Influence of anthropogenic activities on the edaphic microbial biomass (C and N) of old growth forests, in remote places of South-Central Chile

Carlos Belezaca-Pinargote [1], R. Godoy-Bórquez [2], M. Barrientos-González [2]

[1] Universidad Técnica Estatal de Quevedo, Carrera de Ingeniería Forestal, Quevedo, Ecuador.
[2] Universidad Austral de Chile, Facultad de Ciencias, Instituto de Ciencias Ambientales y Evolutivas, Campus Isla Teja s/n, casilla 567, Valdivia Chile.
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Autor para correspondencia, Carlos Belezaca, E-mail: cbelezaca@uteq.edu.ec ORCID: 000-0002-3158-7380

Abstract: *Considering that deforestation affects the composition, dynamics and biogeochemistry of soil, we postulate that anthropogenic intervention in pristine evergreen forests located in remote places of South-Central Chile, involve significant losses of C and N in the edaphic microbial biomass (MB). The objective was to evaluate the impact of the total elimination of forest and understory on the C- and N-MB of soil. Three experimental plots of 900 m^2 were delimited (treatments T1, T2, and T3) of evergreen forest. T1 = total elimination of vegetation cover (clear cutting), T2 = understory elimination, and T3 = pristine forest (control). Monthly and along 15 months (March 2012 – May 2013), three soil independent samples per plot (depth of 0-10 cm) were collected. The soil was sieving and irradiated with microwaves and for the determination of C- and N-MB of soil, the oxidation method of $K_2Cr_2O_6$ (600 nm), and Ninhydrin reagent (570 nm) were employed, respectively. The bare soil (T1) showed the monthly average content of C- and N-MB lower, ranging from 1.03 to 6.68 mg C g s^{-1}, and from 0.003 to 0.014 mg N g s^{-1}, respectively, whose results indicate losses up to 83% of C-MB and 85% of N-MB, versus control site (T3). Understory removal (T2) did not significantly affect the dynamics of C- and N-MB, behaving similar to pristine forest (control). The study provides a baseline in environmental monitoring programs versus anthropogenic impacts in temperate forests of Chile.*

Key Words: *pristine forest, soil biology, temperate rainforest, biogeochemistry.*

1. Introduction

The temperate evergreen forest of old growth, located in remote areas of the Andean foothills of the South Cone of South America, shared by Chile and Argentina, represent particular ecosystems due to the composition and history of their biota, evolved under extreme conditions (Romanya *et al.,* 2005), with low temperatures, high rainfall, reduced entry nitrogen (N) through atmospheric depositions, where its stability and biogeochemistry depend on the internal circulation of nutrients, mainly through the transformation and mineralization of the soil organic matter (SOM), (Godoy *et al.,* 2009; Huygens *et al.,* 2011) and biological nitrogen fixation (Pérez *et al.,* 2004; Reed *et al.,* 2011). The SOM adsorbed by allophane, typical of soils from volcanic ash deposited in high rainfall environments, has a slower dynamic than in non-volcanic soils, due to decomposition factors that protect it, which directly interferes with the main nutrient cycles (C, N and P) (Matus *et al.,* 2008).

A view on the microbiological processes and conservation mechanisms of the N cycle in temperate rainforest soils of Chile, point out that most of the N reservoir is associated with SOM, where the decomposition process is the beginning of many biochemical processes (Huygens *et al.,* 2011). Here, the presence of exo-enzymes produced by the microbial biomass (MB) of the soil in the depolymerization processes of the SOM is relevant, giving rise to internal cycles of the C and N (Huygens *et al.,* 2008a). The reservoir of N in the soil for most of the Chilean temperate forest ecosystems varies from 0.6 to 0.9%, with almost absolute abundance of the organic fractions (mainly humic, huminic and fulvic), (Borie *et al.,* 2002).

The Chilean temperate evergreen forests evolved with exogenous nutrient income restrictions, and under this scenario, the biological activity and especially the MB of the soil, are fundamental for the supply of nutrients, due to its capacity to carry out biochemical transformations (Valenzuela *et al.,* 2001). The BM of the soil, constituted by saprophytic organisms, free living and symbiotic associations, allow the biota to supply the deficiencies of nitrogen, phosphorus and other nutrients (Pérez *et al.,* 2004), estimating that around 97 % of entry of N into pristine terrestrial ecosystems, is the product of biological fixation (Reed *et al.,* 2011).

For the functioning of these ecosystems, the quantity and quality of fine and coarse debris (leaves, trunks, branches, bark, etc.) that accumulates onto and under the forest floor, and their rate of decomposition, are relevant and closely related to the physical-chemical properties of soil (SOM, mineral nutrients, pH, texture, temperature, humidity, etc.), which are considered as important aspects in the determination of MB the soil (Matus *et al.,* 2008; Staelens *et al.,* 2011).

The edaphic MB constitutes the labile fraction of the SOM, and despite representing only between 1 to 4% of the carbon and 2 to 6% of total N of the soil, its presence is vital in the formation of the structure and soil stabilization (Dube *et al.,* 2009). Due to the sensitivity that MB has in responding to changes generated in the soil, and that the C- and N-MB edaphic are part of the total reservoirs of organic C and N, it has been postulated that its presence is a robust indicator to monitor the quality of the soil, as well as to explain in a realistic way the alterations induced by anthropogenic activities in pristine forest ecosystems (Moscatelli *et al.,* 2005; Dale *et al.,* 2008). Its quantification can be determined (among other methods), through the C and N of the MB of soil and the evaluation through time, can contribute to a more detailed knowledge of the processes of immobilization and mineralization of the SOM (Moscatelli *et al.,* 2005; Lillo *et al.,* 2011). However, there is little quantitative information regarding the contribution of the MB of soil to the cycles of the C and N, and as indicators of temporary changes in land use (annual cycle), in evergreen temperate rainforests of old growth located in remote areas of Chile.

Considering that the changes in land use can affect the composition and dynamics of the MB of pristine environments, we postulate that anthropogenic interventions in temperate evergreen forest of old growth of the Center-South of Chile, lead to significant losses of soil C and N- MB and affect the stability of biogeochemical cycles.

2. Materials and Methods

Study area

Forested communities were studied in the Puyehue National Park (PNP) and adjacent property, in the Cordillera de Los Andes, Los Lagos Region, Osorno, Chile (40° 47’ S - 72° 12’ O, entre 716 y 920 masl). The climate is rainy with an average annual rainfall of over 7000 mm and average annual air temperature of 4.5 °C (Oyarzún *et al.,* 2011).

At the beginning of 2011, anthropogenic interventions (felling of the forest) were carried out in a small area of the evergreen forest, located at the southern end of the PNP, with the purpose of using the waters of the Correntoso River (originated in the Paradise Lake), for a power generation project. The present study made a partial use of this intervention, to evaluate the impact of the removal of the vegetation on the edaphic microbial biomass of the pristine forest. The soil where the experiment was established originated from Andesitic-Basaltic volcanic ash deposited on different substrates, classified as trumao "Typic Dystrandepts" (Luzio *et al.,* 2006).

Characteristics of the treatments

Three treatments (scenarios) of land use were proposed, in remote areas of difficult access, whose establishment demanded strong logistics. Three permanent plots of 30 x 30 m (900 m^2) representative of each treatment were delimited. Treatment 1 (T1) was located in an area bordering the PNP, while treatments 2 and 3 (T2 and T3, respectively) were located within the PNP. The plant community in these areas is composed by dominant tree species *Nothofagus betuloides* (Mirb.) Oerst, *Saxegothaea conspicua* Lindl., *Laureliopsis philippiana* (Looser) Shodde with individuals older than 300 years.

T1 consisted in the total elimination of the arboreal and shrub vegetation in November 2011, leaving the soil without vegetal cover. In its original state, it contained a density of 189 trees ha^{-1}, representing 101.5 m^2 of basal area. The plant residues (litter, branches, trunks, etc.) were removed manually, but the stumps were not removed in order to avoid soil disturbance. T2 consisted of the elimination of the understory in August 2011, leaving the upper tree stratum, with a density of 200 trees ha^{-1}, and 110.3 m^2 ha^{-1} of basal area. T3 corresponded to a pristine forest without intervention (control), with a total of 344 trees ha^{-1} that represented 146.33 m^2 ha^{-1} of basal area. Canopy coverage in each treatment was visually estimated according to the methodology used to project the aerial segment of standing trees (radius of canopy). The estimate was made from the base of each tree in four directions (North, South, East and West), (Donoso & Nyland, 2005). Table 1 describes the geographic location and dasonomic characteristics of the study plots, associated with each treatment.

Table 1. Treatments, geographical location and dasonomic characteristics of the study sites in a temperate evergreen old growth forest, Andean Cordillera, South-Central Chile.

Treatments	Geographic location	Altitude (m a.s.l)	Total density (trees ha^{-1}) *	Canopy coverage (%)
T1. Soil without vegetal cover	40º80’65’’ S y 72º85’30’’ W	716	-------	-------
T2. Understory removal	40º79’94’’ S y 72º95’24’’ W	903	200	80
T3. Pristine forest (control)	40º79’85’’ S y 72º95’38’’ W	920	344	95

* Dominant forest species (> 5 cm diameter) in the treatments (Marticorena & Quezada, 1985): *N. betuloides* (Mirb.) Oerst, *S. conspicua* Lindl., *L. philippiana* (Looser) Shodde.

pH, C_{total} y N_{total}

To have an approximation of the chemical characteristics of the soil where the experiment was carried out, in all cases a soil sample was analyzed by treatment. The soil pH was determined by aqueous solution in water with a soil-water ratio of 1: 2.5. The determination of C_{total} was performed with the methodology of oxidation with dichromate in acid medium and colorimetric determination of the reduced chromate. The N_{total} was established by the Kjeldahl digestion method (Sadzawka *et al.,* 2016).

Environmental temperature, soil and precipitation

In order to perform correlations between the variables studied, the air temperature was recorded continuously at 1 m above ground level, and the soil temperature at 10 cm depth was also recorded with a *Datta-logger*, and using a pluviometer the precipitation of the study area was recorded, too.

Collection of soil samples

For each treatment, monthly and along 15 months (March 2012 - May 2013) three soil samples of 1 kg each were collected, from the surface horizon (0 - 10 cm deep) and transferred to the laboratory in refrigerated boxes for immediate analysis, then of sieving the ground.

Extraction of C and N from the MB

We used the method of Islam & Weil (1998) which consists in irradiating soil samples in a microwave at 800 J g-1, for 30 seconds at a temperature not exceeding 80 °C to avoid the solubilization of humic substances. The sudden increase of temperature in the soil generates a rapid oscillation and eventual volatilization of the polar/ion molecules of the cytosol, due to frictional heat (Islam & Weil, 1998; Ferreira de Araujo, 2010). This method, described as cell destroyer and cell contents liberator (C- and N-BM), is a non-toxic alternative to the traditional method of fumigation with chloroform.

In the present study, 10 g of fresh soil was added in glass containers and sterile distilled water was also added until reaching 100% field capacity, finally a thermal shock was applied for 30 seconds. Subsequently, to each soil sample, 50 mL of a 0.5 M K_2SO_4 extractant solution was added and stirred for 45 minutes at 150 r.p.m. Finally, it was filtered, using a Whatman No 2 filter paper and the extracts were stored in freezing at -15 °C, until the analysis of C- and N-MB (Vance *et al.,* 1987).

Analysis of the C of the MB

The colorimetric method was used (Vance *et al.,* 1987), for which 4 mL of each filtrate was deposited in a glass tube for digestion, to which was added 1 mL of 66.7 mM potassium dichromate ($K_2Cr_2O_6$), together with 5 mL of concentrated H_2SO_4. The reaction mixture was subjected to 150 °C for 30 minutes in an oven, then it was put to get cold, initially at room temperature for 30 minutes and then in water for 15 minutes. The final solution was colorimetrically analyzed on a UV/visible light spectrophotometer at 600 nm. The concentration of C in the soil sample was expressed in mg of C per gram of dry soil (mg C g^{-1} ss) and was estimated from the linear function previously obtained from a sucrose standard curve.

Analysis of N of the MB

The methodology proposed by Joergensen & Brookes (1990) was used to determine N from the reaction with Ninhydrin, which considers nitrogen solubilized as amino acids and ammonium. This methodology consisted of depositing in a test tube 0.75 mL of each filtrate, which was added and mixed with 1.25 mL of a sodium citrate buffer solution and then added 1 mL of the Ninhydrin reagent. The test tubes were incubated for 60 minutes in a thermoregulated bath at 100 °C, then cooled and 4 mL of an ethanol: water mixture added. Finally, the product was vigorously agitated, after reading the absorbance in a UV/visible light spectrophotometer at 570 nm. The concentration of N-ninhydrinic was estimated from the linear function previously obtained from a standard curve of ammonium sulfate and was expressed in µg of N per gram of dry soil (µg N g^{-1} ss). The principle of this method is based on the fact that Ninhydrin forms a purple complex with molecules that contain α-amino-nitrogen, such as ammonium and other compounds with α-amino free groups: amino acids, peptides and proteins.

Statistical analysis

Considering that the study was carried out in remote sites, the monthly results of C-MB and N-MB for each treatment are the product of three replications. The final values were subjected to nonparametric statistical analysis, after checking the normality assumptions (Shapiro-Wilk test) and homoscedasticity of variances (Levene test). Subsequently, analysis of variance (ANOVA) was performed, using the Kruskal-Wallis test, followed by a posteriori contrasts (post hoc), according to the Mann-Whitney test, for the comparison of means with significant effects ($P < 0.05$) between treatments. The determination of interactions between variables was carried out using Spearman's nonparametric correlation test (content of C- and N-MB, soil temperature at the time of sample collection, average monthly temperature of the soil, precipitation). The statistical analyzes were carried out in the program STATISTICA 7, version 9 for Windows.

3. Results

Chemical characteristics of the soil

The pH of soil was similar in the three treatments at two depths (0.0-10 cm, 60-80 cm), with ranges from 5.26 to 5.85, placing them in the category of slightly acid soils. The highest values of C_{total} and N_{total}, were found 0.0 – 10 cm deep in T1, with 9.70% and 0.41%, respectively. The soil C/N ratio was higher in the T2 and T3 treatments compared to T1, especially at a greater depth (42.6, 45.0, 21.6 respectively), (Table 2).

Table 2. Chemical characterization of soil in three treatments of a temperate evergreen old growth forest, Andean Cordillera, South-Central Chile.

	Treatments					
	T1		T2		T3	
Depth (cm)	0 – 10	60 – 80	0 – 10	60 – 80	0 – 10	60 – 80
pH en H_2O (1:2,5)	5,51	5,85	5,48	5,54	5,26	5,46
C_{Total} (%)	9,70	2,30	5,30	7,90	4,30	5,10
N_{Total} (%)	0,41	0,11	0,16	0,18	0,16	0,12
C/N ratio	23,6	21,6	32,7	45,0	27,9	42,6

The values correspond to a single reading. T1: Soil without vegetal cover, T2: Understory removal, T3: pristine forest (control). Techniques used: pH measured in distilled water (1: 2.5); C_{total} evaluated by oxidation of sodium dichromate with sulfuric acid; N_{total} estimated by Kjeldahl digestion (Sadzawka *et al.,* 2006).

Carbon of the MB

Significant statistical differences were detected in most of the months evaluated, with the exception of October, November, December (2012), and March 2013 ($P > 0.05$). T1 showed the lowest C-MB values, with ranges from 1.03 to 6.58 mg C g^{-1} ss versus T2 and T3 (control). The contents of C-MB determined in the soil of T2 and T3 behaved statistically similar during the majority of the months studied, except in May 2012. During the 15 months evaluated, in 11 of them (March, April, May, June, July, August, September, 2012, and in January, February, April and May 2013) it was detected a significant decrease in the amount of C-MB soil in the experimental treatment T1, reaching losses greater than 80%, in relation to the control treatment (T3), with ranges of decrease in its content between 44.09 – 83.27%. When comparing T2 with T3 (control), the losses and gains of C-MB were shared in 50% for each one, during the months evaluated, whose situation was observed in the absence of significant statistical differences (Table 3). The average monthly value in the study period was 2.42; 5.81 and 5.82 mg C g^{-1} ss, for the treatments T1, T2 and T3, respectively, where the losses of C in T1 (soil without vegetal cover) were notorious.

Table 3. Carbon content of edaphic microbial biomass (mg C g^{-1} ss), in three treatments soil management in a temperate evergreen old growth forest, Andean Cordillera, South-Central Chile.

Months	Treatments				
	T1		T2		T3
March-2012	2,06±0,31 a	(- 67,90) *	5,18±2,00 b	(- 19,44) *	6,43±2,10 bc
April-2012	2,20±0,53 a	(- 46,98)	4,33±0,65 b	(+ 04,33)	4,15±0,50 bc
May-2012	3,14±1,26 a	(- 47,60)	3,35±1,06 a	(- 44,16)	6,00±1,18 b
June-2012	2,66±0,22 a	(- 64,86)	5,94±2,23 b	(- 21,32)	7,57±3,00 bc
July-2012	2,56±1,11 a	(- 59,55)	5,35±1,89 b	(- 15,48)	6,33±0,74 bc
August-2012	1,03±0,56 a	(- 83,27)	6,36±2,47 b	(+ 3,24)	6,16±2,42 bc
September-2012	2,29±0,62 a	(- 53,36)	6,27±0,84 b	(+ 27,69)	4,91±0,66 bc
October-2012	1,29±0,60	(- 73,12)	5,70±3,77	(+ 18,75)	4,80±1,46
November-2012	3,88±2,27	(- 48,19)	5,84±1,01	(- 22,02)	7,49±3,14
December-2012	6,58±3,44	(- 26,06)	9,79±2,32	(+ 10,00)	8,90±2,69
January-2013	1,40±1,35 a	(- 78,15)	7,41±2,12 b	(+ 15,60)	6,41±1,34 bc
February-2013	1,55±1,33 a	(- 67,16)	4,22±0,66 b	(- 10,59)	4,72±0,69 bc
March-2013	1,34±0,66	(- 50,95)	5,86±1,59	(+ 131,25)	2,72±1,71
April-2013	2,76±0,48 a	(- 44,09)	6,29±1,77 b	(+ 27,58)	4,93±1,44 bc
May-2013	1,52±0,56 a	(- 73,88)	5,29±1,42 b	(- 09,26)	5,83±0,68 bc

Values represent the average of three repetitions with their respective standard deviation. Different letters symbolize statistically different means, averages without letters indicate absence of statistical significance (*P <0.05*) according to the Mann-Whitney test. T1: soil without vegetation cover, T2: Understory removal, T3: pristine forest. *Values within parentheses indicate the percentage of decrease (-) or increase (+) in the content of C-MB in treatments T1 and T2 with respect to treatment T3 (control), respectively.

Nitrogen of the BM

In 12 of the 15 months evaluated significant statistical differences were detected (*P <0.05*). Similar to the case of the C-MB, in the soil from T1, the lowest contents of N-MB were determined and, in most months, they differed statistically from those determined in T2 and T3 (control). The values found in T1 were in the range of 3 – 14 µg N g^{-1} ss.

The decrease of the N-MB in the soil of T1 was significant during the evaluation period, detecting losses that sometimes reached more than 80%, compared to T3 (control), with ranges of 53.33 – 85.71%. When contrasting T2 with T3, it was detected that, in most of the months evaluated, there were no significant statistical differences, except in April 2013, when the amount of N in the soil of T2 (29 µg N g^{-1} ss), was 70.5% higher than T3 (17 µg N g^{-1} ss), (Table 4). The average monthly value in the study period was 8.1; 18 and 19 µg N g^{-1} ss, for T1, T2 and T3, respectively; highlighting clearly the losses of N in T1 (soils without vegetation cover).

Table 4. Nitrogen content of edaphic microbial biomass (µg N g^{-1} ss), in three treatments soil management in a temperate evergreen old growth forest, Andean Cordillera, South-Central Chile.

Months	Treatments				
	T1		T2		T3
March-2012	14 ± 5	(- 22.2) *	20 ± 8	(+ 11.1) *	18 ± 5
April-2012	7 ± 0.1 a	(- 46.2)	14 ± 7 b	(+ 7.6)	13 ± 2 b
May-2012	9 ± 3 a	(- 55.0)	16 ± 5 b	(- 20.0)	20 ± 2 b
June-2012	13 ± 3 a	(- 55.2)	25 ± 7 b	(- 13.8)	29 ± 11 b
July-2012	8 ± 3 a	(- 73.3)	21 ± 4 b	(- 30.0)	30 ± 8 c
August-2012	3 ± 1 a	(- 85.7)	14 ± 6 b	(- 33.3)	21 ± 6 b
September-2012	8 ± 2 a	(- 46.7)	18 ± 2 b	(+ 20.0)	15 ± 2 b
October-2012	4 ± 1 a	(- 80.0)	20 ± 5 b	(0.0)	20 ± 2 b
November-2012	10 ± 1 a	(- 60.0)	20 ± 3 b	(- 20.0)	25 ± 3 b
December-2012	12 ± 1 a	(- 45.5)	26 ± 8 b	(+18.2)	22 ± 5 b
January-2013	8 ± 2	(- 55.6)	13 ± 6	(- 27.8)	18 ± 3
February-2013	3 ± 1 a	(- 84.2)	18 ± 9 b	(- 5.3)	19 ± 1 b
March-2013	6 ± 1 a	(- 45.5)	13 ± 3 b	(+ 18.2)	11 ± 0.1 b
April-2013	11 ± 2 a	(- 35.3)	29 ± 12 b	(+ 70.6)	17 ± 5 a
May-2013	7 ± 2 a	(- 53.3)	14 ± 4 b	(- 6.7)	15 ± 2 b

Values represent the average of three repetitions with their respective standard deviation. Different letters symbolize statistically different means, averages without letters indicate absence of statistical significance (*P <0.05*) according to the Mann-Whitney test. T1: soil without vegetation cover, T2:

Understory removal, T3: pristine forest. *Values within parentheses indicate the percentage of decrease (-) or increase (+) in the content of N-MB in treatments T1 and T2 with respect to treatment T3 (control), respectively.

The biological variables C-MB and N-MB showed a positive and significant correlation ($P < 0.05$, $P < 0.01$, $P < 0.001$) in each treatment studied. The C-MB and N-MB correlated positively and significantly with the soil water content in the treatments studied. It was determined that the C-MB of soil in T1, and N-MB of the control treatment (T3), correlated negatively with the average and maximum monthly environmental temperatures. The soil water content in all the evaluated treatments showed a negative correlation with the average and maximum monthly environmental temperatures. While the C-MB of T1 correlated positively with the monthly precipitation (Table 5).

Table 5. Correlations of physical and biological variables in three treatments soil management in a temperate evergreen old growth forest, Andean Cordillera, South-Central Chile (march 2012 - May 2013).

	C-MB-T1	N- MB-T1	C-MB-T2	N- MB-T2	C-MB-T3	N- MB-T3	WC-T1	WC-T2	WC-T3	Max. Temp.	Average Temp.
N-MB-T1	0.64***										
C-MB-T2	0.074 ns	0.20 ns									
N-MB-T2	0.41**	0.41**	0.34*								
C-MB-T3	0.09 ns	0.23 ns	0.38**	0.08 ns							
N-MB-T3	0.53***	0.19 ns	0.28 ns	0.47***	0.44**						
WC-T1	0.36*	0.46**	0.19 ns	0.33*	0.09 ns	0.22 ns					
WC-T2	0.29 ns	0.25 ns	0.45**	0.30*	0.01 ns	0.15 ns	0.43**				
WC-T3	0.49***	0.33*	0.31*	0.35*	0.42**	0.50***	0.54***	0.43**			
Max. Temp.	-0.38 **	-0.13 ns	-0.00 ns	0.04 ns	-0.13 ns	-0.37*	-0.36*	-0.30*	-0.56***		
Average Temp.	-0.36*	-0.08 ns	-0.01 ns	-0.00 ns	-0.10 ns	-0.37*	-0.38**	-0.33*	-0.59***	0.97***	
Rainfall	0.29*	0.25 ns	-0.14 ns	-0.09 ns	-0.06 ns	0.03 ns	0.27 ns	0.17 ns	0.25 ns	-0.65***	-0.57***

*: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$; ns: not significant. C-MB: carbon from the microbial biomass; N-MB: nitrogen from the microbial biomass; T1: soil without vegetation cover, T2: Understory removal, T3: pristine forest (control). WC: water content; Max. Temp.: maximum monthly environmental temperature; Average temp.: average monthly environmental temperature; Rainfall.: monthly rainfall.

4. Discussion and Conclusions

The results obtained show that the biological dynamics in soils of temperate evergreen old growth forest in southern Chile is affected by changes in land use through intensive anthropic interventions, a situation that has already been documented by other researchers (Rivas *et al.,* 2009) in ecosystems similar to the present study. The total elimination of the vegetal cover made in T1, led to losses higher than 80% in the contents of C- and N-MB, in relation to the treatments T2 (understory removal), and control T3 (pristine forest). The dramatic reduction of C- and N-MB in T1 could be associated with the elimination of vegetation cover, and the consequent lack organic substrates of forest for the development of microbial activity, mainly litter (litter, flowers, fruits, fine branches, etc.) product of the net primary production of the system (Dube *et al.,* 2009). This effect has already been documented in other forest and agricultural ecosystems, both in temperate and tropical regions (Xu *et al.,* 2006; Dube *et al.,* 2009).

On other hand, minor anthropic interventions on forest floor (understory removal), did not imply changes in the dynamics of the C- and N-MB of soil, on the contrary, they showed a behavior similar to the pristine forest, with values 3 and 4 times higher than those obtained in the soil devoid of plant cover (T1), product of greater accumulation of plant material contributed mainly by *N. betuloides* (Mirb.) Oerst, *S. conspicua* Lindl., and *L. philippiana* (Looser) R Shodde, which provide high levels of litter throughout the year (Staelens *et al.,* 2011). The comprehension of the complex nutrient conservation mechanisms that occur in the temperate rain forest ecosystems of old growth in southern Chile, lets to understand that important elements such as nitrogen are immobilized in the MB, within the large reservoir of SOM and gradually released (Huygens *et al.,* 2011). This situation can be explained by the results obtained by Staelens *et al.* (2011), who demonstrated that nitrogen is mineralized much faster in pristine forests, than in those strongly intervened, which would be associated with a greater amount and activity of MB in the soil, constituting an important mechanism of conservation of N (Rivas *et al.,* 2009; Huygens *et al.,* 2011).

The contents of C-MB (3.35 – 9.79, 2.72 – 8.90 mg C g^{-1} ss) and N-MB (13 – 29, 11 – 30 µg N g^{-1} ss) obtained in the soils of T2 and T3, respectively, were higher than those found in T1 (1.03 – 6.58 mg C g^{-1} ss; and 3 – 14 µg N g^{-1} ss, correspondingly), and for the case of C-MB, much higher than those reported by Lillo et al. (2011), who worked on four pristine plant formations, following an altitudinal transect in the Conguillío National Park (38º 40 'S and 71º 45' W), obtained values between 0.92 – 1.26 mg C g^{-1} ss. However, for the situation of the N-MB, the reports of the authors mentioned above were very close to those found in the treatments T2 (understory removal) and T3 (pristine forest) (T3) of the present study.

The marked differences in carbon content can probably be explained by the biotic and abiotic conditions prevailing in each geographical area (Romanya *et al.,* 2005). The dominant forest species in each place exert significant differences, granted by the quantity and quality of litter (Benintende *et al.,* 2008; Dube *et al.,* 2009). The rate of mineralization of the litter and amount of MB would be a function of the recalcitrance of the vegetal remains contributed to the soil, given by the chemical composition of the litter (concentrations of lignin, cellulose, starches, phenols, etc.), microbial byproducts, rizodepositation, formation of humic polymers, and the production of carbonized organic matter (Schmidt *et al.,* 2011), which are mechanisms of selective conservation of C in the soil (Matus *et al.,* 2008; Rivas *et al.,* 2009) .

Losses greater than 80% of C- and N-MB in soil without vegetation cover (logged forests), show that the forest soils of southern Chile subjected to changes in use, negatively affect the flow of nutrients in the ecosystem and, therefore, burst into the balance of the biogeochemical cycles, altering the distribution patterns of N reserves in volcanic soils, with a reduction of nitrogen towards the high molecular weight fractions (humines, humic acid), (Borie *et al.,* 2002). This is an indicator of loss of natural balance, affecting the nitrogen and carbon cycles, respectively (Huygens *et al.,* 2005, 2008b).

The absence of positive and significant correlations of the C-MB and N-MB with physical variables, lets to deduce that they are probably associated to other factors such as the availability of nutrients, quantity and nature of mineral clays, soil biochemistry, aluminum concentration and free iron, etc., not considered in this study, as Neculman *et al.* (2013), and similar to those studied by Lillo *et al.* (2011). However, the positive and significant correlation obtained between the content of C- and N-MB in the treatments T1, T2 and T3, lets to point out that the methodology used in this research is reliable and effective, and can be used to monitor changes in the biology of soils caused by anthropogenic interventions, as explained Amato & Ladd (1988). In this sense, due to the sensitivity of the MB as a bioindicator of environmental changes in different latitudes (Benintende *et al.,* 2008; Dale *et al.,* 2008), it can be very useful in southern Chile.

The results obtained in the present study constitute a baseline of knowledge for monitoring changes in soils of temperate evergreen old growth forest ecosystems and allow to dispute that soil MB is a sensitive indicator against anthropogenic impacts, so they can be incorporated into programs of environmental monitoring, in this type of ecosystem of southern Chile.

5. References

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