

Description of the proposed work and aim of the visit

Carbon (C) sequestered in subsoil has gained increasing importance in recent years due to the high capacity of subsoil horizons to store soil organic matter (SOM) with mean residence time of hundreds to thousands of years (Rumpel and Kögel-Knabner, 2011). Results for acid Cambisols and Podzols (Eursterhues et al., 2005; Kleber et al., 2005) indicate that the C stabilization in the subsoil is mainly due to chemical adsorption on Aluminum and Iron. The chemical composition of C present in subsoil was determined only to a limited extent due to the low C concentrations encountered in the deep horizons of such soil types. In Andisols, the carbon storage potential is higher in any other soil type, due to their high content of short-range order minerals such as allophane, imogolite and ferrihydrite with considerable potential to form stable complexes with organic molecules (Shoji et al., 1993; Parfitt et al., 1997). Due to their high carbon content even in subsoils horizons, Andisols may be privileged models for chemical characterization of stabilized C at depth.

In Chile, approximately 50% of Andisols are located under old-growth forests. These ecosystems are important reserves of temperate forests in the world (Armesto et al., 1998), where the precipitation chemistry still reflects a close approximation of pre-industrial conditions (Boeckx et al., 2005). The productivity of these ecosystems depends on the internal cycling of SOM (Godoy et al., 2009) and their highly efficient mechanisms of a conservative nutrient cycling (Huygens et al 2008). Litter and woody materials (logs and branches) are the main source of organic matter and nutrients to the soil and watercourses in forested mountain areas systems (Vitousek et al., 1995; Schellegel and Donoso, 2007). In these systems, the dynamics of above and belowground litter is strongly influenced by several factors such as: tree species composition, tree density, basal area, age structure, altitude and seasonality (Liu et al., 2003). SOM stabilization may be more influenced by soil properties. Up to now factors influencing SOM dynamics and nutrient cycling in deep soil horizons of Andisols were largely ignored. Therefore, the focus of my investigation will be on the nature and dynamics of SOM in subsoils with contrasting texture. I sampled replicated soil profiles of (1) a well developed soil (silty loam) in San Pablo de Tregüa (39°38'S, 72°05'W) and (2) a sandy scoriaceous porous soil which occur in the dejections cones of the volcanoes in the Puyehue National Park (40° 47'S, 72°12'W). Soil samples were collected at three depths (0-20, 20-60 and 60-80 cm).

SOM stability in the top- and subsoil horizons of two Andisols has been assessed during laboratory incubation. For San Pablo de Tregüa soil, we could confirm our hypothesis of

lower C mineralization in subsoil horizons as compared to the topsoil. We assume that the reduced C respiration in the subsoil was due to greater stability of native SOM. However, we observed no significant differences between the C respiration at the surface and depth for the Puyehue soil. These results suggest that the labile organic matter pool in top and subsoil horizons is equally important possibly due to transfer of C from the topsoil or higher root growth, these differences could be related to a contrasting chemical composition of SOM at both sites.

The bulk characterization of SOM may be achieved by ^{13}C solid-state nuclear magnetic resonance spectroscopy, a method widely used in soil science (Kögel-Knabner, 1997). This method gives a good overview of the bulk chemical composition of a solid sample without prior extraction. Four chemical shift regions can be identified and related to labile and recalcitrant SOM compounds. The application of this method to Chilean soil samples allowed to characterize and compares the chemical composition of SOM stored in top- and subsoil horizons of two soils under similar vegetation but different texture and identified the chemical composition of refractory carbon in top- and subsoil horizons of Andisols. For these reasons, the second part of my work was to determine the chemical composition of C of these soils for identifying the chemical groups involved in the stabilization of SOM in the subsoil and compare their chemical composition with the C of the surface horizons.

Description of the main results obtained

Study site and sampling

1. Study area

The area corresponds to the Andean mountain range located in San Pablo de Tregua (SPT), Panguipulli (39° 38 'S, 72° 05' W) and the Puyehue National Park (PNP), Antillanca (40° 47 'S, 72 ° 12' W) with elevations ranging between 550 and 1,200 meters above sea level. The rainforests of southern Chile have developed under a temperate climate, with low temperatures and high humidity, where the woody vegetation produces a litter with a slow and incomplete decomposition in soil, resulting in an SOC accumulation in the surface (Donoso, 1992). In this mountain forest, the mean precipitation may reach more than 5,000 mm per year, with an average of annual temperature of 5°C (Rosales, 2000; Oyarzun et al., 2004). The vegetation in SPT is constituted by a native evergreen pristine *Nothofagus dombeyi* forest in combination with *Laureliopsis philippiana* and *Saxegothaea conspicua*. In adjacent area it

was a pasture of 50 years old that was eventually grazed with dairy cows. In PNP, the dominant vegetation is a pure evergreen old *Nothofagus betuloides* forest. The soils in both areas are an Andisols characterized mainly by abundant allophane (~50% of the mass of clay) and Al complexed with organic matter (Besoain et al., 1985). In SPT the topography is undulating, while in the PNP the soils are in the piedmont position with north aspect and undulating topography. In the two areas the soils presented a litter layer of variable thickness and thereafter a mineral soil reaching 60 cm deep. Deep layer were the C horizons with abundant coarse sand and volcanic gravel.

1.1 Soil sampling

Two sites were sampled, one in SPT and one in PNP. At each site three soil pits were excavated in a linear transect of about 100 m in SPT and 20 m in PNP. Sampling was conducted with a soil core (8 cm diameter x 5 cm length) in the mineral soil and consisted of a composed sample taken from each layer sampled at 0-20 cm corresponding to A horizons, 20-60 cm corresponding to AC horizons and 60-80 cm corresponding to C horizons. A total of 18 samples were collected. In addition, six samples were taken for the bulk density at 10, 40 and 70 cm does the first, second and third layer, respectively. Soil samples were transported as soon as possible to our lab in isolated container. Each soil sample was sieved to a 2 mm and plant debris and coarse organic materials were removed by hand. The general description of soil profiles are shown in Table 1.

2. ¹³C NMR spectroscopy

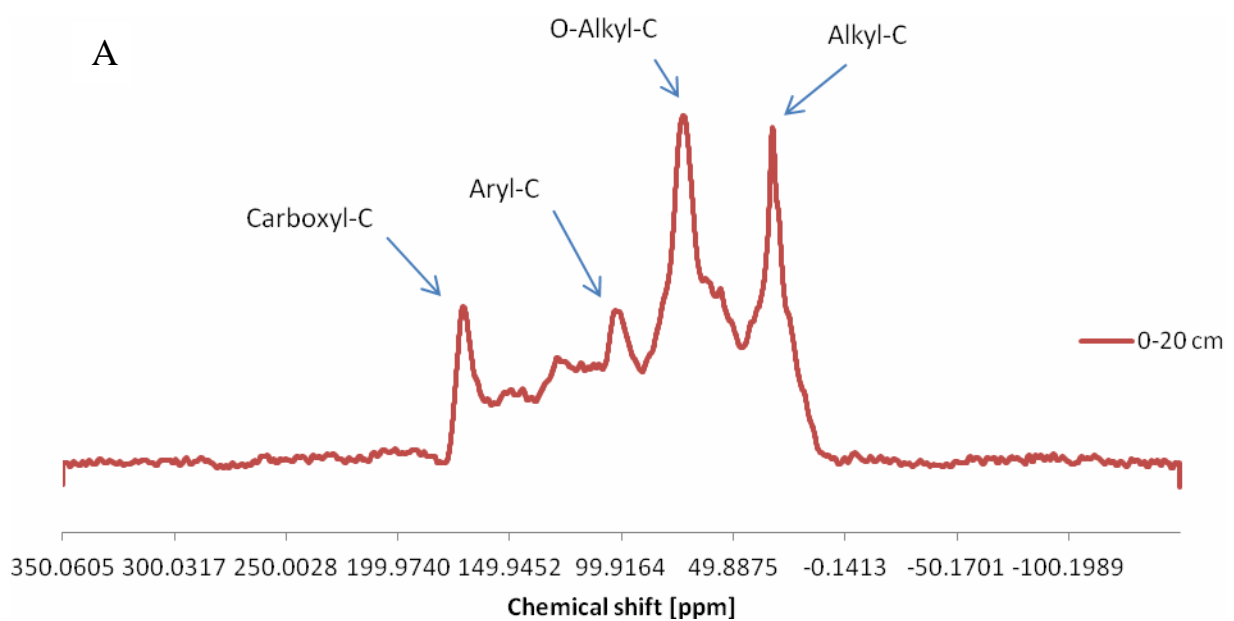
Solid-state ¹³C NMR spectra were obtained before and after oxidation by H₂O₂ with Bruker DSX 200 spectrometer (BioSpin GmbH, Karlsruhe, Germany). It was utilized a ¹³C-resonance frequency of 50.3 MHz with a spinning speed of 6.8 kHz. The chemical shifts were calibrated by tetramethylsilane (0 ppm). C groups were identified by integration of the signal intensity in their respective shift regions. The regions from 45 to 60 ppm and from 60 to 110 ppm were assigned to N-alkyl-C and O-alkyl respectively. Olefinic and aromatic C was detected in regions between 110 and 160 ppm. The region from 160 to 200 ppm was assigned to carbonyl (aldehyde and ketone) and carboxyl/amide C. Finally, resonances in th region -10 to 45 ppm corresponds to alkyl C.

Table 1. Soil characteristics (average of three pedons per site \pm standard error of the mean)

Site	Soil horizons (cm)	Bulk density (Mg m ⁻³)	Total OC (g kg ⁻¹ soil)	$\delta^{13}\text{C}$ (‰)	Total N (g kg ⁻¹ soil)	$\delta^{15}\text{N}$ (‰)	Soil pHwater	Al _p (g kg ⁻¹ soil)	Fe _p (g kg ⁻¹ soil)	C _p (g kg ⁻¹ soil)	Allophane (g kg ⁻¹ soil)
SPT	A (0-20)	0,38 \pm 0,06	200,7 \pm 8,09	-27,43	16,01 \pm 0,79	1,13	4,45 \pm 0,01	14,43 \pm 1,68	15,86 \pm 0,33	111,15 \pm 2,48	26,62 \pm 7,04
	AC (20-60)	0,39 \pm 0,04	92,48 \pm 3,61	-25,68	7,18 \pm 0,47	4,65	5,39 \pm 0,16	12,08 \pm 0,30	13,79 \pm 0,45	53,94 \pm 6,35	188,14 \pm 50,59
	C (60-80)	0,33 \pm 0,01	68,6 \pm 4,20	-25,93	4,18 \pm 0,38	3,85	5,70 \pm 0,12	6,18 \pm 0,30	1,86 \pm 0,52	22,69 \pm 3,68	269,22 \pm 18,52
PNP	A (0-20)	0,41 \pm 0,03	59,82 \pm 13,47	-26,03	4,13 \pm 1,16	-3,38	5,25 \pm 0,08	9,05 \pm 4,20	8,12 \pm 1,32	63,08 \pm 16,73	31,45 \pm 18,44
	AC (20-60)	0,57 \pm 0,03	38,19 \pm 1,16	-25,45	2,25 \pm 0,08	-1,00	5,66 \pm 0,26	5,64 \pm 3,29	3,73 \pm 3,06	28,46 \pm 7,77	58,18 \pm 37,03
	C (60-80)	0,63 \pm 0,05	28,4 \pm 1,61	-25,60	1,69 \pm 0,12	-2,05	5,79 \pm 0,18	2,88 \pm 1,28	1,3 \pm 0,85	15 \pm 8,95	66,47 \pm 15,30

Preliminary results

The chemical composition of SOM has been determined by ^{13}C NMR in both sites at different depths. The results to the topsoil showed a dominant peak at 33, 72 and 171 ppm that correspond to alkyl-C, O-Alkyl-C and Carboxyl-C, respectively (1A). The main intensity of signal corresponds to peak 33 and 72 ppm which is associated a polysaccharides and the presence of proteins and peptides. Finally, the relative intensity of the signal at 171 ppm is derived from carboxyl and amides groups. The medium soil presents the similar intensities in compare to the topsoil, however, higher signals in the peaks 32 and 100 ppm which indicate a high presence of polysaccharides and carbohydrates (2A). The figure 3A shows that the signals presented in the subsoil have different intensities due to a less intensity of polysaccharides in the peak 71 ppm. The signals 72 and 171 ppm that indicates the presence of proteins and peptides and carboxyl groups, respectively, were of less intensity in compare to the surface soil. Figure 2 shows the integrals of proportion of each C group. In the topsoil, this proportion indicated a 49.7% to O-Alkyl-C, 22.8% to Alkyl-C. The Aryl-C and Carboxyl-C groups represented just an 18.6 and 8.6% of the total, respectively. To the medium soil, the proportion of C groups was of 50.5, 28.9, 12.4 and 8.2%. Finally, in the subsoil these values represented a 40.2% of Alkyl-C, 38.8% of O-Alkyl-C. The Aryl-C and Carboxyl-C groups represented a 12.4 and 8.6%.



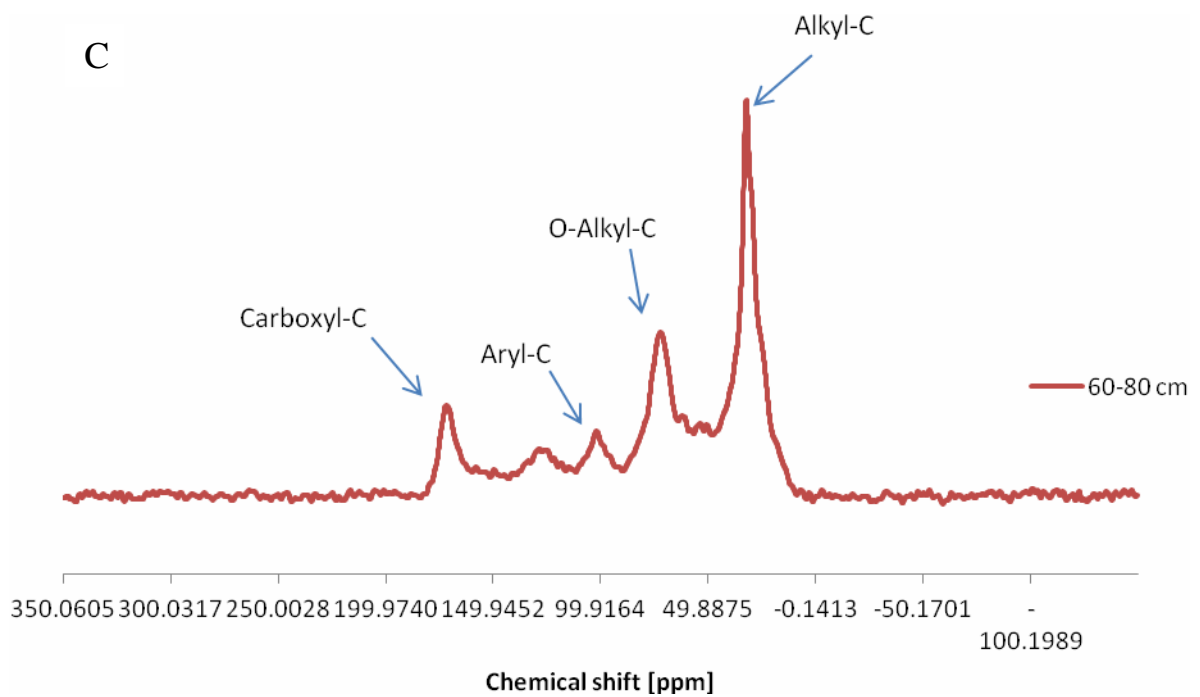
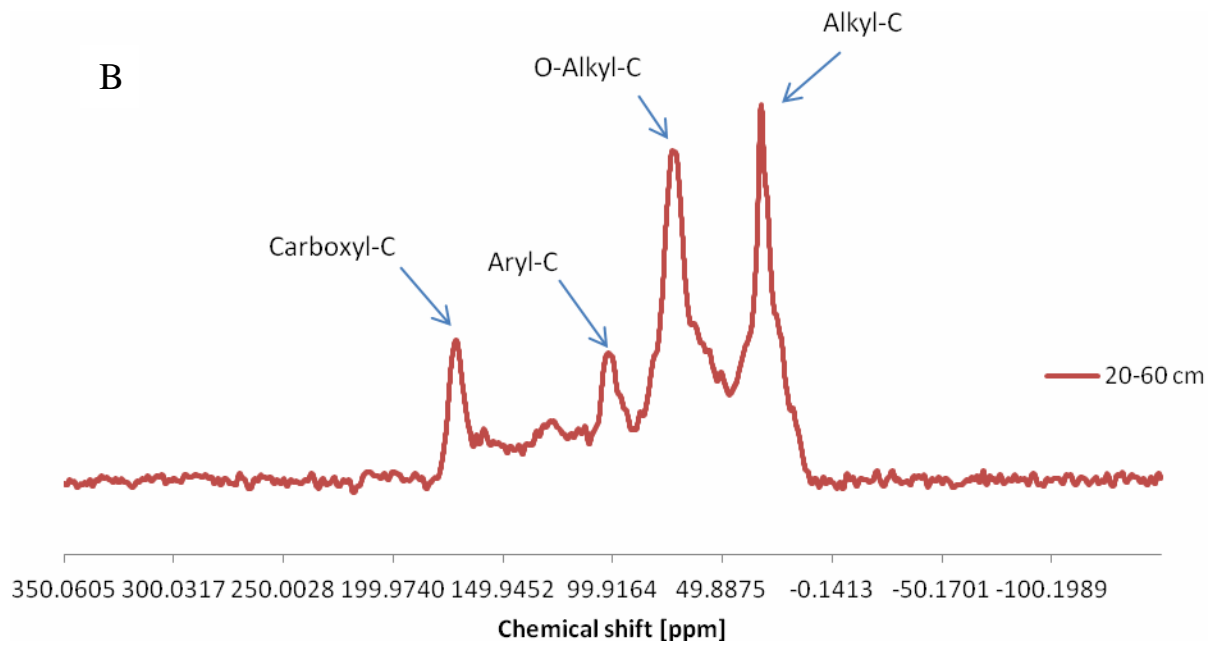


Figure 1. ^{13}C CPMAS NMR spectra of SOM at different depths. (1A) horizons 0-20 cm; (1B) horizon 20-60 cm and (1C) horizon 60-80 cm.

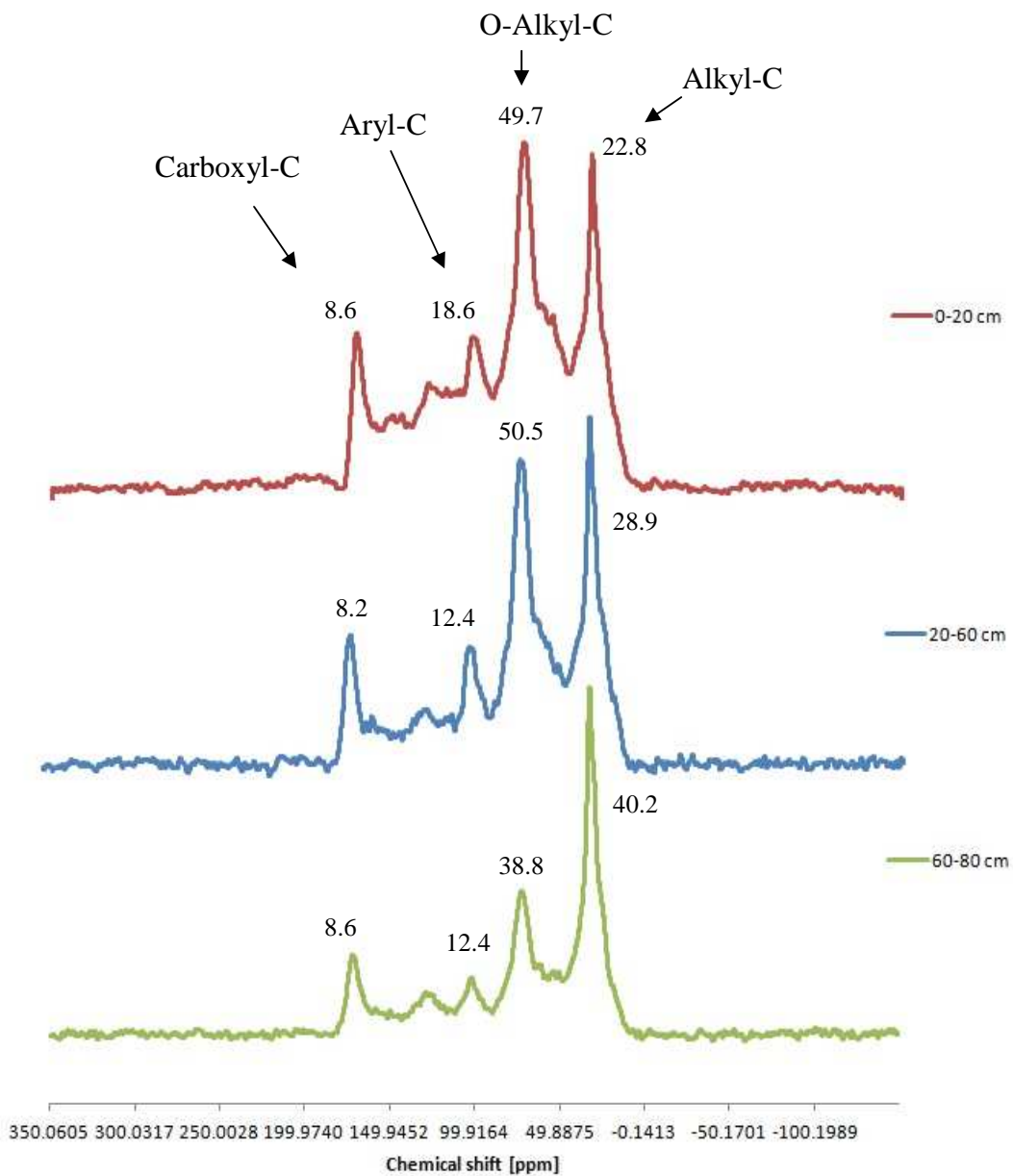


Figure 2. The integrals proportion of SOM at different depth soil, represented in percentage (%).

The preliminary results indicates that a high signal of Alkyl-C and the low presence of O-Alkyl-C in the subsoil in compare to the surface horizons could be related with the high presence of lipids and other aliphatic molecules, which might correspond to preserved plant-

derived molecules. Therefore, these preliminary results suggests a high C preservation in the subsoil with respect to the topsoil due the presence of this C-groups of lower degree of decomposition, generates probably by the absence of fresh C transfer from the topsoil to the subsoil.

Projected publications/articles resulting or to result from your grant

F. Matus, R. Neculman, C. Rumpel, M. Panichini, M.L. Mora. 2011. Soil carbon storage and stabilization in Andisols: A Review. (submitted to Biogeochemistry).

Other comments

These results are only preliminary and are still under discussion together with the results of another site.