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RESEARCH PAPER

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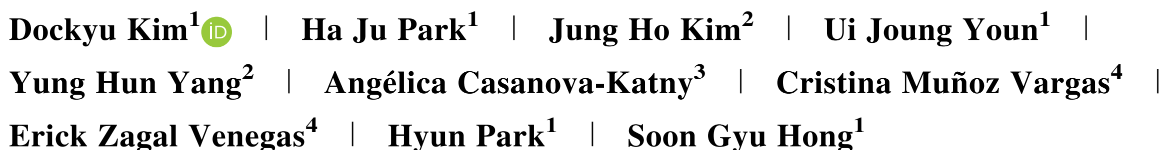
Passive warming effect on soil microbial community and humic substance degradation in maritime Antarctic region



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Although the maritime Antarctic has undergone rapid warming, the effects on indigenous soil-inhabiting microorganisms are not well known. Passive warming experiments using open-top chamber (OTC) have been performed on the Fildes Peninsula in the maritime Antarctic since 2008. When the soil temperature was measured at a depth of 2–5 cm during the 2013–2015 summer seasons, the mean temperature inside OTC (OTC-In) increased by approximately 0.8 °C compared with outside OTC (OTC-Out), while soil chemical and physical characteristics did not change. Soils (2015 summer) from OTC-In and OTC-Out were subjected to analysis for change in microbial community and degradation rate of humic substances (HS, the largest pool of recalcitrant organic carbon in soil). Archaeal and bacterial communities in OTC-In were minimally affected by warming compared with those in OTC-Out, with archaeal methanogenic *Thermoplasmata* slightly increased in abundance. The abundance of heterotrophic fungi *Ascomycota* was significantly altered in OTC-In. Total bacterial and fungal biomass in OTC-In increased by 20% compared to OTC-Out, indicating that this may be due to increased microbial degradation activity for soil organic matter (SOM) including HS, which would result in the release of more low-molecular-weight growth substrates from SOM. Despite the effects of warming on the microbial community over the 8-years-experiments warming did not induce any detectable change in content or structure of polymeric HS. These results suggest that increased temperature may have significant and direct effects on soil microbial communities inhabiting maritime Antarctic and that soil microbes would subsequently provide more available carbon sources for other indigenous microbes.

KEYWORDS
Antarctic soil, degradation, humic substances, microbial community

Abbreviations: EC, electrical conductivity; FA, fulvic acids; HA, humic acids; HS, humic substances; HSQC, heteronuclear single quantum coherence; NMR, nuclear magnetic resonance; OTC, open-top chamber; OTC-In, inside OTC; OTC-Out, outside OTC; OTU, operational taxonomic unit; PLFA, phospholipid-derived fatty acids; SOM, soil organic matter; VWC, volumetric water content.

Dockyu Kim and Ha Ju Park contributed equally to this work.

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1 | INTRODUCTION

The IPCC Fourth Assessment Report models project temperature increases at a rate of 0.34 °C per decade (to 2100) over Antarctica land and grounded ice sheets [1]. Over the past several decades, the maritime Antarctic has undergone rapid warming related to global climate change, and the rising temperature in the region has subsequently led to various changes in environment, such as ice shelf collapse and glacial retreat, as well as biological responses such as enhanced plant populations and growth rates [2] and increased microbial activity. Although the microbes (both eukaryotic fungi and prokaryotic bacteria and archaea) play a critical role as decomposers in soil nutrient cycling for cold ecosystems, little is known about the response of soil microbial communities, such as changes in microbial diversity and functional roles, to the current rapid warming in maritime Antarctic terrestrial ecosystems [3,4].

The effects of warming on soil microbial communities are suggested to be direct and diverse through several lines of experimental evidence [5–7]. For example, fell-field soils from Antarctic Peninsula Signy Island, in which bacteria and fungi were responsible for 81–89% of soil heterotrophic respiration [8], were incubated at different temperatures. When their colony-forming unit values measured, the values increased with increasing temperatures (from 4 to 20 °C), with the effect of increased temperature more significant for bacteria than for fungi [5]. In microcosm experiments with subarctic soil rich in humic substances (HS), such as tundra soil from Alaska, humic acids (HA) content significantly decreased after a 99-day incubation at 5 °C. The relative abundance of bacterial phylum *Proteobacteria* greatly increased, indicating involvement in the degradation of HS; in parallel, the rate of HS degradation for pseudomonad isolates (class *Gammaproteobacteria*) increased with rising temperatures in a range of 0–20 °C [7]. These results suggest that the effect of temperature may be even more significant for bacteria than for fungi in cold soil environments.

Fungal communities can respond differently to changes in soil depth and nutrient condition [9,10]. Previous studies have proposed that in maritime Antarctic soil, warming facilitates the colonization of soil by fungi which play a vital ecological role in decomposition [4]. Warming may therefore be a main driver for the changes in size and structure of fungal community, possibly affecting the degradation of soil organic matter (SOM) across a range of terrestrial and maritime ecosystems in Antarctic Peninsula [5].

Composed of primarily HA and fulvic acids (FA), HS are important natural organic compounds found throughout the environment, including in the maritime Antarctic, and they constitute 60–80% of the total SOM. HS are formed by spontaneous condensation of biomolecules originating from the decay of plants and other organisms [11], and are extremely resistant to biodegradation due to their structural complexity. It is believed that long-term low levels of microbial degradative activities contribute to a large amount of HS stored in the maritime Antarctic region [7]. Since the temperature rising would increase the microbial activities, microbial degradation of HS could be a primary mechanism through which low-molecular-weight organic compounds are supplied to around environment.

Global warming in the maritime Antarctic could lead to changes in various environmental factors such as vegetation diversity and density and soil biochemical and biophysical characteristics [5]. Thus, we hypothesize that passive warming using open-top chambers (OTCs) would result in different effects on the soil microbial communities of fungi, bacteria and archaea between the inner- and outer-side soils of OTCs. These changes in structure and activity of the microbial community would then lead to changes in degradation rate for SOM, including HS, and subsequently changes in the surrounding ecosystem, including vegetation and soil properties. Our experimental investigations using OTCs would provide researchers with information on microbial community responses to global warming in the maritime Antarctic region.

2 | MATERIALS AND METHODS

2.1 | Description for study site

The study was carried out on the Fildes Peninsula (King George Island in the South Shetland Archipelago; Fig. 1), an ice-free maritime region of the western Antarctic Peninsula that has warmed nearly 3 °C in the past 50 years (+0.56 °C per decade from 1951 to 2000), a rate more than three times the global mean [12]. The climate of the Fildes Peninsula is mild by Antarctic standards, with a maritime climate in the summer and polar climate in the winter. Mean air temperatures were 0.6–1.5 °C in summer (December–February) and −6.5 °C in winter (July–August) during 1970–2004. The Fildes Peninsula is overcast more than 70% of the time in any month. Summer rain is common, with a monthly mean of January–February of 40–70 mm (2007, unpublished meteorological data). These particular climate conditions permit the growth of a diverse range of cryptogam species. The local flora covers large areas with well-developed lichen and moss communities (frequently *Sanionia* spp.) and the hair grass *Deschampsia antarctica*, one of the two vascular plants which has colonized the Antarctic region.

2.2 | OTC setup and microclimate measurements

In 2008, ten OTCs were installed in an approximately 200 m^2 area on Juan Carlos Point (62°12′ S 58°59′ W; 37 m above sea level) on the Fildes Peninsula. This experimental site has a

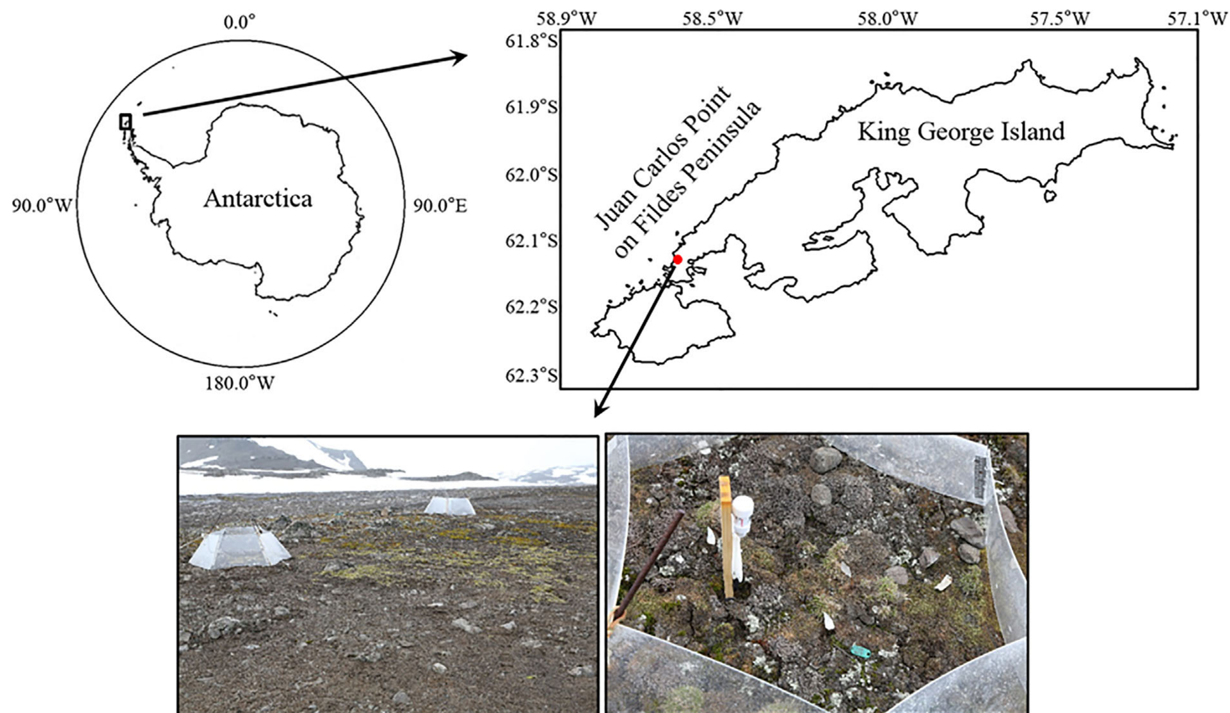

FIGURE 1 Location and photos of open-top chamber (OTC) passive warming experimental sites on the Fildes Peninsula in King George Island, the Antarctic Peninsula

moss-grass community dominated by *D. antarctica* and mosses (*Sanionia georgico-uncinata* and *Polytrichastrum alpinum*) [13]. The chambers are designed to increase the air temperature by preventing the loss of heat by convection. They are of hexagonal outline and assembled of 3 mm thick, transparent acrylic panels of 40 cm tall, which taper at the top and have a 106.4 cm diameter base. Each panel has five holes of 1 cm in diameter to allow for improved airflow and avoid excessive warming. The OTCs were previously tested for 2 years for resistance to Antarctic climatic conditions and are similar in design to others used in Signy and Anchorage Islands, Antarctica [14]. Each corresponding control was demarcated region of the same size and shape as the OTC footprint within ~3.0 m of each OTC. Soil temperature and volumetric water content (VWC) were measured within the inner-side of the OTC (designated OTC-In) and the control site (designated OTC-Out) using an EM-50 ECH2O logger (DECAGON Devices, USA) with an EC-5 sensor, which was installed at a soil depth of 2–5 cm. Measurement was performed during summer seasons from 2013 to 2015. Statistical analysis was carried out with the software Infostat/E. Soil microclimatic variables (temperature and VWC) were analyzed using ANOVA.

2.3 | Soil sampling and analysis

For soil chemical and physical characterization, three OTCs were randomly selected, from which OTC-In and OTC-Out soils were collected at a depth of 2–5 cm during the 2015 summer season and frozen until analysis. Six independent soil samples were used for each analysis. The samples were thawed at ambient temperature, dried in air, and passed through a 2-mm sieve. Soil pH was measured in a soil solution (1:1 soil:water) by the potentiometric method. Electrical conductivity (EC) was measured with a conductivity meter in a saturated paste of soil and water. Total carbon and nitrogen content were measured by the dry combustion method using a CN elemental analyzer (LECO TruSpec CN model 630-100-100, USA). Texture analysis was carried out using the Bouyoucos hydrometer method.

For pyrosequencing and HS content analyses, three different OTCs were randomly selected. From each site, approximately 40 g of OTC-In and OTC-Out soils were collected from the top layer (0–5 cm) in January 2015. The frozen soils were slowly thawed at ambient temperature. Six independent soil samples were used for pyrosequencing and HS content analyses. Soils were pooled to obtain two representative samples (each ~40 g of OTC-In and OTC-Out), which were subjected to HS structure and phospholipid-derived fatty acids (PLFA) analyses.

2.4 | Pyrosequencing and microbial community analysis

DNA was extracted from each OTC-In and OTC-Out soil using a soil DNA extraction kit (MO Bio, USA) according to the

manufacturer's instructions, followed by PCR amplification using the following forward and reverse primers: bacteria (V1–V3 region of 16S rRNA gene), 5′-GAGTTTGATCMTGGCTCAG-3′ and 5′-WTTACCGCGGCTGCTGG-3′; archaea (V4–V5 region of 16S rRNA gene), 5′-CAGCCGCCGCGGTAA-3′ and 5′-YCCGGCGTTGAMTCCAATT-3′; fungi (ITS2 region between 5.8S and 28S rRNA genes), 5′-GCATCGATGAAGAACGCAGC-3′ and 5′-TCCTCCGCTTATTGATATGC-3′. The PCR conditions were as follows: initial denaturation at 95 °C for 5 min, followed by 30 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 30 s, and final elongation at 72 °C for 7 min. The amplified products were purified using resin columns and subjected to pyrosequencing by Chunlab Inc. (Korea) with a 454 GS FLX Titanium Sequencing System (Roche, USA).

Original sequencing reads were separated by the unique barcodes, from which the barcode, linker, and PCR primer sequences were removed. Individual collections of sequences were depleted of non-16S rRNA sequences and chimeras using HMMER 3.0 and BLAST. The resulting trimmed sequences were assigned to taxonomic categories via alignment with the EzTaxon-e or EzFungi databases. For calculation of alpha diversity measures, sequences were clustered and assigned to operational taxonomic units (OTUs) using the CD-HIT algorithm. The OTUs were input to the MOTHUR software platform to generate diversity indices. Taxonomic categories were used to calculate the relative abundance (expressed as a percentage) at the various taxonomic levels in the samples.

2.5 | Determination of microbial biomass changes using PLFA analysis

For total lipid extraction, a mixture of 10 ml chloroform and 20 ml methanol was added to 40 g of frozen soil. An additional 10 ml chloroform was then added and blended for 30 s. The suspension was shaken at 200 rpm and 20 °C for 16 h. After shaking, 10 ml distilled water was added to the suspension, which was centrifuged at 4000 rpm for 10 min to separate chloroform layer. A bottom layer containing chloroform was fully evaporated using a nitrogen evaporator. Resulting total lipids were dissolved in 1 ml chloroform and separated into neutral lipids, glycolipids, and phospholipids on a silicic acid column as previously described [15]. Phospholipids were derivatized by mild alkaline methanolysis method [16] and PLFA were analyzed with a Perkin-Elmer gas chromatograph–mass spectrometer (Clarus 680-SQ 8) equipped with an Elite-5MS capillary column (30 m × 0.25 mm × 0.25 μm, 5% diphenyl, and 95% dimethyl polysiloxane). The peak area of each PLFA was identified and quantified relative to internal standard, heneicosylic acid (C21:0), using MIDI peak identification software (Sherlock Microbial Identification System, MIDI Inc., USA).

Standard fatty acid nomenclature [17] was used (total number of carbon atoms: number of double bonds and their location after "ω" in the fatty acid molecule). The prefixes Me-, cy-, i-, and a- refer to methyl group, cyclopropane group, and iso- and anteiso-branched fatty acids, respectively. The sum of the following PLFAs was used as a measure of each microbial biomass: gram-positive bacteria (i-C15:0, a-C15:0, i-C16:0, i-C17:0, a-C17:0), gram-negative bacteria (cy-C17:0, cy-C19:0, C16:1ω7), actinomycetes (10Me-C16:0, 10Me-C17:0), and fungi (C18:1ω9, C18:2ω6, C18:3ω6).

2.6 | Content and structural analysis of HS components

For HS extraction, top-layer soils (0–5 cm) from OTC-Out and OTC-In were completely dried at 45 °C and passed through a testing sieve to remove coarse moss, lichen debris, and small stones. A 0.5 N NaOH solution was added to the dried soil (25.0 ml per 1.0 g soil), incubated for 3 h with continuous shaking, and then further incubated at 4 °C for 12 h. The NaOH extract was separated from the soil by centrifugation at 5500×*g* for 15 min and acidified to pH 2.0 with 5.0 N HCl. Insoluble HA were separated from the soluble fraction containing FA by centrifugation (4000×*g* for 7 min). The HA pellet and FA solution were lyophilized, and the resulting solid HA and FA were weighed.

The HA pellet and FA solution, both prepared before lyophilization, were used to detect structural changes in HS extracted from OTC-In and OTC-Out soils. HA dissolved in 0.1N NaOH or FA solution was loaded on solid phase extraction tubes containing reversed phase packings (Supelclean ENVI-18 SPE Tube, Sigma–Aldrich Co.) which was conditioned with 2 ml methanol followed by 2 ml of 0.25% formic acid. After washing twice with 10 ml pure water, HA or FA were eluted with methanol and evaporated in a vacuum. The purified HA or FA were solubilized in CD_3OD, evaporated again, and dissolved again (19–24 mg) in 700 μl 0.1N NaOD, and then subjected to nuclear magnetic resonance (NMR) analysis. Gel permeation chromatography and Fourier transform infrared spectra were also obtained to detect changes in molecular mass distribution and structure in HA and FA, respectively [18].

3 | RESULTS

3.1 | Microclimatic data and soil characteristics

In passive warming experiments on Juan Carlos Point (2008–2015), mean daily soil temperatures during the 2013–2015 summer seasons measured 2–5 cm below mosses significantly increased in OTC-In compared to OTC-Out. The

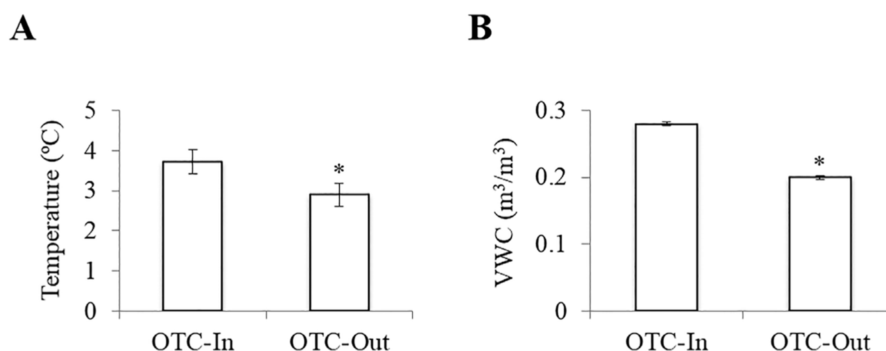

FIGURE 2 Microclimatic characteristics of soils during the passive warming treatment. Values are mean ($n = 206$) for (A) soil temperature and (B) volumetric water content (VWC) during the summer period of 2013–2015. OTC-In, inside OTC; OTC-Out, outside OTC. *Indicates significant differences between the treatments, based on ANOVA analysis

mean soil temperature in OTC-In vs. OTC-Out was 3.72 ± 0.29 °C vs. 2.90 ± 0.29 °C ($F_{1206} = 4.46$, $p \leq 0.0371$) (Fig. 2A). Changes in VWC (m^3/m^3) were also recorded, with OTC-In values increasing significantly. The mean VWC in OTC-In vs. OTC-Out was 0.28 ± 0.003 vs. 0.20 ± 0.003 ($F_{1206} = 667.1$, $p \leq 0.001$) (Fig. 2B).

The soil chemical and physical data were analyzed by Wilcoxon Rank Sum test and any significant differences in the soil characteristic results were not detected between OTC-In and OTC-Out (p-value = 0.09–0.93). Sand and lime were the principal components of both soils, fluctuating between 70–72% sand and 24–25% lime. Low values for total organic carbon (~0.98%) and total nitrogen (~0.09%) were present in both soils with a C/N ratio value of approximately 10.9, and a mean soil pH of 6.52 (Table 1).

3.2 | Phylogenetic analysis for microbial compositional change

Information on microbial diversity and community composition is of great importance for prediction of response to environmental changes (in this case, temperature-associated HS degradation rate). Pyrosequencing of microbial small-subunit ribosomal RNA genes was used to determine changes in microbial communities during the 8-year *in situ* experiments using an OTC passive warming device. Archaeal, fungal, and bacterial sequences in OTC-Out samples were clustered into 34 (±10), 463 (±74), and 1783 (±276) OTUs, respectively, while those in OTC-In samples were 28 (±13), 401 (±25), and 1917 (±399) OTUs. By statistical analysis based on OTU richness (Chao1 and ACE indices) and diversity (Shannon index), archaeal, fungal, and bacterial communities in the OTC-In samples were generally similar to those in the OTC-Out samples (Wilcoxon Rank Sum test, p-value = 0.4–1.0; Table 2).

The changes in relative abundances of bacterial and fungal communities were analyzed by ANOSIM significance test (p-value = 0.9 for bacteria and p-value = 0.4 for fungi). The archaeal community was not analyzed owing to relatively low numbers of phylum. As shown above, significant changes in relative abundances in bacterial and archaeal communities (predominantly phylums *Actinobacteria* and *Thaumarchaeota*, respectively) were not detected between samples. However, among the archaeal phyla, *Euryarchaeota* in OTC-In samples was slightly increased (from 0.9% to 4.8%) compared to OTC-Out samples (Fig. 3A and B). Within *Euryarchaeota*, the largely acidophilic and methanogenic class *Thermoplasmata* [19] increased by 3.8% (from 0.8% in OTC-Out samples to 4.6% in OTC-In samples). Notably, we observed an obvious increase in the relative abundance of dominant fungal phylum *Ascomycota* in these soil communities (from 65% in OTC-Out to 84% in OTC-In; Fig. 3C). At the genus level within *Ascomycota*, the abundances of Verrucariaceae_uc, Ascomycota_uc_g, and Dothideomycetes_uc_g changed most significantly during the experiment period (Fig. 3D).

TABLE 1 Soil chemical and physical characteristics inside the OTCs (OTC-In) and in control plots (OTC-Out)

	Chemical and physical characteristics of soil							
Sample	**EC (μs m^{-1})**	**pH**	**% N**	**% C**	**C/N**	**% sand**	**% lime**	**% clay**
OTC-Out	59.6 (±16.8)	6.5 (±0.2)	0.094 (±0.021)	0.968 (±0.218)	10.3 (±0.4)	71.7 (±2.6)	23.7 (±2.1)	4.5 (±0.5)
OTC-In	64.4 (±23.7)	6.6 (±0.3)	0.092 (±0.018)	0.994 (±0.171)	10.8 (±0.7)	69.6 (±3.1)	25.2 (±2.6)	5.2 (±0.5)

The statistical data are the mean values of three independent analyses for each OTC-Out and OTC-In soil.

TABLE 2 Summary of pyrosequencing results and statistical analyses of microbial communities in soil samples from open-top chamber (OTC) passive warming experimental sites

Target microorganism	Number of reads	OTU richness			OTU diversity (Shannon)
		Observed	Chao1	ACE	
Bacteria					
OTC-Out	8064 ± 1030	1783 ± 276	3016 ± 422	4007 ± 494	6.49 ± 0.08
OTC-In	6888 ± 364	1917 ± 399	3301 ± 1022	4425 ± 1632	6.74 ± 0.24
Archaea					
OTC-Out	5919 ± 3883	34 ± 10	39 ± 11	44 ± 14	1.48 ± 0.48
OTC-In	6167 ± 4824	28 ± 13	31 ± 13	31 ± 13	1.42 ± 0.68
Fungi					
OTC-Out	8826 ± 2049	463 ± 74	576 ± 69	581 ± 69	4.39 ± 0.70
OTC-In	9987 ± 1006	401 ± 25	524 ± 49	505 ± 40	3.98 ± 0.05

The statistical data are the mean values of three independent analyses for each OTC-Out and OTC-In soil.

3.3 | Determination of microbial biomass

To detect the response of the soil microbial community to rising temperatures within the OTCs, total microbial biomass was estimated using the PLFA analytic method (Table 3). In the OTC-Out soil, fungal and bacterial PLFA content was determined to be 6.8 and 5.8 mg kg^{-1} soil (total 12.6 mg kg^{-1}), while fungal and bacterial PLFA content of OTC-In soil was 8.0 and 7.5 mg kg^{-1} soil (total 15.5 mg kg^{-1}). The ratio of fungal:bacterial biomass was

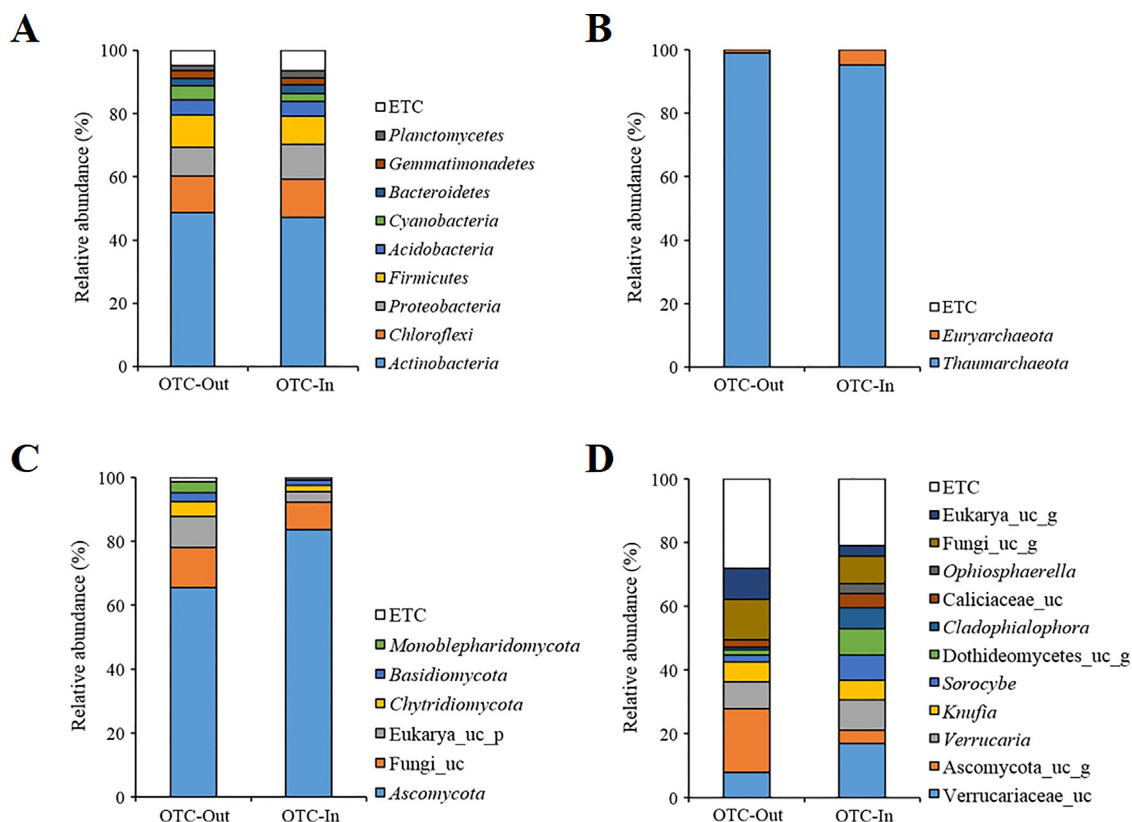

FIGURE 3 Changes in abundance and taxonomic composition of soil microbes induced by the OTC warming experiment. Relative abundances of (A) bacterial phyla, (B) archaeal phyla, (C) fungal phyla, and (D) different genera within the fungal phylum *Ascomycota* are shown. ETC denotes the sum of minor taxa (<1.0% in relative abundance). Relative abundances are the mean values of three independent analyses for each OTC-Out and OTC-In soil

TABLE 3 Quantity of fungal and bacterial phospholipid-derived fatty acids (PLFAs) in soil samples from open-top chamber (OTC) passive warming experimental sites

	Amount of phospholipid fatty acids (mg kg^{-1} soil)	
	OTC-Out	**OTC-In**
Fungi	6.8	8.0
Bacteria	5.8	7.5
Gram+	3.2	4.4
Gram−	1.8	2.0
Actinomycete	0.8	1.1
Total	12.6	15.5
Ratio of fungal/bacterial biomass	1.17	1.07

1.17 in OTC-Out soil and decreased to 1.07 in OTC-In soil (measured as one of fungal PLFA to bacterial PLFA, including Gram-positive bacteria, Gram-negative bacteria, and actinomycetes).

3.4 | Analysis of HA and FA content

From NaOH-soluble HS extracts, the amount of HA and FA fractions were determined to be 4.9 (±0.5) mg and 651.0 (±18.0) mg per 1 g OTC-Out soil, while HA and FA per gram of OTC-In soil were 6.8 (±1.8) mg and 665.0 (±3.6) mg. The HS extracted from OTC-Out was approximately 66% of the dried soil sample, and approximately 67% of OTC-In dried soil samples, demonstrating that total HS content did not differ between the inner and outer sides of OTCs during the 8-year passive warming experiment. Additionally, these data confirm that HS are the largest constituent of SOM and that FA content is much greater than HA content in the surface layer (approximately 65.8% vs. 0.6%). This difference in HS content was observed in previous studies of other soil types, such as subarctic Alaska tundra soil (36% FA and 46% HA, [7]) and Korean forest soil (64% FA and 11% HA, unpublished data in 2014).

3.5 | Analysis of HA and FA structure

^{1}H NMR spectra of HAs extracted from OTC-Out and OTC-In samples were nearly identical, displaying broad signals for aromatic groups at δ_H 6.59–8.51, oxygenated methine and methylene groups at δ_H 3.07–4.47, and aliphatic sp^3 group protons at δ_H 0.91–2.61 due to its high molecular weight (Fig. 4A and B). A heteronuclear single quantum coherence (HSQC) spectroscopy experiment was performed to obtain detailed information of ^{1}H and ^{13}C NMR signals, which was also nearly identical. The HSQC spectra showed a correlation of aliphatic sp^3 protons with carbon signals at δ_C 10–40, while no HSQC correlations were observed for the oxygen-bearing alkyl and aromatic regions (data not shown).

The FAs from OTC-Out and OTC-In samples were comparatively analyzed through the ^{1}H NMR and HSQC experiments, but as in the HAs, no significant structural changes were detected. ^{1}H NMR spectra of FAs showed similar patterns (Fig. 4C and D). When compared to HAs, the HSQC spectra of FAs revealed more clear correlations for aromatic protons at δ_H 6.52–7.27 with carbon signals at δ_C 119–129, oxygenated methine and methylene protons at δ_H 3.07–4.45 with carbon signals at δ_C 51–77, and the remaining aliphatic signals at δ_H 0.98–2.70 with carbon signals at δ_C 10–40 (data not shown).

As a result, 1D and 2D NMR patterns of HAs and FAs from OTC-Out samples were almost identical to those from OTC-In samples. However, structural differences between HAs and FAs were observed regardless of soil source, with functional groups such as −OH, −COOH, $-OCH_3$, and −NH much more clearly observed in the ^{1}H and HSQC NMR spectra of FA than those of HA, a result that may be expected since the molecular weight of FA is lower than that of HA.

4 | DISCUSSION

The Western Antarctic Peninsula is one of the most rapidly warming regions on Earth, and many biotic communities inhabiting this dynamic region are responding to the climatic change. The effect of warming on soil microbes in cold environments is presumed to be direct and diverse through experimental evidence [6], which shows that the effects might be more significant for bacteria than fungi [5,7] or facilitate the colonization of soil by a wider diversity of fungi than is currently observed [4].

It has been well known that greenhouses modify potential limiting factors (such as temperature and moisture) in a complex and interactive manner. In this experiment OTC-In samples showed slightly but significantly increased soil temperature and water availability, two of the most important abiotic factors in determining the distribution of Antarctic organisms [20] and possibly microbial biomass. During the 8-year period of the OTC passive warming experiment in Fildes Peninsula, a meaningful change in the relative abundances of bacteria and archaea was not detected between OTC-In and OTC-Out samples that were collected in 2015. However, the archaeal phylum *Euryarchaeota* slightly increased in OTC-In samples: the increase by 3.8% of the methylotrophic methanogenic class *Thermoplasmata* [19]. *Euryarchaeota* includes many methanogens which produce methane during low-molecular-weight compound degradation, indicating that *Euryarchaeota* strains may participate in the degradation and

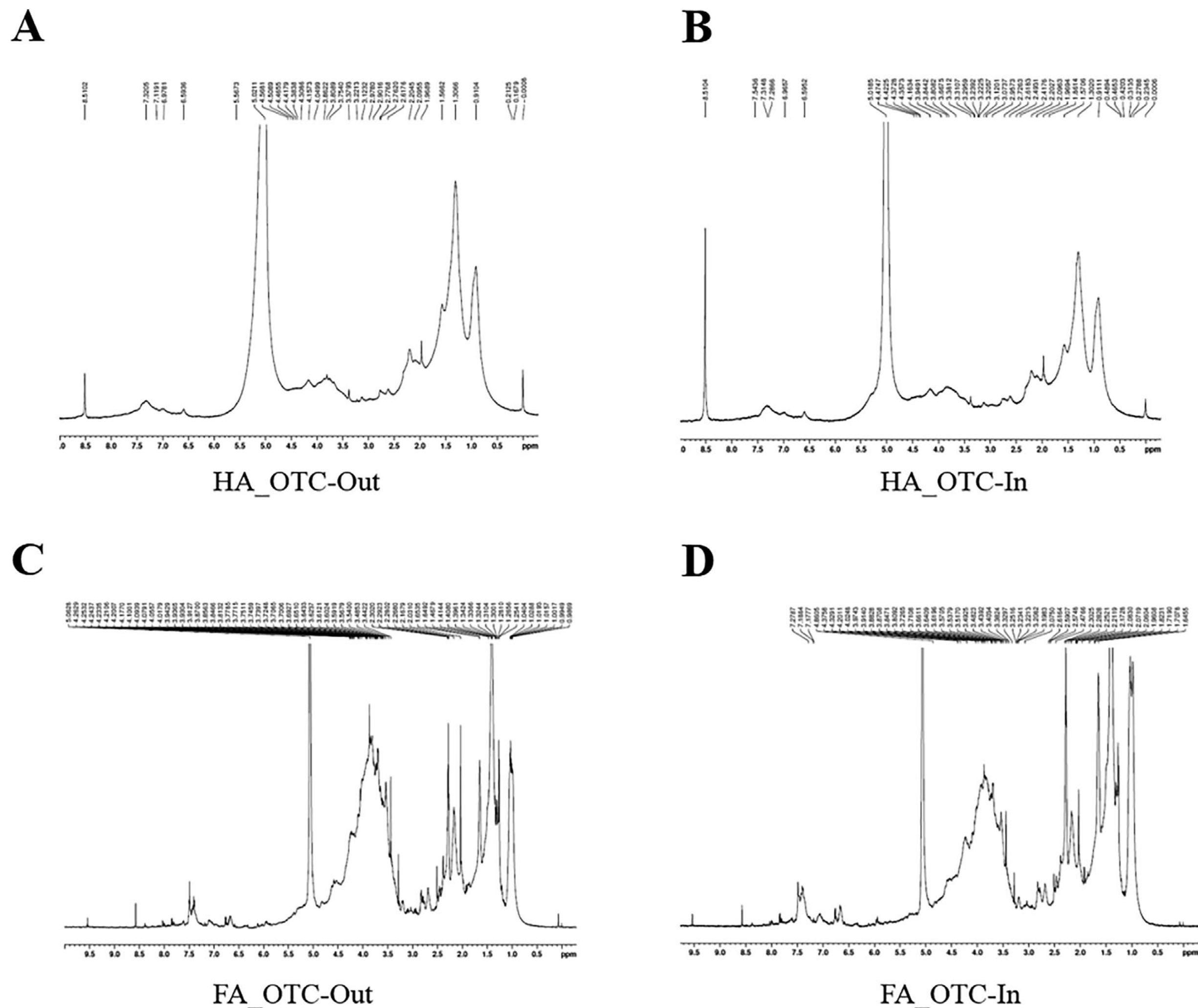

FIGURE 4 ^{1}H-NMR spectral analysis of humic acids (HA) and fulvic acids (FA) in OTC warming experimental soils. A, HA in OTC-Out; (B) HA in OTC-In; (C) FA in OTC-Out; (D) FA in OTC-In

mineralization processes of SOM within this environment. In a previous report, the relative abundance of methanogens in the metabolically versatile class *Methanomicrobia* increased during the thawing of Alaskan frozen permafrost cores [21].

Although the changes in bacterial and archaeal relative abundances were minor, a pronounced increase was observed for *Ascomycota*, a dominant fungal phylum in these soils increasing by approximately 19% in OTC-In. As surface air temperature appears to be an important factor shaping the fungal diversity and composition of maritime Antarctic soil [4], *Ascomycota* might be more vulnerable to the changes in environment than other microbes. Although *Ascomycota* is the largest fungal group and are heterotrophic organisms that obtain nutrients from a variety of organic substrates by secreting powerful digestive enzymes, their role (such as soil organic degradation) in this maritime region could be proposed with further characterization.

The total PLFA content of bacteria and fungi increased in OTC-In (total 15.5 mg kg^{-1} soil) compared to OTC-Out (total 12.6 mg kg^{-1} soil). This suggests that the small temperature effects within OTCs might have led to an increase in both bacterial and fungal biomass, perhaps due to the release of more low-molecular-weight growth substrates in OTC-In versus OTC-out soil. The ratio of fungal:bacterial PLFA content decreased in OTC-In (1.07) compared with OTC-Out (1.17). This decrease in ratio indicates that the increased amount of fungal biomass was slightly lower than the increase of bacterial biomass in OTC-In, in parallel with microbial consumption of the growth substrates. Overall, these changes in microbial biomass generally seem to reflect the response of the soil microbial community to rising temperatures within OTCs, through the change in SOM content.

As shown in Table 3, fungi constitute a greater portion of soil biomass than bacteria in these sites. Based on numerous biochemical and physiological studies, soil heterotrophic fungi are known to play a critical role in the formation, transformation, degradation, and mineralization of HS [11,22]. Several of these soil heterotrophic fungi, such

as white-rot fungi, are able to degrade various aromatic macromolecules such as lignin and HS through a nonspecific and highly oxidative process. Accordingly, we hypothesized that soil microbial responses to global warming may accelerate the degradation of HS, a main source of soil organic carbon and nitrogen, which may induce subsequent biotic and abiotic effects in the maritime Antarctic. This long-term passive warming treatments influenced soil microbial community and increased its biomass. However, we could not detect any significant changes in the amount and structures of HS (HAs and FAs) between OTC-In and OTC-Out samples, nor in chemical and physical characteristics of the soils. These observations indicate that the effects of rising temperature in OTC-In (an approximate 0.8 °C increase for the 2013–2015 summer seasons) were not significant enough to result in a difference in degradation rate or capacity for complex biopolymer HS of soil-born microbes. There might be a possibility that the changes were below the limit of detection for analytical procedures. However, the significant structural differences between HAs and FAs were observed regardless of soil source. Saprotrophic fungi, identified as efficient HS degraders, produce nonspecific oxidizing enzymes (laccases and peroxidases) and break down biopolymers to low-molecular-weight compounds [11]; the structural differences of HAs and FAs suggest that such oxidative low-molecularization of HA to FA (or FA-like degradation metabolites) has consistently occurred in OTC experimental sites, although the degradative rates was not significant between OTC-Out and OTC-In samples. Taken together, the results from this study of passive warming imply that continued climate warming will be likely to have a profound impact on soil microbial biology and soil ecology in the maritime Antarctic. In detail, warming in the natural environment will derive the more accumulation of HS degradative products owing to microbial enhanced degradation-related metabolisms and then the increased input of carbon and nitrogen to these soils will probably lead to increases in soil microbial biomass and plant populations.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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