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PII: S0048-9697(20)35131-7

DOI: https://doi.org/10.1016/j.scitotenv.2020.141602

Reference: STOTEN 141602

To appear in: *Science of the Total Environment*

Received date: 12 September 2019

Revised date: 5 August 2020

Accepted date: 8 August 2020

Please cite this article as: F.J. Díaz-Puente, T. Schmid, M. Pelayo, et al., Abiotic factors influencing soil microbial activity in the northern Antarctic Peninsula region, *Science of the Total Environment* (2020), https://doi.org/10.1016/j.scitotenv.2020.141602

ABIOTIC FACTORS INFLUENCING SOIL MICROBIAL ACTIVITY IN THE NORTHERN ANTARCTIC PENINSULA REGION

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Abstract: Microorganisms play a key role in the carbon (C) cycle through soil organic matter (SOM). The rate of SOM mineralization, the influence of abiotic factors on this rate and the potential behaviour of SOM are of particular interest in the northern Antarctic Peninsula and offshore islands. This is one of the most rapidly warming regions on Earth with numerous ice-free areas, some with abundant wildlife and with the greatest known soil organic carbon (SOC) storage in Antarctica. The latter implies extended Antarctic summer conditions promote increased terrestrial plant growth and soil microbial activity (SMA). SMA, determined by respirometry, is a measure of ecosystem function, and depends on microclimatic conditions and soil environmental properties. SMA and the effect of abiotic variables have been analysed in locations with different soil types, on Cierva Point (Antarctic Peninsula), Deception Island and Fildes Peninsula (King George Island). Soil microbial biomass carbon (SMBC) ranged from 5.66 to 196.6 mg SMBC kg^{-1} and basal respiration (BR) from 2.86 to 160.67 mg CO_2 kg^{-1} d^{-1}. SMBC and BR values were higher in Cierva Point, followed by Fildes Peninsula and Deception Island, showing the same trend of SOM abundance. Except for Cierva Point, low nitrogen, phosphorus and C concentrations were observed. SMBC/total organic carbon (TOC) levels indicated that SOC was recalcitrant and SOM content was closely related to the extent of vegetation cover observed *in situ*. High metabolic quotient values obtained at Cierva Point and Deception Island (median values 7.27 and 6.53 mg C-CO_2 g $SMBC^{-1}$ h^{-1}) and low SMBC/TOC in Cierva Point suggest a poor efficiency of the microbial populations in the consumption of the SOC. High SMBC/TOC values obtained in Deception Island indicates that SMBC may influence SOM stabilization. Mineralization rates were very low (negligible values to 1.44%) and sites with the lowest values had the highest SOM.

Keywords: Microbial activity, Soils, Organic matter, Mineralization, South Shetland Islands.

1. Introduction

Biogeochemical cycling of soil carbon (C) is mainly undertaken by microorganisms, through the process of mineralization of soil organic matter (SOM). Important and measurable proxies for the cycling of C in soils are soil microbial biomass carbon (SMBC) and soil microbial activity (SMA). Both SMA and SMBC are sensitive to environmental changes and have been widely used as indicators to assess soil quality and ecosystem health. The size of the SMBC has been proposed as a sensitive indicator for measuring the adverse effects of contaminants on the soil microbial community with soils contaminated by pesticides (Sousa et al., 2004), deposition of atmospheric pollutants (Klumpp et al., 2003) and heavy metals having lower SMBC (Barajas-Aceves, 2005). SMBC is determined through laboratory-based soil respiration experiments, whereby the metabolic quotient (qCO_2), a measure of the SMA, C-CO_2 emitted, per unit of SMBC, can be determined. Previous research has shown high qCO_2 values can be indicative of microbial populations inefficiently utilising soil organic carbon (SOC), such as during the early stages of pedogenesis, as shown in soil chronosequences (Insam and Haselwandter, 1989) or as soil develops in tailing waste following coal mining activities (Insam and Domsch, 1988). High qCO_2 values are also observed in soils contaminated by organic compounds (Frische and Höper, 2003) or heavy metals (Nordgren et al., 1988). Interpretation of qCO_2 is intuitive, because under conditions of stress, such as the scenarios mentioned above, microorganisms tend to devote resources and energy to maintenance, rather than growth and therefore a decrease in SMBC (Anderson, 2003). In certain cases, no predictable *q*CO2 values have been observed in soils polluted with heavy metals (Nordgren et al., 1988) or in soil chronosequences (Wardle and Ghani, 1995). Wardle and Ghani (1995) found unusually high qCO_2 values in a 120,000 year old vegetated soil in the Franz Joseph Glacier area chronosequence, in New Zealand, and in a later publication the same authors attributed the high qCO_2 values to the unexpected scarcity of nutrients, low phosphorus (P) availability and to the low quality of SOM (Wardle and Ghani, 2018). Due to the potential limitations of the metabolic

quotient and that no one single variable can accurately deal with the often complex eco-physiological state of the soil microbial populations, some authors encourage the use of other complementary variables to cope with the status of the microbial populations. One such variable is the ratio SMBC to total organic carbon (TOC) ratio (Anderson, 2003), where soil microbial populations under stress should present high qCO_2 values and low SMBC/TOC ratios. The SMBC/TOC ratio has been used as an indicator of the quality of the SOM, as microorganisms are unable to incorporate highly recalcitrant SOM as SMBC, obtaining low values of the SMBC/TOC ratio (Insam and Domsch, 1988; Wardle, 1992). However, in heavily weathered tropical soils a high SMBC in relation to low TOC has been reported and suggested to occur because SOM is weathered unless included in the living organisms and microbial immobilization becomes a mechanism of nutrient retention (Luizao et al., 1992).

SMA and SMBC investigations have been undertaken in the McMurdo Dry Valleys (Barrett et al., 2006a Zeglin et al., 2009; Van Horn et al., 2014) and in different regions of East Antarctica (Bölter, 1992; Roser et al., 1992a, 1993b; Zeglin et al., 2009; Ma et al., 2013). However, to-date, studies in the West Antarctica investigating soil microbial activity (SMA) through basal respirometry or soil microbial biomass using substrate induced respiration (SIR) method are limited to just one (Tscherko et al., 2003). The northern Antarctic Peninsula region of West Antarctica is of great interest to study SMA for a number of reasons: a) it is one of the most rapidly warming regions on Earth, with temperatures having risen by up to 0.56 ºC per decade since the 1950s (Turner et al., 2002, 2014); b) the region includes numerous ice-free areas in which soil formation occurs (Bockheim, 2014), including areas recently exposed to pedogenic processes where incipient soils are beginning to form (Bölter, 2011), and areas where periglacial processes are predominant (López-Martínez et al., 2012), with freshwater availability and an active water cycle in summer (Moreno et al., 2012). Such ice-free areas have the greatest storage of SOC within Antarctica. In this region, soil formation is influenced by the lithology, geomorphological setting, physical weathering freeze-thaw cycles, limited chemical weathering and vegetation cover (Cannone et al., 2006; Navas et al., 2008; Balks et al., 2013; Michel et al.,

2014). Furthermore, permafrost has a variable distribution and continuity with an altitudinal trend which results in pedodiversity (Bockheim et al., 2013; Simas et al., 2015).

SOM, quality and quantity, are the most likely factor to influence SMBC and SMA (Wynn-Williams, 1982; Barret et al., 2006a). There are other factors to consider, such as pH (Roser, 1993a and b), electrical conductivity (EC), as well as available nutrients, nitrogen (N) and P (Tscherko et al., 2003; Hopkins et al., 2006). These factors could also limit SOM mineralization, leading to its increase in the soils of the South Shetland Islands.

Due to the dynamic environment and limited previous studies into the role of soil microorganisms in C cycling in West Antarctica, samples were analysed from several sites in the northern Antarctic Peninsula region to determine SMA and SMBC, to study what abiotic factors have the greatest influence on SOM mineralization rates. The objectives of this work were: i) determine SMBC and basal respiration at selected sites in the northern Antarctic Peninsula region; ii) analyse soil microbial population stress; and iii) determine the influence of the main edaphic variables on basal respiration, SMBC and soil microbial population stress indicators, and iv) analyse the potential behaviour of the SOM.

2. Materials and methods

2.1. Description of the study sites, soils and sampling point locations

Study sites were located in the South Shetland Islands and the northern Antarctic Peninsula, including: Deception Island (DI), Fildes Peninsula (FP) on King George Island, and Cierva Point (CP) in the north-western coast of the Antarctic Peninsula (Fig. 1). The region has a cold and moist, maritime climate with mean annual air temperature of −2.2 °C, mean summer air temperatures above 0 °C for up to four months and between 350 to 1,000 mm precipitation per year (Simas et al., 2015).

Soil samples were taken at FP, DI and CP (Figs. 1c to 1e) during a field campaign from mid-December 2012 to the beginning of February 2013. The campaign started on FP where snow cover made the selection of areas of interest somewhat difficult and limited. Sampling was

carried out within areas representing different geomorphological features and surface covers. On DI and FP sampling was constrained to areas where soil forming processes was minimal such as on weathered bedrock with vegetation cover and fine volcanic deposits where bare soil and sparse vegetation was present (Schmid et al., 2017). In contrast, at CP, where there is an abundance of vegetation, samples were taken in areas that had a high vegetation cover and soil forming processes were more advanced. Key characteristics of the sampling sites are included in the supplementary material Table SM1. The location was registered with a GPS (handheld Garmin), and the surface cover type and the vegetation cover percentage were determined in the field for the different sites. Soil surficial samples to a depth 15 cm were taken and stored at 4 ºC. The samples were divided and one half was sieved (<2 mm) and kept in a field moist state for respirometric analysis, while the other half was air-dried at room temperature until a dry weight in equilibrium was attained and then sieved (<2 mm) for physical and chemical analysis.

2.2. Soil chemical and physical properties

Different edaphic variables were measured and included: pH (soil:water-1:2.5); EC (soil:water-1:5), and particle size analysis using the Bouyoucos densimeter procedure (López-Ritas and López-Melida, 1990; International Organization for Standardization, 2016). Water holding capacity (WHC) and moisture content were analysed following standard procedures according to Guitián-Ojea and Carballas (1976).

Oxidizable organic carbon (OC_{ox}) was determined by wet acid oxidation and a recovery factor = 1 was applied (Walkley and Black, 1934; Walkley, 1947). TOC was determined as the difference between total carbon (TC), obtained by means of a LECO CHNS-932 Elemental Analyzer using the Dumas combustion method (LECO Corp., St. Joseph, USA) and Inorganic C, determined by combustion 200 ºC after sample acidification with hydrochloric acid, through a TOC-V-CSH (Shimadzu Scientific Instruments, Kyoto, Japan). Nitrogen Kjeldhal (N(K)) was analysed by concentrated sulphuric acid digestion (Digestion Unit Büchi K-435) and steam distillation (Scrubber Büchi B-414 and Distillation Unit Büchi K-360) to finally determine reduced nitrogen forms by a boric acid titration (Titriator Metrohm 848 Titrino plus). Total

nitrogen (N_{tot}) was also determined with a LECO CHNS-932 Elemental Analyzer and the C/N ratio could then be calculated (TOC/N_{tot}**)**. Soluble P was extracted following the procedure by Olsen and Sommers (1982), the solution was shaken for 30 min at 180 rpm, supernatant filtered, and finally soluble P was determined by a colorimetric reaction (430 nm UV-VIS spectrophotometer), with a nitrovanadomolybdic reactive agent. Cation exchange capacity (CEC) was determined after soil samples were saturated with sodium acetate, gently mixed manually for 5 min and centrifuged (8000 rpm), then the precipitate was washed with ethanol, mixed and centrifuged. This procedure was repeated until the EC of the supernatant was lower than 40 μS cm^{-1}, then sodium was displaced by ammonium acetate and determined in solution by atomic absorption spectrometry.

2.3. Basal respiration, maximum respiratory rate and microbial biomass carbon

Respirometric assays that include SMBC by SIR, maximum respiratory rate (MRR) or BR were performed with a Micro-Oxymax respirometer (Columbus Insts. Ohio, USA). The air sample was dried before CO_2 was determined with an infrared sensor (range 0-1.5% CO_2 accuracy ± 0.01%) and O_2 was measured with a paramagnetic sensor (range 0-21% O_2 accuracy ± 0.007%). In order to measure BR, soil samples were moistened to 70% of their WHC, immediately after which the bottles containing soil samples (10 g dry soil equivalent per bottle and a triplicate of each sample) were connected to the respirometer and the respiration rate was measured hourly. During the assay, the temperature was kept at 22±0.5 ºC. Data used to determine soil respiration rates were based on the average of CO_2 rates obtained between 20 to 30 h after the start of the assay; when more stable respiration rates were reached (Margesin et al., 2000) The obtained value was then used to calculate qCO_2. Furthermore, CO_2 accumulated during a 24 hour incubation period was also determined. The SMBC by SIR was estimated as follows: soil samples were moistened and incubated overnight at 22 ºC. Immediately prior to the analysis, glucose was added to the soil sample with a solution containing sufficient water to moisten samples at 70% of their WHC. During the assay, the temperature was kept at 22±0.5 ºC following the procedure described by Anderson and Domsch (1978). Optimum respiration rate

was tested at 6 g glucose kg^{-1} dry soil. SIR analysis was prepared with 10 g of soil and each assay was carried out in triplicate. SMBC was calculated from the equation of Anderson and Domsch (1978). The MRR was the maximum CO_2 production rate obtained in the first 7 h of the SIR assay. C_{mic}/C_{org} ratio has been evaluated as the ratio of the SMBC to TOC, instead of OC_{ox}, because some of the OC_{ox} values were under the detection limit of the procedure.

2.4. Statistical analysis

The Shapiro-Wilk's test was performed to determine whether the data were normally distributed; while the Levene test was carried out to check the homoscedasticity of the values. When a normal distribution was assumed ($P > 0.05$), one-way analysis of variance (ANOVA) was used to evaluate the existence of significant differences, those assumed when $P < 0.05$, for the analysed variables. Non parametric tests were applied when a normal distribution could not be assumed, then the Kruskal-Wallis test was applied to check differences and ($P < 0.05$) the Mann-Whitney test to form the groups. In the Mann-Whitney test, the significant level was ($P < 0.05$). Tau b coefficient was performed to establish possible relationships between SMA variables and physico-chemical variables, the Correlation was significant at $P < 0.05$ or $P < 0.01$ level.

Multivariate analysis was performed in order to reduce and organize variables into components and to analyse the relation of the variables with the components and the degree of explanation of the variable offered by the components. Kaiser-Meyer-Olkin (KMO) and Bartlett tests were used to check the suitability of the factorial analysis. Hence, most variables were subjected to the communalities analysis and those obtaining a value over 0.6 were selected and, based on the correlation matrix, components were extracted with principal component analysis (PCA). All the statistical analyses were performed with SPSS for Windows versions 11.5.1 and 26.0 under contract 0077403979 (IBM SPSS Inc.).

3. Results

3.1. Vegetation cover, soil physical and chemical characteristics

The soils had the greatest mean average percentage of vegetation cover on CP (47%) followed by KGI (5%) and DI (4%). The vegetation cover consisted mainly of mosses and lichens, but also contained grass (Deschampsia antarctica). Soils at all study sites were poorly developed, and limited to accumulation of organic matter in the upper horizons (Table 1 and Table SM2). Particular lithological and depositional conditions are present where coarse fractions (coarse fragments of greater than 2 mm and sand) predominate, although heterogeneity in fine earth versus coarse fragments were determined. At CP, the particle size distribution shows a remarkable high percentage of fine earth fraction. More heterogeneous values were obtained in DI and FP. Soil textural analysis showed a general predominance of sandy loam classes, with a sand content of over 50% in all samples. FP showed the highest sand content of up to 98% with a more heterogeneous textural classification, from "sandy clay loam" (finer) to "sand" (coarser).

CP had a higher EC than sampling sites at DI or FP with the maximum values reaching 1191, 311 and 211 µS cm^{-1}, respectively. The highest WHC and CEC values were from CP and lowest values at DI with intermediate values at FP (Table 1).

Soil pH values obtained in CP indicate strongly acid conditions, except for one of the samples with a pH value close to 7.4. A wider range of pH was obtained in DI samples, from 4 to 8.8 and pH values closer to neutrality in FP, from 6.5 to 8.4. Apparently, the differences in the parent material of the soils can explain the variability observed in pH. There were statistically significant differences ($P < 0.05$) between pH values analysed in CP and those analysed in FP and in DI.

The highest values of OC_{ox} and TOC were obtained in CP samples. TOC ranges between 4.22% and 46.14%., the TOC values obtained in CP are higher and statistically significant different from the values obtained in samples from DI and FP (Table 1). The lowest values of OC_{ox} were determined in DI samples, some of the samples were under the detection limit, while the highest OC values were obtained in CP samples and there were statistically significant differences among the three sampling points. At CP, OC_{ox} and TOC in soils had the highest values of 16.4% and 46.14% and mean values of 8.65% and 23.74%, respectively

(Table 2). The OC_{ox} and TOC values show statistically significant differences compared to samples from DI and FP (Table SM3). TOC/OC_{ox} values obtained for CP and FP are from 2.41 to 2.90 and 2.52 to 5.54, respectively, compared to those of DI with a much greater range of 1.72 to 28.73.

N and P showed significant differences between CP and those of the other two sites. N(K) analysis showed values of 0.35% to 2.45% at CP while N(K) values obtained in DI range from 0.00% to 0.12% (Table 1). N(K) values obtained for the FP sites range from 0.0 to 0.14%. N(K) values showed statistically significant differences among the sampling sites. The mean TOC/N_{tot} at CP and FP were 13.37 and 20.71 respectively, while the mean TOC/N_{tot} at DI was 3.02, showing statistically significant differences across sites at $P < 0.05$ (Table 1).

Soluble P obtained at CP ranged from 3.29 to 62.48 mg kg^{-1} (Table 1), where the highest soluble P was obtained for CP1 (Table 1) in an abandoned penguin rookery. In comparison soluble P values for DI and FP determined in this work range from 0.18 mg kg^{-1} to 28.78 mg kg^{-1}, and from 0.54 mg kg^{-1} (FP9) to 4.83 mg kg^{-1} (FP7). Soluble P values obtained in CP show statistically significant differences compared to those obtained in DI and FP.

3.2. Variables based on SIR

SMBC values ranged from 5.66 mg SMBC kg^{-1} in DI9 on volcanic soils with sparse moss and lichens on rocks and 6.4 mg SMBC kg^{-1} in CP4 in an abandoned penguin nest to 196.6 mg SMBC kg^{-1} in CP1, a site covered with grass (*Deschampsia antarctica*), moss and lichens (Table 2). SMBC values obtained in FP ranged 45.54 mg SMBC kg^{-1} to 141.31 mg SMBC kg^{-1}. The results show statistically significant differences across the different sampling sites (Table 2). The highest values were obtained in CP with a greater vegetation cover; the lowest values were obtained in the volcanic soil of DI, while FP had an intermediate range of values. A relatively high relationship, at $P < 0.01$, was obtained between SMBC and OC_{ox} ($p=0.51$), TOC ($p=0.49$) and N(K) ($p=0.47$) (Table 3).

MRR values ranged from 0.06 mg CO_2 100 g^{-1} (DI9) to 1.06 mg CO_2 100 g^{-1} (CP1) (Table 2); while the range of values obtained in FP range from 0.15 mg CO_2 100 g^{-1} to 0.86 mg CO_2 100 g^{-1}. There were statistically significant differences for the MRR values obtained at the

three study sites. Furthermore, the distribution of values obtained for MRR is quite similar to the distribution of values obtained for SMBC. Furthermore MRR is related with the same chemical properties as described for SMBC, OC_{ox} (p=0.49) at $P < 0.01$, TOC (p=0.45) at $P < 0.05$ and N(K) (p=0.45) at $P < 0.01$ (Table 3).

3.3. Variables based on basal respirometry

CO_2 accumulated in 24 h incubation period ranged from 2.86 mg CO_2 kg^{-1} d^{-1} (DI5) to 160.67 mg CO_2 kg^{-1} d^{-1} (CP1) (Table 2). The values obtained in CP are higher and over a greater range than the values obtained for the other sampling sites. DI has the lowest values and is followed by FP that has only slightly higher values than DI. There were statistically significant differences in BR across the three sampling sites, and likely relate to differences in soil properties. BR correlated to C_{org} (oxidizable and total) (p=0.58 and 0.60) and nutrients N(K_2) (p=0.59) at $P < 0.01$ and to a lesser extent to available P (p=0.46) and TOC/N_{tot} ratio (p=0.37) at $P < 0.01$(Table 3).

C mineralization rate per day, expressed as C-CO_2 kg^{-1} d^{-1}, with C as a percentage of the TOC, showed close to negligible values from 0.00 to 0.07% in CP, somewhat higher values of 0.13 to 0.89% in FP samples and the highest values of up to 1.44% were obtained in DI. There were statistically significant differences between sites, as C mineralisation rates were negatively related ($P < 0.01$) with SOM (TOC; OC, N(K), TOC/N_{tot} and PO_4^{3-}; as well as CEC) (Table 3).

3.4. qCO_2 and SMBC/TOC ratio

The values ranged from 1.84 mg C-CO_2 g $SMBC^{-1}$ h^{-1} (DI4) to 45.43 mg C-CO_2 g $SMBC^{-1}$ h^{-1} (CP4) (Table 2). CP tended to have lower values compared to DI and FP, with DI having a higher range of values, and FP, the lowest range of values (Table 2). There was no significant difference in qCO_2 values across the study sites.

Regarding SMBC/TOC ratio, CP values are very low, from 0 to 0.37, values for FP ranged from 0.78 to 8.72 and values in DI range from 1.02 to 27.22, where this latter high value was obtained for DI4. There were significant differences ($p < 0.05$) between the low values of SMBC/TOC in CP and the values obtained in DI or FP (Table 2).

3.5. *Relationship between the microbiological and soil variables*

The principal component analysis showed a matrix with four components (Table 4) explaining close to 86% of the variance. PC1 explained 37.52% of the variance and the highest Loading Factors (LF) were related to organic matter, provided to the soil through vegetation, variable vegetation cover (LF= 0.82), and, as a consequence high LF for nutrients and C (Soluble P of 0.92; N(K) of 0.91; TOC and OC_{ox} of 0.92, as well as SMBC 0.66). CEC is a variable that is highly and positively related to SOM content, with LF of 0.81. Furthermore, pH has a high, but negative LF of -0.74. SMA is positively related to this component, where BR LF is 0.85, CO_2 (accum. 24h) is 0.82 and MRR is 0.64. However, SMBC has its highest LF in PC2 with a LF of 0.71 as well as MRR with a LF of 0.74, while %TOC as C- CO_2 d^{-1} has a medium, but positive LF (0.42); qCO_2 has a very high and negative LF of -0.73 and SMBC/TOC LF is high and positive with 0.57. Component 3 explains 13.72% of the variance with the %TOC as C-CO_2 d^{-1} has the highest and positive LF of 0.65 and TOC/N_{tot} has a high, but negative value of -0.71. The 4th component only has a high LF for C/N ratio of 0.40 and a high, but negative LF for EC of -0.73.

Plotting regression factor 2 against regression factor 1 (Fig. 2a), CP shows the highest positive value for factor 2 (CP4) and the highest but negative value for factor 3 (CP4). Most DI and FP scores are around the intersection, most of them with negative values for factor 1, while DI4 and FP9 show high values for factor 2. Plotting Regression factor 3 against Regression factor 4 (Fig. 2b) shows the point with the highest and positive value for factor 4 (CP4), while the highest and positive value for score 3 is DI4. Most FP samples show negative values for the Regression factor 4.

Discussion

4.1. Environmental factors and soil physical and chemical properties

The soils from the different study areas are within ice-free areas that are dominated by paraglacial and periglacial processes and landforms (López-Martínez et al., 2012). The maritime climate ensures summer precipitation, a high relative air humidity (80–90%), and snow melt to

provide high summer water availability to soils. An abundance of water in the summer months is important for soil development as water promotes physical, chemical and biological processes (Bölter, 2011). Although conditions may be somewhat more favourable during the short austral summer, soil formation is limited by low temperatures that oscillate around 0 ºC and frequent freeze-thaw cycles.

These soils are generally regarded as poorly developed; however site specific factors will influence the different soil forming processes. Factors such as vegetation, proximity to the coastline and the influence of seabird colonies as seen in CP will enhance the formation of organic material and activity of microorganisms. These factors are closely related to the nutrients N and P; where CP showed 3 to 20 times and 2 to 18 times higher values, respectively, compared with KGI and DI. The N and P values for the individual sites were within the ranges reported by Haus et al. (2015) and Simas et al. (2015). Furthermore, CP samples were classed as nonsaline to slightly saline with a higher EC compared to the samples from DI or FP classed as nonsaline (Schoeneberger et al., 2012).

4.2. Soil Microbial Biomass Carbon

SMBC values showed important variations between the sampling sites, being higher at CP than in the rest of the sampling locations. This is highly consistent with the abundance of SOM at CP. In a Victoria Land study, reported SMBC values were highly variable, where SMBC values obtained by the fumigation extraction method (Vance et al., 1987) ranged from 8.2 to 506.1 mg SMBC kg^{-1} (Barrett et al., 2006a) and between 15 and 62 mg SMBC kg^{-1} in the McMurdo Dry Valleys (Zeglin et al., 2009). At Windmill Island, SMBC values were as high as 6700 mg SMBC kg^{-1} within active penguin areas 940 mg SMBC kg^{-1} in the surrounding area, 490 mg SMBC kg^{-1} in abandoned penguin areas, and 54 mg SMBC kg^{-1} on beach samples not influenced by penguins (Roser et al., 1993a). Conversely SMBC values obtained in this work from non-ornithogenic beach samples from DI were much lower in general and ranged from 5.7 to 136.1 mg SMBC kg^{-1} (Table 2), since such landforms are virtually free of SOM. Although the method to estimate SMBC is the same as that applied by Roser et al. (1993b), some of the

values obtained by these authors seem to be very high. These authors suggested that the SMBC values reported in the penguin areas were overestimated because soil samples were alkaline, known to favour the growth of bacteria over fungi and consequently the very active bacterial communities likely increased the substrate induced respiratory rate (Roser et al., 1993b). In a study investigating two transects from the Ecology Glacier (King George Island) SIR-derived SMBC values ranged from 45 mg SMBC kg^{-1} (distant to bird influence) and rose up to 1950 mg SMBC kg^{-1} near penguin rockeries or in the proximities of areas with sea bird excrement (Tscherko et al., 2003). Data reported in this paper from samples taken on FP range from 19.7 mg SMBC kg^{-1} to 141.3 mg SMBC kg^{-1}. SMBC values (by way of biovolume method) were reported in the range of 0.5 mg SMBC kg^{-1} (in bare soils) to 1554 mg SMBC kg^{-1} (vegetated soils) near Arctowski Station (King George Island), showed a high positive correlation with the TOC (Bölter, 1995). A further consideration when comparing our SMBC results between locations and with previous studies is that our sampling was largely influenced by local meteorological conditions. Particularly on FP, site selection was restricted to often bare soil surfaces with sparse vegetation. This is relevant when comparing our results with those of other authors and also affects the results obtained in the following subsections.

4.3. Basal Respirometry

The highest hourly BR values (mg CO_2 kg^{-1} h^{-1}) were found in samples from CP, which were approximately four times the median value analyzed in the other locations (DI and FP) (Table 1). Daily BR values from this work present statistically significant differences across the sampling areas. The values were lower than those reported for the Arctowski Glacier area (Tscherko et al., 2003), which were between 100 and 700 mg CO_2 kg^{-1} d^{-1}, values that the authors regard as low values. Nevertheless the values obtained in this work were in the range of 2.9 to 161 mg CO_2 kg^{-1} d^{-1}. These were higher than those obtained during laboratory incubation experiments (10 ºC) from soil sampled from the Garwood Valley, which were between 1.15 and 17.7 mg CO_2 kg^{-1} d^{-1} (Hopkins et al., 2006), and much higher than the values obtained at 20 ºC, between 1 and 2.5 mg CO_2 kg^{-1} d^{-1} in McMurdo Dry Valleys (Barrett et al., 2006a). The rate of

CO_2 production, measured by respirometry, is the result of SOM mineralization, and is generally low in Antarctic soils as the carbon source is low or highly recalcitrant (Pires et al., 2017). As mentioned previously, environmental factors such as temperature in incubation experiments (Hopkins et al., 2006), or available water and/or nutrients (Barret et al., 2006a) influence BR, as well as SMBC, which our results suggest, positively influences BR. Furthermore, the relationship between BR per unit of SMBC could offer an indication of the stress of the microbial population under investigation (Anderson and Domsch, 2010).

4.4. Metabolic quotient and C_{mic}/C_{org}

In the case of the Antarctic soils, high qCO_2 levels were expected due to the scarcity of available nutrients (C, N and P) (Tscherko et al. 2003). The values obtained from this study ranged from 1.84 mg C-CO_2 g $SMBC^{-1}$ h^{-1} (DI4) to 45.43 mg C-CO_2 g $SMBC^{-1}$ h^{-1} (CP4) (Table 2); which are very high as expected of poorly developed soils (Insam and Haselwandter, 1989) and in agreement with results reported in other studies (Tscherko et al., 2003; Ma et al., 2013). Furthermore, qCO_2 values obtained in this study for FP samples ranged from 1.91 mg C-CO_2 g $SMBC^{-1}$ h^{-1} to 9.47 mg C-CO_2 g $SMBC^{-1}$ h^{-1}, while reported values for FP (Tscherko et al., 2003) ranged from close to 2 mg C-CO_2 g $SMBC^{-1}$ h^{-1} to 30 mg C-CO_2 g $SMBC^{-1}$ h^{-1}, which are in general agreement with our results. Some authors have been reported that high qCO_2 can be related to low P concentrations (Tscherko et al., 2003 Wardle and Ghani, 2018), but our data do not directly support this finding. However, some relationship is inferred as indirectly with variables such as SMBC and *q*CO2 related to microbial activity that shows some correlation with P_{tot}.

Values obtained for the SMBC to TOC ratio (Table 2) were very low in samples from CP, and are in agreement with data reported from penguin nests in East Antarctica (Ma et al., 2013). Low SMBC/TOC ratio can be understood as a measure of the quality of the SOM, because recalcitrant SOM resists microbial decomposition and therefore, unable to be incorporated into SMBC (Insam and Domsch, 1988; Wardle, 1992). Our SMBC values obtained in FP and DI were relatively high, whereas reported TOC values in bare, non ornithogenic soils,

(Tscherko et al., 2003) were lower than the observed in this work for DI and FP. However, SMBC in these samples were in the same range as those obtained in this work for DI and FP, suggesting also high values of the SMBC/TOC.

4.5. Factors affecting SMBC and SMA

There are a number of soil physico-chemical characteristics that can influence SMBC and SMA in Antarctic soils such as: pH, available nutrients and OC (Wynn-Williams, 1982; Tscherko et al., 2003; Barrett et al., 2006a; Ma et al., 2013), available water, at least in very dry soils (Zdanowski and Weglenski, 2001; Treonis et al., 2002; Hopkins et al., 2006), or both (Hopkins et al., 2006; Zeglin et al., 2009). Our results support a strong, positive relationship between SMBC and SOM (OC_{ox} or TOC and nutrients, $N(K_2$ and PO_4^{3-}; as well as with CEC).

In this work, variables related to SMA, such as BR, MRR, and CO_2 accumulated in a 24h period correlate positively with the factors associated with SOM, including different forms of carbon (TOC and OC_{ox}), nutrients and CEC. In addition SMBC is also strongly correlated to SMA. This is consistent with results obtained in *in situ* soil respirometry assays (Barrett et al., 2006b), while *in situ* measured CO_2 flux in Rothera Point, Antarctic Peninsula, was associated with the presence of vegetation, (Cannone et al., 2012), which supports our results where vegetation cover and BR correlate with higher levels of SMA. Ganzert et al. (2011) compared the numbers of culturable heterotrophs in vegetated and non-vegetated surficial soil samples on Livingston Island; they found a range of values from 2.3×10^4 to 1.2×10^8 in vegetated samples and a range of values from 1.6×10^3 to 8.8×10^4 in non-vegetated soil. Given the characteristics of the SMA, BR, MRR, and SMBC data obtained from our CP vegetated sites, we would expect higher levels of culturable bacteria at CP, compared with non-vegetated DI and FP sites.

4.6. *Potential behavior of SOM*

We have reported high values of qCO_2, in agreement with results obtained by others authors (Tscherko et al., 2003), thus suggesting a low efficiency of the soil microbial population in the SOM mineralization. These authors also found a negative correlation between P and qCO_2, in our study we have found the highest negative relation of qCO_2 with variables such as SMBC and MRR, as well as with the ratio SMBC/TOC. Values obtained for the SMBC/TOC

are very low in the CP samples and are similar to those reported by Ma et al. (2013) for ornithogenic soils. In our SMBC/TOC values for DI and FP are in the range of values reported in a highly weathered tropical forest (Luizao et al., 1992), this being consistent with the scarcity of available nutrients and SOM in those sites, and most available SOM already immobilized in the SMBC. We have obtained a negative correlation between TOC/N_{tot}, SMBC/TOC and TOC mineralized in a 24h period, which, in agreement with findings of Pires et al. (2017), suggests that in non-vegetated soils with a low TOC/N_{tot}, a higher percentage of TOC is mineralized in 24h period. In this work, the ratio of TOC to OC_{ox} (Table 1) was used to estimate the recalcitrance of the carbon source, considering OC as a relatively labile carbon source against the TOC (Kumpiene et al., 2011). Furthermore, the percentage of TOC mineralized in 24h (C-CO_2 d^{-1} kg^{-1} as % of TOC) is extremely low in CP samples, from 0.00 to 0.07%, while in DI samples values of the C mineralized range from 0.10 to 1.44% and in FP values from 0.13 to 0.89%. These results are in agreement with the results of Pires et al. (2017) which showed a higher percentage of TOC mineralized in samples from mineral soils than in ornithogenic soils. The above mentioned variables could indicate low C-CO_2 emissions from soils and a potential increase in the SOM, but CO_2-C exchange experiments measured *in situ* (Cannone et al., 2012; Thomazini et al., 2016) show that soil temperature and moisture drive ecosystem respiration. Only in poorly drained sites SOM may accumulate (Thomazini et al., 2016) with the soil acting as a C-CO_2 sink, accumulating SOM. This has been documented in bare soils with very low gross primary production where positive net ecosystem exchange has been reported (Thomazini et al., 2016). A further complication is that most *in situ* experiments are undertaken during the middle of the austral summer (Cannone et al., 2012) when temperatures are highest, and therefore CO_2 flux highest and when attempts are made to replicate in in the laboratory environment at more representative Antarctic soil temperatures a decrease in the CO2 flux which has been seen as a consequence of a decrease of temperature (Laudicina et al., 2015; Pires et al., 2017). This decrease in the CO_2 flux happens simultaneously with the increase in organic matter in soils due to senescence as has been described in the Arctic regions (Nordstroem et al., 2001) and is supposed to occur in the Antarctic (Cannone et al., 2012).

5. Conclusions

Soil samples from CP have the highest SOM content which is a function of greater vegetation and close proximity to active and abandoned penguin colonies. Organic matter influences the quantity of the carbon source (TOC and OC_{ox}) as well as nutrients N(K) and PO_4^{3-}; and physical and chemical characteristics such as WHC and CEC. These variables correlate positively with SMA (as seen by BR and MRR) as well as SMBC.

SOM, in our study, shows a certain degree of recalcitrance to microbial degradation as shown by the low SMBC/TOC and the high qCO_2 that were seen in the CP samples. Both variables indicate some inefficiency in the use of the SOC by the soil microorganisms, and the recalcitrance or low quality of the SOM. The higher values of the SMBC/TOC obtained in DI and FP can be explained by the low TOC in these samples and the likely immobilization of C by microorganisms, as a factor that stabilizes the SOM.

The rate of mineralization and the percentage of the TOC mineralized per day are very low in the three sites. However, the mineralization rate is higher in the poorly developed soils of DI, followed by the samples obtained in FP (King George Island), and the lowest mineralization rate was seen in the CP (Antarctic Peninsula) samples. Taking into account the recalcitrance of the SOM, ratio TOC/OC_{ox}, qCO_2 and SMBC/TOC, it can be concluded that heterotrophic soil microbial activity is going to have a relatively low contribution to the C-CO_2 efflux from soil and the organic matter accumulation in soils from West Antarctica.

Acknowledgements: This work has been supported by the projects CTM 2014-57119-R and RTI2018-098099-B-100 of the Spanish R&D National Plan. The authors acknowledge the logistic support provided by the National Antarctic Programmes of Spain, Chile and Argentina. The authors also appreciated the reviews by two anonymous referees and the editor that have contributed to improve the manuscript.

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Table 1.- Soil physical and chemical properties

	Vegetation cover (%)	Fraction < 2mm (%)	Clay (%)	Silt (%)	Sand (%)	WHC (%)	pH	EC (µS cm^{-1})	CEC (cmol kg^{-1})	OC_{ox} (%)	TOC (%)	TOC/N_{tot}	TOC/OC_{ox}	N(K) (%)	PO_4^{3-} (mg kg^{-1})
Cierva Point (Antarctic Peninsula)															
N	6	6	5	5	5	6	6	5	6	6	6	6	6	6	6
Mean	47	92.8	11.6	34.2	54.4	227. 6	4.7[a]	445	76.4	8.65[a]	24.00[a]	13.37[a]	2.69[a]	1.74[a]	31.38[a]
Median	60	92.5	11.0	36.0	53.0	180.6	4.0	269	82.2	8.74	23.74	13.46	2.70	2.03	32.28
Min.	0	86.0	10.0	26.0	50.0	90.3	3.6	204	24.3	1.75	4.22	8.44	2.41	0.37	3.29
Max.	80	100.0	16.0	38.0	64.0	444.8	7.4	1191	108.5	16.4	46.64	18.66	2.90	2.45	62.48
Deception Island															
N	11	11	11	11	11	10	11	11	11	11	11	11	8	11	11
Mean	5	62.7	15.3	19.9	64.6	35.8	7.2[b]	64	5.0	0.0[illegible](b)	0.15(b)	3.20[(b)]	6.54[(a)]	0.03[(b)]	4.47[(b)]
Median	0	63.0	13.0	22.0	63.0	31.0	7.4	42	3.4	0.01	0.05	1.25	3.16	0.01	1.34
Min.	0	28.0	9.0	4.0	53.0	29.0	4.0	20	0.2	0.00	0.20	0.67	1.72	0.00	0.18
Max.	40	92.0	24.0	33.0	81.0	50.7	8.8	311	12.3	0.24	0.63	10.00	28.73	0.12	28.78
Fildes Peninsula (King George Island)															
N	13	13	13	13	13	13	13	13	13	13	13	13	13	13	13
Mean	4	70.5	13.0	10.6	76.5	32.0	7.3[b]	66.7	33.1	0.08[(c)]	0.33(b)	20.71[(a)]	3.53[(a)]	0.04[(c)]	1.95[(b)]
Median	2	70.0	14.0	12.0	80.0	33.3	7.4	54	34.2	0.07	0.25	10.50	3.54	0.04	1.59
Min.	0	49.0	2.0	0.0	52.0	25.4	6.5	27	13.1	0.02	0.10	5.00	2.52	0.00	0.54
Max.	20	99.0	27.0	26.0	98.0	38.3	8.4	211	51.7	0.17	1.09	109.00	5.54	0.14	4.83

PO_4^{3-} -

Different letters a, b, c imply that the groups are statistically different ($P < 0.05$).

Table 2- Soil microbial respirometry data

Statistics	SMBC mg SMBC kg^{-1}	MBR mg CO_2 h^{-1} 100 g^{-1}	BR(h) mg CO_2 kg^{-1} h^{-1}	BR(d) mg CO_2 kg^{-1} d^{-1}	$q(CO_2)$ mg C-CO_2 g $SMBC_c^{-1}$ h^{-1}	C-CO_2 $d^{-1}kg^{-1}$ (% TOC)	SMBC/TOC (%)
Cierva Point (Antarctic Peninsula)							
N	6	6	6	6	6	6	6
Mean	134.96[(a)]	0.79[(a)]	3.25[(a)]	79.64[a]	12.64[a]	0.018[a]	0.011[(a)]
Median	151.46	0.93	3.12	76.49	7.27	0.010	0.06
Min.	6.41	0.04	1.07	23.76	1.86	0.00	0.00
Max.	196.57	1.06	6.99	160.67	45.43	0.07	0.37
Deception Island							
N	11	11	11	11	11	11	11
Mean	33.55[(b)]	0.22[(b)]	0.52[(b)]	13.00[b]	7.16[a]	0.59[b]	5.00[(b)]
Median	15.99	0.14	0.44	8.48	6.53	0.46	2.47
Min.	5.66	0.06	0.22	2.86	1.84	0.10	1.02
Max.	136.11	0.81	0.97	26.43	12.53	1.44	27.22
Fildes Peninsula (King George Island)							

N	13	13	13	13	13	13	13
Mean	75.69$^{(c)}$	0.41$^{(c)}$	1.00$^{(c)}$	25.94^{c}	3.91^{a,b}	0.32^{c}	3.41$^{(c)}$
Median	70.36	0.37	0.99	25.03	3.65	0.24	2.65
Min.	19.67	0.15	0.34	10.90	1.91	0.13	0.78
Max.	141.31	0.86	1.95	58.72	9.47	0.89	8.72

SMBC - soil microbial biomass carbon; MRR - maximum respiratory rate; BR - basal respiration in hourly (h) rate and in daily (d) rate; $q(CO_2)$ - metabolic quotient; TOC - total organic carbon. Different letters (a, b, c) imply that the groups are statistically different ($P < 0.05$).

Table 3. Kendall's Tau b correlation coefficients

	SMBC	**MRR**	**BR**	**% TOC mineralized d^{-1}**	**CO_2 accum. in 24h**
OC_{ox}	0.51	0.49	0.58	-0.65	0.50
TOC	0.49	0.45	0.60	-0.70	0.52
Vegetation cover (%)				-0.53	
EC	0.43	0.44	0.48	-0.37	0.37
CEC	0.40	0.40	0.49	-0.44	0.42
N(k)	0.47	0.45	0.59	-0.53	0.58
PO_4^{3-}			0.46	-0.50	0.39
TOC/N_{tot}	0.35	0.32*	0.37	-0.32	0.31*

SMBC - soil microbial biomass carbon; MRR - maximum respiratory rate; BR - basal respiration; TOC – total organic carbon; OC_{ox} – oxidizable organic carbon; EC – electrical conductivity; CEC – cation exchange capacity; N(K) – nitrogen Kjeldahl; PO_4^{3-} -.
$^{*}P < 0.05$, the remaining values are $P > 0.05$.

Table 4. Correlation matrix.

Variable	**Component**			
	PC1	**PC2**	**PC3**	**PC4**
PO_4^{3-}	0.92	-0.13	0.10	0.08
TOC	0.92	-0.24	0.19	0.20
N(K)	0.91	-0.30	0.19	0.17
Vegetation cover	0.82	-0.07	0.00	0.16
pH	-0.74	0.23	-0.07	0.32
CEC	0.81	-0.08	-0.30	0.36
CO_2 (accum. in 24h)	0.82	0.40	0.05	-0.17
BR (mg CO_2 kg^{-1} h^{-1})	0.85	0.31	0.07	-0.11
EC	0.53	0.24	0.06	-0.73
SMBC	0.66	0.71	-0.08	0.04
MRR	0.64	0.74	-0.02	-0.01
SMBC/TOC	-0.30	0.57	0.59	0.30
%TOC as C-CO_2 d^{-1}	-0.48	0.42	0.65	0.27
OC_{ox}	0.92	-0.26	0.19	0.18
TOC/N_{tot}	0.14	0.22	-0.71	0.40
qCO_2	0.24	-0.73	0.39	-0.01

% of variance explained	37.52	25.40	13.72	9.90

PO_4^{3-}-BR - basal respiration; SMBC - soil microbial biomass carbon; MRR - maximum respiratory rate; $q(CO_2)$ - metabolic quotient.

Fig. 1. The study areas and location of the sampling sites: a) in Antarctica, b) within the South Shetland Islands and the northern Antarctic Peninsula, c) on Fildes Peninsula, d) on Deception Island and e) at Cierva Point.

Fig. 2. Regression analysis of a) PCA Factor 1 vs Factor 2 and b) PCA Factor 3 vs Factor 4 using 26 of the 30 samples (5 of CP, 8 of DI and 13 of FP) and where 4 samples were not taken into account (1 of CP and 3 of DI) because some soil properties values were below the detection limit or not determined (Tables SM2 and SM3).

Declaration of competing interests

☒ 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.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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F.J. Díaz-Puente: Conceptualization, Formal analysis, Investigation, Writing –Original Draft, Writing – Review and Editing.

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Graphical abstract

Highlights

Antarctic Peninsula (AP) region is one of the most rapidly warming regions on Earth
Soil organic carbon in Maritime Antarctic ice-free areas is scarce or recalcitrant.
Soil microbial biomass carbon and basal respirometry values are low.
Due to nutrient scarcity and carbon source recalcitrance qCO_2 values are high.
Greatest soil organic carbon storage in Antarctica is within the AP region