Pedogenic and microbial interrelation in initial soils under

semiarid climate on James Ross Island, Antarctic Peninsula

Region

Lars A. Meier^{1*}, Patryk Krauze^{2*}, Isabel Prater³, Fabian Horn², Carlos E.G.R. Schaefer⁴, Thomas Scholten¹, Dirk Wagner^{2, 5}, Carsten W. Mueller^{3,6}, and Peter Kühn¹ ¹Department of Geosciences, University of Tuebingen, Tuebingen, D-72070, Germany ²GFZ German Research Centre for Geosciences, Section Geomicrobiology, Potsdam, D-14473, Germany ³Lehrstuhl für Bodenkunde, TU München, Freising, D-85354, Germany ⁴Departamento de Solos, Universidade Federal de Viçosa, Viçosa, BR-36571-000, Brazil ⁵Institute for Earth and Environmental Sciences, University of Potsdam, Potsdam, D-14476, Germany ⁶School of Agriculture and Food Sciences, The University of Queensland, St Lucia, Queensland, AU-4072, Australia Correspondence to: Lars A. Meier (lars-arne.meier@uni-tuebingen.de) *shared first authorship

Abstract. James Ross Island (JRI) offers the exceptional opportunity to study microbial driven 42 pedogenesis without the influence of vascular plants or faunal activities (e.g. penguin 43 rookeries). In this study, two soil profiles from JRI (one at St. Martha Cove - SMC, and another 44 at Brandy Bay - BB) were investigated, in order to gain information about the initial state of 45 soil formation and its interplay with prokaryotic activity, by combining pedological, 46 geochemical and microbiological methods. The soil profiles are similar in respect to 47 topographic position and parent material but are spatially separated by an orographic barrier 48 and therefore represent windward and leeward locations towards the mainly south-westerly 49 50 winds. These different positions result in differences in electric conductivity of the soils caused by additional input of bases by sea spray at the windward site, and opposing trends in the depth 51 52 functions of soil pH and electric conductivity. Both soils are classified as Cryosols, dominated by bacterial taxa such as Actinobacteria, Proteobacteria, Acidobacteria, Gemmatimonadates 53 54 and Chloroflexi. A shift in the dominant taxa was observed below 20 cm in both soils as well as an increased abundance of multiple operational taxonomic units (OTUs) related to potential 55 56 chemolithoautotrophic Acidoferrobacteraceae. This shift is coupled by a change in microstructure. While single/pellicular grain microstructure (SMC) and platy microstructure 57 (BB) is dominant above 20 cm, lenticular microstructure is dominant below 20 cm at both soils. 58 The change in microstructure is caused by frequent freeze-thaw cycles and a relative high water 59 content and goes along with a development of the pore spacing and is accompanied by a change 60 in nutrient content. Multivariate statistics revealed the influence of soil parameters such as 61 chloride, sulfate, calcium and organic carbon contents, grain size distribution, and pedogenic 62 oxide ratios on the overall microbial community structure and explained 49.9% of its variation. 63 The correlation of the POR with the compositional distribution of microorganisms as well as 64 the relative abundance certain microorganisms such as potentially chemolithotrophic 65 Acidiferrobacteraceae-related OTUs could hint on an interplay between soil forming processes 66 and microorganisms. 67

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78 **1 Introduction**

79 In extreme environments, like Antarctica, local climatic conditions such as low temperatures, precipitation or irradiance are important and often limiting factors for soil formation. Even 80 though soils in Antarctica are often poorly developed, they can be highly diverse (Michel et al., 81 2014; Simas et al., 2008; Bockheim et al., 2015). Therefore, soil scientific investigations 82 became a relevant topic in Antarctic research, proving that there are actually soils in Antarctica 83 (Jensen, 1916) and identifying soil forming processes (Ugolini, 1964). Antarctic soil research 84 is mostly located in Victoria Land, continental Antarctica, especially in the McMurdo Dry 85 Valleys (Michel et al., 2014; Ugolini and Bockheim, 2008), in the South Shetlands, maritime 86 Antarctica (Simas et al., 2015) and the western Antarctic Peninsula Region (APR) (Haus et al., 87 2015; Hrbáček et al., 2017b; Schaefer et al., 2017; Souza et al., 2014; Pereira et al., 2017). 88 Soils on continental Antarctica are often saline with thick salt horizons (Souza et al., 2014). 89 Due to environmental stressors such as very low temperatures, low water availability, frequent 90 freeze-thaw cycles and limited organic nutrient contents, soils from continental Antarctica show 91 92 limiting conditions for higher organisms (Cary et al., 2010). However, diverse microbial 93 communities thrive in a variety of Antarctic habitats, such as permafrost soils (Cowan et al.,

94 2014).

95 Soils in maritime Antarctica and western APR differ from soils in continental Antarctica according to their stage of development (Balks et al., 2013; Blume et al., 2004; Parnikoza et al., 96 97 2017). They show extensive cryoturbation processes with occasional salt crusts at the soil surface (Balks et al., 2013; Bockheim, 1997). Local conditions determine nutrient availability 98 99 in Antarctic soils (Prietzel et al., 2019). Ca, Mg, K and P contents are generally high in igneous 100 and volcanic rocks, whereas P and N contents are highest in ornithogenic soils. Ornithogenic 101 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 102 is characterized by penguin deposits mainly consisting of guano and often containing a high 103 content of gravel transported by birds (cf. Ugolini, 1972). Soils from the eastern part of the APR 104 (also called Weddell Sea sector) are different, since they are associated with a dry climatic 105 transitional zone between the wet, warmer maritime Antarctica and colder, arid continental 106 Antarctica. Mean temperatures are below 0°C and liquid water supply is sufficient to allow soil 107 108 forming processes (Souza et al., 2014). Souza et al. (2014) also showed that cryoturbation is less pronounced in the eastern APR than in the South Shetlands. The base saturation (>50%) 109 and electric conductivity (EC) are generally high whereas the amount of total organic carbon 110 111 (TOC) is substantially low. Regarding cryoturbation, active layer depth, chemical weathering and soil organic C-content, soils from the eastern APR are comparable to soils from inland areas

of the Ross Sea Region (Balks et al., 2013), though they are formed on different parent material
(Daher et al., 2018). In comparison, eastern APR with its semiarid soils remains one of the least

114 (Daher et al., 2018). In comparison, eastern APR with its semiarid soils remain

studied areas in Antarctica (Souza et al., 2014; Daher et al., 2018).

Since Microorganisms in Antarctica show a broad diversity as revealed by recent molecular 116 phylogenetic and metagenomic methods (Cowan et al., 2014) and contribute to the weathering 117 of minerals in soils (Uroz et al., 2009), they are pivotal to understand initial soil formation. The 118 bacterial phyla Proteobacteria, Acidobacteria, Actinobacteria, Bacteroidetes, Firmicutes and 119 Gemmatimonadates, commonly found in temperate soils, also dominate the microbial 120 communities observed in Antarctic habitats (e.g. Bajerski and Wagner, 2013; Cary et al., 2010; 121 122 Pearce et al., 2012; Chong et al., 2012). The microbial community structure is influenced by local soil chemical parameters, especially pH (e.g. Chong et al., 2010, Siciliano et al., 2014), 123 124 but also by soil physical parameters such as grain size distribution and soil moisture (Ganzert et al., 2011). Chong et al. (2015) proposed, however, that historical contingency and dispersal 125 limitations could have a stronger influence on differences in community distributions at a 126 regional scale (>1000km). At the microscale, microbial activity such as photosynthesis and 127 nitrogen fixation has a distinct influence on soil chemical parameters, e.g. the increase of carbon 128 and nitrogen contents in oligotrophic soils (Ganzert et al., 2011; Cowan et al., 2011; 129 Niederberger et al., 2015). In return, these changes in soil characteristics affect microbial 130 community composition. Conflicting results illustrate the lack in the understanding of drivers 131 of soil microbial diversity in high latitude soils (Cowan et al., 2014). Since most of the non-132 lichenized Antarctic fungi are known to be decomposers and their abundance and distribution 133 is limited by plant derived nutrients, and bio-available Carbon (Arenz et al., 2011), the focus of 134 this study lies on the prokaryotic interplay with soil characteristics and soil formation. 135

Micromorphological studies in the maritime Antarctica and the western APR described 136 sulphurization and phosphatization in ornithogenic soils and mineral transformation on 137 volcanic rocks (Pereira et al., 2013; Schaefer et al., 2008); and paleosols (Kirshner and 138 139 Anderson, 2011; Spinola et al., 2017). Even though micromorphology offers the opportunity to study constituents of soil and their mutual relations in space and time and to identify soil 140 141 forming processes in an undisturbed state (Stoops, 2003), so far no micromorphological study has been published about soil forming processes in the eastern APR that are influenced neither 142 143 by sulfates nor by birds.

Our study sites are located on James Ross Island in the eastern APR (Fig.1) and therefore offer
 a unique setting to study soil formation and microbial communities in a transitional Antarctic

landscape between the wet maritime and dry, colder continental Antarctica. We selected two 146 different soils, representing coastal soils and inland soils of James Ross Island, developed on 147 similar substrate and at similar topographic positions, but differing in local climate conditions 148 and nutrient contents due to their relative position towards the mainly SW-winds. The western 149 study site (Brandy Bay -BB) is located in a windward position and is highly influenced by sea 150 spray, while the eastern study site (Santa Martha Cove - SMC), located behind a mountain 151 range, is located in a leeward position (Prietzel et al., 2019). This setting enables an 152 investigation of interdependencies particularly between prokaryotic life and soil properties, 153 since the selected soils are not influenced by vascular plants, sulfides, and penguin rookeries. 154 With this, the main goal of our study is to identify major soil and microbiological properties in 155 156 an extreme environment by combining pedochemical and micromorphological methods with microbial community studies based on high throughput sequence analyses. Thus, we will gain 157 158 a better general understanding of (i) the main soil forming processes and (ii) the drivers of soil microbial diversity community structure in the eastern APR. This addresses also the question, 159 160 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 161 soil profiles, i.e. due to different local environmental conditions. 162

163 **2. Material and Methods**

164 **2.1. Regional setting of James Ross Island, maritime Antarctica**

165 [Figure 1]

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James Ross Island is situated east of the Antarctic Peninsula and is the largest island in the western Weddell Sea sector (Hjort et al., 1997). The study area is located on Ulu Peninsula in the northern part of JRI (Fig. 1). It represents one of the largest ice-free areas of the APR (Nedbalová et al., 2013; Hrbáček et al., 2017b) with the beginning of its deglaciation 12.9 ± 1.2 ka ago (Nývlt et al., 2014). More than 300 km² of the JRI lowlands are currently ice-free, except for a few glaciers (Engel et al., 2012).

173 The climate on JRI is semi-arid polar-continental (Martin and Peel, 1978). The precipitation,

mostly snow, ranges between 200 to 500 mm of water equivalent per year with the major share

- during winter (Davies et al., 2013; Zvěřina et al., 2014). The thickness of the snow cover does
- not exceed 30 cm, but varies due to strong winds (Hrbáček et al., 2017b; Hrbáček et al., 2016a).
- 177 The annual air temperature ranges between +10 °C and -30 °C on Ulu Peninsula (Hrbáček et
- al., 2016a; Láska et al., 2011). The year 2015 marked the warmest summer ever measured on

- 179 Ulu Peninsula, having a mean seasonal summer temperature (MSST) of 0.0 °C and a maximum
- air temperature of 13.3 °C (Hrbáček et al., 2017a); even though the mean annual air temperature
- 181 (MAAT) decreased slightly from -6.8 °C in 2011 to -7°C in 2015 (Hrbáček et al., 2016b; Láska

182 et al., 2012).

The two study sites are located at Brandy Bay (BB) near the western coast and at St. Martha
Cove (SMC) at the eastern coast of Ulu Peninsula. Both sites are located at similar topographic
positions (small plateaus) and elevation (80 m a.s.l.) with no visible vegetation (Fig. 2 and Fig.
3).

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188 **[Figure 2]**

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BB is located windward towards the mainly south-westerly winds (Hrbáček et al., 2016c; Nývlt
et al., 2016), whereas SMC is located leeward, shielded by the Lachman Crags from the stronger
winds. This results in less precipitation in the eastern part of JRI (Davies et al., 2013). Therefore,
BB can be considered as a characteristic wind-exposed coastal site with high influence of sea
spray, whereas SMC represents a characteristic soil of an inland site with less influence of sea
spray.

The substrate of both study sites is basically composed of coarse-grained cretaceous sandstones and siltstones of the Alpha Member of the Santa Martha Formation (Hrbáček et al., 2017b). The land surface is generally covered by a debris layer of gravels and large clasts mixed with loose sandy regolith, mostly derived from James Ross Volcanic Group basalts, which were deposited as debris flows containing mainly basalt and hyaloclastite breccia and palagonite (Davies et al., 2013; Hrbáček et al., 2017b; Salzmann et al., 2011). No nesting birds are found on JRI.

The continuous permafrost on James Ross Island shows an active layer thickness ranging between 40 and 107 cm related to the topographic position on Ulu Peninsula (Bockheim et al., 205 2013; Borzotta and Trombotto, 2004).

206 2.2 Soil sampling

During the austral summer period in 2016 soil samples from BB and SMC (Fig. 4 and Fig. 5) were taken. Both profiles were dug until a layer of coarse gravel was found. Bulk samples of both profiles were taken in depth increments (0-5cm, 5-10cm, 10-20cm, 20-50cm, >50cm) and were placed into sterile plastic bags, which were frozen immediately. Continuous cooling at -20°C was ensured by a transfer with the research vessels *RV Polarstern* to Germany. For micromorphological analyses, undisturbed and oriented samples were taken in modified Kubiena boxes (10cm x 6cm x 5cm). Samples for micromorphology were taken at depth of 0-

- 10cm, 10-20cm, 30-40cm, 50-60cm and 80-90cm at SMC. BB samples represent the depth of
- 215 10-20cm, 20-30cm und 40-50cm. Soils were described according to Food and Agriculture
- 216 Organization of the United Nations (FAO) (2006) and classified according to the World
- 217 Reference Base for Soil Resources (WRB; IUSS Working Group WRB, 2015).

218 **2.3 Soil physical and chemical analysis**

219 **2.3.1 Grain size distribution**

The samples were saturated (100ml of deionized water) and sonicated (800J ml⁻¹). Coarse-220 medium sand (>200µm), fine sand (63-200µm) and coarse silt (20-63µm) were obtained by wet 221 sieving. The smaller fractions, including medium silt ($6.3-20\mu m$), fine silt ($2-6.3\mu m$) and clay 222 ($<2\mu$ m), were separated by sedimentation. Fractions $>20\mu$ m were dried at 45°C and weighed 223 afterwards. The fractions <20µm were freeze-dried before weighing. The different procedures 224 were chosen due to practical reasons: freeze-drying allows submitting the finer fractions to 225 226 further analyses (particularly carbon and nitrogen content) immediately, while the coarser 227 fractions need milling anyway.

228 2.3.2 pH, EC, C&N contents, major elements and pedogenic oxides

The pH value was obtained using a pH meter (ph197i, WTW, Germany). Electrical conductivity was measured with a conductivity meter (LE703, Mettler-Toledo, USA). Values of pH and electric conductivity were measured from bulk samples < 2mm in deionized water with a sample to water ratio of 1:2.5.

Carbon (C) and nitrogen (N) contents of the bulk soils were analyzed by dry combustion 233 234 (Elementar CNS Vario Max Cube). 300 to 500mg per sample were analyzed in duplicate. In Order to distinguish between the total organic carbon (TOC) content and the total inorganic 235 carbon (TIC), TIC was removed by acid fumigation after Ramnarine et al. (2011). 100 mg of 236 the milled bulk soil samples were moistened with 20 to 40 µl of deionized water and put into a 237 desiccator together with 100ml of 37% HCl. Afterwards, the samples were dried at 40°C. 238 Finally, the samples were measured again by dry combustion (EuroVector EuroEA3000 239 Elemental Analyser) to obtain the TOC content. TIC content was calculated: TIC = C_{tot} - TOC 240 Major elements were analysed with a wavelength dispersive XRF device (AXS S4 Pioneer, 241 Bruker, USA). Prior to preparation, the samples (ratio Li-metaborate to soil 1:5) were ground 242 with an agate mill for 12 minutes. Major elements were used for the calculation of weathering 243 244 indices.

Pedogenic iron-oxides (Fe_d) were determined by dithionite-citrate-hydrogen carbonate extraction (Holmgren, 1967). Poorly to non crystallised Fe-oxides (Fe_o) were determined by acid ammonium extraction (Schwertmann (1964). The extractions were analysed at a wavelength of 238.204 nm by an inductively coupled plasma optical emission spectrometer (Vista Pro CCD Simultaneous ICP-OES, Varian, USA).

250 2.3.3 Ion chromatography

The initial water content in the investigated soil material was too low to extract sufficient amounts of pore water for ion chromatography. Hence, the soil samples were leached, according to Blume et al. (2011). Five grams of soil material were suspended in 25ml deionized water, shaken for 90 minutes and centrifuged at 9000rpm to separate the soil material from the soil solution and sterile filtered through a 0.22µm PES filter (Sartorius AG, Germany).

The ion concentrations in leached water samples were analysed by using two ion 256 chromatography (IC) systems (SYKAM Chromatographie Vertriebs GmbH, Germany). For 257 258 cations, the IC system consisted of a 4.6 x 200 mm Reprosil CAT column (Dr. Maisch HPLC GmbH, Germany), an S5300 sample injector and an S3115 conductivity detector (both SYKAM 259 Chromatographie Vertriebs GmbH, Germany), 175mg L-1 18-Crone-6 and 120µL 260 methanesulfonic acid served as the eluent with a set flow rate of 1.2mL min⁻¹. The injection 261 volume was 50µL. The column oven temperature was set at 30°C. The Cation Multi-Element 262 IC-standard (Carl Roth GmbH + Co. KG, Germany) containing NH4⁺, Ca²⁺, K⁺, Li⁺, Mg²⁺, Na⁺ 263 was measured before every replication series. For anions, the IC system consisted of a SeQuant 264 SAMS anion IC suppressor (Merck KGaA, Germany), an S5200 sample injector, a 3.0 x 265 150mm Sykrogel A 01 column and an S3115 conductivity detector (all SYKAM 266 Chromatographie Vertriebs GmbH, Germany). 6mM Na₂CO₃ with 90µM sodium thiocyanate 267 served as the eluent with a set flow rate of 1 ml min⁻¹ and a column oven temperature of 50°C. 268 The injection volume was 50 µL. The multi-element anion standard containing F⁻, Cl⁻, Br⁻, NO₂⁻ 269 , NO3⁻, PO4³⁻ and SO4²⁻ was measured before every replication series. The standards and 270 samples were measured in triplicates. 271

272 **2.3.4 Weathering indices and pedogenic oxide ratios**

The KN Index A $(SiO_2+CaO+K_2O+Na_2O)/(Al_2O_3+SiO_2+CaO+K_2O+Na_2O)$ was calculated after Kronberg and Nesbitt (1981). The index is based on the relative enrichment of the Al and Si oxide phase and the leaching of Na, K and Ca. It ranges between 0 (prevailing chemical weathering) and 1 (prevailing physical weathering). To get more precise information on the

index of (CIA) 277 ongoing chemical weathering, the chemical alteration [(Al₂O₃/(Al₂O₃+Na₂O+CaO*+K₂O)) x 100] after Nesbitt and Young (1982), in which CaO* 278 represents the amount of silicate-bound CaO, was calculated. The CIA is frequently used as a 279 quantitative measure of feldspar breakdown, assuming that feldspar represents the most 280 abundant and reactive mineral. Higher values indicate increasing weathering intensity. 281 Additionally, the degree of iron release (Fe_d/Fe_t) after Blume and Schwertmann (1969) was 282 calculated, which gives information on the iron release from primary Fe-bearing mineral 283 weathering: a longer or more intensive weathering process is indicated by a higher ratio 284 (Baumann et al., 2014; Mirabella and Carnicelli, 1992). 285

286 2.4 Micromorphology

Samples for thin section preparation were air dried and afterwards embedded with a mixture of 287 resin (Viscovoss N55 S, Vosschemie, Germany), stabilized Styrene (Merck KGaA, Germany) 288 and hardener (MEKP 505 F, Vosschemie, Germany). After hardening, the samples were 289 290 formatted into plane-parallel blocks and halved in the middle using a saw (Woco Top 250 A1, Uniprec Maschinenbau GmbH, Germany), and then one half was ground with the grinding 291 machine (MPS-RC Vacuum, G&N GmbH, Germany) and mounted onto a glass carrier. Then 292 the mounted samples was sawed into slices of about 150µm thickness. Finally, these slices were 293 294 ground to a thickness of 25µm. The preparation followed the instructions given by Kühn et al. (2017). Afterwards, they were analyzed by using a polarizing microscope (ZEISS Axio 295 Imager.A2m, Software AxioVision 4.7.2, Carl Zeiss Microscopy GmbH, Germany) and 296 described following the terminology of Stoops (2003). 297

298 **2.5 Microbial community analysis**

299 2.5.1 Nucleic acids extraction

For each soil sample (maximum amount of 0.5g per sample), triplicates of total genomic DNA were extracted using the FastDNATM Spin Kit for Soil (MO BIO Laboratories Inc., USA). The extracted DNA was stored at -20°C and used as a template for the enumeration of target genes by quantitative PCR (qPCR) and next-generation sequencing (Illumina HiSeq).

304 2.5.2 Quantification of bacterial 16S rRNA gene copy numbers

qPCR was used to quantify total bacterial abundances. All qPCR assays were performed in
 triplicates on a CFX96 Real-time thermal cycler (Bio-Rad Laboratories Inc., CA, USA) and
 contained 10µl SensiFAST SYBR Mix (Bioline GmbH, Germany), 5.92µl PCR water, 0.04µl

of forward and reverse primer (100 μ M) and 4 μ l template. The quantification of the bacterial 16S rRNA gene was based on the primers 341F (5'-CCTACGGGAGGCAGCAG-3') and 534R (5'-ATTACCGCGGCTGCTGG-3') according to Muyzer et al., 1993. After an initial denaturing phase of 3 minutes at 95°C, the cycler included 35 cycles of 3 seconds at 95°C, 20 seconds at 60°C and 60 seconds at 72°C plus the plate read. All cycling programs included a melting curve from 60°C to 95°C with 0.5°C steps per plate read. The analysis of quantification data was performed with the CFX ManagerTM Software (Bio-Rad Laboratories Inc., CA, USA).

315 2.5.3 Illumina HiSeq-Sequencing

Unique combinations of tagged 515F (5'-GTGCCAGCMGCCGCGGTAA-3') and 806R (5'-316 GGACTACHVGGGTWTCTAAT-3') (Caporaso et al., 2010) primers were assigned to each 317 sample (Tab. S1, S2). For each sample, two PCR reactions were prepared and the PCR product 318 pooled after PCR reduce PCR variability. The PCR was performed on a T100[™] Thermal Cycler 319 (Bio-Rad Laboratories Inc., CA, USA) in 25µl reactions, containing 0.125µl OptiTaq DNA 320 321 Polymerase and 2.5 10x Pol Buffer B (Roboklon GmbH, Germany), 1ul MgCl₂ (25mM), 1ul dNTP Mix (5mM), 16.625µl PCR water, each 0.625µl of forward and reverse primer (20µM) 322 323 and 2.5µl genomic DNA. The following cycler program was used: Initial denaturing step for 3 minutes at 95°C followed by 10 cycles of 1 minute at 94°C, 1 minute at 53°C (-0.2°C/cycle) 324 and 1 minute at 72°C, followed by 20 cycles of 1 minute at 94°C, 1 minute at 50°C and 1 minute 325 at 72°C, followed by a final extension step for 10 minutes at 72°C. All barcoded samples were 326 pooled into a single sequencing library by adding an equal amount of DNA (60ng DNA per 327 sample). Subsequently, a purification of the PCR product pool was achieved by using the 328 Agencourt AMPure XP - PCR Purification (Beckman Coulter, Inc., CA, USA). The Illumina 329 HiSeq-sequencing was performed by GATC Biotech AG, Germany. 330

331 2.5.4 Bioinformatics and statistical analysis

Sequencing was performed on an Illumina HiSeq (2 x 300 bp). Dual-indexed reads were 332 demultiplexed using CutAdapt (options: e0.1; trim-n; Martin, 2011). Barcode base pairs were 333 required to have a phred quality score of Q25 and no mismatches were allowed. Read pairs 334 were merged using PEAR (options: Q25; p10⁻⁴; o20; Zhang et al., 2013). The orientation of all 335 sequences were standardized by an own script using the information from demultiplexing. 336 Sequences containing low-quality base pairs were trimmed and filtered using Trimmomatic 337 (quality score of at least Q25 for trailing and leading base pairs, sliding window length of 5 338 basepairs, minimum sequence length of 200; Bolger et al. 2014). QIIME (version 1.9.1) 339

(Caporaso et al., 2010) was employed for microbiome analysis. USEARCH 6.1 (Edgar, 2010) 340 was used for the detection and removal of chimeric sequences. The SILVA database (version 341 128) (DeSantis et al., 2006) was utilized for the clustering of operational taxonomic units 342 (OTUs) (97% sequence similarity) and their taxonomic assignments. Singletons, OTUs 343 assigned to chloroplasts and mitochondria as well as rare OTUs (relative abundance of <0.1% 344 within each sample) were removed. Sample triplicates were merged by the mean value of their 345 relative abundance before visualization of the sequencing data and before analysis of correlating 346 environmental factors. For the processing and visualization of the obtained OTU table, R and 347 PAST3 (Hammer et al., 2001) were used. The hierarchical clustering of the samples using the 348 average linkage method was based on the Bray-Curtis dissimilarity. CANOCO5 (Šmilauer and 349 Lepš, 2014) was used for the canonical correlation analysis (CCA). If the Bonferroni corrected 350 *p*-value was <0.05, a given environmental parameter was included. Demultiplexed raw 351 European 352 sequencing data were submitted to the Nucleotide Archive (http://www.ebi.ac.uk/ena) under accession number: PRJEB29853. 353

354 **3 Results**

355 **3.1 Field properties and soil classification**

Both soils derived from coarse-grained marine sand- and siltstones, which were covered with 356 volcanic clasts. There was a higher contribution of volcanic material in BB than in SMC. The 357 amount of coarse material > 2mm was larger at the profile BB. Deflation processes led to a 358 residual enrichment of larger grains and pebbles at the soil surface of both profiles. The 359 permafrost table was not reached in both soil profiles, but ground ice was visible in a depth of 360 85cm at SMC. Neither SMC nor BB showed any ornithogenic influence. Both sites were 361 unvegetated by cryptogamic or vascular plants. The C-horizon was the only distinct soil horizon 362 occuring at SMC, whereas BB shows two changes within horizontal structures by abrupt 363 textural change below 10 cm and 20 cm. The textural change below 20 cm goes along with a 364 change in textural class; SCL (Sand: 52.5%, Silt: 21.9% and Clay: 25.6%) - CL (Sand: 44%, 365 Silt: 27.2% and Clay: 28.8%). Different from macroscopic features of the soil profiles, both 366 soils showed evidences of a downward transport and accumulation of particles and nutrients, 367 e.g. soluble products most likely originating from sea spray (Tab. 1). Accumulation starts at a 368 depth of 50cm at SMC and below 20cm at BB. Soil color did not change through the profiles. 369 SMC was brown to yellowish brown and BB was brownish yellow. 370

- 371 Both soils were classified as Cryosols (eutric, loamic) according to the WRB (IUSS Working
- 372 Group WRB, 2015).

373 **3.2 Grain size distribution and soil chemistry**

SMC had higher sand contents (mean value 61.7%, Table 1), while BB was characterized by
lower sand contents (mean value 47.4%) and higher silt and clay contents (mean values 25.3%
and 27.2% respectively). The grain size distribution varied only slightly with depth and similar
clay and silt contents were demonstrated for both soils.

- The pH was slightly to moderately alkaline in both profiles and highly alkaline only in the upper 378 5cm of BB. The pH values followed opposing trends with depth, increasing in SMC from 7.7 379 to 8.1 and decreasing in BB from 8.6 to 7.4. The EC ranged between 50-60µS cm⁻¹ in SMC and 380 was substantially higher in BB with a minimum of 350-450µS cm⁻¹ within 5-50cm and its 381 highest values around 900µS cm⁻¹ between 0-5cm and from 50cm downwards. According to 382 the EC values, SMC and the middle part of BB can be considered as being salt-free, whereas 383 the salt content in the upper and lowermost part of BB was low (Food and Agriculture 384 Organization of the United Nations (FAO), 2006). 385
- The total inorganic carbon (TIC) content was low in both soils ranging between 0.1 and 0.3mg
- g^{-1} in SMC and between 0.7 and 2.0mg g^{-1} in BB. The TOC content ranges from 0.8-0.9mg g^{-1}
- for SMC and from 1.4 and 2.6mg g^{-1} for BB and increased there slightly with depth. The N
- content was around 0.4mg g⁻¹ across both soil profiles. The C/N ratio was generally low with
- values below 7.5 in both soils, it decreased with depth in SMC (2.6 2.1) and increased with depth in BB (4.0-7.4).
- Ion concentrations (Tab. 1) were parallel to the depth function of the conductivity in both soils; e.g. higher EC and ion concentration characterized BB. Cl⁻ concentrations decreased with depth in SMC from 20.5 to 3.5 μ mol g⁻¹ soil as well as in BB from 4,522 to 231 μ mol g⁻¹ soil. The highest SO₄²⁻ concentrations were observed in the shallow (SMC: 9.6 μ mol g⁻¹ soil; BB: 621 μ mol g⁻¹ soil) and deepest (SMC: 15.3 μ mol g⁻¹ soil; BB: 451 μ mol g⁻¹ soil) samples. K⁺, Mg⁺ and Ca⁺ concentrations followed the same trend as SO₄²⁻. Br⁻, NO₂⁻, NO₃⁻ and PO₄³⁻. Li⁺ and NH₄⁺ concentrations were below the detection limit.
- 399
- 400 [Table 1]
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402 **3.3 Weathering indices and pedogenic oxide ratios**

Weathering indices were calculated according to the major element contents (Table 3). The KN
Index A was at 0.91-0.92 in SMC and only slightly lower with 0.89 - 0.90 in BB (Table 2). The
CIA varied between 53.9 and 54.8 in SMC and between 56.9 and 58.8 in BB. Both indices
indicated weak chemical weathering with a slightly higher weathering intensity in BB.

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408 [Table 2]

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410 The Fe_d/Fe_t ratio showed a decreasing trend from 0.18 to 0.11 with depth in SMC indicating a

411 decreasing intensity of pedogenic processes with depth. No particular trend was found in BB; 412 but the Fe_d/Fe_t ratio is – similar to the CIA - generally higher around 0.20 except for 0.16 in the

413 upper 5cm.

414 **3.4 Micromorphology**

Formation of platy and lenticular aggregates due to repeated freezing and thawing processes was detected. Neither platy and lenticular platy structures nor the results of translocation (eluviation) processes were observed during fieldwork, but could be confirmed later using micromorphology.

SMC had a weak to moderately developed pedality and a weak to moderate degree of separation (Table 3). Both, pedality and degree of separation are well developed at a depth of 50-60cm and were lowest developed close to the surface and at the bottom of the profile. In contrast, BB had a well-developed pedality and a moderate to high degree of separation with its maximum development close to the bottom of the profile.

424

425 [Table 3]

426

Lenticular and subangular blocky microstructures were present in both profiles, whereas lenticular microstructure was dominant in SMC and subangular blocky microstructure was dominant in BB. Lenticular shaped aggregates were first observed at a depth of 10cm in profile BB, and at 30cm in SMC (Figures 3a and 3b).

431

432 [Figure 3]

433

Translocations features, like cappings consisting of clay and silt particles welded together with
 sand-sized quartz grains were present in the upper part of both profiles. Link cappings occurred

13

in the lower part of both profiles, with lesser and smaller cappings in BB (Fig. 3d). Link
cappings were very rare and occurred only where coarse rock fragments were located close to
each other. Dusty silt and clay pendants occurred only in the lower part of BB (20-50cm) (Fig.
3e). The sphericity of mineral grains was smooth in both profiles. The minerals were slightly
better rounded in BB (subangular to round) than in SMC (subangular to subrounded).

Weathering processes were identified by pellicular and dotted alteration patterns on rock 441 fragments (mostly in sandstone fragments) in both profiles with a higher number of fragments 442 with dotted alteration patterns than with pellicular alteration patterns. The quantity and intensity 443 of dotted alteration patterns decreased with depth. Larger rock fragments were often strongly 444 weathered, so that mainly quartz-minerals were still preserved (Fig. 3f). Besides quartz, 445 glauconite is the main mineral component in the unweathered sandstone fragments. In addition, 446 feldspars and micas occur to a very small extent. The sandstones cemented by fine material and 447 faint Fe coatings are visible around quartz grains. Pellicular alteration pattern was found 448 exclusively on volcanic rock fragments, and only in the uppermost thin section (0-10cm) of 449 450 SMC (Fig. 3g). Fragments showing pellicular alteration patterns occurred in 10-30cm of BB. Even though the number of weathered fragments decreased, pellicular patterns were slightly 451 thicker in slide BBII (20-30cm) than in BBI (10-20cm). However, pellicular alteration patterns 452 did not exceed the state of "pellicular" in any analyzed slide whereas dotted alteration patterns 453 often reach the state of "patchy cavernous residue" (Fig. 3e) and do occur also as dispersed 454 minute residues (Stoops, 2003). 455

456 **3.5 Microbial abundance and community structure**

The enumeration of the 16S rRNA gene revealed a similar trend for both soil profiles (Fig. 4). The highest abundances with 6.6 x 10^8 copies g⁻¹ soil (BB) and 1.7 x 10^8 copies g⁻¹ soil (SMC) were detected in the uppermost depth increment of both soil profiles. Both soils showed a decrease in bacterial abundances with depth. The lowest bacterial abundances in SMC were detected below 50cm depth with 3.7 x 10^5 copies g⁻¹ soil, and in BB in 20-50cm depth with 1.7 x 10^6 copies g⁻¹ soil.

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 (Tab. S4). The number of reads per sample ranged from 54,122 to 916,583 with a mean value of 513,582. A total of 687 OTUs was clustered. After taxonomic classification, 258 putative taxa were obtained. Shannon's H index was used to estimate and
compare the alpha diversity of the different depth increments interval of the soils (Tab. S5).
Both soils showed a similar Shannon's H index, which ranged from 3.7 to 4.7 not following

472 any specific trend.

Bacteria dominated the microbial community in both soil profiles (Fig. 4). Higher abundances 473 of Thaumarchaeota (7.2 - 12.9%) were found in the upper 10cm of the soil profile from SMC 474 (Tab. S4). On a phylum level, the soil profile of SMC was dominated by Proteobacteria (23.4 -475 57.9%) and Actinobacteria (17.7-41.3%) but showed also relative high abundances of 476 Acidobacteria (3.9-14.1%). The microbial community in BB was also mainly composed of 477 Proteobacteria (28.2-30.8%), followed by Actinobacteria (27.6-46.6%), Gemmatimonadetes 478 479 (3.9-24.7%) and Chloroflexi (5.3-10.9%). Bacteroidetes were highly abundant (10.5%) in the top 5 cm of BB. Regarding potential phototrophic organisms in the investigated soils, the 480 amount of chloroplast-related reads was relatively low (<0.2%) in each sample, except for SMC 481 >50 cm (0.03% - 1.30%) and BB 0 - 5 cm (0.87% - 1.01%). Cyanobacteria-related OTUs were 482 483 rare and only showed low relative abundances in SMC 5 - 10cm (0.06%), SMC 10 - 20cm (; 0.62%), SMC >50cm (0.04%). 484

485

486 **[Figure 4]**

487

The distribution of dominant OTUs was reflected by a cluster analysis based on the Bray-Curtis 488 dissimilarity of the investigated depth increments. Samples were clustered according to their 489 origin and depth. On a first level, samples grouped according to depth in upper (0–20cm) and 490 deeper (20-80cm) samples and within these groups they clustered according to location (BB vs. 491 SMC). An exception is the sample from BB from the depths 0-5cm which formed an own 492 cluster (Fig. 5). The deeper samples in both profiles (20-80cm depth) showed high relative 493 abundances of three OTUs related to Acidiferrobacteraceae(1, 2, 3) (SMC: 1.7-14.6%; BB: 2.2-494 9.8%) and one OTU related to Gemmatimonadaceae(1) (SMC: 1.5-3.8%; BB: 14.1-20.3%). 495 High proportions of two OTUs related to Gammaproteobacteria(1, 2) (SMC: 2.8-11.4%; BB: 496 5.4-10.2%) and one OTU related to Gaiellales(2) (SMC: 3.7-5.7%; BB: 7.2-8.3%) were 497 observed in the shallow samples (0-20 cm). BB 0-5 cm was comprised of a strongly different 498 community. The most abundant taxa in this sample were related to *Thermomonas*(1) (6.4%), 499 Sphingomonas (3.7%) and Solirubrobacterales(1) (3.7%). 500

501

502 [Figure 5]

503

The relationship of OTU distribution and environmental parameters was examined by applying 504 a CCA (Fig. 6). Contents of chloride (18.5%), calcium (11.8%), sulfate (5.9%), silt (5.6%), 505 TOC (6%) and the Fe_d/Fe_t-ratio (12.5%) formed the optimal subset to explain variations in 506 community structure of the investigated soil profiles (p < 0.05). The adjusted explained 507 compositional variation was 49.9%. A strong correlation between the unique community of BB 508 0-5cm and the saline conditions was observed, mainly caused by high sulfate and chloride 509 concentrations. The remaining samples were arranged according to sample site and depth as 510 511 already observed in the cluster analysis above.

512

513 [Figure 6]

514 **4 Discussion**

The interaction of biotic and abiotic processes remains one of the fundamental questions in 515 ecosystem research and further the initial development of soils under harsh environmental 516 conditions, such as Antarctica. So far, only a few studies exist for polar environments that 517 integrate pedogenic and microbiological research (e.g. Aislabie et al. 2008, Cowan et al. 2014, 518 Ganzert et al. 2011; Bajerski and Wagner, 2013). James Ross Island offers an exceptional 519 opportunity to improve our understanding of the interrelations between soil formation and 520 microbiological properties in the absence of plants. The present interdisciplinary study gives 521 profound insights in the state of soil formation and microbial community structure in initial 522 soils in the transition zone between maritime and continental Antarctica. 523

James Ross Islands is located in the transition zone between warmer and wetter maritime Antarctica and cold and dry continental Antarctica (Souza et al., 2014). In this area, we studied two representative soils 16km apart, with different exposures to the dominant south-westerly winds. The leeward position of SMC displays formation conditions of a typical inland soil, while BB in its windward position represents coastal soils. As indicated by EC values, BB is influenced by sea spray, while SMC, sheltered behind the Lachman Crags, does not show strong input of soluble salts from sea spray.

The examined soils on JRI were characterized by low TOC (0.9-2.6mg g⁻¹) and low total nitrogen contents (approx. 0.4mg g⁻¹), which is common for Antarctic soil environments (e.g. Cannone et al., 2008), and relative high pH values (7.4- 8.6). The moderately to highly alkaline pH in both soils cannot be explained by the occurrence of CaCO₃, because the soils have a negligible amount with of TIC (≤ 2 mg g⁻¹). Low C contents do not only show the missing influence of penguins, but also indicate a relative juvenility of the soils: This indicates that no

cations have been leached from the topsoil, and therefore the pH remains neutral to basic 537 (Wilhelm et al., 2016). In addition, the content of basalt clasts in the parent material results in 538 increased soil pH values (Simas et al., 2002; Moura et al., 2012). The opposing trends in the 539 depth function of the pH values are caused by the input of soluble salts from sea spray: wind 540 can transport soluble salts from the sea causing an additional input of bases simultaneously 541 increasing the pH at BB, while SMC is not affected (Benassai et al., 2005; Russell et al., 2010; 542 Hara et al., 2004; Udisti et al., 2012). Since the substrate was not colonized by plants, lichens 543 544 or endolithic prokaryotes, and the taxonomic data revealed low abundances of phototrophic organisms, the alkalization of the substrate by the release of hydroxyl ions in the course of 545 photosynthesis has a minor effect on soil pH. On the other hand, the neutral to basic pH does 546 547 not significantly affect the soil microbial community structure, which is in accordance with observations in soils from Livingston Island (South Shetland Archipelago, maritime Antarctica) 548 549 by Ganzert et al. (2011). They explained it by the occurrence of a specific soil microbial community, which thrives under low C and N conditions and is not depending on nutrient input. 550 551 Therefore, pH is mainly driven by the parent material composition combined with the input of soluble salts in these young soils on JRI. 552

The additional input of airborne cations by sea spray led to higher sodium and calcium contents and a rejuvenation of the affected depth increments of the soil profile, which can be seen in the lower CIA values in 0-5 cm soil depth of both soils compared to the lower part of the profiles. Ions, for instance sulfate accumulate close to the permafrost table, which acts as a barrier and therefore explains increasing contents of sulfate with depth. The high amount of sulfate near the surface is most likely caused by sea spray and precipitation, because they are known to carry high amounts of sulfate in coastal areas (Blume et al., 2010).

Chemical weathering, as indicated by the KN-Index A (Kronberg and Nesbitt, 1981), is only of 560 minor importance whereas physical weathering is prevailing. The CIA and pedogenic oxide 561 ratios (POR) confirmed the low degree of soil formation. Pedogenic oxides with specific 562 degrees of crystallization relate to intensity and/or duration of pedogenic processes (Baumann 563 564 et al., 2014; Blume and Schwertmann, 1969; Mirabella and Carnicelli, 1992). The results show that both CIA and both POR are slightly higher at BB compared to SMC. The KN-Index A and 565 the CIA showed a weak chemical weathering of these mineral soils (Michel et al., 2014). Both 566 indices indicated a more intensive chemical weathering at BB and, thus, indicate a slightly 567 stronger pedogenesis at BB than at SMC. This finding could be explained by the sea- and 568 windward position of BB, which results in an increased water availability and a slightly more 569 570 levelled microclimate. Since both soils are located in similar topographic positions and derived

from similar parent material, CIA and POR results allow the interpretation that soils influenced 571 by coastal conditions tend to be more weathered. Besides physical and chemical weathering, 572 microorganisms play an important role in mineral dissolution and oxidation. Adapted 573 microorganisms colonize minerals and are, depending on nutritional requirements, nutrient 574 availability and mineral type, potential contributors to the weathering of minerals (Uroz et al., 575 2009). Taxonomical groups, which are usually connected to microbial weathering, are present 576 in the soils, such as Massilia, Bacillus (Ma et al., 2011) and Polaromonas (Frey et al., 2010). 577 Interestingly, the relative abundances of these taxa changed according to the degree of 578 579 weathering. This could indicate a possible interrelation between the occurrence of these potential weathering-related organisms and the degree of weathering of Antarctic soils. 580

581 Evaluating weathering using the CIA, it must be noted that the value for BB is most likely underestimated. Ion chromatography results show that Na-content is much higher at BB. The 582 583 high amount of Na is most likely caused by sea spray, which is known to carry high amounts of Na (Udisti et al., 2012). Since the calculation of the CIA takes Na into account (Nesbitt & 584 585 Young, 1982), the CIA values would be significantly higher if the additional input of sea salts could be excluded. It is very likely that the actual difference in state of weathering between 586 SMC and BB would be much higher. In conclusion, chemical weathering, even without 587 influence of guano deposits, is of higher importance for the current state of soil formation, than 588 the ongoing cryoturbation. 589

In case of the pedogenic oxide ratios, 12.5% of the total compositional variation could be 590 explained, which indicates a correlation between the microbial community structure and 591 weathering at a very initial stage of soil formation. The pedogenic oxide ratios correlate with 592 the compositional distribution of microorganisms in the investigated soils, and with the relative 593 abundances of one Acidiferrobacteraceae-related OTU. Microorganisms of this family are 594 described as autotrophic sulfur and iron oxidizers, which have the capacity to use ferrous iron, 595 thiosulfate, tetrathionate, sulfide and elemental sulfur as electron donors and oxygen or ferric 596 iron as terminal electron acceptor (Hallberg et al., 2011). The reactive iron could potentially be 597 598 used as terminal electron acceptor in the course of microbial iron cycling (Canfield, 1989). Organic matter, a potential substrate for heterotrophic microbial processes, sorbs on mineral 599 surfaces (Kaiser and Guggenberger, 2000) and could be released in the course microbial 600 oxidation and reduction of reactive iron phases. In addition to the autotrophic processes, the 601 release of sorbed, organic matter from mineral surfaces could be an additional way to increase 602 the pool of biologically available carbon. The availability of such a mechanism potentially has 603

an influence on the microbial community structure and abundances in oligotrophic environments.

Translocation features are common features in permafrost-affected soils. They often occur 606 together with platy rectangular or lenticular aggregates, caused by reoccurring freeze-thaw-607 cycles (Van Vliet-Lanoë, 1985). Platy blocks and lenses dominated the microstructure in the 608 areas between 20 and 50cm of both profiles. They were absent near the surface of both profiles 609 and at the bottom of the profile SMC. These microstructures are known to occur in the transition 610 611 zone between permanently frozen and unfrozen soils (Shur et al., 2005; Van Vliet-Lanoë et al., 2004). Here, the alternating temperature and soil moisture conditions additionally affect the 612 microbial community structure. The frequency of freeze-and-thaw cycles tends to be steady in 613 614 the middle part of a permafrost-affected soil, whereas weather shifts influence the surface, causing several freeze-and-thaw events per day, which do not result in typical microstructure 615 formation due to insufficient water supply (Van Vliet-Lanoë, 1985). Aggregate formation by 616 reoccurring freeze-and-thaw cycles result in a change in pore shape and size (Van Vliet-Lanoe 617 et al., 2004). Especially during the summer season, intensive insolation causes high evaporation, 618 resulting in dry soil surfaces. Changes in pore space affects microbial habitats, due to larger 619 pores and a more sufficient water supply. This has a severe influence on matter fluxes and soil-620 environmental conditions, which is reflected in a changing species distribution and, more 621 specifically, the occurrence of different clusters of highly abundant organisms in both soils. 622 Multivariate statistics were performed for soil depth increments considered as being 623 independent. However, when processes are discussed that link between soil horizons, e.g. water 624 and solute flow through the profiles, we account for the limited number of two soil profiles with 625 great care. We could not detect any environmental factors that increase or decrease the 626 correlation between the chosen depth increments. Nevertheless, freeze-and-thaw cycles 627 definitely also occur in the upper part of the profile, as indicated by the well sorted areas (Van 628 Vliet-Lanoë, 1985), which were described as single grain microstructure. Near the permafrost 629 table aggregates are often formed by frost desiccation and are hence poorly compacted what 630 631 makes them unstable upon moistening, which occurs during thawing events and explains the missing platy microstructure at SMC near the bottom of the profile (Van Vliet-Lanoë, 2010). 632 The fact that lenticular shaped aggregates occur also in the lower part of the profile indicates 633 that the permafrost table is located underneath the layer of coarse gravel at BB. 634

Although the investigated soils were poorly developed, an abundant and diverse prokaryotic community could be observed. Microbial abundances in both soils showed a decreasing trend with depth. Values of up to 10^9 gene copies g⁻¹ soil in the uppermost depth increments are comparable to observed microbial abundances from other cold environments, such as alpine
glacial forelands (Sigler et al., 2002), permafrost-affected soils from arctic regions (Liebner et
al., 2008) and Antarctic glacier forefields (Bajerski and Wagner, 2013).

Both soils were characterized by a highly diverse community dominated by Proteobacteria, 641 Actinobacteria, Gemmatimonadetes, Acidobacteria and Chloroflexi, which is in accordance 642 with the observations in other continental and maritime Antarctic habitats (e.g. Yergeau et al., 643 2007; Cary et al., 2010, Ganzert et al., 2011, Bajerski and Wagner 2013, Wang et al., 2016). 644 645 Substantial differences in geochemical parameters such as conductivity, the change of the community structure on a phylum level were evident as well as the occurrence of depth-646 dependent clusters (0-20 cm; >20 cm) of dominant OTUs (Fig. 8). Whereas the upper 20cm of 647 the soils were dominated by Gammaproteobacteria and Gaiellales, the deeper part of the soils 648 increased abundances of OTUs related to Acidiferrobacteraceae 649 showed and Gemmatimonadaceae. This distinct shift correlates with the occurrence of the microstructure 650 related to freezing and thawing and could be related to its changes of the pore space and the 651 availability of oxygen, water and nutrients. For instance, Gemmatimonadaceae were a common 652 observation in the soils and showed increased abundances in deeper parts of BB. These 653 organisms have a cosmopolitan distribution in terrestrial environments and depend on the soil 654 moisture condition of the respective soil and soil depth (DeBruyn et al., 2011; Bajerski and 655 Wagner, 2013). Only a few isolates have been described for this phylum (e.g. Zeng et al., 2015) 656 and their exact functions in soil ecosystems remain uncertain. The change in relative abundance 657 of these taxa with depth could be coupled to the changing availability of water, which depends 658 on the microstructure. For example, the amount and size of microaggregates have been shown 659 to be important regarding prokaryotic colonization, leading to genetically distinct communities 660 as well as cell densities in different size classes of aggregates (Ranjard et al., 2000). Thus, in 661 addition to environmental parameters, which shape the overall prokaryotic community, the 662 microstructure of the initial soils could have a substantial influence on species distribution. 663

Higher abundances of Bacteroidetes- and especially Flavobacteriaceae-related OTUs were 664 665 observed in the uppermost area of soil from BB, while only showing minor abundances in the deeper soil areas. This area differed from the remaining soil in two regards, namely very high 666 chloride concentrations and a relative high content of coarse sandy material and could select 667 for adapted psychroand halotolerant Bacteroidetes-related organisms, such 668 as 669 Flavobacteriaceae (e.g. Bajerski et al., 2013a). Members of the Flavobacteriaceae family detected in this area, for instance Gillisia sp., were isolated from Antarctic habitats before and 670 671 were shown to be at least moderately tolerant to saline conditions (Bowman and Nichols, 2005).

Putative halotolerant or halophilic Flavobacteriaceae in this area could have a need for high 672 chloride contents. Chloride can be accumulated inside the cell to osmotically balance the 673 cytoplasm with the surrounding habitat (Oren et al., 2002; Müller and Oren, 2003). 674 Furthermore, the detected Bacteroidetes-related organisms could prefer the coarser, sandy 675 microstructure from this depth increment. The preference of microbial groups for certain grain-676 size-dependent microenvironments, for instance the sand-sized fraction being preferred by 677 Bacteroidetes, was shown, e.g. in Typic Hapludalfs from central Denmark (Hemkemeyer et al., 678 679 2018).

Both investigated soils were poor in soil organic C as well as N. Organisms with the ability to 680 use oxygenic photosynthesis to fixate CO₂, such as cyanobacteria, were nearly absent in the 681 682 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, 683 inorganic compounds and metabolic pathways utilizing those may have a more pronounced role 684 in sustaining the microbial ecosystem at this initial stage of the soils. Several of the most 685 686 abundant taxa observed in BB and SMC were putative chemoautotrophs involved in nitrogen, iron and sulfur cycling, such as potential ammonia-oxidizing Thaumarchaeota or sulfur/iron-687 oxidizing Acidiferrobacteraceae. Microorganisms can be seen as the primary pioneers of 688 nutrient-poor environments such as Antarctic soils, and were shown to have the genetic 689 potential to fix C and N (Cowan et al., 2011; Niederberger et al., 2015), thus increasing C and 690 N contents of these oligotrophic soils. The chemoautotrophic Thaumarchaeota oxidize 691 ammonia aerobically to nitrite (Brochier-Armanet et al., 2008; Vajrala et al., 2013) and were 692 observed in many studies located in Antarctica (Magalhães et al., 2014; Ayton et al., 2010). 693 However, ion chromatography showed that amounts of ammonia as well as nitrite and nitrate 694 were negligible in both soils. Ammonia originating from necromass and products in the course 695 of nitrification could be metabolized directly by the present community, so no accumulation of 696 the different intermediates containing nitrogen takes place. These organisms are reported to 697 have the genetic potential to use the hydroxypropionate/hydroxybutyrate pathway for CO₂ 698 699 fixation, which is highly efficient and could provide an ecological advantage in oligotrophic environments (Könneke et al., 2014). Further, a part of the community could use atmospheric 700 701 compounds as energy source. Atmospheric H₂, CO, and CO₂ are scavenged and used as an energy source by microorganisms, especially organisms associated with the phyla 702 703 Actinobacteria, Chloroflexi, Acidobacteria, Planctomycetes, Verrucomicrobia, and Proteobacteria (Greening et al., 2015; Ji et al., 2017) .Operational taxonomic units related to 704 705 the phylum Actinobacteria and the associated orders Acidimicrobiales and Solirubrobacterales

were highly abundant in the investigated soils. Microorganisms in Antarctic soils, especially 706 bacteria related to the phyla Actinobacteria, AD3 and WPS-2, were shown to generate biomass 707 by consuming H₂, CO₂ and CO from the atmosphere (Ji et al., 2017). The gene for 708 chemosynthetic CO₂ fixation, *rbcL1E*, was found in multiple orders, including 709 Pseudonocardiales, Acidimicrobiales and Solirubrobacterales. Similar functional capabilities 710 could be present and active in the investigated soils. Our results show that, in this initial stage 711 of soil development, chemolithoautotrophic lifestyles plays an important role for the generation 712 of biomass and initial accumulation of soil organic carbon and nitrogen. 713

714 **5. Conclusion**

The presented soil and microbiological study on initial soils in the semiarid environment of Antarctica shows the current state of soil formation indicated by main soil and microbiological properties and their interplay. The results allow us to draw the following conclusions:

- Despite similarities in topographic position and substrates, both profiles have distinct differences in chemistry (content of salts indicated by EC, opposing trends in pH and states of weathering, indicated by WI and POR) and microbiology (depth functions of microbial abundances and diversity, e.g. Proteobacteria, Gemmatimonadetes and Thaumarchaeota abundances), which are caused by the different local environmental conditions at each site.
- The EC values of the soils and the depth function of the pH values clearly showed
 different conditions for soil formation at the two sites caused by the more exposed
 location of BB towards the mainly south-westerly winds, resulting in a more intense
 weathering and higher input of salt by sea spray.
- Taking weathering and aggregation as indicators of soil formation, we conclude that
 coastal conditions in contrast to inland conditions favor the formation of soils in
 maritime Antarctica.
- 4. Despite different local environmental conditions at each site, the microbial communities
 differ more distinctly between the depth increments in one profile than between the two
 profiles. Therefore, we conclude that in this initial stage of soil formation factors such
 as weathering and microstructure formation, as well as the resulting parameters (e.g.
 water availability and matter fluxes), are more important drivers of soil microbial
 community composition than chemical parameters such as EC and pH.
- Assuming that prokaryotic life is highly affected by changes in soil structure and vice
 versa, further investigations in this field should include analyses of (micro-) aggregates.

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Thomas Scholten and Carsten W. Mueller. Lars A. Meier and Carsten W. Mueller carried out
fieldwork during the PROANTAR fieldtrip led by Carlos E.G.R. Schaefer in 2016. Lars A.
Meier, Patryk Krauze, Isabel Prater and Fabian Horn did analyses and interpretation. Lars A.

- 744 Meier and Patryk Krauze prepared this manuscript with contributions from all co-authors.
- 745
- 746 *Competing interests.* The authors declare that they have no conflict of interests.
- 747

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1058 Tables

	Depth	$pH_{\rm H20}$	EC	TIC	TOC	Z	C/N	\mathbf{Na}^{+}	¥	\mathbf{Mg}^{+}	\mathbf{Ca}^+	CI.	SO_4^{2-}	Sand	Silt	Clay
														63 - 2000	2-63	Ś
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Sample	[cm]		[µS cm ⁻¹]	$[mg g^{-1}]$	[mg g ⁻¹]	$[mg g^{-1}]$		[µmol g ⁻¹]	[%]	[%]	[%]					
SMC 0-5	0-5	T.T	46	0.1	6.0	0.4	2.6	41.5	2.5	4	10.4	20.6	9.6	61.2	18.9	19.8
SMC 5-10	5-10	8	36	0.1	0.9	0.4	2.5	30.4	2.4	3.6	9.6	13.1	5.7	59.9	19.4	20.7
MC 10-20	10-20	7.9	33	0.3	0.9	0.4	2.3	27.1	2	3.1	8.3	8.7	3.3	63.8	17.1	19.1
MC 20-50	20-50	8	33	0.1	0.8	0.4	2.2	38.6	1.5	2.1	4.9	5.5	3	61.9	17.2	20.8
SMC > 50	>50	8.1	65	0.2	0.9	0.4	2.1	91.5	2.7	3.1	6.3	3.5	15.3	61.7	20	18.3
BB 0-5	0-5	8.6	950	1.4	1.4	0.4	4	1590	23.4	84.6	151	4522	621	49.8	25.2	24.9
BB 5-10	5-10	8.1	561	1.2	2.1	0.4	5.6	470	16.3	57.4	108	702	123	46.4	25.7	27.9
BB 10-20	10-20	T.T	385	0.7	2	0.3	5.9	268	12.2	42.6	93	369	88	52.5	21.9	25.6
BB 20-50	20-50	7.6	505	2	2.5	0.4	6.7	191	18.3	79.8	173	386	163	44	27.2	28.8
BB > 50	>50	7.4	965	1	2.6	0.4	7.4	149	23.9	140	297	231	451	44.3	26.8	28.9

1059Table 1: Soil properties of two soil profiles from St. Marta Cove (SMC) and Brandy Bay (BB) from James Ross Island,1060Antarctica.

1061 1062 1064 1065 Table 2: Weathering indices (WI) and pedogenic oxide ratios (POR) of two soil profiles from St. Marta Cove (SMC) and Brandy Bay (BB) from James Ross Island, Antarctica. CIA = chemical index of alteration; KN-A = Kronberg Nesbitt Index; Fe_d = dithionite-soluble iron; Fe_t = total iron; Fe_o = oxalate-soluble iron.

		V	VI					
	Depth					Fet	Fed	Feo
Sample	[cm]	CIA	KN-A	Fed/Fet	Fe _o /Fe _d	[mg g ⁻¹]	[mg g ⁻¹]	[mg g ⁻¹]
SMC 0-5	0-5	53.9	0.92	0.18	0.56	45.57	7.99	4.48
SMC 5-10	5-10	54.2	0.91	0.18	0.45	44.71	7.83	3.56
SMC 10- 20	10-20	54.8	0.91	0.16	0.53	40.74	6.61	3.48
SMC 20- 50	20-50	54.3	0.91	0.15	0.59	40.76	5.96	3.53
SMC > 50	>50	54.1	0.92	0.11	1.72	42.25	4.83	8.3
	0.5	560	0.00	0.16	0.61	52 77	0.60	5.2
BB 0-2	0-5	56.9	0.89	0.16	0.61	53.77	8.68	5.3
BB 5-10	5-10	58.5	0.89	0.21	0.57	44.09	9.08	5.19
BB 10-20	10-20	58.1	0.9	0.2	0.58	42.57	8.34	4.85
BB 20-50	20-50	58.8	0.9	0.21	0.56	39.82	8.43	4.68
BB > 50	>50	58.2	0.9	0.21	0.54	38.18	7.88	4.24

**]	Deg	ree o	fra	oun	dnes	s ar	nd sj	phei	icity	resu	ılts s	sepa	rate	d b	у "	'/'':	tw	0 0	diff	ere	ent	deg	gree	es v	ver	e n	nair	ıly	pre	sent	; m	eası	ıred
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	ocatio			pen							Х	Х																					
tures	Transle		coatings	cap	(X)	X	Х	(X)		(X)	Х																						
Pedofea	res			li	(X)			Х				Х																					
	featu		цц	8	(X)		Х				Х	Х																					
	imorphic	-	odules	8	Х	Х					(X)	Х																					
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1067 Table 3: Micromorphological features of two soil profiles from St. Marta Cove (SMC) and Brandy Bay (BB) from James

Ross Island, Antarctica

The micromorphological property is shown by the presence (cross) or absence (no cross). (x) = occasional occurring * Microstructures separated by "/": two different microstructures were found. Microstructures separated by "()": one ms shows partly features of another ms







1077Figure 1: Mainmap shows the regional setting of Ulu-Peninsula on James Ross Island, Maritime Antarctica. Black1078circles indicate the location of both study sites, Brandy Bay (BB) and St. Marta Cove (SMC). Sidemap 1-3 provide an1079additional overview over Antarctica, the Antarctic Peninsula Region and James Ross Island.



- 1080
- 1081 Figure 2: Study sites and soil profiles on James Ross Island; a: St. Marta Cove (SMC). It is not covered with vegetation.
- 1082 A 90 cm deep soil profile was taken; b: soil profile St. Marta Cove (SMC). Scale of the tape measure is in cm; c: study
- 1083 site Brandy Bay (BB) is close to snowfield. It is not covered with vegetation. A 60cm soil profile was taken; d: Soil profile
- 1084 Brandy Bay (BB). Scale of the tape measure is in cm.



- 1127 Figure 3: Images of micromorphological featuress found at Brandy Bay (BB) and St. Marta Cove (SMC). Pictures were taking using plane polarized light (ppl) and crossed polarizers (xpl). (a) BB III: highly separated lenticular platy microstructure, platy
- aggregates are indicated by green dotted lines, lenticular ms is indicated by black dotted lines, 2.5x, ppl; (b) SMC IV:
- moderately separated lenticular platy microstructure, indicated by black dotted lines, 2.5x, ppl; (c) SMC I: coarse monic
- ¹¹²⁹ microstructure, 2.5x, pp; (d) BB II: chitonic c/f-related distribution and thin link cappings (li) on quartz grains, 20x, pp; (e)
- 1130 BB III: weathered rock fragment covered by silty capping (cap) and also showing a thick pendent (pen) consisting of silty material and mineral grains, 10x, ppl; (f) SMC I: strongly weathered sandstone fragment with former boundaries, indicated
- by red dotted line, still visible by capping (cap), 5x, ppl; (g) SMC I: weathered volcanic rock fragment with distinct pellicular
- 1132 alteration pattern, 5x, ppl; (h) BB II: weathered and broken volcanic rock fragment with internal volcanic glass and covered by a thin clay capping (cap),(110-120μm), 2.5x, ppl; (i) SMC I: weathered volcanic rock fragment with feldspar phenocrysts;
- 1133 covered by a dusty clay-silt capping (80-100 μ m) (cap), 2.5x, ppl; (i) SMC I:; usage of crossed polarizers makes it possible to
- tell external coating (cap) from altered internal material, border indicated by grey dotted line, 2.5x, xpl.
- 1135



1137 Figure 4: Relative abundances of phyla and bacterial 16S rRNA qPCR gene abundances of soil profiles from Brandy

1138 Bay (BB) and St. Marta Cove (SMC) on James Ross Island, Antarctica. Triplicates are merged. Only phyla with a 1139 relative abundance of at least 5% at a given site are shown. The remaining phyla are summarized as "Others".

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1141 Figure 5: Heatmap based on the relative abundances of the observed operational taxonomic units (OTUs) in soil profiles

1142from Brandy Bay (BB) and St. Marta Cove (SMC) on James Ross Island, Antarctica. Only OTUs with a relative1143abundance of at least 3% in a given sample were included. Samples as well as OTUs were clustered using average1144linkage hierarchical clustering.





Figure 6: Canonical correlation analysis of the microbial composition of soil profiles from Brandy Bay (BB; black symbol) and St. Marta Cove (SMC; yello symbol) based on Bray-Curtis dissimilarities of the OTU data and its associated environmental parameters. If the Bonferroni corrected p-value was below 0.05, a given environmental parameter was included in the visualization. The amounts of chloride, sulfate, silt, Ca and TOC contents, and the Fed/Fet

1150 ratio explained 49.9% of the microbial community composition.