Short Note

Findings and challenges in understanding the impacts of human-induced trampling on Antarctic edaphic microbial communities and their recovery potential

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Abstract

Antarctic terrestrial ecosystems, covering less than 1% of the continent, are under increasing anthropogenic threats, particularly from climate change and tourism. This study focuses on the impact of human trampling on soil microbial communities in Barrientos Island (South Shetlands archipelago), a frequently visited location. Soil samples were collected within and at the edge of two established paths representing varying levels of human disturbance. Physical and chemical analyses revealed significant differences between the paths, potentially creating anaerobic conditions favorable for specific microbial taxa. Thus, microbial communities also differed between the paths. Aerobic heterotrophic taxa were dominant in the less disturbed path, while anaerobic taxa such as Bacteroidetes vadinHA17 thrived in the more compacted path. Although alpha diversity indices did not differ, beta diversity analyses showed notable distinctions, particularly between the paths rather than trampled and untrampled areas. These findings suggest that microbial communities may recover following the stop of human activity, but also that indigenous microbial communities or other overlooked factors may be influencing the recovery potential. This study thus offers a starting point for similar research, as it highlights the need for further investigation to determine whether human trampling or other environmental factors are primarily responsible for these differences.

Keywords: Antarctica; endangered areas; human impact; microbial ecology; soil environment; soil microbial communities; tourism

(Received 30 September 2024; revised 9 December 2024; accepted 11 December 2024)

Introduction

Life in Antarctica is mainly restricted to less than 1% of the total Antarctic surface (a value that is increasing with climate change) in ecosystems devoid of ice that are present from the seashore to a few hundred metres inland or isolated within small ice-free areas surrounded by large extensions of ice (Lee *et al.* 2017). These areas provide shelter for a wide range of terrestrial organisms, from vascular plants, lichen, fungi, mosses and algae, to animals such as arthropods, nematodes and tardigrades (Convey *et al.* 2014). Despite its uniqueness, this biodiversity is under anthropogenic threats, ranging from climate change to tourism (Kennicutt *et al.* 2019). Although this latter activity is tightly regulated through the Protocol on Environmental Protection to the Antarctic Treaty (in

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Cite this article: Fernández-Martínez, M., Galbo Cacha, R. M., López-Archilla, A. I., Tejedo, P., Garrido-Benavent, I., De Los Ríos, A., & Benayas, J. 2025. Findings and challenges in understanding the impacts of human-induced trampling on Antarctic edaphic microbial communities and their recovery potential. *Antarctic Science*, 1–4. https://doi.org/ 10.1017/S0954102024000531 application since 1998), a total of 104 897 tourists visited Antarctica in 2023 (International Association of Antarctica Tour Operators, https://iaato.org/information-resources/data-statistics/). Of them, 98% of total visits occurred in the Antarctic Peninsula and the South Shetland Islands due to their relatively easy access from cruise ships departing from southern South America. Consequently, terrestrial ecosystems have been affected by various human impacts, including trampling on soils and vegetation and damage to their communities (Pertierra *et al.* 2013, Tejedo *et al.* 2016).

Microbial communities are essential components of Antarctic soil ecosystems, playing a key role in sustaining biodiversity, ecosystem functions and succession processes (Cowan 2014, Garrido-Benavent *et al.* 2020). Nevertheless, they have often been overlooked in human impact studies. Hence, it is difficult to determine the extent to which they are affected by human activities such as trampling and how disturbances to the microbial community affect the entire ecosystem.

On the basis of the above, the goal of this study is to understand how soil microbial communities respond to human trampling in Antarctic soils, using one of the most visited locations - Barrientos Island (South Shetland Islands) - as a case study.

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Materials and methods

Soil samples were collected during 2020 from Barrientos Island (Supplemental Information 1), a location frequently visited as a result of cruise-based Antarctic tourism. Barrientos Island is a small island with ice-free soils covered by mosses, lichens and Prasiola spp. algae, along with the presence of remarkable geological formations and several populations of sea mammals and penguin breeding colonies (Tejedo et al. 2016). To visit this site, various paths were created along Barrientos Island, with the main ones being the so-called 'Upper' and 'Lower' paths (hereafter 'UP' and 'LP', respectively; Supplemental Information 1). UP was opened decades ago, while LP was designated in 2006 as the only suitable route to cross Close Area B, which occupies the central part of the island and is covered by a very extensive moss carpet (Secretariat of the Antarctic Treaty 2006). However, Resolution 5 (2012), XXXV Antarctic Treaty Consultive Meeting (ATCM) recommended closure of both paths as of 2013 to cease human repercussions through trampling: the newer LP was suffering from a more intensive human trampling, which multiplied its impacts. According to Tejedo et al. (2016), UP is 670 m long, 3.42 ± 2.74 m wide, traversing areas covered by mosses, with medium-sized soil mineral particles and low water retention, which runs down the slope towards the seashore. On the other hand, LP is 750 m long, 1.68 ± 0.21 m wide, running on the lower part of the slope in parallel to southern beaches (avoiding penguin sub-colonies) but also through a moss-covered area that is thicker and more diverse than the one surrounding UP. The soil consists of sandy material, with small-sized mineral particles, being constantly saturated with water from rains and glacial ice runoff, which turns it into a muddy area. Both UP and LP travel from penguin nesting areas to the seashore, so they are usually traversed by these animals. Organic matter from their faeces and lost feathers is thus frequently introduced in to these soil environments (authors' personal observations).

For sampling, the top 20 cm of soil within the paths (trampled soils; T) and the top 1 m far from the edge of the path (under the moss cover; untrampled soils; U) were extracted with a sterilized soil core sampler at a total of five sampling points (two along UP (UPT/U 5, 7) and three along LP (LPT/U 1, 3, 4); n = 10; Supplemental Information 1 & 2). Some basic soil physical and chemical parameters were obtained *in situ* at 5 and 10 cm depths with a HydraProbe sensor (Stevens Water Monitoring Systems, Inc.). Soil samples were immediately frozen at -80°C and stored until total DNA extraction was performed at Museo Nacional de Ciencias Naturales (Madrid, Spain). For detailed information on sequencing, bioinformatics and statistical procedures, see Supplemental Information 3.

Results and discussion

Physical and chemical measurements showed that the temperature was higher at 10 cm depth compared to at 5 cm depth, as these deeper soils are less affected by changing weather conditions at the surface, while every conductivity and water-related measured parameter was, on average, higher for LP (Supplemental Information 2). These findings differ from those obtained in previous comparable studies, which showed that less trampled soils generally exhibit greater water retention capacity (Sherman *et al.* 2019). The lower position of LP down the slope, therefore receiving water and sediments, along with its sandy texture (more easily compacted by trampling) and proximity to the seashore (potentially contributing

humidity, organic matter and salts), may explain these differences. These factors, in turn, influence the characteristics of soil habitats inhabited by microbial communities.

The principal bacterial families found in the soils were Chitinophagaceae (11.80%, Bacteroidota phylum), Gemmatimonadaceae (8.47%, Gemmatimonadota phylum), Chthoniobacteraceae (6.30%, Verrucomicrobiota phylum), Intrasporangiaceae (2.58%, Actinobacteriota phylum) and Bacteroidetes vadinHA17 (2.23%, Bacteroidota phylum). A significant number of amplicon sequence variants (ASVs) showed unidentified matches with database records (18.70%; Supplemental Information 4). Members of Chitinophagaceae, Gemmatimonadaceae and Chthoniobacteraceae showed high and consistent abundances in every sample from UP. They were also more frequently detected in the untrampled soils of LP. The lower water content measured on average in these UP soils, their loose texture and their organic matter that can derive from the moss cover (including the contribution of penguin faeces) may create better conditions for the development of these aerobic chemoorganoheterotrophic families (Rosenberg et al. 2013). Moreover, the presence of these families relying on organic matter in the trampled soils of UP may suggest a certain resilience of at least the most abundant microbial taxonomic groups if they do not suffer from intense anthropic trampling for a period of time. This is consistent with previous findings in comparable areas reported by Sherman et al. (2019), as they also observed that soil compaction resulting from human trampling affected the taxonomic diversity of microbial communities. In addition, Bacteroidetes vadinHA17 and Rhodocvclaceae families were notably absent from any of the samples taken along UP, but they were abundant in the trampled soils of LP. Unlike Chitinophagaceae, Gemmatimonadaceae and Chthoniobacteraceae, both Bacteroidetes vadinHA17 and Rhodocyclaceae families primarily include heterotrophic, strict or facultative anaerobes that are capable of degrading complex carbon sources (Rosenberg et al. 2013, Oren 2014). While the organic matter may originate from the same sources as in the other sampling points, the remarkably higher water content found in these soils together with the expected greater compaction due to the presence of smallsized mineral particles and the later and more intense trampling along this path probably creates a significant number of anaerobic microhabitats that are rich in recalcitrant organic matter that these families can exploit (Oren 2014, Wei et al. 2019). All of these notable differences in taxa distribution are in accordance with the SIMPER analyses results (Supplemental Information 5) that showed that ASVs from all of these families, along with Ktedonobacteraceae, were major contributors to the significant differences between the samples (top 10 ASV contributions: Chthoniobacteraceae - 3.568%; Gemmatimonadaceae - 3.352%; Chitinophagaceae - 3.303%; Bacteroidetes vadinHA17 - 2.383%; Ktedonobacteraceae - 0.903%).

Despite this different distribution of the taxa, richness (S') and diversity indices (Shannon - H', Simpson - D, and Pielou's phylogenetic diversity index) did not show any significant differences when comparing trampled *versus* untrampled areas. This contrasts with previous findings showing that trampled areas have a higher diversity, such as those of Sherman *et al.* (2019). Similarly, no significant differences were observed when comparing UP with LP. However, beta-diversity analyses (Bray-Curtis dissimilarity index) showed contrasting results (Supplemental Information 5): while no differences were found between trampled and untrampled areas along UP, trampled areas of LP showed significant differences were mainly due



Figure 1. Canonical correspondence analysis (CCA) plot showing the correspondence of the main physical and chemical parameters measured (blue arrows) with the microbial community composition of the samples (turquoise and orange circles and triangles). The most abundant bacterial families appear in light grey font. The analyses covered 84% (CCA1 = 72%, CCA2 = 12%) of the total variance of the data and was significant overall at P < 0.05 (999 permutation tests for the CCA).

to the distinct detection of Bacteroidetes vadinHA17, Rhodocyclaceae and other families adapted to the anaerobic soil conditions created on this path, in contrast to the rest of the analysed soils. The described lack of differences between trampled and untrampled areas of UP again points to the possible recovery of the initial microbial community structure after ceasing human activity on these soils. In contrast, the ANOSIM test indicated that significant differences were found for microbial community composition between UP and LP (P < 0.05) rather than between trampled and untrampled areas. Therefore, these contrasting findings between the two statistical approaches not only may be explained by the soil habitats newly created after trampling, but also may be explained by differences in indigenous microbial community composition. In that sense, UP and LP areas present contrasting diversities in moss cover (Tejedo *et al.* 2016) that could also be reflected in associated soil microbial groups (Duran *et al.* 2021). In any case, these results point to the need for further studies devoted to clarifying whether trampling on Antarctic soils is the primary cause of these differences on soil microbial communities or whether surrounding environmental factors may exert a more important influence.

Results from the exploratory canonical correspondence analysis (CCA; total explained variance of 84% - CCA1 = 72%, CCA2 = 12%; Fig. 1) grouped the four UP samples together (UPT and UPU, again pointing to the possible recovery of the indigenous community), while LP samples were distinct from them and from each other along CCA1. Samples coming from untrampled areas

(LPU) were grouped in the bottom left corner of the ordination, while trampled areas (LPT) appeared to the right of CCA1. Within this group, LP3T stood apart from the other two samples but was more related to the Bacteroidetes vadinHA17 family. Despite the remaining main bacterial families (Chitinophagaceae, Gemmatimonadaceae, Chthoniobacteraceae, Intrasporangiaceae) showing a more even distribution at the centre of the ordination, the distant location of all UP and LP samples may also point to the possible influence of the indigenous microbial community. However, since most of the analysed soil physical and chemical parameters were strongly related to LP samples (probably enhanced by soil compaction derived from trampling) and were strongly correlated to each other, it is impossible to assert with certainty whether a different soil microbial community composition prior to the disturbance was the single cause of the differences between the samples.

Based on these results, it appears that main primordial microbial groups from these soils represent a capacity to recover within less than 7 years in moderately impacted soils, such as those along UP. Conversely, the results also indicate that intense trampling can alter soil habitats (e.g. by compaction) to the extent that certain microbial taxa thrive so efficiently that they prevent the recovery of the original community structure for at least these same 7 years. Therefore, additional assays are needed, including those with longer post-trampling periods, as is research into other potentially overlooked factors influencing Antarctic soil communities. Our results suggest that human activities altering soil ecosystems may thus have irreversible consequences on microbial communities. As these microorganisms support essential metabolic ecosystemic processes, such changes may significantly impact the rest of the biota in these fragile polar areas. Hence, this study contributes to the field by providing a starting point for future research either corroborating or conflicting with these findings, and it also underscores the need for further exploration of the environmental factors that could impact microbial communities in trampled Antarctic areas.

Supplementary material. The supplementary material for this article can be found at http://doi.org/10.1017/S0954102024000531.

Acknowledgements. None.

Financial support. None.

Competing interests. The authors declare none.

Author contributions. Conceptualization: MAF-M, ADIR, JB. Methodology: all coauthors. Investigation: all coauthors. Visualization: MAF-M, RMGC. Validation: all coauthors. Writing - original draft: MAF-M, RMGC, with the help of all coauthors.

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