Response to reviewer comments on manuscript bg-2018-488: "From substrate to soil in a pristine environment – pedochemical, micromorphological and microbiological properties from soils on James Ross Island, Antarctica"

We would like to thank the referees for their helpful and constructive comments, which greatly helped to improve our manuscript. We have prepared a response where we account for all points raised by the referees, as described below. We show the referees' comments in grey text, while our responses are formatted as standard text. Line indications refer to the changes in the revised manuscript.

Anonymous Referee #1

First, we would like to thank you for taking the time to review our manuscript. We were glad about the positive and constructive feedback on our work.

1. The authors should dedicate more discussion to the energy sources of the community

While the paper is generally very detailed, in my opinion more focus needs to be spent on the potential energy sources for the community. The cell counts observed are high for soils with such low organic carbon content.

Could inorganic energy sources such as atmospheric hydrogen, atmospheric CO, and ammonia potentially be sustaining this community? The authors mention that Actinobacteria were present, but other H2-scavenging phyla (Acidobacteria, Chloroflexi) and CO-scavenging phyla (Proteobacteria, Chloroflexi) are known.

Due to the lack of organic carbon as well as the low amount of potential phototrophic organisms in the soils, we suspect inorganic energy sources to be crucial to sustain the microbial ecosystem. This is supported by our data (Tab. S6) and observations of a variety of microorganisms (e.g. Acidiferrobacteraceae, potential ammona-oxidizing Thaumarchaeota), which potentially use such energy sources (e.g. L655ff, L666 – 669).

To underline this, we changed the text accordingly:

"Organisms with the ability to use oxygenic photosynthesis to fixate CO_2, such as cyanobacteria, were nearly absent in the investigated soils. Low abundances of

Cyanobacteria are a common observation for Antarctic soil habitats (Ji et al., 2016). Due to the lack of phototrophic organisms and organic carbon, inorganic compounds and metabolic pathways utilizing those may have a more pronounced role in sustaining the microbial ecosystem at this initial stage of the soils." (L. 685-690)

In addition, we added a more detailed discussion regarding the usage of atmospheric compounds as energy sources:

"Further, a part of the community could use atmospheric compounds as energy source. Atmospheric H_2, CO, and CO_2 are scavenged and used as an energy source by microorganisms, especially organisms associated with the phyla Actinobacteria, Chloroflexi, Acidobacteria, Planctomycetes, Verrucomicrobia, and Proteobacteria (Greening et al., 2015; Ji et al., 2017). Operational taxonomic units related to the phylum Actinobacteria and the associated orders Acidimicrobiales and Solirubrobacterales were highly abundant in the investigated soils." (L. 705-711)

It is also mentioned that potential ammonia-oxidising Thaumarchaeota are present in the community. Based on the physicochemical analysis, how much ammonia is available to sustain them?

Potential ammonia-oxidising Thaumarchaeota have been present throughout the investigated profiles, and even have shown relative high abundances of up to 12.9%. However, ammonia, nitrite and nitrate could not be quantified by ion chromatography in any sample indicating negligible amounts, as written in L386f. It is well known that energy sources are scarce in ice-free areas of Antarctica (Souza et al., 2014; Cary et al., 2010), and might be metabolized quite quickly when they become available (e. g. from degradation of microbial necromass), which would explain the very low amounts as revealed by ion chromatography.

With this in mind, we modified the part discussing the presence of AOA in the discussion as follows:

"However, ion chromatography showed that amounts of ammonia as well as nitrite and nitrate were negligible in both soils. Ammonia originating from necromass and products in the course of nitrification could be metabolized directly by the present community, so no accumulation of the different intermediates containing nitrogen takes place." (L. 699-702)

It is also not clear, based on the results or figures, how abundant Cyanobacteria and algae were in the community. Can the authors dedicate a few sentences in the results to clarifying this? It is stated that phototrophs were ‘nearly absent’, but it would be more informative to state their relatively abundance (even if tiny). It is stated that chloroplast reads were removed, so presumably some chloroplasts were detected.

We agree with the reviewer, that both numbers are informative for the reader and therefore added detailed information on the filtered reads which reveals the number of OTUs associated with both Cyanobacteria and chloroplasts. We also included a table with this information in the supplementary data (Tab. S4). However, we assume that active phototrophic organisms only occur in the uppermost layers of the investigated soils and reads in the deeper layers originate from translocated and phototrophically inactive organisms.

The text was changed as follows:
“In total, 19,732,536 reads were obtained after merging the forward and reverse reads, demultiplexing, filtering, and deletion of chimeric and singleton sequences. Additionally, reads of chloroplast-associated OTUs (36,573), mitochondria-associated OTUs (1,117) as well as rare OTUs (OTUs with a relative abundance of <0.1% in every sample; 4,287,382) were filtered, resulting in 15,407,464 reads (Table S4).” (L. 468-472)

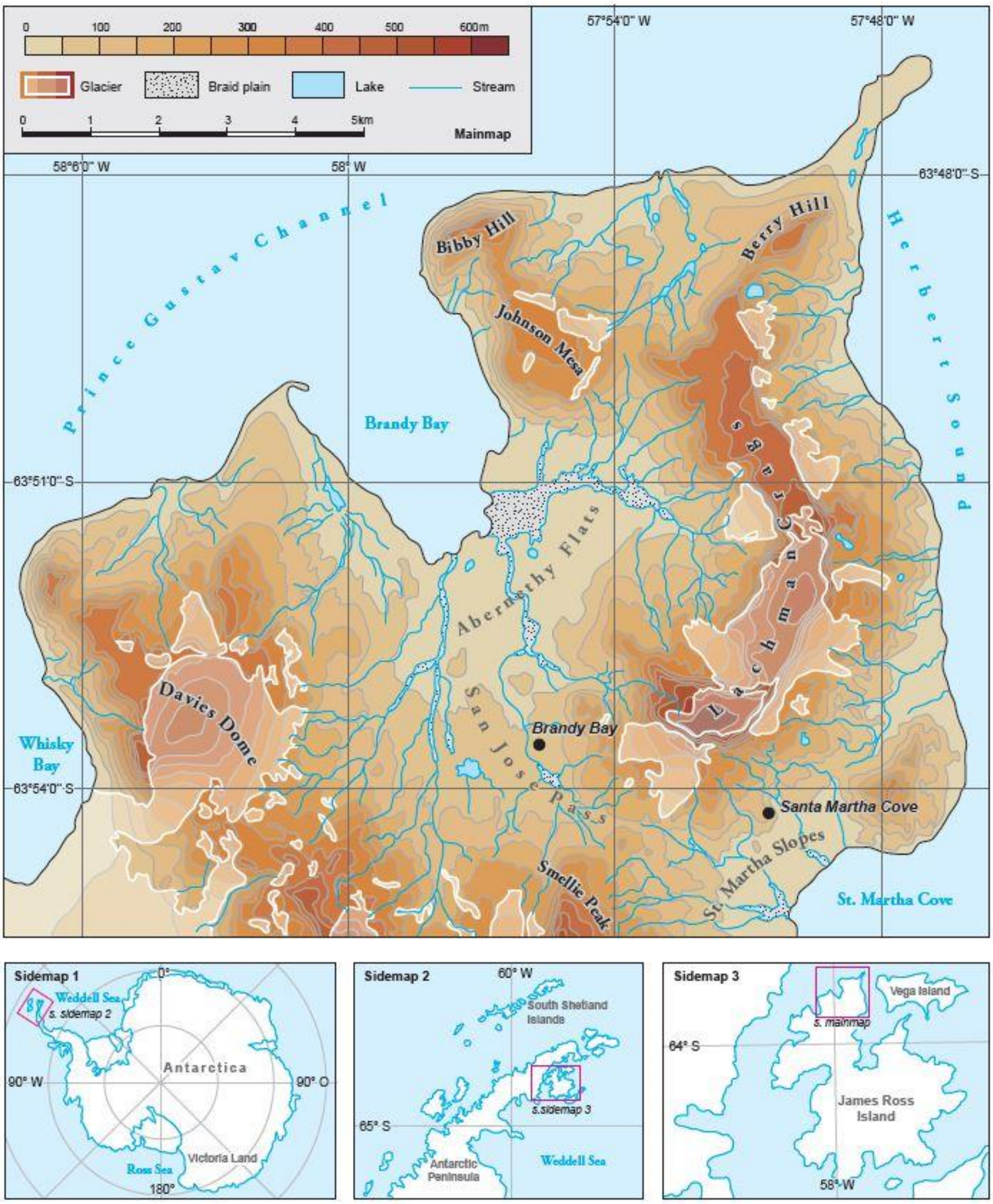
In addition, we included observations on potential photosynthetic organisms in the results:
“Regarding potential phototrophic organisms in the investigated soils, the amount of chloroplast-related reads was relatively low (<0.2%) in each sample, except for SMC >50 cm (0.03% - 1.30%) and BB 0 – 5 cm (0.87% - 1.01%). Cyanobacteria-related OTUs were rare and only showed low relative abundances in SMC 5 – 10cm (0.06%), SMC 10 – 20cm (; 0.62%), SMC >50cm (0.04%).” (L. 485-489)

2. The authors should modify and consolidate the figures and possibly tables

The figures are not always as informative as the text. It is not entirely clear, based on the figure or legend, what the satellite image of Figure 1 and how this relates to the inlet. Could this figure be modified?

Thank you very much for this remark. As the satellite image seems not to be able to reflect the characteristics of the working area, we have decided to replace the satellite image with a map of the area.

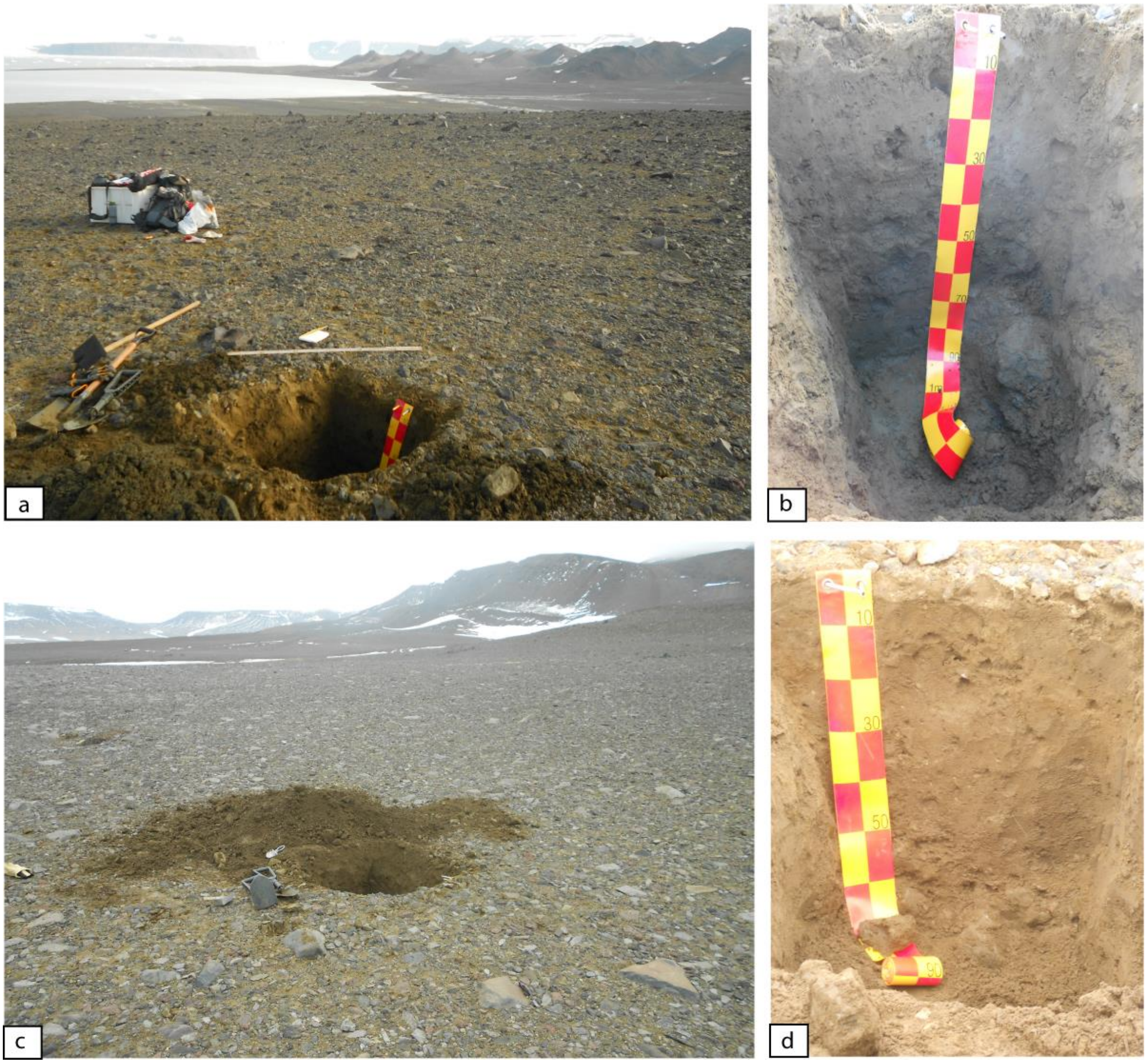
We changed figure 1 as follows:



For Figure 2 to 5, could these photographs be amalgamated into a single multi-panel figure given they show similar things?

Many thanks. Combining the images into one multi-panel figure makes the chapter more compact and helps the reader to get the important information about the study sites.

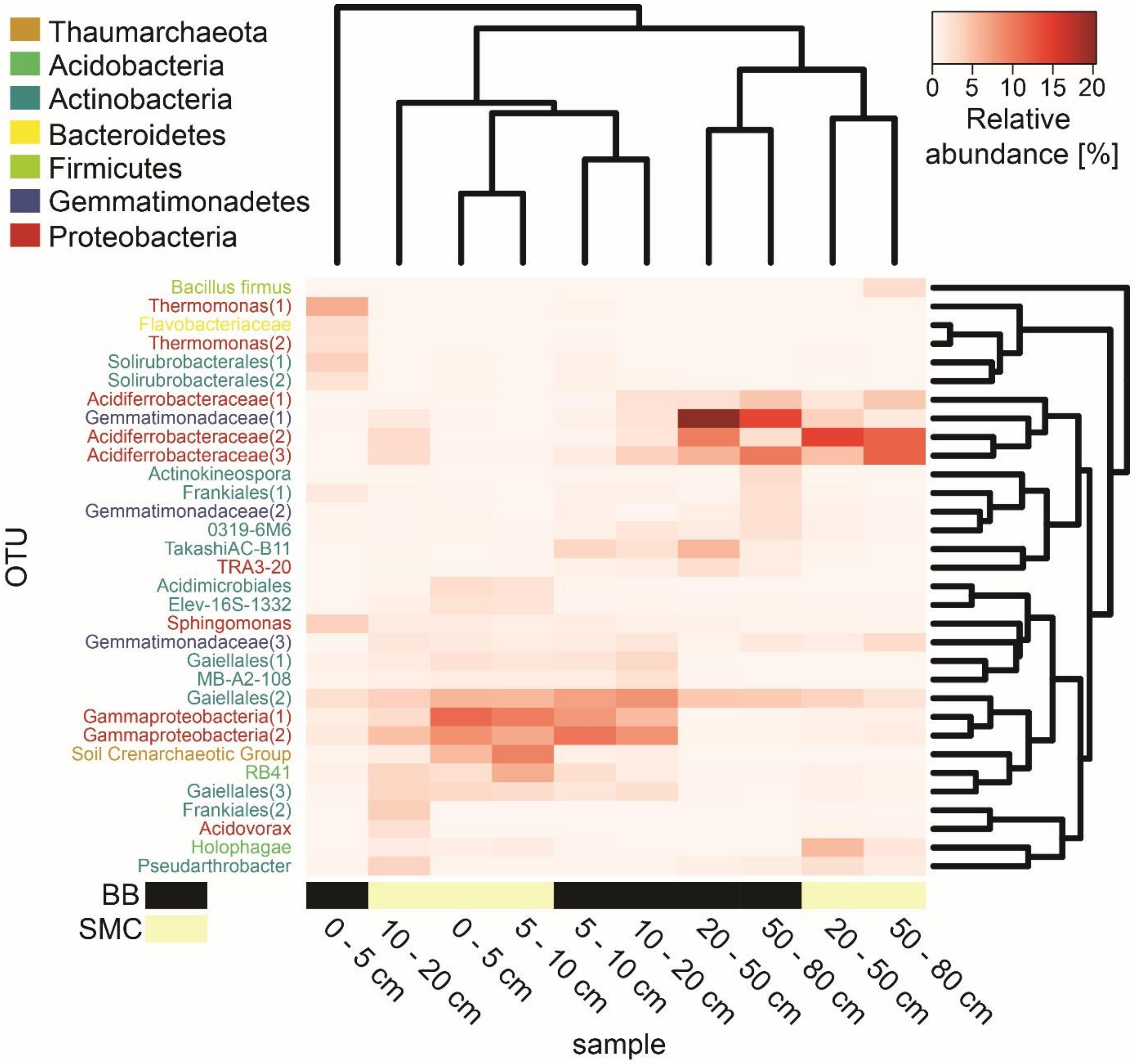
We changed figure 2 as follows:



For Figure 8, while the heatmap is a useful summary, the odd colouring makes it hard to see trends. Could the authors modify this to increase the contrast and make more abundant OTUs darker than lighter. OTUs with 0% relative abundance should be white rather than navy blue.

The heatmap was modified as suggested. Lighter colors represent lower relative abundances, whereas darker colors represent higher relative abundances. We hope that this change improves the overall clarity of the presented data.

We changed the heatmap as follows:



In addition, some of the tables may be more suited for supplementary material.

Thank you very much for the valuable assessment. Since the results in Tables 1, 2, and 4 are discussed directly in the paper, we consider these tables as basic information for our discussion. Table 3, on the other hand, is the basis for the results presented in Table 2. For this reason, we agree with the proposal and move table 3 to the supplementary material. Here it becomes the new Table S1.

L91-93: I disagree with this assessment. Most studied topsoils in Antarctic ice-free regions harbour diverse microbial communities with 16S rRNA gene counts exceeding 107.

We agree that topsoils in Antarctic ice-free regions harbor diverse microbial communities, which are adapted to the present conditions, as we mentioned in e.g. L 92f or L115-121. Our statement in L90ff was targeted at groups such as higher plants, and vertebrates.

To emphasize this, we rephrased the paragraph as follows:

“Due to environmental stressors such as very low temperatures, low water availability, frequent freeze-thaw cycles and limited organic nutrient contents, soils from continental Antarctica show limiting conditions for higher organisms (Cary et al., 2010). However, diverse microbial communities thrive in a variety of Antarctic habitats, such as permafrost soils (Cowan et al., 2014).” (L. 95-99)

L82: Please change ‘proofing’ to ‘proving’

We changed it as follows:

“Therefore, soil scientific investigations became a relevant topic in Antarctic research, proving that there are actually soils in Antarctica (Jensen, 1916) and identifying soil forming processes (Ugolini, 1964).” (L. 87-89)

L99: Clarify what is meant by ‘ornithogenic’ soil given it is a specialised term

Ornithogenic soils are well known in Antarctica. The World Reference Base for Soil Resources (WRB, 2014) defines ornithogenic material (from Greek ornithos, bird, and genesis, origin) as material with strong influence of bird excrement which often has a high content of gravel transported by birds.

The surface of these soils consists often of an indurated guano crust and scattered pebbles are common, since the penguins use them for their nests. The guano acts as additional source of nutrients particularly N and P.

We added the following information:
“Local conditions determine nutrient availability in Antarctic soils (Prietzel et al., 2019). Ca, Mg, K and P contents are generally high in igneous and volcanic rocks, whereas P and N contents are highest in ornithogenic soils. Ornithogenic soils are well known in Antarctica. The World Reference Base for Soil Resources (WRB, 2014) defines ornithogenic material (from Greek ornithos, bird, and genesis, origin) as material, which is characterized by penguin deposits mainly consisting of guano and often containing a high content of gravel transported by birds (cf. Ugolini, 1972)”. (L. 103-109).

L139-143: As this sentence is quite complicated, I recommend breaking it up into two: “These soils are not influenced by vascular plants, sulfides, and penguin rookeries. Our study aims to identify major soil and microbiological properties by combining pedochemical and micromorphological methods with microbial community studies based on high throughput sequence analyses.”

Thanks for providing this helpful comment. We changed this part as follows:
“This setting enables an investigation of interdependencies particularly between prokaryotic life and soil properties, since the selected soils are not influenced by vascular plants, sulfides, and penguin rookeries.

With this, the main goal of our study is to identify major soil and microbiological properties in an extreme environment by combining pedochemical and micromorphological methods with microbial community studies based on high throughput sequence analyses. Thus, we will gain a better general understanding of (i) the main soil forming processes and (ii) the drivers of soil microbial community structure in the eastern APR. This addresses also the question, if the variance of pedogenic and microbiological properties are larger between depth increments within one profile (e.g. with different distances to the permafrost table) or between different soil profiles, i.e. due to different local environmental conditions.” (L. 157-167)

L500: Consider modifying ‘laboratory’ to ‘study site’.

We agree with that comment. The paragraph reads now as follows:

“James Ross Island offers an exceptional opportunity to improve our understanding of the interrelations between soil formation and microbiological properties in the absence of plants.” (L. 524-526)

L659: Please change ‘fixate’ to ‘fix’

We are glad to comply with this remark. We changed this sentence as follows:

“Microorganisms can be seen as the primary pioneers of nutrient-poor environments such as Antarctic soils, and were shown to have the genetic potential to fix C and N (Cowan et al., 2011; Niederberger et al., 2015), thus increasing C and N contents of these oligotrophic soils.” (L. 693-696)

Additional Literature:

Greening, C., Constant, P., Hards, K., Morales, S. E., Oakeshott, J. G., Russell, R. J., Taylor, M. C., Berney, M., Conrad, R., and Cook, G. M.: Atmospheric Hydrogen Scavenging: From Enzymes to Ecosystems, Appl. Environ. Microbiol., 81, 1190-1199, 2015.

Ji, M., van Dorst, J., Bissett, A., Brown, M. V., Palmer, A. S., Snape, I., Siciliano, S. D., and Ferrari, B. C.: Microbial Diversity at Mitchell Peninsula, Eastern Antarctica: A Potential Biodiversity “Hotspot”, Polar Biology, 39, 237-249, 2016.

Prietzel, J., Prater, I., Colocho Hurtarte, L. C., Hrbáček, F., Klysubun, W., and Mueller, C. W.: Site Conditions and Vegetation Determine Phosphorus and Sulfur Speciation in Soils of Antarctica, Geochimica et Cosmochimica Acta, 246, 339-362, https://doi.org/10.1016/j.gca.2018.12.001, 2019.

Ugolini, F. C.: Ornithogenic Soils of Antarctica, in: Antarctic Terrestrial Biology, edited by: Llano, G. A., 1972.