Warming constrains bacterial community responses to nutrient inputs in a southern, but not northern, maritime Antarctic soil

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ABSTRACT

We investigated the effects of increased soil temperature, water and nutrient availability on soil bacterial communities at Wynn Knolls on Signy Island (60 °S) in the northern maritime Antarctic and at Mars Oasis (71 °S) in the southern maritime Antarctic. After 10–12 months, analyses of the concentrations of ester linked fatty acids (ELFAs) in soil indicated that bacterial communities responded positively to single applications of substrates at both locations, with 20% and 49% increases in total Gram positive and Gram negative bacterial markers, respectively, in response to the application of tryptic soy broth (TSB; a complex substrate containing organic carbon and nitrogen, plus other nutrient elements) at Wynn Knolls, and 120% and 44% increases in Gram positive bacterial markers at Mars Oasis in response to the application of TSB and the amino acid glycine (a relatively simple source of organic carbon and nitrogen), respectively. Responses to the warming treatment were not detected at Wynn Knolls, where open top chambers (OTCs) increased mean monthly soil temperatures by up to 0.7 °C, but at Mars Oasis, where OTCs increased monthly soil temperatures by up to 2.4 °C, warming led to 41% and 46% reductions in the concentrations of Gram positive bacterial markers in soil to which glycine and TSB had been applied, respectively. Warming also led to 55% and 51% reductions in the ratio of Gram positive to Gram negative markers in soils at Mars Oasis to which glycine and TSB had been applied. These data suggest that warming may constrain the responses of bacterial communities to carbon and nitrogen inputs arising from dead plant matter entering maritime Antarctic soils in future decades.

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1. Introduction

Mean annual air temperatures in the maritime Antarctic, particularly along the western Antarctic Peninsula, have risen at rates of up to 0.4 °C decade^{-1} over the past 50–100 years (Adams et al., 2009), which is approximately double the rate at which global mean surface temperature has increased over the last 30 years (c. 0.2 °C decade^{-1}; Hansen et al., 2006). The warming of maritime Antarctic terrestrial ecosystems is having widespread effects on the physical environment: along with the collapse of ice shelves and the retreat of glaciers, changes to precipitation patterns have occurred, with increases in snow accumulation having been recorded in recent decades (Thomas et al., 2008; Adams et al., 2009). In addition to these changes to the physical environment, the warming of maritime Antarctic terrestrial ecosystems is also altering the cover of plant species. For example, the population sizes of Deschampsia antarctica and Colobanthus quitensis, the two native Antarctic higher plant species, increased by 7–26 fold between the mid 1950s and early 1990s (Fowbert and Smith, 1994; Smith, 1994), and a new southern limit for flowering plants in Antarctica has recently been established (Convey et al., 2011). The expansion of maritime Antarctic plant populations over future decades is likely to enhance and alter nutrient inputs to soils (Hill et al., 2011), with the input of carbon and nitrogen to these soils most probably leading to significant increases in soil microbial biomass (Davey and Rothery, 1992; Malosso et al., 2005). Similar

increases in nutrient inputs from microbial and cryptogam sources (via photosynthesis and nitrogen fixation) have also been postulated for dry valleys soils in continental Antarctica (Novis et al., 2007; Hopkins et al., 2008). These predictions are supported by studies showing larger microbial biomass in vegetated than in non-vegetated maritime Antarctic soils (Yergeau et al., 2007a).

Warming also influences the size and composition of bacterial communities in maritime Antarctic soils. For example, the deployment of screens over soil at Signy Island in the South Orkney Islands increased the abundances of Cyanobacteria in soil by 2–17 fold, with substantial increases in the biomass of *Phormidium* and *Nostoc* spp. in warmed soils (Wynn-Williams, 1996). Recently, it has been shown that bacteria are more frequent in vegetated soils that have been passively warmed with open top chambers (OTCs) at two sites in the maritime Antarctic, and that warming increases the ratio of Alphaproteobacteria to Acidobacteria (Yergeau et al., 2011). At present, however, these are the only data on soil bacterial responses to warming in natural maritime Antarctic environments, and, importantly, nothing is known of the combined effects of warming and nutrient inputs on the bacterial communities of maritime Antarctic soils. Given that soil bacteria are pivotal to ecosystem processes, such as the mineralisation of nutrients from dead organic matter, the current lack of data on the responses of these microbes to warming and nutrient input in the region represents a significant gap in knowledge.

Here we report the results of an experiment designed to test the hypothesis that warming, in combination with either nutrient input or water application, would influence Antarctic soil bacterial communities. The study was undertaken at two sites with different climates, one on Signy Island in the northern maritime Antarctic, and the other on Alexander Island in the southern maritime Antarctic. Both of the locations studied have warmed in recent decades: air temperature at Signy Island increased by 1 °C between 1950 and 1990 (Quayle et al., 2002) and surface temperatures at a site 240 km to the south of Mars Oasis are estimated to have increased by 2.7 °C between the mid 1940s and the late 2000s (Thomas et al., 2009). We used OTCs to warm soil to temperatures that are predicted to be reached during the next two to five decades at either location, given these current rates of warming (Adams et al., 2009).

2. Materials & methods

2.1. Site descriptions

The experiment was established at Wynn Knolls (60° 41′ 56″ S, 45° 38′ 10″ W) on Signy Island in the South Orkney Islands and at Mars Oasis (71° 52′ 42″ S, 68° 15′ 00″ W) on south-eastern Alexander Island in the southern maritime Antarctic (Fig. 1). Wynn Knolls is situated at *c.* 500 m from the western shoreline of Signy Island at an altitude of 199 m above sea level. The island has an oceanic climate, characterised by dense cloud cover during the austral summer (7.2–7.4 oktas; Collins et al., 1975). Precipitation in the northern maritime Antarctic, where Signy Island is located, varies between 350 mm and 500 mm water equivalents per annum, with much falling as rain in the austral summer (Smith, 1984). Soils on the island are derived from schists, amphibolites and marbles (Collins et al., 1975). The more southerly site, Mars Oasis, is formed from a contact moraine between the George VI ice shelf and the south-eastern coast of Alexander Island. The oasis, consisting of areas of till, fluvial and lacustrine sediments (Sugden and Clapperton, 1981), with streams and ponds forming during the austral summer, is located at an altitude of 20 m above sea level. Soil at the site is derived from the sandstones and mudstones of Two Step Cliffs massif. The western Antarctic Peninsula, and in

particular its southern reaches, have a relatively continental climate, with frequent periods of cloudless skies during the summer. Precipitation in the southern maritime Antarctic is *c.* 350 mm water equivalents per annum, most of which falls as snow (Smith, 1984). The soils at Wynn Knolls and Mars Oasis had similar chemical properties, with mean (±SEM) pH values of 7.5 ± 0.15 and 7.3 ± 0.12, electrical conductivities of 29.4 ± 2.9 μS and 31.9 ± 2.7 μS, NO_3^-–N concentrations of 0.033 ± 0.019 mg kg^{-1} and 0.007 ± 0.007 mg kg^{-1}, and NH_4^+–N concentrations of 0.138 ± 0.065 mg kg^{-1} and 0.095 ± 0.015 mg kg^{-1}, respectively (Dennis et al., 2012). The concentrations of both total organic carbon and total nitrogen in the soils were very small, with organic carbon concentrations of 0.140 ± 0.010% and 0.260 ± 0.020%, and nitrogen concentrations of 0.038 ± 0.004% and 0.020 ± 0.002% in the two soils, respectively (Dennis et al., 2012).

2.2. Manipulation experiment and sampling

Three treatments were applied at each site in factorial combination. Soil was warmed using OTCs, water was applied to soil to simulate short-term changes in moisture availability arising from melting snow and ice, and growth substrates were added to alter carbon and nitrogen availability to soil microbes. The addition of the substrates broadly simulated increases in soil nutrients associated with organic inputs from algae and cyanobacteria, in a way directly analogous to a corresponding experiment in the dry valleys region of continental Antarctica (Hopkins et al., 2008). In order to apply these treatments, 64 circular plots of 1 m diameter were established at each site within a 17 m × 17 m area that was devoid of vegetation. Thirty-two of the plots were covered with conical clear polycarbonate OTCs of 1 m diameter (see Fig. 1b in Marion et al., 1997). The moisture content of the soil in 16 of the chambered plots and in 16 of the control plots was adjusted to 100% of water-holding capacity in the surface 5 cm of soil using water purified with ion exchange columns (Fistreem, UK). For each temperature–water combination, four replicate plots were each
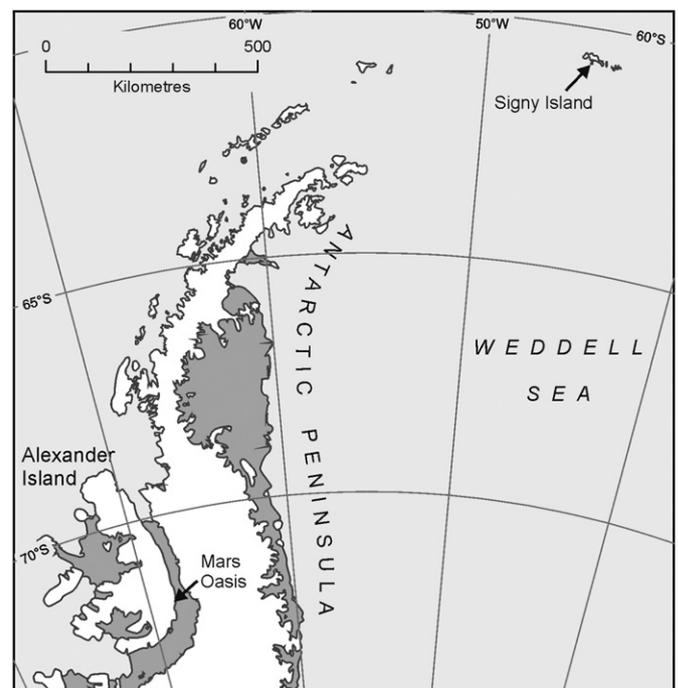

Fig. 1. Map of the maritime Antarctic, indicating the locations of Signy Island, Alexander Island and Mars Oasis.

either non-amended or were amended with glucose, glycine or powdered tryptic soy broth (Difco, USA), at a rate of 2.0 mg C g^{-1} dry wt soil, which was mixed into the soil surface to c. 20 mm depth. The soil in the unamended control plots were mixed in a similar fashion. The experimental layout resulted in 16 temperature–water–substrate combinations, each replicated four times in a randomised design at each site. Soil temperatures (depth c. 10–50 mm) were recorded at each site in three randomly-selected chambered and three randomly-selected control plots using TinyTag Plus 2 TGP-4017 loggers (Gemini Data Loggers Ltd., UK). The plots at Mars Oasis were established in November 2007 and those at Wynn Knolls in January 2008. Soils were collected from the 64 plots at each site after 10 and 12 months, respectively, by filling 50 ml clean plastic tubes with soil (depth c. 0–20 mm), which were then frozen until analysis.

2.3. Analyses of ELFAs

The analysis of ELFAs (ester-linked fatty acids) limited to known microbial fatty acids allows the rapid fingerprinting of lipids in the soil of microbial origin (Drijber et al., 2000; Steger et al., 2003; Hinojosa et al., 2005). ELFAs were extracted from soil (10 g) "spiked" with a 3 μg aliquot of the ELFA 23:0 as an internal standard, and were then derivatised using the method of Schutter and Dick (2000) and analysed by gas chromatography (GC). The GC analyses were conducted on an Agilent Technologies 6890N Network GC system with a flame ionization detector and an Agilent HP-5 column (Agilent Technologies, UK) with helium as the carrier gas. The temperature programmed resolution of individual esters was carried out at 160 °C for 2 min, 160–270 °C at 4 °C min^{-1}, and then at 270 °C for 10 min. ELFAs were identified by comparing retention times with commercial (bacterial acid methyl esters mixture; Supelco, USA) and in-house standards and were quantified by relating the area of individual peaks to that of the internal standard.

2.4. Statistical analyses

Bacterial biomass was estimated by summing the ELFAs (expressed as nmol fatty acid g^{-1} dry wt soil) indicative of Gram positive bacteria (viz., i15:0, a15:0, br16:0, i16:0, br17:0, 10Me16:0, i17:0, a17:0, br18:0, 10Me17:0, 10Me18:0 and 19:1; Lechevalier and Lechevalier, 1988; O'Leary and Wilkinson, 1988) and Gram negative bacteria (viz., 16:1ω9c, 16:1ω7c, cy17:0ω7, cy19:0ω9 and cy19:0ω7; Lechevalier and Lechevalier, 1988; Zelles, 1999). The ratio of Gram positive to Gram negative markers was also calculated. General Linear Models and one-way ANOVA were used to determine the effects of OTCs, water and substrate treatments on the concentrations of ELFAs in the MINITAB 15 package. The effects of the treatments on the concentrations of individual ELFAs were analysed separately when treatment effects were identified on the concentrations of total Gram positive or Gram negative markers.

3. Results

3.1. Effects of OTCs on soil temperature and frequency of freeze–thaw cycles

The deployment of OTCs at Wynn Knolls on Signy Island led to minimum, mean and maximum increases in monthly soil temperatures of 0.3 °C, 0.6 °C and 0.7 °C, respectively, relative to controls (Fig. 2a), whereas at Mars Oasis on Alexander Island, OTC deployment resulted in larger increases in soil temperatures, with minimum, mean and maximum increases in monthly soil temperatures of 0.6 °C, 1.3 °C and 2.4 °C, respectively (Fig. 2b). The largest
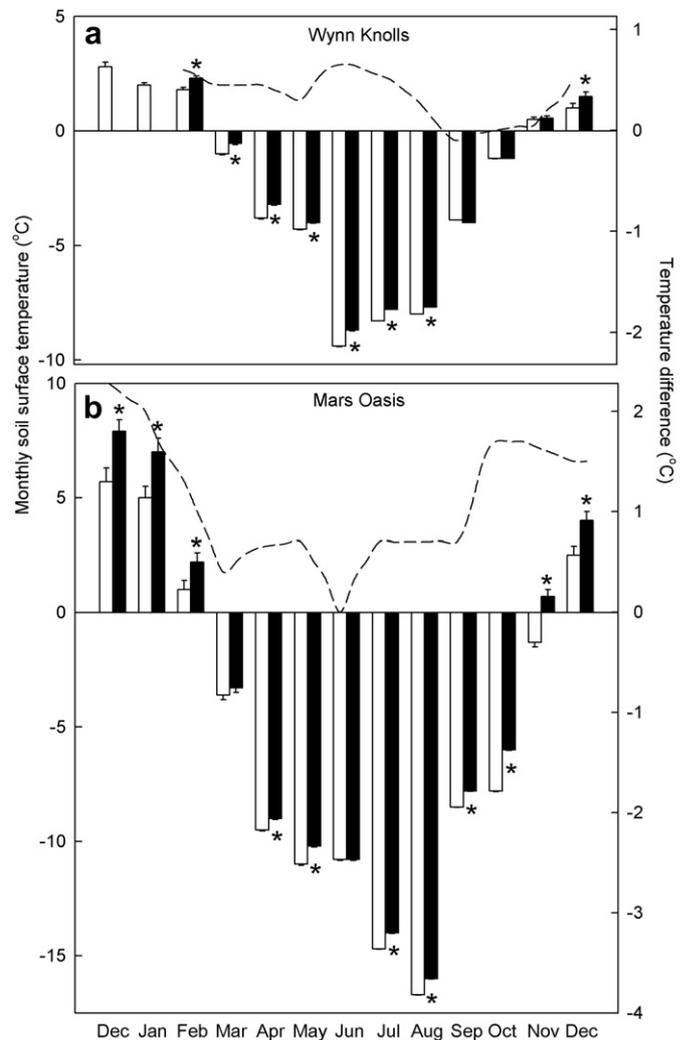

Fig. 2. Mean monthly soil temperatures (left axis) in control plots (open bars) and plots covered with OTCs (closed bars) at (a) Wynn Knolls and (b) Mars Oasis. Values are means of three replicates + SEM. Significant differences between those for control and chambered plots are denoted by *; $P < 0.05$. The dashed line shows the difference in temperature between control and chambered plots (right axis).

increases in monthly soil temperatures arising from OTC deployment at Mars Oasis usually occurred between November and February (Fig. 2b), when the site was free of snow. Although mean (±SEM) summer (Dec–Feb) soil temperatures in control plots did not differ between Mars Oasis and Wynn Knolls (3.6 ± 1.1 °C and 1.9 ± 0.4 °C, respectively; $F_{1,7} = 2.05$, $P > 0.05$), soil temperatures were significantly lower at Mars Oasis than at Wynn Knolls during the other months of the year (−10.3 ± 1.4 °C and −4.4 ± 1.2 °C, respectively; $F_{1,15} = 10.42$, $P < 0.01$).

No effects of OTCs were recorded on the number of monthly freeze–thaw cycles at Wynn Knolls, where there were 0.3–7 cycles month^{-1} in control plots when soil was not permanently frozen (Oct–Apr; data not shown). In contrast, OTC deployment at Mars Oasis significantly decreased the mean (±SEM) number of freeze–thaw events in December 2007 and 2008 from 11.9 ± 1.4 to 3.3 ± 1.3 cycles and from 10.8 ± 1.2 to 6.1 ± 1.3 cycles, respectively, and in January 2008 from 5.8 ± 1.4 to 0.1 ± 1.7 cycles (all $P < 0.05$; data not shown). The mean number of freeze–thaw cycles month^{-1} in control plots during months in which the soil was not permanently frozen was significantly higher at Mars Oasis than it was at Wynn Knolls (11.0 ± 2.8 and 3.8 ± 0.8, respectively; $F_{1,13} = 7.06$, $P < 0.05$).

3.2. Between-site differences

There were apparent differences in the bacterial biomass between the two sites, as indicated by the concentrations of bacterial markers in the soils. The concentrations of Gram positive and Gram negative bacterial markers were 76% and 85% lower at Mars Oasis than at Wynn Knolls, respectively (Fig. 3a, b). However, Gram positive markers were relatively more frequent at Mars Oasis: the ratio of Gram positive to Gram negative markers was 54% greater at this site than it was at Wynn Knolls (Fig. 3c).
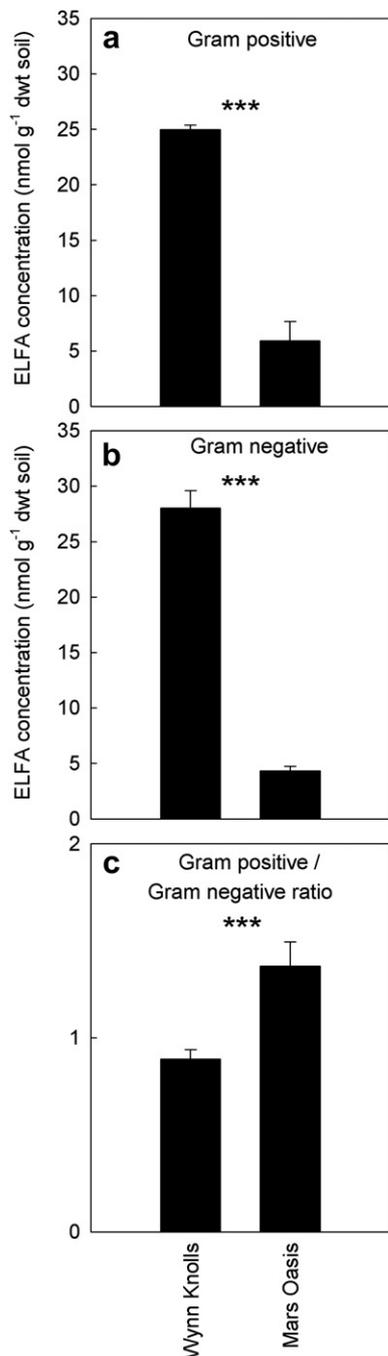

Fig. 3. Concentrations of (a) Gram positive and (b) Gram negative markers, and (c) the ratio between Gram positive and Gram negative markers, at Wynn Knolls and Mars Oasis. Values are means of 64 replicates + SEM (i.e. all other treatments combined). Significant differences between sites are denoted by ***; $P < 0.001$. Note that the y-axis in (c) is dimensionless.

3.3. Responses to substrate amendments and water addition

The addition of substrates to soil had significant effects on bacterial markers. Substrate amendments affected the amount of both Gram positive and Gram negative markers at Wynn Knolls (Table 1), with 20% and 49% increases, respectively, in the concentrations of ELFAs indicative of Gram positive and Gram negative forms in response to the application of TSB to soil at this site, relative to non-amended controls (Fig. 4a, b). In soil to which TSB had been applied, the increases in Gram positive markers were associated with greater concentrations of the ELFAs a15:0 and a17:0 (Fig. 4a, inset), and the increases in Gram negative markers were associated with a greater concentration of 16:1ω7c (Fig. 4b, inset). The addition of substrates to soil did not affect the ratio of Gram positive to Gram negative markers at Wynn Knolls (Fig. 4c). Significant effects of substrate amendments were also detected on the concentrations of Gram positive markers at Mars Oasis (Table 1). At this site, the addition of glycine and TSB to soil led to 44% and 120% increases in the concentrations of ELFAs indicative of Gram positive bacteria, relative to controls (Fig. 4d). These increases were associated with greater concentrations of a15:0 and a17:0 in soil to which glycine and TSB had been applied (Fig. 4d, inset). The concentrations of ELFAs indicative of Gram negative bacteria remained unaffected by the substrate amendments at Mars Oasis (Fig. 4e), but there were 106% and 58% increases in the ratio of Gram positive to Gram negative markers after glycine and TSB had been applied to soil at this site, respectively, relative to non-amended soil (Fig. 4f).

The addition of water had no effect on the concentrations of ELFAs in soil at either Wynn Knolls or Mars Oasis, and there were no interactive effects of water with OTCs or substrates on ELFA concentrations at either site (Table 1).

3.4. Responses to OTCs

Although no main or interactive effects of OTCs were recorded on ELFA profiles at Wynn Knolls, a significant main effect of OTCs, and a significant substrate × OTC interaction term, was detected on ELFA concentrations in soil at Mars Oasis (Table 1). There were no effects of OTCs on Gram positive markers or the ratio of Gram positive to Gram negative markers in control (non-amended) soil or soil to which glucose had been applied at Mars Oasis, with the only

Table 1
F ratios from general linear models testing for the main and interactive effects of substrate amendments, water application and OTCs on the concentrations of Gram positive and Gram negative bacterial markers, and the ratio of Gram positive to Gram negative markers, at Wynn Knolls and Mars Oasis. Significant values are denoted by $^*P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$. Error d.f. in all analyses were 48.

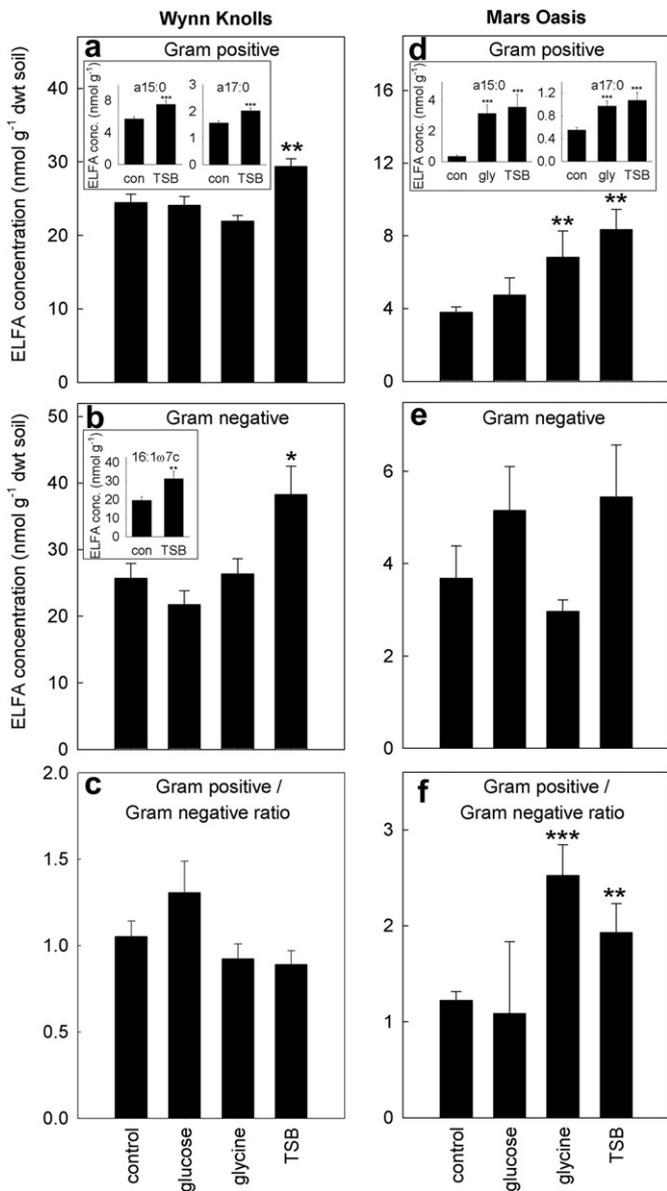|	Gram positive	Gram negative	Gram positive/ Gram negative ratio
Wynn Knolls			
Substrate	8.20***	6.15**	2.62
Water	0.06	1.63	0.92
OTC	0.12	1.19	0.23
Substrate × water	0.73	1.29	0.11
Substrate × OTC	1.42	0.87	0.33
Water × OTC	0.53	1.03	0.36
Substrate × water × OTC	0.92	0.99	1.39
Mars Oasis			
Substrate	8.76***	2.05	11.91***
Water	0.52	1.18	0.14
OTC	11.81**	0.05	17.45***
Substrate × water	0.61	1.09	0.54
Substrate × OTC	2.94*	0.45	5.97**
Water × OTC	0.51	0.44	0.11
Substrate × water × OTC	0.58	1.97	0.29

Fig. 4. The influence of glucose, glycine and TSB application to soil on the concentrations of Gram positive (a, d) and Gram negative (b, e) markers, and the ratio of Gram positive to Gram negative markers (c, f), at Wynn Knolls (left column) and Mars Oasis (right column). Values are means of 16 replicates + SEM (i.e. the heated [OTC]) and unheated treatments have been combined). Those that are significantly different from controls are denoted by *; $P < 0.05$, **; $P < 0.01$ and ***; $P < 0.001$. Note that the y-axes in (c) and (f) are dimensionless. Insets in (a) and (d) show the concentrations of the markers a15:0 and a17:0 in non-amended soil (con) or soil to which glycine (gly) or TSB had been applied at Wynn Knolls and Mars Oasis, respectively. That in (b) shows the concentration of 16:1ω7c in non-amended (con) soil or soil to which TSB had been applied at Wynn Knolls. Notation as in main figures.
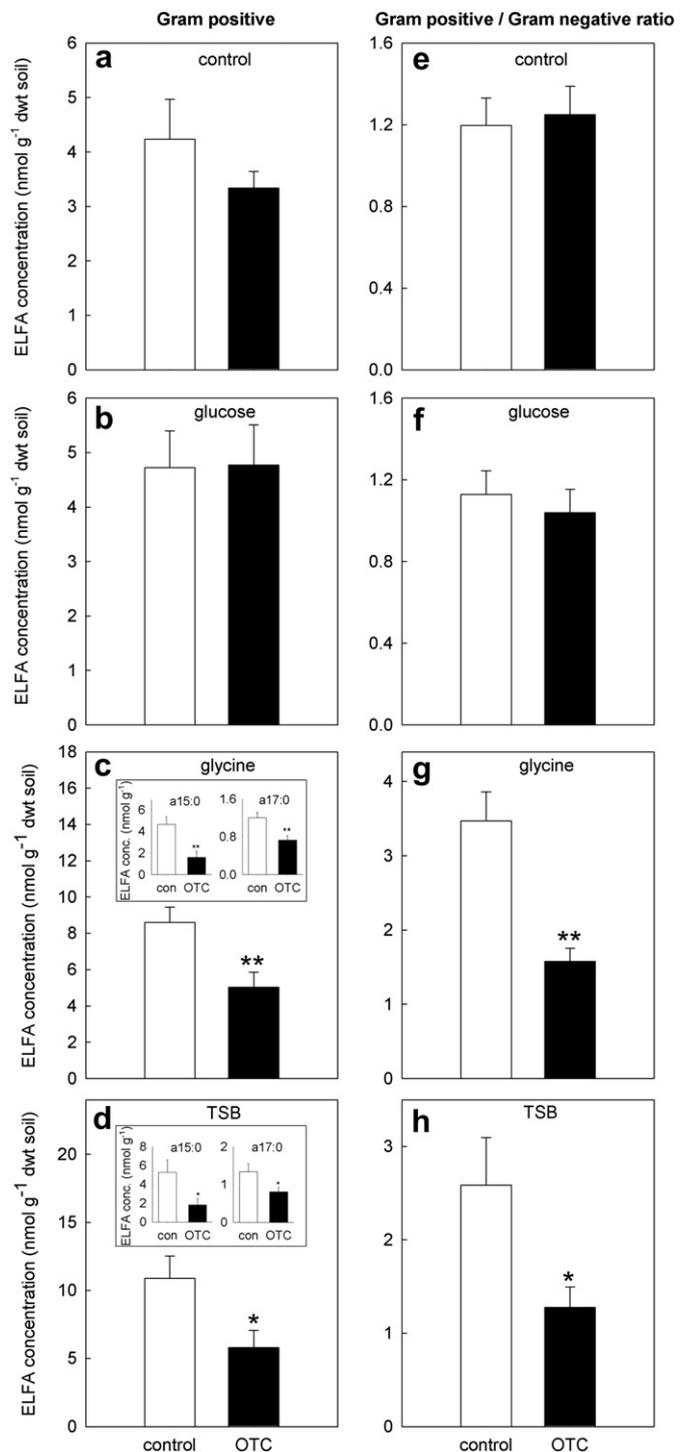
significant effects of OTCs being recorded in soils to which glycine and TSB had been applied (Fig. 5), accounting for the significant OTC × substrate interaction term. In soil to which glycine and TSB had been applied, OTCs led to 41% and 46% reductions in the concentrations of ELFAs indicative of Gram positive markers, respectively (Fig. 5c, d). In both cases, these reductions were associated with lower concentrations of the ELFAs a15:0 and a17:0 in chambered, compared with control, soil (Fig. 5c, d, insets). No significant differences were recorded between the mean (±SEM) concentrations of ELFAs indicative of Gram positive bacteria in warmed soil to which glycine and TSB had been applied

Fig. 5. Mean concentrations of Gram positive markers (left column) and the ratio of Gram positive to Gram negative markers (right column) at Mars Oasis in control soils (open bars) and soils covered with OTCs (closed bars) that were either non-amended (a, e) or to which glucose (b, f), glycine (c, g) or TSB (d, h) had been applied. Values are means of eight replicates + SEM (i.e. by contrast with Fig. 4, the heated [OTC] and unheated treatments have not been combined). Those that are significantly different from controls are denoted by *; $P < 0.05$ and **; $P < 0.01$. Note that the y-axes in (e)–(h) are dimensionless. Insets in (c) and (d) show the concentrations of the markers a15:0 and a17:0 in control (con) or chambered (OTC) soil to which glycine or TSB had been applied, respectively. Notation as in main figures.

(5.02 ± 0.82 and 5.79 ± 1.26 nmol g^{-1} dry wt soil, respectively; Fig. 5c, d) and those in warmed soil to which no substrate had been applied (3.34 ± 0.30 nmol g^{-1} dry wt soil; Fig. 5a; $F_{1,15}$ = 3.76 and 3.64, respectively, both $P > 0.05$). The combination of warming and substrate amendment also influenced the relative abundance of Gram positive markers: in soil to which glycine and TSB had been applied, OTCs reduced the ratio of Gram positive to Gram negative markers by 55% and 51%, relative to controls (Fig. 5g, h). As with the concentrations of Gram positive markers, the mean (±SEM) ratios of Gram positive to Gram negative markers in warmed soil to which glycine and TSB had been added (1.57 ± 0.17 and 1.27 ± 0.22; respectively, Fig. 5g, h) were not significantly different from the ratio recorded in warmed soil to which no substrate had been applied (1.25 ± 0.14; Fig. 5e; $F_{1,15}$ = 2.20 and 0.01, respectively, both $P > 0.05$).

4. Discussion

It is usually assumed that environmental warming will have positive effects on the biota that inhabit the cold and arid soils of the Antarctic (Convey and Smith, 2006; Convey, 2011). This view is supported by data from warming experiments on Eubacteria and Cyanobacteria, and long-term observations of plant populations in the natural environment (Fowbert and Smith, 1994; Smith, 1994; Wynn-Williams, 1996; Yergeau et al., 2011). However, our study does not support this assumption: here, we applied a warming treatment that was consistent with two- to five-decadal changes in temperature in maritime Antarctic ecosystems at Signy Island (1 °C between 1950 and 1990; Quayle et al., 2002) and close to Alexander Island (2.7 °C between the 1940s and 2000s; Thomas et al., 2009), and found no significant effect of the treatment on the biomass of bacteria in non-amended soils at either location after one year. However, we cannot discount the possibility that further years of treatment might lead to positive effects of warming on bacterial biomass in soils to which substrates are not added.

Owing to the frequent occurrence of cloudless skies in the southern maritime Antarctic, Mars Oasis received more thermal input than Wynn Knolls during summer, and the warming effect of the OTCs was consequently amplified at this more southerly site. Here, when substrates were applied to warmed soil in the form of an amino acid (glycine) or a complex mixture of organic carbon, nitrogen and other elements (TSB), there were substantial reductions in the concentrations of Gram positive bacterial markers. This confirmed our hypothesis that a combination of warming and nutrient input would influence soil bacterial communities. These reductions constrained the response of the soil bacterial community to warming, because the concentration of total Gram positive bacterial markers in warmed soil that received glycine or TSB was no greater than in warmed soil that received no nutrient input. The changes were associated with reductions in chambered soil in the concentrations of the ELFAs a15:0 and a17:0, which are frequent in Actinobacteria such as *Arthrobacter* and in aerobic endospore-forming bacteria (Haack et al., 1994; Kieft et al., 1994). These data, along with our observation that the ratio of Gram positive to Gram negative markers declined when glycine- and TSB-amended soil at Mars Oasis was warmed, support previous data indicating that warming alters the composition of Antarctic soil bacterial communities (Wynn-Williams, 1996; Yergeau et al., 2011).

The short-term increase in soil moisture applied in the current study had no apparent effects on soil bacterial communities, probably reflecting the 10- and 12-month periods between the single applications of water and sampling at each site. Similarly, annual applications of water to soils of the dry valleys in the continental Antarctic have no effects on the abundances of nematodes (Simmons et al., 2009), although rapid freezing and

sublimation often limits the residence time of liquid water in these soils (Hopkins et al., 2006a). More frequent water application would most probably have had measurable effects on soil bacterial biomass, since bacteria are more abundant in Antarctic soils that contain more liquid water (Wynn-Williams, 1996; Harris and Tibbles, 1997). However, water availability is not considered to be a major factor controlling the composition of maritime Antarctic soil bacterial communities, and so substantial changes to community composition associated with increased water availability are unlikely (Newsham et al., 2010).

In contrast to the lack of response to water application, the soil bacterial communities at Wynn Knolls and Mars Oasis responded positively to both glycine and TSB amendments, confirming the view that microbial growth and activity is limited by carbon, and in particular, nitrogen inputs in both maritime (Davey and Rothery, 1992; Malosso et al., 2005) and continental Antarctic soils (Hopkins et al., 2006b, 2008; Sparrow et al., 2011). In Sparrow et al. (2011) the effect of substrate additions were detected up to 3 years after addition, suggesting that some substrate effect was likely to have persisted in our current maritime Antarctic sites after 1 year. As with the responses to the warming treatment, these increases were associated with greater concentrations of the Gram positive markers a15:0 and a17:0, but were also associated with increased concentrations of the general Gram negative marker 16:1ω7c in TSB-amended soil at Wynn Knolls. The relative frequency of Gram positive markers in Mars Oasis soil also changed in response to carbon and nitrogen inputs: when glycine and TSB were applied to soil at this site, the ratio of Gram positive to Gram negative markers increased, corroborating data from studies on subarctic heath soils, in which this ratio responded positively to inorganic nutrient (N, P and K) inputs (Schmidt et al., 2000; Rinnan et al., 2007). The greater ratio of Gram positive to Gram negative markers observed in nutrient-amended subarctic heath soils has been attributed to changes mediated by the plant community (Rinnan et al., 2007), but this cannot explain the effects reported here in non-vegetated soils. Rather, we attribute the increase in the relative frequency of Gram positive markers following substrate amendment to the low concentration of nitrogen in Mars Oasis soil, which, on the addition of organic carbon, probably results in increased nitrogen demand of Gram positive bacteria.

Arctic studies indicate comparatively slow responses of soil microbial communities to warming and inorganic nutrient additions in the natural environment, with up to 15 years required for microbial community composition to respond to these treatments (Rinnan et al., 2007; Deslippe et al., 2011). In contrast, the responses to the warming and substrate treatments recorded here were relatively rapid: glycine or TSB amendments led to substantial increases in the concentrations of bacterial markers in one year or less. The discrepancy between the observations from Arctic and Antarctic studies is probably associated with plant cover: plants were absent from the soils studied here, allowing clear treatment effects of warming and substrate amendments on soil bacteria to be identified. In contrast, the majority of the soils that are experimentally warmed in the Arctic are vegetated, with higher plant cover, notably that of deciduous shrubs, responding to warming and N or P fertilization after 2–6 years and 6–15 years of treatment, respectively (Shaver et al., 2001; Walker et al., 2006; Wookey et al., 2009). These increases alter the composition of rhizosphere- and root-inhabiting microbes, but the effects only manifest themselves after 15–18 years of treatment (Rinnan et al., 2007; Deslippe et al., 2011).

The harsher environment at Mars Oasis, with its lower winter temperatures and greater frequency of freeze–thaw cycles during summer, had apparent effects on soil bacterial biomass, with smaller ELFA concentrations indicative of both Gram positive and

Gram negative bacteria at this site compared with Wynn Knolls. This is in broad agreement with studies showing reduced soil bacterial biomass at more southerly locations in the maritime Antarctic (Yergeau et al., 2007a). However, we found that the ratio of Gram positive to Gram negative markers was greater at Mars Oasis, corroborating the view that Gram positive bacteria, which possess thick, interlinked peptidoglycan cell walls, are more frequent in extreme soils (Schimel et al., 2007). For example, Gram positive, and in particular endospore-forming, bacteria are frequent in soil at Mars Oasis, where the analysis of clone libraries indicates that members of the order Actinomycetales are the most frequent bacteria in soil close to the area where the OTCs were deployed (Newsham et al., 2010), and that the frequency of Actinobacteria in soil is approximately double that of a fellfield soil on Signy Island (Yergeau et al., 2007b). More widely, analyses of clone libraries indicate that the most frequent bacteria in soils from three sites in the dry valleys in Victoria Land are the Actinobacteria (Smith et al., 2006).

Using rates of respiration of soil micro-organisms at different temperatures for a range of Arctic soils (Mikan et al., 2002), and the current temperature data (Fig. 2), we estimate the approximate cumulative annual respiration to be about 3.5 times greater at Wynn Knolls than at Mars Oasis, with the warming treatments increasing annual respiration by 10% and 50% at Wynn Knolls and Mars Oasis, respectively. These estimates suggest that although microbial respiration (activity) was greater at Wynn Knolls (the more maritime, wetter and warmer site) than at Mars Oasis, the potential for microbial responses to warming was greater at Mars Oasis than it was at Wynn Knolls, relative to the unwarmed control. This suggests that factors such as liquid water availability or nutrient availability are more likely constraints to microbial responses at the more southerly site. Comparable effects of soil water content on microbial activity were observed in an experiment in an extreme cold and dry site in the dry valleys (Sparrow et al., 2011).

What mechanisms might explain the reductions reported here in bacterial biomass in warmed, substrate-amended soils? One possibility is that the amelioration of the soil environment caused by OTC deployment at Mars Oasis may have increased the frequencies of bacterivorous nematodes in soil. However, this is unlikely, given the very low densities of nematodes in soil at the location where the OTCs were deployed (c. 12 individuals kg^{-1} soil; Convey and Wynn-Williams, 2002) and the finding that OTC deployment decreases the abundance of a microbial-feeding nematode species by 42% in dry valleys soils (Simmons et al., 2009). A further possibility is that the increased temperature of soil in OTCs may directly inhibit the growth of the bacterial community. Again, this is unlikely, given the tendency for microbes in soils exposed to wide seasonal fluctuations in temperature, such as those at Mars Oasis, to be psychrotrophs, which have optimum growth temperatures of >15 °C (Russell, 1990). A more plausible explanation is that the elevated soil temperatures within OTCs decreased soil water matrix potential, which limited the diffusion of the substrates to metabolically active cells and decreased bacterial growth rates in soil (Zak et al., 1999). Whether or not such effects occur when senescent plant material enters southern maritime Antarctic soils is at present an open question, but is one that urgently needs to be addressed.

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