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# Leucine retention by the clay-sized mineral fraction. An indicator of C storage

## Retención de leucina por la fracción mineral del tamaño de arcilla. Un indicador del almacenamiento de C

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ABSTRACT

The chemical interactions between soil clay-sized mineral fractions and soluble organic compounds are mainly the result of soil sorption mechanisms into reactive sites. The ability of clay-size fractions from different Chilean soil groups to protect leucine from microbial decay was used as an index of the retention of soluble organic matter by soil mineral fractions. The reactivity of clay-sized mineral fractions was determined by extractable Al in ammonium acetate 1M, pH 4.8; acid ammonium oxalate 0.2 M; sodium pyrophosphate 0.1 M and pH levels in NaF. Leucine retention provided by the clay-sized mineral fraction was estimated by determining the amount of leucine remaining after a 24 h period of incubation post-application of leucine to soil mineral fractions under controlled conditions. The slope of non-linear regression (ratio between added versus recovered) reflects the different chemical protection capacities of the materials evaluated, where *b* values were 0.001, 0.021, 0.043, 0.124, 0.128 and 0.259 for quartz sand, smectite, kaolinite, halloysite, volcanic-glass and allophane, respectively. We found that the leucine retention index was related to the clay-sized reactive fraction for all the soil groups evaluated ($R^2 > 0.75$). Thus, within the range used (up to 1480 mg $kg^{-1}$ of leucine applied), the recovery was related to the amount of aluminium extracted with ammonium acetate 1M ($R^2 = 0.96$). Clay-sized mineral fractions with greater reactivity, due to their dominant colloid fraction (clay-sized mineral fractions plus residual SOC), expressed bigger leucine protection to microbial decay.

### RESUMEN

Las interacciones químicas entre las fracciones minerales del tamaño de arcilla del suelo y los compuestos orgánicos solubles son principalmente el resultado de los mecanismos de adsorción en los sitios reactivos del suelo. La capacidad de las fracciones de tamaño de arcilla de los diferentes grupos de suelos chilenos para proteger la leucina de la descomposición microbiana fue utilizada como un índice de retención de la materia orgánica soluble por las fracciones minerales del suelo. La reactividad de las fracciones minerales de tamaño de arcilla se determinó mediante el Al extractable en acetato de amonio 1M, pH 4,8; en oxalato ácido de amonio 0,2 M; en pirofosfato de sodio 0,1 M y los niveles de pH NaF. La retención de leucina que proporciona la fracción mineral del tamaño de arcilla se estimó determinando la cantidad de leucina que quedaba después de un período de incubación de 24 horas tras la aplicación de leucina a las fracciones minerales del suelo en condiciones controladas. La pendiente de la regresión no lineal (relación entre la adición y la recuperación) refleja las diferentes capacidades de protección química de los materiales evaluados, donde los valores *b* fueron de 0,001, 0,021, 0,043, 0,124, 0,128 y 0,259 para arena de cuarzo, esmectita, caolinita, halloysita, vidrio volcánico y alofán, respectivamente. Encontramos que el índice de retención de leucina estaba relacionado con la fracción reactiva del tamaño de arcilla para todos los grupos de suelos evaluados ($R^2 > 0,75$). Así, dentro del rango utilizado (hasta 1480 mg $kg^{-1}$ de leucina aplicada), la recuperación estaba relacionada con la cantidad de aluminio extractado con acetato de amonio 1M ($R^2 = 0,96$). Las fracciones minerales del tamaño de arcilla con mayor reactividad, debido a su fracción coloidal dominante (fracciones minerales del tamaño de arcilla más carbono orgánico residual del suelo), expresaron una mayor protección de la leucina frente a la descomposición microbiana.

*Palabras clave*: Indice de leucina, suelos, fracciones de tamaño de arcilla, aminoácidos, adsorción

## INTRODUCTION

Soil organic carbon (SOC) is an extremely valuable natural resource and a key element to soil quality (Kirkby *et al.*, 2013). Irrespective of the climate regulated debate, the SOC stock must be restored, enhanced, and improved (Lal, 2004). Therefore, it is essential to achieve an adequate management of soil organic matter (SOM) in agroecosystems, for the purposes of advancing in food security, mitigating climate change and improving water quality (Lal, 2017). In general, SOC is usually separated into two functional components within the soil: non-labile carbon (stabilized) and labile carbon (Blair *et al.*, 2006). Organic carbon (OC) stabilisation results from the input of the OC portion which becomes resistant to microbial decay and remains in the soil over time (Sollins *et al.*, 1996; Schmidt *et al.*, 2011). Organic-mineral interactions are the most important mechanisms to establish the long-term carbon (C) stabilisation of soils (Dippold *et al.*, 2014; Dignac *et al.*, 2017). The chemical interactions that occur between organic compounds and the soil matrix mainly result from sorption reactions (Wiesmeier *et al.*, 2019). Several studies (Zhang *et al.*, 2012; Saidy *et al.*, 2013; Gao *et al.*, 2017; Apostel *et al.*, 2017) have researched the soil sorption ability to retain different organic compounds and its relationship with the physicochemical parameters that influence the SOC storage (Rassmussen *et al*., 2018; Wiesmeier *et al.*, 2019). Whatever the precise mechanism of sorption or stabilisation, the reaction between soluble organic matter and soil colloids (clays, oxides, oxyhydroxides and the same SOM) results in changes of the original degradability of these compounds by microbial biomass (Kleber *et al.*, 2015). Reactivity of the soil colloidal fraction can be used as an index of the soils capacity to accumulate organic matter, assuming this is one of the principal mechanisms capable of explaining the diverse content of organic matter in soils.

Soil sorption processes occur in the hydroxyls found in clay colloid edges (Oburger *et al.*, 2009; Kleber *et al*., 2015). The interactions between the oxygen borders associated with the silica tetrahedral layer and soluble organic C were shown to be weaker than those among hydroxyl groups associated with the aluminium octahedron (Kahle *et al*., 2004; Matus *et al*., 2014). Thus, compounds such as phosphate or even organic acids have been shown to be more adsorbed in soils with higher reactive sites (Andisols) than in soils with intermediate levels of reactive sites (Podzols) and much less so in soils with few reactive sites (Inceptisols) (Oburger *et al*., 2009; Matus *et al*., 2014; Vázconez and Pinochet, 2018). Also, Matus *et al*. (2006) reported that Al extractable in acid ammonium acetate was the primary factor explaining the soil C variation in similar soils rather than the climatic variables and clay content. In addition, Valle *et al*. (2015) confirmed that Al extractable was a better indicator of soil reactivity in Andisols than the clay content. Therefore, when this parameter was related to SOM content we could confirm that the extractable aluminium is the most important index of the SOM value (Clunes *et al*., 2014; Rasmussen *et al*., 2018). Soil adsorption sites mainly depend on the clay mineralogical composition (Lal, 2017; Wiesmeier *et al*., 2019). The ligand adsorption capacity of OC compounds in clays are related to the clay ability to form these bonds, being smaller in kaolinite and greater in clay 2:1 type smectite and vermiculite (Zhang *et al*., 2012; Kleber *et al*., 2015). Moreover, in short range-order aluminosilicate clays as an allophane and imogolite, the ability to form bonds with different OC compounds and to protect these compounds from degradation can be greater (Matus *et al*., 2014; Wiesmeir *et al*., 2019).

Soluble OC compounds such as amino acids (alanine, leucine, etc.) are usually easily degraded by soil microbial biomass (Oburger *et al*., 2009; Apostel *et al*., 2017). However, there is certain protection which can be provided by an interaction of organic compounds with clays (Lützow *et al*., 2006; Nowak *et al*., 2011) for a short time in soil solutions before microorganisms quickly use them to grow (Kemmitt *et al*., 2008), allowing these compounds to remain within the soil system (Dippold *et al*., 2014). This research proposes that the protection provided by soil colloids fraction of a neutral amino acid with a carboxyl group in its structure (Gao *et al*., 2017) and quickly absorbed by microorganisms, such as leucine (Reischke *et al*., 2014), would be reflected in the amount of leucine recovered after 24 h of incubation. This retention index would thus be related to the reactivity of the colloids fraction found in each soil. Therefore, this study aimed to compare the colloid material fractions (dominant clay + silt fractions plus the residual OC) of main soil groups present in Chile, regarding their capacity to chemically protect leucine from microbial degradation.

## MATERIALS AND METHODS

### Characterisation of soils and clay-sized fraction materials

In this study, the mineral fraction of the soil was represented by the "clay-sized fraction" separated through sequential physical and chemical dispersion methods. To obtain clay-sized fractions, five great soil groups were used: i) Inceptisol (Typic Xerochrepts) ii) Alfisol (Ultic Haploxeralfs) iii) Ultisol (Acrudoxic Hydric Hapludands) iv) Entisol (Vitrandic Udorthent) and v) Andisol (Acrudoxic Hapludand) (CIREN, 1981; Pino *et al*., 2002; CIREN, 2003; Soil Survey Staff, 2010). Predominant clay mineralogy in each of the soils mentioned above is smectite, kaolinite, halloysite, volcanic-glass and allophane, respectively (Besoain, 1985; CIREN, 2003). Disturbed soil samples were collected, sieved (< 2 mm), air-dried (25 °C) and characterised by their reactivity, determined by measuring their extractable Al content. Al was extracted using ammonium acetate 1M, pH 4.8 (soil-solution ratio 1:10; $Al_a$) (Sadzawka *et al*., 2006), acid ammonium oxalate 0.2 M (soil solution ratio 1: 50; $Al_o$), and sodium pyrophosphate 0.1 M (soil solution ratio 1:100; $Al_p$) (Sadzawka, 1990). Also, Fe was extracted using acid ammonium oxalate 0.2 M (soil solution ratio 1: 50), pH in NaF (soil solution ratio 1:50) was measured (Sadzawka, 1990).

## Physical soil colloid fraction separation

Soil samples weighing 60 g (dry basis) were digested with hydrogen peroxide (100 volumes) to eliminate organic material (Sadzawka, 2006). The hydrogen peroxide was applied daily at 20 °C (room temperature) and then the samples were placed in a water bath (around 80 °C) until the material stopped reacting. The sand was physically separated with a 63 µm sieve (Forshythe, 1974). The remaining supernatant suspension was dispersed with ultrasonic vibrations for five minutes (Schallfix, ultrasound stem, 120.000 rpm) and silt and clay soil fractions were separated by decantation (Mayer *et al*., 2002). Subsequently, in the separated clay-sized material the residual organic C contents were measured using Walkley and Black methodology by wet digestion (Sadzawka, 1990).

## Incubation of soil colloid fraction with leucine

One (± 0.01) g (dry basis) of clay-sized fractions was incubated for 24 h in the dark at 20 °C with increasing leucine rates of 186, 360, 740, 1120 and 1480 mg $kg^{-1}$ soil, which were prepared by adding 0.0093, 0.018, 0.037, 0.056 and 0.074 g of leucine to a 1 M HCl solution. From these solutions, 0.5 mL were applied to 1 g of clay material. A control was included using quartz sand inert material (as a non-retentive substrate) to evaluate leucine recovery in an inert material.

After 24 hours of incubation, the remaining leucine was extracted; its amount was determined using the colourimetric method measured at 570 nm. This method is based on the colourimetric determination when the ninhydrin reaction reaches a pH of 5.0 (Sparks*,* 1996). This method was used because the screening for adsorption on a < 2 µm fraction of 0–10 cm depths showed that L-lysine, L-histidine, and L-arginine were adsorbed by similar amounts while L-glutamic acid and L-leucine were not adsorbed in the pH range of 5.7–7.2 (Bartlett and Doner, 1988). Thus, all samples were adjusted to a pH of 5.0 for further testing. The sample preparation for colourimetric analyses was slightly modified from the original method, following the procedure described in Rothamsted's review (2005). This consisted of taking 1 mL of the sample solution and mixing it with 2 mL of citric acid and 1 mL of the combined solution (a mixture of the sodium acetate buffer, ninhydrin reagent, distilled water and tin chloride).

The amount of leucine recovered from the different clay-sized fractions in the soils was fit to a non-linear regression between the amount of leucine applied and the amount of leucine recovered. The slope obtained from this relation represents the leucine retention rate (Leucine retention index: LRI).

Furthermore, for the purposes of this research, the total amount of the reactive colloidal fraction of the soils evaluated was calculated as follows:

Total colloidal fraction reactivity = $Al_a$ + residual SOC content

Where, $Al_a$ (g 100 $g^{-1}$) is Al extracted using ammonium acetate 1M, pH 4.8 and residual SOC content (g 100 $g^{-1}$) is the organic C content measured in the separated clay-sized fraction.

## Statistical analysis

The data were analysed using a completely randomized design with three replicates. Non-linear regressions were used (best-fit curve). All of the clay-sized fraction material samples, including all of the leucine rates, were incubated separately and the amount of leucine remaining was determined after incubation times. Normality of the residuals of the model was checked with the D'Agostino-Pearson normality test. The coefficient of determination ($R^2$) was calculated as indicators of a good fit. The statistical program GraphPad Prism v.5.0 was used.

## RESULTS

## Retention of leucine in clay-sized fractions

The amount of leucine recovered from different clay-sized fractions of the soils evaluated was fit to a non-linear regression between the amount of leucine applied and the amount of leucine recovered (Figure 1). Quartz sand was used as a control because it is a non-reactive material and all of the leucine applied would be in the solution and its disappearance would therefore be produced by microbial decay during the incubation. Results showed that adding up to 1480 mg $kg^{-1}$ of leucine did not produce an increase in the amount of leucine found in the solution after the incubation period (only 1.9 mg $kg^{-1}$ was recovered, regardless of the amount of leucine applied).

The slope ($b$) of the equation reflects the different chemical retention capacities of the clay-sized fractions

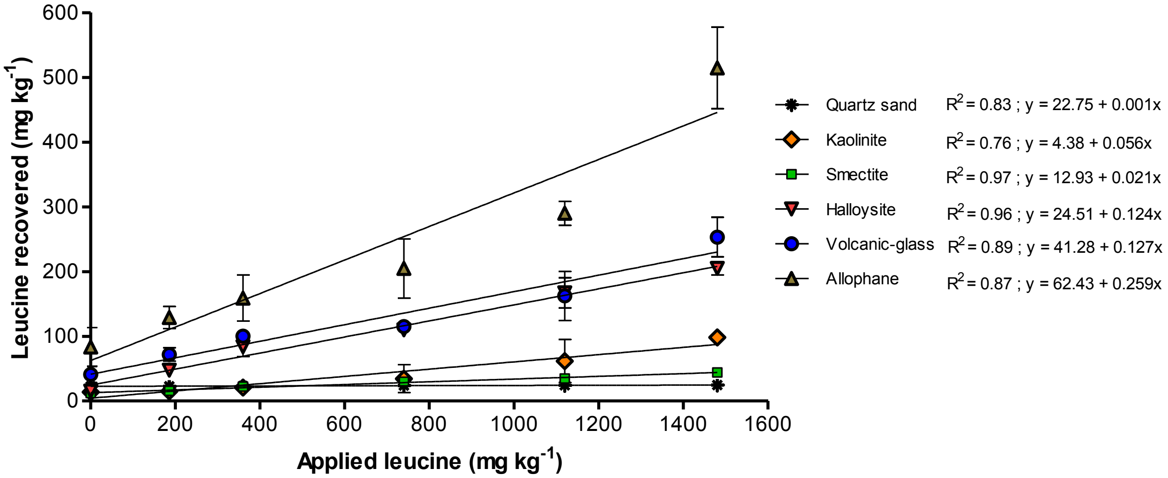

**Figure 1.** Relationship between applied and recovered leucine rates for each clay-sized mineral fraction of soils versus quartz sand. All regressions present statistical differences ($p < 0.0001$) for each treatment ($n = 18$).

**Figura 1**. Relación entre la leucina aplicada y la taza recuperada de leucina para cada fracción mineral de tamaño de arcillas de los suelos versus arena de cuarzo. Todas las regresiones fueron diferentes estadísticamente ($p < 0,0001$), para cada tratamiento ($n = 18$).

evaluated, where *b* values were 0.001, 0.021, 0.043, 0.124, 0.128 and 0.259 for quartz sand, smectite, kaolinite, halloysite, volcanic-glass and allophane, respectively. Although Inceptisol clay-size fraction (smectite) presented the lowest slope value, the leucine retention (recovering 31.1 mg kg$^{-1}$ of leucine) was 16 times higher than the value obtained from the quartz sand (control). A second level of leucine retention (recovering 63.2 mg kg$^{-1}$ of leucine), as shown by the slope, was presented by the clay fraction from the Alfisol (Figure 2). Ultisol showed the third level of leucine retention, recovering 183.5 mg kg$^{-1}$ of leucine. The fourth level of leucine retention (recovering 188.9 mg kg$^{-1}$ of leucine) was shown by the clay-sized fraction in the Entisol. Finally, the material with the highest leucine retention (recovering 383.3 mg kg$^{-1}$ of leucine) was found in the clay-sized fraction in the Andisol (Figure 1). These results allowed to propose a leucine retention index (LRI) using the variation on the slope of the regression between recovered leucine and applied leucine.

## Mineral reactivity of clay-sized fractions

The soil clay-sized fractions ability to retain ions and organic compounds in solution was assessed using different chemical indicators mainly associated with extractable Al to represent the mineral reactivity of each soil type (Figure 2). The reactivity shown by the extracted Al contents was correlated positively to the pH NaF, because the different soils with different mineralogy evaluated in this study were able to generate very distinct amounts of reactive OH (Figure 2).

The clay-sized fraction from Andisol and Entisol showed high reactivity, subsequently, the Ultisol, Inceptisol and finally Alfisol proved to be derived from less reactive materials. Al extracted by acid oxalate ($Al_o$) from the Andisol was 4-folds more than the Ultisol, 6-folds that of the Entisol and much higher than that obtained from the smectite clay soil (Colina, 22 times lower) or the kaolinitic Alfisol (29 times lower). Al extracted by pyrophosphate ($Al_p$) also showed a similar trend, where the reactive Al of the Andisol was 3 times the value obtained by the Entisol and the Ultisol, subsequently the Alfisol (79 times) and Inceptisol (215 times). The Al extracted by ammonium acetate ($Al_a$), Andisol had the highest reactivity, followed by Ultisol (5 times less) and Entisol (9 times), with Inceptisol (951 times) and Alfisol (1260 times) showing much lower levels of extractable Al (Figure 2). The Fe extracted by acid oxalate from the Andisol was 1-fold more than the Ultisol, 3-folds that of the Entisol and about 5-folds the value for Inceptisol and Alfisol (data not shown).

## Leucine retention index (LRI) and reactivity parameters of clay-size fractions of soils

To evaluate this relationship in all of the studied cases, a non-linear regression was adjusted. LRI was better related to reactive $Al_a$ (determined in both the soil and the clay-size fraction) and with reactive hydroxyls measured in pH NaF (Figure 3), where the soil series that presented the highest LRI was Puyehue.

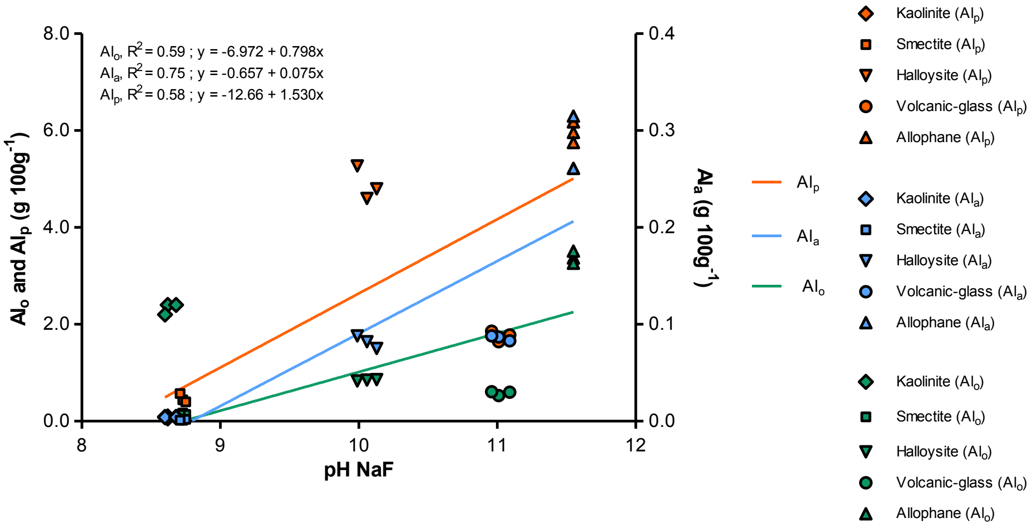

**Figure 2.** Relationship between the pH in NaF and Al extracted by sodium pyrophosphate (Al$_p$), acid oxalate (Al$_o$), and ammonium acetate (Al$_a$). Blue symbols mean Al$_a$, green symbols mean Al$_o$ and orange symbols mean Al$_p$. (n = 3).

**Figura 2.** Relación entre pH en NaF y Al extractado en el pirofosfato de sodio (Al$_p$), oxalato ácido (Al$_o$) y acetato de amonio (Al$_a$). Los símbolos azules son Al$_a$, símbolos verdes son Al$_o$ y símbolos naranjas son Al$_p$. (n = 3).

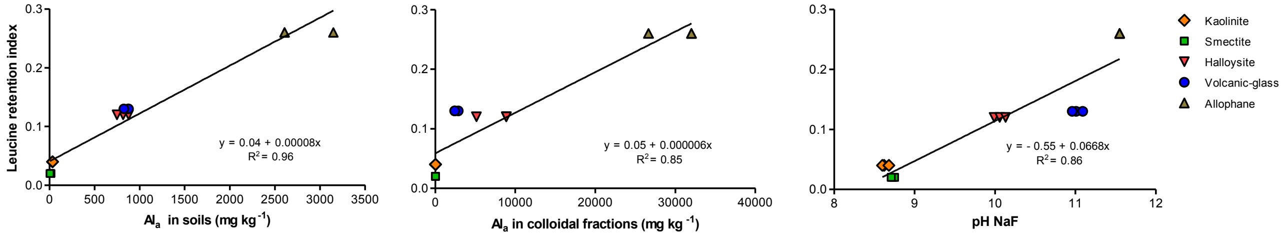

**Figure 3.** Relationship between Clay-sized fractions and parameters that determine the reactivity of leucine retention (leucine retention index) measured in soils and slopes. Extracted Al$_a$: Extracted Al ammonium acetate.

**Figura 3.** Relación entre las fracciones de tamaño de arcilla y los parámetros que determinan la reactividad medida en los suelos y las pendientes de retención de leucina (índice de retención de leucina). Al$_a$: Al extractado en acetato de amonio.

## DISCUSSION

### Soil Reactivity Indicators

Several researchers have used similar indicators to characterise different types of soils, reactivity and mineralogy, including soils of volcanic origin (Matus *et al.*, 2008; Oburger *et al.*, 2009; Matus *et al.*, 2014; Valle *et al.*, 2015; Rassmussen *et al.*, 2018; Cotrufo *et al.*, 2019). The pH in NaF has been used to estimate the hydroxyl reactivity of soil colloidal material, in particular its specific adsorption (Fieldes and Perrot, 1966; Kleber *et al.*, 2005; Oburger *et al.*, 2009). It has been demonstrated that there is a relationship between the OH reactivity of clay soils and their ability to specifically adsorb (covalent bonds) phosphates (Gilkes and Hughes, 1994; Valle *et al.*, 2015), retain and accumulate C stores (Kramer and Chadwiks, 2016) and to adsorb boron in volcanic-influenced soils (Terraza *et al.*, 2018). For this reason, the pH NaF is a reliable indicator of the presence of allophonic materials and is also used to evaluate the degree of short-range minerals in soils (Valle *et al.*, 2015; Terraza *et al.*, 2018; Enang *et al.*, 2019) because clays with high reactivity, such as allophane, produce an alkaline reaction with sodium fluoride, releasing OH (Egawa *et al.*, 1960; Fieldes and Perrot, 1966). The pH values in 1N NaF above 9.4 indicate the presence of allophane and organic substance complexes with active aluminium (amorphous), as is the case with Andisols (Gaitan and Lopez, 2007). Results showed that clay-size fraction (which represent the mineral part of the colloidal fraction in this research) derived from volcanic ash, which was the dominant clay allophane, presented pH NaF values greater than 11.0. These results suggest that leucine retention is produced by the ligand exchange between clay materials hydroxyl edges and the OH present within the leucine molecule.

The "reactive" Al ($Al_a$, $Al_o$ and $Al_p$) of clay-sized mineral fraction of soils is used as an indirect measurement of the reactivity of OH sites in the soil (Matus *et al*., 2014; Valle *et al*., 2015; Kramer and Chadwick, 2016; Enang *et al*., 2019). Since Al presents a coordination of six, it generates an octahedron structure with hydroxyls along its edges (Kleber *et al*., 2015). Ligand exchange can occur on OH borders, retaining anions in a specific way in their exchange complex (Oburger *et al*., 2009). There is a positive relationship between a stable organic C content and the amount of hydroxyl ions released after a reaction with a NaF solution, suggesting that ligand exchange between the protonated surface of hydroxyl sites and organic functional groups is the main mechanism of C stabilisation in soils (Kleber *et al*., 2005; Kramer and Chadwick, 2016). In Chile, the "reactive" $Al_a$ is the most used indicator for soil reactivity in volcanic soils and to characterize different soil mineral materials (Rodríguez, 1993; Matus *et al*., 2008; Valle and Carrasco, 2018). In addition, considering its different extraction methods, it has been used as a relevant parameter to determine C storage in soils given its positive correlation with SOC (Matus *et al*., 2014; Clunes *et al*., 2014; Valle and Carrasco, 2018).

## Retention of leucine in clay-sized fraction materials

Soil aggregation influences SOM stabilisation because it provides accessibility of microorganisms to organic compounds (Lal, 2004). Stabilisation is defined as the protection of OM from mineralisation (Lützow *et al*., 2006). The quartz material was non-reactive (Figure 2) and that there was no interaction between the leucine amino acid used and the quartz material used (Dipplod *et al*., 2014). The results suggest that leucine decayed in all of the soils sampled, regardless of their chemical retention capacity because after adding an amino acid (labile carbon source) their adsorption depends on the acidity conditions of the medium, adsorbate properties, concentration applied and the microbial use prior to the sorption of the mineral surface (Gao *et al*., 2017). Also, the electrostatic interactions between adsorbates, particle surfaces, and adsorbate molecules are important in amino acid adsorption to minerals and that these interactions also depend on charged clay surfaces (Ding and Henrich´s, 2002; Apostel *et al*., 2017). The lower leucine retention by Inceptisol is consistent with the dominant clay type smectite (Table 1), present in the alluvial soils of central Chile (Besoain, 1985). The clay-sized fraction from the Alfisol soil dominated by kaolinite and representative of the great granitic group of Chilean soils (Besoain, 1985; Rodriguez, 1993). This soil showed twice the recovery of clay-sized fraction from the Inceptisol. Subsequently and with a change in the slope, the next recovery could be associated with the paracrystalline clay 1:1, dominant halloysite presented by the great group of red clay soils such as Ultisol (Besoain, 1985). Finally, the clay-sized fraction in Entisol and Andisol are dominated by the non-crystalline allophane clays present in the volcanic ash soils (Besoain, 1985), which presented the highest retention of amino acids of all the soils evaluated (Figure 1), which is consistent with their high reactivity.

The clear difference between the clay-sized fractions of the soils evaluated and their capacity to retain an easily degradable soluble C compound such as leucine, allow us to propose an indicator of C retention in Chilean soils given the recovery slope determined in this research.

## Relationship between colloidal fractions parameters and leucine retention index

Chesire *et al*. (2000) used differences in IR spectroscopy to determine the nature of the OM attached to the clay, showing that the clay-sized fractions of the soil surface horizons had different C contents, depending on the input from vegetation or clay's greater adsorptive capacity in the soil. This OM might present colloidal characteristics and OH border presence due to their complex structures (Cotrufo *et al*., 2019). All soil clay-sized fraction materials evaluated after digestion with hydrogen peroxide showed a residual SOC content, probably due to incomplete dispersion during the digestion process of organic matter (Figure 4). This content of SOC residual remaining after digestion did not follow the same trend and was higher in the Entisol clay-sized fraction, followed by Andisol, Ultisol, Alfisol and finally Inceptisol. Thus, the clay-size fractions evaluated may have been affected regarding their ability to retain leucine by the presence of residual SOC. Therefore, and for the experimental conditions of this research, $Al_a$ plus residual SOC content was considered a measurement of the entire "colloidal fraction" and was correlated with the retention index (Figure 4). The $Al_a$ plus the residual SOC content from the clay-size fractions was considered as the "total colloidal fraction reactivity" which was related to the LRI suggesting that the residual SOC content did not affect clay-sized fractions' ability to retain leucine (Figure 4). In this way, we can assume that the hydroxyls that bond with leucine molecules originate from the mineral fraction (clay-sized fraction) of each soil evaluated. Similarly, the structural organisation of the organo-mineral complexes appeared to be a major factor in the accumulation of organic compounds in the outer layers of the complexes (Bonnard *et al*., 2012; Kleber *et al*., 2015). Thus, it is appro-

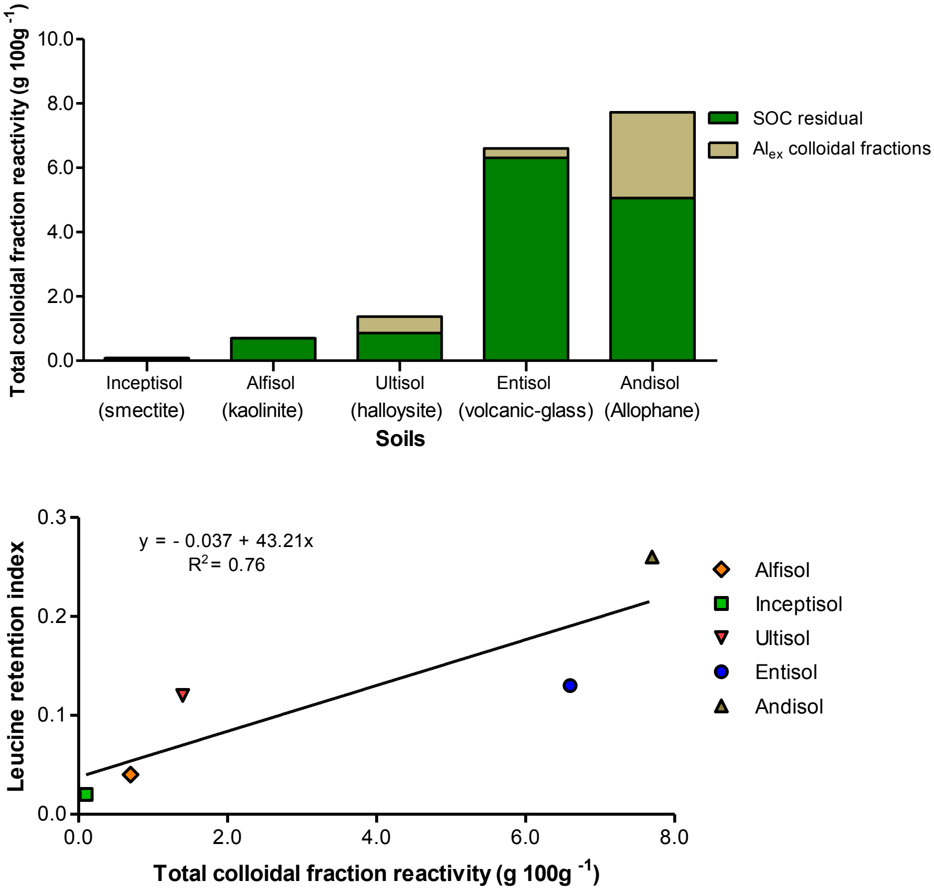

**Figure 4**. Relationship between the Al extracted by ammonium acetate plus the residual SOC content from clay-sized mineral fractions ("Total colloidal fraction reactivity") according to the determination of leucine retention index of the soils evaluated.

**Figura 4.** Relación entre el Al extractado en acetato de amonio más el contenido residual de COS de las fracciones de tamaño de arcilla ("Fracción de reactividad coloidal total") según la determinación del índice de retención de leucina de los suelos evaluadas.

priate to consider the ligand exchange due to positively charged amino groups on negatively charged mineral, since the main sorption mechanism for amino acids in soils (especially for neutral amino acids like Alanine and Leucine) is clearly related to the specific surface area of the sorbents, showing a stronger interaction with Al-OH-groups as $Al(OH)_3^-$ (Dipplod *et al*., 2014). However, regardless of the strength of the binding between negatively charged mineral fraction (clay-sized) by amino groups and the positively charged mineral fraction surface of the carboxylic groups, both bindings should be considered to evaluate an efficient binding of amino acids over the mineral fractions in the soil (Gao *et al*., 2017). This sorption mechanism can be protecting the amino acids from microbial degradation.

Based on the results of this study, it is proposed that the mineralogical composition of each clay-sized fraction determines the leucine retention capacity of soils. Furthermore, leucine retention is produced mainly by hydroxyl edges in the colloidal fractions, that is when considering both the clay-sized fraction and the residual SOC in a pool. Also, iron oxides such as goethite are very strong sorbents in soils with a higher portion of OH-groups functional for ligand exchange with the carboxyl groups of amino acids (Dippold *et al*., 2014; Kleber *et al*., 2015). Therefore, it is important to consider the different reactive soil fractions and the adsorption mechanisms involved in each fraction, in order to accurately evaluate the retention of carbon compounds by the soil. Chesire *et al*. (2000) suggested that added polysaccharide was adsorbed by clays by the hydrogen bonding involved in OH groups since there was no retention of residual nitrogen during oxidation. Similarly, Kemmitt *et al.* (2008) suggested that the mineralisation of OM is regulated by an abiotic destabilising process that transforms non-bioavailable substrates into bioavailable substrates without affecting the functionality of the microbial population. Furthermore, the OC associated with the mineral fraction does not inhibit additional sorption of dissolved OC, since the sorption process depends on the chemical characteristics of OC compounds and the mineral fraction properties (Jagadamma *et al*., 2014), hence the stability of the soluble OC of the soil varies with the clay mineralogy and its interaction with hydrated iron oxides (Saidy *et al*., 2012; 2013).

## CONCLUSIONS

A leucine retention index (LRI) produced by soil colloidal fractions after incubation was proposed and related to extractable Al, the pH in NaF and residual SOC. This is indicative of the reactivity of soil colloidal fraction represented by clay-sized fractions and residual SOC soils. A higher material reactivity produced more leucine retention by colloidal fractions, causing retention of soluble organic compounds and providing protection against the degradation of soil organic carbon.

The LRI proposed provides an estimate of the soil capacity to retain soluble carbon compounds that are easily degradable. However, further research is needed to extrapolate the results to other soil types under different analysis conditions (e.g. land uses), as well as assessing the effects of use change dynamics on carbon sequestration capacity and how these changes are related to the biodiversity of soil microorganisms. We consider that it is necessary to separate the mineral fraction of the soil associated with soil organic matter (Particular Organic Matter; POM and Mineral Associated-Organic Matter pools; MAOM) and different scales of aggregates. Furthermore, it is essential to evaluate the microbial activity during incubation of reactive fractions with the amino acid leucine, to estimate “losses by consumption” during the retention period.

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