Soil microbial community structure and enzymatic activity along a plant cover gradient in Victoria Land (continental Antarctica)

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ABSTRACT

In continental Antarctica, autotrophs are exclusively represented by cyanobacteria, algae, lichens and mosses. Consequently, Antarctic soil communities are expected to be rather simple and primarily dominated by microorganisms. Recently, a change in abundance of mosses and lichens has been observed in continental Antarctica in response to an increase of the active permafrost layer, but the implication of this change to soil micro-organisms remains little known. Here we aim to clarify to what extent the abundance of mosses and lichens affects soil biogeochemistry in Victoria Land, with a particular focus on soil microbial abundance and associated soil enzymatic activity. To achieve this aim, we assessed the structure of soil microbiome and the activity of hydrolytic C, N, and P enzymes along a gradient in soil physico-chemical conditions and plant cover. Moss cover strongly relates to the amount of soil organic carbon (SOC), soil water and nutrient content. Soils with higher content of organic carbon were characterized by higher microbial biomass and showed a relatively higher abundance of fungi as compared to bacteria. More specifically, PLFAs biomarkers for Actinomycetes and Gram-positive bacteria were mainly associated to soils with lower SOC. In order to sustain a higher microbial biomass, total activity of hydrolytic enzymes increased with increasing SOC content. Eco-enzymatic stoichiometry, based on C to P and C to N ratios, indicates a higher investment in N- and P-hydrolytic enzymes (ratio < 1), particularly at low SOC content. Oppositely, an increase in C-hydrolytic enzyme activity (ratio ≈ 1) was observed with increasing accumulation of organic carbon. Such a result seems to indicate a stronger role of soil pH at low SOC on enzymatic stoichiometry (abiotic control) whereas with increasing accumulation of organic matter the enzymatic stoichiometry is more affected by microbial metabolism (biotic control).

*Keywords:*

Mosses, fungi, bacteria, PLFAs, stoichiometry

1. **Introduction**

The few areas in Antarctica that are not permanently covered by snow or ice (c. 0.4%) harbor soils that are in early development. These soils are exposed to extreme climatic conditions, in particular low temperatures and low water availability (Cannone et al., 2008; Chan et al., 2013; Kennedy, 1993), which limit the presence of plants. For this reason, Antarctic soil communities are expected to be rather simple and dominated by microorganisms (Lee et al., 2012; Yergeau et al., 2012). An additional consequence of the scattered occurrence

of plant communities is that abiotic factors are suggested to have a major control on microorganisms in Antarctic soils (Barrett et al., 2006; Hogg et al., 2006), similarly to what has been observed in glacier forefields at early stages of soil formation (Schulz et al., 2013; Tscherko et al., 2005).

In contrast to maritime Antarctica, where vascular plants play a significant role on soil microbial structure and function (Roberts et al., 2009; Teixeira et al., 2010; Teixeira et al., 2013), in continental Antarctica cyanobacteria, algae, lichens and mosses are the only autotrophs currently capable of colonizing the soils due to harsh climatic conditions, geological history and geographical isolation (Cannone and Seppelt, 2008; Rogers, 2007). Recently, in response to an increase of the active layer thickness of permafrost, a change in the abundance of mosses and lichens has been observed in continental Antarctica (Guglielmin and Cannone, 2012; Guglielmin et al., 2014). While for arctic and subarctic permafrost soils an increase of active layer thickness has been associated to a change in structure and function of microbial communities (e.g., Mackelprang et al., 2016; Shi et al., 2015; Tveit et al., 2013), studies linking moss and lichen abundance to soil microbes in continental Antarctica are limited, particularly regarding the implications to microbial function (e.g. Yergeau et al., 2012). In light of the rapid responses of soil microbes to abiotic and biotic changes (Kennedy, 1996; Yergeau et al., 2012) and the overall sensitivity of polar ecosystems to global environmental changes (Barrett et al. 2006; Beyew et al., 1999; Wall, 2007), it is crucial to understand the relationship between aboveground plant community and belowground soil microbes in order to better predict soil biogeochemical processes in continental Antarctica.

The activity of extracellular soil enzymes can directly provide information on microbial metabolism. Indeed, extracellular enzymes are the drivers of soil organic matter decomposition (Sinsabaugh et al., 2008), hence their activity can be interpreted as an indication of microbial nutrient limitation (Allison and Vitousek, 2005; Schimel and Weintraub, 2003). The rationale for interpreting relative carbon (C), nitrogen (N) and phosphorous (P) limitations from the relative activity of C, N and P-acquiring enzymes (i.e. soil enzyme stoichiometry) is based on the necessity of microbial communities to maintain a stoichiometric nutrient balance in relation to the amount of nutrients in soil and the environmental conditions (Sinsabaugh et al., 2009). In addition, the activities of extracellular soil enzymes are also dependent on a set of abiotic factors such as soil pH (Sinsabaugh et al., 2008). Indeed, soil pH can affect enzyme activities, for example, by selecting the composition of soil microbial communities, by affecting the sorption of enzymes on soil particles, or by changing the solubility of targeted substrates (Lammel et al., 2018; Turner, 2010; Xun et al., 2015).

In this study, we aim to clarify to what extent moss and lichen cover relates to soil microbes in Victoria Land with a particular focus on soil microbial abundance and associated extracellular enzymatic activities. To this aim, we selected our study sites under a diverse array of lithological and geographic conditions, resulting in a gradient in soil physico-chemical conditions (in particular soil pH) and plant cover. Specifically, we want to answer the following three major questions: 1) How do microbial biomass and microbial community structure change along a gradient of plant cover? 2) Does the activity of extracellular C, N, and P-acquiring enzymes differ in relation to soil physico-chemical conditions and microbial abundance? 3) On the basis of eco-enzymatic stoichiometry, which nutrient is mainly limiting the microbial metabolism?

1. **Material and methods**

*2.1. Study area*

The study sites are located along the coast of Victoria Land (Ross sector, continental Antarctica) along a latitudinal gradient (73ºS – 77ºS) from Apostrophe Island to Finger Point (Fig. S1). All study sites are ice-free areas located along the coast, have a maximum elevation range of 250 m (Table S1), and have a continuous permafrost with an active layer thickness varying from zero to more than 90 cm depending on year and location. Overall the studied soils can be classified as Gelisols (Bockheim et al., 2015). A more detailed description of topographical and physical characteristics of the study sites can be found in Table S1 (Cannone et al., 2008). At each study site, one or more permanent plots, depending on the variability of vegetation and soil conditions, were installed since 2002-2003 as part of a long-term monitoring program (Cannone, 2006; Guglielmin et al., 2014).

The vegetation of Victoria Land is composed exclusively of cryptogams (e.g. Cannone and Seppelt, 2008) with four main vegetation types that were dominated by mosses, mosses encrusted by epiphytic lichens, macrolichens, or scattered epilithic lichens and mosses. The climate of the area is characterized by mean annual air temperature of -13.8 °C (Guglielmin and Cannone, 2012) and very low precipitation (always in form of snow) ranging from 100 to 200 mm y-1 as measured at Mario Zucchelli Station (c. 74°S) (Monaghan et al., 2006). Further south (c. 77°S), the climate is drier and colder with a mean annual air temperature of -17.5 °C at Marble Point (Guglielmin et al., 2011).

* 1. *Soil sampling and estimation of plant cover*

At each permanent plot, during the austral summer 2012-2013, soil samples were collected from the upper soil layer (0–2 cm deep) where most of the organic matter is deposited. The samples were then shipped and stored at -20 °C until analyses.

Cover estimation of mosses, lichens, cyanobacteria and green algae is based on vegetation data from Cannone (2006) and Cannone et al. (2008). Plants with a cover high enough to be visually estimated in all the permanent plots were lichens and mosses (Table S1). Cyanobacteria were abundant in only one site and green algae in two sites (total cover < 6%) (Cannone et al., 2008).

*2.3 Soil samples processing and chemical analyses*

After thawing, soil samples were sieved (2 mm mesh) and divided in subsamples for different analyses. One set of subsamples was oven-dried at 105°C to determine dry weight and water content. Soil pH was measured by adding 20 ml of distilled water to 10 g of air-dried soil. Another set of subsamples was used to determine dissolved nutrient concentrations. Briefly, c. 10 g of air-dried soil was extracted in 100 ml de-ionized water, shaken for 2h, and filtered using glass microfiber filters (Whatman GF/D). The obtained extracts were analysed colorimetrically for N-NH4 by the salicylate method, N-NO3 by the cadmium reduction method, P-PO4 by the molybdenum blue method using a continuous flow analyser (FlowSys, Systea, Roma, Italy). An additional set of soil subsamples was used to determine organic carbon concentration (SOC) as difference between total carbon concentration (catalytically aided combustion oxidation at 900°C) and inorganic carbon concentration (pre-acidification, oven temperature 250 °C) using a Shimadzu TOC-Vcsh (Shimadzu Corporation, Kyoto, Japan) connected with a solid sample module (Shimadzu SSM-5000A). In parallel, soil organic matter (SOM) was determined by loss on ignition (LOI) after burning soil subsamples at 550°C for 4 hours. In light of the positive relationship between SOC and SOM concentration (SOM = 0.97 + 0.96·SOC; r2 = 0.78, p < 0.001, n = 15), hereafter we refer to SOC concentration as determined by combustion catalytic oxidation method. All nutrient concentrations were corrected for the dry weight of the soil sample. Major soil biogeochemical characteristics as measured at the permanent plots can be found in Table S2.

*2.4 Determination of microbial biomass and microbial community structure in soil samples*

Total microbial biomass and structure of microbial community were assessed using phospholipid fatty acid (PLFA) analysis. PLFAs were extracted following a modified Bligh and Dyer (1959) method (Börjesson et al., 1998). Total lipids were extracted overnight from 5 g freeze-dried soil in a solvent phase of 3.0 ml 50mM phosphate buffer (pH = 7.0), 3.8 ml chloroform (CHCl3), 7.6 ml methanol (MeOH), and 4 ml Bligh and Dyer reagent (CHCl3: MeOH: P-buffer; 1: 2: 0.8 (v/v/v)). Extracted lipids were subsequently added to Discovery® DSC-Si SPE Tubes (Sigma-Aldrich) and then separated into neutral lipids, glycolipid, and phospholipid by sequential addition of chloroform, acetone and methanol solutions. PLFA 19:0 (Larodan Malmö, Sweden) was added as internal standard to the phospholipid fraction. PLFAs were trans-esterified to fatty acid methylesthers (FAMEs) using 1 ml 0.2M methanolic-KOH (Chowdhury and Dick, 2012; Sundh et al., 1997). PLFAs were analysed on a gas chromatograph according to Steger et al. (2003). Fungal biomass was calculated based on the fatty acid 18:2ω6c and 18:1ω9c (Frostegård and Bååth, 1996; White et al., 1996). The biomass of Gram-positive (G+) bacteria was associated to the amount of the following biomarkers: a15:0, a17:0, i14:0, i15:0, i16:0, i17:0; for Gram-negative bacteria (G-) the biomarkers: 16:1ω9c, 16:1ω11c, 18:1ω5c, 18:1ω7c, cy17:0, cy19:0; and for Actinomycetes the biomarkers: 10Me16:0 and 10Me18:0 (Brockett et al., 2012; Frostegård and Bååth, 1996; Willers et al., 2015). Total bacterial biomass was the sum of G+, G- and Actinomycetes biomass. The ratio of fungal to total bacterial biomass (F:B ratio) in soil samples was calculated from PLFAs data.

*2.5 Soil enzymatic activity*

Soil samples were assayed for the potential activity of the following hydrolytic enzymes: β-glucosidase (BGL using 4-methylumbelliferyl (MUB)-β-D-glucopyranoside as substrate); α-glucosidase (AGL using 4-MUB-α-D-glucopyranoside), xylosidase (XYL using 4-MUB-β-D-xylopyranoside), phosphatase (PHO using 4-MUB phosphate), leucine-aminopeptidase (LEU using L-leucine-7-amido-4-methylcoumarin (MUC)), alanine-aminopeptidase (ALA using L-alanine-7-amido-4-MUC), arginine-aminopeptidase (ARG using L-arginine-7-amido-4-MUC), glycine-aminopeptidase (AMI using glycine-7-amido-4-MUC) and chitinase (CHI using 4-MUB-N-acetyl-β-D-glucosaminide). The protocol followed Saiya-Cork et al. (2002) with minor modifications. Specifically, 1 g of soil was added to 7 ml of milliQ water and shaken for c. 30 minutes at room temperature. After centrifugation, 250 µl of supernatant slurry were transferred in a 96-well microplate where 50 µl of specific enzyme substrate (1000 µM; see above) was added. Assays were conducted in triplicate at natural soil pH and incubated at 10 °C. During incubation, the enzymatic kinetic was followed for 5 hours by measuring the fluorescence every 30 minutes (emission wavelength at 450-nm and excitation wavelength at 330-nm; BioTek SynergyMX). In parallel, for each soil sample a specific calibration curve was created using 250 µl of surnatant slurry and 50 µl of methylumbelliferone (MUF) or 7-amino-4-methylcoumarin (MCU) at different concentrations. The blank standard was prepared by adding 50 µl of milliQ water to 250 µl of corresponding supernatant slurry. The final activity of each enzyme was reported as nmol of MUF/MCU released per gram of dry soil per hour (nmol g-1 h-1) and as nmol of MUF/MCU released per gram of soil organic carbon (dry weight) per hour (nmol g-1 SOC h-1) (German et al., 2011).

Based on their biogeochemical role, the selected hydrolytic enzymes can be grouped into the following categories (Kelley et al., 2011; Sinsabaugh et al., 2008; Stone et al., 2012): 1) starch degradation (α-glucosidase); 2) xylan (hemicellulose) degradation (xylosidase); 3) cellulose degradation (β-glucosidase); 4) chitin degradation (chitinase); 5) aminoacids degradation (leucine-, alanine-, glycine-, and arginine-aminopeptidase); and 6) organic phosphorus degradation (phosphatase). According to this classification, we calculated the ratio of β-glucosidase activity relatively to α-glucosidase + xylosidase activities, as well as the ratio of chitinase to the sum of activities of aminoacids hydrolyzing enzymes (i.e. leucine aminopeptidase + alanine aminopeptidase + glycine aminopeptidase + arginine aminopeptidase) as indication of the chemical complexity of soil organic matter so that an increase of these ratios can be associated to the decomposition of chemically more complex organic matter (Geyer et al., 2013; Kelley et al., 2011; Sun et al., 2018).

In accordance to Sinsabaugh et al. (2009), eco-enzymatic stoichiometry of extracellular C, N and P hydrolyzing enzymes was calculated as ratios of ln(BGL+XYL+AGL): ln(CHI+LEU+ALA+GLY+ARG) and ln(BGL+XYL+AGL):ln(PHO) activities for all soil samples. Although total C, N and P enzyme activities are not quantified, these ratios can give some insights on the enzyme resources directed towards the acquisition of organic P (i.e. C:P ratio) and N (i.e. C:N ratio) relative to C (Sinsabaugh et al., 2008).

*2.6. Statistical analyses*

We used univariate and multivariate statistical analyses in order to elucidate the relationships among microbial abundance, microbial community structure, enzyme activities and environmental parameters. Univariate statistical analyses, such as Pearson’s correlation and linear regression, were done using Statistica v. 11 (StatSoft). A principal component analysis, using Statistica v. 11 (StatSoft), was applied to differentiate the permanent plots based on vegetation cover and major soil chemical parameters. In addition, we performed a non-metric multidimensional scaling (NMDS), a unconstrained ordination technique, with the metaMDS function in the vegan package of R software (R Core Team, 2017) in order to relate PLFA biomarkers to the main environmental parameters, namely soil pH, nutrient availability (i.e., nitrate, ammonium, and phosphate concentrations), soil organic carbon content, and total vegetation cover.

1. **Results**

*3.1 Plant cover, soil chemistry and soil microbial community*

Total plant cover was positively associated to soil organic carbon content (SOC) (Fig. 1). More specifically, this trend was largely driven by moss cover which was strongly related to SOC (r*2* = 0.77, p < 0.001, n = 15), while lichen cover did not relate to SOC content (r*2* = 0.01, p = 0.75, n = 15) (Fig. S2). Overall, principal component analysis (PCA) of permanent plots based on vegetation cover and soil chemistry showed that greater vegetation cover (mosses + lichens) was associated not only to a greater SOC content, but also to greater nutrient availability and soil water content, but to lower soil pH (Fig. S3). Soils with higher SOC content were also positively related (n = 15) to the concentration of dissolved organic C (DOC) (r2 = 0.90, p < 0.001), inorganic N (r2 = 0.53, p < 0.01), and phosphate (r2 = 0.40, p < 0.05) (Fig. S4).

Total microbial biomass (PLFAs) increased with increasing plant cover, although the relationship was marginally significant (Fig. 1). This trend was driven by lichen cover which was strongly related to PLFAs (r2 = 0.40, p = 0.01, n = 15), while moss cover did not relate to PLFAs content (r2 = 0.06, p = 0.37, n = 15) (Fig. S2). More specifically, fungal biomass showed a statistically significant correlation with plant cover (r2 = 0.29, p < 0.05, n = 15), whereas no significant relationship was found between bacterial biomass and plant cover (r*2* = 0.19, p = 0.10, n = 15).

From our data we could not establish a relationship between fungal biomass and bacterial biomass with SOC content (p > 0.05, n = 15), but there was a positive linear relationship between the F:B ratio and SOC (r*2* = 0.42, p < 0.01, n = 15) (Fig. S5). Overall, bacterial biomass was higher than fungal biomass with a mean fungal to bacterial ratio (F:B) of 0.74 (range = 0.16 - 1.87) (Fig. S5). The NMDS ordination of PLFAs biomarkers (Fig. 2) showed that the scores of fungal biomarkers were associated to relatively high values of plant cover and contents of SOC and inorganic nitrogen. Actinomycetes and Gram-positive bacteria biomarkers, on the other hand, were associated mainly to the lower end of SOC and plant cover gradients. The ordination scores of PLFAs biomarkers associated to Gram-negative bacteria had a more widespread distribution along the selected environmental gradients.

*3.2 Enzymatic activity and eco-enzymatic stoichiometry*

After normalization with the correspondent SOC content, the activity of hydrolytic enzymes involved in the degradation of both C and P compounds was significantly related to total microbial biomass, whereas the total activity of N-hydrolyzing enzymes, although positively related to total microbial biomass, was not statistically significant (Fig. 3). More specifically, both fungal and bacterial biomass were positively correlated to C (r2 = 0.41 and r2 = 0.38, respectively, p < 0.05, n =15) and P (r2 = 0.44 and r2 = 0.45, respectively, p < 0.01, n = 15) enzymatic activities, whereas no significant relationship was found with N enzymatic activity (r2 = 0.23 and r2 = 0.14 for fungal and bacterial biomass, respectively; p > 0.05, n =15).

The specific activity of each hydrolytic enzyme was positively related to soil pH with the only exception of β-glucosidase (BGL) and chitinase (CHI) (Fig. 4). Overall, total activity of C-hydrolyzing enzymes (y = 0.08x - 0.25; r*2* = 0.32, p= 0.029, n = 15), N-hydrolyzing enzymes (y = 0.33x – 1.31; r*2* = 0.85, p< 0.001, n = 15), as well as the activity of P-hydrolyzing enzyme (y = 0.17x – 0.80, r*2* = 0.59, p< 0.001, n =15) showed a steeper increase, per unit of pH, in the case of P- and, particularly, N-degrading enzymes.

Regarding eco-enzymatic stoichiometry, both the BGL:(XYL+AGL) ratio and the CHI:(LEU+ALA+GLY+ARG) ratio increased with increasing amount of SOC (Fig. 5). We also observed that the total C:P and C:N activity ratios (logarithmic scale) were positively related (Fig. 6). On the whole, the mean (± S.D.) C:N ratio (0.58 ± 0.23) was significantly lower (t-Student = -3.9, p < 0.001, n = 15) than the mean C:P ratio (0.88 ± 0.20) so suggesting a greater resource allocation towards the acquisition of N compounds compared to P compounds (1:1 line, Fig. 6). Only at higher SOC content both stoichiometric ratios tended to 1, indicating an increase of resource allocation towards C acquisition with increasing SOC accumulation.

1. **Discussion**

The main aim of this study was to relate the abundance of mosses and lichens to soil microbes in continental Antarctica (Victoria Land). In particular, we focused on microbial community abundance and structure, as well as enzymatic activity, the latter being used as a proxy of microbial metabolism (Hill et al., 2018). To reach this goal, we adopted a sampling design consisting in soil collection along a gradient of plant cover.

Concerning the first research question (i.e. the relationship between plant cover, microbial abundance and microbial community structure), our data indicate that plant cover exerts a positive effect on microbial biomass through the accumulation of soil organic matter (Gajananda, 2007; Geyer et al., 2013). Such positive feedback can be explained by the fact that soils richer in organic matter possess higher nutrient availability and more stable physical conditions for microbial metabolism, in particular for what concerns water availability (De los Rios et al., 2014; Pointing et al., 2009; Smith, 1999; Yergeau et al., 2007). Increasing microbial abundance along the gradient of SOC content was, however, accompanied by a change in microbial community structure. Indeed, although bacteria were overall dominant, fungal biomass increased more than bacterial biomass with increasing amount of SOC, as indicated by increased F:B ratios along the SOC gradient. Because soils characterized by low or very low organic matter content do not provide a high number of suitable niches for fungi (Adams et al., 2006; Dennis et al., 2012; Siciliano et al., 2014), the observed shift in the F:B ratio can be explained by considering that fungi are more dependent on fixed organic C sources as compared to bacteria. Similar results in spatial fungal abundance were also observed along retreated glacial chronosequences where fungi become more abundant in late-successional soils in response to accumulation of soil organic matter (e.g. Allison et al., 2007; Brown and Jumpponen, 2014; Dong et al., 2016; Fernandez-Martinez et al., 2017).

A more detailed analysis of microbial community structure based on PLFA biomarkers showed a higher abundance of microbial species with a ruderal life-strategy, such as Actinomycetes and Gram-positive bacteria, in soils with lower SOC content, a result that may reflect not only a different adaptation of microbes to quality and quantity of soil organic matter (Bragazza et al., 2015; Bird et al., 2011; Drigo et al., 2010; Fanin and Bertrand, 2016; Fierer et al. 2003; Kramer and Gleixner, 2008; Paterson et al., 2007), but also a different microbial resistance to environmental stress. Indeed, microbes in soils with lower SOC content are expected to experience less stable micro-habitat conditions so that stress-resistant microbes, such as Gram-positive bacteria, can be here more competitive in light of their higher inherent resistance to, for example, water stress (Hopkins et al., 2014; Schimel et al., 2007) .

Concerning the second research question (i.e. the relationship between soil enzymatic activity and soil biogeochemistry), the positive correlation between total microbial biomass and total enzymatic activity per unit of SOC indicates a higher investment in extracellular enzymes in order to sustain a greater microbial biomass (Capek et al., 2015; Geyer et al., 2017; Hopkins et al., 2008; Stock et al., 2019; Turner et al., 2002). However, the relative investment in specific hydrolytic enzymes changes in relation to the amount of SOC. When plant-derived organic matter starts accumulating in soils, we can observe an increase of BGL:(XYL+AGL) ratio, which may be explained by a higher activity of β-glucosidase involved in the release of glucose from cellulose (Geyer et al., 2013; Stone et al., 2012). Similarly, the ratio of chitinase activity to total activities of aminoacids-degrading enzymes, i.e. CHIT:(LEU+ARG+ALA+GLY) ratio, increased with increasing amount of SOC, a trend in line with the accumulation of plant-derived organic matter (Zeng et al., 2015), as well as with the greater abundance of fungi (Kelley et al., 2011) and microfauna (Smykla et al., 2018), containing chitin, in response to the accumulation of organic matter. Overall, these results indicate that microbes modify the relative investment in extracellular enzymes in relation to the chemical composition of available organic matter (Antony et al., 2014; Chukov et al., 2015).

Concerning the third research question (i.e. the role of nutrient limitation on microbial metabolism), the eco-enzymatic stoichiometry of microbial nutrient acquisition based on C:N and C:P ratios showed a relatively higher activity of N- and P-hydrolyzing enzymes compared to C-hydrolyzing enzymes, particularly at low SOC content. This result indicates a major limiting role of P and, particularly, N as compared to C and it is in agreement with the results of fertilization experiments in similar polar deserts showing a C and N co-limitation on biotic soil activity (Ball et al., 2018; Ball and Virginia, 2014; Hopkins et al., 2008). However, in consideration of the wide range of soil pH here investigated, we hypothesize that a relatively higher activity of N and P enzymes is primarily controlled by soil pH, particularly in soils with low SOC content. Indeed, after normalization of enzymatic activity to SOC content, the so called specific enzyme activity (Sinsabaugh et al., 2008), we observed that the increase of activity per unit of pH change is steeper for N- and P-hydrolyzing enzymes than for C-hydrolyzing enzymes. Such response is in accordance with previous studies about the role of soil pH in affecting hydrolytic enzymes and the stronger positive effect of soil pH on P- and N-degrading enzymes compared to C-degrading enzymes (Geyer et al., 2017; Sinsabaugh et al., 2008; Stark et al., 2014; Stock et al., 2019). This means that the activity of N- and P-degrading enzymes in soils with low SOC is more directly controlled by soil pH, i.e. by abiotic conditions, and that only with the accumulation of plant-derived SOC the effect of local soil pH is reduced so that a stronger control by soil microbial community (i.e. biotic conditions) takes place (Castle et al., 2017; Schimel and Schaffer, 2012; Zeglin et al., 2009).

1. **Conclusions**

Our study provides a mechanistic insight on the biogeochemical feedback between the abundance of mosses and lichens and soil microbes in Victoria Land (continental Antarctica). Overall, moss cover is the major driver of accumulation of SOC in these types of soils. Higher content of SOC positively affects the microbial biomass, in particular the relative abundance of fungi. Total activity of hydrolytic enzymes involved in C, N and P degradation reflects the amount of microbial biomass with a primary abiotic role of soil pH in controlling the specific enzymatic activity in soil with low SOC. Overall, our data indicate that any change of plant cover, in particular of mosses, in Victoria Land can affect soil biogeochemistry in the light of the strict relationships between aboveground and belowground living components also in soils that are in early development states.

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**Appendix A. Supplementary data**

Supplementary data related to this article can be found.

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**Fig. 1.** Soil organic carbon (SOC) and total microbial biomass (based on PLFAs content) in relation to total plant cover (lichens + mosses) at each permanent plot. Dashed line represents the linear regression for SOC (y = 0.15 + 0.022x, r*2* = 0.35, p = 0.020, n = 15), whereas solid line is the regression for total PLFAs (y = 8.15 + 0.12x, r*2* = 0.26, p = 0.052, n = 15).

**Fig. 2.** Non-metric multidimensional scaling (NMDS) ordination of microbial PLFAs biomarkers as measured in 15 permanent plots. The vectors in the ordination diagram represent the gradient of selected chemical and biological variables, namely soil organic carbon (SOC), soil pH, soil dissolved nitrate, ammonium and phosphate concentrations, as well as vegetation cover. Asterisks indicate independent and statistically significant (p < 0.05) variables. Stress value = 0.095.

**Fig. 3.** Total activity of enzymes targeting carbon (C) (dashed line), nitrogen (N) (dotted line), and phosphorus (P) (solid line) compounds in relation to total microbial biomass (based on PLFAs) after normalization with soil organic carbon (SOC) content (n = 15). Regression analysis was significant for C (r2 = 0.50, p < 0.01) and P enzymatic activity (r2 = 0.57, p < 0.01), but not for N enzymatic activity (r2 = 0.22, p = 0.08).

**Fig. 4.** Activity of C-, N- and P-hydrolyzing enzymes in relation to soil pH. Enzyme activity was normalized for the corresponding content of soil organic carbon (SOC). Enzyme abbreviations and associated linear regressions are: AGL = α-glucosidase, XYL = xylosidase, BGL = β-glucosidase, ALA = alanine-aminopeptidase, ARG = arginine-aminopeptidase, GLY = glycine-aminopeptidase, CHI = chitinase, LEU =leucine-aminopeptidase, and PHO = phosphatase. Linear regression for each enzyme was: AGL (y = 0.13x – 1.08, r*2* = 0.50, p < 0.01), BGL (y = 0.03x – 0.18, r*2* = 0.06, p = 0.38), XYL (y = 0.16x – 1.71, r*2* = 0.47, p < 0.01), ALA (y = 0.37x – 2.14, r*2* = 0.86, p < 0.001), ARG (y = 0.30x – 1.99, r*2* = 0.71, p < 0.001), GLY (y = 0.40x – 2.53, r*2* = 0.88, p < 0.001), CHI (y = 0.07x – 0.72, r*2* = 0.16, p = 0.14), LEU (y = 0.33x – 1.78, r*2* = 0.86, p < 0.001), PHO (y = 0.17x – 0.80, r*2* = 0.59, p < 0.001). Number of soil samples n = 15.

**Fig. 5.** Ratio of the activities of enzymes associated to C-compounds degradation (dashed line: r2 = 0.66, p < 0.01, n =15) and N-compounds degradation (solid line: r2 = 0.38, p < 0.05, n = 15) in response to increasing amount of soil organic carbon. For C-hydrolyzing enzymes the ratio refers to β-glucosidase relatively to the sum of xylosidase and α-glucosidase activities, whereas for N-hydrolyzing enzymes the ratio refers to the activity of chitinase relatively to the sum of activities of protein-hydrolyzing enzymes (i.e. leucine-, alanine-, glycine-, and arginine aminopeptidase).

**Fig. 6.** Relationship between the eco-enzymatic C:N and C:P stoichiometry. The area of each circle is proportional to the amount of organic carbon in the corresponding soil sample. Dashed line is the linear regression analysis (y = 0.74x + 0.44, r*²* = 0.62, p < 0.001, n = 15).