Increased temperatures alter viable microbial biomass, ammonia oxidizing bacteria and extracellular enzymatic activities in Antarctic soils

Sebastian Barnard[1†], Marc W. Van Goethem[1†#], Storme Z. de Scally[1], Don A. Cowan[1], Peet Jansen van Rensburg[2], Sarina Claassens[3], Thulani P. Makhalanyane[1,4*]

[1] Centre for Microbial Ecology and Genomics, Department of Biochemistry, Genetics and Microbiology, University of Pretoria, Pretoria 0028, South Africa

[2] Focus Area Human Metabolomics, North-West University, Potchefstroom Campus, Private Bag X6001, Potchefstroom 2520, South Africa

[3] Unit for Environmental Sciences and Management, North-West University, Potchefstroom Campus, Private Bag X6001, Potchefstroom 2520, South Africa

[4] Marine Microbiomics Programme, Department of Biochemistry, Genetics and Microbiology, University of Pretoria, Pretoria 0028, South Africa

[†] Contributed equally

Present address: Environmental Genomics and Systems Biology Division, Lawrence Berkeley National Laboratory, Berkeley, California, USA

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*** Corresponding author:** Thulani P. Makhalanyane

Email: thulani.makhalanyane@up.ac.za

Tel: (+27) 012 420 6976

Abstract

The effects of temperature on microorganisms in high latitude regions, and their possible feedbacks in response to change, are unclear. Here, we assess microbial functionality and composition in response to a substantial temperature change. Total soil biomass, *amo*A gene sequencing, extracellular activity assays and soil physicochemistry were measured to assess a warming scenario. Soil warming to 15°C for 30 days triggered a significant decrease in microbial biomass compared to baseline soils (0°C; $P < 0.05$) after incubations had induced an initial increase. These changes coincided with increases in extracellular enzymatic activity for peptide hydrolysis and phenolic oxidation at higher temperatures, but not for the degradation of carbon substrates. Shifts in ammonia-oxidising bacteria (AOB) community composition related most significantly to changes in soil carbon content ($P < 0.05$), which gradually increased in microcosms exposed to a persistently elevated temperature relative to baseline incubations, while temperature did not influence AOBs. The concentration of soil ammonium (NH_4^+) decreased significantly at higher temperatures subsequent to an initial increase, possibly due to higher conversion rates of NH_4^+ to nitrate by nitrifying bacteria. We show that higher soil temperatures may reduce viable microbial biomass in cold environments but stimulate their activity over a short period.

Keywords: ammonia oxidation, Antarctica, functionality, nitrogen cycling, microbial communities, PFLA

Introduction

In cold terrestrial environments, where severe environmental conditions restrict biodiversity and ecosystem function, microbial communities form the basis of food webs (Makhalanyane *et al.*, 2015). In these regions, the cycling of important elements including carbon and nitrogen is principally driven by microorganisms (Cary *et al.*, 2010, Cowan *et al.*, 2014). The past few decades have provided substantial insights regarding the microbial communities in these depauperate environments and have revealed that these communities are diverse (Niederberger *et al.*, 2012), harbour a wide capacity for nutrient sequestration (Yergeau *et al.*, 2007, Cowan *et al.*, 2014, Ji *et al.*, 2017), and are functionally redundant and resilient (de Scally *et al.*, 2016, Wei *et al.*, 2016). While climate change, particularly warming, is expected to substantially alter the dynamics associated with Antarctic soil microbiomes, with expected decreases in biogeochemical cycling (Makhalanyane *et al.*, 2016), very few studies have provided mechanistic evidence supporting this assertion. Changes in the net capacity for biogeochemical cycling of carbon and nitrogen in these nutrient constrained environments is likely to impact Antarctic terrestrial biodiversity and ecosystem services (Wall, 2012).

The oxidation of ammonia to nitrate by nitrification is a critical two-step process in the global nitrogen (N) cycle (Wuchter *et al.*, 2006). Nitrification is the biological oxidation of inorganic soil ammonia (NH_3) to nitrate (NO_3^-) via nitrite (NO_2^-). The process is driven by ammonia-oxidising bacteria (AOB) and archaea (AOA) under aerobic conditions and is essential for ecosystem functionality in soils (Prosser, 1990). Notably, nitrification supplies the NO_3^- required for primary production (Bollmann & Conrad, 1998, Smith *et al.*, 2014). The first step of aerobic nitrification is the rate-limiting step of ammonia-oxidation (Kowalchuk & Stephen, 2001). The oxidation of NH_3 to hydroxylamine (NH_2OH) is driven exclusively by chemolithotrophic ammonia-oxidising archaea (AOA) and AOB that produce the ammonia monooxygenase subunit A enzyme (*amoA*) (Prosser & Nicol, 2008). This process has been well characterised in temperate soils (Offre *et al.*, 2009, Rasche *et al.*, 2010, Aigle *et al.*, 2019), and acidic agricultural soils (Kemnitz *et al.*, 2007, Gubry-Rangin *et al.*, 2010). In other extreme environments, such as terrestrial hot springs, AOA and AOB have been demonstrated to perform crucial functions (Reigstad *et al.*, 2008, Schleper, 2010, Szukics *et al.*, 2010, Straka *et al.*, 2019). In contrast to our understanding on carbon cycling in Antarctic soils (Burkins *et al.*, 2001, Kong *et al.*,

2012, Shanhun *et al.*, 2012), surprisingly little is known regarding the ecology and functioning of AOA and AOB populations in cold terrestrial environments.

AOA and AOB are present in a wide range of soil types (Leininger *et al.*, 2006, Prosser & Nicol, 2012, Giguere *et al.*, 2018) and both keystone groups have the potential to contribute to ammonia-oxidation in extreme edaphic habitats (Chan *et al.*, 2013, Yung *et al.*, 2014, Tolar *et al.*, 2016). Surveys of *amoA* gene diversity in soils and marine ecosystems have shown that *Thaumarchaeota* (AOA) are much more common than bacterial ammonia oxidisers (Leininger *et al.*, 2006, Shen *et al.*, 2008, Auyeung *et al.*, 2015, Prosser *et al.*, 2020) while AOB (principally two monophyletic clades within the *Beta-* and *Gamma-Proteobacteria*) are prevalent across different ecosystems (Jung *et al.*, 2011, Xia *et al.*, 2011, Banning *et al.*, 2015). Regardless, the rates of ammonia-oxidation are thought to be dependent on both biotic and abiotic features: for example, the ratio of AOB to AOA within a soil community (Tourna *et al.*, 2008, Liu *et al.*, 2015) has been shown to influence the rate of nitrification by up to three orders of magnitude (Hu *et al.*, 2015). There is also a growing body of evidence that soil warming negatively affects the relative abundances of AOB and AOA, such that population changes have the potential to substantially influence local nitrification rates (Jung *et al.*, 2011, Auyeung *et al.*, 2015), particularly in cold soil environments.

Since Antarctic desert soils have been shown to harbour detectable populations of both AOA and AOB (Magalhães *et al.*, 2014), these ecosystems would be excellent models for exploring the responses of these organisms to warming. Increases in mean or peak temperatures in Antarctic soils have the potential to affect the growth and activities of ammonia oxidisers in different ways as previous studies have shown using non-Antarctic soils (Auyeung *et al.*, 2015). Small temperature increases are likely to increase the functionality of AOB in line with Arrhenius behaviour, while larger increases, where peak soil temperatures exceed the temperature limits of the organisms or their ammonia-oxidising enzymes, would have a deleterious effect on *in situ* nitrification. Alternatively, the loss of thermosensitive keystone taxa due to persistent or peak soil warming might result in shifts from cold-adapted to more thermotolerant species (Philippot *et al.*, 2013). We hypothesised that exposure to warming over the short-term may alter AOB community composition with concomitant increases in enzymatic activities and total soil biomass. Using replicated microcosm experiments,

we simulated a soil warming scenario of 15°C, which is markedly higher than current mean annual air temperatures in the Taylor Valley but is consistent with temperatures observed on north-facing slopes in the austral summer. We assessed functional responses to Antarctic soil warming by monitoring ammonia-oxidising bacterial diversity (using *amoA* gene specific PCR and sequencing), microbial community composition (using phospholipid fatty acid analysis; PLFA) and extracellular enzymatic activities.

Materials and Methods

Sample Collection and Soil Physicochemical Analyses

Bulk surface soil (~6 kg) was aseptically collected from the Taylor Valley, Eastern Antarctica (77.658°S, 163.092°E) in January 2014 as detailed previously (de Scally *et al.*, 2016). Soils were stored in a polyethylene bag below freezing during transit to the laboratory (Centre for Microbial Ecology and Genomics, University of Pretoria, South Africa), and at -80°C until processing. At the laboratory, a 2-mm sieve was used to remove small stones from the composite soils. Soil physicochemical analyses were performed using standardised procedures (Sparks *et al.*, 1996) at the Central Analytical Facility, Stellenbosch University, South Africa as described previously (de Scally *et al.*, 2016). Total soil carbon (TC), total soil nitrogen (TN), nitrogen available as ammonium (NH_4^+-N) and nitrogen available as nitrate (NO_3^--N) were obtained by combustion analysis on a LECO TruSpec® Elemental Determinator (LECO Corporation, St. Joseph, MI, USA). Soil pH was measured in triplicate using ~2 g soil samples by preparing 1:2.5 soil to deionised water [w:v] slurry solutions with a Crison Bench pH meter (Crison, Alella, Spain) as detailed previously (Eckert & Sims, 1995, Kamutando *et al.*, 2017).

Experimental manipulations

Microcosm experiments were used to determine the effect of exposure to an elevated temperature on Antarctic soil AOB composition and function. For acclimatization and establishment, soils were incubated at -20°C for two days prior to the start of the experiment. After this period, 50 g of soil was added twenty-four sterile 50 ml Schott bottles, each representing a microcosm. Three replicates of

50 g each were also collected 10 days before the start of the experiment as detailed previously (de Scally *et al.*, 2016). These samples represent the control microbial community composition (i.e. pre-incubation controls). Next, 12 microcosms were incubated in a Labotec IncoCool (Labotec, Johannesburg, South Africa) incubator set to 0°C (baseline temperature) while the remaining 12 microcosms were initially incubated at 1.5°C, with the temperature gradually increased by 1.5°C per day to a final constant temperature of 15°C. This time point was Day 0, at which point three soil microcosms were randomly selected from both baseline and elevated temperature groups and sampled for subsequent analyses. Over the course of the experiment, we randomly selected triplicate soil microcosms from each treatment. Samples were collected 5, 15 and 30 days after Day 0 to provide a final sample size of 27 soil samples.

Metagenomic DNA Isolation, PCR amplification and sequencing

DNA was extracted from 2 g soil from each sample (n=27, 12 of each temperature regime, with three controls) using the PowerSoil® DNA Isolation Kit (MoBio, Carlsbad, USA) as per the manufacturer's instructions. DNA was amplified by single-step PCR using the HotStarTaq Plus Master Mix Kit (Qiagen, Chatsworth, USA) using the universal bacterial primer set bac-*amoA*-1F and bac-*amoA*-2R (Rotthauwe *et al.*, 1997), which target regions flanking the bacterial ammonia monooxygenase (*amoA*) gene to produce 458bp amplicons. The process was repeated using the universal archaeal primer set arch-amoA-1F and arch-amoA-2R, which produce a 635 bp fragment as described previously (Francis *et al.*, 2005). Amplicons from each sample were pooled in equal concentrations and purified using Agencourt AMPure Beads (Agencourt Bioscience Corporation, USA). The samples were then submitted for 2 x 250 bp paired-end sequencing on an Illumina MiSeq platform (www.mrdnalab.com, Shallowater, TX, USA).

Sequence Data Processing and Phylogenetic Tree Building

Forward and reverse paired sequences were merged after their barcodes had been removed using mothur (Schloss *et al.*, 2009). Sequences with ambiguous bases (internal *N*'s) and those shorter than 150 bp in length were discarded (Schmieder & Edwards, 2011). Sequences were then denoised in mothur, chimeras were removed using Uchime v.4.2.40 (Edgar *et al.*, 2011), and operational taxonomic units (OTUs) were clustered at 3% divergence (97% similarity as per Alves *et al.* (2018))

against the entire NBCI nt-database using NCBI Blast+ 2.9 (Altschul *et al.*, 1990). Matches were considered significant if *E*-values were below 1 X 10^{-3} and bit scores above 60. We confirmed the OTU descriptions by identifying amplicon sequence variant (ASVs) using dada2 (Callahan *et al.*, 2016) within a functional gene pipeline designed for *amoA* phylogeny (Aigle *et al.*, 2019), however, since these results were not significantly different we use the OTU descriptions throughout the manuscript.

We built a Neighbour-Joining tree (Saitou & Nei, 1987) using our most common *amoA*-AOB sequences and included 100 reference *amoA*-AOB sequences from NCBI, which were collated and aligned in MAFFT v7 (Katoh & Standley, 2013). The tree also included an *amoA*-AOA sequence as the outgroup. The final tree is based on 1,000 replications of the tree build and bootstrap values support the phylogenetic placement of the branches. The final tree was visualised in iTOL v3 (Letunic & Bork, 2006).

Statistical Analyses

Statistical analyses were performed in R v.3.4.3 using the *vegan* package (Oksanen *et al.*, 2007), in QIIME v.1.9.1 (Caporaso *et al.*, 2010) and in STAMP (Statistical Analysis of Metagenomic Profiles) v.2.1.3 (Parks *et al.*, 2014) to test for significant differences in the taxonomic profiles of each temperature regime (baseline *vs*. elevated). Significant differences in community composition between the treatments (baseline vs. elevated) were tested using the *adonis* function (PERMANOVA) after 999 permutations in R (Anderson & Walsh, 2013), while the within group variation was assessed using the *betadisper* function. Differences in OTU counts were tested using ANOVA. A redundancy analysis (RDA) was used to examine explanatory soil physicochemical variables which could explain differences in community composition using OTU relative abundance data. Kruskal-Wallis tests were used to determine significant differences between abiotic variables across the temperature treatments.

Extracellular Enzymatic Assays

To quantify proxies for microbial nutrient acquisition and turnover processes, we measured various extracellular enzymatic activities [alkaline phosphatase (AP)], leucyl aminopeptidase (LAP), N-

acetyl-β-glucosaminidase (NAG), β-1,4-glucosidase (BG), β-D-xylosidase (BX), phenol oxidase (PO) and phenol peroxidase (PPO), as described previously (de Scally *et al.*, 2016). We used L-3,4-dihydroxyphenylalanine (L-DOPA) as a substrate analogue for colorimetric assays, and 4-methylumbelliferyl (MUB) and L-Leucine-7-amido-4-methylcoumarin (AMC) substrate analogues for fluorescent assays (Sinsabaugh *et al.*, 2008). Briefly, sample slurries were prepared by mixing 3 g of soil material with 100 ml Tris-Cl buffer (0.1 M, pH 7.9). Next, 200 µl slurry aliquots and 50 µl of the substrate-analogue dilution were mixed in 96-well microplate wells, providing eight technical replicates for each enzymatic assay (n=8) per sample. Plates were then incubated for 2 hours in the dark at 0°C for baseline soil communities and at 15°C for elevated-temperature soil communities. We measured absorbance on a Multiskan GO spectrophotometer (ThermoScientific, Louis, USA) and fluorescence using a Spectramax® Paradigm Multi-Mode Microplate Reader (Molecular Devices, USA). Enzymatic activities were calculated using the methodology described by Sinsabaugh and co-workers 2008).

Phospholipid Fatty Acid Analysis

We performed phospholipid fatty acid (PLFA) analyses at the Unit for Environmental Sciences and Management (North-West University, South Africa) to both quantify total microbial biomass and to profile microbial community composition. Triplicate soil samples were used whereby total lipids were extracted from approximately 5 g of lyophilised soil according to a modified Bligh and Dyer procedure using a single-phase chloroform-methanol-phosphate buffer system in a ratio of 1:2:0.8 (v/v/v) (Peacock & White, 2016). Silicic acid column chromatography was used to fractionate lipids into polar, neutral and glycolipids. For this study, only the polar lipid fractions were used to prepare fatty acid methyl esters (FAMEs) by mild alkaline methanolysis. FAMEs were separated, quantified and identified using a combination of gas chromatography and mass spectrometry (Peacock & White, 2016). Microbial biomass was estimated as the total extractable PLFA and community composition was based on the relative concentrations of individual fatty acids within each of the three replicate samples.

Results

Most soil samples were slightly alkaline (mean soil pH 7.7). Soil pH ranged from slightly acidic (6.9) to moderately alkaline (9.2; Table 1). TC was significantly higher in soils exposed to elevated temperatures than baseline soils (mean elevated 0.3% *vs* mean baseline 0.2%; ANOVA, $P < 0.05$). TN remained unchanged across treatments (TN; 0.02% *vs* 0.03%). A striking observation was that soil NH_4^+-N was depleted in elevated-temperature soils compared to baseline soils (3.7 mg/kg *vs* 7.8 mg/kg; Fig. 1*A*), although this was not statistically supported. In contrast, soil nitrate content remained unchanged independent of differences in incubation temperature (1.4 mg/kg *vs* 1.4 mg/kg; Fig. 1*B*; Table 1).

We analysed PLFAs as a measure of total cellular biomass, which we then used to quantify changes in microbial community composition across the treatments (Table 2). The initial PLFA at Day -10 estimated basal microbial community biomass at 204 pmol/g. The FAME groups, as defined by Boschker and Middelburg 2002), indicated that bacterial signatures were dominated by Gram-negative taxa (166 pmol/g), with lower proportions of both 'general' bacteria (33 pmol/g) and eukaryotes (6 pmol/g). Persistent exposure to elevated temperature was linked to significant decreases in total biomass (~183 pmol/g on average after 30 days of incubation; ANOVA, $P < 0.05$). In contrast, the effect of exposure to an temperature of 0°C significantly increased soil biomass in the baseline samples relative to the initial community biomass (~225 pmol/g on average after 30 days; ANOVA, $P < 0.05$; Fig. 2). The increases in microbial biomass were also characterized by reduced variation among the triplicates. The contribution of each fatty acid methyl ester to the overall community biomass is presented in Table 2.

Next we determined the activity of seven extracellular enzymes, as accepted proxies for microbial metabolism (Sinsabaugh *et al.*, 2008). Enzymatic activity was detectable for only three of the seven enzymes tested. Notably, phenol peroxidase (PPO) showed the highest overall activity (118 nmol h^{-1} g^{-1}), followed by phenol oxidase (PO; 67 nmol h^{-1} g^{-1}) and leucine aminopeptidase (LAP; 48 nmol h^{-1} g^{-1}). All three enzymatic activities were significantly increased by incubation at 15°C compared to the baseline soils (ANOVA, $P < 0.05$; Fig. 3).

Sequencing generated 3,079,284 *amoA*-AOB gene sequences (mean length 491 bp) across the 27 soil microcosm samples (12 from each temperature treatment, and 3 controls). We chose to analyse one of three technical replicates from each microcosm at each time point for both temperature treatments to eliminate samples with a poor representation of *amoA*-AOB sequences (low sequencing depth). For all downstream analyses, we randomly rarefied each of the remaining communities (n=9) to 96,308 *amoA* sequences, which was the lowest sequence number from the retained samples. After removing singletons and OTUs present only in a single sample, the dataset comprised 74 unique *amoA*-AOB OTUs across the 9 soil microcosms. We found no significant differences in AOB community composition relating to the effect of temperature (PERMANOVA, $P > 0.05$).

Since temperature had no significant impact on community composition, we used redundancy analysis (RDA) to investigate whether soil physicochemistry could be more important. RDA showed that soil carbon (TC) significantly affected the composition of the ammonia oxidising community, based on *amoA* gene sequences (Kruskal-Wallis test; $P < 0.05$; Fig. 4). This result is due to the increase in soil TC (%) in samples exposed to elevated temperature. Our sequence analysis showed that two unique AOBs dominated all soil communities, accounting for 95% of all reads (Fig. 4*B*). Both *amoA*-AOB OTUs showed highest sequence homology to *Nitrosospira multiformis* (Norton *et al.*, 2008), although they only shared 93.7% identity across the full-length *amoA* gene sequence. Most *amoA*-AOB sequences (64.6%) were most closely related to the environmental clone D-4 (NCBI accession: HM481196.1), while 30% were assigned as environmental clone D-1 (HM481193.1) which were identified in Chinese wetland soils (Li *et al.*, 2011). Placing our three most abundant *amoA*-AOB gene sequences within the context of publicly available *amoA*-AOB genes indicated that the Antarctic strains form a separate clade within the phylogenetic tree (Fig. S1).

Only 13 OTUs were present in all nine of the soils analysed. Within the temperature treatment groups, we found that the baseline communities had 67 *amoA*-AOB OTUs (γ-diversity), while elevated-temperature communities had 64 *amoA*-AOB OTU. In total, 57 OTUs were shared between the groups. The pre-treatment (Day -10) control community harboured 44 *amoA*-AOBs OTUs. By

the end of the experiment (Day 30), the baseline soil communities had an average of 44 ± 7.6 *amoA*-AOB OTUs per sample, whereas the elevated soil communities had 36.5 ± 5.8 *amoA*-AOB OTUs.

Discussion

The objective of this study was to investigate the effects of temperature increases on biogeochemical cycling, with a focus on nitrogen cycling, and microbial biomass dynamics in Antarctic soils. Consequently, we constructed Antarctic soil microcosms and simulated short-term temperature increases, focusing on ammonia-oxidizing bacteria. Although previous studies have assessed community level responses to perturbation (de Scally *et al.*, 2016) and demonstrated that Antarctic soil microbial communities are generally resilient and functionally redundant. However, the extent to which these community level responses may be true for important functional processes is ambiguous. Moreover, the effects of increased temperatures on functional processes in extreme systems, such as Antarctic terrestrial soils, is underexplored. Using combined results from physicochemical analysis, functional activity measurements and microbial community composition we show that augmented soil temperatures may reduce microbial biomass levels. However, this lower microbial biomass appears to coincide with increases in some functional activities, at least in the short term immediately after the temperature change. Through measuring ammonia-oxidizing bacteria, our findings support previous studies showing that these systems may be functionally redundant.

During the manipulation experiments, we observed significant changes for some soil physicochemical characteristics between the different temperature regimes. Most notably, TC increased significantly in the soil microcosms exposed to elevated temperatures compared to their corresponding baseline soils. This may due to differing rates of cellular respiration, which have previously been shown to be increased at higher soil temperatures across a range of different soil types (Karhu *et al.*, 2014). While Karhu and colleagues (2014) included a wide range of Arctic and boreal soils, the precise response of Antarctic soils to temperature remain unclear. Several samples with low levels of TC similar to oligotrophic Antarctic soils were included and showed enhanced responses to increased temperatures, while other soils appeared to exhibit compensated responses

(Karhu *et al.*, 2014). These variable responses may be due to soil microbial communities (Doetterl *et al.*, 2015, Wieder *et al.*, 2015, Bradford *et al.*, 2016, Cavicchioli *et al.*, 2019). In a scenario where Antarctic soils are dominated by chemoautotrophs or photoautotrophs; higher temperatures would result in overall cell activity increases leading to higher CO_2 fixation due to autotrophic activity. As our experimental simulation was under austral summer conditions (with persistent light) this autotrophic activity would result in increased TC consistent with our result. Conversely, the reduced respiration rates in low temperature soils may deplete soil carbon available for bacterial production (Anderson, 2003). Our data suggest that a result of exposure to 15°C may be reduced soil biomass. This is likely due to several related reasons including increased CO_2 production, nutrient depletion and cell death. The results from extracellular enzymatic assays (β-glucosidase and β-xylosidase), which showed little to no activity for C-acquiring enzymes at higher temperatures, support our conclusion.

The exposure of Antarctic soil microcosms to elevated temperatures led to a significant decrease in total microbial biomass (as indicated by PLFA analysis). Significant decreases in soil microbial biomass are consistent across soil types during incubations at temperatures between 5 and 25°C (Curtin *et al.*, 2012). We interpret this finding as the direct result of microbial mortality at higher temperatures, which results in accelerated degradation of cellular components. However, this interpretation assumes that a high percentage of the community is psychrophilic (Cowan *et al.*, 2014) and that these taxa may not be functionally resistant to temperature increases as shown by previous studies (de Scally *et al.*, 2016). Under this scenario, the initial decrease in biomass could be caused by the lysis of dead microbial cells, with the surviving population scavenging liberated nutrients for cellular respiration (Burkins *et al.*, 2001). There is some evidence suggesting that autotrophic carbon fixation may be upregulated in Antarctic soils as a result of warming (Hopkins *et al.*, 2006, Novis *et al.*, 2007), which may also explain the observed increase in TC. However, we cannot exclude the possibility that the sampling time regime we implemented (sampling over days rather than hours), may fail to capture more nuanced cellular kinetic processes occurring within the first few hours of warming.

Analysis of signature lipid biomarkers (Willers *et al.*, 2015) provided estimates of soil microbial community composition. The phylogenetic composition of these experimental manipulations was broadly consistent to previous studies, in which Gram-negative *Proteobacteria*, *Bacteroidetes* and *Acidobacteria* were found to typically dominate surface soils of the Antarctic Dry Valleys (Wang *et al.*, 2015, Van Goethem *et al.*, 2018). The low levels of eukaryote-assigned FAMEs also supports previous literature which reports low proportions of eukaryotic phyla in Antarctic open soil communities (Makhalanyane *et al.*, 2015, Niederberger *et al.*, 2015, Obbels *et al.*, 2016, Adriaenssens *et al.*, 2017). Irrespective of the experimental temperature manipulations, the proportion of eukaryotes remained low, suggesting that their overall biomass was not influenced by an altered climatic regime. However, we cannot distinguish between eukaryotic taxa, and so there may be a possibility that these shifts may be clearer at lower taxonomic ranks.

Interestingly, our *amoA* gene sequence data showed no significant changes in ammonia-oxidising bacterial community composition between the high temperature and baseline microcosms, even though enzymatic activities and PLFA profiles were affected by incubation temperature. The absence of significant changes in *amo*A-specific community composition may be due to the dominance of only two OTUs in all soils which together accounted for upwards of 95% of total *amo*A sequences. Both OTUs were most assigned as *Nitrosomonas multiformis* (*Betaproteobacteria*). *N. multiformis* commonly dominates ammonia-oxidising bacterial populations in soil (Garbeva *et al.*, 2007). Our *N. multiformis* OTUs matched AOBs previously identified in Chinese wetland soils (Li *et al.*, 2011), although our sequences formed a separate clade within the phylogenetic tree. The sequence similarity across *amo*A genes was below 95%, typically assumed to be the genus level threshold. Whether these are novel AOB which may be unique to Antarctic soils, is unclear. It may well be that ammonia-oxidizing archaea play a larger role in Antarctic soil nitrogen cycling than previously assumed. While we did not retrieve sufficient sequence data to test and support this assertion, previous studies have shown that AOA are core constituents of Antarctic soils, despite comprising only a low proportion of taxa (Jung *et al.*, 2011). It is reasonable to predict that in these oligotrophic soils, AOA may be the primary drivers of nitrification as in other arid soils (Banning *et al.*, 2015).

By the end of the manipulation experiment, soils exposed to elevated temperatures had lower concentrations of nitrogen, as ammonium, compared to baseline soils. Values for soil $N\text{-}NH_4^+$ were similar to those reported for maritime Antarctic soil core microcosms following six months of exposure to elevated temperatures (Yergeau & Kowalchuk, 2008). We had expected concomitant increases in the concentration of $N\text{-}NO_3^-$, given our expectation that ammonia would be converted to nitrate via nitrification (Prosser, 1990). Instead, we found that the proportion of soil nitrogen available as nitrate showed little quantitative change across the two temperature regimes.

The analyses of *amo*A gene sets, total soil biomass, extracellular enzymatic activity measurements and soil physicochemical analyses together show the response of Antarctic functional guilds to increased temperature. Our experimental manipulation suggests that increased temperatures may trigger short term shifts in viable microbial biomass. We show, for the first time, that these shifts may coincide with substantial increases in peptide hydrolysis and phenolic oxidation. This is in contrast to activities related to the degradation of carbon substrates. We also found that the composition of AOBs in these depauperate soils was significantly shaped by TC, which increased throughout the experimental manipulation. We suspect that warming likely results in increased conversion rates of NH_4^+ to nitrate, decreasing available NH_4^+ levels in soils. Our experimental manipulation study evidence of potential effects of warming on Antarctic functional processes and activities. We suggest that these results have implications for future climate scenarios, as increased mean temperatures may result in decreases in soil biomass in hyper-arid Antarctic soils. However, whether the effects of warming shown here may hold for natural cold desert systems is unclear. Transplant experiments in other systems, over a longer period with increased sampling to assess the kinetic variations during the earlier stages, may provide answers on how warming may ultimately affect microbial communities. These data may, in turn, provide a holistic understanding on the impacts of warming on functional processes and whether microbial communities may exacerbate the effects of change.

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Data availability

The sequence datasets generated in this study can be found on the NCBI Sequence Read Archive under the accession number PRJNA557209.

Conflict of Interest

The authors declare no conflict of interest.

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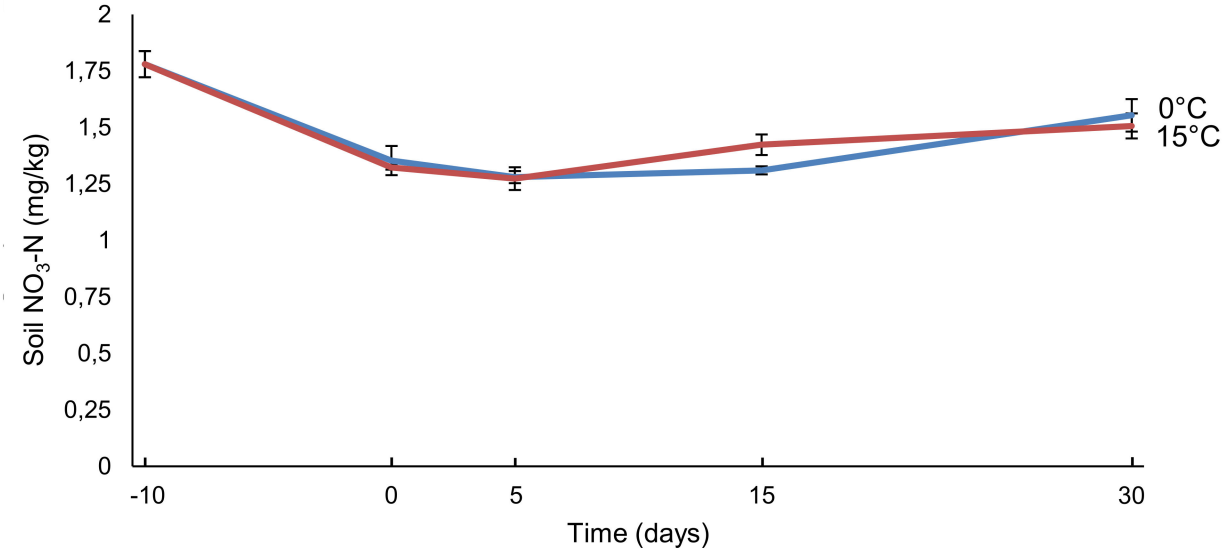
List of Figures and Tables

Supplementary Material


2
1,75
1,5
1,25
1
0,75
0,5
0,25
0
Soil NO_3-N (mg/kg)
-10
0
5
15
30
Time (days)
0°C
15°C

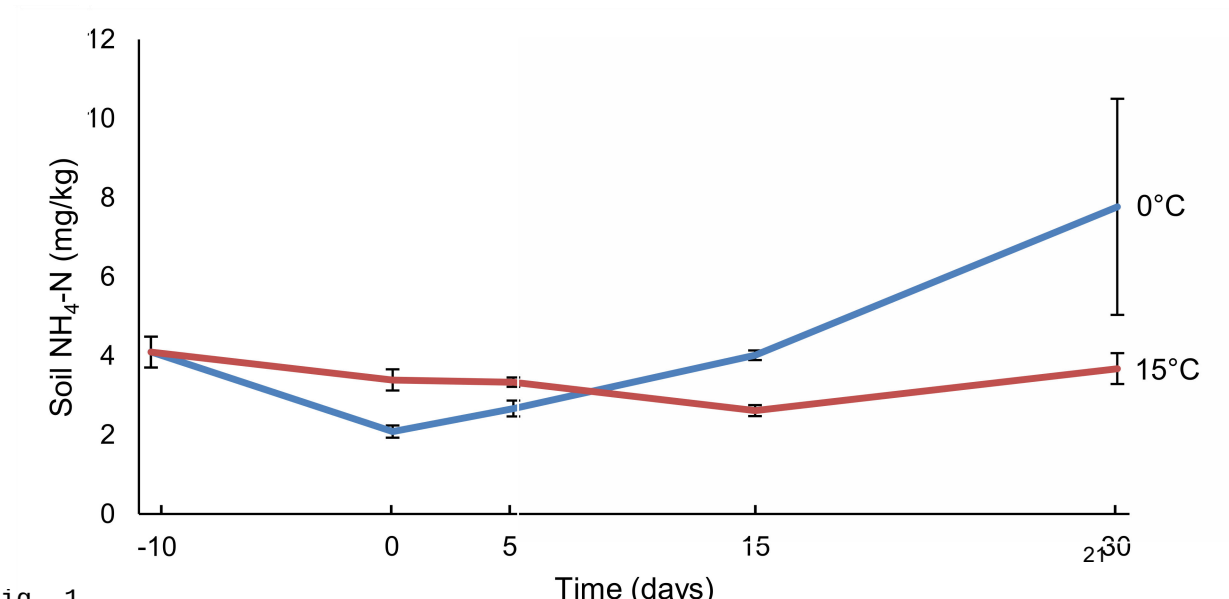

12
10
8
6
4
2
0
Soil NH_4-N (mg/kg)
-10
0
5
15
30
Time (days)
0°C
15°C

Fig. 1

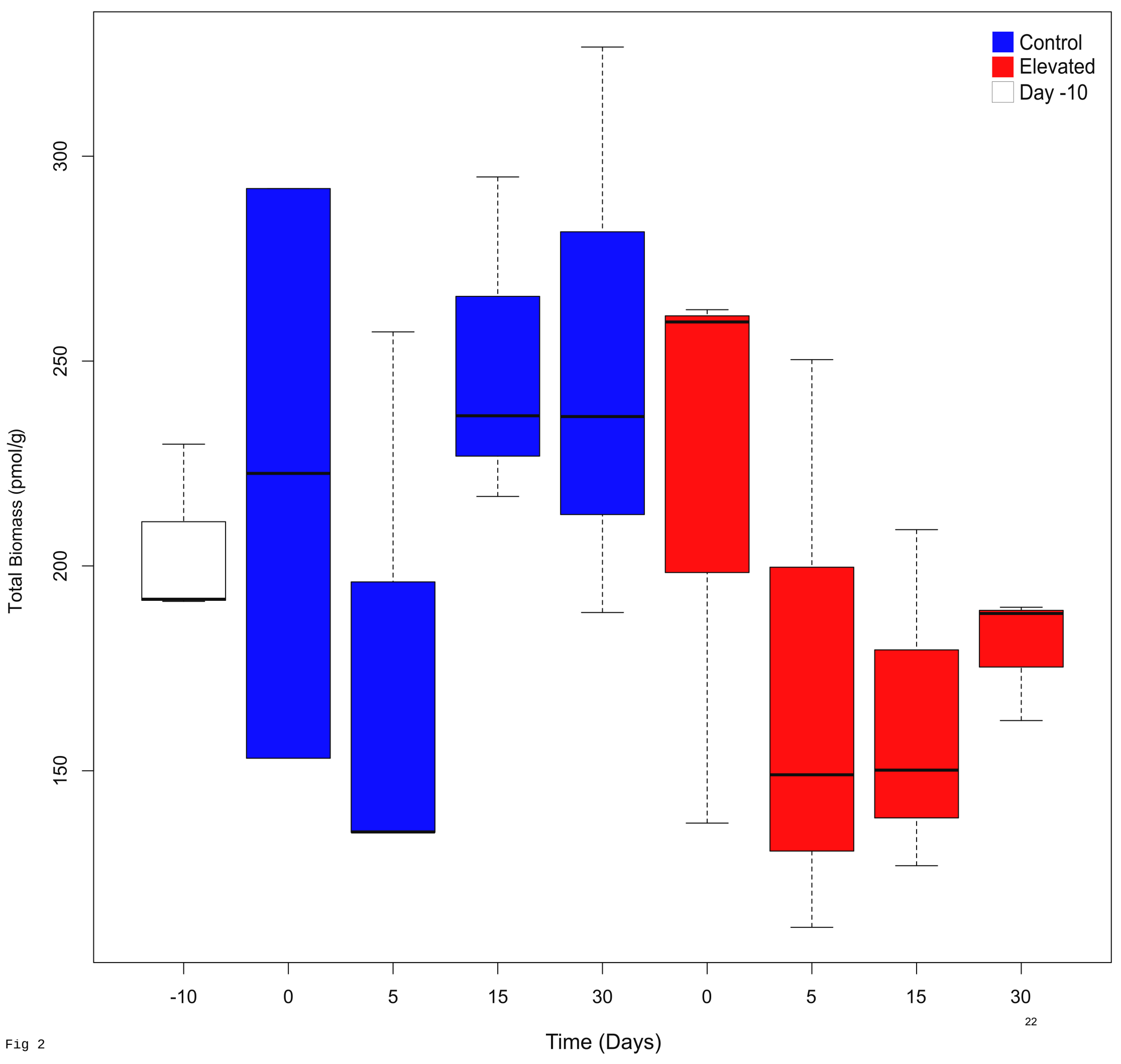
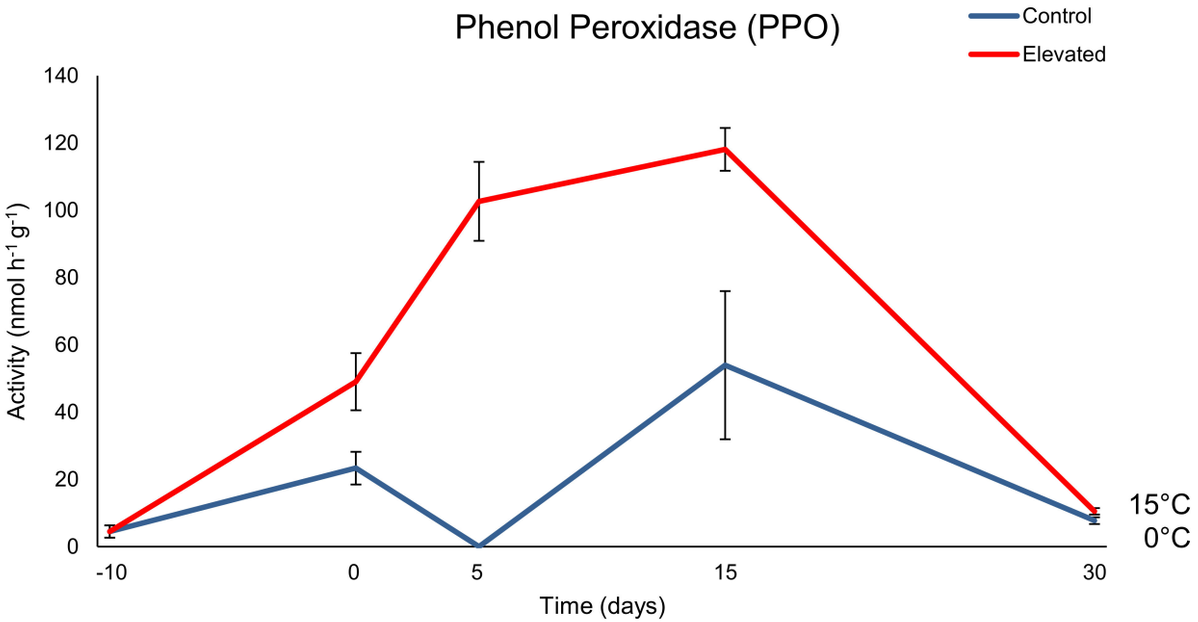
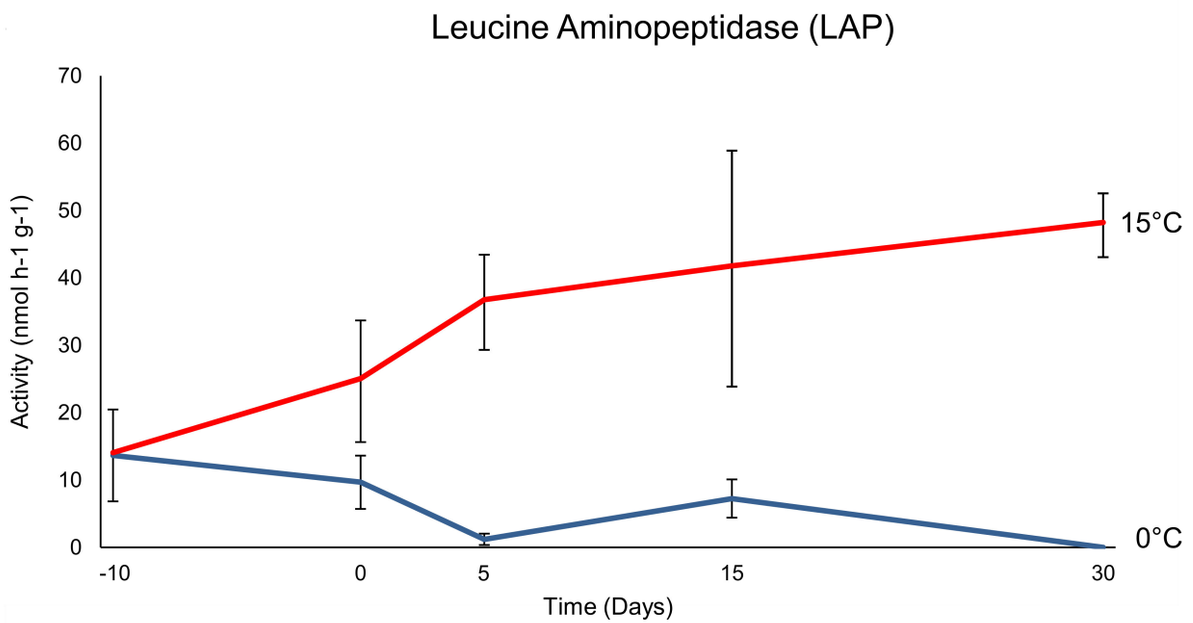

Control
Elevated
Day -10
Total Biomass (pmol/g)
300
250
200
150
-10
0
5
15
30
0
5
15
30
Time (Days)

Fig 2


Phenol Peroxidase (PPO)
Control
Elevated
Activity (nmol h-1 g-1)
140
120
100
80
60
40
20
0
-10
0
5
15
30
Time (days)
15°C
0°C


Leucine Aminopeptidase (LAP)
Activity (nmol h-1 g-1)
70
60
50
40
30
20
10
0
-10
0
5
15
30
Time (Days)
15°C
0°C

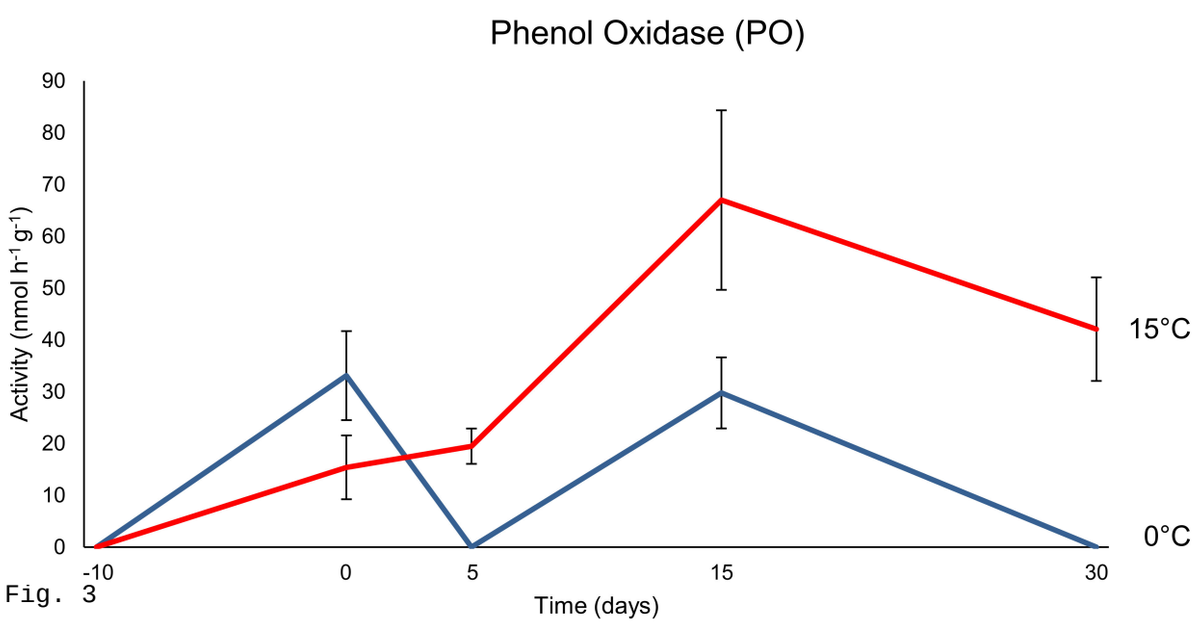

Phenol Oxidase (PO)
Activity (nmol h-1 g-1)
90
80
70
60
50
40
30
20
10
0
-10
0
5
15
30
Time (days)
15°C
0°C

Fig. 3

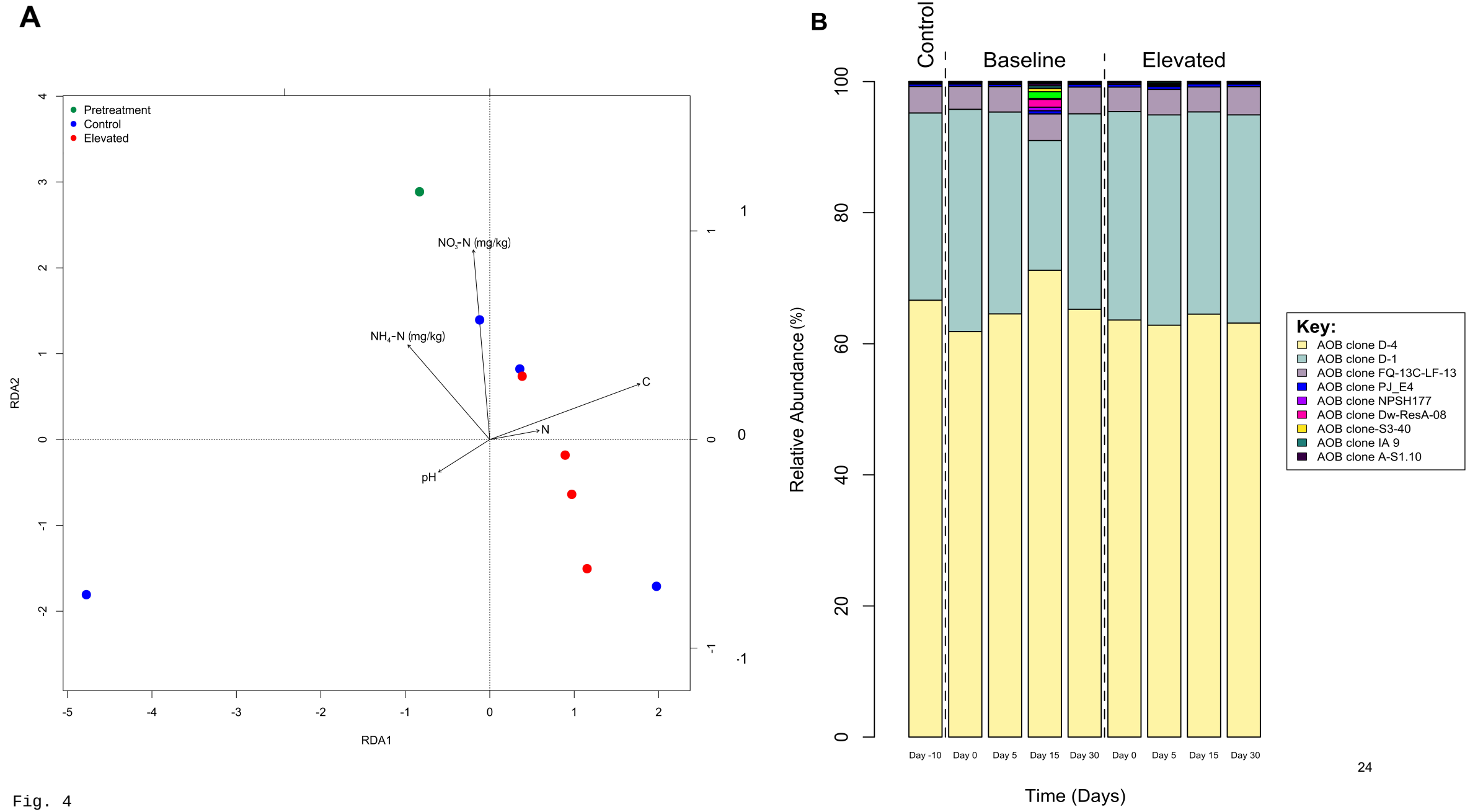
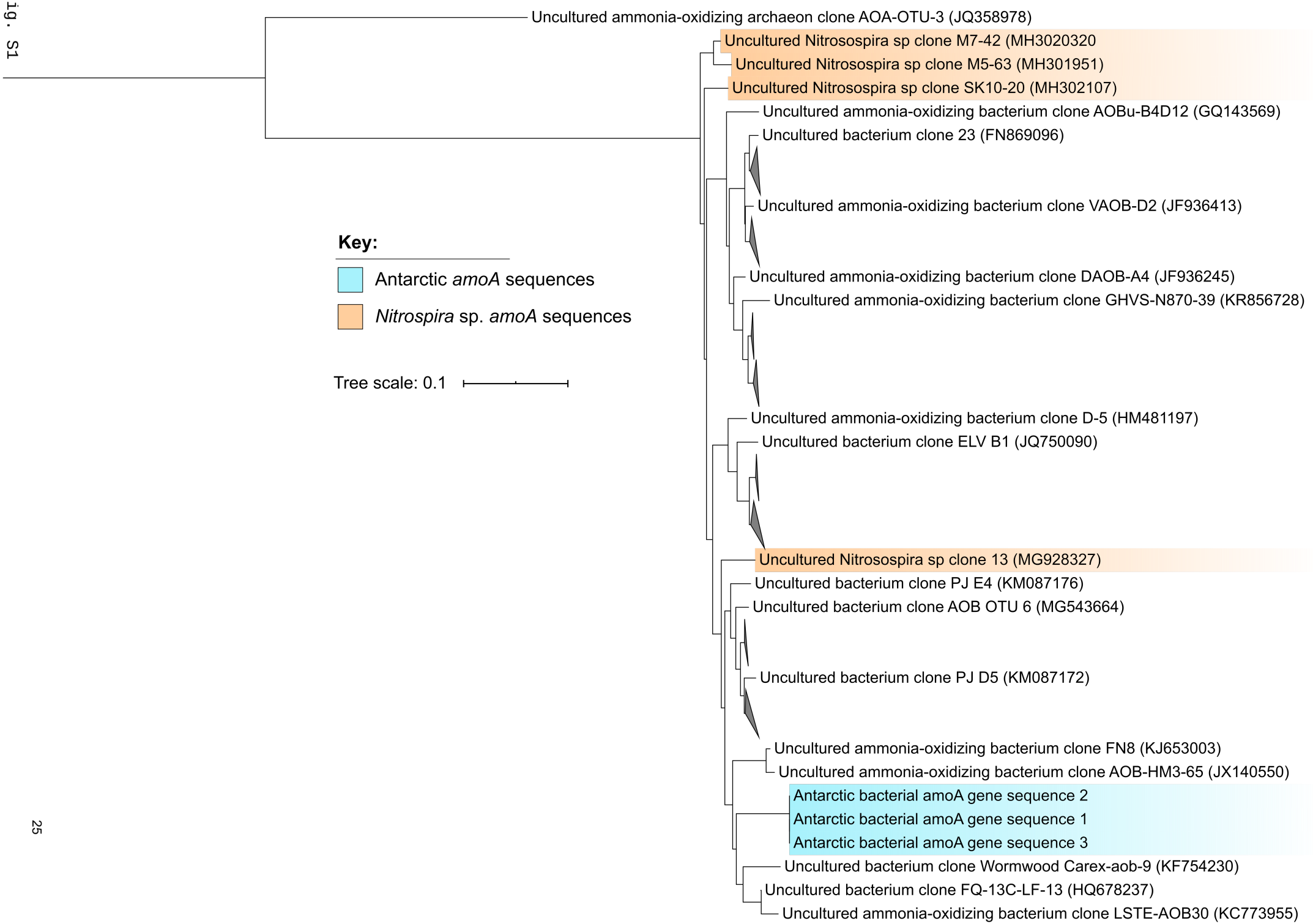

A
Pretreatment
Control
Elevated
NO3-N (mg/kg)
NH4-N (mg/kg)
C
N
pH
RDA1
RDA2
B
Control
Baseline
Elevated
Relative Abundance (%)
Day -10
Day 0
Day 5
Day 15
Day 30
Day 0
Day 5
Day 15
Day 30
Time (Days)
Key:
AOB clone D-4
AOB clone D-1
AOB clone FQ-13C-LF-13
AOB clone PJ_E4
AOB clone NPSH177
AOB clone Dw-ResA-08
AOB clone-S3-40
AOB clone IA 9
AOB clone A-S1.10

Fig. 4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Uncultured Nitrosospira sp clone M7-42 (MH3020320
Uncultured Nitrosospira sp clone M5-63 (MH301951)
Uncultured Nitrosospira sp clone SK10-20 (MH302107)
Uncultured ammonia-oxidizing bacterium clone AOBu-B4D12 (GQ143569)
Uncultured bacterium clone 23 (FN869096)
Uncultured ammonia-oxidizing bacterium clone VAOB-D2 (JF936413)
Uncultured ammonia-oxidizing bacterium clone DAOB-A4 (JF936245)
Uncultured ammonia-oxidizing bacterium clone GHVS-N870-39 (KR856728)
Uncultured ammonia-oxidizing bacterium clone D-5 (HM481197)
Uncultured bacterium clone ELV B1 (JQ750090)
Uncultured Nitrosospira sp clone 13 (MG928327)
Uncultured bacterium clone PJ E4 (KM087176)
Uncultured bacterium clone AOB OTU 6 (MG543664)
Uncultured bacterium clone PJ D5 (KM087172)
Uncultured ammonia-oxidizing bacterium clone FN8 (KJ653003)
Uncultured ammonia-oxidizing bacterium clone AOB-HM3-65 (JX140550)
Antarctic bacterial amoA gene sequence 2
Antarctic bacterial amoA gene sequence 1
Antarctic bacterial amoA gene sequence 3
Uncultured bacterium clone Wormwood Carex-aob-9 (KF754230)
Uncultured bacterium clone FQ-13C-LF-13 (HQ678237)
Uncultured ammonia-oxidizing bacterium clone LSTE-AOB30 (KC773955)
Key:
Antarctic amoA sequences
Nitrospira sp. amoA sequences
Tree scale: 0.1