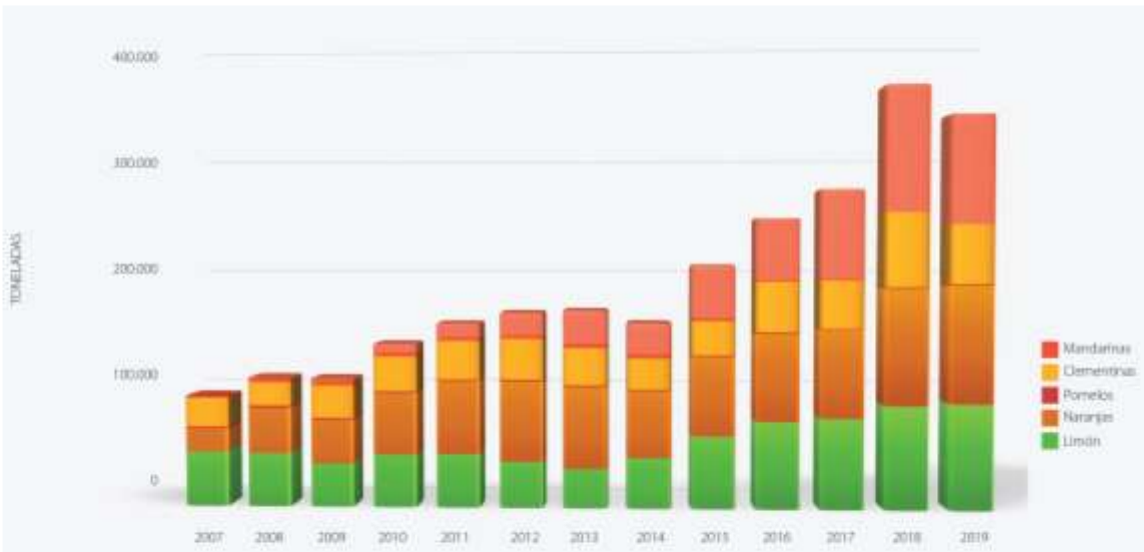


Figure 2.2: Evolution of citrus exports in Chile (Source www.comitedecitricos.cl/#)

Amongst the different agro-industry wastes generated during harvesting or industrial processing, citrus fruit wastes are a source of abundant, low-cost and immediately available renewable energy (Lopez-Velazquez et al., 2013). These wastes are composed of seeds, peels and pulp and are the main by-product of juice industries, accounting for about 50 wt% of the raw fruit processed (Alvarez et al., 2018). Citrus peel wastes contains insoluble carbohydrates (cellulose, pectin), other sugars (glucose, fructose, sucrose, galactose), acids (mostly citric and malic acids, but also tartaric, benzoic, oxalic and succinic acids), lipids, mineral elements (nitrogen, calcium and potassium), volatile compounds, flavonoids, limonoids, enzymes (pectinesterase, phosphatase, peroxidase), pigments and carotenoids, polyphenolics, nitrogen constituents and vitamins (Zema et al., 2018). In the last decades to avoid environmental pollution from citrus processing industry an alternative use of their residues have been evaluated (Alvarez et al., 2018; Sharma et al., 2017; Zema et al., 2018). Among the most traditional option for disposal is its application to soil, providing a source of slow-release nutrients and microelements, as substrate for compost production, as cattle feed, or in the cosmetic industry. Newly, these residues have been used in the energy industry for bio-methane or bioethanol production, in the food industry; for production of pectin and dietary fibers and in the and pharmaceutic industries; for extraction of flavonoids, flavorings agents and citric acid (Rezzadori et al., 2012; Sharma et al., 2017)

As mentioned above, the fresh citrus by-products have been used as livestock feed, due to contain high carbohydrate and low lignin, which is ideal for the digestion system of ruminant animals (Bampidis and Robinson, 2006). Moreover, its nutritive value, which is similar to that of barley grain or sugar beet pulp was used successfully as a substitute for alfalfa in guinea pig production (Mínguez and Calvo, 2018). On the other hand, one of the popular application of these residues in the pharmaceutical industry, is the use of citrus press cake extracts, on the cellular melanin content reduction in the human skin. Kim et al., (2013) reported that the ethylacetate extracts of citrus-press cakes is a potential anti-melanogenic agent and may be effective for topical application for treating hyperpigmentation disorders. Another by-product from citrus wastes are the essential oils, well known due to their strong antimicrobial, antioxidant, and anti-inflammatory properties and have a number of potential applications, such as ingredients in food additives, preservatives against spoilage, pharmaceuticals, and cosmeceuticals (Sharma et al., 2017)

Citrus waste is one of the most important sources of the lignocellulosic biomass because it is rich in carbohydrates and had low lignin content and other low molecular weight hydro-carbons which contain many hydroxyl functional groups. Environment friendly biomaterials derived from the agricultural waste have emerged as a promising substitute for the removal of dyes and heavy metals (e.g. Zn, Ni, Cu, Pb and Cr) from wastewater effluents (Mahato et al., 2020).

As a result of increasing amount of citrus fruits processed yearly and the environmental issues associated, composting of organic residues from the citrus processing industry, has been widely investigated in recent years due to have shown the positive agronomic effects. (Bernal-Vicente et al., 2008). Among agronomic uses of citrus wastes is the direct land spreading, improving organic matter content of soil and consequently enhancing its fertility. In addition, macronutrients such as N, P and K contained in the biomass can be releases. However, additional supply with mineral fertilizers is generally necessary to achieve the nutrient content required by crops (Zema et al., 2018). Guerrero et al., (1995), evaluated the industrial orange wastes as organic soil fertilizer in lettuce. The results showed that there was an increase in dry-matter at increasing doses of either pulp or peel wastes applied. In addition, a fairly strong positive relationship between nutrients obtained (N-P-K) and the type of wastes applied. Gelsomino et al., (2010) suggested that at field scale, orange compost can be used as organic fertilization; while in nursery crops it can be mixed with commercial potting substrates selected in relation to plant sensitivity, due to orange compost addition to nursery crops selectively induced pH and electrical conductivity increases, affected plant growth responses.

Nevertheless, excessive citrus waste application rates to soil can have adverse impacts on soil biota and ecological functions, caused by toxic compounds that may directly affect soil ecology, such as, limonene, tannins, flavonoids, and saponins (Abolusoro et al., 2010). These organic components could be released into the soil through leaching and decomposition and may pose direct ecotoxicological effects on soil fauna and flora (Ruiz et al., 2008). Several studies have demonstrated that the use of citrus peel or citrus extracts can be negatively affected soil biota. Mvumi et al. (2017) showed that the citrus extract had adverse effects on the survival, fecundity, and avoidance behavior of earthworms. They attributed these adverse effects to acidic pH and potentially toxic organic compounds in citrus waste such as limonene. Other studies with D-limonene, have been demonstrated to be fatal against a variety of organisms which include ants, mosquitoes, flies, crickets, and termites (Abolusoro et al., 2010; Ware and Whitacre, 2004)

CHAPTER III

Citrus residue enhances the effectiveness of beef cattle manure improving the phosphorus availability in acidic Andisol

Article accepted in Communications in Soil Science and Plant Analysis

Citrus residue enhances the effectiveness of beef cattle manure improving the phosphorus availability in acidic Andisol

Abstract

Phosphorus availability and acid pH are major limiting factors for grass and crop production in the Andisols of Southern Chile. Sustainable management requires both the efficient use of fertilizers and the recycling of agricultural waste. The aim of this study was to investigate the effect of citrus waste, alone or in combination with beef cattle manure (CM), on phosphorus availability. Controlled laboratory incubations were carried out for up to six weeks. Lemon (L) and mandarin (M) fruit wastes were added to an Andisol, amended or not with dried CM. Fruit parts (Peel, Juice or whole fruit), and for whole fruit the size of the fruit pieces added to soil, were compared. Water-extractable (WE) P, phosphatase activity (P-ase), and pH were monitored. In general, the size and distribution of the citrus fruit wastes gave similar trends in WE-P, P-ase, and pH. Lemon induced larger effects on WE-P and pH than did mandarin. CM caused sustained soil pH increment, increased soil WE-P and P-ase. Citrus addition produced short-lived acidification followed by soil pH elevation and immediate increases in WE-P and P-ase. The combined amendment with citrus wastes enhanced the positive effect of CM on soil WE-P and soil pH, with only a small effect on P-ase. These findings indicate that citrus wastes would intensify the beneficial effects of CM amendment on soil P availability and additionally the liming effect on acid soils.

Keywords: Organic residues, Cost effective soil amendment, Liming effect, Sustainable agriculture, Water-extractable phosphorus, Phosphatase activity

3.1 Introduction

Phosphorus (P) is an essential element for plant growth and is important to guarantee crop quality and yields. Global crop production increased markedly during the 20th century largely because of the use of fertilizers derived from phosphate rock. However phosphate rock is a limited, non-renewable resource (Roberts and Johnston, 2015). There is an ongoing debate about the longevity of global P resources, particularly high-quality rock phosphate reserves. Some studies have suggested that global P production will peak in 2033 (Cordell et al. 2014; Cordell and Neset 2011; Cordell et al. 2009), whereas Van Vuuren et al. (2010) conclude that only about 40–60% of the available P resources would be extracted by 2100 and the International Fertilizer Development Center (IFDC) maintains that phosphate rock concentrate reserves will be available for the next 300-400 years (Van Kauwenbergh, 2010). Regardless of the precise chronology of a future shortage, a considerable improvement in phosphorus fertilizer use efficiency is required to maintain cost-effective agricultural productivity, environmental quality and food security. Among the strategies for improving P use efficiency, the implementation of best management practices (BMPs) (Roberts and Johnston 2015), the reduction of soil P leaching and erosion (Rittmann et al. 2011; Scalenghe et al. 2014), the recycling and recovery of materials containing P (Kratz et al., 2019), the optimization of manure application rates (Sattari et al., 2012) and understanding the P forms in manure to maximize its benefits (He et al., 2016; Pagliari et al., 2020; Pagliari and Laboski, 2012) should all be considered.

Acid soils are characterized by high organic P content, and sparingly soluble inorganic P with only a tiny fraction in soil solution as the orthophosphate anion. This anion is strongly adsorbed on organo-mineral surfaces, including oxides, allophane, and phyllosilicates or precipitated as inorganic salts principally with aluminium (Al) and iron (Fe) in acid soils

(Gérard 2016; Violante et al. 2002; Violante and Pigna 2002). Plants have developed several strategies in response to the P deficiency and Al toxicity in acid soils (Barra et al. 2018; Ma et al. 2001; Ryan et al. 2001; Wang et al. 2007). Root exudation of organic anions increase P availability to plants by mobilizing phosphate bound to sorption sites in soil (Bolan et al., 1994) including Fe and Al-(hydr)oxides (Gérard, 2016; Gerke et al., 2000, 1994), or by the formation of complexes with Fe and Al in solution. Another strategy to contend with P deficiency, is the release of acid phosphatase by plants in response to P stress. These enzymes hydrolyze a range of organic-P forms thereby enhancing plant P uptake (Richardson et al., 2011).

Southern Chile is a region devoted to grassland production to support the beef and dairy cattle industry and more recently has seen the expansion of citrus orchards. Citrus production and processing for juice generate a large amount of waste (Sharma et al. 2017; Zema et al. 2018) mainly consisting of peel and pressed pulp or whole fruits discarded due to poor quality (e.g., damaged fruit) or to regulations to limit production (Ruiz and Flotats, 2014). Citrus fruits are classified as acid fruits as their juice has pH 2.0-3.0 due to citric and malic acids. Citric acid is the most abundant acid in the endocarp, whereas oxalic, malic, malonic, and citric acids are present in citrus peel (Ladaniya, 2008). Recycled citrus fruit processing waste could constitute a soil organic amendment in the context of sustainable and cost-effective management of P fertilization. It has been demonstrated that citrus waste, used as a soil amendment, improves crop yields (Guerrero et al. 1995; Tuttobene et al. 2009) however to date, no study has focused on its effect on soil P fertility.

The importance of manure as a soil amendment has been known since ancient times. In organic and sustainable agriculture, cattle manure (CM) represents a source of macro and micronutrients for crop and grass production and simultaneously guarantees the incorporation of organic matter improving physical, chemical and biological properties of soils (Fuentes et al. 2008; Diacono and Montemurro 2010; He and Zhang 2014; Liu et al. 2017; Cai et al. 2019). According to Pagliari et al. (2020) most of P in cattle manure is inorganic and labile (with up to 48% being $NaHCO_3$–extractable and 70% H_2O–extractable) and should therefore be readily available for plants (He et al. 2016; Tiecher et al. 2014).

Not only does manure provide available nutrients, but it may contribute to microbial and biochemical activity. Marinari et al. (2000) found that stabilized dairy cattle manure amendment led to large increases in acid phosphatase activity and this was attributed to a priming effect leading to the mineralization of soil organic matter thereby releasing nutrients. Waldrip et al. (2012) evaluated the effect of organic dairy manure on soil phosphatase activity in an acid silt loam soil and found a positive correlation between P-manure addition and acid phosphatase activity, although there were exceptions to this trend. The authors suggested that during early plant growth, the root induced the production of acid phosphatase or stimulated the extracellular soil phosphatase activity by rhizospheric processes to meet plant nutrient demand. In contrast, poultry manure was found to have little effect on phosphatase activity of the same soil (Waldrip et al. 2011).

The objective of this study was to investigate the additional advantage of applying citrus waste along with cattle manure to assess whether organic matter, and in particular the organic acids, present in citrus wastes, could improve the P availability for crops in an acid soil of Southern Chile. Our starting hypothesis was that a combination of citrus wastes and cattle manure could be the basis of a cost-effective soil amendment improving the fertility of acid soils. We paid attention to the possibility that oxyanions present in citrus waste could inhibit phosphatase activity and hence limit the mineralisation or soil organic phosphorus.

3.2 Materials and methods

3.2.1 Soil and beef cattle manure (CM) sampling

The soil studied was an Andisol collected from Southern Chile, Barros Arana locality 39º06'12''S, 72º37'42''W. The soil has silt loam texture and is classified as Andosol (IUSS Working Group, 2014) or Typic Hapludand (Soil Survey Staff, 2014). The soil was collected from 0 to 20 cm depth after removing a thin layer of surface litter, air dried and sieved <2 mm. The non-composted beef cattle manure (cow-pats) was collected from Santa Elena farm where animals graze outside, located in the same locality. It was air-dried for 5 days, milled and sieved <2 mm for subsequent analysis and use.

3.2.2 Chemical characterization of soil and CM

Both soil and cattle manure were characterized using routine methods according to Sadzawka et al. (2006). Soil pH was determined by potentiometer in a soil: solution (H_2O) suspension (ratio: 1:2.5). Soil extractable phosphorus was extracted with bicarbonate solution (Olsen-P) and quantified using the method of Murphy and Riley (1962). Total phosphorus was determined by alkaline digestion with sodium hypobromite (NaBrO) with the method of Dick and Tabatabai (1977). Total carbon and nitrogen were determined using an elemental analyser (CHN NA1500; Carlo Erba Elemental Analyzer, Stanford, CA, USA). Exchangeable cations (Ca, Mg, Na and K) were extracted with 1 M NH_4Ac at pH 7.0 and quantified using flame atomic absorption spectrophotometry (FAAS, Unicam SOLAAR 969). Exchangeable aluminium was extracted with 1 M KCl and analysed by flame atomic absorption spectrometry (FAAS). Selected physicochemical properties of the soils and cattle manure are shown in Table 3.1.

Table 3.1: Chemical properties of the Andisol Barros Arana from Southern Chile and beef cattle manure. Data are means ± SD of three replicates.

Parameters	**Values**	
	Barros Arana soil	**Beef cattle manure**
pH	5.6 ± 0.0	7.0 ± 0.0
Total carbon (%)	11.7 ± 0.2	41.1 ± 0.2
Total nitrogen (%)	0.9 ± 0.0	2.1 ± 0.0
C/N	12.5 ± 0.2	19.2 ± 0.5
Olsen P (mg kg^{-1})	6.0 ± 1	497 ± 10
Total P (mg kg^{-1})	1981 ± 11	3710 ± 26
K (mmol kg^{-1})	3.5 ± 0.1	45.2 ± 0.5
Na (mmol kg^{-1})	1.8 ± 0.1	49.6 ± 0.1
Ca (mmol kg^{-1})	40.7 ± 0.4	182 ± 1
Mg (mmol kg^{-1})	9.3 ± 0.1	67.5 ± 0.2
Al (mmol kg^{-1})	1.1 ± 0.1	1.2 ± 0.1

Soil and beef cattle manure data are presented on a dry matter basis

3.2.3 Characterization of lemon and mandarin fruits

Fresh fruits of lemon (C*itrus limon* L. Osbeck) and mandarin (*Citrus reticulata*, Blanco, 1837) were purchased from commercial retailers and healthy fruits were selected for uniformity of shape and colour. The fruits were cut in half and squeezed to extract juice. The juice was filtered to remove pulp and seeds and then centrifuged at 3000 *g* for 10 min. Supernatant was diluted 1:50 for citrate determination and 1:10 for the other anions. The diluted samples were membrane filtered (Sartorius Minisart ®, 0.2 µm). The peels of the squeezed fruit samples were cut in small pieces. Ten grams of peel were put in a flask with 25 ml of ultrapure water, shaken for one hour then membrane filtered. Extracts were analysed using High Performance Liquid Chromatography (HPLC, Shimadzu LC 20, DAD SPD-M20A detector) using a 4.6 x 250 mm, 5 µm Symmetry C18 column.

In order to determine the P content on lemon and mandarin peel, each fruit was washed with distilled water and then cut into small pieces, placed in paper bags and dried at 65 °C for 48 h according to Sadzawka et al. (2004). In addition, total carbon and nitrogen were determined using an elemental analyser (CHN NA1500; Carlo Erba Elemental Analyzer, Stanford, CA, USA). The pH was measured in juice and in the water extracts of peel by potentiometry.

3.2.4 Treatments of CM-amended soil with chopped lemon or mandarin fruits

Two series of incubation experiments were conducted to evaluate the effect of lemon or mandarin addition on the P release and the acid phosphatase activity (P-ase activity). In the first experiment, 50 g of soil or a mixture of soil with 5% CM was placed in plastic pots of 100 ml capacity. The addition of this rate of manure represents about the same amount of total P and four times as much Olsen P as initially present in soil. Peel and adhering pulp (named Peel, P) were cut with a sharp knife into small pieces (< 5 mm). Peel, juice (J) or whole fruit (WF) were added to each pot and carefully mixed with soil. There were two rates of addition of citrus to soil, either 20% or 40%, expressed as equivalent fresh mass of the whole fruit. Thus, for example, 20 g whole fruit was added to 50 g soil in the 40% treatments and this is equivalent to 12.2 g peel or 7.5 ml juice.

The soil with or without cattle manure, but no addition of citrus, was incubated as a control. Each treatment was prepared in triplicate and samples were incubated at 25 °C, with moisture content at 80% of soil water holding capacity and aerobic condition for up to 6 weeks. Moisture content was calculated considering both juice and added distilled water. Pots were loosely covered and opened daily to allow air exchange. The mass was monitored daily, but no water addition was required to maintain moisture content.

At intervals during the 6-week incubation period (1 hour (h), 1 day (d), 3 d, 1 week (w) 2 w and 4 w) subsamples were taken from each container and analysed for P-ase activity (0.2 g), water extractable P and pH (0.3 g). There were 4 repetitions of each sample, and each of these was a composite sample of four subsamples. The P-ase activity was determined using the method of Tabatabai and Bremner (1969) modified by Rubio et al. (1990) for soil with high organic matter content. In brief, 200 mg soil sample was incubated with 200 µl of 30 mM *p*-nitrophenyl phosphate disodium (Sigma Aldrich), and 800 µl of 0.1 M sodium acetate buffer, pH 5.5, at 25 °C for 30 hours. After incubation, the enzymatic reaction was stopped by adding 200 µl of 2 M $CaCl_2$ and 800 µl of 0.2 M NaOH. The suspension was then centrifuged at 15 000 *g* for 5 min at 25 °C, and the absorbance of the supernatant solution at 405 nm was measured in microplates using a UV-Vis spectrophotometer (Multiskan GO, Thermo Scientific). Water-extractable P was measured using the malachite green method (Ohno and Zibilske, 1991). A soil suspension by adding 1.5 ml deionized water to 0.3 g of soil solid, shaking vigorously for 5 min and allowing to settle for 2 h. Finally, the pH of this settled soil suspension was measured.

Preliminary experiments showed that Olsen P was too large to be sensitive to changes in fractionation induced by incubation or citrus addition.

Preliminary experiments showed that dilution was sufficient to ensure that there was no interference from citrate in the phosphate assay and that the addition of $CaCl_2$ was sufficient to limit colour release when the enzyme assay was stopped with alkaline solution.

3.2.5 Treatments of CM-amended soil with coarsely chopped lemon or mandarin fruits

A second experiment was carried out using more coarsely chopped fruits (4-5 cm in size, ⅛ lemon or ¼ mandarin) and the soil amended with 5% of CM. This scale was chosen to be intermediate between the controlled laboratory experiment and a future field study. In these experiments soil pots were set up in three layers: at the base, layer C with about 15 g of soil, or CM-amended soil, over layer B of approximately 20 g of soil, or CM-amended soil, plus chopped lemon or mandarin, and in the top layer A, with 15 g of soil or CM-amended soil. The layers were separated with a nylon mesh with 35-µm pore diameter. Plastic pots with only soil were used as controls, following the same procedure described above. All samples were prepared in triplicate. The samples were stored for four weeks at 25°C under aerobic conditions, and at 80% of soil water holding capacity. At the end of the incubation period, soil was collected from each layer to obtain composite samples and analysed to determine pH, water extractable P, P-ase activity

3.2.6 Statistical analysis

The statistical analysis was carried out using XLSTAT version 2016.02.284551 (Addinsoft, New York, USA). Data were first tested for deviation from normality using the Shapiro–Wilk test and the homogeneity of within-group variances was assessed with Levene´s test. Data collected were subjected to analysis of variance (ANOVA) and the means were separated by the Duncan test at $p \leq 0.05$. In the P-ase data the differences between control and CM was assessed by paired t-test ($p \leq 0.05$).

3.3 Results

3.3.1 Chemical characterization of soil and beef cattle manure samples

Chemical properties of the Andisol Barros Arana are reported in Table 1. The soil had a total carbon content of about 12%, a moderate acidity (pH 5.6) and low levels of Ca, Mg, K, and Na. The Al content of exchange complex was low, with Al saturation less than 1%. The Olsen-P content in soil was very low (6 mg kg^{-1}) despite the high content of total P (1981 mg kg^{-1}).

Beef cattle manure was characterized by neutral pH, 41.1% C, C/N ratio equal to 19.2, high total P (3710 mg kg^{-1}) as well as Olsen-P (500 mg kg^{-1}) and high exchangeable cations contents of but a low Al saturation (1.2 mmol kg^{-1}).

3.3.2 Characterization of lemon and mandarin fruits

The organic anion contents of lemon and mandarin peel and juice are shown in Table 3.2. The amount of organic anion depended on both the fruit and fruit part. In lemon, citrate was the main organic anion found in juice and peel (21.0 and 8.1 g kg^{-1}, respectively) followed by oxalate, malate and succinate. In contrast, malate was the main anion found in the peel of mandarin (3.6 g kg^{-1}), and citrate in mandarin juice (2.4 g kg^{-1}). The pH values of juice ranged between 1.9 and 3.6. Lemon juice had the lowest pH observed (1.9), whereas that of mandarin juice was slightly higher, 2.7. The pH values of lemon and mandarin peel were 2.9 and 3.6, respectively. The C/N ratio in lemon peel was greater than in mandarin peel and the P contents of lemon and mandarin peel were 1.0 and 1.8 gkg^{-1}, respectively.

Table 3.2.- Composition of peel and juice of lemon and mandarin. For water-extractable organic anions (citrate, oxalate and malate) were quantified by HPLC

Fruit	Part of fruit	Citrate	Oxalate	Succinate	Malate	P	C/N	pH
		g kg^{-1} of fresh mass				g kg^{-1} DW		
Lemon	Peel	8.1 ± 0.0	0.5 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	1.0 ± 0.1	33.4 ± 0.9	2.9 ± 0.1
	Juice	21.0 ± 0.0	0.1 ± 0.0	0.01 ± 0.00	0.3 ± 0.0	-	-	1.9 ± 0.0
Mandarin	Peel	1.4 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	3.6 ± 0.2	1.8 ± 0.0	27.0 ± 0.3	3.6 ± 0.0
	Juice	2.4 ± 0.0	0.1 ± 0.0	0.02 ± 0.00	0.9 ± 0.0	-	-	2.7 ± 0.0

3.3.3 Soil amended with finely chopped citrus

3.3.3.1 Effect of amendments on soil pH

Beef cattle manure (CM) addition led to an increase in soil pH. Although pH fluctuated during the incubation period, the manure amended soil was on average about 0.2 units more alkaline than the unamended soil. Citrus addition caused an initial acidification of both soil and manure-amended soil (Table 3). This decrease of pH was greater for lemon than for mandarin addition, and greater for juice than peel, and increased with increased addition of peel (Table 3.3).

The lemon peel treatments, 20% and 40%, led to immediate (after one hour) decreases in pH of 0.8 and 0.9 units, respectively, with respect to the soil control. Manure counterbalanced to some extent the initial acidifying effect of citrus addition. Soil amended with cattle manure plus 20% and 40% LP decreased the pH by 0.4 and 1.3 units, respectively, compared with CM-soil control. With the larger addition of peel, the liming effect of manure was cancelled. The greatest pH change occurred when juice or whole fruit were added to soil or CM-amended soil. For soil, the addition of juice and whole fruit caused a decrease around 1.7 units, whereas for CM-amended soil this decrease was 1.5 for juice and 1.9 for whole fruit, and as for peel. After two weeks pH increased with all lemon treatments in both soil and CM-amended soil reaching in average a pH value of 6.2 (Table 3.3). The acidifying effect of lemon addition decreased with time, and indeed, after about 2 weeks, citrus amended soil were more alkaline than S or S+CM controls.

Mandarin treatments had less impact on soil pH (S or S+CM controls), relative to lemon treatments, and so the range of pH values measured was smaller. Each of the mandarin additions caused a decrease in pH with respect to S and S+CM at each incubation time for the first 2 weeks (Table 3.3). For soil (without manure) the immediate (1 hour) decrease in pH was 0.26 units for 20% peel and 0.5 units for 40% peel without significant ($p \leq 0.05$) difference. Incorporation of juice and whole fruit led to larger decreases in pH, 0.5 and 0.6 units, respectively. The effects of mandarin additions to CM-amended soil were very similar to those observed for soil, with a general pH increased caused by CM. The short-term acidification observed for citrus addition was delayed, in comparison with lemon, leading to a surprising net acidification of the CM-amended soils for the 1-day and 3-day incubation periods with respect to soil+mandarin.

Table 3.3.- Changes in soil pH recorded during incubation period after application of peel (P), juice(J) and whole fruit (WF) of lemon (L) or mandarin (M) to soils or soil amended with beef cattle manure (CM). Data are means ± SD of four replicates. Different lower case letters indicate significant differences among incubation times within the same lemon or mandarin treatment, whereas different capital letters indicate significant differences among lemon and mandarin treatments in the same incubation time at Duncan post-hoc test ($p \leq 0.05$).

Treatments				**pH**			
Incubation Period	**1h**	**1d**	**3d**	**1w**	**2w**	**4w**	**6w**
S	6.0 ± 0.0 ^{aA}	6.2 ± 0.1 ^{aA}	6.3 ± 0.1 ^{aA}	6.4 ± 0.0 ^{aA}	6.1 ± 0.0 ^{aA}	5.5 ± 0.0 ^{bB}	5.5 ± 0.0 ^{bB}
S,CM	6.2 ± 0.0 ^{aA}	6.2 ± 0.2 ^{aA}	6.3 ± 0.1 ^{aA}	5.9 ± 0.1 ^{bB}	6.2 ± 0.0 ^{aA}	6.0 ± 0.0 ^{aA}	6.2 ± 0.0 ^{aA}
				Lemon			
S, 20% LP	5.2 ± 0.0 ^{cB}	5.6 ± 0.0 ^{bB}	5.6 ± 0.0 ^{bB}	5.7 ± 0.1 ^{bBC}	6.0 ± 0.0 ^{aA}	6.3 ± 0.0 ^{aA}	6.3 ± 0.0 ^{aA}
S, 40%LP	5.1 ± 0.0 ^{bC}	5.4 ± 0.1 ^{bB}	5.2 ± 0.1 ^{bBC}	5.5 ± 0.0 ^{bC}	6.0 ± 0.0 ^{aA}	6.3 ± 0.0 ^{aA}	6.3 ± 0.0 ^{aA}
S, 40% LJ	4.3 ± 0.1 ^{dD}	4.8 ± 0.1 ^{cC}	5.0 ± 0.1 ^{cC}	5.5 ± 0.1 ^{bC}	6.3 ± 0.1 ^{aA}	6.1 ± 0.0 ^{aA}	5.7 ± 0.0 ^{bB}
S, 40% LWF	4.3 ± 0.0 ^{cD}	4.5 ± 0.1 ^{cC}	4.6 ± 0.0 ^{cC}	5.0 ± 0.1 ^{bD}	6.2 ± 0.0 ^{aA}	6.3 ± 0.0 ^{aA}	6.3 ± 0.0 ^{aA}
S, CM, 20% LP	5.9 ± 0.1 ^{cB}	5.7 ± 0.1 ^{cB}	6.0 ± 0.1 ^{bA}	6.0 ± 0.1 ^{bB}	6.4 ± 0.0 ^{aA}	6.3 ± 0.0 ^{aA}	6.2 ± 0.0 ^{aA}
S, CM, 40% LP	5.1 ± 0.1 ^{cC}	5.3 ± 0.1 ^{bcB}	5.5 ± 0.1 ^{bB}	5.7 ± 0.0 ^{bBC}	6.3 ± 0.0 ^{aA}	6.4 ± 0.0 ^{aA}	6.3 ± 0.1 ^{aA}
S, CM, 40% LJ	4.8 ± 0.1 ^{cC}	5.0 ± 0.1 ^{cBC}	5.5 ± 0.1 ^{bB}	5.9 ± 0.0 ^{aB}	6.3 ± 0.0 ^{aA}	6.1 ± 0.0 ^{aA}	6.2 ± 0.0 ^{aA}
S, CM, 40% LWF	4.3 ± 0.1 ^{cD}	4.6 ± 0.1 ^{cC}	4.7 ± 0.1 ^{cC}	5.5 ± 0.1 ^{bC}	6.2 ± 0.1 ^{aA}	6.3 ± 0.0 ^{aA}	6.3 ± 0.0 ^{aA}
				Mandarin			
S, 20% MP	5.7 ± 0.1 ^{bB}	5.6 ± 0.0 ^{bB}	5.1 ± 0.0 ^{cC}	4.9 ± 0.0 ^{cD}	5.7 ± 0.0 ^{bB}	6.2 ± 0.0 ^{aA}	6.0 ± 0.0 ^{aA}
S, 40% MP	5.5 ± 0.1 ^{bB}	5.5 ± 0.0 ^{bB}	4.7 ± 0.1 ^{cC}	4.9 ± 0.0 ^{cD}	5.7 ± 0.0 ^{bB}	6.2 ± 0.0 ^{aA}	6.0 ± 0.1 ^{aA}
S, 40% MJ	5.5 ± 0.1 ^{bB}	5.4 ± 0.0 ^{bB}	5.1 ± 0.2 ^{bC}	4.7 ± 0.0 ^{cD}	5.4 ± 0.0 ^{bC}	5.9 ± 0.0 ^{aA}	6.1 ± 0.0 ^{aA}
S, 40% MWF	5.4 ± 0.0 ^{cB}	5.2 ± 0.0 ^{cB}	5.3 ± 0.1 ^{cC}	5.2 ± 0.1 ^{cD}	5.8 ± 0.0 ^{bB}	6.1 ± 0.0 ^{aA}	6.1 ± 0.1 ^{aA}
S, CM, 20% MP	5.9 ± 0.1 ^{bB}	5.6 ± 0.1 ^{cB}	5.9 ± 0.1 ^{bA}	6.1 ± 0.0 ^{abA}	6.3 ± 0.0 ^{aA}	6.2 ± 0.0 ^{aA}	6.1 ± 0.0 ^{aA}
S, CM, 40% MP	5.8 ± 0.0 ^{bB}	5.3 ± 0.1 ^{cB}	5.7 ± 0.0 ^{bB}	5.6 ± 0.0 ^{bBC}	6.0 ± 0.1 ^{aA}	6.4 ± 0.0 ^{aA}	6.1 ± 0.0 ^{aA}
S, CM, 40% MJ	5.7 ± 0.1 ^{bB}	5.2 ± 0.0 ^{cB}	5.6 ± 0.1 ^{bB}	6.2 ± 0.0 ^{aA}	6.3 ± 0.0 ^{aA}	6.2 ± 0.0 ^{aA}	6.1 ± 0.0 ^{aA}
S, CM, 40% MWF	5.5 ± 0.0 ^{bB}	5.1 ± 0.0 ^{cB}	5.0 ± 0.0 ^{cC}	5.7 ± 0.0 ^{bBC}	5.9 ± 0.1 ^{bB}	6.3 ± 0.0 ^{aA}	6.3 ± 0.0 ^{aA}

3.3.3.2 Effect of amendment on water-extractable phosphorus

The extractable soil P content was significantly ($p \leq 0.05$) affected by the incorporation of citrus fruits and cattle manure (Figure 3.1). There was an initial flush of P solubility at the start of the incubation experiment, and manure addition increased this flush. Thereafter P tended to decrease with time, but remained on average larger in manure amended soil. Citrus amendment caused large flush of P. Lemon addition led to larger increases in P than did mandarin. In general, the greatest increase of WE P content was observed when whole fruit was added, followed by peel.

When whole fruit was added to CM-amended soil WE P content increased 7-fold with respect to control (from 180 to 1230 µg kg^{-1}), indicating a synergistic effect between citrus and manure additions. It must be noted that a different scale had to be used to present data with CM amendment after 1 hour, because of the magnitude of this flush. Lemon peel addition (20% and 40%) increased P content approximately 2.5-fold on control soil; meanwhile for CM-amended soil, P increased approximately 3-fold. Interestingly, the lowest phosphorus values were obtained when juice treatment was applied, but the effects of juice and peel were additive. After the initial flush of P, extractable P levels fell back rapidly, but remained greater in citrus-amended soils throughout the incubation period.

The mandarin treatments led to smaller enhancements of water-extractable P; the incorporation of 40% peel or whole fruit increased the WE P content of both control and CM-amended soil (1.8-fold and 2.5-fold respectively). No particular differences were found when 20% peel was added. The whole fruit treatment produced slightly greater increases, 2.3 fold on S and 2.9-fold S+CM. Although the P content fell markedly after 1 day, both the CM and 40% whole fruit additions maintained large increases in the P content, 8.0 and 17.9-fold respectively. At the end of the 6-week incubation, the highest P contents were found in the CM-amended soil with lemon treatments containing peel (either peel or whole fruit)

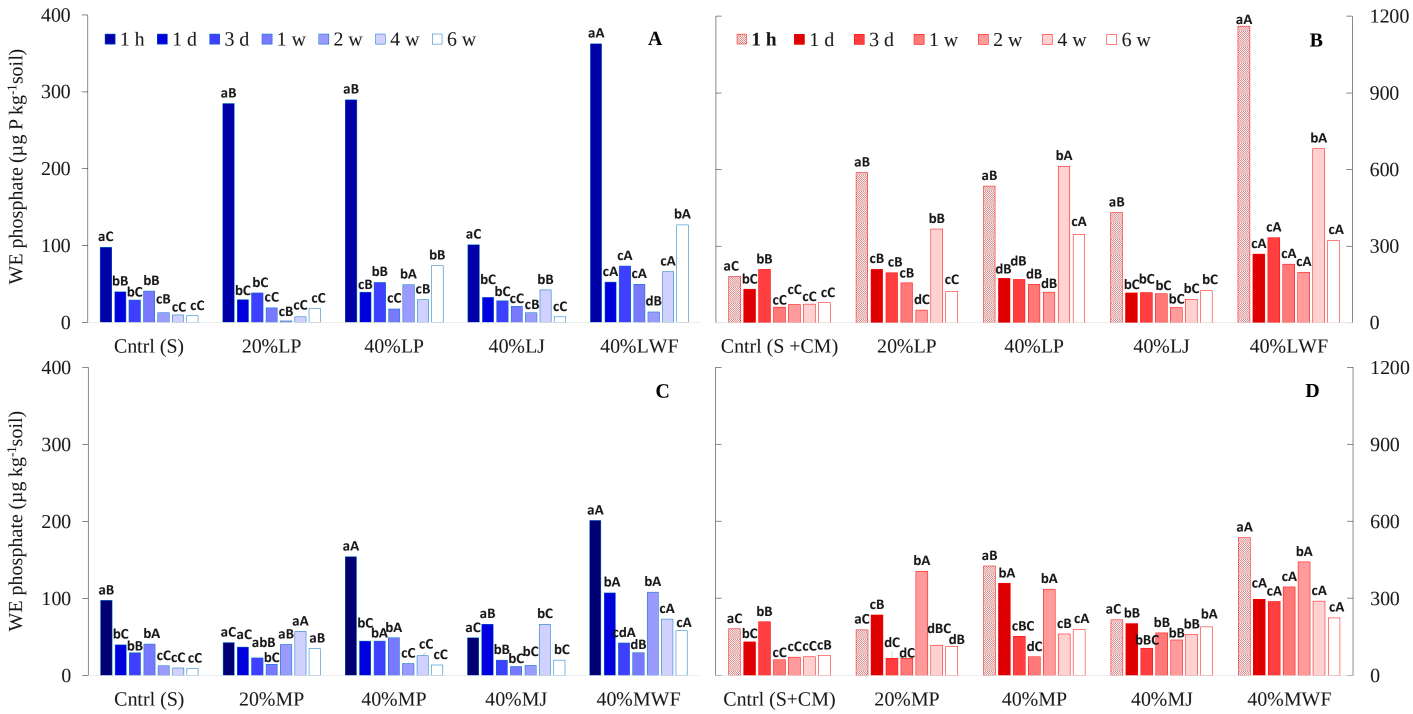


Figure 3.1. Water extractable P content after lemon amendment (A, B) and mandarin (C, D) of incubation experiment for different incubation periods. Different letters indicate statistically significant differences ($p \leq 0.05$) lower case letters, between lemon or mandarin treatments; upper case letters, between soil and CM treatment for the same lemon or mandarin treatments. A different scale is used for data with CM amendment and a 1-h incubation period, and this is emphasised using hatched filling of the bars.

3.3.3.3 Effect of amendment on phosphatase activity

Phosphatase activity in soil was fairly constant and showed no consistent variation during the incubation period with little effect of manure addition (Figure 3.2). Citrus fruit treatments containing peel significantly ($p \leq 0.05$) increased acid phosphatase activity in soil, with or without cattle manure, at each stage of the incubation (Figure 2A). After 1 hour, treatments containing lemon peel (peel and whole fruit) increased acid phosphatase activity that reached on average values of about 1.7 nkat PNP g soil^{-1}, which were maintained until the end of incubation period. In contrast, the juice treatment caused a marked, but short-lived decrease in phosphatase activity from 1.0 to 0.6 nkat PNP g soil^{-1}. Similar behaviour was observed for mandarin treatments, where the incorporation of peel and whole fruit tended to enhance the phosphatase activity in both soil and CM-amended soil. The initial (1-hour) increases due to peel or whole fruit addition were small and not always significant ($p \leq 0.05$) in soil, but more marked for CM-amended soil (1.9 and 1.8 nkat PNP g soil^{-1} for Peel and WF, respectively). Mandarin juice, in line with lemon juice, led to decreased phosphatase activity, that was particularly marked after 1 hour. In contrast to lemon juice, the decrease in P-ase activity induced by mandarin juice was observed throughout the incubation period.

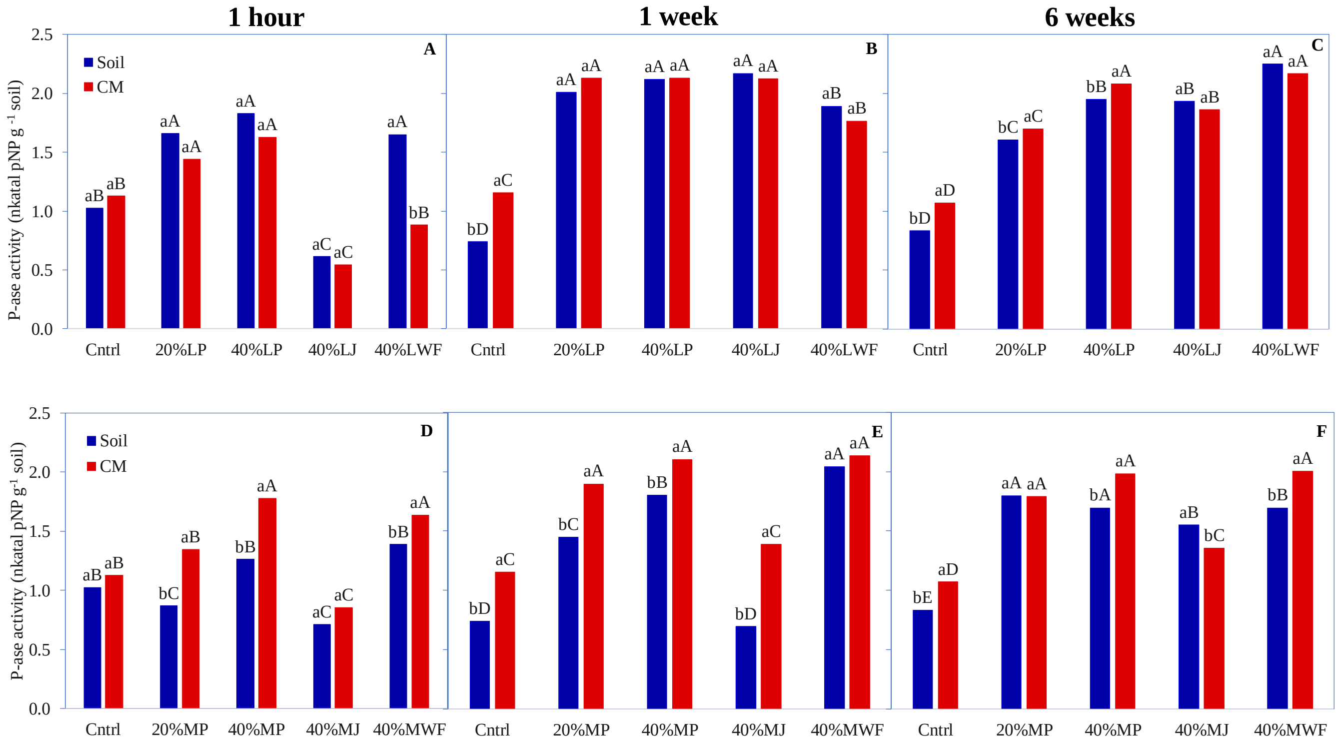


Figure 3.2. Changes in acid phosphatase activity in soil and CM-amended with addition of lemon (A, B, C) and mandarin (D, E, F) after three different incubation periods (1 hour, 1 week, 6 weeks). Different letters indicate statistically significant differences ($p \leq 0.05$) lower case letters, between lemon or mandarin treatments; upper case letters, between soil and CM treatment for the same lemon or mandarin treatments.

3.3.4 Soil amended with coarsely chopped fruit

3.3.4.1 Effect on water-extractable phosphorus

The trends in WE P were remarkably similar to those measured in the first incubation experiment (Figure 3.1), but with greater amount of extractable P in layer B, suggesting a scaling-up effect. The highest WE P content was observed in the soil surrounding the fruit (layer B) in both soil (S) and

CM-amended soil (CM-S) (Figure 3.3). For S, WE P increased from 170 to 240 µg kg^{-1} with chopped lemon and from 43 to 110 µg kg^{-1} with chopped mandarin. For CM-amended soil, the WE P content increased from 250 to 450 µg kg^{-1} in the lemon treatment and from 50 to 250 µg kg^{-1} in mandarin treatment (Figure 3).

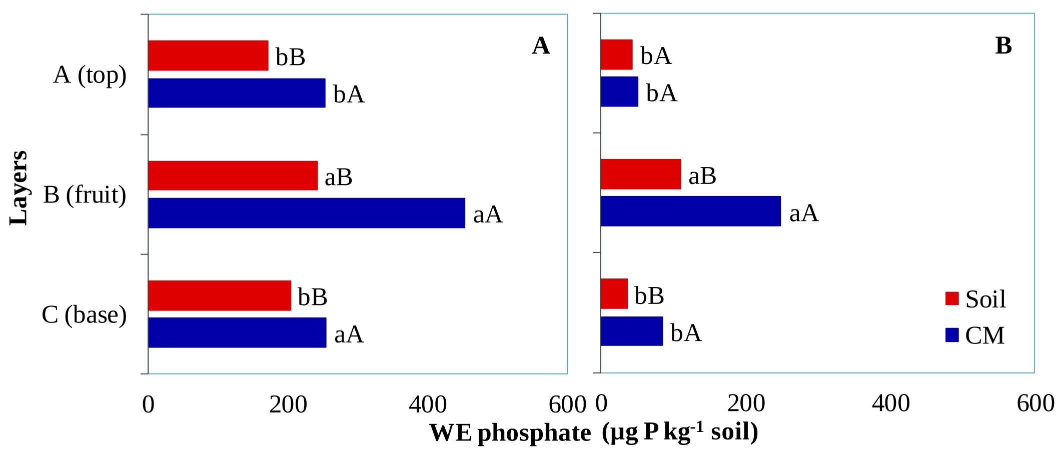


Figure 3.3. Water extractable P content from coarsely chopped lemon (A) and mandarin (B) incubation experiments, for each of the layers, from top to base. Different letters indicate statistical significant differences ($p \leq 0.05$). Lower case letters, between layers for the same soil; upper case letters, between soil and CM.

3.3.4.2 Effect on pH

Soil amendment was also performed using larger pieces of chopped fruit (>4-5 cm) to be closer to real conditions. Soil pH in the top layer (A) most closely resembled the control soil and greatest effects were observed for the middle layer (B), where soil was in direct contact with chopped fruit. Soil pH in layer B increased by 0.7 and 0.6 units following lemon- and mandarin- chopped fruit amendments, respectively, compared with the layer A (Table 3.4). The incorporation of lemon and mandarin also increased pH of the CM-amended soil by 0.5 unit, respectively. In layer C the increase in pH was significant ($p \leq 0.05$) but smaller than in layer B. Thus in soil amended with lemon and mandarin, pH increased by 0.4 and 0.6 units, respectively, while the pH increases in CM-amended soil were smaller, 0.3 units for lemon and not significant ($p \leq 0.05$) for mandarin (Table 3.4).

3.3.4.3 Effect on phosphatase activity

Phosphatase activity tended to be increased by fruit addition, and as observed for pH and WE P, the effects were greatest in layer B (Table 3.4). Values in the top layer were least influenced by citrus addition, and the effect was intermediate in the lowest layer. Activity was lower in CM-amended soil, and the increase induced by citrus was greater for lemon than mandarin.

Table 3.4: Values of pH and phosphatase activity of the soil collected from three layers in pots of the second experiment. Different letters indicate statistical significant differences ($p \leq 0.05$). Lower case letters, between layers for the same soil; upper case letters, between soil and CM.

Treatment	Layer	pH	Phosphatase activity (nkatal PNP g soil^{-1})
S+L	A	5.4 ± 0.0^{cB}	1.5 ± 0.1^{bA}
	B	6.1 ± 0.1^{aB}	2.0 ± 0.1^{aA}
	C	5.8 ± 0.0^{bB}	2.0 ± 0.1^{aA}
S+CM+L	A	6.1 ± 0.1^{cA}	1.2 ± 0.1^{cB}
	B	6.6 ± 0.1^{aA}	2.1 ± 0.1^{aA}
	C	6.3 ± 0.0^{bA}	1.7 ± 0.1^{bB}
S+M	A	5.4 ± 0.0^{bB}	1.7 ± 0.1^{abA}
	B	6.0 ± 0.1^{aB}	1.9 ± 0.1^{aA}
	C	6.0 ± 0.0^{aB}	1.7 ± 0.1^{bA}
S+CM+M	A	6.1 ± 0.0^{bA}	1.0 ± 0.1^{cB}
	B	6.5 ± 0.1^{aA}	2.0 ± 0.1^{aA}
	C	6.1 ± 0.0^{bA}	1.4 ± 0.1^{bB}

L: lemon; CM: beef cattle manure; M: mandarin

3.4 Discussion

Applications of citrus fruit (all treatments) caused an initial decrease in soil pH. However, with increasing incubation time, pH rose to an average of 6.10. The initial soil pH decrease can be explained by the acidity *per se* of the amendment. The subsequent increase in pH may be attributed to the decomposition of organic matter, including the decarboxylation of the organic anions (Naramabuye and Haynes, 2006a). Large increases in pH have been reported during the composting of citrus wastes (between days 45 and 50) (Van Heerden et al., 2002). Several studies have shown an increase in pH following crop residue addition to soil, particularly in acid soil (Butterly et al. 2013; Vanzolini et al. 2017; Xu et al. 2006; Xu and Coventry 2003; Yan et al. 1996). For example, Mokolobate and Haynes (2002) compared the liming effect of four organic residues applied to an acid soil and concluded that addition of organic residues to acid soils could be a practical strategy to increase soil pH, thus decreasing concentrations of phytotoxic Al without lime applications. In addition, the application of manure to soils also supplies large amounts of organic matter, containing carboxyl and phenolic hydroxyl groups, that play essential roles in buffering soil acidity and increasing the pH of acid soils (Whalen et al., 2000). We observed increased soil pH in both incubation experiments, caused by both cattle manure and citrus residue additions.

One concern with the addition of citrus fruits to these soils was that the oxyanions they would release could inhibit phosphatase activity, thereby reducing the availability of organic P to crops. The observations of this study allow us to dismiss these fears: in the long term phosphatase activity was enhanced by both amendments. This finding contrasts with the observation of Waldrip et al. (2012) that cattle manure had large effect on acid phosphatase activity, and is closer to the finding of Waldrip et al. (2011) who report no significant effect of poultry manure on phosphatase activity. In our case, the acid phosphatase activity was found to be more strongly influenced by the citrus treatments than by cattle manure addition in both incubation experiments. There was no synergistic effect between manure and citrus fruit on phosphatase activity. The greater effect of citrus in comparison with manure could be explained by the stimulation of microbial growth due to the incorporation of readily degradable organic substrates (Wang et al. 2012; Intanon et al. 2015; Dumontet et al. 2017). As in this study, Pascual et al. (2002) report that phosphatase activity was highest in soil amended with fresh organic wastes, rather than with composted organic wastes, at least in the short term. They attributed this increase to increases in microbial biomass and activity. This observation is coherent with the relative effects of citrus and manure additions since cattle manure was stabilized prior to incorporation into soil, whereas citrus residues were applied fresh. Several studies have found high phosphatase activity during the first month of composting of organic wastes, however at the end of the process it is drastically decreased, which could be attributed to a reduction in easily degradable compounds (Ros et al. 2006; Raut et al. 2008; Albrecht et al. 2010). However, even though phosphatase activity indicates the potential for mineralization of organic P, we found no correlation between enzymatic activity and water extractable phosphorus in soils (data not shown). This could be explained by increased microbial activities, involving a higher P demand and release of phosphatases when organic waste, rich in easily available C and N, is incorporated. However, as the organic matter is consumed, microbial activity would decrease, decreasing both P demand and phosphatase activity (Criquet and Braud, 2008).

It is well known that cattle manure amendments can increase P availability along with the improvement of various soil physical, chemical and biological properties (Parham et al. 2002, 2003; Ros et al. 2006; He and Zhang 2014; Tiecher et al. 2014; Cai et al. 2019). In this study, the use of CM increased not only the WE P content but also increased the phosphatase activity and soil pH. This study shows that the use of citrus residues also improved these soil properties. The relationship between low-molecular-mass organic anions and inorganic phosphorus release from soils has been widely investigated (Kpomblekou-A and Tabatabai 2003; Harrold and Tabatabai 2006; Kizewski et al. 2010; Taghipour and Jalali 2013; Wang et al. 2015; Santos, Hesterberg, et al. 2017; Santos, Silva, et al. 2017). It is generally assumed that the mechanisms of release inorganic phosphorus involving low-molecular-mass organic anions include the dissolution of sparingly soluble minerals containing phosphorus, the change pH of soil solution and the capacity to chelate metal cations, notably Fe, Al, or Ca, and the blocking of P-adsorption sites on soil (Jones 1998; Ryan et al. 2001; Bais et al. 2006). In both incubation experiments increased WE P content after addition of citrus fruit was observed. We know of no studies on the use of citrus residues for mobilizing soil-P, nevertheless it is reasonable to suppose that the organic acids released from citrus lead to increased P solubility (Table 3.2). In the first incubation experiment, treatments with L-WF and CM, increased soil P release 7- fold respect to control after 1 hour. However, a marked decrease after one day was observed; this is probably due to the rapid microbial degradation of organic anions (Jones, 1998; Daniel Menezes-Blackburn et al., 2016).

Most importantly, in the medium term, after six weeks' incubation, treatments with cattle manure, 40% peel, and whole fruit always showed the largest WE P content. Similarly, a 2-fold increase of soil WE P with respect to control soil was observed in the incubation experiment with coarsely chopped fruit (Figure 3.2). This is coherent with reported increases in biomass production after addition of orange waste, for example that of durum wheat and lettuce, (Tuttobene et al. 2009; Guerrero et al. 1995) and points to improved P nutrition as a major.

3.5 Conclusions

Our findings showed that the addition of citrus residues in combination with beef cattle manure to soil had a sustained positive effect on phosphatase activity and an additional synergistic effect on soil P availability, assessed as WE P. The increase in phosphatase activity caused by organic residues used in this experiment could indicate an enhancement of soil fertility and thus the sustainability of agricultural production. There was no evidence that increased solubility of soil P inhibited phosphatase activity, even during the short-term flush of P solubilization due to citrate and other oxyanions in the citrus amendment. The addition of citrus waste enhanced the liming effect of manure throughout the 6-week experiment, a positive effect in this acid soil. The time trends observed demonstrate the importance of medium term experiments to avoid hasty conclusions based on short-lived flushes (<1 week). Furthermore, the "liming effect" produced by this type of residues could be used as a biotechnological strategy to decrease the lime requirements in such acid soils.

This controlled laboratory study is a promising basis for further longer-term and field based investigations of a mixed citrus-manure amendment. Furthermore, it is necessary to evaluate composting the organic matrices used for amplifying the obtained advantages, as well as, assess phytotoxicity reduction, stability of the organic substances in the soil and its effect on soil chemistry and microbiology.

CHAPTER IV

Assessment of the combined effects of cattle manure and lemon peel waste on soil-plant biochemical properties and phosphorus acquisition by ryegrass

Article submitted in Applied Soil Ecology

Assessment of the combined effects of cattle manure and lemon peel waste on soil-plant biochemical properties and phosphorus acquisition by ryegrass

Abstract

Agro-industrial waste-derived amendments may be used as substitutes for synthetic fertilizers to improve soil physicochemical and biological properties and crop production. Microbial activity plays an essential role in the beneficial effects or organic amendments on soil quality. In this study, we investigated the combined effects of two different agro-industrially derived amendments, cattle manure (CM) and lemon peel (LP), on soil biological properties, plant nutrition and antioxidant responses in ryegrass to evaluate their putative use as organic phosphorus fertilizers. A combined amendment of CM+LP was more effective than each one used separately. For example, microbial biomass was more than 4 times that of the control. This can be largely attributed to the fungal community because the richness biodiversity index was greater than that of the bacterial and fungal biomass was greater than bacterial biomass. The bacterial biodiversity index of the treated soils was lower than that of the control but was less dominant and more changeable with soil chemical parameters such as Al and pH. Interestingly, ryegrass yield and phosphorus uptake significantly increased with the addition of combined CM+LP (43% and 44%, respectively), as observed from treatments with synthetic triple superphosphate fertilizer (TSP). Less oxidative stress was observed in treatments with CM+LP supplementation than in treatments with TSP. In conclusion, the combined application of CM+LP showed a beneficial effect on P uptake and oxidative stress with a concomitant increase in plant productivity, indicating that both amendments could be used as organic fertilizers to improve the soil fertility and sustainability of agricultural production systems.

Keywords: organic residues; synergistic effect soil health; plant nutrition; oxidative stress

4.1. Introduction

Currently, worldwide, awareness is rising on the reuse, recovery and recycling of materials from the agricultural industry due to their ability to improve soil physical, biological and chemical characteristics and to supply an optimal combination of macro- and micronutrients for crops (Cercioglu, 2017; Diacono and Montemurro, 2010; Gopinath et al., 2008). In particular, the phosphorus scarcity has promoted changes in agricultural processes, since both prices and demand to meet food security needs, have increased since the mid-20th century (Neset et al., 2016). The use of organic fertilizers, including crop residues, cattle manure and biochar, can reduce the dependence on inorganic fertilizers. Furthermore, the sustainable management of wastes reduces waste levels and is a strategy of the circular economy, encouraged by the European Commission through its "Circular Economy Action Plan" (European Commission, 2015).

Livestock industry is a major activity in southern Chile, accounting for 79% of the national total head of livestock (ODEPA 2019), with an annual production of 42 million tons of manure (SAG 2006). In addition, approximately 90% of phosphorus contained in cattle manure is inorganic , which is the principal reason for its use as a source of P to compensate for the scarcity of phosphate rock (Cordell and Neset, 2014). This is especially important in soils where P is a limiting factor for plant production, as is the case for Andisol soils. In southern Chile, approximately 60% of agricultural soils are classed as Andisols, which are characterized by low pH and low available P concentrations, despite high total P levels (Borie et al., 1989; Escudey et al., 2001; Mora et al., 2017; Redel et al., 2016).

It is known that organic anions compete with phosphate on the soil anion exchange complex (Jara et al., 2006; Violante, 2013; Violante et al., 2002) and this is one of the reasons that plant roots exudate organic acids to compensate poor P nutrition, thus mobilizing phosphate from the soil, making it available for plant uptake (Waithaisong et al., 2015). We recently demonstrated that phosphate solubility is enhanced by combining CM and citrus residues as an amendment for acid soil. That study focused on the chemical effect of the citrus amendment, namely their high organic anion contents, mainly citrate and malate, with smaller amounts of oxalate and succinate. Sustained (6-week) increases in water extractable phosphorus, phosphatase activity and pH were reported when citrus residues were applied alone or in combination with cattle manure (CM) in an Andisol (Paredes et al., 2021). These results suggest that the use of these residues used in combination may be a strategy to improve soil quality (liming effects and hence Al toxicity) along with improving soil P availability. The aim of this study is to elucidate these effects emphasizing the role of changes in soil microbial activity. We adopted a two-prone approach. The first approach was to determine the effects of these organic residues (CM and LP) on the soil microbial community, assessed by microbial biomass C, N and P, and respiration. The second approach was to evaluate the improvement of soil quality for plant growth by measuring soil available nutrient status, plant phosphate uptake, and antioxidant responses in ryegrass grown in a greenhouse.

4.2. Materials and methods

4.2.1 Sampling and chemical characterization of soil and cattle manure samples

The soil used for the experiment was an Andisol collected from the Barros Arana locality of southern Chile located at 39°06'12"S, 72°37'42"W. The soil has a silt loam texture and is classified as an Andosol (IUSS Working Group, 2014) or Typic Hapludand (Soil Survey Staff, 2014). The soil was collected from 0 to 20 cm depth after removing a thin layer of surface litter, air dried and 2 mm sieved. Semi-fresh cattle manure was collected from Santa Elena Farm located in the same locality.

The samples were air-dried for 5 days, milled and sieved through a 2-mm sieve for subsequent analysis. Soil and CM, and soil after composting were characterized using routine methods according to Sadzawka et al. (2006). The chemical analysis of composted soil was carried out according to Sadzawka et al. (2006). pH was determined in H_2O with a 1:2.5 soil sample:water solution ratio. Total C and N were determined by dry combustion using a CHN autoanalyzer (CHN NA 1500, Carlo Erba). The available P was extracted with sodium bicarbonate (0.5 M $NaHCO_3$ at pH 8.50) using the Olsen method and quantified spectrophotometrically at 880 nm by the methylene blue complex method (Murphy & Riley, 1962). Basic exchangeable cations (Ca, Mg, Na and K) were extracted with 1 M ammonium acetate (pH 7) and determined by atomic absorption spectrophotometry (AAS). Exchangeable Al was extracted with 1 M potassium chloride and determined by EAA.

4.2.2 Characterization of lemon peel

The peels of the squeezed fruit samples were cut into small pieces. Ten grams of peel was placed in a flask with 25 ml of ultrapure water, shaken for one hour and membrane filtered. Extracts were analyzed by HPLC with a Shimadzu LC 20 DAD SPD-M20A detector. Reversed-phase separations were carried out using a 4.6 x 250 mm, 5 µm Symmetry C18 column. For chemical analysis, lemon peel was cut into small pieces and dried at 65 °C for 48 h according to Sadzawka et al. (2004). In addition, total carbon and nitrogen were determined using an elemental analyzer (CHN NA1500; Carlo Erba Elemental Analyzer, Stanford, CA, USA).

4.2.3 Incubation experiment

Soil and amendments were incubated under controlled conditions. Cattle manure (CM) was applied at a rate of 5%, and lemon peel (LP) was applied at a rate of 40%. The experiment included the following treatments: soil + CM; soil + LP; soil + CM + LP; soil without amendment was used as a control (Control). There were 12 experimental units with three replicates for each of the treatments. Organic materials were thoroughly mixed with air-dried soil samples and placed in plastic bags with small perforations. Soils were maintained at a water content of 70% of field capacity at 25 °C. Respiration was monitored for one month (see below) and at the end of this period, soil microbial biomass (C, N and P) and of the composition of soil microbial community were assayed. Incubation was continued for 3 months prior to the greenhouse experiment, and for clarity this soil is referred to as composted soil. 100-g samples were used for the shorter incubation experiment and 600-g for the longer composting prior to the pot experiment. The bags were mixed several times during the process to promote decomposition of organic matter.

4.2.3.1 Soil microbial biomass C, N and P

Microbial biomass C and N were determined by the fumigation–extraction method using ethanol-free $CHCl_3$ and 50 mM K_2SO_4 as an extractant (Vance et al., 1987), whereas microbial biomass P was determined according to Brookes et al. (1982). K_2SO_4-extractable C was quantified using an automated combustion total organic C (TOC) analyzer (TOC-V CPH Shimdzu, Japan), and the difference in C content between fumigated and nonfumigated samples was corrected using a KEC factor (the percentage of total microbial C extracted by K_2SO_4) of 0.45 to estimate SMB-C (Wu et al., 1990). N contents of fumigated and unfumigated soil extracts were determined using Kjeldahl digestion and MBN was calculated as the difference in NO_3^- content between fumigated and nonfumigated samples using an extraction efficiency factor (KEN) of 0.54 (Brookes et al., 1985). SMB-P was determined according to Brookes et al. (1982). The microbial biomass P (SMB-P) was calculated as the difference in P content between fumigated and nonfumigated samples using an extraction efficiency factor (KEP) of 0.40 accounting for the efficiency of Pi extraction from lysed microbial cells (Brookes et al. 1982); in addition, the calculation took into account P recovery measured as the proportion of the P spike recovered in each nonfumigated soil sample.

4.2.3.2 Analysis of Microbial Community composition by PCR-DGGE

The microbial community composition of the soil was evaluated at the end of the incubation experiments (30 d) by DGGE using specific primer sets for bacteria and fungi. Total DNA from soil was extracted using a Power Soil DNA Isolation Kit (QIAGEN, United States) according to the manufacturer's instructions. DNA was quantified, and its purity was evaluated using the A260/A280 and A260/A230 ratios provided by MultiskanTM GO software. Then, fragments of the 16S rRNA gene were amplified by touch-down polymerase chain reaction (PCR) with primer set EUBf933-GC (5' -GCA CAA GCG GTG GAG CAT GTG G G-3')/EUBr1387 (5' -GCC CGG GAA CGT ATT CAC CG-3') (Iwamoto et al). For fungal community analysis, fragments of the 18S rRNA gene were amplified by nested PCR. First, fragments were obtained by touchdown PCR using primer set NS1 (5′-GTA GTC ATA TGC TTG TCT C-3′)/NS8 (5′-TCC GCA GGT TCA CCT ACG GA-3′) followed by a second PCR with primer sets NS7-GC (5′-GAG GCA ATA ACA GGT CTG TGA TGC-3, GC-clamp: CGC CCG GGG CGC GCC CCG GGC GGG GCG GGG GCA CGG GGG)/F1Ra (5′-CTT TTA CTT CCT CTA AAT GAC C 3. All PCR amplifications were carried out with reagents supplied with GoTaq® Flexi DNA Polymerase (Promega, Co. Madison, WI, USA) (Durán et al., 2019).

DGGE analysis was performed using a DCode system (Bio-Rad Laboratories, Inc.). Twenty-five microliters of PCR product were loaded onto a 6% (w/v) polyacrylamide gel with a 40–70% gradient (urea and formamide). The electrophoresis was run for 16 h at 75 V. The gel was then stained with SYBR Gold (Molecular Probes, Invitrogen Co.) for 30 min and photographed on a UV transilluminator.

4.2.4 Microbial respiration

Microbial respiration was measured based on the alkali absorption of CO_2 at 25 °C for 30 days. Soil (control) or amended with CM (5%), LP (40%) or a mixture of both (as for the incubation experiments) was placed (20 g) in a 1 L flask with a vial containing 10 mL of 0.25 N NaOH and a vial containing 10 mL distilled water to maintain a humid atmosphere. The flask was hermetically sealed and incubated at 25 °C. Vials containing NaOH were periodically replaced. The CO_2 captured in the vial with NaOH solution was determined by titrating the remaining alkali with 0.25 N HCl after the precipitation of carbonate with 0.1 mol L^{-1} $BaCl_2$ (Iannotti et al., 1994)

.

4.2.5 Greenhouse experiment

A greenhouse assay was carried out using the composted soil (3-month incubation as above). In addition to the control, (SC), CM, LP and CM+LP treatments, triple superphosphate (TSP) addition was included as a chemical control. The amount of TSP added was equal to the measured increase in Olsen P after CM+LP amendment and 3-month composting. The TSP was milled and mixed with the soil prior to sowing the ryegrass seeds. Seventy seeds of ryegrass (*Lolium perenne* L.) cv. Nui were sown in each pot containing 0.6 kg of soil. After germination, the assay was thinned to 60 seedlings per pot, and 50 mg N kg^{-1} of soil (as urea) was applied to the SC and TSP treatments, whereas 30 mg N kg^{-1} of soil was applied to the LP, CM and CM+LP treatments. Three pots were used as replicates for each treatment (15 experimental units). During the growth period, the plants were watered daily with distilled water. Harvesting was carried out after 30 days once the shoot biomass of the TSP and CM treatments had reached 30 cm in plant height. The fresh shoots and roots were collected. Roots were washed first with tap water, then with distilled water to remove adhering soil. Plant material was stored at -20 or -80 °C for biochemical analyses, or dried at 65 °C for 48 h to determine dry weight (DW) and chemical parameters.

4.2.5.1 Plant chemical analyses

The dried shoot and root tissues were ground to a fine powder, ashed in a muffle furnace at 500 °C for 8 h, digested with 2 M HCl and paper filtered (Whatmann WHAT99-292-125). In the extract, P, Al, Ca, Mg, K and microelements were determined according to Sadzawka et al. (2004). The P concentration was measured spectrophotometrically using the molybdovanadate method. Al, Ca, Mg, K and microelements were measured by flame atomic absorption spectrophotometry FAAS. Moreover, total C and N were determined using an elemental analyzer (CHN NA1500; Carlo Erba Elemental Analyzer, Stanford, CA, USA).

4.2.5.2 Lipid peroxidation assay

The level of lipid peroxidation products was used as an oxidative stress indicator. In fresh shoot material, lipid peroxidation was measured using the thiobarbituric acid reacting substances (TBARS) assay according to Du and Bramlage (1992). The absorbance was measured at 532, 600 and 440 nm in order to correct for the interference generated by TBARS-sugar complexes, and the results were expressed in nmol of malondialdehyde (MDA) per g^{-1} of fresh weight.

4.2.5.3 Superoxide dismutase activity

The activity of superoxide dismutase (SOD; EC. 1.15.1.1) was assessed since this enzyme represent the first barrier of protection from oxidative damage (Yu and Rengel, 1999). The total SOD was determined by measuring the photochemical reduction of nitroblue tetrazolium (NBT) according to

Donahue et al. (1997). The amount of enzyme was defined as a 50% inhibition of the NBT reduction corresponding to one SOD unit, and enzyme activity was expressed on both a fresh weight and protein basis. The amount of protein in the crude enzyme extract was measured spectrophotometrically using bovine serum albumin (BSA) as the standard following the method developed by Bradford (1976).

4.2.6 Statistical analysis

Data were analyzed using a one-way ANOVA followed by Tukey's post hoc procedure. Different letters were used to display post hoc differences. The DGGE banding profile were clustered as a dendrogram by using Phoretix 1D analysis software (Clarke, 1993) (Total Lab Ltd., United Kingdom). In silico analysis was also used to estimate bacterial and fungal diversity by richness (S), the Shannon–Wiener index, and dominance by the Simpson index (D), represented by 1-D or 1-λ (Sagar and Sharma, 2012). Data normality of microbial community composition was analyzed according to Kolmogorov's test. Similarities between bacterial communities were visualized in PCO using Primer 7 software (Primer-E Ltd., Ivybridge, United Kingdom). Differences were considered significant when the P-value was lower than or equal to 0.01.

4.3. Results

The composition of the initial soil, lemon peel and cattle manure are given in Table 4.1

4.3.1 Incubation experiment

The cumulative CO_2 flux was significantly elevated in all amendments relative to the control, with LP having a larger effect than CM (Fig. 4.1). The production of CO_2 increased from 62 µg C-CO_2 g soil^{-1}, representing the basal respiration of the control soil, to 254 µg C-CO_2 g soil^{-1} in the CM+LP treatment (4-fold increase) on the first day. The peak was reached after 5 days in the soils treated with

CM+LP (inset to Figure 4.1). Values measured at the end of incubation decreased, approaching to control levels.

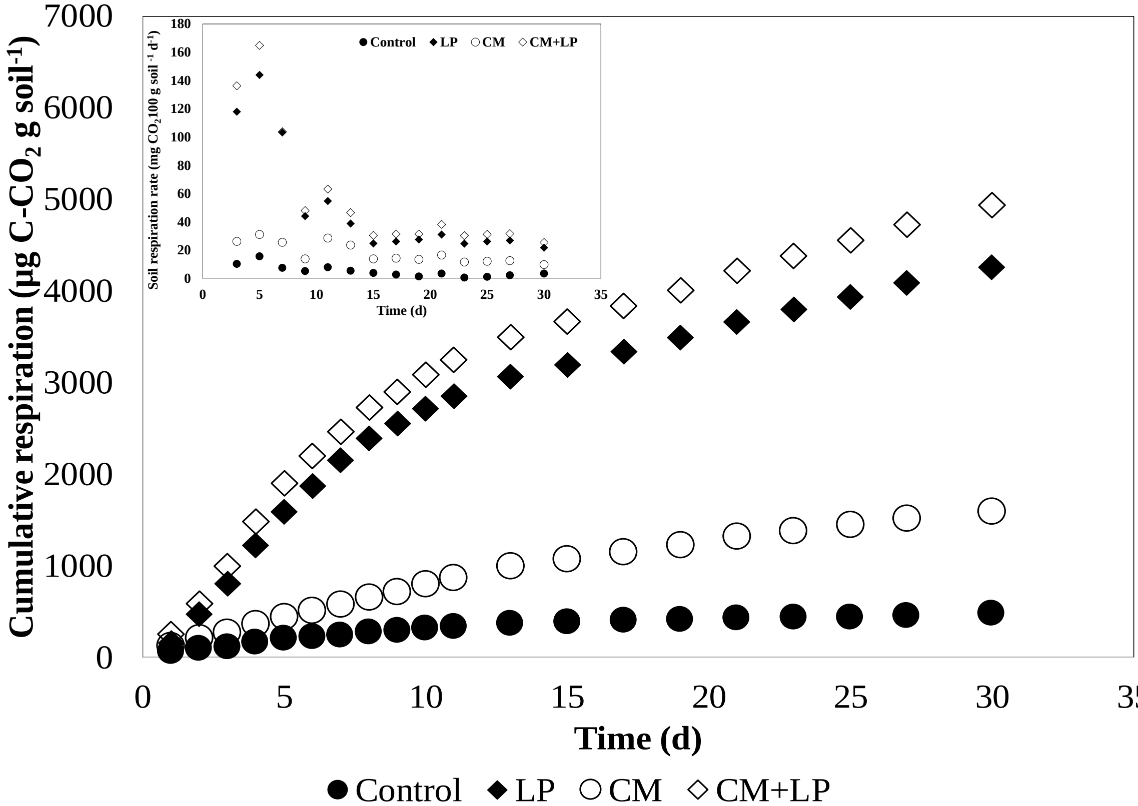


Figure 4.1: Cumulative CO_2 production in response to different soil treatments using cattle manure (CM), lemon peel (LP) or both (CM+LP) for a 1-month period. The insert shows the daily rate, to emphasis the greatest respiration in all systems after 5 days.

Values of SMB-C, SMB-N and SMB-P were higher in soil amended with CM and LP together than in the other treatments (Fig 4.2). SMB-C was significantly increased by both CM and LP, and their effects are additive leading to a more than 4-fold relative to that of the control soil (Fig 4.2A). SMB-N was decreased by CM and no significant effected of LP alone was observed, however in combination there was a synergistic effect resulting in an increase from 79 mg kg^{-1} to 221 mg kg^{-1} relative to the control soil (Fig. 4.2B). The CM amendment led to a 3-fold increase in SMB-P (Fig.

4.2C), and the combined addition of LP enhanced this increase 2-fold, although alone it had no significant effect.

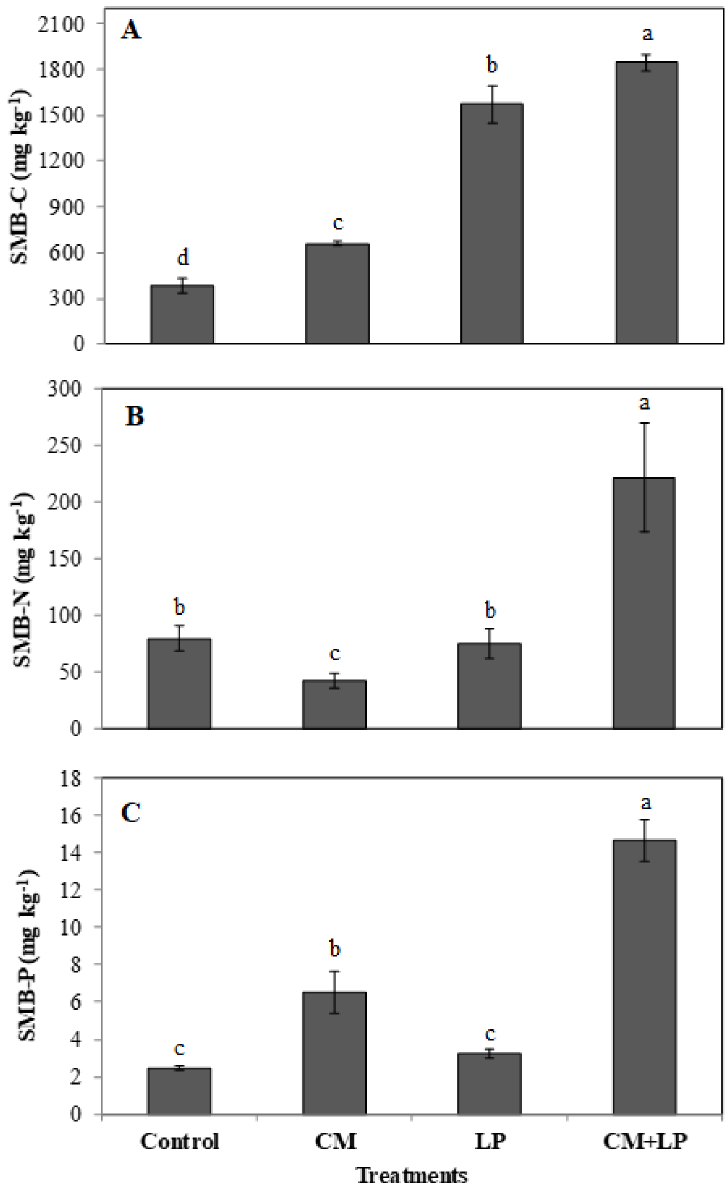


Figure 4.2. Soil microbial biomass (A) C, (B) N, and (C) P in response to different soil treatments using cattle manure (CM), lemon peel (LP) or both (CM+LP). Data are means of three replicates ± SD. Different letters indicate significant differences (p≤0.05) between treatments.

Figure 4.3 shows the effects of treatments on the bacterial community composition as revealed by the DGGE analysis. Both the dendrogram and PCA show that treatments involving LP (both LP and CM+LP) were grouped separately with respect to treatments without LP (control and CM), showing different community composition at distance=3. However, the biodiversity index (measuring richness and dominance), showed that treatments with organic amendment showed less richness of species (S) and individuals (N) than the control but were less dominant (1- λ) (Supplementary Figure A1). When we compared the richness index to chemical properties, we found an inverse correlation with pH and corresponding positive relations with exchangeable Al content (Supplementary Figure A2). In contrast, dominance followed an inverse trend (less dominance with a low Al and high pH).

Fungal community compositions were not related to amendments, with all treatments grouped together except in the case of CM+LP (Figure 4.4). This treatment showed considerable levels of diversity and dominance relative to the rest of the treatments, but the results were not correlated with chemical properties.

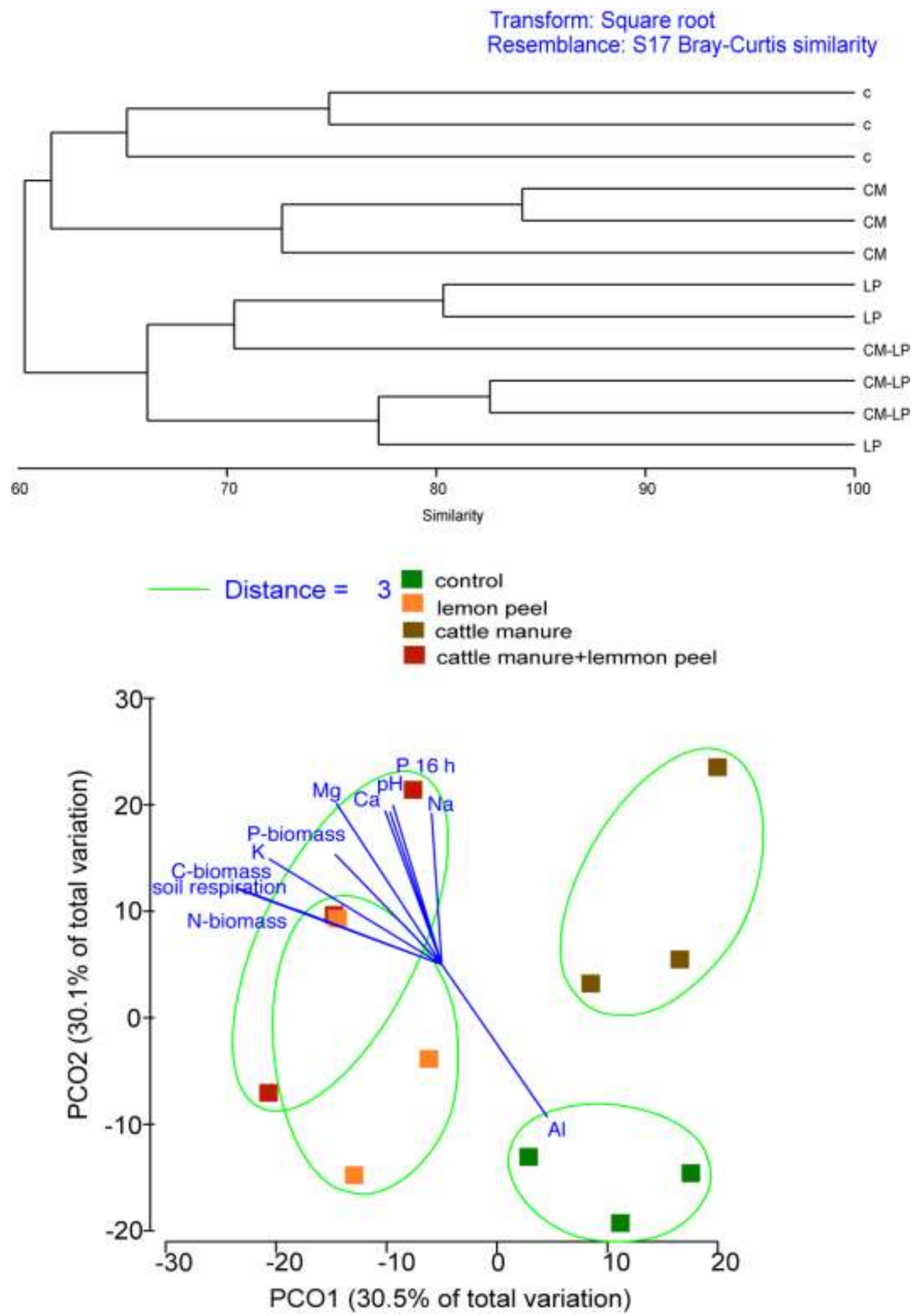


Figure 4.3. Dendrogram and principal component analyses of DGGE profiles of bacteria (16S rRNA gene) after 30 days of incubation experimentation.

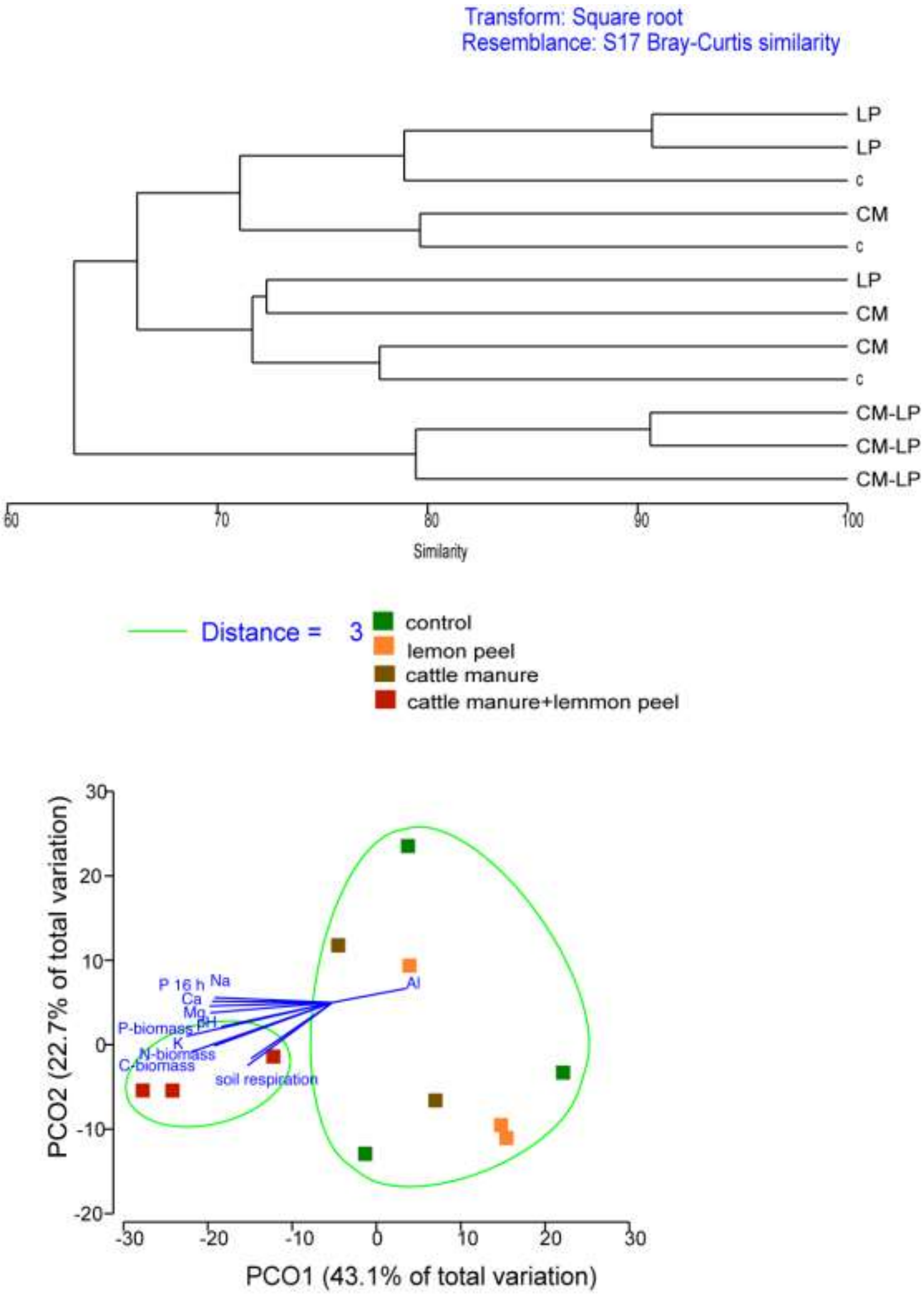


Figure 4.4. Dendrogram and principal component analyses of DGGE profiles of fungi (18S rRNA gene) after 30 days of incubation

Table 4.1: Chemical properties of soil, cattle manure and lemon peel. Mean values (n = 3) ± SD. For Soil and cattle manure (CM) phosphorus concentration (P) corresponds to Olsen P, while for lemon peel (LP) is total P. Water-extractable organic anions (citrate, oxalate and malate) were quantified by HPLC.

	P	Ca	Mg	Na	K	Al	C/N	pH	Citrate	Oxalate	Malate
	mg kg^{-1}								g kg^{-1}		
Soil	6 ± 1	814 ± 8	113 ± 1	41 ± 2	137 ± 4	10 ± 1	12.5 ± 0.2	5.6 ± 0.0	-	-	-
CM	497 ± 10	3642 ± 16	824 ± 2	1141 ± 2	1767 ± 20	11 ± 1	19.2 ± 0.5	7.0 ± 0.0	-	-	-
LP	845 ± 12	5403 ± 551	777 ± 276	260 ± 36	9222 ± 2122	2.9 ± 0.5	33.4 ± 0.9	2.9 ± 0.1	8.7 ± 0.0	0.5 ± 0.0	0.2 ± 0.0

4.3.2 Greenhouse experiments

The content of available nutrients was significantly modified three months after the addition of cattle manure and lemon peel to the soil. There was a significant increase in Olsen P, and exchangeable cation (Ca, Mg, Na, and K) contents and pH (Table 4.2). The content of Olsen P increased 1.5-fold when LP was applied, whereas that of soil treated with LP and CM increased 2.6-fold relative to the soil control. Exchangeable Ca, Mg, Na and K contents were significantly higher ($P < 0.05$, Tukey test) when LP and CM had been applied. There was no effect of amendments on N and C concentrations for either treatment. pH was significantly influenced by CM or LP, but the combined effect of cattle manure/lemon peel increased pH by 0.8 units compared to the control soil.

Table 4.2: Soil chemical properties at the end of composting (3 months).

	Olsen P	Ca	Mg	Na	K	Al saturation	pH	C/N
	(mg kg^{-1})					(%)		
Control	5.4 ± 0.3^{c}	890 ± 22^{d}	102 ± 5^{d}	23 ± 2^{d}	98 ± 4^{d}	3.4 ± 0.1^{a}	5.2 ± 0.1^{b}	12.7^{a}
LP	6.0 ± 0.2^{b}	1046 ± 20^{c}	144 ± 7^{c}	35 ± 2^{c}	551 ± 12^{b}	0.4 ± 0.1^{b}	5.7 ± 0.2^{a}	12.7^{a}
CM	8.6 ± 0.5^{a}	1420 ± 16^{b}	235 ± 1^{b}	156 ± 2^{b}	414 ± 4^{c}	0.1 ± 0.0^{bc}	5.7 ± 0.1^{a}	12.7^{a}
CM+LP	9.4 ± 0.3^{a}	1558 ± 50^{a}	273 ± 10^{a}	166 ± 5^{a}	962 ± 70^{a}	0.01 ± 0.0^{c}	5.9 ± 0.1^{a}	12.6^{a}

The application of CM and CM+LP resulted in 39 and 44% higher ryegrass yields than those of the soil control, respectively (Fig. 4.5A). Interestingly, the same yield was obtained with the CM+LP treatment and chemical fertilizer (TSP). The treatments involving organic amendments increased shoot P content by 25% with CM and 43% with CM+LP, while mineral fertilizer treatment (TSP) increased by 32% relative to the soil control. However, no significant differences were observed in the amount of P in shoots with both treatments (CM and CM+LP) relative to the TSP treatment, whose values were 0.92, 0.95 and 0.99 g pot^{-1}, respectively (Fig. 4.5B).

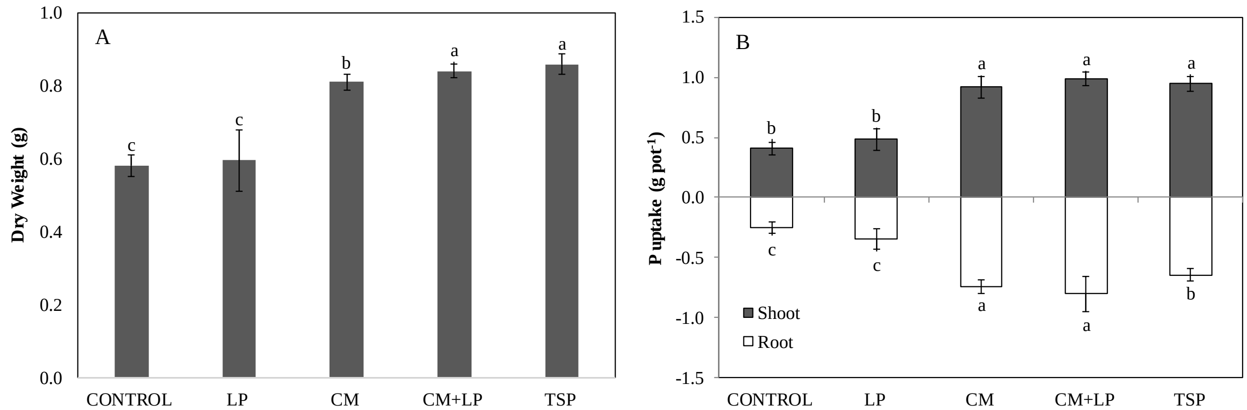


Figure 4.5. Dry weight of shoot (A) and **s**hoot and root P uptake of ryegrass plants under cattle manure (CM), lemon peel (LP) or both (CM+LP) and triple superphosphate (TSP) treatments. Data are means of three replicates ± SD. Different letters indicate significant differences ($p \leq 0.05$) between treatments.

In addition, macronutrients were significantly higher in the treatment that received TSP than in the control (Table 4.3). In contrast, the addition of LP significantly decreased values of Ca (51%), Mg (38%) and Al (70%). Although there were few differences in the macronutrient levels in shoots from the CM+LP treatment relative to the control, significant differences were observed in the K uptake, which increased 66%, whereas those of Ca and Al decreased by 12 and 49%, respectively (Table 4.3).

Table 4.3. Effect of treatments on shoot plant uptake. Mean values (n = 3) ± SD.

Plant part	Treatment	Ca	Mg	Na	K	Al
		mg pot^{-1}				µg pot^{-1}
Shoot	Control	$5.9^{b} \pm 0.3$	$2.0^{c} \pm 0.1$	$1.2^{c} \pm 0.1$	$26.5^{bc} \pm 1.6$	$31.4^{b} \pm 4.3$
	TSP	$11.2^{a} \pm 1.4$	$4.2^{a} \pm 0.3$	$5.9^{a} \pm 0.6$	$30.7^{b} \pm 2.0$	$38.1^{a} \pm 3.8$
	LP	$2.8^{c} \pm 0.4$	$1.2^{d} \pm 0.3$	$0.9^{c} \pm 0.3$	$23.3^{c} \pm 4.2$	$9.5^{d} \pm 1.1$
	CM	$4.9^{b} \pm 0.3$	$2.6^{b} \pm 0.2$	$2.8^{b} \pm 0.4$	$40.6^{a} \pm 2.9$	$13.8^{c} \pm 1.9$
	CM+LP	$5.1^{b} \pm 0.5$	$2.5^{b} \pm 0.2$	$3.0^{b} \pm 0.3$	$43.9^{a} \pm 2.3$	$16.1^{c} \pm 4.2$
Root	Control	$1.5^{b} \pm 0.1$	$0.5^{b} \pm 0.0$	$0.4^{c} \pm 0.1$	$2.7^{bc} \pm 0.2$	$617^{c} \pm 106$
	TSP	$2.8^{a} \pm 0.6$	$0.9^{a} \pm 0.1$	$0.9^{a} \pm 0.0$	$2.6^{c} \pm 0.3$	$2066^{a} \pm 286$
	LP	$2.8^{a} \pm 0.4$	$0.4^{b} \pm 0.1$	$0.2^{c} \pm 0.1$	$3.2^{b} \pm 0.8$	$555^{c} \pm 155$
	CM	$1.4^{b} \pm 0.3$	$0.9^{a} \pm 0.1$	$0.6^{b} \pm 0.0$	$6.8^{a} \pm 0.3$	$1391^{b} \pm 126$
	CM+LP	$2.9^{a} \pm 0.1$	$0.8^{a} \pm 0.2$	$0.5^{b} \pm 0.1$	$6.7^{a} \pm 1.3$	$1437^{b} \pm 276$

Control: soil control; LP: lemon peel; CM: cattle manure and TSP: triple superphosphate

Table 4.4: Soil chemical properties after harvest. Mean values (n = 3) ± SD.

	Olsen P	Ca	Mg	Na	K	Al saturation	pH	C/N
	mg kg^{-1}					%		
Control	4.4 ^{c} ± 0.5	835 ^{d} ± 35	85.8 ^{d} ± 2.5	21.5 ^{c} ± 1.3	39.1 ^{d} ± 0.0	14.1 ^{a} ± 0.5	5.1 ^{d} ± 0.0	11.9 ^{a} ± 0.2
TSP	4.4 ^{c} ± 0.3	860 ^{d} ± 13	78.9 ^{d} ± 5.5	14.6 ^{d} ± 3.5	37.8 ^{d} ± 2.3	12.0 ^{b} ± 0.5	5.4 ^{c} ± 0.0	11.9 ^{a} ± 0.2
LP	3.7 ^{c} ± 0.4	963 ^{c} ± 11	111 ^{c} ± 1	39.1^{b} ± 2.3	395 ^{b} ± 7	9.6 ^{c} ± 0.5	5.7 ^{b} ± 0.1	12.0 ^{a} ± 0.1
CM	7.7 ^{a} ± 0.3	1321 ^{b} ± 14	227 ^{b} ± 6	158 ^{a} ± 4	283 ^{c} ± 16	5.7 ^{d} ± 0.5	5.7 ^{b} ± 0.0	12.2 ^{a} ± 0.2
CM+LP	6.6 ^{b} ± 0.5	1435 ^{a} ± 21	243 ^{a} ± 6	163 ^{a} ± 10	602 ^{a} ± 7	4.2 ^{e} ± 0.5	6.0 ^{a} ± 0.0	12.2 ^{a} ± 0.3

Control: soil control; LP: lemon peel; CM: cattle manure and TSP: triple superphosphate

Lipid peroxidation in shoot showed a significant decrease in response to organic amendment applied (Fig. 4.6A). When plants were grown in LP and CM treatments, the amount of TBARS decreased 1.3 fold, while those of CM+LP and TSP decreased 1.7 fold. There was no significant difference in the amount of TBARS in CM+LP treatment in comparison to mineral fertilizer (TSP treatment). Meanwhile, regarding the enzymatic antioxidant activity, a differential SOD activity in response to organic amendments applied was observed in shoot (Fig. 4.6B). Control shoots exhibited the highest SOD activity compared to the other treatments. The application of LP caused a decrease in SOD activity by 6% in comparison to the control. While, when CM and CM+LP were applied, SOD activity was significantly reduced by 20 %. However, there was no significant difference in SOD activity among the CM, CM+LP and TSP treatments.

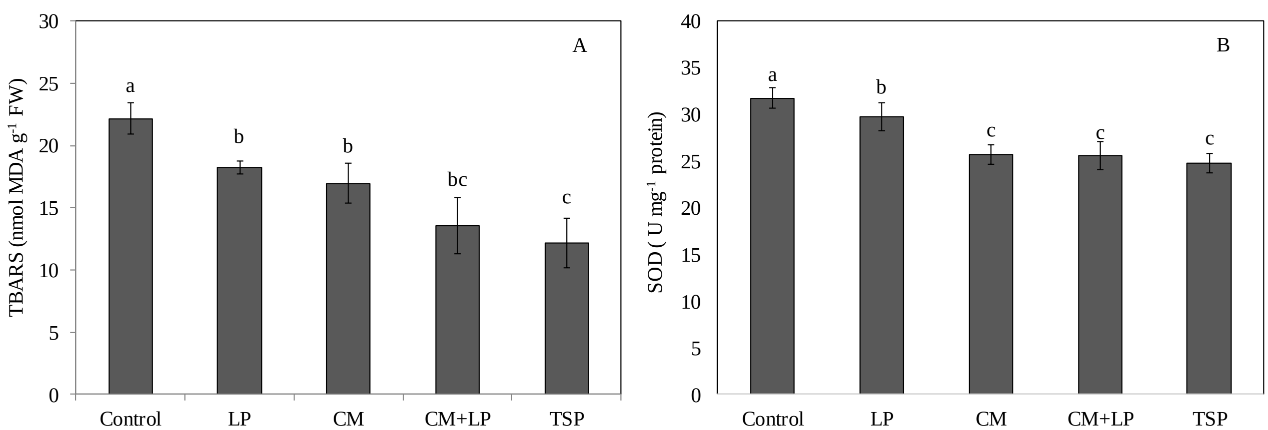


Figure 4.6. Shoot lipid peroxidation (TBARS) (A) and superoxide dismutase activity (SOD) (B) of ryegrass plants under cattle manure (CM), lemon peel (LP) or both (CM+LP) and triple superphosphate (TSP) treatments. Data are means of three replicates ± SD. Different letters indicate significant differences ($p \leq 0.05$) between treatments.

4.4. DISCUSSION

4.4.1 Incubation experiment

The addition of organic amendments such as CM and LP increased microbial activity as determined by CO_2 evolution and microbial biomass, probably due to the stimulation of microbial activity and growth as CM and LP were bio-degraded, providing nutrients and readily available C sources. However, analysis of the microbial community composition by DGGE, showed that the bacterial community was more influenced than the fungal community. Similarly greater stability of fungal composition than that of bacteria have been observed by other studies (Barnard et al., 2013; Durán et al., 2018), nevertheless the fungal community showed high richness indexes in treatments with organic amendment but considerable dominance relative that of the control. One explanation is that representative fungal groups had beneficial effects on plants but this hypothesis would have to be verified by future research. This is in agreement with the findings of (Paula et al., 2020) who report that fungal activity was enhanced on addition of an organic amendment (spent mushroom substrate) in a 14-week incubation. Bacterial communities of the amended soils were less dominant but showed a lower richness index than in the control soil, possibly because bacterial communities displayed higher resilience to the changes introduced by organic amendments.

It is well established that organic soil amendments can release nutrients for crop uptake and increase soil organic matter content, thereby influencing soil biological properties and organic matter fractions (Nguyen and Marschner, 2016). Chu et al. (2007) observed in a field experiment that organic manure had a significantly greater impact on microbial biomass C and microbial activity than mineral fertilizers, while microbial metabolic activity was significantly higher under balanced fertilization than under nutrient-deficient fertilization. Soil microorganisms can survive in the soil even in an inactive status but they react immediately to residue addition (Gatiboni et al., 2011).

SMB was more strongly affected by the combined CM+LP than by each treatment alone. Significant differences among all treatments suggest that CM and LP amendments had a considerably positive effect on the size and activity of the microbial community. Soil microbial biomass has been used as an indicator for ecosystem nutrient limitations (Güsewell, 2004; Xu et al., 2013), since soil microbes are more efficient in taking nutrients up than plants (Bardgett et al., 2003). In nutrient-depleted ecosystems, the relatively high fraction of specific nutrients in microbial biomass implies strong limitations of this element for plants (Jonasson et al., 1999).

4.4.2 Greenhouse experiments

Generally, treatments improved soil nutrient status. In particular, Olsen P levels increased by 11% in soil treated with LP peel and 73% when CM+LP was applied in combination. Similarly, exchangeable cations (Ca, Mg, Na and K) significantly increased following the addition of these residues to the soil (Table 4.2). Organic residues have been reported to increase P availability in soils with high P retention (Guppy et al., 2005), probably due to the release of byproducts of residue decomposition, such as low-molecular-weight (LMW) organic acids. Organic oxyanions compete for soil P sorption sites, resulting in increased soil P availability (Kang et al., 2009; Yu et al., 2013). Moreover, organic amendments increase soil fertility due to the addition of soluble base cations, which are released from the mineralization of organic residues. In this case, both residues are an essential source of cations; in particular, CM contributed Ca and Mg (Table 4.1), whereas LP released K and organic acids (Table 4.1). Liu et al. (2012) and Hargreaves et al. (2008) reported an increase in cation exchange capacity when composting at different rates was applied to soil. In addition, Cellier et al. (2014) evaluated the use of compost to restore soil fertility and improve plant nutrition in Mediterranean areas affected by fires.

The soil liming effect due to the addition of residues (CM and LP) increased soil pH thus decreasing concentrations of phytotoxic Al (Haynes and Mokolobate, 2001). The liming effect of organic amendment observed after composting was maintained during plant growth. The value of pH in amended soil was greater by 0.9 units than in the control soil and this led to a 70% decrease in Al concentrations. Considering the fertility constraints of volcanic soils, these residues could be used as complementary fertilizers by improving the chemical properties of these soils. Moreover, after harvesting, soil pH, Al saturation, Olsen P and exchangeable cations were significantly higher in the organic amended soil than in the mineral fertilizer treated soil (Table 4.4). This confirms the important role that organic residues can play in buffering pH and soil nutrition (Neina, 2019; Nguyen and Marschner, 2016).

In this study, no significant differences in shoot dry weight were found between the CM+LP and synthetic fertilizer (TSP) treatments. A similar result was reported by Tuttobene et al. (2009), who demonstrated that the use of dried orange waste produced wheat yields similar to those got with mineral fertilizers. In contrast, a study carried out by Gopinath et al. (2008) showed that wheat yields in all treatments involving organic residues were markedly lower than those obtained through mineral fertilizer treatment.

There were no significant differences in either shoot or root P uptake by ryegrass between organic and mineral amendments. This suggests that CM and CM+LP supplied P to the soil and also improved its availability by reducing sorption (Hartono et al., 2005; Yu et al., 2013). It is known that environmental stresses such as Al toxicity, low pH and P-deficiency among others can cause oxidative stress damage in plants. In our study, the application of CM or CM+LP reduced lipid peroxidation and SOD activity (Fig 4.6). These results could be explained by the decrease of exchangeable Al, the increase of P availability and the liming effect, caused by the addition of the organic residues used in this study. In agreement with our results, Bowden et al. (2010) observed a decrease in lipid peroxidation with a significant reduction on SOD and APX activities in corn leaf, attributable to the application to organic amendments and the sufficient N supply. This could reduce the production of reactive oxygen species (ROS), and therefore, the need of the plant to increase the enzymatic antioxidant protection to control lipid peroxidation. On the other hand, organic amendments have been shown to have a protective effect against metal toxicity by increasing enzyme activities such as SOD, CAT, and POD, as a part of the defense mechanisms in plants (Nigam et al., 2019; Ramzani et al., 2017). However, in this study, we demonstrate that the use of combined CM+LP may improve plant nutrition and maintain low levels of damage and antioxidants at lower levels.

4.5. Conclusions

The combined addition of two different organic wastes improved both soil and plant biochemical properties and caused a significant increase in P nutrition with a concomitant effect on ryegrass yields. It is important to emphasize that the main change in the soil observed can be attributed to the increase in pH (15%) and decrease in Al saturation (70%) when CM+LP was applied. Furthermore, Olsen P concentrations significantly increased (73%). Therefore, from an agronomic point of view, these residues could be used as organic fertilizers or for complementary fertilization because they have dual advantages: a very significant P source and a liming effect on soil, leading to a decrease in the cost and quantity of chemical fertilizers used. Hence, using both combined residues as organic fertilizer results in higher soil fertility and productivity without affecting the environment.

CHAPTER V

General discussion, concluding remarks and future directions

5.1 General discussion

The current assessment of phosphorus (P) status in the world, indicates that increasing global P demand, dwindling global P reserves, and increasing geopolitical constraints could affect the supply of P and impact upon global food security (Chowdhury et al., 2017). Hence, strategies for sustainable P management should be promoted, including P recycling and reducing demand (Powers et al., 2019). Moreover, as mentioned above phosphorus plays an important role in plants development and growth, hence, an adequate soil P availability is required for optimum crop production. This thesis evaluated the effect to applied cattle manure (CM) alone or in combination with citrus residues, on soil P availability, soil biological properties and plant nutrition in ryegrass.

Our findings shown in Chapters III and IV, the combined application of CM and citrus residues improved soil biochemical properties and induced a significant increase in P nutrition along with a positive effect on ryegrass yield. In the incubation experiments (Chapter III), the results showed an initial large flush of water extractable soil P (WE-P) content after CM and citrus amendment to soil, this flush larger being in manure-amended soil. Besides, the citrus amendment also caused an increase in WE-P content and this increase was greatest when whole fruit was added, followed by peel. We do not know of previous studies focused on the mobilization of soil-P by citrus residues, however, it is reasonable to suppose that the increased P solubility is caused by both, organic acids released from citrus and the addition of readily decomposable organic matter. The relation between organic acids and phosphorus release has been widely reported, as organic acid exudation is one of the mechanisms used by roots plants for mobilizing unavailable P in soil (McKay Fletcher et al., 2019; Richardson et al., 2011; Wang et al., 2007, 2008). If we considered that millimolar concentrations of organic anion are required in the soil solution to effectively increase soluble P concentrations (Ryan et al., 2001), in this study we showed high concentrations of water-extractable organic acids, being the citric acid the highest concentration, and it was found in the peel and juice of the lemon (292 and 308 mM, respectively). The use of organic amendments has been suggested by many authors as an strategy to improve the chemical and biological properties of soil (Mokolobate and Haynes, 2002; Naramabuye and Haynes, 2006a; Parham et al., 2002; Waldrip et al., 2012; Whalen et al., 2000). We found that the combined amendment of CM and citrus residues significantly increased both phosphatase activity and soil pH. This finding is in agreement with the findings of Waldrip et al. (2012), where cattle manure had a large effect on acid phosphatase activity. However, in our study, acid phosphatase activity was more strongly influenced by the citrus treatments than the CM amendment. This effect could be explained by the stimulation of microbial growth due to the incorporation of readily degradable organic substrates, as suggested by Marinari et al. (2000) who attributed the increase of acid phosphatase to a priming effect for mineralization of native soil OM and subsequent release of soluble nutrients for microbial growth.

Despite the acidity of citrus waste, addition of each of the citrus treatments were found to increase soil pH after the first week. This was attributed to the decomposition of organic matter, including the decarboxylation of the organic anions (Naramabuye and Haynes, 2006a). According to Xu et al. (2006), the addition of plant residues to soil leads to increases in soil pH, depending on their cation and nitrogen contents , rates of application and decomposition, and on the initial pH and buffer capacity of the soil. In addition, it is well established that the addition of organic manure to acidic soils increases the soil pH considerably. It has been suggested that the mechanism that best describes the neutralization of soil acidity by animal manure application is microbial decarboxylation of calcium-organic matter complex that lead to the release and subsequent hydrolysis of calcium ions (Ano and Ubochi, 2007). In addition to the direct and lasting increase in soil organic matter content following manure amendment, there is evidence that manure can ameliorate Al toxicity, and reduce soil acidity, probably by complexation (Naramabuye and Haynes, 2006b).

The beneficial effect of organic amendments on soil biota is well known (Jilani et al., 2007; Zhang et al., 2012) and their effect well documented through the analysis of different parameters, as soil respiration, enzyme activity, microbial biomass and DNA analysis (Dumontet et al., 2017; Intanon et al., 2015; Marschner et al., 2015). In this study, we observed that the soil respiration was significantly increased relative to the control following each of the amendments, but lemon peel (LP) had a larger effect than cattle manure (CM) (Fig. 4.1). Furthermore, significant differences in soil microbial biomass were observed, suggesting a positive effect on both the size and activity. This increase was influenced by the readily metabolizable nutrients found in CM and LP, meeting the needs of microbial biomass. This result is consistent with the observations of Liu et al. (2010), who reported that the microbial biomass was greater when organic manure was applied along with inorganic fertilizers. Use of organic materials including crop residues, manures and composts has been suggested to enhance soil nutrient bioavailability and to increase fertilizer use efficiency (Gichangi et al., 2009; Malik et al., 2013). These materials stimulate the synthesis of soil microbial biomass and labile microbial metabolites rich in phosphorus, which act as a labile pool of P in soil protected against fixation. During the process of biomass turnover, this P may be released slowly and taken up by the plants more efficiently (Khan and Joergensen, 2009).

The microbial community analysis showed a greater stability the composition of the fungal community in comparison with the bacterial community and showed considerable levels of diversity and dominance relative to the rest of the treatments. This trend was consistent with Paula et al. (2020), who report that fungal activity was enhanced on addition of an organic amendment in an incubation experiment. On the other hand, bacterial communities of the amended soils were less dominant that the non-amended soil, but showed a lower richness index than the control soil, possibly because bacterial communities displayed higher resilience to the changes introduced by organic amendments. A long-term fertilization study carried out by Chu et al. (2007), indicated that treatments with organic fertilizer had few effects on the richness and diversity of soil general bacterial community, indicating that the bacteria in organic compost themselves had no significant effect on soil bacterial community. Our study also assessed the effect of two types different agro-industrially derived amendments, cattle manure (CM) and lemon peel (LP), on plant nutrition and antioxidant responses in ryegrass. We observed that the combined application of CM and LP significantly increased Olsen P and exchangeable cations. As was previously mentioned, organic residues increase soil fertility due to release of by-products of organic matter decomposition (organic acids and soluble base cations). In addition, total plant biomass production was as good for plants receiving either CM+LP or an equivalent amount of available P as synthetic fertilizer (STP). This observation is consistent with the findings of Waldrip et al. (2011), where it was observed that poultry manure amendment increased early growth of perennial ryegrass, with no statistically significant difference in biomass production between plants receiving inorganic fertilizer and poultry manure. Furthermore, CM+LP also improved oxidative stress as assessed by the superoxide dismutase activity by reducing lipid peroxidation in ryegrass shoot. We assume that this was caused by the decrease in exchangeable Al, the increase in P availability and the liming effect, produced by the addition of organic residues. We conclude that the use of these residues in combination (CM and LP) is an efficient strategy to offset chemical P fertilizer requirements, by improving P availability and by decreasing the toxic effects of acidity of soil.

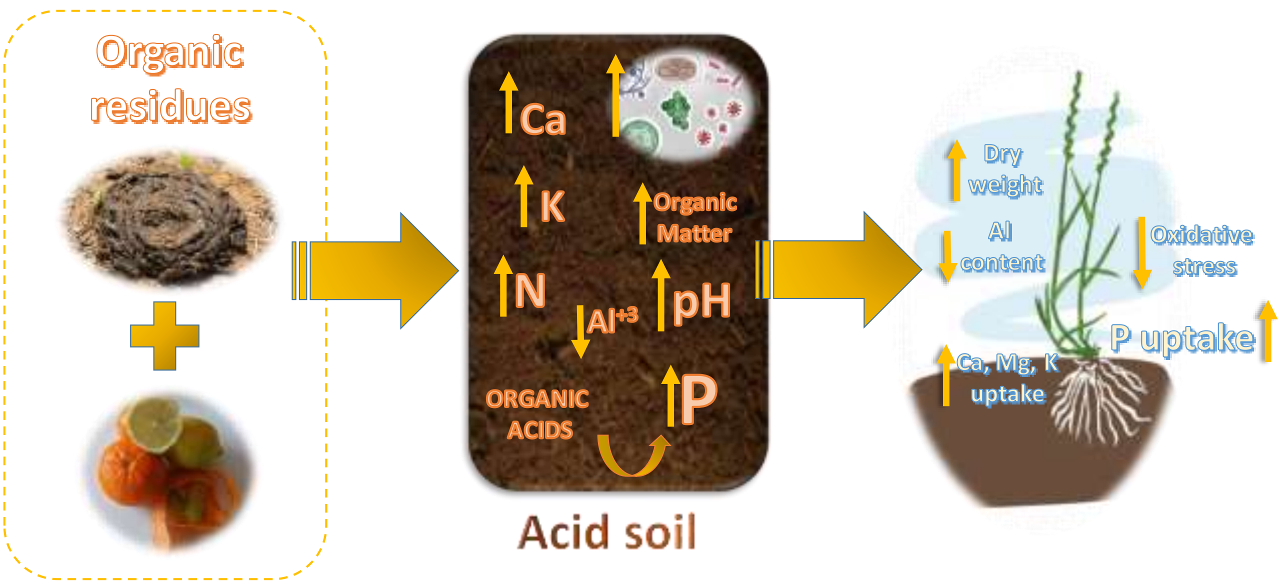
5.2 Concluding remarks and future directions

New, alternative sources of P are urgently required to alleviate the potential geopolitical problems associated with available phosphorus (P) reserves, along with the high P demand for satisfying food requirements. The use of agroindustrial by-products, such as cattle manure (CM) and lemon peel (LP) are a sustainable strategy to cope with this issue. Our findings showed that the addition of citrus residues in combination with cattle manure significantly increased soil phosphatase activity and available P content. The increase in P availability is probably caused by organic acids present in the citric amendment and by the stimulation of microbial biomass. Moreover, the liming effect produced by the CM was enhanced by the addition of the citrus amendment. Further, our study reveals that the combined use of CM and LP enhanced the microbial biomass, particularly the fungal community, because both richness biodiversity and biomass were greater for the fungal community in comparison with the bacterial community.

In the greenhouse study, we found that the use of CM+LP gave fodder ryegrass yield and phosphorus uptake equal to that with an equivalent addition of inorganic fertilizer (triple superphosphate) as well as a less oxidative stress. It is important to emphasize that this results can be attributed to the increase in soil pH and decrease in Al saturation, when both residues where applied in combination. Overall, our study showed that there is a good potential for using the combination of cattle manure and lemon peel, with improvements in soil microbial and chemical properties, assessed by soil microbial biomass, pH and the proportion of available soil P. These improvements have a direct impact on phosphorus uptake and plant biomass.

Consequently, we suggest that more research is required in order to elucidate the dynamics of nutrient release from residues and organic amendments, and thus promote their widespread use in agricultural soils. In particular, the ideal ratio citrus waste-cattle manure must be determined. Moreover, an industrial strategy such as composting must be evaluated to convert these residues into a convenient and cost-effective product with high nutrient content with reduced environmental impact produced by fresh residues. Assays with 13C-labelled residues to evaluate the decomposition processes and quantity of residue carbon incorporated into soil organic carbon pools would also be informative.

5.3. Graphical summary



REFERENCES

Abaye, D.A., Lawlor, K., Hirsch, P.R., Brookes, P.C., 2005. Changes in the microbial community of an arable soil caused by long-term metal contamination. Eur. J. Soil Sci. 56, 93–102. https://doi.org/10.1111/j.1365-2389.2004.00648.x

Abdala, D.B., da Silva, I.R., Vergütz, L., Sparks, D.L., 2015. Long-term manure application effects on phosphorus speciation, kinetics and distribution in highly weathered agricultural soils. Chemosphere 119, 504–514. https://doi.org/10.1016/j.chemosphere.2014.07.029

Abolusoro, S.., Oyedunmade, E.., Olabiyi, T.., 2010. Evaluation of sweet orange peel aqueous extract (Citrus sinensis) as root – knot nematode suppressant. J. Trop. Agric. Food, Environ. Ext. 9, 170–175.

Achat, D.L., Bakker, M.R., Saur, E., Pellerin, S., Augusto, L., Morel, C., 2010. Quantifying gross mineralisation of P in dead soil organic matter: Testing an isotopic dilution method. Geoderma 158, 163–172. https://doi.org/10.1016/j.geoderma.2010.04.027

Adeleke, R., Nwangburuka, C., Oboirien, B., 2017. Origins, roles and fate of organic acids in soils: A review. South African J. Bot. 108, 393–406. https://doi.org/10.1016/j.sajb.2016.09.002

Agegnehu, G., Bass, A.M., Nelson, P.N., Bird, M.I., 2016. Benefits of biochar, compost and biochar-compost for soil quality, maize yield and greenhouse gas emissions in a tropical agricultural soil. Sci. Total Environ. 543, 295–306. https://doi.org/10.1016/j.scitotenv.2015.11.054

Albrecht, R., Petit, J. Le, Calvert, V., Terrom, G., Périssol, C., 2010. Changes in the level of alkaline and acid phosphatase activities during green wastes and sewage sludge co-composting. Bioresour. Technol. 101, 228–233. https://doi.org/10.1016/j.biortech.2009.08.017

Alobwede, E., Leake, J.R., Pandhal, J., 2019. Circular economy fertilization: Testing micro and macro algal species as soil improvers and nutrient sources for crop production in greenhouse and field conditions. Geoderma 334, 113–123. https://doi.org/10.1016/j.geoderma.2018.07.049

Alvarez, J., Hooshdaran, B., Cortazar, M., Amutio, M., Lopez, G., Freire, F.B., Haghshenasfard, M., Hosseini, S.H., Olazar, M., 2018. Valorization of citrus wastes by fast pyrolysis in a conical spouted bed reactor. Fuel 224, 111–120. https://doi.org/10.1016/j.fuel.2018.03.028

Ano, A.O., Ubochi, C.I., 2007. Neutralization of soil acidity by animal manures: Mechanism of reaction. African J. Biotechnol. 6, 364–368. https://doi.org/10.4314/ajb.v6i4.56212

Araujo Galvão, G.D., De Nadae, J., Clemente, D.H., Chinen, G., De Carvalho, M.M., 2018. Circular Economy: Overview of Barriers. Procedia 10th CIRP Conf. Ind. Prod. Syst. 73, 79–85. https://doi.org/10.1016/j.procir.2018.04.011

Arriaga, F., Lowery, B., 2003. Soil physical properties and crop productivity of an eroded soil amended with cattle manure. Soil Sci. 168, 888–899. https://doi.org/10.1097/01.ss.0000106403.84926.7e

Bais, H.P., L.Weir, T., Perry, L.G., Gilroy, S., Vivanco, J.M., 2006. The Role of Root Exudates in Rhizosphere Interactions with Plants and Other Organisms. Annu. Rev. Plant Biol. 57, 233–266. https://doi.org/10.1146/annurev.arplant.57.032905.105159

Balemi, T., Negisho, K., 2012. Management of soil phosphorus and plant adaptation mechanisms to phosphorus stress for sustainable crop production: A review. J. Soil Sci. Plant Nutr. 12, 547–561. https://doi.org/10.4067/s0718-95162012005000015

Bampidis, V.A., Robinson, P.H., 2006. Citrus by-products as ruminant feeds: A review. Anim. Feed Sci. Technol. 128, 175–217. https://doi.org/10.1016/j.anifeedsci.2005.12.002

Bandyopadhyay, K.K., Misra, A.K., Ghosh, P.K., Hati, K.M., 2010. Effect of integrated use of farmyard manure and chemical fertilizers on soil physical properties and productivity of soybean. Soil Tillage Res. 110, 115–125. https://doi.org/10.1016/j.still.2010.07.007

Bardgett, R.D., Streeter, T.C., Bol, R., 2003. Soil microbes compete effectively with plants for organic-nitrogen inputs to temperate grasslands. Ecology 84, 1277–1287. https://doi.org/10.1890/0012-9658(2003)084[1277:SMCEWP]2.0.CO;2

Barnard, R.L., Osborne, C.A., Firestone, M.K., 2013. Responses of soil bacterial and fungal communities to extreme desiccation and rewetting. Int. Soc. Microb. Ecol. 7, 2229–2241. https://doi.org/10.1038/ismej.2013.104

Barra, P.J., Viscardi, S., Jorquera, M.A., Duran, P.A., Valentine, A.J., Mora, M. de la L., 2018. Understanding the strategies to overcome phosphorus-deficiency and aluminum-toxicity by ryegrass endophytic and rhizosphere phosphobacteria. Front. Microbiol. 9, 1–12. https://doi.org/10.3389/fmicb.2018.01155

Bernal-Vicente, A., Ros, M., Tittarelli, F., Intrigliolo, F., Pascual, J.A., 2008. Citrus compost and its water extract for cultivation of melon plants in greenhouse nurseries. Evaluation of nutriactive and biocontrol effects. Bioresour. Technol. 99, 8722–8728. https://doi.org/10.1016/j.biortech.2008.04.019

Bhattacharyya, R., Kundu, S., Prakash, V., Gupta, H.S., 2008. Sustainability under combined application of mineral and organic fertilizers in a rainfed soybean-wheat system of the Indian Himalayas. Eur. J. Agron. 28, 33–46. https://doi.org/10.1016/j.eja.2007.04.006

Blackwell, M., Darch, T., Haslam, R., 2019. Phosphorus use efficiency and fertilizers: Future opportunities for improvements. Front. Agric. Sci. Eng. 6, 332–340. https://doi.org/10.15302/J-FASE-2019274

Blanco-canqui, H., Hergert, G.W., Nielsen, R.A., 2014. Cattle Manure Application Reduces Soil Compactibility and Increases Water Retention after 71 Years. Soil Sci. Soc. Am. J. 79, 212–223. https://doi.org/10.2136/sssaj2014.06.0252

Bogaard, A., Fraser, R., Heaton, T.H.E., Wallace, M., Vaiglova, P., Charles, M., Jones, G., Evershed, R.P., Styring, A.K., Andersen, N.H., Arbogast, R.M., Bartosiewicz, L., Gardeisen, A., Kanstrup, M., Maier, U., Marinova, E., Ninov, L., Schäfer, M., Stephan, E., 2013. Crop manuring and intensive land management by Europe’s first farmers. Proc. Natl. Acad. Sci. U. S. A. 110, 12589–12594. https://doi.org/10.1073/pnas.1305918110

Böhme, L., Langer, U., Böhme, F., 2005. Microbial biomass, enzyme activities and microbial community structure in two European long-term field experiments. Agric. Ecosyst. Environ. 109, 141–152. https://doi.org/10.1016/j.agee.2005.01.017

Bolan, N.S., Naidu, R., Mahimairaja, S., Baskaran, S., 1994. Influence of low-molecular-weight organic acids on the solubilization of phosphates. Biol. Fertil. Soils 18, 311–319. https://doi.org/10.1007/BF00570634

Borie, F., Rubio, R., Morales, A., Curaqueo, G., Cornejo, P., 2010. Arbuscular mycorrhizae in agricultural and forest ecosystems in Chile. J. Soil Sci. Plant Nutr. 10, 185–206. https://doi.org/10.4067/S0718-95162010000100001

Borie, F., Zunino, H., 1983. Organic matter-phosphorus associations as a sink in P-fixation processes in allophanic soils of Chile. Soil Biol. Biochem. 15, 599–603. https://doi.org/10.1016/0038-0717(83)90056-1

Borie, F., Zunino, H., Martínez, L., 1989. Macromolecule-P associations and inositol phosphates in some Chilean volcanic soils of temperate regions. Commun. Soil Sci. Plant Anal. 20, 1881–1894. https://doi.org/10.1080/00103628909368190

Bowden, C.L., Evanylo, G.K., Zhang, X., Ervin, E.H., Seiler, J.R., 2010. Soil carbon and physiological responses of corn and soybean to organic amendments. Compost Sci. Util. 18, 162–173. https://doi.org/10.1080/1065657X.2010.10736952

Bowles, T.M., Acosta-Martínez, V., Calderón, F., Jackson, L.E., 2014. Soil enzyme activities, microbial communities, and carbon and nitrogen availability in organic agroecosystems across an intensively-managed agricultural landscape. Soil Biol. Biochem. 68, 252–262. https://doi.org/10.1016/j.soilbio.2013.10.004

Bradford, M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–254. https://doi.org/10.2135/cropsci1994.0011183X003400020031x

Brookes, P.C., Landman, A., Pruden, G., Jenkinson, D.S., 1985. Chloroform fumigation and the release of soil nitrogen: A rapid direct extraction method to measure microbial biomass nitrogen in soil. Soil Biol. Biochem. 17, 837–842. https://doi.org/10.1016/0038-0717(85)90144-0

Brookes, P.C., Powlson, D.S., Jenkinson, D.S., 1982. Measurement of microbial biomass phosphorus in soil. Soil Biol. Biochem. 14, 319–329. https://doi.org/10.1016/0038-0717(82)90001-3

Büchi, L., Wendling, M., Amossé, C., Jeangros, B., Sinaj, S., Charles, R., 2017. Long and short term changes in crop yield and soil properties induced by the reduction of soil tillage in a long term experiment in Switzerland. Soil Tillage Res. 174, 120–129. https://doi.org/10.1016/j.still.2017.07.002

Butterly, C.R., Baldock, J.A., Tang, C., 2013. The contribution of crop residues to changes in soil pH under field conditions. Plant Soil 366, 185–198. https://doi.org/10.1007/s11104-012-1422-1

Cai, A., Xu, M., Wang, B., Zhang, W., Liang, G., Hou, E., Luo, Y., 2019. Manure acts as a better fertilizer for increasing crop yields than synthetic fertilizer does by improving soil fertility. Soil Tillage Res. 189, 168–175. https://doi.org/10.1016/j.still.2018.12.022

Cai, A., Zhang, W., Xu, M., Wang, B., Wen, S., Shah, S.A.A., 2018. Soil fertility and crop yield after manure addition to acidic soils in South China. Nutr. Cycl. Agroecosystems 111, 61–72.

https://doi.org/10.1007/s10705-018-9918-6

Calabi-Floody, M., Velásquez, G., Gianfreda, L., Saggar, S., Bolan, N., Rumpel, C., Mora, M.L., 2012. Improving bioavailability of phosphorous from cattle dung by using phosphatase immobilized on natural clay and nanoclay. Chemosphere 89, 648–655. https://doi.org/10.1016/j.chemosphere.2012.05.107

Cellier, A., Gauquelin, T., Baldy, V., Ballini, C., 2014. Effect of organic amendment on soil fertility and plant nutrients in a post-fire Mediterranean ecosystem. Plant Soil 376, 211–228. https://doi.org/10.1007/s11104-013-1969-5

Cercioglu, M., 2017. The Role of Organic Soil Amendments on Soil Physical Properties and Yield of Maize (Zea mays L.). Commun. Soil Sci. Plant Anal. 48, 683–691. https://doi.org/10.1080/00103624.2017.1298787

Chaudry, M.A., Badshah, A., Bibi, N., Zeb, A., Ahmed, T., Ali, S., Ter Meulen, U., 2004. Citrus waste utilization in poultry rations. Arch. fur Geflugelkd. 68, 206–210.

Chen, C.R., Sinaj, S., Condron, L.M., Frossard, E., Sherlock, R.R., Davis, M.R., 2003. Characterization of phosphorus availability in selected New Zealand grassland soils. Nutr. Cycl. Agroecosystems 65, 89–100. https://doi.org/10.1023/A:1021889207109

Chowdhury, R.B., Moore, G.A., Weatherley, A.J., Arora, M., 2017. Key sustainability challenges for the global phosphorus resource, their implications for global food security, and options for mitigation. J. Clean. Prod. 140, 945–963. https://doi.org/10.1016/j.jclepro.2016.07.012

Christensen, M.L., Sommer, S.G., 2013. Manure Characterisation and Inorganic Chemistry, in: Animal Manure Recycling: Treatment and Management. John Wiley & Sons, pp. 41–65. https://doi.org/10.1002/9781118676677.ch4

Chu, H., Lin, X., Fujii, T., Morimoto, S., Yagi, K., Hu, J., Zhang, J., 2007. Soil microbial biomass, dehydrogenase activity, bacterial community structure in response to long-term fertilizer management. Soil Biol. Biochem. 39, 2971–2976. https://doi.org/10.1016/j.soilbio.2007.05.031

Clarke, K.R., 1993. Non-parametric multivariate analyses of changes in community structure. Aust. J. Ecol. 18, 117–143. https://doi.org/10.1111/j.1442-9993.1993.tb00438.x

Condron, L.M., Newman, S., 2011. Revisiting the fundamentals of phosphorus fractionation of sediments and soils. J. Soils Sediments 11, 830–840. https://doi.org/10.1007/s11368-011-0363-2

Cordell, D., Drangert, J.O., White, S., 2009. The story of phosphorus: Global food security and food for thought. Glob. Environ. Chang. 19, 292–305. https://doi.org/10.1016/j.gloenvcha.2008.10.009

Cordell, D., Neset, T.S.S., 2014. Phosphorus vulnerability: A qualitative framework for assessing the vulnerability of national and regional food systems to the multi-dimensional stressors of phosphorus scarcity. Glob. Environ. Chang. 24, 108–122. https://doi.org/10.1016/j.gloenvcha.2013.11.005

Cordell, D., Rosemarin, A., Schröder, J.J., Smit, A.L., 2011. Towards global phosphorus security: A systems framework for phosphorus recovery and reuse options. Chemosphere 84, 747–758. https://doi.org/10.1016/j.chemosphere.2011.02.032

Criquet, S., Braud, A., 2008. Effects of organic and mineral amendments on available P and phosphatase activities in a degraded Mediterranean soil under short-term incubation experiment. Soil Tillage Res. 98, 164–174. https://doi.org/10.1016/j.still.2007.11.001

Demelash, N., Bayu, W., Tesfaye, S., Ziadat, F., Sommer, R., 2014. Current and residual effects of compost and inorganic fertilizer on wheat and soil chemical properties. Nutr. Cycl. Agroecosystems 100, 357–367. https://doi.org/10.1007/s10705-014-9654-5

Diacono, M., Montemurro, F., 2010. Long-term effects of organic amendments on soil fertility. A review. Agron. Sustain. Dev. 30, 401–422. https://doi.org/10.1051/agro/2009040

Dick, W., Tabatabai, M., 1977. An alkaline oxidation method for the determination of total phosphorus in soils. Soil Sci. Soc. Am. J. 41, 511–514. https://doi.org/https://doi.org/10.2136/sssaj1977.03615995004100030015x

Dinesh, R., Dubey, R.P., Ganeshamurthy, A.N., Prasad, S.G., 2000. Organic manuring in rice based cropping system : Effects on soil microbial biomass and selected enzyme activities. Curr. Sci. 79, 1716.

Ding, X., Han, X., Liang, Y., Qiao, Y., Li, L., Li, N., 2012. Changes in soil organic carbon pools after 10 years of continuous manuring combined with chemical fertilizer in a Mollisol in China. Soil Tillage Res. 122, 36–41. https://doi.org/10.1016/j.still.2012.02.002

Ding, X., Liang, C., Zhang, B., Yuan, Y., Han, X., 2015. Higher rates of manure application lead to greater accumulation of both fungal and bacterial residues in macroaggregates of a clay soil. Soil Biol. Biochem. 84, 137–146. https://doi.org/10.1016/j.soilbio.2015.02.015

Donahue, J.L., Okpodu, C.M., Cramer, C.L., Grabau, E.A., Alscher, R.G., 1997. Responses of antioxidants to paraquat in pea leaves: Relationships to resistance. Plant Physiol. 113, 249–257. https://doi.org/10.1104/pp.113.1.249

Du, Z., Bramlage, W.J., 1992. Modified Thiobarbituric Acid Assay for Measuring Lipid Oxidation in Sugar-Rich Plant Tissue Extracts. J. Agric. Food Chem. 40, 1566–1570. https://doi.org/10.1021/jf00021a018

Dumontet, S., Cavoski, I., Ricciuti, P., Mondelli, D., Jarrar, M., Pasquale, V., Crecchio, C., 2017. Metabolic and genetic patterns of soil microbial communities in response to different amendments under organic farming system. Geoderma 296, 79–85. https://doi.org/10.1016/j.geoderma.2017.02.025

Dunjana, N., Nyamugafata, P., Shumba, A., Nyamangara, J., Zingore, S., 2012. Effects of cattle manure on selected soil physical properties of smallholder farms on two soils of Murewa, Zimbabwe. Soil Use Manag. 28, 221–228. https://doi.org/10.1111/j.1475-2743.2012.00394.x

Durán, P., Barra, P.J., Jorquera, M.A., Viscardi, S., Fernandez, C., Paz, C., Mora, M. de la L., Bol, R., 2019. Occurrence of soil fungi in antarctic pristine environments. Front. Bioeng. Biotechnol. 7, 1–12. https://doi.org/10.3389/fbioe.2019.00028

Durán, P., Tortella, G., Viscardi, S., Barra, P.J., Carrión, V.J., De La Luz Mora, M., Pozo, M.J.,

2018. Microbial community composition in take-All suppressive soils. Front. Microbiol. 9, 1–15. https://doi.org/10.3389/fmicb.2018.02198

Eghball, B., 1999. Liming effects of beef cattle feedlot manure or compost. Commun. Soil Sci. Plant Anal. 30, 2563–2570. https://doi.org/10.1080/00103629909370396

Eghball, B., Wienhold, B.J., Gilley, J.E., Eigenberg, R.A., 2002. Mineralization of Manure Nutrients Mineralization of manure nutrients. J. Soil Water Conserv. 57, 470–473.

El-Nagar, D.A., Mohamed, R.A.A., 2019. Characterization and Impact of Cattle Manure Particle Size on Physical Properties of Sandy Soils. J. Geosci. Environ. Prot. 7, 180–194. https://doi.org/10.4236/gep.2019.78013

Escudey, M., Galindo, G., Förster, J.E., Briceño, M., Chang, A., Fo, J.E., 2001. Chemical forms of phosphorus of volcanic ash-derived soils in Chile. Commun. Soil Sci. Plant Anal. 32, 601–616. https://doi.org/http://dx.doi.org/10.1081/CSS-100103895

European Commission 2015. COM(2015) 614 final – Closing the loop – An EU action plan for the circular economy. Available at: https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:52015DC0614. (accessed 11 May 2021).

European Economic and Social Committee, 2016. Circular Economy Package — Waste-based and Organic Fertilisers. Available at: http://www.eesc.europa.eu/en/our-work/opinions-information-reports/opinions/circular-economy-package-waste-based-and-organic-fertilisers. (accessed 11 May 2021).

Flachowsky, G. and Hennig, A., 1990. Composition and Digestibility of Untreated and Chemically Treated Animal Excreta for Ruminants A Review. Biol. Wastes 31, 17–36. https://doi.org/https://doi.org/10.1016/0269-7483(90)90041-P

Foster, N.W., Service, C.F., Marie, S.S., 2002. Forest Ecosystems: Nutrient Cycling. Encycl. Soil Sci. https://doi.org/10.1081/E-ESS-120001709

Fuentes, B., Bolan, N., Naidu, R., Mora, M. de la L., 2006. Phosphorus in Organic Waste-Soil Systems. J. Soil Sci. Plant Nutr. Plant. Nutr 6, 64–83. https://doi.org/10.4067/S0718-27912006000200006

Fuentes, B., Jorquera, M., Mora, M. de la L., 2009. Dynamics of phosphorus and phytate-utilizing bacteria during aerobic degradation of dairy cattle dung. Chemosphere 74, 325–331. https://doi.org/10.1016/j.chemosphere.2008.08.045

Fuentes, B., Mora, M. de la L., Bolan, N., Naidu, R., 2008. Assessment of phosphorus bioavailability from organic wastes in soil, in: Developments in Soil Science. pp. 363–411. https://doi.org/10.1016/S0166-2481(07)32016-3

Gatiboni, L.C., Jefferson Luis Meirelles Coimbra, Denardin, R.B.N., Wildner, L.D.P., 2011. Microbial Biomass and Soil Fauna During the Decomposition of Cover Crops in No-Tillage System. Rev. Bras. Cienc. do Solo 35, 1151–1157.

Gelsomino, A., Abenavoli, M.R., Princi, G., Cacco, G., Sorgonà, A., Attinà, E., 2010. Compost from fresh orange waste: A suitable substrate for nursery and field crops? Compost Sci. Util. 18, 201–210. https://doi.org/10.1080/1065657X.2010.10736956

Gérard, F., 2016. Clay minerals, iron/aluminum oxides, and their contribution to phosphate sorption in soils - A myth revisited. Geoderma 262, 213–226. https://doi.org/10.1016/j.geoderma.2015.08.036

Gerke, J., 2015. Phytate (Inositol Hexakisphosphate) in Soil and Phosphate Acquisition from Inositol Phosphates by Higher Plants. A Review. Plants 4, 253–266. https://doi.org/10.3390/plants4020253

Gerke, J., Römer, W., Beißner, L., 2000. The quantitative effect of chemical phosphate mobilization by carboxylate anions on P uptake by a single root. II. The importance of soil and plant parameters for uptake of mobilized P. J. Plant Nutr. Soil Sci. 163, 213–219. https://doi.org/10.1002/(SICI)1522-2624(200004)163:2<213::AID-JPLN213>3.0.CO;2-0

Gerke, J., Römer, W., Jungk, A., 1994. The excretion of citric and malic acid by proteoid roots of Lupinus albus L.; effects on soil solution concentrations of phosphate, iron, and aluminum in the proteoid rhizosphere in samples of an oxisol and a luvisol. Zeitschrift für Pflanzenernährung und Bodenkd. 157, 289–294. https://doi.org/10.1002/jpln.19941570408

Ghosh, P.K., Ramesh, P., Bandyopadhyay, K.K., Tripathi, A.K., Hati, K.M., Misra, A.K., Acharya, C.L., 2004. Comparative effectiveness of cattle manure, poultry manure, phosphocompost and fertilizer-NPK on three cropping systems in vertisols of semi-arid tropics. I. Crop yields and system performance. Bioresour. Technol. 95, 77–83. https://doi.org/10.1016/j.biortech.2004.02.011

Gianinazzi, S., Gollotte, A., Binet, M.N., van Tuinen, D., Redecker, D., Wipf, D., 2010. Agroecology: The key role of arbuscular mycorrhizas in ecosystem services. Mycorrhiza 20, 519–530. https://doi.org/10.1007/s00572-010-0333-3

Gichangi, E.M., Mnkeni, P.N.S., Brookes, P.C., 2009. Effects of goat manure and inorganic phosphate addition on soil inorganic and microbial biomass phosphorus fractions under laboratory incubation conditions. Soil Sci. Plant Nutr. 55, 764–771. https://doi.org/10.1111/j.1747-0765.2009.00415.x

Gopinath, K.A., Saha, S., Mina, B.L., Pande, H., Kundu, S., Gupta, H.S., 2008. Influence of organic amendments on growth, yield and quality of wheat and on soil properties during transition to organic production. Nutr. Cycl. Agroecosystems 82, 51–60. https://doi.org/10.1007/s10705-008-9168-0

Guerrero, C.C., Carrasco de Brito, J., Lapa, N., Oliveira, J.F.S., 1995. Re-use of industrial orange wastes as organic fertilizers. Bioresour. Technol. 53, 43–51. https://doi.org/10.1016/0960-8524(95)00050-O

Guppy, C.N., Menzies, N.W., Moody, P.W., Blamey, F.P.C., 2005. Competitive sorption reactions between phosphorus and organic matter in soil: A review. Aust. J. Soil Res. 43, 189–202. https://doi.org/10.1071/SR04049

Güsewell, S., 2004. N:P ratios in terrestrial plants: Variation and functional significance. New Phytol. 164, 243–266. https://doi.org/10.1111/j.1469-8137.2004.01192.x

Hansen, J.C., Cade-menun, B.J., Strawn, D.G., 2004. Phosphorus Speciation in Manure-Amended Alkaline Soils. J. Environ. Qual. 33, 1521–1527.

https://doi.org/https://doi.org/10.2134/jeq2004.1521

Hargreaves, J.C., Adl, M.S., Warman, P.R., 2008. A review of the use of composted municipal solid waste in agriculture. Agric. Ecosyst. Environ. 123, 1–14. https://doi.org/10.1016/j.agee.2007.07.004

Harrold, S.A., Tabatabai, M.A., 2006. Release of Inorganic Phosphorus from Soils by Low-Molecular-Weight Organic Acids. Commun. Soil Sci. Plant Anal. 37, 1233–1245. https://doi.org/10.1080/00103620600623558

Hartono, A., Funakawa, S., Kosaki, T., 2005. Phosphorus sorption-desorption characteristics of selected acid upland soils in Indonesia. Soil Sci. Plant Nutr. 51, 787–799. https://doi.org/10.1111/j.1747-0765.2005.tb00113.x

Hati, K.M., Mandal, K.G., Misra, A.K., Ghosh, P.K., Bandyopadhyay, K.K., 2006. Effect of inorganic fertilizer and farmyard manure on soil physical properties, root distribution, and water-use efficiency of soybean in Vertisols of central India. Bioresour. Technol. 97, 2182–2188. https://doi.org/10.1016/j.biortech.2005.09.033

Hati, K.M., Swarup, A., Mishra, B., Manna, M.C., Wanjari, R.H., Mandal, K.G., Misra, A.K., 2008. Impact of long-term application of fertilizer, manure and lime under intensive cropping on physical properties and organic carbon content of an Alfisol. Geoderma 148, 173–179. https://doi.org/10.1016/j.geoderma.2008.09.015

Haygarth, P.M., Bardgett, R.D., Condron, L.M., 2013. Phosphorus and nitrogen cycles and their management, Soil Conditions and Plant Growth. John Wiley & Sons. https://doi.org/10.1002/9781118337295

Haynes, R.J., Mokolobate, M.S., 2001. Amelioration of Al toxicity and P deficiency in acid soils by additions of organic residues: A critical review of the phenomenon and the mechanisms involved. Nutr. Cycl. Agroecosystems 59, 47–63. https://doi.org/10.1023/A:1009823600950

He, Y.T., Zhang, W.J., Xu, M.G., Tong, X.G., Sun, F.X., Wang, J.Z., Huang, S.M., Zhu, P., He, X.H., 2015. Long-term combined chemical and manure fertilizations increase soil organic carbon and total nitrogen in aggregate fractions at three typical cropland soils in China. Sci. Total Environ. 532, 635–644. https://doi.org/10.1016/j.scitotenv.2015.06.011

He, Z., Pagliari, P.H., Waldrip, H.M., 2016. Applied and Environmental Chemistry of Animal Manure: A Review. Pedosphere 26, 779–816. https://doi.org/10.1016/S1002-0160(15)60087-X

He, Z., Zhang, H., 2014. Applied manure and nutrient chemistry for sustainable agriculture and environment. Appl. Manure Nutr. Chem. Sustain. Agric. Environ. 1–379. https://doi.org/10.1007/978-94-017-8807-6

Hijbeek, R., van Ittersum, M.K., ten Berge, H.F.M., Gort, G., Spiegel, H., Whitmore, A.P., 2017. Do organic inputs matter – a meta-analysis of additional yield effects for arable crops in Europe. Plant Soil 411, 293–303. https://doi.org/10.1007/s11104-016-3031-x

Houlbrooke, D., Longhurst, B., Orchiston, T., Muirhead, R., 2011. Characterising dairy manures and slurries, Prepared for Surface Water Integrated Management (SWIM).

Iannotti, D.A., Grebus, M.E., Toth, B.L., Madden, L. V., Hoitink, H.A.J., 1994. Oxygen

Respirometry to Assess Stability and Maturity of Composted Municipal Solid Waste. J. Environ. Qual. 23, 1177–1183. https://doi.org/10.2134/jeq1994.00472425002300060007x

Intanon, S., Hulting, A.G., Myrold, D.D., Mallory-Smith, C.A., 2015. Short-term effects of soil amendment with meadowfoam seed meal on soil microbial composition and function. Appl. Soil Ecol. 89, 85–92. https://doi.org/10.1016/j.apsoil.2015.01.009

Jamraiz Kiani, M., Kaleem Abbasi, M., Rahim, N., 2005. Use of organic manure with mineral N fertilizer increases wheat yield at Rawalakot Azad Jammu and Kashmir. Arch. Agron. Soil Sci. 51, 299–309. https://doi.org/10.1080/03650340500069114

Jara, A.A., Violante, A., Pigna, M., de la Luz Mora, M., 2006. Mutual Interactions of Sulfate, Oxalate, Citrate, and Phosphate on Synthetic and Natural Allophanes. Soil Sci. Soc. Am. J. 70, 337. https://doi.org/10.2136/sssaj2005.0080

Jiang, H., Han, X., Zou, W., Hao, X., Zhang, B., 2018. Seasonal and long-term changes in soil physical properties and organic carbon fractions as affected by manure application rates in the Mollisol region of Northeast China. Agric. Ecosyst. Environ. 268, 133–143. https://doi.org/10.1016/j.agee.2018.09.007

Jilani, G., Akram, A., Ali, R.M., Hafeez, F.Y., Shamsi, I.H., Chaudhry, A.N., Chaudhry, A.G., 2007. Enhancing crop growth, nutrients availability, economics and beneficial rhizosphere microflora through organic and biofertilizers. Ann. Microbiol. 57, 177–184. https://doi.org/10.1007/bf03175204

Jonasson, S., Michelsen, A., Schmidt, I.K., 1999. Coupling of nutrient cycling and carbon dynamics in the Arctic, integration of soil microbial and plant processes. Appl. Soil Ecol. 11, 135–146. https://doi.org/10.1016/S0929-1393(98)00145-0

Jones, D.L., 1998. Organic acids in the rhizosphere - A critical review. Plant Soil 205, 25–44. https://doi.org/10.1023/A:1004356007312

Kandil, E.E., Abdelsalam, N.R., Mansour, M.A., Ali, H.M., Siddiqui, M.H., 2020. Potentials of organic manure and potassium forms on maize (Zea mays L.) growth and production. Sci. Rep. 10, 1–11. https://doi.org/10.1038/s41598-020-65749-9

Kang, J., Hesterberg, D., Osmond, D.L., 2009. Soil Organic Matter Effects on Phosphorus Sorption: A Path Analysis. Soil Sci. Soc. Am. J. 73, 360–366. https://doi.org/10.2136/sssaj2008.0113

Khan, K.S., Joergensen, R.G., 2009. Changes in microbial biomass and P fractions in biogenic household waste compost amended with inorganic P fertilizers. Bioresour. Technol. 100, 303–309. https://doi.org/10.1016/j.biortech.2008.06.002

Kim, S.S., Kim, M.J., Choi, Y.H., Kim, B.K., Kim, K.S., Park, K.J., Park, S.M., Lee, N.H., Hyun, C.G., 2013. Down-regulation of tyrosinase, TRP-1, TRP-2 and MITF expressions by citrus press-cakes in murine B16 F10 melanoma. Asian Pac. J. Trop. Biomed. 3, 617–622. https://doi.org/10.1016/S2221-1691(13)60125-2

Kizewski, F.R., Boyle, P., Hesterberg, D., Martin, J.D., 2010. Mixed Anion (Phosphate / Oxalate) Bonding to Iron (III) Materials. J. Am. Chem. Soc. 132, 2301–2308. https://doi.org/10.1021/ja908807b

Kpomblekou-A, K., Tabatabai, M.A., 2003. Effect of low-molecular weight organic acids on phosphorus release and phytoavailabilty of phosphorus in phosphate rocks added to soils. Agric. Ecosyst. Environ. 100, 275–284. https://doi.org/10.1016/S0167-8809(03)00185-3

Kratz, S., Vogel, C., Adam, C., 2019. Agronomic performance of P recycling fertilizers and methods to predict it: a review, Nutrient Cycling in Agroecosystems. Springer Netherlands. https://doi.org/10.1007/s10705-019-10010-7

Kundu, S., Bhattacharyya, R., Prakash, V., Gupta, H.S., Pathak, H., Ladha, J.K., 2007. Long-term yield trend and sustainability of rainfed soybean-wheat system through farmyard manure application in a sandy loam soil of the Indian Himalayas. Biol. Fertil. Soils 43, 271–280. https://doi.org/10.1007/s00374-006-0102-9

Ladaniya, M., 2008. Fruit Biochemistry, in: Elsevier Inc (Ed.), Citrus Fruit, Biology Technology and Evaluation. Atlanta, USA. https://doi.org/10.1016/B978-012374130-1.50008-5.

Lekasi, J.K., Tanner, J.C., Kimani, S.K., Harris, P.J.C., 2003. Cattle manure quality in Maragua District, Central Kenya: effect of management practices and development of simple methods of assessment. Agric. Ecosyst. Environ. 94, 289–298. https://doi.org/https://doi.org/10.1016/S0167-8809(02)00037-3

Lemanowicz, J., Siwik-ziomek, A., Koper, J., 2014. How Fertilization with Farmyard Manure and Nitrogen Affects Available Phosphorus Content and Phosphatase Activity in Soil. Polish J. Environ. Stud. 23, 1211–1217.

Liu, E., Yan, C., Mei, X., He, W., Bing, S.H., Ding, L., Liu, Q., Liu, S., Fan, T., 2010. Long-term effect of chemical fertilizer, straw, and manure on soil chemical and biological properties in northwest China. Geoderma 158, 173–180. https://doi.org/10.1016/j.geoderma.2010.04.029

Liu, H., Huang, Y., Duan, W., Qiao, C., Shen, Q., Li, R., 2020. Microbial community composition turnover and function in the mesophilic phase predetermine chicken manure composting efficiency. Bioresour. Technol. 313, 123658. https://doi.org/10.1016/j.biortech.2020.123658

Liu, J., Schulz, H., Brandl, S., Miehtke, H., Huwe, B., Glaser, B., 2012. Short-term effect of biochar and compost on soil fertility and water status of a Dystric Cambisol in NE Germany under field conditions. J. Plant Nutr. Soil Sci. 175, 698–707. https://doi.org/10.1002/jpln.201100172

Liu, Z., Rong, Q., Zhou, W., Liang, G., 2017. Effects of inorganic and organic amendment on soil chemical properties, enzyme activities, microbial community and soil quality in yellow clayey soil. PLoS One 12. https://doi.org/10.1371/journal.pone.0172767

Lopez-Velazquez, M.A., Santes, V., Balmaseda, J., Torres-Garcia, E., 2013. Pyrolysis of orange waste: A thermo-kinetic study. J. Anal. Appl. Pyrolysis 99, 170–177. https://doi.org/10.1016/j.jaap.2012.09.016

Ma, J.F., Ryan, P.R., Delhaize, E., 2001. Aluminium tolerance in plants and the complexing role of organic acids. Trends Plant Sci. 6, 273–278. https://doi.org/10.1016/S1360-1385(01)01961-6

Mahato, N., Sharma, K., Sinha, M., Baral, E.R., Koteswararao, R., Dhyani, A., Hwan Cho, M., Cho, S., 2020. Bio-sorbents, industrially important chemicals and novel materials from citrus processing waste as a sustainable and renewable bioresource: A review. J. Adv. Res. 23, 61–82. https://doi.org/10.1016/j.jare.2020.01.007

Malhotra, V.H., Sandeep, S., Pandey Renu, 2018. Phosphorus Nutrition: Plant Growth in Response to Deficiency and Excess, in: Plant Nutrients and Abiotic Stress Tolerance. Springer Nature Singapore, India, p. 171.190. https://doi.org/10.1007/978-981-10-9044-8

Malik, M.A., Khan, K.S., Marschner, P., Fayyaz-ul-Hassan, 2013. Microbial biomass, nutrient availability and nutrient uptake by wheat in two soils with organic amendments. J. Soil Sci. Plant Nutr. 13, 955–966. https://doi.org/10.4067/S0718-95162013005000075

Marinari, S., Masciandaro, G., Ceccanti, B., Grego, S., 2000. Influence of organic and mineral fertilisers on soil biological and physical properties. Bioresour. Technol. 72, 9–17. https://doi.org/https://doi.org/10.1016/S0960-8524(99)00094-2

Marschner, P., Hatam, Z., Cavagnaro, T.R., 2015. Soil respiration, microbial biomass and nutrient availability after the second amendment are influenced by legacy effects of prior residue addition. Soil Biol. Biochem. 88, 169–177. https://doi.org/10.1016/j.soilbio.2015.05.023

Mcdowell, R.W., Stewart, I., 2005. Phosphorus in Fresh and Dry Dung of Grazing Dairy Cattle, Deer, and Sheep: Sequential Fraction and Phosphorus-31 Nuclear Magnetic Resonance Analyses. J. Environ. Qual. 34, 598–607. https://doi.org/10.2134/jeq2005.0598

McKay Fletcher, D.M., Shaw, R., Sánchez-Rodríguez, A.R., Daly, K.R., van Veelen, A., Jones, D.L., Roose, T., 2019. Quantifying citrate-enhanced phosphate root uptake using microdialysis. Plant Soil. https://doi.org/10.1007/s11104-019-04376-4

Menezes-Blackburn, D., Inostroza, N.G., Gianfreda, L., Greiner, R., Mora, M.L., Jorquera, M.A., 2016. Phytase-producing Bacillus sp. inoculation increases phosphorus availability in cattle manure. J. Soil Sci. Plant Nutr. 16, 200–210. https://doi.org/10.4067/S0718-95162016005000016

Menezes-Blackburn, D., Jorquera, M.A., Gianfreda, L., Greiner, R., de la Luz Mora, M., 2013. A novel phosphorus biofertilization strategy using cattle manure treated with phytase–nanoclay complexes. Biol. Fertil. Soils 50, 583–592. https://doi.org/10.1007/s00374-013-0872-9

Menezes-Blackburn, Daniel, Paredes, C., Zhang, H., Giles, C.D., Darch, T., Stutter, M., George, T.S., Shand, C., Lumsdon, D., Cooper, P., Wendler, R., Brown, L., Blackwell, M., Wearing, C., Haygarth, P.M., 2016. Organic Acids Regulation of Chemical-Microbial Phosphorus Transformations in Soils. Environ. Sci. Technol. 50, 11521–11531. https://doi.org/10.1021/acs.est.6b03017

Mínguez, C., Calvo, A., 2018. Effect of supplementation with fresh orange pulp (Citrus sinensis) on mortality, growth performance, slaughter traits and sensory characteristics in meat guinea pigs. Meat Sci. 145, 51–54. https://doi.org/10.1016/j.meatsci.2018.06.003

Miyittah, M., Inubushi, K., 2003. Decomposition and CO2-C evolution of okara, sewage sludge, cow and poultry manure composts in soils. Soil Sci. Plant Nutr. 49, 61–68. https://doi.org/10.1080/00380768.2003.10409980

Mokolobate, M.S., Haynes, R.J., 2002. Comparative liming effect of four organic residues applied to an acid soil. Biol. Fertil. Soils 35, 79–85. https://doi.org/10.1007/s00374-001-0439-z

Mora, M. de la L., Demanet, R., Acuña, J.J., Viscardi, S., Jorquera, M., Rengel, Z., Durán, P., 2017. Aluminum-tolerant bacteria improve the plant growth and phosphorus content in ryegrass grown in a volcanic soil amended with cattle dung manure. Appl. Soil Ecol. 115, 19–26. https://doi.org/10.1016/j.apsoil.2017.03.013

Murphy J, Riley JP, 1962. A modified single solution method for the determination of phosphate in natural waters. Anal. Chem. Acta 27, 31–36. https://doi.org/10.1016/S0003-2670(00)88444-5

Mvumi, B.M., Gwenzi, W., Mhandu, M.G., 2017. Ecotoxicological effects of citrus processing waste on earthworms, Lumbricus terrestris L. Ind. Crops Prod. 110, 123–129. https://doi.org/10.1016/j.indcrop.2017.09.003

Naramabuye, F.X., Haynes, R.J., 2006a. Effect of organic amendments on soil pH and Al solubility and use of laboratory indices to predict their liming effect. Soil Sci. 171, 754–763. https://doi.org/10.1097/01.ss.0000228366.17459.19

Naramabuye, F.X., Haynes, R.J., 2006b. Short-term effects of three animal manures on soil pH and Al solubility. Aust. J. Soil Res. 44, 515–521. https://doi.org/10.1071/SR05062

Neina, D., 2019. The Role of Soil pH in Plant Nutrition and Soil Remediation. Appl. Environ. Soil Sci. 2019. https://doi.org/10.1155/2019/5794869

Neset, T.S., Cordell, D., Mohr, S., VanRiper, F., White, S., 2016. Visualizing Alternative Phosphorus Scenarios for Future Food Security. Front. Nutr. 3. https://doi.org/10.3389/fnut.2016.00047

Nguyen, T.T., Marschner, P., 2016. Soil respiration, microbial biomass and nutrient availability in soil after repeated addition of low and high C/N plant residues. Biol. Fertil. Soils 52, 165–176. https://doi.org/10.1007/s00374-015-1063-7

Nigam, N., Yadav, V., Khare, P., Singh, R.P., Das, P., Shanker, K., Sharma, R.S., 2019. Exploring the benefits of biochar over other organic amendments for reducing of metal toxicity in Withania somnifera. Biochar 1, 293–307. https://doi.org/10.1007/s42773-019-00023-4

Odepa. Oficina de Estudios y Políticas Agrarias 2019. Ministerio de Agricultura. Gobierno de Chile. Ficha Nacional. Available at: https://www.odepa.gob.cl/estadisticas-del-sector/ficha-nacional-y-regionales/attachment/ficha-nacional-2019. (accessed 11 May 2021).

Ohno, T., Zibilske, L.M., 1991. Determination of Low Concentrations of Phosphorus in Soil Extracts Using Malachite Green. Soil Sci. Soc. Am. J. 55, 892–895. https://doi.org/10.2136/sssaj1991.03615995005500030046x

Pagliari, P.H., Laboski, C.A.M., 2012. Investigation of the Inorganic and Organic Phosphorus Forms in Animal Manure. J. Environ. Qual. 41, 901–910. https://doi.org/10.2134/jeq2011.0451

Pagliari, P.H., Wilson, M., Waldrip, H.M., He, Z., 2020. Nitrogen and Phosphorus Characteristics of Beef and Dairy Manure. Anim. Manure Prod. Charact. Environ. Concerns, Manag. 45–62. https://doi.org/10.2134/asaspecpub67.c4

Parham, J.A., Deng, S.P., Da, H.N., Sun, H.Y., Raun, W.R., 2003. Long-term cattle manure application in soil. II. Effect on soil microbial populations and community structure. Biol. Fertil. Soils 38, 209–215. https://doi.org/10.1007/s00374-003-0657-7

Parham, J.A., Deng, S.P., Raun, W.R., Johnson, G. V., 2002. Long-term cattle manure application in soil. I. Effect on soil phosphorus levels, microbial biomass C, and dehydrogenase and phosphatase activities. Biol. Fertil. Soils 35, 328–337. https://doi.org/10.1007/s00374-002-0476-2

Pascual, J.A., Moreno, J.L., Hernández, T., García, C., 2002. Persistence of immobilised and total urease and phosphatase activities in a soil amended with organic wastes. Bioresour. Technol. 82, 73–78. https://doi.org/10.1016/S0960-8524(01)00127-4

Paula, F.S., Tatti, E., Thorn, C., Abram, F., Wilson, J., O'Flaherty, V., 2020. Soil prokaryotic community resilience, fungal colonisation and increased cross-domain co-occurrence in response to a plant-growth enhancing organic amendment. Soil Biol. Biochem. 149, 107937. https://doi.org/10.1016/j.soilbio.2020.107937

Pizzeghello, D., Berti, A., Nardi, S., Morari, F., 2014. Phosphorus-related properties in the profiles of three Italian soils after long-term mineral and manure applications. Agric. Ecosyst. Environ. 189, 216–228. https://doi.org/10.1016/j.agee.2014.03.047

Poll, C., Thiede, A., Wermbter, N., Kandeler, A., Sessitsh, E., 2003. Micro-scale distribution of microorganisms and microbial enzyme activities in a soil with long-term organic amendment. Eur. J. Soil Biol. 54, 715–724. https://doi.org/10.1046/j.1365-2389.2003.00569.x

Powers, S.M., Chowdhury, R.B., MacDonald, G.K., Metson, G.S., Beusen, A.H.W., Bouwman, A.F., Hampton, S.E., Mayer, B.K., McCrackin, M.L., Vaccari, D.A., 2019. Global Opportunities to Increase Agricultural Independence Through Phosphorus Recycling. Earth's Futur. 7, 370–383. https://doi.org/10.1029/2018EF001097

Qian, P., Schoenau, J.J., Wu, T., Mooleki, P., 2004. Phosphorus amounts and distribution in a Saskatchewan soil after five years of swine and cattle manure application. Can. J. Soil Sci. 84, 275–281. https://doi.org/https://doi.org/10.4141/S03-016

Ramzani, P.M.A., Coyne, M.S., Anjum, S., Khan, W.-D., Iqbal, M., 2017. In situ immobilization of Cd by organic amendments and their effect on antioxidant enzyme defense mechanism in mung bean (Vigna radiata L.) seedlings. Plant Physiol. Biochem. 118, 561–570. https://doi.org/10.1016/j.plaphy.2017.07.022

Rasoulzadeh, A., Yaghoubi, A., 2010. Effect of cattle manure on soil physical properties on a sandy clay loam soil in Effect of cattle manure on soil physical properties on a sandy clay loam soil in North- West Iran. J. Food, Agric. Environ. 8, 976–979.

Raut, M.P., Prince William, S.P.M., Bhattacharyya, J.K., Chakrabarti, T., Devotta, S., 2008. Microbial dynamics and enzyme activities during rapid composting of municipal solid waste - A compost maturity analysis perspective. Bioresour. Technol. 99, 6512–6519. https://doi.org/10.1016/j.biortech.2007.11.030

Rayne, N., Aula, L., 2020. Livestock manure and the impacts on soil health: A review. Soil Syst. 4, 1–26. https://doi.org/10.3390/soilsystems4040064

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. J. Soil Sci. Plant Nutr. 16, 490–506. https://doi.org/10.4067/S0718-95162016005000041

Redel, Y.D., Rubio, R., Rouanet, J.L., Borie, F., 2007. Phosphorus bioavailability affected by tillage and crop rotation on a Chilean volcanic derived Ultisol. Geoderma 139, 388–396. https://doi.org/10.1016/j.geoderma.2007.02.018

Rezzadori, K., Benedetti, S., Amante, E.R., 2012. Proposals for the residues recovery: Orange waste as raw material for new products. Food Bioprod. Process. 90, 606–614. https://doi.org/10.1016/j.fbp.2012.06.002

Richardson, A.E., Lynch, J.P., Ryan, P.R., Delhaize, E., Smith, F.A., Smith, S.E., Harvey, P.R., Ryan, M.H., Veneklaas, E.J., Lambers, H., Oberson, A., Culvenor, R.A., Simpson, R.J., 2011. Plant and microbial strategies to improve the phosphorus efficiency of agriculture. Plant Soil 349, 121–156. https://doi.org/10.1007/s11104-011-0950-4

Rittmann, B.E., Mayer, B., Westerhoff, P., Edwards, M., 2011. Capturing the lost phosphorus. Chemosphere 84, 846–853. https://doi.org/10.1016/j.chemosphere.2011.02.001

Roberts, T.L., Johnston, A.E., 2015. Phosphorus use efficiency and management in agriculture. Resour. Conserv. Recycl. 105, 275–281. https://doi.org/10.1016/j.resconrec.2015.09.013

Ros, M., Pascual, J.A., Garcia, C., Hernandez, M.T., Insam, H., 2006. Hydrolase activities, microbial biomass and bacterial community in a soil after long-term amendment with different composts. Soil Biol. Biochem. 38, 3443–3452. https://doi.org/10.1016/j.soilbio.2006.05.017

Rubio, R., Moraga, E., Borie, F., 1990. Acid phosphatase activity and vesicular-arbuscular mycorrhizal infection associated with roots of four wheat cultivars. J. Plant Nutr. 13, 585–598. https://doi.org/10.1080/01904169009364102

Ruiz, B., Flotats, X., 2014. Citrus essential oils and their influence on the anaerobic digestion process: An overview. Waste Manag. 34, 2063–2079. https://doi.org/10.1016/j.wasman.2014.06.026

Ruiz, N., Lavelle, P., Jiménez, J., 2008. Soil macrofauna field manual, Food and Agriculture Organization of the United Nations. https://doi.org/http://www.fao.org/3/i0211e/i0211e.pdf

Rurangwa, E., Vanlauwe, B., Giller, K.E., 2020. The response of climbing bean to fertilizer and organic manure in the Northern Province of Rwanda. Exp. Agric. 56, 722–737. https://doi.org/10.1017/S0014479720000277

Ryan, P.R., Delhaize, E., Jones, D., 2001. Function and mechanism of organic anion exudation from plant roots. Annu. Rev. Plant Physiol. Plant Mol. Biol. 52, 527–560.

Sadzawka, A., Carrasco, M.A., Gez, R., Mora, M.D.L.L., Flores, H., Neaman, A., 2006. Métodos de análisis recomendados para los suelos de Chile. Revision 2006. Ser. Actas-Instituto Investig. Agropecu.

Sadzawka, A., Grez, R., Carrasco, M., Flores, H., Mora, M., Rojas, C., 2007. Métodos De Análisis De Tejidos Vegetales, 2 ed. ed, Serie Actas - Instituto de Investigaciones Agropecuarias.

SAG. Servicio Agrícola y Ganadero. 2006. Diagnóstico de la problemática ambiental de los residuos generados por la producción de aves y vacunos de leche en Chile y capacitación en la evaluación de planteles pecuarios. Available at: https://www.sag.gob.cl/sites/default/files/residuos_aves_vacunos_leche.pdf. (accessed 11 May 2021).

Sagar, R., Sharma, G.P., 2012. Measurement of alpha diversity using Simpson index (1/λ): the jeopardy. Environ. Skept. Critics 2012, 23–24.

Santos, S.R., Hesterberg, D., Alleoni, L.R.F., 2017a. Phosphate Speciation and Citrate-Induced Mobilization of P in an Acric Oxisol. Commun. Soil Sci. Plant Anal. 48, 1977–1988. https://doi.org/10.1080/00103624.2017.1395450

Santos, S.R., Silva, E. de B., Alleoni, L.R.F., Grazziotti, P.H., 2017b. Citric acid influence on soil phosphorus availability. J. Plant Nutr. 40, 2138–2145. https://doi.org/10.1080/01904167.2016.1270312

Sattari, S.Z., Bouwman, A.F., Giller, K.E., van Ittersum, M.K., 2012. Residual soil phosphorus as the missing piece in the global phosphorus crisis puzzle. Proc. Natl. Acad. Sci. 109, 6348–6353. https://doi.org/10.1073/pnas.1113675109

Sawyer, J., 2009. Soil Fertility : Current Topic Soil, Iowa State University Extension. https://doi.org/http://www.agronext.iastate.edu/soilfertility/currenttopics/TFI-NRCS-ISU_NutrientManagementPlanningEducation_6-7-13.pdf

Scalenghe, R., Edwards, A.C., Barberis, E., Ajmone-Marsan, F., 2014. Release of phosphorus under reducing and simulated open drainage conditions from overfertilised soils. Chemosphere 95, 289–294. https://doi.org/10.1016/j.chemosphere.2013.09.016

Schachtman, D.P., Reid, R.J., Ayling, S.M., 1998. Phosphorus Uptake by Plants: From Soil to Cell. Plant Physiol. 116, 447–453. https://doi.org/10.1104/pp.116.2.447

Schloter, M., Dilly, O., Munch, J.C., 2003. Indicators for evaluating soil quality. Agric. Ecosyst. Environ. 98, 255–262. https://doi.org/10.1016/S0167-8809(03)00085-9

Shahzad, T., Chenu, C., Genet, P., Barot, S., Perveen, N., Mougin, C., Fontaine, S., 2015. Contribution of exudates, arbuscular mycorrhizal fungi and litter depositions to the rhizosphere priming effect induced by grassland species. Soil Biol. Biochem. 80, 146–155. https://doi.org/10.1016/j.soilbio.2014.09.023

Sharma, K., Mahato, N., Cho, M.H., Lee, Y.R., 2017. Converting citrus wastes into value-added products : Economic and environmently friendly approaches. Nutrition 34, 29–46. https://doi.org/10.1016/j.nut.2016.09.006

Sharpley, A.N., Mcdowell, R.W., Kleinman, P.J.A., 2004. Amounts , Forms , and Solubility of Phosphorus in Soils Receiving Manure. Soil Sci. Soc. Am. J. 68, 2048–2057. https://doi.org/https://doi.org/10.2136/sssaj2004.2048

Sharpley, A.N., Moyer, B., 2000. Phosphorus Forms in Manure and Compost and Their Release during Simulated Rainfall. J. Environ. Qual. 29, 1462–1469. https://doi.org/https://doi.org/10.2134/jeq2000.00472425002900050012x

Shen, J., Yuan, L., Zhang, J., Li, H., Bai, Z., Chen, X., Zhang, W., Zhang, F., 2011. Phosphorus dynamics: From soil to plant. Plant Physiol. 156, 997–1005. https://doi.org/10.1104/pp.111.175232

Simpson, R.J., Oberson, A., Culvenor, R.A., Ryan, M.H., Veneklaas, E.J., Lambers, H., Lynch, J.P., Ryan, P.R., Delhaize, E., Smith, F.A., Smith, S.E., Harvey, P.R., Richardson, A.E., 2011.

Strategies and agronomic interventions to improve the phosphorus-use efficiency of farming systems, Plant and Soil. https://doi.org/10.1007/s11104-011-0880-1

Singh, G., Surinder, J., Singh, Y., 2007. Manuring and residue management effects on physical properties of a soil under the rice – wheat system in Punjab , India. Soil Tillage Res. 94, 229–238. https://doi.org/10.1016/j.still.2006.07.020

Tabatabai, M.A., Bremner, J.M., 1969. Use of p-nitrophenyl phosphate for assay of soil phosphatase activity. Soil Biol. Biochem. 1, 301–307. https://doi.org/10.1016/0038-0717(69)90012-1

Taghipour, M., Jalali, M., 2013. Effect of low-molecular-weight organic acids on kinetics release and fractionation of phosphorus in some calcareous soils of western Iran. Environ. Monit. Assess. 185, 5471–5482. https://doi.org/10.1007/s10661-012-2960-y

Tang, Y., Zhang, H., Schroder, J.L., Payton, M.E., Zhou, D., 2007. Animal Manure Reduces Aluminum Toxicity in an Acid Soil. Soil Sci. Soc. Am. J. 71, 1699–1707. https://doi.org/10.2136/sssaj2007.0008

Tiecher, T., Zafar, M., Mallmann, F.J.K., Bortoluzzi, E.C., Bender, M.A., Ciotti, L.H., Santos, D.R. dos, 2014. Animal manure phosphorus characterization by sequential chemical fractionation, release kinetics and 31P-NMR analysis. Rev. Bras. Ciência do Solo 38, 1506–1514. https://doi.org/10.1590/S0100-06832014000500016

Toor, G.S., Hunger, S., Peak, J.D., Sims, J.T., Sparks, D.L., 2006. Advances in the Characterization of Phosphorus in Organic Wastes: Environmental and Agronomic Applications. Adv. Agron. 89, 1–72. https://doi.org/10.1016/S0065-2113(05)89001-7

Turner, B and Leytem, A., 2004. Phosphorus Compounds in Sequential Extracts of Animal Manures: Chemical Speciation and a Novel Fractionation Procedure. Environ. Sci. Technol. 38, 6101–6108. https://doi.org/https://doi.org/10.1021/es0493042

Turner, B.L., McKelvie, I.D., Haygarth, P.M., 2002. Characterisation of water-extractable soil organic phosphorus by phosphatase hydrolysis. Soil Biol. Biochem. 34, 27–35. https://doi.org/10.1016/S0038-0717(01)00144-4

Tuttobene, R., Avola, G., Gresta, F., Abbate, V., 2009. Industrial orange waste as organic fertilizer in durum wheat. Agron. Sustain. Dev. 29, 557–563. https://doi.org/10.1051/agro/2009014

van der Wiel, B.Z., Weijma, J., van Middelaar, C.E., Kleinke, M., Buisman, C.J.N., Wichern, F., 2019. Restoring nutrient circularity: A review of nutrient stock and flow analyses of local agro-food-waste systems. Resour. Conserv. Recycl. X 3, 100014. https://doi.org/10.1016/j.rcrx.2019.100014

Van Heerden, I., Cronjé, C., Swart, S.H., Kotzé, J.M., 2002. Microbial, chemical and physical aspects of citrus waste composting. Bioresour. Technol. 81, 71–76. https://doi.org/10.1016/S0960-8524(01)00058-X

Van Kauwenbergh, S.J., 2010. World Phosphate Rock Reserves and Resources Technical Bulletin IFDC-T-75, IFDC-T-75. https://doi.org/ISBN 978-9-88999-167-3

Van Vuuren, D.P., Bouwman, A.F., Beusen, A.H.W., 2010. Phosphorus demand for the 1970-2100 period: A scenario analysis of resource depletion. Glob. Environ. Chang. 20, 428–439. https://doi.org/10.1016/j.gloenvcha.2010.04.004

Vance, E.D., Brookes, P.C., Jenkinson, D.S., 1987. An extraction method for measuring soil microbial biomass C. Soil Biol. Biochem. 19, 703–707. https://doi.org/10.1016/0038-0717(87)90052-6

Vanzolini, J.I., Galantini, J.A., Martínez, J.M., Suñer, L., 2017. Changes in soil pH and phosphorus availability during decomposition of cover crop residues. Arch. Agron. Soil Sci. 63, 1864–1874. https://doi.org/10.1080/03650340.2017.1308493

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. J. Soil Sci. Plant Nutr. 16, 294–304. https://doi.org/10.4067/S0718-95162016005000024

Velásquez, Gabriela, Ngo, P.T., Rumpel, C., Calabi-Floody, M., Redel, Y., Turner, B.L., Condron, L.M., Mora, M. de la L., 2016. Chemical nature of residual phosphorus in Andisols. Geoderma 271, 27–31. https://doi.org/10.1016/j.geoderma.2016.01.027

Violante, A., 2013. Elucidating Mechanisms of Competitive Sorption at the Mineral/Water Interface, Advances in Agronomy. Elsevier. https://doi.org/10.1016/B978-0-12-405942-9.00003-7

Violante, A., Pigna, M., 2002. Competitive Sorption of Arsenate and Phosphate on Different Clay Minerals and Soils. Soil Sci. Soc. Am. J. 66, 1788–1796. https://doi.org/10.2136/sssaj2002.1788

Violante, A., Pigna, M., Ricciardella, M., Gianfreda, L., Chimico-agrarie, D.S., Federico, N., 2002. Adsorption of phosphate on variable charge minerals and soil as affected by organic and inorganic ligands. Dev. Soil Sci. 28, 279–295.

Waithaisong, K., Robin, A., Martin, A., Clairotte, M., Villeneuve, M., Plassard, C., 2015. Quantification of organic P and low-molecular-weight organic acids in ferralsol soil extracts by ion chromatography. Geoderma 257–258, 94–101. https://doi.org/10.1016/j.geoderma.2014.12.024

Waldrip, H.M., He, Z., Erich, M.S., 2011. Effects of poultry manure amendment on phosphorus uptake by ryegrass, soil phosphorus fractions and phosphatase activity. Biol. Fertil. Soils 47, 407–418. https://doi.org/10.1007/s00374-011-0546-4

Waldrip, H.M., He, Z., Griffin, T.S., 2012. Effects of organic dairy manure on soil phosphatase activity, available soil phosphorus, and growth of Sorghum-Sudangrass. Soil Sci. 177, 629–637. https://doi.org/10.1097/SS.0b013e31827c4b78

Wang, B.L., Shen, J.B., Zhang, W.H., Zhang, F.S., Neumann, G., 2007. Citrate exudation from white lupin induced by phosphorus deficiency differs from that induced by aluminum. New Phytol. 176, 581–589. https://doi.org/10.1111/j.1469-8137.2007.02206.x

Wang, J.J., Li, X.Y., Zhu, A.N., Zhang, X.., H.W.Zhang, Liang, W.., 2012. Effects of tillage and residue management on soil microbial communities in North China. Plant Soil Environ. 58, 28–33. https://doi.org/10.1016/j.ecolind.2011.05.009

Wang, Y., Chen, X., Whalen, J.K., Cao, Y., Quan, Z., Lu, C., Shi, Y., 2015. Kinetics of inorganic and organic phosphorus release influenced by low molecular weight organic acids in calcareous, neutral and acidic soils. J. Plant Nutr. Soil Sci. 178, 555–566. https://doi.org/10.1002/jpln.201500047

Wang, Y., He, Y., Zhang, H., Schroder, J., Li, C., Zhou, D., 2008. Phosphate Mobilization by Citric, Tartaric, and Oxalic Acids in a Clay Loam Ultisol. Soil Sci. Soc. Am. J. 72, 1263–1268. https://doi.org/10.2136/sssaj2007.0146

Wang, Y., Lambers, H., 2020. Root-released organic anions in response to low phosphorus availability: recent progress, challenges and future perspectives. Plant Soil 447, 135–156. https://doi.org/10.1007/s11104-019-03972-8

Ware, G.W., Whitacre, D.M., 2004. An Introduction to Insecticides, in: The Pesticide Book. MeisterPro Information Resources, Ohio. https://doi.org/https://ipmworld.umn.edu/ware-intro-insecticides

Whalen, J.K., Chang, C., 2001. Phosphorus Accumulation in Cultivated Soils from Long-Term Annual Applications of Cattle Feedlot Manure. J. Environ. Qual. 30, 229–237. https://doi.org/10.2134/jeq2001.301229x.

Whalen, J.K., Chang, C., Clayton, G.W., Carefoot, J.P., 2000. Cattle Manure Amendments Can Increase the pH of Acid Soils. Soil Sci. Soc. Am. J. 64, 962–966. https://doi.org/10.2136/sssaj2000.643962x

Williams, D.M., Blanco-Canqui, H., Francis, C.A., Galusha, T.D., 2017. Organic farming and soil physical properties: An assessment after 40 years. Agron. J. 109, 600–609. https://doi.org/10.2134/agronj2016.06.0372

Wong, M.T.F., Nortcliff, S., Swift, R.S., 1998. Method for determining the acid ameliorating capacity of plant residue compost, urban waste compost, farmyard manure, and peat applied to tropical soils. Commun. Soil Sci. Plant Anal. 29, 2927–2937. https://doi.org/10.1080/00103629809370166

Wu, J., Joergensen, R.G., Pommerening, B., Chaussod, R., Brookes, P.C., 1990. Measurement of soil microbial biomass C by fumigation-extraction: An automated procedure. Soil Biol. Biochem. 22, 1167–1169. https://doi.org/10.1016/0038-0717(90)90046-3

Xu, J.M., Tang, C., Chen, Z.L., 2006. The role of plant residues in pH change of acid soils differing in initial pH. Soil Biol. Biochem. 38, 709–719. https://doi.org/10.1016/j.soilbio.2005.06.022

Xu, R.K., Coventry, D.R., 2003. Soil pH changes associated with lupin and wheat plant materials incorporated in a red-brown earth soil. Plant Soil 250, 113–119. https://doi.org/10.1023/A:1022882408133

Xu, X., Thornton, P.E., Post, W.M., 2013. A global analysis of soil microbial biomass carbon, nitrogen and phosphorus in terrestrial ecosystems. Glob. Ecol. Biogeogr. 22, 737–749. https://doi.org/10.1111/geb.12029

Yan, F., Schubert, S., Mengel, K., 1996. Soil pH increase due to biological decarboxylation of organic anions. Soil Biol. Biochem. 28, 617–624. https://doi.org/10.1016/0038-0717(95)00180-8

Yang, R., Mo, Y., Liu, C., Wang, Y., Ma, J., 2016. The Effects of Cattle Manure and Garlic Rotation on Soil under Continuous Cropping of Watermelon (Citrullus lanatus L .). PLoS One 11, 1–15. https://doi.org/10.1371/journal.pone.0156515

Yu, Q., Rengel, Z., 1999. Micronutrient deficiency influences plant growth and activities of superoxide dismutases in narrow-leafed lupins. Ann. Bot. 83, 175–182. https://doi.org/10.1006/anbo.1998.0811

Yu, W., Ding, X., Xue, S., Li, S., Liao, X., Wang, R., 2013. Effects of organic-matter application on phosphorus adsorption of three soil parent materials. J. Soil Sci. Plant Nutr. 13, 1003–1017. https://doi.org/10.4067/S0718-95162013005000079

Zaller, J.G., Köpke, U., 2004. Effects of traditional and biodynamic farmyard manure amendment on yields , soil chemical , biochemical and biological properties in a long-term field experiment. Biol. Fertil. Soils 40, 222–229. https://doi.org/10.1007/s00374-004-0772-0

Zema, D.A., Calabrò, P.S., Folino, A., Tamburino, V., Zappia, G., Zimbone, S.M., 2018. Valorisation of citrus processing waste : A review. Waste Manag. 80, 252–273. https://doi.org/10.1016/j.wasman.2018.09.024

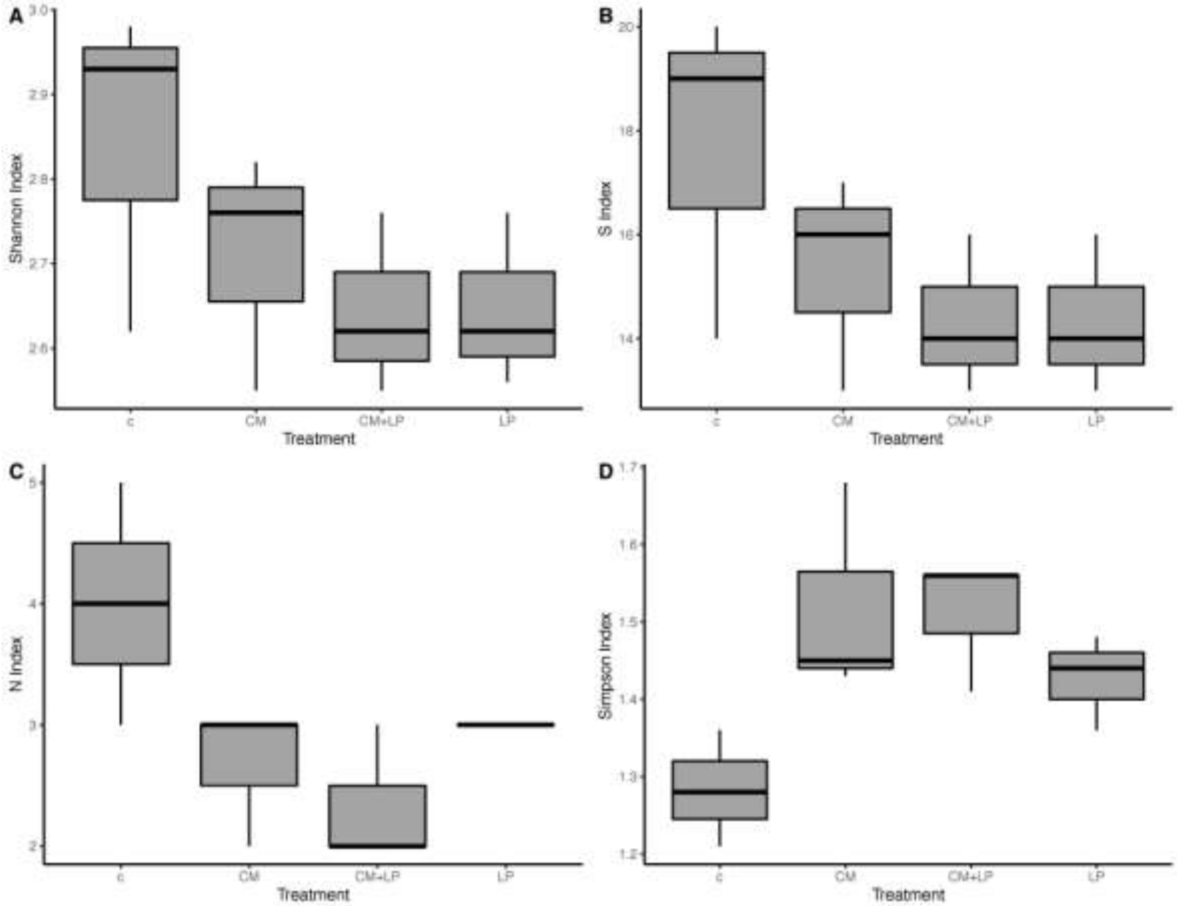
Zhang, F., Shen, J., Zhang, J., Zuo, Y., Li, L., Chen, X., 2010. Rhizosphere Processes and Management for Improving Nutrient Use Efficiency and Crop Productivity. Implications for China, 1st ed, Advances in Agronomy. Elsevier Inc. https://doi.org/10.1016/S0065-2113(10)07001-X

Zhang, Q.C., Shamsi, I.H., Xu, D.T., Wang, G.H., Lin, X.Y., Jilani, G., Hussain, N., Chaudhry, A.N., 2012. Chemical fertilizer and organic manure inputs in soil exhibit a vice versa pattern of microbial community structure. Appl. Soil Ecol. 57, 1–8. https://doi.org/10.1016/j.apsoil.2012.02.012

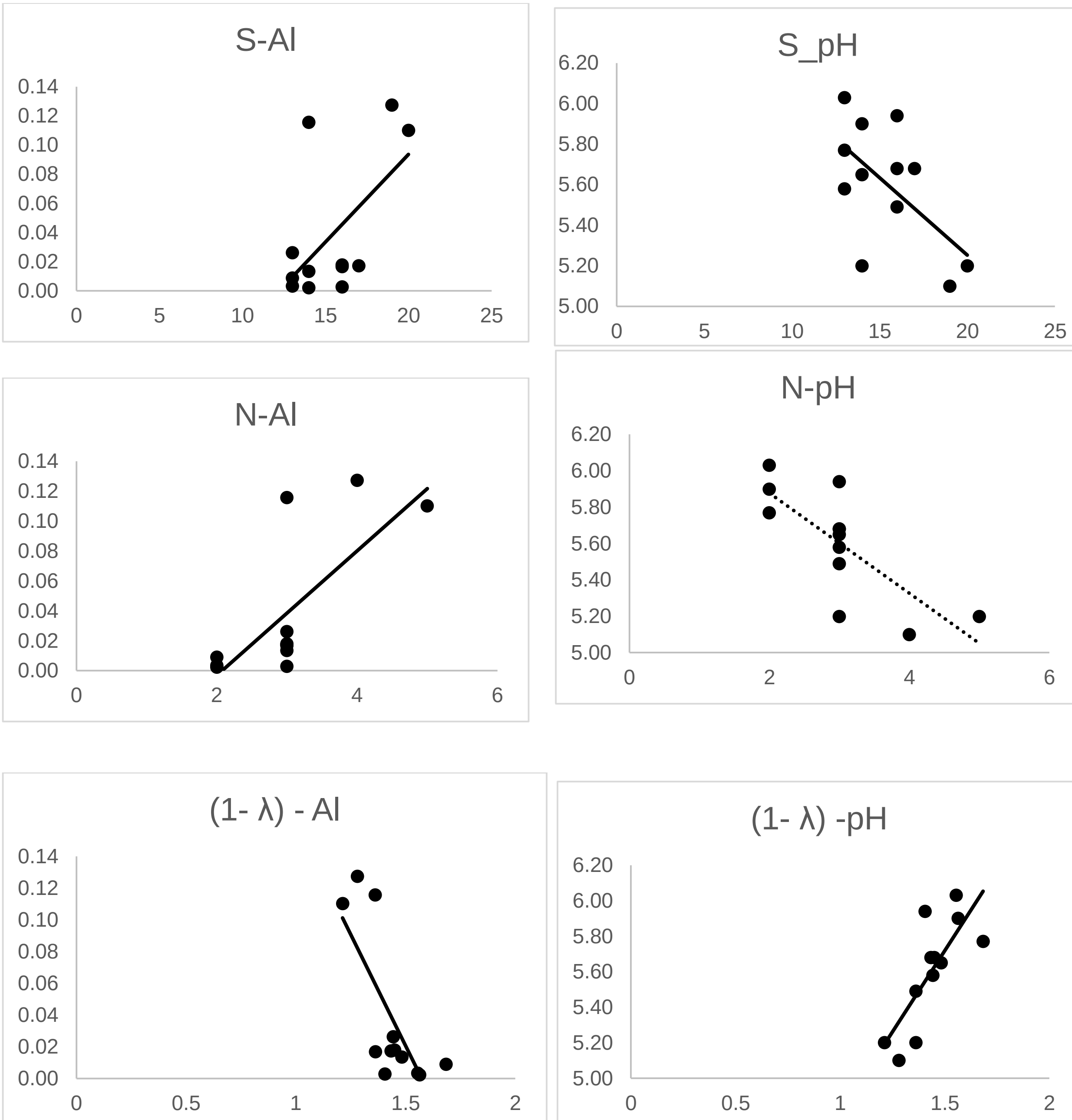
Zhao, Y., Wang, P., Li, J., Chen, Y., Ying, X., Liu, S., 2009. The effects of two organic manures on soil properties and crop yields on a temperate calcareous soil under a wheat-maize cropping system. Eur. J. Agron. 31, 36–42. https://doi.org/10.1016/j.eja.2009.03.001

Zhou, K., Sui, Y., Liu, X., Zhang, X., Jin, J., Wang, G., Herbert, S.J., 2015. Crop rotation with nine-year continuous cattle manure addition restores farmland productivity of artificially eroded Mollisols in Northeast China. F. Crop. Res. 171, 138–145. https://doi.org/10.1016/j.fcr.2014.10.017

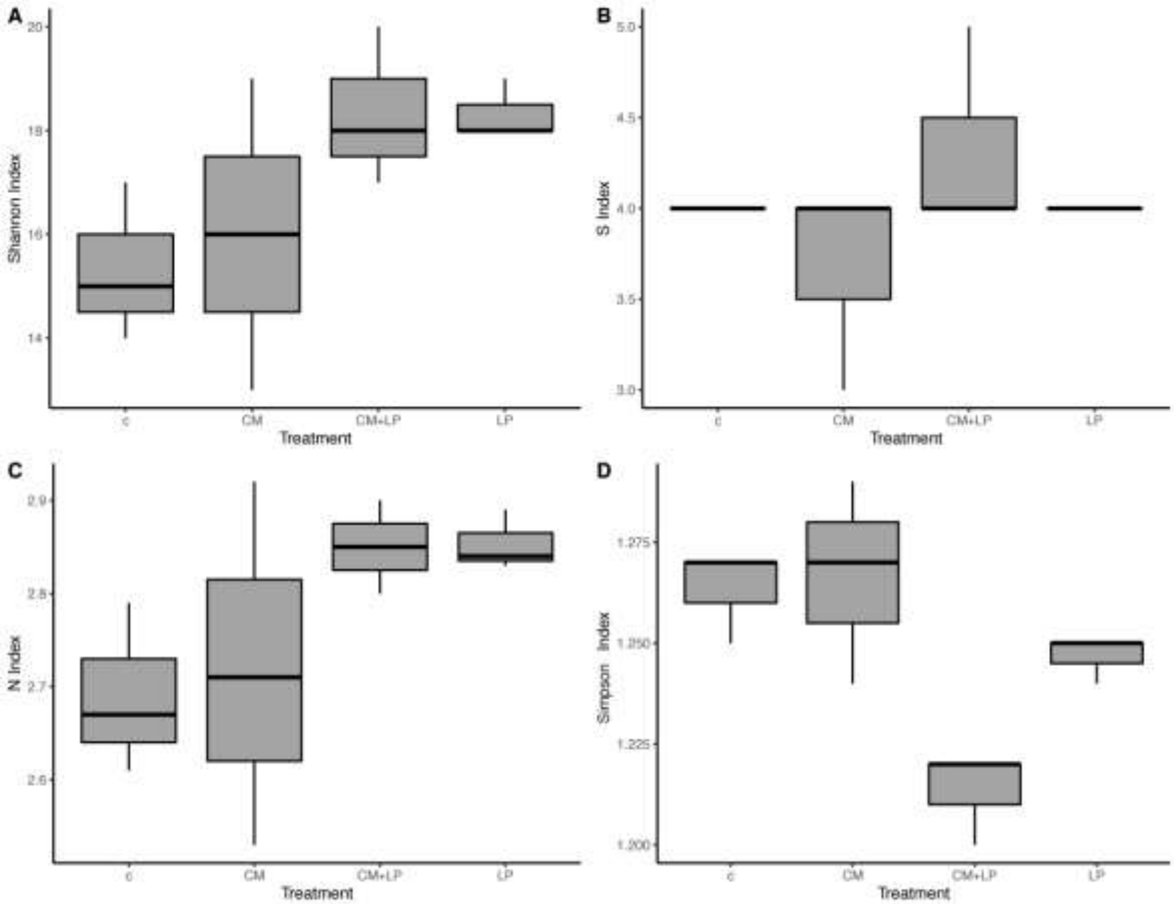
SUPPLEMENTARY MATERIAL PAPER 2



Supplementary Figure 1. Biodiversity index of bacteria



Supplementary Figure 2. Correlation between biodiversity of the bacterial community index S (species), d (individual), H’ (Shannon), and Simpson (expressed as 1- λ) with Al and pH



Supplementary Figure 3. Biodiversity index of fungi

CECILIA DE LOURDES PAREDES NEGRON <cecilia.paredes@ufrontera.cl>

Fwd: Communications in Soil Science and Plant Analysis - Decision on Manuscript ID LCSS-2020-1206.R1

5 mensajes

MARIA DE LA LUZ MORA GIL <mariluz.mora@ufrontera.cl> 16 de abril de 2021, 12:16
Para: CECILIA DE LOURDES PAREDES NEGRON <cecilia.paredes@ufrontera.cl>

---------- Mensaje reenviado ---------
De: **MARIA DE LA LUZ MORA GIL** <mariluz.mora@ufrontera.cl>
Fecha: El vie, 16 de abr. de 2021 a la(s) 12:16
Asunto: Re: Communications in Soil Science and Plant Analysis - Decision on Manuscript ID LCSS-2020-1206.R1
Para: <LCSS-peerreview@journals.tandf.co.uk>

Thanks you very much

We will wait for the proof
Regards

Maria Luz Mora

El El vie, 16 de abr. de 2021 a la(s) 11:55, Communications in Soil Science and Plant Analysis <onbehalfof@manuscriptcentral.com> escribió:

> 16-Apr-2021
>
> Dear Dr Mora:
>
> Ref: Citrus residue enhances the effectiveness of beef cattle manure improving the phosphorus availability in acidic Andisol
>
> Our referees have now considered your paper and have recommended publication in Communications in Soil Science and Plant Analysis based upon the scientific merit of data presented in the manuscript. We are pleased to accept your paper. We will conduct the final editing of your manuscript, at that time we may request changes to be made and we will contact you if that is necessary. After the final editing of your manuscript we will forward the paper to the publisher for copy editing and typesetting. The reviewer comments are included at the bottom of this letter.
>
> You will receive proofs for checking, and instructions for transfer of copyright in due course. Accepted papers will be transmitted for production. The first and most important task for authors at that point will be to complete an online author agreement form. Please make sure you complete it as soon as you receive the publisher notice about it.
>
> The publisher also requests that proofs are checked and returned within 48 hours of receipt.
>
> Thank you for your contribution to Communications in Soil Science and Plant Analysis and we look forward to receiving further submissions from you.
>
> Sincerely,
> Dr Mills
> Editor in Chief, Communications in Soil Science and Plant Analysis
> LCSS-peerreview@journals.tandf.co.uk
>
> Reviewer(s)' Comments to Author:
>
> Please double check the manuscript for abbreviations. Abbreviations must be spelled out the first time they are mentioned in the abstract and starting again with the introduction section this includes elements, chemical names, etc.
> Double check that all references are cited within the text, and that all citations within the text have a corresponding reference. Double check the spelling of the author names.

--
Dr. María de la Luz Mora
Director
Scientific and Technological Bioresource Nucleus BIOREN-UFRO
Universidad de La Frontera
Tel: +56 45 2734191 | mariluz.mora@frontera.cl| bioren.cl
Francisco Salazar 01145 Temuco, Chile

--
Dr. María de la Luz Mora
Director
Scientific and Technological Bioresource Nucleus BIOREN-UFRO
Universidad de La Frontera
Tel: +56 45 2734191 | mariluz.mora@frontera.cl| bioren.cl
Francisco Salazar 01145 Temuco, Chile

CECILIA DE LOURDES PAREDES NEGRON <cecilia.paredes@ufrontera.cl> 17 de abril de 2021, 10:38
Para: DOCTORADO EN CIENCIAS DE RECURSOS NATURALES <doctoradocrn@ufrontera.cl>, GLADYS INES OYARZUN HERNANDEZ <gladys.oyarzun@ufrontera.cl>, andres.quiroz@ufrontera.cl, Mariluz Mora <mariluz.mora@ufrontera.cl>

Estimado Dr. Quiroz:

Esperando que se encuentre bien junto a su familia, me dirijo a usted para reenviar el correo de la revista Communications in Soil Science and Plant Analysis, indicando que nuestro manuscrito fue aceptado. Requisito necesario para poder agendar el examen privado.Le informo además que nos encontramos trabajando en dar respuesta a los comentarios de los revisores del segundo manuscrito y que en estos días se reenviará para su revisión. Adjunto además la tesis con las últimas modificaciones realizadas.

Muchas gracias desde ya, y quedo a la espera de la fecha para dar el examen correspondiente.

Sin otro particular se despide afectuosamente

Cecilia
[El texto citado está oculto]
--
Cecilia Paredes Negrón
Ingeniero Ambiental
Magister en Ciencias de Recursos Naturales
Laboratorio de Suelos y Plantas
Instituto de Agroindustrias
Universidad de La Frontera
Fono: 045-2325494

---

2 adjuntos

Tesis Doctorado_Cecilia Paredes.pdf
1427K

Tesis Doctorado_Cecilia Paredes.docx
20086K

CECILIA DE LOURDES PAREDES NEGRON <cecilia.paredes@ufrontera.cl> 17 de abril de 2021, 11:04
Para: Staunton Siobhan <siobhan.staunton@inrae.fr>

Dear Siobhan:

We have good news, the manuscript was accepted!!. You can see below the email sent from the journal. Thank you very much for your support and I will be eternally grateful for helping me and accompanying me in this long process.
Now I´m waiting for the private exam date. These days you probably will receive my thesis manuscript from the Doctoral program and they will inform the respective date.
I hope to send you the second manuscript later, because I have been working on the final details.

With love

Cecilia

---------- Forwarded message ---------
De: **MARIA DE LA LUZ MORA GIL** <mariluz.mora@ufrontera.cl>
Date: vie, 16 abr 2021 a las 12:16
Subject: Fwd: Communications in Soil Science and Plant Analysis - Decision on Manuscript ID LCSS-2020-1206.R1
To: CECILIA DE LOURDES PAREDES NEGRON <cecilia.paredes@ufrontera.cl>

[El texto citado está oculto]

[El texto citado está oculto]

Staunton Siobhan <siobhan.staunton@inrae.fr> 18 de abril de 2021, 14:49
Para: CECILIA DE LOURDES PAREDES NEGRON <cecilia.paredes@ufrontera.cl>

Wonderful news. At last. Thank YOU for your work. There is always an element of chance in submissions, and this had an unfairly rough traetment.

I hope Carlos is much better.

With love,

Siobhan

[El texto citado está oculto]

ANDRES QUIROZ <quirozandre@gmail.com> 19 de abril de 2021, 9:37
Para: CECILIA DE LOURDES PAREDES NEGRON <cecilia.paredes@ufrontera.cl>

Hola Cecilia

Espero que estés muy bien
Gracias por los documentos.

Saludos

AQ
[El texto citado está oculto]

Dr. Andrés Quiroz
Profesor Titular
Laboratorio de Ecología Química
Departamento de Ciencias Químicas y Recursos Naturales
Universidad de La Frontera
Temuco-Chile

Citrus residue enhances the effectiveness of beef cattle manure improving the phosphorus availability in acidic Andisol

Cecilia Paredes[1], Siobhan Staunton[2], Giuseppe Di Rauso Simeone[3], Maria A. Rao[3], Antonio Violante[3] and María de la Luz Mora[4*]

[1]Programa de Doctorado en Ciencias de Recursos Naturales, Universidad de La Frontera, Temuco, Chile.

[2]INRAE-IRD-Cirad-SupAgro-Université de Montpellier, Eco&Sols, Place Viala, 34060 Montpellier, France.

[3]Dipartimento di Agraria, Università di Napoli Federico II, Portici (Napoli), Italy.

[4]Center of Plant, Soil Interaction and Natural Resources Biotechnology, Scientific and Biotechnological Bioresource Nucleus, Universidad de La Frontera, Temuco, Chile.

***Correspondence:**

María de la Luz Mora

E-mail: mariluz.mora@ufrontera.cl

Abstract

Phosphorus availability and acid pH are major limiting factors for grass and crop production in the Andisols of Southern Chile. Sustainable management requires both the efficient use of fertilizers and the recycling of agricultural waste. The aim of this study was to investigate the effect of citrus waste, alone or in combination with beef cattle manure (CM), on phosphorus availability. Controlled laboratory incubations were carried out for up to six weeks. Lemon (L) and mandarin (M) fruit wastes were added to an Andisol, amended or not with dried CM. Fruit parts (Peel, Juice or whole fruit), and for whole fruit the size of the fruit pieces added to soil, were compared. Water-extractable (WE) P, phosphatase activity (P-ase), and pH were monitored. In general, the size and distribution of the citrus fruit wastes gave similar trends in WE-P, P-ase, and pH. Lemon induced larger effects on WE-P and pH than did mandarin. CM caused sustained soil alkalization, increased soil WE-P and P-ase. Citrus addition produced a short-lived acidification followed by net alkalization and immediate increases in WE-P and P-ase. The combined amendment with citrus wastes enhanced the positive effect of CM on soil WE-P and alkalization, with only a small effect on P-ase. These findings indicate that citrus wastes would intensify the beneficial effects of CM amendment on soil P availability and additionally the liming effect on acid soils.

Keywords: Organic residues, Cost effective soil amendment, Liming effect, Sustainable agriculture, Water-extractable phosphorus, Phosphatase activity

Introduction

Phosphorus (P) is an essential element for plant growth and is important to guarantee crop quality and yields. Global crop production increased markedly during the 20th century largely because of the use of fertilizers derived from phosphate rock. However phosphate rock is a limited, non-renewable resource (Roberts and Johnston 2015). There is an ongoing debate about the longevity of global P resources, particularly high-quality rock phosphate reserves. Some studies have suggested that global P production will peak in 2033 (Cordell et al. 2014; Cordell and Neset 2011; Cordell et al. 2009), whereas Van Vuuren et al. (2010) conclude that only about 40–60% of the available P resources would be extracted by 2100 and the International Fertilizer Development Center (IFDC) maintains that phosphate rock concentrate reserves will be available for the next 300-400 years (Van Kauwenbergh 2010). Regardless of the precise chronology of a future shortage, a considerable improvement in phosphorus fertilizer use efficiency is required to maintain cost-effective agricultural productivity, environmental quality and food security. Among the strategies for improving P use efficiency, the implementation of best management practices (BMPs) (Roberts and Johnston 2015), the reduction of soil P leaching and erosion (Rittmann et al. 2011; Scalenghe et al. 2014), the recycling and recovery of materials containing P (Kratz et al. 2019), the optimization of manure application rates (Sattari et al. 2012) and understanding the P forms in manure to maximize its benefits (Pagliari and Laboski 2012, He et al. 2016, Pagliari et al. 2020) should all be considered.

Acid soils are characterized by high organic P content, and sparingly soluble inorganic P with only a tiny fraction in soil solution as the orthophosphate anion. This anion is strongly adsorbed on organo-mineral surfaces, including oxides, allophane, and phyllosilicates or precipitated as inorganic salts principally with aluminium (Al) and iron (Fe) in acid soils (Gérard 2016; Violante et al. 2002; Violante and Pigna 2002). Plants have developed several strategies in response to the P deficiency and Al toxicity in acid soils (Barra et al. 2018; Ma et al. 2001; Ryan et al. 2001; Wang et al. 2007). Root exudation of organic anions increase P availability to plants by mobilizing phosphate bound to sorption sites in soil (Bolan *et al.* 1994) including Fe and Al-(hydr)oxides (Gerke et al. 1994, 2000, Gérard 2016), or by the formation of complexes with Fe and Al in solution. Another strategy to contend with P deficiency, is the release of acid phosphatase by plants in response to P stress. These enzymes hydrolyze a range of organic-P forms thereby enhancing plant P uptake (Richardson et al. 2011).

Southern Chile is a region devoted to grassland production to support the beef and dairy cattle industry and more recently has seen the expansion of citrus orchards. Citrus production and processing for juice generate a large amount of waste (Sharma et al. 2017; Zema et al. 2018) mainly consisting of peel and pressed pulp or whole fruits discarded due to poor quality (e.g., damaged fruit) or to regulations to limit production (Ruiz and Flotats 2014). Citrus fruits are classified as acid fruits as their juice has pH 2.0-3.0 due to citric and malic acids. Citric acid is the most abundant acid in the endocarp, whereas oxalic, malic, malonic, and citric acids are present in citrus peel (Ladaniya 2008). Recycled citrus fruit processing waste could constitute a soil organic amendment in the context of sustainable and cost-effective management of P fertilization. It has been demonstrated that citrus waste, used as a soil amendment, improves crop yields (Guerrero et al. 1995; Tuttobene et al. 2009) however to date, no study has focused on its effect on soil P fertility.

The importance of manure as a soil amendment has been known since ancient times. In organic and sustainable agriculture, cattle manure represents a source of macro and micronutrients for crop and grass production and simultaneously guarantees the incorporation of organic matter improving physical, chemical and biological properties of soils (Fuentes et al. 2008; Diacono and Montemurro 2010; He and Zhang 2014; Liu et al. 2017; Cai et al. 2019). According to Pagliari et al. (2020) most of P in cattle manure is inorganic and labile (with up to 48% being $NaHCO_3$–extractable and 70% H_2O–extractable) and should therefore be readily available for plants (He et al. 2016; Tiecher et al. 2014).

Not only does manure provide available nutrients, but it may contribute to microbial and biochemical activity. Marinari et al. (2000) found that stabilized dairy cattle manure amendment led to large increases in acid phosphatase activity and this was attributed to a priming effect leading to the mineralization of soil organic matter thereby releasing nutrients. Waldrip et al. (2012) evaluated the effect of organic dairy manure on soil phosphatase activity in an acid silt loam soil and found a positive correlation between P-manure addition and acid phosphatase activity, although there were exceptions to this trend. The authors suggested that during early plant growth, the root induced the production of acid phosphatase or stimulated the extracellular soil phosphatase activity by rhizospheric processes to meet plant nutrient demand. In contrast, poultry manure was found to have little effect on phosphatase activity of the same soil (Waldrip et al. 2011).

The objective of this study was to investigate the additional advantage of applying citrus waste along with CM to assess whether organic matter, and in particular the organic acids, present in citrus wastes, could improve the P availability for crops in an acid soil of Southern Chile. Our starting hypothesis was that a combination of citrus wastes and CM could be the basis of a cost-effective soil amendment improving the fertility of acid soils. We considered the possibility that oxyanions present in citrus waste could inhibit phosphatase activity and hence limit the mineralisation or soil organic phosphorus.

Materials and methods

Soil and beef cattle manure (CM) sampling

The soil studied was an Andisol collected from Southern Chile, Barros Arana locality 39º06'12"S, 72º37'42"W. The soil has silt loam texture and is classified as Andosol (IUSS Working Group, 2014) or Typic Hapludand (Soil Survey Staff, 2014). The soil was collected from 0 to 20 cm depth after removing a thin layer of surface litter, air dried and sieved <2 mm. The non-composted CM (cow-pats) was collected from Santa Elena farm where animals graze outside, located in the same locality. It was air-dried for 5 days, milled and sieved <2 mm for subsequent analysis and use.

Chemical characterization of soil and CM

Both soil and CM were characterized using routine methods according to Sadzawka et al. (2006). Soil pH was determined by potentiometer in a soil: solution (H_2O) suspension (ratio: 1:2.5). Soil extractable phosphorus was extracted with bicarbonate solution (Olsen-P) and quantified using the method of Murphy and Riley (1962). Total phosphorus was determined by alkaline digestion with sodium hypobromite (NaBrO) with the method of Dick and Tabatabai (1977). Total carbon and nitrogen were determined using an elemental analyser (CHN NA1500; Carlo Erba Elemental Analyzer, Stanford, CA, USA). Exchangeable cations (Ca, Mg, Na and K) were extracted with 1 M NH_4Ac at pH 7.0 and quantified using flame atomic absorption spectrophotometry (FAAS, Unicam SOLAAR 969). Exchangeable aluminium was extracted with 1 M KCl and analysed by flame atomic absorption spectrometry (FAAS). Selected physicochemical properties of the soils and CM are shown in Table 1.

Characterization of lemon and mandarin fruits

Fresh fruits of lemon (C*itrus limon* L. Osbeck) and mandarin (*Citrus reticulata*, Blanco, 1837) were purchased from commercial retailers and healthy fruits were selected for uniformity of shape and colour. The fruits were cut in half and squeezed to extract juice. The juice was filtered to remove pulp and seeds and then centrifuged at 3000 *g* for 10 min. Supernatant was diluted 1:50 for citrate determination and 1:10 for the other anions. The diluted samples were membrane filtered (Sartorius Minisart ®, 0.2 μm). The peels of the squeezed fruit samples were cut in small pieces. Ten grams of peel were put in a flask with 25 ml of ultrapure water, shaken for one hour then membrane filtered. Extracts were analysed using High Performance Liquid Chromatography (HPLC, Shimadzu LC 20, DAD SPD-M20A detector) using a 4.6 x 250 mm, 5 μm Symmetry C18 column.

In order to determine the P content on lemon and mandarin peel, each fruit was washed with distilled water and then cut into small pieces, placed in paper bags and dried at 65 °C for 48 h according to Sadzawka et al. (2004). In addition, total carbon and nitrogen were determined using an elemental analyser (CHN NA1500; Carlo Erba Elemental Analyzer, Stanford, CA, USA). The pH was measured in juice and in the water extracts of peel by potentiometry.

Treatments of beef cattle manure-amended soil with chopped lemon or mandarin fruits

Two series of incubation experiments were conducted to evaluate the effect of lemon or mandarin addition on the P release and the acid phosphatase activity (P-ase activity). In the first experiment, 50 g of soil or a mixture of soil with 5% beef cattle manure (CM) was placed in plastic pots of 100 ml capacity. The addition of this rate of manure represents about the same amount of total P and four times as much Olsen P as initially present in soil. Peel and adhering pulp (named Peel, P) were cut with a sharp knife into small pieces (< 5 mm). Peel, juice (J) or whole fruit (WF) were added to each pot and carefully mixed with soil. There were two rates of addition of citrus to soil, either 20% or 40%, expressed as equivalent fresh mass of the whole fruit. Thus, for example, 20 g whole fruit was added to 50 g soil in the 40% treatments and this is equivalent to 12.2 g peel or 7.5 ml juice.

The soil with or without CM, but no addition of citrus, was incubated as a control. Each treatment was prepared in triplicate and samples were incubated at 25 °C, with moisture content at 80% of soil water holding capacity and aerobic condition for up to 6 weeks. Moisture content was calculated considering both juice and added distilled water. Pots were loosely covered and opened daily to allow air exchange. The mass was monitored daily, but no water addition was required to maintain moisture content.

At intervals during the 6-week incubation period (1 hour (h), 1 day (d), 3 d, 1 week (w) 2 w and 4 w) subsamples were taken from each container and analysed for P-ase activity (0.2 g), water extractable P and pH (0.3 g). There were 4 repetitions of each sample, and each of these was a composite sample of four subsamples. The P-ase activity was determined using the method of Tabatabai and Bremner (1969) modified by Rubio et al. (1990) for soil with high organic matter content. In brief, 200 mg soil sample was incubated with 200 µl of 30 mM *p*-nitrophenyl phosphate disodium (Sigma Aldrich), and 800 µl of 0.1 M sodium acetate buffer, pH 5.5, at 25 °C for 30 hours. After incubation, the enzymatic reaction was stopped by adding 200 µl of 2 M $CaCl_2$ and 800 µl of 0.2 M NaOH. The suspension was then centrifuged at 15 000 *g* for 5 min at 25 °C, and the absorbance of the supernatant solution at 405 nm was measured in microplates using a UV-Vis spectrophotometer (Multiskan GO, Thermo Scientific). Water-extractable P was measured using the malachite green method (Ohno and Zibilske 1991). A soil suspension by adding 1.5 ml deionized water to 0.3 g of soil solid, shaking vigorously for 5 min and allowing to settle for 2 h. Finally, the pH of this settled soil suspension was measured. Preliminary experiments showed that Olsen P was too large to be sensitive to changes in fractionation induced by incubation or citrus addition.

Preliminary experiments showed that dilution was sufficient to ensure that there was no interference from citrate in the phosphate assay and that the addition of $CaCl_2$ was sufficient to limit colour release when the enzyme assay was stopped with alkaline solution.

Treatments of beef cattle manure-amended soil with coarsely chopped lemon or mandarin fruits

A second experiment was carried out using more coarsely chopped fruits (4-5 cm in size, ⅛ lemon or ¼ mandarin) and the soil amended with 5% of beef cattle manure (CM). This scale was chosen to be intermediate between the controlled laboratory experiment and a future field study. In these experiments soil pots were set up in three layers: at the base, layer C with about 15 g of soil, or CM-amended soil, over layer B of approximately 20 g of soil, or CM-amended soil, plus chopped lemon or mandarin, and in the top layer A, with 15 g of soil or CM-amended soil. The layers were separated with a nylon mesh with 35-µm pore diameter. Plastic pots with only soil were used as controls, following the same procedure described above. All samples were prepared in triplicate. The samples were stored for four weeks at 25°C under aerobic conditions, and at 80% of soil water holding capacity. At the end of the incubation period, soil was collected from each layer to obtain composite samples and analysed to determine pH, water extractable P, P-ase activity

Statistical analysis

The statistical analysis was carried out using XLSTAT version 2016.02.284551 (Addinsoft, New York, USA). Data were first tested for deviation from normality using the Shapiro–Wilk test and the homogeneity of within-group variances was assessed with Levene´s test. Data collected were subjected to analysis of variance (ANOVA) and the means were separated by the Duncan test at $p \leq 0.05$. In the P-ase data the differences between control and CM was assessed by paired t-test ($p \leq 0.05$).

Results

Chemical characterization of soil and beef cattle manure samples

Chemical properties of the Andisol Barros Arana are reported in Table 1. The soil had a total carbon content of about 12%, a moderate acidity (pH 5.58) and low levels of Ca, Mg, K, and Na. The Al content of exchange complex was low, with Al saturation less than 1%. The Olsen-P content in soil was very low (6 mg kg^{-1}) despite the high content of total P (1981 mg kg^{-1}).

Beef cattle manure was characterized by neutral pH, 41.1% C, C/N ratio equal to 19.2, high total P (3710 mg kg^{-1}) as well as Olsen-P (500 mg kg^{-1}) and high exchangeable cations contents of but a low Al saturation (1.2 mmol kg^{-1}).

Characterization of lemon and mandarin fruits

The organic anion contents of lemon and mandarin peel and juice are shown in Table 2. The amount of organic anion depended on both the fruit and fruit part. In lemon, citrate was the main organic anion found in juice and peel (21.0 ± 0.01 and 8.07 ± 0.01 g kg^{-1}, respectively) followed by oxalate, malate and succinate. In contrast, malate was the main anion found in the peel of mandarin (3.60 ± 0.20 g kg^{-1}), and citrate in mandarin juice (2.38

± 0.001 g kg^{-1}). Lemon juice had the lowest pH observed, 1.92, whereas that of mandarin juice was slightly higher, 2.65. The pH values of lemon and mandarin peel were 2.86 and 3.58, respectively. The C/N ratio in lemon peel was greater than in mandarin peel and the P contents of lemon and mandarin peel were 0.97 and 1.17 g kg^{-1}, respectively.

Soil amended with finely chopped citrus

Effect of amendments on soil pH

Beef cattle manure (CM) addition led to an increase in soil pH. Although pH fluctuated during the incubation period, the manure amended soil was on average about 0.2 units more alkaline than the unamended soil. Citrus addition caused an initial acidification of both soil and manure-amended soil (Table 3). This decrease of pH was greater for lemon than for mandarin addition, and greater for juice than peel, and increased with increased addition of peel (Table 3).

The lemon peel treatments, 20% and 40%, led to immediate (after one hour) decreases in pH of 0.8 and 0.9 units, respectively, with respect to the soil control. Manure counterbalanced to some extent the initial acidifying effect of citrus addition. Soil amended with CM plus 20% and 40% LP decreased the pH by 0.4 and 1.3 units, respectively, compared with CM-soil control. With the larger addition of peel, the alkalising effect of manure was cancelled. The greatest pH change occurred when juice or whole fruit were added to soil or CM-amended soil. For soil, the addition of juice and whole fruit caused a decrease around 1.7 units, whereas for CM-amended soil this decrease was 1.46 for juice and 1.90 for whole fruit, and as for peel, the larger addition of whole fruit cancelled the alkalinising effect of CM. After two weeks pH increased with all lemon treatments in both soil and CM-amended soil reaching in average a pH value of 6.2 (Table 3). The acidifying effect of lemon addition decreased with time, and indeed, after about 2 weeks, citrus amended soil were more alkaline than S or S+CM controls.

Mandarin treatments had less impact on soil pH (S or S+CM controls), relative to lemon treatments, and so the range of pH values measured was smaller. Each of the mandarin additions caused a decrease in pH with respect to S and S+CM at each incubation time for the first 2 weeks (Table 3). For soil (without manure) the immediate (1 hour) decrease in pH was 0.26 units for 20% peel and 0.5 units for 40% peel without significant ($p \leq 0.05$) difference Incorporation of juice and whole fruit led to larger decreases in pH, 0.52 and 0.64 units, respectively. The effects of mandarin additions to CM-amended soil were very similar to those observed for soil, with a general alkalinisation caused by CM. The short-term acidification observed for citrus addition was delayed, in comparison with lemon, leading to a surprising net acidification of the CM-amended soils for the 1-day and 3-day incubation periods with respect to soil+mandarin.

Effect of amendment on water-extractable phosphorus

The extractable soil P content was significantly ($p \leq 0.05$) affected by the incorporation of citrus fruits and CM (Figure 1). There was an initial flush of P solubility at the start of the incubation experiment, and manure addition increased this flush. Thereafter P tended to decrease with time, but remained on average larger in manure amended soil. Citrus amendment caused large flush of P. Lemon addition led to larger increases in P than did mandarin. In general, the greatest increase of WE P content was observed when whole fruit was added, followed by peel.

When whole fruit was added to CM-amended soil WE P content increased 7-fold with respect to control (from 180 to 1230 μg kg^{-1}), indicating a synergistic effect between citrus and manure additions. It must be noted that a different scale had to be used to present data with CM amendment after 1 hour, because of the magnitude of this flush. Lemon peel addition (20% and 40%) increased P content approximately 2.5-fold on control soil; meanwhile for CM-amended soil, P increased approximately 3- fold. Interestingly, the lowest phosphorus values were obtained when juice treatment was applied, but the effects of juice and peel were additive. After the initial flush of P, extractable P levels fell back rapidly, but remained greater in citrus-amended soils throughout the incubation period.

The mandarin treatments led to smaller enhancements of water-extractable P; the incorporation of 40% peel or whole fruit increased the WE P content of both control and CM-amended soil (1.8-fold and 2.5-fold respectively). No marked differences were found when 20% peel was added. The whole fruit treatment produced slightly greater increases, 2.3 fold on S and 2.9-fold S+CM. Although the P content fell markedly after 1 day, both the CM and 40% whole fruit additions maintained large increases in the P content, 8.0 and 17.9-fold respectively. At the end of the 6-week incubation, the highest P contents were found in the CM-amended soil with lemon treatments containing peel (either peel or whole fruit).

Effect of amendment on phosphatase activity

Phosphatase activity in soil was fairly constant and showed no consistent variation during the incubation period with little effect of manure addition (Figure 2). Citrus fruit treatments containing peel, significantly ($p \leq 0.05$) increased acid phosphatase activity in soil, with or without CM, at each stage of the incubation (Figure 2A). After 1 hour, treatments containing lemon peel (peel and whole fruit) increased acid phosphatase activity that reached on average values of about 1.7 nkat PNP g $soil^{-1}$, which were maintained until the end of incubation period. In contrast, the juice treatment caused a marked, but short-lived decrease in phosphatase activity from 1.0 to 0.6 nkat PNP g soil^{-1}. Similar behaviour was observed for mandarin treatments, where the incorporation of peel and whole fruit tended to enhance the phosphatase activity in both soil and CM-amended soil. The initial (1-hour) increases due to peel or whole fruit addition were small and not always significant ($p \leq 0.05$) in soil, but more marked for CM-amended soil (1.9 and 1.79 nkat PNP g soil^{-1} for Peel and WF, respectively). Mandarin juice, in line with lemon juice, led to decreased phosphatase activity, that was particularly marked after 1 hour. In contrast to lemon juice, the decrease in P-ase activity induced by mandarin juice was observed throughout the incubation period.

Soil amended with coarsely chopped fruit

Effect on pH

Soil amendment was also performed using larger pieces of chopped fruit (>4-5 cm) to be closer to real conditions. Soil pH in the top layer (A) most closely resembled the control soil and greatest effects were observed for the middle layer (B), where soil was in direct contact with chopped fruit. Soil pH in layer B increased by 0.73 and 0.56 units following lemon- and mandarin- chopped fruit amendments, respectively, compared with the layer A (Table 4). The incorporation of lemon and mandarin also increased pH of the CM-amended soil by 0.49 and 0.45 units, respectively. In layer C the increase in pH was significant ($p \leq 0.05$) but smaller than in layer B. Thus in soil amended with lemon and mandarin, pH increased by 0.40 and 0.57 units, respectively, while the pH increases in CM-amended soil were smaller, 0.25 units for lemon and not significant ($p \leq 0.05$) for mandarin (Table 4).

Effect on water-extractable phosphorus

The trends in WE P were remarkably similar to those measured in the first incubation experiment (Figure 1), but with greater amount of extractable P in layer B, suggesting a scaling-up effect. The highest WE P content was observed in the soil surrounding the fruit (layer B) in both soil (S) and CM-amended soil (CM-S) (Figure 3). For S, WE P increased from 170 to 240 µg kg^{-1} with chopped lemon and from 43 to 110 µg kg^{-1} with chopped mandarin. For CM-amended soil, the WE P content increased from 250 to 450 µg kg^{-1} in the lemon treatment and from 50 to 250 µg kg^{-1} in mandarin treatment (Figure 3).

Effect on phosphatase activity

Phosphatase activity tended to be increased by fruit addition, and as observed for pH and WE P, the effects were greatest in layer B (Table 4). Values in the top layer were least influenced by citrus addition, and the effect was intermediate in the lowest layer. Activity was lower in CM-amended soil, and the increase induced by citrus was greater for lemon than mandarin.

Discussion

Applications of citrus fruit (all treatments) caused an initial decrease in soil pH. However, with increasing incubation time, pH rose to an average of 6.10. The initial soil pH decrease can be explained by the acidity *per se* of the amendment. The subsequent increase in pH may be attributed to the decomposition of organic matter, including the decarboxylation of the organic anions (Naramabuye and Haynes 2006). Large increases in pH have been reported during the composting of citrus wastes (between days 45 and 50) (Van Heerden et al. 2002). Several studies have shown an increase in pH following crop residue addition to soil, particularly in acid soil (Butterly et al. 2013; Vanzolini et al. 2017; Xu et al.

2006; Xu and Coventry 2003; Yan et al. 1996). For example, Mokolobate and Haynes (2002) compared the liming effect of four organic residues applied to an acid soil and concluded that addition of organic residues to acid soils could be a practical strategy to increase soil pH, thus decreasing concentrations of phytotoxic Al without lime applications. In addition, the application of manure to soils also supplies large amounts of organic matter, containing carboxyl and phenolic hydroxyl groups, that play essential roles in buffering soil acidity and increasing the pH of acid soils (Whalen et al. 2000). We observed increased soil pH in both incubation experiments, caused by both CM and citrus residue additions. We conclude that the combined addition of manure and citrus residue could be a cost-effective solution to mitigate the acidity of soil and thus alleviate Al toxicity, which are the most important limiting factors for crop production in this type of soil.

One concern with the addition of citrus fruits to these soils was that the oxyanions they would release could inhibit phosphatase activity, thereby reducing the availability of organic P to crops. The observations of this study allow us to dismiss these fears: in the long term phosphatase activity was enhanced by both amendments. This finding contrasts with the observation of Waldrip et al. (2012) that cattle manure had large effect on acid phosphatase activity, and is closer to the finding of Waldrip et al. (2011) who report no significant effect of poultry manure on phosphatase activity. In our case, the acid phosphatase activity was found to be more strongly influenced by the citrus treatments than by CM addition in both incubation experiments. There was no synergistic effect between manure and citrus fruit on phosphatase activity. The greater effect of citrus in comparison with manure could be explained by the stimulation of microbial growth due to the incorporation of readily degradable organic substrates (Wang et al. 2012; Intanon et al. 2015; Dumontet et al. 2017). As in this study, Pascual et al. (2002) report that phosphatase activity was highest in soil amended with fresh organic wastes, rather than with composted organic wastes, at least in the short term. They attributed this increase to increases in microbial biomass and activity. This observation is coherent with the relative effects of citrus and manure additions since CM was stabilized prior to incorporation into soil, whereas citrus residues were applied fresh. Several studies have found high phosphatase activity during the first month of composting of organic wastes, however at the end of the process it is drastically decreased, which could be attributed to a reduction in easily degradable compounds (Ros et al. 2006; Raut et al. 2008; Albrecht et al. 2010). However, even though phosphatase activity indicates the potential for mineralization of organic P, we found no correlation between enzymatic activity and water extractable phosphorus in soils (data not shown). This could be explained by increased microbial activities, involving a higher P demand and release of phosphatases when organic waste, rich in easily available C and N, is incorporated. However, as the organic matter is consumed, microbial activity would decrease, decreasing both P demand and phosphatase activity (Criquet and Braud 2008).

It is well known that cattle manure amendments can increase P availability along with the improvement of various soil physical, chemical and biological properties (Parham et al. 2002, 2003; Ros et al. 2006; He and Zhang 2014; Tiecher et al. 2014; Cai et al. 2019). In this study, the use of CM increased not only the WE P content but also increased the phosphatase activity and soil pH. This study shows that the use of citrus residues also improved these soil properties. The relationship between low-molecular-mass organic anions and inorganic phosphorus release from soils has been widely investigated (Kpomblekou-A and Tabatabai 2003; Harrold and Tabatabai 2006; Kizewski et al. 2010; Taghipour and Jalali 2013; Wang et al. 2015; Santos, Hesterberg, et al. 2017; Santos, Silva, et al. 2017). It is generally assumed that the mechanisms of release inorganic phosphorus involving low-molecular-mass organic anions include the dissolution of sparingly soluble minerals containing phosphorus, the change pH of soil solution and the capacity to chelate metal cations, notably Fe, Al, or Ca, and the blocking of P-adsorption sites on soil (Jones 1998; Ryan et al. 2001; Bais et al. 2006). In both incubation experiments increased WE P content after addition of citrus fruit was observed. We know of no studies on the use of citrus residues for mobilizing soil-P, nevertheless it is reasonable to suppose that the organic acids released from citrus lead to increased P solubility (Table 2). In the first incubation experiment, treatments with L-WF and CM, increased soil P release 7- fold respect to control after 1 hour. However, a marked decrease after one day was observed; this is probably due to the rapid microbial degradation of organic anions (Jones 1998, Menezes-Blackburn et al. 2016).

Most importantly, in the medium term, after six weeks incubation, treatments with CM, 40% peel, and whole fruit always showed the largest WE P content. Similarly, a 2-fold increase of soil WE P with respect to control soil was observed in the incubation experiment with coarsely chopped fruit (Figure 2). This is coherent with reported increases in biomass production after addition of orange waste, for example that of durum wheat and lettuce, (Tuttobene et al. 2009; Guerrero et al. 1995) and points to improved P nutrition as a major.

Conclusions

Our findings showed that the addition of citrus residues in combination with beef cattle manure to soil had a sustained positive effect on phosphatase activity and an additional synergistic effect on soil P availability, as assessed with WE P. The increase in phosphatase activity caused by organic residues used in this experiment could indicate an enhancement of soil fertility and thus the sustainability of agricultural production. There was no evidence that increased solubility of soil P inhibited phosphatase activity, even during the short-term flush of P solubilization due to citrate and other oxyanions in the citrus amendment. The addition of citrus waste enhanced the liming effect of manure throughout the 6-week experiment, a positive effect in this acid soil. The time trends observed demonstrate the importance of medium term experiments to avoid hasty conclusions based on short-lived flushes (<1 week). Furthermore, the "liming effect" produced by this type of residues could be used as a biotechnological strategy to decrease the lime requirements in such acid soils.

This controlled laboratory study is a promising basis for further longer-term and field based investigations of a mixed citrus-manure amendment. Furthermore, it is necessary to evaluate composting the organic matrices used for amplifying the obtained advantages, as well as, assess phytotoxicity reduction, stability of the organic substances in the soil and its effect on soil chemistry and microbiology.

Conflict of interest statement

All authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author contributions

The work presented here was carried out in collaboration between all authors. CP and MLM defined the research theme. CP performed all the experiments under the supervision of SS and MLM. CP, SS, MR, AV and MLM wrote the manuscript. All authors have contributed to, seen and approved the manuscript.

Funding

This work was supported by the FONDECYT 1181050

Acknowledgments

We would like to thank the Scientific and Technological Bioresource Nucleus (BIOREN-UFRO) and Soil and Plant Laboratory of Universidad de La Frontera for providing access to specialized equipment.

References

Albrecht, R., Petit, J. Le, Calvert, V., Terrom, G., and Périssol, C., 2010. Changes in the level of alkaline and acid phosphatase activities during green wastes and sewage sludge co-composting. *Bioresource Technology*, 101 (1), 228–233.

Bais, H.P., L.Weir, T., Perry, L.G., Gilroy, S., and Vivanco, J.M., 2006. The Role of Root Exudates in Rhizosphere Interactions with Plants and Other Organisms. *Annu. Rev. Plant Biol.*, 57, 233–266.

Barra, P.J., Viscardi, S., Jorquera, M.A., Duran, P.A., Valentine, A.J., and Mora, M. de la L., 2018. Understanding the strategies to overcome phosphorus-deficiency and aluminum-toxicity by ryegrass endophytic and rhizosphere phosphobacteria. *Frontiers in Microbiology*, 9, 1–12.

Bolan, N.S., Naidu, R., Mahimairaja, S., and Baskaran, S., 1994. Influence of low-molecular-weight organic acids on the solubilization of phosphates. *Biology and Fertility of Soils*, 18 (4), 311–319.

Butterly, C.R., Baldock, J.A., and Tang, C., 2013. The contribution of crop residues to changes in soil pH under field conditions. *Plant and Soil*, 366 (1–2), 185–198.

Cai, A., Xu, M., Wang, B., Zhang, W., Liang, G., Hou, E., and Luo, Y., 2019. Manure acts as a better fertilizer for increasing crop yields than synthetic fertilizer does by improving soil fertility. *Soil and Tillage Research*, 189, 168–175.

Cordell, D., Drangert, J.O., and White, S., 2009. The story of phosphorus: Global food security and food for thought. *Global Environmental Change*, 19 (2), 292–305.

Cordell, D. and Neset, T.S.S., 2014. Phosphorus vulnerability: A qualitative framework for assessing the vulnerability of national and regional food systems to the multi-dimensional stressors of phosphorus scarcity. *Global Environmental Change*, 24 (1), 108–122.

Cordell, D., Rosemarin, A., Schröder, J.J., and Smit, A.L., 2011. Towards global phosphorus security: A systems framework for phosphorus recovery and reuse options. *Chemosphere*, 84 (6), 747–758.

Criquet, S. and Braud, A., 2008. Effects of organic and mineral amendments on available P and phosphatase activities in a degraded Mediterranean soil under short-term incubation experiment. *Soil and Tillage Research*, 98 (2), 164–174.

Diacono, M. and Montemurro, F., 2010. Long-term effects of organic amendments on soil fertility. A review. *Agronomy for Sustainable Development*, 30 (2), 401–422.

Dick, W. and Tabatabai, M., 1977. An alkaline oxidation method for the determination of total phosphorus in soils. *Soil Science Society of America Journal*, 41, 511–514.

Dumontet, S., Cavoski, I., Ricciuti, P., Mondelli, D., Jarrar, M., Pasquale, V., and Crecchio, C., 2017. Metabolic and genetic patterns of soil microbial communities in response to different amendments under organic farming system. *Geoderma*, 296, 79–85.

Fuentes, B., de la Luz Mora, M., Bolan, N.S., and Naidu, R., 2008. Chapter 16 Assessment of phosphorus bioavailability from organic wastes in soil. *Developments in Soil Science*, 32, 363–411.

Gérard, F., 2016. Clay minerals, iron/aluminum oxides, and their contribution to phosphate sorption in soils - A myth revisited. *Geoderma*, 262, 213–226.

Gerke, J., Römer, W., and Beißner, L., 2000. The quantitative effect of chemical phosphate mobilization by carboxylate anions on P uptake by a single root. II. The importance of soil and plant parameters for uptake of mobilized P. *Journal of Plant Nutrition and Soil Science*, 163 (2), 213–219.

Gerke, J., Römer, W., and Jungk, A., 1994. The excretion of citric and malic acid by proteoid roots of Lupinus albus L.; effects on soil solution concentrations of phosphate, iron, and aluminum in the proteoid rhizosphere in samples of an oxisol and a luvisol. *Zeitschrift für Pflanzenernährung und Bodenkunde*, 157 (4), 289–294.

Guerrero, C.C., Carrasco de Brito, J., Lapa, N., and Oliveira, J.F.S., 1995. Re-use of industrial orange wastes as organic fertilizers. *Bioresource Technology*, 53 (1), 43–51.

Harrold, S.A. and Tabatabai, M.A., 2006. Release of Inorganic Phosphorus from Soils by Low-Molecular-Weight Organic Acids. *Communications in Soil Science and Plant Analysis*, 37 (9–10), 1233–1245.

He, Z., Pagliari, P.H., and Waldrip, H.M., 2016. Applied and Environmental Chemistry of Animal Manure: A Review. *Pedosphere*, 26 (6), 779–816.

He, Z. and Zhang, H., 2014. Applied manure and nutrient chemistry for sustainable agriculture and environment. *Applied Manure and Nutrient Chemistry for Sustainable Agriculture and Environment*, 1–379.

Van Heerden, I., Cronjé, C., Swart, S.H., and Kotzé, J.M., 2002. Microbial, chemical and physical aspects of citrus waste composting. *Bioresource Technology*, 81 (1), 71–76.

Intanon, S., Hulting, A.G., Myrold, D.D., and Mallory-Smith, C.A., 2015. Short-term effects of soil amendment with meadowfoam seed meal on soil microbial composition and function. *Applied Soil Ecology*, 89, 85–92.

Jones, D.L., 1998. Organic acids in the rhizosphere - A critical review. *Plant and Soil*, 205 (1), 25–44.

Van Kauwenbergh, S.J., 2010. *World Phosphate Rock Reserves and Resources Technical Bulletin IFDC-T-75*. IFDC-T-75.

Kizewski, F.R., Boyle, P., Hesterberg, D., and Martin, J.D., 2010. Mixed Anion (

Phosphate / Oxalate) Bonding to Iron (III) Materials. *Journal of the American Chemical Society*, 132 (7), 2301–2308.

Kpomblekou-A, K. and Tabatabai, M.A., 2003. Effect of low-molecular weight organic acids on phosphorus release and phytoavailabilty of phosphorus in phosphate rocks added to soils. *Agriculture, Ecosystems and Environment*, 100 (2–3), 275–284.

Kratz, S., Vogel, C., and Adam, C., 2019. *Agronomic performance of P recycling fertilizers and methods to predict it: a review*. Nutrient Cycling in Agroecosystems. Springer Netherlands.

Ladaniya, M., 2008. Fruit Biochemistry. *In*: Elsevier Inc, ed. *Citrus Fruit, Biology Technology and Evaluation*. Atlanta, USA.

Liu, Z., Rong, Q., Zhou, W., and Liang, G., 2017. Effects of inorganic and organic amendment on soil chemical properties, enzyme activities, microbial community and soil quality in yellow clayey soil. *PLoS ONE*, 12 (3).

Ma, J.F., Ryan, P.R., and Delhaize, E., 2001. Aluminium tolerance in plants and the complexing role of organic acids. *Trends in Plant Science*, 6 (6), 273–278.

Marinari, S., Masciandaro, G., Ceccanti, B., and Grego, S., 2000. Influence of organic and mineral fertilisers on soil biological and physical properties, 72, 9–17.

Menezes-Blackburn, D., Paredes, C., Zhang, H., Giles, C.D., Darch, T., Stutter, M., George, T.S., Shand, C., Lumsdon, D., Cooper, P., Wendler, R., Brown, L., Blackwell, M., Wearing, C., and Haygarth, P.M., 2016. Organic Acids Regulation of Chemical-Microbial Phosphorus Transformations in Soils. *Environmental Science and Technology*, 50 (21), 11521–11531.

Mokolobate, M.S. and Haynes, R.J., 2002. Comparative liming effect of four organic residues applied to an acid soil. *Biology and Fertility of Soils*, 35 (2), 79–85.

Murphy J and Riley JP, 1962. A modified single solution method for the determination of phosphate in natural waters. *Analytical Chemistry Acta*, 27, 31–36.

Naramabuye, F.X. and Haynes, R.J., 2006. Effect of organic amendments on soil pH and Al solubility and use of laboratory indices to predict their liming effect. *Soil Science*, 171 (10), 754–763.

Ohno, T. and Zibilske, L.M., 1991. Determination of Low Concentrations of Phosphorus in Soil Extracts Using Malachite Green. *Soil Science Society of America Journal*, 55 (3), 892.

Pagliari, P.H. and Laboski, C.A.M., 2012. Investigation of the Inorganic and Organic Phosphorus Forms in Animal Manure. *Journal of Environmental Quality*, 41 (3), 901–910.

Pagliari, P.H., Wilson, M., Waldrip, H.M., and He, Z., 2020. Nitrogen and Phosphorus Characteristics of Beef and Dairy Manure. *Animal Manure: Production, Characteristics, Environmental Concerns, and Management*, 45–62.

Parham, J.A., Deng, S.P., Da, H.N., Sun, H.Y., and Raun, W.R., 2003. Long-term cattle manure application in soil. II. Effect on soil microbial populations and community structure. *Biology and Fertility of Soils*, 38 (4), 209–215.

Parham, J.A., Deng, S.P., Raun, W.R., and Johnson, G. V., 2002. Long-term cattle manure application in soil. I. Effect on soil phosphorus levels, microbial biomass C, and dehydrogenase and phosphatase activities. *Biology and Fertility of Soils*, 35 (5), 328–337.

Pascual, J.A., Moreno, J.L., Hernández, T., and García, C., 2002. Persistence of immobilised and total urease and phosphatase activities in a soil amended with organic wastes. *Bioresource Technology*, 82 (1), 73–78.

Raut, M.P., Prince William, S.P.M., Bhattacharyya, J.K., Chakrabarti, T., and Devotta, S., 2008. Microbial dynamics and enzyme activities during rapid composting of municipal solid waste - A compost maturity analysis perspective. *Bioresource Technology*, 99 (14), 6512–6519.

Richardson, A.E., Lynch, J.P., Ryan, P.R., Delhaize, E., Smith, F.A., Smith, S.E., Harvey, P.R., Ryan, M.H., Veneklaas, E.J., Lambers, H., Oberson, A., Culvenor, R.A., and Simpson, R.J., 2011. Plant and microbial strategies to improve the phosphorus efficiency of agriculture. *Plant and Soil*, 349 (1–2), 121–156.

Rittmann, B.E., Mayer, B., Westerhoff, P., and Edwards, M., 2011. Capturing the lost phosphorus. *Chemosphere*, 84 (6), 846–853.

Roberts, T.L. and Johnston, A.E., 2015. Phosphorus use efficiency and management in agriculture. *Resources, Conservation and Recycling*, 105, 275–281.

Ros, M., Pascual, J.A., Garcia, C., Hernandez, M.T., and Insam, H., 2006. Hydrolase activities, microbial biomass and bacterial community in a soil after long-term amendment with different composts. *Soil Biology and Biochemistry*, 38 (12), 3443–3452.

Rubio, R., Moraga, E., and Borie, F., 1990. Acid phosphatase activity and vesicular-arbuscular mycorrhizal infection associated with roots of four wheat cultivars. *Journal of Plant Nutrition*, 13 (5), 585–598.

Ruiz, B. and Flotats, X., 2014. Citrus essential oils and their influence on the anaerobic digestion process: An overview. *Waste Management*, 34 (11), 2063–2079.

Ryan, P.R., Delhaize, E., and Jones, D., 2001. Function and mechanism of organic anion exudation from plant roots. *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, 52, 527–560.

Sadzawka, A., Carrasco, M.A., Gez, R., Mora, M.D.L.L., Flores, H., and Neaman, A.,

2006. Métodos de análisis recomendados para los suelos de Chile. Revision 2006. *Serie Actas-Instituto de Investigaciones Agropecuarias*.

Sadzawka, A., Grez, R., Carrasco, M., Flores, H., Mora, M., and Rojas, C., 2004. Métodos De Análisis De Tejidos Vegetales, 53.

Santos, S.R., Hesterberg, D., and Alleoni, L.R.F., 2017. Phosphate Speciation and Citrate-Induced Mobilization of P in an Acric Oxisol. *Communications in Soil Science and Plant Analysis*, 48 (17), 1977–1988.

Santos, S.R., Silva, E. de B., Alleoni, L.R.F., and Grazziotti, P.H., 2017. Citric acid influence on soil phosphorus availability. *Journal of Plant Nutrition*, 40 (15), 2138–2145.

Sattari, S.Z., Bouwman, A.F., Giller, K.E., and van Ittersum, M.K., 2012. Residual soil phosphorus as the missing piece in the global phosphorus crisis puzzle. *Proceedings of the National Academy of Sciences*, 109 (16), 6348–6353.

Scalenghe, R., Edwards, A.C., Barberis, E., and Ajmone-Marsan, F., 2014. Release of phosphorus under reducing and simulated open drainage conditions from overfertilised soils. *Chemosphere*, 95, 289–294.

Sharma, K., Mahato, N., Cho, M.H., and Lee, Y.R., 2017. Converting citrus wastes into value-added products : Economic and environmently friendly approaches. *Nutrition*, 34, 29–46.

Tabatabai, M.A. and Bremner, J.M., 1969. Use of p-nitrophenyl phosphate for assay of soil phosphatase activity. *Soil Biology and Biochemistry*, 1 (4), 301–307.

Taghipour, M. and Jalali, M., 2013. Effect of low-molecular-weight organic acids on kinetics release and fractionation of phosphorus in some calcareous soils of western Iran. *Environmental Monitoring and Assessment*, 185 (7), 5471–5482.

Tiecher, T., Zafar, M., Mallmann, F.J.K., Bortoluzzi, E.C., Bender, M.A., Ciotti, L.H., and Santos, D.R. dos, 2014. Animal manure phosphorus characterization by sequential chemical fractionation, release kinetics and 31P-NMR analysis. *Revista Brasileira de Ciência do Solo*, 38 (5), 1506–1514.

Tuttobene, R., Avola, G., Gresta, F., and Abbate, V., 2009. Industrial orange waste as organic fertilizer in durum wheat. *Agronomy for Sustainable Development*, 29 (4), 557–563.

Vanzolini, J.I., Galantini, J.A., Martínez, J.M., and Suñer, L., 2017. Changes in soil pH and phosphorus availability during decomposition of cover crop residues. *Archives of Agronomy and Soil Science*, 63 (13), 1864–1874.

Violante, A. and Pigna, M., 2002. Competitive Sorption of Arsenate and Phosphate on Different Clay Minerals and Soils. *Soil Science Society of America Journal*, 66 (6), 1788–1796.

Violante, A., Pigna, M., Ricciardella, M., Gianfreda, L., Chimico-agrarie, D.S., and Federico, N., 2002. Adsorption of phosphate on variable charge minerals and soil as affected by organic and inorganic ligands. *Developments in Soil Science*, 28, 279–295.

Van Vuuren, D.P., Bouwman, A.F., and Beusen, A.H.W., 2010. Phosphorus demand for the 1970-2100 period: A scenario analysis of resource depletion. *Global Environmental Change*, 20 (3), 428–439.

Waldrip, H.M., He, Z., and Erich, M.S., 2011. Effects of poultry manure amendment on phosphorus uptake by ryegrass, soil phosphorus fractions and phosphatase activity. *Biology and Fertility of Soils*, 47 (4), 407–418.

Waldrip, H.M., He, Z., and Griffin, T.S., 2012. Effects of organic dairy manure on soil phosphatase activity, available soil phosphorus, and growth of Sorghum-Sudangrass.

Soil Science, 177 (11), 629–637.

Wang, B.L., Shen, J.B., Zhang, W.H., Zhang, F.S., and Neumann, G., 2007. Citrate exudation from white lupin induced by phosphorus deficiency differs from that induced by aluminum. *New Phytologist*, 176 (3), 581–589.

Wang, J.J., Li, X.Y., Zhu, A.N., Zhang, X.., H.W.Zhang, and Liang, W.., 2012. Effects of tillage and residue management on soil microbial communities in North China. *Plant Soil and Environment*, 58 (1), 28–33.

Wang, Y., Chen, X., Whalen, J.K., Cao, Y., Quan, Z., Lu, C., and Shi, Y., 2015. Kinetics of inorganic and organic phosphorus release influenced by low molecular weight organic acids in calcareous, neutral and acidic soils. *Journal of Plant Nutrition and Soil Science*, 178 (4), 555–566.

Whalen, J.K., Chang, C., Clayton, G.W., and Carefoot, J.P., 2000. Cattle Manure Amendments Can Increase the pH of Acid Soils. *Soil Science Society of America Journal*, 64 (3), 962–966.

Xu, J.M., Tang, C., and Chen, Z.L., 2006. The role of plant residues in pH change of acid soils differing in initial pH. *Soil Biology and Biochemistry*, 38 (4), 709–719.

Xu, R.K. and Coventry, D.R., 2003. Soil pH changes associated with lupin and wheat plant materials incorporated in a red-brown earth soil. *Plant and Soil*, 250 (1), 113–119.

Yan, F., Schubert, S., and Mengel, K., 1996. Soil pH increase due to biological decarboxylation of organic anions. *Soil Biology and Biochemistry*, 28 (4–5), 617–624.

Zema, D.A., Calabrò, P.S., Folino, A., Tamburino, V., Zappia, G., and Zimbone, S.M., 2018. Valorisation of citrus processing waste : A review. *Waste Management*, 80, 252–273.

Figure legends

Figure 1. Water extractable P content after lemon amendment (A, B) and mandarin (C, D) of incubation experiment for different incubation periods. Different letters indicate statistically significant differences ($p \leq 0.05$) lower case letters, between lemon or mandarin treatments; upper case letters, between soil and CM treatment for the same lemon or mandarin treatments. A different scale is used for data with CM amendment and a 1-h incubation period, and this is emphasised using hatched filling of the bars.

Figure 2. Changes in acid phosphatase activity in soil and CM-amended soil with addition of lemon (A, B, C) and mandarin (D, E, F) after three different incubation periods (1 hour, 1 week, 6 weeks). Different letters indicate statistically significant differences ($p \leq 0.05$) lower case letters, between lemon or mandarin treatments; upper case letters, between soil and CM treatment for the same lemon or mandarin treatments.

Figure 3. Water extractable P content from coarsely chopped lemon (A) and mandarin (B) incubation experiments, for each of the layers, from top to base. Different letters indicate statistical significant differences ($p \leq 0.05$). Lower case letters, between layers for the same soil; upper case letters, between soil and CM.

Table 1: Chemical properties of the Andisol Barros Arana from Southern Chile and beef cattle manure. Data are means ± SD of three replicates.

Parameters	**Values**	
	Barros Arana soil	**Beef cattle manure**
pH	5.58 ± 0.03	7.01 ± 0.01
Total carbon (%)	11.67 ± 0.19	41.06 ± 0.19
Total nitrogen (%)	0.93 ± 0.02	2.14 ± 0.04
C/N	12.5 ± 0.2	19.2 ± 0.5
Olsen P (mg kg^{-1})	6 ± 1	497 ± 10
Total P (mg kg^{-1})	1981 ± 11	3710 ± 26
K (mmol kg^{-1})	3.5 ± 0.1	45.2 ± 0.5
Na (mmol kg^{-1})	1.8 ± 0.1	49.6 ± 0.1
Ca (mmol kg^{-1})	40.7 ± 0.4	182 ± 0.8
Mg (mmol kg^{-1})	9.3 ± 0.1	67.5 ± 0.2
Al (mmol kg^{-1})	1.1 ± 0.1	1.2 ± 0.1

Soil and beef cattle manure data are presented on a dry matter basis.

Table 2.- Composition of peel and juice of lemon and mandarin. For water-extractable organic anions (citrate, oxalate and malate) were quantified by HPLC

Fruit	Part of fruit	Citrate	Oxalate	Succinate	Malate	P	C/N	pH
			g kg^{-1} of fresh mass			g kg^{-1} DW		
Lemon	Peel	8.07 ± 0.01	0.45 ± 0.00	0.10 ± 0.01	0.19 ± 0.00	0.97 ± 0.13	33.4 ± 0.9	2.86 ± 0.06
	Juice	21.0 ± 0.01	0.10 ± 0.00	0.01 ± 0.00	0.25 ± 0.00	-	-	1.92 ± 0.01
Mandarin	Peel	1.40 ± 0.001	0.16 ± 0.00	0.28 ± 0.01	3.60 ± 0.20	1.17 ± 0.02	27.0 ± 0.3	3.58 ± 0.02
	Juice	2.38 ± 0.001	0.05 ± 0.00	0.021 ± 0.00	0.93 ± 0.00	-	-	2.65 ± 0.02

Table 3.- Changes in soil pH recorded during incubation period after application of peel (P), juice(J) and whole fruit (WF) of lemon (L) or mandarin (M) to soils or soil amended with beef cattle manure (CM). Data are means ± SD of four replicates. Different lower case letters indicate significant differences among incubation times within the same lemon or mandarin treatment, whereas different capital letters indicate significant differences among lemon and mandarin treatments in the same incubation time at Duncan post-hoc test ($p \leq 0.05$).

Treatments				**pH**			
Incubation Period	**1h**	**1d**	**3d**	**1w**	**2w**	**4w**	**6w**
S	5.99 ± 0.03 aA	6.23 ± 0.05 aA	6.25 ± 0.07 aA	6.36 ± 0.04 aA	6.06 ± 0.02 aA	5.48 ± 0.02 bB	5.50 ± 0.01 bB
S,CM	6.23 ± 0.03 aA	6.24 ± 0.15 aA	6.32 ± 0.07 aA	5.93 ± 0.12 bB	6.20 ± 0.02 aA	6.04 ± 0.04 aA	6.15 ± 0.02 aA
				Lemon			
S, 20% LP	5.23 ± 0.04 cB	5.63 ± 0.03 bB	5.58 ± 0.01 bB	5.69 ± 0.07 bBC	6.01 ± 0.04 aA	6.31 ± 0.01 aA	6.26 ± 0.02 aA
S, 40%LP	5.06 ± 0.03 bC	5.40 ± 0.06 bB	5.21 ± 0.07 bBC	5.51 ± 0.02 bC	6.03 ± 0.03 aA	6.34 ± 0.01 aA	6.32 ± 0.03 aA
S, 40% LJ	4.28 ± 0.05 dD	4.76 ± 0.06 cC	5.02 ± 0.08 cC	5.52 ± 0.07 bC	6.26 ± 0.13 aA	6.07 ± 0.01 aA	5.65 ± 0.03 bB
S, 40% LWF	4.27 ± 0.02 cD	4.49 ± 0.05 cC	4.56 ± 0.04 cC	5.04 ± 0.13 bD	6.18 ± 0.04 aA	6.34 ± 0.02 aA	6.34 ± 0.04 aA
S, CM, 20% LP	5.87 ± 0.10 cB	5.56 ± 0.05 cB	5.98 ± 0.06 bA	6.02 ± 0.08 bB	6.38 ± 0.03 aA	6.28 ± 0.01 aA	6.24 ± 0.01 aA
S, CM, 40% LP	5.05 ± 0.12 cC	5.29 ± 0.08 bcB	5.49 ± 0.09 bB	5.74 ± 0.03 bBC	6.31 ± 0.03 aA	6.36 ± 0.02 aA	6.32 ± 0.05 aA
S, CM, 40% LJ	4.77 ± 0.05 cC	4.98 ± 0.06 cBC	5.49 ± 0.06 bB	5.91 ± 0.03 aB	6.34 ± 0.04 aA	6.14 ± 0.03 aA	6.16 ± 0.02 aA
S, CM, 40% LWF	4.28 ± 0.10 cD	4.64 ± 0.09 cC	4.70 ± 0.07 cC	5.46 ± 0.05 bC	6.17 ± 0.05 aA	6.26 ± 0.01 aA	6.29 ± 0.01 aA
				Mandarin			
S, 20% MP	5.73 ± 0.09 bB	5.61 ± 0.02 bB	5.14 ± 0.02 cC	4.92 ± 0.02 cD	5.69 ± 0.02 bB	6.21 ± 0.04 aA	5.95 ± 0.02 aA
S, 40% MP	5.49 ± 0.05 bB	5.48 ± 0.09 bB	4.73 ± 0.11 cC	4.88 ± 0.01 cD	5.65 ± 0.05 bB	6.17 ± 0.01 aA	5.99 ± 0.05 aA
S, 40% MJ	5.47 ± 0.05 bB	5.40 ± 0.08 bB	5.13 ± 0.23 bC	4.71 ± 0.01 cD	5.35 ± 0.04 bC	5.85 ± 0.01 aA	6.09 ± 0.03 aA
S, 40% MWF	5.35 ± 0.04 cB	5.22 ± 0.03 cB	5.27 ± 0.11 cC	5.18 ± 0.13 cD	5.78 ± 0.04 bB	6.14 ± 0.03 aA	6.08 ± 0.06 aA
S, CM, 20% MP	5.88 ± 0.08 bB	5.59 ± 0.08 cB	5.90 ± 0.07 bA	6.10 ± 0.02 abA	6.33 ± 0.04 aA	6.23 ± 0.01 aA	6.07 ± 0.02 aA
S, CM, 40% MP	5.79 ± 0.04 bB	5.29 ± 0.07 cB	5.73 ± 0.03 bB	5.62 ± 0.02 bBC	6.04 ± 0.08 aA	6.37 ± 0.03 aA	6.27 ± 0.02 aA
S, CM, 40% MJ	5.74 ± 0.05 bB	5.23 ± 0.01 cB	5.60 ± 0.12 bB	6.17 ± 0.03 aA	6.27 ± 0.04 aA	6.20 ± 0.01 aA	6.10 ± 0.02 aA
S, CM, 40% MWF	5.53 ± 0.01 bB	5.05 ± 0.04 cB	5.04 ± 0.04 cC	5.65 ± 0.02 bBC	5.90 ± 0.08 bB	6.33 ± 0.01 aA	6.28 ±0.01 aA

Table 4: Values of pH and phosphatase activity of the soil collected from three layers in pots of the second experiment. Different letters indicate statistical significant differences ($p \leq 0.05$). Lower case letters, between layers for the same soil; upper case letters, between soil and CM.

Treatment	Layer	pH	Phosphatase activity (nkatal PNP g soil^{-1})
S+L	A	5.40 ± 0.02 cB	1.49 ± 0.10 bA
	B	6.12 ± 0.06 aB	1.98 ± 0.06 aA
	C	5.79 ± 0.03 bB	2.00 ± 0.06 aA
S+CM+L	A	6.08 ± 0.06 cA	1.21 ± 0.05 cB
	B	6.57 ± 0.06 aA	2.08 ± 0.15 aA
	C	6.33 ± 0.01 bA	1.69 ± 0.07 bB
S+M	A	5.39 ± 0.04 bB	1.74 ± 0.09 abA
	B	5.96 ± 0.05 aB	1.88 ± 0.08 aA
	C	5.97 ± 0.02 aB	1.73 ± 0.06 bA
S+CM+M	A	6.06 ± 0.02 bA	0.99 ± 0.06 cB
	B	6.51 ± 0.10 aA	1.97 ± 0.05 aA
	C	6.08 ± 0.01 bA	1.43 ± 0.06 bB

L: lemon; CM: beef cattle manure; M: mandarin

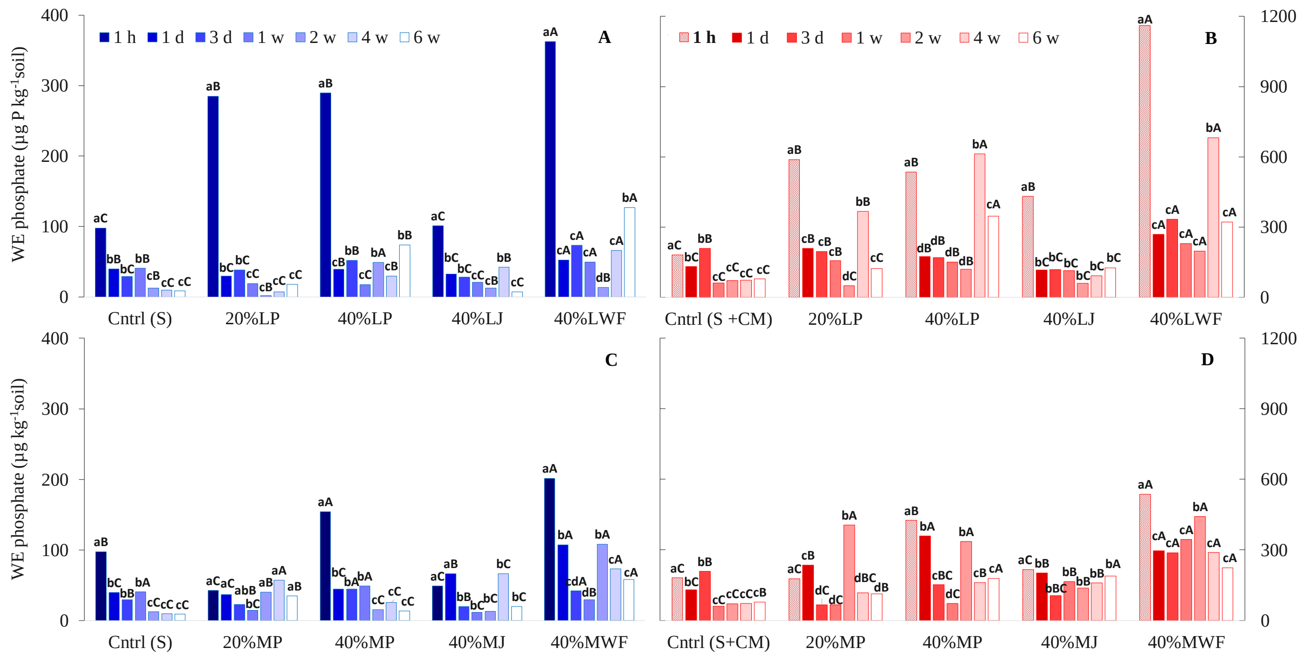

A
B
C
D
1 h
1 d
3 d
1 w
2 w
4 w
6 w
WE phosphate (µg P kg-1soil)
WE phosphate (µg kg-1soil)
0
100
200
300
400
0
300
600
900
1200
Cntrl (S)
20%LP
40%LP
40%LJ
40%LWF
Cntrl (S +CM)
20%LP
40%LP
40%LJ
40%LWF
Cntrl (S)
20%MP
40%MP
40%MJ
40%MWF
Cntrl (S+CM)
20%MP
40%MP
40%MJ
40%MWF

Figure 1

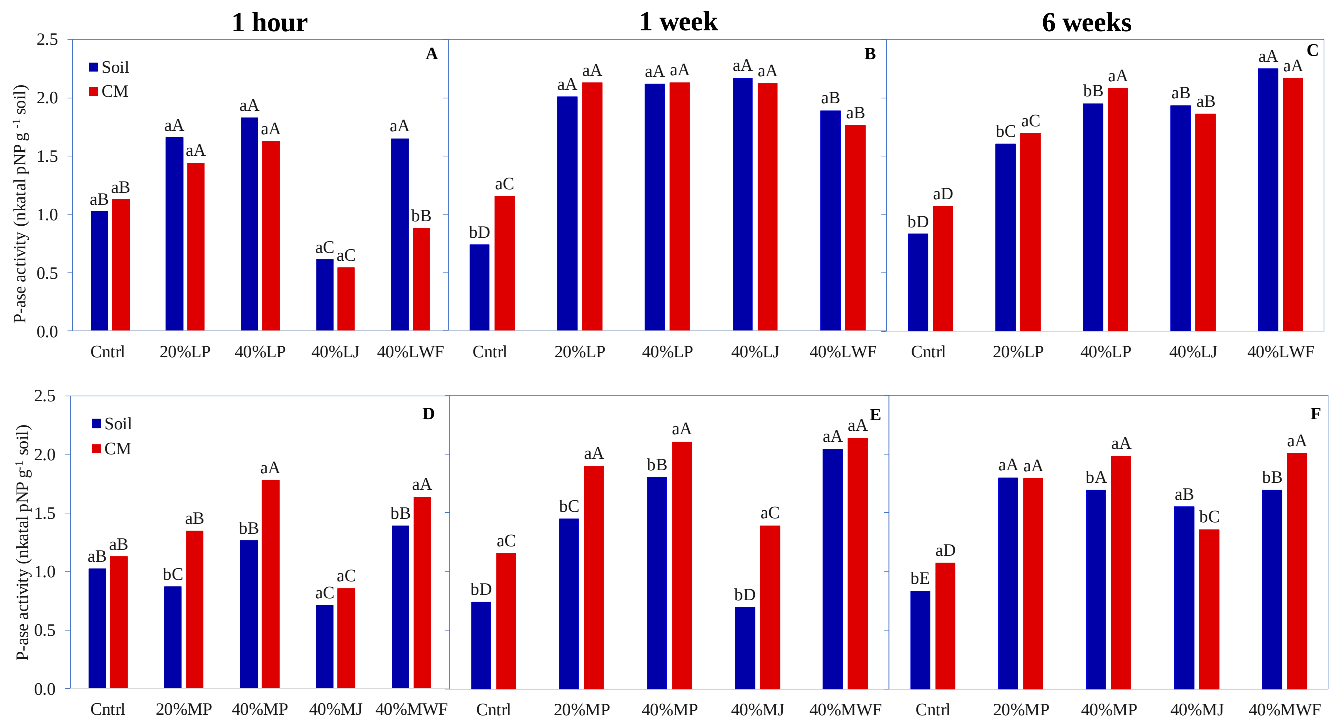

1 hour
1 week
6 weeks
P-ase activity (nkatal pNP g-1 soil)
Soil
CM
A
B
C
D
E
F
Cntrl
20%LP
40%LP
40%LJ
40%LWF
20%MP
40%MP
40%MJ
40%MWF
0.0
0.5
1.0
1.5
2.0
2.5

Figure 2

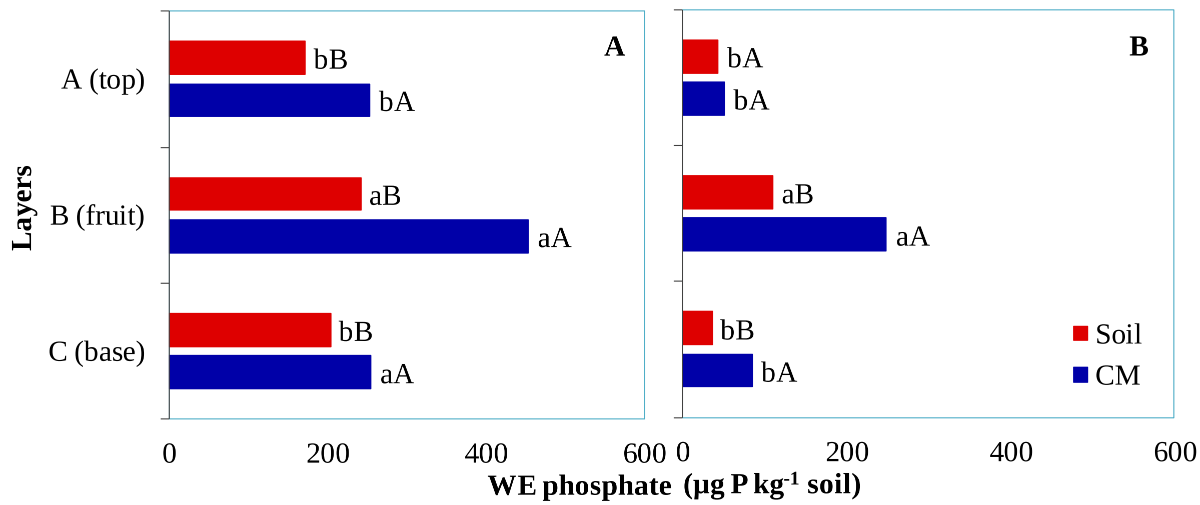

A
B
A (top)
B (fruit)
C (base)
Layers
bB
bA
aB
aA
bB
aA
bA
bA
aB
aA
bB
bA
Soil
CM
0
200
400
600
0
200
400
600
WE phosphate (µg P kg-1 soil)

Figure 3

Applied Soil Ecology

Assessment of the combined effects of beef cattle manure and lemon peel waste on soil-plant biochemical properties and phosphorus uptake by ryegrass

--Manuscript Draft--

Manuscript Number:	APSOIL-D-20-00825R1
Article Type:	Research Paper
Section/Category:	Microorganism-related Submissions
Keywords:	organic residues soil health plant nutrition oxidative stress
Corresponding Author:	Maria de la Luz Mora Universidad de la Frontera Temuco, CHILE
First Author:	Cecilia Paredes, PhD Student
Order of Authors:	Cecilia Paredes, PhD Student
	Siobhan Staunton
	Paola Durán
	Rodrigo Rodríguez
	Maria de la Luz Mora
Abstract:	Agro-industrial waste-derived amendments may be used as substitutes for synthetic fertilizers to improve soil physicochemical and biological properties and crop production. Microbial activity plays an essential role in the beneficial effects or organic amendments on soil quality. In this study, we investigated the combined effects of two different agro-industrially derived amendments, beef cattle manure (CM) and lemon peel (LP), on soil biological properties, plant nutrition and antioxidant responses in ryegrass to evaluate their putative use as organic phosphorus fertilizers. A combined amendment of CM+LP was more effective than each one used separately. For example, microbial biomass was more than 4 times that of the control. This can be largely attributed to the fungal community because the richness biodiversity index was greater than that of the bacterial and fungal biomass was greater than bacterial biomass. The bacterial biodiversity index of the treated soils was lower than that of the control but was less dominant and more changeable with soil chemical parameters such as Al and pH. Interestingly, ryegrass yield and phosphorus uptake significantly increased with the addition of combined CM+LP (43% and 44%, respectively), with respect to treatments with synthetic supertriple phosphate fertilizer (STP). Less oxidative stress was observed in treatments with CM+LP supplementation than in treatments with supertriple phosphate fertilizer. In conclusion, the combined application of CM+LP showed a beneficial effect on P uptake and oxidative stress with a concomitant increase in plant productivity, indicating that the combined amendment could be used as an organic fertilizer to improve the soil fertility and sustainability of agricultural production systems.
Suggested Reviewers:	Daniel Menezes-Blackburn Sultan Qaboos University danielblac@squ.edu.om
	Nanthi Bolan The University of Newcastle nanthi.bolan@newcastle.edu.au
	Zhongqi He USDA-ARS zhongqi.he@ars.usda.gov
Response to Reviewers:	Responses to Reviewers' comments:

Reviewer #1:
The topic of this work is interesting to researchers in relevant fields. The experiments were soundly fitting the goals. The authors presented their data quite straightforward. I have a few concerns on terminology and editorial consistency. Citation of a few more relevant papers may enhance your introduction and discussion, and so that greater impacts of your work.

1. L1. Title and main text. Can you be more specific of the type of cattle manure as beef manure or dairy manure?
ANSWER: DONE. Following the recommendations of Reviewer, we specify the type of cattle manure in the title and main text.

2. L55-56. (ODEPA 2019) and SAG 2006 are not listed in the references. Should double check all citations.
ANSWER: DONE. We apologize for this mistake. The references were included in line 554 and 585 and all citations were double checked

3. A recent book "Animal Manure: Production, Characteristics, Environmental Concerns and Management. ASA Special Publication 67. ASA and SSSA, Madison, WI. Contracted March 2017) may be citable at the beginning. And several chapters are specifically helpful for your discussion.
ANSWER: Thanks very much for literature provided, it certainly will strengthen our manuscript.

4. L54. Are you really citing statistics of "Livestock industry" or "Animal Industry"? Please note that "livestock" does not include "poultry". Refer to chapter 1 Animal manure production and utilization: Impact of modern concentrated animal feeding.
ANSWER: The statistics correspond to "Livestock industry", because it does not consider the poultry manure data. In addition, we cited the reference in L54

5. L56. 90% of phosphorus contained in cattle manure is inorganic" seems in conflict with the estimation/measurements in most research papers. Need more elaboration on the concern. Chapter 4 Nitrogen and phosphorus characteristics of beef and dairy manure.
ANSWER: DONE. A new sentence has been included in the introduction to clarify the estimation L56-59, making reference to the literature indicated by the reviewer

6. L336. The paper "Effects of poultry manure amendment on phosphorus uptake by ryegrass, soil phosphorus fractions and phosphatase activity. Biol Fertil Soils 47:407-418) may be helpful for a in=depth discussion of your green-house observations/data.
ANSWER: DONE. Thank you for making this suggestion. A new sentence has been included in the discussion to develop the understanding of the beneficial effects of organic amendments on P nutritional status of the plant. L385-388.

7. L373 and similar cases. Be consistent. (Fig 6) should be Fig. 6. In some places, Figure x is used, rather than Fig. X.
ANSWER: DONE. To be consistent, we homogenize as follow: Fig. X

Reviewer #2:
The Authors present a second manuscript on a very elegant idea well aligned with the circular economy and the need to revitalize soil biological activity. The experimental design accesses the effect of using manure, lemon peel and the combination of both treatments on soil dynamic properties. The effect of the combined treatment on soil respiration, and other soil biotic and abiotic parameters were determined. The results clearly show the advantages of using the combined manure and lemon peel to improve soil characteristics and nutrient balance. The manuscript is well written and has a good narrative. The results have potential to have a big impact on soil management.
ANSWER: Thanks for your review and we also very much appreciate your positive comments of our research.

Reviewer #3.
In this study, the authors of this research paper have investigated the combined effects of two different agro-industrially derived amendments, cattle manure (CM) and lemon peel (LP) on soil biological properties, plant nutrition, and antioxidant responses in ryegrass to evaluate their putative use as organic phosphorus fertilizers. The authors attempted to link soil microorganisms' role. Their activity in the assimilation of phosphorus by plants studied soil processes and indicators directly related to the assimilation of soil nutrients, including phosphorus.

1.There is no literature data on which groups of microorganisms are involved in the biodegradation of lemon peel and manure.
ANSWER. DONE. We added lines 76-82, highlighting the importance of cellulolytic and xylanolytic enzymes produced by the mesophilic microorganisms.

2. What types of bacteria and fungi are involved in the decomposition of agricultural residues like cattle manure and lemon peel have not been studied.
ANSWER. DONE. Please see lines 76-82

3. Indicating only the biodiversity index of microorganisms is not enough since you set the task to show the role of microorganisms in the biodegradation of organic residues.
ANSWER. Denaturing gradient gel electrophoresis (DGGE) is a rapid fingerprint analysis of microbial community composition, diversity, and dynamics. However, is not possible to identify the existence of groups microorganisms, but in our study allows to elucidate the effects of organic amendment addition emphasizing the role of changes in soil microbial activity (lines 85-86)

4. Why the low level of the biodiversity index of the fungal community must be explained, as you know, the role of the fungal community in the biodegradation of lemon peel is higher than that of bacteria. Fungi have a specific set of enzymes that decompose lemon peel.
ANSWER. DONE. We agree with the reviewer. According to our results, fungal community showed high richness indexes in treatments with organic amendment and considerable dominance relative that of the control. We clarified and explain about the specific set of enzymes that decompose lemon peel (lines 317-330). Moreover, a new sentence has been included in the conclusion highlighting the importance of fungal community in organic amendment decomposition (lines 415-416).

5.In the discussion, it would be necessary to cite the composition and role of autochthonous and zymogenic microflora in soil and manure and not the role of them.
ANSWER. DONE. We added lines 76 to 82

6. As the data in table 2 show, at the end of the experiment, within three months, there was a decrease in soil pH. This indicator can be considered unfavorable; plant roots can better absorb nutrients at a neutral pH value. Besides, at such pH values, the proportion of fungi, including pathogenic fungi, can increase.
ANSWER. DONE. We clarified this important point that you suggest. In order to this we added lines 373-375 indicating that the incorporation of organic amendment the major biodiversity of fungi microbial community increase of beneficial microorganisms due to when plants were grown in LP and CM treatments, the amount of TBARS decreased 1.3 fold.

Finally, we want to thank the reviewers for all recommendations and suggestions, which have greatly enhanced our manuscript.

Temuco, Chile, April 2021

Dra. Judith Ascher-Jenull
Editor-in-Chief
Applied Soil Ecology

Dear Dra. Judith,

Please find attached the manuscript titled: "Assessment of the combined effects of beef cattle manure and lemon peel waste on soil-plant biochemical properties and phosphorus uptake by ryegrass" by Cecilia Paredes, Siobhan Staunton, Paola Durán, Rodrigo Rodríguez and María de la Luz Mora, to be evaluated for publication in Applied Soil Ecology.

All the Editor suggestions, as well as those proposed by the Reviewers 1 and 3, were considered in the revised version of the manuscript. A correction list indicating all the changes and corrections that we had made are included in this new submission.

We hope that our revised manuscript receives a good reception in Applied Soil Ecology.

Kind regards,

Dra. María de la Luz Mora
Universidad de La Frontera
Temuco, Chile.
Email: mariluz.mora@ufrontera.cl
Phone: +56 (45) 2325450

Responses to Reviewers' comments:

Reviewer #1:

The topic of this work is interesting to researchers in relevant fields. The experiments were soundly fitting the goals. The authors presented their data quite straightforward. I have a few concerns on terminology and editorial consistency. Citation of a few more relevant papers may enhance your introduction and discussion, and so that greater impacts of your work.

1. L1. Title and main text. Can you be more specific of the type of cattle manure as beef manure or dairy manure?

ANSWER: DONE. Following the recommendations of Reviewer, we specify the type of cattle manure in the title and main text**.**

2. L55-56. (ODEPA 2019) and SAG 2006 are not listed in the references. Should double check all citations.

ANSWER: DONE. We apologize for this mistake. The references were included in line 554 and 585 and all citations were double checked

3. A recent book "Animal Manure: Production, Characteristics, Environmental Concerns and Management. ASA Special Publication 67. ASA and SSSA, Madison, WI. Contracted March 2017) may be citable at the beginning. And several chapters are specifically helpful for your discussion.

ANSWER: Thanks very much for literature provided, it certainly will strengthen our manuscript.

4. L54. Are you really citing statistics of "Livestock industry" or "Animal Industry"? Please note that "livestock" does not include "poultry". Refer to chapter 1 Animal manure production and utilization: Impact of modern concentrated animal feeding.

ANSWER: The statistics correspond to "Livestock industry", because it does not consider the poultry manure data. In addition, we cited the reference in L54

5. L56. 90% of phosphorus contained in cattle manure is inorganic" seems in conflict with the estimation/measurements in most research papers. Need more elaboration on the concern. Chapter 4 Nitrogen and phosphorus characteristics of beef and dairy manure.

ANSWER: DONE. A new sentence has been included in the introduction to clarify the estimation L56-59, making reference to the literature indicated by the reviewer

6. L336. The paper "Effects of poultry manure amendment on phosphorus uptake by ryegrass, soil phosphorus fractions and phosphatase activity. Biol Fertil Soils 47:407-418) may be helpful for a in=depth discussion of your green-house observations/data.

ANSWER: DONE. Thank you for making this suggestion. A new sentence has been included in the discussion to develop the understanding of the beneficial effects of organic amendments on P nutritional status of the plant. L385-388.

7. L373 and similar cases. Be consistent. (Fig 6) should be Fig. 6. In some places, Figure x is used, rather than Fig. X.

ANSWER: DONE. To be consistent, we homogenize as follow: Fig. X

Reviewer #2:

The Authors present a second manuscript on a very elegant idea well aligned with the circular economy and the need to revitalize soil biological activity. The experimental design accesses the effect of using manure, lemon peel and the combination of both treatments on soil dynamic properties. The effect of the combined treatment on soil respiration, and other soil biotic and abiotic parameters were determined. The results clearly show the advantages of using the combined manure and lemon peel to improve soil characteristics and nutrient balance. The manuscript is well written and has a good narrative. The results have potential to have a big impact on soil management.

ANSWER: Thanks for your review and we also very much appreciate your positive comments of our research.

Reviewer #3.

In this study, the authors of this research paper have investigated the combined effects of two different agro-industrially derived amendments, cattle manure (CM) and lemon peel (LP) on soil biological properties, plant nutrition, and antioxidant responses in ryegrass to evaluate their putative use as organic phosphorus fertilizers. The authors attempted to link soil microorganisms' role. Their activity in the assimilation of phosphorus by plants studied soil processes and indicators directly related to the assimilation of soil nutrients, including phosphorus.

1.There is no literature data on which groups of microorganisms are involved in the biodegradation of lemon peel and manure.

ANSWER. DONE. We added lines 76-82, highlighting the importance of cellulolytic and xylanolytic enzymes produced by the mesophilic microorganisms.

2. What types of bacteria and fungi are involved in the decomposition of agricultural residues like cattle manure and lemon peel have not been studied.

ANSWER. DONE. Please see lines 76-82

3. Indicating only the biodiversity index of microorganisms is not enough since you set the task to show the role of microorganisms in the biodegradation of organic residues.

ANSWER. Denaturing gradient gel electrophoresis (DGGE) is a rapid fingerprint analysis of microbial community composition, diversity, and dynamics. However, is not possible to identify the existence of groups microorganisms, but in our study allows to elucidate the effects of organic amendment addition emphasizing the role of changes in soil microbial activity (lines 85-86)

4. Why the low level of the biodiversity index of the fungal community must be explained, as you know, the role of the fungal community in the biodegradation of lemon peel is higher than that of bacteria. Fungi have a specific set of enzymes that decompose lemon peel.

ANSWER. DONE. We agree with the reviewer. According to our results, fungal community showed high richness indexes in treatments with organic amendment and considerable dominance relative that of the control. We clarified and explain about the specific set of enzymes that decompose lemon peel (lines 317-330). Moreover, a new sentence has been included in the conclusion highlighting the importance of fungal community in organic amendment decomposition (lines 415-416).

5.In the discussion, it would be necessary to cite the composition and role of autochthonous and zymogenic microflora in soil and manure and not the role of them.

ANSWER. DONE. We added lines 76 to 82

6. As the data in table 2 show, at the end of the experiment, within three months, there was a decrease in soil pH. This indicator can be considered unfavorable; plant roots can better absorb nutrients at a neutral pH value. Besides, at such pH values, the proportion of fungi, including pathogenic fungi, can increase.

ANSWER. DONE. We clarified this important point that you suggest. In order to this we added lines 373-375 indicating that the incorporation of organic amendment the major biodiversity of fungi microbial community increase of beneficial microorganisms due to when plants were grown in LP and CM treatments, the amount of TBARS decreased 1.3 fold.

Finally, we want to thank the reviewers for all recommendations and suggestions, which have greatly enhanced our manuscript.

Highlights

- **Cattle manure and lemon peel had synergistic beneficial effects on soil quality**
- **Organic amendment enhanced fungal abundance and activity**
- **Organic amendments led to lasting liming effect and enhanced available P**
- **Lemon peel enhanced respiration more than cattle manure**
- **Lemon Peel and manure had a protective effect on plant oxidative stress**

Assessment of the combined effects of beef cattle manure and lemon peel waste on soil-plant biochemical properties and phosphorus uptake by ryegrass

Cecilia Paredes[1], Siobhan Staunton[2], Paola Durán[3,4], Rodrigo Rodríguez[1,4] and María de la Luz Mora[3*]

[1]Programa de Doctorado en Ciencias de Recursos Naturales, Universidad de La Frontera, Temuco, Chile.

[2]INRAE, UMR Eco&Sols, INRA-IRD-Cirad-SupAgro-Université de Montpellier, Place Viala, 34060 Montpellier, France.

[3]Center of Plant, Soil Interaction and Natural Resources Biotechnology, Scientific and Biotechnological Bioresource Nucleus, Universidad de La Frontera, Temuco, Chile.

[4]Biocontrol Research Laboratory, Universidad de La Frontera, Temuco, Chile.

***Correspondence:**

María de la Luz Mora

E-mail: mariluz.mora@ufrontera.cl

Abstract

Agro-industrial waste-derived amendments may be used as substitutes for synthetic fertilizers to improve soil physicochemical and biological properties and crop production. Microbial activity plays an essential role in the beneficial effects or organic amendments on soil quality. In this study, we investigated the combined effects of two different agro-industrially derived amendments, beef cattle manure (CM) and lemon peel (LP), on soil biological properties, plant nutrition and antioxidant responses in ryegrass to evaluate their putative use as organic phosphorus fertilizers. A combined amendment of CM+LP was more effective than each one used separately. For example, microbial biomass was more than 4 times that of the control. This can be largely attributed to the fungal community because the richness biodiversity index was greater than that of the bacterial and fungal biomass was greater than bacterial biomass. The bacterial biodiversity index of the treated soils was lower than that of the control but was less dominant and more changeable with soil chemical parameters such as Al and pH. Interestingly, ryegrass yield and phosphorus uptake significantly increased with the addition of combined CM+LP (43% and 44%, respectively), with respect to treatments with synthetic supertriple phosphate fertilizer (STP). Less oxidative stress was observed in treatments with CM+LP supplementation than in treatments with supertriple phosphate fertilizer. In conclusion, the combined application of CM+LP showed a beneficial effect on P uptake and oxidative stress with a concomitant increase in plant productivity, indicating that the combined amendment could be used as an organic fertilizer to improve the soil fertility and sustainability of agricultural production systems.

Keywords: organic residues; soil health; plant nutrition; oxidative stress

1. Introduction

Currently, worldwide, awareness is rising on the reuse, recovery and recycling of materials from the agricultural industry due to their ability to improve soil physical, biological and chemical characteristics and to supply an optimal combination of macro- and micronutrients for crops (Cercioglu, 2017; Diacono and Montemurro, 2010; Gopinath et al., 2008). In particular, the phosphorus scarcity has promoted changes in agricultural processes, since both prices and demand to meet food security needs, have increased since the mid-20th century (Neset et al., 2016). The use of organic fertilizers, including crop residues, cattle manure and biochar, can reduce the dependence on inorganic fertilizers. Furthermore, the sustainable management of wastes reduces waste levels and is a strategy of the circular economy, encouraged by the European Commission through its "Circular Economy Action Plan" (European Commission, 2015).

Livestock industry (Pagliari et al., 2020) is a major activity in southern Chile, accounting for 79% of the national total head of livestock (ODEPA 2019), with an annual production of 42 million tons of manure (SAG 2006). In addition, the labile P fraction (H_2O-P and $NaHCO_3$-P) has been reported to vary between 53% and 88% in cattle manure (P. H. Pagliari et al., 2020). This fraction is particularly important because it is easily available for plant growth. Hence, is the principal reason for use cattle manure as a source of P to compensate the scarcity of phosphate rock (Cordell and Neset, 2014). This is especially important in soils where P is a limiting factor for plant production, as is the case for Andisol soils. In southern Chile, approximately 60% of agricultural soils are classed as Andisols, and are characterized by low pH and low available P concentrations, despite high total P levels (Borie et al., 1989; Mora et al., 2017; Redel et al., 2016).

It is known that organic anions compete with phosphate on the soil anion exchange complex and this is one of the reasons that plant roots exudate organic acids to compensate poor P nutrition, thus mobilizing phosphate from the soil, making it available for plant uptake (Waithaisong et al., 2015). We recently demonstrated that phosphate solubility is enhanced by combining CM and citrus residues as an amendment for acid soil. That study focused on the chemical effect of the citrus amendment, namely their high organic anion contents, mainly citrate and malate, with smaller amounts of oxalate and succinate. Sustained (6-week) increases in water extractable phosphorus, phosphatase activity and pH were reported when citrus residues were applied alone or in combination with beef cattle manure (CM) in an Andisol (Paredes et al., 2021). These results suggest that the use of these residues used in combination may be a strategy to improve soil quality (liming effects and hence Al toxicity) along with improving soil P availability. On the other hand, the activity of microorganisms (procaryotes and fungi) is essential for degradation of organic amendment and nutrient release for plant uptake, either by allochthonous microbes, including the addition of pure cultures, soil transplanting, and microorganisms residing in organic amendment or by stimulating key resident autochthonous microorganisms (Fuentes et al., 2009; Liu et al., 2020; Paula et al., 2020; Wang et al., 2019). Thus, their persistence and variation has been influenced by C/N ratio, pectin degradation rate and temperature (Wang et al., 2019). Understanding the dynamics of microbial communities in these systems is, therefore, desirable for designing successful management strategies aiming to optimize nutrient availability and improve plant productivity. The aim of this study is to elucidate these effects emphasizing the role of changes in soil microbial activity. We adopted a two-prong approach. The first approach was to determine the effects of these organic residues (CM and LP) on the soil microbial community, assessed by microbial biomass C, N and P, and respiration. The second approach was to evaluate the improvement of soil quality for plant growth by measuring soil available nutrient status, plant phosphate uptake, and antioxidant responses in ryegrass grown in a greenhouse.

2. Materials and methods

2.1 Sampling and chemical characterization of soil and CM samples

The soil used for the experiment was an Andisol collected from the Barros Arana locality of southern Chile located at 39°06'12"S, 72°37'42"W. The soil has a silt loam texture and is classified as an Andosol (IUSS Working Group, 2014) or Typic Hapludand (Soil Survey Staff, 2014). The soil was collected from 0 to 20 cm depth after removing a thin layer of surface litter, air dried and sieved <2 mm. Semi-fresh CM was collected from Santa Elena Farm located in the same locality.

The samples were air-dried for 5 days, milled and sieved through a 2-mm sieve for subsequent analysis. Soil and CM, and soil after composting were characterized using routine methods according to Sadzawka et al. (2006). The chemical analysis of composted soil was carried out according to Sadzawka et al. (2006). pH was determined in H_2O with a 1:2.5 soil sample:water solution ratio. Total C and N were determined by dry combustion using a CHN autoanalyzer (CHN NA 1500, Carlo Erba). The available P was extracted with sodium bicarbonate (0.5 M $NaHCO_3$ at pH 8.50) using the Olsen method and quantified spectrophotometrically at 880 nm by the methylene blue complex method (Murphy & Riley, 1962). Basic exchangeable cations (Ca, Mg, Na and K) were extracted with 1 M ammonium acetate (pH 7) and determined by atomic absorption spectrophotometry (AAS). Exchangeable Al was extracted with 1 M potassium chloride and determined by EAA.

2.2 Characterization of lemon peel (LP)

The peels of the squeezed fruit samples were cut into small pieces. Ten grams of peel was placed in a flask with 25 ml of ultrapure water, shaken for one hour and membrane filtered. Extracts were analyzed by HPLC with a Shimadzu LC 20 DAD SPD-M20A detector. Reversed-phase separations were carried out using a 4.6 x 250 mm, 5 µm Symmetry C18 column. For chemical analysis, LP peel was cut into small pieces and dried at 65 °C for 48 h according to Sadzawka et al. (2004). In addition, total carbon and nitrogen were determined using an elemental analyzer (CHN NA1500; Carlo Erba Elemental Analyzer, Stanford, CA, USA).

2.3 Incubation experiment

Soil and amendments were incubated under controlled conditions. CM was applied at a rate of 5%, and LP was applied at a rate of 40%. The experiment included the following treatments: soil + CM; soil + LP; soil + CM + LP; soil without amendment was used as a control (Control). There were three replicates for each of the treatments. Organic materials were thoroughly mixed with air-dried soil samples and placed in plastic bags with small perforations. Soils were maintained at a water content of 70% of field capacity at 25 °C. Respiration was monitored for one month (see below) and at the end of this period, soil microbial biomass (C, N and P) and of the composition of soil microbial community were assayed. Incubation was continued for 3 months prior to the greenhouse experiment, and for clarity this soil is referred to as composted soil. 100-g samples were used for the shorter incubation experiment and 600-g for the longer composting prior to the pot experiment. The bags were mixed several times during the process to promote decomposition of organic matter.

2.3.1 Soil microbial C, N and P

Microbial biomass C and N were determined by the fumigation–extraction method using ethanol-free $CHCl_3$ and 50 mM K_2SO_4 as an extractant (Vance et al., 1987), whereas microbial biomass P was determined according to Brookes et al. (1982). K_2SO_4-extractable C was quantified using an automated combustion total organic C (TOC) analyzer (TOC-V

CPH Shimdzu, Japan), and the difference in C content between fumigated and nonfumigated samples was corrected using a KEC factor (the percentage of total microbial C extracted by K_2SO_4) of 0.45 to estimate SMB-C (Wu et al., 1990). N contents of fumigated and unfumigated soil extracts were determined using Kjeldahl digestion and MBN was calculated as the difference in NO_3^- content between fumigated and nonfumigated samples using an extraction efficiency factor (KEN) of 0.54 (Brookes et al., 1985). SMB-P was determined according to Brookes et al. (1982). The microbial biomass P (SMB-P) was calculated as the difference in P content between fumigated and nonfumigated samples using an extraction efficiency factor (KEN) of 0.40 accounting for the efficiency of Pi extraction from lysed microbial cells (Brookes et al. 1982); in addition, the calculation took into account P recovery measured as the proportion of the P spike recovered in each nonfumigated soil sample.

2.3.2 Analysis of Microbial Community composition by PCR-DGGE

The microbial community composition of the soil was evaluated at the end of the incubation experiments (30 d) by DGGE using specific primer sets for bacteria and fungi. Total DNA from soil was extracted using a Power Soil DNA Isolation Kit (QIAGEN, United States) according to the manufacturer's instructions. DNA was quantified, and its purity was evaluated using the A260/A280 and A260/A230 ratios provided by MultiskanTM GO software. Then, fragments of the 16S rRNA gene were amplified by touch-down polymerase chain reaction (PCR) with primer set EUBf933-GC (5' -GCA CAA GCG GTG GAG CAT GTG G G-3')/EUBr1387 (5' -GCC CGG GAA CGT ATT CAC CG-3') (Iwamoto et al). For fungal community analysis, fragments of the 18S rRNA gene were amplified by nested PCR. First, fragments were obtained by touchdown PCR using primer set NS1 (5′-GTA GTC ATA TGC TTG TCT C-3′)/NS8 (5′-TCC GCA GGT TCA CCT ACG GA-3′) followed by a second

PCR with primer sets NS7-GC (5′-GAG GCA ATA ACA GGT CTG TGA TGC-3, GC-clamp: CGC CCG GGG CGC GCC CCG GGC GGG GCG GGG GCA CGG GGG)/F1Ra (5′-CTT TTA CTT CCT CTA AAT GAC C 3. All PCR amplifications were carried out with reagents supplied with GoTaq® Flexi DNA Polymerase (Promega, Co. Madison, WI, USA) (Durán et al., 2019).

DGGE analysis was performed using a DCode system (Bio-Rad Laboratories, Inc.). Twenty-five microliters of PCR product were loaded onto a 6% (w/v) polyacrylamide gel with a 40–70% gradient (urea and formamide). The electrophoresis was run for 16 h at 75 V. The gel was then stained with SYBR Gold (Molecular Probes, Invitrogen Co.) for 30 min and photographed on a UV transilluminator.

2.4 Microbial respiration

Microbial respiration was measured based on the alkali absorption of CO_2 at 25 °C for 30 days. Soil (control) or amended with CM (5%), LP (40%) or a mixture of both (as for the incubation experiments) was placed (20 g) in a 1 L flask with a vial containing 10 mL of 0.25 N NaOH and a vial containing 10 mL distilled water to maintain a humid atmosphere. The flask was hermetically sealed and incubated at 25 °C. Vials containing NaOH were periodically replaced. The CO_2 captured in the vial with NaOH solution was determined by titrating the remaining alkali with 0.25 N HCl after the precipitation of carbonate with 0.1 mol L^{-1} $BaCl_2$ (Iannotti et al., 1994).

2.5 Greenhouse experiment

A greenhouse assay was carried out using the composted soil (3-month incubation as above). In addition to the control, (SC), CM, LP and CM+LP treatments, triple superphosphate (TSP) addition was included as a chemical control. The amount of TSP added was equal to the measured increase in Olsen P after CM+LP amendment and 3-month composting. The TSP was milled and mixed with the soil prior to sowing the ryegrass seeds. Seventy seeds of ryegrass (*Lolium perenne* L.) cv. Nui were sown in each pot containing 0.6 kg of soil. After germination, the assay was thinned to 60 seedlings per pot, and 50 mg N kg^{-1} of soil (as urea) was applied to the SC and STP treatments, whereas 30 mg N kg^{-1} of soil was applied to the LP, CM and CM+LP treatments. Three pots were used as replicates for each treatment. During the growth period, the plants were watered daily with distilled water. Harvesting was carried out after 30 days once the shoot biomass of the TSP and CM treatments had reached 30 cm in plant height. The fresh shoots and roots were collected. Roots were washed first with tap water, then with distilled water to remove adhering soil. Plant material was stored at -20 or -80 °C for biochemical analyses, or dried at 65 °C for 48 h to determine dry weight (DW) and chemical parameters.

2.5.2 Plant chemical analyses

The dried shoot and root tissues were ground to a fine powder, ashed in a muffle furnace at 500 °C for 8 h, digested with 2 M HCl and paper filtered (Whatman WHAT99-292-125). In the extract, P, Al, Ca, Mg, K and microelements were determined according to Sadzawka et al. (2004). The P concentration was measured colorimetrically using the molybdovanadate method. Al, Ca, Mg, K and microelements were measured by flame atomic absorption spectrophotometry FAAS. Moreover, total C and N were determined using an elemental analyzer (CHN NA1500; Carlo Erba Elemental Analyzer, Stanford, CA, USA).

2.5.3 Lipid peroxidation assay

The level of lipid peroxidation products was used as an oxidative stress indicator. In fresh shoot material, lipid peroxidation was measured using the thiobarbituric acid reacting substances (TBARS) assay according to Du and Bramlage (1992). The absorbance was measured at 532, 600 and 440 nm in order to correct for the interference generated by TBARS-sugar complexes, and the results were expressed in nmol of malondialdehyde (MDA) per g^{-1} of fresh weight.

2.5.4 Superoxide dismutase activity

The activity of superoxide dismutase (SOD; EC. 1.15.1.1) was assessed since this enzyme represent the first barrier of protection from oxidative damage (Yu and Rengel, 1999). The total SOD was determined by measuring the photochemical reduction of nitroblue tetrazolium (NBT) according to Donahue et al. (1997). The amount of enzyme was defined as a 50% inhibition of the NBT reduction corresponding to one SOD unit, and enzyme activity was expressed on both a fresh weight and protein basis. The amount of protein in the crude enzyme extract was measured spectrophotometrically using bovine serum albumin (BSA) as the standard following the method developed by Bradford (1976).

2.5 Statistical analysis

Data were analyzed using a one-way ANOVA followed by Tukey's post hoc procedure. Different letters were used to display post hoc differences. The DGGE banding profile were clustered as a dendrogram by using Phoretix 1D analysis software (Clarke, 1993) (TotalLab Ltd., United Kingdom). In silico analysis was also used to estimate bacterial and fungal diversity by richness (S), the Shannon–Wiener index, and dominance by the Simpson index (D), represented by 1-D or 1-λ (Sagar and Sharma, 2012). Data normality of microbial community composition was analyzed according to Kolmogorov's test. Similarities between bacterial communities were visualized in PCO using Primer 7 software (Primer-E Ltd., Ivybridge, United Kingdom). Values are given as means ± standard errors. Differences were considered significant when the P-value was lower than or equal to 0.01.

3. Results

The composition of the initial soil, LP and CM are given in Table 1.

3.1 Incubation experiment

The cumulative CO_2 flux was significantly elevated in all amendments relative to the control, with LP having a larger effect than CM (Fig. 1). The production of CO_2 increased from 62 μg C-CO_2 g soil^{-1}, representing the basal respiration of the control soil, to 254 μg C-CO_2 g soil^{-1} in the CM+LP treatment (4-fold increase) on the first day. The peak was reached after 5 days in the soils treated with CM+LP (inset to Fig. 1). Values measured at the end of incubation decreased, approaching to control levels.

Values of SMB-C, SMB-N and SMB-P were higher in soil amended with CM and LP together than in the other treatments (Fig. 2). SMB-C was significantly increased by both CM and LP, and their effects are additive leading to a more than 4-fold relative to that of the control soil (Fig. 2A). SMB-N was decreased by CM and no significant effected of LP alone was observed, however in combination there was a synergistic effect resulting in an increase from 79 mg kg^{-1} to 221 mg kg^{-1} relative to the control soil (Fig. 2B). The CM amendment led to a 3-fold increase in SMB-P (Fig. 2C), and the combined addition of LP enhanced this increase 2-fold, although alone it had no significant effect.

In Fig. 3 it shows the effects of treatments on the bacterial community composition as revealed by the DGGE analysis. Both the dendrogram and PCA show that treatments involving LP (both LP and CM+LP) were grouped separately with respect to treatments without LP (control and CM), showing different community composition at distance=3. However, the biodiversity index (measuring richness and dominance), showed that treatments with organic amendment showed less richness of species (S) and individuals (N) than the control but were less dominant (1- λ) (Supplementary Fig. 1). When we compared the richness index to chemical properties, we found an inverse correlation with pH and corresponding positive relations with exchangeable Al content (Supplementary Fig. 2). In contrast, dominance followed an inverse trend (less dominance with a low Al and high pH).

Fungal community compositions were not related to amendments, with all treatments grouped together except in the case of CM+LP (Fig. 4). This treatment showed considerable levels of diversity and dominance relative to the rest of the treatments, but the results were not correlated with chemical properties.

3.3 Greenhouse experiments

The content of available nutrients was significantly modified three months after the addition of CM and LP to the soil. There was a significant increase in Olsen P, and exchangeable cation (Ca, Mg, Na, and K) contents and pH (Table 2). The content of Olsen P increased 1.5-fold when LP was applied, whereas that of soil treated with LP and CM increased 2.6-fold relative to the soil control. Exchangeable Ca, Mg, Na and K contents were significantly higher ($P < 0.05$, Tukey test) when LP and CM had been applied. There was no effect of amendments on N and C concentrations for either treatment. pH was significantly influenced by CM or LP, but the combined effect of CM+LP increased pH by 0.8 units compared to the control soil.

The application of CM and CM+LP resulted in 39 and 44% higher ryegrass yields than those of the soil control, respectively (Fig. 5A). Interestingly, the same yield was obtained with the CM+LP treatment and chemical fertilizer (STP). The treatments involving organic amendments increased shoot P content by 25% with CM and 43% with CM+LP, while mineral fertilizer treatment (STP) increased by 32% relative to the soil control. However, no significant differences were observed in the amount of P in shoots with both treatments (CM and CM+LP) relative to the STP treatment, whose values were 0.92, 0.95 and 0.99 g pot^{-1}, respectively (Fig. 5B). In addition, macronutrients were significantly higher in the treatment that received STP than in the control (Table 4). In contrast, the addition of LP significantly decreased values of Ca (51%), Mg (38%) and Al (70%). Although there were few differences in the macronutrient levels in shoots from the CM+LP treatment relative to the control, significant differences were observed in the K uptake, which increased 66%, whereas those of Ca and Al decreased by 12 and 49%, respectively (Table 3).

Lipid peroxidation in shoot showed a significant decrease in response to organic amendment applied (Fig. 6A). When plants were grown in LP and CM treatments, the amount of TBARS decreased 1.3 fold, while those of CM+LP and STP decreased 1.7 fold. There was no significant difference in the amount of TBARS in CM+LP treatment in comparison to mineral fertilizer (STP treatment). Meanwhile, regarding the enzymatic antioxidant activity, a differential SOD activity in response to organic amendments applied was observed in shoot (Fig. 6B). Control shoots exhibited the highest SOD activity compared to the other treatments. The application of LP caused a decrease in SOD activity by 6% in comparison to the control. While, when CM and CM+LP were applied, SOD activity was significantly reduced by 20 %. However, there was no significant difference in SOD activity among the CM, CM+LP and STP treatments.

4. DISCUSSION

4.1 Incubation experiment

The addition of organic amendments such as CM and LP increased microbial activity as determined by CO_2 evolution and microbial biomass, probably due to the stimulation of microbial activity and growth as CM and LP were bio-degraded, providing nutrients and readily available C sources. The analysis of the microbial community composition by DGGE showed the greater influence of the fungal rather than the bacterial community, since that fungal community showed high richness indexes in treatments with organic amendment and considerable dominance relative that of the control. This is in agreement with the findings of Paula et al. (2020) who report that fungal activity was enhanced on addition of an organic amendment (spent mushroom substrate) in a 14-week incubation and could be attributed to the important role of pectinolytic, cellulolytic and xylanolytic enzymes by the mesophilic fungi which had greater success in soil colonization (Dhillon et al., 2004; Mamma et al., 2008; Paula et al., 2020). On the other hand, fungal community were more stable in considering chemical soil parameters evolution. This greater stability of fungal composition than that of bacteria have been observed by other studies (Barnard et al., 2013; Durán et al., 2018). Respect to bacterial community of the amended soils were less dominant but showed a lower richness index than in the control soil, and was more influenced than the fungal community considering the influence of soil parameters evolution.

It is well established that organic soil amendments can release nutrients for crop uptake and increase soil organic matter content, thereby influencing soil biological properties and organic matter fractions (Nguyen and Marschner, 2016). Chu et al. (2007) observed in a field experiment that organic manure had a significantly greater impact on microbial biomass

C and microbial activity than mineral fertilizers, while microbial metabolic activity was significantly higher under balanced fertilization than under nutrient-deficient fertilization. Soil microorganisms can survive in the soil even in an inactive status but they react immediately to residue addition (Gatiboni et al., 2011).

SMB was more strongly affected by the combined CM+LP than by each treatment alone. Significant differences among all treatments suggest that CM and LP amendments had a considerably positive effect on the size and activity of the microbial community. Soil microbial biomass has been used as an indicator for ecosystem nutrient limitations (Güsewell, 2004; Xu et al., 2013), since soil microbes are more efficient in taking up nutrients than plants (Bardgett et al., 2003). In nutrient-depleted ecosystems, the relatively high fraction of specific nutrients in microbial biomass implies strong limitations of this element for plants (Jonasson et al., 1999).

4.2 Greenhouse experiments

Generally, treatments improved soil nutrient status. In particular, Olsen P levels increased by 11% in soil treated with LP peel and 73% when CM+LP was applied in combination. Similarly, exchangeable cations (Ca, Mg, Na and K) significantly increased following the addition of these residues to the soil (Table 2). Organic residues have been reported to increase P availability in soils with high P retention (Guppy et al., 2005), probably due to the release of byproducts of residue decomposition, such as low-molecular-weight (LMW) organic acids. Organic oxyanions compete for soil P sorption sites, resulting in increased soil P availability (Kang et al., 2009; Yu et al., 2013). Moreover, organic amendments increase soil fertility due to the addition of soluble base cations, which are released from the mineralization of organic residues. In this case, both residues are an essential source of cations; in particular, CM contributed Ca and Mg (Table 1), whereas LP released K and organic acids (Table 1). Liu et al. (2012) and Hargreaves et al. (2008) reported an increase in cation exchange capacity when composting at different rates was applied to soil. In addition, Cellier et al. (2014) evaluated the use of compost to restore soil fertility and improve plant nutrition in Mediterranean areas affected by fires.

The soil liming effect due to the addition of residues (CM and LP) increased soil pH thus decreasing concentrations of phytotoxic Al (Haynes and Mokolobate, 2001). The liming effect of organic amendment observed after composting was maintained during plant growth. The value of pH in amended soil was greater by 0.8 units than in the control soil and this led to a 99% decrease in Al concentrations. Considering the fertility constraints of volcanic soils, these residues could be used as complementary fertilizers by improving the chemical properties of these soils. Moreover, after harvesting, soil pH, Al saturation, Olsen P and exchangeable cations were significantly higher in the organic amended soil than in the mineral fertilizer treated soil (Table 4). This confirms the important role that organic residues can play in buffering pH and soil nutrition (Neina, 2019; Nguyen and Marschner, 2016) and in the increase of beneficial microorganisms due to when plants were grown in LP and CM treatments, the amount of TBARS decreased 1.3 fold.

In this study, no significant differences in shoot dry weight were found between the CM+LP and synthetic fertilizer (STP) treatments. A similar result was reported by Tuttobene et al. (2009), who demonstrated that the use of dried orange waste produced wheat yields similar to those got with mineral fertilizers. In contrast, a study carried out by Gopinath et al. (2008) showed that wheat yields in all treatments involving organic residues were markedly lower than those obtained through mineral fertilizer treatment.

There were no significant differences in either shoot or root P uptake by ryegrass between organic and mineral amendments. This suggests that CM and CM+LP supplied P to the soil and also improved its availability by reducing sorption (Hartono et al., 2005; Yu et al., 2013). Additionally, our data for shoot and root P uptake from ryegrass, were agreed with the study carried out by Waldrip et al. (2011), who showed that the use of poultry manure promoted higher root and shoot P uptake and increased root P concentrations. In our case, root P uptake under CM+LP treatment, was even higher than with the mineral fertilizer treatment (Fig. 2B). It is known that environmental stresses such as Al toxicity, low pH and P-deficiency among others can cause oxidative stress damage in plants. In our study, the application of CM or CM+LP reduced lipid peroxidation and SOD activity (Fig. 6). These results could be explained by the decrease of exchangeable Al, the increase of P availability and the liming effect, caused by the addition of the organic residues used in this study. In agreement with our results, Bowden et al. (2010) observed a decrease in lipid peroxidation with a significant reduction on SOD and APX activities in corn leaf, attributable to the application to organic amendments and the sufficient N supply. This could reduce the production of reactive oxygen species (ROS), and therefore, the need of the plant to increase the enzymatic antioxidant protection to control lipid peroxidation. On the other hand, organic amendments have been shown to have a protective effect against metal toxicity by increasing enzyme activities such as SOD, CAT, and POD, as a part of the defense mechanisms in plants (Nigam et al., 2019; Ramzani et al., 2017). However, in this study, we demonstrate that the use of combined CM+LP may improve plant nutrition and maintain low levels of damage and antioxidants at lower levels.

5. Conclusions

The combined addition of two different organic wastes improved both soil and plant biochemical properties and caused a significant increase in P nutrition with a concomitant effect on ryegrass yields. It is important to emphasize that the main change in the soil observed can be attributed to the increase in pH (15%) and decrease in Al saturation (99%) when CM+LP was applied. Furthermore, Olsen P concentrations significantly increased (73%). Therefore, from an agronomic point of view, these residues could be used as organic fertilizers or for complementary fertilization because they have dual advantages: a very significant P source and a liming effect on soil, leading to a decrease in the cost and quantity of chemical fertilizers used. Hence, using both combined residues as organic fertilizer results in higher soil fertility and productivity without affecting the environment highlighting the important role of fungal community in organic amendment decomposition.

Author contributions

The work presented here was carried out through collaboration between all authors. CP and MLM defined the research theme. CP performed all of the experiments under the supervision of MLM. R. R carried out DGGE analysis under PD's supervision. CP, SS, PD, and MLM wrote the manuscript. All of the authors have contributed to, seen and approved the manuscript.

Funding

This work was supported by FONDECYT 1181050.

6. References

Bardgett, R.D., Streeter, T.C., Bol, R., 2003. Soil microbes compete effectively with plants for organic-nitrogen inputs to temperate grasslands. Ecology 84, 1277–1287.

https://doi.org/10.1890/0012-9658(2003)084[1277:SMCEWP]2.0.CO;2

Barnard, R.L., Osborne, C.A., Firestone, M.K., 2013. Responses of soil bacterial and fungal communities to extreme desiccation and rewetting. Int. Soc. Microb. Ecol. 7, 2229–2241. https://doi.org/10.1038/ismej.2013.104

Borie, F., Zunino, H., Martínez, L., 1989. Macromolecule-P associations and inositol phosphates in some Chilean volcanic soils of temperate regions. Commun. Soil Sci. Plant Anal. 20, 1881–1894. https://doi.org/10.1080/00103628909368190

Bowden, C.L., Evanylo, G.K., Zhang, X., Ervin, E.H., Seiler, J.R., 2010. Soil carbon and physiological responses of corn and soybean to organic amendments. Compost Sci. Util. 18, 162–173. https://doi.org/10.1080/1065657X.2010.10736952

Bradford, M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–254. https://doi.org/10.2135/cropsci1994.0011183X003400020031x

Brookes, P.C., Landman, A., Pruden, G., Jenkinson, D.S., 1985. Chloroform fumigation and the release of soil nitrogen: A rapid direct extraction method to measure microbial biomass nitrogen in soil. Soil Biol. Biochem. 17, 837–842. https://doi.org/10.1016/0038-0717(85)90144-0

Brookes, P.C., Powlson, D.S., Jenkinson, D.S., 1982. Measurement of microbial biomass phosphorus in soil. Soil Biol. Biochem. 14, 319–329. https://doi.org/10.1016/0038-0717(82)90001-3

Cellier, A., Gauquelin, T., Baldy, V., Ballini, C., 2014. Effect of organic amendment on soil fertility and plant nutrients in a post-fire Mediterranean ecosystem. Plant Soil 376, 211–228. https://doi.org/10.1007/s11104-013-1969-5

Cercioglu, M., 2017. The Role of Organic Soil Amendments on Soil Physical Properties and Yield of Maize (Zea mays L.). Commun. Soil Sci. Plant Anal. 48, 683–691. https://doi.org/10.1080/00103624.2017.1298787

Chu, H., Lin, X., Fujii, T., Morimoto, S., Yagi, K., Hu, J., Zhang, J., 2007. Soil microbial biomass, dehydrogenase activity, bacterial community structure in response to long-term fertilizer management. Soil Biol. Biochem. 39, 2971–2976. https://doi.org/10.1016/j.soilbio.2007.05.031

Clarke, K.R., 1993. Non- parametric multivariate analyses of changes in community structure. Aust. J. Ecol. 18, 117–143. https://doi.org/10.1111/j.1442-9993.1993.tb00438.x

Cordell, D., Neset, T.S.S., 2014. Phosphorus vulnerability: A qualitative framework for assessing the vulnerability of national and regional food systems to the multi-dimensional stressors of phosphorus scarcity. Glob. Environ. Chang. 24, 108–122. https://doi.org/10.1016/j.gloenvcha.2013.11.005

Dhillon, S.S., Gill, R.K., Gill, S.S., Singh, M., 2004. Studies on the utilization of citrus peel for pectinase production using fungus Aspergillus niger. Int. J. Environ. Stud. 61, 199–210. https://doi.org/10.1080/0020723032000143346

Diacono, M., Montemurro, F., 2010. Long-term effects of organic amendments on soil fertility. A review. Agron. Sustain. Dev. 30, 401–422. https://doi.org/10.1051/agro/2009040

Donahue, J.L., Okpodu, C.M., Cramer, C.L., Grabau, E.A., Alscher, R.G., 1997. Responses of antioxidants to paraquat in pea leaves: Relationships to resistance. Plant Physiol. 113, 249–257. https://doi.org/10.1104/pp.113.1.249

Du, Z., Bramlage, W.J., 1992. Modified Thiobarbituric Acid Assay for Measuring Lipid Oxidation in Sugar-Rich Plant Tissue Extracts. J. Agric. Food Chem. 40, 1566–1570.

https://doi.org/10.1021/jf00021a018

Durán, P., Barra, P.J., Jorquera, M.A., Viscardi, S., Fernandez, C., Paz, C., Mora, M. de la L., Bol, R., 2019. Occurrence of soil fungi in antarctic pristine environments. Front. Bioeng. Biotechnol. 7, 1–12. https://doi.org/10.3389/fbioe.2019.00028

Durán, P., Tortella, G., Viscardi, S., Barra, P.J., Carrión, V.J., De La Luz Mora, M., Pozo, M.J., 2018. Microbial community composition in take-All suppressive soils. Front. Microbiol. 9, 1–15. https://doi.org/10.3389/fmicb.2018.02198

European Commission 2015. COM(2015) 614 final – Closing the loop – An EU action plan for the circular economy. Available at: https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:52015DC0614. (accessed 16 April 2021).

Fuentes, B., Jorquera, M., Mora, M. de la L., 2009. Dynamics of phosphorus and phytate-utilizing bacteria during aerobic degradation of dairy cattle dung. Chemosphere 74, 325–331. https://doi.org/10.1016/j.chemosphere.2008.08.045

Gatiboni, L.C., Jefferson Luis Meirelles Coimbra, Denardin, R.B.N., Wildner, L.D.P., 2011. Microbial Biomass and Soil Fauna During the Decomposition of Cover Crops in No-Tillage System. Rev. Bras. Cienc. do Solo 35, 1151–1157.

Gopinath, K.A., Saha, S., Mina, B.L., Pande, H., Kundu, S., Gupta, H.S., 2008. Influence of organic amendments on growth, yield and quality of wheat and on soil properties during transition to organic production. Nutr. Cycl. Agroecosystems 82, 51–60. https://doi.org/10.1007/s10705-008-9168-0

Guppy, C.N., Menzies, N.W., Moody, P.W., Blamey, F.P.C., 2005. Competitive sorption reactions between phosphorus and organic matter in soil: A review. Aust. J. Soil Res. 43, 189–202. https://doi.org/10.1071/SR04049

Güsewell, S., 2004. N:P ratios in terrestrial plants: Variation and functional significance.

New Phytol. 164, 243–266. https://doi.org/10.1111/j.1469-8137.2004.01192.x
Hargreaves, J.C., Adl, M.S., Warman, P.R., 2008. A review of the use of composted municipal solid waste in agriculture. Agric. Ecosyst. Environ. 123, 1–14. https://doi.org/10.1016/j.agee.2007.07.004
Hartono, A., Funakawa, S., Kosaki, T., 2005. Phosphorus sorption-desorption characteristics of selected acid upland soils in Indonesia. Soil Sci. Plant Nutr. 51, 787–799. https://doi.org/10.1111/j.1747-0765.2005.tb00113.x
Haynes, R.J., Mokolobate, M.S., 2001. Amelioration of Al toxicity and P deficiency in acid soils by additions of organic residues: A critical review of the phenomenon and the mechanisms involved. Nutr. Cycl. Agroecosystems 59, 47–63. https://doi.org/10.1023/A:1009823600950
Iannotti, D.A., Grebus, M.E., Toth, B.L., Madden, L. V., Hoitink, H.A.J., 1994. Oxygen Respirometry to Assess Stability and Maturity of Composted Municipal Solid Waste. J. Environ. Qual. 23, 1177–1183. https://doi.org/10.2134/jeq1994.00472425002300060007x
Jonasson, S., Michelsen, A., Schmidt, I.K., 1999. Coupling of nutrient cycling and carbon dynamics in the Arctic, integration of soil microbial and plant processes. Appl. Soil Ecol. 11, 135–146. https://doi.org/10.1016/S0929-1393(98)00145-0
Kang, J., Hesterberg, D., Osmond, D.L., 2009. Soil Organic Matter Effects on Phosphorus Sorption: A Path Analysis. Soil Sci. Soc. Am. J. 73, 360–366. https://doi.org/10.2136/sssaj2008.0113
Liu, H., Huang, Y., Duan, W., Qiao, C., Shen, Q., Li, R., 2020. Microbial community composition turnover and function in the mesophilic phase predetermine chicken manure composting efficiency. Bioresour. Technol. 313, 123658.

https://doi.org/10.1016/j.biortech.2020.123658
Liu, J., Schulz, H., Brandl, S., Miehtke, H., Huwe, B., Glaser, B., 2012. Short-term effect of biochar and compost on soil fertility and water status of a Dystric Cambisol in NE Germany under field conditions. J. Plant Nutr. Soil Sci. 175, 698–707. https://doi.org/10.1002/jpln.201100172
Mamma, D., Kourtoglou, E., Christakopoulos, P., 2008. Fungal multienzyme production on industrial by-products of the citrus-processing industry. Bioresour. Technol. 99, 2373–2383. https://doi.org/10.1016/j.biortech.2007.05.018
Mora, M. de la L., Demanet, R., Acuña, J.J., Viscardi, S., Jorquera, M., Rengel, Z., Durán, P., 2017. Aluminum-tolerant bacteria improve the plant growth and phosphorus content in ryegrass grown in a volcanic soil amended with cattle dung manure. Appl. Soil Ecol. 115, 19–26. https://doi.org/10.1016/j.apsoil.2017.03.013
Murphy J, Riley JP, 1962. A modified single solution method for the determination of phosphate in natural waters. Anal. Chem. Acta 27, 31–36. https://doi.org/10.1016/S0003-2670(00)88444-5
Neina, D., 2019. The Role of Soil pH in Plant Nutrition and Soil Remediation. Appl. Environ. Soil Sci. 2019. https://doi.org/10.1155/2019/5794869
Neset, T.S., Cordell, D., Mohr, S., VanRiper, F., White, S., 2016. Visualizing Alternative Phosphorus Scenarios for Future Food Security. Front. Nutr. 3. https://doi.org/10.3389/fnut.2016.00047
Nguyen, T.T., Marschner, P., 2016. Soil respiration, microbial biomass and nutrient availability in soil after repeated addition of low and high C/N plant residues. Biol. Fertil. Soils 52, 165–176. https://doi.org/10.1007/s00374-015-1063-7
Nigam, N., Yadav, V., Khare, P., Singh, R.P., Das, P., Shanker, K., Sharma, R.S., 2019.

Exploring the benefits of biochar over other organic amendments for reducing of metal toxicity in Withania somnifera. Biochar 1, 293–307. https://doi.org/10.1007/s42773-019-00023-4

Odepa. Oficina de Estudios y Políticas Agrarias 2019. Ministerio de Agricultura. Gobierno de Chile. Ficha Nacional. Available at: https://www.odepa.gob.cl/estadisticas-del-sector/ficha-nacional-y-regionales/attachment/ficha-nacional-2019. (accessed 16 April 2021).

Pagliari, P., Wilson, M., He, Z., 2020. Animal Manure Production and Utilization: Impact of Modern Concentrated Animal Feeding Operations. Anim. Manure Prod. Charact. Environ. Concerns, Manag. 1–14. https://doi.org/10.2134/asaspecpub67.c1

Pagliari, P.H., Wilson, M., Waldrip, H.M., He, Z., 2020. Nitrogen and Phosphorus Characteristics of Beef and Dairy Manure. Anim. Manure Prod. Charact. Environ. Concerns, Manag. 45–62. https://doi.org/10.2134/asaspecpub67.c4

Paredes, C., Staunton, S., Di Rauso Simeone, G., Rao, M.A., Violante, A, Mora, M.L., 2021. Citrus residue enhances the effectiveness of beef cattle manure improving the phosphorus availability in acidic Andisol. Comm. Soil. Sci. Plant Anal *accepted manuscript*

Paula, F.S., Tatti, E., Thorn, C., Abram, F., Wilson, J., O'Flaherty, V., 2020. Soil prokaryotic community resilience, fungal colonisation and increased cross-domain co-occurrence in response to a plant-growth enhancing organic amendment. Soil Biol. Biochem. 149, 107937. https://doi.org/10.1016/j.soilbio.2020.107937

Ramzani, P.M.A., Coyne, M.S., Anjum, S., Khan, W. ud D., Iqbal, M., 2017. In situ immobilization of Cd by organic amendments and their effect on antioxidant enzyme defense mechanism in mung bean (Vigna radiata L.) seedlings. Plant Physiol.

Biochem. 118, 561–570. https://doi.org/10.1016/j.plaphy.2017.07.022

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. J. Soil Sci. Plant Nutr. 16, 490–506. https://doi.org/10.4067/S0718-95162016005000041

Sadzawka, A., Carrasco, M.A., Gez, R., Mora, M.D.L.L., Flores, H., Neaman, A., 2006. Métodos de análisis recomendados para los suelos de Chile. Revision 2006. Ser. Actas-Instituto Investig. Agropecu.

Sadzawka, A., Grez, R., Carrasco, M., Flores, H., Mora, M., Rojas, C., 2004. Métodos De Análisis De Tejidos Vegetales 53.

SAG. Servicio Agrícola y Ganadero. 2006. Diagnóstico de la problemática ambiental de los residuos generados por la producción de aves y vacunos de leche en Chile y capacitación en la evaluación de planteles pecuarios. Available at: https://www.sag.gob.cl/sites/default/files/residuos_aves_vacunos_leche.pdf. (accessed 16 April 2021).

Sagar, R., Sharma, G.P., 2012. Measurement of alpha diversity using Simpson index (1/λ): the jeopardy. Environ. Skept. Critics 2012, 23–24.

Tuttobene, R., Avola, G., Gresta, F., Abbate, V., 2009. Industrial orange waste as organic fertilizer in durum wheat. Agron. Sustain. Dev. 29, 557–563. https://doi.org/10.1051/agro/2009014

Vance, E.D., Brookes, P.C., Jenkinson, D.S., 1987. An extraction method for measuring soil microbial biomass C. Soil Biol. Biochem. 19, 703–707. https://doi.org/10.1016/0038-0717(87)90052-6

Waithaisong, K., Robin, A., Martin, A., Clairotte, M., Villeneuve, M., Plassard, C., 2015.

Quantification of organic P and low-molecular-weight organic acids in ferralsol soil extracts by ion chromatography. Geoderma 257–258, 94–101. https://doi.org/10.1016/j.geoderma.2014.12.024

Waldrip, H.M., He, Z., Erich, M.S., 2011. Effects of poultry manure amendment on phosphorus uptake by ryegrass, soil phosphorus fractions and phosphatase activity. Biol. Fertil. Soils 47, 407–418. https://doi.org/10.1007/s00374-011-0546-4

Wang, J., Liu, Z., Xia, J., Chen, Y., 2019. Effect of microbial inoculation on physicochemical properties and bacterial community structure of citrus peel composting. Bioresour. Technol. 291, 121843. https://doi.org/10.1016/j.biortech.2019.121843

Wu, J., Joergensen, R.G., Pommerening, B., Chaussod, R., Brookes, P.C., 1990. Measurement of soil microbial biomass C by fumigation-extraction: An automated procedure. Soil Biol. Biochem. 22, 1167–1169. https://doi.org/10.1016/0038-0717(90)90046-3

Xu, X., Thornton, P.E., Post, W.M., 2013. A global analysis of soil microbial biomass carbon, nitrogen and phosphorus in terrestrial ecosystems. Glob. Ecol. Biogeogr. 22, 737–749. https://doi.org/10.1111/geb.12029

Yu, Q., Rengel, Z., 1999. Micronutrient deficiency influences plant growth and activities of superoxide dismutases in narrow-leafed lupins. Ann. Bot. 83, 175–182. https://doi.org/10.1006/anbo.1998.0811

Yu, W., Ding, X., Xue, S., Li, S., Liao, X., Wang, R., 2013. Effects of organic-matter application on phosphorus adsorption of three soil parent materials. J. Soil Sci. Plant Nutr. 13, 1003–1017. https://doi.org/10.4067/S0718-95162013005000079

Figure legends

Fig. 1: Cumulative CO_2 production in response to different soil treatments using beef cattle manure (CM), lemon peel (LP) or both (CM+LP) for a 1-month period. The insert shows the daily rate, to emphasis the greatest respiration in all systems after 5 days.

Fig. 2. Soil microbial biomass (A) C, (B) N, and (C) P in response to different soil treatments using beef cattle manure (CM), lemon peel (LP) or both (CM+LP). Data are means of three replicates ± SD. Different letters indicate significant differences ($p \leq 0.05$) between treatments.

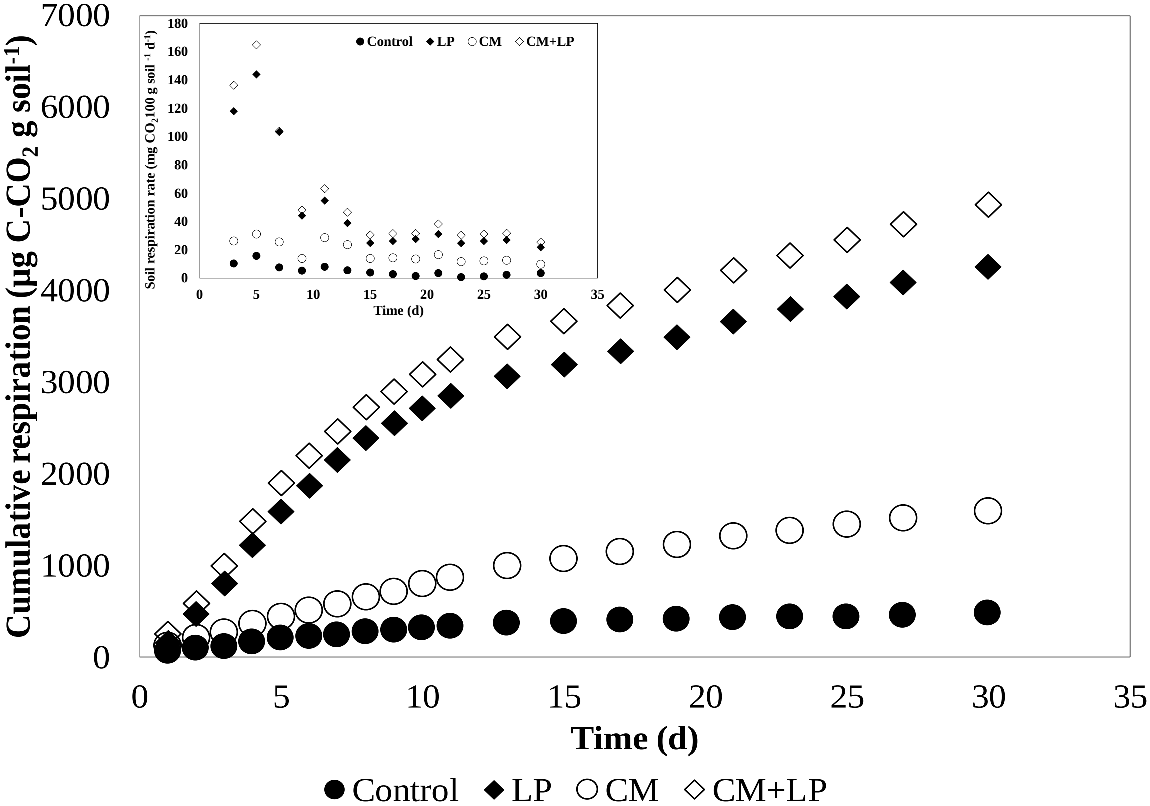
Fig. 3. Dendrogram and principal component analyses of DGGE profiles of bacteria (16S rRNA gene) after 30 days of incubation experimentation.

Fig. 4. Dendrogram and principal component analyses of DGGE profiles of fungi (18S rRNA gene) after 30 days of incubation.

Fig. 5. Dry weight of shoot (A) and **s**hoot and root P uptake of ryegrass plants under beef cattle manure (CM), lemon peel (LP) or both (CM+LP) and supertriple phosphate (STP) treatments. Data are means of three replicates ± SD. Different letters indicate significant

differences ($p \leq 0.05$) between treatments.

Fig. 6. Shoot lipid peroxidation (TBARS) (A) and superoxide dismutase activity (SOD) (B) of ryegrass plants under beef cattle manure (CM), lemon peel (LP) or both (CM+LP) and supertriple phosphate (STP) treatments. Data are means of three replicates ± SD. Different letters indicate significant differences ($p \leq 0.05$) between treatments.



Cumulative respiration (µg C-CO2 g soil-1)
7000
6000
5000
4000
3000
2000
1000
0
0
5
10
15
20
25
30
35
Time (d)
Control
LP
CM
CM+LP
Soil respiration rate (mg CO2 100 g soil -1 d-1)
180
160
140
120
100
80
60
40
20
0
Time (d)

Fig. 1

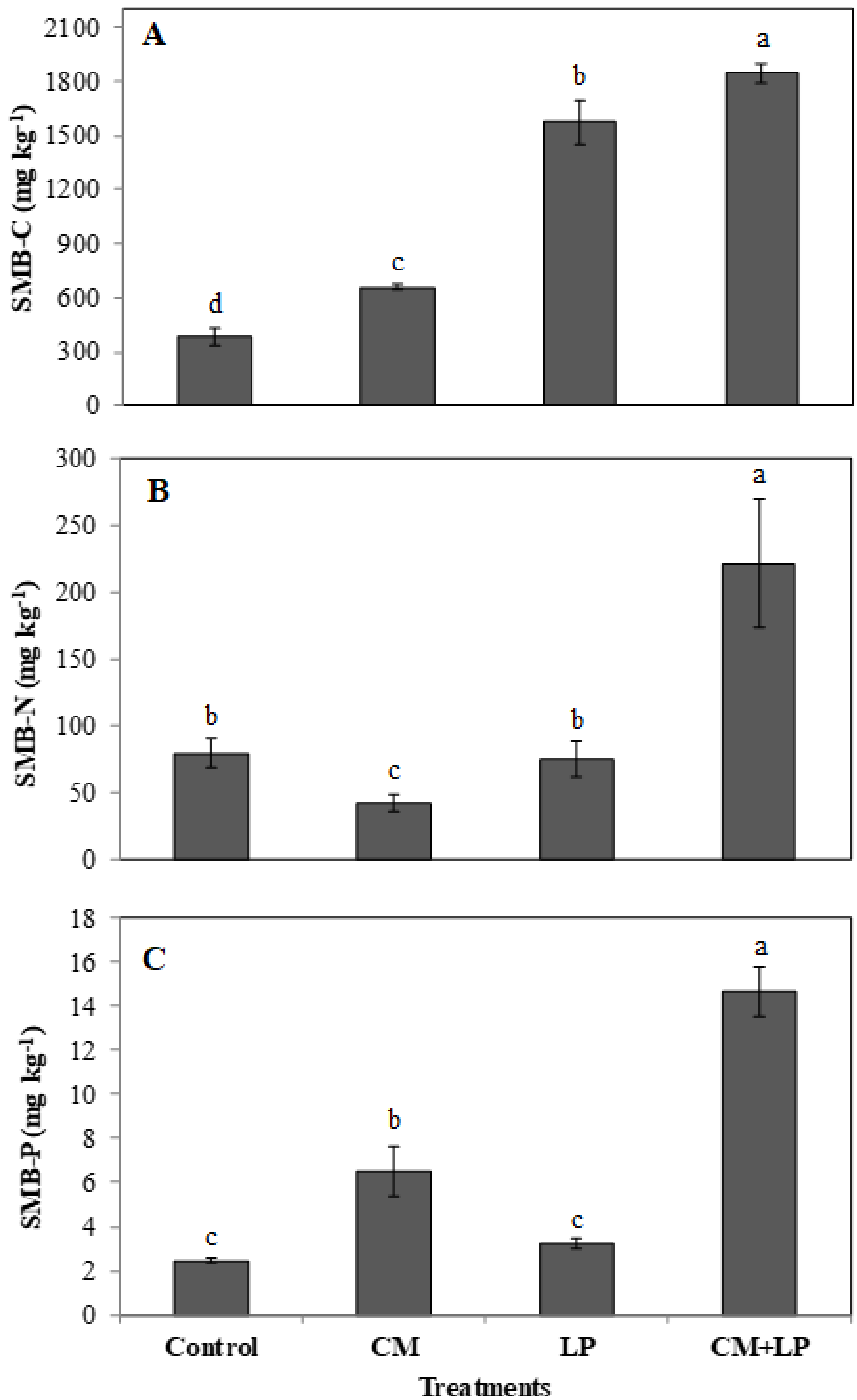

A
B
C
SMB-C (mg kg-1)
SMB-N (mg kg-1)
SMB-P (mg kg-1)
Control
CM
LP
CM+LP
Treatments

Fig. 2

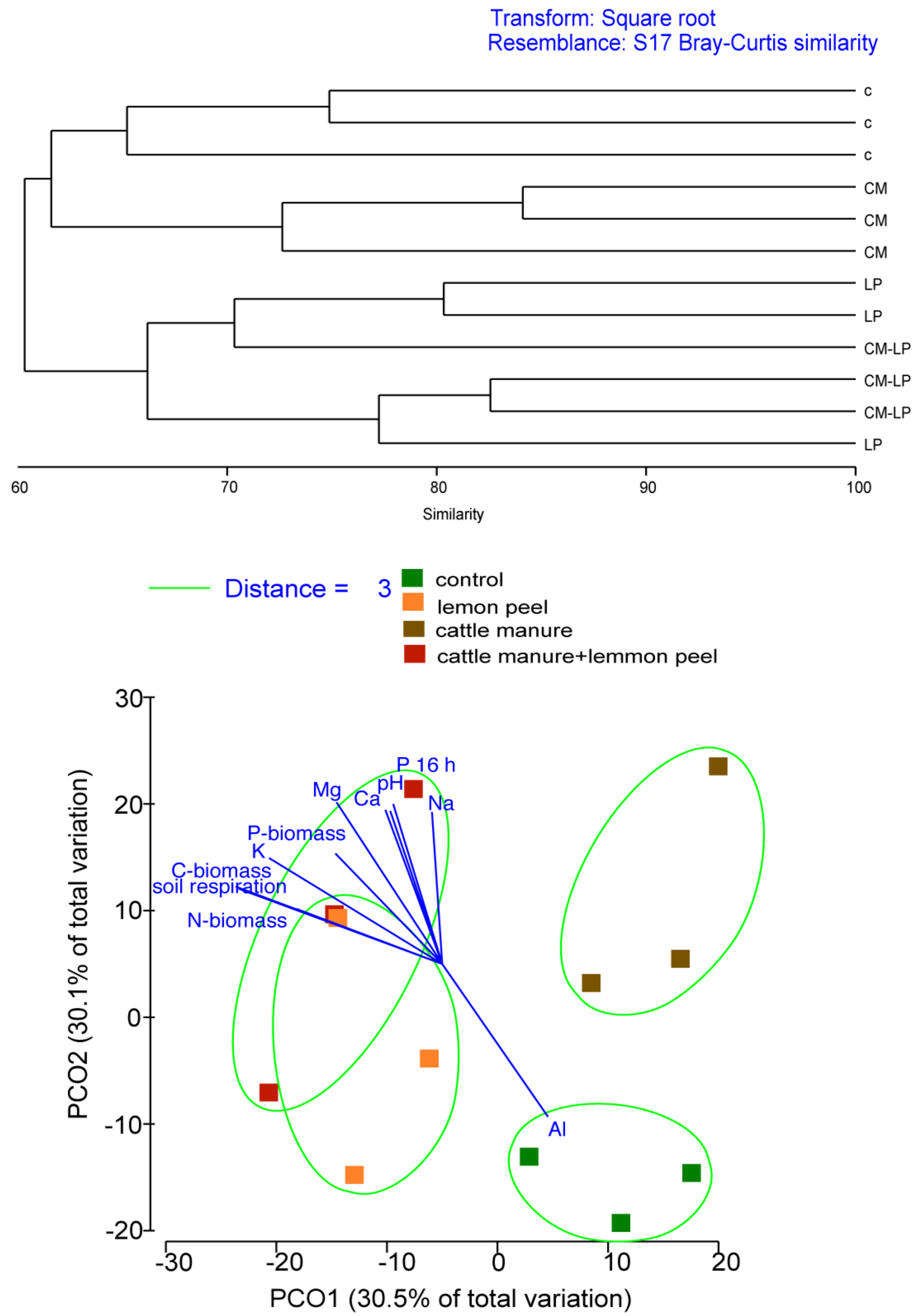

Transform: Square root
Resemblance: S17 Bray-Curtis similarity
c
c
c
CM
CM
CM
LP
LP
CM-LP
CM-LP
CM-LP
LP
60
70
80
90
100
Similarity
Distance = 3
control
lemon peel
cattle manure
cattle manure+lemmon peel
P 16 h
pH
Mg
Ca
Na
P-biomass
K
C-biomass
soil respiration
N-biomass
Al
30
20
10
0
-10
-20
-30
-20
-10
0
10
20
PCO2 (30.1% of total variation)
PCO1 (30.5% of total variation)

Fig. 3

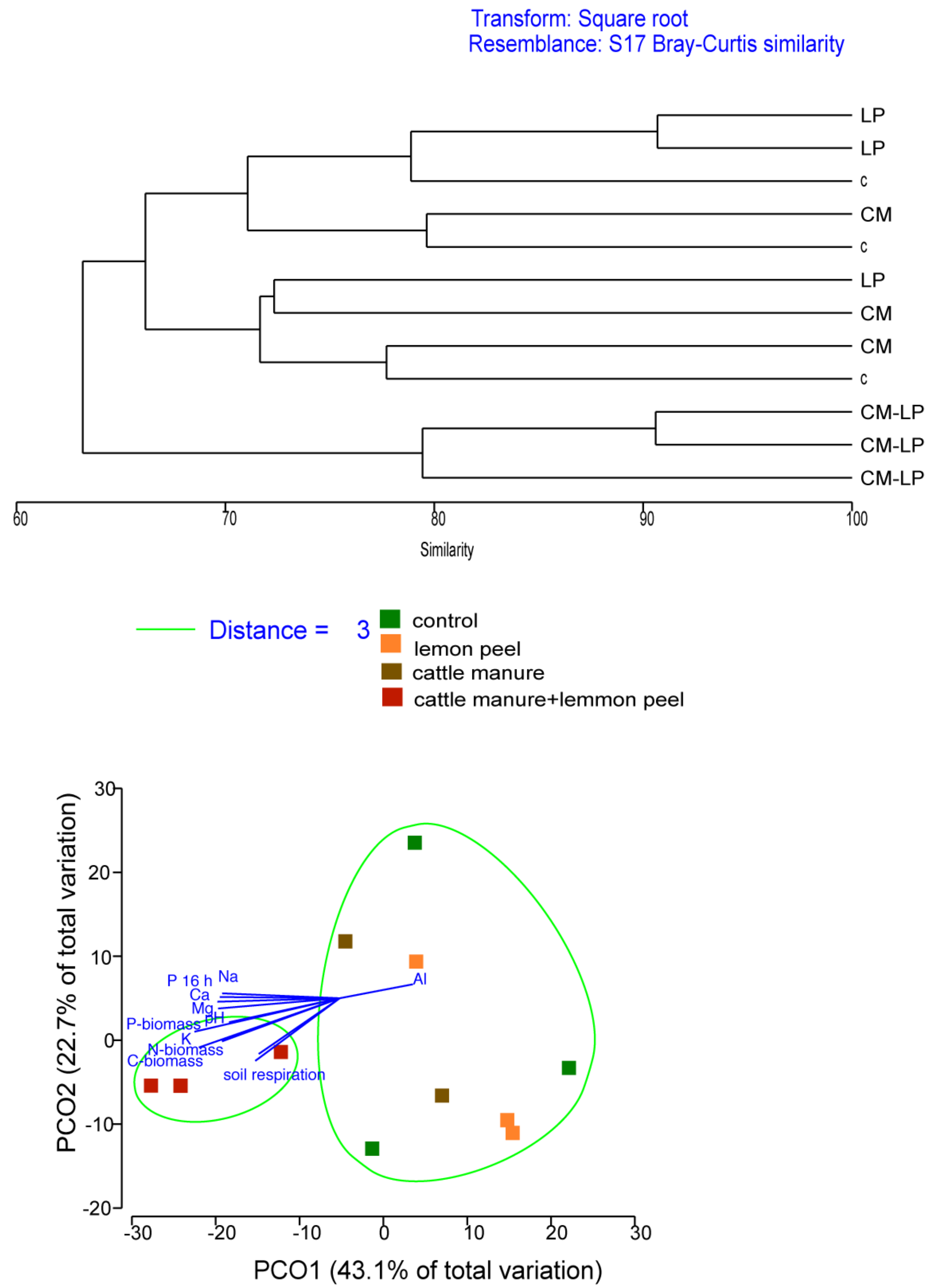

Transform: Square root
Resemblance: S17 Bray-Curtis similarity
LP
LP
c
CM
c
LP
CM
CM
c
CM-LP
CM-LP
CM-LP
60
70
80
90
100
Similarity
Distance = 3
control
lemon peel
cattle manure
cattle manure+lemmon peel
PCO2 (22.7% of total variation)
PCO1 (43.1% of total variation)
P 16 h
Na
Ca
Mg
pH
P-biomass
K
N-biomass
C-biomass
soil respiration
Al
30
20
10
0
-10
-20
-30
-20
-10
0
10
20
30

Fig. 4

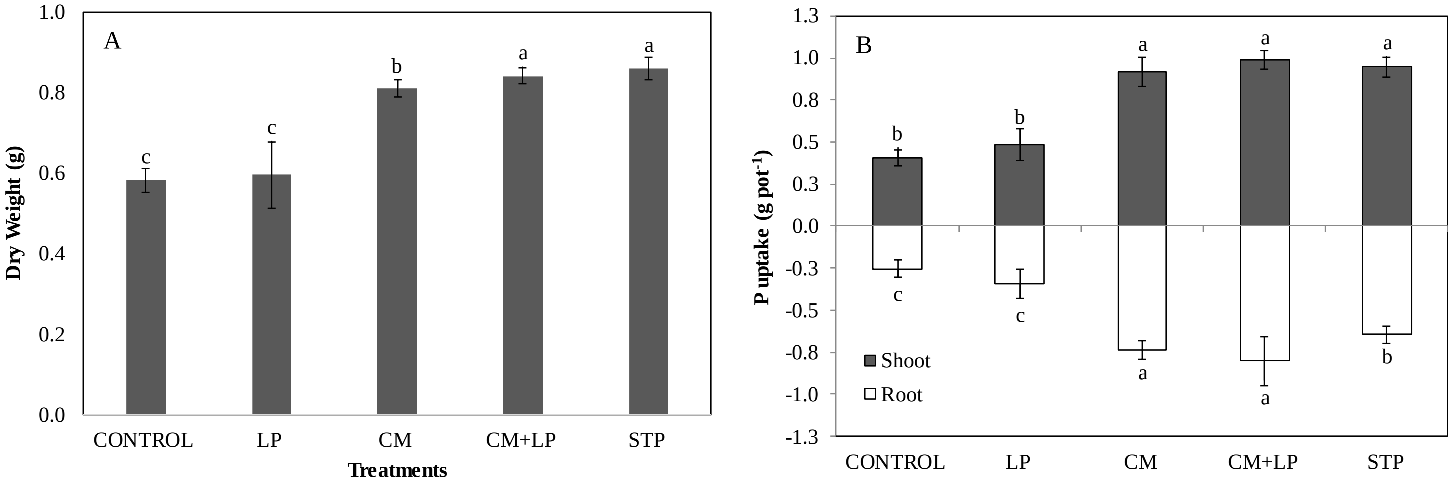

A
Dry Weight (g)
1.0
0.8
0.6
0.4
0.2
0.0
c
c
b
a
a
CONTROL
LP
CM
CM+LP
STP
Treatments
B
P uptake (g pot-1)
1.3
1.0
0.8
0.5
0.3
0.0
-0.3
-0.5
-0.8
-1.0
-1.3
b
b
a
a
a
c
c
a
a
b
Shoot
Root
CONTROL
LP
CM
CM+LP
STP

Fig. 5

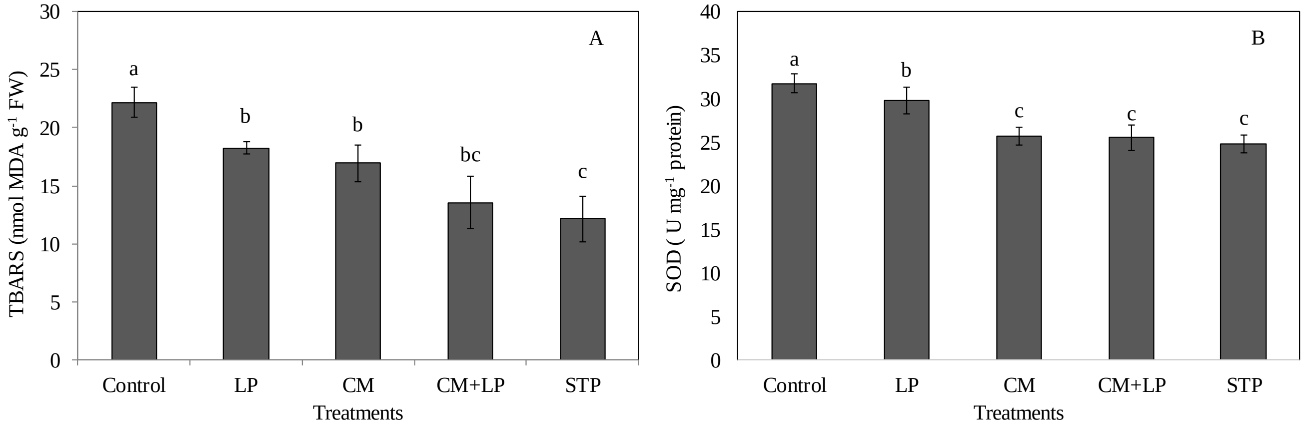

A
TBARS (nmol MDA g-1 FW)
30
25
20
15
10
5
0
a
b
b
bc
c
Control
LP
CM
CM+LP
STP
Treatments
B
SOD (U mg-1 protein)
40
35
30
25
20
15
10
5
0
a
b
c
c
c
Control
LP
CM
CM+LP
STP
Treatments

Fig. 6

Table 1: Chemical properties of soil, CM and LP. Mean values (n = 3) ± SD. For Soil and CM phosphorus concentration (P) corresponds to Olsen P, while for LP is total P. Water-extractable organic anions (citrate, oxalate and malate) were quantified by HPLC.

	P	Ca	Mg	Na	K	Al	C/N	pH	Citrate	Oxalate	Malate
	mg kg^{-1}								g kg^{-1}		
Soil	6 ± 1	814 ± 8	113 ± 1	41 ± 2	137 ± 4	10 ± 1	12.5 ± 0.2	5.58 ± 0.03	-	-	-
CM	497 ± 10	3642 ± 16	824 ± 2	1141 ± 2	1767 ± 20	11 ± 1	19.2 ± 0.5	7.01 ± 0.01	-	-	-
LP	845 ± 12	5403 ± 551	777 ± 276	260 ± 36	9222 ± 2122	2.90 ± 0.50	33.4 ± 0.9	2.86 ± 0.06	8.7 ± 0.01	0.45 ± 0.01	0.19 ± 0.01

CM: beef cattle manure, LP: lemon peel

Table 2: Soil chemical properties at the end of composting (3 months).

	Olsen P	Ca	Mg	Na	K	Al saturation	pH	C/N
	(mg kg^{-1})					(%)		
Control	5.41 ± 0.28[c]	890 ± 22[d]	102 ± 5[d]	23 ± 2[d]	98 ± 4[d]	3.39 ± 0.1[a]	5.17 ± 0.06[c]	12.71[a]
LP	6.03 ± 0.23[b]	1046 ± 20[c]	144 ± 7[c]	35 ± 2[c]	551± 12[b]	0.38 ± 0.1[b]	5.65 ± 0.20[b]	12.74[a]
CM	8.55 ± 0.48[a]	1420 ± 16[b]	235 ± 1[b]	156 ± 2[b]	414± 4[c]	0.14 ± 0.0[bc]	5.71 ± 0.05[b]	12.78[a]
CM+LP	9.40 ± 0.34[a]	1558 ± 50[a]	273 ± 10[a]	166 ± 5[a]	962± 70[a]	0.01 ± 0.0[c]	5.94 ± 0.08[a]	12.59[a]

Control: soil control; LP: lemon peel; CM: beef cattle manure

Table 3. Effect of treatments on shoot plant uptake. Mean values (n = 3) ± SD.

Plant part	Treatment	Ca	Mg	Na	K	Al
		mg pot^{-1}				µg pot^{-1}
Shoot	Control	$5.85^{b} \pm 0.27$	$1.96^{c} \pm 0.14$	$1.16^{c} \pm 0.06$	$26.47^{b} \pm 1.59$	$31.40^{a} \pm 4.29$
	STP	$11.18^{a} \pm 1.39$	$4.24^{a} \pm 0.28$	$5.94^{a} \pm 0.60$	$30.66^{b} \pm 2.03$	$38.13^{a} \pm 3.76$
	LP	$2.84^{c} \pm 0.40$	$1.21^{d} \pm 0.29$	$0.86^{c} \pm 0.27$	$23.34^{b} \pm 4.22$	$9.48^{c} \pm 1.13$
	CM	$4.94^{b} \pm 0.32$	$2.63^{b} \pm 0.24$	$2.75^{b} \pm 0.37$	$40.63^{a} \pm 2.90$	$13.75^{b} \pm 1.86$
	CM+LP	$5.14^{b} \pm 0.51$	$2.53^{b} \pm 0.24$	$3.04^{b} \pm 0.27$	$43.88^{a} \pm 2.31$	$16.07^{b} \pm 4.17$
Root	Control	$1.45^{b} \pm 0.10$	$0.49^{b} \pm 0.02$	$0.39^{c} \pm 0.05$	$2.73^{bc} \pm 0.20$	$616.93^{c} \pm 105.75$
	STP	$2.78^{a} \pm 0.55$	$0.92^{a} \pm 0.07$	$0.91^{a} \pm 0.04$	$2.60^{b} \pm 0.32$	$2065.62^{a} \pm 286.28$
	LP	$2.84^{b} \pm 0.40$	$0.39^{b} \pm 0.10$	$0.20^{d} \pm 0.05$	$3.15^{c} \pm 0.76$	$555.00^{c} \pm 154.49$
	CM	$1.36^{a} \pm 0.31$	$0.87^{a} \pm 0.09$	$0.58^{b} \pm 0.00$	$6.77^{a} \pm 0.26$	$1390.76^{b} \pm 126.29$
	CM+LP	$2.89^{a} \pm 0.13$	$0.83^{a} \pm 0.16$	$0.51^{b} \pm 0.05$	$6.73^{a} \pm 1.29$	$1436.80^{b} \pm 275.67$

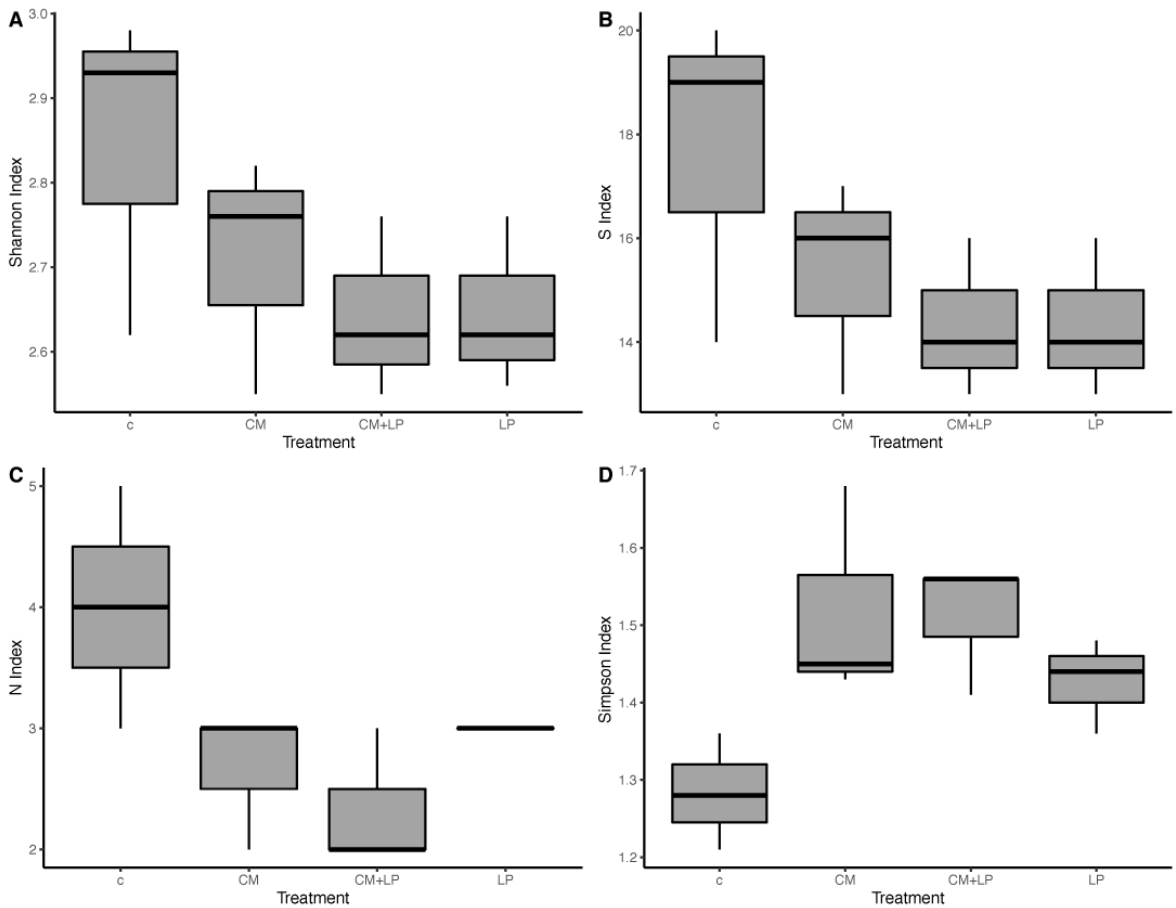
Control: soil control; LP: lemon peel; CM: beef cattle manure and STP: supertriple phosphate

Table 4: Soil chemical properties after harvest. Mean values (n = 3) ± SD.

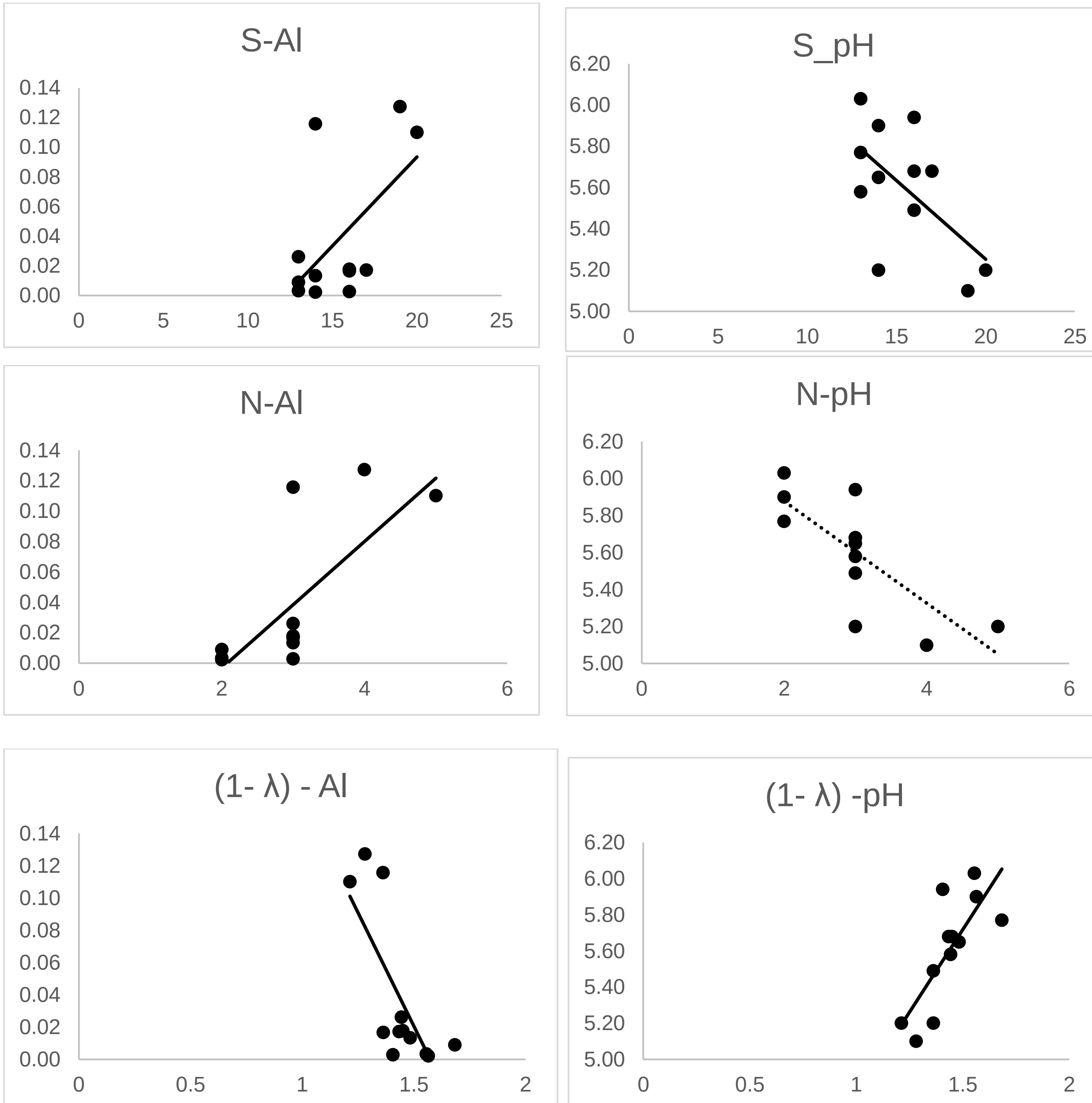
	Olsen P	Ca	Mg	Na	K	Al saturation	pH	C/N
	mg kg^{-1}					%		
Control	4.35 ± 0.54c	$835.3^{d} \pm 35.0$	$85.81^{d} \pm 2.54$	$21.47^{c} \pm 1.33$	$39.10^{d} \pm 0.00$	$14.09^{a} \pm 0.52$	$5.07^{d} \pm 0.02$	$11.85^{a} \pm 0.22$
STP	4.35 ± 0.33c	$860.0^{d} \pm 13.1$	$78.89^{d} \pm 5.50$	$14.57^{d} \pm 3.51$	$37.80^{d} \pm 2.26$	$11.99^{b} \pm 0.52$	$5.35^{c} \pm 0.03$	$11.92^{a} \pm 0.21$
LP	3.73 ± 0.40c	$962.7^{c} \pm 11.0$	$110.6^{c} \pm 0.70$	$39.10^{b} \pm 2.30$	$394.9^{b} \pm 6.77$	$9.59^{c} \pm 0.52$	$5.65^{b} \pm 0.05$	$11.99^{a} \pm 0.12$
CM	7.65 ± 0.31a	$1320.7^{b} \pm 13.6$	$227.3^{b} \pm 6.14$	$157.9^{a} \pm 3.51$	$282.8^{c} \pm 15.8$	$5.70^{d} \pm 0.52$	$5.65^{b} \pm 0.01$	$12.22^{a} \pm 0.22$
CM+LP	6.56 ± 0.49b	$1435.3^{a} \pm 21.0$	$242.8^{a} \pm 5.59$	$162.5^{a} \pm 10.4$	$602.1^{a} \pm 6.77$	$4.20^{e} \pm 0.52$	$5.98^{a} \pm 0.04$	$12.20^{a} \pm 0.33$

Control: soil control; LP: lemon peel; CM: beef cattle manure and STP: supertriple phosphate

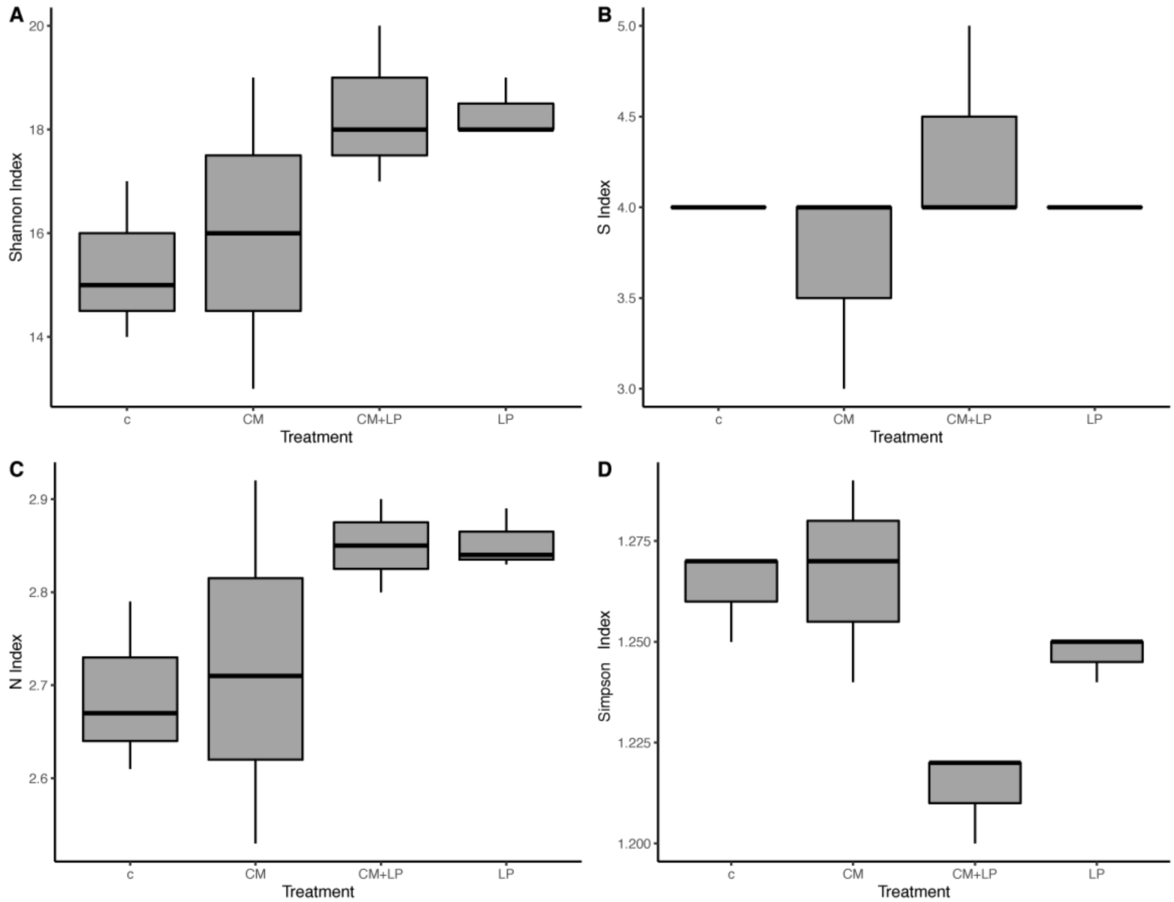
SUPPLEMENTARY MATERIAL



Supplementary Fig. 1. Biodiversity index of bacteria



Supplementary Fig. 2. Correlation between biodiversity of the bacterial community index S (species), d (individual), H' (Shannon), and Simpson (expressed as 1- λ) with Al and pH



Supplementary Fig. 3. Biodiversity index of fungi

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: