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Bacterial community composition and diversity respond to nutrient amendment but not warming in a maritime Antarctic soil

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Abstract

A resumption of climate warming in maritime Antarctica, arising from continued greenhouse gas emissions to the atmosphere, is predicted to lead to further expansions of plant populations across the region, with consequent increases in nutrient inputs to soils. Here, we test the main and interactive effects of warming, applied with open top chambers (OTCs), and nutrient amendment with tryptic soy broth (TSB), an artificial growth substrate, on bacterial community composition and diversity using Illumina sequencing of 16S rRNA genes in soil from a field experiment in the southern maritime Antarctic. Substantial effects of TSB application on bacterial communities were identified after 49 months, including reduced diversity, altered phylogenetic community assembly processes, increased *Proteobacteria* to *Acidobacteria* ratios, and significant divergence in community composition, notably increases in the relative abundances of the Gram positive genera *Arthrobacter*, *Paeniglutamibacter* and *Planococcus*. Contrary to previous observations from other maritime Antarctic field warming experiments, we recorded no effects of warming with OTCs, or interactive effects of OTCs and TSB application, on bacterial community composition or diversity. Based on these findings, we conclude that further warming of the maritime Antarctic is unlikely to influence soil bacterial community composition or diversity directly, but that increased nutrient inputs arising from enhanced plant growth across the region may affect the composition of soil bacterial communities, with possible effects on ecosystem productivity.

Introduction

Surface air temperatures in the maritime Antarctic during the latter half of the 20th Century rose at a faster rate than in any other region of the Southern Hemisphere (0.2–0.5 °C per decade) [1]. Although a recent analysis of temperature records indicates that warming of the region slowed in the late 1990s [2], climate change models forced with only moderate greenhouse gas emission scenarios predict rises in surface air temperatures in maritime Antarctica of 2–4 °C before the end of the 21st Century [3, 4]. Based on observations made between the 1950s and late 1990s, further rises in air temperature in the region can be expected to lead to substantial impacts in the physical environment, including glacial retreat and ice shelf disintegration [5, 6]. However, it is apparent that climate warming will also influence the ecology of maritime Antarctic terrestrial ecosystems, with accelerated plant growth rates, expansions in native plant populations and increases in soil microbial diversity being predicted as the region warms [7–9].

One consequence of expanding plant populations in a warmer maritime Antarctic will be that nutrient inputs to the soils of the region will increase [10]. Previous studies have simulated these increased nutrient inputs by applying artificial growth substrates, such as glucose, glycine, ammonium chloride and tryptic soy broth (TSB), to Antarctic soils [11, 12]. The application of these substrates consistently results in increased concentrations of total ester linked fatty acid (ELFA) markers in soil, indicative of a larger microbial community [12, 13]. However, despite a larger biomass of microbes in nutrient-amended soils, it is less clear how substrate amendment influences soil microbial community composition and diversity. For example, in a study using ELFA markers, reductions in richness (measured by the Shannon diversity index) were reported for Continental Antarctic Dry Valleys soils to which glucose and ammonium chloride had been added [12], but no effects of the same substrates were found on soil microbial community composition in another study in the same region [11].

Nutrient amendment combined with warming has been shown to influence the composition of maritime Antarctic soil bacterial communities. In a study at Mars Oasis on Alexander Island in the southern maritime Antarctic, Dennis et al. [13] added glucose, glycine and TSB to soil in factorial combination with warming, applied using open top chambers (OTCs). After one year, TSB and glycine application in combination with warming reduced the concentrations in soil of the fatty acids a15:0 and a17:0, which are frequent in Gram positive *Actinobacteria* such as *Arthrobacter* [14], and consequently halved the ratio of Gram positive to Gram negative bacteria, relative to soils that had been amended with the substrates but had not been warmed [13]. The composition of Antarctic soil bacterial communities has also been reported to be affected by warming alone. A study using 454 pyrosequencing of 16S rRNA genes indicated that warming with OTCs for three years alters soil bacterial communities at two locations in the maritime Antarctic and one in the cool southern temperate zone, with consistent increases across all three locations in *Alphaproteobacteria* to *Acidobacteria* ratios [15]. Given that increases in the abundances of *Proteobacteria* are associated with enhanced rates of C mineralisation [16], these higher *Alphaproteobacteria* to *Acidobacteria* ratios were posited to lead to enhanced C turnover in warmer Antarctic soils [15].

With the exception of two studies [15, 17], previous research into the effects of warming and nutrient application on soil bacteria in the maritime Antarctic has assessed changes to communities by measuring ELFA concentrations in soil [11–13]. Owing to the inability of ELFAs to distinguish between any microbial groups other than the Gram positive bacteria, Gram negative bacteria and fungi, it remains unclear from these previous studies precisely how nutrient amendment or warming influence the taxonomic composition of bacterial

communities in maritime Antarctic soils. Here, we therefore report a study that used Illumina sequencing of bacterial 16S rRNA genes, which provides a more precise assessment of changes to soil bacterial community composition than the use of ELFA markers, to determine the effects of TSB application and warming on the taxonomic composition of soil bacterial communities at the same experiment studied by Dennis et al. [13].

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Materials and Methods

Field experiment and sampling

The soil warming experiment was located at Mars Oasis (71° 52' 42" S, 68° 15' 00" W) on the south-eastern coast of Alexander Island in the southern maritime Antarctic (see [13] for map). The oasis consists of an upper and lower terrace, with the lower site, where the experiment was established, consisting of a level expanse of soil composed of till, fluvial and lacustrine sediments [18]. The soil has a mean pH (H₂O) value of 8.0, and mean total C and N concentrations of 0.30% and 0.02%, respectively [13]. The extensive, homogeneous expanse of soil on which the warming experiment was deployed enabled a high number of replicates of each treatment to be applied, reducing heterogeneity between replicate soils [*c.f.* 15]. Vegetation is absent from the soil on which the experiment was deployed, enabling the effects of treatments on microbial communities to be tested without the confounding influence of plants [*c.f.* 19]. Microarthropods are only present in soil close to pools or under rocks [20], and higher animals, including seals and nesting birds, are absent from the oasis. Access to Mars Oasis was by fixed-wing aircraft, fitted with skis, from Rothera Research Station on Adelaide Island.

100 In late November 2007, 64 plots of 1 m diameter were established in an area measuring 17 m × 17 m, with 32 of the plots being covered with fibreglass conical polycarbonate OTCs of 1 m diameter (see Fig. 1b in [21]). OTCs were used to effect increases in soil temperatures, recorded at *c.* 10–50 mm depth using TinyTag Plus 2 loggers (Gemini Data Loggers Ltd., Chichester, UK). The experiment was designed to test the effects of warming and its interactions with TSB, glycine, glucose and water application on soil microbial communities [13]. However, the analyses here are restricted to soils that received a factorial combination of warming and TSB. On 27 November 2007, 10 December 2009 and 21 December 2010, powdered TSB (Becton Dickinson, Franklin Lakes, NJ, USA) was mixed into soil in eight plots with sterile spoons to *c.* 50 mm depth, raising soil C and N concentrations to 2.3 mg g⁻¹ dwt soil and *c.* 0.22 mg g⁻¹ dwt soil, respectively [22]. Unamended soil, to which substrates were not added, was also mixed with sterile spoons to *c.* 50 mm depth, again in eight plots.

110 Twelve of the 28 soils for the present study were sampled on 26 November 2007, shortly before the commencement of the treatments, and 16 were sampled on 21 December 2011, after 49 months of treatment. Those collected in 2011 consisted of eight unamended soils, from four chambered and four unchambered plots, and eight TSB-amended soils, again from four chambered and four unchambered plots. Those from 2007 were a sub-set of soils from the same plots that were sampled in 2011, with three, rather than four, replicate plots per treatment. Sampling took place, prior to the application of substrates in both years, by filling clean 50 ml capacity plastic tubes with soil (depth *c.* 0–50 mm). The soils were kept at *c.* -3 °C for 24 h before being returned to Rothera Research Station, where moisture concentrations in sub-samples were determined gravimetrically (105 °C for 3 h) and the remaining soils were frozen at -20 °C, prior to transport to the UK and subsequent storage at the same temperature.

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DNA extraction, 16S rRNA gene amplification and sequencing

The 28 soil samples were thawed on ice and total DNA was extracted from 1.1 g (fwt) sub-samples under sterile conditions using a Power Soil DNA kit (Qiagen, Manchester, UK). The DNA extracts were eluted in 50 μ l of 10 mM TRIS-HCl (pH 8.5) and were then dried and subsequently rehydrated. The hypervariable regions V3 and V4 of 16S rRNA genes were PCR amplified using the primers 341F (5'- CCTACGGGNGGCWGCAG -3') and 805R (5'-WTTACCGCGGCTGCTGG-3') [23]. The resulting amplicons were purified and subjected to index PCR using a Nextera XT Index kit (Illumina, San Diego, CA, USA). The index-tagged amplicons were purified, normalized, pooled and sequenced using the Illumina MiSeq platform (2 \times 300 bp) (Illumina, Inc.) at the Graduate School of Public Health in Seoul National University.

Sequence processing

The 2,906,421 paired-end 16S rRNA gene sequences (mean length 452 bp) that were generated were merged using the PANDAseq assembler with default settings [24]. The merged sequences were further processed in mothur [25]. A set of unique sequences was generated by binning identical sequences, and was aligned against SILVA version 123 (<http://www.arb-silva.de/>). The aligned sequences were preclustered (2 bp difference) using a mothur implementation of the single-linkage preclustering algorithm [26]. Chimeric sequences were checked and removed using the Chimera Uchime algorithm in *de novo* mode [27]. The quality filtered bacterial 16S rRNA gene sequences were taxonomically classified against an EzTaxon-extended database [28] using the naïve Bayesian classifier (80% bootstrap cutoff with 1000 iterations) [29]. Sequences were clustered into operational taxonomic units (OTUs) at 3% dissimilarity using the opti-clust algorithm [30], with singleton OTUs being removed prior to subsequent analyses. The sequences were randomly sub-sampled (rarefied) to 16,870 sequences per sample to standardise sequencing depth across samples.

Phylogenetic community assembly

A maximum-likelihood tree was constructed with sequences of representative OTUs using the FastTree program [31]. The phylogenetic assembly within each community was calculated using the standardized effect size of mean nearest taxon distance (SES.MNTD) in the Picante R package (null model 'taxa.labels' with 999 randomizations) [32]. The β -nearest taxon index (β NTI) was also calculated in order to infer the relative influences of ecological processes governing the phylogenetic assembly of communities [33–35]. For this, we calculated between-community mean nearest taxon distance (β MNTD) in the Picante R package, which is the difference in standard deviation units between observed β MNTD and the mean of the null distribution of β MNTD, yielding a measure of the degree of phylogenetic similarity between closely related OTUs in two communities.

Statistical analyses

The effects of TSB application and OTCs on soil bacterial community composition were determined by Bray-Curtis dissimilarity matrices [36] calculated from square-root transformed OTU abundances in the PRIMER v6 software package [37]. General linear models (GLMs) in the MINITAB 17 package were used to test for main and interactive effects of OTCs and TSB application on (i) soil moisture concentration, (ii) the Shannon diversity index, (iii) SES.MNTD and β NTI, (iv) the ratio of Gram positive to Gram negative taxa, (v) the

relative abundances of individual phyla and genera and (vi) the ratios of total *Proteobacteria* to *Acidobacteria* and *Alphaproteobacteria* to *Acidobacteria*. Relative abundance data, which were expressed as percentages, were square root transformed prior to analyses. Analyses at the genus level were restricted to the 19 genera present at relative abundances of $\geq 0.5\%$. The relative abundances of Gram positive taxa were calculated by summing the abundances of the *Actinobacteria*, *Firmicutes* and *Saccharibacteria_TM7* [38], whilst those of Gram negative bacteria were calculated by summing the abundances of all other named phyla.

Statement of data availability The 16S rRNA amplicon sequences generated in this study have been deposited in the NCBI SRA under project accession number PRJNA492190. Environmental data are available from the corresponding author upon reasonable request.

Results

Soil temperatures and moisture concentrations

Mean monthly temperatures at 10–50 mm depth in unchambered soil at Mars Oasis ranged between 6.7 °C (December) and -16.9 °C (August), with the OTCs effecting mean temperature increases at this depth of 2.1–2.3 °C, relative to control plots, between November and January (Table 1). The OTCs predominantly affected late spring and early summer soil temperatures, with smaller increases (0.3–1.5 °C) being recorded in surface soil temperatures between February and October (Table 1). Absolute minimum and maximum temperatures recorded in unchambered soils were -33.7 °C and 20.3 °C, and those in chambered soils were -32.3 °C and 30.4 °C, respectively. Soil moisture concentration (mean 2.6%) was unaffected by OTCs in 2007 ($F_{1,8}=1.63$, $P>0.24$) or 2011 ($F_{1,20}=0.03$, $P>0.86$). TSB and its interaction with OTCs also did not influence soil moisture concentration in 2007 (both $F_{1,8}<1.11$, $P>0.32$) or 2011 (both $F_{1,20}<0.24$, $P>0.63$).

Soil bacterial community composition, diversity and phylogenetic assembly

Nonmetric multiple dimension scaling (NMDS) ordination based on Bray-Curtis dissimilarity indicated significant effects of TSB application on soil bacterial community composition, with the community composition of the eight TSB-amended soils sampled in 2011 showing significant divergence from the other 20 soils that were sampled (Fig. 1a). These analyses showed no apparent effect of OTCs on the composition of the soil bacterial community (Fig. 1a). GLMs indicated that there were no main or interactive effects of either OTCs or TSB amendment on the Shannon diversity index before the commencement of treatments in 2007 (all $F_{1,8}<2.60$, $P>0.145$). However, the same analyses indicated a highly significant main effect of TSB application on the Shannon index in 2011 ($F_{1,11}=20.77$, $P=0.001$), with TSB amendment resulting in a 22% reduction in the mean (\pm S.E.) value of the index, from 6.78 (\pm 0.20) to 5.31 (\pm 0.24) (Fig. 1b). Rarefaction curves similarly showed lower OTU richness in soils sampled in 2011 to which TSB had been applied (Online Resource, ESM Fig. 1). Significant effects of TSB application were also found on phylogenetic assembly processes: although there were no main or interactive effects of either treatment on SES.MNTD or β NTI in 2007 (all $F_{1,8}=3.63$, $P>0.05$), mean (\pm S.E.) values of SES.MNTD declined in 2011 from -13.08 \pm 0.97 in unamended soils to -20.33 \pm 0.99 in TSB-amended soil ($F_{1,11}=21.87$, $P=0.001$; Fig. 1c), and mean (\pm S.E.) values of β NTI fell from 1.70 \pm 0.57 in soils that did not receive TSB to -3.83 \pm 0.89 in amended soils ($F_{1,17}=17.54$, $P=0.001$; Fig. 1d). There

200 were no main effects of OTCs, or interactive effects of OTCs and TSB application, on the Shannon diversity index, SES.MNTD or β NTI in 2011 (all $F_{1,17} < 1.72$, $P > 0.20$; Fig. 1b–d).

Relative abundances of Gram positive and Gram negative bacteria

205 Gram positive bacterial taxa constituted the majority of OTUs recorded in soil in 2007 (mean relative abundance \pm S.E. of $70.70 \pm 3.71\%$), with 99% of Gram positive OTUs belonging to the *Actinobacteria*. There were no main or interactive effects of TSB application or OTCs on the ratio of Gram positive to Gram negative bacteria, either in 2007 ($F_{1,8} < 2.08$, $P > 0.187$) or in 2011 ($F_{1,12} < 0.84$, $P > 0.377$; Online Resource, ESM Fig. 2).

Relative abundances of bacterial phyla

210 There were no main or interactive effects of either treatment on the abundances of any bacterial phyla in 2007 (all $F_{1,8} < 2.76$, $P > 0.135$). In 2011, there was a main effect of TSB application on the abundance of one Gram positive phylum, the *Firmicutes*, with a mean (\pm S.E.) increase in the relative abundance of the phylum from $0.03 (\pm 0.01)\%$ in unamended soil to $12.82 (\pm 5.17)\%$ in TSB-amended soil ($F_{1,12} = 17.04$, $P = 0.001$; Fig. 2a).

215 One Gram negative phylum, the *Bacteroidetes*, also increased in abundance in TSB-amended soil in 2011, with an approximate tripling in its mean (\pm S.E.) abundance from $2.97 (\pm 0.43)\%$ to $9.97 (\pm 2.77)\%$ in TSB-amended soil ($F_{1,12} = 7.50$, $P = 0.018$; Fig. 2b). However, the abundances of the majority of Gram negative phyla declined in TSB-amended soils: TSB application led to significant reductions in the abundances of seven of these phyla, viz., the *Deltaproteobacteria*, *Chloroflexi*, *Acidobacteria*, *Cyanobacteria*, *Gemmatimonadetes*, *Planctomycetes* and *Verrucomicrobia*, with 87–96% reductions in their abundances in soil to which TSB had been applied, 220 relative to unamended soils (all $F_{1,20} = 9.42$ – 209.36 , $P = 0.010$ – < 0.001 ; Fig. 2c–i). There were no main or interactive effects of OTCs and TSB application on the abundances of any phyla in 2011 (Fig. 2a–i; all $F_{1,20} = 2.51$, $P > 0.139$).

Relative abundances of bacterial genera

225 No main or interactive effects of either treatment were recorded on the abundances of any bacterial genera in 2007 (all $F_{1,8} < 3.17$, $P > 0.11$). In contrast, in 2011, there were highly significant main effects of TSB application on the abundances of nine Gram positive genera (Fig. 3a–i). Those of *Arthrobacter*, *Paeniglutamicibacter* and *Planococcus* each increased from 0.003 – 0.025% in unamended soil to 6.15 – 17.49% in soil to which TSB had been applied (all $F_{1,12} > 11.43$, $P < 0.005$; Fig. 3a–c). In contrast, the abundances of the Gram positive genera 230 *Conexibacter*, *Gaiella*, *Ilumatobacter*, *Pseudonocardia*, *Rubrobacter* and *Modestobacter* each decreased by 93–98% in TSB-amended soil, relative to unamended soil (all $F_{1,12} > 22.68$, $P < 0.001$; Fig. 3d–i). The abundances of two Gram negative genera, *Pedobacter* and *Pedobacter_g3*, both increased from 0.01 – 0.07% in unamended soil to 2.85 – 2.86% in soil to which TSB had been applied (both $F_{1,12} > 11.05$, $P < 0.006$; Fig. 3j, k). In contrast, the abundances of four other Gram negative genera, viz., *Nostoc*, *Blastocatella*, *Flavisolibacter* and *Tepidisphaera*, 235 each decreased in soil by 87–98% in response to TSB application compared with unamended soil (all $F_{1,20} > 9.86$, $P < 0.009$; Fig. 3l–o). There were no main effects of OTCs, or interactive effects of OTCs and TSB application, on the abundances of any bacterial genera in 2011 (all $F_{1,12} < 2.31$, $P < 0.155$; Fig. 3a–o). One way ANOVA similarly indicated no differences between the relative abundances of *Arthrobacter*, *Paeniglutamicibacter*,

240 *Planococcus* or any other Gram positive genera in chambered, TSB-amended soil and unchambered, amended soil (all $F_{1,6} < 0.89$, $P > 0.381$; Fig. 3a–i).

Proteobacteria to Acidobacteria ratios

245 In 2007, there were no main or interactive effects of the two treatments on the ratios of total *Proteobacteria* to *Acidobacteria* or *Alphaproteobacteria* to *Acidobacteria* (all $F_{1,8} < 0.48$, $P > 0.51$). In 2011, TSB application led to a highly significant ($F_{1,12} = 16.72$, $P < 0.002$) mean increase (\pm S.E.) in the ratio of *Proteobacteria* to *Acidobacteria*, from 1.70 (\pm 0.20) in unamended soil to 114.30 (\pm 44.60) in soil to which the substrate had been applied (Online Resource, ESM Fig. 3), and a similarly highly significant ($F_{1,20} = 46.50$, $P < 0.001$) increase in the ratio of *Alphaproteobacteria* to *Acidobacteria*, from 1.24 (\pm 0.16) in unamended soil to 28.48 (\pm 6.14) in soil to which TSB has been applied (Online Resource, ESM Fig. 4). OTCs, or the interaction between OTCs and TSB application, did not influence the ratios of total *Proteobacteria* to *Acidobacteria* or *Alphaproteobacteria* to *Acidobacteria* in 2011 (both $F_{1,12} < 0.66$, $P > 0.432$; Online Resource, ESM Figs. 3, 4).

Discussion

255 The analyses here indicate substantial effects of nutrient amendment on bacterial community composition, diversity and phylogenetic assembly processes in a southern maritime Antarctic soil. In contrast, warming with OTCs, or the interaction between OTCs and substrate amendment, had no discernible influences on the community parameters measured here. These observations are not consistent with previous studies showing main effects of warming with OTCs, and interactive effects of warming with OTCs and substrate amendment, on maritime Antarctic soil bacterial communities [13, 15]. For example, in a study of soils from the same experiment as that sampled here, TSB application to chambered soil led to decreases after one year in the concentrations of ELFA markers for Gram positive bacteria such as *Actinobacteria*, relative to TSB-amended soil that had not been warmed, and consequently halved the ratio of Gram positive to Gram negative bacteria [13]. Here, in TSB-amended soil, we found no evidence of significant effects of OTCs on the ratio of Gram positive to Gram negative bacteria after four years, suggesting that the previously-reported influence of warming on this parameter [13] is transient in nature. The analyses here also failed to corroborate a previous study showing increases in the ratio of *Alphaproteobacteria* to *Acidobacteria* in two maritime Antarctic and one cool temperate zone soil that had been warmed with OTCs for three years [15]. It is possible that differences in soil water availability may explain the disparity between the two studies. Whilst there were no effects of the treatments applied here on soil moisture concentrations, it is plausible that the lower moisture concentrations in soils at Mars Oasis relative to those studied by Yergeau et al. [15] (see Table 3 in [39]) may have constrained microbial responses to warming [40]. Differences in soil chemistry might also explain the disparity between the studies. In the soils studied by Yergeau et al. [15], C and N concentrations were substantially higher (4–36% and 0.4–3.0%, respectively) than in soil at Mars Oasis, and pH values, which have a strong effect on the abundances of *Acidobacteria* in soil [41, 42] were also much lower (4.1–6.1) [39]. However, the *Alphaproteobacteria* to *Acidobacteria* ratio of 1.2 recorded here in unamended soil was the same as that in the soils studied by Yergeau et al. [15], and, in agreement with previous research [16], the ratios of *Proteobacteria* to *Acidobacteria* in soil at Mars Oasis were responsive to nutrient amendment, with one to two orders of magnitude increases in these parameters in response to TSB application. We hence cannot fully explain the absence of an effect of OTCs on

the *Alphaproteobacteria* to *Acidobacteria* ratio in the present study. Further research is therefore needed to
280 confirm, as suggested previously [15], that elevated *Alphaproteobacteria* to *Acidobacteria* ratios are consistent
features of warmed maritime Antarctic soils. Warming with OTCs in the current study also had no effect on the
relative abundance of *Cyanobacteria* in soil, or on that of *Nostoc*, a frequent genus in this phylum. These
observations suggest that the previously-reported changes to the morphology of Cyanobacterial cells, including
285 those of *Nostoc*, at the surfaces of warmed Antarctic soils arise from treatment-induced changes to the
morphology of cells [17], rather than alterations to soil microbial community composition.

The findings here indicate that OTCs, which increase mean monthly surface soil temperature at Mars
Oasis by up to 2.3 °C and, as previously reported from Antarctica [43], result in absolute maximum soil surface
temperatures rising to *c.* 30 °C, have no measurable effects on soil bacterial community composition, assembly
processes or diversity after four years of treatment. Whilst other studies have identified significant effects of
290 long-term warming on Low Arctic soils [44], our observations broadly support those from sub-Arctic soil
warming experiments showing no effects of 1 °C increases in mean soil temperature [45], applied with OTCs,
on the ratio of Gram positive to Gram negative bacteria or the phylogenetic composition of soil bacterial
communities [19, 46]. Recent data further support the view that the increases in soil temperature elicited by
OTCs may be insufficient to change soil microbial community composition, with transects through geothermal
295 habitats in Iceland showing that increases in soil temperature of *c.* 7–19 °C are necessary to force detectable
changes in soil bacterial community composition [47]. Similarly, previous studies along a latitudinal transect
between Mars Oasis and Signy Island in the South Orkney Islands (60 °S), at which mean annual temperatures
(MATs) are -11 °C and -4 °C, respectively, show MAT to be the best predictor for soil microbial alpha and beta
diversity, with significant increases in diversity in warmer habitats [9]. Along an even wider climatic gradient,
300 between the Ellsworth Mountains (MAT -25 °C) [48] in the continental Antarctic and the Falkland Islands
(MAT 7.5 °C) [49], increased soil bacterial diversity has been recorded in more northerly habitats [42], with
MAT having recently been identified as the main driver of this pattern in diversity [50].

Despite warming with OTCs failing to elicit a response in soil bacterial community composition and
diversity after 49 months, the application of TSB to soil at Mars Oasis led to significant divergence in bacterial
305 community structure from that in unamended soil, and significant reductions in community diversity. Although
it is possible that these responses may have been partly owing to the removal prior to diversity analyses of
singleton OTUs of rare taxa (some of which may have been oligotrophs), our observations corroborate previous
studies showing that altered bacterial community structure and lower diversity, which might affect functional
stability and resilience to perturbations [51], are consistent features of soils to which nutrients are applied. For
310 example, the annual application of 10 g m⁻² N and 5 g m⁻² P (as NH₄-NO₃ and P₂O₅, respectively) [52] to Low
Arctic soils for >20 years leads to declines in the Shannon index [53, 54]. However, the analyses reported here
also show that changes to phylogenetic community assembly processes occur in nutrient-amended soils, with
SES.MNTD declining from -13 in unamended soils to -20 in soils to which TSB had been applied. The more
negative values in amended soil indicate that the bacterial taxa were more closely related than expected under a
315 random model of community assembly, i.e., that they were phylogenetically more clustered [55], with the
clustering likely associated with environmental filtering imposed by nutrient application. Similarly, βNTI
declined from 1.7 in unamended soil to -3.8 in soil to which TSB had been applied. In unamended soil, the mean
proportion of pairwise βNTI comparisons fell within the null distribution ($|\beta\text{NTI}| < 2$), indicating that

320 phylogenetic community composition was attributable to stochastic assembly, with random ecological drift governing bacterial community dynamics. In contrast, in amended soil, the mean β NTI value of <-2 indicated significantly less than expected phylogenetic turnover, i.e., homogeneous selection [35], showing that nutrient addition imposed a strong homogeneous selective pressure on bacterial community assembly.

325 The analyses here indicated substantial increases in the relative abundances in TSB-amended soil of the Gram positive phylum *Firmicutes* and the Gram positive genera *Paeniglutamicibacter*, *Planococcus* and *Arthrobacter*, taxa previously shown to be frequent in soil at Mars Oasis [42, 56, 57]. In contrast, TSB application led to consistent decreases in the relative abundances of Gram negative *Bacteroidetes*, and seven other Gram negative phyla, including *Deltaproteobacteria*, in soil to which TSB had been added. These observations are strikingly different to those from experiments on Low Arctic soils, where the annual application of N and P increases the abundances of Gram negative phyla, typically members of the *Alpha-*, *Beta-* and *Gammaproteobacteria*, and decreases those of Gram positive *Actinobacteria* [53, 54]. At present, it is unclear why Low Arctic and southern maritime Antarctic soils should respond so differently to nutrient application. It is possible that differences in the concentrations and elemental compositions of the nutrients applied to the soils in the two regions account for these disparities. However, it is also plausible that differences in the environmental conditions between the two regions might account for the different responses of soil bacterial communities to nutrient additions. In the less extreme, vegetated soils of Alaska, in which temperatures fall to $-14\text{ }^{\circ}\text{C}$ at *c.* 100 mm depth during midwinter [58], it is possible that Gram negative bacterial taxa are able to take advantage of nutrient inputs. In contrast, in the harsher environment of soils at Mars Oasis, the midwinter temperatures of which approach $-34\text{ }^{\circ}\text{C}$ at 10–50 mm depth, Gram positive bacteria, which possess thick, peptidoglycan-rich cell walls, enabling their survival in extreme habitats, including high-altitude, hyperarid soils in the Chilean Andes and soils of the continental Antarctic McMurdo Dry Valleys [59–62], may have a competitive advantage over Gram negative taxa and might hence be responsive to nutrient inputs.

330 Studies in the sub- and Low Arctic have found lengthy response times to nutrient amendments, with yearly treatments, which lead to approximate increases of 0.4 mg C and $0.03\text{ mg N g}^{-1}\text{ soil}$, not eliciting responses in soil bacterial community composition until 15–24 years after nutrient applications begin [19, 53, 54]. Whilst it is possible that the rapid responses to nutrient amendments recorded here in bacterial community composition and diversity are caused by the 5–7 times higher increases in C and N concentrations in soil at Mars Oasis (2 mg C and *c.* $0.2\text{ mg N g}^{-1}\text{ dwt soil}$, respectively) [22], the findings here support the view that the decadal changes to soil microbial communities recorded in Arctic soils in response to nutrient amendment may indeed be secondary effects caused by gradual changes to plant biomass and community composition [19]. We hence advocate further studies in barren soils at high latitudes, where the effects of nutrient inputs from expanding plant populations are most likely to be amplified, to identify whether or not the same increases in soil C and N concentrations recorded in sub- and Low Arctic soils [19, 53, 54] elicit similar rapid changes to soil microbial communities.

355 **Conclusions**

Contrary to previous research [13, 15], the current study indicates no effects of increases of up to $2.3\text{ }^{\circ}\text{C}$ in mean monthly soil temperatures on the bacterial community composition of a maritime Antarctic soil. From the analyses here, it thus seems unlikely that further warming in the region, predicted to occur before the end of the

21st Century under moderate greenhouse gas emission scenarios [3, 4], will have primary effects on soil
360 bacterial community composition. However, we cannot discount the possibility that warming may have
secondary effects on soil bacterial communities of the region *via* its positive effects on plant growth [7, 8] and
subsequent increases in nutrient inputs to soils [10]. Such increases have the capacity to alter soil microbial
community composition, such as *Proteobacteria* to *Acidobacteria* ratios and the mineralisation of limiting
365 nutrients [16], which, coupled with increases in soil microbial biomass [12, 13], may ultimately lead to
increased productivity at the ecosystem level.

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References

1. Adams B et al. (2009) The Instrumental Period. In: Turner J, Bindschadler R, Convey P, di Prisco G,
380 Fahrbach E, Gutt J, Hodgson D, Mayewski P, Summerhayes C (eds.) Antarctic Climate Change and the
Environment. Scientific Committee on Antarctic Research, Scott Polar Research Institute, Cambridge,
UK, pp 183–298
2. Turner J, Lu H, White I, King JC, Phillips T, Hosking JS, Bracegirdle TJ, Marshall GJ, Mulvaney R,
Deb P (2016) Absence of 21st century warming on Antarctic Peninsula consistent with natural
variability. *Nature* 535:411–415
3. Bracegirdle TJ, Connolley WM, Turner J (2008) Antarctic climate change over the Twenty First
385 Century. *J Geophys Res* 113:D03103
4. Bracegirdle TJ, Stephenson DB (2012) Higher precision estimates of regional polar warming by
ensemble regression of climate model predictions. *Clim Dyn* 39:2805–2821
5. Vaughan DG, Marshall GJ, Connelley WM, Parkinson C, Mulvaney R, Hodgson DA, King JC, Pudsey
390 CJ, Turner J (2003) Recent rapid regional climate warming on the Antarctic Peninsula. *Climatic
Change* 60:243–274
6. Cook AJ, Fox AJ, Vaughan DG, Ferrigno JG (2005) Retreating glacier fronts on the Antarctic
Peninsula over the past half-century. *Science* 308:541–544
7. Fowbert JA, Smith, RIL (1994) Rapid population increases in native vascular plants in the Argentine
395 Islands, Antarctic Peninsula. *Arctic Alpine Res* 26:290–296
8. Royles J, Amesbury MJ, Convey P, Griffiths H, Hodgson DA, Leng MJ, Charman DJ (2013) Plants
and soil microbes respond to recent warming on the Antarctic Peninsula. *Curr Biol* 23:1702–1706

- 400 9. Newsham KK, Hopkins DW, Carvalhais LC, Fretwell PT, Rushton SP, O'Donnell AG, Dennis PG (2016) Relationship between soil fungal diversity and temperature in the maritime Antarctic. *Nat Clim Change* 6:182–186
10. Hill PW, Farrar J, Roberts P, Farrell M, Grant H, Newsham KK, Hopkins DW, Bardgett RD, Jones DL (2011) Vascular plant success in a warming Antarctic may be due to efficient nitrogen acquisition. *Nat Clim Change* 1:50–53
- 405 11. Hopkins DW, Sparrow AD, Shillam LL, English LC, Dennis PG, Novis P, Elberling B, Gregorich EG, Greenfield LG (2008) Enzymatic activities and microbial communities in an Antarctic dry valley soil: responses to C and N supplementation. *Soil Biol Biochem* 40:2130–2136
12. Dennis PG, Sparrow AD, Gregorich EG, Novis PM, Elberling B, Greenfield LG, Hopkins DW (2013) Microbial responses to carbon and nitrogen supplementation in an Antarctic dry valley soil. *Antarct Sci* 25:55–61
- 410 13. Dennis PG, Newsham KK, Rushton SP, Ord VJ, O'Donnell AG, Hopkins DW (2013) Warming constrains bacterial community responses to nutrient inputs in a southern, but not northern, maritime Antarctic soil. *Soil Biol Biochem* 57:248–255
14. Haack SD, Garchow H, Odelsen DA, Forney LJ, Klug MJ (1994) Accuracy, reproducibility, and interpretation of fatty acid methyl ester profiles of model bacterial communities. *Appl Environ Microbiol* 60:2483–2493
- 415 15. Yergeau E, Bokhorst S, Kang S, Zhou J, Greer CW, Aerts R, Kowalchuk GA (2012) Shifts in soil microorganisms in response to warming are consistent across a wide range of Antarctic environments. *ISME J* 6:692–702
16. Fierer N, Bradford MA, Jackson RB (2007) Toward an ecological classification of soil bacteria. *Ecology* 88:1354–1364
- 420 17. Wynn-Williams DD (1996) Response of pioneer soil microalgal colonists to environmental change in Antarctica. *Microb Ecol* 31:177–188
18. Sugden DE, Clapperton CM (1981) An ice-shelf moraine, George VI Sound, Antarctica. *Ann Glaciol* 2:135–141
- 425 19. Rinnan R, Michelsen A, Bååth E, Jonasson S (2007) Fifteen years of climate change manipulations alter soil microbial communities in a subarctic heath ecosystem. *Glob Change Biol* 13:28–39
20. Convey P, Smith RIL (1997) The terrestrial arthropod fauna and its habitats in northern Marguerite Bay and Alexander island, maritime Antarctic. *Antarct Sci* 9:12–26
- 430 21. Marion GM, Henry GHR, Freckman DW, Johnstone J, Jones G, Jones MH, Le Vesques E, Molau U, Mølgaard P, Parsons AN, Svoboda J, Virginia RA (1997) Open-top designs for manipulating field temperature in high-latitude ecosystems. *Glob Change Biol* 3:20–32
22. Benhua S, Dennis PG, Laudicina VA, Ord VJ, Rushton SP, O'Donnell AG, Newsham KK, Hopkins DW (2014) Biogeochemical responses to nutrient, moisture, and temperature manipulations of soil from Signy Island in the maritime Antarctic. *Antarct Sci* 26:513–520
- 435 23. Klindworth A, Pruesse E, Schweer T, Peplies J, Quast C, Horn M, Glöckner FO (2013) Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. *Nucleic Acids Res* 41:e1

24. Masella AP, Bartram AK, Truszkowski JM, Brown DG, Neufeld JD (2012) PANDAseq: paired-end assembler for illumina sequences. *BMC Bioinform* 13:31
- 440 25. Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, Lesniewski RI, Oakley BB, Parks DH, Robinson CJ, Sahl JW, Stres B, Thallinger GG, van Horn DJ, Weber CF (2009) Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Appl Environ Microbiol* 75:7537–7541
- 445 26. Huse SM, Welch DM, Morrison HG, Sogin ML (2010) Ironing out the wrinkles in the rare biosphere through improved OTU clustering. *Environ Microbiol* 12:1889–1898
27. Edgar RC, Haas BJ, Clemente JC, Quince C, Knight R (2011) UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics* 27:2194–2200
28. Kim OS, Cho YJ, Lee K, Yoon SH, Kim M, Na H, Park SC, Jeon YS, Lee JH, Yi H, Won S, Chun J (2012) Introducing EzTaxon-e: a prokaryotic 16S rRNA gene sequence database with phylotypes that represent uncultured species. *Internatl J Systematic Evolut Microbiol* 62:716–721
- 450 29. Wang Q, Garrity GM, Tiedje JM, Cole JR (2007) Naïve Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Appl Environ Microbiol* 73:5261–5267
30. Westcott SL, Schloss PD (2017) OptiClust, an improved method for assigning amplicon-based sequence data to operational taxonomic units. *mSphere* 2:e00073-17
- 455 31. Price MN, Dehal PS, Arkin AP (2010) FastTree 2-Approximately Maximum-Likelihood Trees for Large Alignments. *PLoS ONE* 5:e9490
32. Kembel SW, Cowan PD, Helmus MR, Cornwell WK, Morlon H, Ackerly DD, Blomberg SP, Webb CO (2010) Picante: R tools for integrating phylogenies and ecology. *Bioinformatics* 26:1463–1464
33. Stegen JC, Lin X, Konopka AE, Fredrickson JK (2012) Stochastic and deterministic assembly processes in subsurface microbial communities. *ISME J* 6:1653–1664
- 460 34. Stegen JC, Lin X, Fredrickson JK, Chen X, Kennedy DW, Murray CJ, Rockhold ML, Konopka A (2013) Quantifying community assembly processes and identifying features that impose them. *ISME J* 7:2069–2079
35. Dini-Andreote F, Stegen JC, van Elsas JD, Salles JF (2015) Disentangling mechanisms that mediate the balance between stochastic and deterministic processes in microbial succession. *Proc Natl Acad Sci USA* 112:E1326–E1332
- 465 36. Bray JR, Curtis JT (1957) An ordination of the upland forest communities of Southern Wisconsin. *Ecol Monogr* 27:325–349
37. Clarke KR, Gorley RN (2006) *PRIMERv6: User Manual/Tutorial*. PRIMER-E: Plymouth, UK
- 470 38. Winsley TJ, Snape I, McKinlay J, Stark J, van Dorst JM, Ji M, Ferrari BC, Siciliano SD (2014) The ecological controls on the prevalence of the candidate division TM7 in polar regions. *Front Microbiol* 5:345
39. Yergeau E, Bokhorst S, Huiskes AHL, Boschker HTS, Aerts R, Kowalchuk GA (2007) Size and structure of bacterial, fungal and nematode communities along an Antarctic environmental gradient. *FEMS Microbiol Ecol* 59:436–451
- 475

40. Christiansen CT, Haugwitz MS, Priemé A, Nielsen CS, Elberling B, Michelsen A, Grogan P, Blok D (2017) Enhanced summer warming reduces fungal decomposer diversity and litter mass loss more strongly in dry than in wet tundra. *Glob Change Biol* 23:406–420
41. Sait M, Davis KER, Janssen PH (2006) Effect of pH on isolation and distribution of members of Subdivision 1 of the phylum *Acidobacteria* occurring in soil. *Appl Environ Microbiol* 72:1852–1857
42. Yergeau E, Newsham KK, Pearce DA, Kowalchuk GA (2007) Patterns of bacterial diversity across a range of Antarctic terrestrial habitats. *Environ Microbiol* 9:2670–2682
43. Bokhorst S, Huiskes A, Convey P, Sinclair BJ, Lebouvier M, Van de Vijver B, Wall DH (2011) Microclimate impacts of passive warming methods in Antarctica: implications for climate change studies. *Polar Biol* 34:1421–1435
44. Deslippe JR, Hartmann M, Simard SW, Mohn WW (2012) Long-term warming alters the composition of Arctic soil microbial communities. *FEMS Microbiol Ecol* 82:303–315
45. Dorrepaal E, Toet S, Van Logtestijn RSP, Swart E, Van De Weg MJ, Callaghan TV, Aerts R (2009) Carbon respiration from subsurface peat accelerated by climate warming in the subarctic. *Nature* 460:616–619
46. Weedon JT, Kowalchuk GA, Aerts R, Freriks S, Röling WFM, van Bodegom PM (2017) Compositional stability of the bacterial community in a climate-sensitive sub-Arctic peatland. *Front Microbiol* 8:317
47. Radujković D, Verbruggen E, Sigurdsson BD, Leblans NIW, Janssens IA, Vicca S, Weedon JT (2018) Prolonged exposure does not increase soil microbial community compositional response to warming along geothermal gradients. *FEMS Microbiol Ecol* 94. doi: 10.1093/femsec/fix174
48. Bockheim JG, Schaefer CEGR (2015) Soils of Ellsworth Land, the Ellsworth Mountains. In: Bockheim JG (ed.) *The soils of Antarctica*. World Soils Book Series, Springer, Switzerland, pp 169–181
49. Bokhorst S, Huiskes A, Convey P, van Bodegom PM, Aerts R (2008) Climate change effects on soil arthropod communities from the Falkland Islands and the maritime Antarctic. *Soil Biol Biochem* 40:1547–1556
50. Dennis PG, Newsham KK, Rushton SP, O'Donnell AG, Hopkins DW (2019) Soil bacterial diversity is positively associated with air temperature in the maritime Antarctic. *Sci Rep* doi: 10.1038/s41598-019-39521-7
51. Chong CW, Silvaraj S, Supramanian Y, Snape I, Tan IKP (2018) Effect of temperature on bacterial community in petroleum hydrocarbon-contaminated and uncontaminated Antarctic soil. *Polar Biol* 41:1763–1775
52. Mack MC, Schuur EAG, Bret-Harte MS, Shaver GR, Chapin FS III (2004) Ecosystem carbon storage in Arctic tundra reduced by long-term nutrient fertilization. *Nature* 431:440–443
53. Campbell BJ, Polson SW, Hanson TE, Mack MC, Schuur EAG (2010) The effect of nutrient deposition on bacterial communities in Arctic tundra soil. *Environ Microbiol* 12:1842–1854
54. Koyama A, Wallenstein MD, Simpson RT, Moore JC (2014) Soil bacterial community composition altered by increased nutrient availability in Arctic tundra soils. *Front Microbiol* 5:516
55. Webb CO, Ackerly DD, McPeck MA, Donoghue MJ (2002) Phylogenies and community ecology. *Ann Rev Ecol Syst* 33:475–505

56. Newsham KK, Pearce DA, Bridge PD (2010) Minimal influence of water and nutrient content on the bacterial community composition of a maritime Antarctic soil. *Microbiol Res* 165:523–530
57. Chong CW, Convey P, Pearce DA, Tan IKP (2012) Assessment of soil bacterial communities on Alexander Island (in the maritime and continental Antarctic transitional zone). *Polar Biol* 35:387–399
- 520 58. Shaver G (2005) Daily summary of 10 cm soil temperatures in the Arctic LTER moist acidic experimental plots from 1998 to present, Toolik Lake Field Station, Alaska. Environmental Data Initiative. <http://dx.doi.org/10.6073/pasta/89b6208bc6631129949eeca791063ed3>. Accessed 12 September 2018
- 525 59. Smith JJ, Tow LA, Stafford W, Cary C, Cowan DA (2006) Bacterial diversity in three different Antarctic cold desert mineral soils. *Microb Ecol* 51:413–421
60. Babalola O, Kirby BM, Le Roes-Hill M, Cook AE, Cary SC, Burton SG, Cowan DA (2009) Phylogenetic analysis of actinobacterial populations associated with Antarctic Dry Valley mineral soils. *Environ Microbiol* 11:566–576
- 530 61. Costello EK, Halloy SRP, Reed SC, Sowell P, Schmidt SK (2009) Fumarole-supported islands of biodiversity within a hyperarid, high-elevation landscape on Socompa Volcano, Puna de Atacama, Andes. *Appl Environ Microbiol* 75:735–747
62. Solon AJ, Vimercati L, Darcy JL, Arán P, Porazinska D, Dorador C, Farías ME, Schmidt SK (2018) Microbial communities of high-elevation fumaroles, Penitentes, and dry tephra “soils” of the Puna de Atacama volcanic zone. *Microb Ecol* 76:340–351
- 535

Table 1 Mean monthly soil temperatures at 10–50 mm depth in unchambered plots at Mars Oasis and mean monthly increases in soil surface temperatures effected by open top chambers. Data were recorded from December 2007–December 2008 and December 2009–November 2011. Values are means of three replicates.

Month	Mean monthly soil surface temperature (° C)	Mean monthly increase in soil surface temperature (° C)
Jan	6.09	2.20
Feb	1.64	1.31
Mar	-4.93	0.72
Apr	-12.78	0.32
May	-14.04	1.01
Jun	-12.93	0.26
Jul	-16.54	0.63
Aug	-16.89	1.06
Sep	-14.53	1.04
Oct	-9.08	1.47
Nov	-2.25	2.32
Dec	6.67	2.10

545 **Figure legends**

Fig. 1 (a) NMDS ordination based on Bray-Curtis dissimilarities of bacterial communities in Mars Oasis soils receiving a factorial combination of tryptic soy broth (TSB) and warming, applied with open top chambers (OTCs), (b) Shannon index of bacterial community diversity, (c) standardized effect size of mean nearest taxon distance (SES.MNTD) and (d) β -nearest taxon index (β NTI) in TSB-amended and warmed soils sampled from Mars Oasis. Note that data in (a) are shown for 2007, prior to treatments being applied to soils, and 2011. Those in (b)–(d) are for 2011 only. Values in (b)–(d) are means of four replicates \pm SEM. Main and interactive effects of TSB and OTCs are shown in each pane. *Abbreviation*: n.s., not significant.

550 **Fig. 2** Relative abundances of nine bacterial phyla in soil at Mars Oasis in 2011 that had received a factorial combination of TSB and warming (with OTCs). Values are means of four replicates \pm SEM. Abbreviations and notation as in Fig. 1. Note that y-axes are not identically scaled.

560 **Fig. 3** Relative abundances of 15 bacterial genera in soil at Mars Oasis in 2011 that had received a factorial combination of TSB and warming (with OTCs). Values are means of four replicates \pm SEM. Abbreviations and notation as in Fig. 1. Note that y-axes are not identically scaled.