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Silvopastoral use of *Nothofagus antarctica* forests in Patagonia: impact on soil microorganisms

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Abstract Soil microorganisms contribute to soil carbon storage, soil respiration and nutrient cycling. In south Patagonia, there are scarce studies on carbon (C) and nitrogen (N) in soil microbial biomass (MB). The aim of this work was to evaluate C and N content in soil MB, and soil microbial respiration according to different sites and forest uses: *Nothofagus antarctica* primary forests (PF), silvopastoral forests (SPF) and open site (OS). For MB determination, soil samples (0–10 cm depth) were collected in spring (November) and summer (February) over two years. Soil microbial biomass C (SMB-C) was determined using the chloroform fumigation-extraction method and soil microbial biomass N (SMB-N) by a modification of the fumigation–incubation method. Significant differences were observed in SMB-C according to site, forest use and year. Morro Chico ranch presented the lowest SMB-C (721.4 μg C g^{-1}) whereas Tres Marías had the highest (1216.6 μg C g^{-1}). For forest use, the gradient PF > SPF > OS was observed. SMB-N also presented differences and SPF had significantly lower SMB-N (110.9 μg N g^{-1} soil) than PF (137.5 μg N g^{-1} soil) and OS (141.76 μg N g^{-1} soil). The total cumulative respiration was 9657, 10,560 and 12,780 mg C kg^{-1} for SPF, OS and PF, respectively. Our results support the hypothesis that silvopastoral use of *N. antarctica* forests affects SMB-C. This should be considered if *N. antarctica* trees are going to be remove to install silvopastoral systems, because this clearly affects soil microorganisms, and in consequence, soil carbon dynamics.

Keywords Soil organic carbon · Decomposition · Microbial biomass · Soil biology

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Introduction

Nothofagus antarctica forest extends from 46° to 56° South Latitude in Argentina and Chile. In south Patagonia the main use of these forests is as silvopastoral systems, where natural grasses under the tree canopy are grazed by cattle and sheep (Peri and Ormaechea 2013). Nowadays, there is a lack of information about soil biology in these austral environments in comparison with other ecosystems. Furthermore, under a climate change scenario of increasing mean maximum annual temperatures in the region by 2–3 °C by 2080 (Kreps et al. 2012), the grazing pressure could increase in *N. antarctica* forests. Therefore, evaluation of changes in soil biology owing to silvopastoral practices in this ecosystem is required in order to predict future impact of management intensification.

Soil microorganisms are recognized as a central part of many soil processes, since they contribute to soil carbon storage, soil respiration and in the cycling of C, nitrogen (N) and phosphorus (P) (Van Veen et al. 1987; Bahaus and Pare 1998). The quantity and composition of microbial biomass change according to physical and chemical variations in soils (Wolters and Joergensen 1991; Bauhus and Khanna 1994) and with forest management (Ohtonen et al. 1992; Bauhus and Barthel 1995). Thus, amounts of microbial C and N can be used as sensitive indicators for changes or disturbances in soil ecosystems (Bahaus et al. 1998). Moreover, in a review about soil organic carbon (SOC) storage and its best indicators, Weismeier et al. (2019) pointed out that carbon in soil microbial biomass (SMB-C) pool size and the SMB-C and SOC ratio are sensitive indicators that reflect cropping and management effects. Monitoring these biologic soil variables can thus serve to predict SOC changes.

Soil respiration presents the second largest input of CO_2 into the atmosphere (Bohn 1982), and changes occurring in soil respiration relating to land-use change may greatly influence global warming (Kuzyakov and Gavrichkova 2010; Sainju et al. 2008). Soil respiration directly reflects microbial activity and indirectly the availability of labile substrates (Noellemeyer et al. 2008). The metabolic quotient (qCO_2), which relates the microbial potential respiration with microbial C biomass, provides information on the metabolic efficiency of soil microorganisms (Pirt 1975; Anderson and Domsch 1990).

In Patagonia, there are some background about the soil microbial biomass and potential respiration in grasslands or northern sites (Satti et al. 2003; Dube et al. 2009; Gonzalez Polo et al. 2019; Toledo et al. 2021), although, in south Patagonia, forest is less diverse and information about soil biology is missing. Likewise, silvopastoral use may implicate thinning practices for keeping intermediate crown cover values and grazing, and there are evidences reporting that canopy opening modifies the biogeochemical cycle of elements (Caldentey et al. 2001; Jussy et al. 2004). Thinning practices in *N. antarctica* forests for silvopastoral uses may modify soil C and N dynamics through changes in micro- environmental factors and lower potential nutrients return from litterfall (Peri et al. 2016a, b) or due to increasing losses of nitrate by leaching (Feller et al. 2000; Mazzarino et al. 1998a). In general, silvopastoral systems are considered sustainable production systems, characterized by a great biodiversity and multifunctionality compared with other livestock production. Thus, silvopastoral systems aim to increase the provision of ecosystem services (ES) (e.g., timber, non-forest products, livestock), with the minimum impact on regulation (e.g., carbon sequestration, water and erosion regulation) and support services (e.g., soil formation, primary production, nutrient cycling), and maintain the cultural services associated with forests systems (Peri et al. 2016b; Chillo et al. 2021).

At present, there is a lack of information about SMB-C and SMB-N in south *N. antarctica* forests and it is unknown if the silvopastoral use affects this variables. Thus, the aim of this study was to measure SMB-C and SMB-N and microbial respiration in *N. antarctica* forests and their variation according to sites, year and land use. We hypothesize that silvopastoral practices in *N. antarctica* forests decrease SMB-C and respiration with possible consequences on soil organic matter accumulations on the long-term.

Material and methods

Study sites

The study was carried out in *N. antarctica* forests located in the SW of Santa Cruz province, Argentina (Fig. 1). Three different sites (ranches) were selected: Morro Chico (51° 57′ 24″ SL; 71° 31′ 48″ WL), Cancha Carrera (51° 13′ 21″ SL; 72° 15′ 34″ WL) and Tres

Fig. 1 Location of the study sites, in SW of Santa Cruz province, Patagonia, Argentina. Map obtained from Instituto Geográfico Nacional (Argentina) www.ign.gob.ar

Marías (51° 19′ 05 SL″; 72° 10′ 47 WL″). In all sites, three land use were evaluated: mature (120–150 years) *N. antarctica* stands under silvopastoral use (SPF), with a crown cover of 50–60% and grazed by cattle or sheep; primary forest (PF) with higher crown cover (80–90%) without domestic animals grazing; and open site (OS) which was located just beside the forest limit. Understory vegetation, especially in SPF, is dominated by grasses such as *Agrostis capilaris*, *Bromus setifolium*, *Dactylis glomerata*, *Deschampsia flexuosa*, *graminoids* of the genus *Carex* and, in a lower proportion, *Festuca gracillima*. The OS had a vegetation cover of 95% mainly constituted by grasses like *A. capillaris*, *F. gracillima*, *F. magellanica*, and *Carex sp*.

The climate is cold temperate with a mean annual temperature of 7 °C and 390 mm of mean annual rainfall. Monthly precipitation and mean temperature were obtained from the Agrometeorological Station Davis MB5 LR Vantage Pro 2 located at the National University of the Patagonia Austral, in Río Turbio (51° 31′ 58″ SL; 72° 15′ 45″ WL).

The soils of the study site are mollisols with sandy loamy texture and slightly acid pH.

Soil characteristics and microbial biomass measurements

From each site, soil samples were collected in 1000 m^2 plots along five 25 m transects at 10 m spacing, for each land use: primary forest, silvopastoral forest and open site. At each transect, one composite sample of five soil cores was collected (10 cm depth) to characterize the soil (N = five composite samples × 3 land use × 3 sites = 45). Soil samples were sieved by a 2 mm-mesh and air dried to measure pH in water (1:2.5), electrical conductivity (1:5 soil:water solution) and extractable P by the ascorbic-molybdate method (Kuo 1996). For total C and N determinations soils were sieved by 0.5 mm-mesh and measured by dry combustion (Thermo Electron, FlashEA 1112). Soil bulk density was determined with a metal cylinder of 10 cm depth. Gravimetric soil water content was estimated to express the biological variables per gram of dry soil.

For SMB-C and SMB-N, another three composite samples of five soil cores were collected over two years, in spring (November, 2014 and 2015) and summer (February, 2015 and 2016), from the transects described above (N = 3 composite samples × 3 land use × 3 sites × 2 seasons × 2 years = 108). These samples were refrigerated (4 °C) until further processing. In laboratory, soil samples were sieved by a 2 mm-mesh and moisture content was determined on three sub-samples of 20 g by drying to constant weight at 105 °C. Likewise, in summer of the second year, another extra soil samples were taken for potential microbial respiration measurements, following the same procedure and number of samples described for SMB-C.

SMB-C was determined using the chloroform fumigation-extraction method (Brookes et al. 1985; Vance et al. 1987), where each soil sample was dividend in three sub-samples (for laboratory set-up) of 100 gr, being 50 gr for control and another 50 g for the chloroform aggregate. SMB-N was determined by a modification of the fumigation–incubation method (Vitousek and Matson 1985; Mazzarino et al. 1998a, b). Soil samples were divided in three subsamples of 60 g, and then divided again in two subsamples of 30 g each (one for control and one for fumigation). Liquid chloroform (1 mL) was added directly in fumigated samples, stirred and left for 20 h in sealed beakers in desiccators. Chloroform was then removed using a vacuum pump. Fumigated and non-fumigated samples were incubated at field capacity (60% moisture) for 10 days at 25 °C. After incubation, fumigated and control samples were extracted with 2 M KCl and analyzed for NH_4-N by the Berthelot reaction (Keeney and Nelson 1982). SMB-C and SMB-N were calculated as the differences in organic C and N between fumigated and non-fumigated (control) samples, divided by a correction factor. Non-extractable amounts of microbial C and N were compensated for by a correction factor of kC = 0.45 (Jenkinson and Ladd 1981) and kN = 0.54 (Brookes et al. 1985; Joergensen and Mueller 1996), respectively. For potential microbial respiration measurements, in laboratory soil samples were divided in three sub-samples of 70 g each. Microbial respiration was measured as CO_2 emission in incubation at 25 °C for 252 days and at field capacity (60% moisture). Evolved CO_2 was trapped with NaOH traps and titrated with HCl (0.2 M) after Ba_2Cl addition. Vials containing NaOH were replaced and analyzed at 7, 14, 28, 41, 63, 84, 105, 140, 202 and 252 days; potential microbial respiration was estimated as the cumulative CO_2 evolution during the incubation period. Using values of respiration and SMB-C the metabolic quotient for CO_2

(qCO$_2$ = unit CO$_2$-C emitted/unit SMB-C) was determined as described by Anderson and Domsch (1986).

Statistical analysis

Statistical analyses were performed using INFOSTAT software (Di Rienzo et al. 2018). Soil variables were analyzed with a two-way analysis of variance (two-way ANOVA). The SMB-C and SMB-N data were analyzed with multifactorial ANOVA, with the factors being the sites (ranches), land use (PF, SPF and OS), season (summer and spring) and year. Soil microbial respiration was analyzed by two-way ANOVA with site and land use as factors.

Significant differences between means were separated by Tukey's test with a significance level of $p < 0.05$. Pearson correlation coefficients were used to detect significant correlations between SMB-C and SMB-N with soil nutrients.

Results

Total rainfall was 349, 413 and 255 mm year^{-1} for 2014, 2015 and 2016, respectively (Fig. 2A). The spring of the first year of study (November 2014) presented higher rainfall than spring of the second year. Contrary, in summer time, the second year had higher values of rainfall than the first year of measurements (Fig. 2A). Mean temperature in November was similar for both years, but in January and March, the second year had 2 C° more than the first year (Fig. 2B).

Except for P, significant differences were found in soil characteristics among sites (Table 1). Tres Marías presented higher contents of C and N, meanwhile Morro Chico had the lowest pH value (5.31) and the highest bulk density (0.98 g cm^{-3}). Likewise, Cancha Carreras showed higher values of C:N and C:P ratios meanwhile Morro Chico presented the lowest C:P ratio. Land use also presented significant differences in P, pH, C:N ratio and C:P ratio (Table 1). PF had more soil P and higher C:N ratio than other land use soils. OS presented the lowest C:N ratio and the highest C:P ratio (Table 1).

C and N in microbial biomass

Significant differences were found in SMB-C according to site, land use and year (Table 2). Morro Chico presented the lowest SMB-C (721.4 μg C g^{-1}) whereas Tres Marías had the highest (1216.6 μg C g^{-1}). For land use, the gradient PF > SPF > OS was observed (Table 2). Likewise, significant differences were found in SMB-C according to year, being higher in the second year (Table 2). In contrast, no significant differences were found between seasons (Table 2). Furthermore, significant interactions were found: land use x year and season x land use x year (Table 2).

For SMB-N, significant differences were found according to site, land use, year and season (Table 3). SMB-N was higher in Tres Marías (164.18 μg N g^{-1} soil) and lower in Morro Chico (82.4 μg N g^{-1} soil). In land use, SPF had significantly lower SMB-N (110.9 μg N g^{-1} soil) than PF (137.5 μg N g^{-1} soil) and OS (141.76 μg N g^{-1} soil) (Table 3). As in SMB-C, SMB-N was higher in the second year of measurements, and contrary to SMB-C, in SMB-N differences were found according to season, being values in summer significantly higher than spring (Table 3).

Across all sites, SMB-C showed significant positive correlations with soil C, P, N and pH (Table 4). The highest correlation value was for soil P (r = 0.77) followed by C (r = 0.67) and N (r = 0.58). SMB-N also showed significant positive

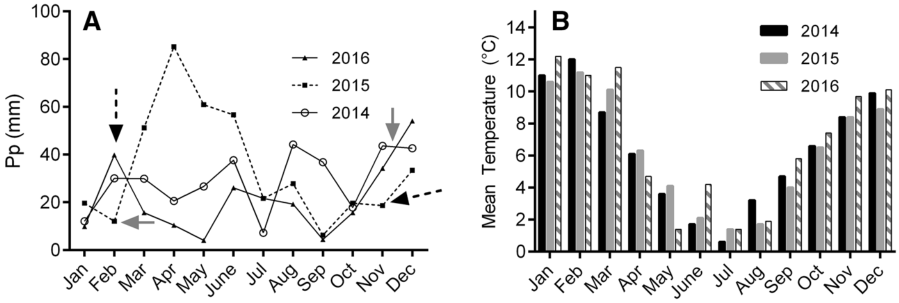

Fig. 2 **A** Monthly precipitation (pp) and **B** mean temperature from the study sites for 2014, 2015 and 2016. Grey arrows indicate the moment of sampling in the first year of study and dotted black arrows indicate sampling in the second year

Table 1 Soil characteristics from sites and land uses studied in *Nothofagus antarctica* forests located in Santa Cruz, south Patagonia, Argentina

	C (g kg^{-1})	N (g kg^{-1})	Olsen-P (g kg^{-1})	pH	C:N	C:P	Bulk density (g cm^{-3})
Site							
Cancha Carreras	63.49 a	4.65 a	0.019 a	5.73 a	13.65 a	3341.58 ab	0.74 a
Tres Marías	104.53 b	8.29 b	0.033 a	5.79 a	12.61 b	3167.57 a	0.61 a
Morro Chico	40.18 a	3.11 a	0.019 a	5.51 b	12.92 b	2114.74 b	0.98 b
Significance	< 0.0001	< 0.0001	ns	0.037	0.0024	0.028	0.0004
Land use							
Primary forest	77.48 a	5.53 a	0.043 a	5.90 a	14.2 a	1801.86 a	0.70 a
Silvopastoral forest	65.67 a	5.03 a	0.023 b	5.82 a	13.05 b	2898.69 a	0.87 a
Open site	65.06 a	5.48 a	0.005 b	5.31 b	11.87 c	13,012.0 b	0.77 a
Significance	ns	ns	0.0002	< 0.0001	< 0.0001	< 0.0001	ns

Different letters indicate significant differences, ns = not significant, (n = 45)

Table 2 Statistical analysis (ANOVA) of microbial biomass carbon (µg C g^{-1} soil) according to different sites, land use (PF, SPF, OS), season and year of measurement, in *Nothofagus antarctica* forests in south Patagonia, Argentina (n = 108)

Variability source	Total square sum	Freedom degrees	Mean square	F value	*p* value
Model	30,532,037	35	872,343.91	20.48	< 0.0001
Site	4,724,826.53	2	2,362,413.27	55.45	< 0.0001
Land use	16,260,514.5	2	8,130,257.25	190.83	< 0.0001
Season	289,198.87	1	289,198.87	6.79	0.05
Year	3,577,111	1	3,577,111	83.96	< 0.0001
Significant interactions					
Season × year					< 0.0001
Land use × year					< 0.0001
Season × land use × year					0.0014

	Tukey test Mean	α = 0.05 n	
Site			
Morro Chico	721.41	36	a
Cancha Carreras	1082.86	36	b
Tres Marías	1216.6	36	c
Land use			
Open site	482.12	36	a
Silvopastoral forest	1130.64	36	b
Primary forest	1408.11	36	c
Season			
Summer	955.21	34	a
Spring	1058.7	34	a
Year			
First year	824.96	18	a
Second year	1188.95	18	b

Table 3 Statistical analysis (Two-way ANOVA) of soil microbial biomass nitrogen (μg N g^{-1}soil) according to different sites, land use (primary forest, silvopastoral forest and open site), season (summer and spring) and year of measurement in south Patagonia, Argentina (n = 108)

Variability source	Total square sum	Freedom degrees	Mean square	F value	*p* value
Model	342,558.75	35	11,812.37	13.3	< 0.0001
Site	111,710.71	2	55,855.36	62.87	< 0.0001
Land use	16,165.55	2	8082.78	9.1	0.0004
Season	33,468.93	1	33,468.93	37.67	< 0.0001
Year	17,958.49	1	17,958.49	20.22	< 0.0001
Significant interactions					
Site × land use					0.0312
Site × season					0.0024
Land use × season					0.0001
Land use × year					0.0007
Site × land use × season					0.0011

	Tukey test	α = 0.05	
	Mean	n	
Site			
Morro Chico	82.39	36	a
Cancha Carreras	147.11	36	b
Tres Marías	164.18	36	b
Land use			
Silvopastoral forest	110.93	36	a
Primary forest	137.47	36	b
Open site	141.76	36	b
Season			
Summer	145.58	34	a
Spring	109.69	34	b
Year			
First year	114.72	18	a
Second year	155.98	18	b

correlations with soil variables although values were slightly lower (Table 4).

Soil potential respiration

Soil potential respiration differed among sites and land use (Fig. 3A, B). In general, respiration was higher in Tres Marías and lower in Morro Chico (Fig. 3A). Likewise, significant differences were found in days 7, 28, 41, 140 and 202, where PF had the highest values and OS the lowest (Fig. 3B). The total cumulative respiration was 8474, 11,234 and 13,283 mg C kg^{-1} for Morro Chico, Cancha Carreras and Tres Marías, respectively (Fig. 3C), meanwhile according to land use it was 9657, 10,560 and 12,780 mg C kg^{-1} for SPF, OS and PF, respectively (Fig. 3D).

The qCO$_2$ calculated differed significantly according to land use, and values were 0.07 CO$_2$-C emitted/

Table 4 Pearson linear correlation coefficients (r) among soil chemistry variables and soil microbial biomass in sites of south Patagonia, Argentina

	Microbial biomass-C	Microbial biomass-N
C	0.67 (0.00012)	0.6 (0.00091)
P	0.77 (< 0.001)	0.65 (0.00025)
N	0.58 (0.0016)	0.41 (0.04)
pH	0.67 (0.00014)	0.63 (0.00042)
Bulk density	ns	ns

In parenthesis is shown the *p* value, ns = not significant unit of SBM-C /day for PF and SPF and 0.17 CO_2-C emitted/unit of SBM-C /day for OS, respectively (Fig. 3F). In contrast, no significant differences were found in qCO_2 according to sites (Fig. 3E).

Discussion

In this study, SMB-C varied according to site, land use and year. Tres Marías ranch presented the highest values, probably because this site also had higher

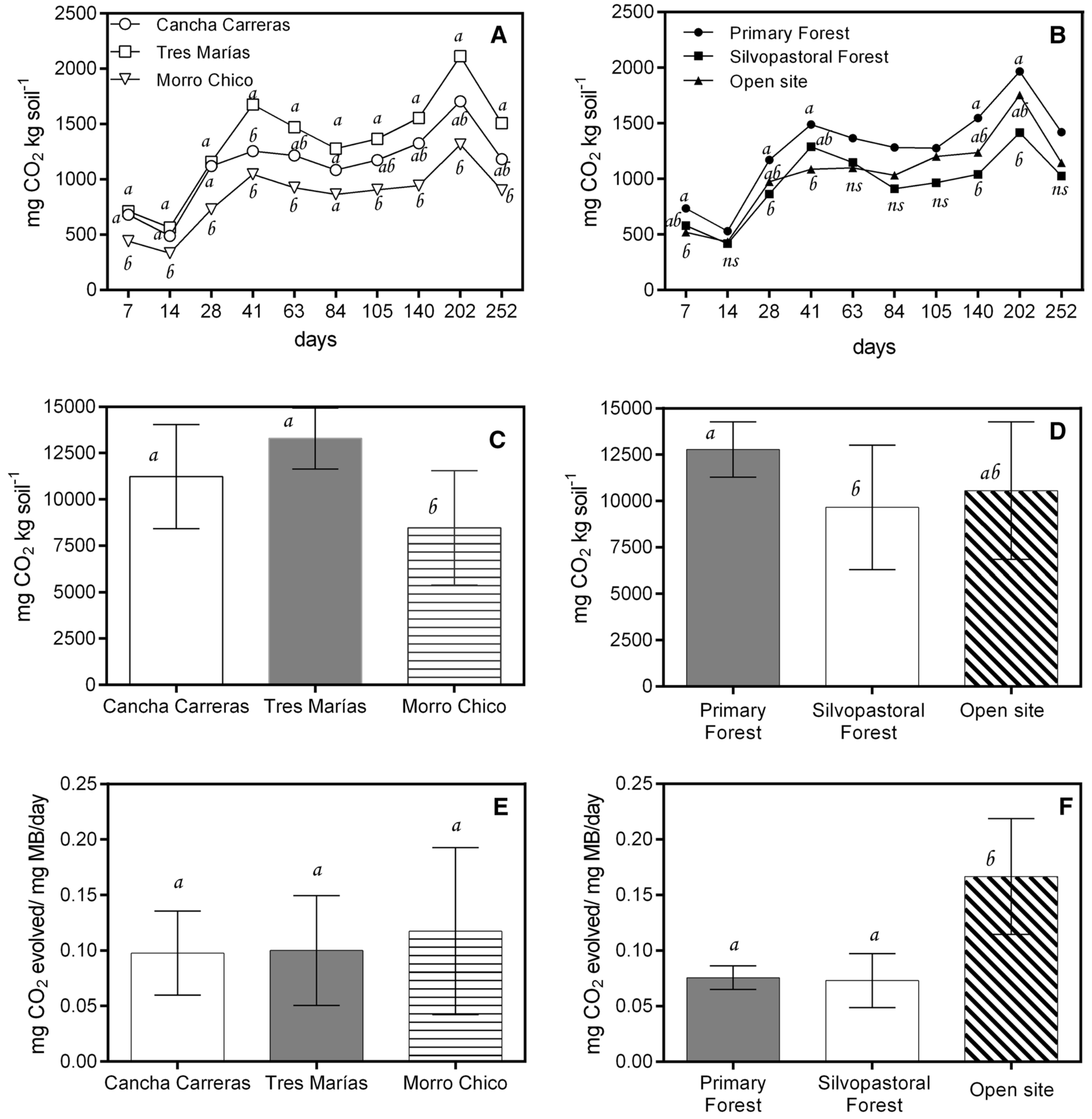

Fig. 3 Accumulated potential respiration (**A**), by date and forest condition (**B**), total accumulated respiration by site (**C**) total accumulated respiration by forest condition (**D**) metabolic quotient by site (qCO2) (**C**) and metabolic quotient by forest condition (**D**) for different soil in south Patagonia, Argentina: primary *N. antarctica* forest (PF), silvopastoral *N. antarctica* forest (SPF) and adjacent open site (OS). Vertical bars indicate standard deviation of the mean. Different letters indicate significant differences

values in soil C, N and P, and there were strong positive correlations among these soil variables and SMB-C. This is in concordance with Toledo et al (2021) who presented similar correlations in an environmental gradient in south Patagonia, and with Satti et al. (2003), who informed positive correlations between SMB-N and soil C and N, in northern Patagonian forests.

Our results support the hypothesis that silvopastoral use of *N. antarctica* forests affects soil microbial biomass, since SPF presented significantly lower SMB-C than PF. Many factors could explain this decrease. To install SPF, some trees are removed to stimulate grass growth, and this involves microclimatic changes like more light that reaches the ground, less soil moisture, etc. (Bahamonde et al. 2012). All these changes could affect SMB-C. Toledo et al (2021) reported higher SMB-C in wetter sites in an environmental gradient in south Patagonia. In our study, PF with higher tree cover, probably conserve better soil moisture than SPF, and this could result in higher SMB-C. Otherwise, Peri et al. (2016a, b) reported that the removal of trees through thinning practices for silvopastoral use of *N. antarctica* forest in Patagonia caused a decrease of 35–50% in the contribution of litter to soil forest. This minor litterfall contribution may change the quality and availability of substrate for soil microorganisms in SPF. Furthermore, differences in SMB-C between PF and SPF could be related with soil P, since PF had almost twice more soil P than SPF. This higher soil P in PF is probably due to more abundant tree roots and the fact that *Nothofagus* usually presents associations with ectomycorrhizas which could facilitate P acquisition (Diehl et al. 2008). Our results are concordant with Viruel et al (2020) who reported that livestock silvopastoral system significantly affected soil bacterial communities in dry Chaco, Argentina. These authors reported higher soil microbial biomass values under tree canopy, compared with values between tree rows, in a *Prosopis alba*/*Chloris gayana* silvopastoral system in dry Chaco. Furthermore, Viruel et al (2020) indicated that *Prosopis* and *Leucanea* improved TN and SOC under tree canopy, but this effect decrease between tree rows, where TN and SOC values were lower than in pure pasture samples. They attribute this to resource depletion under grasses installed between tree rows, and a resource accumulation under trees (Viruel et al. 2020). Something similar could be happening in our study, where grasses in SPF could produce a resource depletion compared with PF. SMB-C values found in our study in forest sites were similar to those reported by Dube et al. (2009) in a *N. pumilio* forest (5–10 cm depth) in northern Chilean Patagonia (1011.8 µg C g^{-1}). They were also in the range of those reported by Joergensen et al. (1995) for beech soils in Germany for a pH of around 6 (958 µg C g^{-1}), and lower than those reported by Ross et al. (1996) in a *N. solandri* forest in New Zealand in 0–10 cm depth (1670 µg C g^{-1}).

In our study, OS presented lower values in SMB-C compared with other land uses. This agree with many authors that claim that the implementation of agroforestry or silvopastoral systems increase C storage in soils in silvopasture compared with treeless pastures (Haile et al. 2008; Takimoto et al. 2009; Howlett et al. 2011). Thus, severe harvesting of *N. antarctica* trees to install grazing areas could serially impact on soil microorganisms abundance, and in consequence, in soil carbon. According to this, Gargaglione et al (2014) comparing grasses growing under *N. antarctica* silvopastoral systems vs. grasses in open sites reported that trees may ''facilitate'' N absorption by grasses, increasing herbaceous N content. The higher SMB-C in forests in our study could be related with more favorable conditions for microorganisms in forest soils compared with grasslands, since grassland soils displayed a soil C:P ratio ten times higher than forest sites, which probably denotes a P deficiency for microorganisms in OS. Despite of the presence of tree ectomycorrhizas which could turn P more available in forest environments, differences in SMB-C between forest and OS could be related with the quality of the detritus that reaches the soil. Litter fall from *N. antarctica* trees, present higher N and P concentrations than litter from grasses in OS (Bahamonde et al. 2012). The SMB-C values found in OS in our study were lower than those reported by Dube et al. (2009) for a degraded grassland (1169 µg C g^{-1}) in northern Chilean Patagonia and higher than those reported by Gonzalez Polo et al. (2019) for a grassland (149 µg C g^{-1}) in Río Negro, north Patagonia, Argentina. These differences between north and south Patagonia could be related to differences in climatic conditions (higher temperature and annual precipitations) or differences in litter quality.

Microbial potential respiration also was affected by site and land use. As expected, the site with higher SMB-C, Tres Marías, presented the highest respiration. This is concordant with Toledo et al (2021) who reported that soil potential respiration in Patagonian grasslands was higher in those sites with more SMB-C and better soil conditions. In our study, total accumulative respiration showed the gradient PF > OS > SPF indicating that in SPF microorganisms were less active, probably owing to some limiting factor like less soil P or moisture, or lower quality or availability of substrate (Zheng et al. 2009).

Another interesting result found in our study was that the metabolic quotient did not vary according to sites, although it varied according to land use. We found that qCO_2 was significantly higher in OS compared with forest sites, indicating that in grasslands microorganisms were less efficient probably due to stress caused by nutrient limitations, lower quality substrates or water deficit, since these sites are exposed to strong winds from the SW that usually reaches 100 km/h. Nutrient limitations were quite visible for soil P, where OS presented the highest C:P ratio. In concordance, Spohn and Chodak (2015) reported that qCO_2 was strongly correlated with the C:P ratio in beech soils. These authors claimed that P limitation of the microorganisms possibly caused the positive correlation of the qCO_2 and the soil C:P ratio. Likewise, Manzoni et al. (2010, 2012) reported that microorganism's release more C in the form of CO_2 when substrate is nutrient poor according to microorganisms' nutritional requirements. Thus, we suspect that the higher qCO_2 in OS were closely related to the C:P ratio in soils that produced P restriction in OS microorganisms.

Regarding to SMB-N we found that this varied according to site, land use, season and year. Morro Chico presented the lowest SMB-N, probably related with their lower soil N content. Likewise, SPF had significantly lower SMB-N than other environments. Satti et al. (2003) reported that microbial N pool was closely linked to the quantity and quality of the soil carbon and its availability to heterotrophic microorganisms. However, in our study, no significant differences were found in soil C or N according to land use, indicating that in this case, may be another factor is limiting microorganisms. As was explained with SMB-C, probably this decrease is related with a P or water limitation or lower litter quality in SPF.

Finally, we observed higher SMB-C and SMB-N in the second year of measurements, probably related to differences in rainfall between years. It is well known that microbial biomass may change with precipitation or temperature (Cregger et al. 2012; Liu et al. 2016). In Patagonian ecosystems, soil profiles recharge water in winter, and this is usually the main reserve to plant growth in the following growing season (Fabricante et al. 2009; Jobbágy and Sala 2000). In our study, winter in 2015 had considerably more precipitation than winter in 2014, and this probably resulted in a higher grass production and soil microbial turnover during spring and summer of 2016. In addition, in February of 2016, rainfall doubled compared with February of 2015, and this could also explain the increase in SMB-C and SMB-N observed in the second year of our study.

Conclusions

In this study we report the first data of SMB-C and SMB-N and potential respiration for south *N. antarctica* forests and their silvopastoral use. The silvopastoral use of *N. antarctica* forests decreased SMB-C and this should be taken into account if *N. antarctica* trees are going to be remove to install silvopastoral systems, because, depending of thinning intensity, this clearly could affect soil microorganisms, and in consequence, soil carbon storage. Furthermore, SMB-C, SMB-N and respiration varied according to site and year, indicating that in these austral systems, as in many other global ecosystems, microorganisms abundance clearly change according to soil and climate conditions.

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