Antarctic microbial communities are functionally redundant, adapted and resistant to short term temperature perturbations

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Abstract

Climate change has the potential to induce dramatic shifts in the biodiversity and functionality of soil microorganisms in polar hyperarid ecosystems. In these depauperate soil ecosystems, microbial communities are vital as they represent the dominant input sources of essential nutrients. However, the effects of changing climate on extreme edaphic environments, such as the McMurdo Dry Valleys of Antarctica, remain poorly understood. To better understand these effects, we constructed soil microcosms and simulated temperature shifts over a 40-day period. Soil physicochemical analysis revealed low levels of key nutrients, with mean organic carbon and nitrogen contents of <0.1% and 11.55 ppm, respectively. We also applied 16S rRNA gene amplicon sequencing to determine taxonomic composition and enzyme assays to measure *in situ* activity. Our data showed a prevalence of ubiquitous soil taxa (Actinobacteria, Chloroflexi and *Deinococcus-Thermus*), with a smaller proportion of autotrophic phyla (i.e. Cyanobacteria). None of the major phyla showed relative abundance changes in response to temperature. We found very low extracellular enzyme activity levels across all samples and observed no significant differences among temperature treatments. Functional predictions (using PICRUSt) revealed the putative presence of key genes implicated in the cycling of carbon (*ppc, rbcl*) and nitrogen (*nifH*, *nirK*), in stress response and in DNA repair throughout all treatments. Overall, our results suggest that short-term temperature fluctuations do not alter microbial biodiversity and functionality in Antarctic soils. This study provides the first evidence that microbial communities within this edaphic extreme environment may be functionally redundant, adapted and resistant to short term climatic perturbations.

1. Introduction

Global temperatures are expected to rise by up to 6.4°C within the next 100 years, largely due to the effects of anthropogenic climate change (IPCC, 2007; Singh et al., 2010; Clements et al., 2014). Rising temperatures lead to the melting of surface and shallow sub-surface ice, resulting in the mobilisation of water and nutrients in polar terrestrial habitats, which are large carbon reservoirs (Singh et al., 2010; Isbell et al., 2015). Microbial communities are the dominant biota within these terrestrial habitats and are also known mediators of key biogeochemical cycles, particularly carbon and nitrogen turnover (Singh et al., 2010; Cowan et al., 2014). It is therefore hypothesised that changes in soil microbial community diversity and function may lead to alterations in essential ecosystem cycles (Singh et al., 2010; Gutknecht et al., 2012). However, it remains unclear how soil microbial communities may respond to temperature increases (Cowan et al., 2014), especially within climatically sensitive regions such as the McMurdo Dry Valleys (MDVs) of Antarctica (Cowan and Ah Tow, 2004; Barrett et al., 2006; Pointing et al., 2009).

The MDV mineral soils represent an ideal ecosystem in which to assess the effects of climatic changes due to their trophic simplicity and microbially driven nature (Cowan and Ah Tow, 2004; Pointing et al., 2009). However, field studies are limited within the MDVs due to the challenging environmental conditions and strict regulations designed to protect the Antarctic region (Cowan and Ah Tow, 2004). Microcosms, which are laboratory based studies, allow the creation of artificial environments within which the effects of perturbations may be assessed under controlled conditions (Grenni et al., 2012; Martínez et al., 2014). Although microcosms cannot account for the complete complexity of the natural environment (Grenni et al., 2012), such studies have shown reliable results (Grenni et al., 2012; Blake et al., 2015) which are comparable to studies performed in the field (Treonis et al., 2002; Martínez et al., 2014).

The microbial diversity of MDV soils is surprisingly high (Aislabie et al., 2006; Niederberger et al., 2008; Dreesens et al., 2014; Richter et al., 2014). The microbial communities in these edaphic regions have also been shown to harbour the capacity for complex metabolic functions (Yergeau et al., 2007a; Hopkins et al., 2008; Chan et al., 2013), with multiple genes for key pathways in carbon and nitrogen cycling (Yergeau et al., 2007a; Chan et al., 2013). Soil respiration (Hopkins et al.,

2006), extracellular enzyme activities (Hopkins et al., 2008) and acetylene reduction (a valid proxy for nitrogen fixation) (Niederberger et al., 2008) have all been detected in MDV soils. These studies indicate that microbial communities within the MDV region have the capacity to contribute to functional ecosystem processes (Barrett et al., 2006; Cowan et al., 2014).

Microbial communities within Antarctic soils may respond relatively rapidly to environmental changes (Stomeo et al., 2012; Tiao et al., 2012). For instance, the repositioning of a mummified seal carcass led to rapid (within three years) changes in the underlying soil microbial community structure (Tiao et al., 2012). In addition, a number of studies assessing microbial diversity and abiotic factors along Antarctic latitudinal gradients have shown that community structure and functionality may be potentially altered in response to temperature (Rinnan et al., 2009; Newsham et al., 2015), moisture (Stomeo et al., 2012) and soil physiochemical status (Lee et al., 2012; Stomeo et al., 2012). However, how MDV soil microbial communities may respond directly to climatic perturbations, as well as how rapidly, is still unclear (García-Palacios et al., 2015).

Two hypotheses concerning the predicted response of microbial communities to climatic change have been proposed. The community diversity hypothesis (Isbell et al., 2015) suggests functional redundancy among organisms and that losses in diversity will not lead to decreases in function. Alternatively, the keystone species hypothesis (Pold and Deangelis, 2013) proposes that loss of a few key taxa will lead to lower functionality and thus there is little functional redundancy. In support of this hypothesis, studies have found that microbial abundance and diversity may increase in response to temperature changes, while maintaining functional capacity (Yergeau and Kowalchuk, 2008; Newsham et al., 2015). Similarly, a decline in microbial biomass in response to temperature can be associated with an increase in soil respiration (Laudicina et al., 2015). However, other studies have shown that higher temperatures lead to a decrease in microbial species richness (Jung et al., 2011; Dennis et al., 2013; Philippot et al., 2013). This may, in turn, lead to a reduction in functional gene abundance (Jung et al., 2011) and microbial activity (Hopkins et al., 2006; Philippot et al., 2013). Such conflicting results indicate that the exact effects of climatic changes on microbial diversity and functionality, and subsequent ecosystem processes, remain unresolved (García-Palacios et al., 2015).

Here, we examine the influence of short term temperature fluctuations on Antarctic soil microbial community diversity and potential functionality. Microcosms were constructed using soil samples collected from the MDVs. Three temperature treatments were then applied to the microcosms for a period of forty days, where a baseline sample was taken prior to the start of the experiment and samples were recovered periodically. We used 16S rRNA gene amplicon sequencing to assess microbial diversity changes. The activity of extracellular enzymes potentially involved in microbial nutrient acquisition were combined with PICRUSt and KEGG functional gene and pathway predictions to assess potential microbial functionality.

2. Materials and Methods

2.1 Soil sample collection and physicochemical analysis

Approximately 4kg of mineral soil was collected in the vicinity of Spalding Pond in the Taylor Valley region of the McMurdo Dry Valleys, Antarctica (77.65808° S, 163.09204° E) in January 2015 (Figure 1). Soil was collected aseptically by retrieving the top 5 cm of soil within a 20 x 20 cm sampling area. Soil was placed in sterile plastic bags and stored at -30°C. All necessary permits were obtained through Antarctica New Zealand and the New Zealand Ministry of Foreign Affairs and Trade (MFAT) to allow the removal of the soil. The soil was then shipped on dry ice to the Centre for Microbial Ecology and Genomics (CMEG) at the University of Pretoria, where it was stored at -80°C. Soil physiochemical analyses were performed at the Department of Plant Production and Soil Science of the University of Pretoria on three replicate soil samples prior to the microcosm experiment. Soil particle size was determined using the hydrometer method, as previously described (Bouyoucos, 1962). Percentage organic carbon analysis was performed according to the Walkley and Black method (Walkley and Black, 1934). Soil pH was determined using a glass electrode with a water-to-soil ratio of 2.5:1, according to specifications previously provided (Coleman, 1967). Ion concentrations were determined using potassium chloride (Bremner and Keeney, 1966), ammonium acetate (Chapman, 1965) and P- Bray I (Bray and Kurtz, 1945) extractions. The cation exchange capacity of the soil was determined according to a previously described method (Gillman et al., 1983).

2.2 Microcosm set-up

To determine the effect of increasing and fluctuating temperatures on Antarctic soil microbial communities, a microcosm experiment was designed. Soil samples were randomly assigned to three temperature treatment groups. Temperature simulations were applied over a period of forty days, wherein samples were taken 10 days prior to the start of the experiment and in 5 day intervals thereafter (Figure S1, S2). The treatment groups consisted of a control temperature group, at 0°C for the entire experiment and two temperature treatment groups. One treatment group, called "stable" was at a constant elevated temperature of 15°C. The other treatment group, called "fluctuating" varied from 0 to 15°C with 1.5°C increments per day, remained at 15°C for 10 days and was then decreased from 15 to 0°C with 1.5°C increments per day (Figure S1). The start of the experiment is indicated as day 0, where the control and fluctuating treatment groups were at 0°C, whereas the stable treatment group was at 15°C. Ten days prior to the start of the microcosm experiment, the stable treatment group was increased from 0 to 15°C with 1.5°C increments per day (Figure S1, day -10). Further details of the experimental procedure are present in Supplementary Methods and Materials and are available on the online version of this manuscript.

2.3 Microcosm experiment analysis

2.3.1 Microbial community analysis using Illumina-based amplicon sequencing

DNA extractions were performed using a MoBio PowerSoil® DNA Isolation Kit (MoBio, Carlsbad, USA) according to the manufacturer's instructions. The replicates of each treatment group taken at each sampling point were pooled prior to sequencing to obtain an equimolar concentration of each replicate in the final reaction. DNA samples were then sent to MRDNA (www.mrdnalab.com, Shallowater, USA) for sequencing. The primers used for PCR amplification were 515F (5′-GTGCCAGCMGCCGCGGTAA-3′) and 806R (5′-GGACTACVSGGGTATCTAAT-3′) (Caporaso et al., 2011), which specifically target both the bacterial and archaeal V4 hypervariable region of the 16S rRNA gene. The samples had a barcode added to the forward primer to allow for multiplex sequencing and paired-end sequencing was performed on an Illumina MiSeq platform according to the manufacturer's instructions.

Sequencing analyses were performed using the Quantitative Insights into Microbial Ecology (QIIME) software package (Caporaso et al., 2010). Sequences were demultiplexed, quality filtered and split into samples according to barcode sequences. Quality filtering involved removing sequences with less than 400 nucleotides, greater than 1000 nucleotides, an average quality score of less than 25, homopolymer runs larger than 6 bases, greater than 6 ambiguous bases, all primer mismatches and any barcode errors greater than 1.5. Chimeras were identified and removed using the USEARCH method and the Greengenes reference library (Desantis et al., 2006). Operational taxonomic unit (OTU) definition and taxonomic assignment was performed to the default of 97% sequence identity using the Greengenes reference library. Singletons were removed by specifying two as the minimum number of times an OTU must appear to be retained. The dataset was rarefied to 22,400 sequences per sample.

2.3.2 Microbial community potential functionality analysis

PICRUSt functional predictions

Functional pathway and marker gene prediction was performed using an OTU table generated from QIIME, PICRUSt software and KEGG orthology (Langille et al., 2013). Following the online protocol, the OTU table was normalized by copy number to ensure that the abundances of each OTU were normalised to the number of 16S rRNA genes occurring within a specific OTU. The normalised OTU table was then used to convert the 16S rRNA gene sequencing data into a predicted metagenome for each sample using the reference database. PICRUSt can only assign function to OTUs occurring within the reference (Greengenes) database and therefore New.ReferenceOTUs. and New.CleanUp.ReferenceOTUs were removed, decreasing the number of OTUs identified in the original dataset from 8,843 to 769. After a predicted metagenome was created from PICRUSt, cellular pathway and marker gene assignment to each sample was performed using KEGG orthology (Kanehisa and Goto, 2000; Kanehisa et al., 2014).

Extracellular enzyme assays

Potential activities of seven extracellular enzymes involved in carbon [β-glucosidase (BX), β-xylosidase (BG) and phenol oxidase/peroxidase (PO/PPO)], nitrogen [N-acetyl glucosaminidase

(NAG), leucine aminopeptidase (LAP)] and phosphorus [alkaline phosphatase (AP)] acquisition were assessed. Substrate analogues linked to 4-methylumbelliferone (MUB) or 7-aminocoumine (AMC) for the fluorescent assays and L-3,4- dihydroxyphylalanine (L-DOPA) for the colorimetric assays were used. The experimental procedure was performed as previously described (Sinsabaugh et al., 2008). Briefly, 5g of samples were mixed with 100 mL of 0.1 M Tris buffer (pH 9.9) and the resulting sample slurry was stirred to ensure homogenisation. Aliquots of 200 µL were added to 96-well microplates, with four replicated wells per sample per enzyme assay. Plates were then incubated for 3 hours at 7°C in the dark. Fluorescence and absorbance measurements were then taken on a Spectramax® Paradigm Multi-Mode Microplate Reader (Molecular Devices, USA) and a ThermoScientific Multiskan GO spectrophotometer (ThermoScientific, USA), respectively. Enzyme activities were calculated according to equations previously described (Sinsabaugh, 1994; German et al., 2011).

2.4 Statistical analysis

All statistical analyses were performed using either the R statistical package version 3.2.2 (R Core Team, 2013), or PAST version 2.17 (Hammer et al., 2004). Microbial diversity was analysed using the R statistical package. Alpha-, beta- and gamma- diversity were calculated using the *vegan* package in R. Significant differences in diversity metrics between treatment groups and sampling days were determined using a one-way ANOVA analysis via the *aov* function of the *vegan* package in R, as well as post-hoc TukeyHSD tests. Venn diagrams were generated with both the *vegan* package in R to create a biological distance matrix using Bray-Curtis similarity and the *gplots* package to create the final diagram. Permutational multivariate analysis of variance (PERMANOVA) was used to detect significant differences in microbial community composition between treatment groups and sampling days. PERMANOVA was performed using the *adonis* function in R with 999 random permutations to determine statistical significance. Analysis of similarity (ANOSIM) was performed on taxonomic data to analyse the similarity between treatment groups using the *anosim* function of the *vegan* package in R with 999 random permutations.

A two-dimensional non-metric multidimensional scaling (NMDS) ordination of sequencing data was generated using Bray-Curtis similarity and the *meta.nmds* function of the *vegan* package in R. A

redundancy analysis (RDA) was performed on sequencing data using the *rda* function of the *vegan* package in R. Enzyme assays were added to the RDA ordination plot as vectors using the *envfit* function of the *vegan* package in R, and significant drivers were determined via permutational analysis at a cut-off p-value of 0.05. Statistically significant differences ($p < 0.5$) between days and treatment groups for each enzyme assay were determined using one-way ANOVA in PAST. To determine where significant differences occurred, post-hoc TukeyHSD analyses were performed. Statistically significant differences between treatment groups for pathway data were determined using PERMANOVA via the *adonis* function in R. A heatmap of pathway data was created using the *ggplot2, reshape2, plyr* and *scales* packages in R.

2.5 Accession numbers

The sequence data generated from this study have been deposited in the Short Read Archive (SRA) of the National Centre for Biotechnology Information (accession number SRP067320).

3. Results and discussion

3.1 Physiochemical analysis of soil

Soil physiochemical data is summarised in Table 1. The percentage of sand was very high (97%) whereas the silt and clay proportion were comparatively low (3% combined). The abundance of organic matter was also low, with a mean organic carbon content of 0.093% (w/w). The average pH value of the soil was 9.9 and the cation exchange capacity (CEC) of the soil was 8.29 cmol/kg, which reflects the dominance of the sand fraction in these soils. Sodium (Na) and calcium (Ca) were found to have the highest concentrations (127.41 and 110.94 ppm, respectively) whereas magnesium (Mg) and phosphorus (P) were found to have the lowest concentrations (17.42 and 7.69 ppm, respectively). The inorganic nitrogen composition of the soil, indicated by the ammonium (NH_4) and nitrate (NO_3) concentrations, was found to be 11.55 ppm. These findings are consistent to previous soil analysis from Dry Valley soils and confirm that these soils are highly oligotrophic (Lee et al., 2012; Makhalanyane et al., 2013). The physiochemical characteristics and resulting oligotrophic nature of MDV soils strongly influences the microbial diversity present (Stomeo et al., 2012).

3.2 Microbial community diversity analysis

The total number of OTUs identified from the sequencing analysis at a taxonomic assignment similarity cut-off of 97%, following singleton removal, was 8,834. The percentage of OTUs unique to each temperature group across the experimental time period was as follows; the control temperature treatment group harboured the highest number of unique OTUs (30%), whereas the fluctuating and stable treatment groups had slightly lower percentages of unique OTUs, with 24% and 21%, respectively. The percentage of OTUs shared among all three treatment groups (13%) was less than the percentage of OTUs unique to each group. Despite the differences observed among treatment groups in terms of OTU abundance, none were statistically significantly different based on PERMANONVA analysis (999 random permutations). An ANOSIM analysis of OTU similarity among groups indicated that the groups were almost identical ($R=0.0065$, $p > 0.5$). A two-dimensional (2D) non-metric multidimensional scaling (NMDS) ordination of OTU data (Figure S3) shows no clustering of samples according to treatment group.

Alpha-diversity was determined as both the number of observed OTUs and the Shannon diversity index (H' index) for each sample (Table 2). A high H' index was found for all three of the temperature treatment groups. This result, along with the high number of observed OTUs, indicates a high species richness within each treatment group (Niederberger et al., 2008). These findings are similar to previous studies on Antarctic soil microbial diversity, where H' index values and the number of observed OTUs range between approximately 1.5 to 7 (Niederberger et al., 2008; Teixeira et al., 2010; Pessi et al., 2012; Wang et al., 2015) and between approximately 10 to > 4500 (Teixeira et al., 2010; Lee et al., 2012; Wang et al., 2015), respectively. No statistically significant differences in alpha diversity were found between temperature treatments using one-way ANOVA.

Beta-diversity was calculated using the Whittaker or γ/α diversity measure (Table 2) and one-way ANOVA showed no statistically significant differences in beta-diversity between temperature treatment groups. Gamma diversity for the three treatment groups (Table 2) and each sampling day (Table S1) remained relatively high, and was substantially higher than previously detected in Antarctic soils. For example, a study surveying soil microbial diversity across several MDV regions

found gamma diversity values in the range of 136-218 (Sokol et al., 2013), several orders of magnitude lower than detected in this study.

No statistically significant differences in diversity measures were observed in response to the temperature simulations. This finding is contrary to expectations that soil warming is likely to lead to relative changes in compositional microbial diversity (Zogg et al., 1997; Pessi et al., 2012; Okie et al., 2015). However, previous studies have also shown that microbial diversity may remain relatively unaffected by increased temperatures. For example, a study which assessed the effects of experimental warming on microbial communities in temperate mountain forest soils found that warming did not affect the abundance of most microbial groups (Schindlbacher et al., 2011). In the present study, the maintenance of high diversity values across all three temperature treatments is in line with the community diversity hypothesis (Pold and Deangelis, 2013). These results suggest that the microbial communities present may be resistant to the short-term imposed temperature perturbations, resulting in the non-responsive nature of these communities.

Little variation in microbial taxonomy was observed among treatment groups and sampling days**.** Figures 3 and 4 show the relative abundance of OTUs classified to phylum level and are plotted according to either treatment (Figure 3) or sampling day (Figure 4). These figures show that the majority of phyla identified are at a relatively low abundance, with only three phyla occurring at a frequency of more than 15%. The most abundant phyla in all samples were affiliated with bacteria, including Actinobacteria, Chloroflexi and *Deinococcus-Thermus*. These phyla are ubiquitously distributed in cold environments and particularly in Antarctic soils (Yergeau et al., 2007b; Fierer et al., 2012; Cowan et al., 2014). The abundance of the three dominant phyla at Class level did not differ among the three treatments, or sampling days (Figures S4-S6). Interestingly, Proteobacteria and Gemmatimonadetes occurred with a mean abundance of only ±6% each throughout the three treatments, in contrast to previous studies where they were found to dominate Antarctic soils (Makhalanyane et al., 2013). However, Proteobacteria are known to prefer more nutrient rich habitats (Yergeau et al., 2012), which may explain their lower abundance in the nutrient poor soils analysed in this study.

Cyanobacteria (0.2%) were found in almost all samples at all sampling points, although their abundance did not differ across days or treatments. Other identified phyla (Figures 3 and 4) occurred at an abundance of less than 4% across all treatments and sampling days. The percentage of unassigned sequences was low, with a mean of 0.57%, indicating good taxonomic assignment of the sequencing data.

The dominant bacterial phyla identified in this study are all known to harbour members highly resistant to extreme environmental conditions (Margulis and Chapman, 2009; Oren and Papke, 2010). The majority of phyla identified also contained members with heterotrophic physiologies (Oren and Papke, 2010; Shivlata and Tulasi, 2015; Thiel et al., 2015), capable of degrading complex organic compounds (Oren and Papke, 2010). Autotrophic taxa were also identified within all treatment groups, which is consistent to previous findings in Antarctic soils (Makhalanyane et al., 2015).

Archaea were found to be present at a very low abundance, with only two phyla identified, Crenarchaeota and Euryarchaeota. Euryarchaeota was present in only one sample, at an abundance of 0.007%. Crenarchaeota were found to occur consistently in all samples at a mean abundance of 0.42%. The low abundance of these taxa in Antarctic soils is consistent with previous findings from cold desert soils (Pointing et al., 2009).

Overall, no significant shift in the relative abundance of bacterial and archaeal taxa was observed, in contrast to observations from more benign Antarctic soils (Yergeau and Kowalchuk, 2008). However, in comparison, Dry Valley soils are severely oligotrophic and water limited (Cowan et al., 2014). It has previously been proposed that nutrient and water limitation may limit the ability of Antarctic soils to respond to changes due to warming (Wynn-Williams, 1996; Treonis et al., 2002). Our results suggest that this limitation also extends to microbial communities in these habitats.

3.3 Microbial community potential functionality analysis

PICRUSt and KEGG orthology assigned putative metabolic pathways and their associated marker genes involved in an array of cellular functions to each sample by creating predicted metagenomes. While these predictions are only indicative of putative functional capacity, previous

studies have shown that PICRUSt analysis yields reliable results (Xu et al., 2014; Hill et al., 2015). Figure 5 shows a heatmap of predictive cellular pathways identified within all samples, with those considered most relevant to this study highlighted with a red box (environmental adaptation, DNA repair, and metabolism). No statistically significant differences were found between temperature treatment groups for any of the predictive pathways identified (PERMANOVA, $P > 0.05$) (Figure 5). Similarly, the graphical differences observed in Figure 5 between sampling dates within each treatment group were found to be non-significant (PERMANOVA, $P > 0.05$). Rather, a consistent putative activity for all identified pathways can be seen throughout all temperature treatment groups. Key pathways (highlighted with a red box in Figure 5) along with their associated functions and marker genes, are indicated in Table 3. Figure 5, in conjunction with Table 3, shows that cellular processes involved in stress adaptation to both cold and heat stress were potentially present in all samples. Carbon fixation potential was also predicted for all samples, as was the potential for nitrogen metabolism, including nitrogen fixation, denitrification and ammonification.

Cellular pathways and marker genes identified using PICRUSt software (Langille et al., 2013) and KEGG orthology (Kanehisa et al., 2014) correlated with the characteristics of the observed taxa. For example, the prediction of pathways involved in carbohydrate, energy and amino acid metabolism (Table 3) was consistent with the predicted heterotrophic physiologies of the majority of identified taxa, such as *Deinococcus-Thermus*, Proteobacteria, Actinobacteria, Chloroflexi and Gemmatimonadetes (Margulis and Chapman, 2009; Oren and Papke, 2010; Shivlata and Tulasi, 2015; Thiel et al., 2015). As heterotrophic microorganisms are known to degrade complex substrates (Kuffner et al., 2012), these correlations suggest the involvement of the identified taxa in these pathways. Putative pathways and marker genes involved in carbon and nitrogen cycling were consistent with the continuous presence of both autotrophic and heterotrophic taxa. Some identified members of Actinobacteria, such as the genus *Streptomyces* and family Frankiaceae are capable of nitrogen fixation (Shivlata and Tulasi, 2015). Two identified orders of the phylum Chloroflexi, Chloroflexales and Herpetosiphonales, as well as the class Alphaproteobacteria of the phylum Proteobacteria, contain members capable of carbon assimilation (Campbell et al., 2014; Imam et al., 2015). The photoautotrophic phylum Cyanobacteria, which was also consistently

present throughout the three treatment groups at a low mean abundance (Figures 3,4), is capable of carbon acquisition and therefore potentially plays a role in energy metabolism (Pointing et al., 2009; Durall and Lindblad, 2015).

The putative presence of genes involved in environmental adaptation and stress response is correlated to the predicted stress-tolerant physiologies of the observed taxa. For example, members of the phyla *Deinococcus-Thermus* and Actinobacteria are known to be highly desiccation and stress tolerant (Oren and Papke, 2010; Shivlata and Tulasi, 2015; Thiel et al., 2015), suggesting a correlation between the presence of these taxa and the putative identification of environmental adaptation as well as replication and repair pathways (Table 3). While these predictions are only indicative of the potential presence of the identified genes and cellular pathways, their abundance is consistent with previous functional diversity surveys within Antarctic soils (Pearce et al., 2012; Chan et al., 2013). The consistent presence of stress-tolerant taxa and marker genes throughout induced temperature fluctuations in this study may indicate resistance of the soil microbial communities to short-term temperature perturbations.

Activities were detected for four of the seven extracellular enzymes analysed, with little variability in activity between the three temperature treatments (control, fluctuating and stable). Phenol oxidase (PO) showed the highest overall activity (up to 60 nmol h^{-1} g^{-1}, Figure 6A), followed by phenol peroxidase (PPO), where a slightly lower activity was detected (53 nmol h^{-1} g^{-1}; Figure 6B). No statistically significant differences were found for the PO and PPO assays between the three temperature treatment groups ($F_{2,6}$, $p > 0.05$). When compared to the redundancy analysis ordination of the bacterial community structure, the PO activity showed a significant linkage to the bacterial clustering (R^2 =0.7784, $p < 0.01$), although the direction of the PO arrow did not point to any specific temperature treatment group (Figure 7). This may potentially indicate that the microbial communities present were consistently producing these extracellular enzymes, with no change in production in response to temperature fluctuations.

No significant difference was found between the temperature treatments for the AP assay ($F_{2,6}$, $p > 0.05$). The average observed AP activity was consistently low throughout temperature treatments, where the highest observed activity was 4 nmol h^{-1} g^{-1} (Figure 6C). LAP activity was consistently

observed for all samples at all sampling days (except day 10), although at lower rates (Figure 6D) and no statistically significant differences between temperature treatment groups were observed. However, permutational analysis indicated that LAP was significantly linked to the ordinated bacterial community structure (R^2 = 0.4891, P < 0.01) and the direction of the arrow of the LAP activity pointed mainly towards fluctuating treatments samples (Figure 7). No activities were detected for the enzymes β-xylosidase (BG), β-glucosidase (BX) or N-acetyl glucosaminidase (NAG) (Table 4).

Although metabolic potential for C, N and P transformation have been previously observed in MDV soils, the low extracellular enzyme activities observed in this study may be due to the highly oligotrophic nature of the soil, in which the extremely low organic carbon and nitrogen supply may have limited enzyme biosynthesis (Hopkins et al., 2008). Increased temperature (either the stable or fluctuating increase) had no direct effect on any enzyme activity tested, which is in contrast to an empirical model prediction based on *in situ* measurement of potential microbial activities in Arctic soils (Wallenstein et al., 2009). However, the lack of an observable temperature effect was consistent with previous field studies conducted in the MDVs (Hopkins et al., 2008) and the Arctic (Melle et al., 2015; Seo et al., 2015). The lack of an observable temperature effect on microbial activities may also be a consequence of the very low soil organic carbon availability, as in alpine ecosystems, limited labile carbon availability can reduce the temperature sensitivity of organic matter mineralization (Song et al., 2010).

The significant link of LAP activity, an enzyme involved in the acquisition of nitrogen, and the bacterial community structure (Figure 7), may indicate particular nitrogen limited conditions. Moreover, LAP was especially linked with the fluctuating temperature treatments indicating that enzyme activities might be enhanced when soil conditions are changing. This is similar to a previous study done in alpine, arctic and sub-arctic soils, where the highest enzyme activities were found to rise in spring, during conditions of increasing temperatures and thaw (Jefferies et al., 2010). Therefore, the effect of temperature changes on microbial activities may occur during changing soil conditions, although such changes may need longer time periods (i.e. longer than 40 days) to be observed.

The lack of observable changes in microbial diversity measures, taxonomic identities, extracellular enzyme activities as well as putative pathways and marker genes, suggests that MDV microbial communities may be adapted and resistant to short term changes. However, it is possible that the apparent stability detected in this study could be masking several other changes. For example, an initial increase in microbial activity in response to increased temperature, may be followed by a subsequent decrease due to the depletion of labile substrates, such as organic carbon (Bradford et al., 2008). This decrease may either be due to continued carbon losses or the thermal acclimation of microbial communities (Karhu et al., 2014; Hartley et al., 2008). As the duration of the study may have been too short to detect changes in activity following substrate depletion, we propose a hypothesis for how an associated decrease in microbial activity may have occurred in this case. Studies on low organic carbon content (<2%), alkaline soils, have shown that microbial communities acclimate to temperature increases via a compensatory response (Karhu et al., 2014). Due to the lack of a detectable community shift, as well as the consistent presence of putative functional potential, we propose that over a longer time period, in a natural environment where substrate would not be artificially depleted, the communities detected in this study may have undergone thermal acclimation (Kuffner et al., 2012; Karhu et al., 2014). MDV microbial communities are already adapted to the stressful oligotrophic conditions present within the Antarctic soil environment (Chan et al., 2013; Cowan et al., 2014), suggesting this adaptation may be maintained under changing nutrient conditions. Although it is also possible that changing substrate conditions may have resulted in microbial community and physiological shifts, or that communities may have switched to utilising more recalcitrant substrates, the detection of these changes is beyond the scope of the present study. Therefore, future work should focus on assessing functional changes at the cellular level, via methods such as metatranscriptomics and metaproteomics, in order to rapidly detect any temperature induced changes.

4. Summary

This study aimed to investigate how increased and fluctuating temperatures may affect MDV soil microbial community structure and predicted functionality on short time-scales. Using 16S rRNA gene amplicon sequencing, enzymatic assays and PICRUSt functional annotation, we have shown

that microbial community structure and both measured and predicted functionality was not significantly altered in response to short term temperature fluctuations. Soil physiochemical analysis indicated that soil characteristics were similar to those previously observed within the MDVs (Toner et al., 2013; Levy et al., 2014), as the soil was alkaline, saline and contained low levels of key nutrients such as organic carbon and nitrogen (Lee et al., 2012; Makhalanyane et al., 2013). A few dominant phyla, which typically exhibit heterotrophic physiologies and stress-tolerant lifestyles, were consistently present in all treatment groups, a finding which was complemented by results obtained from enzymatic assays and PICRUSt putative functional predictions. The lack of observed changes in community composition and functionality suggests that these communities may be resistant to short term temperature perturbations, and that there may be considerable functional redundancy within the microbial community.

5. Acknowledgements

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6. Conflict of interest

The authors declare no conflict of interest.

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replicates; error bars represent the standard error. Only four of the seven enzymes analysed are present as the remaining three showed no activity in all samples.

Figure 7: A redundancy analysis (RDA) ordination of phylogenetic data with extracellular enzymes added as vectors indicating significant drivers of the observed clustering (p-value < 0.05). The three treatment groups are indicated by the key in the top left corner. LAP and PO refer to two enzymes, namely leucine aminopeptidase and phenol oxidase, respectively.

Supplementary Information

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Table S1: Gamma-diversity of temperature treatment samples according to sampling day.

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Figure S1: Line chart indicating the experimental design of the microcosm experiment. The experiment was conducted over a 30-day period, with the start indicated at day 0. Day -10 refers to a period of 10 days prior to the start of the experiment. The three treatment groups and their temperatures at specific time-points are indicated.

Figure S2: Soil microcosms for the three temperature treatment groups. (A) Control group, (B) Fluctuating group and (C) Stable group.

Figure S3: Abundance of the phylum *Deinococcus-Thermus* summarised to class level across three treatment groups and eight sampling points indicated in percentages. Only one class, Deinococci, was identified. The three different temperature treatment groups are shown by C, F and S which represent control (0°C), fluctuating (0°C-15°C) and stable (15°C) respectively. Numbers -10 to 30 indicate the sampling days, where day 0 is the start of the temperature experiment and day -10 is ten days prior to the start.

Figure S4: Abundance of the phylum Actinobacteria summarised to class level across three treatment groups and eight sampling points indicated in percentages. The three different temperature treatment groups are shown by C, F and S which represent control (0°C), fluctuating (0°C-15°C) and stable (15°C) respectively. Numbers -10 to 30 indicate the sampling days, where day 0 is the start of the temperature experiment and day -10 is ten days prior to the start.

Figure S5: Abundance of the phylum Chloroflexi summarised to class level across three treatment groups and eight sampling points indicated in percentages. The three different temperature treatment groups are shown by C, F and S which represent control (0°C), fluctuating (0°C-15°C)

and stable (15°C) respectively. Numbers -10 to 30 indicate the sampling days, where day 0 is the start of the temperature experiment and day -10 is ten days prior to the start.

Table 1: Soil characteristics identified from soil physiochemical analyses of pre-treatment samples.

Soil Characteristics	Pooled replicates
Percentage sand	97 (±0.42)
Percentage silt	1 (±0)
Percentage clay	2 (±0)
Percentage carbon	0.0935 (±0.009)
pH	9.9 (±0.015)
Cation exchange capacity (cmol/kg)	8.29 (±1.3)
NH_4 and NO_3 (ppm)	11.55 (±0.39)
K (ppm)	70.89 (±16.6)
Ca (ppm)	110.94 (±23.4)
Na (ppm)	127.41 (±13.8)
Mg (ppm)	17.42 (±3.4)
P (ppm)	7.69 (±0.2)

The values were obtained from soil analysed prior to the microcosm experiment and are reported as a mean of three replicates. Standard deviations for each analysis are indicated in brackets.

Table 2: Measures of α- β- and γ- diversity for temperature treatment samples taken at several time-points.

	α-diversity		β-diversity	γ–diversity
Samples	Observed OTUs	Shannon diversity (H')	Whittaker	
C-10	1556	5,232013	2,926735	
C0	1307	5,311446	3,484315	
C5	1411	5,152953	3,227498	
C10	761	5,162895	5,984231	
C15	776	4,953165	5,868557	
C20	569	4,715299	8,003515	
C25	581	4,665257	7,83821	
C30	1071	4,674236	4,252101	
Control mean	1004	4,983408	5,198145	4554
F-10	1424	4,99119	2,752809	
F0	711	4,955225	5,513361	
F5	997	4,931967	3,931795	
F10	524	4,830524	7,480916	
F15	1022	4,933382	3,835616	
F20	439	4,632857	8,929385	
F25	969	5,097072	4,045408	
F30	945	4,711199	4,148148	
Fluctuating mean	879	4,885427	5,07968	3920
S-10	1166	4,924196	3,105489	
S0	1258	5,265043	2,878378	
S5	602	4,826812	6,01495	
S10	379	4,589837	9,55409	
S15	866	5,140891	4,181293	
S20	553	4,896036	6,54792	
S25	921	4,99164	3,931596	
S30	859	4,757152	4,215367	
Stable mean	826	4,923951	5,053635	3621

C, F and S refer to the control, fluctuating and stable temperature treatment groups, respectively. Numbers (-10 to 30) indicate the days samples were taken throughout the microcosm temperature experiment.

Table 3: Pathways, their associated cellular processes and marker genes identified from PICRUSt and KEGG orthology using 16S rRNA gene sequencing data

Pathway	Cellular process(es) involved	Marker gene(s) identified
Replication and repair	Replication and repair of damaged DNA – base excision repair, homologous recombination, nucleotide excision repair	Base excision repair (*alkA* – glycosylase, *xthA* – exodeoxyribonuclease, *polA* – DNA polymerase I) *
Environmental Adaptation	Transcription factors involved in stress response as well as cold and heat adaptation	Adaptive stress response (transcriptional regulators - *ada-alk, adaA, ada*), cold shock protein (*cspA*), heat and stress response (transcription factor - *ctsR*)*
Energy metabolism	Carbon fixation, photosynthesis, as well as nitrogen and methane metabolism	Carbon fixation (RuBisCo - *rbcl, rbcs, ppc*), nitrogen fixation (nitrogenase - *nifH, anfG*), ammonification (nitrate reductase- *nirK*) and denitrification (nitrite reductase - *nirB*)*
Carbohydrate metabolism	Nucleotide and sugar metabolism, such as fructose, glucose and mannose metabolism.	E.g. Sugar metabolism - phosphofructokinase (*fruK*) *
Amino acid metabolism	Biosynthesis and degradation of amino acids. Including both hydrophobic amino acids (such as glycine, leucine, isoleucine), and polar/ hydrophilic amino acids (such as lysine, arginine and aspartate).	E.g Amino acid synthesis (*patA* - aminotransferase), amino acid degradation (*icuD* - dehydrogenase) *

*Not all possible marker genes for all pathways are indicated, as not all were determined in the analysis.
Relevant genes for several pathways are indicated with their associated function.

Table 4: Soil extracellular enzymes analysed and their corresponding functions.

Extracellular enzyme	***Abbreviation***	***Function***
β-Xylosidase	BX	Degradation of hemicellulose; aids in microbial acquisition of carbon
β-Glucosidase	BG	Degradation of cellulose; aids in microbial acquisition of carbon
β-*N*-acetyl-glucosaminidase	NAG	Degradation of β-1,4-linked glucosamine polymers such as chitin; aids in microbial acquisition of nitrogen
Leucine aminopeptidase	LAP	Removal of hydrophobic amino acids from peptides; aids in microbial acquisition of nitrogen
Alkaline phosphatase	AP	Degradation of phosphomono- and di-esters; aids in microbial acquisition of phosphorus
Phenol oxidase	PO	Polyphenol oxidation or lignin degradation; aids in microbial nutrient acquisition
Phenol peroxidase	PPO	Degradation of lignin and aromatic compounds; aids in microbial nutrient acquisition

Figure 1

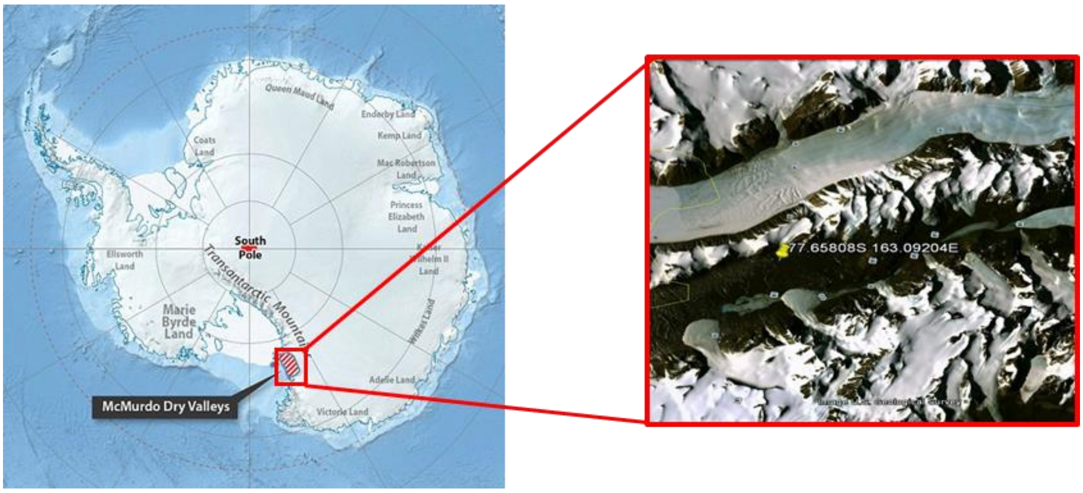

Queen Maud Land
Enderby Land
Kemp Land
Mac Robertson Land
Princess Elizabeth Land
Coats Land
South Pole
Ellsworth Land
Marie Byrde Land
Transantarctic Mountains
Wilkes Land
Adelie Land
Victoria Land
McMurdo Dry Valleys
77.65808S 163.09204E

Figure 2

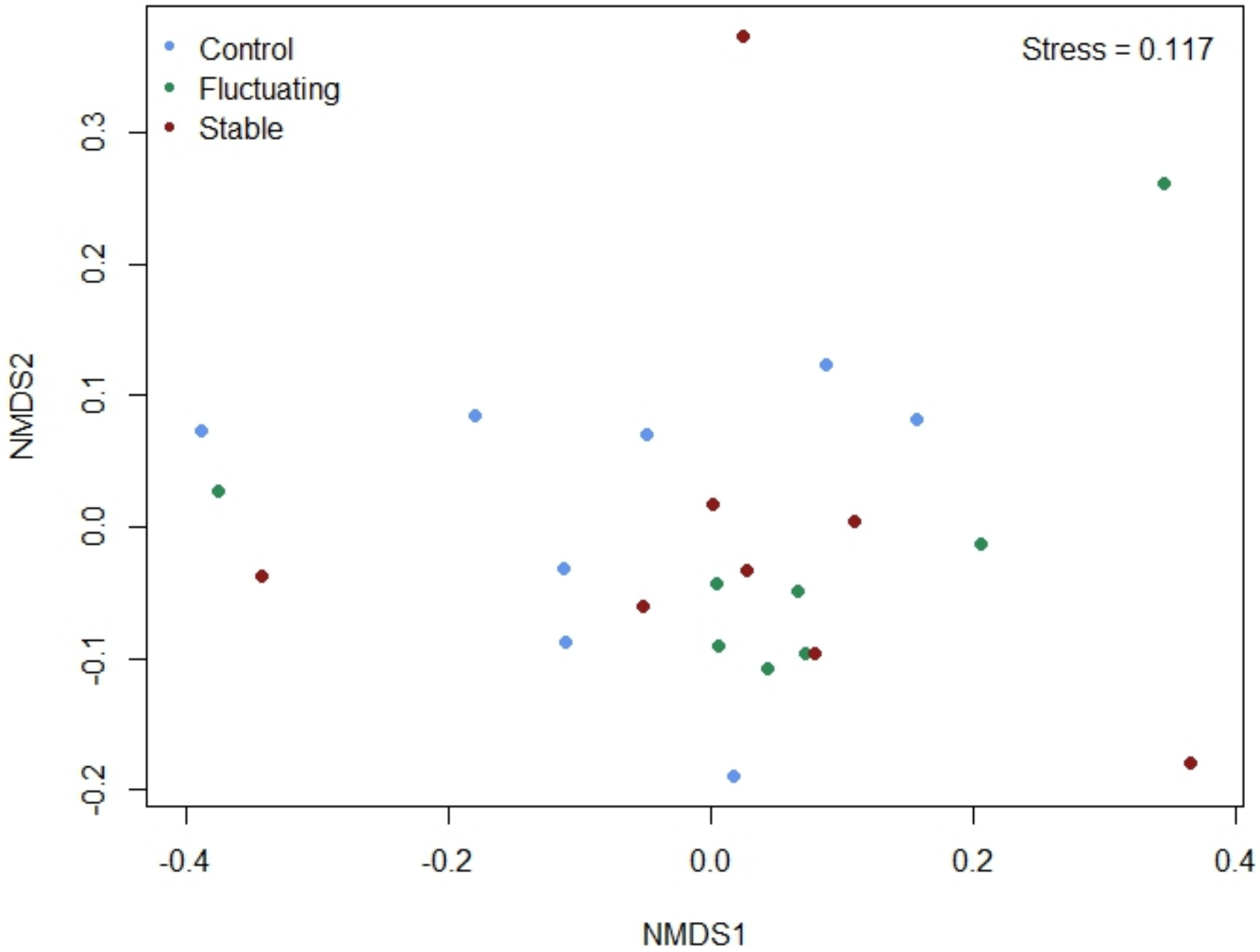

Control
Fluctuating
Stable
Stress = 0.117
NMDS2
NMDS1
0.3
0.2
0.1
0.0
-0.1
-0.2
-0.4
-0.2
0.0
0.2
0.4

Figure 3

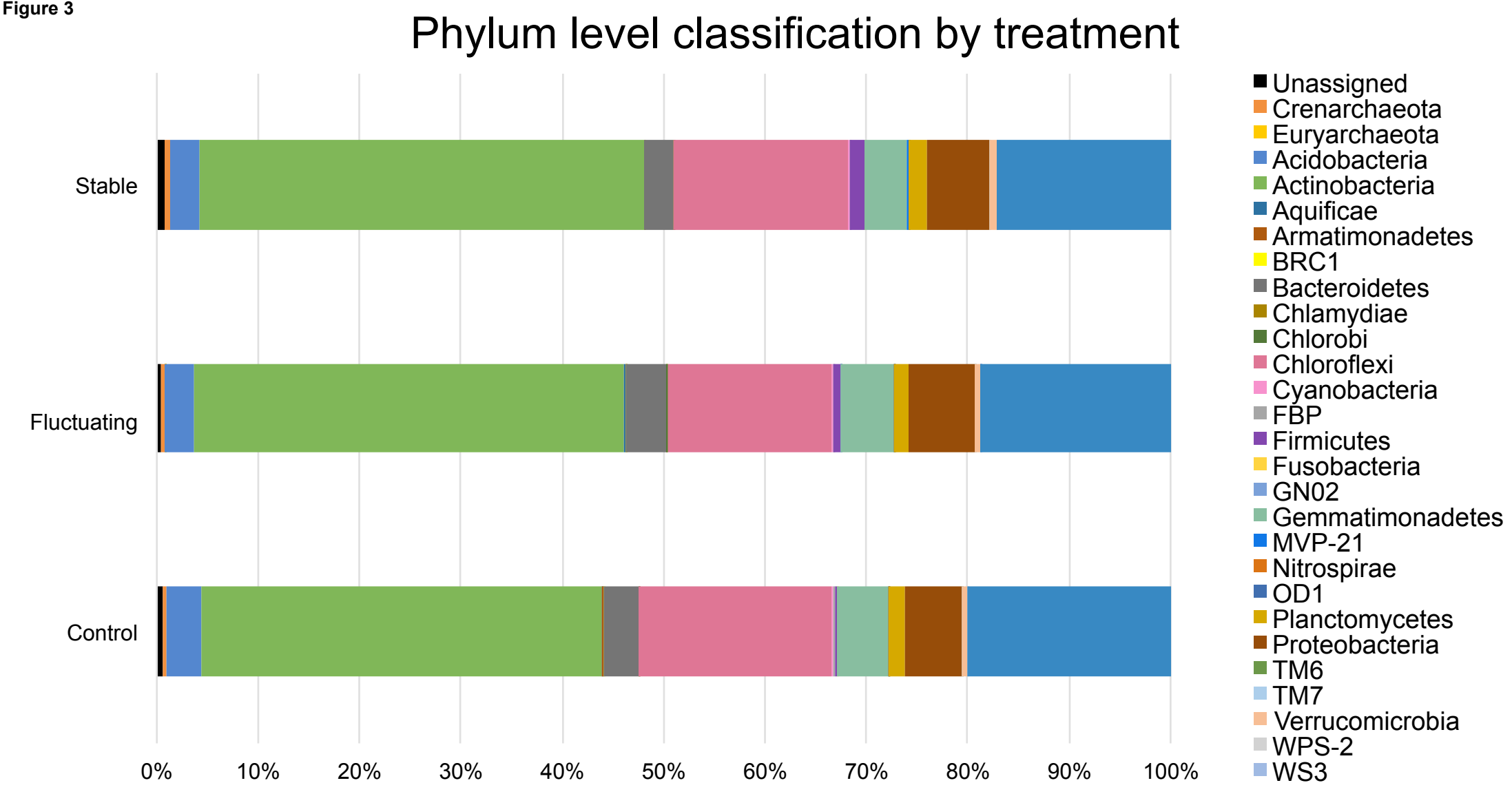

Phylum level classification by treatment
Stable
Fluctuating
Control
0%
10%
20%
30%
40%
50%
60%
70%
80%
90%
100%
Unassigned
Crenarchaeota
Euryarchaeota
Acidobacteria
Actinobacteria
Aquificae
Armatimonadetes
BRC1
Bacteroidetes
Chlamydiae
Chlorobi
Chloroflexi
Cyanobacteria
FBP
Firmicutes
Fusobacteria
GN02
Gemmatimonadetes
MVP-21
Nitrospirae
OD1
Planctomycetes
Proteobacteria
TM6
TM7
Verrucomicrobia
WPS-2
WS3

Figure 4

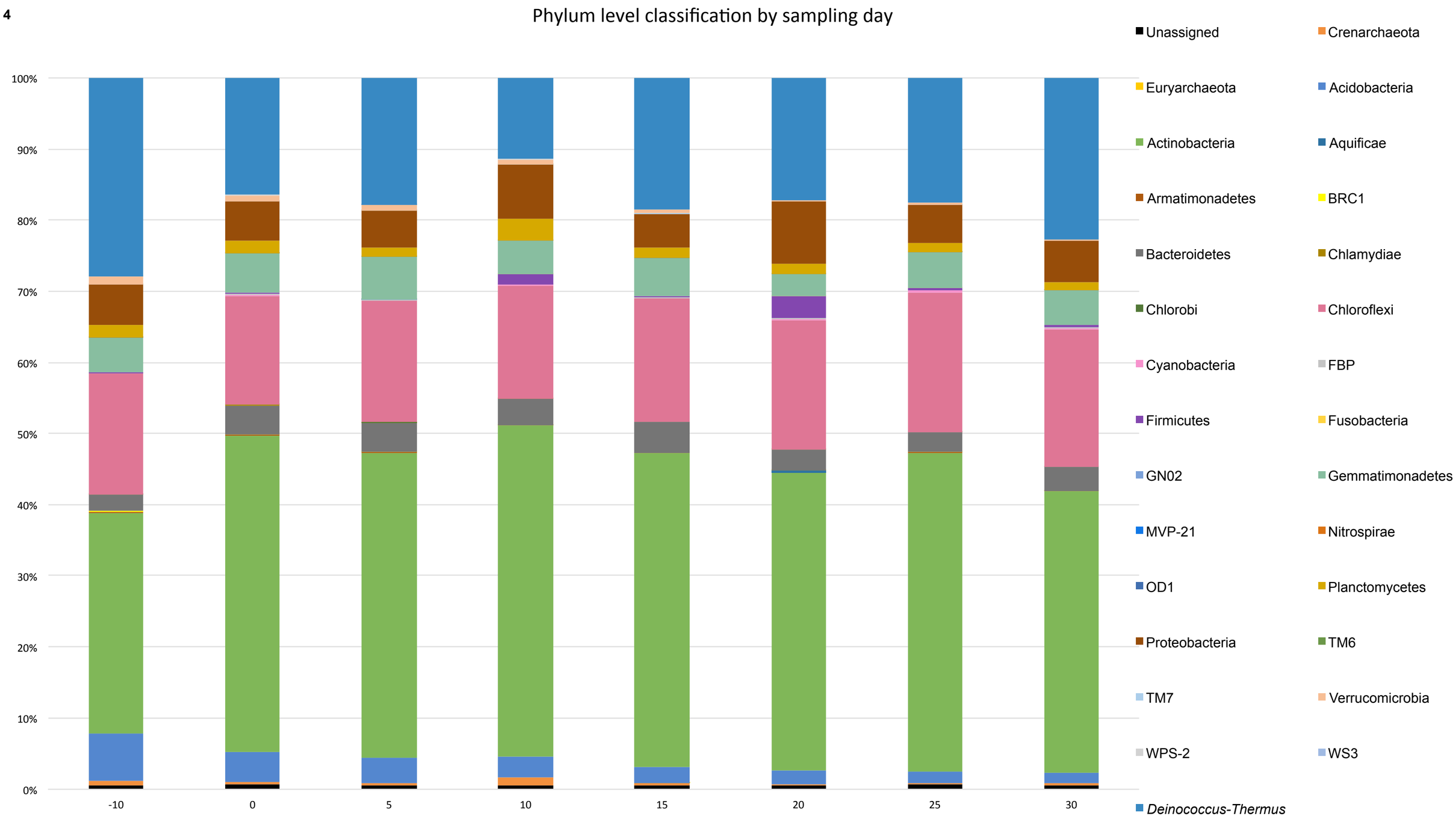

Phylum level classification by sampling day
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
-10
0
5
10
15
20
25
30
Unassigned
Crenarchaeota
Euryarchaeota
Acidobacteria
Actinobacteria
Aquificae
Armatimonadetes
BRC1
Bacteroidetes
Chlamydiae
Chlorobi
Chloroflexi
Cyanobacteria
FBP
Firmicutes
Fusobacteria
GN02
Gemmatimonadetes
MVP-21
Nitrospirae
OD1
Planctomycetes
Proteobacteria
TM6
TM7
Verrucomicrobia
WPS-2
WS3
Deinococcus-Thermus

Figure 5

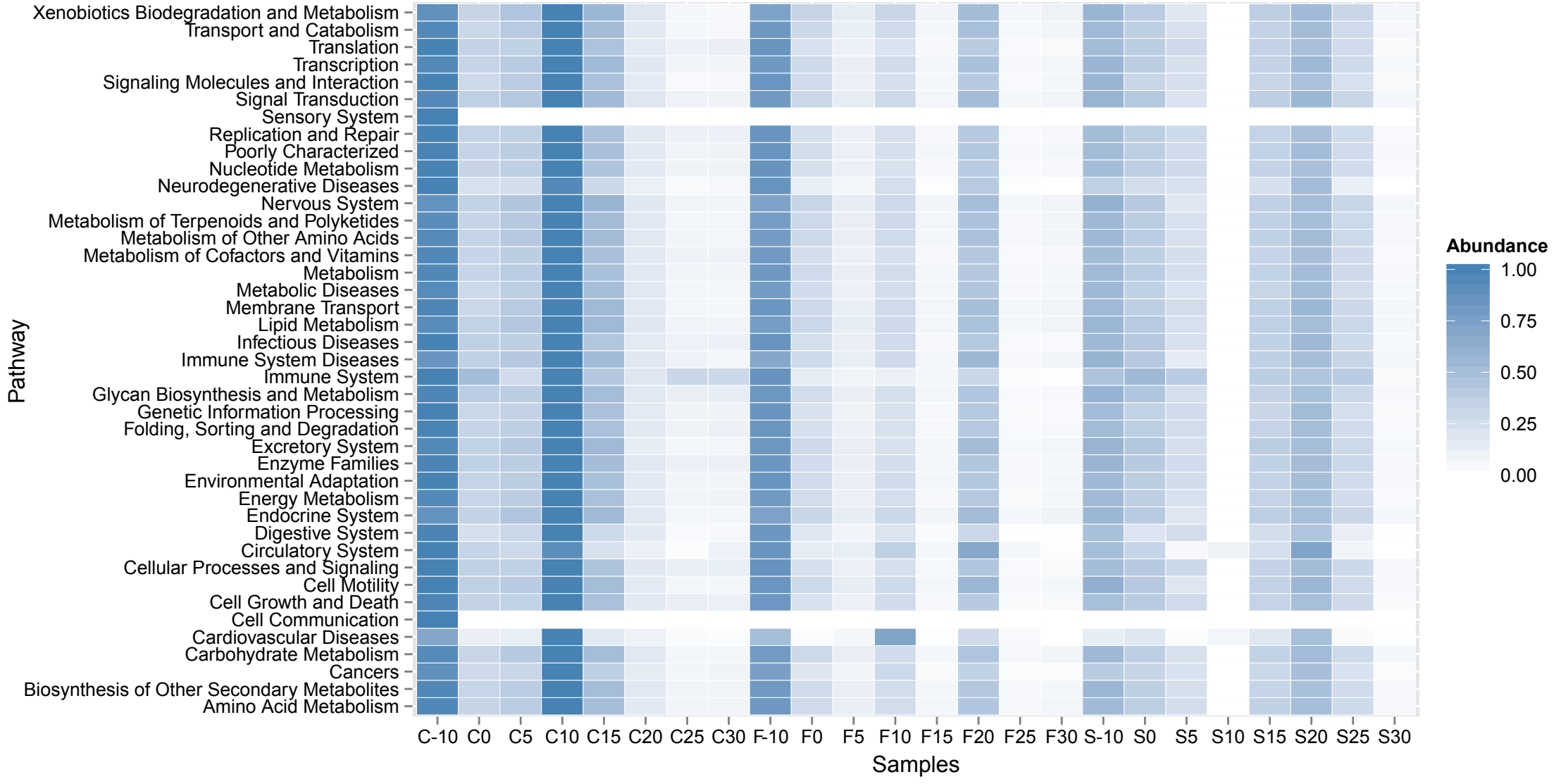

Xenobiotics Biodegradation and Metabolism
Transport and Catabolism
Translation
Transcription
Signaling Molecules and Interaction
Signal Transduction
Sensory System
Replication and Repair
Poorly Characterized
Nucleotide Metabolism
Neurodegenerative Diseases
Nervous System
Metabolism of Terpenoids and Polyketides
Metabolism of Other Amino Acids
Metabolism of Cofactors and Vitamins
Metabolism
Metabolic Diseases
Membrane Transport
Lipid Metabolism
Infectious Diseases
Immune System Diseases
Immune System
Glycan Biosynthesis and Metabolism
Genetic Information Processing
Folding, Sorting and Degradation
Excretory System
Enzyme Families
Environmental Adaptation
Energy Metabolism
Endocrine System
Digestive System
Circulatory System
Cellular Processes and Signaling
Cell Motility
Cell Growth and Death
Cell Communication
Cardiovascular Diseases
Carbohydrate Metabolism
Cancers
Biosynthesis of Other Secondary Metabolites
Amino Acid Metabolism
Pathway
C-10 C0 C5 C10 C15 C20 C25 C30 F-10 F0 F5 F10 F15 F20 F25 F30 S-10 S0 S5 S10 S15 S20 S25 S30
Samples
Abundance
1.00
0.75
0.50
0.25
0.00

Figure 6

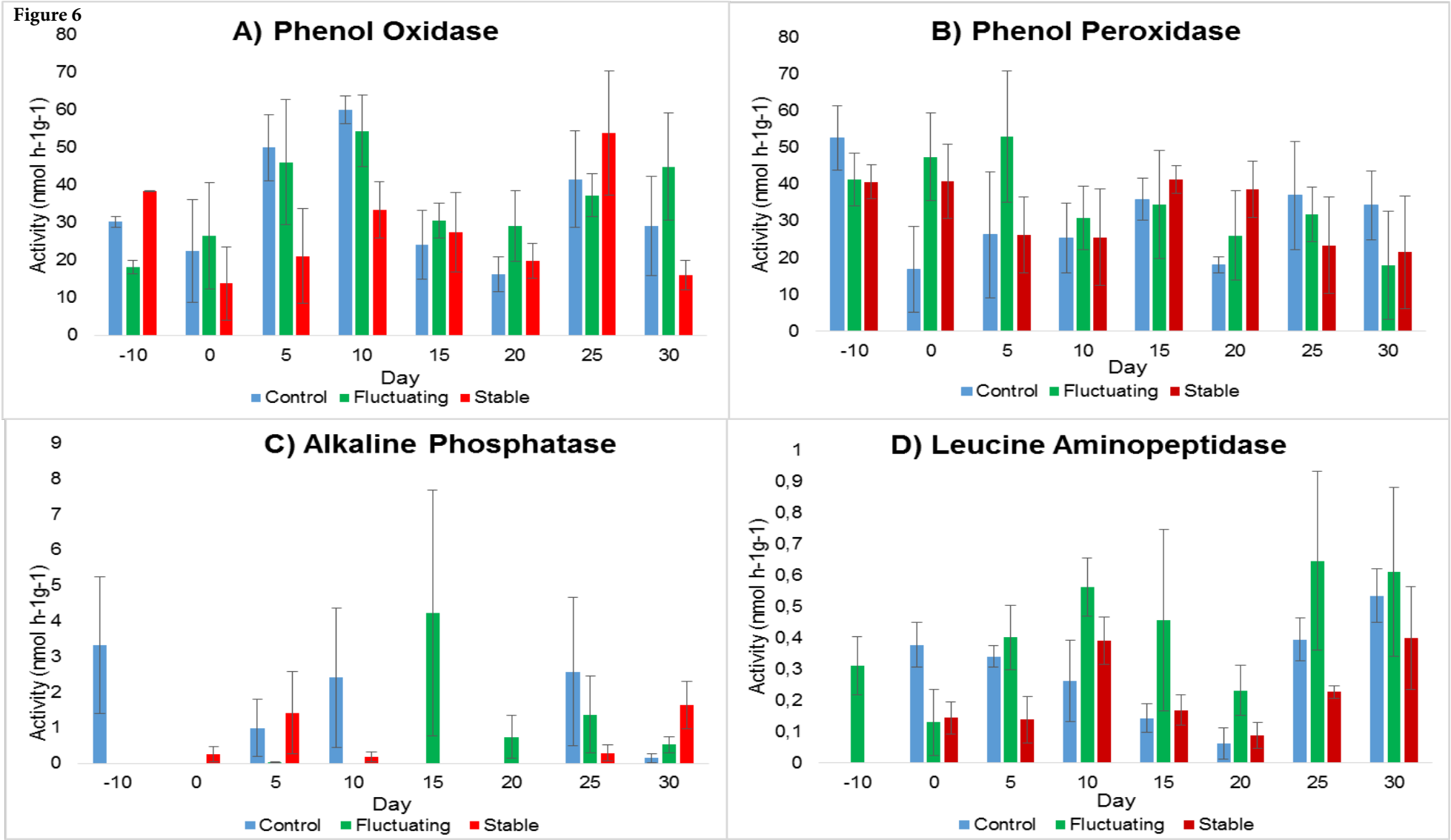

A) Phenol Oxidase
Activity (nmol h-1g-1)
Day
Control
Fluctuating
Stable
B) Phenol Peroxidase
Activity (nmol h-1g-1)
Day
Control
Fluctuating
Stable
C) Alkaline Phosphatase
Activity (nmol h-1g-1)
Day
Control
Fluctuating
Stable
D) Leucine Aminopeptidase
Activity (nmol h-1g-1)
Day
Control
Fluctuating
Stable

Figure 7

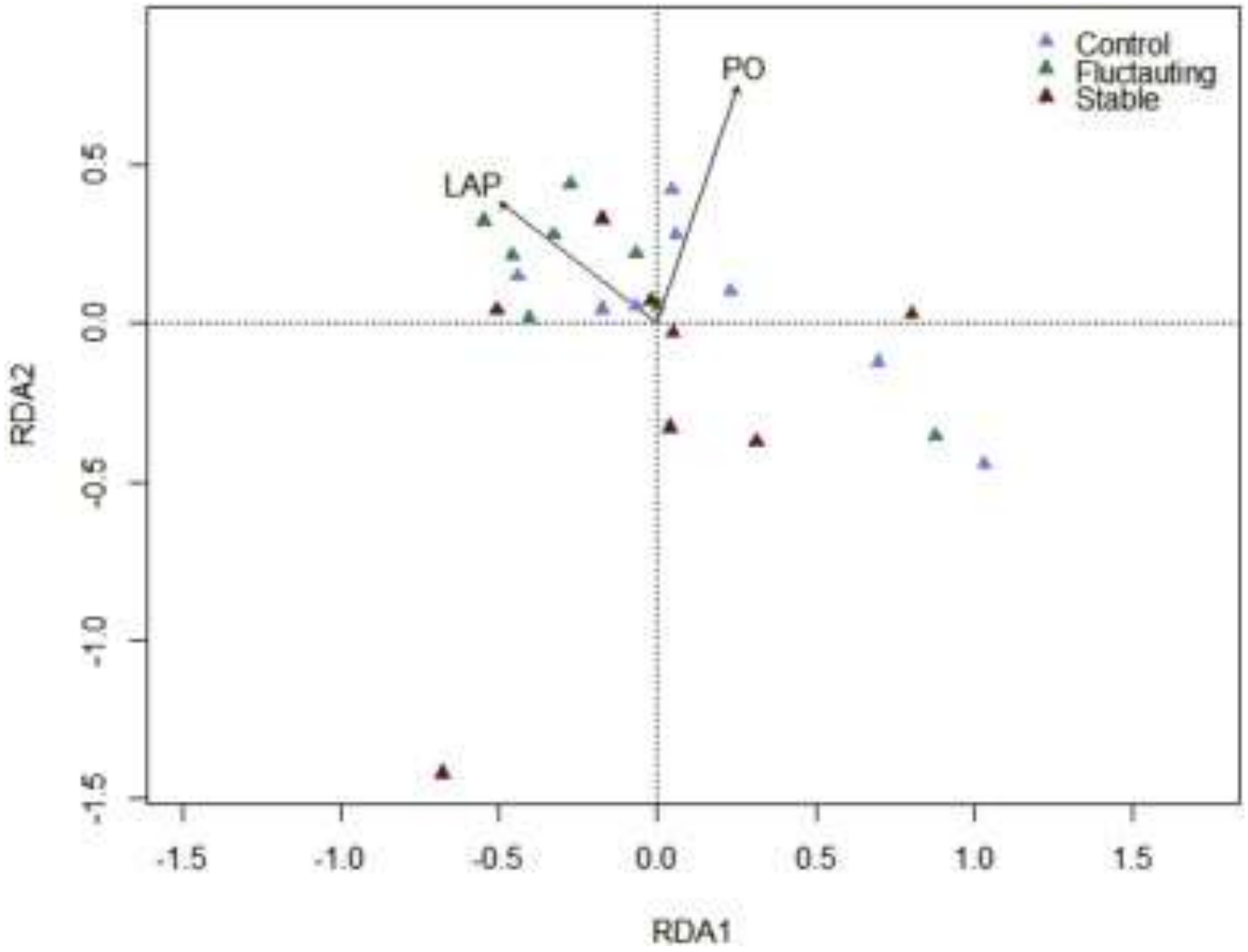

Control
Fluctauting
Stable
PO
LAP
RDA2
RDA1
0.5
0.0
-0.5
-1.0
-1.5
-1.5
-1.0
-0.5
0.0
0.5
1.0
1.5

Table S1: Gamma-diversity of temperature treatment samples according to sampling day.

Day	γ-diversity
-10	2784
0	2261
5	2150
10	1225
15	1853
20	1176
25	1752
30	1969

Figure S1

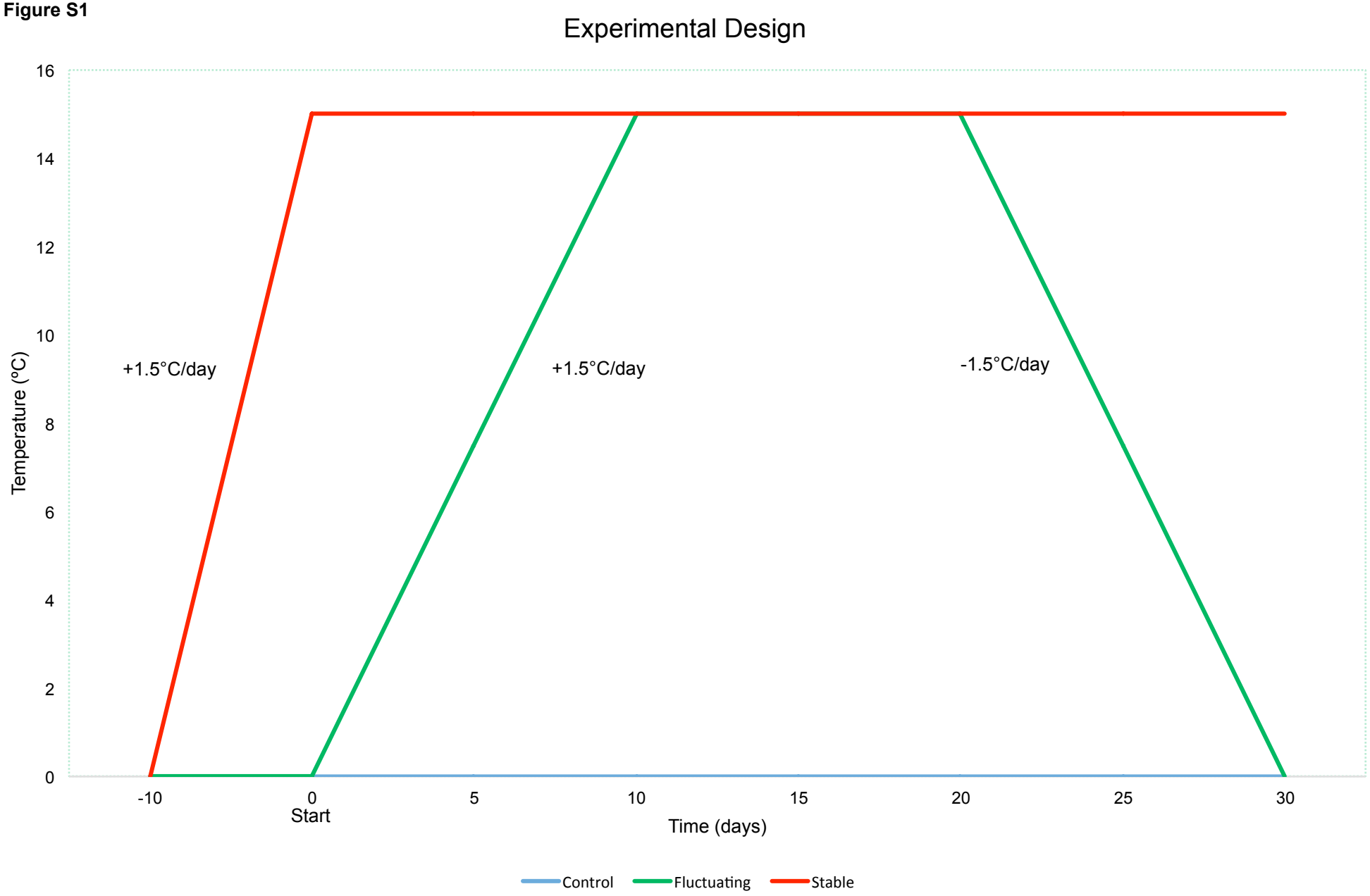

Experimental Design
16
14
12
10
8
6
4
2
0
Temperature (°C)
+1.5°C/day
+1.5°C/day
-1.5°C/day
-10
0
Start
5
10
15
20
25
30
Time (days)
Control
Fluctuating
Stable

Figure S2

Figure S3

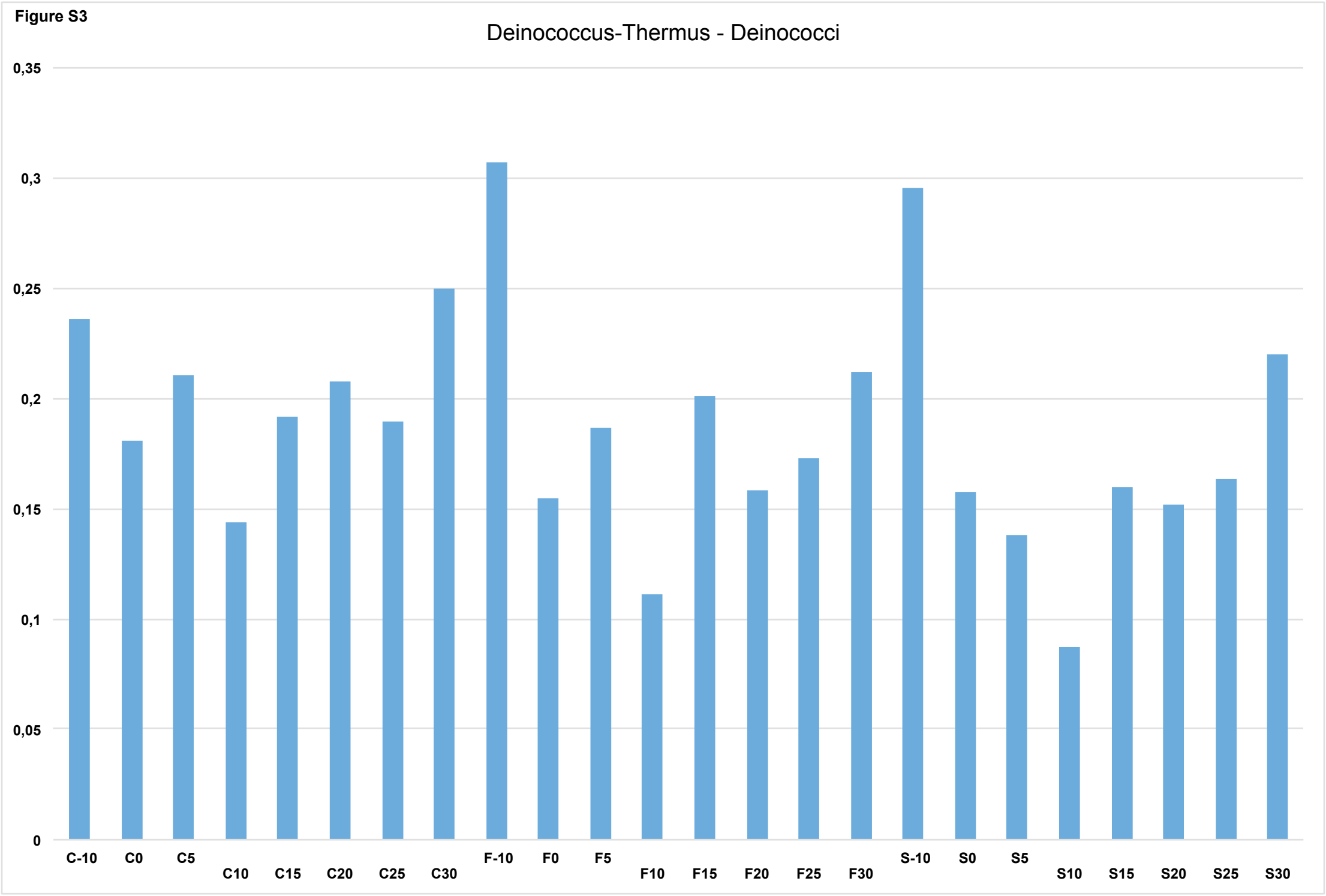

Deinococcus-Thermus - Deinococci
0,35
0,3
0,25
0,2
0,15
0,1
0,05
0
C-10
C0
C5
C10
C15
C20
C25
C30
F-10
F0
F5
F10
F15
F20
F25
F30
S-10
S0
S5
S10
S15
S20
S25
S30

Figure S4

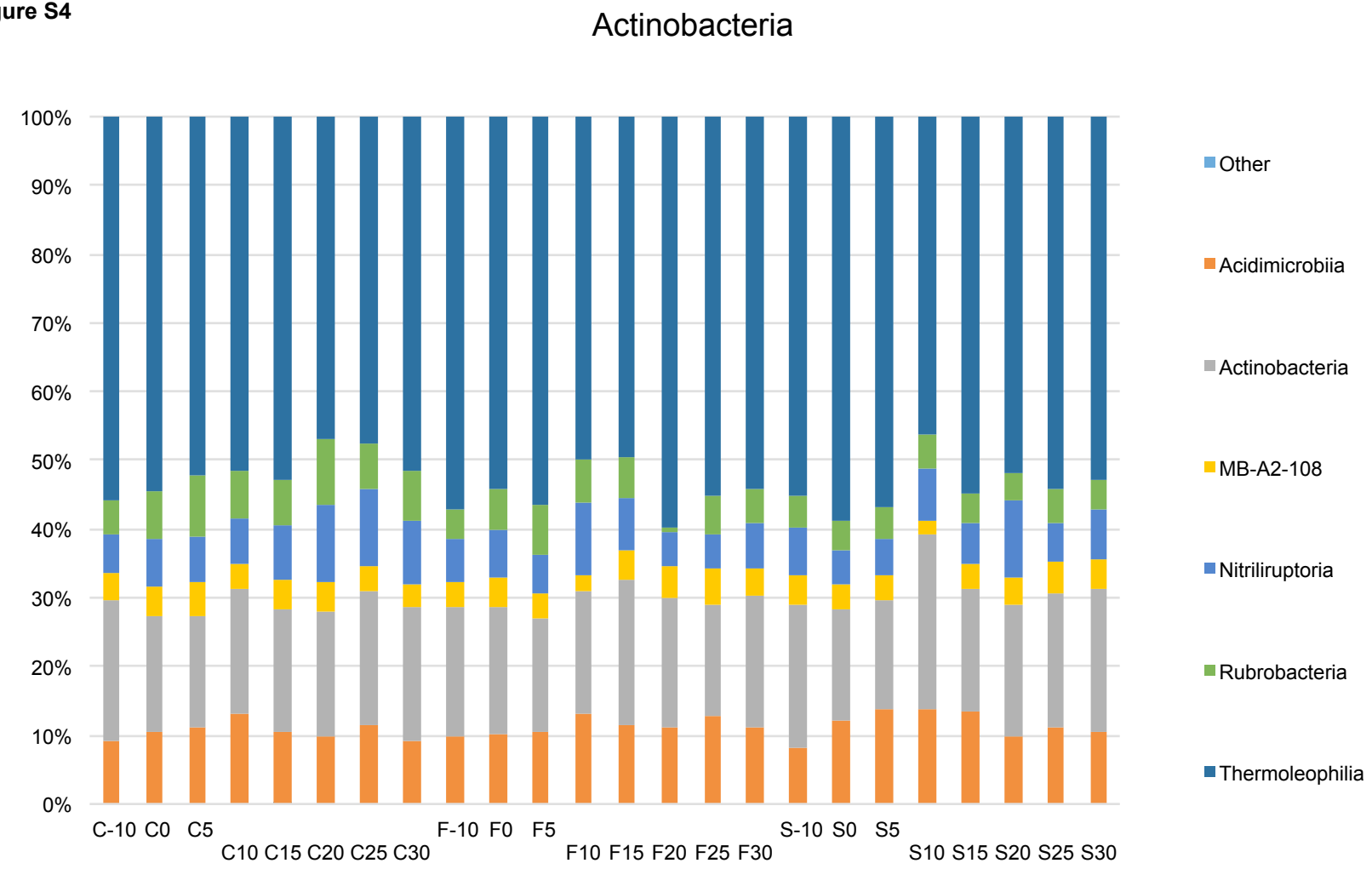
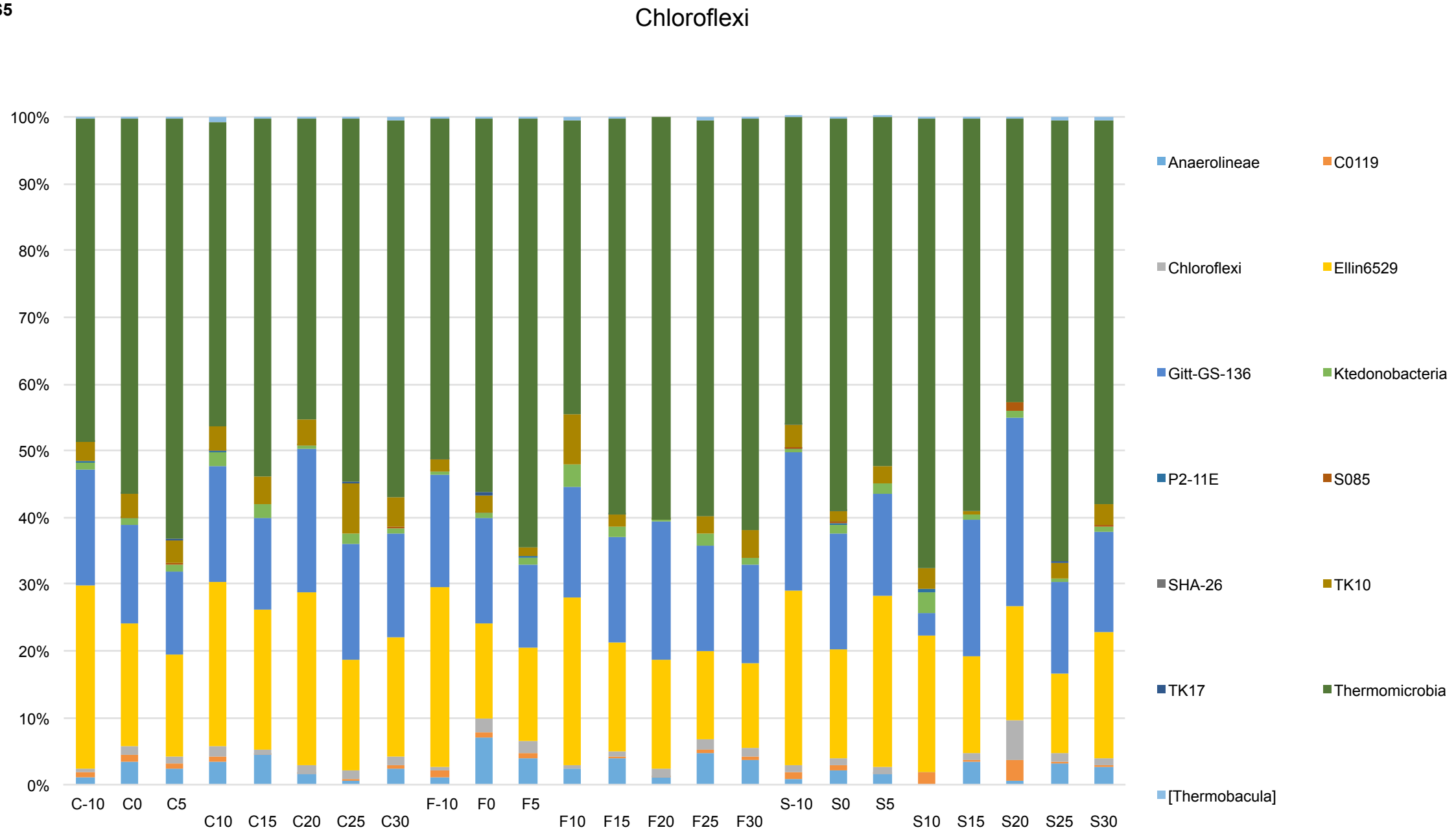

Actinobacteria
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
C-10 C0 C5 C10 C15 C20 C25 C30 F-10 F0 F5 F10 F15 F20 F25 F30 S-10 S0 S5 S10 S15 S20 S25 S30
Other
Acidimicrobiia
Actinobacteria
MB-A2-108
Nitriliruptoria
Rubrobacteria
Thermoleophilia

Figure S5


Chloroflexi
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
C-10
C0
C5
C10
C15
C20
C25
C30
F-10
F0
F5
F10
F15
F20
F25
F30
S-10
S0
S5
S10
S15
S20
S25
S30
Anaerolineae
C0119
Chloroflexi
Ellin6529
Gitt-GS-136
Ktedonobacteria
P2-11E
S085
SHA-26
TK10
TK17
Thermomicrobia
[Thermobacula]