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CARBON STABILISATION AND DESTABILISATION PROCESSES OPERATING IN THE TOP- AND SUBSOIL OF ANDISOLS UNDER TEMPERATE RAINFOREST, SOUTHERN CHILE

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...dedicada a mis padres, Patricia y Hernan ...y abuelos, Elsa y Alfredo

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Summary and outline of this theses

Soil carbon (C) sequestration is a strategy to capture atmospheric CO₂ and transfer it into stable soil organic carbon (SOC) forms. There are a numbers of mechanisms of C stabilisation, the processes that made SOC resistant to decomposition. For example: physical and chemical adsorption, physical protection by inaccessibility or spatial impedance to the substrate by decomposer, selective preservation of SOC by their recalcitrance and the environmental climatic conditions. The studies of C stabilisation and destabilisation (the processes that make organic substrate available for decomposition) across the soil profile are still scarce in volcanic soils. Few studies have been carried out to investigate those processes in Andisols despite the fact that the C storage potential of Andisols is higher than in any other soil type. Stable C occurs in this soil type due to its association with aluminum- and iron-oxides together with amorphous clay minerals like allophane and imogolite type materials. Recent investigations have shown that the lack of fresh C substrate in the subsoil limits the biological activity, and would influence preservation of native SOC in soil depth. This would regulate the mechanisms of the stabilisation of SOC in the subsoil if fresh C is not available. In this thesis, I investigated the factors and mechanisms that control the C stabilisation in the top- and subsoil horizons of volcanic temperate old growth rainforest soils in Southern Chile. This mountain rainforest of southern Chile still maintain pristine conditions and has been developed under a temperate climate, with low temperatures and high precipitation, where woody vegetation produces a litter with slow decomposition rates resulting in the accumulation of C on the surface soil, depending of climate conditions and soil texture. Therefore, these temperate rainforests provide a unique opportunity under pristine conditions to develop a baseline of the patterns and processes in the biogeochemical C cycling.

The outline of this thesis begins with a general introduction. In Chapter I, we address the general objectives of our thesis with regards to SOC stabilisation/destabilisation processes operating in temperate rainforest Andisols of southern Chile.

In Chapter II, we made a literature review for the factors and mechanisms involved in the stabilisation/destabilisation of SOC in volcanic soils. From this review emerge interesting results depending on the combination of several factors: Al and Fe activity, soil pH, allophane and imogolite type materials and the environmental conditions (vegetation type, precipitation and temperature). In this chapter, it's hypothesized that soil pH and the competition between Al- soil organic matter (SOM) complex and allophane formation is the most important control between stabilisation processes. Moreover, higher metal:C_p ratio in the subsoil might induce higher SOM saturation with Al and Fe than in the top soil, meaning both metals will not be able to stabilise more C and therefore, the Al and Fe in excess would be available to react with silica gel for the synthesis of allophane and imogolite type materials.

In Chapter III, we show that the C stabilisation capacity evolves with soil age relative to the evolution of the mineral phase. Mineralogical changes during pedogenesis are related to the composition and ¹⁴C activity as an indicator for the mean residence time of SOC in temperate old-growth forest of Southern Chile. The allophane formation at older stages of soil development leads to SOM stabilisation. In younger soils and at lower pH, metal–SOM complex formation may be more important. C stock distribution within the soil profile changes with soil age, with higher contribution of subsoil C to C stocks in younger Andisols. It is suggested that the combination of several factors (Al, Fe, and C extracted in

Na-pyrophosphate, metal/ C_p ratio, and pH of the soil) which change through pedogenesis control the SOM stabilisation.

In Chapter IV, we focused on priming effect, the C released from native SOM from the fresh substrate addition at different soil depth to study the biological activity related to the stabilising capacity of these Andisols in two different sites with old-growth forest of Southern Chile. The limitation of the biological activity by lack of fresh C substrate is an important mechanism that would influence the preservation of native SOC in the subsoil if C substrate is not available. In this chapter, we hypothesized that SOM in these soils would be highly stable due to strong mineral interactions and therefore would not be possible the C release to upon *priming effect*. Moreover, the different stabilisation agents would induce contrasting potential SOC mineralisation and contrasting response to priming effect. Results showed a lower potential SOC mineralisation in subsoil of both Andisols compared to the surface soil in accordance with the increasing ¹⁴C age at depths. Addition of cellulose and nutrients induced net negative priming in the topsoil, whereas priming of most subsoil SOC remained positive after 80 days of incubation. We suggest, that adsorption to the mineral phase is of greater importance for SOC stabilisation in Andisols than physical inaccessibility. Destabilisation of SOC by priming is possible and seems to be controlled by soil pH.

Finally, In Chapter V we present a general discussion and conclusions where all factors and mechanisms we studied in SOC stabilisation/destabilisation in temperate oldgrowth rainforest Andisols of southern Chile are discussed. Also, we present the future directions concerning to the C stabilisation processes that operate in top- and subsoil. The main conclusion of this thesis are: (1) The pedogenic SOM stabilisation is relevant in these soils acting simultaneously by soil pH, non-crystalline clay and competition of metals (Al)

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and SOM for the formation of Al (and Fe)-SOM complexes or allophane type materials in the top and subsoils. However, the C complex formation it seems to be more important in the surface soils with low pH (< 5.8) than the allophane formation in the subsoils with high pH (> 5.8) top, Then, the latter mechanism is the cause of C stabilisation the subsoils rather than C complex formation. (2) In general, the SOM in these soils type is highly stable due to strong mineral interactions, therefore, is not possible to release C upon *priming*. However, the different stabilisation agents would induce contrasting potential C mineralisation and contrasting response to *priming* in deeper horizons.

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CHAPTER I

General Introduction

1.1 General introduction

SOC reservoir corresponds to 615 Gigatons (Gt) C in the top 0.2 m and 2,300 Gt C at depths of up to 3 meters (Jobbágy et al., 2000; Schmidt et al., 2011). SOC reservoir sizes in deep volcanic soil are particularly important due to the high SOC mean residence time (MRT), which increase strongly with soil depth, reaching a range of 500–5,000 years (Neculman et al., 2013). These amounts are higher and more long-lasting than those stored in the atmosphere or terrestrial biomass and may be influenced by human activity (Lal, 2004), which suggest that an increasing of SOC storage may counteract increasing CO₂ concentrations (Lal, 2004).

SOC stabilisation is defined as mechanistic processes that lead to protection of SOC from mineralisation (Sollins et al., 1996). One of the stabilisation mechanisms is the selective preservation of SOC due to recalcitrance, including plant litter, microbial byproducts, rhizodeposition of organic compounds, humic polymers, and charred organic matter (Matus et al., 2009; Schmidt et al., 2011). Physical protection of SOM has to do with the spatial inaccessibility of organic matter for decomposers due to for example occlusion, encapsulation, intercalation between minerals and aggregates, hydrophobicity, while the chemical stabilisation with the interaction of mineral surfaces (Fe-, Al-, Mn-oxides,) and metal phyllosilicates ions (González and Laird, 2003). However, the factors and mechanisms that control the C stability in deep soils are still poorly understood (Schmidt et al., 2011; Chabbi et al., 2009; Fontaine et al., 2007) and even less for deep volcanic soils. For instance, Eusterhues et al. (2005) have indicated that adsorption of SOM with minerals is the most important mechanism of stabilisation in the subsoil, whereas, Fontaine et al. (2007) showed that this mechanism could be overcome by the 'priming'

effect, i.e. the decomposition of native SOM due to the incorporation of fresh C energy releasing C otherwise stabilised.

Forest ecosystems play a central role in the global C cycle and ecology and C storage in these systems has important, practical implications from the point of view of global change and production of woods resources (Schulze, 2000). Soil C storage is controlled primarily by the input of net primary production (above- and below-ground compartment) and its decomposition rate (Larcher, 2003; Canadell et al., 1996; Lauenroth and Gill, 2003). In temperate environments, SOM decomposes because of the activity of heterotrophic soil microbes, which represent about 2-4% of SOC (Jenkinson et al., 1981).

Old-growth forests of Southern Chile represent an important reserve of temperate forests in the world (Armesto et al., 1998, 2009). The precipitation chemistry in mountain area of Chile still reflects a close approximation of pre-industrial conditions (Boeckx et al., 2005). In this case, the productivity of the ecosystem depends on the internal cycling of SOM (Godoy et al., 2009) and highly efficient mechanisms of a conservative nutrient cycling (Huygens et al., 2008). The ecosystem C and N cycles are strongly coupled by several ways and influencing the regulation of C and N pools and fluxes (Schulze, 2000). These ecosystems developed on Andisols, a soil type formed due to volcanic activity.

The annual cycle of input of litterfall (fine and coarse) derived from above-and belowground parts (Schelegel and Donoso, 2008; Staelens et al., 2011) feeds back to the heterotrophs of the ecosystems which use the energy stored in the organic matter and recycle nutrients as a major resource for further plant growth and organisms and to contribute to stability of the forest ecosystems (Schulze, 2000; Huygens et al., 2008). The decomposer chain have several groups of organisms competing for plant organic matter (bacteria, fungi and soil animals) and the variation of fine litter decomposition by mycroflora and functional group activities are a crucial aspect to the control of SOM decomposition (Valenzuela et al., 2001). Decomposition is affected by a number of external factors: for example, microclimatic conditions, the amount and nature of clay, microbial activity, soil pH, and free Al and Fe (Sollins et al., 1996). Other external conditions such as temperature and precipitation also play a key role in regulating SOM decomposition and stabilisation processes, especially in temperate old growth forest in Andean mountain region (Oyarzún et al., 2004, 2011). In these ecosystems, SOC stabilisation mechanisms have recently received a lot of attention due to its importance in the global terrestrial C cycling in the top soil (Schmidt et al., 2011). However, although in deep soils these mechanisms were found to be soil type dependent (Kögel-Knabner et al., 2008), they have mainly been studied in non-volcanic soils.

1.1.1 Stabilisation vs destabilisation mechanisms operating in top- and subsoil horizons

A detailed concept of stabilisation of SOM was first presented by Sollins et al. (1996) and recently reviews by Schmidt et al. (2011). A more expanded concept of the term stabilisation not only includes the potential loss of SOM by respiration, but also soil erosion, and leaching of soluble organic compounds. Von Lützow et al. (2006) defined the stability as the set of processes or mechanisms that lead to protection of SOC from mineralisation. Destabilisation is defined as an increase in the potential loss of SOM (Sollins et al., 1996). These processes depend on several factors, mainly the input and chemical composition of the organic substrate, as well as soil texture, moisture and temperature. The losses by erosion are also important because it may affect stabilisation and destabilisation processes via preferential export and/or deposition of mineral and organic compounds (Heimsath et al., 2000; Sparling et al., 2003) relevant aspect in old growth forest ecosystems under high level of precipitation regimes in mountain area of Southern Chile

(Oyarzún et al., 2004, 2011). Within the mechanisms of stabilisation we can find: i) chemical adsorption, which is the result of chemical bonding between SOM and the surface of mineral soil (Fe-Al-Mn-oxides, phyllosilicates) and metal ions (Tipping, 2002). These interactions can be mechanism such as ligand-exchange, weak interactions (van der Waals forces and hydrogen bridges) and interactions of metal ions with organic substances (Theng, 1979; Oades, 1989; Vermeer and Koopal, 1998; Vermeer et al., 1998; Matus et al., 2009). ii) The physical protection of SOM, which produces a spatial inaccessibility to microorganisms in the decomposition of SOM. This inaccessibility may be due to the occlusion of organic matter in soil aggregates (Adu and Oades, 1978), encapsulation in organic macromolecules, and intercalation with minerals (Schmidt et al., 2011). iii) The selective preservation of SOM due to the accumulation of recalcitrant molecules. Von Lützow et al. (2006) postulated as the primary recalcitrance the plant litter and rizhodeposits, while the secondary recalcitrance involves microbial products, polymers and humic charred products. Krull et al. (2003) suggested that the selective preservation due to the recalcitrance of SOM is the only mechanism that protects the stabilisation of SOM for longterm periods, whereas others indicated that only interaction with the mineral phase may be able to protect organic compounds for millenia (e.g. Kögel-Knabner et al., 2008). Eusterhues et al. (2005) indicated that in the subsoil the stabilisation of SOM is dominated by chemical sorption to minerals, such as aluminum (Al) and iron (Fe) oxides and clay minerals. In this context, Rumpel et al. (2012) indicated that a high means residence time of organic C is related to its degree of stabilisation and that SOM in deep soil, with high mean residence times may be used as a model for the study of stabilised C. Destabilisation mechanisms that control the mean residence time of SOM include the so-called 'priming effect'. The priming effect involves mineralisation of native SOM when fresh SOM is added (Jenkinson, 1971). Priming may be positive or negative (Hamer and Marschner, 2005; Guenet et al., 2010). Positive priming is related to the lack of fresh C,

which could provide energy necessary to degrade native SOM (Fig. 1) (Kuzyakov et al., 2000). The *priming effect* (PE) was first observed and defined as the decomposition of extra native C after the addition of fresh substrate in the soil by Bingemann et al. (1953). It is the result of an increase in microbial activity due to high available energy and nutrients released from the fresh organic matter (Bingemann et al., 1953; Sorensen et al., 1974). Fontaine et al. (2003) proposed two potential mechanisms that lead to a *priming effect*. The first mechanism considers that extracellular enzymes produced in the decomposition of fresh substrate for specific microorganisms (r strategists), which are efficient in the degradation of SOM, depending on the biochemical similarities between the fresh substrate and SOM, while the second mechanism depends exclusively on the competition for the decomposition of fresh substrate. The use of this substrate would generate a significant increase in the populations of K strategists, and hence the rate of decomposition of SOM. In this case, the intensity of the *priming effect* depends on the degree of competition for energy acquisition between microorganisms strategists r y K.



Figure 1. Scheme of the priming effect. Interaction between the decomposition of added fresh C substrate and native SOM: (a) Extra CO_2 evolved from native SOM. (After Kuzyakov et al., 2000).

Studies of destabilisation mechanisms such as PE are still scarce in subsoil horizons, Fontaine et al. (2007) showed that the stability of C in deep Ultisol of grassland is maintained due to a lack of fresh substrate as an energy source for microorganisms, preventing their mineralisation. However, it should be noted that the addition of fresh substrate C can also cause a reduction or immobilization of the C added in this case it becomes negative *priming effect* (Kuzyakov, 2000).

Stabilisation of SOM in deep soil may be influenced by the same mechanisms operating in topsoils. However, specific environmental conditions, such as lack of oxygen may induce greater importance of other parameters, such as redox potential, *e.g.* Fe (III) oxide, which acts as electron acceptor under abiotic conditions, could facilitate anaerobic respiration, which results in release of Fe (II) in solution (Weber et al., 2006). pH may also be of greater importance in subsoil under such conditions.

The above mentioned findings are important for SOM storing capacity in soils, as subsoils; due to their low C content compared to topsoils have the highest potential to accumulate further C. Therefore, the mechanisms of C stabilisation need to be elucidated in detail for the different soil types. Mechanisms that act in deeper horizons can be different but may have the same relevance in comparison to those in the topsoil.

1.1.2 Chilean allophanic soils under old growth temperate rain forest

In Chile, many soils are Andisols (Soil Survey Staff, 2008), developed from volcanic parent materials. In general, the soils are derived from volcanic ash and pumice materials (CIREN, 2001), overlying andesitic and basaltic lavas and fluvio-glacial sediments. The organic matter in Chilean Andisols can reach up to 31% (Matus et al., 2008b) and their features are: low pH,

amorphous clay minerals like allophane (about 50% of clay weight) and halloysite and other short-range order materials (Besoaín et al., 1985) in the top layer. However, SOM distribution and accumulation in deep layer of these soils have been rarely studied (Matus et al., 2009). Andisols contain also considerable amounts of reactive Al and amorphous Fe (Torn et al., 1997) and poorly crystallized minerals and oxides, which cause other unique characteristics, e.g. low bulk density and high phosphate retention (Parfitt, 1980; Borie and Rubio, 2003). There is also evidence that Al (extracted with Na-pyrophosphate) influences the SOM content in volcanic soils, since a positive and highly significant relationship has been found between these two variables (Matus et al., 2008; Percival et al., 2000). The stabilising effect of Al is due to complexation with SOC in the soil solution and subsequent precipitation as insoluble Al–SOM complexes, suppressing microbial enzyme activities and substrate degradation (Matus et al., 2009; Panichini et al., 2012).

The forest ecosystems, especially old growth temperate rain forests are the main C sinks (Grove, 2001), where the accumulated amounts are significant and the soil C residence time can reach centuries (Luyssaert et al., 2008). The rain forests of southern Chile have developed under low temperatures and high humidity. Under such conditions the woody vegetation produces litter, which slowly and incompletely decomposes in soil, resulting in accumulation of SOM on the soil surface (Staelens et al., 2011). However, intense rainfall can leach nutrient, clay and Al and Fe oxides from the surface into the subsoil (Pritchett, 1990). Although, the latter is believe to be produced *in situ*, the migration of allophane from top- to deep horizons has not been described. Leaching generates greater acidity in the surface soil horizons, with a soil pH ranging between 5.0 and 5.2. Subsoils are slightly less acidic. The response to climate change of pristine temperate rain forest remains uncertain. In mountain forest, the mean precipitation may reach more than 5,000 mm per year, with an average of annual temperature of 5°C (Oyarzún et al., 2004). The

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high rainfall is important since recent investigations have shown a dissimilatory nitrate reduction to ammonium (DNRA) via anaerobic organisms resulting in a highly efficient retention of N (Huygens et al., 2008). With regard to phosphorus, volcanic soils are characterized by a high adsorption capacity of this element with a consequent low availability, which can be overcome by mycorrhizal phosphate acquisition in the topsoil (Borie and Rubio, 2003). In a recent study, Matus et al. (2009), suggest that the mechanisms that protect SOC in the subsoil appear to be the same as in the topsoil. It seems that microbial biomass was provided with sufficient energy to sustain microbial populations as active as in the top soil suggesting the existence of a finite capacity to store SOM (protective capacity) in these soils. The latter is relevant, because the soils can act as source or sink, depending on the degree of C saturation. It appears that porous volcanic soils (very sandy soils) are saturated with SOM in the topsoil and this may favor the vertical transport of organic material to subsoil (Matus et al., 2009). In the word of Matus et al. (2008b) *"improved knowledge of these factors and mechanisms is essential to determine whether the nutrient will react to global change impact and how we are globally going to face this problem in allophanic soils...*".

1.2 Hypotheses

Old-growth forests in Chile are an important part of the global temperate forest reserve. They are characterized by a comparably high efficiency in nutrient cycling, particularly under extreme climatic conditions like high rainfall (>5000 mm yr⁻¹). Temperate forest ecosystems store large amounts of C, which are controlled by the rates of net primary production and decomposition of SOM. Recent studies indicate that especially C-accumulation in deeper soil depths contributes significantly to C sequestration in old-growth forests ecosystems. Therefore, in this thesis the hypotheses are:

- The pedogenic SOM stabilisation as observed by ¹⁴C age is modulated simultaneously by soil pH, non-crystalline clay through competition of metals (Al) and SOM for the formation of Al (and Fe)-SOM complexes or allophane type materials
- 2) The SOM in these soils is highly stable due to strong mineral interactions. Therefore it is not be possible to release C following addition of labile C.

1.3 General goal

The objective of this study is to identify the factors and mechanisms that control the C stabilisation in the top- and subsoil horizons of volcanic temperate old growth rainforest soils.

1.4 Specific goals

The goals of this thesis were to:

- (1) examine the distribution and chemical composition of SOM in the top- and subsoil horizons of two pristine temperate old growth rain forests Andisols of different age.
- (2) determinate the pedogenic contribution of Al and Fe SOM-complexes and allophane formation to C stabilisation measured by ¹⁴C activity in top- and subsoil horizons of temperate old growth rain forest.
- (3) evaluate the potential C mineralisation and PE following addition of cellulose in the topand subsoil horizons of Andisols obtained of two pristine temperate old growth rain forest.

CHAPTER II

Soil carbon storage and stabilisation in Andic soils: a review

Submitted to Catena

2.1 Introduction

Volcanic ash soils present a disproportionate amount of soil C in the soil organic matter (SOM) (Batjes, 1996; Eswaran et al., 1993), as they occupy only 0.84% of the global land area, but they may contain several time the C amount of non-volcanic soils (Dahlgren et al., 2004). These soils store about 5% of global soil C (Eswaran et al., 1993), because of its mineralogical properties for C sequestration.

Andic soils including Andisols according to USDA classification (Soil Survey Staff, 2008) or Andosols (FAO-ISRIC-ISSS, 1998) covers more than 120 million hectares (Figure 2 of Dahlgren et al., 2004) and they display unique morphological, physical and chemical properties attributed to the composition of their mineral phase. The minerals consists of short range ordered (SRO) materials like allophane, imogolite, ferrihydrite and Al- and Fe–humus complexes lacking of long range crystal atomic order (Harsh et al., 2002). Under the name of Andisols and based on the mineralogical composition of the A horizons, two groups are found: 1) non-allophanic Andisols dominated by Al–humus complexes and 2:1 layer silicates and 2) allophanic Andisols dominated by allophane and imogolite (Shoji et al., 1985). The allophanic Andisols contain appreciable quantity of allophane and imogolite type materials and they are distributed in areas having recent volcanic ash and thick deposition of Holocene and/or late-Pleistocene tephras developed under pH > 5 (Shoji et al., 1982). While, non-allophanic Andisols are developed from older tephra deposits and very often in high altitude mountains. Non-allophanic are developed under more acid soil pH conditions (pH < 5) and exposed to deposition of exogenous

materials, such as the loess from China dominated by 2:1 clay minerals (Bautista-Tulin and Inoue, 1997; Mizota et al., 1990; Inoue and Naruse, 1987).

The present review aims specifically to updating and integrate fundamental aspects of SOM composition and stabilisation for Andic soils (hereafter referred to as Andisols), understanding for stabilisation the SOM substrate that does not yield further biodegradation (Sollins et al., 1996). In particular, we want to point out differences and similarities between Andisols and other soil types, in order to gain information why Andisols are able to store more organic matter than the existing content of any other soil type. We have reviewed recent literature on the nature of SOM and its relation to stabilisation processes operating in top- and subsoil. As a result, this review is outlined in three sections. First we focus in discussing the nature and origin of SOM in Andisols. There is no signal of preservation of recalcitrant plant-derived compounds in allophanic soils (Suárez-Abelenda et al., 2011; Buurman et al., 2007); consequently the processes of incorporation of C compounds as well as their decomposition products in deep soil horizons are poorly understood. In the second part we discuss the interactions of SOM and mineral phase because destabilisation of adsorbed dissolved organic matter (DOM) following microbial processing has been proposed as a crucial mechanism of soil C transport into the subsoil (Kaiser and Kalbitz, 2012). Finally in the third section the C storage capacity of Andisols is evaluated. The existing theories do not take into account the mechanism and factors for the successful competition between SOM and metals (Al and Fe) that counteract allophane formation. In particularly we take some example from Chilean Andisols (Garrido and Matus, 2012).

2.2 Nature and origin of organic matter in Andisols

2.2.1 Chemical compositions

Most studies on the chemical composition of SOM in Andisols were carried out after alkaline extraction of humic and fulvic acids such as NaOH and Na₄P₂O₇ extractable (Nierop et al., 2005). The chemical properties of humic substances were found to be different for Andisols and adjacent non-andic soils. The Andic soils accumulate more unsaturated C than the non-andic soil showing higher carboxyl functional groups, possibly involving stable complexes with the Al of allophane materials, and methoxyled groups from poorly degraded lignin deposited from the above vegetation (Conte et al., 2003). Humic material extracted from Andisols of Japan has been reported to show a higher degree of condensation compared to those of non-andic soils (Yonebayash and Hattori, 1988; Kuwatsuka et al., 1978). The high aromaticity of these fractions could be related to the presence of charred plant remaining due to regular burning of the vegetation and melanic epipedon characteristic (Shindo et al., 2004; Golchin et al., 1997). The NaOH extractable SOM of volcanic soils unaffected by burning was found to be dominated by polysaccharide derived compounds, whereas relative enrichment of lignin, proteins and lipids was noted in Na₄P₂O₇ which is more aggressive reagent for extractable SOM of topsoil (Nierop et al., 2005). These and other studies concluded that recalcitrant plantderived compounds are scarcely preserved in comparison with the stabilisation of an easily degradable microbial-derived material volcanic soils (Suárez-Abelenda, et al., 2011; Buurman and Nierop, 2007; Buurman et al., 2007; González-Pérez et al., 2007; Nierop et al., 2005; Naafs et al., 2004). This is in line with recent publication by Hernández et al.

(2012). They reported that the content of non-crystalline minerals resulted in a higher C stabilisation and there was a not significant relationship between the amorphous minerals and aromatic compounds. Allophane nanoparticles enhanced the protection of microbial metabolites produced during the decomposition. This suggests that the chemical protection is not acting as it could be expected, because the short residence time of different plantderived organic matter of different forest vegetation type and the contribution of microbial sugars and N-compounds to SOM including recalcitrant condensed organic compounds in the soil profile (Buurman et al., 2007). In line with these findings, Barbera et al., (2008) found labile (oxidisable with H₂O₂) organic matter to include aromatic compounds (like charcoal). This is in accordance with the general literature on SOM stabilisation in nonvolcanic soils, where chemical recalcitrance of plant litter compounds is no longer regarded as SOM stabilisation mechanism (Dungait et al., 2012; Kleber et al., 2011; Marschner et al., 2008). Even black C, a recalcitrant SOM component, which is generally preserved in other soil types for more than centuries (Hammes et al., 2008; Rumpel et al., 2008) does not seem to accumulate in Andisols (Cusack et al., 2013). This may be due to absence of interaction of this component with soil minerals (Rivas et al., 2012). Biomarker studies showed that root derived suberin is of great importance for organic matter found in the A horizon of Andisols and at depth, while in summer leaching of microbial residues and aliphatic compounds may also contribute (Naafs et al., 2004; Naafs and Van Bergen, 2002). The latter authors suggested that intact biopolyesters or molecules may be protected chemically by trapping in the insoluble organic macromolecular network (Naafs and Van Bergen, 2002). Recently, Nierop and Jansen (2009) reported that solvent extractable lipids preserve their plant-derived signature throughout the soil profile, even if the bulk SOM composition no longer resembles the vegetation growing on these soils. This is in accordance with new

studies on the hydrophobic (HB) and hydrophilic (HI) SOM components (Spaccini et al., 2006). The hydrophobic to hydrophilic ratio ranged from 1.48 to 3.44 in the forested sites, but cultivation substantially reduced HB/HI ratio, except for Andisols. There is a contribution of Alkyl C in the subsoil evaluated by acid dichromate oxidation that increases C storage (Rivas et al., 2012). It seems that Alkyl structures can be protected from acid oxidation due to their hydrophobic nature, possibly by encapsulation into their hydrophobic network (Knicker and Hatcher, 2001).

Allophane nanoparticles (Calabi-Floody et al., 2011) have been proposed as a complex fractal structure protecting available C from microorganisms and enzymes (Chevallier et al., 2010; Woignier et al., 2007). The contribution of lignin structures to humic substances can be enhanced by secondary compounds biosynthesis from soil biota (Nierop and Buurman, 2007). Moreover the importance of amorphous materials on the molecular composition and stabilisation of SOM is not clear and need to further attention. The composition of SOM stabilised by mineral interactions in subsoils of allophanic as well as non-allophanic Andisols was found to be enriched in N-containing compounds and different from those of the A horizon (Rumpel et al., 2012). This could be either due to a specific origin or in situ ageing of SOM in subsoils. Evidence from studies on volcanic soils suggests that the presence of SRO minerals has a controlling effect on soil C:N, most likely through the stabilising effect of highly reactive non-crystalline clay minerals on SOM. Increasing C:N ratio with soil depth was reported for Andisols dominated by SRO minerals (Schrumpf et al., 2006; Lilienfein et al., 2004; Conte et al., 2003; Kawahigashi et al., 2003). In contrast, most other (non-andic) soil types show decreases in C:N with depth (Rumpel and Kögel-Knabner, 2011), which is attributed to decomposition of plant-derived compounds and greater contributions of microbial products with depth in the absence of stabilising SRO minerals (Baldock et al., 1992; Nadelhoffer and Fry, 1988). NMR spectral differences between organic matters collected from different horizons are consistent with enrichment of material that has undergone less microbial processing in the deeper Andisols. During plant litter decomposition and SOM formation, the dominant signal in the O-alkyl C region from plant cellulose and hemicellulose is progressively replaced by the alkyl C structures (nonpolar aliphatic compounds) such as waxes, resins and lipids; their accumulation in soils is attributed to selective preservation of recalcitrant plant compounds and/or to new production during microbial processing (Grandy and Neff, 2008). Detailed knowledge about organic matter input and degradation at different depths in Andisols is lacking.

As mentioned above, there is a wealth of studies reporting the composition of NaOH soluble SOM in Andisols, whereas relatively few studies are concerned with the SOM composition in solid (physical) SOM fractions. It was only in recent years that the composition of the mineral phase interacting with DOM and solid SOM stabilisation in the subsoil was taken into account. In the following, we will review literature dealing with such interactions and their effect on SOM stabilisation.

2.2.2 Distribution across the soil profile

The origin of SOM within an Andisol profile is uncertain. The strong adsorption potential of the mineral phase of Andisols assumes that DOM is of limited importance (Dahlgren et al., 2004; Aran et al., 2001). The soil-forming processes on non-allophanic soils developed under Japanese grass as affected by Japanese oak invasion was investigated by Dahlgren et al., (1991). They concluded that translocation of metals such as Al and Fe,

and DOM is not a main process in their Andisols. Metal humus complexes and hydroxy-Al interlayered 2:1 minerals, rather than SRO were formed. However, recently Kaiser and Kalbitz (2012) proposed a new conceptual model in which the DOM through soil profile not only result from physico-chemical chromatography concept (fractional sorption and coprecipitation) but also from microbial processing and subsequent delivery of organic matter previously sorbed in overlaying horizons. This model also apply for Andisols and can explain the radiocarbon age of SOM in the subsoil that contrasts sharply with the assumption of roots being a major source of deep soil C (Rumpel et al., 2002). The nature and origin of DOM in the soil and stream water was studies by Qualls and Haines (1991) in non andic soils (Inceptisol and Ultisols). Most of the DOM was in the humic fractions (hydrophobic acids and phenols) comprising 35 to 57 % of the DOC in all samples. The composition of DOM can vary largely in accordance to the nature of the forest vegetation. These results corroborate a recent study by Kramer et al., (2012), who suggested that organic matter storage in subsoils of Andisols is controlled by the transport and adsorption of oxidised compounds, which strongly resemble DOM from plant litter. The authors therefore suggest that long-term C storage in the subsoils of Andisols occurs through SRO minerals of plant-litter derived aromatic acids transported via preferential flow. A preferential flow path in the delivery of C to deep soils was observed in Hawaiian rain forest volcanic soils (Marin-Spiotta et al., 2011). However, it seems that the dominant drainage process in Andisols is matrix flow and that preferential flow occurs only at some times per year and accounted between 16-27% of the annual total drainage (Eguchi and Hasegawa, 2008; Hasegawa and Sakayori, 2000). Therefore, it is unclear; how plant litter derived organic acids could quantitatively contribute to the high amounts of stabilised SOM in Andisols. The soil architecture need to be taken into account in future studies, and in particular water flow in Andisols under different land-use and vegetation need to be characterised. As matrix flow seems to dominate drainage of Andisols, it need to be clarified if horizontal stratification is of the same importance for subsoil C stabilisation as in non-andic soil types (Chabbi et al., 2009).

Bioturbation may be important for vertical SOM translocation (Tonneijck and Jongmans, 2008), in spite of acidic soil conditions. Roots are usually thought to be the main contributor to deep soil C (Rasse et al., 2006; Jobbágy and Jackson, 2000). However, in Andisols root activity could be limited by acid pH and Al activity (Dahlgren et al., 2004). The importance of the three different precursors of SOM (root-derived organic matter, DOM and organic matter transported by bioturbation) in deeper soil horizons need to be addressed in future studies.

The broad implication of our literature review is that there is no indication of preservation of recalcitrant plant-derived compounds, but rather stabilisation of easily degradable microbial derived materials (Buurman et al., 2007). Second a new conceptual model suggests that DOM and wet extractable organic matter is adsorbed and desorbed and microbial process the SOM which is delivered in the subsoil (Kaiser and Kalbitz, 2012). There is a gap of knowledge wheatear the transport and adsorption of plant-derived organic matter is controlled by preferential (Kramer et al., 2012) or matrix flow in the subsoils of Andisols (Eguchi and Hasegawa, 2008). Bioturbation may be important for translocation of plant-derived organic matter too (Tonneijck and Jongmans, 2008).

2.3 Interactions between organic matter and the mineral phase

2.3.1 Physico-chemical stabilisation

Under humid weathering conditions, the composition of the colloidal fraction (< 2 μ m) forms a continuum between pure Al–humus complexes (which is very rare) and pure allophane/imogolite, depending on the pH and organic matter characteristics (Panichini et al., 2012; Mizota and Van Reeuwijk, 1989). This has been observed in New Zealand, African and Japanese volcanic ash derived soils (Parfitt et al., 2009; Shoji et al., 1993; Wada, 1989; Wada et al., 1987; Parfitt et al., 1983). Al- and Fe-humus complexes are formed mainly in environments rich in organic C and low pH, but allophane is restricted to pH > 5 (Garrido and Matus, 2012; Shoji and Fujiwara, 1984).

In allophanic Andisols, the allophane (model $Al_2Si_2O_5 \cdot nH_2O$) is a primary SRO clay that occurs as spherical nanoparticles 4–5 nm in diameter with high specific surface area (~800 g m-2) (Calabi-Floody et al., 2011; Harsh et al., 2002; Parfitt et al., 2002). Imogolite, as allophane is a SRO (para) crystalline structure occurring in a very small tubes (several µm in length), and often forms bundles of two to several hundred tubes. The tubes diameter is 1 nm for inside and 2 nm for outside. Imogolite has Al: Si ratio of two (Yoshinaga and Aomine, 1962), although it has been recently suggested that poorly ordered proto-imogolite, rather than Al-rich allophanes and imogolites are present in Andisol horizons (Levard et al., 2012).

The mineralogical properties of allophanic Andisols, particularly SRO (allophane and imogolite type materials) present a high reactive surface area and they are regarded as the major agent for C stabilisation (Parfitt, 2009; Parfitt et al., 2002; Torn et al., 1997; Sollins et al., 1996; Zunino et al., 1982). This feature as well as the abundance of charged sites on the clay surface facilitates the adsorption of organic molecules which is an important mechanism leading to physicochemical protection of SOM. Recently it has been shown that allophane, which makes up most of the nanoclay fraction (< 100 nm) of Andisols contains high concentration of SOM resistant to chemical oxidation (Calabi-Floody et al., 2011). However, up to now the SOM compounds associated to allophane as well as their turnover time within different horizons of the soil profile are unknown.

The main controlling mechanism of sorption of humic substances by variable-charge minerals is ligand exchange reaction (Spark, 2003; Percival et al., 2000; Yuan et al., 2000). Matus et al. (2009) used a model developed by Yuan et al. (2000) to explain Al-SOM complex formation in allophanic soils (Figure 1). Hydroxyl (OH)Al(H₂O) groups attract or lose protons depending on soil pH. The reaction produces a negative charge that is compensated by electrostatic interactions with Na⁺ or cations such as Al³⁺ ions. Thus, humic acid sorption is promoted by the presence of electrolytes like NaCl and CaCl₂ (although Ca²⁺ is scarce in allophanic soils). Soil organic matter binds to spherules of allophane and can interact again with a cation to form a new complex, mediated by electrostatic attraction. This mechanism is the precursor of further electrostatic interactions for a new nucleus of Al-SOM complexes that subsequently precipitate. Huygens et al. (2005) showed, however that electrostatic interaction with amorphous Al and clay minerals was the main mechanism for SOM stabilisation in Andisols from Southern Chile.



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Figure 1 Transversal view of a external hypothetical allophane spherule of 3.5-5 nm and humic acids (HA) with its carboxylate groups (-COOH) showing ligand exchange mechanism which is bound to Na^+ or Ca^{2+} electrolyte by electrostatic interactions (interpreted from the model of Yuan et al., 2000; Matus et al., 2009).

Amongst the hypotheses that explain SOM accumulations in Andisols are: 1) Cold climates decrease the duration and intensity of biological activity (Boudot et al., 1986). 2) Phosphorus as rate-limiting factor for organic matter mineralization (Munevar and Wollum, 1977) and 3) Mineral associations which reduces SOM mineralization (Zunino et al., 1982). The hypothesis that Al oxides rather than the clay content and climatic factors are the most important stabilising agent for SOM variation in temperate allophanic soils representing different soils orders and land-uses on a regional scale was tested first by Percival et al. (2000) in New Zealand and by Matus et al. (2006; 2008b) in Chilean volcanic soils. They found that the proportion of silt and clay, the climatic factors (precipitation and temperature) and Fe oxides, did not predict the soil C variation. In contrast, the extractable Al in Na-pyrophosphate (Percival et al., 2000) was highly correlated with the SOM. Recently Tsui et al. (2013) studied the climate, vegetation types and soil mineralogy along elevation gradients in subtropical volcanic ash derived soils from Taiwan. They found that the elevation was a good predictor of SOM stock. Experimental evidence for the stabilisation potential of amorphous Al oxides was provided recently by Schneider et al. (2010) and Miyazawa et al. (2013). Schneider et al. (2010) concluded that the mineral availability for sorption of DOM as well as the solution chemistry determines sorptive interactions and thus the stability and composition of the sorbed DOM against microbial decay.

Most studies on C storage in Andisols are concerned with stabilisation mechanisms. Destabilisation mechanisms are generally less studied (Sollins et al., 2009). Soil solution
studies in volcanic deposits have shown the formation of amorphous materials (active Al and Fe compounds) and SOM accumulation characterized by high concentration of Fe, Al, and DOM in A horizons with little translocation of these components to B horizons (Dahlgren et al., 1991; Ugolini et al., 1988). Therefore, it has been long hypothesized that non-crystalline materials (e.g., allophane, imogolite, and ferrihydrite) in B horizons have formed in situ, rather than by translocation (Dahlgren et al., 2004). Recently however, the transport of SRO mineral-SOM complexes to a greater soil depth has been observed in Andisols after conversion of tropical forest into grassland (Osher et al., 2003). In high precipitation region, C losses from the soil appear to occur via downward transport, either as colloids or in solution. However, almost no paper deals with this crucial issue. Latest literature indicates that sorbed organic compound can be desorbed as consequence of protective site saturation (Kaiser and Kalbitz, 2012). This is due to complexation reactions of Al with the soil organic C in the soil solution and subsequent precipitation of insoluble complexes Al-SOM (Rasmussen et al., 2006). Moreover, change in solution chemistry due to agricultural practices can lead to dissociation of SRO mineral-organic matter complexes and C loss through mineralisation (Osher et al., 2003).

The empirical evidence of the importance of Al for stabilisation and destabilisation of SOM at field scale need to be completed by studies concerning the type of organic matter sorbed under specific conditions for instance between tropical and temperate volcanic soils. Of particular interest is the hydrophobic black C in Andisol. As above mentioned this material is richest in aliphatic compounds (Rivas et al., 2012) that can be protected from oxidation due to their hydrophobic nature.

2.3.2 Physical protection

Location of physically protected organic matter in stable aggregates reduces its accessibility for microbes, fungi or enzymes (Sollins et al., 1996) and has been reported as the main mechanism controlling the stability of SOM in Andisols (Huygens et al., 2005) as well as other non-andic soils (Dungait et al., 2012). Several authors noted extremely high stability of soil aggregates isolated from Andisols (e.g. Candan and Broquen, 2009; Hoyos and Comerford, 2005). Baumgarten et al. (2013) reported that Al-humus-complexes promote the soil aggregation of hydrophobic organic compounds of alu-andic Andisols, which present the ratio between Na-pyrophosphate extractable Alp (Al-humus complexes, Parfitt and Kimble, 1989) and acid ammonium oxalate extractable Alo (Al dissolved from imogolite, allophane, and Al-humus complexes) > 0.5 (Hernández et al. 2012).

Land-use has a tremendous impact on SOM physical protection in the soil structure of Andisols (Dec et al. 2012; Dörner et al., 2011; 2010; Seguel and Horn, 2005; Ellies et al., 2000; Hartge et al., 1978). One characteristic of Andisols is that the aggregate hierarchy model does not apply. In this model the binding agents act at different hierarchical stages of aggregation (Tisdall and Oades, 1982). Microaggregates (20–250 μ m) made up of primary silt and clay particles are bound together by persistent binding (humified organic matter and polyvalent metal cation complexes). They in turn are bound together into macroaggregates (>250 μ m) by temporary (i.e. fungal hyphae and roots) and transient (i.e., microbial- and plant-derived polysaccharides) binding agents. Because of this hierarchical order microaggregate stability is higher and less dependent on agricultural management than macroaggregate stability. Woignier et al. (2008) described allophane as porous material between 3-100 nm, able to bind and form new aggregates. They also empathized that these

characteristics confer low diffusivity and permeability at the microaggregate level, which would be the reason why allophanic soils have a high capacity to stabilise SOM and they do not respond to the hierarchical aggregation model. This hypothesis was recently supported by the study of Chevallier et al. (2010), who showed that the biodegradability of SOM in Andisols was closely related to the allophane content and the existence of fractal clusters forming a 'Nan labyrinth' that protects SOM within the microaggregates.

For determination of the quantity of SOM physically protected against decomposition in Andisols, the sequential density fractionation approaches have been developed (Panichini et al., 2012; Basile-Doelsch et al., 2007; Sollins et al., 2006). In general, organic matter associated to the mineral phase is found in the heavy density fraction (> 2.0 g cm3) of soils (Turchenek and Oades, 1979). While the contribution of the C associated with the heavy fraction increased with soil depth, up to 50% of organic C may be present in light and POM fractions of the A horizons of Andisols under forest (Panichini et al., 2012; Rumpel et al., 2012; Spycher et al., 1983; Sollins et al., 1983). This is more than typically associated with the light fraction of non-andic soil types even in A horizons and may be explained by the presence of light mineral material. Andisols have the particularity to contain very light minerals such as allophane, imogolite type materials and volcanic glass, which warrens for the use of lower densities. In general, the density for the separation of the light fraction of Andisols lies between 1.35 and 1.90 g cm-3 (Prior et al., 2007; Sollins et al., 2009; Huygens et al., 2008; Basile-Doelsch et al., 2007; Sollins et al., 2006; Huygens et al., 2005). In order to study the amount of organic matter associated to specific minerals, Basile-Doelsch et al. (2007) focused on the heavy fraction (> 1.9 g cm-3) of an Andic subsoil horizon with high variety of minerals and separated it into organomineral fractions which were analysed for their mineral composition and their

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organic matter content. The results showed that imogolite type minerals bound three to six times more organic matter than an orthoclase and iron oxides. Contrasting results were obtained for sequential density fractionation of A horizons of Andisols, where mineralogy was not related to SOM composition (Sollins et al., 2009; 2006). Despite of these conflicting results, the idea to look at organic matter dynamics in relation to mineral aggregation assemblage of Andisols seems to be a valuable approach considering that both factors influence each other. For example, it has been shown in Japanese soils that the formation of allophane is impeded by a high content of Fe and Al humates (Miyazawa et al., 2013; Shoji et al., 1993). The organic matter accumulation can inhibit the iron oxides crystallization processes (Pizarro et al., 2003) and this is very much related with the mean residence times of organic matter in Andisols which changes relative to mineral transformations (Mikutta et al., 2010; Egli et al., 2007; Torn et al., 1997).

2.3.3 Stabilisation mechanisms as influenced by land-use

A detailed knowledge on selective soil chemical and mineralogical characteristics on organic matter accumulation in relation to soil productivity and managements of volcanic soils has been reviewed extensively in excellent presentations (e.g. Dahlgren et al., 2004; Parfitt, 2009). Here we will mainly focus on the effect of land-use conversion on SOM stability.

Land-use changes are hypothesised to have little impact on C stabilisation potential of Andisols, although soil management was found to change basic soil properties such as bulk density, pH, and microbial community structure (Rahman et al., 2008). Absence of such effects on SOM were thought, as it was previously mentioned, to be a resulted of their high structural stability, absence of aggregate hierarchy (Candan and Broquen, 2009; Hoyos and Comerford, 2005) and slow stabilisation of fresh organic matter (Paul et al., 2008; Buurman et al., 2007; López-Ulloa et al., 2005). However, Johnson-Maynard et al., (1997) indicated chemical changes properties in Andisols accompanied by successional communities of bracken fern (Pteridium aquilinum [L.] Kuhn) from adjacent undisturbed forest of Abies grandis [Dougl. ex D.Don] Lindl. The pH and organic C from bracken fern were reduced significantly than forest and they induced a shift from allophanic to nonallophanic properties. Land-use changes were also found to affect SOM storage as well as microbial biomass activity (Dube et al., 2009; Huygens et al., 2005). Generally, conversion of grassland to forest plantation led to decrease of SOM stocks, associated with disturbance of the site when the forest is established (Dube et al., 2009). Conversion from native forest to grassland led to an increase of SOM stock in two adjacent volcanic soils (Huygens et al., 2005). The directions of change were similar as those observed by Guo and Gifford (2002) for a range of non-andic soil types. In Andisols from Chile, five years of pastures (alfalfa or white clover) promoted an increase of SOM labile fractions in comparison with annual crop rotation (Zagal et al., 2013; 2009).

Effects of agricultural practices on SOM in Andisols may be long lasting. A recent study evidenced that SOM storage was reduced and its chemistry changed even as long as 200 years of abandonment of agricultural activities (Cusack et al., 2013). Andisols may lose their Andic properties upon successive rewetting and drying. Volcanic materials behave as gels during drying with a large irreversible shrinkage that can modify the soil physical properties (Woignier et al., 2008). Management techniques, such as no-tillage or minimum tillage thus need to be implemented and developed to preserve as much as possible Andic properties and initial SOM content upon cultivation. Moreover, enzymes and microbial

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byproducts may be adsorbed on SRO mineral surfaces and it can be deactivated for its function (Saggar et al., 1994). In Andisols, microbial decomposition of organic matter may be limited by Al toxicity (Tate and Theng, 1980; Illmer et al., 2003) and low pH values. Recently it was reported that extremely acid soil pH conditions leading possibly Al toxicity along with high microporosity may lead to a preservation of plant-derived aliphatic C in Andisols (Tonneijck et al., 2010).

2.3.4 Influence of time, climate and vegetation on soil organic matter stabilisation

SOM storage and residence times of SOM in Andisols are changing through time. This is related to weathering and ageing processes affecting the mineral phase and in turn SOM stabilisation (Torn et al., 1997). The ¹⁴C ages of humic acids extracted from A horizons of Andisols ranged from modern to 30,000 years before present (YBP), with the majority in the range 1000–5000 YBP (Inoue and Higashi, 1988). Torn et al. (1997) studied the soil organic matter accumulation along a 4-million year soil chronosequence formed on basaltic lava in Hawaii. Noncrystalline materials concentration were related to a maximum accumulation of SOM after 150,000 years and then decreased along more stable crystalline minerals leading a reduced SOM. Mineralogy changes of Andisols were studied along weathering sequences in southern Italy. The main mineral transformations during 115,000 years of soils development were from volcanic glass to imogolite and kaolinite. The highest C accumulation was observed in young soils with an age < 2000 years (Egli et al., 2008). They noted however, that after 15,000 years, C accumulation seemed to be at the maximum. Lilienfein et al. (2003) studied the rates of increase in allophane, specific surface area changes, and organic matter accretion in a young andesitic chronosequence

from 77 to 1,200 years in California. The C accumulation rates were highest in initial stages of soil development and ceased after 600 years. Concomitantly, allophane concentrations increased to a maximum rate of 0.14 g kg-1 year-1. Evolution of the chemical composition of stabilised organic matter through time has been intensively studied using Andisols from Hawaii covering 0.3-4100 ky (Mikutta et al., 2009; Chorover et al., 2004) and from New Zealand (0-10 ky) (Prior et al., 2007). In both sequences SOM composition changes have been recorded. In early weathering stages, the mineral phase was composed of primary, low-surface area minerals (olivine, pyroxene, feldspar) associated with low amounts of SOM and a high contribution of microbial carbohydrates. From 2000-40,0000 years SOM content increased sharply, was associated with a concomitant increase of poorly crystalline minerals and showed a higher contribution of lignin in organomineral complexes. The third weathering stage was characterised by transformation of poor crystalline into well crystalline mineral phase and decrease in SOM associated with soil minerals. Andisols formed under conditions of rapid chemical weathering primarily from colored basaltic parent materials at pH > 5, release high concentrations of cations (e.g K, Mg, Al, Fe and Si). Al and Fe hydroxides and silicate ions in soil solution precipitate from the supersaturated solution to give SOR or poorly crystalline alumino-silicates allophane and imogolite type materials. Andisols will be dominated by allophane and imogolite type materials or by Fe- and Al-organic complexes (humus-complexes) upon pedogenic changes and soil pH. At pH > 5 the carbonic acid weathering influences the soil to allophane formation allowing the Al to polymerize and co-precipitate with Si, whereas at pH < 5 the formation of metal (Al and Fe)-humus complexes will be favored (Neculman et al., 2013). The storing capacity of Andisols is function of the surface area available for sorption of SOM rather than the amount of clay content (Baldock and Nelson, 2000; Saggar et al.,

1994). Thus, the high specific surface areas of noncrystalline constituents in Andic soils would be expected to have high potential to stabilise SOM.

As C stabilisation in Andisols depends on the composition of the mineral phase (Rasmussen et al., 2006), it may be influenced by climate and vegetation through acceleration of weathering processes. Vegetation inputs that bring much organic matter directly into the soil cause black deep high-organic matter across the soil profile in Japanese soils. Regular burning may intensify the black colour and increase humus aromaticity and mean residence time (Iimura et al., 2010) at top and subsoils (Rivas et al., 2012). Broquen et al. (2005) studying a bioclimatic sequence of Argentinean Andisols, observed that vegetation types resulted in a sensible contribution of grass-shrub steppe to the formation of humic substances than under Nothofagus spp. forest indicating that the differences in type of organic matter accumulating in various soils might have on expression on weathering, being higher under forest soil than under grass-shrub steppe. These authors indicate a high C mineralisation and moderately acid soil pH under xeric moisture regime, whereas under udic moisture regime and lower annual temperature, they found the highest SOM storage, lowest pH and highest aluminum activity. Thus climate and vegetation may in addition to chemical weathering processes influence abiotic parameters which determine microbial activity and hence destabilisation of SOM. However, the response of SOM stabilisation and destabilisation processes to climate and vegetation change has not yet been addressed in detail.

In the following section we will review literature on the C storage capacity of Andisols associated with the stabilisation mechanisms, the priming action linked with the pedogenic process.

2.4 Carbon storage capacity

Andisols containing high amounts of Al and Fe present high stabilisation capacity (Eswaran et al., 1993; Jansen et al., 2011). The role of soil mineralogy as key variables controlling the reduced C mineralization have been investigated in SOR-bearing soils (Rasmussen et al., 2006; Boudot et al., 1989; 1988; Zunino et al 1982). Amorphous SOR materials associated with P deficiency may additionally retard SOM decomposition (Zunino et al 1982). As previously mentioned, the protective capacity of SOM is continuously renewed resulting from pedogenic processes including: 1) burial of soils by repeated falling volcanic ash (Imaya et al 2010); 2) chemically protective C pool interacting with cations and noncrystalline inorganic materials (e.g., allophane, imogolite, ferrihydrite) (Shoji et al., 1993) and 3) physically protective C pool through the macro- and micro-aggregates formation (Dahlgren et al., 2004; Shoji et al., 1993). Stabilising mechanism, the ability of clays to encapsulate SOM, the burial organic compounds within aggregates, can made inaccessible to decomposers organisms, contributing significantly to SOM accumulation (Dahlgren et al., 2004).

The prediction of the SOM storing capacity of volcanic soils and how this evolves along the time in interaction with mineral phase is a key issue. The storing capacity and eventually a maximal (saturation) of chemically protected C pool are closely related to the steady-state of SOM concept. The latter is mainly controlled by the input C rate, climate and soil mineralogy, while, C saturation depends on the chemically protective sites occupied with SOM. The C saturation never has been applied to volcanic soils, although the storing capacity evolution under similar climate conditions is much slower and less intense in non-andic compared to those changes in Andisols (Egli et al., 2008; Dahlgren et al., 2004). Evidence of chemical saturation has been presented by Arai and Tokuchi (2010). They found no significant differences of SOM in the fine fraction (silt plus clay) of 55-year-old C. japonica plantation (with greater SOM stock) than natural forest composed mainly of Abies firma and Tsuga diversifolia in Japanese volcanic soils. The SOM differences resulted from larger C accumulation in the coarser and OM fractions.

As in non-andic soils, the importance to distinguish whether Andisols has reached a new steady-state of SOM or a maximal SOM accumulation is crucial, since a soil approaching to this limit will accumulate small amount of SOM due to the reduced protective sites. Under such conditions detached SOM from mineral phase, the DOC, can be transported to the subsoil. Although several mechanism have been proposed for sequestering C in different C-sink through changes in management as temporary measures (Dungait et al, 2012; Jiménez and Lal, 2006), the soil becomes a C-sink or C-source depending on the extent to which the reactive fractions approach to the C saturation (Matus et al., 2008a). It can be hypothesised that the short residence time of different plant-derived organic matter and the contribution of microbial sugars and N-compounds, including recalcitrant condensed aromatics compounds in the soil profile of Andisols is compatible with the idea of a limited SOM retention capacity. As it was said formerly, the DOM and organic colloids transported through soil profile can be assumed as results of the matrix flow and from the limited retention of SOM in their reactive fraction (Kaiser and Kalbitz, 2012).

The process that control the soil C stability along the soil profile and the influences pattern of nutrient limitation in response to several factors including the effect of climate change such as the elevated CO_2 and temperature, remains elusive. The signature $\delta^{13}C$ of

respired CO₂ suggests greater decomposition of recalcitrant soil C compounds with increasing temperature, indicating a priming action. The priming effects is and acceleration or retardation of the native SOM mineralization. Preferential C uses by soil microorganism leads a change of the turnover by an extra C mineralisation from native SOM of amended fresh C soils compared with the control without addition (Sparling et al., 1982). In young volcanic top soils, Al and Fe oxides are the most important agents for SOM stabilisation compared with old volcanic deep soils containing allophane-like materials (Neculman et al., 2013; Garrido and Matus, 2012). Rasmussen et al. (2007; 2006) studied the importance of the mineralogical composition of soil on the priming action in temperate rain forests and they found that the amorphous clay-like (volcanic soils) trends to exhibit a negative priming (retardation) in the top soils. Chemical interaction between SOM and metals (Al and Fe oxides) promotes SOM-complexes and stable macro- and micro-aggregates providing physical protection of SOM (Neculman et al., 2013; Panichini et al., 2012). This is important because complexed SOM increase the mean residence time and microbial biomass prefer fresh substrate consumption thereby limiting CO₂ losses from native SOM. Like non-andic soil, the decomposition of SOM may be energy limited (Fontaine et al., 2007). For example, Crow et al. (2009) evidenced priming action of topsoil SOM due to doubling the litter addition on Andisols under forest, accounting for 11.5-21.6% of the annual CO₂ flux. In general, the information on the proportion of SOM stored in Andisols due to priming effects is scarce.

Organic matter storage capacity of Andisols regardless the land-use or climate was found to vary mainly by the interactions with soil minerals, in particular Al oxides in moderately acid Andisols (Matus et al. 2006) and podozols (Bardy et al., 2007). Recently Miyazawa et al. (2013) confirmed that the Al obtained after pyrophosphate extraction and

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pH (H_2O) values are directly related to the variation of organic C concentration in Japanese Andisols. As discussed above, the mineral assemblage as well as SOM associated to it may evolve with time, and their storage capacity (Mikutta et al., 2009; Torn et al., 1997).

2.4.1 Evaluation and controls of carbon storage capacity

The evaluation of C sequestration capacity in Andisols can be approached from several points of view. For instance the humic acids in Japanese volcanic ash soils are considered to accumulate large amount of stable macromolecules as Al- and Fe-humic acid complexes which are resistant against decomposition and leaching (Wada and Higashi, 1976). The disappearance of the melanic epipedon with a decreasing aromatic C and increasing alkyl C proportion of humic acids was a good indicator of C sequestration in Japanese soils over 30 years of conversion from grassland to forest (Jimura et al., 2010). Sequestering soil C not only relies upon the storing capacity given by the mineral interaction and time, but also from the availability of stabilising elements, nitrogen (N), phosphorus (P) and sulphur (S) which are known to be essential components of the stable SOM. The hypothesis that the stable pool of SOM with constant ratios of C:N:P:S for nonandic and volcanic soils can be used to evaluate the strategies for C sequestration of soil was recently tested by Kirkby et al. (2011). This is of particular interest for Andisols that show a large reservoir of organic P, but with serious limitations of available inorganic P in forests and agricultural soils (Borie et al., 1989). Further research is needed to more accurately determine the amount of organic P in stable SOM pool and how C:N:P:S relates to the SOM storing capacity.

Recently has been proposed that the molar metal (Alp and Fep):Cp ratio can be used as an indicator of chemical C pedogenic transformations of Andisols (Garrido and Matus, 2012) (See below). According to these authors any soil exceeding a molar metal: C ratio of >0.18, the SOM will be fully complexed (saturated) with Al and Fe and it cannot longer be available for metal complexation. This ratio is remarkable similar to the value of 0.17 estimated from a theoretical humus complex unit of a melanic Japanese Andisols formed by 40 C unit and three carboxyl groups complexed with Al and one exhibiting a negative charge (Nanzyo, 2002). Similar metal: C ratio has also been estimated in Spodsols. A strong linear relationship between Alp+Fep and Cp (R2 = 0.97; P < 0.001) was established by Dahlgren and Ugolini (1991). Their data indicated a slope of 0.18, similar to the early estimation of 0.16 by Higashi et al (1981). Once the complexing capacity of humic substances is saturated with metals, the Al in excess will react with Si to form allophanic materials and this may explain the poor relationship between allophane content and SOM in Matus et al., (2006) and Percival et al. (2000) (Garrido and Matus, 2012). In non-saturated humus complexes such relationships do exists because SOM bind to non-crystalline type materials (Garrido and Matus, 2012). Given the large variability of soil acidity (e.g. 4.9-6.5) of Andisol, the complexing ability of humic substances may vary, making the saturation ratio somehow variable. However, the metal:C ratio are remarkable similar between Spodsols and Andisols and for any given Andisol and horizon, a ratio may exist which corresponds to the saturation of the humic complex with metals (Dahlgren and Ugolini, 1991). Furthermore, from our result presented by Rivas et al. (2012) it can be calculated the relationship between the radiocarbon age and the ratio values (Alp+Fep:Cp) (Figure 2). This result suggest that: 1) the oldest subsoil showed a higher molar metal:C ratio, i.e. toward the saturation, while the youngest topsoil was far from this maximal limit and 2) the stabilising capacity of Andisols evolves with time and C stabilisation is controlled by the presence of allophane in the subsoils.

Summarising, the chemical stabilised C pool in young Andisols is reduced along pedogenic process to a more stable mineral phase interacting with newly formed SOM. Negative priming action can counteracts the reduced storing capacity of C saturated soil by preventing the mineralization of native SOM. SOM in the chemically saturated C pool can no longer be protected undergoing rapid decomposition, otherwise physically stabilised by soil structure.



Figure 2 Relationship between the radio carbon dating and the molar ratio; of Al_p and Fe_p extracted in Na-pyrophosphate and the soil organic C (SOC) at different soil depth of an Andisol from Chile. Horizontal bars are the standard error of the mean (data no published from Rivas et al., 2012).

2.5 Conclusions

• The chemical composition and stabilisation processes of SOM in Andisols were different from those of non-andic soil types.

• The C storage capacity of Andisols was found to be higher than those of other soil types and especially related to the Na-pyrophosphate extractable Al and Fe. This is in particular due to the combination of specific properties of Andisols with Al, and Fe activity, and the presence of allophane and imogolite type materials.

• The chemical characterization of SOM fractions of Andisols indicated the stabilisation of microbial-derived products rather than plant-derived organic matter compounds.

• The physical protection of SOM in macro and microaggregates or micropores played a key role too.

• The impact of soil disturbances and climate change on the storage capacity of soil organic matter of Andisols requires further attention because physical and chemical properties of Andisols may be irreversible once the soil is altered.

• The relative contribution of root material, DOM and bioturbation for SOM transport, drainage and SOM accretion across the soil profile upon cultivation need to be further investigated. How implementing management techniques such as no-tillage can preserve these Andic properties is unclear.

• The C storage capacity, given by the organo-mineral interactions can eventually be saturated with SOM affecting the transport of DOM and C delivery in deep soils. This is in connection with the soil architecture in particular with the water flow in Andisols under different land-use and vegetation. However, as matrix flow seems to dominate drainage of

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Andisols, it is not clear whether horizontal stratification is of the same importance for subsoil C stabilisation as in non-andic soil types.

• Priming effects may occur but it is unknown whether affect SOM mineralisation negatively across the soil profile. There is evidence showing that priming effect on topsoil SOM of Andisols under forest may account for about 1/5 of the annual CO₂ flux of the soil. The intensity of priming effects of Andisols has not been tested to the extent than those of non-andic soils.

• The maximum C storage capacity of Andisols was found to be associated to Al- and Fe-pyrophosphate. SOM stabilisation and destabilisation is a function of soil pH, the competition of Al for SOM and the C age for allophane formation. Destabilisation processes of SOM in Andisols have been barely addressed. This is also influenced by their specific abiotic parameters, such as pH and SRO minerals, which can limit biological activity.

• The humus-complexes model with a metal:soil C ratio (0.16-0.18), representing the upper boundary for SOM complexation in Andisols, require further attention. Any metal above this limit will not be complexed with SOM, but it will be available for allophane and imogolite formation, even further than pH 5. Laboratory experiments need to be setup to confirm this hypothesis.

• Finally, it is essential to develop a body of working hypotheses that express the current understanding and point to critical experiments to validate these ideas. The prospective of the new insight could be used to frame such hypotheses that highlight some of the major areas of uncertainty within known mechanisms and factors. Some of these are stated in the next:

2.6 Future directions

• The hypothesis that Andisols store much SOM for a long time requires further attention. Based on the current knowledge, research for SOM in Andisols also needs quantification of SOM stabilisation on different time scales throughout soil development. Changes in SOM composition following changes of the mineral phase through time also need to be addressed in further studies. The hypothesis that the organic matter types most susceptible to be stabilised by different organo-mineral interactions in various parts of the soil profile should be addressed as well as their susceptibility to destabilisation processes on different time scales.

• In order to understand the processes and to be able to develop management practices we need to know more about the nature of stabilised organic matter. In particular, we need to address the question, if similar SOM-types are stabilised in tropical and temperate Andic soils and if this is similar compared to non-andic soil types.

• The interaction between soil biology and SOM chemistry need to be studied in the field as well as in laboratory experiments. We hypothesized that SOM mineralization triggered through priming effects may be important across the soil profile. This is supported by the new conceptual model of transportation and destabilisation which also applies for Andisols. The nature of the microbial communities of Andisols needs to be examined and compared to microbial communities present in non-andic soil types.

CHAPTER III

Organic matter stabilisation in two Andisols of contrasting age under temperate rain forest

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3.1 Introduction

Andisols (Soil Survey Staff, 2008) are regarded as privileged study case for soil C stabilisation mechanisms because of their high C storage capacity due to their elevated content of short-range order minerals such as allophane and imogolite type materials (Parfitt, 2009). However, Andisols capacity to preserve SOM is not unlimited (Buurman et al., 2007; Garrido and Matus, 2012; Panichini et al., 2012). Several mechanisms of SOM stabilisation against decomposition have been described in temperate soils including recalcitrance of organic compounds, spatial SOM inaccessibility for decomposer organisms and metal ions interacting with the mineral phase (von Lutzöw et al., 2006).

It is well known that the minerals of Andisols evolves with time and may influence C stabilisation (Torn et al. 1997; Lilienfein et al. 2003). In particular, the transformation of noncrystalline (allophane imogolite and ferrihydrite) into crystalline minerals (halloysite, kaolinite, gibbsite, goethite and haematite) may lead to C destabilisation because of their lower adsorption capacity compared to non-crystalline minerals (Torn et al., 1997).

Contrasting stage of soil development may therefore be one of the reasons for the observed discrepancies in different studies concerning the main agents for SOM stabilisation (Matus et al., 2009). Changes in the mineralogy and C stabilisation potential with time may also influence SOM composition (Prior et al., 2007; Mikutta et al., 2009) and may be closely related to general soil chemistry.

Shoji and Fujiwara (1984) were the first proposing that the formation of allophanic and non-allophanic clay and metal (Fe and Al)-SOM complexes is controlled mainly by the soil pH which is closely related to the pedogenic evolution (Huggett, 1998). Many papers rely upon this

assumption that is usually not tested (e.g. Takahashi and Shoji, 2002). Generally, pH <5 is considered as a boundary between SOM-complexes and allophane type material formation (Mizota and Van Reeuwijk, 1989). In presence of colored basaltic basic parent materials at high pH (>5.0) carbonic acid weathering influences the formation of the allophanic Andisols. The HCO₃⁻ anion does not form chelates with Al, allowing the Al to polymerize and coprecipitate with silica in the pH range of 5.0 to 7.0 (Dahlgren and Walker, 1993). In Andisols with relatively low pH (<5.0) the acid weathering inhibits the polymerization of aluminum hydroxide, and thus the formation of hydrous Al-silicates, favoring the formation of metal (Al and Fe)-organic complexes (Ugolini and Dahlgren, 1987). Recently Garrido and Matus (2012) and Panichini et al. (2012) reported that the soil pH was inversely related to metal-SOM complexes and directly proportional to allophane content of Chilean volcanic soils. In addition to pH, competition of metals (Al and Fe) by SOM and silica may influence the formation of SOM stabilisation agents in Andisols. Excess of Al relative to the complexing capacity of the humic substances favors allophane formation. The metal: C_p ratio ($C_p = C$ soluble in pyrophosphate) may be an indicator for the pedogenic direction of soil weathering to allophane or metal-humus complex formation (Higashi et al., 1981; Garrido and Matus, 2012).

In active volcanic regions like Central Southern Chile, soil may not be older than a few ten thousand years (Holocene/late Pleistocene) due to continuous burial following new eruptions. Therefore, minerals may remain non-crystalline, leading to high stabilisation potential for organic matter. In the present study we hypothesised that pedogenic SOM stabilisation is modulated simultaneously by soil pH, non-crystalline clay and depth through competition of metals (Al) and SOM for the formation of Al (and Fe)-SOM complexes or allophane type materials.

The aims of this study were: (1) to examine the distribution and chemical composition of SOM in top- and subsoil horizons of two pristine rain forests Andisols of different age and (2) to

examine the contribution of Al and Fe SOM-complexes and allophane to C stabilisation in topand subsoil horizons.

3.2 Materials and methods

3.2.1 Study area

The study sites are part of the Andean mountain range and located in San Pablo de Tregua (SPT), a long term ecological research Station, Panguipulli (39° 35' S and 72° 07' W, at 720 m above sea level) (Anderson et al., 2012) and the Puyehue National Park (PNP), Antillanca (40° 47' S and 72° 12' S, at 800 m above sea level). The rainforests in southern Chile have not been under chronic air pollution inputs and have remained floristically stable throughout the Holocene/late Pleistocene (Perakis and Hedin, 2002). The ecosystems selected in this study have developed under a temperate climate, with low average annual temperatures (5 °C) and high humidity with average annual precipitation >5,000 mm (Oyarzún et al., 2004; 2011). The vegetation at SPT consists of a native evergreen pristine Nothofagus dombeyi (Mirb.) Oerst., forest in mixture with Laureliopsis philippiana Looser and Saxegothaea conspicua Lindl (Oyarzún et al., 2011). At PNP, the dominant vegetation is pure evergreen old Nothofagus betuloides forest, (Oyarzún et al., 2004). The soils in both areas are Andisols (Soil Survey Staff, 2008) from the same parent materials. At SPT, the soils are derived from volcanic ash and pumice materials (CIREN, 2001), overlying andesitic and basaltic lavas and fluvio-glacial sediments. These soils are older than PNP and have a silty to sandy-loam texture throughout the complete soil profile. At PNP, the soil material is derived from andesitic and basaltic tuff scoria (CIREN, 2001). However, the soil texture is sandy loam to coarse sand throughout the complete

soil profile. The topography at SPT is undulating, while at PNP the soils occur in the plane after steeped piedmont with northern aspect. At both study sites, the soils had a litter layer of variable thickness (5 and 10 cm) and the mineral soil showed three horizons down to a depth of 100 cm.

3.2.2 Soil sampling

At each site, two 10 x 10 m plots were selected and a soil pit was excavated in the center of each plot. Sampling was conducted using a steal stainless cylinder (8 cm diameter x 5 cm length), which was pushed into the soil profile wall at 0-30, 30-60 and 60-100 cm, corresponding to A, B₁ and B₂ horizons for SPT and A, AC and C horizons for PNP, respectively. Two replicates per horizon at each site, in total 12 soil samples were collected. A soil sample using similar soil core cylinder was extracted from each horizon for bulk density determination. Soil samples were transported soon after sampling to the laboratory in appropriate containers. Moist soil samples were cleaned removing visible leaves, roots and organic debris and thereafter they were passed through a 2 mm sieve, air dried and stored at 5 °C for further analyses.

3.2.3 pH, Al, Fe and Si extractions and allophane determination

The pH was measured in demineralised water using a 1:2.5 soil:solution ratio as used for similar soils by Matus et al. (2008). The following extractions were conducted: (1) Al_p and Fe_p were extracted by shaking for 16 hours with 0.1 M Na-pyrophosphate (soil: solution ratio of 1:100) at pH 10. Thereafter, the suspension was centrifuged for 15 minutes at 1,270 *g*, with three drops of superfloc flocculant (N-100, Cytec, Canada.) and the supernatant was filtered. The suspension and un-dissolved materials were removed through filtration with acid-washed paper

with 7–11 μ m pore size and the solution passed again through 0.45 μ m millipore acid-washed filter paper; (2) Al_o, Si_o and Fe_o were extracted with 0.2 M ammonium oxalate oxalic acid (soil:solution ratio 1:50) at pH 3 and the suspension was shaken for 4 hours in dark and thereafter centrifuged for 15 minutes at 1,270 g, with three drops of superfloc. The supernatant was filtered as described above and transferred to a plastic container and stored in dark. The concentration of Al, Fe and Si in both extracts was measured in duplicates using atomic absorption spectrometry. The allophane content was calculated indirectly using the equation of Parfitt and Wilson (1985), modified by Mizota and Van Reeuwijk (1989):

Allophane (%) =
$$\frac{\operatorname{Si}_{\circ}(\%) \times 100}{\mathrm{y}}$$
 (1)

Where
$$y = 23.4 - 5.1 \times \left[\frac{(Al_o(\%) - Al_p(\%))}{Si_o(\%)} \right]$$
 (2)

3.2.4 C content analysis

The C content of bulk soil was analyzed using a CHN NA1500, Carlo Erba elemental analyzer (Stanford, USA) by the dry combustion method. The soil C stock (Mg ha⁻¹) was calculated according to the following equation:

$$C_{\text{stock}} = C_{\text{content}} \times d \times dt \times 10$$
(3)

Where $C_{content}$ is the soil C content (g kg⁻¹ dry soil), d is the bulk density (Mg m⁻³) and dt, the depth thickness (m)

3.2.5 SOM-complexes determination

The C_p extracted with Na-pyrophosphate was defined as C associated to Al_p and Fe_p complexed with SOM. The C_p in the supernatant of pyrophosphate extracts was analyzed with a total organic C analyzer (TOC-V CPH, Shimadzu, Kyoto, Japan). The $(Al_p+Fe_p):C_p$ molar ratio of 0.12 was considered as the maximum value to reach the SOM saturation with metals (Higashi et al., 1981).

3.2.6 Radiocarbon analysis

The ¹⁴C activity of ground bulk soil samples for the two sites and all depths was determined with an accelerator mass spectrometry (AMS) at the ¹⁴C Poznan Radiocarbon Laboratory, Poland. For these measurements, CO₂ was produced from soil samples by combustion at 900°C and reduced to graphite. The measured ¹⁴C activity was corrected for isotope fractionation (Stuiver and Polach, 1977) and it was expressed in percent modern C (pMC). Radiocarbon age is expressed in years before present (years BP).

3.2.7 Solid state ¹³C NMR spectroscopy

The bulk characterization of SOM was carried out by solid state ¹³C nuclear magnetic resonance (NMR) spectroscopy. This method gives a good overview of the bulk chemical composition of a solid soil sample. Four chemical shift regions can be identified and related to labile and recalcitrant SOM compounds. Solid-state ¹³C NMR spectra were obtained after demineralisation of soil samples with hydrofluoric acid (HF) (Rumpel et al., 2006) and the HF

residue was analysed using a DSX 200 spectrometer (Bruker BioSpin GmbH, Karlsruhe, Germany). The instrument operated at a ¹³C resonance frequency of 50.3 MHz and a spinning speed of 6.8 kHz was applied. The ¹³C chemical shifts are given relative to tetramethylsilane and were calibrated using glycine (COOH, 176.08 ppm). Functional C group content was determined using integration of the respective chemical shift regions using the instrument software (Forte et al., 2006). Resonances in the region -10 to 45 ppm correspond to Alkyl C and the regions from 45 to 60 ppm and from 60 to 110 ppm were assigned to N-alkyl-C and *O*-alkyl. Olefinic and aromatic C was detected in regions between 110 and 160 ppm. The region from 160 to 220 ppm was assigned to carbonyl (aldehyde and ketone) and carboxyl/amide C (Kogel-Knabner et al., 1997).

3.2.8 Data analysis

Normal distribution (Gaussian) of all variables was tested using a skewness test value of 0.5 (Webster and Oliver, 2001). If the skewness value was ≥ 0.5 (i.e., non Gaussian), we log-transformed the variables. ANOVA test was used and comparison of the mean values was conducted by Tukey-Kramer test. The relationships amongst several soil properties were explored by linear regression analyses using SPSS statistical software v11.0 package (SPSS Inc, Chicago, IL, USA) at a significant level of *P*<0.05.

3.3 Results

3.3.1 Chemical soil parameters

The pH of both soils ranged between 4.5 ± 0.01 and 5.8 ± 0.2 increasing with depth (P<0.05). The pH value for the top- and subsoil of SPT was lower than for PNP (Table 1). The bulk density in PNP increased slightly with soil depth from 0.41 ± 0.03 to 0.63 ± 0.1 Mg m⁻³ while in SPT the bulk densities ranged from 0.34 ± 0.01 to 0.43 ± 0.01 Mg m⁻³ and decreased slightly in the subsoil. The metal-SOM complexes were evaluated by Al_p and Fe_p extraction, which reflects the complexation ability of SOM by these metals. For both sites, the Al_p and Fe_p contents decreased with soil depth. In SPT, Al_p varied from 6.2 ± 0.3 to 14.4 ± 1.7 g kg⁻¹ and in PNP between 2.9 ± 1.3 and 9.1 ± 4.2 g kg⁻¹. For Fe_p, these values ranged from 1.9 ± 0.5 to 15.9 ± 0.3 and from 1.3 ± 0.9 to 8.1 ± 1.3 , respectively. Finally, the allophane content was higher in SPT than that in PNP and highest in the deepest horizons at both sites. The allophane content in SPT varied from 26.6 ± 7.0 g kg⁻¹ in the topsoil to 269.2 ± 18.5 g kg⁻¹ in the subsoil and for PNP these values ranged between 31.5 ± 18.4 and 66.5 ± 15.3 g kg⁻¹. Metal:C_p ratios indicate SOM-complex or allophane formation. This ratio was higher in deep horizons (>30 cm) these values averaged 0.20. In SPT, the ratio was 0.13 in the topsoil and increased to 0.16 in the subsoil (Table 1).

Site	Soil horizons (cm)	Soil pH _{water} ^a	Bulk density Mg m ⁻³	Al_p^{b} (g kg ⁻¹ soil)	Fe _p	C _p	Al _o	Fe _o	Si _o	Allophane ^c	Metal:C _p ratio ^d
SPT	A (0-30)	4.5 ± 0.01	0.43 ± 0.01	14.4 ± 1.7	15.9±0.3	74.4±12.5	21.5 ± 3.5	33.3±2.7	3.4 ± 0.1	26.6 ± 7.0	0.13
	B ₁ (30-60)	5.4±0.20	0.42 ± 0.01	12.1±0.3	13.8±0.5	45.6±7.9	59.1±13.2	50.9±3.1	16.2±0.5	188.1 ± 50.6	0.19
	B ₂ (60-100)	5.7±0.10	0.34 ± 0.01	6.2±0.3	1.9±0.5	25.2±2.5	74.5 ± 5.7	$33.9{\pm}14.3$	24.4 ± 0.3	269.2 ± 18.5	0.12
PNP	A (0-30)	5.3±0.10	0.41 ± 0.03	9.1±4.2	8.1±1.3	39.4±13.0	16.5 ± 8.2	20.2±3.2	2.9±0.1	31.5 ± 18.4	0.14
	AC (30-60)	5.7±0.30	0.57 ± 0.03	5.6±3.3	3.7±3.1	13.7±6.2	19.9 ± 5.5	17.6±1.5	5.7 ± 0.2	58.2±37.0	0.22
	C (60-100)	5.8±0.20	0.63±0.10	2.9±1.3	1.3±0.9	8.9±3.9	19.3±4.0	16.6±1.4	6,2±0.1	66.5±15.3	0.17

Table 1 Soil chemical properties. Data represent the mean±standard deviation (n=2) of two replicates field samples of two pristine rain forest soils (SPT = San Pablo de Tregua and PNP = Puyehue National Park). Each data point is the average of duplicates.

^a Soil:water ratio 1:2.5

^b Subscript 'p' means Na-pyrophosphate extraction and subscript 'o', means oxalate extraction (for further description see text)
^cAllophane content was calculated indirectly using the equation of Parfitt and Wilson (1985), modified by Mizota and Van Reeuwijk (1989)
^d(Al_p+Fe_p):C_p ratio

3.3.2 Soil C content and ¹⁴C activity

The soil C content in SPT varied between 68.6 ± 4.2 g kg⁻¹ in the subsoil up to 200.7 ±8.1 g kg⁻¹ in the topsoil (Table 2). Soil C in PNP was threefold lower than soil C found in SPT, varying between 28.4 ± 1.6 and 59.8 ± 13.4 g kg⁻¹ in the subsoil and topsoil (Table 2). Previous analysis of some soil samples studied here indicated that the inorganic C as carbonates was negligible (<0.01 g kg⁻¹). Table 2 also shows the ¹⁴C activity and the corresponding radiocarbon ages for both soils. In general, a decrease of ¹⁴C activity with increasing soil depth was observed. The ¹⁴C values in SPT ranged between 109.0 pMC in the topsoil to 55.6 pMC in the subsoil. For PNP, these values varied between 101.7 to 93.5 pMC. The radiocarbon ages of all horizons in SPT were between modern and 4,720 years BP while in PNP the ¹⁴C ages ranged between modern and 545 years BP.

Table 2 Soil C, ¹⁴C activity and ¹⁴C age of the bulk soil samples. Data represent the mean \pm standard deviation (n=2) of two replicates field for samples (except ¹⁴C activity) of two pristine rain forest soils (SPT = San Pablo de Tregua and PNP = Puyehue National Park). Each data point is the average of duplicates.

Site	Soil horizons (cm)	Soil C (g kg ⁻¹ soil)	¹⁴ C activity (pMC) ^a	¹⁴ C age (vears BP) ^b	Cp/Soil C
			u - /	() the second se	
SPT	A (0-30)	200.7±8.1	109.0	Modern	0.37
	B ₁ (30-60)	92.5±3.6	85.1	1300	0.49
	B ₂ (60-100)	68.6±4.2	55.6	4720	0.37
PNP	A (0-30)	59.8±13.4	101.7	Modern	0.66
	AC (30-60)	38.2 ± 1.2	98.3	135	0.36
	C (60-100)	$28.4{\pm}1.6$	93.5	545	0.31

^apMC= percent modern C

^byears BP= years before present

Soil C stock was 464 ± 20 in SPT and 210 ± 37 Mg ha⁻¹ in PNP (Fig. 1). The relative proportion of the soil C stock stored in B₁ and B₂ horizons for SPT was 25% and 20%, respectively, while the topsoil comprised 55% of total C. For PNP, these proportions were 35%, 31% and 34% for A, AC and C horizons, respectively. The relative contribution C_p complexed with metals to the soil C, ranged from 0.37, i.e. 37% in the subsoil to 0.49, i.e. 49% in the topsoil for SPT and between 0.31 and 0.66, i.e. 31% and 66 % for top- and subsoil at PNP, respectively (Table 2).



Figure 1. Soil C-stock. Data represent the means and standard deviation of two replicates field samples of two pristine rain forest soils (SPT = San Pablo de Tregua and PNP = Puyehue National Park). Each data point is the average of duplicates.

3.3.4 Chemical composition of SOM analyzed by solid state ¹³C NMR spectroscopy

The chemical composition of SOM was determined by solid state ¹³C NMR spectroscopy for both sites in all three soil depths (Fig. 2 and Table 3). The spectra of the topsoil showed peaks mainly at 32, 73 and 172 ppm that correspond to alkyl-C, *O*-alkyl-C and carboxyl-C, respectively. The signal intensity peaks in the alkyl C and *O*-alkyl C region of the spectra are most likely derived from lipids, polysaccharides and proteins. The peak 172 ppm in the carboxyl C region (Fig. 2 and Table 3) may be derived from carboxylic and amides groups. Spectra from B₁ and AC horizons (30-60 cm) were similar to those of the topsoil (0-30 cm). In deep horizons (60-100 cm) of both soils, slightly lower signal intensity from polysaccharides was observed. The chemical composition of bulk SOM of the horizons of both soils was similar.



Figure 2. Solid state ¹³C NMR spectra of SOM bulk samples at different soil depths of two pristine rain forest soils (SPT = San Pablo de Tregua and PNP = Puyehue National Park).

uni iorest.						
Site	Horizons	Carboxyl C	Aryl C	<i>O</i> -Alkyl C	Alkyl C	
	(cm)					
SPT	A (0-30)	8.9	18.6	49.7	22.8	
	B ₁ (30-60)	8.2	12.4	50.5	28.9	
	B ₂ (60-100)	8.6	12.4	38.8	40.2	
PNP	A 0-30)	8.9	16.5	47.8	27.0	
	AC (30-60)	6.4	13.6	44.6	35.3	
	C 60-100)	8.6	15.2	42.8	33.6	

Table 3 ¹³C-CPMAS NMR for chemical shifts^a assignments in HF treated samples (% of total integrated region) of San Pablo de Tregua (SPT) and Puyehue National Park (PNP) rain forest.

^aCarboxyl (160-220 ppm), Aryl C (110-160 ppm), *O*-alkyl C (45-110 ppm) and Alkyl C (-10-45 ppm).

3.3.5 Relationship amongst the soil properties

We found a significant and positive relationship between C_p and soil C as well as between C_p and Al_p , and between C_p and Fe_p (Fig. 3). However, the same relationship with soil pH was negative in the range pH 4.5-6.0 (Fig. 4a). Significant positive regressions for both soils were recorded between pH and allophane content (Fig. 4b) while inverse relationships were obtained for Al_p : Al_o and pH (Fig. 4c). Finally, we found an inverse relationship between the ¹⁴C activity and the allophane content for the two soils (Fig. 5), although in PNP a trend was observed only.

3.4 Discussion

3.4.1 Soil C stock and age

On a global average, Andisols contain 30.6 kg m⁻² C down to 100 cm depth (Eswaran et al., 1993) which is low in comparison to SPT (46 kg m⁻²) but much higher compared to PNP (21 kg m⁻²). In the present study, the higher SOM stocks at SPT compared to PNP may be mainly related to the finer texture and greater soil age at this site (Table 2). The greater soil age at SPT may have led to a soil high in silt and clay, Fe_p, Al_p, C_p, Si_o and allophane content (Table 1) as also found by Lilienfein et al. (2003). The ¹⁴Cage of SOM stored in subsoils, as an indicator for soil age, ranged between 545 to 4,720 years BP (Table 2), similar to the values recorded by Rivas et al. (2012) for volcanic soils of the same region in the Andean mountain. Many authors have studied the C accumulation in volcanic soil chronosequences (e.g. Lilienfein et al., 2003; Mikutta et al., 2009; Peña-Ramírez et al., 2009). The highest C accumulation rate was noted in young volcanic soils from southern Italy (Egli et al. 2008) with an age <2,000 years, which increased to a maximum after 15,000 years. Lilienfein et al. (2003) working in a chronosequence in California found an increase in the rates of allophane formation and organic matter accretion with time. The soil C accretion rates were highest in initial stages of soil development simultaneously with allophane accumulation and ceased after 600 years. The allophane concentrations increased to a maximum rate of 0.14 g kg⁻¹ year⁻¹ and were highest in the oldest soils.



Figure 3. Relationship between C_p and (a) soil C, (b) Al_p and (c) Fe_p . Data represent the means and standard deviation of two replicates field samples of two pristine rain forest soils (SPT = San Pablo de Tregua and PNP = Puyehue National Park). Each data point is the average of duplicates.



Figure 4. Relationship between soil pH and (a) C_p , (b) allophane, (c) $Al_p:Al_o$ ratio and (d) allophane:SOM ratio. Data represent the means and standard deviation of two replicates field samples of two pristine rain forest soils (SPT = San Pablo de Tregua and PNP = Puyehue National Park). Each data point is the average of duplicates.

Our data showed that in SPT the highest C stocks occurred in topsoil (55%), whereas at PNP, the subsoil comprised the highest C stock. These data suggest, that in young volcanic soils from southern central Chile, C accumulation occurred similarly in topand subsoil horizons at earlier stages of soil development and that topsoils are more prone to C accumulation in later stages of soil development.



Figure 5. Relationship between allophane and 14 C activity of two pristine rain forest soils (SPT = San Pablo de Tregua and PNP = Puyehue National Park).

3.4.2 Solid state CPMAS ¹³C NMR

SOM composition as determined by solid state ¹³C NMR spectroscopy was similar at both sites with few differences between top- and subsoil (Fig. 2, Table 3). The higher contribution of alkyl-C in subsoil horizons is most probably assigned to aliphatic compounds due to the high amounts of Al that may lead to preservation of aliphatic compounds (Tonneijck et al., 2010). In general our solid-state ¹³C NMR spectra were fairly similar for both soils and all depths (Table 3). This contrast to a study by Rumpel et al. (2012) who applied analytical pyrolysis to density fractions from top- and subsoil horizons of Andisols. The authors reported clear differences between organic matter composition from top- and subsoil horizons in Andisols under forest on the Island of Tenerife. The contrasting results may be related to the two different methods. Solid state ¹³C NMR spectroscopy gives an overview over the chemical composition of the whole untreated sample (Kögel-Knabner, 1997), whereas pyrolysis detects specific compounds depending on the applied pyrolysis conditions (Dignac et al., 2005). Moreover, both methods could yield biased results, when organic matter is in close interaction with the mineral phase. Pyrolysis results could be biased by retention of molecules by the mineral phase (Zegouagh et al., 2004), whereas NMR results may be biased due to C loss after the HF pretreatment (Gelinas et al., 2001). C-loss after HF treatment was around 50% in SPT and between 14 and 21% in PNP. However, studies with temperate forest soils did not indicate strong differences in chemical SOM composition between HF-treated and untreated samples even with a 90% C-loss (Eusterhues et al., 2007). Although bias due to methological issues cannot be excluded, we suggest, that the SOM in the two soils may have a rather similar composition despite their contrasting age and different content of C stabilising mineral compounds.

3.4.3 Relationship between C and mineral parameters

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). Al- and Fe-SOM complexes are typically defined as the Al and Fe fraction extracted by Na-pyrophosphate (McKeague, 1967). Bardy et al. (2007) demonstrated that Na-pyrophosphate successfully removed the Al linked to the organic matter in tropical Podzols of Amazonia.

The atomic metal: C_p ratio of 0.12 has been defined as the limit to judge whether a soil tends to the formation of allophane or SOM-complex (Higashi et al., 1981; Panichini et al., 2012). A value > 0.12 implies that SOM-complexes are saturated with metals (Al and Fe) leaving the extra Al (which interacts with Si) for allophone type materials formation.
Similar theoretical values can be calculated from the model of Nanzyo (2002). Our results indicated a metal: C_p ratios of 0.13-0.14 for the topsoil with low pH and 0.16-0.20 in deep soils with high pH (Table 1). Origin and type of SOM as well as pH influence the complexing ability of Al and Fe in different horizons, making metal: C_p ratio an indicator somehow variable (Dahlgren and Ugolini, 1991). However, for any given horizon, a ratio may exist which corresponds to the saturation of the humic complex with metals (Higashi et al., 1981).

The inverse relationship between soil pH and C_p supported the hypothesis that the metal-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, low pH <6 may have caused a release of Al into soil solution favoring the formation of Al-SOM complexes and their dominance. In contrast pH >6 it could favor the allophane polymerization and allophane-SOM sorption (Fig. 4). 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).

The present results indicate, that C stabilisation in Andisols need to be explained by combining several factors, which evolve during pedogenesis. The binary composition theory dictates that the formation of allophane-imogolite dominates at pH >5 ($Al_p:Al_o < 0.5$) whereas SOM-complexes dominate at pH <5 ($Al_p:Al_o > 0.5$). Several studies use $Al_p:Al_o$ indicators (e.g. López-Ulloa et al., 2005). However, two volcanic soils displaying similar pH may present different $Al_p:Al_o$ ratio as shown in Fig. 4c while they display similar C_p (Fig. 4a) making this C pool a more reliable indicator for SOM complex formation since it accounts for SOM bound to Al and Fe. Nonetheless, as previously discussed, the

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dominance of SOM-complex is strongly influenced by soil pH and on elemental ratios in soil solution. SOM competes with Si for metals (e.g. Al and Fe) in the soil solution, favouring the formation of hydrous-Al silicates by polymerisation and SOM stabilisation by sorption with metals. Our data supported the following interpretation. At pH <6, Fe and Al hydroxide may form complexes with SOM, reducing the rate of allophane-SOM formation and increasing C_p in response to a metal: C_p ratio <0.12 (SOM-complex non-saturated with metals). At pH >6 any weathered Al may react with silica gel to form allophane to promote the allophane-SOM formation in response to a metal: C_p ratio >0.12 (SOM-complex saturated with metals).

The metal:C_p ratio may also be affected by the soil age. The positive and strong relationship between ¹⁴C-based SOM age and allophane content suggests that C stabilisation could be controlled by the presence of allophane (Matus et al., 2008). Horizons with young SOM showing more acidic pH in the topsoil presented low allophane content with lower metal:C_p ratio at pH <6, while the opposite was true for deep soil horizons with old SOM. Moreover, from Table 1 it can be noted that both Al_p and Fe_p as much as C_p declined in deep soil profile at both sites, while allophane increased (Fig. 4d), supporting the hypothesis that allophane may plays a key role in SOM stabilisation in deep (Matus et al., 2008) and oldest soils, increasing their storage capacity (Mikutta et al., 2009). However, in young soils where the allophane content is low, the C stabilisation is controlled mainly by the presence of Al and Fe in the soil through metal-SOM complexes. These results are in accordance with Lilienfein et al. (2003), who found that in oldest soils, the allophane concentration was higher in comparison with the youngest soils.

3.5 Conclusions

We examined the C storage as well as the stabilisation mechanisms in two pristine temperate rain forest Andisols with contrasting radiocarbon age. Our data suggest that allophane formation at older stages of soil development leads to SOM stabilisation. In younger soils and at lower pH, metal-SOM complex formation may be more important. Contrasting stabilisation agents do not influence SOM composition. C stock distribution within the soil profile changes with soil age, with higher contribution of subsoil C to C stocks in younger Andisols. We suggest that the combination of several factors (Al, Fe and C extracted in Na- pyrophosphate, metal:C_p ratio and pH of the soil) which change through pedogenesis control the SOM stabilisation.

CHAPTER IV

Potential mineralisation and priming of carbon stored in top- and subsoil horizons of two Andisols under temperate old-growth rain forest

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4.1 Introduction

In view of future climate change and ecosystem disturbance, controls of soil organic carbon (SOC) turnover must be clearly identified, because any change in the soil carbon balance may affect global climate (Davidson and Janssens, 2006). The dynamics of the stable SOC pool with residence times of more than centuries is of particular interest. Stable SOC cannot be isolated by a physical or chemical separation method (Falloon et al., 1998). It may be present at high concentrations in bare fallows (Rühlmann et al, 1999) and subsoil horizons (Rumpel and Kögel-Knabner, 2011). Moreover, SOC in volcanic soils may be more stable than in any other soil type due to strong interactions with the mineral phase (Zunino et al., 1982; Parfitt et al., 2002). Longterm SOC stabilisation in volcanic soils may have several reasons related mainly to their specific mineral composition, in particular their high content of short-range order minerals. A recent study suggested minimal loss of SOC from those soils under climate warming scenarios (Rasmussen et al., 2006). Apart from increasing temperature, SOC mineralisation in volcanic soils was shown to be affected by changes in pH and to a lesser extent by addition of mineral nutrients (Miyazawa et al., 2013). Mineralisation increase was shown to be stronger in topsoil compared to subsoil related most probably to shortage of easily decomposable compounds (Miyazawa et al., 2013). The presence of such compounds could stimulate the mineralisation of otherwise stable SOC by the so-called priming effect (PE), defined as change in the mineralisation rate of SOC in the presence of fresh organic matter (Jenkinson, 1971). Indeed, while priming was shown to accelerate the mineralisation of chemically recalcitrant organic matter compounds, such as black carbon (Hamer et al., 2004; Nocentini et al., 2010) and subsoil SOC, stabilised by interactions with the mineral phase (Fontaine et al., 2007), it seems that stable SOC is as vulnerable to

priming as more labile C (Guenet et al., 2012). However, the mineral assemblage of soil was shown to strongly influence SOC mineralisation (Rasmussen et al., 2006) and could thus also lead to contrasting priming effects in different soil types (Sinyakina and Kuzyakov, 2002).

In this study, we investigated potential SOC mineralisation and priming in volcanic soils, where SOC has previously been shown be stabilised by humus complexes as well as adsorption to allophane minerals (Neculman et al., 2013). As SOC dynamics in top- and subsoil horizons has different controls (Salomé et al., 2010) and the agents controlling SOC stabilisation through mineral interactions in Andisols were shown to be different in different parts of the profile and to change with soil age (Torn et al., 1997; Garrido and Matus, 2012; Panichini et al., 2012; Neculman et al., 2013), we studied all horizons from two soil profiles of contrasting age.

We hypothesized that SOC in these two soils under temperate old-growth rain forest would be highly stable due to strong mineral interactions and that it may therefore not be possible to release SOC upon priming. We further hypothesized that different stabilisation agents in the two soils would induce contrasting potential SOC mineralisation and contrasting response to priming.

The aims of the present study were to evaluate (1) potential SOC mineralisation, (2) to compare it to its 14 C activity as an indicator for its stability *in situ* and (3) to evaluate possible priming effects after addition of 13 C-labelled cellulose in two Andisols under temperate old-growth rain forest of southern Chile.

4.2 Materials and methods

4.2.1 Study sites

The study sites were previously described by Neculman et al. (2013) and correspond to two pristine temperate old-growth rainforests in southern Chile located in San Pablo de Tregüa (SPT), Panguipulli (39° 35' Latitude South and 72° 07' Longitude West, at 720 m above sea level) and Puyehue National Park (PNP), Antillanca (40° 47' Latitude South S and Longitude West 72° 12' S, at 800 m above sea level). The ecosystems have developed under a temperate humid climate (annual average temperature 5 °C and precipitation >5,000 mm) (Oyarzún et al., 2004; 2011). The vegetation at SPT consists of a native evergreen pristine *Nothofagus dombeyi* (Mirb.) Oerst., forest in mixture with *Laureliopsis philippiana* Looser and *Saxegothaea conspicua* Lindl (Oyarzún et al., 2011). At PNP, the dominant vegetation is pure evergreen old *Nothofagus betuloides* (Mirb.) Oerst., forest (Oyarzún et al., 2004). The soils of both sites are classified as Andisols (Soil Survey Staff, 2008) derived from basaltic coloured rock. At SPT, the soils are older than at PNP and have a silty to sandy-loam texture throughout the soil profile while at PNP, texture is sandy loam to coarse sand across the soil profile. The topography at SPT is undulating, while at PNP the soils occur in the plane. A litter layer of variable thickness (5 and 10 cm) was found at both study sites.

4.2.2 Soil sampling

At each site, two 10 x 10 m permanent plots were selected and a soil pit was excavated in the center of each plot. Sampling was conducted using a steal stainless cylinder (8 cm diameter x 5 cm length), which was pushed into the soil profile wall at 0-30, 30-60 and 60-100 cm, corresponding to A, B_1 and B_2 horizons for SPT and A, AC and C horizons for PNP, respectively. Two replicates per horizon in each soil pit at both sites, in total 12 soil samples were collected and they were transported soon after sampling to the laboratory in an appropriate container. Visible leaves, roots and organic debris were removed from moist soil and the cleaned soil samples were passed through a 2 mm sieve, air dried and stored at 5 °C before incubation. The C content, isotopic composition as well as the radiocarbon age of the soils and other soil characteristics such as allophane content and the amorphous Al and Fe oxides associated-C (C_p) are presented in Table 1.

4.2.3 Incubation experiment

The experimental units consisted of 500 ml flasks. We put 10 g (dry weight) of soil in these flasks and adjusted them to a water potential of pF 3 by adding distilled water until the soils moisture content corresponded to 60% of their water holding capacity. All flasks were preincubated for 14 days at 22°C. After the preincubation, 1 g ¹³C-labelled cellulose (extracted from uniformly ¹³C labelled wheat, δ^{13} C=1.860 ‰) per kg dry soil was added to half of the flasks and mixed with the soil (soil with cellulose). Non-amended soil (control soils) was also mixed to apply the same physical disturbance. Two nutrient solutions (NH₄NO₃, KH₂PO₄) were added to the cellulose treatments to give final C-cellulose:N and C- cellulose:P ratio of 15:1 and 80:1 in order to avoid limitation of cellulolytic micro-organisms (e.g., Hodge et al., 2000). The SOC mineralisation and δ^{13} C of mineralised CO₂ were monitored by sampling the headspace of the flasks at days 1, 3, 14, 21, 35, 49, 63 and 80. At the beginning of the experiment and after each measurement, the flasks were flushed with CO₂-free air. We considered 24 samples, one per depth, per plot (i.e. two field replicates) and per site. Soil moisture was maintained throughout the experiment by adding deionised water if necessary just after CO₂ measurements.

Site	Soil horizons	Soil Texture ¹	Allophane ²	Soil C	Soil pH	¹⁴ C activity	¹⁴ C age	$\delta^{13}C$	C _p
	(cm)	(g kg soil ⁻¹)	(g kg soil ⁻¹)	(g kg soil ⁻¹)		$(pMC)^3$	(years BP) ⁴	‰	(g kg ⁻¹ soil)
SPT	A (0-30)	513	27±7	200.7±8.1	4.5±0.01	109.0	Modern	27.4	74.4±12.5
	AC (30-60)	550	188±51	92.5±3.6	5.4±0.20	85.1	1300	25.7	45.6±7.9
	C (60-100)	349	269±19	68.6±4.2	5.7±0.10	55.6	4720	25.9	25.2±2.5
PNP	A (0-30)	402	32±18	59.8±13.4	5.3±0.10	101.7	Modern	26.0	39.4±13.0
	B ₁ (30-60)	166	58±37	38.2±1.2	5.7±0.30	98.3	135	25.5	13.7±6.2
	B ₂ (60-100)	221	67±15	28.4±1.6	5.8±0.20	93.5	545	25.6	8.9±3.9

Table 1 Elemental analysis, stable isotope composition and ¹⁴C activity of the bulk soil samples. Data are presented as mean±standard deviation (n=3) (From Neculman et al., 2013). SPT= San Pablo de Tregüa, Panguipulli and PNP=Puyehue National Park, Antillanca, Southorn Chilo

¹Clay and silt (CIREN, 2001). ²Calculated as in Parfitt and Wilson (1985), modified by Mizota and Van Reeuwijk (1989). ³pMC= percent modern C. ⁴years BP= years before present.

4.2.4 Measurement of CO_2 concentration, $\delta^{13}C$ and total carbon mineralisation

The CO₂ concentration (μ mol CO₂ mol⁻¹ air), thereafter μ mol mol⁻¹, was measured with a MICROGC (Agilent, Santa Clara,USA) and carbon isotope ratios of CO₂ were measured by using a gas chromatograph 5890 GC (Hewlett-Packard, Palo Alto, California, USA) equipped with a 3-meter full column filled with a Prorapak QS stationary phase (80-100 mesh) and coupled to an isotope ratio mass spectrometer (Isochrom III, Micromass-GVI Optima). The proportion of cumulative C mineralisation after 80 days of incubation of the control and amended ¹³C-labelled cellulose soils was fitted to a double exponential model (Thuriès et al., 2000):

$$y = y_{\max 1} (1 - e^{-k_1 t}) + y_{\max 2} (1 - e^{-k_2 t})$$
(1)

where: y is the total amount of CO_2 in the flask's atmosphere (sample or control) in mg C-CO₂ kg⁻¹ dried soil, y_{max 1} is the potential amount of C-CO₂ y₁ evolved from the labile C pool, y_{max} ₂ is the potential C-CO₂ y₂ evolved from the stabilised C pool, k₁ and k₂ are the rate constants of labile and stabilised C pool, respectively and t is the time in days.

4.2.5 Quantification of the priming effect

The PE induced by cellulose was calculated by comparing the amount of 12 C-CO₂ in samples with cellulose to the amount of 12 C-CO₂ in the control treatments. The PE intensity was calculated according to Guenet et al. (2010) using the following equation:

$$PE = \alpha x Q_{sample} - Q_{control}$$
⁽²⁾

where Q is the amount of CO_2 in the flask's headspace (sample or control) in mg C-CO₂ kg⁻¹ dried soil, and

$$\alpha = (A_{substrate} - A_{sample})/(A_{substrate} - A_{control})$$
(3)

where $A_{substrate}$, A_{sample} and $A_{control}$ represent the isotopic abundance of cellulose of CO_2 in the headspace of the sample and of CO_2 in the headspace of the control.

The isotopic abundances were calculated from the $\delta^{13}\text{C-CO}_2$ with the following equation:

$$A = IR/(1 + IR)$$
(4)

where IR is the isotopic ratio ${}^{13}C/{}^{12}C$, calculated as:

¹³C/¹²C =
$$[(\delta^{13}C - CO_2/1000) + 1] \times 0.0112372$$
 (5)

and 0.0112372 is the IR of international standard.

4.2.6 Data analysis

We used the Mann and Whitney test to analyze for significant differences (p<0.05) of cumulative SOC mineralisation after 80 days of incubation from the different treatments. Relationships between data sets were explored by regression analyses. All statistical analyses were performed using the Excel software.

4.3 Results

4.3.1 Carbon mineralisation rates

Cumulative C-CO₂ mineralisation showed a common pattern for both sites in all depths (Fig. 1 and 2). A plateau was reached between 60 and 80 days of incubation, although the cumulative mineralisation of CO₂ in general changed its pattern after 20 days in the top soils and later in subsoil horizons. After 80 days of incubation, the total amount of SOC mineralised ranged between 151 ± 3.3 and 3026 ± 66.8 mg C kg⁻¹ soil (Table 2). The total SOC mineralised from subsoil was lower compared to SOC mineralised from topsoil and in a similar range for both sites. It increased towards the soil surface. For topsoil of SPT, the cumulative amount of 2906.8 \pm 60.5 mg CO₂-C kg⁻¹ after 80 days was much higher than for topsoil of PNP (Fig 1, 2 and Table 2). At the end of the experiment, between 0.51 and 1.45% of SOC present in the two soils was mineralised. Higher proportions of SOC were mineralised from topsoil horizons as compared to subsoils and these differences were more pronounced for SPT than PNP (Table 2). Comparing the proportion of SOC mineralised in soil horizons at the two sites, most SOC was mineralised from subsoil horizons of PNP and topsoil of SPT. Cellulose amendment increased carbon mineralisation in all soil horizons, except for topsoil of SPT.



Figure 1 Cumulative carbon mineralisation over 80 days of *in vitro* incubation at 22 °C of a pristine temperate old-growth rain forest of San Pablo de Tregua (SPT) ¹³C-labelled cellulose amended and non-amended Andisols at different depths: (a) A(0-30 cm), (b)AC (30-60 cm and (c) C(60-100 cm).



Figure 2 Cumulative carbon mineralisation over 80 days of *in vitro* incubation at 22 °C of a pristine temperate old-growth rain forest of Puyehue National park (PNP) ¹³C-labelled cellulose amended and non-amended Andisols at different depths: (a) A(0-30 cm), (b)AC (30-60 cm and (c) C(60-100 cm).

Following cellulose and nutrient amendment, there was a PE for both soils after 80 days of incubation (Table 2). After 80 days of incubation, the addition of cellulose and nutrients had induced a negative PE in the A horizons of both sites. In general the priming intensity was greater for topsoil sampled at SPT compared to topsoil sampled at PNP. Negative priming was also observed for the 30-60 cm horizon from PNP, whereas we observed positive PE for all subsoil horizons sampled at SPT and the deepest horizon of PNP.

Table 2 Cumulative C mi labelled ¹³ C cellulose ove Tregüa, Panguipulli and P	neralisation and net prin r 80 days of in vitro mi NP=Puyehue National l	ning effect in two neralisation at 22 Park, Antillanca, S	Andisols a °C. SPT= S outhern Ch	mended with San Pablo de iile.	1 ?
		· · ·			
	Control	Soil + c	ellulose	Priming	

		Control		Soil + cellulos	e Priming
		(mg CO ₂ - C kg ⁻¹ soil)	% of soil C	(mg CO ₂ - 0	C kg ⁻¹ soil)
SPT	(A)0-30	2906.8 ± 60.5	1.45	3025.9 ± 66.8	-315.7 ± 61.2
	(AC) 30-60	341.7 ± 19.2	0.37	794.5 ± 21.1	50.1 ± 12.6
	(C)60-100	100.9 ± 3.1	0.15	346.6 ± 18.9	59.0 ± 3.1
PNP	(A)0-30	734.2 ± 13.8	1.26	1017.2 ± 5.0	-100.9 ± 10.4
	(B1) 30-60	324.4 ± 7.3	0.85	689.6 ± 18.2	-17.8 ± 2.3
	(B2) 60-100	151.1 ± 3.3	0.53	532.8 ± 52.7	62.3 ± 11.4

4.4 Discussion

4.4.1 Factors explaining potential C mineralisation in top and subsoil horizons of two Andisols of different age

The potential SOC mineralisation presented strong differences related to sampling depths. It was highest in surface soil horizons and gradually decreasing with depth at both sites. Despite the strong difference in the amount of SOC mineralised especially from topsoil samples, the proportion of SOC mineralised was in a similar range of both soils. Salomé et al. (2010) observed much higher SOC mineralisation (in % of total SOC mineralised) in subsoil samples for a similar incubation setup. Disturbance through sieving was found by these authors to strongly increase the SOC mineralisation from subsoil samples leading to similar amounts of SOC mineralised from top and subsoil samples of a Eutric Cambisol (Salomé et al., 2010). This was explained by physical separation of microbes and substrate (Xiang et al., 2008), leading to long residence times of subsoil OC *in situ*. In our case, it was interesting to note that SOC mineralisation expressed as % of initial SOC was still much lower in subsoil horizons compared to the topsoil despite disturbance through sieving. These data indicate that in Andisols in contrast to physical solution and contrast to physical solution of soils (Moni et al., 2010), SOC stabilisation controls may not be strongly related to physical inaccessibility.

Comparing the two Andisols of different age, the oldest soil SPT presented the greatest SOC mineralisation in topsoil only. In fact, there was a close relationship between the ¹⁴C activity of SOC and its potential mineralisation in the laboratory incubation (Fig. 3). This relationship indicates lower SOC mineralisation in samples with low ¹⁴C activity,

which thus has long mean residence times. Our results support the above statement about the importance of other than physical protection controlling SOC stabilisation. The pH may be important, as in Andisols a negative correlation between SOC content and pH was noted (Miyazawa et al., 2013) and Al toxicity is often cited as a factor explaining SOC accumulation in Andisols (Tonneijck et al., 2010). However, despite the negative correlation between bulk SOC content and pH in our soils, we could not establish any correlation between pH and potential SOC mineralisation. In a previous study, we could relate increasing stabilisation of SOC with increasing soil age to the amount of allophane in the samples (Neculman et al., 2013). In this study, the amount of allophane was correlated negatively with SOC mineralisation, whereas no relationship was observed with the amount of Al-humus complexes. Therefore, we suggest that with increasing soil age, the formation of allophane related to SOM content and pH could lead to stronger protection of SOC from mineralisation than Fe/Al humus complexes.



Figure 3. Relationship between C mineralised after 80 days and ¹⁴C activity

4.4.2 Priming following cellulose and nutrient addition

In general, positive PE was observed for subsoil horizons, while topsoil horizons showed a negative PE. These results are contrasting to the ones obtained by Salomé et al. (2010) for a Eutric Cambisol and also to our initial hypothesis. In some cases it may thus be possible to overcome strong SOC stabilisation by input of energy rich material particularly in subsoil horizons. Our results further suggest that fresh organic matter input into topsoil leads to increase of carbon storage. This is in agreement with observations on SOC stocks, which showed that in these young volcanic soils, topsoils are more prone to SOC accumulation than subsoils (Neculman et al., 2013). In the case of a negative PE, SOC decomposition might not be limited by nutrients or energy or the energy added is not sufficient to induce SOC degradation (Jenkinson, 1971). Negative priming in topsoil was also observed by Guenet et al., (2010) for bare fallow sites containing high concentrations of stable SOC. They explained negative priming by preferential substrate utilization, where soil microorganisms switch from poorly decomposable SOC to more easily degradable when fresh substrate is added (Sparling et al., 1982). Preferential substrate utilization may occur only if nutrient availability is sufficient (Cheng, 1999), as it may have been the case for Andisols due to addition of nutrients with easily decomposable cellulose. In our case the amount to SOC subjected to priming was related to soil pH, with priming becoming positive at pH 5.5 (Fig. 4). Soil pH was observed to strongly influence decomposition processes through its combined effect on enzyme activities (Sinsabaugh et al., 2008), microbial communities and Al toxicity (Walse et al., 1998). The regulatory role of pH for SOM turnover was recently confirmed by Leifeld et al. (2013), who stated that pH may have a stronger effect on the turnover of old stabilised SOC than on residue decomposition.

Our results suggest, that the pH effect on turnover of SOM is related to priming. At pH < 5, microorganisms switched their substrate utilization and no longer degraded SOC. At pH > 5.5, cellulose and nutrient addition to subsoil horizons induced a positive PE, indicating that microorganisms may be able to decompose stable SOC if they are provided with energy.



Figure 4. Relationship between amount of C subject to priming and soil pH

4.5 Conclusions

We investigated the potential SOC mineralisation and possible PE in Andisols under old growth forest in southern Chile using laboratory incubation and ¹³C-labelled cellulose addition to top- and subsoil horizons. Our results suggest a lower potential SOC mineralisation in subsoil compared to topsoil. The proportion of SOC mineralised from disturbed samples was in accordance with ¹⁴C activity, indicating that most likely interaction with the mineral phase is in these soils of greater importance than physical inaccessibility as SOC stabilisation mechanism. The positive priming effect induced by addition of fresh organic matter in subsoil samples shows that physico-chemical stabilisation may be overcome by energy addition. However, pH values > 5.5 are required for positive priming effect. At lower pH values, priming is negative and addition of labile substrates leads to SOC accumulation.

CHAPTER V

General discussion, concluding remarks and future directions

5.1 General discussion

Currently the studies of C stabilisation and destabilisation across the soil profile are still scarce for volcanic soils despite the fact that the C storage potential in Andisols is higher than in any other soil type. Studies concerned with SOM in Andisols have been carried out to investigate mainly processes of C stabilisation through association with Aland Fe- oxides together with amorphous clay minerals like allophane and imogolite type materials, which is one of the main mechanisms of the stability of soil organic C in allophanic soils. Under this scenario, the studies of C stabilisation/destabilisation in Andisols under temperate old-growth rain forest are a unique opportunity to develop a baseline of the patterns and processes in the biogeochemical C cycle.

In this thesis, we analyzed the contribution of allophane and metal–SOM complexes to soil C stabilisation in two Andisols under temperate rain forest of Southern Chile. Results supported the hypothesis that allophane plays a key role for SOM stabilisation in deep and oldest soil, while SOM stabilisation by metal (Al and Fe) complexation is more important in the surface horizons and in younger soils. The metal/C_p ratio (C_p extracted in Na-pyrophosphate), soil pH, and radiocarbon age seemed to be important indicators for formation of SOM–metal complexes or allophane in top- and subsoils of Andisols. However, the changes in main mineral stabilisation agents with soil age do not influence SOM composition. Finally, we suggested that the combination of several chemical parameters (Al_p, Fe_p and C_p, metal/C_p ratio, and pH) which change through soil age controls SOM stabilisation in these type of ecosystems. Recent investigations also have shown that fresh C substrate addition can induce a SOM destabilisation in the top and subsoil. In deep horizons, because old SOM may limits the biological activity, influencing directly the preservation of native C at depth.

In thesis, we hypothesized also that the mechanisms of C stabilisation in the subsoil may only be effective if fresh C is not available. We determined the influence of fresh added substrate (13 C-labelled cellulose) on soil microbial activity through the release of C-CO₂ under controlled conditions. Results showed a lower potential C mineralisation in subsoil of both Andisols compared to the surface soil in accordance with the increasing 14 C age at depths, indicating that most likely interaction with the mineral phase is in these soils of greater importance than physical inaccessibility as SOC stabilisation mechanism. Addition of cellulose and nutrients induced net negative priming in the topsoil, whereas priming of subsoil SOM remained positive after 80 days of incubation. The positive priming effect induced by addition of fresh organic matter in subsoil samples shows that physico-chemical stabilisation may be overcome by energy addition. However, pH values > 5.5 are required for positive priming effect. At lower pH values, priming is negative and addition of labile substrates leads to SOC accumulation.

5.2 Concluding remarks

Our results indicated the gap of knowledge of the mechanisms involved in C stabilisation in the top- and deep Andisols in the temperate old-growth rainforest ecosystems. The main conclusion was that the quantity, chemical composition and stabilisation processes of SOM in Andisols were different from those operating in non-andic soil types. Also, the C storage capacity of Andisols was found to be higher than those

of other soil types and most probably this is related to the Na-pyrophosphate extractable Al and Fe.

We showed that development of SOM stocks in deep soils depend on allophane type material formation in older development stages determining longer MRT of SOM. In contrast, the SOM stocks for top soils depend mainly of the presence of Al and Fe where metal-SOM complex formation dominates. Moreover, our results indicated that SOM stabilisation in temperate Andisols under pristine rainforest is influenced by addition of labile substrate. In topsoil, negative priming may lead to accretion of SOM, while the decomposition of subsoil SOM is likely to be enhanced by positive priming. These different directions could be directly related to different contribution of metal-SOM

5.3 Future directions outlined from this thesis

Results of this thesis support the hypothesis that the metal- SOM complexes and allophane are complementary processes and depend mainly on soil pH, hence, the competition between Al-SOM-complex and allophane type materials formation is the most important control amongst stabilisation processes in forest allophanic soils. Moreover, higher metal:C_p ratio in the subsoil might induce higher SOM saturation with Al and Fe than in the top soil, meaning both metals will not be able to stabilise more C and therefore, the Al and Fe in excess will be available to react with silica gel for the synthesis of allophane and imogolite type materials. However, we believe that is absolutely necessary further research in the laboratory to test this hypothesis.

The key aspects of the interactions between SOM and soil mineral phase in Andisols is to elaborate a conceptual model, which the metal: C_p ratio extracted in Na pyrophosphate, soil pH and allophane type materials evolving with time are important aspects to be considered for SOM stabilisation through soil profile of Andisols. Again laboratory experiments setup needs to be performed to validate the conceptual model.

The interaction between soil biology and SOM chemistry need to be studied in the field as well as in laboratory experiments. There are no papers that the priming effect has been studied under field conditions. Finally, the changes in SOM composition following changes of the mineral phase through time also need to be addressed in further studies, but across a large variety of soils on a regional scale.

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