Soil enzymes and biological activity at different levels of organic matter stability

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Abstract

Soil biological activity has important implications for soil carbon (C) sequestration. However, very little is known about the environmental factors, particularly the effect of soil mineralogy on availability of C for soil microorganisms. In this study, we have investigated the influences of soil type (clay mineralogy) on C mineralization and its effects on biological activity at different levels of soil organic matter stability. Two soils an allophanic, derived from recent volcanic ash and a kaolinitic, resulting from metamorphic parent materials were physically fractioned in to light (LF, coarse sand 250-2000 μm), intermediate (IF, fine sand 53-250 μm) and mineral (MF, silt and clay < 53 μm) fractions. Several biological and biochemical analyses at Ah horizons of mineral soil and physical fractions were conducted: soil respiration, enzymatic activities, carbohydrates and microbial biomass, amongst others soil variables. The results indicated that the bulk soil and physical fractions had a significant impact on cumulative C mineralized after 30 days of incubation and soil enzyme activities. More than 76% of total C-CO₂ variation was explained by stepwise multiple regression analysis including factors such as soil enzymes (β -glucosidase, dehydrogenase and phosphatase) and inorganic P. Soil ATP extraction was a good indicator of microbial activity, because of a positive and significant correlation among ATP and i) C-CO₂ and ii) metabolic quotient (soil respiration rate divided by microbial biomass). We also found an inverse and significant relationship between Al pyrophosphate (Al bound to SOM) and the C-CO₂ in volcanic soil, whereas the same correlation did not occur in kaolinitic soil. Our results confirmed a greater stabilization capacity of MF in allophanic than in kaolinitic soils due to the amorphous minerals clay materials.

Keywords: Soil physical fractionation, carbon mineralization, volcanic soils, mineral interaction

1. Introduction

It is important to know how soil responds to environmental changes to predict the response of soil ecosystem to soil C sequestration under climatic change scenarios (Post *et al.*, 2009; Nie *et al.*, 2013; Zhao *et al.*, 2014). For instance, the addition of fresh labile C that generally induces an acceleration in the turnover of native soil organic matter (SOM), the priming effects, might cause an extra mineralization of C, greater than fresh C incorporated into the soil.

From a physical point of view, the habitat for soil microorganisms is influenced not only by the soil pore size distribution in the soil matrix, but also by the mineral composition and availability of C (Van Gestel *et al.*, 1996; Barberán *et al.*, 2014). It is well known that soil microbes are unable to assimilate solid SOM directly, but rather simple and dissolved compounds for their growth and development. Consequently, soil microbes produce extracellular enzymes to make readily usable dissolved compounds. For example, β -glucosidase, a hydrolytic enzyme that acts on β -1,4 linkage of oligomers, yield organic substances of low molecular weight and soluble compounds (Hammel, 1997). Another example, is shortage of the available nitrogen (N) in soil limits the microbial activity, so soil microbes produce oxidative enzymes to degrade recalcitrant SOM and release the occluded N (Carreiro *et al.*, 2000; Waldrop *et al.*, 2004). Dornbush *et al.* (2007) found that the N content of grass litters had a positive correlation with the activity of β -glucosidase family enzymes. The release of microbial enzyme in response to the quantity and quality of SOM in different soil types needs to be better understood (Rasmussen *et al.*, 2006; Kuzyakov, 2010). Soil physical fractionation and soil mineralogy might indicate different C stability and functionalities for microbial communities. Different methods of soil fractionation have been developed to study the mechanisms that control SOM

stability and complexity. The most popular one, has been fractionation by using liquids with different densities for non-volcanic soils (Christensen 1992; Jastrow 1996; Six *et al.*, 2002; Jolivet *et al.*, 2003). In volcanic soils, the density fractionation has been scarcely reported (Panichini *et al.*, 2012), due to intrinsic gel soil characteristics of the volcanic materials. However, the common method that is often applied in both soil types is the wet sieving (Kemper and Koch, 1966).

The present study was performed in the Andes and Coastal range old growth forest of *Nothofagus* spp. in southern Chile in allophanic soil derived from a volcanic ash and kaolinitic soil derived from metamorphic materials. 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 mineral composition at different level of SOM stability can provide valuable information to study the nutrient conservation dynamics in pristine ecosystems respiration.

The aim of this study was to determine the biological activity in various physical fractions isolated from Ah mineral horizons from soils with contrasting mineralogy and stabilization capacity of SOM.

2. Materials and Methods

2.1. Site characterization

The present study was developed in volcanic soil derived from recent volcanic ash in the Andes range and in a kaolinitic soil in the Coastal range. Both sites belong to temperate rain forest ecosystems in southern Chile. The volcanic soil is located in the Puyehue National Park (PNP) (40°26'–40°58'S and 72°22'–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 >4,000 mm and mean annual temperature (MAT) 9.2 °C (Oyarzún et al., 2004). These soils 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 an important amount of Al- and Fe-SOM complexes (Matus et al., 2008). The second area of study is Alerce Costero National Park (PAC) (40°12' S and 73°26' W, 1000 m.a.s.l.) in the summit of Cordillera de la Costa. The soil is developed from metamorphic-schist materials 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 *Nothofagus nitida* (Phil). The sampling site was a huge area dominated by *N. nitida*, *Saxegothaea conspicua* (Lindl.) and *Laureliopsis philippiana* (Looser) Schodde (Monimiaceae). The mean annual precipitation is > 4,000 mm and annual temperature fluctuates between 9.5 and 12.1 °C.

2.2. Soil sampling

Top Ah mineral horizon (5-10 cm) were sampled after organic litter horizon was removed.

The soils were 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 macronutrients (Table 1) (Matus et al., 2011; Garrido and Matus, 2012).

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 _{water}	5.7	4.5
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 (potassiumchloride)	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	kaolinit
Texture ⁴	SL	CL

¹Soil organic carbon

²Cation exchange capacity

³Luzio et al. (2003); Neculman et al. (2014)

⁴SL = silty loam and CL = clay loam

2.3. Physical fractionation of soil organic matter (SOM)

We used Balesdent et al. (1991) method for physical fractionation. From each soil, three physical fractions were obtained in triplicate after removing the floatable in water organic materials: Light fraction (LF, coarse sand > 250 µm), intermediate fraction (IF, fine sand 50-250) and mineral fraction (MF, silt and clay < 53 µm). 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 53 μm sieve. 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 oven dried at 35 °C.

2.4. Incubation of bulk soils physical fractions

About 10 g (dry weight basis) of bulk soil and physical fractions (previously inoculated) were placed in 250 ml flask (Schott) with a tight rubber stopper and incubated at 60% of field capacity (-33 kPa) for seven days at 26 ± 2 °C in dark. We assume that this procedure allowed removing the particulate organic matter 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. 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:

$$\text{C-mineralization rate} = \frac{\text{Ln}(C_{t_2}) - \text{Ln}(C_{t_1})}{t_2 - t_1}$$

where, C_1 and C_2 is the cumulative C mineralization (mg C- CO_2) at t_1 and t_2 , respectively.

Mineral N (ammonium and nitrate) 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. Inorganic phosphorus was determined by the method Olsen (Olsen *et al.*, 1954). Olsen-P was measured by extracting with 0.5 M NaHCO_3 adjusted to pH 8.5. (Matus *et al.*, 2011; Garrido and Matus, 2012)

2.5. Biological and biochemical analysis

2.5.1. Microbial biomass C quotient

Microbial biomass C (MB-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 ($q\text{CO}_2$) was calculated as the soil respiration rate divided by microbial biomass C (Anderson and Domsch, 1990) and served as indicator of microbial metabolic C use efficiency present in the sample.

2.5.2. Enzyme activity

We determined 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 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 the soil sample (in triplicate), which was incubated at 37 °C for 30 min. Both activities were determined by the released NH_4 (Nannipieri *et al.*, 1980). Phosphatase and β -glucosidase activities were determined using p-nitrophenyl phosphate disodium or p-nitrophenyl- β -D-glucopyranoside as substrates, respectively (Masciandaro *et al.*, 1994). Two ml of 0.1 M maleate buffer (pH 6.5 for both phosphatase and β -glucosidase activities) and 0.5 ml 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 , then 2 ml of 0.5 M NaOH were added and the mixture centrifuged at 2000 g for 5 min. To stop the β -glucosidase assay, trishydroxymethylaminomethane was used according to Tabatabai (1982). The amount of p-nitrophenol was determined using a spectrophotometer at 398 nm (Tabatabai and Bremner, 1969).

2.5.3. Carbohydrates

Total carbohydrates were determined by the direct method described by Safařík & Šantrůčková (1992). About 0.25 g of soil sample was transferred to glass tubes and 1 mL of 72% sulfuric acid plus 20 mL of water were added. The glass tubes were placed in water bath at 104 °C for 5 h. Thereafter, once the tubes were cooled down, the suspension was filtered (Whatman No. 40) and the supernatant was transferred to 50 mL ball flask. One milliliters of the extract was added to 4 mL of anthrone reagent

(4 g in 200 mL anthrone sulfuric acid), stirred and warmed 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. The absorbance was measured at 540 nm.

2.5.4. ATP

ATP was extracted from soil using Webster *et al.* (1984) procedure and measured as recommended in Ciardi and Nannipieri (1990). Twenty milliliters of a phosphoric acid was added to 1 g of soil, 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.).

2.6. Data analysis

The ANOVA test and correlations amongst different soil properties were carried out by JPM software. All analyses were conducted with a significant *P* value of 0.05. Regression analysis to test the effect of soil properties on C mineralization 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 significantly 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 (Garrido and Matus, 2012). 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 significantly. 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).

3. Results

3.1. 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). The allophanic soils in PNP is silty loam (Neculman *et al.*, 2012), whereas in kaolinitic metamorphic soil it is clay loam (Luzio *et al.*, 2003). 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 complexed Al 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).

3.2. Incubation

In all soils and fractions, the C mineralization was between 162 and 1000 mg C-CO₂ kg⁻¹ soil after 30 days of incubation and it was always lower in PAC soil (Figure 1). Depending on the fraction, mineralization decreased as follow: LF > IF > MF in both soils (Figure 1A and 1B). In general, the mineralized C tended to stabilize after 15 days of incubation (Figure 1C and 1D).

3.3. Enzymes activity, carbohydrates and other soil parameters

Enzymes secreted by soil microorganisms and various related soil properties at different fractions provided information about the potential activity of the soil microbial community. All soil enzymes, β -glucosidase (βG), dehydrogenase (DH), phosphatase (P_{ase}) and urease (U) and carbohydrates (total, soluble and reducing sugars), inorganic N (ammonium and nitrate), inorganic P (Pi) and soil respiration were normally distributed [Shapiro-Wilk test > 0.05 or skewness test value < 0.5 , Webster and Oliver (2001)]. The effect of Ah mineral horizons and physical fraction was analyzed by two way ANOVA. Significant effect was obtained for the interaction (soil $\times$ fraction) for βG , DH, U and reducing sugars (Table 3). However, soil respiration, the C-CO₂, phosphatase and Pi were only significant for soil and fraction. The enzyme activity after 30 days of incubation is shown in Figure 2A and 2B. The βG and DH followed similar pattern in the bulk soil and fractions. Both enzymes were higher in PNP bulk soil in comparison with PAC soil (Table 2).

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

¹LF = 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; ²Soil organic carbon by Walkley and Black (1934);

³Aluminium extracted with Na-pyrophosphate; ⁴C in the microbial biomass.

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 (Figure 2C). Phosphatase activity decreased as the fraction size diminished in both soils (Figure 2D).

Although the total and soluble carbohydrates were not significant, they showed consistent trend with βG and DH (Figure 3A and 3B). This was particularly true for MF of reducing sugars.

3.4. Simple 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). The 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 interesting results emerge. 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. Ammonium was inversely correlated with nitrate and highly correlated with soluble carbohydrates. In order to look at the response of C-CO₂ in the physical fraction isolated from PNP and PAC soils, a stepwise multiple regression (SMR) analysis was conducted. Table 5 shows the soil attributes for significant variables influencing C-CO₂ as calculated by increasing R² in forward step. Similar results were obtained for bulk soils (not shown). The C-CO₂ variation due to several soil properties in Table 5 was similar to the relevant coefficient of correlation obtained in Table 4. Dehydrogenase, urease and phosphatase were common variables and they explained >76% the C-CO₂ variation in PNP and PAC soils. Soluble and total carbohydrates were relevant variables in PAC soil, as well.

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

Table 3. Significant effect of Ah mineral soil (allophanic and kaolinitic) and physical fraction (LF, IF and MF) on various biological and soil properties in temperate rain forest in Puyehue National Park (PNP) and Alerce

Source of Variation	DF	βG		DH		U		P _{ase}		RdSug		Pi		C-CO ₂	
		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

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

Figure 3. Average of enzymes activity after 30 days of incubation. (A) Total carbohydrate (CHOT), (B) soluble carbohydrate (CHOs) and (C) Reducing sugar, in bulk soil and fractions from temperate rain forest in Puyehue National Park (PNP) and Alerce Costero National Park (PAC).

3.5. Carbon stabilization

The soil metabolic quotient (qCO_2), the respiration rate $C-CO_2$ mineralized between 15 and 30 days divided by microbial biomass C was used as indicator of C metabolic efficiency for microorganisms (Anderson and Domsch, 1990). There was a highly significant correlation with qCO_2 and ATP and ATP, in turn, was

highly correlated with the cumulative $C-CO_2$ at 15-30 days of incubation too (Figure 4).

The C stabilization is evaluated by the relationship between Al_p (Al bound to SOM) 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 (Figure 5).

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-NO ₃	N-NH ₄	Pi	C-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-NO ₃	0.01	0.12	-0.06	0.20	-0.24*	-0.69**	-0.19	1.0			
N-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-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-NO₃; Ammonium =N-NH₄; Pi = Olsen P; ** $P < 0.001$; * $P < 0.05$.

Table 5. Contribution of various soil parameter on soil C respiration of physical fractions using 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	0.00	1.00
	DH	0.44	1.28	5.46	0.02
	N-NH ₄	0.58	-0.26	15.75	0.00
	βG	0.69	2.52	20.11	0.00
	U	0.73	-0.07	7.67	0.01
	P _{ase}	0.76	0.39	3.98	0.05
PAC					
	Intercept		-26.065	0.00	1.00
	SolCH	0.58	-0.368	60.46	0.00
	P _{ase}	0.67	0.52	4.12	0.05
	Pi	0.74	0.34	10.32	0.00
	TotCH	0.76	0.033	10.00	0.00
	U	0.78	0.06	6.47	0.02
	DH	0.80	0.97	3.39	0.05

Figure 4. Relationship between ATP and (A) C-CO₂, (B) qCO₂ at 15-30 days of incubation from the bulk soil and the physical fractions isolated from Puyehue National Park (PNP) and Alerce Costero National Park (PAC). * $p < 0.05$ and ** $P < 0.01$

Figure 5. Relationship between Al pyrophosphate and C-CO₂ at 15-30 days of incubation in the bulk soil and in the physical fraction isolated from Puyehue National Park (PNP) and Alerce Costero National Park (PAC). * $p < 0.05$ and ** $P < 0.01$

4. Discussion

4.1. Mineral control on biological activity and C stabilization

The present research was carried out in two soils from forest ecosystems developed from volcanic ash (allophanic) and metamorphic schist (kaolinitic). The influence of soil mineralogy on C stabilization in the bulk soil and different physical fractions was evaluated. The biological activity in response to the soil mineral composition and physical fractions were assumed to represent the level of SOM 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 the fractions and these result were consistent with those obtained with multiple regression analysis. Both, β G and DH activity were highly correlated with the release of C-CO₂, $r=0.63$ and $r=0.66$, respectively. In general, the respiration was correlated with phosphatase, reducing sugar and inorganic P, indicating the importance of β G and DH activity for degrading labile organic compounds at expenses of inorganic P in both soils. The amount of β -glucosidase and dehydrogenase decreased in MF of PNP, while the opposite pattern was found in the same fractions in PAC soil. Soil organic matter in the MF of PNP is complexed with Al oxides and short-range ordered (SRO) minerals, (allophane like-materials, Garrido and Matus, 2012; Neculman *et al.*, 2012). Recently, it has been recognized that SOM can be micro-encapsulated inside of highly and this is an important mechanism of C stabilization (Asano and Wagai, 2014; Chevallier *et al.*, 2010). This contrasts with the lower stabilization capacity in kaolinitic, 1:1 clay mineral soil.

Multiple regression analysis indicated that β -glucosidase and dhydrogenase, together with urease,

phosphatase and Pi explained >76 % the variation of soil C respiration in both amended soils. Spite of the reduced C mineralization in PAC, the greatest activity of β -glucosidase, and dehydrogenase was registered in the MF due to a reduced stabilization capacity (Luzio *et al.*, 2003) (see below). The latter was attributed to the inhibitory effect of free Al reflected in a 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).

Our findings appear to be consistent with the model of cellular metabolism, where N limits microbial biomass to build proteins, but the rates of protein synthesis are limited by the high P demands as supported by the high correlation of C-CO₂ and phosphatase activity. Incorporation of these physiological processes may improve models of carbon cycling and understanding of the effects of nutrient availability on soil C turnover across terrestrial ecosystems.

4.2. Relationship between metabolic quotient ($q\text{CO}_2$) and C stabilization

The metabolic quotient ($q\text{CO}_2$) or the specific respiration rate, the total amount of C-CO₂ mineralized between 15 and 30 days divided by microbial biomass C was positive and highly correlated with ATP in the bulk and physical fractions. The ATP was also correlated with the C-CO₂ on 15-30 days of incubation. It is possible that the differences in the $q\text{CO}_2$ along the ATP variation can be due to the microbial composition in different fractions. Microbial community structure is affected by particle size significantly, yielding higher diversity of microbes in small size fractions than in coarse size fractions (Sessitsch *et al.*, 2001). The mineralogical composition of soil showed an important role in the C mineralization, since there was an inverse and significant relationship between Al_p (Al bound to SOM) and the C-CO₂ for PNP

but not for PAC soil (Figure 5). This relationship was expected to occur only in volcanic soils, because of the potential of Al_p to complex SOM (Matus *et al.*, 2014). However, in kaolinitic soils, more crystalline clay exerted a small stabilizing effect. In previous studies, Al-SOM complexes were the primary factor explaining 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 complexes and allophane formation are inverse, although complementary processes are mainly regulated by soil pH (Garrido and Matus, 2012; Panichini *et al.*, 2012). In the present study, the PNP in volcanic soil with pH 6.2 could promote the allophane polymerization and thereafter SOM sorption. It is also well known that other factors, 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.* (personal communication) 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 simple C substrates (glucose and citrate) added to kaolinite and illite-mica were more decomposable than those added to ferric hydroxide. Zunino *et al.* (1982) noted a decrease in C mineralization when allophanic materials were added to non-volcanic soil. The chemical bonding between SOM and mineral surfaces decreased the availability of C to microorganisms (Guggenberger and Kaiser, 2003). Clay minerals have specific surface areas; therefore, it may be expected that clay type influences the capacity of soils to hold organic C.

5. Conclusions

We examined C stabilization capacity of two pristine temperate rain forest soils; allophanic and kaolinitic soils. The soils were physically fractionated in light (LF, coarse sand > 250 μ m), intermediate (IF, fine sand 50-250 μ m) and mineral (MF, silt and clay < 53 μ m). Biological activity was evaluated. Soil type and the physical fractions had a significant impact on all enzyme activities. More than 76% of total C-CO₂ in PNP and 80% in PAC soil were explained by the combination of β -glucosidase, dehydrogenase, urease and phosphatase using stepwise multiple regression analysis. Mineral fraction of PNP containing allophanic-like materials, unlike PAC with dominant kaolinitic clay, showed the lowest enzymatic activity, except for urease and phosphatase. The ATP was highly correlated with i) C-CO₂ at 15-30 d of incubation and ii) qC-CO₂ (respiration rate divided by microbial biomass). These results were in line with an inverse relationship found between Al_p (Al bound to SOM) and C-CO₂, indicating the large stabilization capacity of allophanic compared with kaolinitic soils.

Acknowledgements

The authors thank to CONICYT-Chile for their financial contributions through FONDECYT Projects 1130193 and Insertion Project of Advanced Human Capital in the Chilean Private Sector, 781 301 003.

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