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Short-term impacts of polyethylene and polyacrylonitrile microplastics on soil physicochemical properties and microbial activity of a marine terrace environment in maritime Antarctica[☆]

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ABSTRACT

Evidence of microplastic (MP) pollution in Antarctic terrestrial environments reinforces concerns about its potential impacts on soil, which plays a major role in ecological processes at ice-free areas. We investigated the effects of two common MP types on soil physicochemical properties and microbial responses of a marine terrace from Fildes Peninsula (King George Island, Antarctica). Soils were treated with polyethylene (PE) fragments and polyacrylonitrile (PAN) fibers at environmentally relevant doses (from 0.001% to 1% w w⁻¹), in addition to a control treatment (0% w w⁻¹), for 22 days in a pot incubation experiment under natural field conditions. The short-term impacts of MPs on soil physical, chemical and microbial attributes seem interrelated and were affected by both MP dose and type. The highest PAN fiber dose (0.1%) increased macro and total porosity, but decreased soil bulk density compared to control, whereas PE fragments treatments did not affect soil porosity. Soil respiration increased with increasing doses of PAN fibers reflecting impacts on physical properties. Both types of MPs increased microbial activity (fluorescein diacetate hydrolysis), decreased the cation exchange capacity but, especially PE fragments, increased Na⁺ saturation. The highest dose of PAN fibers and PE fragments increased total nitrogen and total organic carbon, respectively, and both decreased the soil pH. We discussed potential causes for our findings in this initial assessment and addressed the need for further research considering the complexity of environmental factors to better understand the cumulative impacts of MP pollution in Antarctic soil environments.

1. Introduction

Microplastic (MP) pollution is a consequence of inappropriate use and disposal of plastics and a recognized global environmental concern as it becomes widespread in several marine and terrestrial ecosystems (Campanale et al., 2022; Horton et al., 2017; Lebreton et al., 2019), posing potential risks to living organisms (Khalid et al., 2021; Ma et al., 2023; Yang et al., 2021). MP occurrence in terrestrial environments is more representative in areas of high population density and intensive anthropic activities, but it can also reach remote regions (Büks and Kaupenjohann, 2020). Field research and atmospheric/oceanic

modeling have shown that global dispersion can support the occurrence of MPs in areas with low population density in coastal and high-altitude areas, from low to high latitudes (Evangelou et al., 2020; Godoy et al., 2022; Isobe et al., 2017; Lusher et al., 2015; Onink et al., 2019). Hence, despite geographic isolation, low human occupation, and protection under international law, the Antarctic continent is not exempt from MP pollution (Rota et al., 2022).

Plastic pollution has been reported in Antarctica since the 1980s (van Franeker and Bell, 1988), and has become the most common and enduring evidence of past and recent human activities at some coastal Antarctic sites (Bargagli and Rota, 2023). Most debris reported from

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islands around continental Antarctica are historic remnants of man-made debris since the beginning of human exploration, increasing recognition and concern over its impact on marine birds and seals (Convey et al., 2002). Recently, MPs have been detected in several compartments of the Antarctic marine (Kelly et al., 2020; Munari et al., 2017; Suaria et al., 2020) and terrestrial (Aves et al., 2022; González-Pleiter et al., 2020; Perfetti-Bolaño et al., 2022) environments, including the biota inhabiting these ecosystems (Bergami et al., 2020; Bessa et al., 2019; Fragão et al., 2021). External sources of MP contamination by long-distance transport are usually considered (González-Pleiter et al., 2020; Isobe et al., 2017; Lozoya et al., 2022), but it is evident that the highest concentrations of MPs in Antarctica occur near anthropic activities (Lacerda et al., 2019; Munari et al., 2017).

Coastal research stations act as localized sources of MPs for water and marine sediments, especially fibers originating from washing clothes (Cincinelli et al., 2017; Munari et al., 2017), and wind action can contribute to their dispersion and deposition with snow in inland terrestrial environments (Aves et al., 2022; González-Pleiter et al., 2020). The Fildes Peninsula (King George Island) hosts six permanent scientific bases and the sole airport of South Shetlands Islands, therefore the anthropic pressure is enormous (Lu et al., 2012) and includes the widespread presence of MPs (Lozoya et al., 2022). The Peninsula was subject of the first assessment of soil MPs in Antarctica, which suggested land use and occupation as the main sources of MP fragments (Perfetti-Bolaño et al., 2022). Human occupation in Antarctica occurs mainly in ice-free areas of periglacial environments, which represent less than 0.5% of the continent's area (Brooks et al., 2019). In these sensitive environments, soil acts as the main mediator of terrestrial ecological processes in Antarctica, mediating the cycling of nutrients and chemical, hydrological, and biological processes (Lopes et al., 2021; Simas et al., 2007; Thomazini et al., 2015).

However, MPs can change soil properties and may affect their ability to perform multifunctional ecosystem functions (Chia et al., 2022; Qiu et al., 2022; Wan et al., 2023). MPs impacts soil physical properties such as aggregation, bulk density, porosity, and water dynamics (Machado et al., 2018; Qi et al., 2020; Zhang et al., 2019), as well as soil chemical properties, such as soil pH, nutrient availability and adsorption reactions (Boots et al., 2019; Li and Liu, 2022; Zhang et al., 2020a). Consequently, MPs triggers responses in the soil microbiota (Rillig et al., 2021; Zhao et al., 2021). Furthermore, as MPs are heterogeneous contaminants, their effects on soil properties are different in relation to shape, size, polymeric composition and concentration (Lehmann et al., 2021; Wan et al., 2023). For example, PE MPs tend to decrease soil pH (Boots et al., 2019; Yu et al., 2020) and microbial activity (Fei et al., 2020) while polypropylene (PP) MPs present an opposite behavior (Liu et al., 2017; Zhao et al., 2021). Alternatively, fibers often promote negative impacts on soil structure but other shapes do not have such obvious responses (Lehmann et al., 2021).

The effects of MPs are also dependent on soil type and environmental conditions. Inherent soil characteristics affect the interaction between MPs and natural soil particles, which tend to be more efficient in soils with higher clay and organic matter contents (Guo et al., 2022; Ingrassia et al., 2022; Liang et al., 2021). The exposure of MP polluted soils to different temperature and moisture conditions also mediates the effects on soil properties such as aggregation, microbial activity and organic matter decomposition (Liang et al., 2019; Lozano et al., 2021a; Zhang et al., 2019). In general, periglacial soils of Maritime Antarctic coastal areas are poorly weathered, poorly structured and coarse (Bockheim, 2014), being constantly subject to physical (e.g. cryoturbation) (Chaves et al., 2017; Michel et al., 2014), biological and chemical dynamics (e.g. ornithogenic influence) (Guo et al., 2018; Lopes et al., 2021; Simas et al., 2007). However, despite the evidence of MPs occurring in terrestrial environments of Maritime Antarctica (González-Pleiter et al., 2020; Perfetti-Bolaño et al., 2022), potential impacts on their soils are still largely unknown.

Here we used well-established soil physical, chemical, and microbial

response parameters to perform the first exploratory assessment of potential disturbances caused by the presence of two usual MP types (PAN fibers and PE fragments) in an Antarctic marine terrace bare soil on the Fildes Peninsula (King George Island, Maritime Antarctica) through a pot experiment under field conditions. Field experimentation was prioritized to account for actual daily temperature and moisture variations, which drive microbial metabolism and nutrient cycling in Antarctic soils (Pires et al., 2017; Thomazini et al., 2020), seeking to provide insights and guide further research based on representative natural conditions. With this experimental setup we expected that significant impacts on soil properties would be observed on a short-term scale and the effects would be distinct for the different MP types (polymer and shape) and applied doses.

2. Material and methods

2.1. Soil and site description

The tested soil was sampled in February 2022 on the Fildes Peninsula, Maritime Antarctica (62°13'S; 58°57'W), in a marine terrace environment susceptible to flooding by ice melt drainage streams, with sparse mossy vegetation, approximately 50 m from the coastline and 13 m a.s.l. (Fig. S1). This area was selected for the experiment because this type of environment is susceptible to MP contamination in Antarctica, both by the runoff of fresh meltwater streams and by coastal maritime influence (González-Pleiter et al., 2020; Perfetti-Bolaño et al., 2022). Fildes Peninsula is one of the main entrances for ships and airplanes to the Maritime Antarctica region, and a major hub of anthropic activities. Soils in the study area are mostly formed by transported sediments of basaltic and andesitic origin, resulting from periglacial and isostatic uplift processes (Michel et al., 2014), which are common conditions on marine terraces in the Maritime Antarctic region (López-Martínez et al., 2012). The clay mineralogy in the area is predominantly composed of chlorites, micas and smectites (Pelayo et al., 2022). The experiment was performed during the Antarctic summer (Zacharias and Setzer, 2004), in February and March 2022. The actual mean daily air temperature in the area ranged between -1.1 and 5.5 °C, with cumulative liquid precipitation of 16.4 mm distributed over 13 days, cumulative snow precipitation of 4 cm distributed over 3 days, and mean UVB radiation index of 1 and maximum of 3 (Chilean meteorological service - station 950001).

The soil (~25 kg) was sampled with a hoe and a metal trowel at a 0–15 cm depth, in the transition between a poorly structured sandy surface horizon, and a clayey subsurface horizon with a stronger developed structure of plastic consistency and characteristic features of occurrence of expansive clays (Fig. S1). After sampling, the soil was sieved in the field at ambient temperature through a 2 mm mesh to remove granules and pebbles, and the fraction that passed through the sieve was used for the experiment. The physical and chemical characteristics of the soil used in the experiment are presented in Table S1.

2.2. Microplastic addition and experimental set up

The experiment tested the effect of two types of MPs: polyacrylonitrile (PAN) fibers and polyethylene (PE) fragments (Fig. S2). PE is primarily used in single-use plastic packaging and is one of the most common polymeric composition of MPs found in coastal environmental samples, where fragments are generally high in low density polymers like PE (Erni-Cassola et al., 2019; Hale et al., 2020; Koutnik et al., 2021). Fibers are the most common shape found even in Antarctic ecosystems (Fragão et al., 2021; González-Pleiter et al., 2020; Hale et al., 2020), where PAN fibers, used as synthetic wool for clothing, have been found in Antarctic biota (Bessa et al., 2019) and shown high toxicity for soil nematodes (*C. elegans*) (Kim et al., 2020). The PAN fibers were obtained by manually cutting 100% "Mollet" acrylic threads (Círculo S/A, São Paulo, Brazil; product No. 781), with mean size of 2818 µm (±1193 µm; min = 635 µm, max = 5463 µm; n = 55). The virgin PE fragments were

acquired from Bianquímica (São Paulo, Brazil; product No. MLB1282396142), with mean size of 407 μm ($\pm 369 \mu\text{m}$; min = 31 μm , max = 2644 μm ; n = 205). Both MP types were microwaved for 3 min (500 W) to minimize the risk of microbial contamination (Machado et al., 2019), and stored in sterilized glass bottles until their addition to the soil. Microwaving did not cause visible damage or melting of the MPs according to a stereomicroscope inspection.

Fragment doses were 0.01, 0.1 and 1.0% wet weight, and fiber doses were 0.001, 0.01 and 0.1% wet weight, so that the soil presented minimal differences in volume with MP particles effectively blended to the soil matrix (Machado et al., 2018). The doses were calculated by wet weight since the experiment was set up in situ without access to drying oven resources. This range of doses has been widely reproduced in experiments with soil MPs, which can cause noticeable changes in soil properties (Colzi et al., 2022; Li and Liu, 2022; Pignattelli et al., 2020). Considering real conditions found in environmental samples of highly polluted sites (Fuller and Gautam, 2016; Scheurer and Bigalke, 2018; Wang et al., 2020), these doses simulate the worst future scenarios of MP pollution in Antarctic terrestrial environments (Perfetti-Bolaño et al., 2022).

The MPs were weighed ($\pm 0.001 \text{ g}$) and mixed at the predetermined doses with 3 kg of soil in a 100 L polyethylene bag by vigorous stirring supported by a metal spatula. After mixing, each experimental unit was prepared with 700 g of spiked wet soil, which were stored without compaction in conical polypropylene pots (h = 8 cm, upper diameter = 11 cm, lower diameter = 9.5 cm) with bottom holes (3 mm diameter) to allow water percolation. The soil moisture during the experiment set up was 27%. Four replicates for each treatment plus the control (without MP addition) were prepared, totaling 28 experimental units that were partially buried in the same soil sample site, side by side, in random positions, to simulate real field conditions (Fig. S1E). After 22 days, the pots were removed and stored frozen until the analyses were carried out. To minimize the risks of contaminating the Antarctic environment and deep soil layers, each experimental unit was covered with a holed plastic lid allowing gas exchange and the entry of precipitation water, and had its bottom covered with permeable paper. In addition, a soil layer ($\sim 3 \text{ cm}$) immediately in contact with the experimental units ($\sim 0.7 \text{ m}^2$) was collected after the experiment and transported to the laboratory for further disposal.

2.3. In situ CO_2 flux measurements

To estimate the microbial activity, the CO_2 emissions ($\mu\text{mol m}^{-2} \text{ s}^{-1}$) were measured on the soil surface of the experimental units using an automated soil CO_2 flux system LI-8100 A portable analyzer (LI-COR Biosciences, Lincoln, USA) coupled to a dynamic automatic survey chamber. The cylindrical chamber (854.2 cm^3 , base area 83.7 cm^2) was positioned in PVC collars (10 cm in diameter) previously inserted in the upper part of each experimental unit. The flux measurements lasted 45 s, with pre- and post-purges of 10 s, and the CO_2 concentrations were recorded inside the chamber at intervals of 1 s. The CO_2 emission data were collected with three measurement replicates for each experimental unit. Fifteen data collection events were carried out in total, and the average of daily measurements was used to calculate the mean CO_2 flux for each treatment throughout the experiment. The soil temperature was measured in each experimental unit using a digital thermometer ($\pm 0.1 \text{ }^\circ\text{C}$) at each data collection event. Since soil temperature is one of the most influential variables related to CO_2 fluxes (Carvalho et al., 2013; Thomazini et al., 2020), a correlation analysis (r, Pearson) was performed on the daily data of these two variables to assess whether changes in CO_2 fluxes derived from fluctuations in soil temperature.

2.4. Soil analysis

The physical and chemical properties of the soil were determined by methods according to Embrapa guidelines (2017; described in detail in

Table S2).

After thawing, undisturbed subsamples were obtained from each experimental unit using a volumetric cylinder, while the remaining soil from the samples was air-dried. The undisturbed samples are representative of the newly formed structure in the experimental units during the experiment. The soil bulk density was determined by the volumetric cylinder method, while the particle density was assessed by the volumetric flask method, and the total soil porosity was calculated using the indirect method through the ratio of the latter values. The hydraulic conductivity in a saturated medium was determined by the induced flow method, with a constant head of 2 cm. The soil microporosity was assessed using a tension table at -6 kPa , and the soil macroporosity was determined by the difference between micro and total soil porosity. The physical analyzes were carried out in three replicas, since the fourth undisturbed sample replica was intended for the production and analysis of thin sections (which is subject of another study).

Soil pH was determined in distilled water with a glass electrode in a 1:2.5 suspension (w v^{-1}). The potential acidity ($\text{H}^+ + \text{Al}^{3+}$) was extracted by 1 M ammonium acetate solution at pH 7. Exchangeable cations (Ca^{2+} , Mg^{2+} , and Al^{3+}) were extracted with 1 M KCl solution and the available P (AP), Na^+ and K^+ were extracted with Mehlich-1 extractor. The elemental concentrations in the extracts were determined by atomic absorption (Ca^{2+} , Mg^{2+} , and Al^{3+}), flame emission spectrometry (K^+ and Na^+) and photocolourimetry (AP). From these results, the cation exchange capacity (CEC) was calculated. The total organic carbon (TOC) content was determined by titration after wet oxidation with potassium dichromate (Yeomans and Bremner, 1988), and the total nitrogen (TN) content was determined by sulfuric acid digestion followed by Kjeldahl distillation (Bremner, 1960). The C:N ratio was calculated on a mass basis. The general microbial activity of the soil was assessed in 1 g of dry soil by the hydrolysis of fluorescein diacetate (FDA) method, optimized by Green et al. (2006).

2.5. Statistical analysis

The effects of the treatments on soil parameters were evaluated by the analysis of variance (ANOVA) on a factorial design, considering the MP type, MP dose and the interaction of the two factors as a source of data variation. In this case, only the doses shared between the two MP types were considered (0.01 and 0.1%) as categorical variables. Then, one-way ANOVAs were performed on linear models for the separate PE fragments or PAN fibers treatments, and the treatments were differentiated from the control by grouping the means when ANOVA was significant according to Fisher's least significant difference test. Model residuals were verified for ANOVA assumptions of normality and homogeneity of variance. Since they did not meet ANOVA assumptions, statistical inferences on mean CO_2 flux and soil temperature data were supported by the Kruskal-Wallis test, with the same post hoc test for grouping means. A multivariate principal component analysis (PCA) was performed to evaluate relationships between the response parameters based on effects caused by the treatments, whereas the variables coordinates were used to assess correlations with the principal components (Abdi and Williams, 2010). The data suitability for PCA was verified using the Bartlett sphericity test and the Shapiro Wilk normality test. A significance level of 0.05 was considered for all tests, and all statistical analyses and graphs were achieved in R environment (R 4.2.2 with Rstudio 2021.09.0 interface) (R Core Team, 2023; RStudio Team, 2020). Linear models were achieved with the `lm` function, ANOVA with the `anova` function, and Kruskal-Wallis test with the `kruskal.test` function, from the R base package. Fisher's least significant difference test was achieved with the `LSD.test` function from the "agricolae" package. PCA was performed using the `prcomp` function with scaled data on treatment means, and the package "factoextra" was used to extract the variables coordinates and generate the variables correlation plot. Packages "dplyr", "Rmisc", "ggplot2" and "ggthemes" were used for data manipulation and graphs.

3. Results

3.1. Impacts on soil physical properties

Except for the total porosity, all soil physical parameters presented a significant dose-dependent response regardless of the MP type, but only total and macroporosity presented a significant type-dependent response, apart from the dose (Table S3). The interaction effect was significant for the soil bulk density and macroporosity. PE fragments caused significant differences only for soil bulk density and hydraulic conductivity (Table S5). All PE fragment treatments decreased soil bulk density by approximately 6% compared to the control, regardless of the dose (Fig. 1B). The 0.01 and 1% PE fragments doses decreased the hydraulic conductivity up to 94% compared to the control (Fig. 1A). PAN fibers caused significant differences in all physical parameters depending on the applied doses (Table S5, Fig. 1). Macroporosity decreased up to 25% compared to the control when PAN fibers doses were equal or lower than 0.01% and increased by up to 17% when treated with the 0.1% dose (Fig. 1E). The soil total porosity was affected in the same way as macroporosity, but to a lesser extent, and decreased up to 5% compared to the control when PAN fiber doses were equal or below 0.01% (Fig. 1C). Soil bulk density was only affected by the 0.1% dose of PAN fibers, decreasing by up to 6% compared to the control. Hydraulic conductivity did not differ from the control at the 0.1% PAN fibers dose but decreased it by up to 97% at the 0.01% dose.

3.2. Impacts on soil chemical properties

The MP doses and types affected differently soil chemical properties. Soil pH, TN and C:N ratio presented a significant dose-dependent response, regardless of the MP type. The TN, C:N ratio, Na^+ and sodium saturation presented a significant type-dependent response, regardless of the dose (Table S4). The interaction effect between MP type and dose was significant for Ca^{2+} , Na^+ , AP and TN.

Among the soil chemical parameters evaluated, only Mg^{2+} , K^+ and $\text{H}^+ + \text{Al}^{3+}$ contents were not significantly affected by the PAN fibers or PE fragments treatments (Table S6; Figs. 2 and 3). For PAN fibers

treatments, the significant effects on pH, AP, TN, C:N ratio, Na^+ and sodium saturation were different according to the dose, but the significant effects observed for Ca^{2+} and CEC were the same regardless of the applied dose. The significant effects caused by PE fragments also differed according to the dose for pH, TOC, C:N ratio, CEC, Na^+ and sodium saturation, but did not differ according to the dose for Ca^{2+} .

The Ca^{2+} contents decreased by up to 12% compared to the control for any PAN fibers treatment, and by up to 15% for any PE fragments treatment (Fig. 2C). CEC decreased by up to 4% for any PAN fiber treatment compared to the control, and by up to 7% for the 1% dose of PE fragments (Fig. 2F). Both MP types induced similar behavior on pH, Na^+ and sodium saturation – low doses increased pH and Na^+ , while high doses decreased the pH and caused non-significant changes in the exchangeable Na^+ levels (Fig. 2D, E and 3D). Only PAN fibers treatments significantly increased the AP levels by increasing doses (Fig. 3E). PAN fibers caused a significant increase in TN contents up to 84% compared to the control at a 0.1% dose (Fig. 3B), and the PE fragments significantly increased the TOC contents by up to 130% compared to the control at a 1% dose (Fig. 3A). Thus, the highest concentrations of the two types of MPs had opposite effects on the C:N ratio – PAN fibers at 0.1% decreased C:N ratio by up to 66%, while PE fragments at 1% increased it by up to 91% compared to the control (Fig. 3C).

3.3. Impacts on soil respiration and microbial activity

Effects of PAN fibers were significant on mean CO_2 fluxes and different for the tested doses, increasing the CO_2 fluxes as the dose increased, resulting in fluxes up to 20% higher for the 0.1% PAN fibers treatment compared to the control (Table S7, Fig. 4A). Alternatively, PE fragments treatments did not significantly affect mean CO_2 fluxes. The treatments did not cause significant effects on mean soil temperature (Table S7, Fig. 4B), and no significant correlation ($r = -0.16$) was observed between soil temperature and mean CO_2 fluxes. No significant effects on soil microbial activity measured by FDA hydrolysis depending on MP dose, MP type or on the interaction between the two factors were observed (Table S4). However, both PE fragments and PAN fibers treatments caused significant effects on FDA hydrolysis (Table S6).

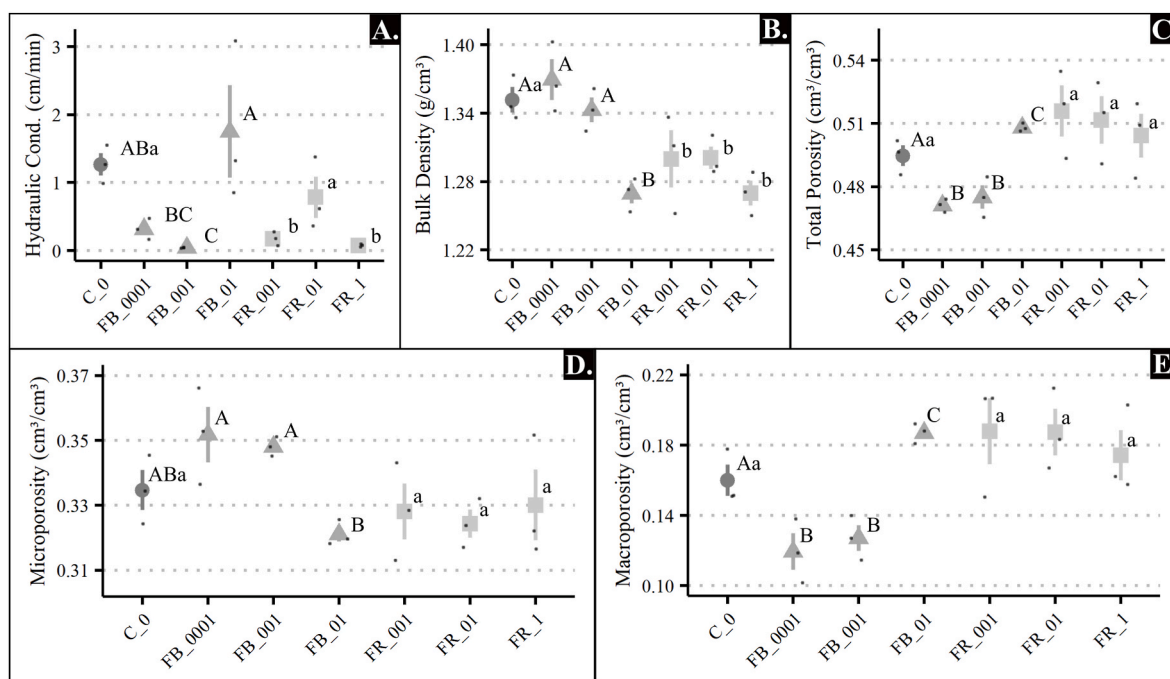


Fig. 1. Effects of MP treatments on soil physical properties: A. saturated hydraulic conductivity; B. soil bulk density; C. soil total porosity; D. microporosity; E. macroporosity. Treatments are shape and color coded in grayscale, from darkest to lightest: control (C, circle); fibers (FB, triangle); and fragments (FR, square). Means with the same letters belong to the same group, by Fischer's LSD ($\alpha = 0.05$).

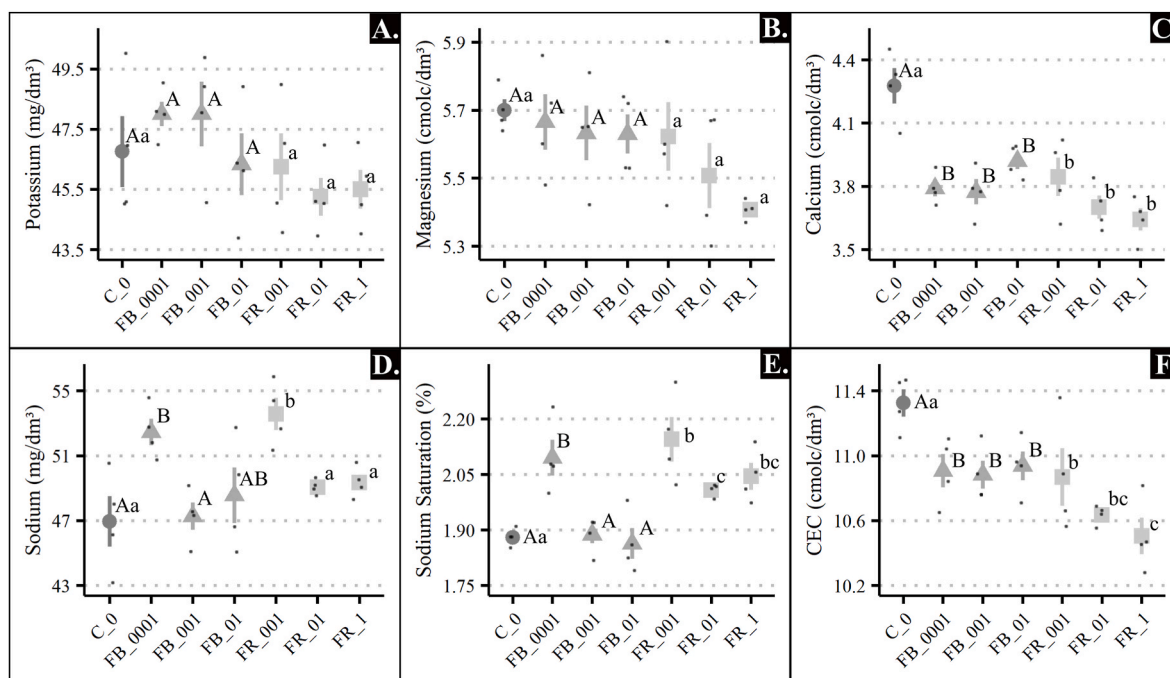


Fig. 2. Effects of MP treatments on soil chemical properties: A. K^+ ; B. Mg^{2+} ; C. Ca^{2+} ; D. Na^+ ; E. sodium saturation; F. CEC. Treatments are shape and color coded in grayscale, from darkest to lightest: control (C, circle); fibers (FB, triangle); and fragments (FR, square). Means with the same letters belong to the same group, by Fischer's LSD ($\alpha = 0.05$).

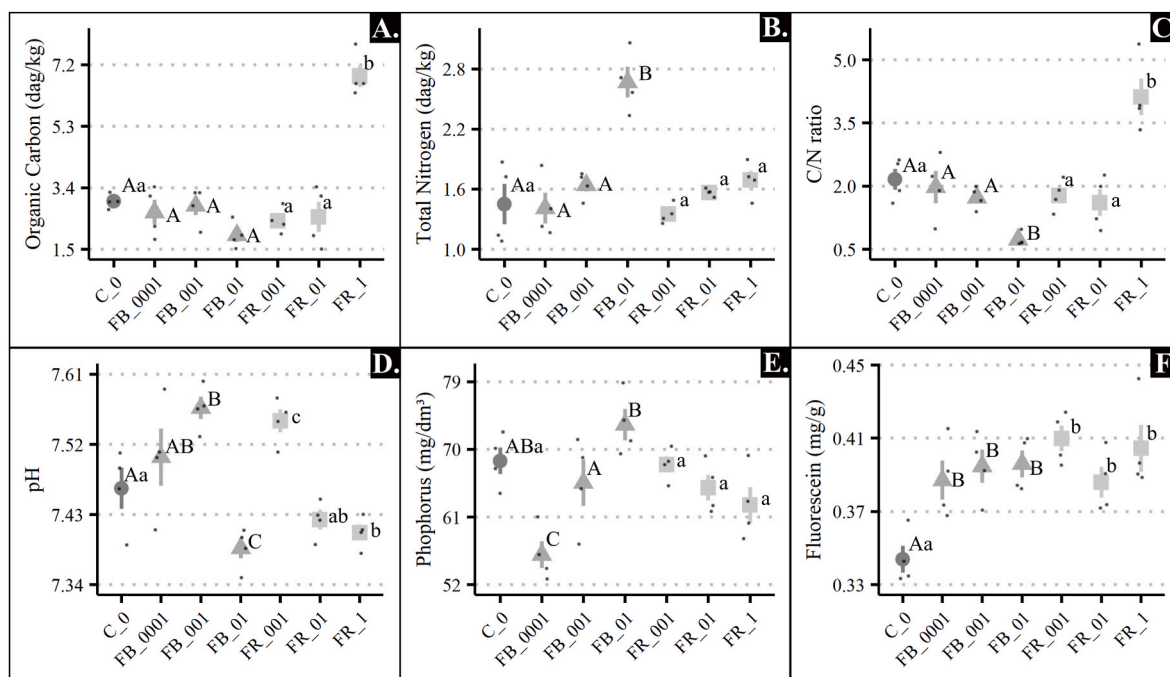


Fig. 3. Effects of MP treatments on soil chemical properties: A. TOC; B. TN; C. C:N ratio; D. soil pH; E. AP; F. FDA (fluorescein released). Treatments are shape and color coded in grayscale, from darkest to lightest: control (C, circle); fibers (FB, triangle); and fragments (FR, square). Means with the same letters belong to the same group, by Fischer's LSD ($\alpha = 0.05$).

Regardless of the dose, FDA hydrolysis increased by up to 18% for PE fragments treatments and by up to 15% for PAN fibers treatments compared to the control (Fig. 3F).

3.4. Principal component analysis

Five principal components (PC1 to PC5) had eigenvalues greater

than 1, although PC1 (38%) and PC2 (29%) cumulatively explained 67% of the data variance (Table S8) and were addressed in the variable correlation plot (Fig. S3). PC1 shows a strong correlation with porosity (0.83) and macroporosity (0.88), and an expected strong negative correlation with bulk density (-0.95) and microporosity (-0.86). Mean CO_2 fluxes (0.83) are also strongly positive correlated with PC1, and consequently with porosity, but pH (-0.72), Mg^{2+} (-0.76) and K^+

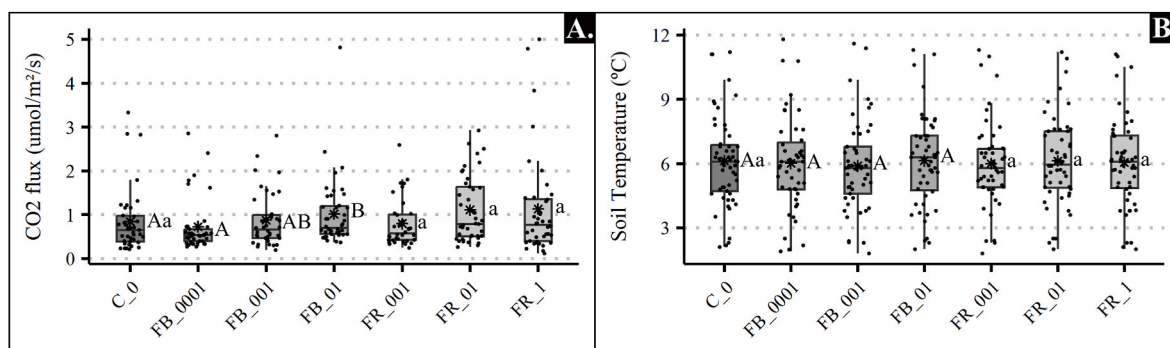


Fig. 4. Effects of MP treatments on mean soil CO₂ fluxes (A.), mean soil temperature (B.) during the 22 days incubation period. Treatments are color coded in grayscale, from darkest to lightest: control (C); fibers (FB); and fragments (FR). Means with the same letters belong to the same group, by Fischer's LSD ($\alpha = 0.05$).

(-0.95) presented a strong negative correlation, although the latter two did not show significant differences between treatments. PC2 was positively correlated with hydraulic conductivity (0.92), Ca²⁺ (0.77), AP (0.77) and CEC (0.71), but negatively correlated with sodium saturation (0.74). PC3 (14%), PC4 (9%) and PC5 (6%) cumulatively explained 29% of the data variance (Table S8) but did not show high correlations with any variables, except Na⁺ and PC3 (-0.73), which may indicate high random data variability associated with uncontrolled field conditions.

4. Discussion

This study showed that MPs affect soil attributes on an Antarctic marine terrace, according to their type and dose. Except for total porosity, all physical parameters were significantly affected by MP dose, although for soil bulk density and macroporosity the influence of doses was dependent on the MP type. However, total porosity exclusively depended on MP type, and was not significantly affected by PE fragments. These results contradict the findings of Lozano et al. (2021b), who suggested little influence of MP concentration on soil aggregation, and discrepancies may rely on the different soil types tested. Antarctic marine terrace soils of the Fildes Peninsula present a weakly developed structure with high sand content and presence of expansive clays (Michel et al., 2014), and soils with these characteristics are strongly susceptible to MP impacts on soil aggregation and hydraulic properties (Guo et al., 2022; Ingraffia et al., 2022). Therefore, the inherent physical characteristics of the soil tested here may enhance the aggregating or disaggregating effect of MP particles incorporation at increasing levels, since this is little expected from their natural condition of unconsolidated constituents (Bockheim, 2014). Even so, specific impacts considering MP types separated are influenced by the applied dose (Lozano et al., 2021b), which was corroborated by this study and several others (Liu et al., 2017; Machado et al., 2018; Zhang et al., 2020b).

PAN fibers affected soil structure, which led to pronounced changes in soil porosity and saturated hydraulic conductivity, but PE fragments physically interacted with the soil matrix more harmoniously and presented no significant changes in soil porosity. PAN fibers seem to obstruct macropores at low doses ($\leq 0.01\%$), decreasing soil macroporosity and the hydraulic conductivity related to it (Centeno et al., 2020). MP fibers are known to decrease aggregation and aggregate stability by creating fracture points or decreasing cohesion (Ingraffia et al., 2022; Lozano et al., 2021b; Zhang and Liu, 2018), and can favor the contact between MP particles rather than between soil particles at high concentrations (Guo et al., 2022; Machado et al., 2018). Therefore, the 1% PAN fibers treatment decreased the microporosity probably reflecting a poor aggregation, but increased macroporosity by creating interconnected pores between the fiber particles. However, increasing macroporosity didn't increase the hydraulic conductivity, since these pores formed by MP particles are less efficient to conduct water than the natural ones, due to the hydrophobicity of MPs and their ability to bind

soil particles/aggregates (Guo et al., 2022). Since no significant effects on soil porosity were observed in PE fragment treatments, the replacement of natural soil pores by inefficient pores formed between plastic particles (Guo et al., 2022) probably addresses the decrease in hydraulic conductivity. It is also unlikely that the decrease in soil bulk density by PE fragments treatments was promoted by significant changes in soil structure, but rather by their lower density ($\sim 0.95 \text{ g cm}^{-3}$) compared to soil mineral particles ($\sim 2.65 \text{ g cm}^{-3}$) and PAN fibers ($\sim 1.18 \text{ g cm}^{-3}$).

Our results on soil physical properties are in line with the hypothesis of shape dissimilarity – the more dissimilar the shape of a contaminant in relation to the matrix, the greater the impacts promoted on the surrounding environment (Rillig et al., 2019). The absence of vegetation and roots in the tested soil may have amplified this effect, increasing the impacts of linear shaped fibers on soil physical properties. This hypothesis has often been corroborated in studies assessing the impacts of MP fibers on soil aggregation, although the chemical composition and particle size seem to affect the soil aggregation directly or indirectly (Lehmann et al., 2021; Lozano et al., 2021b; Machado et al., 2018).

Soil aggregation and water dynamics can modulate microbial functions and vice versa (Han et al., 2021; Rillig et al., 2017; Vu et al., 2022), which has frequently been noted among the effects driven by MPs in soils (Liang et al., 2021; Machado et al., 2018; Rillig et al., 2021; Yu et al., 2020). Carbon mineralization by CO₂ emissions is strictly related to microbial metabolism and controls carbon stocks in Maritime Antarctica, where it is sensitive to soil temperature and moisture (Carvalho et al., 2013; Thomazini et al., 2020). Hence, porosity and soil aggregation affect soil respiration by conditioning water and oxygen availability for soil microbiota (Yang et al., 2019b). The daily measurements of soil respiration presented no significant correlation with soil temperature, but mean CO₂ fluxes were strongly associated with the effects of MPs on soil physical properties according to PCA. This suggests that greater microbial activity and CO₂ fluxes were favored by MPs increasing porous spaces, air/water circulation and oxygen diffusivity (Rillig et al., 2021).

The increased microbial activity may also indicate a disturbance caused by the MP addition as a representative source of organic carbon – associated with FDA hydrolysis by PCA – and nitrogen in the low organic matter soil tested. Polyethylene MPs are composed of $\sim 87\%$ carbon which was quantified by the analytical method employed here, contributing to increase TOC levels up to 130% compared to the control at the 1% dose. This input would represent $\sim 8.7 \text{ g}$ of PE carbon source and an even greater increase in TOC levels was to be expected, although it is unclear whether the TOC analysis quantified all carbon originating from MPs. As the degradation of PE MPs is unlikely to occur in this short-term scale (Huang et al., 2021; Yang et al., 2023), the representative input of a poorly available carbon source may triggered a positive priming effect and favored the decomposition of native soil organic matter, contributing to a TOC increase lower than expected. The magnitude of the priming effect is positively correlated with MP

degradability (Zhang et al., 2023), but it could be representative in this soil with high relative availability of limiting nutrients (e.g., AP, low C:N ratio).

Nonetheless, PAN fibers comprise ~28% nitrogen, and the 0.1% dose represents a significant input to total soil nitrogen. Therefore, the C:N ratio of soils treated with PE fragments and PAN fibers presented opposite trends, which can lead to changes in the structure of soil microbial communities (Zhang et al., 2014). Although the addition of PAN fibers also represents a carbon input, low C:N ratios tend to increase organic matter decomposition and carbon mineralization in Maritime Antarctic soils (Pires et al., 2017), which may have favored significant increases in CO₂ fluxes. In contrast, the higher carbon input increased C:N ratio in soils treated with 1% PE fragments and could have affected the mineralization rate, causing data variability and no significant differences in mean CO₂ fluxes.

The effects on soil pH were dose-dependent and similar for both PE fragments and PAN fibers, increasing pH at a 0.01% dose but decreasing it above 0.1% dose. Soil pH was strongly negatively related with physical properties and CO₂ fluxes according to PCA, similar to previous reports (Yang et al., 2019a). Increasing CO₂ fluxes at MP doses of 0.1% or higher could favor carbonic acid formation, potentially decreasing pH. Furthermore, soil pH regulates and is affected by soil microbiota (Rong et al., 2021; Yang et al., 2019b), playing a key role in shaping the plastisphere community (Feng et al., 2022; Li et al., 2021; Rillig et al., 2023). Since our treatments affected microbial activity and C:N ratio, the effects observed on soil pH could possibly be linked to impacts on microbial functions. However, further research is encouraged to better address these effects, as soil pH influences biotic processes in the coastal land environments of Maritime Antarctica by affecting soil microbial communities, nutrient cycling and vegetation cover (Park et al., 2012; Thomazini et al., 2015; Tscherko et al., 2003).

Effects of MPs on pH can be promoted by changes in adsorption and cation exchange dynamics (Boots et al., 2019; Feng et al., 2022). Both PE fragments and PAN fibers affected cation exchange dynamics, decreasing CEC compared to the control. The CEC of the studied soil is chiefly composed of Mg²⁺ and Ca²⁺, so its decrease was mainly caused by impacts on exchangeable contents of these cations. Sorption processes involving MPs are mainly controlled by surface mechanisms, although MPs in the size range of this study have a low specific surface area (Li and Liu, 2022; Luo et al., 2020; Xu et al., 2019). Despite the negative charge surface at the pH range of the studied soil (Godoy et al., 2019), the lower capacity of MPs to adsorb cations compared to natural soil particles probably address the CEC decrease, which was more representative for the higher dose applied (PE fragments at 1%). MPs decreasing soil adsorptive capacity can affect the mobility and availability of divalent metallic cations (Cao et al., 2023; Feng et al., 2022; Zhang et al., 2020b), which could be worrisome in Fildes Peninsula – where soils are historically and continually affected by heavy metals enrichment (Amaro et al., 2015; Lu et al., 2012; Marina-Montes et al., 2020).

Interestingly, sodium saturation increased especially in soils treated with the lowest doses of both MP types, which indicate a greater presence of Na⁺ ions in the soil exchange complex. The Na⁺ ion is easily leachable as it has a larger hydrated radius and fewer specific interaction with soil-charged particles than divalent cations, although characteristics of soil porosity and pH also affect soil sorption dynamics in the presence of MPs (Luo et al., 2020). Our results demonstrated a selective interaction of MPs with Na⁺ ions, and further investigation into the adsorption behavior under these conditions is needed to better understand this interaction.

Otherwise, MPs adsorptive functions do not seem to be a main factor affecting soil P dynamics, but secondary effects may play a more representative role (Li and Liu, 2022). Changes promoted in soil pH affect phosphate solubilization, which is mainly controlled by mineral or exchangeable calcium sources within this pH range (Penn and Camberato, 2019). Impacts of MPs on soil microbiota also affect enzymes

that promote P mineralization and availability, which can reflect in AP content (Dong et al., 2021; Feng et al., 2022). Here, only the lowest dose of PAN fibers treatment significantly decreased the soil AP content, which were strongly associated with hydraulic conductivity according to PCA. The effects on AP possibly addressed impacts of PAN fibers on physical and hydrological conditions mediating pH and microbial functions, but specific mechanisms involved need to be further explored accounting to the sources and speciation of P, as coastal areas in Maritime Antarctica are highly affected by the runoff of penguin and marine birds guano (Simas et al., 2007).

Different types and doses of MPs affected Antarctic marine terrace soil properties, but the generalization of the results presented here must be addressed with care. Given the short-term character of our experiment, significant effects on soil physical properties refer to the artificial structure newly built in the experimental units during the incubation period. Nevertheless, periglacial soils are subject to freezing and thawing activity which can induce aggregation over short periods, even on a scale of days (Vliet-Lanoë, 2010). Liquid water infiltrates the soil during snow melting periods and it freezes during sub-zero conditions causing gel-like clays and organic particles to precipitate, promoting cohesion and flocculation into aggregates with voids formed by desiccation crack networks (Campbell and Claridge, 1987; Vliet-Lanoë, 2010). These processes are even more evident in summer due to increased liquid water availability and more frequent freeze-thaw cycles driven by daily temperature fluctuations (Almeida et al., 2017; Chaves et al., 2017), potentially favoring MP particles integration into soil aggregates (Fig. S4). Over time, cryoturbation can promote particle movement, fragmentation and sorting along the active layer of permafrost (Bockheim and Tarnocai, 1998). The low temperatures make polymers more brittle and increase susceptibility of MPs to fragment (Chubarenko, 2022). In a scenario of MP pollution, cryoturbation processes could contribute to vertical migration and fragmentation of MPs possibly affecting soil properties at greater depths, which should be further investigated.

Likewise, MPs degradation is not expected to occur in about three weeks and was not assessed in this study, but the addition of recalcitrant organic carbon sources may be sufficient to trigger the disturbance impacts on microbial functions. However, Antarctic hydrocarbon-degrading microorganisms can metabolize carbon from polypropylene MPs when exposed exclusively to this carbon source (Habib et al., 2020). This is plausible to occur in the Fildes Peninsula region due to its history of anthropogenic impacts and prevalent hydrocarbon pollution (Wu et al., 2023). Moreover, Maritime Antarctic environments are vulnerable to climate change (Rosa et al., 2023; Thomazini et al., 2020), which favors soil exposure promoted by glacial retreat and creates new ice-free areas to be colonized by the Antarctic fauna, flora and microbiota (Francelino et al., 2011) affecting soil ecosystems and carbon mineralization (Pires et al., 2017; Simas et al., 2007). The ornithogenic influence, common in Maritime Antarctica coastal areas, accelerate organic matter degradation, mineralization of carbon from recalcitrant sources, and colonization by vegetation (Pires et al., 2017; Simas et al., 2007; Thomazini et al., 2015). As MPs are present in penguin scats (Bessa et al., 2019; Fragão et al., 2021), their interaction with ornithogenic processes is presumable and can contribute to mediate the effects of soil MPs observed here. Therefore, considering the complexity of these multiple environmental factors is needed and encouraged in future assessments to better understand the ecological and long-term consequences of MP pollution in these ecosystems.

5. Conclusions

We reported impacts of PAN fibers and PE fragments MPs on several physical, chemical and microbial attributes of an Antarctic marine terrace soil in a short-term pot experiment under field conditions. PAN fibers significantly affected soil porosity and hydraulic conductivity, but PE fragments did not change soil porosity. Both MP types increased

microbial activity regardless of the dose, but only the highest PAN fibers dose (0.1%) increased CO₂ fluxes, compared to the control, when soil porosity increased. The addition of PE and PAN at high doses (1% and 0.1%, respectively) represented a significant input of C and N to the soil with low organic matter content, affecting the C:N ratio. Soil pH increased at low doses and decreased at high doses for both MP types, while CEC decreased with the addition of PAN fibers regardless of dose but decreased with increasing doses of PE fragments. PE fragments and PAN fibers showed specific effects that remain unclear on exchangeable Na⁺ and AP contents, respectively. The effects on soil properties observed here are likely interrelated and foster further investigation. Complex environmental factors should be considered to better address their causes and long-term ecological implications, including the interaction of MPs with Antarctic soil microbiota under climate change conditions and co-contamination with heavy metals and/or organic pollutants, besides to cumulative effects on environments undergoing ornithogenesis and cryoturbation dynamics.

CRedit authorship contribution statement

Caik Oliveira de Miranda: Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **José João Leis Leal de Souza:** Writing – review & editing, Supervision, Methodology, Data curation, Conceptualization. **Carlos Ernesto Gonçalves Reynaud Schaefer:** Writing – review & editing, Supervision, Project administration, Funding acquisition. **Esperanza Huerta Lwanga:** Writing – review & editing, Supervision. **Fernando Nadal Junqueira Villela:** Investigation.

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.

Data availability

No data was used for the research described in the article.

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Appendix A. Supplementary data

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