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EFFECT OF SOIL MINERAL COMPOSITION ON AVAILABLE CARBON AND PRIMING EFFECT IN PRISTINE OLD GROWTH TEMPERATE RAIN FORESTS

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Esta tesis fue realizada bajo la supervisión del Dr. Francisco Matus B., 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

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…dedicada a mi hija Amelia

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Summary and outline of this thesis

This thesis is composed of seven chapters about the effect of biological activity on available carbon (C) for soil microorganisms and priming effects (PE). The PE is the short-term turnover of native soil organic matter (SOM) originated by moderate and strong soil disturbance, particularly when the soil is supplied with fresh C input. In Chapter I we give a general introduction that provides an overview of the main coincidences and divergences found in the literature on PE. We hypothesized that soil mineral composition and chemical stabilization of SOM controls the response of microbial community to the available fresh C inducing the PE. The general objective is to determine the rhizophere PE (RPE) in soil with different stabilization capacities in anallophanic soil from volcanic origin and kaolinitic soils derived from metamorphic rock, both developed under temperate rain forests. Chapter II presents an overview of the limitations, artifacts and biases including relevant variables in the experimental designs on PE. The knowledge of coincidences and disagreement of PE are discussed. The main conclusion of this chapter is that there is a gap of information to predict the PE and particularly the RPE. There is a missing link of successional microbe community relative to the C use efficiency of metabolic pathways in soils with different stabilization capacities. The perspectives of these assumptions are further discussed. Chapter III is a published invited review focus on the available C in interaction with soil mineral phase at rhizosphere level, where most biological activity occurs. Available C for soil microorganism's consumption depends on the mineral composition and the type of exudates, and its relative concentrations in the soil solution. Rhizosphere priming effects may occur, but it is unknown whether it can influence the SOM mineralization across the soil profile. There is evidence that RPE on the topsoil of volcanic soils under forest cover may account for approximately one fifth of the annual CO_2 soil respiration. Chapter IV describes the influence of available C for soil microorganisms in soil of contrasting capacity to store SOM (allophanic and kaolinitic). We determine the enzymatic activities, C-mineralization, reducing sugars, carbohydrates, microbial biomass in various soil physical fractions (light, LF >250 µm; intermediate, IF 53-250 µm and mineral, MF < 53 µm) and bulk soils. Physical fractions had a significant impact on enzymatic activities. Mineral fraction of allophanic soils showed the lowest activity, except for urease. In contrast in kaolinitic (metamorphic) soil, the greater activity detected for enzymes was in the LF, given the most labile available C for soil microbes. Differences in enzymatic activities in soil fractions showed the functionality and efficiency of microbial community to mineralize different SOM complexity. Chapter V shows the importance of Al addition on the potential Al inhibition on soil microbial activity. In this Chapter we hypothesize that that a molar ratio Al:C> 0.1 in the soil solution causes an inhibition or

reduction of the bioavailability of C for growth of soil microorganisms (Schwesig et al. 2003; Scheel et al. 2008), because the Al is capable of binding DOM and when the complexing capacity is exceeded the complexes precipitates, so the excess Al inhibits the microbial growth. In contrast, when the molar ratio Al:C <0.1, the Al complexes formation reduces the potential risk for inhibition of microbial growth.The results showed that the C mineralization of DOM and mineral soil decreased as Al:C ratio for values greater than 0.12. Flow cytometry analysis for cells counting was in close correlation with the C mineralization. Al addition caused a complexation of Althat allowed the growth of microorganism comparable to the control. In chapter VI the impact of root carbon influxes of C4 plants (maize) was evaluated on RPE in different physical fractions isolated from allophanic and metamorphic soils (Experiment 1). The results supported the hypothesis that the magnitude of RPE is strongly influenced by the clay type. The priming was lower in the MF fraction of allophanic soil attributed to a decreased availability of C. The RPE in kaolinitic metamorphic soils was two folds the amount of C in the allophanic soil (14 % against 33 %, respectively). In allophanic soils, as expected, there was a negative correlation between the active Al (extracted with pyrophosphate) versus the RPE and ^{13}C derived C. This result indicates the strong influence of mineral composition on SOM mineralization across all plant growing stages. In a second approaches (Experiment 2) the impact of roots on RPE at flowering (maximum C influx from roots) were evaluated once the release of fresh C from roots was stopped. Our results support previous one experiment, indicating that soil bearing 1:1 clay mineralized more C (25 times) than allophanic soil due to its adsorption capacity. Chapter VII provides a general discussion, and concluding remarks, including recommendations for future work.

The main conclusions are: there is lack of knowledge to explain the factor and mechanism that induce the PE. One problem in the evaluation of PE is the little importance of the role of N mineralization processes on RPE. The organic C derived from microbial activity and root exudates is probably the most mobile and bioavailable fraction of C in the rhizosphere interacting with mineral phase. For instance, the Al humus-complex formation needs further attention because Al may be toxic for soil microorganisms, but also it can remove the Al from soil solution when the molar ratio Al:C < 0.12.

The metal–humus complexes, Al hydroxides, and pH were identified as key physiochemical variables accounting for variation in dissolved organic C (DOC) and microbial activity. This indicated that soil properties can suppress microbial growth and respiration associated to the C availability.

On the other hand, the hypothesis that the mineral composition of soils determines the magnitude of RPE was confirmed. We found a strong, but inverse relationship between active Al and RPE in

allophanic soils. The same relationship was not found in the kaolinitic PAC soil. The magnitude of RPE was associate to the input of root exudates from maize plants as indicated by the release of ^{12}C-CO_2 in different developing stages. Thus, RPE was controlled by plant derived C and clay mineralogy. Mineral fraction of allophanic soil produces a less SOM decomposition compared with kaolinitic soil with more crystalline clay.

The RPE carry out the implicit assumption that carbon use efficiency did not vary between amended and control, so few paper include a complete C balance to elucidate this assumption.

It is important to identify the successional microbial communities involved in PE, since they respond differently to various substrates and they ultimately determine the preferential C utilization for growth and production of C-CO_2.

The hypothesis that RPE is driven by low molecular weight organic substances from root exudates requires further research. We have also hypothesized that SOM mineralization due to RPE may be important across the soil profile. New conceptual models explaining colloidal transports in soil with different mineralogy support this.

Further studies on the effects of C availability on soil C turnover should go beyond merely evaluating the flow rate of C or C pool size. It should also pay close attention to SOM composition and structure as well as to changes in enzyme and microbial activities, as the more complex dynamics driving whether the priming effect does or does not occur with C and N amendments.

The temperate rain forest soils exposed to large fluctuations of O_2 across the year is of particularly importance on RPE as this factor affects the anoxi/oxic and biotic/abiotic processes. Mineral fraction may contain anaerobic microsites (O_2 depleted) due to the root and microorganism respiration.

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CHAPTER I

General Introduction

1.1 General Introduction

A major uncertainty in soil carbon (C) studies is to elucidate what are the mechanisms underlying when the fresh C is incorporated into the soil on the turnover of soil organic matter (SOM). The priming effect (PE) is the increases of native SOM mineralization after the input of fresh C residue (Bingeman et al., 1953). In other words, the PE is the extra mineralization of C from SOM after the addition of fresh organic matter compared with the control. The PE not only occurs for C, but also N. Coupling C dynamics in plant and soil remains as one of the least understood components of the global C cycle (Paterson and Sim, 2013, Cheng et al., 2014, Hill et al., 2015). Priming may occur directly as a result of nutrient mining by microbial communities, or by the assumption that exudates provide a readily bioavailable supply of energy for the decomposition of native SOM (co-metabolism) (Keiluweit et al., 2015).

1.1.1 Priming effect

The influence of root activity on SOM decomposition is central to study these soil and ecosystem C dynamics (Paterson, 2009; Kuzyakov, 2010; Fontaine et al., 2011). The main constrains is to quantify the soil C fluxes and to distinguish the C fluxes from live roots (autotrophic respiration) and from the decomposition of recent root debris and exudates and true SOM decomposition (heterotrophic respiration) (Paterson and Sim, 2013). This problem has been resolved using isotope (stable and radioactive) techniques. On the other hand, many factors contribute to PE. First the occlusion of SOM in soil aggregates, restricting the access of roots, microbes and their enzymes, contributes to the spatial and temporal variability of SOM turnover and ultimately on RPE. Abiotic factors such as temperature and moisture are sensitivity for SOM turnover too (Thiessen et al., 2013). To understand the potential C sequestration mechanism that integrates different soil types (Sommer and Bossio, 2014), plant species and soil microbial activities is not fully understood as well.

The quantification of rhizodeposition and identification of individual compounds and their functionality is not very well developed (Paterson et al., 2007; Reichstein et al., 2014). The critical link between vegetation and soil C and N cycling, where living roots stimulate the decomposition of SOM, is

still poorly understood (Wild et al., 2014). Various mechanisms have been proposed to explain the wide range of reported effects, but none of them has been well established. As a consequence, the priming effect is generally not included in current ecosystem and global scale models of C-cycling (Kuzyakov and Domanski, 2000; Wild et al., 2014; Hill et al., 2015).

Fontaine et al. (2007), showed that fresh plant-derived C (cellulose) produces a real priming effects by stimulating microbial turnover on old recalcitrant C (2,567 ± 226 y). The fresh C-input in the top soil produced an increase in the turnover rate of old recalcitrant C compared with the control without addition. Furthermore, when the fresh C was added to the subsoil, the turnover of the recalcitrant C proceeded at comparable rates as the topsoil. This pointed out that the presence of fresh C supplies promotes the C destabilization. These effects need to be explored in contrasting soil types and field conditions. Soil type plays an important role in the intensity and direction of priming effects by increasing or decreasing the soil C losses. However, the mechanisms are poorly understood. Phyllosilicate clays and hydrous oxides are recognized to be important minerals involved in SOM stabilization, particularly in volcanic soils with high SOM storage capacity (Matus et al., 2014).

The PE defined by Bingeman et al. (1953) (i.e. the increase in SOM mineralization following the input of fresh C residue) can be positive (Dalenberg and Jager, 1989; Hamer and Marschner, 2002; Hamer and Marschner, 2005; Zimmerman et al., 2011) or negative (Gontikaki et al., 2013). Other studies have found that addition of litter may have neutral effect (Martens et al., 2009; Sullivan and Hart, 2013). Nottingham et al. (2009), showed an additional CO_2 evolved after amendment of fresh organic matter without an increase of microbial turnover, the apparent priming effect. The PE may also occur by the input of high or low C molecular weight of fresh organic matter (Kuzyakov and Domanski, 2000, Cheng et al., 2014). Three hypotheses have been proposed to explain the PE: (1) soil microbes would prefer the readily available substrate and thereafter the native SOM (preferential use of substrates) (Sparling et al., 1982) (2) increased degradation of SOM due to microbial growth and consequently an increased enzymatic activity which is capable to decompose both, SOM and a more labile organic compounds (co-metabolic decomposition of SOM) (Kuzyakov, 2010) and (3) The PE depends on the dynamics of soil microorganisms (Hamer and Marschner, 2005; Blagodatsky et al., 2010) based on the succession communities during decomposition. Fresh organic matter can be consumed by r-strategist's communities, increasing its population releasing enzymes as they degrade the fresh substrate. In contrast the K-strategists consume old C releasing CO_2 into the atmosphere (Fontaine et al., 2003). In general the PE vary

depending on the available substrate, the substrate quality (Kuzyakov, 2010) and the environmental conditions such as soil types, which have been little addressed in the literature (Falloon and Smith, 2000).

1.1.2 Rhizosphere priming effect

Belowground C input by tree roots has been shown to increase the decomposition of recalcitrant soil organic matter (Carney et al., 2007; Dijkstra and Cheng, 2007; Bengtson et al., 2012). Experiments doubling the CO_2 concentrations have led to a long-term net loss of C from soil in spite of higher plant growth, due to enhanced microbial degradation of SOM (Carney et al., 2007). In a one-year greenhouse study, SOM decomposition was significantly greater in soils planted with trees compared to unplanted controls (Dijkstra and Cheng, 2007). These and other studies indicate that interactions between soil and tree roots accelerate SOM decomposition. The mechanism underlying this is the so called 'rhizosphere priming effect' (RPE) which is not completely understood (Fontaine et al., 2003; Kuzyakov et al., 2009; Paterson, 2009). The degradation of humified soil organic matter is a highly energy demanding process which does not release as much C as required for its degradation, and therefore proceeds at slow rates in the soil (Kuzyakov, 2002; Kuzyakov et al., 2009). If energy limitation is alleviated by the input of easily available C, microbes may be able to enhance soil organic matter degradation in order to gain limiting nutrients (Paterson, 2009), which may be one possible explanation for the RPE. In contrast, some studies suggest that the RPE does not come from increased breakdown of recalcitrant soil organic matter, but from improved turnover of the microbial biomass and subsequent higher mineralization rates of microbial endocellular reserves (De Nobili et al., 2001; Weintraub et al., 2007) . Thus, the rhizosphere priming effect may be more complex than previously thought. Blagodatskaya and Kuzyakov (2008), recently suggested the presence of a short-term priming effect driven by the activation of endogenous microbial resources, which in turn facilitates a long term priming effect, involving the activity of specialist soil microbes capable of degrading complex soil C and N resources. However, so far there is little scientific data to back up this theory and the experimental evidence that unravels the priming mechanism in natural systems, highlighting the need for further research.

1.1.3 Energy balance

The net flux of C in terrestrial ecosystems is the balance between the assimilation of C through photosynthesis and the out-flux of C mainly by ecosystem respiration. Forest ecosystems take up a larger amount of CO_2 during photosynthesis that is released through respiration, thus these systems act as a sink for C (Dixon et al., 1994; Oren et al., 2001; Luyssaert et al., 2008). Since forest ecosystems contain a large part of the earth's terrestrial C pool (Pan et al., 2011), even small imbalances in photosynthesis and respiration could have an effect on the CO_2 and global change.

The mechanisms controlling the exchange of C between soils, plants and the atmosphere are poorly understood. The SOM content of a soil depends on the balance between belowground inputs, mainly as detritus material and root exudates versus the outputs, mainly as CO_2. Carbon sequestration in soils is a climate mitigation strategy based on the assumption that the flux of C from the air to the soil can be increased while the release of C from the soil back to the atmosphere is decreased (Leifeld et al., 2005). This transformation has the potential to reduce atmospheric concentrations of CO_2, thereby slowing global warming and mitigating climate change (Leclère et al., 2014).

1.1.4 Relationship between priming effect and soil C stabilization

When the fresh organic C is incorporated into the soil, the total CO_2 respired can be greater than theCO_2 released from the fresh C added. The primed fraction is a C destabilization mainly of SOM from mineral phase. Von Lützow et al. (2007), described three main mechanisms of stabilization of SOM: (1) interactions with minerals such as clays or with metal ions, (2) protection of microbial enzymes due to occlusion in the soil aggregates and (3) SOM complex molecules which are resistant to mineralization by soil microorganisms (chemical stabilization). Priming effect has been linked to amorphous mineral materials (Xue et al., 2005; Rasmussen et al., 2006). In young volcanic soils, Al and Fe oxides are the most important agents for SOM stabilization compared with old volcanic soils containing allophane-like materials (Neculman et al., 2013). Rasmussen et al. (2007), studied the importance of the mineralogical composition of soil on PE in temperate rain forests and they found that the amorphous clay trends to

exhibit negative PE in the top soils. Chemical interaction between organic matter and metals promote SOM-complexes and stable macro- and micro-aggregates providing physical protection of SOM (Panichini et al., 2012). This is important because complexed SOM increase the mean residence time and microbial biomass prefer fresh substrate consumption thereby limiting CO_2losses from native SOM.

The present thesis was developed in the Andean and coastal range forest ecosystems in southern Chile in old growth *Nothofagus* species in volcanic (allophanic) and kaolinitic metamorphic derived soil, respectively. These ecosystems are important reservoir of temperate forests in the world (Armesto et al., 1998). The combination of low nutrient input, low temperatures and high SOM stabilization capacity (Matus et al., 2008), are excellent scenarios to test the PE in temperate rain forest. Two sites were selected. The first study area was a volcanic soil in the Andes range that present exceptional characteristics for store SOM in Puyehue National Park (PNP) (Godoy et al., 2001; Oyarzún et al., 2004; Matus et al., 2006). They display unique morphological, physical and chemical properties attributed to the composition of their mineral phase consisting of short range ordered (SRO) materials like allophane, imogolite, ferrihydrite and Al- and Fe– humus complexes (Shoji et al., 1993; Matus et al., 2006). The precipitation chemistry in the region is one of the cleanest in the world (Godoy et al., 2001; Oyarzún et al., 2004) with a mean annual precipitation between 4,000 and 7,000 mm per year. The second area of study was in Alerce Costero National Park (PAC) in the Cordillera de la Costa range. The soil is developed from metamorphic- schist- (Ultisol) with dominant presence of kaolinite (Luzio et al., 2003). The PAC is an ancient forest *Fitzroya cupressoides* (Mol.) Johnst., in mix with *Weinmannia trichosperma* Cav., and *Notohofagus nitida* (Phil). The mean annual precipitation > 4,000 mm and mean annual temperature of 12.1 °C.

1.2 Hypotheses

Soil mineral composition, soil physical fractions and chemical SOM stabilization control the response of microbial community to priming effect intensity by regulating the organic C availability. We predict that the resistance of organic C to decomposition in different physical fractions contributes differently to the magnitude of priming effect.

1.3 General objective

The main objective of the present thesis is to determine the PE under different C availability for microbial community in volcanic (allophanic) and kaolinitic soil derived from metamorphic materials with different stabilization capacity.

CHAPTER II

Limitations, artifacts and biases evaluating the carbon priming effect

2.1 Abstract

Priming effect (PE) is a short-term turnover of native soil organic matter originated by moderate and strong soil perturbance such as the addition of fresh organic materials. Limitations, artifacts and biases are produced by including or excluding relevant variables in the experimental designs, which may lead an apparent or real PE. The latter is an acceleration or retardation of soil organic matter turnover due to increased activity or amount of microbial biomass. Most studies are laboratory experiments and few are devoted to investigate the mechanisms of such phenomena. The aim of this review is intent to integrate the main conceptual limitations, artifacts and biases associated with lack of knowledge and possible causes and processes leading to priming actions using the references on agricultural ecosystems and model experiments. The evaluation of PE was done by approaching to the conceptual mechanisms that influence different intensities of PE linked to carbon use efficiency (CUE) and preferential carbon substrate utilization (PSU). Apparent priming rise from isotopic pool substitution and from possible change of CUE. Other limitations and artifacts for apparent and real priming effect are presented in Tables. Our main conclusion is that there is gap of information which is expressed in a lack of nuclear and auxiliary hypotheses that predict the PE. There is a missing links between the interaction of soil microbe's successional communities and the C use efficiency and how this is related to the metabolic pathway in soil with different stabilization capacities. It is important to identify the microbial communities involved in PE, since they respond in a different way to various substrates addition and they are finally who determines the C use efficiency and preferential C utilization for growth and production of CO_2. The perspectives to test of these assumptions are further discussed.

Keywords: Priming effect, microbial biomass, carbon mineralization, priming effect artifacts, carbon use efficiency.

2.2 Introduction

Continuous addition of energy-rich fresh organic matter (FOM) allows acceleration or desaccelerating the soil organic matter (SOM) mineralization, the so called positive and negative priming effect (PE), respectively (Bingeman et al., 1953; Jenkinson et al., 1985; Kuzyakov and Domanski, 2000; Kuzyakov, 2010; Fontaine et al., 2011). The mineralization of C in non-amended soils is an oxidative process mediated mainly by the heterotrophic microbial biomass acting on native SOM, i.e. positive priming (Hamer and Marschner, 2002; Zimmerman et al., 2011). In contrast in amended soil the microbial biomass can lead a consumption of FOM and no mineralization of native SOM, i.e. negative priming effect (Sparling et al., 1982; Guenet et al., 2010a; Lu et al., 2014). The latter has been linked to the stabilization of SOM by mineral components (SOM which is not further mineralized, (Sollins et al., 1996). Rasmussen et al. (2006; 2007) studied the importance of the mineralogical composition of soil on PE in temperate rain forests and they found that the amorphous clay-like (volcanic soils) trends to exhibit negative PE. Amorphous materials, like SOM complex may lead negative priming (Xue et al., 2005; Rasmussen et al., 2006), although Neculman et al. (2013) working in volcanic soils found both, positive and negative priming effect. Positive PE with different duration may be induced by incorporation of organic and inorganic fertilizers (Jenkinson et al., 1985; Kuzyakov and Domanski, 2000; Schulz and Glaser, 2012), input of plant residues (Dalenberg and Jager, 1989; Shen and Bartha, 1997; Perelo and Munch, 2005), and by root exudation of organic substances (Cheng and Coleman, 1990; Blagodatsky et al., 2010; Hatton et al., 2015). The duration of PE has been associated to the substrate quality, few days for labile compound and several months for more recalcitrant ones like polymerized substrates (Kuzyakov, 2010). Many models with different levels of detail have attempted to describe the positive PE ignoring the importance of negative PE, because it is difficult to demonstrate this effect experimentally. This becomes even more complex when we consider the various interactions that occur in the soil at temporal level dynamics, environmental fluctuations, available resources, climatic factors, and anthropic intervention.

Three main hypotheses offer a mechanistic explanation of PE based on the activation of microbial populations due to incorporation of readily available substrates. Jenkinson et al. (1985), proposed low molecular weight compounds often leads to an activation of soil microorganisms to degrade SOM. Kuzyakov and Domanski (2000), indicated the production of microbial enzyme under energy-rich FOM substrates allows and extra decomposition of SOM, namely coevolution degradation. The third theory was

proposed by Fontaine et al. (2003) suggesting that the PE is due to a succession of soil microorganisms strategists. The r-strategist microorganisms, quickly growth on energy-rich FOM and thereafter, the k-strategists, slowly decompose more complex substrates of long latency periods. For a review on the factors and mechanisms inducing a PE it is recommended to consult the following excellent review: Kuzyakov and Domanski (2000); Blagodatskaya and Kuzyakov (2008) and Kuzyakov (2010).

Evaluation of PE has not been an easy task; methodological constrains such as to assess the PE in the field, the complexity for integrating the interaction of plant and soil microorganisms, the use of label isotope to distinguish different C pools and the lack of knowledge for the processes that concurs simultaneously, limits a global explanation of this phenomenon. These together with artefactual restriction like the overestimation of the C input substrate, the stochiometry C:N:P ratio of the substrate and the initial soil nutrients and biases produced by non-uniform label substrates, all induce different priming intensities (Kuzyakov and Bol, 2006).

In this review were analyzed more than sixty papers directed to evaluate the PE mainly in incubation under controlled conditions in which soil temperature and moisture were not changed. Few papers have evaluated the temperature and soil moisture controlling the PE (Dijkstra and Cheng, 2007, Bader and Cheng, 2007; Thiessen et al., 2013; Pausch et al., 2013), however, in the present review we will focuses to integrate the main methodological and conceptual limitations, artifacts and biases other than temperature and soil moisture controls associated with the evaluation of priming effects. An integrative and conceptual approach for the mechanisms that influence different intensities of PE linked to CUE and PSU by soil microbial biomass is further discussed.

In this review we present three sections. First we aim to discuss and gain insight about the apparent and real PE and how this is mainly related to substrate added. Second we present the methodological and conceptual constraints in evaluating the PE and finally a conceptual proposal; how the priming action is linked with the substrate use efficiency and the metabolic pathway of microbial biomass.

2.3 Apparent and real PE, what does it mean?

What is a real and what is apparent PE?. The real priming effect is produced by an increase in the release of CO_2 from recalcitrant SOM (Jenkinson et al., 1985; Hamer and Marschner, 2002; Kuzyakov and

Domanski, 2000). SOM first is destabilized and depolymerized by soil microbes and exoenzymes in the soil matrix. An apparent priming effect is produced by the circulation of the C substrate, in which native microbial biomass starts to renew its metabolites and release unlabeled CO_2(Bingeman et al., 1953; Dalenberg and Jager, 1989; De Nobili et al., 2001; Blagodatskaya and Kuzyakov, 2008). The replacement pool of ^{12}C in the microbial biomass by ^{13}C, rather than the decomposition of SOM has been identified as a cause of error in the priming studies, using substrates marked isotopically called "false priming" by (Fontaine et al., 2011).

Apparent priming effects have been shown with the addition of low amounts of glucose (De Nobili et al., 2001; Blagodatskaya et al., 2011) and high amounts of glucose (Dalenberg and Jager, 1989). Therefore, the nature of the incorporated fresh C (soluble or polymerized) appears to determine the substrate utilization by microbes (turnover or the microbial production of enzymes degrading SOM). Given that the soluble C and nutrients diffuse rapidly in the soil matrix, these substrates can activate many dormant soil microbes, which release unlabeled C as CO_2, which leads to the apparent priming effect (Mondini et al., 2006).One of the first researchers who propose the apparent positive priming was Jenkinson et al. (1985) even when they worked with N, the PE are caused by isotope substitution of labeled N. For C, the excess of unlabeled CO_2 production is often called apparent PE, because it originates from an isotope substitution in microbial biomass C and not from native SOM mineralization (Bingeman et al., 1953; Dalenberg and Jager, 1989). If we also taken into account that soluble C diffuses rapidly in the soil matrix, it activates dormant microbial bodies renewing their metabolites and releasing unlabeled C-CO_2 (Fontaine et al., 2003). This apparent PE acceleration in the turnover of microbial biomass as revealed by an increase of respiratory activity may be observed for a long-term (several days or weeks) (Jenkinson et al., 1985; Dalenberg and Jager, 1989; Wu et al., 1993; Degens and Sparling, 1996, Chander and Joergensen, 2001) or for several minutes to hours (De Nobili et al., 2001). The occurrence of apparent PE depends on the nature of the C input rate (Wu et al., 1993) as the supply of soluble C (sugars) with mineral nutrients, while the real PE is observed with the supply of polymerized C as cellulose, ryegrass, vegetable waste, roots extracts, root exudates, biochar, fungus debris and even with allophone clay minerals (Dalenberg and Jager, 1989; Zunino et al., 1982; Fontaine et al., 2004; Fontaine et al., 2007; Blagodatskaya and Kuzyakov, 2008).

Blagodatskaya and Kuzyakov (2008), assumed that one of causes of real acceleration of SOM mineralization requires an excess of fresh organic matter with an ample C:N ratio. The real PE is

associated with nutrient limitation on soil microbial activity. The microorganisms require C and other nutrients in specific stoichiometric proportions. Soil microbes can adjust their own C to N ratio by releasing CO_2 (Dakora and Phillips, 2002; Manzoni et al., 2008; Manzoni et al., 2012) or they can immobilize inorganic soil N to decompose N-depleted FOM (Craine et al., 2007). Thus, CUE has been inversely related with the stoichiometry C:N of the substrate in terrestrial and sediment (Sinsabaugh et al., 2013) and therefore CUE can be related to the PE. CUE values can vary between 0.3 and 0.6 and (Sinsabaugh et al., 2013) factors other than stoichiometry C:N substrate are also involved (soil type, temperature and water content) (Albrizio and Steduto, 2003; Chambers et al., 2004; Metcalfe et al., 2010; Steinweg et al., 2008; Manzoni et al., 2012; Koranda et al., 2013). However, CUE, such an important parameter never has been related with PE although both processes are intimately associated. The mineralization of SOM (faster or slower) will thus depend on the availability of nutrients and the C:N ratio of the active SOM pool (Kuzyakov and Domanski, 2000) and CUE (Manzoni et al., 2012). Nicolardot et al. (2001), classify FOM added to soil, according to C:N ratio, the substances with a ratio greater than 10 causes negative PE due to the immobilization of N and substances with a ratio < 8 causes a positive PE. It is known that C:N ratio of the microbial biomass is about 8, thus the threshold value would be a C:N ratio of about between 20-25, where about 50% of C will be incorporated into microbial biomass for growth and the other half was used as energy and release as CO_2 (Sinsabaugh et al., 2013).

According to Kuzyakov and Domanski (2000), the mechanisms and causes for a real and apparent positive PE is show in Fig 1. A real positive PE may be due to: i) an acceleration of SOM mineralization as substrate and energy source, due to the addition of mineral-N fertilizers, that produce more microbial activity from native SOM and ii) an acceleration of SOM native mineralization and N immobilization through increasing microbial activity and production of extracellular enzymes, because of the addiction of easily decomposable organic substances and mineral-N fertilizers. Apparent PE originates from an isotope substitution in microbial biomass C and not from native SOM mineralization. Soluble C accelerates the turnover of microbial biomass increasing respiration for long- or short-term (De Nobili et al., 2001).

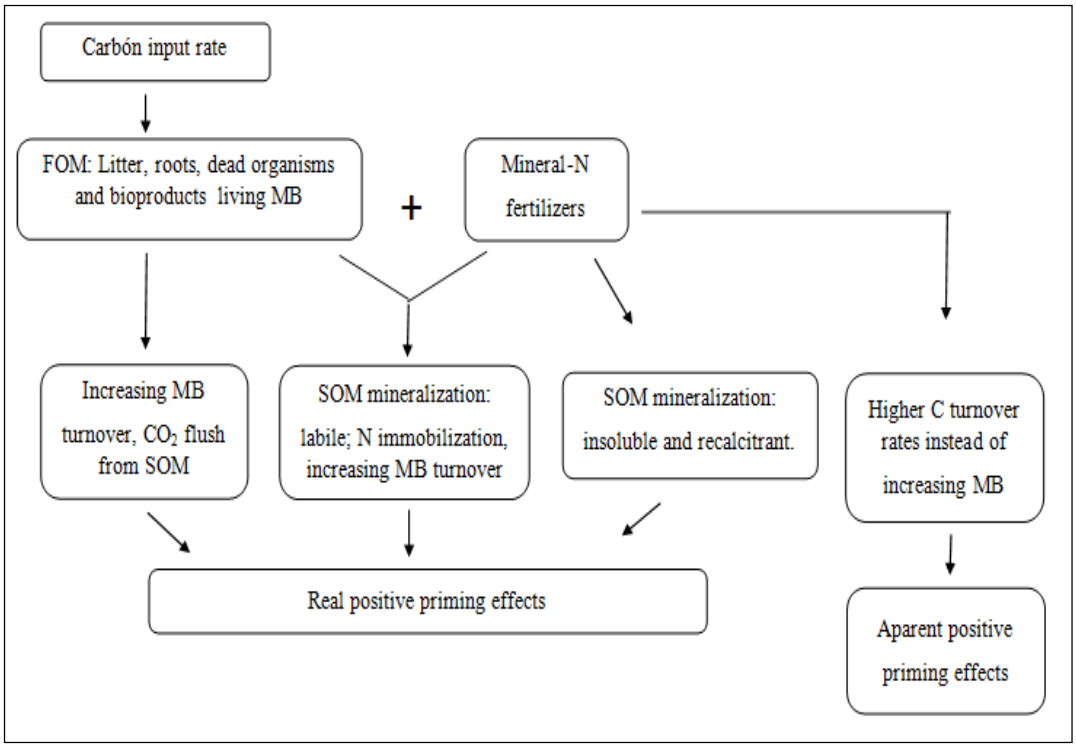

Fig 1. Conceptual scheme of mechanisms and causes of a real and apparent priming effect for C. MB= microbial biomass, SOM = soil organic matter, FOM = fresh organic matter, (Kuzyakov, 2010).

The real negative PE is usually considered as an outcome of preferential FOM utilization by decomposers originally growing on SOM (Sparling et al., 1982; Cheng, 1999; Kuzyakov, 2002; Guenet et al., 2010b). In negative priming affects the nutrient losses of the soil organic matter are replaced. Real negative PE has also been observed induced by stable SOM (Guenet et al., 2010b) or highly reactive minerals in soil (Jenkinson et al., 1985; Leifeld et al., 2002; Neculman et al., 2013). A real negative PE that has been experimentally assessed is produced by: i) C input with a C:N ratio < 16, which induce C-immobilization in microbial biomass and ii) mineral-N fertilizers, due to the N-immobilization in microbial biomass, owing to the greater availability of a substrate rich in C only (Kuzyakov and Domanski, 2000) (Fig 2.). According to Blagodatskaya and Kuzyakov (2008) an apparent negative PE can be produced by incomplete decomposition of the FOM during the incubation. The latter has been attributed to the toxicity of the substrate (Kuzyakov and Domanski, 2000; Guenet et al., 2010a; Guenet et al., 2012).

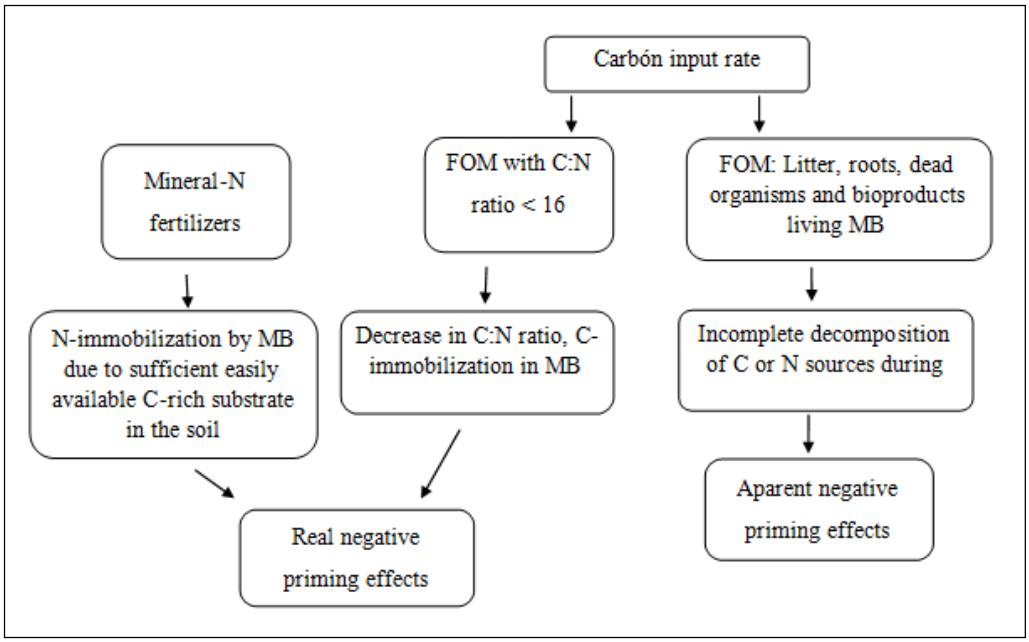

Fig 2. Conceptual scheme of mechanisms and causes of a real and apparent negative priming effect for C. MB = microbial biomass, SOM = soil organic matter, FOM = fresh organic matter, (Blagodatskaya and Kuzyakov, 2008).

Mineral adsorption that physically and chemically protects SOM may enhance the negative PE. SOM and FOM added interact with stabilizing agents such as mineral components for sorption (Sollins et al., 1996, Guggenberger and Kaiser, 2003). SOM degradation has been shown to be inhibited strongly in silicate minerals by sorption mechanism (Zimmerman et al., 2004; Mikutta et al., 2007). Three mechanisms of SOM stabilization have been described: i) chemical stabilization with soil minerals (e.g. clay and silt particles) (Hassink and Whitmore, 1997), ii) physical protection by aggregates, which form physical barriers between the microorganism and substrates (Six et al., 2002) and iii) the biochemical stabilization, due to its own chemical composition (e.g. recalcitrant compounds such as lignin and polyphenols) and through chemical complexing processes (e.g. condensation reactions) in the soil (von Lützow et al., 2007). In allophanic volcanic soils SOM stabilization mechanisms involve: i) the formation of organo mineral complexes and ii) the association of SOM to the allophane type materials, each protecting SOM from decomposition through mechanisms that are still subject of debate (Dahlgren et al., 2004). PE has been linked to amorphous mineral materials (Xue et al., 2005; Rasmussen et al., 2006; Neculman et al., 2013). In young volcanic soils, Al and Fe oxides are the most important agents for SOM

stabilization compared with old volcanic soils which contain allophane-like materials (Neculman et al., 2013). Rasmussen et al. (2006; 2007), studied the importance of the mineralogical composition of soil on PE in temperate rain forests and they found that the amorphous clay-like (volcanic soils) trends to exhibit negative PE in the top soils. Chemical interaction between organic matter and metals promote SOM-complexes and stable macro- and micro-aggregates providing physical protection of SOM (Panichini et al., 2012). This is important because complexed SOM increase the mean residence time and microbial biomass prefer fresh substrate consumption thereby limiting CO_2 losses from native SOM. That results suggest that the whole SOM level is controlled by organo-mineral interactions other than the adsorption mechanism because the specific surface area and adsorptive capacity are clearly different among soil minerals (Wagai et al., 2009). This may lead different PE intensities according to the soil stabilization capacity of SOM.

Summarizing, if we fail to clearly distinguish the real and apparent priming effects, it has important implications because we cannot understand clearly the processes determining C sequestration in soil. So far, no global explanations have been suggested to distinguish from apparent and real PE. It is not clear whether the prime source of CO_2 is SOM or endogenous microbial metabolism (Bell et al., 2003). The difficulties associated to distinguish the apparent from real priming are also because both phenomena are occurred simultaneously (Mondini et al., 2006). This suggest that the contribution of real and apparent priming effect can be estimated by distinctive substrate-SOM-originated from microbial biomass pools and from newly formed SOM (Schneckenberger et al., 2008). Further studies with different labeling of the three major groups (substrate added, microbial biomass and recalcitrant organic soil matter) are necessary to distinguish where the PE occurs (Kuzyakov and Bol, 2006) and to estimate precisely the true effects of priming.

2.4 Limitations, artifacts and biases in evaluating the priming effect

PE has been difficult to evaluate because of several methodological limitations that reduce the possibilities to assess clearly the priming effect. The main limitation reported by several authors, is the excess of CO_2 produced by extra SOM mineralization during incubation process which is difficult to observe in the field [e.g. Bruun and EL-Zehery, 2012)]. PE cannot be assessed if the C-input is not labelled to distinguish the

origin and magnitude of CO_2 released from native SOM. While this is not an easy accessible technique, double labeling with ^{14}C and ^{13}C is even more difficult. PE cannot be shown until the native organic matter is labeled by stable isotopes and not only by using labeled FOM. Very few studies have labeled the very stable C pools and partially humified SOM (Bell et al., 2003). This limitation occurs because the methods for performing separate measurements of CO_2 from the different pools are not simple (Kuzyakov, 2002), as well as the size of the SOM in different ecosystems is so broad and variable, making it difficult to reach consensus on the magnitude and direction of both CO_2 and its effect on the rates of decomposition of SOM (Hungate et al., 1995). While there are many studies using ^{14}C and ^{13}C isotopes to assess the transformation and turnover of SOM (Paterson, 2009), it is not clear whether the CO_2 involved in the effect comes from the more labile or recalcitrant materials (Blagodatskaya et al., 2011). The models do not evaluate the strength of the effect of a mixture of more or less stable and labile C (Guenet et al., 2012).

The mathematical models that describe the mechanisms of PE intensities differ greatly in their concepts, complexity, equations and parameters incorporated (Wutzler and Reichstein, 2008; Manzoni and Porporato, 2009). Furthermore, the dynamics of organic matter mineralization in the short term in different C pools makes simulations very complicated (Vanlauwe et al., 1998). This is even more complex if the influence of changes in land use, agricultural practices, climate and soil vulnerability are included in PE models (Falloon and Smith, 2000). An important obstacle for clear understanding of PE is the discrepancy of proposed mechanisms. For example, the preferential C substrate utilization, PSU (Sparling et al., 1982) and coevolution decomposition enzymes degradation (Kuzyakov and Domanski, 2000) are two extreme different explanations for the same phenomenon. (Sparling et al., 1982) indicated that the more labile substrates present in the soil as rhizodeposits (high-energy) and other more polymerized as SOM, the soil microbes prefer for the use of more readily available substrate. The second mechanism raised by (Kuzyakov and Domanski, 2000) is that SOM mineralization is because FOM in amended soil induces an increase in enzymatic activity which is capable of decompose both SOM and FOM, the so called co-metabolic decomposition of SOM. De Nobili et al. (2001) suggested that the addition of FOM trigger the activity of the microorganisms in latency stage producing the substrate decomposition and consequently their consumption. Fontaine et al. (2003), proposed a mechanism based on microbial competition in which the addition of rich energy FOM with low nutrient availability, favors the growth of k-strategist microorganisms that are able to mineralize the SOM, while r-strategy of rapid growth, consume labile C and nutrients in the soil solution. It is difficult to classify these strategists in different

soil types and substrate added, because of the technical limitation to measure the status of these communities which are very sensitive to biological disturbances (Moyes et al., 2010) (Table 1).

Table 1. Limitations associated to the evaluation of PE and their possible causes

Limitation	Possible causes	Reference
Difficulty to observe the effect in the field.	To recovery labeled substrates under field conditions and non-control of soil moisture and temperature.	(Westerman and Kurtz, 1973; Hamer and Marschner, 2002; Luo et al., 2004; Kuzyakov, 2010; Bruun et al., 2010)
Experiments did not consider the simultaneous interaction of plants, microorganisms and soil fauna.	The circulation of C in soil is influenced by many interacting factors: litter quality, root density, microbial community structure, physical and chemical soil properties,type of vegetation, plant nutritional status and growth rate. Difficult to consider the interaction of all these inducing the PE and tracing the soil C flow.	(Kuzyakov and Domanski, 2000; Lambers et al., 2009; Blagodatskaya et al., 2011; Stewart et al., 2015)
Carbon labeled is required	Analytical studies use labeled organic matter with stable or radioactive isotopes to differentiate between the sources and release of CO_2.	(Hamer and Marschner, 2002; Bell et al., 2003; Kuzyakov and Bol, 2006; Amelung et al., 2008; Cheng, 2009)
Non-uniform labeled materials.	Application of ^{14}C or ^{13}C is a prerequisite; very sensitive to the experimental conditions and non-uniformed labeled materials.	(Hungate et al., 1995; Kuzyakov and Bol, 2006; Paterson, 2009)
Difficulty to distinguish more or less stable and labile C deposits.	The contribution of individual sources of soil CO_2efluxes and no single and fully satisfactory partitioning method do yet exists.	(Kuzyakov, 2002; Blagodatskaya et al., 2011; Guenet et al., 2012)
Discrepancy between mathematical and conceptual models that explain the mechanism or intensities of PE.	Lack of knowledge for understanding the processes that regulate the PE. The models recognize the importance for incorporating the diversity of soil microorganisms, but it is still a difficult goal to achieve. The study of the relationship between the PE and availability of nutrients from soil has proved to be a challenge.	(Fontaine et al., 2003; Wutzler and Reichstein, 2008; Manzoni and Porporato, 2009; Moyes et al., 2010)
Land use-managements, climate and soil vulnerability are not considered.	Landscape and soil complexity are difficult to model including this factor at different scales.	(Falloon and Smith, 2000)

The above mentioned limitations support the conclusions that the consequences associated with PE in the C balance of soils and their impact on C sequestration is potentially huge and it has been studied very little (Loiseau and Soussana, 1999; Fontaine and Barot, 2005; Paterson, 2009).

By artifacts we mean any distortion or biases in priming evaluation caused by including or excluding determinant variables in the experimental designs, which may lead to misinterpretation or erroneous conclusions (Table 2). It is common to see conceptual errors, for example, giving little emphasis on the role of N that controls the processes of mineralization of SOM in the incubation (Blagodatskaya and Kuzyakov, 2008). Recent studies have not considered the potential importance of P for PE, especially in P-limited soil incubations (Cleveland and Liptzin, 2007; Cleveland and Townsend, 2006). On the other hand, in most mathematical models the mineralization of the SOM is represented by the first order kinetics (Friedlingstein et al., 2006). This has been criticized (Fontaine and Barot, 2005, Wutzler and Reichstein, 2008), because it does not represent the existing relationship between FOM (e.g. roots exudates, litter, etc.) and the mineralization of SOM simultaneously. This relationship could be an important mechanism of SOM stabilization in deeper soil layers (Fontaine et al., 2007), which is why they require clear-comparison between the first-order kinetics and any of the alternative decomposition formulations. This produces a biases in the interpretation of the PE due to conceptual gaps.

Besides this, little attention has been paid for the negative PE produced by substrate toxicity incorporated or selective use of C sources by microbial communities (Szolnoki et al., 1963; Degens, 1998; Kuzyakov, 1997).

Other types of errors are commonly produced by not adequate control of experimental variables which generates inaccuracies in the evaluation of PE. It is common for example, not properly separate the CO_2 output sources, which may come from the FOM incorporated into the soil or from the sum of the other sources including labeled SOM, microbial biomass and plant debris (Guenet et al., 2012). The term "apparent" priming has been used for the extra CO_2 evolution derived from experimental errors due to the use of non-uniformly labeled substrate, incomplete trapping of evolved CO_2, addition high amount of substrate to the soil (Jenkinson, 1965; Jenkinson et al., 1985; Brookes et al., 1990; Conde et al., 2005), or greater decomposition of organic matter in response to soil moisture (Niklaus and Falloon, 2006).

These inaccuracies in measurement also occur having no clear scales and limits of determinations. Laboratory conditions usually omit many soil-plant processes, which can also control the PE and SOM

dynamics such as the effect of plant exudates on microbial populations, mineral nutrients, SOM and FOM (Kuzyakov and Domanski, 2000; Kuzyakov, 2002). If the PE is influenced by the interactions of roots exudates, these can shape fundamentally plant functions such as allocation of C and nutrient absorption (Pausch et al., 2013). That is why researchers are required to perform more realistic systems to quantify the relative importance of the mechanisms studied at larger scale.

Table 2. Artifact and biases associated to the evaluation of priming effects and their possible causes.

Artifacts and biases	Possible causes	Reference
Conceptual gaps	Misinterpretations results and conclusions.	(Blagodatskaya and Kuzyakov, 2008; Neill and Guenet, 2010)
Potential importance of phosphorus (P)	Is unknown the P role on the processes associated with the stability of SOM. The effect is evaluated from C perspective rather than P and other nutrients.	(Cleveland and Townsend, 2006; Cleveland and Liptzin, 2007)
Little attention to the negative PE.	Effect occurring only in short term or produced by substrate toxicity.	(Szolnoki et al., 1963; Degens and Sparling, 1996; Kuzyakov, 1997)
Inadequate variable controls in the experiments.	C input too high or too low. It did not resemble field conditions. Use of non-uniformly labeled substrate in the incubations.	(Jenkinson, 1965; Brookes et al., 1990; Conde et al., 2005; Niklaus and Falloon, 2006; Guenet et al., 2012)

PE caused by incorporating single C substrates, such as glucose, cellulose and pyruvate does not reflect the C input and quality under field conditions (Neill and Gignoux, 2006). Experiments performed incorporating litter (Kuzyakov, 1997; Bell et al., 2003) or glucose (Vasconcellos, 1998; Aoyama et al., 2000) effectively induce a PE, but most soils come from cropping and they not considers natural ecosystems under pristine unpolluted conditions or soils with extreme environments conditions (Bell et al., 2003; Hamer and Marschner, 2005). There is a number of substrates applied into the soils that does not resemble natural conditions (Hamer and Marschner, 2002; Hernández and Hobbie, 2010) including the quality of the substrates and its C:N ratio (Craine et al., 2007). Finally very few studies have investigated the consequence of PE intensity due to the incorporation of water-soluble substrates (Shen and Bartha, 1997; Falchini et al., 2003) which effectively induce positive PE (Traoré et al., 2000).

Activation of different microorganisms through the incorporation of different C substrates, as well as the adaptation of these microorganisms to consume them, are important factors controlling the priming effect (Kuzyakov, 2010). PE was detected after addition of plant residues (Malosso et al., 2004; Rasmussen et al., 2007), simple sugars (Jones et al., 2004;Zyakun and Dilly, 2005), amino acids (Hamer and Marschner, 2002; Hamer and Marschner, 2005), glucose (Szolnoki et al., 1963; Zunino et al., 1982; Vasconcellos, 1998; Aoyama et al., 2000), root extracts (Mary et al., 1992) and the root exudates (Dijkstra and Cheng, 2007) , fungal debris (Zunino et al., 1982a) and biochar (Zimmerman et al., 2011) (Table 3). In different environment plant residues contain different amounts of carbohydrates, nitrogen compounds, lipids, and lignin as main organic compounds (Kögel-Knabner, 2002). More labile compounds such as glucose are rapidly assimilated by microorganisms (Perelo and Munch, 2005; Fischer et al., 2010), unlike more polymerised compounds as the litter which is composed of a complex mixture of compounds which are broken through exoenzymes which are produced by fungi and actinomycetes and require greater energy for mineralization (Tavares and Nahas, 2014).

Few studies have used complex substrates close to natural inputs such as straw or roots (Blagodatskaya and Kuzyakov, 2008) and fewer have examined how PE and ultimately storing soil C vary as a function of the quantity and quality input substrate (Mary et al., 1992; Blagodatskaya et al., 2007). Moreover, these studies have yielded conflicting results because of the range of treatments used: mineral organic fertilizer (Jenkinson et al., 1985; Bol et al., 1999; Olayinka, 2001; Leifeld et al., 2002; Fangueiro et al., 2007), exudation organic substances by the roots (Paterson, 2003; Cheng and Kuzyakov, 2005; Cheng, 2009), or plant residue inputs (Bell et al., 2003; Perelo and Munch, 2005).

Table 3. Carbonaceous substrates used for incubation to determine the priming effect

Substrate used	**References**
Alanine, catecol, fructose, catecol, oxalic acid	(Hamer and Marschner, 2002; Hamer and Marschner, 2005; Goulden et al., 1996)
Celullose	(Fontaine et al., 2004; Guenet et al., 2010a, Fontaine et al., 2011; Guenet et al., 2012)
Sugars	(Vasconcellos, 1998; Shen and Bartha, 1997; Jones et al., 2004; Zyakun and Dilly, 2005; Werth et al., 2006; Nottingham et al., 2009)

Root extracts and exudates	Mary et al., 1992; 1993; Traoré et al., 2000; Strobel, 2001; Kuzyakov, 2002; Fu and Cheng, 2002; Curtis et al., 2002; Paterson, 2003; Cheng and Kuzyakov, 2005; Dijkstra and Cheng, 2007; Cheng, 2009; Balogh et al., 2011.
Fungal debris	Zunino et al., 1982b; Jeffery et al., 2011; Luo et al., 2011.
Glucose	Szolnoki et al., 1963; Vasconcellos, 1994; Aoyama et al., 2000; Fontaine et al., 2003; Bol et al., 2003; Bell et al., 2003; Perelo and Munch, 2005; Curtis et al., 2005; Conde et al., 2005; Blagodatskaya et al., 2007; Blagodatskaya and Kuzyakov, 2008; Fischer et al., 2010; Blagodatsky et al., 2010; Jeffery et al., 2011; Blagodatskaya et al., 2011; Bityutskii et al., 2012; Sullivan and Hart, 2013; Bastida et al., 2013; Zunino et al., 1982a.
Plant residues	Sachtler, 1959; Reid and Goss, 1983; Kuzyakov, 1997; Vanlauwe et al., 1998; Stemmer et al., 1998; Kögel-Knabner, 2002; Bell et al., 2003; Malosso et al., 2004; Waldrop and Firestone, 2004; Fontaine et al., 2004; Conde et al., 2005; Dijkstra et al., 2006; Rasmussen et al., 2007; Fontaine et al., 2007; Bader and Cheng, 2007; Blagodatskaya and Kuzyakov, 2008; Nottingham et al., 2009; Cheng, 2009; Neill and Guenet, 2010; Guenet et al., 2012; Conrad et al., 2012; Thiessen et al., 2013.
Biochar	Kuzyakov et al., 2009; Luo et al., 2011; Zimmerman et al., 2011; Cross andSohi, 2011; Awad et al., 2012; Mukherjee and Zimmerman, 2013; Saarnio et al., 2013.

2.5 Linking the priming action with substrate applied and C metabolic pathway; a proposal

Few data are available on successional changes in the composition of microbial community during PE (Guenet et al., 2012). The relationship between primed organic matter and microbial growth is still unclear (Blagodatskaya et al., 2007). The link amongst substrate applied, microbial structural community and C metabolic pathways is lacking, as this can provide a greater insight whether PE is controlled by specific group of soil organisms (Manzoni et al., 2012; Sullivan and Hart, 2013) and what are the factors and mechanisms involved that control C use efficiency, CUE. The mechanism that microbial community controls the magnitude and direction of priming (Kuzyakov, 2010) and how soil mineral properties influences the available C substrate for microbial community is uncertain (Hamer and Marschner, 2005).

2.5.1 Priming effect and preferential use of carbon substrate

Preferential substrate utilization (PSU) of roughly polymerized C substrate or more complex substrates such as rhizodeposicion, with an isotopic signature that are preferred by specific microorganisms indicates a decomposition of the substrates differentiated in time (Kuzyakov, 2002). The term PSU has been used frequently in studies of rhizosphere PE (Kuzyakov, 2002; Cotrufo et al., 2005; Kuzyakov and Bol, 2006), applying the easily degradable substrates such as glucose or sucrose ^{13}C which is preferentially mineralized by microorganisms. Other terms such as selective use of organic compounds has been also used (ŠantRůČková et al., 2000) or differential decomposition (Xu et al., 2010). The PSU is conflicting, since poses the question why microbial biomass is stimulated to breakdown of native SOM instead of FOM labile C substrate (Dalenberg and Jager, 1981; Wu et al., 1993; Fontaine and Barot, 2005). One explanation when a more labile substrate is added into the soil, microorganisms prefer the use of fresh energy-rich materials than most recalcitrant one such as SOM temporarily. This is because most active and fastest growing of microbial communities, r-strategists, uses this newly added substrate and later the k-strategist uses the more recalcitrant one (Fontaine et al., 2003). This is consistent with PSU which generates a temporal decrease in mineralization stabilized substrates (Sparling et al., 1982; Billes et al., 1988; Cheng, 1999). When the labile substrates is depleted the more recalcitrant ones is consumed, restoring the normal balance of substrate utilization (Stenström et al., 2001). The PSU depends on the soil nutrients, because the FOM is generally poor in nutrients and its degradation requires obtaining other additional nutrients (Cheng, 1999; Cleveland and Liptzin, 2007; Reed et al., 2011). Hence the rate of degradation of C substrate mainly depends on the composition of the microbial community which directly affects the activity of the enzyme on C consumption (Schimel and Gulledge, 1998). Mondini et al. (2006) also indicated that the SOM decomposition through addition enzyme released also depends on the interactions between FOM and miners decomposers (Fontaine et al., 2003; Blagodatskaya et al., 2007). An important limitation to the use the C substrates by soil microorganisms is its availability in the soil solution where they are consumed by most microorganisms. Given the scarcity of soluble nutrients may be favored microbial species able to extract nutrients from SOM (k-strategist microbes) relative to the species that feed on the fresh-C and immobilize nutrients from the soil solution (r-strategist) (Fontaine et al., 2003). Thus, when the nutrient availability is low, the mineralization of the SOM is more intense and decreases the formation of native organic matter through the fresh-C humification. On the other hand

when soluble nutrients are enough for soil microbes, the mineralization of SOM decreases and a larger amount of nutrient are stored in the SOM which is known as the "mechanism nutrient Bank", that regulates the amount of plant nutrients requirements (Fontaine et al., 2011).

2.5.2 Carbon use efficiency and nutrient availability

Soil C destabilization following mineralization entails the released of C by microbial biomass upon its C consumption for maintenance and reproduction (Van Veen et al., 1985), i.e. the carbon utilization efficiency (CUE) (Steinweg et al., 2008). The microbial biomass production from anabolism of detritus organic matter determines the conversion of C from the total available C for consumption (Six et al., 2006; Miltner et al., 2012).Part of the C consumed is assimilated into microbial biomass (anabolic metabolism) or is respired to generate energy for cellular processes (catabolic metabolism) (Clifton, 1496; Keiblinger et al., 2012). This is an important parameter in many models of C and is generally the same value (0,4) applied in all ecosystems and environmental conditions (Manzoni and Porporato, 2009; Steinweg et al., 2008). The PE has been associated with PSU (Sparling et al., 1982; Billes et al., 1988; Cheng, 1999; Kuzyakov, 2002). PSU depends on substrate quality (labile versus recalcitrant) (Stenström et al., 2001) and nutrients availability in the soil. FOM is generally poor in nutrients and its degradation requires obtaining other additional nutrients such as N immobilization (Cheng, 1999; Cleveland and Liptzin, 2007; Reed et al., 2011). Soil microbes can adjust their own C:N ratio by releasing CO_2 (Manzoni et al., 2008; 2012) or they can immobilize inorganic soil N to decompose N-depleted substrates (Craine et al., 2007). Thus, CUE has been inversely related with the stoichiometry C:N of the substrate in terrestrial and sediment (Sinsabaugh et al., 2013) and therefore CUE can be related to the PE. CUE values can vary between 0.3 and 0.6 (Sinsabaugh et al., 2013) and factors other than stoichiometry C:N substrate are also involved in its variation (soil type, temperature and water content) (Jenkinson et al., 1990; Albrizio and Steduto, 2003; Chambers et al., 2004; Steinweg et al., 2008; Metcalfe et al., 2010; Manzoni et al., 2012; Koranda et al., 2013). However, CUE, such an important parameter never has been related with PE although both processes are intimately associated.

It is crucial to determine whether CUE vary for amended soils and control soil which can be explained by the relationship between the substrate and several circulation metabolic pathways such as

pentose phosphate, glycolysis, Krebs cycle (Hamer and Marschner, 2005; Dijkstra et al., 2011a,b). When a C substrate is added to the soil, microbes may use several pathways indicating that there is no unique standard (Medlyn and Dewar, 1999; Steinweg et al., 2008; Manzoni and Porporato, 2009) CUE. With variations in the substrate quality, CUE varies by the energy cost of the intracellular and extracellular catabolism (Blagodatskaya and Kuzyakov, 2008). Limitation of nutrients reduces CUE uncoupling catabolism and anabolism in metabolic pathways that release energy and increase the production of extracellular enzymes and polysaccharides (Larsson et al., 1995; Russell and Cook, 1995). In contrast when C source is a labile substrate, CUE decreases because maintenance processes increase (Hart et al., 1994; Ziegler et al., 2005). In many experiments, the PE is determined by the implicit assumption that CUE value does not vary between the amended FOM soil and the control without addition. It should be noted that different CUE values may cause different CO_2 release from the same available C for microorganism consumption and this cannot necessarily be attributed to native SOM mineralization (positive priming). The latter may induce to an interpretation of an apparent PE if the full C-balance is not performed (Fontaine et al., 2004; Kuzyakov, 2011). It is difficult to accept however, similar CUE values between the control and amended soil. This is because CUE may change by the initial C: N ratio of the substrate (Manzoni et al., 2008). For example, Sullivan and Hart (2013) found the PE was inversely related with inorganic N pool in the soil and it could be due to a change in CUE values rather than PE. Thus the relationship between PE and CUE should be expected inversely proportional if there is no different CUE between the control and amended soil.

Until now it is believed that CUE vary depending on the availability of nutrients (Kroer, 1994; Manzoni et al., 2012), but recent studies (Frey et al., 2013) show that the microbial communities acclimatized to lack of available nutrient and more efficiently utilize the recalcitrant C (Bradford et al., 2008; Zhou et al., 2012). Furthermore microbial communities appear to respond differently to the substrates incorporated and this should be the reason why the application of glucose to the soil did not change CUE, but with the addition of more complex substrates such as cellulose, CUE decreased (Steinweg et al., 2008). Microbial communities do a primary control on the amount of CO_2 released to the atmosphere, however very few studies have examined the factors controlling CUE (Frey et al., 2013). Thus small variations in the value of the CUE may be significant effects on soil CO_2 flux (Six et al., 2006) affecting a direct estimation of PE.

2.6 Conclusions and perspectives

Several conclusions can be drawn:

1) There is lack of knowledge to explain the factor and mechanism that induce the PE. There are not nuclear and auxiliary hypotheses that predict the PE, even under controlled conditions. There is a missing links between the interaction of soil microbe's succesional communities and the C use efficiency and how this is related to the metabolic pathway in soil with different stabilization capacities.

2) Until now, none clear hypothesis has been suggested to distinguish from real and apparent PE. The main question to distinguish between the two effects, it is not clear if the main source of CO_2 is microbial metabolism SOM or endogenous CO_2 from microbial activity. Other difficulties associated with distinguishing real and apparent PE are reflected by the fact that both phenomena occur simultaneously and can be observed for a long period or for several minutes to hours. Thus, the only thing that seems to be determinant is the nature of the fresh C incorporated, which in turn determine the microbial substrate use in the soil matrix, which can lead to different intensities of PE according to the soil stabilization capacity.

3) The main limitation to evaluate the PE, is the observation in this field due to many factors interacting and its controls is very complicated. Excess CO_2 produced by the additional SOM mineralization should be assessed by labeling substrate to distinguish the origin and magnitude of PE. This technique is not easily accessible, especially if we want to label the very stable C pools and partially humified SOM. Also the size of the SOM in different ecosystems is so wide and variable, making it difficult to reach a consensus on the magnitude and direction of the PE.

4) The artifacts in the evaluation of PE, which are leading to a misinterpretation of PE, are primarily conceptual, and give little importance to the role of N mineralization processes in the SOM, not considering the importance of P.

5) Because PE is mainly evaluated under controlled conditions, many artifacts may occur simultaneously for examples the soil sampling season, the quantities and quality of substrate applied do not resemble the natural conditions from which information can be drown.

6) Studies on the PE are produced conflicting results, because the experiments still use very little polymerized substrates in their incubations, such as glucose, which is very difficult to find in natural conditions and may induces a rapid microbial growth and an apparent PE. Few studies used substrates that are more complex closer to natural inputs, without the addition of nitrogen fertilizer and few others have examined the intensities of PE on the basis of the quantity and quality of the substrate, associated with the mineralogical composition of soil or different SOM stabilization capacities.

7) Preferential use of substrates by microorganisms is a key parameter in controlling the intensities of PE. The degradation rate of C substrate depends primarily on the composition of the microbial community that directly affects the activity of the enzyme and the consumption C in soils of different mineralogical composition, which can generate adsorption or desorption processes of fresh added substrate into the soil.

8) The carbon use efficiency of soil microbes never has been related with PE although both processes are intimately associated. It is crucial to determine whether CUE varies associated with the preferential substrate utilization and several circulation metabolic pathways.

2.6.1 Future directions:

1) In most experiments, the PE is determined by comparing the respired $^{12}CO_2$ SOM amended soils from $^{12}CO_2$ respired from the control soil without substrate addition. Thus, PE carry out the implicit assumption that carbon use efficiency did not vary between amended and control soil. It is very important to verify this assumption, because different efficiencies may lead different CO_2 released from the two soils inducing erroneous interpretations.

2) Is important test the PE intensity with different substrate qualities and initial stoichiometry C:N ratio of the substrate.

3) Is necessary to evaluate the relationship between PE and microbial carbon use efficiency values in soils with different stabilization capacities on SOM to determine the effect on the PE intensities.

4) Finally, it is important to identify the successional microbial communities involved in PE, since they respond differently to various substrates and they are ultimately determine the C use efficient and preferential C utilization for growth and production of CO_2.

CHAPTER III

Soil carbon controlled by plant, microorganism and mineralogy interactions

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3.1 Abstract

Rhizosphere, a thin area of soil surrounding roots receiving carbon (C) exudation from plants, represents a site of intense competition for available C and nutrient between surface-reactive particles and soil microorganisms. This competition can reduce the amount of available C to a critical level, it becomes limiting for microbial growth and soil organic matter decomposition. On the other hand, acceleration or retardation of decomposition of soil organic C caused by root activity is termed rhizosphere priming effect (RPE). This effect has been increasingly recognized to play a crucial role on native C destabilization as is influenced by fresh C availability, microbial activity and soil mineralogy such as crystallinity of clay minerals and Al-, Fe-oxides. Combining microbial ecology and soil mineral interactions, we can understand how soil characteristics and climate change can influence below ground competition and finally RPE. In this review, we focus on the competition for available C in soil, limiting our analyses to the interaction at rhizospheric space, where most processes between microorganisms and mineral phase occurs.

Keywords: Rhizosphere-priming effect, DOC, mineral interaction, carbon exudates

3.2 Introduction

Carbon input from plant to soil through root exudation is one of the major sources of available carbon (C) for microorganisms (Luo et al., 2014). Exudates from living roots stimulate a quick response of soil microbes with acceleration of native soil organic C mineralization, the so-called rhizosphere priming effect (RPE). Soil microbial activities are driven primarily by readily available or labile C provided by root turnover and root exudates influxes (Dijkstra et al., 2013). Rhizosphere priming can also be affected by nutrient availability and substrate quality (Murphy et al., 2015). These interactive effects may be of particular relevance in understanding microbial growth and nutrient supply in response to increased atmospheric CO_2 concentration and temperature. It is well established that increase in atmospheric CO_2 increases photosynthesis, hence root exudation (Drigo et al., 2008). Competition between microorganisms and soil reactive particles for nutrient and dissolved organic C (DOC) is often neglected in the rhizosphere. Traditionally, it has been assumed that microorganisms can access quickly to labile C released into soil, although a number of biotic and abiotic processes can regulate the relative C in soil solution. In addition to microbial uptake, available C can decrease in the rhizosphere due to diffusion and convection process (Raynaud, 2010), and adsorption by soil particles with formation of soil aggregates (Albalasmeh and Ghezzehei, 2014). Weathered Al and Fe can complex DOC quickly in periods of time ranging from seconds to hours (Sparks, 2003). Microbial uptake of low molecular weight organic substances (LMWOS) (e.g., organic acids, amino acids and polysaccharides) from the soil solution takes place within minutes (Jones et al., 2004). In contrast, the half-life time of the same processes for C from LMWOS adsorbed onto mineral clay is longer – from several hours to months or even decades (van Hees et al., 2005).

We often assumed that microorganisms are better competitors than plant roots for nutrients (Kuzyakov and Xu, 2013), because: i) they are involved in the mineralization process, ii) they present a great surface area: volume ratio and iii) they have rapid growth rates (Peng et al., 2008). Microorganisms have numerous strategies to increase their resource acquisition and competition for available C using biotic (e.g. extracellular enzymes release) and abiotic (e.g. redox and metal complexation) mechanisms (Hibbing et al., 2010). It is difficult to assess direct competition between microorganisms and minerals for soil C because of the multiple loops and pathways, through which C circulates amongst different C pools. Furthermore, abiotic factors such as soil pH, amorphous Fe and Al oxides and hydroxides concentration (Kaiser and Guggenberger, 2000), and/or clay types (McDowell and Sharpley, 2003) contribute to protection of soil organic matter (SOM) from microbial decay (Jastrow et al., 2007). Consequently, the

effects of available C on microbial activity in mineral soils at rhizosphere scale are poorly understood. Here we discuss the impact of fast growing microorganisms competing for soluble C from root exudates substrate in the rhizosphere on priming intensity. Root exudates can be released shortly after C fixation (15 minutes to hours) (Matus et al., 2014b) and soil mineralogy regulates labile C gradient concentration in the soil solution by adsorption processes and indirectly by pore size distribution. The aim of this study is to discuss the biotic and abiotic factors controlling RPE and availability of C for soil microorganism assimilation. We have reviewed recent literature concerning RPE and chemical composition of SOM related to soil mineralogy operating mainly in temperate mineral soils. As a result, this review has three sections. First, we focus on available C and rhizosphere priming and its dependence on labile organic C compound in open interaction with soil surface minerals. In the second section, we discuss the interactions between mineralogy, plant and microorganism control on available C and the implications of these interactions on RPE. Finally, in the third section, RPE as an important mechanism of soil C transport into the subsoil is discussed.

3.3 Available carbon and rhizophere priming

Carbon input by plants into the soil is the primary source of SOM (Kuzyakov, 2002). This input and the availability of C induce fast turnover near the roots, because rhizosphere space is not limited by available C (Kuzyakov, 2002), but C is available in hot spots (Kuzyakov and Blagodatskaya, 2015). The RPE intensity is generally short lived changed by the transformation of C following the addition of moderate levels of carbonaceous and nitrogenous materials, including C rhizodeposition from roots (Kuzyakov, 2002). It is increasingly accepted that RPE intensity depends on the availability of fresh C energy and succession of soil microbial community (Fontaine et al., 2004). Root exudates consist of a complex mixture of C rich substrates, such as sugars (50–70%), carboxylic acids (20–30%) and amino acids (10–20%) (de Graaff et al., 2010). Plant roots exert significant control over the flow rate of C (Lal, 2004). Plants spend a large part of their photosynthetic C in the development and maintenance of the rhizosphere (de León-González et al., 2006). The fate of C exudates released into the soil solution depends on root fluxes, soil mineral adsorption, diffusion across soil pores, and microbial utilization (Kuzyakov et al., 2003). For example, and as previously mentioned, LMWOS are rapidly metabolized, whereas high molecular -weight compounds have to be hydrolysed to low molecular-weight products before they can

be taken up by microbial cells (Weintraub et al., 2007). Neutral sugars can be quickly assimilated by microorganisms, because they are not electrically charged, with a half-life of only 20 to 40 minutes in soil (Fischer et al., 2010). While neutral sugars are fast metabolized, carboxylic acids (e.g. citric and malic acids) bearing charge, can rapidly be adsorbed by soil minerals (Dakora and Phillips, 2002). Half-life of organic acids and amino acids in the soil have been measured between 2 h and 12 h (Jones and Darrah, 1994; Jones and Hodge, 1999). Organic acids, such as citrate, may be strongly sorbed into soil components (clay minerals) with the reduction of the relative mineralization rates (Bruun et al., 2010). Within 10 minutes, 99% and 83% of the added citrate was adsorbed by Fe oxides and kaolinite, respectively. Citrate decomposition was reduced for about 99% and 75% in the presence of Fe oxides and kaolinite, respectively (Jones and Edwards, 1998). Therefore, the organo-mineral complex formation is of a critical importance of controlling organic C availability to soil microorganisms (Lopez-Sangil and Rovira, 2013). Mineralization does not depend on the C input rate only, but also on the substrate quality, the initial N: C: P, and the chemical composition of the substrate. Microorganisms controls the available C by quickly uptake of free sugars from the soil, but represents a small proportion being immediately available in hot spots (Kuzyakov and Blagodatskaya, 2015). Most SOM is present in forms that are unavailable to microorganisms (Fontaine et al., 2003). The short-lived RPE in the rhizosphere related to the fast consumption of C during initial stage of mineralization is poorly understood. In summary, it is important, therefore, to consider microorganisms and soil mineralogy competing for fresh organic C for microorganism assimilation. The interactions of these factors can accelerate (positive) or reduce (negative) the RPE by the decomposition of native soil organic matter (Dijkstra et al., 2009). The magnitude and direction of RPE are determined by the amount and type of C exudates released from the root, as well as intrinsic soil mineralogy. At the most basic level, understanding the general characteristics of C fluxes within the soils is difficult because of the diversity of microbial communities, the complexity of mineral soil interactions and the inherent environmental heterogeneity of soil ecosystems.

3.4 Mineralogy, plant and microorganisms control the available carbon

Very little is known about adsorption or desorption of different pools of organic components related to RPE in different soil types due to different mineralogy (Fischer et al., 2010). Rasmussen et al. (2007) studied the importance of the mineralogical composition on C mineralization in temperate rain forests.

They found soil with amorphous clay (volcanic soils) showed a negative priming (Xue et al., 2005, Rasmussen et al., 2006) attributed to the strong adsorption of organic compounds, probably by ligand exchange reaction (Matus et al., 2014a). Soil mineralogy controls the available C in the soil solution not only by adsorption processes, but indirectly limiting diffusion of oxygen and water due to the importance of soil minerals in the formation of soil aggregates (Six et al., 2004). This makes available C in soil less vulnerable to microbial degradation (Kalbitz and Kaiser, 2008).There is a physical occlusion of C in the interstitial space mesopores (2-50 μm) in mineral soils (Zimmerman et al., 2011). The interactions between mineral phase and organic matter can lead to a stabilization of available C trapped or physically protected in soil micropores (< 2 μm) (Baldock and Skjemstad, 2000). Apart from physical protection, as it was already mentioned, the intermolecular interactions between C and surface of clay particles and oxides of Fe and Al play an important role too (Matus et al., 2008). Amorphous Al and Fe oxides are the most reactive components of acidic and neutral soils (Matus et al., 2014a) and have a key function in chemical speciation, bioavailability of nutrients and detoxification (Olaniran et al., 2013).

The role of Al and its effects on water extractable organic matter was evaluated in two old growth temperate rain forests (Merino et al., personal communication). Mineralization of soluble C was not affected by increasing Al addition rate and potential toxicity by Al^{3+} was not observed after 15 days of incubation experiment. This study suggests that Al3+ is toxic when Al:C ratio < 0.12 (Scheel et al., 2008). However, the effects of Al on natural DOC degradation in mineral soils of forests are poorly understood and it is necessary to study Al-humus formation and Al3+ detoxification for their effect on C availability. Indeed, the quantitative information on the degradation of available C has been obtained by studying the decomposition of LMWOS differing for the position of labelled C (Apostel et al., 2015). Alanine ^{14}C labelled in C-1, C-2 and C-3 position was adsorbed by iron oxides, clay minerals (2:1 and 1:1) and activated charcoal. The highest sorption capacity resulted in the low C utilization by soil microbes. Mineralization of alanine peaked within the first 5 h and it was always the highest for C-1 position (–COOH group), whereas C-2 and C-3 mineralization exceeded the mineralization rate of C-1 after 10–50 h. The metabolic pathways, i.e. glycolysis depended on C oxidation and the Krebs cycle of sorbed Alanine at initial stage of decomposition (Dippold et al., 2014). This raises the question whether these low molecular weight compounds are used by the overall soil microflora or by different microbial groups. On the other hand, plants may alter the dynamics of microbial C fluxes and C use efficiency by balancing the catabolic and anabolic metabolism in the rhizosphere. Carbon utilization efficiency by soil microbes was 1.5 times higher in root-free soil than in the rhizosphere soil (Blagodatskaya et al., 2014).

Extracellular soil enzyme activities play a key role in RPE by breaking down native organic matter (depolymerization, e.g., peroxydase) producing soluble simple compounds for microbial assimilation (Sinsabaugh et al., 2009; Sinsabaugh, 2010; Nannipieri et al., 2012). Once organic C is inside the microbial cell, it can be mineralized to CO_2 with the production of ATP (Maire et al., 2013). Under aerobic conditions, the oxidative metabolism of organic compound produces more energy, ATP and CO_2 than the anaerobic metabolism (Zhu et al., 2014).The literature on soil enzymology is extensive (Nannipieri et al., 2012) as well as the enzyme-organo-mineral interaction such as the enzyme adsorption to minerals (Nannipieri et al., 1996) and enzyme–clay interaction (Gianfreda and Bollag, 1994), but the role of enzymes-organo mineral on RPE is poorly understood. Some priming studies have looked directly at enzyme activities, but the results have been contradictory (Table 1). If biotic processes are modified by soil mineralogy, the latter also plays a control on C turnover with an important catalytic role in accelerating abiotic polymerization of phenolic compounds and amino acids to the formation of humic substances. Silicates and Fe oxides can catalyze redox reactions and promote SOM oxidation (Derry et al., 2005). Besides, enzyme like reactions can affect C turnover in soil (Acker and Auld, 2014). For instance, humid tropical forests have the fastest rates of organic matter decomposition, which often coincides with fluctuating oxygen (O_2) availability in surface soils. Alike tropical soils, humid temperate rain forest soils are typically rich in short-range ordered iron oxide Fe^{3+} minerals. Those soils show fluctuating oxygen availability over scales of hours to weeks where Fe^{3+} is reduced to ferrous $Fe^{2+}$and subsequently re-oxidized via biotic or abiotic reactions (Dubinsky et al., 2010; Hall et al., 2013). This mechanism stimulates organic matter decomposition via: organic matter oxidation, likely driven by reactive oxygen species; and acidification (Hall and Silver, 2013). Dissimilatory Fe reducing bacteria are well known to oxidize soil organic matter and can account for the majority of C oxidation under anaerobic conditions (Dubinsky et al., 2010). Abiotic processes have been underestimated across soil profile and should be addressed in future research.

3.5 Rhizosphere priming effect and molecular transport to the subsoil

Complexation of labile compounds with metal such as Al and Fe Oxides provides a direct mechanism for translocation of organic C within the soil profile (Kaiser and Guggenberger, 2000). Sorption by mineral surfaces can protect simple molecules from microbial degradation to some extent van Hees et al. (2002);

Jones and Edwards (1998) compared degradation and sorption of citrate and glucose, and similar studies by Jones and Hodge (1999) were carried out for glutamate, glycine and lysine. Results from both investigations indicated that the reduction of the availability of C for microbial assimilation depended on the type of root exudates and the mineral type. Adsorption of simple molecules onto clay surfaces is almost irreversible, that means short term mineralization of these organic acids while they are available (Boudot, 1992). The studies of Jones (1999); Jones and Hodge (1999) and Owen and Jones (2001) have shown a rapid mineralization of free amino acids (glycine, glutamate and lysine) by microflora from soil solution. Preferential C uses by soil microorganisms of these compounds can lead to a change in the SOM turnover induced by fresh C input (Sparling et al., 1982) from not exudate. We have already mentioned that Rasmussen et al. (2006; 2007) studies in which mineralogical composition of surface forest soil induced a negative priming because of amorphous minerals. Besides, the chemical interaction of SOM, Al- and Fe-oxides can protect SOM against microbial degradation, since it promotes SOM-humus complexes and stable soil aggregation thereby providing physical protection of SOM aggregation (Panichini et al., 2012). It has been long hypothesized that non-crystalline minerals like allophane as been formed in situ, rather than by translocation (Dahlgren et al., 2004). However recently, the transport of mineral-SOM complexes to deeper soil has been reported by conversion of tropical forest into grassland (Osher et al., 2003). In high precipitation regions, C losses from the soil appear to occur via downward transport, either as colloids or in solution. There are almost no studies that address the organic translocation in the subsoil. Kaiser and Kalbitz (2012)Indicates that adsorbed organic compounds can be desorbed because of protective site saturation. This effect is due to complex reactions of Al with soil organic C from the soil solution and the subsequent precipitation of insoluble complexes of Al-SOM (Rasmussen et al., 2006). It is needed empirical evidence showing the importance of labile compounds available for microorganism and transport. This is important for determining the type of organic matter that is sorbed under specific conditions.

Table 1. Organic compounds and enzymes identified in root exudates used in studies of priming effect.

Organic compounds	C-input g C kg^{-1}soil	Ezyme activity	References
Citric acid	0.3–0.6	Phosphomonoesterase[a], Urease, phosphodiesterase	(Lundström et al., 2000; Clemens et al., 2002; Renella et al., 2007; Luo et al., 2014)
Oxalic acid	0.6	Phosphomonoesterase	(Lundström et al., 2000; Zheng et al., 1998;Yang et al., 2000; Luo et al., 2014)
Acetate	8	β-glucosidase, phosphomonoesterase	(Allison, 2005)
Malic acid	6–41		(Clemens et al., 2002; Chowdhury et al., 2014; Rukshana et al., 2012)
Glucose	0.6–25	Phosphomonoesterase, urease, casein-hydrolyzingenzymes	(Luo et al., 2014; Strickland et al., 2012; Nannipieri et al., 1983)

[a]We have replaced the term acid or alkaline phosphatase by "phosphomonoesterase", since the term phosphatase includes several enzymes in biochemistry e.g. phosphomonoesterases, phosdiesterases, etc (Nannipieriet al., 2011).

3.6 Concluding remarks

- Root exudates into the soil solution can be: 1) consumed by soil microorganism and degraded, 2) mineralized abiotically by mineral catalytic effect or, 3) leached from the soil profile, 4) sorpbed to the solid phase or even taken up by plants. . .The low molecular weight of organic substances assimilated directly from soil solution might affect the intensity of RPE, but this process depends on spatial heterogeneity of the rhizosphere.
- Available C for microflora consumption depends on the mineral composition and the type of exudates, and their relative concentrations.
- The microbial interactions with the mineral phase can affect reactions and the process of soil C. Organic C derived from microbial activity and root exudates is probably the most mobile and bioavailable fraction of C in the rhizosphere. The retention and mobility of organic compounds depend on soil properties and can affect the availability of soluble C for soil microbes.

- The effects of root exudates and DOC on RPE across the soil profile need to be further investigated, because the primed compounds may occur in hotspots at root scale.
- Aluminium humus-complex formation needs further attention because Al may be toxic for soil microorganisms, but also it can detoxify the soil solution and enhance C assimilation.
- The saturation of the C-storage capacity of soil, mainly due to organo-mineral interactions, can affect the transport of DOC to deeper soil horizons.
- Rhizosphere priming effects may occur, but it is unknown whether it affects SOM mineralization across the soil profile. There is evidence that RPE on the topsoil SOM of Volcanic soils under forest cover may account for approximately 1/5 of the annual CO_2 evolution from the soil.

3.7 Future directions

- The hypothesis that RPE is driven by low molecular weight organic substances from root exudates requires further research. Based on current knowledge, research regarding SOM competition between soil microflora and mineral phase over time also needs to be addressed in future studies.
- The hypothesis that DOC is the most susceptible to stabilize through different organo-mineral interactions through the soil profile requires further studies.
- We have hypothesised that SOM mineralization due to RPE may be important across the soil profile. This is supported by new conceptual models explaining colloidal transports in soil with different mineralogy.
- The composition of microbial communities during RPE needs to be monitored in soil with different mineralogy. In high reactive soil e.g. allophanic, RPE will be less intense than soils with crystalline clays.

CHAPTER IV

Soil microorganisms and enzyme activity at different levels of organic matter stability

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4.1 Abstract

Biodiversity of soil microbial community has important implications for the stability and functioning of ecosystem processes. Yet, very little is known about the environmental factors, particularly the effect of mineralogy that define the microbial activity in temperate rain forest of south of Chile. In this study, we determined the influenced of soil type (clay mineralogy) and its effects on the biological activity at different level of soil organic matter stability. Two soils an allophanic derived from recent volcanic ash and kaolinitic soil derived from metamorphic parent materials were physically fractioned in light (LF coarse sand 250-2000 µm), intermediate (IF 53-250 µm) and mineral fraction (MF < 53 µm). Several biological and biochemical analyses such as soil respiration, enzymatic activities (β-glucosidase, dehydrogenase, urease and acid phosphatase), carbohydrates (total and soluble), reducing sugars, microbial biomass, ATP and other nutrient (inorganic P and mineral N) were determined. The results indicated that soil and fractions had a significant impact on all enzymatic activities and C mineralized during 30 d of incubation. More than > 76 % of total C-CO_2 was explained including β-glucosidase, dehydrogenase, phosphatase and inorganic P by using stepwise multiple regression analysis. ATP was positive and highly correlated with C-CO_2 and metabolic quotient (soil respiration rate divided by microbial biomass). We found an inverse relationship between Al pyrophosphate (Al bound to SOM) and C-CO_2 evolved between 15 and 30 d of incubation in the volcanic soils. The same correlation did not occur in the kaolinitic soil. The result indicates that there is a great stabilization capacity in the MF of allophanic compared with kaolinitic soil.

4.2 Introduction

It is important to know how soil microorganisms respond to environmental changes of available carbon (C) to predict the response of soil ecosystem under climatic change scenarios (Post et al., 2009; Nie et al., 2013; Zhao et al., 2014). This requires evaluating the physical changes at microbial scale and how these changes affect the response, function and distribution of soil microorganism communities (Xiong et al., 2012; Gifford et al., 2014). It seems very difficult now to study the changes at species level given the extreme microbial diversity in soil and its rapid turnover (Evans and Wallenstein, 2014). For instance, the addition of fresh labile C that generally induces an acceleration in the turnover of native SOM, the priming effects, might produce an amount of extra C mineralization, greater than the fresh C incorporated into the soil. This phenomenon has been scarcely studied in soils with different mineralogy (Rasmussen et al., 2006; Kuzyakov, 2010).

From a physical point of view the habitat for soil microorganisms is distributed in different soil compartments (physical fractions) that depends of pore size distribution, mineral composition and availability C (Van Gestel et al., 1996; Barberán et al., 2014). Species–energy is a commonly invoked theory predicting a positive relationship between species richness and available C. It is well known that soil microbes are unable to directly assimilate complex and solid SOM, but rather simple and dissolved compounds for growth and metabolisms. Consequently, soil microbes produce extracellular enzymes to make readily usable dissolved compounds. For example, β-glucosidase, a hydrolytic enzyme can act on β-1,4 linkage of oligomers to produces low molecular weight and soluble compounds (Dashtban et al., 2010; Floudas et al., 2012). Therefore, when available N in soil limits the microbial activity, microorganism may even produce oxidative enzymes, to degrade recalcitrant organic matter and liberate occluded N (Brzostek and Finzi, 2010; Yang et al., 2014). Dornbush (2007) found that the N content of grass litters had a positive correlation with the activity of β-glucosidese family enzymes. Microbial enzyme production in response to the quantity and quality of SOM in different soil compartments needs to be better understood to reveal ultimately the impact of SOM stability on C mineralization dynamics. Physical fraction and soil mineralogy might indicate different C stability and functionalities for microbial communities. Different methods have been developed, but the most popular ones are those using liquids with different density to identify the mechanisms that control the changes in C and N dynamics pools (Christensen, 1992; Jolivet et al., 2003; Jastrow, 1996; Six et al., 2002). However,

one of the simplest method that can be applied in volcanic and non-volcanic soils is the simple physical fractionation by wet sieving (Kemper and Koch, 1966).

The present study was developed in the Andean and coastal range forest ecosystems in southern Chile in old growth *Nothofagus* species in volcanic (allophanic) and metamorphic (kaolinitic) soils, respectively. These sites offer an ideal opportunity to investigate the influence of soil mineralogy on C stabilization in different physical fractions. The biological activity in response to soil different minerals composition and different level of SOM complexity (stability) can provide a valuable information on C mineralization dynamics.

The aim of this study is to determine the biological activity in various physical fractions in soil of contrasting SOM stabilization capacity (allophanic and kaolinitic soils). We also determine the enzymatic activity, abundance of fungi and bacteria in the same soil and physical fractions.

4.3 Materials and Methods

4.3.1 Site characterization

The present study was developed in an allophanic soil developed from recent volcanic ash in the Andean range and in a metamorphic kaolinitic soil from a coastal range. Both sites belongs to temperate rain forest ecosystems in southern Chile. The combination of low nutrient input and different SOM stabilization capacities are consider interesting attributes to evaluate the stabilization capacity on soil respiration using various biological and biochemical tools. The volcanic soil is located in the Puyehue National Park (PNP) (40° 58' S and 71°50' W) at 800 m.a.s.l. (Godoy et al., 2001; Oyarzún et al., 2004; Matus et al., 2006) in a virgin forest (*Nothofagus betuloides* (Mirb.) with a mean annual precipitation > 3.500 mm and mean annual temperature (MAT) 9.2 °C (Oyarzún et al., 2004). They displays unique morphological, physical and chemical properties attributed to the composition of their mineral phase consisting of short range ordered (SRO) materials like allophane, imogolite, ferrihydrite and an important amount of Al- and Fe–humus complexes (Matus et al., 2008). The second area of study is Alerce Costero National Park (PAC) (40°12´ S-73°26´W, 1000 m.a.s.l.) in the Cordillera de la Costa. The soil is developed from metamorphic-schist with dominant presence of kaolinite (Luzio et al., 2003). The PAC is an ancient forest (*Fitzroya*

cupressoides (Mol.) Johnst., mixed with *Weinmannia trichosperma* Cav., and *Notohofagus nitida* (Phil). The mean annual precipitation > 4,000 mm and mean annual temperature of 12.1 °C.

4.3.2 Soil Sampling

Soils were sampled in the Ah mineral horizon (5-10 cm) after removal of organic litter horizon at the two sites. The soil was transported immediately to the laboratory under cold conditions, homogenized, sieved to < 2 mm and characterized by soil pH in water, soil organic C (SOC), total N, inorganic P (Olsen) and other macro-nutrients (Table 1).

Table 1 Soil characteristics used in this study

Analysis	PNP	PAC
SOC[1] (%)	11.0	9.9
N total (%)	0.7	0.4
C to N	16.3	23.6
P (mg kg^{-1}) Olsen	9.0	4.0
K (mg kg^{-1})	207.0	86.0
$pH_{wáter}$	6.2	4.3
Al pyrophosphate (g kg^{-1})	11.0	5.7
Cations (cmol(+)kg^{-1} soil)		
K	0.5	0.2
Na	0.1	0.1
Ca	2.8	0.5
Mg	0.7	0.6
Al (potasium chloride)	1.2	18.4
Saturation de Al (%)	22.4	93.5
CEC[2] (cmol(+)kg^{-1} soil)	5.3	19.7
Base sat. (cmol(+)kg^{-1} soil)	4.1	1.3
Clay type[3]	allophane	kaolinite
Texture[4]	SL	CL

[1] Soil organic carbon
[2] Cation exchange capacity
[3] Luzio et al. (2003); Neculman et al. (2014)
[4] SL = silty loam and CL = clay loam

4.3.3 Physical fractionation of soil organic matter (SOM)

We use Balesdent et al. (1991) method for physical fractionation. From each soil, three physical fractions were obtained in triplicate after removal the organic materials floatable in water: Light fraction (LF, coarse sand > 250 μm), intermediate (IF, fine sand 50-250) and mineral (MF, silt and clay < 50 μm) fractions. Briefly, a portion of 50 g of moist soil sample was suspended in 180 mL of demineralized water in a 500 mL capped plastic bottle with flat bottom containing 10 glass beads (5 mm diameter).

After 16 h shaking (40 cycles min^{-1}) the soil suspension was poured into a 250 μm sieve. Material remaining on the sieve consisted of large and small visible fragments of plant and animal structures plus coarse sand size particles. The material retained by the sieve was placed in a glass beaker and washed several times with water. We collect any floating material. Soil material < 250 μm consisted in fine sand particles and MF. The last fraction was separated using 50 μm sieves. The MF is assumed to be micro-aggregates composed of silt and clay, whereas IF is assumed to be the fine sand size grain and LF is composed from coarse sand. All soil samples were dried at 35 ºC to avoid damage in microbial biomass.

4.3.4 Incubation

About 10 g (dry weight basis) of whole soil or physical fractions (previously inoculated) were placed in 250 ml flask (Schott) with a tight rubber stopper and incubated for seven days at 26±2 °C in dark at 60% of field capacity (-33 kPa). This procedure allowed removing the POM fraction. At the end of pre-incubation, cellulose substrates (1 mg C g^{-1} soil) was added.

All soils were again incubated with three replicates with 10-ml of 0.5 M NaOH in duplicated. At each sampling 1, 3, 7, 15 and 30 days of incubation, the NaOH was potentiometrically titrated back with 0.5 M HCl. The C mineralization rates (d^{-1}) were determined as:

$$\text{C-mineralization rate} = \frac{\text{Ln}\ (Ct_2) - \text{Ln}\ (Ct_1)}{t_2 - t_1}\ , \qquad (1)$$

where, Ct_1 and Ct_2 is the cumulative C mineralization (mg C–CO_2) at t_1 and t_2, respectively.

4.3.5 Soil analyses

The mineral N (NH_4 and NO_3) was extracted with 1 M KCl solution for 1 h at a soil–solution ratio of 1:10. Mineral N was measured by the Kjeldahl method with magnesium oxide (MgO) and Devarda's alloy (Binkley and Vitousek 1989). Inorganic phosphorus was determined by the method Olsen. Olsen-P was measured by extracting with 0.5 M NaHCO3 adjusted to pH 8.5, according to the (Olsen et al., 1954) method. To determine Al and C associated with pyrophosphate (pH 10), we performed an extraction with 0.1N sodium pyrophosphate on air dried soil. The extract obtained was determined by aluminum (van Reeuwijk, 2002) with atomic absorption and organic C (C_p) with WB method. We used a soil: solution ratio 1:100 that was shaken for 16 h. The suspension was centrifuged (15 min at 2,500 rpm, with three drops of superfloc) and the supernatant was filtered 0.45 µm in polycarbonate filter.

4.3.6 Biological and biochemical analysis

Biomass C was determined by fumigation of the sample with ethanol-free $CHCl_3$ and extraction with 0.5 M K_2SO_4, according to Vance et al. (1987). Soil metabolic quotient (qCO_2) was calculated as the soil respiration rate divided by microbial biomass C (Anderson and Domsch, 1990) and served as indicator of metabolic C use efficiency of microbial biomass present in the sample.

We determine the activity of four enzymes: β-glucosidase, dehydrogenase, urease and acid phosphatase. The reference method for the determination of these enzyme activities is described by Tabatabai (1982) and Eivazi and Tabatabai (1988). Dehydrogenase activity was determined by the reduction of 2-p-iodo-nitrophenyl-phenyltetrazolium chloride (INT) to iodo-nitrophenyl formazan (INTF). Dehydrogenase activity was measured in 0.25 g soil, following incubation in the dark with 0.2 ml of 0.4% INT in distilled water for 20 h at 22°C. The INTF was extracted with 10 ml of methanol by shaking vigorously for 1 min and filtering through a Whatman N° 5 filter paper. The INTF was measured

spectrophotometrically at 490 nm. Urease activities were determined in 0.1 M phosphate buffer at pH 7; 1 M urea. Two ml of buffer were added to 0.25 g of the soil sample (in triplicated), which was incubated at 37 °C for 30 min. Both activities were determined by the NH_4 released (Nannipieri et al., 1980) . Phosphatase and β-glucosidase activities were determined using p-nitrophenyl phosphate disodium (PNPP, 0.115 M) or p-nitrophenyl-b-d-glucopyranoside (PNG, 0.05 M) as substrates, respectively (Masciandaro et al., 1994). These assays are based on the release and detection of p-nitrophenol. Two ml of 0.1 M maleate buffer (pH 6.5 for both phosphatase and b-glucosidase activities) and 0.5 ml of substrate were added to 0.25 g of sample and incubated at 37°C for 90 min. The reaction was stopped by cooling down; 0.5 M $CaCl_2$ and 2 ml of 0.5 M NaOH were then added and the mixture centrifuged at 2000 g for 5 min. To stop the b-glucosidase assay, trishydroxymethyl aminomethane (THAM) was used according to Tabatabai (1982). The amount of p-nitrophenol was determined using a spectrophotometer at 398 nm (Tabatabai and Bremner, 1969).

4.3.7 Carbohydrates

Carbohydrate (CHO) and total soluble carbohydrates were analyzed by the method described by Safařík & Šantrůčková (1992). 0.25 g sample and were translated to glass tubes were weighed, it was added 1 mL of 72% sulfuric acid plus 20 mL of water, placed in bath marie at 104 ° C for 5 h, then (Whatman No. 40) was filtered to a ball 50 mL flush. The extract was taken was added 1 mL and 4 mL of anthrone reagent (4 g in 200 mL anthrone sulfuric acid), stirred and placed in water bath at 80 ° C for 10 min. It was cooled on ice for 10 min and then the absorbance was measured at 660 nm. The amount of reducing sugars released by microorganism was determined by the 3,5-dinitrosalicylic acid (DNS) method (Miller, 1959). DNS reagent was prepared by adding 1 g of DNS and 30 g of sodium potassium tartaric acid to 80 mL of 0.5 N NaOH. The solution was kept at 45°C for the complete dissolution of the reagent and then cooled down at room temperature and diluted with distilled water to 100 mL. The solution was stored for two weeks at 4°C. For the measurement, 0.4 mL of DNS reagent was added to 1 g of soil in tested tubes and they were kept at 95°C for 5 min using Eppendorf Termomixer Comfort. The absorbance was measured at 540 nm.

4.3.8 ATP determination

ATP was extracted from soil using Webster et al. (1984) procedure and measured as recommended in Ciardi and Nannipieri (1990). Twenty millilitres of a phosphoric acid extractant was added to 1 g of soil, and the suspension was shaken in a cool bath, filtered through Whatman paper and an aliquot was used to measure the ATP by luciferin–luciferase assay in a luminometer (Optocomp 1, MGM Instruments, Inc.).

4.3.9 Data analysis

The statistical package (S) MATR was used to examine univariate relationships among soil properties. ANOVA test and correlations amongst different soil properties were conducted by JPM software and SPSS 21. All analyses were conducted with a significant *P* value of 0.05. Regression analysis to test the effect of soil properties on C mineralization (attributed to the SOM stabilization) were conducted following the approach used by Matus et al. (2006). The multiple regression analysis was carried out to test whether the combined effects significantly increased R^2 values compared with those of simple linear correlations. Multiple regression has been criticized because of the inclusion of a multiplicative term (or interaction), which is difficult to interpret (Garrido and Matus, 2012). In the present study, this disagreement was avoided using an additive model in which no multiplicative terms were included; we assumed that the effect of an independent variable strongly reflected the change of the dependent variable, regardless of the level of other effects (Friedrich, 1982). The multiple regression analysis allowed us to perform a stepwise history and forward elimination. Mallow's criterion was used instead of the error mean square to select the best model. A model was selected when Mallow's criterion approached the probability value (0.05), and the number of parameters in the equation significantly increased the R^2 values. All data were tested for the normality of the distribution using a skewness test value of 0.5 (Webster and Oliver, 2001). If the skewness value was >0.5, we concluded that the distribution was not normal. All analyses were computed using JMP statistical software (SAS Institute, Cary, NC, and U.S.A).

4.4 Results

4.4.1 Soil properties

The total recovery from LF, IF and MF to total soil was close to 100%. The proportion of MF in PAC soils was 55% compared with 34% of PNP soils (Table 2).

Table 2 Soil and physical fractions characterization of studied temperate rain forest in Puyehue National Park (PNP) and Alerce Costero Nationl Park (PAC).

Forest	Fraction[1]	Proportion	SOC	Al_P[3]	MB-C[5]	ATP
		%	g kg^{-1}	g kg^{-1}	g kg^{-1}	ng g^{-1}
PNP	LF	45.1±5	271 ±4	4.9 ± 1.3	3.8±0.8	3.27±0.4
	IF	20.6±3	113 ±32	7.2 ± 0.2	2.5±0.5	0.92±0.0
	MF	34.3±3	205±6	9.8 ± 1.7	3.8±1.2	0.07±0.02
	Bulk	100	99 ±15	11.0 ± 1.5	3.8±0.5	2.48±0.1
PAC	LF	15.1±2	95 ±12	2.0 ± 0.3	0.9±0.6	1.88±0.04
	IF	30.4±1	88 ±20	1.2 ± 0.0	2.1±0.4	0.99±0.1
	MF	54.5±5	119±6	2.5 ± 2.4	2.5±0.4	0.04±0.01
	Bulk	100	110±7	5.7 ± 0.2	3.4±0.8	2.32±0.2

[1]LF = light fraction, coarse sand 250-2000 µm; IF = intermediate fraction, fine sand 53-250 µm+particulate organic matter (POM) and MF= mineral fraction, silt and clay < 53 µm;
[2]Soil organic carbon by Wlkley and Black (1934);
[3]Aluminium extracted with Na-pyrophosphate;
[5]C in the microbial biomass.

The allophanic soils in PNP is silty loam (CIREN, 1999), while in kaolinitic metamorphic soil it is clay loam (Luzio et al., 2003). Differences in recovery of MF in both soils were consistent with soil texture. The SOC in both bulk soils was similar however, the MF in PAC soil showed almost half the amount of SOC found in PNP and it was always lower in the other fractions too. The Al_p (pyrophosphate) is an indicator of Al complexed with organic matter, particularly this indicator is important in volcanic soils. As expected, Al_p in PNP doubled the amount found in PAC soil. The MB C obtained by fumigation and extraction varied between 0.9 and 3.8 g C kg^{-1} soil and were similar in all fraction and bulk soil, except in LF of PAC soil. ATP followed the similar trends as for MB (Table 2).

4.4.2 Incubation

In all soils and fractions the mineralization of C reached between 162 and 1000 mg $C\text{-}CO_2$ kg^{-1} soil after 30 days of incubation and it was always lower in PAC soil (Fig 1) Depending on the fraction the mineralization decreased LF > IF> MF in both soils (Fig 1A and 1B) . In general, the mineralized C tended to stabilize after 15 days of incubation (Fig 1C and 1D).

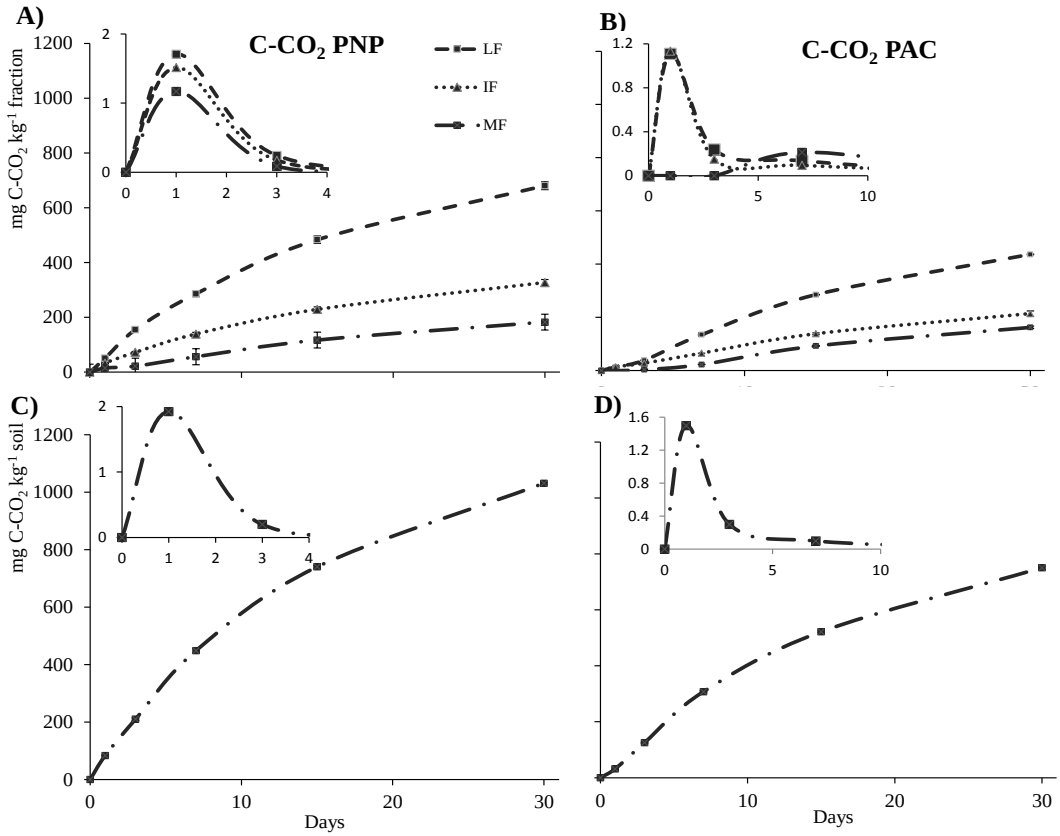


Fig.1. Carbon mineralization in the (A-B) soil fractions and (C-D) bulk soil from temperate rain forest of Puyehue National Park (PNP) and Alerce Costero Nationl Park (PAC).

4.4.3 Enzymes activity, carbohydrates and other soil parameters

The enzymes secreted by soil microorganisms in different fractions provide information about the potential activity of the soil microbial community. We were interested in the enzyme and organisms that participate in the degradation of plant residues mainly constituted by cellulose. All microbiological and soil parameters [β-glucosidase, dehydrogenase, phosphatase, urease, carbohydrates (total, soluble and reducing sugars), inorganic N (ammonium and nitrate), inorganic P and C-CO_2] in the bulk soil and fractions were normally distributed (Shapiro-Wilk test > 0.05). The effect of soil type and fraction was analyzed by two way ANOVA. Significant effect for the interaction (soil x fraction) were obtained for all enzymes activities and reducing sugars (Table 3). Only the effect of soil and fractions (except soil for phosphatase) were significant for inorganic P and C-CO_2.

The average of enzymes activity after 30 days of incubation is presented in Fig 2A and 2B. The β-glucosidase (βG) and dehydrogenase (DH) followed similar pattern in the bulk soil and in the fractions. Both enzymes were higher in PNP bulk soil, probably by the influence of LF (45 % of total weight) in comparison with 15 % in PAC soil (Table 2).

This situation was different in the fractions where βG and DH were significantly higher in MF of PAC soil. Urease did not follow consistent trends (Fig 2C). Phosphatase activity decreased as the fraction size decreased in both soils (Fig 2D).

Although the total and soluble carbohydrates were not significant by the effect of soil and fraction, they were quite consistent with the pattern found for βG and DH (Fig 3A and 3B). This was particularly true for the MF of reducing sugars where the most bacteria reside (Fig 3C).

Table 3 Significant effect of soil type (allophanic and kaolinitic) and physical fraction (LF, IF and MF) on various biological and inorganic soil properties of studied temperate rain forest in Puyehue National Park (PNP) and Alerce Costero Nationl Park (PAC).

		βG		DH		U		P_{ase}		RdSug		Pi		$C\text{-}CO_2$	
Source of Variation	DF	F-ratio	Prob > F	F-ratio	Prob > F	F-ratio	Prob > F	F-ratio	Prob > F	F-ratio	Prob > F	F-ratio	Prob > F	F-ratio	Prob > F
Soil (A)	1	0.02	0.87	1.88	0.17	1.96	0.17	9.99	0.01	16.10	0.001	0.225	0.64	8.29	0.01
Fraction (B)	2	3.63	0.03	3.54	0.03	0.834	0.43	11.55	< 0.0001	4.01	0.02	11.54	< 0.001	28.00	< 0.0001
AxB	2	35.52	< 0.0001	33.20	< 0.0001	10.74	< 0.001	1.32	0.27	14.02	< 0.0001	0.23	0.80	2.20	0.12

βG=β-Glucosidase, DH = Dehydrogenase, U = Urease, P_{ase}= Acid phosphatase, RdSug= Reducing sugars, Pi = Olsen P.

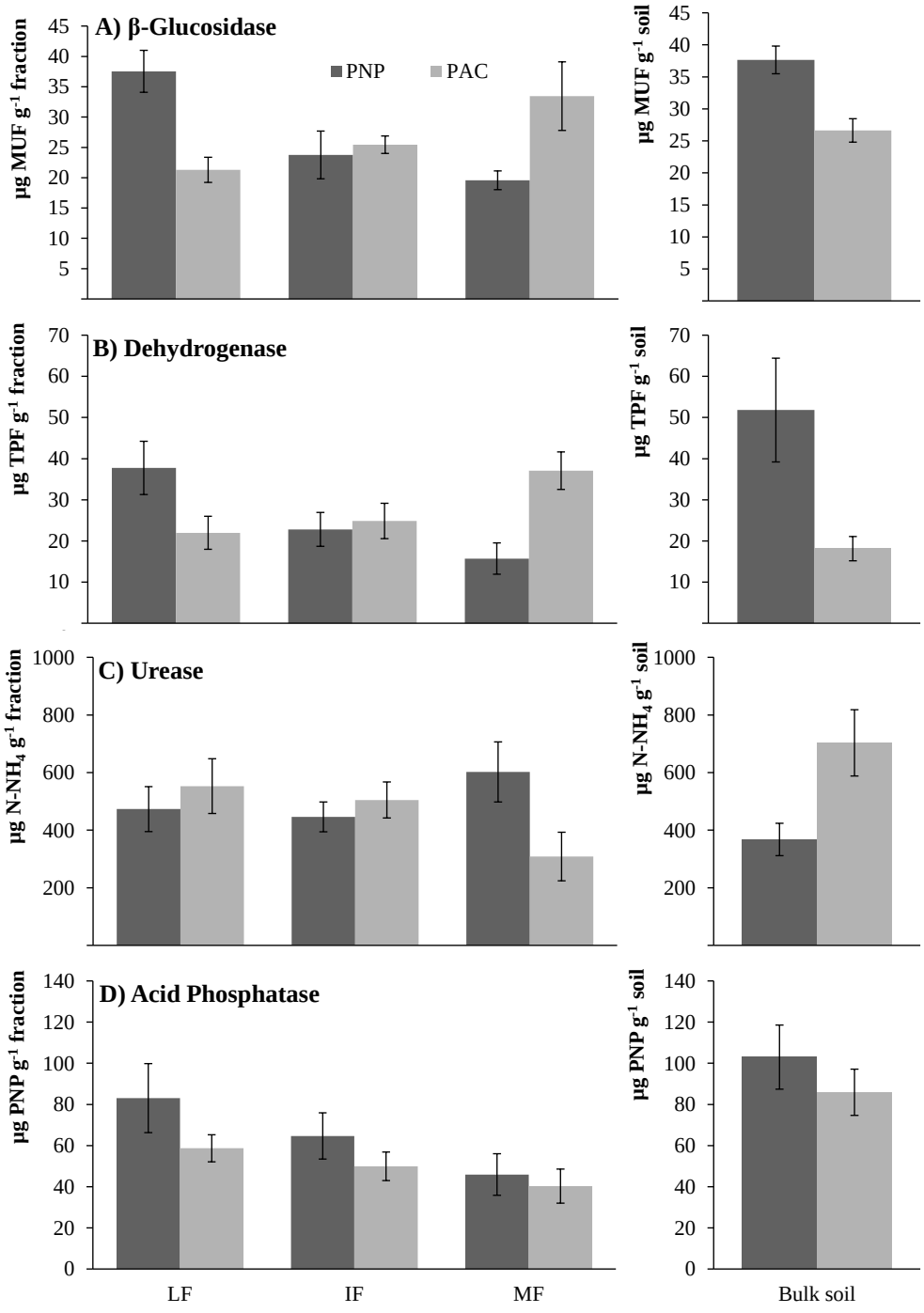


Fig 2. Average of enzymes activity after 30 days of incubation. 2A β-glucosidase (βG), 2B dehydrogenase (DH), 2C urease and 2D acid phosphatase, in bulk soil from temperate rain forest of Puyehue National Park (PNP) and Alerce Costero National Park (PAC).

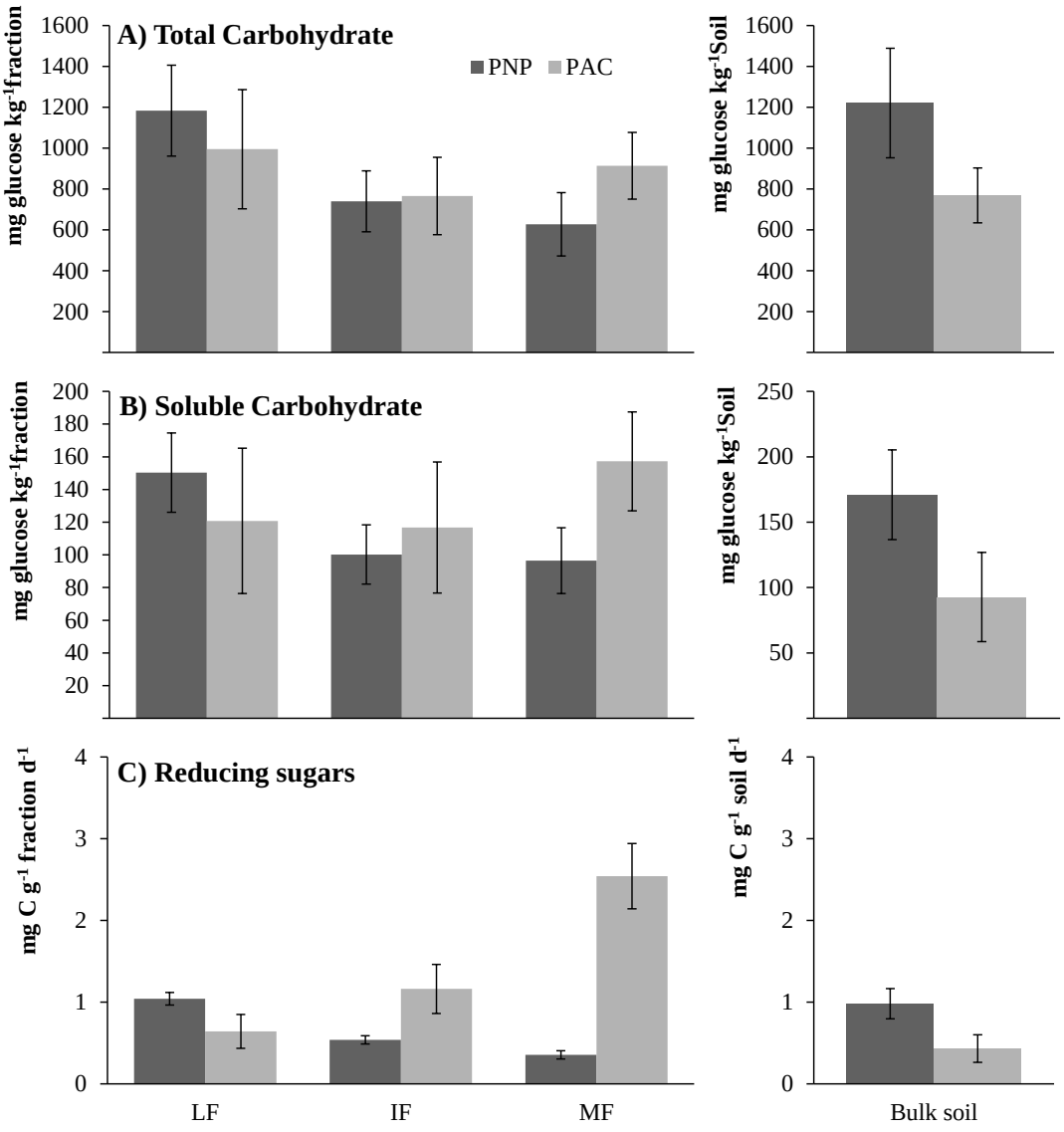


Fig. 3. Average of enzymes activity after 30 days of incubation. 3A Total Carbohydrate (CHOt), 3B Soluble Carbohydrate (CHOs) and Reducing sugar, in bulk soil from temperate rain forest of Puyehue National Park (PNP) and Alerce Costero National Park (PAC).

According to Anderson and Domsch (1990), the soil metabolic quotient (qCO_2), the respiration rate (15-30 days) divided by microbial biomass C can be envisaged as indicator of C metabolic efficiency for microorganisms. We also extracted the ATP from soils and it was highly correlated with qCO_2. (Fig 4A).Therefore, ATP was expected to be highly correlated with the cumulative C-CO_2 at 15-30 days of incubation (Fig 4B).

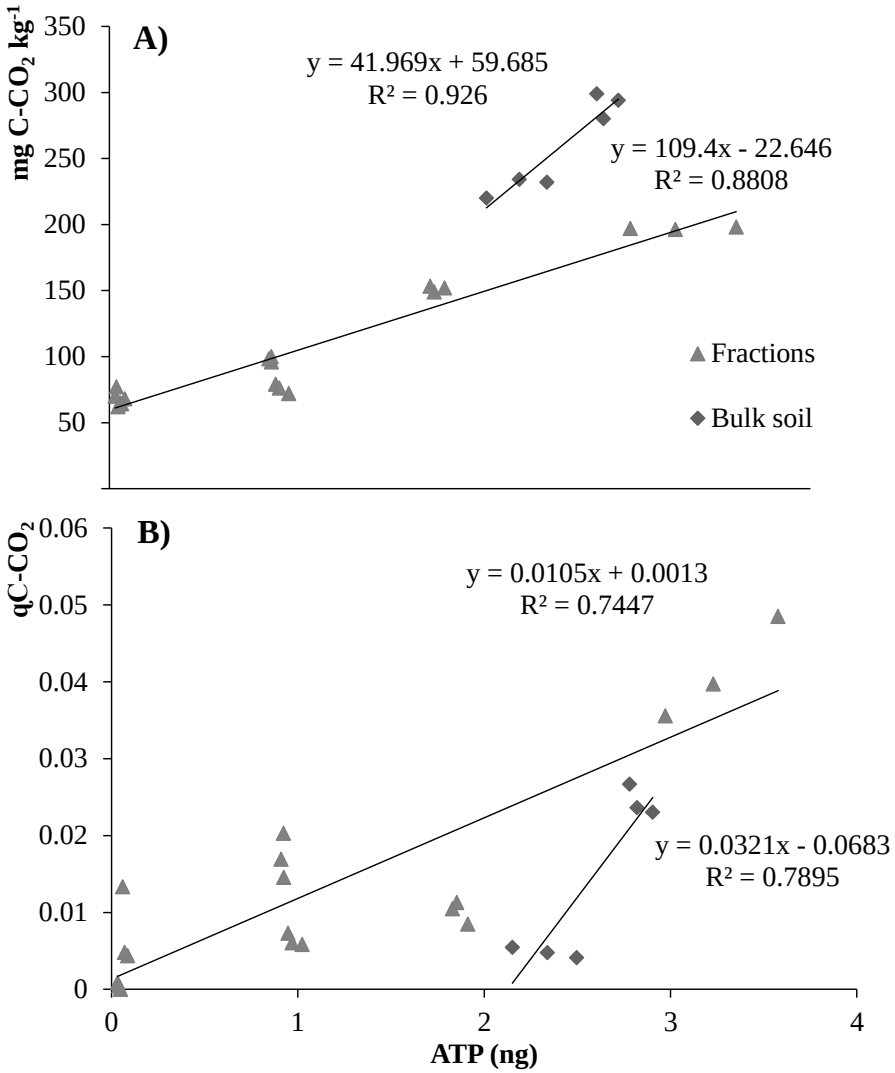


Fig 4. Relationship between ATP from soils with the cumulative C-CO_2 (A) and with qCO_2. (B) at 15-30 days of days of incubation in the soil fraction forest in Puyehue National Park (PNP) and Alerce Costero National Park (PAC).

4.4.4 Correlation matrix and multiple regression analysis

The coefficient of correlations amongst various soil properties were similar in both soils, therefore, only the results for PNP are shown (Table 4). βG and DH were highly correlated each other (r= 0.53, P < 0.01) and both enzymes were correlated with soluble carbohydrates and reducing sugars. From Table 4 emerge interesting results. Soil respiration was positive and highly correlated with βG, DH, phosphatase, reducing sugars and inorganic P, therefore, reducing sugars were expected to be correlated with βG, DH and phosphatase. Finally, ammonium was highly correlated with soluble carbohydrates and inversely correlated with nitrate.

Table 4 Coefficient of correlation (R) amongst various soil properties in different physical fractions (LF, IF and MF) of allophanic soil in temperate rain forest in Puyehue National Park (PNP).

Variable	βG	DH	U	P_{ase}	TotCH	SolCH	RdSug	$N\text{-}NO_3$	$N\text{-}NH_4$	Pi	$C\text{-}CO_2$	
βG	1.0											
DH	0.53**	1.0										
U	-0.11	-0.16	1.0									
AP_{ase}	0.16	0.63**	-0.04	1.0								
TotCH	0.35**	0.24*	0.18	0.24*		1.0						
SolCH	0.21*	0.26*	0.00	0.16		0.32*	1.0					
RdSug	0.75**	0.62*	0.01	0.47**		0.66**	0.49**	1.0				
$N\text{-}NO_3$	0.01	0.12	-0.06	0.20		-0.24*	-0.69**	-0.19	1.0			
$N\text{-}NH_4$	0.04	0.06	0.16	-0.03		0.22*	0.52**	0.23*	-0.52**	1.0		
Pi	0.14	0.74**	-0.19	0.80**		0.04	0.26*	0.35**	0.11	0.03	1.0	
$C\text{-}CO_2$	0.63**	0.66**	-0.38*	0.48**		0.21*	0.09	0.54**	0.21*	-0.33**	0.50**	1.0

LF = light fraction; IF = intermediate fraction; MF mineral fraction; βG = β-Glucosidase, DH = Dehydrogenase; U = Urease; P_{ase}= Acid phosphatase; TotCH = Total carbohydrates; SolCH = Soluble carbohydrates; RdSug = Reducing sugars; Nitrate = $N\text{-}NO_3$; Ammonium = N-NH4; Pi = Olsen P; ** P < 0.001; * P < 0.05.

Stepwise multiple regression analysis (SMRA) was conducted in PNP and PAC in all fraction and bulk soils. Table 5 shows the estimated linear parameters for only significant independent variables that influenced the $C\text{-}CO_2$ as calculated by significant R^2 in forward step. Similar result were obtained for bulk soils and they are not shown. The effect of soil properties on $C\text{-}CO_2$ variation in Table 5 were similar for the relevant variables obtained from the correlation matrix in Table 4. In both soils, DH, urease and

phosphatase were common variables and the linear model explained in 76% and 80 % the C-CO_2 in PNP and PAC soil, respectively. Soluble and total carbohydrates were relevant variables in PAC soil as well.

Table 5. Contribution of various soil parameters on R^2 from a step wise multiple regression analysis from temperate rain forest in Puyehue National Park (PNP) and Alerce Costero National Park (PAC).

Forest	Parameter	R^2	Estimate	F-ratio	Prob> F
PNP					
	Intercept		7.32	0	1
	DH	0.44	1.28	5.46	0.02
	N-NH_4	0.58	-0.26	15.75	0
	βG	0.69	2.52	20.11	0
	U	0.73	-0.07	7.67	0.01
	P_{ase}	0.76	0.39	3.98	0.05
PAC					
	Intercept		-26.065	0	1
	SolCH	0.58	-0.368	60.46	0
	P_{ase}	0.67	0.52	4.12	0.05
	Pi	0.74	0.34	10.32	0
	TotCH	0.76	0.033	10	0
	U	0.78	0.06	6.47	0.02
	DH	0.8	0.97	3.39	0.05

4.4.5 Carbon stabilization

The C stabilization was evaluated by the relationship between Al_p, the Al extracted by pyrophosphate (organic C complexed with Al) and the C-CO_2 mineralized between 15-30 days of incubation. For PNP soil, there was an inverse and significant relationship, which was not the case for PAC soil where not correlation was found at all (Fig 5).

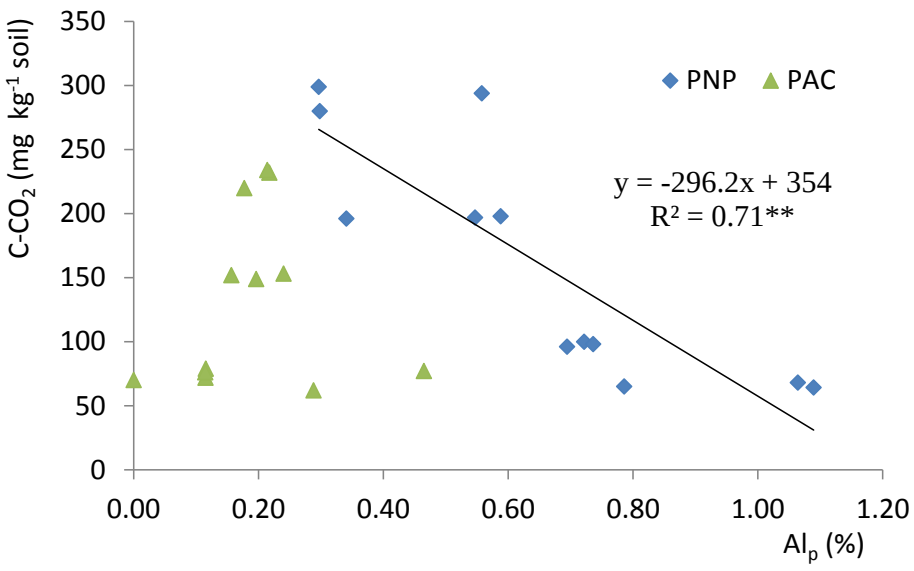


Fig 5. Relationship between Al pyrophosphate and ^{12}C-CO_2 in the soil fraction forest in Puyehue National Park (PNP) and Alerce Costero National Park (PAC)

4.5 Discussion

The present research was developed in two forest ecosystems standing on soils developed from volcanic ash (allophanic) and from metamorphic mica-schist (kaolinitic). The influence of soil mineralogy on C stabilization in the bulk soil and different physical fractions was evaluated using biological and biochemical methods. The biological activity in response to the soil mineral composition and physical fractions were assumed to represent the level of SOM complexity (stability) that provided valuable information on C mineralization stabilization mechanism.

The enzyme activity in the soil amended with cellulose were significant different by the effect of soil type and fractions and these result were consistent with multiple regression analysis. In general β-glucosidese and dhydrogenase, the two degrading enzymes of labile organic matter were present in both soils and fractions and together with urease, phosphatase and Pi explained > 76 % the variation in soil C respiration. The pattern of β-glucosidase and dehydrogenase in the physical fraction of PNP was opposite to the pattern found in the same fraction in PAC soil. In PNP the greater activity detected for both enzymes was in the LF, given the most labile available C (Chiellini et al., 2003). In contrast, the lower activity of

these enzymes in PNP was detected in the MF. In this fraction SOM is complexed with Al oxides and short-range ordered (SRO) minerals, as allophane like-materials (Neculman et al., 2012; Garrido and Matus, 2012), There is also micro-encapsulation of organic matter inside of highly stable micro-aggregates (Asano and Wagai, 2014; Chevallier et al. 2010).

The greater activity of β-glucosidase, and dehydrogenase was registered in the mineral fraction of PAC soil due to a reduced stabilization capacity in association with a more crystallinity clay, such kaolinite and the halloysite precursors (Luzio et al., 2003) (see below). In both cases however, the mineralized C was reduced in MF, being lower in PAC soil. The latter was attributed to the inhibitory effect of free Al by the lower pH of PAC (4.6) compared with PNP (6.2) soil. The percentage of Al saturation in PAC soil was extremely high (Table 1).

4.5.1 Stabilization capacity

Both, βG and DH activity were highly correlated with the release of C-CO_2, r=0.63 and r=0.66, respectively. In general, the respiration was correlated with phosphatase, reducing sugar and inorganic P, indicating the importance of βG and DH activity for degrading labile organic compounds in both soils. The mineralogical composition of soil showed an important role since there was an inverse relationship between Al_p (Al complexed with SOM) and the C-CO_2 mineralized between 15-30 days of incubation. This relationship was expected to occur in volcanic soils only, because of the potential of allophanic soils to form Al-SOM complexes by ligand exchange (Matus et al., 2014). In PNP soil Al bound to organic matter was twice the amount in PAC soil. In metamorphic kaolinitic soils, the more crystallinity clay exerts the stabilization effects, but this effect compared with allophanic soils is reduced (See Chapter 5 of this thesis). In previous studies, Al-SOM complexes was the primary factor explaining the soil C variation in similar soils rather than climatic variables and clay content (Percival et al., 2000; Matus et al. 2006). Neculman et al. (2012) found an inverse relationship between soil pH and C pyrophosphate, supporting the hypothesis that the Al-SOM complex and allophane formation are complementary processes mainly regulated by soil pH (Shoji and Fujiwara, 1984; Garrido and Matus. 2012; Panichini et al. 2012). In the present study, the PNP volcanic soil with pH 6.2 could favor the allophane polymerization and allophane-SOM sorption rather than Al-SOM complexation. It is also well known that factors, others than pH such

as the stability constants of metals and/or the concentration of competing Fe and Al aqueous species also influence the degree of complexation (Dahlgren et al., 2004). Marino et al. (Chapter five of this thesis) established that MF of PNP soil had the lowest C priming in comparison with the same fraction in PAC soil. This indicated the reduced availability of organic C for soil microbes due to clay mineralogy (allophane and imogolite, with high potential for Al-SOM complexes formation). Jones and Edwards (1998), reported that the simple C substrates (glucose and citrate) added to kaolinite and illite-mica were more decomposable than those added to ferric hydroxide. Early, Zunino et al. (1982) noted a decreased priming effect when allophanic materials was added to non-volcanic soil. The chemical bonding between SOM and mineral surfaces decreases the availability of C to microorganisms (Guggenberger and Kaiser, 2003). Clay minerals have specific surface areas; therefore, it may be expected that clay type influence the capacity of soils to hold organic C.

4.6 Conclusions

We examined the C storage as well as the stabilization capacity in two pristine temperate rain forest (allophanic and kaolinitic) soils. Soils were physically fractionated and three fractions were obtained: Light (LF, coarse sand > 250 μm), intermediate (IF, fine sand 50-250 μm) and mineral fraction (MF, silt and clay < 50 μm). The biological activity was evaluated with different biological and biochemical tools, such as soil respiration, microbial biomass, enzymes activity, carbohydrate, ATP extraction and other soil parameters. Soil type and the physical fractions had a significant impact in all enzymes. The differences in enzyme activity in the different fractions of both soils shows the functionality and efficiency of microbial community to mineralize different fractions of SOM. More than > 76 % of total C-CO_2 was explained including β-glucosidase, dehydrogenase, phosphatase and inorganic P by using stepwise multiple regression analysis. β-glucosidase, and dehydrogenase in the MF of PAC soil was higher compared with PNP soils, attributed to the differences in mineralogy of soil that influences the degradation of fresh cellulosic substrate. Mineral fraction of PNP contains allophanic materials and showed the lowest enzymes activity, except for urease and phosphatase. In contrasts, PAC is a kaolinitic dominant clay soil having reduced stabilization capacity. ATP was highly correlated with i) C-CO_2 at 15-30 d of incubation and ii) metabolic quotient (respiration rate divided by microbial biomass). These results were in line with

an inverse relationship found between Al pyrophosphate (Al bound to SOM) and C-CO_2, indicating the large stabilization capacity of allophanic soils compared with kaolinitic soils where no correlation was found.

CHAPTER V

Effect of aluminium on dissolved organic matter in an allophanic and kaolinitic temperate rain forest soil

For submit to Geoderma

5.1 Abstract

Aluminium (Al) and it influence on the mineralization of dissolved organic matter (DOM) and thus on carbon (C) sequestration in forest soils of different mineralogy is poorly understood. The hypothesis evaluated was that Al added in the soil solution produces toxicity to soil microorganisms if the Al go beyond a Al:C molar ratio > 0.1. Further concentration than this value, the complexes of Al-DOM precipitate, leaving Al in excess in the soil solution. Ratio values < 0.1, the Al bindings the organic matter remains in solution because is not yet saturated leading to a reduction of Al toxicity. We investigated the extent and mineralization rates of dissolved organic matter (DOM) and Ah mineral soil horizons inducing various Al:C ratios in allophanic and kaolinitic soils from temperate rain forest. Dissolved organic C extracted was incubated with initial molar Al:C ratios from <0.08 to 0.1 for 15 days. Mineralization was quantified by measurement of evolved C-CO_2. Increasing the initial Al:C ratios up to 0.12 led to a considerable decrease in mineralization (up to 37%compared with control samples). However, the mineralization rates were unaffected at the beginning (1-7 days) of incubation and they were even higher that the control. Ratios of Al:C>0.12 did not further decrease the mineralization, but led to increasing concentrations of free Al in solution, as indicated by the plateau reached for Al:C ratios at higher doses of Al. These results were also supported by the cell counting using flow cytometry analysis, measuring the relative florescence by confocal microscopy and using ART-FTIR. In conclusion, Al influences considerably the mineralization of DOM into the soil solution. Declining Al concentrations would increase the mineralization of DOM only if the Al:C ratio turn out to be less than 0.12 in the range of the Al complexation capacity of DOM.

Key words: Dissolved organic matter, molar Al:C ratio, carbon mineralization

5.2 Introduction

Soil microorganisms consume very fast most soluble organics carbon (C) of dissolved organic matter (DOM) and nutrients directly from the soil solution (Fischer et al., 2010).A continuum C decomposability of DOM can strongly be reduced by conversion of DOM to solid phase by interactions with soil minerals (Scheel et al., 2008; Baldock and Skjemstad 2000).

Dissolved organic matter in temperate forest floor can be considerable (12–50 g C m^{-2} yr^{-1}), but leaching from the subsoil has been found to be relatively small 0.5–7 g C cm^{-2} yr^{-1}(Michalzik et al., 2001; Solinger et al., 2001; Guggenberger and Zech, 1993). The reduced leaching has been attributed to the strong adsorption potential by mineral phase and closely related to the presence of aluminium (Al) bound to organic matter in soil solution. This is particularly true in volcanic soils, where Al and DOM precipitate as insoluble complexes that stop DOM leaching (Merino et al. 2015; Rasmussen et al. 2006).The role of Al–humus complexes in the soil organic C accumulation has been recently emphasized as an important mechanism controlling organic dynamics in Andisol (Takahashi and Dahlgren, 2016). It has been reported that the solubility of Al is controlled by Al-organic complexes (Yagasaki et al., 2006), which is directly related to the toxicity effects of Al for soil microorganism and plants (Takahashi et al., 2003). Al toxicity on microorganisms as well as declined enzyme accessibility due to complexation and precipitation are the reasons for a reduced degradability of DOM (e.g. Jansen et al., 2002; Kaiser 1998).

Since, the Al in soil is highly dependent on its speciation (free, colloidal complexed or precipitated), soil pH plays a key roles in the Al mobility and ultimately on DOM decomposition. At pH ~5 Al might be involved in adsorption reactions and Al toxicity, while at pH 6-7 flocs are formed by Al in presence of low molecular weight organic acids (acetate, oxalate, salicylate, lactate), that can even be degraded because speciation of Al is limited to uncondensed monomers and small oligomers (Masion et al., 2000; Matzner et al., 1998).

In summary Al speciation and Al-organic complexes formation depends: 1) on soil pH that negatively impact soil microbial activity (Schwesig et al.. 2003); 2) the atomic Al:C ratio that controls the formation of soluble DOM-complexes and its subsequent precipitation (Scheel et al., 2007; Schwesig et al., 2003; Garrido and Matus 2008) and 3) the inhibitory effects of Al concentrations on C mineralization (e.g. Blaser and Klemmedson 1987; Brunner and Blaser 1989). The excess of Al relative to the complexing ability for binding DOM, a molar Al:C ratio > 0.1 (Schwesig et al., 2003) leads to a complexes organic

matter precipitation, up to 50% reduction of C mineralization and an increases of free Al in the soil solution (Schwesig et al., 2003).

In south of Chile, most temperate rain forest (>800 m.a.s.l.) stands on volcanic soil (allophanic, pH > 5.5) and metamorphic soil (kaolinitic, pH < 5) (Godoy et al., 2001; Perakis and Hedin, 2001). In spite of these differences, there are common characteristics of these ecosystems such as high Al content, low temperature < 12 °C, high precipitation (> 4,000 mm) and significant losses of DOM in stream water (Perakis and Hedin, 2001; Perakis and Hedin, 2002; Hedin et al., 1995). It is unclear whether these losses can be explained by a reduction in the mineralization of C, by direct effects of Al toxicity in the case of kaolinitic soil and on by decreasing the bioavailability of organic C for soil microorganisms due to Al-DOM complex formation, in the case of allophanic soil (Scheel et al., 2008). The latter authors concluded that the toxicity effects did not cause the C stabilization upon Al addition, but a reduced bioavailability of organic matter after its precipitation.

In the present study we hypothesize that Al^{+3} added in the soil solution causes toxicity to soil microorganisms for Al concentration further than a molar Al:C ratio > 0.1 (complexing ability Al) (Schwesig et al., 2003). Ratios > 0.1 led to increasing concentration of free Al in solution (Al toxicity), indicating that the Al complexation capacity of DOM was exceeded. In contrast, ratios < 0.1, the Al-organic complexes remains still in solution because it is not yet saturated, resulting in a diminution of Al toxicity or detoxification of the soil solution where the C mineralization rates are unaffected.

The objective of this study is to evaluate the effects of Al concentrations on potential C biodegradation at different Al:C ratios from two temperate rain forests soils from south of Chile.

5.3 Materials and Methods

5.3.1 Site characterization

Rain forests in south of Chile have remained floristically stable throughout the Holocene/late Pleistocene (Perakis and Hedin 2002). Two ecosystems were selected in this study: Puyehue National Park (PNP) (40°47′S and 72°12′S at 800 m.a.s.l.) in the Andean Ranges and Alerce Costero National Park (PAC) (40°12′S and 73°26′W in the summit plateau at 1,000 m.a.s.l.) in the Coastal Ranges. Puyehue National Park present a temperate climate, with low average annual temperatures (12 °C) and high humidity (occasional snowfall) with average annual precipitation > 4,000 mm (Oyarzún et al., 2011). The vegetation

at PNP is pure evergreen old forest of *Nothofagus betuloides* (Oyarzún et al., 2004) growing on volcanic deposits. The soil is classified as Andisol, Typic dystrandepts (Soil Survey Staff 1975), derived from andesitic and basaltic tuff scoria with presence of allophane clay minerals (Neculman et al., 2012). Soil texture is a sandy loam to coarse sand throughout the complete profile. The topography at PNP occurs in the plane after steeped piedmont with northern aspect. Alerce Costero National Park is in the Coastal range with maritime, super humid temperate climate. Mean annual precipitation on the summits is about 4,000 mm (including occasional snowfall), with a marked summer (Almeyda and Saez, 1958). Mean an annual temperature is 12 °C. Most forest stands on the summit plateau are dominated by *Fitzroya cupressoides* (Mol.) Johnst, *Weinmannia trichosperma* Cav., and/or *Nothofagus nitida* (Phil.) Krasser. The soils from summit plateau are developed from Palaeozoic micaschists minerals with variable quartz content (Oyarzún, 1985). The soil is silty clay loam well drained, moderately deep formed *in situ* from ancient tectonic uplift of Coastal Range on metamorphic complex. The soil is classified as Ultisol Typic haploumults (Soil Survey Staff, 2008) with the strong presence of kaolinite (Luzio et al., 2003). At both study sites, soils had a litter layer of variable thickness (5 and 10 cm).

5.3.2 Soil sampling

We sampled the top soil horizons (5-15 cm) of Ah horizons at the two forest sites after removal the organic litter horizon. The soils were transported immediately to the laboratory under cold conditions, homogenized, sieved to < 2 mm and stored at 5 ºC for further analyses. DOM was obtained from about 100 g of soil (dry basis) suspended in 200 ml of $CaCl_2$ (0.01 M) and stirred for 10 min at 180 rpm at room temperature. Soil suspension was centrifuged at 4,000 rpm for 10 min and filtered 0.40 µm polycarbonate filter (Zsolnay, 2003). The supernatant (DOM) and mineral soil were characterized for pH in water (2:1 water: soil ratio), soil organic C (SOC) (TOC-VCSH, Shimadzu, Kyoto, Japan), total N (kjeldahl steam distillation, VAPODEST), inorganic P-Olsen (Table 1).

Table 1. Characterization of dissolved organic matter (DOM) extracted from surface (5-15 cm) volcanic (allophanic) soil from Puyehue National Parks (PNP) and metamorphic (kaolinitic) soil from Alerce Costero National Park (PAC) of temperate rain forests.

Analysis[2]	Units	PNP	PAC
DOM			
SOC	mgL^{-1}	5.5 ± 0.01	4.6 ± 0.01
Al_p	mgL^{-1}	9.6± 0.06	10.8± 0.06
Al_p:C_p		0.078±0.01	0.105±0.01
EC	dSm^{-1}	1.9± 0.06	2.0 ± 0.08
pH		4.3 ± 0.04	3.8 ± 0.05
Ah horizons			
Al_k	$g\ kg^{-1}$	0.38 ±0.02	3.81 ± 0.60
SOC	$g\ kg^{-1}$	110 ± 0.80	99 ± 0.30
Al_p	$g\ kg^{-1}$	11.0± 2.0	5.7± 0.0
Al_p:C_p		0.267± 0.00	0.173± 0.00
EC	dSm^{-1}	0.08 ± 0.00	0.03 ± 0.00
pH		5.7 ±0.13	4.5± 0.04
Clay type[1]		Allophane	kaolinite
Texture[2]		SL	CL

Al_p:C_p: Aluminium and carbon extracted by Na-pyrophosphate; EC: Electrical Conductivity.

[1] Luzio et al. (2003); Neculman et al. (2014)

[2] SL = silty loam and CL = clay loam

5.3.3 Al extraction

Dissolved organic matter and the top soil subsamples were analyzed in duplicated as follow: a) Extraction Al with 0.1 M Na-pyrophosphate (pH 10) soil: solution ratio 1:100; shaking for 16 h. Soil suspension and DOM were centrifuged (15 min at 2,500 rpm, with three drops of superfloc) and the supernatant was

filtered 0.40 µm polycarbonate filter and b) Extraction with KCl 1 M (Al_k) in a soil suspension conformed by soil: solution 1:6. The suspension was shaken for 30 min and centrifuged for 15 min at 3,000 rpm. The supernatant was filtered through acid-washed paper to a 100 ml flask. All Al extracts were analyzed in duplicates using atomic absorption spectrometry.

5.3.3 Incubation experiments

Twenty millilitres of DOM extracted from each soil were transferred into 500-ml incubation flasks. Four millilitres of Al–$AlCl_3 \cdot 6H_2O$ (analytical grade, Fluka) were added in increasing concentration at 0, 0.2, 0.4, 2, 4 and 8 mg L^{-1} equivalents to a molar Al:C ratio of 0.011, 0.022, 0.11, 0.22 and 0.44, respectively (Schwesig et al., 2003). This allowed a final molar Al:C ratio in a similar range as those found in natural DOM (Table 1). Dissolved organic matter was stirred and left standing over 48 hours at 5 ± 1 °C and again stirred occasionally. Al extracted by pyrophosphate and dissolved organic C in the DOM solution was measured after 48 hours of Al addition. Dissolved organic matter were incubated in dark at 26±2 °C with 10-ml of 0.5 M NaOH in duplicated. At each sampling 0.5, 1, 3, 7 and 15 days of incubation, the NaOH was potentiometrically titrated back with 0.5 M HCl. The pH and osmolality were measured in DOM in parallel incubations. Hydrolysis of $AlCl_3$ did not result in a reduction of pH and ionic strength, which may also influence C mineralization. Therefore, it was not necessary to controls the pH and osmolality for the specific effects of Al concentration.

The C mineralization rates (d^{-1}) were determined at intervals 1-7 and 7-15 days as:

$$\text{C-mineralization rate} = \frac{\text{Ln}\ (Ct_2) - \text{Ln}\ (Ct_1)}{t_2 - t_1}\ , \qquad (1)$$

where, Ct_1 and Ct_2 is the cumulative C mineralization (mg C–CO_2) t_1 (1-7 d) and t_2 (7-15 d), respectively.

We also incubated Ah horizons of mineral soil from the two sites. Two portions of 10 g (dry basis) of moist soil were watered with 2-ml Al treatments up to 60 % of field capacity to reach the same Al concentration as for DOM. One portion of soil was confined into incubation flask and the other used in parallel incubation for soil pH and osmolality measurements. The mineral soils were incubated at the same temperature in duplicated and sampled as previously described. Al extracted by pyrophosphate was measured after 48 h of Al addition. The controls pH and osmolality were not necessary.

5.3.4 Flow cytometry

We conducted a living cells counting (most fungal cells) in a 1.5 ml of DOM at 4 days of incubation using a flow cytometry (FACs Canto II-Becton Dickinson) with a flow rate of 60 µL min^{-1}. The measurements were performed several times during 20 seconds for all treatments. The particles cells numbers were analysed with FlowJo, Tree Star Inc., Ashland, OR, USA software and expressed in relative scale.

5.3.5 ATR-FTIR analysis

This analysis was conducted in the dry Ah horizons. Soil subsamples previously prepared for metal complexes formation with different concentration of Al were analyzed directly on ATR-FTIR spectrophotometer (Tensor 27, Bruker). The scans were done on the mid-infrared from 3,500 to 500 cm^{-1}, 4 cm^{-1} resolutions and each spectrum was the result of 64 co-added scans.

5.3.6 Confocal laser scanning microscopy

The formation of Al-SOM complex were observed in a screening that generated fluorescence emission at 405, 488 and 633 nm, observed in a Fluoview FV1000 Confocal Laser Scanning Biological Microscope (Olympus, Japan). The FV10-ASW 2.0c and ImagePro softwares were used for emission intensities; here we report a relative fluorescence per μm^2. Optical sections were scanned 10-times in the x-y direction up to 100 µm with a step size of 1 µm. The data for relative fluorescence with or without $AlCl_3$ addition were analyzes for Student's t-tests using ten independent replicates.

5.3.7 Data analysis

Data were log–transformed when they were found to be abnormally distributed. Multiple regression ANOVA, correlations and mean comparison (paired t test) were conducted by SPSS statistical software v11.0 package (SPSS Inc., Chicago, IL, USA) at a significance level of $P< 0.05$.

5.4 Results

5.4.1 Site characterization

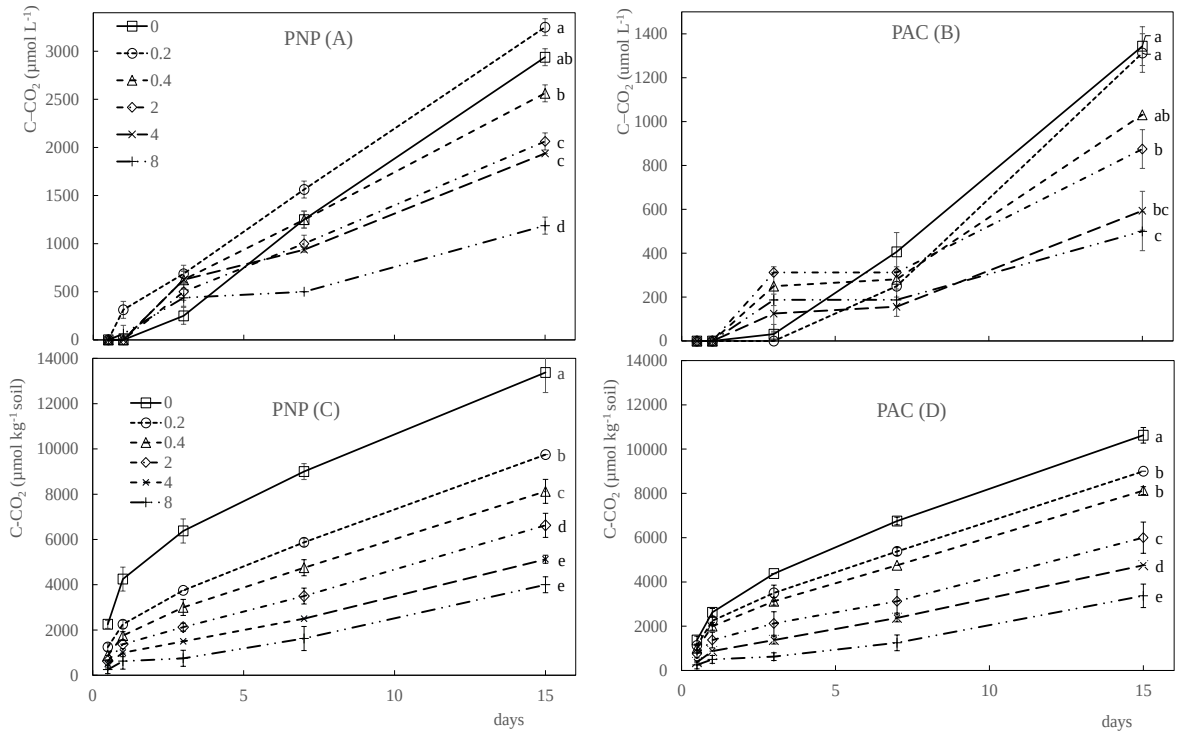
The allophanic soils in PNP is silty loam (CIREN, 1999), while in kaolinitic metamorphic soil is clay loam (Luzio et al., 2003) (Table 1). The SOC in PNP and PAC soils as well as in DOM were similar. SOC in DOM varied between 4.6±0.01 and 5.5±0.01 mg L^{-1} for PAC and PNP respectively, while in Ah horizons, 99±0.3 and 110±0.8 g kg^{-1}. The Al_p (pyrophosphate) is an indicator of Al complexed with organic matter, particularly important in volcanic soils. As expected, Al_p in PNP bulk soil doubled the amount found in PAC soil, 5.7±0.01 versus 11.1±2.0 g kg^{-1}, although these values were similar (10 mg L^{-1}) for DOM. Soil Al extracted in potassium chloride is considered free exchangeable Al in mineral soils. This value was 10 times higher in PAC (3.8± 0.6 g kg^{-1}) than in PNP (0.38±0.02 g kg^{-1}) reflecting the acid pH (4.5±0.04) in PAC soil with respect to (5.7±0.13) in PNP soils.

5.4.2 Carbon mineralization

ANOVA test indicated significant differences ($P < 0.05$) for mineralized C after 15 days of incubation for both DOM and Ah mineral soils (Fig 1). In general, mineralized C of DOM decreased as Al concentration increased, except after 3 days of incubation where Al treatments tended to mineralize even more than the control. The pH and osmolality did not affect the C mineralization and remained almost the same during all incubation (Table 2). The C–CO_2in PNP soil was higher than that in PAC soil and decreased steadily by Al addition (Fig. 1). Depending on the amount of Al concentration, two distinct groups of mineralized C were identified. Adding 0.2 or 0.4 g Al L^{-1} to the DOM from the two sites resulted in only small differences of mineralization compared with zero treatment. In contrast, Al addition > 2 mg Al L^{-1} caused strong C–CO_2 reduction. These differences were not found for Ah mineral horizon incubated.

Carbon mineralization rates (1–7 and 7–15 days) ranged between 0.01±0.00 and 0.94±0.04 µmol $kg^{-1}d^{-1}$ for DOM and between 0.02±0.001–0.12±0.04 µmol $kg^{-1}d^{-1}$for Ah mineral horizon (Table 3). DOM showed significant differences between sites and Al addition (Table 4). Carbon mineralization rates at 1-7 d were not significant, but they were highest at 7-15 d of incubation between 0.2 and 4 mg L^{-1} in both sites. Carbon mineralization rates at 1-7 d for Ah mineral soils were significant between 0.2 and 0.4 mg L^{-1} in PNP and tended to increase in PAC site (Table 5). At 7-15 d of incubation, the same trends was observed in PAC, while no significant differences were detected in PNP site. At the end of incubation, the mineralization rates were similar in both sites.

Figure 1. Carbon mineralization of dissolved organic matter (DOM) respectively from allophanic and kaolinitic soils of temperate rain forest from (A) Puyehue National Park (PNP) and (B) Alerce Costero



National Park (PAC) and from Ah mineral horizons from (C) PNP and (D) PAC after Al addition (Al–$AlCl_3$). Different letters indicates significant differences.

Table 2. pH and electrical conductivity (EC) at 3 and 15 days of incubation of dissolved organic matter (DOM) and Ah mineral horizon from volcanic (allophanic) soil from Puyehue National Parks (PNP) and metamorphic (kaolinitic) soil from Alerce Costero National Park (PAC) of temperate rain forests.

		pH		EC (dSm^{-1})	
Site	$Al–AlCl_3$[1]	3 days	15 days	3 days	15 days
			DOM		
PNP	0	4.41	3.89	1.88	1.90
	0.2	4.38	4.38	1.72	1.70
	0.4	4.46	4.39	1.71	1.70
	2	4.32	4.36	1.72	1.70
	4	4.44	4.39	1.92	1.90
	8	4.55	4.46	1.72	1.70
PAC	0	3.86	3.09	1.95	1.90
	0.2	3.93	3.95	1.79	1.80
	0.4	3.77	3.83	1.78	1.70
	2	3.81	3.83	1.60	1.60
	4	3.88	3.86	1.78	1.80
	8	3.87	3.89	1.76	1.70
			Ah horizon		
PNP	0	5.75	5.66	0.08	0.10
	0.2	5.87	5.70	0.09	0.13
	0.4	5.71	5.73	0.07	0.13
	2	5.87	5.86	0.08	0.14
	4	5.93	5.80	0.08	0.10
	8	5.85	5.79	0.08	0.12
	0	4.69	4.58	0.02	0.03
PAC	0.2	4.68	4.50	0.02	0.02
	0.4	4.65	4.48	0.02	0.02
	2	4.69	4.60	0.02	0.02
	4	4.92	4.53	0.02	0.02
	8	4.72	4.53	0.02	0.02

[1]$Al–AlCl_3$for DOM was added in mg L^{-1} and for Ah mineral soil in mg kg^{-1}

Table 3. Carbon mineralization rates[1] at 1–7 and 7–15 days of incubation of dissolved organic matter (DOM) and Ah soil horizons from volcanic (allophanic) from Puyehue (PNP) and metamorphic (kaolinitic) at Alerce Costero (PAC) rain forest National Parks

	PNP		PAC	
Al–$AlCl_3$[2]	1–7 d	7–15 d	1–7 d	7–15 d
	DOM (µmol C-CO_2 L^{-1} d^{-1})			
0	0.79±0.05	0.06±0.002	0.30±0.04	0.07±0.01
0.2	0.94±0.04	0.04±0.006	0.01±0.01	0.08±0.01
0.4	0.93±0.02	0.04±0.006	0.79±0.05	0.08±0.02
2	0.90±0.03	0.04±0.002	0.83±0.11	0.07±0.01
4	0.93±0.02	0.04±0.003	0.68±0.11	0.06±0.02
8	0.87±0.06	0.05±0.007	0.76±0.00	0.05±0.01
	Ah horizon (µmol C-CO_2 kg^{-1} d^{-1})			
0	0.09±0.01	0.02±0.001	0.11±0.01	0.03±0.001
0.2	0.11±0.02	0.02±0.001	0.10±0.05	0.03±0.001
0.4	0.12±0.04	0.03±0.000	0.10±0.01	0.03±0.001
2	0.09±0.02	0.03±0.001	0.10±0.00	0.04±0.003
4	0.09±0.04	0.04±0.002	0.10±0.03	0.04±0.004
8	0.05±0.02	0.05±0.010	0.05±0.02	0.06±0.007

[1]See equation 1 in the text

[2]Al–$AlCl_3$ for DOM was added in mg L^{-1} and for Ah mineral soil in mg kg^{-1}

Table 4. Summary of multiple regression to test for significant effects of Al addition (Al–$AlCl_3$) on carbon mineralization rates at 1–7 and 7–15 days of incubation of dissolved organic matter (DOM) from volcanic (allophanic) soil from Puyehue National Parks (PNP) and metamorphic (kaolinitic) soil from Alerce Costero National Park (PAC) temperate rain forest.

		1–7 days		7–15 days	
Source variation	DF	F ratio	*P*	F ratio	*P*
Site (A)	1	30.40	0.150	2.24	< 0.0001
Al–$AlCl_3$ (B)	1	0.977	0.306	0.98	0.335
A x B	1	4.597	0.273	1.27	0.045

[1]See Table 1

Table 5. Summary of multiple regression to test for significant effects of Al addition (Al–$AlCl_3$) on carbon mineralization rates at 1–7 and 7–15 days of incubation of Ah mineral horizon from volcanic (allophanic) soil from Puyehue National Parks (PNP) and metamorphic (kaolinitic) soil from Alerce Costero National Park (PAC) temperate rain forest.

		1–7 days		7–15 days	
Source variation	DF	F ratio	*P*	F ratio	*P*
Site (A)	1	0.261	0.754	0.312	0.583
Al-$AlCl_3$ (B)	1	26.72	< 0.0001	20.65	0.0002
A x B	1	0.251	0.622	1.694	0.208

5.4.3 Al:C ratio

Aluminium addition to DOM increases the Al_p:C_p ratio from 0.08 to 0.18(Fig 2A). Any addition > 2 mg L^{-1} resulted in minor differences. The results were similar for mineral soils (Fig 2B). The molar Al:C ratio increased from 0.17 to 1.38.

For DOM there was an inverse relationship between mineralized C at 15 d of incubation and molar Al_p:C_p ratio. The cumulative C–CO_2 were unaffected only if the Al addition reached Al:C ratio between

0.08 and 0.12 (Fig.3A). Ratio > 0.12 cause a further decrease in the mineralization. The C mineralization for Ah mineral horizon sharply decreased from the beginning (Fig. 3B).

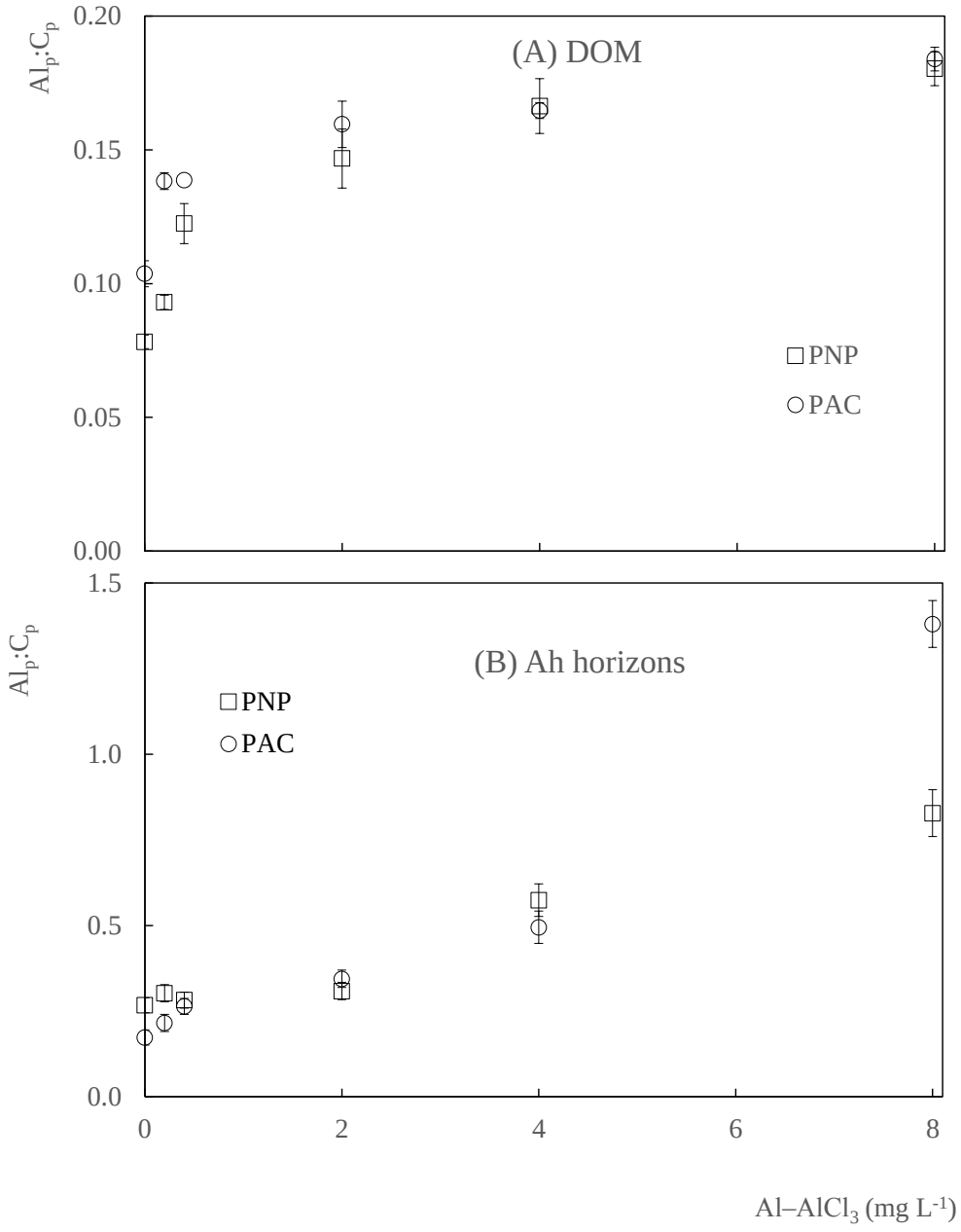


Fig. 2. Relationship between Al ($Al–AlCl_3$) after 48 h addition and molar $Al_p:C_p$ ratio (pyrophosphate Al and C) in (A) dissolved organic matter (DOM) and (B) Ah mineral horizons from allophanic and kaolinitic soils of Puyehue National Park (PNP) and Alerce Costero National Park (PAC), respectively.

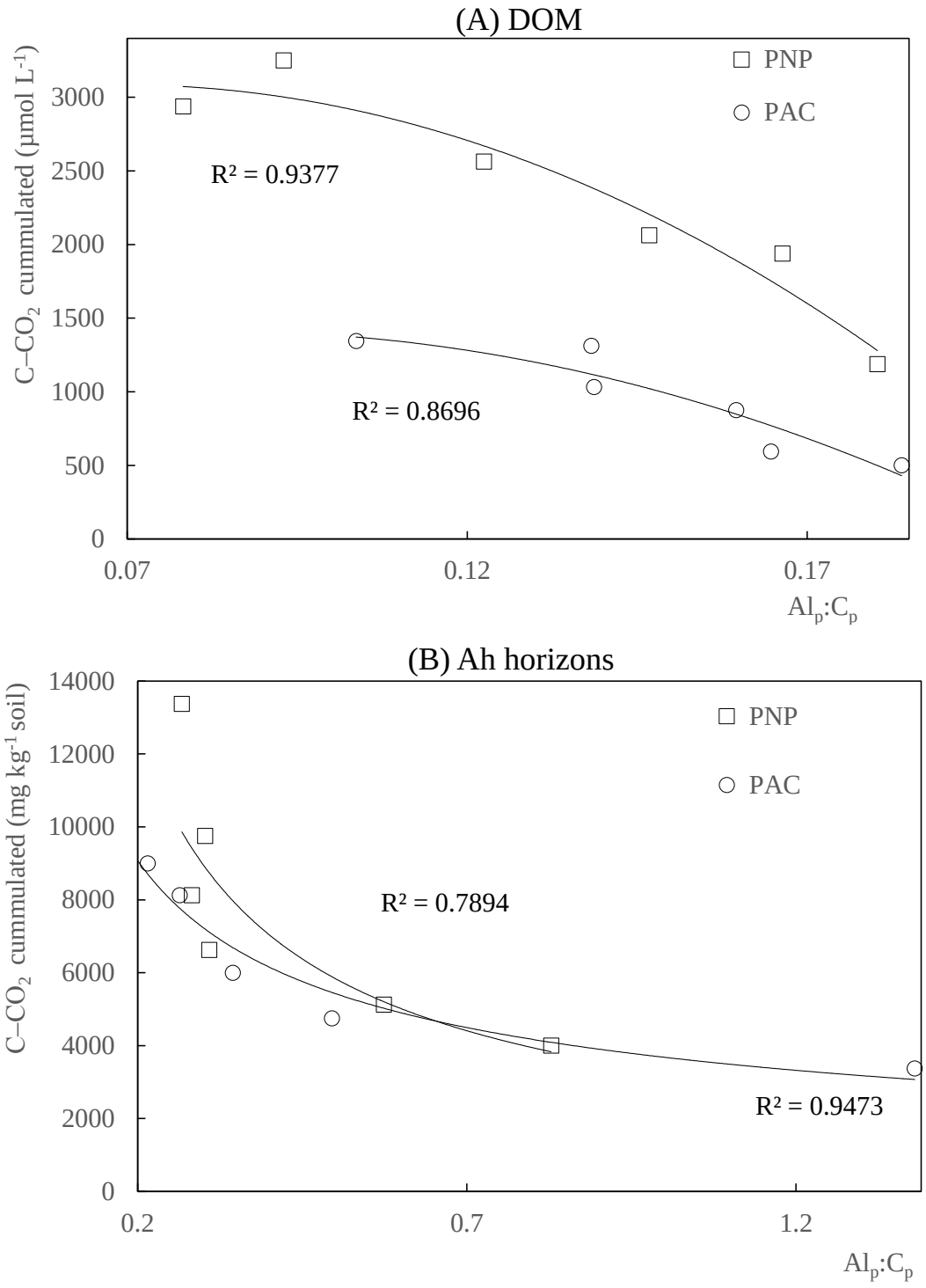


Fig. 3. Relationship between molar Al_p:C_p ratio after 48 h and cumulative C-CO_2 after 15 days in (A) dissolved organic matter (DOM) and (B) Ah mineral horizons from allophanic and kaolinitic soils of Puyehue National Park (PNP) and Alerce Costero National Park (PAC), respectively

5.4.4 Flow cytometry of DOM

The relationship between cell counting by flow cytometry analysis and the cumulative C-CO_2 at 15 days of incubation is shown in Fig 4A. Both, PNP and PAC sites exhibited highly significant correlation. The cell counting was also plotted with Al-$AlCl_3$ addition (Fig 4B). The relative counting cells with respect to the control without Al addition tended to be similar in both soils up to 0.4 mg Al L^{-1}, thereafter the relative proportion declined until 40-50 % of original value.

5.4.5 ART-FTIR

The ART–FTIR spectra of PNP and PAC soils samples are nearly comparable, although the transmittance intensities in PNP were generally higher than those in PAC (Fig. 5). Particularly, in the region of 1010–970 cm^{-1} the major differences are observed between sites.The spectra from Al treatments showed relatively little information in the region 2,000–3,500 cm^{-1}stretching bands. This bands are assigned to aliphatic C-H stretch (~2,950–2,870 cm^{-1}), and OH/NH stretch (~3,320 cm^{-1}). The Al treatments had a pronounced spectral effect at 1,634 and 970 cm^{-1} bands in both soils. These bands are assigned to polysaccharides and cellulose–like compounds (~1,070 cm^{-1}) and amide C=O stretch, aromatic C=C stretch, carboxylate CO stretch and/or conjugated ketone C=O stretch (~1,605 cm^{-1}) (Soong et al., 2014). ART-FTIR transmittance were lower by Al addition at the four major bands.

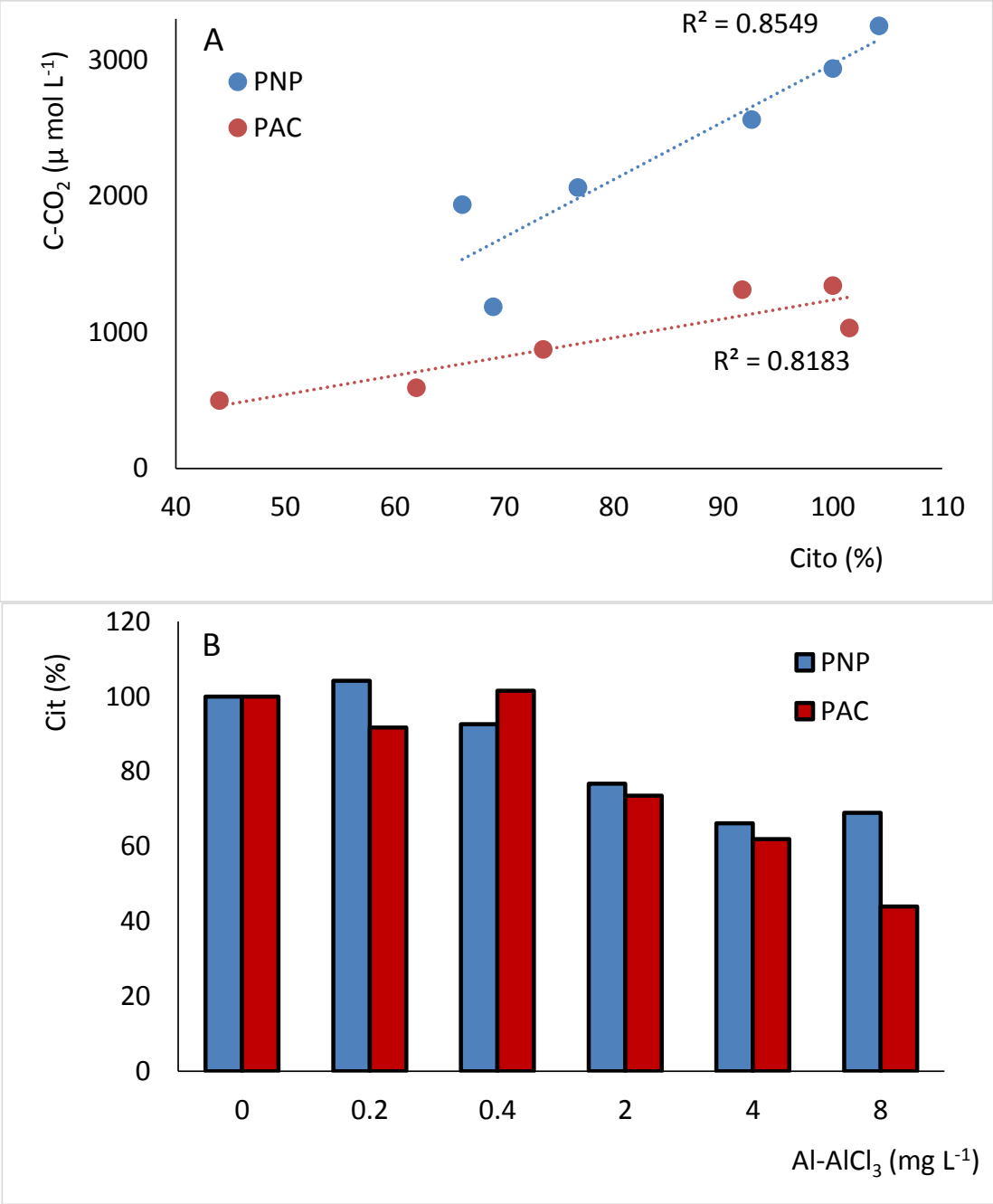


Fig. 4. (A)Relationship between relative flow cytometry cell counting (Cit) and cumulative C-CO_2 after 15 days of incubation experiment (P <0.0001) and (B) effect of Al (Al–$AlCl_3$) after 48 h addition on cell counting from allophanic and kaolinitic soils of Puyehue National Park (PNP) and Alerce Costero National Park (PAC), respectively.

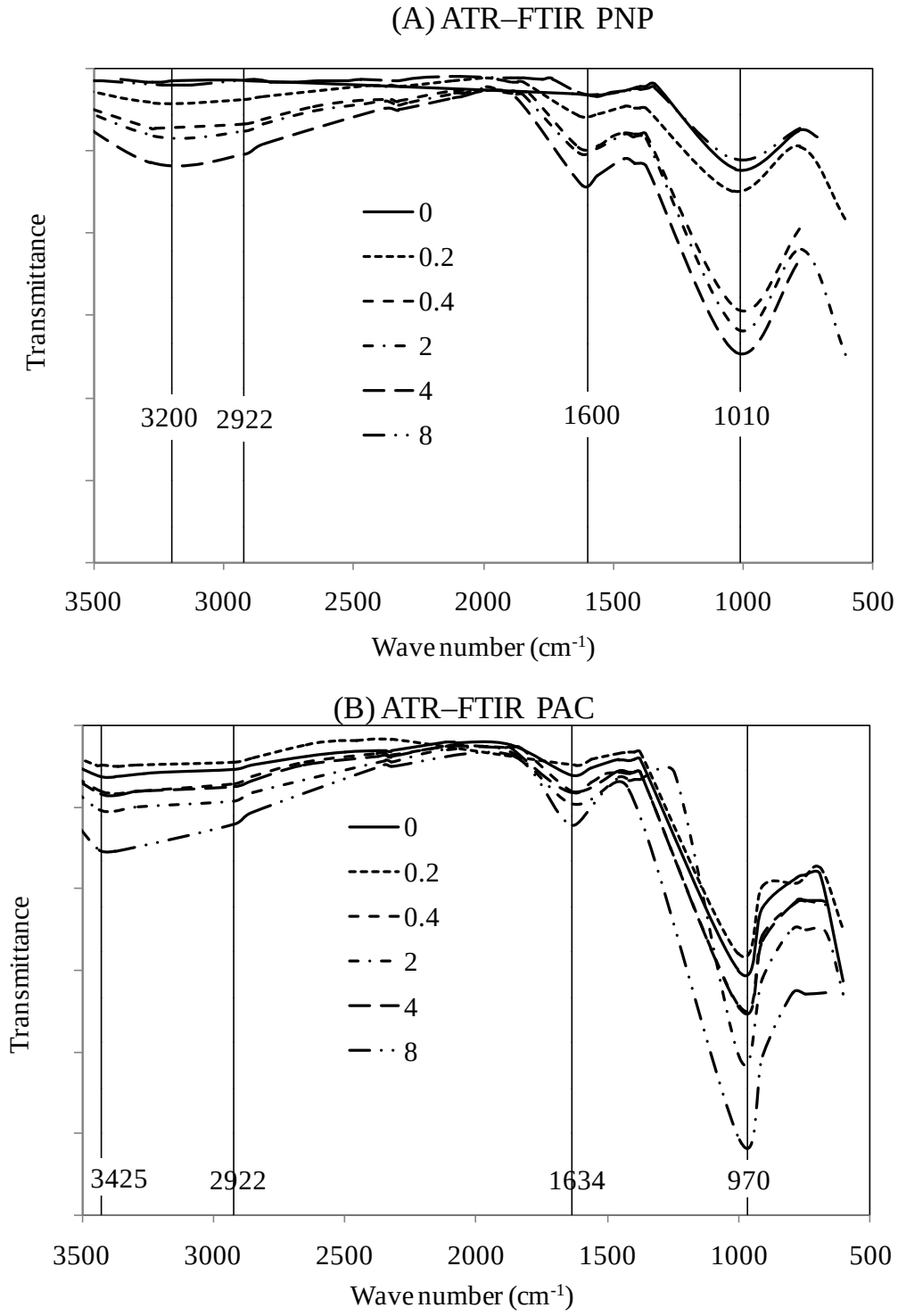


Fig. 5. ART-FIR spectra 48 h after Al addition (Al–$AlCl_3$) to allophanic and kaolinitic soils of temperate rain forest from Puyehue National Park (PNP) and Alerce Costero National Park (PAC), respectively.

5.4.6 Confocal

The formation of Al-SOM complex in Ah mineral soil samples were also observed by fluorescence emission using a confocal laser microscope. The FV10-ASW 2.0c and Image Pro softwares were used for emission intensities (Fig. 6). The average of relative fluorescence per μm^2 was obtained from ten random optical scan in the x-y direction. The relative fluorescence with or without $AlCl_3$ addition were analyzes for Student's t-tests and significant differences ($P < 0.05$) were found. PNP soil (Fig 6 A-C) showed more fluorescence intensity than PAC soil (Fig 6D-F). Clearly, in PNP the control soil without Al addition exhibited florescence, but the intensity was significantly lower compared to 2 and 8 mg Al L^{-1}. Similar results were found for PAC soils although no significant differences were found between 2 and the maximum doses of A.

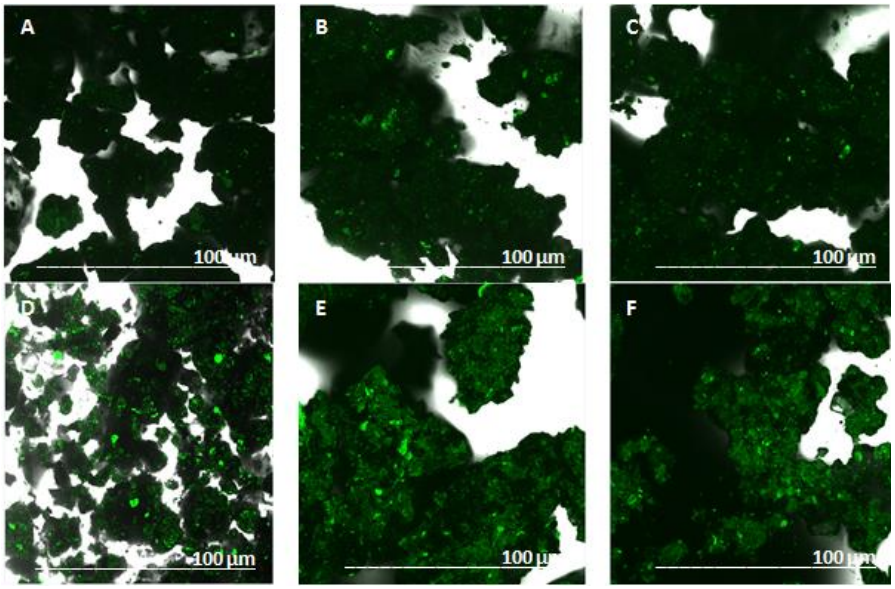


Fig. 6. Fluorescence of added Al ($Al–AlCl_3$) addition (assumed as complexed with organic matter) from allophanic and kaolinitic soils of temperate rain forest from Puyehue National Park (PNP) and Alerce Costero National Park (PAC), respectively. (A) PAC soils without addition, (B) PAC with 2 mg L^{-1}, (C) PAC with 8 mg L^{-1}, (D) PNP soil without addition, (E)PNPwith 2 mg L^{-1} and (F) PAC with 8 mg L^{-1}.

5.5 Discussion

The hypothesis that the mineralization rate is influenced by the effects of potentially toxic Al after Al-humus precipitation for Al:C ratio > 0.1 (Schwesig et al., 2003) was supported in the present study. This effect took place at the beginning of incubation experiment, where Al treatments tended to mineralize even more than the control. This effect was also supported by the C mineralization rate particularly for DOM and Ah horizon of PNP soil. In PAC soil the mineralization rates tended to be even higher for the maximum doses at the end of incubation.

The Al complexed with DOM is soluble for low Al concentrations (Jansen et al., 2003).Aluminium concentration between 0.2 and 0.4 mg L^{-1} caused the formation of mineral organic complexes as evidenced by the sharp increases of Al_p:C_p in DOM and less clear results for Ah mineral horizons. Further Al additions (> 2 mg L^{-1}) did not produce more Al complexes and this was interpreted as full complexing capacity of Al. The amount of DOM precipitated by Al increased strongly up to initial Al:C ratio in the solution from 0.08, whereas a further increase of the Al:C ratio to 0.12 did not strongly change the amount Al-organic complexes. Ratios < 0.12 large proportion of Al is bound in soluble Al-organic forms. This confirms the results of Schwesig et al. (2003); Nierop et al. (2002) and Sheel et al. (2007; 2008). They found a large increase in the fraction of C precipitating for Al:C ratios exceeding 0.03. So at small Al:C ratios the Al is already bound to DOM but the amount is too small to induce precipitation. We can say: at low Al concentration, the toxicity can be ´scrubbed´ from the soil solution, while Al complexes are being saturated and the C mineralization are not further reduced. Formation of mineral-C complexes also depends on the specific form of organic matter (Heckman et al., 2013; Scheel et al., 2007). Aliphatic and higher molecular weight of organic compounds may be preferentially precipitated by Al in the solution. Carboxylic or phenolics groups play key role in the formation of metal organic matter complexes. Our results indicate that differences in origin of organic matter and soil pH may be combined to vary the complexing ability of humic substances between soils, making the ratio somehow variable. However, for any given soil and horizon, a ratio may exist which corresponds to the saturation of complexed DOM.

As previously mentioned, one of the main factors limiting microbial growth is the presence of exchangeable free Al in the soil solution. Al extracted with potassium chloride (Al free) was highest in PAC soil, while in PNP it was almost inexistent. The increased Al_k in PAC is explained by a lower initial

pH 4.5 and relatively lower precipitated of Al-organic complexes. The opposite was true for PNP with pH 5.7 (Jansen et al., 2005; Nierop et al., 2002; Kalbitz and Kaiser, 2007; Zysset and Berggren, 2001).

The ART-FTIR analysis showed lower transmittance at four major bands across soil samples at 3,425–3,200 (OH/NH stretch), 2,922 (aliphatic C-H stretch), 1,600–1,635 (amide C=O stretch, aromatic C=C stretch, carboxylate C–O stretch and/or conjugated ketone C=O stretch), and 1,070 cm^{-1} (cellulose like compounds) (Soong et al., 2014). Transmittance intensities in PNP were generally higher than those in PAC and this was an unexpected results, since Al_p in PNP was highest. In acidic soils, however the surface complexation can take place between dissolved organic compounds and hydrous Al oxide particles in solution (Eikebrokk, 1996; Jekel, 1986). This perhaps could account for more complexes formation than pyrophosphate dissolution at pH 10 in PNP. On the other hand, spectroscopic analyses have indicated preferential precipitation of aromatic compounds (Römkens and Dolfing, 1998; Blaser et al., 1999; Sharpless and McGown, 1999; Scheel et al., 2007). In our study, this could be the case for slightly stretching bands at 1,634-1,600 cm^{-1}. However, the more pronounced stretching bands (1,010–970 cm^{-1}) corresponded to polysaccharides and cellulose–like compounds. These results are consistent with the preferential binding of Al to polysaccharides of natural organic matter (Masion et al., 2000). In general, our results were supported by the cell counting and relative florescence observed by confocal microscopy. Soil microorganisms under low Al concentration were comparable with those growing in the control soil without al addition in both, Ah mineral horizons and dissolved organic C.

5.6 Conclusions

Organic C mineralization of DOM from rain forest allophanic and kaolinitic soils were unaffected by potentially toxicity of Al up to Al:C ratio of 0.12. Rations < 0.12 Al speciation and DOM allow a `detoxification´ of Al in soil solution. This took place for C mineralization at the beginning (1-7 d) of incubation experiments. These result were also supported by the cell counting and the florescence observed by confocal microscopy. As long as the Al concentration increases ahead of Al:C ratio > 0.12, the Al-organic matter precipitated allowing a `detoxification´ of Al in soil solution.

CHAPTER VI

Effect of carbon availability on rhizosphere priming in soils of contrasting mineralogy

In preparation

6.1 Abstract:

A major uncertainty in soil carbon studies is how inputs of fresh plant–derived carbon affect the turnover of soil organic matter (SOM) by the so-called rhizosphere priming effect (RPE). The RPE is defined as the acceleration or retardation of decomposition of SOM by the root activity. Soil type and soil mineralogy influence the priming effect and the underlying mechanisms are poorly understood. In the present study we evaluated the impact of root carbon influxes of C4 plants (maize) on RPE from soil fractions (light, 250-2000, intermediate 50-250 µm and mineral < 50 µm fraction) isolated from temperate rain forests soil; allophanicand kaolinitic soil derived from metamorphic materials. The soil fractions were confined in small capsules in contact with roots in a pot experiment. Soil C respiration was partitioned and ^{13}C natural abundance measured at four phenological stages. We also evaluate the magnitude of RPE on root colonized capsules in a second experiment where the photosynthesis was stopped at flowering. The pots were harvested and the capsules were incubated for 24 h to examine the RPE without rhizodeposits. The results supported the hypothesis that the magnitude of RPE is lower in the mineral fraction as the C availability decreases. This was particularly true in the mineral fraction of allophanic soil. The RPE in kaolinitic metamorphic soils, with more crystalline clay, mineralized two fold the amount of C in the allophanic soil (14 % against 33 %). There was a negative and strong correlation between C and Al (extracted by pyrophosphate) and i) RPE and i) ^{13}C derived C in allophanic soils. The result suggests a strong influence of mineral composition on mineralization of SOM. The present results also show that the priming increased as the phenological stage progressed; revealing the importance of fresh C materials on recalcitrant SOM decomposition. This was supported by the second experiment where the RPE dramatically decreased 24 h after photosynthesis ceased. Basal respiration and RPE were reduced up to 33 times compared with the pot with plant growing. We concluded that RPE depended on the fresh available C and soil mineralogy.

6.2 Introduction

In annual plant species, 20 - 60% of the photosynthetically fixed C is translocated to the roots, and a considerable proportion of this C (up to 70%) can be released into the rhizosphere. This process is called rhizodeposition (Liljeroth et al., 1994; Kuzyakov and Domanski, 2000). The C inputs by plant roots generally stimulate a quick response of soil microbes by accelerating the decomposition of native soil organic matter (SOM), the so-called rhizosphere priming effect (RPE) (Dijkstra and Cheng, 2007, Ekberg et al., 2007). For example, the decomposition of SOM of planted soils was 50 % to 350% over the control unplanted soil (Cheng and Kuzyakov, 2005; Dijkstra and Cheng, 2007; Carney et al., 2007b) producing a positive RPE. Plants accelerate the SOM decomposition (Kuzyakov et al., 2009; Paterson, 2009) due to an increase of readily available labile organic C (LOC) provided by root turnover and root-exudate influxes (Dijkstra et al., 2013). The LOC concentration in soil rhizosphere declines rapidly because of 1) microorganism assimilation, 2) solute gradient diffusion into soil aggregates (Raynaud, 2010), 3) complexation with Al and Fe and 4) adsorption onto clay minerals (Albalasmeh and Ghezzehei, 2014). Soil organic matter stability, the resistance of organic compounds to the decomposition, depends on its association with clay type, occlusion within aggregates, and the biochemical composition of SOM (Mikutta et al., 2006). The SOM pool can be operationally divided into physical fractions with different stabilities, from fast to slow turnover C, i.e. from labile to mineral fraction bound to SOM, controlling the C availability for soil microorganisms (von Lützow et al., 2007; Heitkamp et al., 2011). As far as we know, there is only one study in which priming effect was tested in different soil fractions with homogeneous substrates. Ohm et al. (2007) tested the priming effect on sand (63-2000 µm), silt (2-63 µm) and clay (<2 µm) by adding fructose and alanine. Both substrates strongly increased the mineralization over the control soil without addition, indicating that the priming effect might be also caused by substrate limitation. Rasmussen et al. (2007) studied the effect of temperature sensitivity and soil mineral assemblage on priming of temperate forest soil. Regression analysis indicated that priming effect was controlled by short range order minerals, Fe oxyhydroxides, and Al–humus complexes amongst other parameters.

Volcanic and metamorphic derived soils differ in their stabilization capacity and influence available C for soil microbial community, because of the importance of clay mineralogy on decomposition of SOM. The mineralogy of volcanic soil (allophane, imogolite and oxide-hydroxy of Al and Fe, Soil Survey Staff

(2008) causes unique characteristics, e.g., moderately low pH, low bulk density, high phosphate retention, high water holding capacity, high specific surface area of mineral fraction and high complexation capacity between Al and SOM (Besoain and Sepulveda, 1985; Matus et al., 2008; Casanova et al., 2013). More than half of volcanic soils in Chile occurs in the East side of the country in Cordillera de los Andes (and steeped piedmonts) developed under *Notophagus* spp. On the other hand, metamorphic soil (1:1 kaolinite and halloysite dominant clay, Luzio et al., 2003) derived from bed rock in Cordillera de la Costa are developed under forest vegetation (*Fitzroya cupressoides*) in moderately deep soils derived from schist with texture that range from moderately fines in surface and fines in depth. They present relatively high levels of free Al, which increases along the soil profile. Iron levels are extremely high in these soils. The organic matter content is high at surface horizons and decreases sharply with depth (IREN and UACh, 1978).

In this Chapter the magnitude of RPE was assessed in allophanic and metamorphic soils in experiment carried on France (Clermont Ferrand), where maize (*Zea mays*) grown in contact with physical fractions isolated from these soils. The idea was to determine the effect of C4 plants on RPE by varying the C availability in soils with different mineralogy.

The hypothesis of this study was that the organic C availability affects the intensity of rhizosphere priming in soils with contrasting mineralogy.

The aims were to evaluate the RPE in the bulk soil and various physical fractions isolated from soils with different mineralogy. We did two experiments; in the first one, we determine the effects of phenological stages of maize plants on the rhizosphere priming and in the second experiment, the RPE was evaluated on bulk and soil fraction colonized by roots after fresh organic C released by plants was ceased.

6.3 Methodology

6.3.1 Site and soil sampling

Two soils were selected in this study. One soil was an Andisol formed from recent volcanic ash (Typic dystrandepts) (Soil Survey Staff, 2008) from a virgin forest (*Nothofagus betuloides* (Mirb.) in Puyehue National Park (PNP) (40°26 '– 40° 58'S and 72°22'–71°50 'W) between 2,236–2,250 m.a.s.l. The mean annual precipitation is (MAP) > 3.500 mm and mean annual temperature (MAT) is 9.2 °C (Oyarzún et al., 2005a).The soils are derived from basaltic scoria with presence of allophane clay minerals (Matus et al., 2014b). The other soil was taken from Alerce Costero National Park (PAC) (40°73° 27'W-11'S) between 650–1048 m.a.s.l. in Cordillera de la Costa. The soil is developed from metamorphic- schist- (Ultisol) with presence of kaolinite and halloysite (Luzio et al., 2003). The PAC presents an ancient forest (*Fitzroya cupressoides* (Mol.) Johnst., *Weinmannia trichosperma* Cav., and *Notohofagus nitida* (Phil). The MAP is > 4,000 mm and the MAT 9.5-12.1 °C. Both soils were sampled in the first mineral horizon (5-10 cm) after removal of the organic litter horizon. The soils were transported to the laboratory under cold conditions, homogenized, sieved to <2 mm and characterized (Table 1).

6.3.2 Pyrophosphate extraction

To determine Al and C associated with pyrophosphate (pH 10), we performed an extraction with 0.1N sodium pyrophosphate on air dried soil. The extract obtained was determined by aluminum (van Reeuwijk 2002) with atomic absorption and organic C (C_p) with WB method. We used a soil: solution ratio 1:100 that was shaken for 16 h. The suspension was centrifuged (15 min at 2,500 rpm, with three drops of superfloc) and the supernatant was filtered 0.45 µm in polycarbonate filter.

6.3.3 Pot experiment

Rhizosphere priming was evaluated in various physical fractions and bulk soil of PNP and PAC by growing maize (*Zea mays* L, cultivar Rugosa) in pots of 2 L. Soil fractionation was conducted according to Balesdent et al. (1991). Briefly, 50 g moist soil sample was suspended in 180 mL of demineralized water in 500 mL capped plastic bottles containing 10 glass beads (5 mm diameter). After 16 h shaking (40 cycles min^{-1}) the soil suspension was poured into a 250 μm sieve. The materials retained in the sieve (sand and organic debris) were washed several times. Three fractions were obtained: light (LF, 250-2000 μm), intermediate (IF, 53-250 μm) and mineral (MF, < 53 μm). All soil samples were dried at 35 ºC and characterized (Table 2). Twenty ml of physical fraction and bulk soil were inoculated with 1% of bulk soil and confined in PVC capsules (2 cm diameter x 6.4 cm length). The PVC capsules were sealed on the top with a nylon mesh of 1.0 mm opening to allow root growth. The bottom of the capsules were also sealed with a nylon mesh of 30 μm to prevent the root passages. Each pot was half filled with puzzolane (air-dried sieved <5 mm inert materials), where three capsules of the same fraction size and bulk soil were vertically and carefully uniformly deposited. The rest of the pots were completed with puzzolane. Each pot was replicated four times. The soil moisture contents of the pots and capsules were adjusted to 80 % of water holding capacity before maize planting. Each pot received four pre-germinated seeds. In total, 48 (planted and non-planted) pots that received the capsules with the physical fraction and 16 (planted and unplanted) pots with the bulk soil were cropped in both soils. Eight planted and non-planted pots with air-dried puzzolane were also regarded. All pots were placed in a growth chamber (25±0.5 ºC day and night with PAR of 1.400 μmol m^{-2} s^{-1}). The plants were grown with a photoperiod of 12-hours, day/night. The plants were fertilized with nitrogen (55 mg N cm^{-2}, equivalent to 70 kg N ha^{-1}), phosphorus (100 kg P_2O_5 ha^{-1}) and potassium (200 kg K_2O ha^{-1}) on 15 and 32 days after showing. At the first fertilization time, sulfur (20 kg S ha^{-1}), magnesium (60 kg MgO ha^{-1}) and micro-nutrients in solution were applied. The water content of each pot was controlled gravimetrically and was adjusted with deionized water during 131 days of experiment. All pots were distributed in a randomized block design within the grow chamber and the pots were occasionally rotated. Sanitary treatments were not necessary, because the plants were healthy.

6.3.4 Evaluations

The RPE was evaluated at extended leaves (EL, two leaves-32 days after sowing), vegetative (V, early whorl-44 days after sowing), flowering (F, late whorl-61 days after showing) and maturity (M, tassel-131 days after sowing) by collecting the C-CO_2 respiration from each pot in sealed black PVC gas chambers. Two chambers were constructed; a low volume chamber (8.5 L, 14 cm diameter x 55 cm length) for small plants growing at the first three phenological stages and high volume chamber (13.5 L, 14 cm diameter x 100 cm length) were used at maturity. All chambers were equipped with a small electrical fan to mix the air. In all the phenological stages of measurement, all pots were placed within the chambers and incubated for 24 h. All chambers were caped with a screw lid provided with valve to take gas samples. The absence of light stopped the photosynthesis. After 24 h, the respired C-CO_2 gas was sampled with a syringe and injected into the infra-red gas analyzer. If the C-CO_2 concentration was >1,500 ppm a gas dilution was required with free air CO_2. The diluted gas samples were injected into the PICARRO (G2301) to measure the isotopic signal of C-CO_2 respired from maize plants ($\delta^{13}C$ -12.27 ‰) and SOM ($\delta^{13}C$ -23.47 ‰ for PNP bulk soil and $\delta^{13}C$ -23.53 ‰ for PAC bulk soil).

In the second experiment also later of C-CO_2 measurements in chambers at flowering stage, the pot and plants were left few hours to recovery and the plants and capsules were harvested. The colonized roots in the capsules containing the bulk and soil fractions were cleaned and incubated in dark for 24 h (20 °C) in a sealed glass jar of 250 ml, provided with a rubber lead to take gas samples. The respired C-CO_2 from each glass jar was measured and the isotopic signal.

6.3.5 Calculations

Soil-derived $^{12}CO_2$-C (R_s, mg C-CO_2 kg^{-1} dry soil day^{-1}) was separated from plant-derived CO_2-C (R_p, mg CO_2-C kg^{-1} dry soil day) using mass balance equations.

$$R_t = R_p + R_s \quad (1)$$

$$R_t x\, A_t^{13} = R_p x\, A_p^{13} + R_s x A_s^{13} \quad (2)$$

where A_s^{13} is the ^{13}C abundance of soil, A_p^{13}, the ^{13}C abundance of plant, R_t the total CO_2 emitted by the pot (soil plus plant) and A_t^{13} its ^{13}C abundance. The R_p corresponds to CO_2-C coming out from the whole plant respiration, mycorrhizae and microbial respiration of rhizodeposits and plant litter. The rhizosphere priming effect (RPE, mg CO_2-C kg^{-1} dry soil day^{-1}) induced by the plant was calculated as:

$$RPE = (R_p, \text{planted soil}) - (R_s, \text{control soil}) \quad (3)$$

6.3.6 Carbon balance

The C balance was calculated as the difference in the amount of C-CO_2 that is derivate from ^{13}C plant (C-input) and ^{12}C derived from the control soil plus the primed ^{12}C pool (C-output)

6.3.7Data analyses

Two way ANOVA for (soil type and soil physical fractions) and three ways (soil type, soil physical fractions and planted and unplanted soil) were analyzed using JPM software and SPSS 21. All analyses were conducted with a significant *p* value of 0.05, previous test for normality. The abnormally distributed were log transformed when necessary.

6.4 Results

6.4.1 Bulk soil and soil physical fraction respiration

Bulk soil and soil fraction respiration $^{12}C\text{-}CO_2$ from planted and unplanted pots fluctuated between 2.15 mg C kg^{-1} dry soil and 624.48 mg C kg^{-1} dry soil and no significant differences were detected amongst development stages (data not shown). Soil respiration of bulk soil at maturity fluctuated between 50 and 136 mg C kg^{-1} and more $^{12}C\text{-}CO_2$ evolved from PNP (twice) than PAC pots ($P < 0.05$) (Fig 1A). Note that the soil respiration in PNP and PAC are similar in bare soil (control), whereas this amount was twofold in planted soils (Fig 1A). The amount of $^{12}C\text{-}CO_2$ in the plant + soil fraction in both soils ranged from about 110 mg C kg^{-1} up to 600 mg C kg^{-1} fraction (Fig. 1B). The C mineralization was always higher in planted pots and it decreased as the size of the fraction decreased in PNP, while it increased in PAC soil (Fig.1B). Mineral fraction of PAC soil exhibited the highest C mineralization.

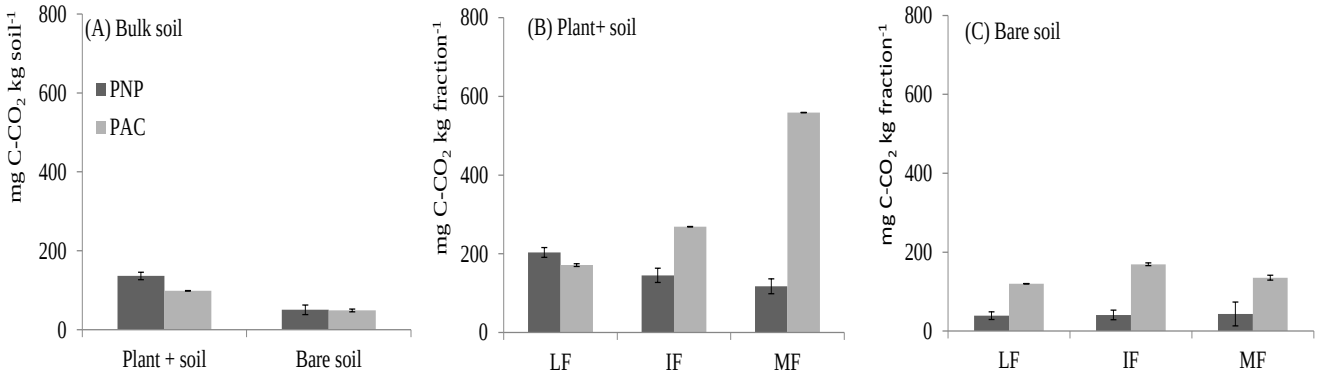


Figure 1. Basal soil respiration from Puyehue National Park (PNP) and Alerce Costero National Park (PAC) of (A) planted (maize) bulk and bare soil pot at maturity phenological stage; (B) soil physical fractions and in planted pot and (C) bare pot. Bars indicate standard error of mean. Significant differences at $p < 0.05$.

The $^{13}C\text{-}CO2$ derived from maize plants in the bulk soil and in various physical fractions showed significant differences between the two soils (Fig. 2). The ^{13}C derived from maize plants in the bulk soil was significantly higher in PNP than in PAC (Fig. 2A), following the same trends as for the total $^{12}CO_2$ in Fig 1. Identical trends, as in Fig 1 were observed for LF, IF and MF in both soils (Fig 2B).

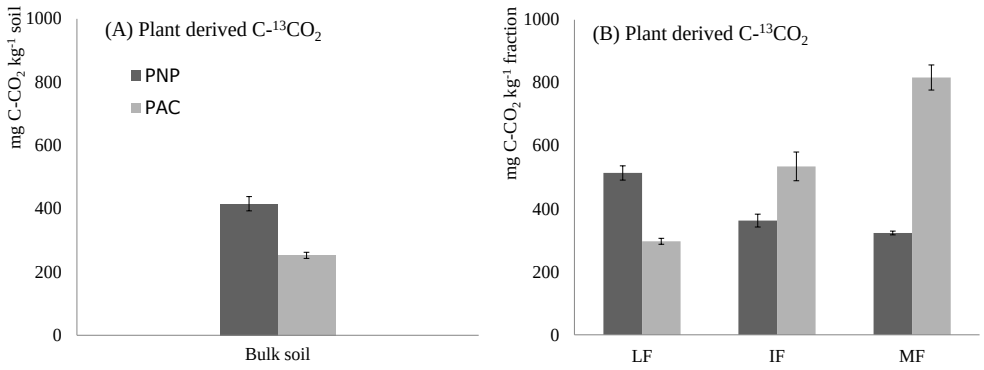


Figure 2. Maize derived C-$^{13}CO_2$ at maturity from Puyehue National Park (PNP) and Alerce Costero National Park (PAC) of (A) bulk soils and (B) various physical fractions. Bars indicate standard error of the mean. Significant differences at $p< 0.05$.

6.4.2 Rhizosphere priming effect (RPE)

In general, there was a positive RPE in all soils (43-419 % over the control) (Fig. 3). The RPE was higher in PNP than in PAC of bulk soil (Fig. 3A). In PNP, priming decreased as the size fraction decreased (Fig. 3B), whereas the opposite was true in PAC soil (Fig. 3C).

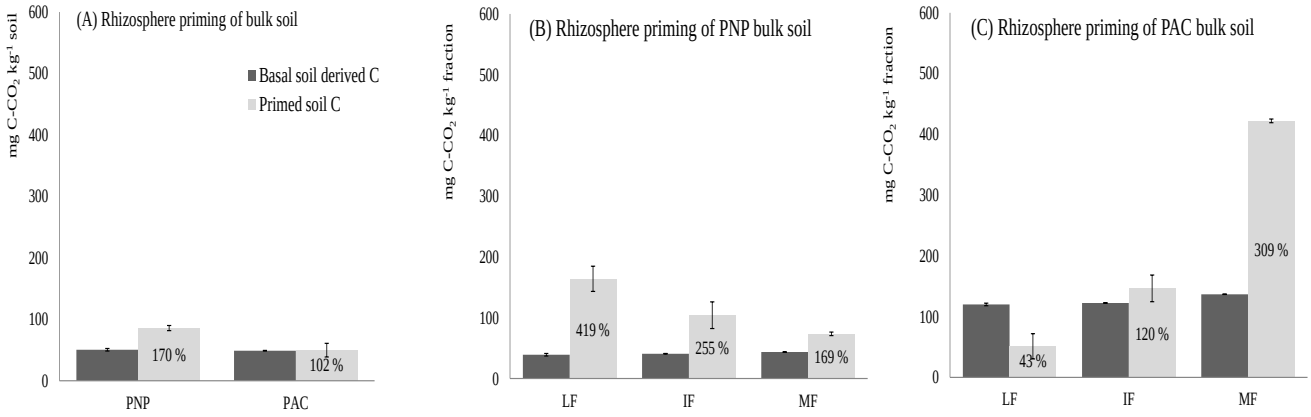


Figure 3. Rhizosphere priming effect of maize plant at maturity growing stage for (A) bulk soil and soil fractions from (B) Puyehue National Park (PNP) and (C) Alerce Costero National Park (PAC). Bars indicate standard error of mean. Significant differences at $p< 0.05$.

The contribution of primed fraction from the total CO_2 released of bulk and mineral fraction during the four phenological stages are showed in Fig. 4. The RPE in bulk soil of PNP did not show a clear trend, although small differences were detected. The basal respiration of SOM decreased along the growing stages from 49 % to 7 %. In the same soil, plant derived C was twofold at maturity compared with other development stages (Fig 4A). As in PNP, PAC bulk soil RPE contribution did not show trends across phenological stages (Fig. 4B), whereas RPE of MF increased from 2 % to 14 % in PNP and from 6% to 33 % in PAC soil (Fig. 4C and 4D). The contribution of basal respiration in PAC soil was 68 % of the total CO_2 released and decreased to 4 % at maturity stage. In contrast, PNP did not show any trends. In PNP the plant derived C was rather constant, unlike PAC soil increased from 27 % to 76 % at maturity.

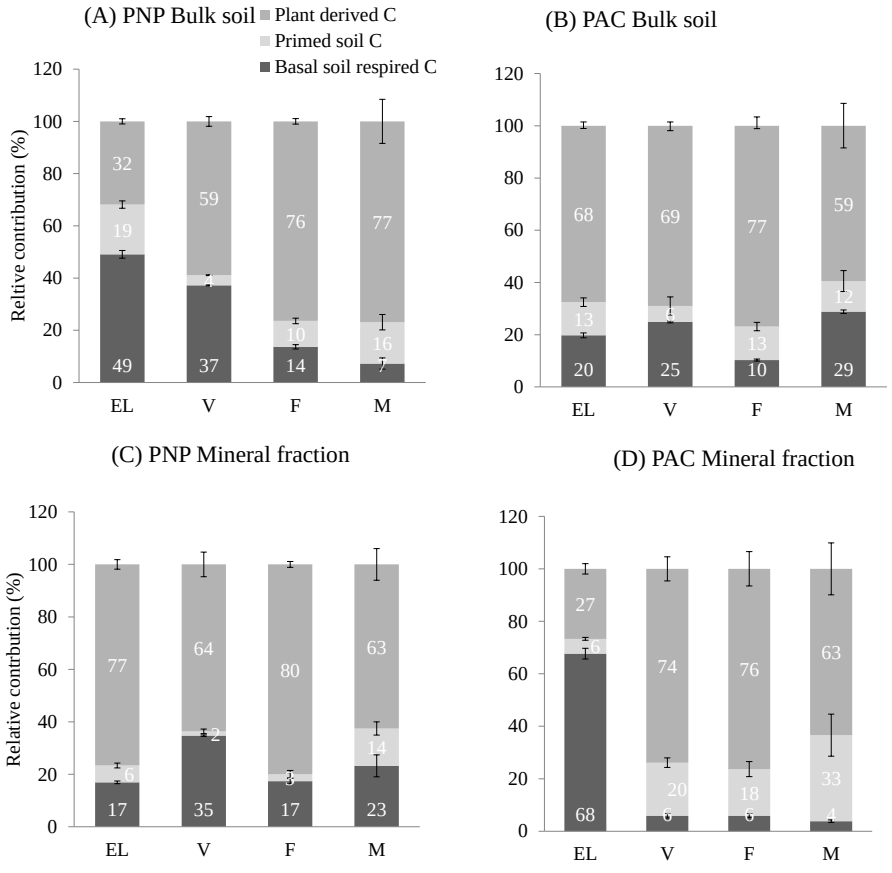


Figure 4. Contribution of basal soil respiration, rhizosphere priming effect and plant derived C of maize plant at maturity in a pot experiment containing (A-B) the bulk soil and soil and (C-D) soil fractions from Puyehue National Park (PNP) and and Alerce Costero National Park (PAC). Bars indicate standard error of the mean.

6.4.3 Relationship between RPE, plant derived C and Al–pyrophosphate

There was a positive, but highly significant relationship between the RPE and ^{13}C-CO_2 plant derivate C in both PNP and PAC soils (Fig. 5). The magnitude of RPE on SOM decomposition was higher in PAC than in PNP soil, as indicated by the higher slope (two folds) in PAC than in PNP soil. In general the plant derived C explained > 40 % the variation of RPE and these results were highly consistent with Fig. 1,2 and 3.

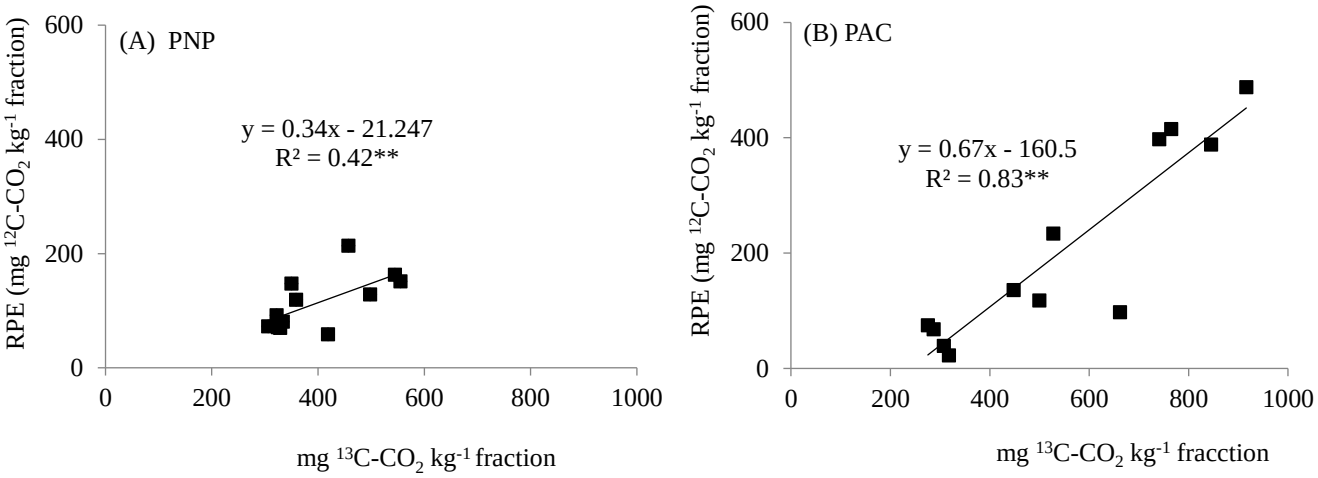


Figure 5. Relationship between rhizosphera priming effect (RPE) and ^{13}C-CO_2 in all soils from Puyehue National Park (PNP) and Alerce Costero National Park (PAC). * P < 0.05; ** P < 0.01.

We also found an inverse, but highly significant relationship between Al_p and C_p extracted by pyrophosphate, RPE and plant derived C in PNP soil (Fig. 6). There was no significant relationship in metamorphic PAC soil, because these soils exhibited scarce Al_p (Table 1). The correlation found in PNP indicates that highly reactive soils having high Al-SOM complexes reduce RPE and ^{13}C-CO_2 derived C, probably because of the great C sequestration capacity of allophanic soils.

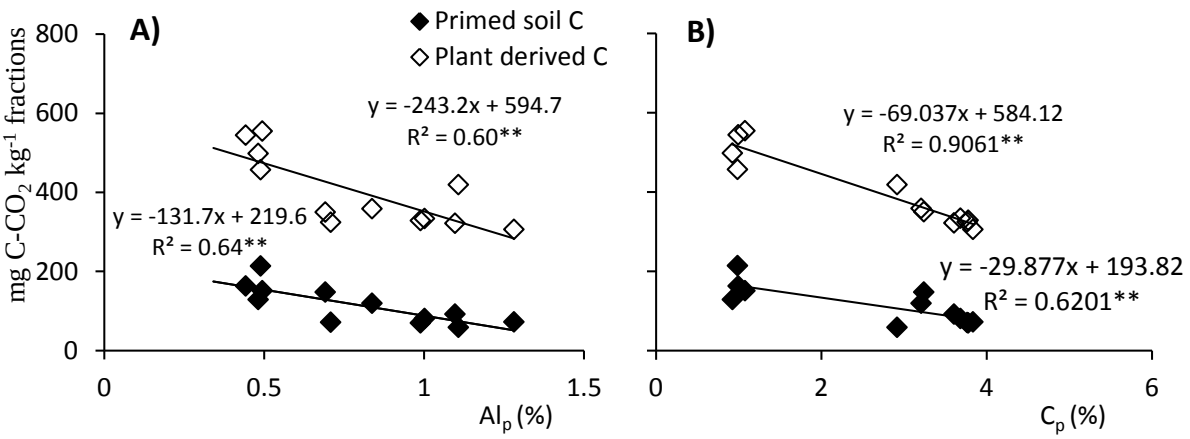


Figure 6. Relationship between Al and C pyrophosphate with RPE and ^{13}C-CO_2 plant derived in the soil fraction of Puyehue National Park (PNP). * $P < 0.05$; ** $P < 0.01$.

6.4.4 Rhizosphere priming effect (RPE) in colonized roots

There was a positive RPE in all physical fractions (17–610 % over the control) (Fig. 7). The RPE was generally higher in PAC than in PNP, except for LF in colonized roots and MF in the pot experiment at flowering (Fig. 7). In all fractions RPE measured at flowering decreased more than 12 times after 24 h of incubation. The colonized roots of physical fractions followed identical trends as those found in Experiment 1 (c.f. Fig 7B and 7D).

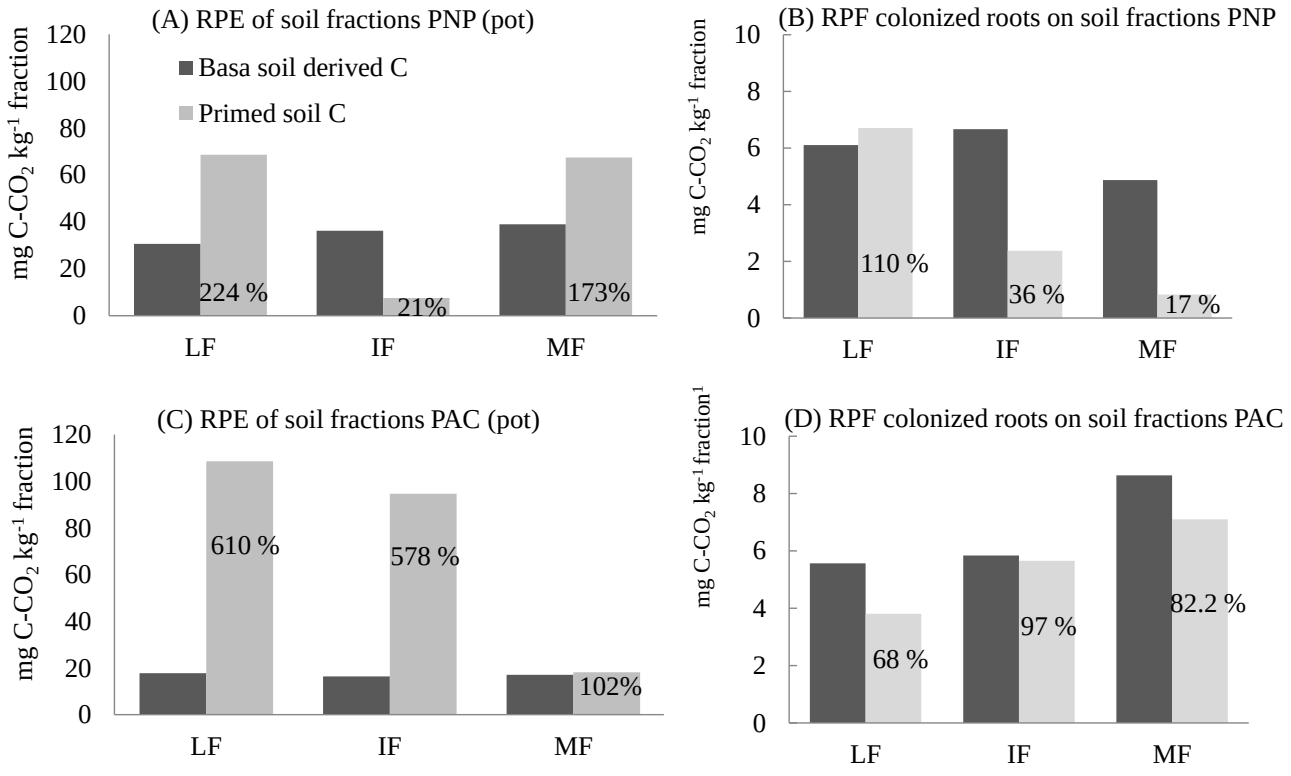


Figure 7. (A and C) basal and RPE from mineralized C derived from maize plants at flowering in Puyehue National Park (PNP) and Alerce Costero National Park (PAC) in the pot experiment of PVC capsules containing the physical fraction and (B and D) from PVC capsules colonized by maize roots harvested and incubated for 24 h. Note the ordinate presents different scales. Significant differences at $p< 0.05$.

6.4.5 Carbon balance

The difference in the amount of $C\text{-}CO_2$ that is derivate from ^{13}C plant (C-input) and ^{12}C derived from the control soil plus the primed ^{12}C pool (C-output) was regarded as the C balance (Fig 8). This procedure was applied for the two soils and fractions in two phenological stages. In general the C balance was positive at maturity and near to zero and negative in early stage of plant growing. The C balance followed similar pattern as those found for RPE in PNP and PAC soil. Clearly PNP bulk soil is able to sequester more C than PAC in bulk soil. However at maturity MF pf PAC soils is able to sequester more C than PNP soil.

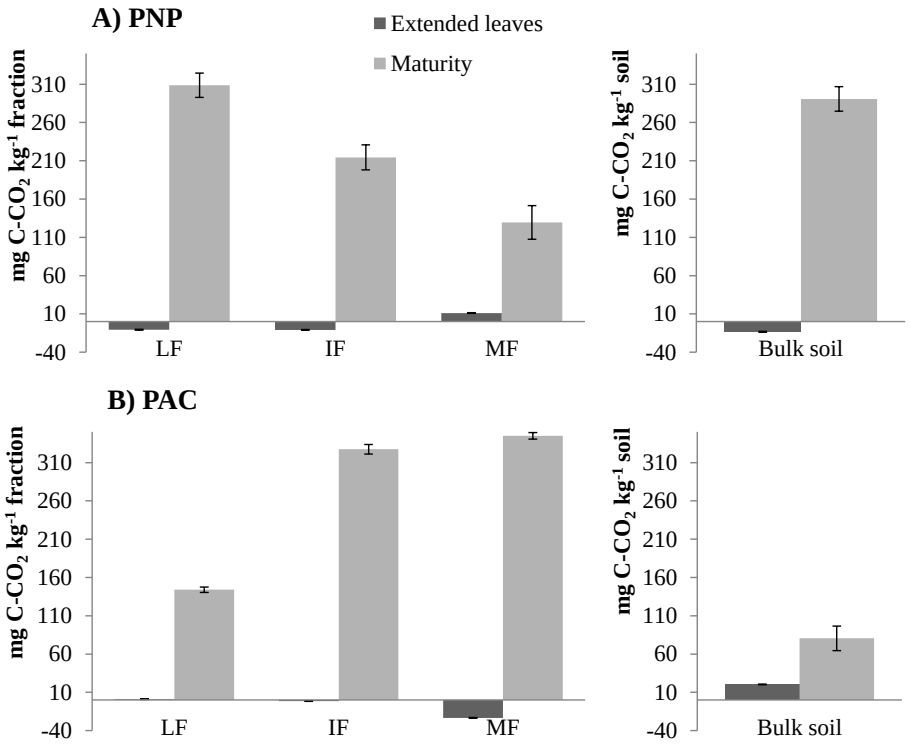


Figure 8. Difference between the amounts of CO_2 derived from plant (input) and soil (output) in Puyehue National Park (PNP) and Alerce Costero National Park (PAC) of (A) bulk soils and (B) various physical fractions. Bars indicate standard error of the mean. Significant differences at $p< 0.05$.

6.5 Discussion

In the first experiment, our results indicate that the phenological stages of maize plants positively influenced the RPE during 131 days of experiment in all bulk soils and physical fractions. We assume that the increasing root rhizodeposits caused the increase of CO_2 released from native SOM. The input of LOC substances to soils can significantly change the degradability, otherwise resistant native SOM. Fontaine et al. (2007) showed that the stability of organic matter in deep soil layers (2500 year-old C), most bound to minerals is controlled by the supply of fresh plant-derived C. For example, C mineralization from recalcitrant organic matter, such as biochar, has been reported to increase because of LOC addition (Hamer et al., 2004; Kuzyakov et al., 2009). Our results supported the idea that magnitude of RPE is controlled by the clay mineralogy and the availability of fresh organic C. The slope of RPE and ^{13}C derived plant

relationship was lower in amorphous clay of PNP soils indicating strong capacity of clay mineral to stabilize the LOC, which, in turn, controls the growth of microbes.

The physical fraction size also influenced positively the mineralization of C in the two soils. Mineral fraction had the lowest primed C in PNP (Fig 3B) and the highest in PAC (Fig 3C). This was attributed to the reduced availability of organic C for soil microbes due to the clay mineralogy (allophane and high potential for Al-SOM complexes formation in PNP compared with PAC soil with kaolinite and halloysite clay) (Matus et al., 2008; Luzio et al., 2003). Table 1 and 2 show that the quantity of Al bound to SOM in PNP soil was twice the amount in PAC soil. Mineral fraction of PAC soils contain kaolinite and halloysitye, which are more crystalline clay (Luzio et al., 2003). Jones and Edwards (1998) reported that the simple C substrates (glucose and citrate) added to kaolinite and illite-mica were more decomposable than those added to ferric hydroxide. The chemical bonding between SOM and mineral surfaces decreases the availability of C to microorganisms (Guggenberger and Kaiser, 2003). Clay minerals have different specific surface areas; therefore, it may be expected that clay type influence the capacity of soils to hold organic C.

The ^{13}C-CO_2 of planted soils in PNP decreased as the particle became small. These results agree with RPE in the mineral fraction indicating the potential C storage of these soils. In contrast, PAC soil showed the opposite trends because the LOC is less retained by kaolinite and halloysite minerals (Merino et al., 2015). It is also probable that these minerals are easily saturated with organic matter (Feng et al., 2014).The trends of REP in the present study were also supported because the proportion of C mineralization was always higher in PAC soil than in PNP due to mineral composition. These results were also supported by a highly negative correlation between Al_p and RPE in PNP. The Al_p can be regarded as the active Al which is capable to bound SOM, suggesting that PNP sequester, more fresh C than PAC soil, where no correlation was found.

Ludwig et al. (2005) found that the storage of maize-derived C in particle size fractions of the Ap horizon decreased in the order clay (0.65 kg C m^2) > fine and medium silt (0.43 kg C m^2) > coarse silt (0.33kg C m2) > fine sand (0.13 kg C m^2) and > coarse sand (0.06kg C m^2). Results of these studies emphasize that C in the clay fraction is more resistant to microbial decomposition compared to that in other soil particle size fractions. However, (Ohm et al. (2007)) reported a strong priming effect in sand (63-2000 µm), silt (2-63 µm) and clay (<2 µm) by the addition of fructose and alanine resulted in different priming of recalcitrance SOM.

In the second experiment the RPE was greatly reduced 24 h after root plants C influxes were ceased (Fig.7). This means that the fresh C rhizodeposits from plants were consumed after 24 h of incubation. We also found that the RPE on SOM decomposition was higher in metamorphic (1:1 clay) compared with allophanic soil. However, the primed MF was dramatically reduced in both soils, once the fresh C input released from maize roots ceased. Dijkstra et al. (2006) found that differences of RPE on SOM decomposition could be largely explained by biomass of soybean and sunflower, particularly leaf biomass, explaining 49-74 % of RPE in SOM. Cheng (2009) used a natural ^{13}C tracer to separately measure SOM-derived CO2 from root-derived CO2. Results indicated that the magnitude of the RPE on SOM decomposition varied from 0 to > 380 % of the unplanted control (soybean and wheat), and it was largely influenced by plant species and phenology. The variations in plant growing stages may strongly affect C availability for soil microbial community and impact the RPE, as showed in Experiment 1. The quality and quantity of root exudates vary considerably according to the plant species and the physiological status (Nguyen (2009)Johnen and Sauerbeck (1977). Nguyen (2003) estimated that the amount of C released by roots exudation ranged from 0.2% to 7% of the root dry matter per day and he observed that root exudation was the highest at flowering in cereal plants.

In these second experiment living roots and their rhizodeposits stimulated microbial activity and SOM decomposition up to 10 folds compared with bare soil. This calculation can be obtained when the photosynthesis was stopped and capsule of colonized roots were incubated for 24 h. It is well know that RPE varies widely among plant species (e.g. Pausch et al., 2013; Cheng, 2009). Pausch et al. (2013) found that the RPE was positive under all plants (soybean, sunflower and wheat) and it ranged from 43% to 136% above the unplanted control. At flowering, RPE was the highest indicating the importance of plant C influxes on RPE. In other studies with maize plant RPE was found negative on SOM decomposition (Kuzyakov and Cheng, 2004). The contribution of arbuscular mycorrhizal fungi, exudates, and depositions of litter on RPE has also been emphasized in grassland soils (Shahzad et al., 2015).

In summary organic rhizodeposition by maize roots were ceased at flowering and LOC was consumed after 24 h photosynthesis stop. The LOC strongly reduce the RPE. In both experiments our results indicated that the RPE was controlled by plant derived C and clay mineralogy.

6.6 Conclusions

The hypothesis that the mineral composition of soils determines the magnitude of RPE was confirmed. This was supported because mineral fraction of allophanic soils and the potential of active Al for SOM complexes formation decreased the availability of fresh organic C and, in turn, the RPE compared with more crystallinity clay of kaolinitic soil. The RPE increase as the C input from ^{13}C-plant increase. There was an inverse relationship between Al extracted by pyrophosphate and RPE and negative relationship between Al and plant derived ^{13}C. Similar results were also obtained with the C extracted by pyrophosphate, indicating the ability of allophanic soils to sequester C. The magnitude of RPE is associate to the input of C from maize roots across phenology, which in turn is controlled by the clay mineral and active Al in volcanic soil. Finally the positive C balance in the bulk soils of PNP soil was far than in PAC soils, however in the mineral fraction the opposite was true This C input modulate the magnitude of RPE depending on the reactivity of the mineral phase to stabilize the labile C pool.

CHAPTER VII

General discussion, concluding remarks and future directions

7.1 General discussion

A complete understanding of the mineral control on terrestrial carbon (C) dynamics lacking (Torn et al. 1997; Wagai et al. 2009; Wagai and Kitayama 2012). Soil organic C from soil organic matter (SOM) is strongly influenced by soil mineral phase components (clay minerals) and soil aggregation, which increases the mean residence time of organic C (Asano and Wagai, 2014). For instance, SOM complexation with Al and Fe in aqueous or solid phase produces a strong stabilizing effect, controlling the soil C sequestration and available of C for soil microbes (Merino et al., 2015; Zunino et al., 1982b). In contrast, the addition of fresh labile C or available C in presence of plants generally induces an acceleration in the turnover of native SOM, the so called rhizosphere priming effect (RPE). This is considered as a destabilization of native organic C, which has been scarcely studied in soils with contrasting mineralogy.

There are several mechanisms of stabilization of SOM that controls the available C for the microbial communities that prevent further SOM biodegradation (Sollins et al., 1996) where humification dominates over mineralization. These mechanisms include recalcitrance of organic compounds, spatial SOM inaccessibility for decomposers and soil mineral interaction with SOM (von Lützow et al., 2007). Several studies have suggested that the amount of amorphous clay minerals, oxides or hydroxides of aluminium (Al) and iron (Fe) and organo-mineral complexes in volcanic soils are the key agents for the enormous organic C accretion in comparison with non-volcanic soils (Matus et al., 2014). In non-volcanic soils, SOM interacts with crystalline clay minerals such as 2:1 and 1:1 that lead to a SOM accumulation ten times lower than that of volcanic soils (Jones and Snigh, 2014). In general, there is an important contribution of Al and Fe (as colloidal phase) to complexes SOM in all soils, but non crystalline amorphous type materials (i.e. allophane, imogolite, ferrihydrite, Al- and Fe-humus complex) are particular important in volcanic-allophanic soils (Shoji et al., 1988; Nanzyo, 2002; Tsai et al., 2010). Matus et al. (2008) found a positive relationship between SOC and Al and Fe pool complexed with SOM in several Chilean volcanic soils. About 40–60% SOC has been found bound to Al and Fe (Garrido and Matus, 2012).

Another factor that controls the SOM stabilization and C availability is the soil aggregation. Although, we recognize the importance of this factor on the physical protection of SOM, soil aggregates and its influence on C mineralization and RPE is beyond the scope of this study.

To better understand the dynamics of SOM and C availability for soil microbes, SOM should be physically fractionated in different particle size to explore the contribution of various size fractions to C mineralization (Oades, 1993; Kleber et al., 2007; Six et al., 2002; Sollins et al., 1996; Sollins et al., 2009). The specialized decomposers in specific soil fractions depend on the characteristics of SOM. Soil microbes differs in its response to the addition of fresh organic matter (Poll et al., 2003), producing different mineralization rates and different intensities of RPE (Ohm et al., 2007).

The above information indicates that volcanic and non-volcanic soils differs in their stabilization capacity and we might gain information on RPE if we use the fractionation technique using soil of different mineralogy. In Chapter I we present a general introduction where is emphasized the uncertainty of priming effect due to the available C for soil microbes. Coupling C dynamics in plant and soil remains as one of the least understood components at global scale because the interaction complexity (Paterson and Sim, 2013; Cheng et al., 2014; Hill et al., 2015). For instance, the priming may occur resulting from mining of soil nutrients or by the exudates provided by root plants as supply of easily energy for the decomposition of native SOM (Keiluweit et al., 2015).

In the present thesis, we hypothesized that soil mineral composition and chemical SOM stabilization control the biological activity and priming effect intensity by regulating the organic C availability. We predict that the resistance of organic C to decomposition in different physical fractions (light > 250 μm; intermediate 53-250 μm and mineral < 53 μm) contributes differently to the magnitude of priming effect and this view has not been explored.

The main general objective is to determine the priming effects under different C availability for microbial community in volcanic (allophanic) and kaolinitic soils of metamorphic origin.

In Chapter II we intent to integrate the main conceptual limitations, artifacts and biases associated with lack of knowledge and possible causes and processes leading the priming effect. The scientific community concurs that the factors and mechanism controlling the priming effect are not fully understood. For instance, there is vague knowledge about the real and apparent priming effects. The apparent priming is fast growing of microorganism releasing CO_2 other that C released from native SOM (the real priming

effect). Another important factor that is being more and more considered in the literature is the role of N in the priming effect, particularly when the quality of the C input rate is regarded based on C to N ratio (Chena et al., 2013).

There are intriguing mechanisms that are not yet being well explored, such as the role of unknown CO_2 respiration by extracellular exoenzymes in the sterilized soils. Another important issue little explored is the needs to present C balance including the priming effect in connection with the carbon use efficiency. In the present thesis we did not examine the successional microbial communities involved in the priming effects, since they respond differently to various substrates and they ultimately determine the C use efficiency overall the growth processes.

In Chapter III, the rhizosphere, a thin area of soil surrounding roots receiving C exudation are intense spots of competition for available C and nutrient between surface-reactive particles and soil microorganisms. This poses the question what is the critical level that becomes limiting for microbial growth and soil organic matter decomposition. On the other hand, complexation of labile compounds with metal such as Al and Fe oxides provides a direct evidence of translocation of organic C within the soil profile (Kaiser and Guggenberger, 2000). Transport of mineral-SOM complexes to deeper soil has been reported by conversion of tropical forest into grassland (Osher et al., 2003). In high precipitation regions, C losses from the soil appear to occur via downward transport, either as colloids or in solution.

The enzymatic activity in different physical fractions presented in Chapter IV (see below) indicated the mineral fraction, where allophane and kaolinite clay occurs, the C available for soil microbial assimilation is reduced due to the clay type of root exudates (van Hees et al., 2002; Jones and Edwards, 1998). Preferential C uses by soil microorganisms of simple compounds such aminoacids, citric acids, etc. can lead to a change in the SOM transport and turnover induced by fresh C input (Sparling et al., 1982). In this Chapter we conclude that there is a lack of studies in which mineralogical composition is taken into account (Rasmussen et al., 2007; 2006). We intent to go deeper in Chapter IV where the soil was physically fractionated and the biological activity evaluated in each fraction.

In Chapter IV several biological and biochemical analyses such as soil respiration, enzymatic activities (β-glucosidase, dehydrogenase, urease and acid phosphatase), carbohydrates (total and soluble), reducing sugars, microbial biomass, ATP and other nutrient (inorganic P and mineral N) were determined. We learned that the mineral interactions with low molecular weight organic compound and neutral sugars

leads a readily consumption of C that did not interacts with mineral phase. In both studied soils (allophanic and kaolinitic,) the C-CO_2 respired was explained (>76 %) by the activity of β-glucosidase, dehydrogenase and phosphatase as indicated by in multiple regression analysis. Theses enzymes activities were lower in the mineral fraction < 53 µm of allophanic, unlike kaolinitic clay, while the ATP was highly correlated with C-CO_2 and *q*C-CO_2, the metabolic respiration, indicating that our results were consistent with the complexation capacity of soil as shown in Chapter V.

There is a poor understanding of the influence of Al oxides on mineralization of C in forest soils. In Chapter V the effect of Al on dissolved organic matter (DOM) in an allophanic and kaolinitic temperate rain forest soil was evaluated. Here we hypothesized that Al added in the soil solution causes inhibition for soil microorganisms growth when the molar Al:C ratio > 0.1 (Schwesig et al., 2003). This is because it is assumed that the Al is able to complex the DOM and when this capacity is surpassed, the excess of Al can inhibit the microbial growth. In contrast, for Al:C ratio < 0.1, the forming complexes reduces the "free Al" in the soil solution allowing microbial growth and even a greater C mineralization.The objective of this study was to evaluate the effect of Al concentrations on the potential for C biodegradation at different Al:C ratio in the two studied soils. The extent and mineralization rates of DOM and Ah mineral soil horizons at various Al:C ratios was investigated. Dissolved organic C extracted was incubated with several Al doses from 0, 0.2, 0.4, 2, 4 and 8 mg L^{-1} equivalents to a molar Al:C ratio of 0.011, 0.022, 0.11, 0.22 and 0.44, respectively (Schwesig et al., 2003). Mineralization was quantified byC-CO_2 evolution. Indeed the hypothesis was confirmed since the C mineralization rates were unaffected by the Al addition until Al:C ratio 0.12. This took place for C mineralization at the beginning (1-7 d) of incubation experiments. These result were also supported by the cell counting and the florescence observed by confocal microscopy. Ratio > 0.12 inhibited the microbial growth as indicated by the lower rates of C mineralization. We conclude that the Al complexation with DOM is quite important and it regulates the C availability for microbial growth in accordance with Chapter IV. The mineral controls on soil organic matter biodegradation in soils with different mineralogy is studied in Chapter VI, where the RPE was evaluated in different soil fractions.

In Chapter VI we presented two experiments in the first one we demonstrated that the RPE was reduced by the greater soil capacity to sequester C. This was attributed to a decreased in C availability for soil microorganisms that depended on clay mineralogy. This experiment was conducted across several phenological stages from maize (*Zea maiz* L) plants growing in pots, where plant roots colonized the bulk

soil and various physical fractions from allophanic and kaolinitic soil. In the second experiment we compared the impact of roots on RPE in different physical fractions and bulk soil in a pot experiment harvested at flowering (C influx from roots was stopped). The results indicated that RPE dramatically decreased after 24 h post-harvest, when the C rhizodeposit ceased. Basal respiration and RPE were reduced between 8 and 33 times with respect to the soils with plants. Our results indicate that PAC soil mineralized more C than PNP and that RPE was larger in this soil because of its reduced sequestration capacity (attributed to more crystallinity of clay minerals). A positive C balance, the difference in the amount of C-CO_2 that is derivate from ^{13}C plant (C-input) and ^{12}C derived from the control soil plus the primed ^{12}C pool (C-output) in the bulk soils of PNP (allophanic) soil was far than in PAC (kaolinitic) soils, however in the mineral fraction the opposite was true This C input modulate the magnitude of RPE depending on the reactivity of the mineral phase to stabilize the labile C pool. We concluded that RPE depended on the fresh available C input and soil mineralogy.

7.2 Concluding remarks

Several conclusions can be drawn:

- There is lack of knowledge to explain the factor and mechanism that induce the PE. The variability of the SOM in different ecosystems is so high that makes difficult to reach a consensus on the magnitude and direction of PE.
- The main artifacts in the evaluation of PE are primarily conceptual.
- There is little importance to the role of N and P on PE.
- Available C for microflora consumption depends on the mineral composition and the type of exudates, and their relative concentrations.
- The microbial interactions with the mineral phase can affect the reactions and the process of soil C. Organic C derived from microbial activity and root exudates is probably the most mobile and bioavailable fraction of C in the rhizosphere. The retention and mobility of organic compounds depend on soil properties and can affect the availability of soluble C for soil microbes.

- Aluminium humus-complex formation needs further attention, because Al may be toxic for soil microorganisms, but it can also enhance C assimilation in the soil solution.
- Organic C mineralization of DOM from allophanic and kaolinitic soils were unaffected by potentially toxicity of Al up to a molar ratio Al:C of 0.12. Metal:C ratios < 0.12, the Al speciation and DOM complexes formation removes Al in the soil solution allowing the microbial growth.
- Metal–humus complexes, Al hydroxides, and pH are identified as key physiochemical variables accounting for variation in DOC and microbial activity across the sites, This demonstrates that soil properties might suppress the microbial growth and respiration, all associated to C availability.
- The hypothesis that the mineral composition of soils determines the magnitude of RPE was confirmed. This was supported because mineral fraction of allophanic soil and the potential active Al for SOM complexes formation decreased the availability of fresh organic C and RPE compared with kaolinitic soil.
- There was an inverse relationship between Al extracted by pyrophosphate and RPE.
- The magnitude of RPE is associated to the input of C from maize roots across its phonology, which in turn is controlled by the clay mineral and active Al.
- The RPE was controlled by plant derived C and clay mineralogy. Mineral fraction of allophanic soil produces a less SOM decomposition compared with kaolinitic soil with more crystalline clay.
- The RPE decreases when the input C from fresh ^{13}C-plant derived ceased. The C input modulates the magnitude of RPE, depending on the reactivity of the mineral phase to stabilize the SOM.

7.3 Future directions outlined from this thesis

- The PE carry out the implicit assumption that carbon use efficiency did not vary between amended and control soil. It is very important to verify this assumption by making the soil C balance, because different efficiencies may lead different CO_2 respired.
- Is important to identify the successional microbial communities involved in PE, since they respond differently to various substrates and they determines the C use efficient and preferential C utilization for growth and production of CO_2.

- The hypothesis that RPE is driven by low molecular weight organic substances from root exudates requires further research. Based on current knowledge the SOM competition between soil microorganism and mineral phase over the time also needs to be addressed.
- The hypothesis that DOC is the most susceptible to stabilize through different organo-mineral interactions through the soil profile requires further studies.
- We have hypothesized that SOM mineralization due to RPE may be important across the soil profile. This is supported by new conceptual models explaining the colloidal transports in soil with different mineralogy.
- The RPE has been studied from microorganism and nutrient availability point of view. Microorganism's accessibility to recalcitrant SOM with different availability of C plays a crucial role on RPE.
- Further studies on C availability on soil C turnover should go deeper. It should be pay close attention on SOM composition and structure as well as the changes in enzyme and microbial activities on PE.
- Temperate rain forest soils exposed to large fluctuations of O_2 across the year is of particularly importance for RPE. These processes are affected by anoxi-abiotic processes. Mineral fraction may contain anaerobic microsites (O_2 depleted) due to the root and microorganism respiration.

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