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Effects of land use types and spatial heterogeneity on soil microbial biomass and activity along environmental gradients in Austral ecosystems

Santiago Toledo [a,*], Guillermo Martínez Pastur [b], Julián Rodríguez Souilla [b], Pablo L. Peri [a,c]

[a] *Instituto Nacional de Tecnología Agropecuaria (INTA), Mahatma Gandhi 1322, Río Gallegos, Santa Cruz 9400, Argentina*

[b] *Centro Austral de Investigaciones Científicas (CADIC), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Houssay 200, Ushuaia, Tierra del Fuego 9410, Argentina*

[c] *Universidad Nacional de la Patagonia Austral (UNPA), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Lisandro De La Torre 860, Río Gallegos, Santa Cruz 9400, Argentina*

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ABSTRACT

Different land use systems can alter the structure and function of soil microorganisms, affecting microbial-mediated processes and potentially undermining consequences for soil fertility and ecosystem health. The effects of land use types (LUTs) on soil microbial attributes remain poorly understood in ecosystems. Therefore, the objectives were: (i) to evaluate the responses of microbial biomass carbon (MBC) and nitrogen (MBN), MBC:MBN, soil basal respiration (SBR), microbial metabolic quotient (qCO_2), and microbial quotient (qMC) to different LUTs, including primary forests (PF), silvopastoral systems (SPS) and grasslands, across an environmental gradient from coastal to inland sites of Southern Patagonia; and (ii) to assess the influence of *Nothofagus antarctica* tree canopies (canopy vs inter-canopy) on these microbial attributes. SPS maintained similar values of MBC, MBC:MBN, and qMC compared to PF, but with significant increasing SBR (40%) and qCO_2 (55%) values. Grasslands decreased MBN (71 mg kg^{-1}), SBR (44 mg C kg^{-1}), and qCO_2, but increased the MBC:MBN compared with PF. Inland sites at higher altitudes showed reductions in MBC (45%), MBN (28%), and MBC:MBN, and increases in qCO_2 (77%) compared to inland and coastal areas at lower altitudes. Moreover, microbial variables (MBC, MBN, SBR, qCO_2, and qMC) were consistently higher under tree canopies than in inter-canopy areas. These findings highlight the importance of conserving forest stands under SPS use to sustain soil microbial functions. Soil microbial attributes could serve as sensitive and effective bioindicators of soil quality and health, offering valuable tools for land use monitoring in ecosystems.

1. Introduction

Soil microorganisms play a fundamental role in essential biological processes and ecosystem services, including the decomposition and mineralization of organic matter, nutrient cycling, soil fertility, and soil health (Fierer et al., 2021). These processes, in turn, influence plant performance, plant community diversity and composition, plant growth, and primary production in terrestrial ecosystems (Saccá et al., 2017). Moreover, microorganisms play essential roles in soil carbon cycling through microbial growth, biomass formation, CO_2 fluxes, and carbon (C) sequestration, thereby contributing to climate change feedback mechanisms (Jansson and Hofmockel, 2020). Anthropogenic activities such as deforestation and land-use changes cause reductions in C stock due to reduced vegetation cover (Bargali et al. 2019; Manral et al., 2020; Babur et al., 2022; Fartyal et al., 2025). Vegetation cover influences the soil aggregates, fertility, and composition of microbial community, which in turn affect the soil microbial biomass and microbial efficiency in carbon utilization (Bargali et al., 2018; Babur et al., 2021). The microbial processes of nutrient cycles are affected by forest vegetation due to the differences in quality and quantity of litters, root exudates and the soil properties (Manral et al., 2023; Babur et al., 2021, 2022). The development of soil health indicators to assess the effects of conservation and management strategies by incorporating microbial variables is receiving increasing attention globally.

Several studies have explored the biogeographic patterns of soil microbial variables across environmental gradients of precipitation and

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* Corresponding author.
E-mail address: toledo.santiago@inta.gob.ar (S. Toledo).

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temperature, as well as regarding vegetation, soils, and ecosystem types (Delgado-Baquerizo et al., 2016; Babur et al., 2021; Toledo et al., 2023). However, the mechanisms through which edaphic, vegetation, and climatic factors interact to shape local microbial communities remain only partially understood (Waldrop et al., 2017). Understanding these interactions is crucial for identifying the major environmental drivers of soil microbial communities and their roles in ecosystem functioning (Saccá et al., 2017; Jansson and Hofmockel, 2020), particularly in vulnerable forest ecosystems subject to climate change and land-use intensification (Gargaglione et al., 2022; Baldrian et al., 2023; Peri et al., 2024a). The environmental heterogeneity that influences microbial community structure and diversity is primarily driven by climate, soil physical and chemical properties, and plant composition (Ma et al., 2019; Kang et al., 2021; Toledo et al., 2023). Environmental gradients can shape different soil microbial communities and their functions, ultimately leading to shifts in belowground ecosystem processes such as decomposition and nutrient cycling (Schimel et al., 2007; Praeg et al., 2020; Hartmann and Six, 2023). Furthermore, the potential impact of different land-use types on soil microbial biomass and activity in both coastal and inland ecosystems remain largely unexplored (Manral et al., 2023; Praeg et al., 2020; Dong et al., 2022). This environmental heterogeneity also plays a crucial role in shaping variations in soil fertility across landscapes and at microenvironmental scales, such as tree canopy cover (Ortiz et al., 2023; Peri et al., 2024b).

Individual plants influence the physical, chemical, and biological soil properties beneath their canopies, primarily through the deposition and subsequent accumulation of litter and biomass, which enrich soil organic matter (Gargaglione et al., 2022; Marquez et al., 2022). In turn, localized soil nutrient patches affect the nutrient uptake and performance of individual plants (Viruel et al., 2021; Adomako et al., 2022) and, ultimately, the growth and activity of soil microorganisms within ecosystems (Toledo et al., 2022). Thus, spatial heterogeneity in forest soils arises from differences in the quality and quantity of litter inputs, fluctuating abiotic conditions (e.g., precipitation, light availability, temperature) under tree canopies, variations in the composition and amount of stemflow, and spatial disparities in water and nutrient uptake by tree roots, among other factors (Toro-Manríquez et al., 2019; Martínez Pastur et al., 2023). Moreover, understanding the distribution of microbial communities is important for developing ecosystem management strategies, particularly regarding land-use intensification (Álvarez et al., 2024; Christel et al., 2024). There is evidence that canopy openings modify microenvironmental soil conditions by increasing soil temperature fluctuations and soil water content (Bahamonde et al., 2012; Gargaglione et al., 2014; Gonzalez-Polo et al., 2019; Ortiz et al., 2023). However, the effect of tree cover on soil microbial attributes—mainly assessed through respiration and microbial biomass—remains inconsistent, with studies reporting no effect, as well as both decreases and increases in microbial activity under tree canopies (Peri et al., 2016; Gonzalez-Polo et al., 2019; Viruel et al., 2021; Babur et al., 2022; Toledo et al., 2022). Therefore, understanding small-scale spatial variations in microbial biomass and activity at the stand level is essential for accurately assessing soil C turnover through microbial growth, biomass formation, and heterotrophic respiration-driven CO_2 fluxes, and climate change regulations in terrestrial ecosystems (Jansson and Hofmockel, 2020; Babur et al., 2022).

Currently, the preservation of native forests is a key goal in forest management, aiming to safeguard biodiversity and maintain the multiple ecosystem services (ES) they provide to society (Martínez Pastur et al., 2020; Rosas et al., 2020; Ulyshen et al., 2023). Moreover, the conversion of forests and grasslands into agricultural lands is a major global concern in the context of environmental degradation and climate change (Kooch et al., 2016; Praeg et al., 2020; Peri et al., 2024a). While the significant impacts of such transformations on plant and animal biodiversity at the landscape level are well recognized (Rosas et al., 2020; Ulyshen et al., 2023), their effects on soil microbial attributes remain poorly understood (Christel et al., 2024). Land-use changes primarily lead to significant alterations in carbon (C) and nitrogen (N) inputs and dynamics, as well as in soil organic carbon (SOC) stocks (Kooch et al., 2016; Chen et al., 2017), which in turn drive soil physical, chemical, and microbial processes (Manral et al., 2023; Kooch et al., 2019). Consequently, grassland and forest soils present contrasting characteristic, particularly in structure, quantity and quality of organic matter, which provide different nutrient and energy sources for microorganisms (Lee et al., 2020; Sun et al., 2020; Dang et al., 2024).

Forest ecosystems are often used as benchmarks for assessing changes in soil microbial variables following land-use transitions (Ma et al., 2015, 2019, 2024; Toledo et al., 2024). Evidence suggests that converting native forests to other land uses leads to shifts in the abundance, diversity, and composition of soil organisms, which can significantly alter key ecosystem processes (Gargaglione et al., 2022; Álvarez et al., 2024). As a response to these challenges, silvopastoral systems (SPS) have emerged as sustainable production alternatives, offering high biodiversity and multifunctionality compared to conventional livestock production systems (Peri et al., 2016). SPS aim to enhance the provision of ES (e.g., timber, non-forest products, livestock) while minimizing negative impacts on regulatory functions (e.g., carbon sequestration, water and erosion regulation) and supporting services (e.g., soil formation, primary production, nutrient cycling), as well as maintaining the cultural values associated with forest ecosystems (Peri et al., 2016; Gonzalez-Polo et al., 2019). Furthermore, both the establishment of silvopasture and its conversion into other land-use systems modify above- and belowground productivity, rooting depth and distribution, and the quantity and quality of organic matter inputs (Marquez et al., 2022; Ortiz et al., 2023). SPS have become a viable alternative for livestock production, enhancing overall productivity, carbon storage, and soil health while reducing heat stress for pastures and livestock, thus providing ecological and economic benefits for local farmers (Peri et al., 2016, 2024a). However, several studies indicate that livestock systems can alter the structure and functionality of SPS ecosystems, affecting biogeochemical cycles, ecological interactions, and biological composition, including soil microbial variables and biochemical properties (Bahamonde et al., 2012; Gargaglione, 2014, 2022; Viruel et al., 2021).

Understanding soil microbiological changes following the implementation of land management systems provides a scientific basis for decisions aimed at promoting sustainable livestock and forest use (Fierer et al., 2021; Baldrian et al., 2023; Hartmann and Six, 2023). This study aims to explore how soil microbial attributes respond to different land-use types across environmental gradients and under the influence of tree canopies, providing key insights for soil conservation in the context of intensified productive systems. The specific objectives of this study were: (i) to evaluate microbial variables (biomass, activity, and microbial coefficient) across different land-use types (primary forests, silvopastoral systems, and grasslands) along an environmental gradient. (ii) To assess the biomass and activity of soil microbial communities under the influence of Nothofagus antarctica canopies. (iii) To analyze the relationships between microbial variables (biomass, activity, and microbial coefficient) and key environmental factors (climate, vegetation, and soil properties) across the environmental gradient in different ecosystem types. We hypothesize that: (i) soil microbial attributes—such as biomass, activity, and microbial coefficients—will respond differently depending on land-use type (primary forests, silvopastoral systems, and grasslands) across an environmental gradient; and (ii) the canopy of Nothofagus antarctica will significantly influence the biomass and activity of soil microorganisms. This knowledge is fundamental for monitoring soil microbial variables in sustainability assessments and understanding how these patterns shift following land-use conversion in Austral ecosystems.

2. Materials and methods

2.1. Study area

This study was conducted in three areas across the natural distribution of *Nothofagus antarctica* (commonly named ñire) forests in Tierra del Fuego, Argentina (Fig. 1). The sampling areas were carried out in three sites along an environmental gradient (54.07 to 54.26° S, and 66.82 to 67.69° W): (i) El Roble ranch (inland at lower altitude = In-LA), with continental conditions inland (minimum 2 °C and maximum 12 °C); (ii) San Pablo ranch, with maritime influence near the coasts of the Atlantic ocean (minimum 0 °C and maximum 10 °C); and (iii) Las Hijas ranch (inland at higher altitude = In-HA) located in a inland landscape with intermediate characteristics. Ñire forests mainly grow in the ecotonal zones between forests and steppes, where livestock ranching is the predominant land use (Martínez Pastur et al., 2021). These forests have lower timber value than other *Nothofagus* forests, but greater understory species richness and biomass, which supports their use for livestock grazing under silvopastoral management, with special interest for conservation (Peri et al., 2016; Rosas, 2020).

The climate in Tierra del Fuego (Argentina) is characterized by short and cool summers, and long snowy winters. Precipitation in the study area ranged from 366 mm yr^{-1} at inland site to 397 mm yr^{-1} in the coast. The mean annual temperature is 5.2 ± 0.1 °C (1.6 °C in the coldest and 9.6 °C in the warmest months) and frost may occur at any time of the year. *Nothofagus* forest soils in Tierra del Fuego have been classified as podzols according to IUSS Working Group WRB (IUSS Working Group WRB, 2014). The landscape occupied by these ecosystems is predominantly of glacial origin, composed of loess and alluvial deposits in the foothill region. Soils in this area have a silty loam texture and slightly acidic pH, and are characterized by high permeability, good drainage, high water retention capacity, and abundant organic matter content (Martínez Pastur et al., 2022).

2.2. Experimental design and sample collection

We followed a two-factor random design, with environmental gradient (EG) and land use types (LUTs) as main factors. The three land use types represent a management intensity gradient: a non-managed native *N. antarctica* pure forest (primary forest, PF) as a control, a

Fig. 1. Location of sampling sites in the environmental gradient across the natural distribution range of *Nothofagus antarctica* forests in Tierra del Fuego, Argentina. Sampling was conducted at three sites: (i) El Roble Ranch (Inland, lower altitude = In-LA), (ii) San Pablo Ranch (Coastal areas), and (iii) Las Hijas Ranch (Inland, higher altitude = In-HA).

thinned forest for silvopastoral purposes (SPS), and grasslands under pastoral uses (G) (Fig. 2A). The PF present >80% of canopy cover, while SPS present a remnant canopy cover of 40–50% with higher understory cover (80–100%) compared to PF (60–80%). The adjacent grasslands (G) presented high vegetation cover (95%), constituted mainly by grasses. Livestock mainly included cattle and occasionally sheep (~8 in km^{-2} and 6 in km^{-2}, respectively), which graze freely in big paddocks (>1000 ha each). *N. antarctica* forests are significantly degraded in certain places of Patagonia, and consequently, silvopastoral systems have become a productive alternative to develop the sustainability of the ecosystems.

The final sampling design (Fig. 2A) included 36 plots (3 areas across EG × 3 LUTs × 4 replicates). In addition, we evaluated the influence of canopies of *N. antarctica* trees (under tree canopy = TC and inter canopy = IC between trees) as a second factor of study (Fig. 2B).

At each plot, three composite soil samples (0–10 cm) were randomly collected using soil auger cores (5.6 cm in diameter) after removing the litter layer (Fig. 2C). The surface soil layer (0–10 cm) was considered for the analysis because most of the microbial activities remain confined to this region (Bargali et al., 2018, 2019; Gargaglione et al., 2022). Besides, to analyze the influence of the crown at forest environments, we collected six soil samples (0–10 cm depth) in each plot of PF and SPS: three samples under the canopy and three in the inter-canopy areas. Soil samples were placed in plastic bags and stored in refrigerated containers until transport to the laboratory for the analyses.

Soil microbial variables were sampled and determined during February 2023 (coincident with the peak of the growing season). For this, we used a subsample of 600 g from the soil samples, which were sieved (2 mm mesh) and then refrigerated at 4°C for one week until microbial variable analyses.

2.3. Environmental and vegetation characteristics

The climate parameters for each site were derived from the WorldClim 2 data set (Fick and Hijmans, 2017) and topography from the Shuttle Radar Topography Mission (Farr et al., 2007). Data contains global geographical surfaces for different climatic and topographic parameters that describe mean annual precipitation (MAP, $mm.yr^{-1}$),

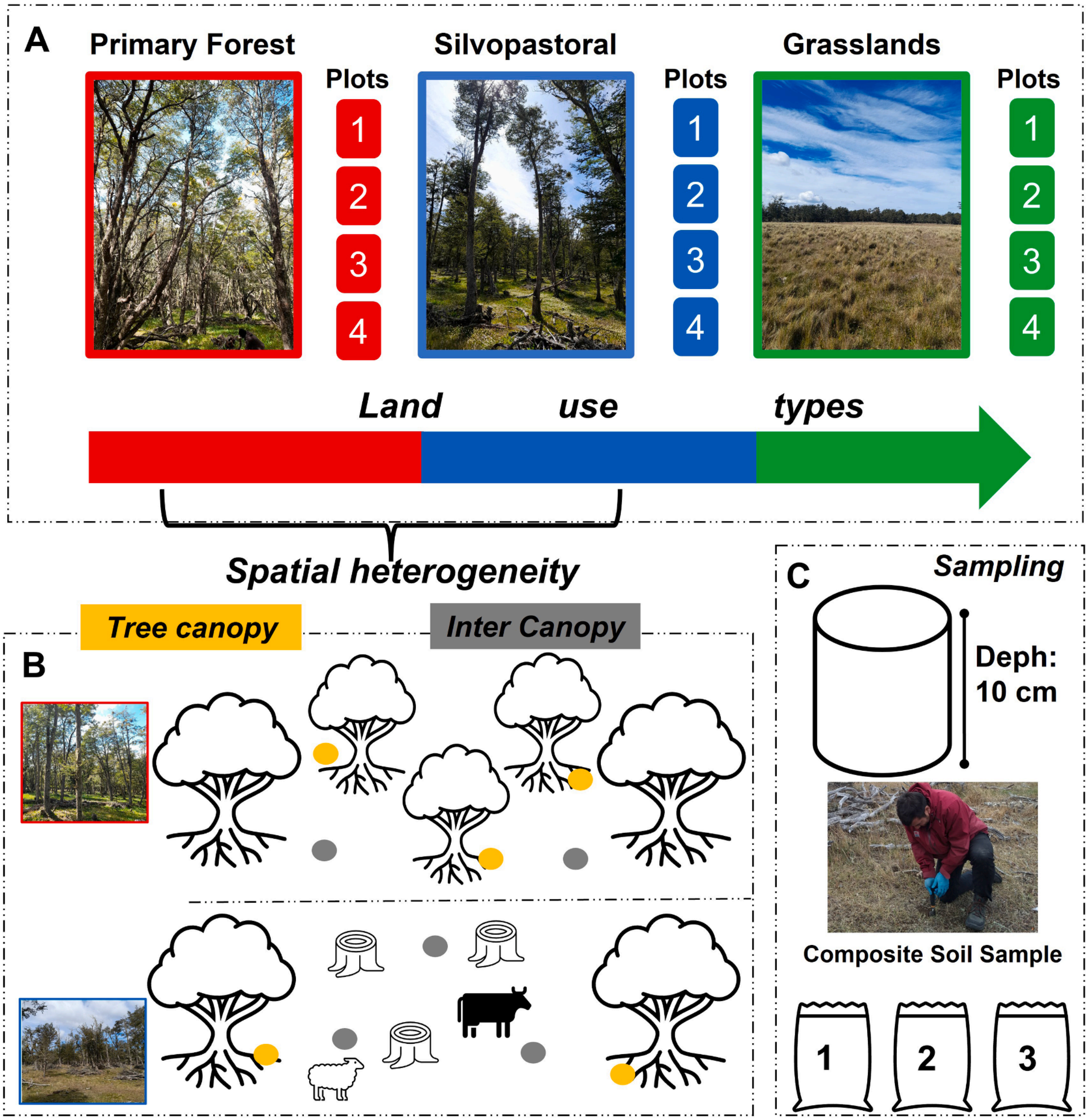

Fig. 2. Details of the (A) three land use types: PF= primary forests (red), SPS= silvopastoral systems (blue), and (G) grasslands (green); (B) analysis of the influence of tree canopies (yellow) and inter canopies (gray) in the *N. antarctica* stands with and without management (PF and SPS); and (C) soil sampling design at each plot.

mean annual temperature (MAT, °C), altitude (m.a.s.l.), expressed at a resolution of 0.0083° (approximately 1 km).

The forest structure was characterized by two sub-plots, using the point sampling method (BAF = 1–5) (Bitterlich, 1984) with a Criterion RD-1000 (Laser Technology, NY, USA). At each sampling-point we measured: (i) dominant height (DH, m), using a TruPulse 200 laser clinometer and distance rangefinder (Laser Technology, NY, USA) by averaging the height of the two taller trees, (ii) tree density (TD, $n.ha^{-1}$), (iii) basal area (BA, $m^2.ha^{-1}$), and (iv) hemispherical photos (Nikon 35 mm and Sigma 8 mm lens) to obtain crown cover (CC, %) using Gap Light Analyzer software (Frazer et al., 2001). At the three ecosystem types (PF, SPS, G) we also measured the understory characteristic using the point-intercept method (Levy and Madden, 1933) with 50 intercept points every 1 m. At each point, we recorded vascular plants (dicots, monocots, and ferns) including tree regeneration less than 1.30 m height. We also recorded the presence data of other species occurrence but were not intercepted by the points. These data were used to determine dominant species and vegetation covers (VC, %), following Moore (1983) and Correa (1998). Understory vegetation biomass (UVB, kg ha^{-1}) was determined at the peak of the growing season in 1 m^2 located in the center area of each plot by clipping up to the soil surface all aboveground biomass in two quadrats of 0.2 × 0.5 m. Then, samples were dried (60 °C) for 48-h to obtain dry matter plant biomass (total, alive, and dead components).

Soil samples were used for different laboratory analyses, including: (i) Soil organic carbon (SOC, %) were derived from the dry combustion (induction furnace) method, using a conversion factor of 0.58 (Peri et al., 2018); (ii) Kjeldahl digestion was used to determine soil total nitrogen (Soil N, %) (Bremner and Mulvaney, 1983); and (iii) Olsen methodology was used to determine soil available phosphorus (Soil P, ppm) (Olsen, 1954).

2.4. *Soil microbial biomass and activity*

Microbial biomass carbon (MBC, mg C kg^{-1} dry soil) was estimated using the chloroform-fumigation extraction method (Vance et al., 1987), calculated as: MBC = (OCf - OCnf)/kEc; Where: OCf = organic C extracted from fumigated samples, OCnf = *C* extracted from non-fumigated samples, and kEc = fumigation efficiency constant = 0.45. Microbial biomass nitrogen (MBN, mg N kg^{-1} dry soil) was determined by a modification of the fumigation–incubation method (Joergensen and Mueller, 1996). Liquid chloroform (1 ml) was added directly to 30 g of moist soil samples, stirred, and left for 20-h in sealed beakers located in desiccators. Chloroform was then removed using a vacuum pump. Fumigated and non-fumigated samples were incubated at field capacity for 10 days at 25 °C. After incubation, samples were extracted with 2 M KCl and analyzed for NH_4-N by the Berthelot reaction. MBN was calculated as the difference in N between fumigated and non-fumigated samples, divided by a correction factor (kN). Non-extractable amount of microbial N was compensated for by a correction factor of kN = 0.54.

Soil basal respiration (SBR, mg C kg^{-1} soil day^{-1}) was estimated by quantifying the carbon dioxide (CO_2) released in the process of basal respiration (heterotrophic) during 7 days of incubation (Robertson et al., 1999). This was done by placing 75 g fresh soil (moistened to 60 % of field capacity) into 1.5 l capacity glass containers with hermetic lids, together with a smaller flask containing 20 ml 0.2 M NaOH to capture the released CO_2. The released CO_2 was determined by titration with 0.1 M HCl, after precipitation of the barium carbonate formed by adding barium chloride ($BaCl_2$) aqueous solution to the NaOH solution, utilizing phenolphthalein as an indicator. Soil microbial metabolic quotient (qCO_2, μg C mg^{-1} MBC d^{-1}) was defined as soil basal respiration per unit of biomass and was calculated as the C-CO_2 evolution of SBR per unit MBC and per unit time (Anderson and Domsch, 1990). Finally, we calculated the microbial quotient: ratio of MBC to total C (qMC = MBC/SOC), expressed as percentage (Anderson and Domsch, 1990) and a ratio of MBC:MBN.

2.5. *Statistical analyses*

Statistical analyses were performed using INFOSTAT software. Data were tested for normal distribution (Shapiro-Wilk test) and homogeneity of variances (Levene's test). The microbial variables (MBC, MBN, MBC:MBN, SBR, qCO_2, qMC) were analyzed with a two-way analysis of variance (ANOVA), using environmental gradient sites (In-LA, In-HA, and Coastal) and land use types (PF, SPS, G) as main factors. In addition, two-way ANOVA was used to determine the effects of canopies of *N. antarctica* trees (TC and IC) on physicochemical (SOC, Soil N, and Soil P) and microbial (MBC, MBN, SBR, MBC:MBN ratio, qCO_2, and qMC) variables at different land use (PF and SPS). Significant differences were compared with the Tukey test at a significance of $p < 0.05$. A principal component analysis (PCA) was used to analyze the relationships among microbial variables (MBC, MBN, SBR, MBC:MBN ratio, and qCO_2), and to visualize the changes and differences in microbial attributes between different land use types, and the influence of canopies of *N. antarctica* trees (tree canopy and inter-canopy) compared to open-lands (grasslands). Additionally, to select explanatory variables by considering land use types and influence of crown cover by the comparison of Pearson's correlation coefficients, which were used to explore the relationships among microbial variables (MBC, MBN, SBR, MBC:MBN ratio, and qCO_2) and soil physicochemical properties (SOC, Soil N, and Soil P), as well as climatic (MAP, MAT, and altitude) and vegetation variables (DH, TD, BA, CC, UVB, and VC).

3. Results

3.1. *Characterization of environmental and vegetation variables*

Climate characterization of sampled areas showed that the MAP varied from 366 to 397 mm yr^{-1}, and MAT fluctuated from 5.03 °C in inland at higher altitude (Las Hijas Ranch) to 5.60 °C in the coast area. Altitudes varied from 53 to 130 m.a.s.l. for the inland and costal sites, respectively (Table 1). Dominant height was higher in forests located at coast areas (10.3 m) compared to inland at lower altitudes (8.3 m) and inland at higher altitudes (8.8 m). Tree density and canopy cover were higher in primary forests stands (>2200 $trees.ha^{-1}$, >78%) than in SPS (343–537 $trees.ha^{-1}$, <69 %). Understory vegetation biomass was higher in grasslands compared with PF and SPS across the studied environmental gradient (Table 1). The dominant plant species were *Poa pratensis, Agrostis stolonifera, Festuca* spp. associated with *Deschampsia flexuosa, Taraxacum officinale*, and *Trifolium repens*. Vegetation cover was lower at high altitudes (Las Hijas ranch), reaching up to 92 to 136%, and higher in inland at low altitudes and coast areas, showing values between 116 and 181% (grasslands), 148 and 238% (silvopastoral systems), and 160 and 221% (primary forests) (Table 1). SOC, SN, and SP contents were higher at the coast compared to the other areas (inland). Additionally, higher SN and SP contents were measured in silvopastoral systems compared to grasslands (Table 1).

3.2. *Response of soil microbial variables to environmental gradients and land use types*

Soil microbial variables significantly varied ($p < 0.05$) depending on the environmental gradients and land use types (Table 2). Soil MBC and qMC followed similar trend: inland at high altitude < inland at low altitude < coast. MBN measured in inland at high altitude (116 mg kg^{-1} dry soil) was significantly lower than inland at low altitude and coastal areas (28% and 44%, respectively). Also, inland at higher altitude area significantly decreased MBC:MBN compared to inland and coast areas at low altitudes. However, there was no effects of the studied environmental gradient over SBR (Table 2). Regarding the land use types, MBN, SBR and qCO_2 were significantly higher in forests (PF and SPS)

Table 1

Characterization of the study sites along environmental gradients at different ecosystem types in Tierra del Fuego (Argentina). Geographic location, climate (MAP=mean annual precipitation, MAT=mean annual temperature), forest structure and understory plants, and soil chemical (SOC: soil organic carbon, soil nitrogen (N), and soil phosphorus (P)) in the different land use types (*G* = grasslands, SPS = silvopastoral systems, PF = primary forests). Values are mean and standard deviation (±SD).

Environmental factors	Inland at lower altitude			Inland at higher altitude			Coasts		
	G	SPS	PF	G	SPS	PF	G	SPS	PF
Latitude (S)	−54.093	−54.090	−54.071	−54.208	−54.214	−54.213	−54.265	−54.262	−54.263
Longitude (W)	−67.685	−67.693	−67.659	−67.272	−67.295	−67.280	−66.828	−66.827	−66.831
Climate									
MAP (mm.yr^{-1})	379.9 ± 20.3			366.6 ± 12.1			397.0 ± 31.2		
Altitude (m.a.s.l)	57.3 ± 5.5			130.5 ± 6.5			53.5 ± 4.5		
MAT (°C)	5.25 ± 0.5			5.03 ± 0.3			5.60 ± 0.4		
Vegetation									
Dominant height (m)	–	8.5 ± 1.7	8.3 ± 2.1	–	10.1 ± 2.4	8.8 ± 1.6	–	11.6 ± 1.5	10.3 ± 2.0
Tree density (n ha^{-1})	–	537 ± 47	2239 ± 52	–	343 ± 22	2384 ± 61	–	395 ± 31	2404 ± 59
Basal area (m^{-2} ha^{-1})	–	22 ± 3.5	40 ± 6.1	–	17 ± 1.9	34 ± 5.4	–	20 ± 2.2	42 ± 7.0
Canopy cover (%)	7.1 ± 1.1	52.7 ± 7.4	78.9 ± 8.4	14.0 ± 2.3	46.4 ± 3.9	86.4 ± 7.5	2.1 ± 1.0	69.8 ± 4.6	89.2 ± 6.4
Understory biomass (kg ha^{-1})	5736 ± 258	1677 ± 72	1148 ± 57	1994 ± 102	1710 ± 51	1139 ± 39	6357 ± 852	5429 ± 369	1116 ± 28
Dominant species	*Festuca gracillima; Empetrum rubrum; Deschampsia flexuosa; Trisetum spicatum*	*Poa pratensis; Azorella trifurcata; Hieracium pillosella; T. spicatum*	*P. pratensis; Festuca magellanica; Taraxacum officinale; Rumex acetosella*	*Agrostis stolonifera; Festuca ovina; F. magellanica; P. pratensis*	*P. pratensis; A. stolonifera; T. officinale; F. magellanica*	*F. magellanica; P. pratensis; D. flexuosa; T. officinale*	*P. pratensis;Carex curta; Alopecurus magellanicus; Deschampsia flexuosa*	*P. pratensis; Bromus unioloides; T. officinale; Trifolium repens*	*T. officinale; Osmorhiza depauperata; T. repens; P. pratensis*
Vegetation cover (%)	116 ± 12	148 ± 9	160 ± 11	136 ± 5	92 ± 7	99 ± 8	181 ± 18	238 ± 24	221 ± 22
Soil									
SOC (%)	5.8 ± 0.6	4.9 ± 0.5	7.4 ± 1.4	7.6 ± 1.3	6.7 ± 0.5	7.8 ± 1.3	7.9 ± 1.62	7.3 ± 2.1	7.5 ± 0.3
Soil N (%)	0.26 ± 0.04	0.29 ± 0.05	0.37 ± 0.09	0.29 ± 0.06	0.39 ± 0.08	0.45 ± 0.07	0.41 ± 0.13	0.51 ± 0.14	0.40 ± 0.09
Soil P (ppm)	17.4 ± 1.0	18.0 ± 1.9	20.1 ± 1.9	16.3 ± 0.4	22.2 ± 4.4	23.2 ± 1.8	20.9 ± 1.9	24.7 ± 3.2	21.4 ± 4.9

Table 2

Two-way ANOVA for soil biological variables along the studied environmental gradient (EG): inland at lower altitude (In-LA), inland at higher altitude (In-HA), and coast with different land use types (LUTs): grasslands (G), silvopastoral systems (SPS), and primary forests (PF) as main factors in Tierra del Fuego (Argentina). MBC = microbial biomass carbon (mg kg^{-1} dry soil), MBN = microbial biomass nitrogen (mg kg^{-1} dry soil), SBR = soil basal respiration (mg C-CO$_2$ kg^{-1} dry soil d^{-1}), MBC:MBN ratio, qCO$_2$ = metabolic quotient (μgC-CO$_2$ mg^{-1} MBC d^{-1}), qMC = microbial quotient (%). Mean values and standard deviation are shown. Values followed by different letters in each column showed significant differences among environmental gradient (In-LA, In-HA, and coast) and land use types (G, SPS, and PF) using Tukey's test at 5% probability ($p < 0.05$).

Treatments	Levels	MBC	MBN	SBR	MBC:MBN	qCO$_2$	qMC
Environmental gradient	In-LA	794±114 b	149±83 a	81±49 a	7.4 ± 3.8 b	0.10±0.07 a	1.28±0.18 b
	In-HA	547±127 a	116±52 b	78±52 a	5.7 ± 2.3 a	0.16±0.10 b	0.88±0.20 a
	Coast	985±186 c	167±51 a	95±40 a	6.8 ± 2.6 b	0.09±0.02 a	1.64±0.31 c
	F(p)	*55.1(< 0.05)*	*14.4(< 0.05)*	*1.3(0,26)*	*10.9(< 0.05)*	*5.2(< 0.05)*	*65.2(< 0.05)*
Land use types (LUTs)	G	754±261 a	71±27 a	44±25 a	10.8 ± 2.6 b	0.06±0.03 a	1.27±0.49 a
	SPS	783±253 a	151±38 b	123±50 c	5.3 ± 1.3 a	0.17±0.09 c	1.26±0.41 a
	PF	790±171 a	211±52 c	88±25 b	3.9 ± 0.7 a	0.11±0.04 b	1.27±0.27 a
	F(p)	*0.3(0,72)*	*94.8(< 0.05)*	*25.1(< 0.05)*	*163.8(< 0.05)*	*20.7(< 0.05)*	*0.03(0,97)*
Interactions: EG x LUTs							
F(p)		*2.82(< 0.05)*	*5.90(< 0.05)*	*2.44(< 0.05)*	*6.95(< 0.05)*	*3.08(< 0.05)*	*4.06(< 0.05)*

compared to grasslands, while MBC:MBN was significantly lower in forests (PF and SPS) compared to grasslands. We did not detect effects of land use types on MBC and qMC. Significant interactions were detected between the factors for all microbial variables (see Table 2).

The interactions for MBC showed that PF and grasslands did not differ in this fraction of soil carbon between coast and inland areas at low altitudes, but land use types significantly decreased MBC at the inland at high altitude. However, MBC in SPS showed similar values in both inland areas but differed significantly with the coast area (Fig. 3A). SBR determined that while in the inland areas, grassland had lower SBR values than PF and SPS, there was no difference among treatments in coast area (Fig. 3B). MBN was significantly higher in forests (PF and SPS) than in grasslands at inland and coast areas, while no detectable differences were observed for MBN between PF and SPS at the inland at higher altitudes and in coasts (Fig. 3C). The metabolic quotient in the coast area did not change across the land use types (Fig. 3D), however, qCO$_2$ at inland at lower altitudes was significantly lower in the grasslands compared with forests (PF and SPS), and also had differences under SPS use in inland at high altitudes compared with grasslands and PF (Fig. 3D). Besides, there were significant differences among land use types and studied areas in the MBC:MBN ratio, where grasslands had higher values than forests (PF and SPS) at inland and coast areas. However, in the inland at low altitude, the MBC:MBN ratio was significantly higher in grassland than in forests and had higher values in PF

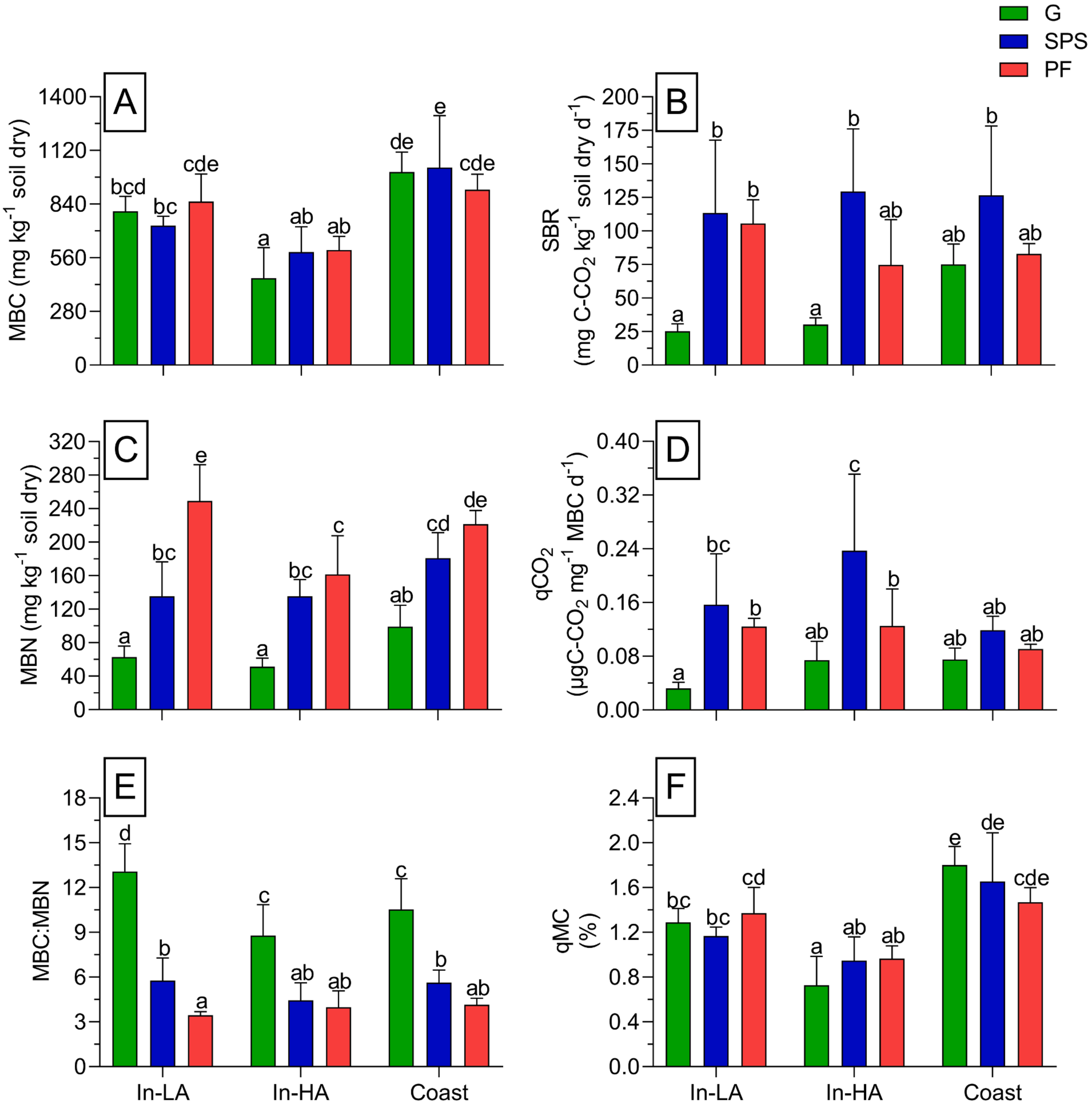

Fig. 3. Two-way ANOVA for the soil microbial variables in the three land use types (*G* = grasslands, SPS = silvopastoral systems, PF = primary forests) across the environmental gradient (In-LA = inland at lower altitude, In-HA = inland at higher altitude, and coast areas) in Tierra del Fuego, Argentina. (A) microbial biomass carbon (MBC, mg kg^{-1} dry soil), (B) soil basal respiration (SBR, mg C-CO$_2$ kg^{-1} dry soil d^{-1}), (C) microbial biomass nitrogen (MBN, mg kg^{-1} dry soil), (D) microbial metabolic quotient (qCO$_2$, μgC-CO$_2$ mg^{-1} MBC d^{-1}), (E) MBC:MBN ratio, and (F) microbial quotient (qMC, %). Mean values and standard deviation are presented, where different letters indicate significant differences using Tukey's test at 5% probability ($p < 0.05$).

than in SPS (Fig. 3E). Moreover, qMC in the grasslands was significantly higher in coast and inland areas at lower altitude than at inland at higher altitudes. However, qMC in SPS showed similar values at both inland areas, significantly differing from coast areas which had the higher values. In forests, qMC showed similar values at lower altitude areas (coast and inland) that significantly differ when are compared to inland at higher altitudes (Fig. 3F).

3.3. Response of soil microbial variables to crown cover of the overstory

Soil characteristics showed differences between areas independently of the overstory canopy, both at PF and SPS. SOC and SN were higher under the tree canopy compared to inter canopies areas, where SOC increased 25%, and SN 29% (Table 3). The soils biological properties showed a significant variation between treatments (Table 3). Microbial variables were higher in the soils under the canopies compared to inter-canopy areas, where MBC increased 16%, MBN 25%, SBR 44 %, qCO$_2$ 39%, and qMC 15%. However, MBC:MBN was higher (14%) in the inter-canopy areas compared to under the canopy microsite for both treatments (PF and SPS). However, significant interactions were detected between factors (forest types and the influence of canopies) for SBR and qCO$_2$ variables (Table 3).

The interaction between forest types and tree canopy for MBC, MBC:MBN ratio, and qMC showed that primary forest and silvopastoral systems did not significantly differ in this fraction of soil carbon between canopy treatments (Fig. 4A, E and F). However, MBC:MBN ratio in silvopastoral systems showed higher values TC and IC areas compared to primary forests, which presented the lower values (Fig. 4E). On the other hand, SBR and MBN clearly showed a spatial heterogeneity between crown cover and land use (PF and SPS), with significant differences

Table 3

Two-way ANOVA of influence of tree canopy (TC = under de tree canopies, IC = inter-canopy areas) at different forest treatments (PF = primary forests and SPS = silvopastoral systems) for the physicochemical (soil organic carbon (SOC, %), soil nitrogen (N, %), soil phosphorus (P, ppm), and soil moisture (%) and microbial variables (microbial biomass carbon (MBC, mg kg^{-1} dry soil), microbial biomass nitrogen (MBN, mg kg^{-1} dry soil), soil basal respiration (SBR, mg C-CO_2 kg^{-1} dry soil d^{-1}), MBC:MBN ratio, metabolic quotient (qCO_2, μgC-CO_2 mg^{-1} MBC d^{-1}), and microbial quotient (qMC, %) in *Nothofagus antarctica* forests of Tierra del Fuego. Mean values and standard deviation are presented. Values followed by different letters in each column showed significant differences among TC and IC in PF and SPS using Tukey's test at 5% probability ($p < 0.05$).

Factors	Levels	SOC	Soil N	Soil P	Soil Moisture		
PF	TC	7.9 ± 0.5ab	0.34±0.07a	23.7 ± 3.0b	33.8 ± 10.1b		
	IC	6.3 ± 0.7a	0.36±0.09a	19.3 ± 2.8a	28.3 ± 8.5a		
SPS	TC	8.7 ± 3.3b	0.48±0.19b	19.1 ± 2.2a	35.7 ± 12.0b		
	IC	6.7 ± 1.9a	0.34±0.08a	24.1 ± 4.7b	27.1 ± 8.9a		
	F(p)	*14.1 (<0.05)*	*8.7(<0.05)*	*19.5(<0.05)*	*5.7(<0.05)*		
		MBC	**MBN**	**SBR**	**MBC:MBN**	**qCO_2**	**qMC**
Tree canopy cover	TC	854±264b	206±48b	135±40b	4.2 ± 1.2a	0.18±0.09b	1.37±0.42b
	IC	719±119a	155±49a	76±19a	4.9 ± 1.2b	0.11±0.04a	1.16±0.19a
	F(p)	*6.3(<0.05)*	*26.9(<0.05)*	*97.3(<0.05)*	*7.8(<0.05)*	*18.7(<0.05)*	*6.3(<0.05)*
Interactions *F(p)*		*0.03(0.85)*	*0.12(0.73)*	*16.5(<0.05)*	*0.49(0.48)*	*4.83(<0.05)*	*0.03(0.85)*

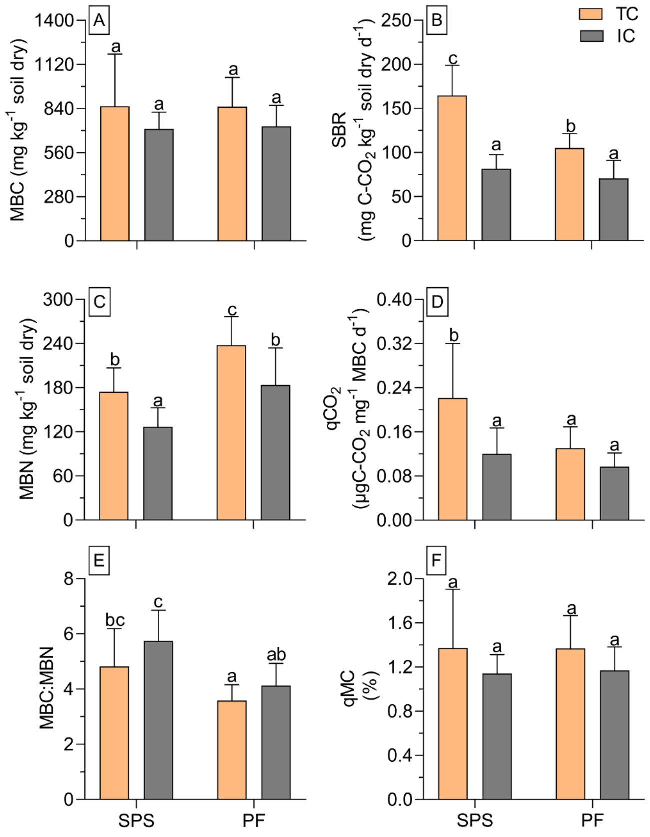

Fig. 4. Two-way ANOVA for the soil microbial variables under de tree canopies (TC) and inter canopy areas (IC) at different treatments (PF = primary forests and SPS = silvopastoral systems) in *Nothofagus antarctica* forests of Tierra del Fuego (Argentina). (A) microbial biomass carbon (MBC, mg kg^{-1} dry soil), (B) soil basal respiration (SBR, mg C-CO_2 kg^{-1} dry soil d^{-1}), (C) microbial biomass nitrogen (MBN, mg kg^{-1} dry soil), (D) microbial metabolic quotient (qCO_2, μgC-CO_2 mg^{-1} MBC d^{-1}), (E) MBC:MBN ratio, and (F) microbial quotient (qMC, %). Mean values and standard deviation are presented, where different letters indicate significant differences using Tukey's test at 5% probability ($p < 0.05$).

between TC levels in primary forests and SPS, where under TC soils had higher values of SBR and MBN than IC areas (Fig. 4B and C). SBR in SPS was significantly higher under TC than in primary forests, while MBN at the different land use types showed higher values under TC, that significantly differed compared to SPS (Fig. 4B and C). The qCO_2 in SPS was significantly higher under TC soils than in IC areas, however, no differences were found for qCO_2 between TC treatments in PF (Fig. 4D).

3.4. Relationships of soil microbial variables with environmental factors

From the principal components analysis (PCAs), differences in soil microbial variables determined a clear separation between land use types, such as grasslands and primary forests (Fig. 5A), and under the tree canopy microsite of forests (PF and SPS) compared with grasslands (Fig. 5B). The first two components of the PCA explained 84 % of data variance (45% and 39% for PC1 and PC2, respectively) with separation along the first axis. This was explained by higher values of MBC:MBN in grassland sites, while that forests and tree canopy *N. antarctica* forests and SPS differences were related to higher values of MBC, MBN, and SBR (Fig. 5A and B). On the other hand, SPS and the inter-canopies had an intermediate response related to higher qCO_2 values (see Fig. 5A and B).

The results of Pearson's correlation coefficient (Table 4) showed a negative and significant ($p < 0.05$) correlation between MBC ant qMC with altitude ($r: = -0.78$). In contrast, MAT ($r = 0.73$ *and* 0.74; $p < 0.05$) and vegetation cover ($r = 0.70$ *and* 0.65; $p < 0.05$), and SOC ($r = 0.54$ *and* 0.52; $p < 0.05$) had a positive correlation with MBC. SBR had a positive correlation with dominant height of *N. antarctica* trees ($r = 0.59$; $p < 0.05$), SOC ($r = 0.54$; $p < 0.05$), and soil nitrogen ($r = 0.53$; $p < 0.05$). However, qCO_2 did not showed significant correlation with climate, vegetation and soil variables. There were a negative and significant ($p < 0.05$) correlation between MBN with dominant height ($r = 0.65$), tree density ($r = 0.71$), basal area ($r = 0.84$), and canopy cover ($r = 0.78$). On the other hand, MBC:MBN had a negative ($p < 0.05$) correlation with dominant height of *N. antarctica* trees ($r = -0.83$), tree density ($r = -0.66$), basal area, and canopy cover ($r = -0.83$). However, understory biomass ($r = 0.65$; $p < 0.05$) had a positive correlation with MBC:MBN.

4. Discussion

4.1. Soil microbial variables along the natural gradient

Microbial biomass and associated coefficients increased in coastal and In-LA soils compared to In-HA. Toledo et al. (2024) observed that differences in climate conditions, including temperature and altitude, influence soil microbial variables. Changes in microbial biomass and associated coefficients may be attributed to shifts in land use patterns. Generally, the physico-chemical properties of soil vary in spatially and temporally due to differences in topography, climate, weathering processes, vegetation types (Fartyal et al., 2025), and microbial activities (Bargali et al., 2018; Babur et al., 2021). In the highly dissected landscapes, bioclimatic conditions change rapidly over short distances, resulting in pronounced heterogeneity of soil types and their physical

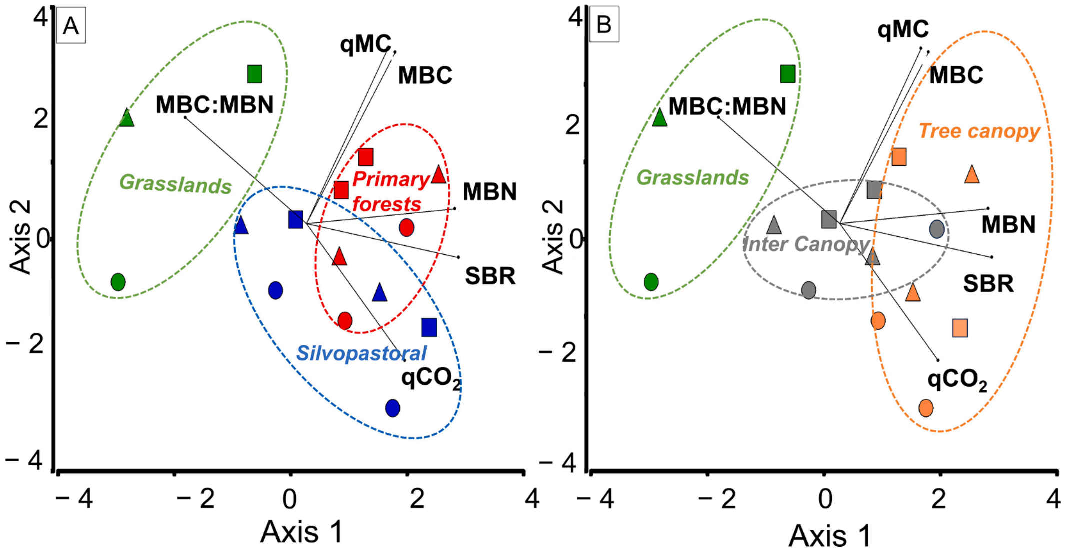

Fig. 5. Principal components analysis including soil microbial variables (microbial biomass carbon and nitrogen: MBC and MBN; soil basal respiration: SBR, microbial metabolic quotient: qCO_2, MBC:MBN ratio, and microbial quotient: qMC) for the three land use types: primary forests (red), silvopastoral system (blue), and grasslands (green), and tree canopy (yellow) and inter canopy (gray) in *N. antarctica* forests of Tierra del Fuego, Argentina.

Table 4
Pearson's correlation indices for microbial biomass carbon and nitrogen (MBC and MBN), soil basal respiration (SBR), microbial metabolic quotient (qCO_2), MBC:MBN ratio, and microbial quotient (qMC) with climate, vegetation, soil and biological variables for the ecosystems of Tierra del Fuego (Argentina). (*) Significance with a $p < 0.05$.

Variables	MBC	SBR	qCO_2	MBN	MBC: MBN	qMC
Mean annual precipitation	0.46	0.07	−0.15	0.13	−0.02	0.48
Altitude	−0.78*	−0.09	0.38	−0.34	−0.16	−0.78*
Mean annual temperature	0.73*	0.10	−0.31	0.27	0.07	0.74*
Dominant height	0.16	0.59*	0.46	0.65*	−0.83*	0.08
Tree density	0.04	−0.01	−0.08	0.71*	−0.66*	0.01
Basal area	0.14	0.25	0.11	0.84*	−0.83*	0.08
Canopy cover	0.17	0.32	0.14	0.78*	−0.83*	0.10
Understory biomass	0.45	−0.05	−0.30	−0.34	0.65*	0.46
Vegetation cover	0.70*	0.23	−0.28	0.49	−0.14	0.65*
Soil Organic Carbon	0.54*	0.54*	0.21	0.35	−0.13	0.52*
Soil nitrogen	0.49	0.53*	0.27	0.26	−0.08	0.48
Soil phosphorus	0.14	0.06	−0.01	0.24	−0.31	0.13
MBC		0.35	−0.26	0.47	0.11	0.99*
SBR			0.79*	0.57*	−0.54*	0.32
qCO_2				0.24	−0.55*	−0.27
MBN					−0.75*	0.42
MBN:MBC						0.16

and chemical properties (Manral et al., 2023, Bargali et al., 2019), which in turn influences vegetation composition and litter input (Babur et al., 2022). In our study, low-altitude coastal sites were warmer and received more precipitation than the In-LA and In-HA regions. Furthermore, the relatively stable climatic conditions in the evaluated coastal sites, due to maritime influence, stimulated an increase in microbial biomass (C and N), which shifted the MBC:MBN ratio and altered microbial metabolic. Our correlation analysis showed that altitude, mean annual temperature (MAT), vegetation cover, and SOC were key determinants of MBC variation.

Delgado-Baquerizo et al. (2016) and Babur et al. (2021) demonstrated that microbial biomass (C and N) increases with rising mean annual precipitation (MAP) and MAT across rangelands, forests, and other biomes. In contrast, Ma et al. (2019) and Toledo et al. (2024) identified MAP as the primary factor regulating the growth of soil microbial communities. It is possible that the gradient in our study does not encompass a sufficiently wide range of MAP (369 to 387 mm yr^{-1}) to capture this effect. Singh et al. (2012) reported that at high elevations, bacterial species richness increases due to "lottery" recruitment driven by extreme temperature fluctuations, stronger UV radiation, and limited nutrient availability. However, the altitudinal differences in our study were small (<80 m.a.s.l.), suggesting that the influence of altitude on soil microbial variables may be limited. Nevertheless, Shen et al. (2015) reported distinct microbial community structures along a small-scale elevation gradient in tundra ecosystems (<100 m), driven by spatial heterogeneity in soil C and N contents. Other studies have found similar results, indicating that microbial community is primarily influenced by soil N content, SOC, and MAT (Takriti et al., 2018; Ma et al., 2019; Kang et al., 2021). These findings align with our study, which showed that soil microbial biomass responses varied according to biome characteristics along the environmental gradient, from coastal to inland sites.

Several studies suggest that regional microclimate determines vegetation attributes (e.g., tree height, canopy cover, and understory vegetation cover in coastal sites), leading to changes in soil characteristics such as higher SOC and soil nitrogen in coastal areas (Chen et al., 2016; Praeg et al., 2020; Toledo et al., 2024). These microclimate-induced variations directly and indirectly influence soil microbial variables. We observed an increase in MBC and MBN along the environmental gradient (In-HA < In-LA < coastal sites). Soil microbiome responses likely result from complex climate-plant-soil-microorganism interactions, where higher MAT and vegetation cover promote efficient soil carbon use (low qCO_2 values) and support greater microbial biomass (MBC and MBN). Additionally, the MBC:MBN ratio is associated with the fungal-to-bacterial ratio (Jansson and Hofmockel, 2020), and shifts in MBC:MBN values may indicate changes in microbial community composition (Cleveland and Liptzin, 2007; Deng et al., 2016; Kooch et al., 2019). In our In-HA site, low MBC:MBN values suggest bacterial dominance, whereas increased MBC:MBN in the inland and coastal sites may indicate a shift toward fungal dominance (Sun et al., 2020).

Lin et al. (2012) found that microbial community differences between forest sites (coastal and inland) were driven by geographic distance, which shaped distinct soil bacterial communities. In our study, inland and coastal ecosystems were separated by 25 to 50 km. At intermediate spatial scales (10–3000 km), some studies have reported significant distance effects and environmental conditions shaping soil microbial composition (Cao et al., 2016; Ivashchenko et al., 2021). Therefore, both geographic distance and environmental conditions likely play key roles in shaping microbial communities at this spatial scale, influencing microbial variables to varying degrees.

4.2. Responses of soil microorganisms to land use types

This study demonstrated that forest management alternatives under silvopastoral practices tend to maintain or increase microbial variables compared to undisturbed forests. However, greater land use intensification (conversion of forests to grasslands) resulted in a decrease in microbial parameters and an increase in MBC:MBN ratio. These microbial component impacts suggest that land use changes could have significant consequences on soil nutrient cycling dynamics and, over time, influence the growth of dominant vegetation in each land use type. In this study, showed a clear separation between undisturbed forests and grasslands but did not detect a difference between silvopastoral and forest sites. Several studies showed in different biomes that land use changes, such as the conversion of forests to grasslands, or natural ecosystems to croplands or silvopastoral systems, resulted in changes in microbial biomass (Manral et al., 2023; Lee et al., 2020; Gargaglione et al., 2022). Soil microbial biomass, as a source or sink of labile nutrients, plays a vital role in the decomposition of soil organic matter and nutrient availability (Hartmann and Six, 2023), being one of the most sensitive soil bioindicators to climate, disturbances, and management (Marcos and Olivera, 2016; Schloter et al., 2018; Kooch et al., 2019). Although our results do not show significant changes in microbial biomass (MBC) among the different land use types, we observe a decrease in MBN in the land use gradient PF $>$ SPS $>$ grasslands. These responses in microbial soil biomass may be related to vegetation variables and soil properties (Gonzalez-Polo et al., 2019; Gargaglione et al., 2022; Christel et al., 2024), but this depends on interaction with the climatic conditions (Toledo et al., 2023, 2024). Results have confirmed a decrease in nutrient availability and SOC in soils following the conversion of natural forests, which was directly reflected in microbial biomass (Khan et al., 2016; Peng et al., 2021; Gargaglione et al., 2022). However, the different land use types show low variation in these variables compared to the undisturbed forests: vegetation cover only showed a 10% decrease in MBC of grassland soils, and SOC decreased by 5%. This contrasts with Gargaglione et al. (2022), who found significant changes in soil nutrient values and microbial biomass when comparing *N. antarctica* forests and grasslands in southern Patagonia. However, in our study, the response of MBC seems to depend on interactions with the site's climatic conditions (MAT and altitude), which is confirmed by the results of the interactions between land use types and the environmental gradient.

In these Austral cold ecosystems, MBN seems to be a microbial bioindicator parameter more sensitive than MBC to reflect changes. MBN may reflect the close relationship between this microbial component and nutrient availability for plants. Gonzalez-Polo et al. (2019) showed that the turnover of nutrients through the soil microbial biomass is an important process that influences overall nutrient availability. Gargaglione et al. (2022) found that land use changes from native *N. antarctica* forests to grasslands and silvopastoral systems in Patagonian ecosystems resulted in a decrease in microbial biomass (C and N), and this was associated with soil physicochemical parameters. In contrast, our results show that variations in MBN are related to forest structure (canopy cover, dominant height, basal area, and tree density), which changes with the intensification of land use change from forests to grasslands. These forest structure variables can be related to tree biomass, which results in the main organic source material for soil nutrient contents that benefits the growth of microorganisms in the ecosystem (Toro-Manríquez et al., 2019; Martínez Pastur et al., 2023; Toledo et al., 2024). Indeed, it was reported that soil nitrogen was closely related to natural vegetation and aboveground biomass (Bahamonde et al., 2015, 2018; Martínez Pastur et al., 2023). Therefore, as pointed out by Qiu et al. (2023) soil microbial stoichiometry could reflect relative soil nutrient limitations and provide insights into the contribution of nitrogen liberation from microbial biomass. In this study, soil microbial C:N ratio was similar in silvopastoral and forest soils, but higher than in grasslands. Lower microbial MBC:MBN ratio indicated a high potential for N release from biomass, while a higher microbial MBC:MBN ratio found in grasslands demonstrated the potential for N immobilization in biomass (Mou et al., 2023). Furthermore, MBN results contribute to the knowledge on how microorganisms control the availability of soil nitrogen and, therefore, regulate ecosystem functions such as plant productivity (Das et al., 2022; Hartmann and Six, 2023). This is particularly interesting for the cold ecosystems of Patagonia and highlights the importance of the labile fractions of microbial N to maintain ecosystems productivity and minimize nutrient soil losses.

Observed decrease in MBN in silvopastoral and grassland land uses compared with undisturbed forests could be due to the fact that active grasses are a highly competitive sink for inorganic nutrients, with high uptake rates and extensive root systems (Kuzyakov and Xu, 2013; Gargaglione et al., 2014, 2022; Martínez Pastur et al., 2024). After removing trees from primary forests to convert them into SPS or grasslands, this allows for greater growth of grass species and other plants. Thus, grasses would restrict microbial growth through competition for available soil nutrients. This competitive dynamic between plants and soil microbes for nutrients has been demonstrated in other ecosystems (Adomako et al., 2022; Das et al., 2022; Toledo et al., 2023). According to this, Gargaglione et al. (2014) found that soils under *N. antarctica* SPS have greater N availability. These authors reported that trees may facilitate N absorption by grasses, thereby increasing herbaceous N content. Additionally, in the establishment of SPS, some trees are removed to stimulate grass growth, which leads to microclimatic changes, such as increased light reaching the soil (Bahamonde et al., 2012).

The land use types showed an asymmetry response of microbial activity. While silvopastoral systems increased the SBR compared to primary forests, grasslands caused a great SBR reduction. Gargaglione et al. (2022) found that microbial activity was lower in silvopastoral systems and grasslands compared to primary forests. Our study shows that in grasslands, microorganisms were less active, probably due to some limiting factor such as low soil nitrogen, SOC, substrate quality or availability (Deng et al., 2016; Gonzalez-Polo et al., 2019; Marquez et al., 2022; Martínez Pastur et al., 2024). On the other hand, litter fall from *N. antarctica* deciduous trees contains higher N and P concentrations than litter from grasses in grasslands (Bahamonde et al., 2012). This could enhance microbial activity in LUTs that maintained a forested cover, such as silvopastoral systems and primary forests. Certainly, in our study, we found that SBR is positively correlated with SOC, SN, and dominant tree height (as a proxy of site quality). Furthermore, differences in SBR could be related to soil nutrient contents (N and P). Thus, the higher SBR values in silvopastoral systems in our study might be related to more favorable conditions (higher radiation, light, temperature), and substrate quality for microorganisms compared with grasslands and primary forests. Gargaglione et al. (2022) found that differences in microbial variables between primary forests and grasslands could be related to the quality of the detritus that reaches the soil. Consistent with our assumption, Fierer et al. (2012) demonstrated that the catabolic activity of soil microbial biomass increases with the additional N input in the natural ecosystem. Possibly, as conversion of primary forests to silvopastoral systems or grasslands generates higher qCO_2 values, which indicates that the vegetation had a higher abundance of easily metabolizable C, whereas the MBC use efficiency was lower (Liu et al., 2023). Therefore, large amounts of absorbed carbon are released by respiration (e.g. silvopastoral systems) rather than accumulated by an increase in MBC (Gargaglione et al., 2022). Additionally, this would increase the rate of carbon decomposition and a decrease carbon storage (Christel et al., 2024; Peri et al., 2024a). Changes in qCO_2 are also associated with changes in the soil microbial community and diversity (Liu et al., 2023). In our study, it was supported by relationships between SBR and MBC:MBN, and qCO_2. A previous study showed that the conversion of forest resulted in higher qCO_2 due to bacterial activity, whereas fungi that have lower qCO_2 are more dominant in the forest stands (Xu et al., 2017).

From the perspective of microbial community, fungi are regarded as K-strategists (Dang et al., 2024; Ma et al., 2024), which are characterized by larger individual sizes and a lower qCO_2 (Lu et al., 2024; Wang et al., 2024). Finally, our study shows that changes in land use generate a shift in microorganism communities (e.g. grasslands are dominated by fungi with a higher MBC:MBN). Also, our results show that LUT modifies the C use efficiency of microorganisms in these cold ecosystems (Gargaglione et al., 2022; Toledo et al., 2024) determined with different qCO_2 values for silvopastoral systems and grasslands compared to primary forests. While conversion to grasslands decreased SBR and increased MBC:MBN, silvopastoral systems increased SBR, but did not modify MBC:MBN. Kooch et al. (2019) and Jansson and Hofmockel (2020) showed that changes in MBC:MBN ratio indicate shifts of microbial community composition, and that the shifts in the fungi:bacteria ratio was correlated with the MBC:MBN. In our study, we determined greater MBC:MBN values in high use intensification grasslands soils, suggesting an increase of fungi or a decrease in soil bacteria (Jansson and Hofmockel, 2020; Sun et al., 2020; Álvarez et al., 2024). The changes in microbial community could influence C use efficiency used by microbes for growth (Álvarez et al., 2024; Toledo et al., 2024). Although, there were no significant differences among forests and silvopastoral following land use changes, the results demonstrate that soil microorganisms can maintained microbial community and MBC but show an increase in SBR. Therefore, large amounts of absorbed C are released by respiration rather than accumulated by an increase in MBC. Possibly, this is because the high plant aboveground biomass in silvopastoral systems can transfer carbon into the soil in the form of leaf litter and root exudates, but soil microbes accelerate the decomposition through soil respiration (Gargaglione et al., 2022).

In our study, SPS have been found to have minimal impact on soil microbial variables. Martínez Pastur et al. (2022) documented that the silvopastoral systems promote changes in forests under natural dynamics, creating a balance between losses (e.g., tree and biodiversity components) and gains (e.g., environmental and forage components). In this framework, silvopastoral systems promote the establishment of new productive environments with intermediary characteristics between primary forests and grasslands, as it was shown in the soil microbial properties obtained in our results. On the other hand, the positive correlation between microbial variables with soil properties and vegetation characteristics highlights the potential use of some biological variables (MBN, SBR, MBC:MBN and qCO_2) as early bioindicators of disturbance to assess the effects of LUTs on soil processes (Schloter et al., 2018; Fierer et al., 2021).

4.3. Effects of crown cover of the overstory on soil microorganisms

Our study shows that *N. antarctica* forests generate spatial heterogeneity in canopy cover, influencing the physical, chemical, and biological characteristics of the soil. These results can be attributed to the deposition of aerial and belowground residues (e.g., leaf and root exudates, leaves, stems, flowers, seeds, and roots) from the forest component, contributing to SOC accumulation. Chen et al. (2017) reported that continuous litter inputs and extensive root systems increase SOC and soil nitrogen levels. Similarly, Dhillon and Van Rees (2017) highlighted a positive correlation between tree attributes (e.g., height, diameter, and canopy size) and litter inputs with SOC accumulation.

In this context, the gradual decomposition of roots, leaves, and branches enhances soil organic matter over time (Toro-Manríquez et al., 2019; Martínez Pastur et al., 2023). These authors also reported a decrease in carbon and nitrogen content as sampling points moved away from the tree crown. Our study further demonstrated that *N. antarctica* tree canopy induces changes in the soil microbiome in both forest types (PF and SPS). Specifically, tree canopy cover increased microbial biomass, activity, and microbial community coefficients. These findings are consistent with Viruel et al. (2021), who reported higher microbial biomass under tree canopies compared to inter-canopy areas in silvopastoral systems. Furthermore, they noted that trees improved SOC and soil nitrogen under canopies, but this effect diminished in inter-canopy areas due to resource depletion under grasses growing between tree rows (Viruel et al., 2021).

Thinning practices, which open the canopy, promote understory growth and enhance timber quality (Bahamonde et al., 2018), generating positive synergies with nutrient and water cycles (Gargaglione et al., 2014; Martínez Pastur et al., 2023). The resulting heterogeneity in the soil microbiome due to tree canopy cover highlights the importance of maintaining forest cover in these southern ecosystems, as both aboveground and belowground components play a crucial role in sustaining soil microbial functionality and, consequently, soil fertility. Guillot et al. (2019, 2021) reported spatial heterogeneity in microbial biomass in agroforestry systems, with higher microbial activity near tree lines. According to these authors, forest components modify the soil microclimate by maintaining soil moisture and reducing temperatures, thereby increasing microbial activity.

Microclimatic modifications, combined with organic matter inputs, can drive changes in soil microbiota. Peri et al. (2010) found a higher proportion of roots concentrated under the stem base, which supports microbial development by providing labile C through root exudation, serving as an energy source for soil microbiota (Babur et al., 2021, 2022). Khan et al. (2016) further highlighted the strong correlation between SOC, soil nitrogen, and microbial biomass. Also, higher microbial quotient (qMC) values indicate that organic C is more readily accessible to soil microbiota (Gargaglione et al., 2014; Ortiz et al., 2023; Toledo et al., 2024).

Consistent with our results, several studies have reported improvements in soil quality with the inclusion of forest components in integrated production systems, such as silvopastoral systems (Martínez Pastur et al., 2022, 2024; Peri et al., 2016, 2024b). However, the influence of tree canopy on soil microbial variables depends on forest type (PF vs SPS), indicating an interaction between these factors. For instance, our findings showed that the observed variation in soil basal respiration (SBR) and the microbial biomass C:N ratio (MBC:MBN) had a greater impact on SPS compared to PF. In PF, where tree cover is denser, soil moisture retention is likely higher than in SPS, potentially explaining the increased microbial biomass nitrogen (MBN).

Conversely, Peri et al. (2016) reported that tree removal through thinning in Patagonian silvopastoral systems reduced litterfall by 35–50%, altering the quality and availability of substrates for soil microorganisms. These responses may be further influenced by grazing activity (Martínez Pastur et al., 2022), where plant biomass removal leads to lower soil organic matter content, increased competition with understory plants, and higher aerial biomass in SPS (Gargaglione et al., 2022). Consequently, soil microorganisms may be required to degrade more recalcitrant organic materials to obtain energy and nutrients. Similarly, Sun et al. (2020) reported that microorganisms release more C as CO_2 when substrates are nutrient-poor and do not meet their nutritional requirements. Therefore, we hypothesize that the higher microbial metabolic quotient (qCO_2) observed under tree canopies in SPS is closely related to the soil C:N ratio, which may result in nitrogen limitations in inter-canopy areas. To accurately evaluate soil nutrient dynamics in forested conditions (PF vs SPS), soil sampling and measurements must be conducted with careful consideration of the spatial heterogeneity induced by tree canopies.

One limitation of this study is that we did not evaluate the specific composition or diversity of soil microbial communities, such as bacterial or fungal taxa related to land-use change. Consequently, we could not establish direct links between microbial community dynamics and observed changes in labile soil carbon stocks or carbon losses. This limits our ability to infer the mechanisms behind carbon cycling processes or microbial efficiency in carbon utilization across different land-use systems. Future research that incorporates molecular approaches (e.g., high-throughput sequencing) is needed to identify key microbial taxa involved in soil C transformations to better understand how land-use intensification may alter microbial-mediated ecosystem functions. Despite this limitation, the study provides a valuable first step in assessing soil microbial responses to land use and canopy heterogeneity in forest ecosystems.

5. Conclusions

This study showed that forest management alternatives under silvopastoral practices tend to maintain microbial variables (MBC, MBC:MBN, and qMC) and increase SBR compared to primary forests. However, greater land use intensification (e.g. conversion of primary forests to grasslands) resulted in a decrease in microbial parameters (MBN, SBR, and qCO_2) and an increase in MBC:MBN. The response of microbial biomass and activity depends on interactions with the site's climatic conditions along the studied environmental gradient, which spans from coastal to inland biomes. These findings suggest that silvopastoral systems could be a viable alternative for enhancing microbial attributes, providing a significant source of ecosystem services, and supporting sustainable forest management. Furthermore, our study was the first to show that tree species, such as *N. antarctica*, function as a "resource island" and create spatial heterogeneity in soil properties (physicochemical and biological) across the gradient of tree crown cover. In a global context, our findings showed that soil microbial biomass, activities, and microbial coefficients could be good soil quality and health bioindicators following land use changes. This can support early assessments of soil degradation and guide sustainable land management practices in forested landscapes worldwide and in cold regions, which are highly sensitive to climate change.

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CRediT authorship contribution statement

Santiago Toledo: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis. **Guillermo Martínez Pastur:** Writing – review & editing, Resources, Project administration, Methodology. **Julián Rodríguez Souilla:** Resources, Formal analysis, Data curation. **Pablo L. Peri:** Writing – review & editing, Investigation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.temicr.2025.100028.

Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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