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Microbial and CO_2 Responses to Water Stresses Show Decreased Productivity and Diversity Through Time

David Michael Robinson
Brigham Young University

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David Michael Robinson

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Zachary Aanderud, Chair
Byron Adams
John Chaston

Department of Plant and Wildlife Sciences

Brigham Young University

ABSTRACT

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David Michael Robinson
Department of Plant and Wildlife Sciences, BYU
Master of Science

Some bacterial taxa when stimulated by water additions will break dormancy, grow, and become dominant members of the community and contribute significant pulses of CO_2 associated with the rewetting event. These pulses of activity are associated with high levels of bacterial productivity in soils. (Aanderud et al. 2011) We examined the bacterial taxa that resuscitate and become metabolically active following two forms of water stress (soil drying-rewetting and freeze-thaw cycles) and we captured and measured the CO_2 emanating from those soils. Specifically, We used target metagenomics, which uses a specific gene pool within bacteria that is associated with a function of an ecological process, in this case active (16S rRNA communities) bacteria and all bacteria (16S rRNA communities) during drying-rewetting and freeze-thaw cycles. We measured an array of community dynamics (i.e., evenness, richness, diversity, relative abundance of taxa, and network analyses between taxa) as dry soils are rewetted and as frozen soils thaw multiple times in three cold desert soils. Soils from all three locations exhibited some similar bacterial taxa and gene function but were large in part their own community derived from the evolutionary history of the continent in which they reside.

Keywords: dormancy, turnover, PiCRUST

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I thank my parents for seeing me through this program and my lab mates for daily support. I wouldn't have made it even remotely close without them. I also thank my committee for guiding me through this process.

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INTRODUCTION

Dormancy and Pulses

Under stressful conditions bacteria have few options for survival, either contend with environmental stresses or enter a reversible state know as dormancy. Dormancy is a bet-hedging strategy that allows microbes to go into a temporary state of low metabolic activity. After entering dormancy, bacteria may wait for more favorable conditions to break dormancy and become metabolically active. Dormancy, as a survival mechanism, is common. In soils, under prevailing conditions, about 90% of the bacterial communities present are inactive, 50% of all bacterial taxa are surviving in dormancy, and at least 25% of all soil genomes contain genes that enable individuals to be resuscitated from a dormant state (Alvarez et al., 1998; Lennon and Jones, 2011; Wang et al., 2014a Aanderud 2015). As bacteria break dormancy there is a burst of activity often referred to as a "pulse." In arid systems, the limiting resource is usually water. Pulses of water resuscitate dormant microorganisms in the soil. In the Sonoran Desert where pulses of water are often few and far between, soil crusts contributed to 80% of the soil level CO_2 fluxes in the atmosphere suggesting that even top layers of soils should be accounted for when measuring CO_2 efflux.(Jessica M Cable, 2003) In temperate coniferous soil in Nova Scotia, the effect of water pulses on decomposition activity was measured in both shallow and deep soil cores by looking at responding CO_2 to changes in temperature. In shallow samples, soil responds with threshold behavior of a higher amount of CO_2 in correlation to water, while deeper samples respond more to temperature than moisture but a pulse of activity still exists. CO_2 increases with increasing amounts of water. (Kellman L, 2013) In multiple systems at multiple depths, pulses of activity contribute to ecosystem function around them. Although very short, pulses may play a large role in ecosystem net production by altering soil respiration rates. CO_2 output is a product

of plant and microbial metabolism but not all pulses are created equal. Pulses of activity can vary in response according to pH, temperature, soil characteristics, water additions, vegetation type, and surface depth, among hosts of other factors we may not fully recognize yet. Multiple environmental stresses cause bacteria to enter dormancy (i.e. temperature, moisture, nutrient availability, chemical changes etc.), but two stresses revolving around soil moisture are universal to all systems and elicit immense pulses of activity every time. Those universal stresses are wet-dry and freeze-thaw cycles.

Rewetting and Freeze Thaw are Huge

The rewetting of dry soils and thawing of frozen soils are common occurrences in almost all terrestrial systems creating immense selective pressure on bacterial community composition and activity. For example, rainfall events rewetting soils may induce pulses of soil respiration that can account for 5–10% of annual net ecosystem CO_2 production in mid-latitude forests (Lee et al. 2004) and up to 90% of the late-season ecosystem respiration in semi-arid grasslands. The mechanisms by which they do this also exist in desert soils (Xu and Baldocchi 2004). Rewetting events in grasslands, coniferous forests, deciduous forests, arid lands, etc. can instantaneously create a response starting with changes in C and N mineralization.(Austin AT) Water availability can vary depending on soil type. If soil has a higher water retention, then nutrients have more time to be moved before bacteria become dormant again, increasing the amount of nitrogen mineralization. These discrete water additions account for a large part of nutrient cycling and availability, cascading effects up the ecosystem.

During freeze-thaw and wet-dry cycles, microbes are put in and out of dormancy and over time it causes them to become stressed to the point of lower abundances and production. It

also selects for a community that is composed more of generalist bacteria such as Actinobacteria that are capable of producing spores to survive. Throughout time gene function is diverted from metabolism to replication and repair which suggests, along with CO_2 levels that productivity decreases with frequent stress cycles. The rare responders of the community which are largely responsible for the nutrient cycling of the system are greatly reduced or in some cases altogether lost. Drying-rewetting cycles as a type of stress are the most frequent environmental stressors for soil microorganisms. About one third of the earth experiences conditions that are either arid, semi-arid or seasonally arid and many other places such as grasslands, dry tropical forests, chaparral, etc., experience drying/rewetting effects.(Gurevitch 2007) Soil microorganisms use many different pathways to cope with the stress of wet-dry cycles. During periods of drought, bacteria accumulate solutes to equilibrate with the surrounding water potential and this helps them from desiccating to death. This life strategy requires a significant amount of energy and is metabolically taxing, using between 7-20% (Firestone 1984) of total bacterial C and 11-30% bacterial N(Tibbett et al 2002). Resources are devoted to survival rather than growth metabolism which puts them into a state of dormancy and little production, too long in this state of survival can lead to death. Upon rewetting, the osmolytes need to be released before the water crosses the membrane causing heavy damage or rupture. The microorganisms respond to this by respiring, polymerizing, or transporting them across the membrane, this releases cell biomass, sometimes up to 50%. Freeze-thaw events cause high metabolic stress in similar ways to wet-dry stresses. When bacteria are frozen, water potential is taken away from the surrounding environment and bacteria are required to form osmolytes to survive the change in water potential in order to keep from desiccating. When soils are thawed, a rush of water becomes available and the osmolytes need to be released to prevent cell rupture. Both of these water stresses select for specific

communities causing some bacteria to enter dormancy and some to thrive. The two stresses occur at totally different times in the season but fundamentally the stresses are marked by a decrease in soil moisture. Since much of the stress centers around osmotic stresses of contending with the absence of water and the subsequent release of soil moisture, the two types of stress may actually select for similar types of bacteria that are able to break dormancy and become active. Further, the genes associated with drying-rewetting and freeze-thaw may also be similar since it requires them to survive environmental responses as drought tolerators. We would expect to see bacteria that express genes allowing them to change osmotically, go in and out of dormancy, membrane transport, etc. It is important to look at bacteria that not only influence C and N pools but also to add the environmental change to fully understand how bacterial communities influence function though time rather than just certain instances.

Cold deserts offer an ideal backdrop to compare bacterial responses to dry and frozen soil being rewetted or thawed. Cold deserts occur across every continent and cover approximately 21.4% of terrestrial biomes. In cold deserts, bacteria live in conditions often suboptimal for metabolic activity due to soil temperature and moisture extremes. Cold desert bacteria must contend with some of the coldest and driest locations on earth. In the dry valley soils of Antarctica, the soil microorganisms have developed with average temperatures of -20 C and moisture of about 10 cm a year. (Burkins 2001) During the Antarctic summer, soils in the dry valleys reach temperatures high enough to cause meltwater in the valleys which largely isn't available to the soil systems. In contrast to Antarctica, in the Colorado Plateau of Southeastern Utah temperatures can be below 0 C and up to 45 C. Precipitation events vary with 71% being below 5mm, frequent rainfall events cause rapid movements in and out of dormancy.(Bowling 2011) Rainfall events in cold desert soils create large pulses of activity (Huxman et al. 2004).

Within hours of a rewetting event, CO_2 production can achieve rates that are 475% higher than pre-wetting conditions (Fierer and Schimel 2003). Bacteria communities that break dormancy have short and discrete events of high productivity mostly from rare responders. Freeze-thaw cycles also regulate water availability leading to these pulses of activity. Freeze-thaw cycles can either create water limitations or free up water to be used by the microbes, functioning much like a wetting event but under colder temperatures.

In our study, we tracked changes in bacterial activity following soil water stress in soils across three cold deserts from three different continents. We measured shifts in the active bacteria community, measured as 16s rRNA communities, and subsequent pulses of CO2 during drying-rewetting and freeze-thaw cycles. Specifically, we focused on capturing similarities between bacterial communities while soils were dry and frozen compared to when soils were wet and thawed to capture patterns of bacterial activity and dormancy. We also measured gene function as PICRUST (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States) to identify potential genes allowing bacteria to be responsible for the break from dormancy. We aimed to identify whether bacterial response to water was universal or localized, if bacterial adaptations to water stress were the same with similar but geographically isolated systems, and if responses to water stress resulted in an increased or decreased amount of productivity and diversity among all systems.

MATERIALS AND METHODS

2.1 Soil Cores and Site Description

Our sampling locations included Great Basin, Utah, Gurbantunggut Desert China, and the McMurdo Dry Valleys, Antarctica. All soils were removed as intact soil cores with a total soil volume of 691.36 cm^2 using PVC pipe (7.6 cm width × 15.24 cm height). The Great Basin Desert soils were removed from Rush Valley, UT (40°05'27.43"N - 112°18'18.24"W). Soil surface composition at the Great Basin site were dominated by the lichens *Collematenax* and *Toninia sedifolia* in shrub interspaces, and the moss *Syntrichia ruralis*, beneath shrubs. The shrub community was dominated by *Artemisia tridentata, ssp. Wyomingensis*, and a native perennial grass *Elymus elymoides*. Mean annual precipitation at the Great basin site specifically is 27.57 cm $year^{-1}$ (± 1.39, *n*=30) and mean annual temperature is 8.87°C (± 0.056, *n*=30). Soils were derived from Lake Bonneville sediments with a Taylorsflat series silt loam texture. The series consists of well-drained, fine-loamy, mixed, mesic Xerollic Calciorthids with 3 to 15% calcium carbonate. Surface soils are alkaline and had a pH of 7.6. The Antarctic soils were removed from the McMurdo Dry Valleys Long-Term Ecological Research Station (MCM LTER) site located in Taylor Valley, Antarctica (77°37'S163°00'E). Mean precipitation for Taylor Valley is >10cm and mean annual temperature is 19°C(± 3). Soils in the Dry Valleys are derived from tills enriched by granites, sandstones, dolerites, and meta-sedimentary rocks that range from Holocene to Miocene in age, they are alkaline, coarse textured, low in organic matter, and contain high concentrations of soluble salts and high pH of 9.3. Soil chemistry and texture reflect a legacy of glacial outwash and mountain erosion. The lower depth of the soils is very weathered and are reflected by a very fine material. There exists no vegetation in the Dry Valleys but there are a mosses and lichens. The Chinese soils were collected in the Junggar Basin of the

Xinjiang Uygur Autonomous Region of China at the desert research site operated by the Xinjiang Institute of Ecology and Geography in the National Academy of Chinese Sciences (They said no to GPS but I have emailed Xioying to ask again). Mean annual precipitation 135mm per year. The mean annual temperature is 7.26 °C with a neutral pH of 7.2. Natural vegetation is dominated by Haloxylon Ammenodendron and Haloxylon Persicum, as wellas shribs and small semi-shrub including Ephedra Distachya L., Calligonnum Leucocladum, Artemisia Capestris subsp. Indora Nyman and Seriphidium Terrae-Albae.

2.2 Drying-rewetting and freeze-thawing cycles

We exposed intact soil cores to either drying and rewetting events of freezing-thawing events to identify patterns of response to desiccation and water additions. Intact soil cores allowed us to remove potential variation in soil conditions and create specific shifts in soil moisture. The cycle for drying and rewetting included: If Soil cores were above 5% VWC they were allowed to finish drying down to 5% VWC before they were wetted to 30% VWC and allowed to dry down to 5% VWC at room temperature. Freeze-thaw cores were first wetted to 30% VWC then were manipulated inside of a ScienTemp freezer. The cycle for freezing and thawing included:The samples were first stepped up to 5°C with the following protocol, 24 hours at -10°C, 24 hours at 0°C and then put to 5°C where they were manipulated. The cycle undergone was 24 hours at 5°C, 24 hours at 0°C and 72 hours at -2°C then repeated back up the ladder. Samples were taken at -2°C and 5°C.

The Wet-dry and freeze-thaw cycles simultaneously occur over extremely large expanses of varying terrain, to reasonably get a representative sample we randomly took our cores from a 100x100 meter area. Eight intact soil cores from each site were exposed to 2 cycles wet-dry and

freeze-thaw cycles. Wet dry-cycles were determines by volumetric water content while freeze-thaw cycles were determined by temperature variation that we decided to reflect in situ conditons shared by the three locations. Antarctic cores were removed in January of 2016, Great Basin cores were removed in May of 2016 and China cores were removed in July of 2016. Antarctic cores were stepped down following the protocol 24 hours at 4°C, 24 hours at 0°C, 24 hours at -10°C, and then down to -20°C. They were kept at -20°C in transit and then were stored at -20°C for two months before the manipulation started. Utah and Antarctic water manipulations were completed at Brigham Young University in Provo, Utah. China water manipulations were conducted at the Xinjiang Institute for Ecology and Geography in Urumqi, China.

2.3 Soil respiration and moisture

Soil CO_2 and moisture during the water manipulations were conducted with real-time sensor technology to capture changes in trace gas flux, temperature and volumetric water content percentages. We measured CO_2 (ppmv)using non-dispersive infrared absorption with 3% CO_2 GMP 220's(Vaisala, Helsinki, Finland) while simultaneously monitoring soil moisture(m^3 H_2O m^{-3} soil), electrical conductivity, and soil temperature(°C). CO_2 sensors were placed at a 2cm depth to log CO_2 data in real time. The placement of the sensors was chosen to capture gas from the entire core just previous to entering the atmosphere. Acclima TDT soil moisture sensors spanned the entire 15.24cm column to log soil moisture through the entire core. In addition to Volumetric Water content the Acclima TDT sensors also measured soil temperature and bulk relative permittivity . Data from the sensors were logged every 15 seconds then compiled over a 7.5 minute time period and logged on a Campbell Scientific CR1000 Data Logger.

2.4 Soil sampling for bacteria community analysis during water manipulations

The three locations experience significant differeces in their wet-dry cycles and relatively similar conditions during freeze thaw cycles. A large wetting event would wet the soils to about 30% Volumetric Water Content (Miller, 2008). Samples from the cores were taken at 5% VWC and at 30% VMC to reflect in field extremes. (Miller 2008) Antarctic wet-dry samples were taken at 5°C instead of room temperature to simulate actual in field wetting conditions. Water additions to the soil were determined by inserting the sensor and converting the volumetric water content to gravimetric water content then making up the difference to 30%. Soil cores from in field were allowed to finish drying down to 5% VWC before they were wetted to 30% VWC, this was tracked with the Acclima sensors. Freeze-thaw cores were first wetted to 30% VMC then were manipulated inside of a ScienTemp. The cycle undergone was 24 hours at 5°C, 24 hours at 0°C and 72 hours at -2°C then repeated back up the ladder. Samples were taken at -2°C and 5°C with the electrical conduit pipe so the frozen core could be penetrated. All cores underwent two cycles and samples correspond with the high and low points of moisture/temperature in their respective cycles. Water additions were all done with type two purified water from the laboratory tap. The laboratory wet-dry samples were allowed to dry at room temperature (23°C) to ensure temperature conditions were the same. Antarctic samples were dried down at 5°C to ensure preservation of normal taxa. Moisture content of the cycles were determined by looking at the extremes of what the cycles could be in the desert systems. Generally, Soil samples were taken from the cores using electrical conduit pipe that underwent a sterilization procedure. The pipes were first cleaned out with a pipe cleaner to ensure no excess debris remained inside the pipe. They were then soaked in 95% ethanol and then sterilized under flame. To remove RNA contamination, they were sprayed again using RNase away and allowed

to dry in a sterile environment. Sub-samples were taken using sterile conduit and placed into whirlpacks and immediately stored in a ThermoFisher -80°C freezer until total RNA extraction. All sub-samples were taken in replicates of 4 for each sampling cycle point. Prior to extracting samples with MoBio and Qiagen Total RNA kits the samples were homogenized to ensure a general microbial representation throughout the core.

2.5 RNA extractions and bacterial communities

To evaluate the changes of community composition between wet-dry and freeze-thaw cycles, we used target-metagenomics on the 16S rRNA gene. Bacterial communities were evaluated based on 4 samples from different core replicates per sampling point. In total we had 4 sampling points with 4 replicates in 2 different cycles treatments (4 replicates x 3 sampling points x 3 locations x 2 cycle treatments= 72 samples) RNA was from 4 g of soil using most of the protocol from the PowerSoil Total RNA Isolation Kit (MoBio, Carlsbad, CA) and the Total RNA Isolation kit(Qiagen). More soil than the 2-gram recommendation was used due to low biomass in soil samples soil samples(we used 4 grams). Samples were also incubated for 30 minutes at room temperature rather than at -20C. Samples were all also vortexed for 5 minutes longer than protocol recommendation during the bead beating step. To convert total RNA to cDNA we used the ThermoFischer Super Script III cDNA conversion kit according to manufacturer's protocol. We amplified the V4 region of the 16S rRNA gene using the bacterial specific primer set 515F and 806R with a 12-nt error correcting Golay barcodes (Fierer et al., 2009; Aanderud et al., 2013). We used the following thermal cycle for PCR reactions: an initial denaturation step at 95ºC for 5 minutes followed by 35 cycles of denaturation at 95ºC for 15 seconds, annealing at 55ºC for 30 seconds, and an extension at 68ºC for 1 minute per kb, then a

4ºC hold. We cleaned up the PCR reaction using the ExoSap PCR Cleanup Product Reagent (ThermoFisher Scientific). Prior to sequencing preparation the samples were checked for content using gel electrophoresis. Samples then underwent normalization using the SequelPrep Normalization Plate Kit according to manufacturer's protocol(Invitrogen). After normalization samples were submitted to the BYU sequencing center and amplified using the Illumina Hi SEQ 2500. We analyzed all sequences using *mothur* (v. 1.39.1) to remove barcodes and short reads, chimeras, and non-bacterial sequences (Schloss et al., 2009). Specifically, we excluded sequences < 260 bp with homopolymers longer than 8 bp, removed chimeras using UCHIME (Edgar et al., 2011), and eliminated mitochondria, archaeal, and eukaryotic 16S rRNA gene sequences based on reference sequences from the Ribosomal Database Project (Cole et al., 2009). We then aligned sequences against the Greengenes database with the SEED aligner, created operational taxonomic units (OTUs) based on uncorrected pairwise distances using a minimum coverage of 93% and minimum pairwise sequence similarity of 97%, and determined the phylogenetic identity of OTUs with the Greengenes database. (http://greengenes.lbl.gov)

2.6 Bacterial Community Analysis

We analyzed bacterial communities by first, using ordination based heatmaps, sequence count abundance distributions of top taxa, and diversity plots(Shannon, Richness, Simpson, Inverse-Simpson). To analyze shifts in bacterial communities following the water stress manipulation, we used Principal Coordinates Analysis (PCoA) and permutational multivariate analyses of variance (PERMANOVA, Anderson 2001). The PCoA was based on a Bray-Curtis distance matrix using the 'phyloseq' package in R (McMurdie and Holmes 2013). The PCoA aided in the visualization of communities, but we tested for the main effects and interactions

between location, time point in cycle, and Volumetric Water Content with PERMANOVA using the *adonis* function also in the *phyloseq* package of R. Heatmaps were made using taxonomic trends of relative recoveries and some of the major families and orders were shown in a heat map with hierarchal clustering using the *heatmap* function in the ggplot package in R. We quantified the alpha diversity of communities as the inverse Shannon index and richness as the total number of OTUs based on 1000 iterations of 900 random resampled seqeunces from each replicate. Taxonomic trends of rare responders in some of the major families were shown in heat maps with hierarchal clustering using the *heatmap* function in the ggplot package in R. Rank families were determined by calculating relative recoveries in Excel by dividing the read counts of the OTUs by the total number of sequences.

RESULTS

3.1 Pulses of Ecosystem Activity Freeze-Thaw

Samples from China and the Utah during freeze-thaw cycles produced slowly elevated CO_2 over 3-5 days as fluxes reached a maximum of at most 1098 ppm in China and 315 ppm in Utah, both pulses following an increase in temperature. In contrast, Antarctic soils generated cycles with higher maximums following soil freezing (335 – 265 ppm at -2°C) and freeze-thaw pulses peaked more rapidly in soil samples from Antarctica (2 days) than China and Utah soils. Our results suggest that the effects of soil thawing on soil respiration are unique but not universal among ecosystems. Desert soils in Utah and China respond according to moisture and temperature(P= .017). Antarctic soils have perhaps evolved differently due to constant low temperatures and moisture levels. They also peak when temperature is at its coldest(-2C, 295-230PPM)(P= .017).

3.2 Pulses of Ecosystem Activity Wet-Dry

During wet-dry cycles, samples from the USA and China produced slowly elevated CO_2 over 14-16 days as fluxes reached a maximum of at most 2384 ppm in China and 1507ppm in Utah. In contrast, Antarctic soils generated cycles with higher maximums following soil drying (321 – 293 ppm at 6%VWC) and wet-dry pulses peaked more rapidly in soil samples from Antarctica (1 day) than China and USA soils.

3.3 *Antarctica Wet-Dry and Freeze-Thaw*

Antarctic samples marginally change according to treatment type with greater changes occurring during freeze-thaw cycles rather than wet-dry cycles. This inference was based on 415,799 quality sequences with 87% or greater coverage and 16,389 unique otus. In Antarctic Wet Dry cycles samples separated in ordination space according to moisture although there is still some overlap between samples making the changes between communities very small. PERMANOVA results support this interpretation, Shannon diversity between the Antarctic samples is somewhat significant(P=.082) but wet-dry and cycle stage(Wet 1, Wet 2, Dry 1, Dry 2) are not. During freeze-thaw cycles samples are separated in ordination space according to freeze or thaw and Shannon diversity changes accordingly. PERMANOVA results support this interpretation showing that there is an effect from Shannon diversity(P=.049) and freeze or thaw cycles(P=.045).

The response of these familial groups were freeze-thaw and wet-dry specific due to the clustering hierarchal rank. The recovery of rare bacteria in wet, dry, and thaw communities were very closely related to each other and thus grouped together. In the case of the freeze 1 and freeze 2 treatments they varied farther in space allowing wet 1 and wet 2 in between them. The

differences between wet-dry and freeze-thaw cycles were very large. For example, with Conexibacteraceae in frozen and wetted samples were at least 1.5x higher in abundance that dry and thawed samples. In contrast during thawed and dried samples Rhodobacteraceae were at least 1.5x higher in thawed and dried samples than in frozen and wetted samples. (CONEXIBACTERACEAE, PATULIBACTERACEAE, and SOLIRUBROBACTERACEAE are nitrate reducers which show up in wetted and frozen samples which also correlates with CO2 production)

Antarctic PiCRUST samples reveal gene content that is indistinguishably similar in all categories. The PiCRUST algorithm uses existing sequenced genomes to predict function composition of genomes using marker gene databases of reference genomes. It then ises the ancestral state reconstruction algorithm to which gene families are present in order to then estimate a composite genome for the samples(Morgan Langille 2014). Due to the lack of reference genomes for Antarctic soil samples this present a problem of prediction. PiCRUST is able to predict estimates of percentages of genes but is uncapable of predicting differences between samples with any certainty even with plausible NTSI Scores differences between percentages are ~.005%.

3.4 Utah Wet Dry and Freeze Thaw

Utah samples change in ordination space according to treatment type with greater changes occurring during freeze-thaw cycles rather than wet-dry cycles. This inference was based on the 415,799 quality sequences with 87% or greater coverage and 16,389 otus. In Utah wet-dry samples separated in ordination space with the largest distance being between Wet 1 and Wet 2 showing a large variance of community. Dry cycle samples sit between Wet 1 and Wet 2

and are very close together in ordination space, showing similarity of communities between the dry samples. PERMANOVA results support this conclusion with Shannon diversity being significant(P=.007) and wet or dry being significant(P=.036). There is no significance of cycle stage or combination of variables. Freeze-thaw cycles in Utah are all very close together in ordination space but far enough away to have significant changes in Shannon diversity. PERMANOVA results support this conclusion with Shannon diversity being significant(P=.003) there is also a marginally significant interaction between Shannon diversity x Cycle stage(P=.088).

The response of these familial groups was freeze-thaw and wet-dry specific due to the clustering hierarchal rank. The recovery of rare bacteria was more similar in wet dry cycles than in freeze-thaw cycles, hierarchal distances in freeze thaw cycles are much larger than wet-dry. Wet-dry cycles are separated in dry cycles, perhaps suggesting basal communities of wet cycles. Wet cycles also show higher abundances of about 2x more of Sphingomonadaceae than dry cycles, Sphingomonadaceae is a know responder to moisture. The differences between wet-dry and freeze-thaw cycles were very large. For example, Phormidiaceae is 1.5-2x more abundant in thaw cycles than in freeze cycles while Rubrobacteraceae has higher abundances in frozen samples.

Predicted metabolic functions of the microbial communities in Utah wet-thaw and freeze-dry cycles were determined by using PICRUSt (v1.01). To measure accuracy of the prediction we used the Nearest Sequenced Taxon Index, for Utah out score is .22 +/- .019 which is within the limits for an accurately predicted phylogenetically diverse sample. Utah Wet-Thaw samples show similarity in gene count percentages across rolled up categories. Among those categories similar trends repeatedly occur. In replication and repair and increase in gene percentage(~.8%

for both categories) occurs for both Thaw 2 and Wet 2 suggesting that over time damages occur to the microbes. There is also a decrease in membrane transport over time, Wet 2 and Thaw 2 decreasing(~.5%). Not all categories show decreases or similar trends throughout time, energy metabolism shows increases from Wet 1 to Wet 2(6.04% - 6.29%) and Decreases from Thaw 1 to Thaw 2(6.40% - 6.28%).

Utah freeze-dry cycles show similar gene percentages for most categories but diffences do exist among key categories. Membrane Transport shows a decrease from Dry 1 to Dry to(11.69% - 11.28%) and an increase from Freeze 1 to Freeze 2(11.58% - 11.73%). In the Metabolism category Dry 1 and Dry 2 increase(2.55% - 2.65%) while Freeze 1 and Freeze 2 decrease(2.56% - 2.49%). Transcription sees decreases from Dry 1 to Dry 2(2.39% - 2.28%) and a minute decrease from Freeze 1 to Freeze 2(2.408% - 2.405%)

3.5 China Wet Dry and Freeze Thaw

In China Samples are separated in ordination space and based on wet-dry cycles. The two different Wet1 and Wet 2 are separated the farthest away in ordination space with Dry 1 in between them. There is no dry cycle 2, it had to be eliminated based on low sequence reads. In the PERMANOVA results Shannon diversity is significant(P=.002), Wet-Dry is marginally significant(P=.078). There are significant Shannon x WetDry and Shannon x CycleStage effects(P=.001, P=.008), showing that more significant than the moisture levels themselves were the stages of the cycles through time. In freeze-thaw cycles samples are separated very little in ordination space, denoting similar community types. However, samples are separated in space based on treatment type with very little overlap. PERMANOVA results support this

interpretation with Shannon diversity being marginally significant(P=.074) but having strong Cycle Stage significance(P=.005) and Shannon x Cycle Stage interactions(P=.001).

The response of these familial groups manifests itself more in the stage of the wet-dry and freeze-thaw cycles rather than the individual treatments themselves. The recoveries of the samples show groupings between the 1st and 2nd part of each treatment. Wet 1 and wet 2 show differences of bacteria that are able to handle stress. For example Micrococcacaea is perhaps over 2x higher in wet 2 than it is in wet 1 and almost all other families of bacteria from wet 1 drop off in abundances to wet 2. Micrococcaceae are known for taking advantage of transient water input in arid soils.(Schwartz et al., 2014) Freeze-thaw samples are also separated between treatments 1 and 2. Thaw 2 and Freeze 2 show higher abundances of 1.5-2x more bacteria than their counterparts.

Predicted metabolic functions of the microbial communities in China wet-thaw and freeze-dry cycles were determined by using PICRUSt (v1.01). To measure accuracy of the prediction we used the Nearest Sequenced Taxon Index, for China our score is .20 +/- .027 which is within the limits for an accurately predicted phylogenetically diverse sample. Among the various categories most are very even in their expression of gene percentages, the most notable differences being in Membrane Transport, Amino Acid Metabolism, and Replication and Repair. In Membrane transport there is an increase from Thaw 1 to Thaw 2(11.73% - 11.94%) and a decrease from Wet 1 to Wet 2(12.06% - 11.94%). In Amino Acid Metabolism there are increases in both Wet 1 and Wet 2(10.90% - 10.99%) as well as Thaw 1 and Thaw 2(10.93% - 11.04%). For Replication and Repair there is a decrease from Thaw 1 to Thaw 2(6.71% - 6.63%) and an increase from Wet 1 to Wet 2(6.61% - 6.63%).

China Freeze-Dry cycles showed similar patterns among the different categories with significant differences in Membrane Transport, Translation, Xenobiotics Biodegradation and metabolism, and Cellular Processes and Signaling. In the Membrane Transport category there is and increase from Freeze 1 to Freeze 2(11.5% - 11.78%) but Dry 1(12.07%) is higher than the Freeze treatments. Translation has a slight decrease from Freeze 1 to Freeze 2(4.31% - 4.29%) while Dry 1(4.05%) is lower than both of the Freeze treatments. Xenobiotics Biodegradation and Metabolism has an increase from Freeze 1 to Freeze 2(3.39% - 3.58%) while Dry 1(3.92%) is higher than both of them. Cellular processes and signaling has a decrease from Freeze 1 to Freeze 2(3.14% - 2.96%) while Dry 1(3.01%) is in between the Freeze treatments.

DISCUSSION

Moisture induced pulses of ecosystem activity are very well documented in soil ecology. CO2 contribution is usually surmised between bacterial response and the piston effect, or the release of CO2 from pores in the soil that have been trapped from the previous activity of bacteria in the soil. Bacterial pulses of activity are usually produced following a couple different movements; First the increased moisture allows them to break free from desiccation stress, thereby shedding metabolites and second; nutrients are redistributed which in most cases can lead to increases in abundances. Increased frequency of wet-dry cycles can lead to overall health stress due to changes in cell walls and metabolic pressure causing them to die. This can also lead to increases of CO_2 in subsequent cycles, bacteria are well known for feeding on dead microbes which allows them to more readily assimilate nutrients into their system.

High frequencies of freeze-thaw cycles with sub zero temperatures and relatively fast warming rates are particularly damaging for microorganisms, due to extracellular ice formation,

leading to the concentration of soil solutes and resulting in protein denaturation, membrane damage, cell dehydration, osmotic stress and lower metabolic rates due to colder temperatures. (Nedwell, 1999; Rodrigues & Tiedje, 2008). Freeze-thaw events also distribute nutrients likea wet dry event, when water becomes available it moves bringing nutrients along with it. In order for bacterial communities to survive some of them need to become inactive to survive the changing conditions. Just like in wet-dry cycles bacteria will become stressed to the point of death rather than just inactivity which allows the next cycle to feed on the dead and produce another much smaller pulse of activity.

Microbial communities in China and Utah responded very similarly to freeze-thaw treatments. Soils in China and Utah show peaks during thawing events and lows during freezing events. The CO_2 response to these treatments is somewhat lagged due to the microbial community turnover but responses are triggered by temperature and changing moisture conditions. Soils in both locations need to be warmed for ~6 hours before a large CO2 response is documented. Our results suggest that the effects of soil thawing on soil respiration are unique but not universal among ecosystems. Desert soils in Utah and China respond according to moisture and temperature because their ecosystem has a much higher variance of temperature than does Antarctica, China and Utah can both see temperatures of over 100 degrees F in the summer and sub-zero temperatures in the winter. Even though moisture and temperature comditons were as close as they could be the responses are still different, this is in part due to differences in available carbon and nitrogen levels or soil chemistry. Soils in China average a pH level of 7.1 which soils in Utah average about 7.5.Carbon and Nitrogen levels are also much lower in China(Carbon- .12% Nitrogen- .002%) than Utah(C- 1.5% Nitrogen- .06%). However lower CN ratios in China would leave one to believe that CO2 production would be lower due to

less nutrient input in the system and we see the opposite. Perhaps evolutionary history of intermittent water dispersal in China selects for bacteria that are much more productive in short spans of time due to limiting water.

Antarctic soils have perhaps evolved differently due to constant low temperatures and moisture levels. They also peak when temperature is at its coldest(-2C, 295-230PPM) this would suggest that Antarctic bacteria are more evolved to handle cold weather and perhaps have rare taxa that are coming into effect and doing a large amount of work during those times. Nutrient content and pH levels of Antarctica are much different than the other two places. The pH of the soils are highly alkaline at an average of 9.3 with Nitrogen and Carbon levels reflecting the heterogeneity of the soils. Carbon in Antarctica on average is .26% while Nitrogen can vary between below detectable limits to .052% of the soils.

Microbial communities in China and Utah also responded very similarly to wet-dry treatments while Antarctic soils responded in an opposite way. Our results suggest that the effects of soil wet-dry cycles on soil respiration are unique but not universal among ecosystems. Desert soils in Utah and China respond according to elevated levels of moisture. Although China has a higher peak of CO_2 response it has a longer lag time before it gets there. It takes Utah soils ~24 hours to reach peak time while in China it takes ~48 hours. The initial response from China is due to a piston effect where trapped CO_2 from previous responses is being flushed out. While it is being flushed out the bacterial community probably turnover to one that gets activated and continues the upward trend of the CO_2 from the periods of 24-48 hours then crashes. The first peak in China has a much slower drop off (~13 days) than the second(~36 hours) suggesting that China is much more adapted to interspersed water additions. The repeated cycles of wet and dry are stressors for some of the bacteria that are adapted so move in and out of dormancy. In Utah

both peaks of CO_2 have long drop off times(~15 days and ~10.5 days) which suggests that the bacteria are somewhat more adapted to back to back wetting and drying conditions. Antarctic soils have perhaps evolved differently due to constant low moisture levels. Peaks in Antarctica occur during low levels of VWC ~5%. Although the three deserts are all classified as cold deserts their responses are not universal even when conditions are somewhat similar. The hypothesis that every bacteria is everywhere waiting for the correct conditions to come out is probably not true.

Although Antarctic PCoA plots have the largest range in variation among the three locations, this is happening with a very small subset of samples. In both cases it's the responder of the first pulse treatment, i.e. wet 1 and thaw 1. The community separates in ordination space from the cluster of the other communities. During wet-dry cycles only wet 1 separates itself from the rest of the community and following treatments are very close together. The stress of constant wet dry cycles leads to the community being taken over by stronger generalist bacteria and we even see a dropout from the bacterial family Intrasporangiaceae which is the only increase from the previous wet cycles, all other bacteria decrease in abundance. This leads to a higher evenness among the community, much like the first cycle. When Antarctic bacteria become stressed out they revert to a basal community of generalists that are able to function under the harsh conditions. We see this in the relative recovery heatmaps, samples group in similarity, being more similar to the other samples in their cycle. They also become less even overtime, shifting to families that are typically found in generalist environments like Rubrobateraceae, Xenoccacaea, Sporichthyaceae, Patulibacteraceae, Soilrubrobacteraceae and Micrococcaceae. All of these families of bacteria are gram-positive bacteria with a high GC content allowing them to survive harsh conditions when other cannot, they also have high

tolerance to ionizing radiation. The other rare responders (Rhodobiaceae and Shpingomonadaceae) lie in wait until they are able to recover and come back out again. Those two families of bacteria PICRUST for Antarctic bacteria reach the limitation of the algorithm showing figures that say they are flatlined in their gene change

CONCLUSION

In estimating nutrient cycling for cold desert systems we cannot treat deserts with similar conditions as having similar outcomes. Even minute soil changes lead to structural changes in the evolutionary history of microbial communities. It is also important to note that bacteria do not respond the same to wet-dry and freeze-thaw stresses although both result in increased desiccation stress. Microbial communities in relation to nutrient cycling, more specifically carbon budgets, need to be analyzed seasonally.

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TABLES

Table 1. Times for Drying-Rewetting and Freezing-Thawing

	Drying-rewetting			Freezing-thawing		
	Antarctica	China	USA	Antarctica	China	USA
Cycle 1						
Peak CO2 (ppm)	298.19+- 2.57	2384.2+- 15.23	1507.20 +- 11.04	322.87 +- 2.82	1075.11 +-9.18	306.37 +- 2.76
Time to peak CO2 (day)		2 Days	.5 Day	2.5 Days	1 Day	.5 Days
Duration of cycle (days)	36.5 Days	15.5 Days	15 Days	7 Days	6 Days	6.5 Days
Daily mean CO2 (ppm)	???	???	???			
Cycle 2						
Peak CO2 (ppm)	321.1816 +- 2.67	2231.97 +-14.97	1222.96 +- 9.29	302.20	979.18	286.89 +- 2.39
Time to peak CO2 (day)		60 Hours	1 Day	1 Day	2.5 Days	.5 Days
Duration of cycle (days)	55.5 Days	15 Days	17.5 Days	6 Days	4.5 Days	6.5 Days
Daily mean CO2 (ppm)						

Table 2. Wet-Dry Permanova Antarctica

```
Call:
adonis(formula = community_bray ~ Shannon * WetDry * CycleStage,      data = communitydf)

Permutation: free
Number of permutations: 999

Terms added sequentially (first to last)

                  Df SumsOfSqs  MeanSqs F.Model      R2 Pr(>F)
Shannon            1   0.25522 0.255220  3.7014 0.25369  0.082 .
WetDry             1   0.04555 0.045547  0.6606 0.04527  0.702
CycleStage         2   0.39128 0.195638  2.8373 0.38893  0.113
Shannon:WetDry     1   0.07044 0.070436  1.0215 0.07001  0.522
Shannon:CycleStage 2   0.17460 0.087298  1.2661 0.17355  0.426
Residuals          1   0.06895 0.068953         0.06854
Total              8   1.00603                  1.00000
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
>
```

Table 3. Freeze-Thaw Permanova Antarctica

```
Call:
adonis(formula = community_bray ~ Shannon * FreezeThaw * CycleStage,      data = communit
ydf)

Permutation: free
Number of permutations: 999

Terms added sequentially (first to last)

                  Df SumsOfSqs  MeanSqs F.Model      R2 Pr(>F)
Shannon            1   0.23343 0.233428  4.4748 0.21896  0.049 *
FreezeThaw         1   0.28913 0.289130  5.5426 0.27121  0.045 *
CycleStage         3   0.41551 0.138504  2.6551 0.38976  0.144
Shannon:FreezeThaw 1   0.04476 0.044763  0.8581 0.04199  0.564
Shannon:CycleStage 1   0.03108 0.031082  0.5958 0.02916  0.720
Residuals          1   0.05217 0.052165         0.04893
Total              8   1.06608                  1.00000
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Table 4. Wet-Dry Permanova Utah

```
Call:
adonis(formula = community_bray ~ Shannon * WetDry * CycleStage,      data = communitydf)

Permutation: free
Number of permutations: 999

Terms added sequentially (first to last)

                   Df SumsOfSqs  MeanSqs F.Model      R2 Pr(>F)
Shannon             1   0.28818 0.288177 2.98154 0.25187  0.007 **
WetDry              1   0.22356 0.223562 2.31302 0.19540  0.036 *
CycleStage          2   0.17928 0.089638 0.92741 0.15669  0.575
Shannon:WetDry      1   0.07912 0.079116 0.81855 0.06915  0.645
Shannon:CycleStage  2   0.18070 0.090349 0.93477 0.15793  0.563
Residuals           2   0.19331 0.096654         0.16895
Total               9   1.14414                  1.00000
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
>
```

Table 5. Wet-Dry Permanova Utah

```
Call:
adonis(formula = community_bray ~ Shannon * FreezeThaw * CycleStage,      data = communitydf)

Permutation: free
Number of permutations: 999

Terms added sequentially (first to last)

                   Df SumsOfSqs MeanSqs F.Model      R2 Pr(>F)
Shannon             1   0.32503 0.32503 2.58565 0.18704  0.003 **
FreezeThaw          1   0.18351 0.18351 1.45984 0.10560  0.131
CycleStage          2   0.19344 0.09672 0.76940 0.11131  0.828
Shannon:FreezeThaw  1   0.19541 0.19541 1.55454 0.11245  0.088 .
Shannon:CycleStage  2   0.21187 0.10593 0.84272 0.12192  0.712
Residuals           5   0.62853 0.12571         0.36168
Total              12   1.73779                 1.00000
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Table 6. Wet-Dry Permanova China

```
Call:
adonis(formula = community_bray ~ Shannon * WetDry * CycleStage,      data = communitydf)

Permutation: free
Number of permutations: 999

Terms added sequentially (first to last)

                  Df SumsOfSqs MeanSqs F.Model      R2 Pr(>F)
Shannon            1   0.38574 0.38574  3.7098 0.20765  0.002 **
WetDry             1   0.16873 0.16873  1.6227 0.09083  0.078 .
CycleStage         1   0.38140 0.38140  3.6681 0.20532  0.001 ***
Shannon:WetDry     1   0.27644 0.27644  2.6586 0.14881  0.008 **
Shannon:CycleStage 1   0.12542 0.12542  1.2063 0.06752  0.291
Residuals          5   0.51989 0.10398         0.27987
Total             10   1.85762                 1.00000
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
>
```

Table 7. Wet-Dry Permanova China

```
Permutation: free
Number of permutations: 999

Terms added sequentially (first to last)

                  Df SumsOfSqs  MeanSqs F.Model      R2 Pr(>F)
Shannon            1   0.15829 0.158290  1.5940 0.08834  0.074 .
FreezeThaw         1   0.13620 0.136203  1.3716 0.07601  0.146
CycleStage         2   0.40754 0.203768  2.0520 0.22743  0.005 **
Shannon:FreezeThaw 1   0.14298 0.142984  1.4399 0.07979  0.119
Shannon:CycleStage 2   0.45040 0.225199  2.2678 0.25135  0.001 ***
Residuals          5   0.49652 0.099303         0.27709
Total             12   1.79193                  1.00000
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
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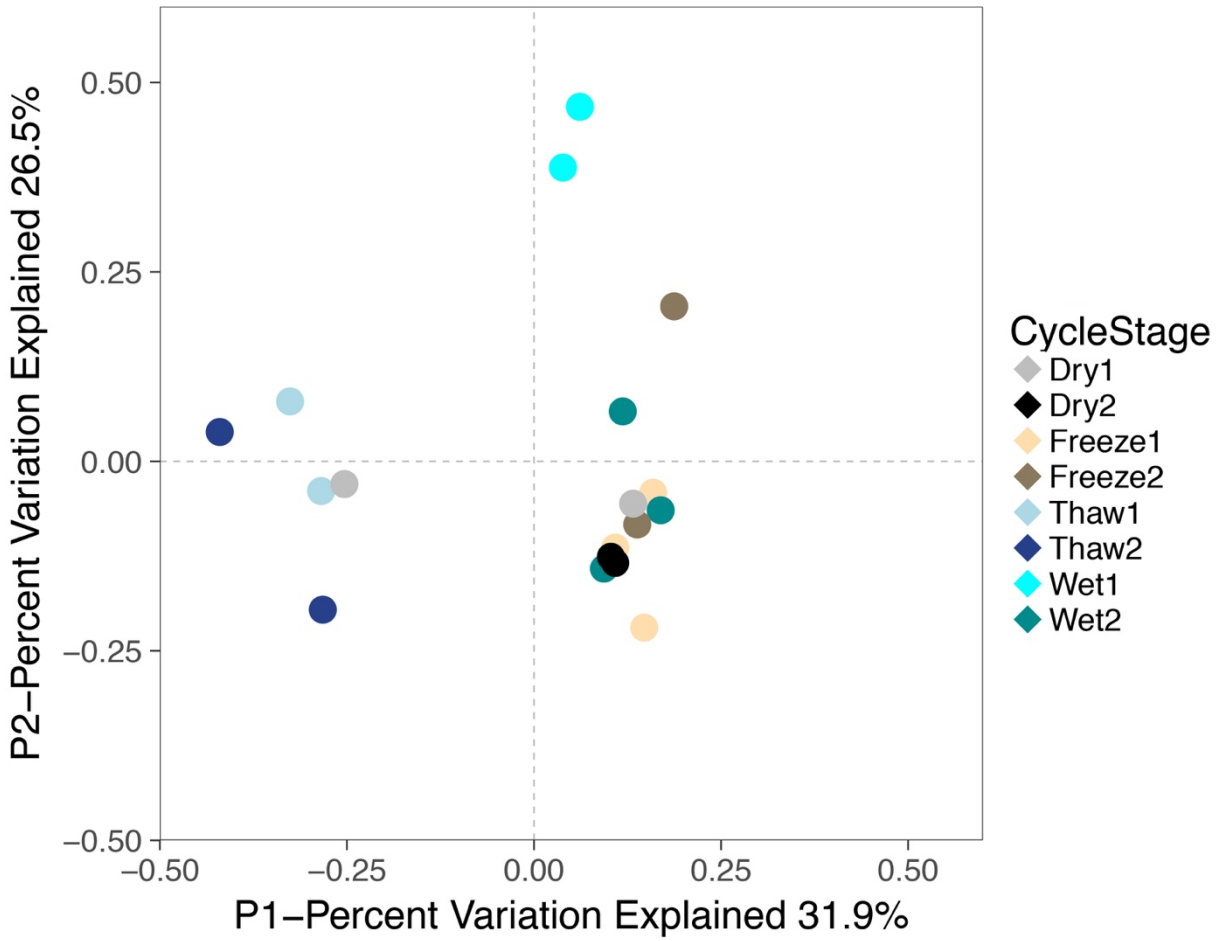

Figure 1. PCoA Antarctica Freeze-Thaw and Wet-Dry

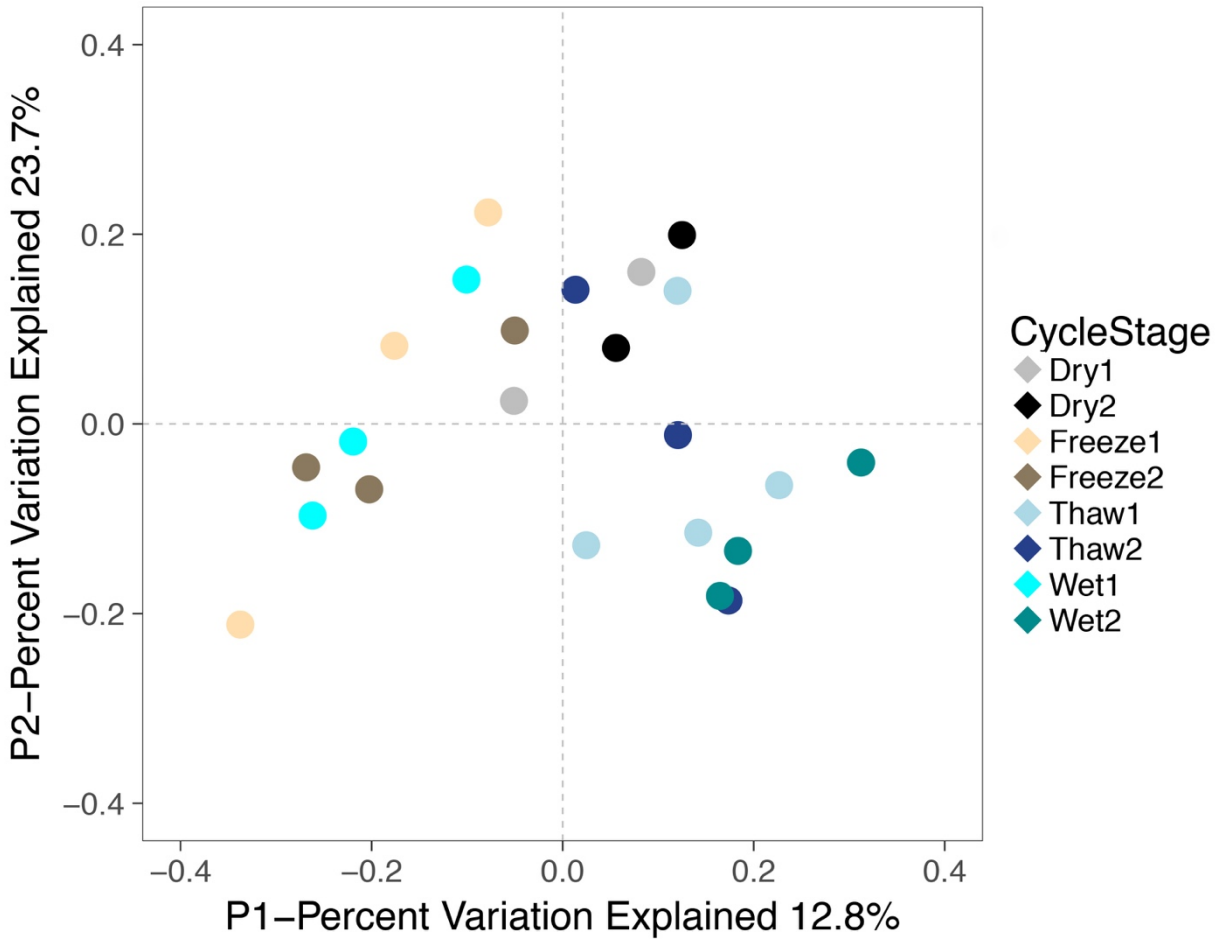

Figure 2. Utah PCoA Freeze-Thaw and Wet-Dry

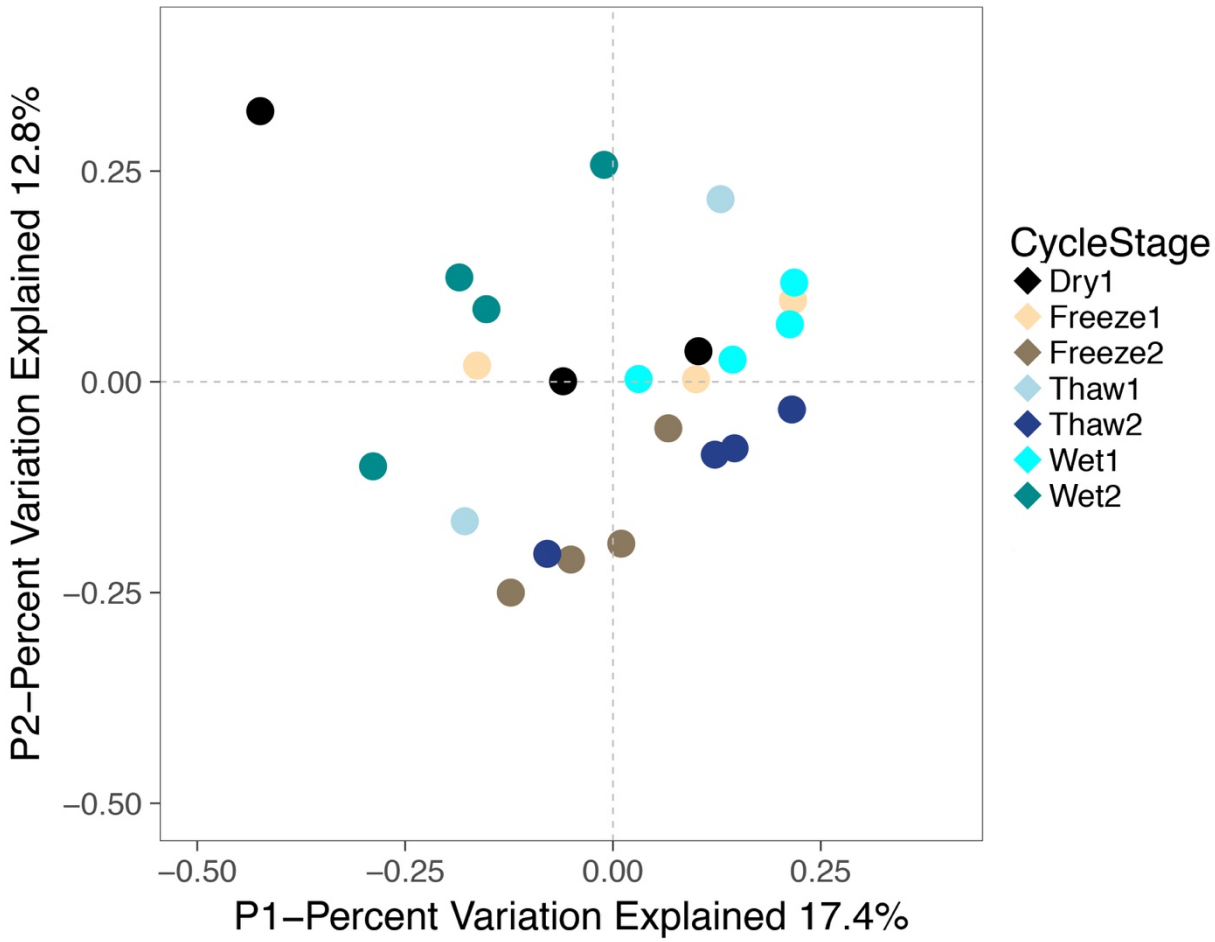

Figure 3. PCoA China Freeze-Thaw and Wet-Dry

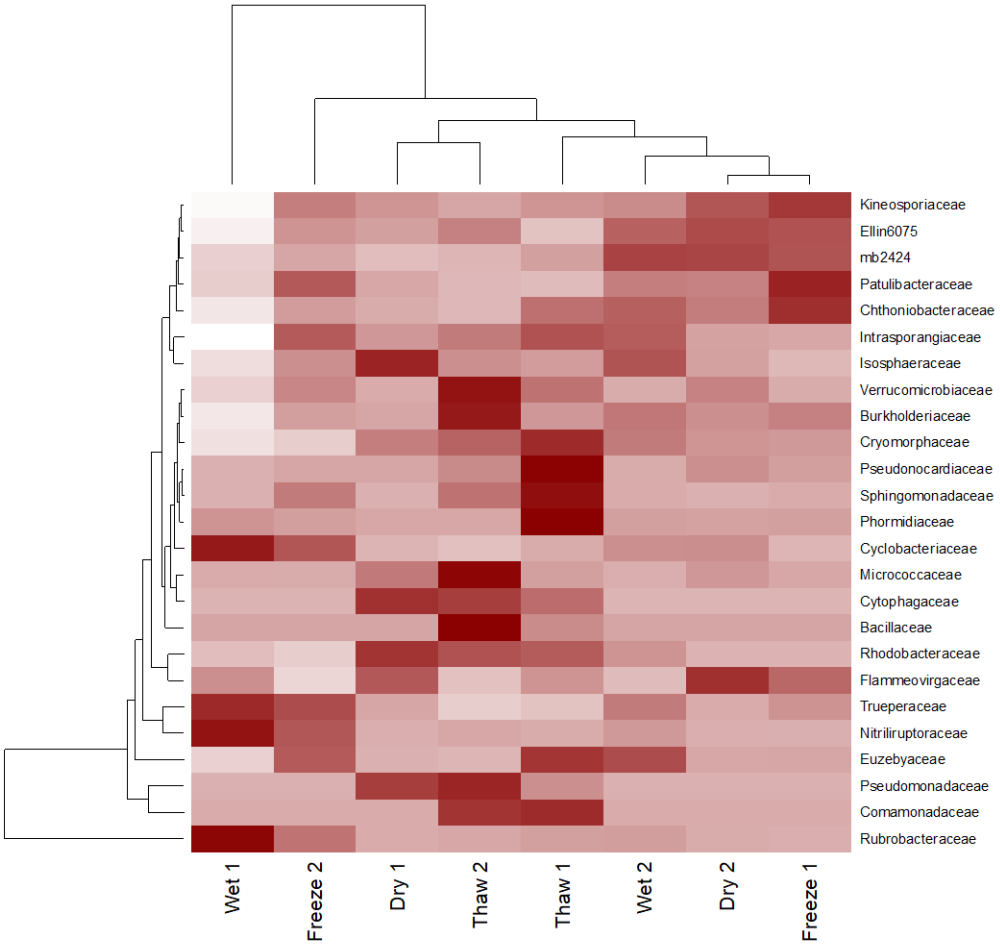

Figure 4. Antarctica Relative recovery Heatmap

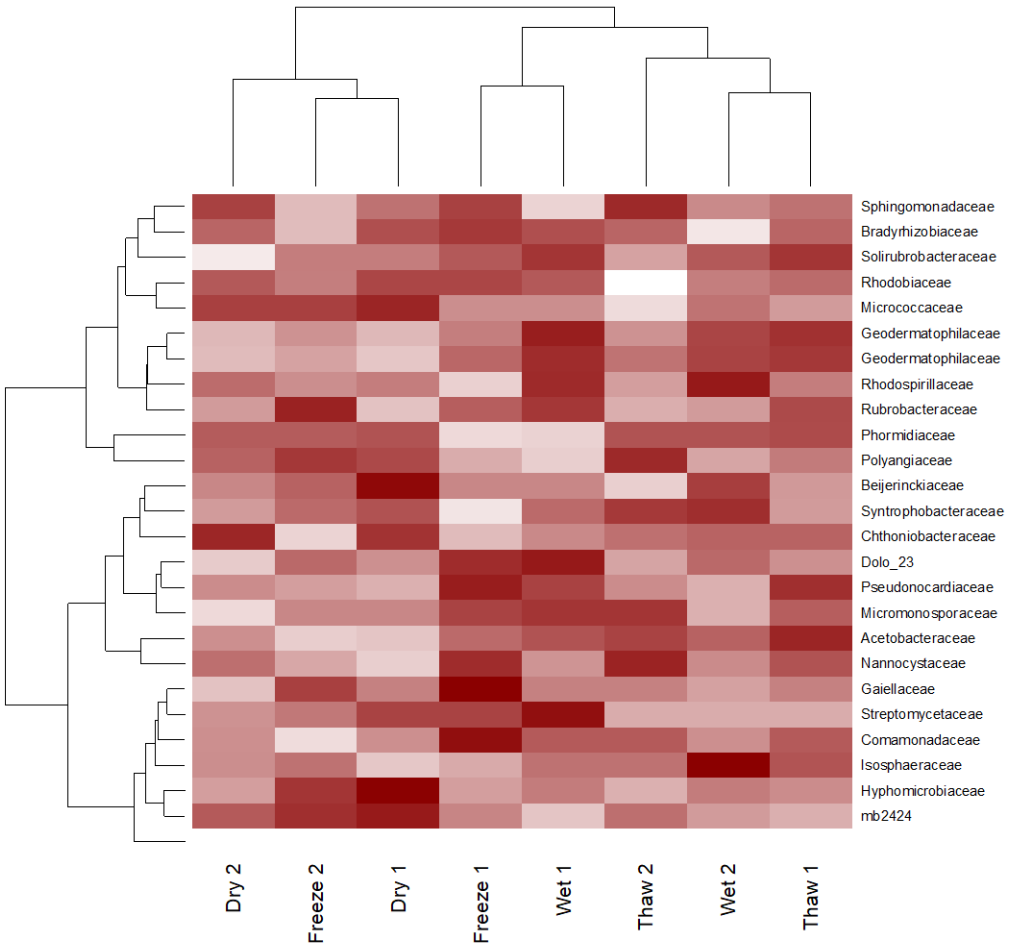

Figure 5. Utah Relative Recovery Heatmap

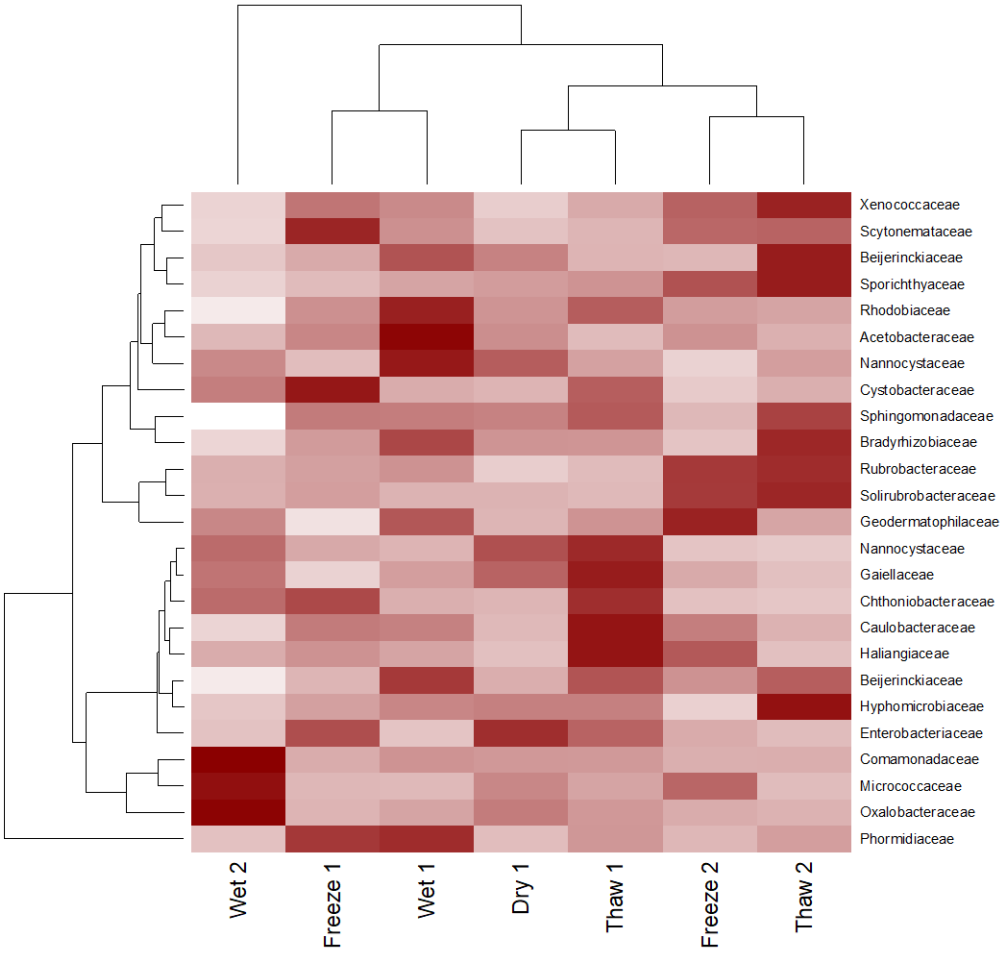

Figure 6. China Relative Recovery Heatmap

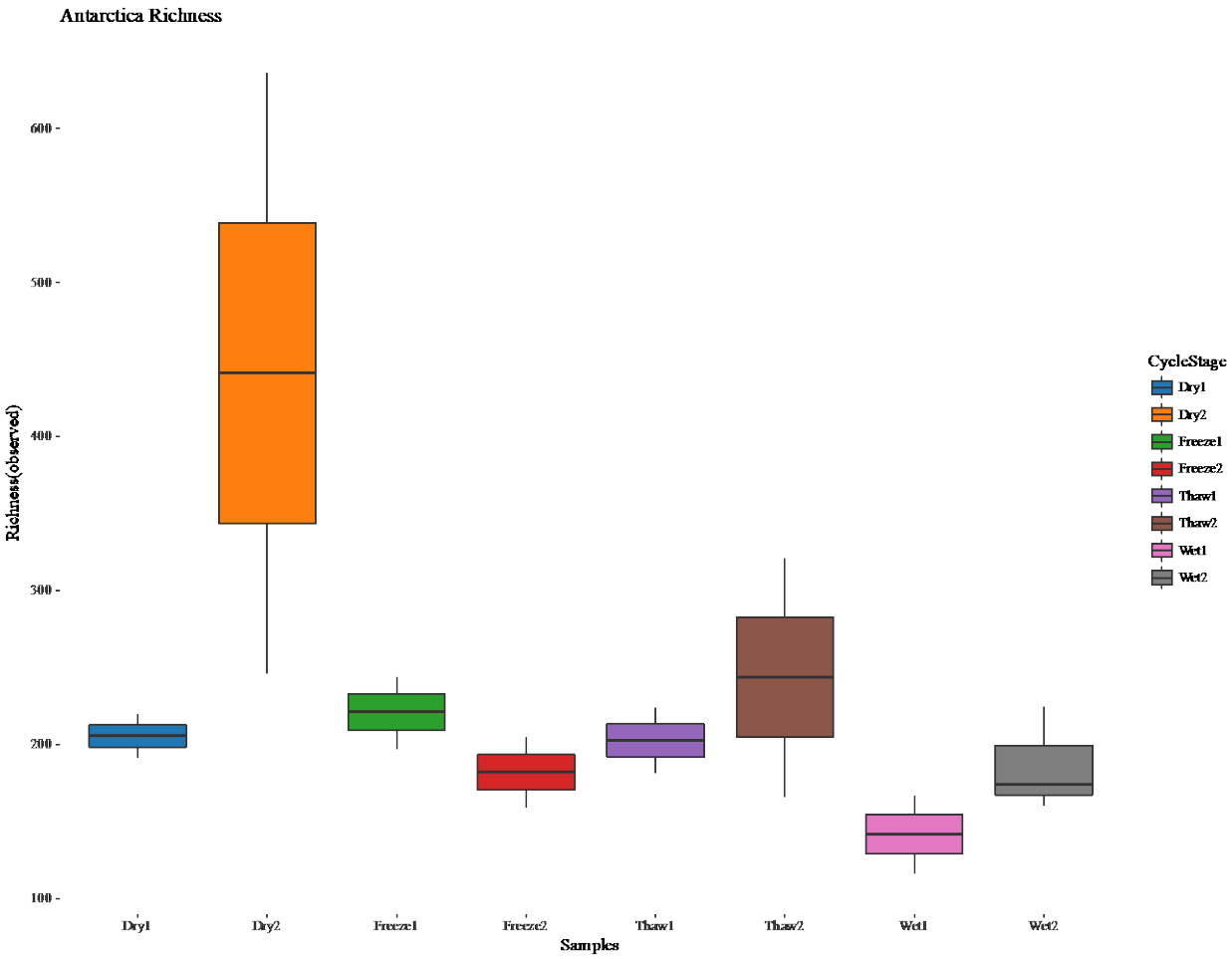

Figure 7. Antarctica Richness Boxplots

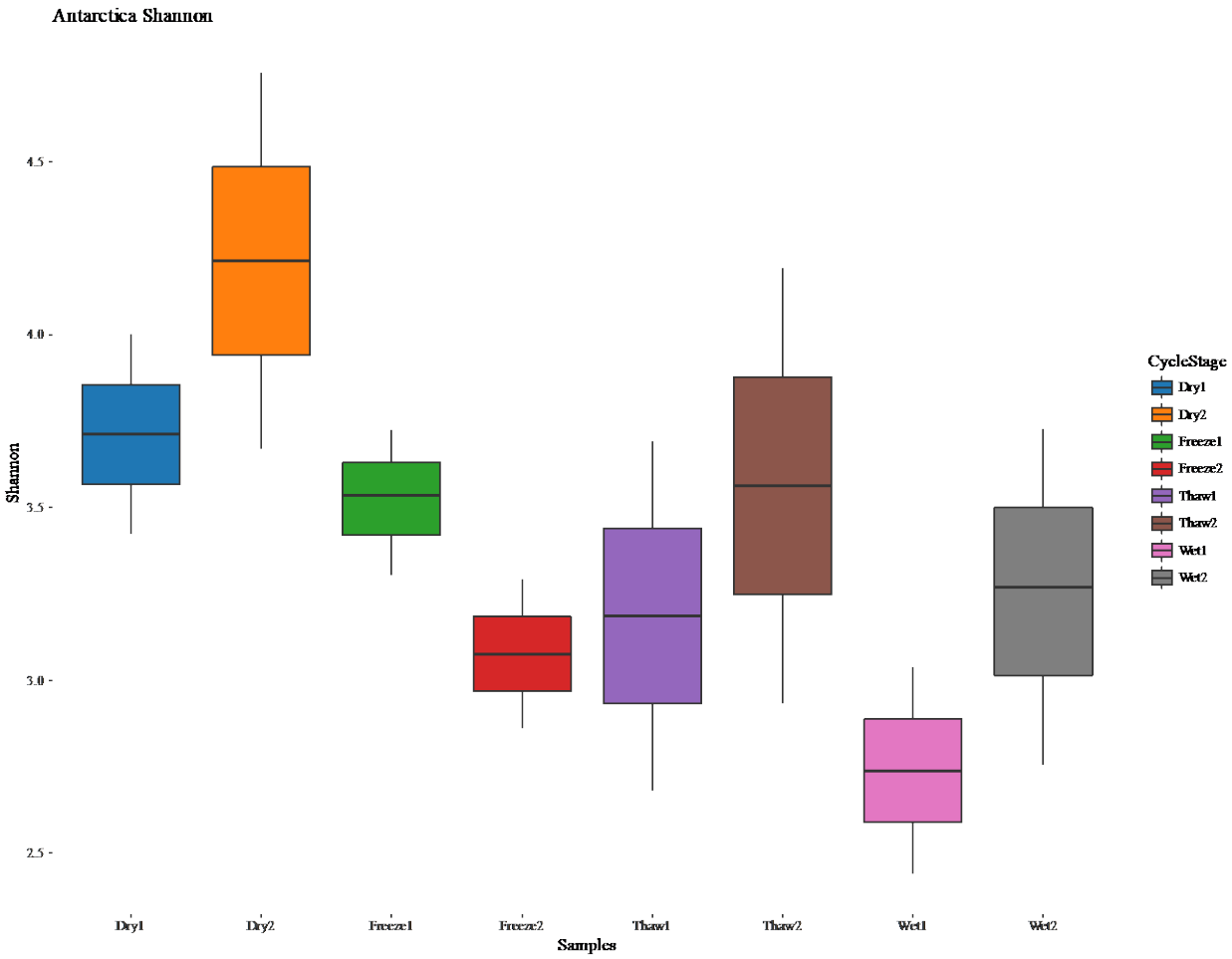

Figure 8. Antarctica Shannon Boxplots

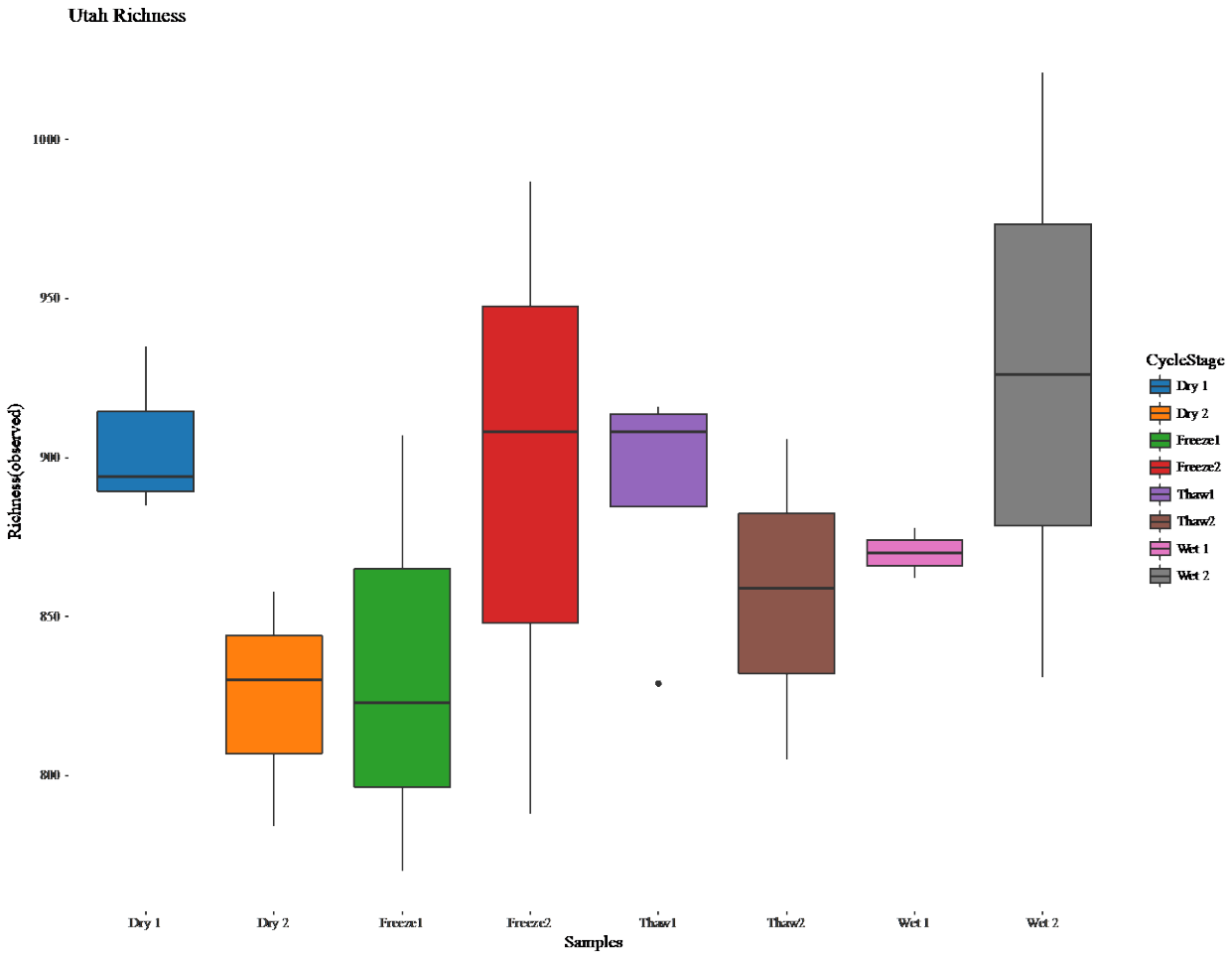

Figure 9. Utah Richness Boxplots

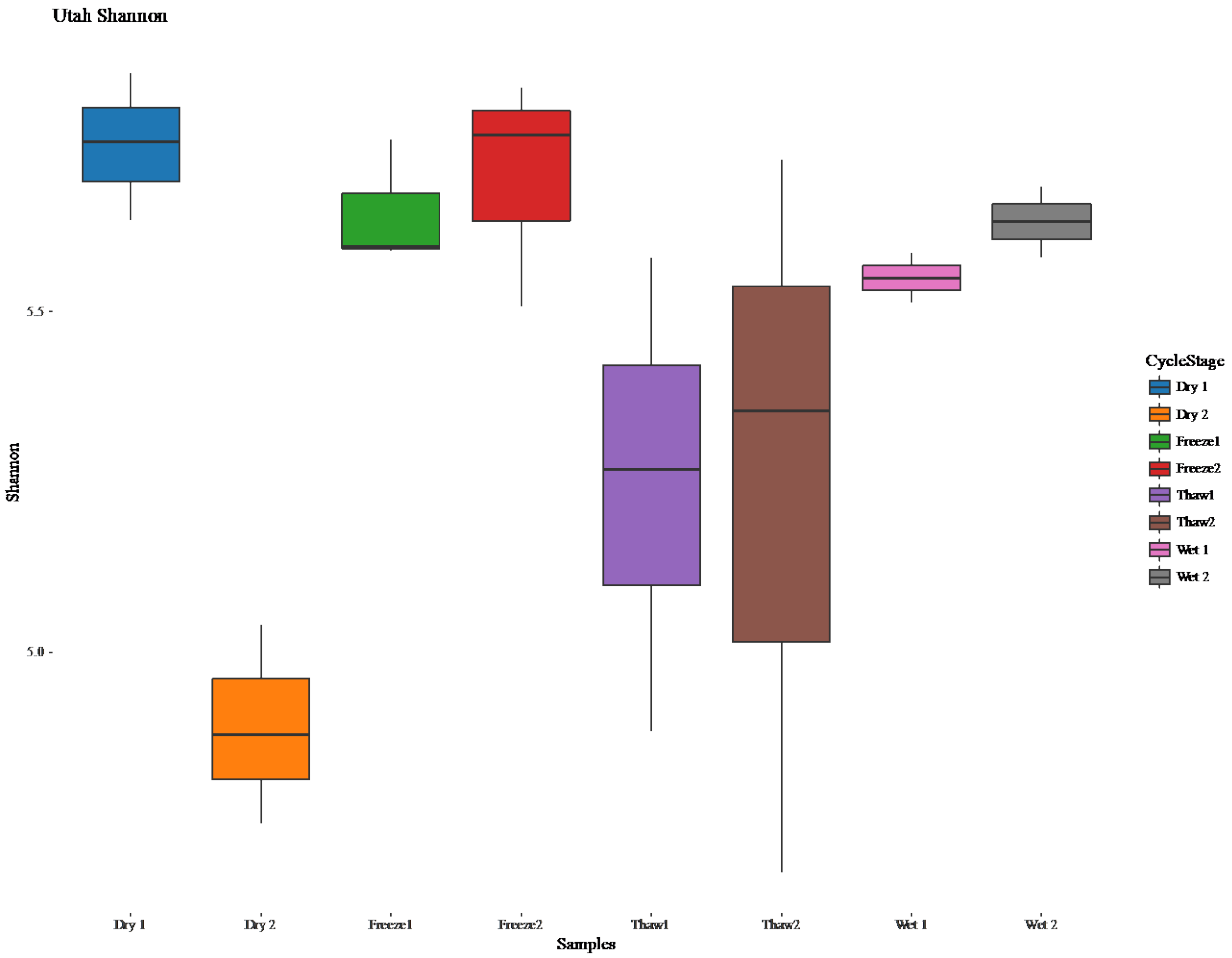

Figure 10. Utah Shannon Boxplot

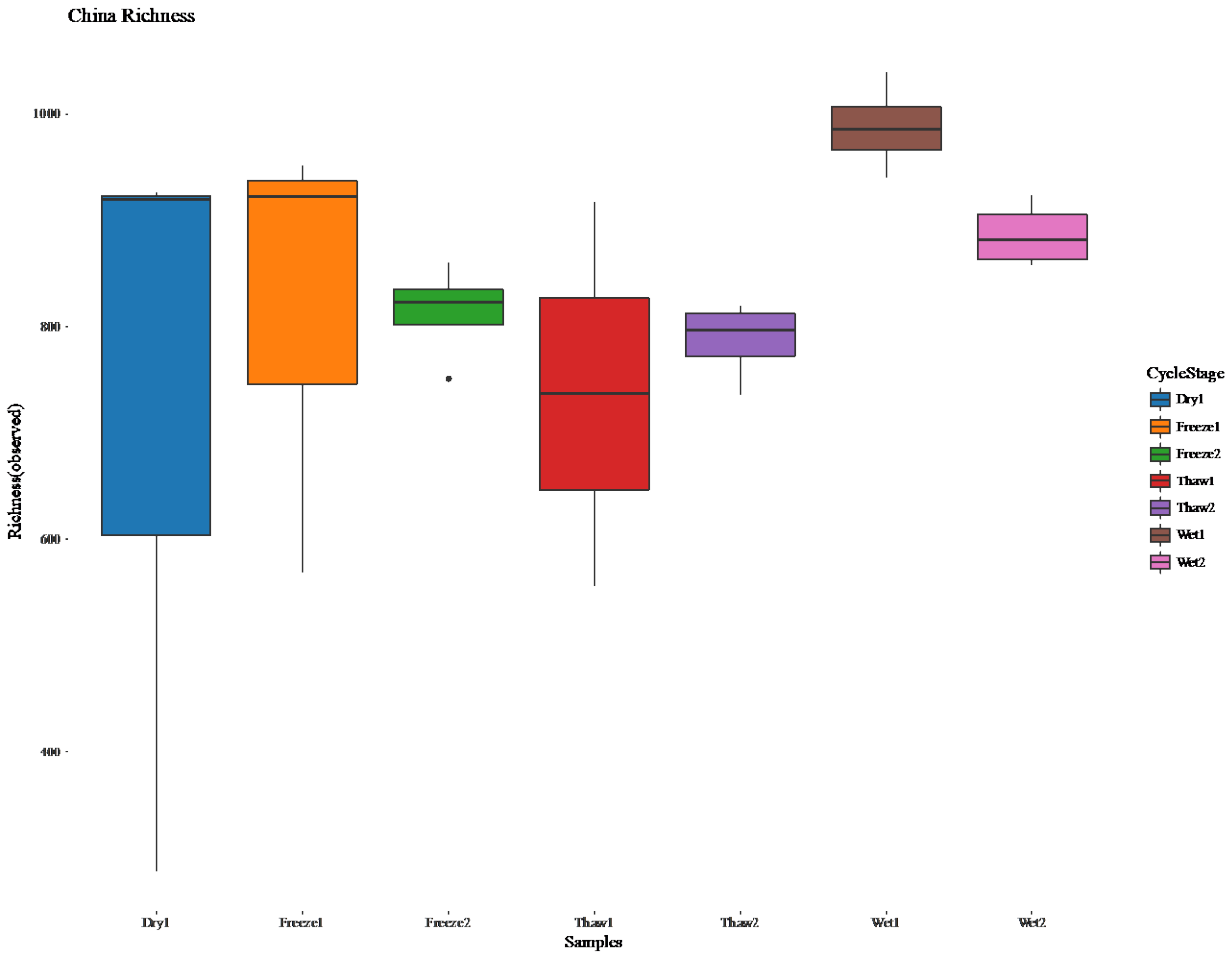

Figure 11. China Richness Boxplots

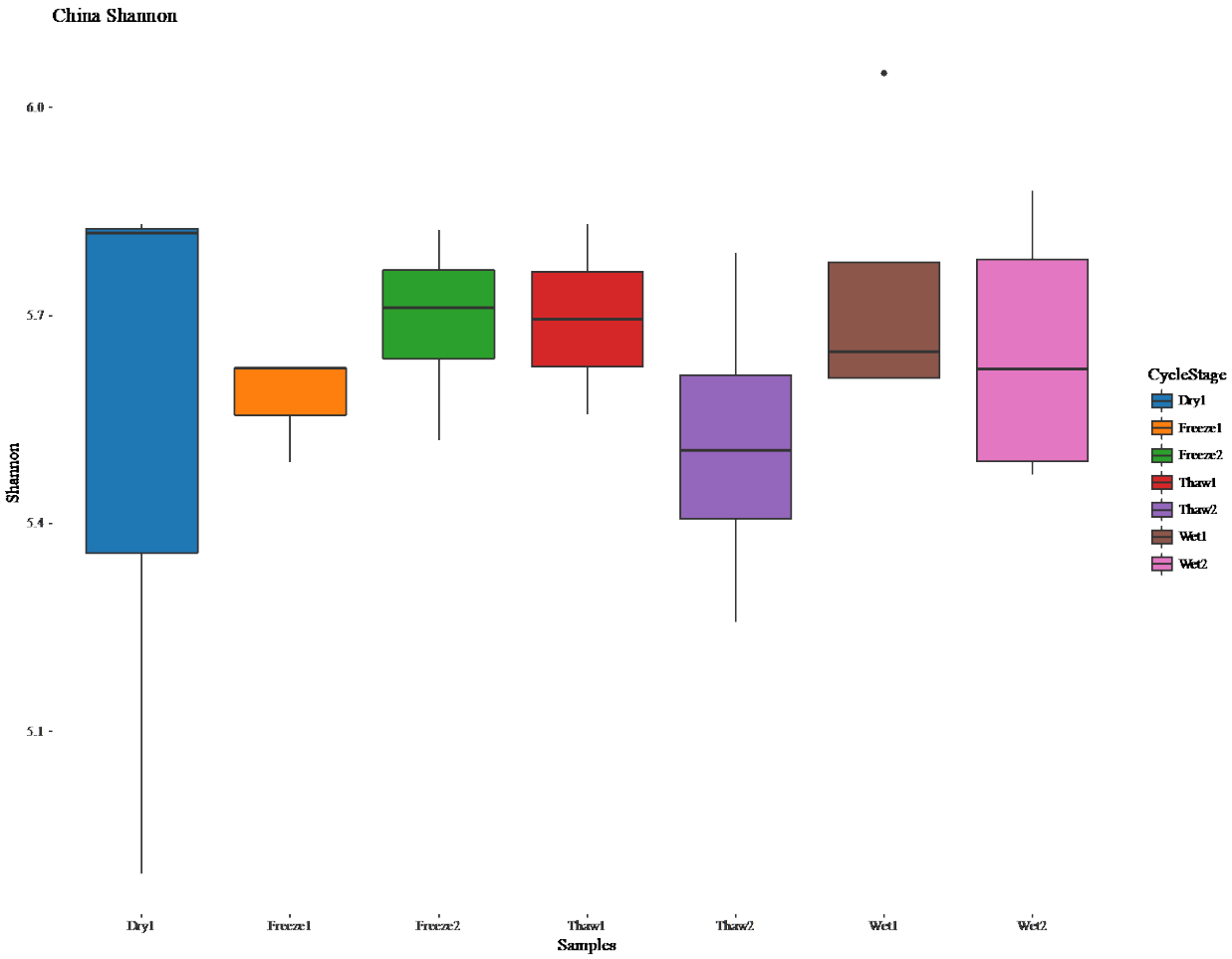

Figure 12. China Shannon Boxplots

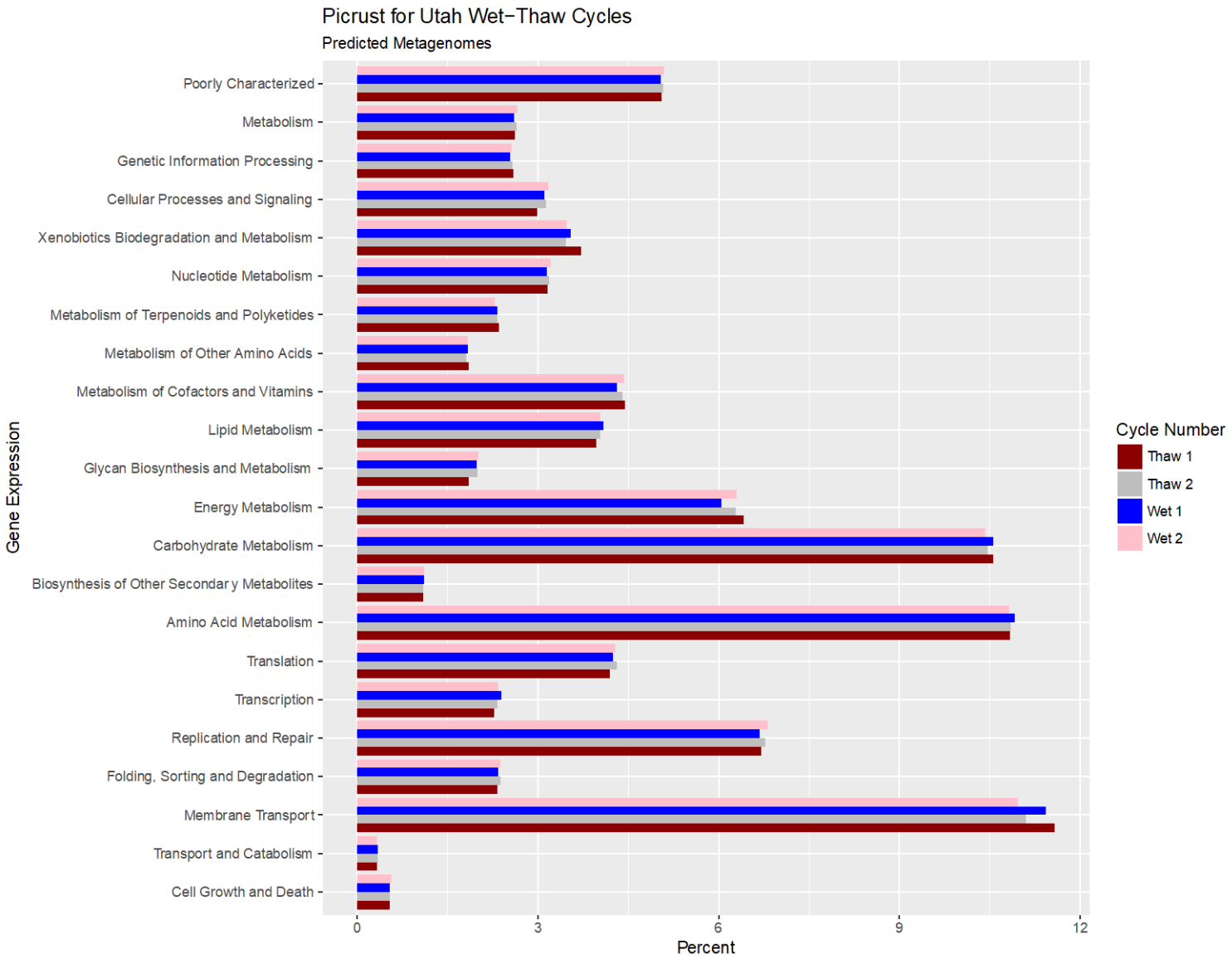

Figure 13. PiCRUST Utah Thaw-Wet Cycles

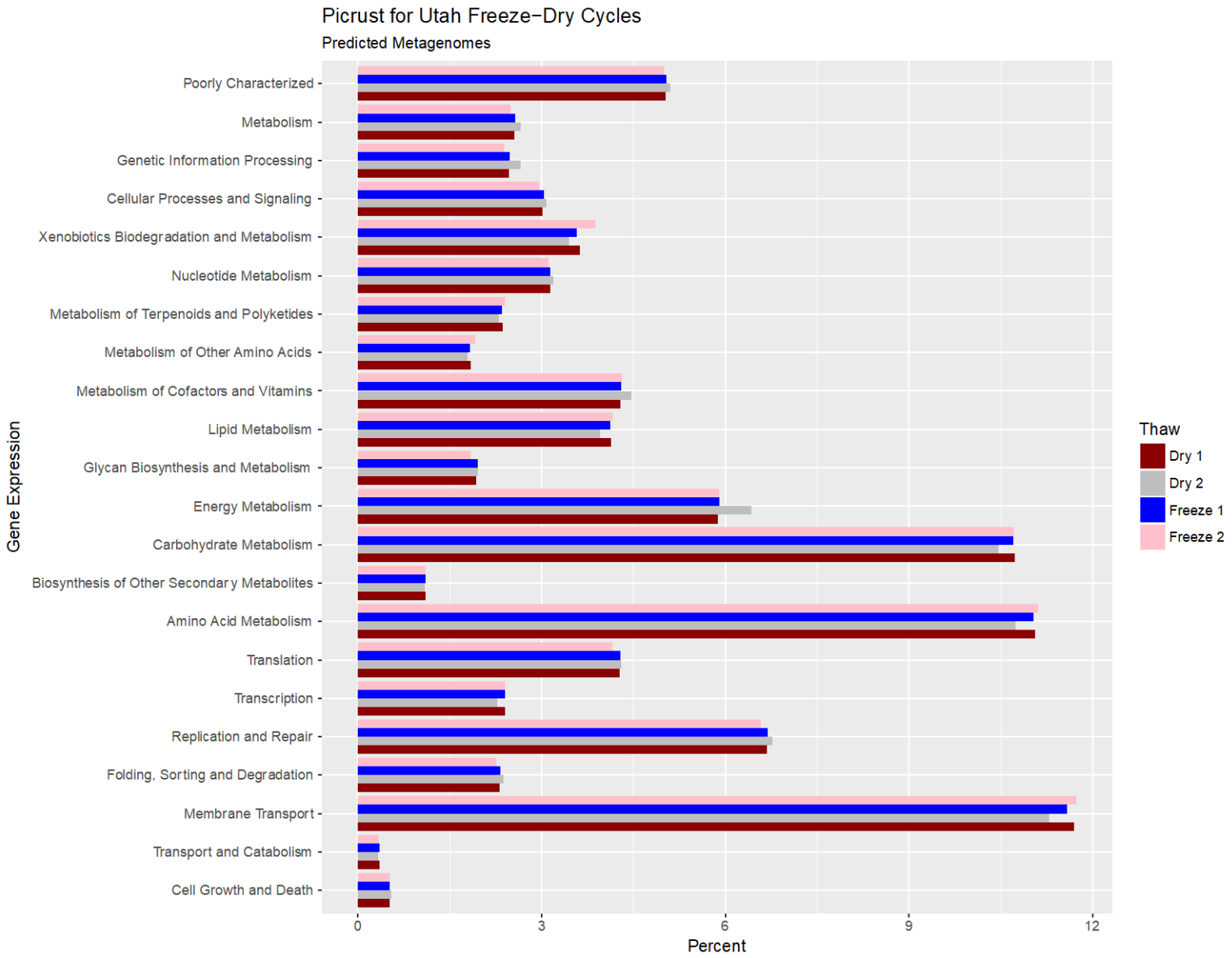

Figure 14. PiCRUST Dry-Freeze Cycles

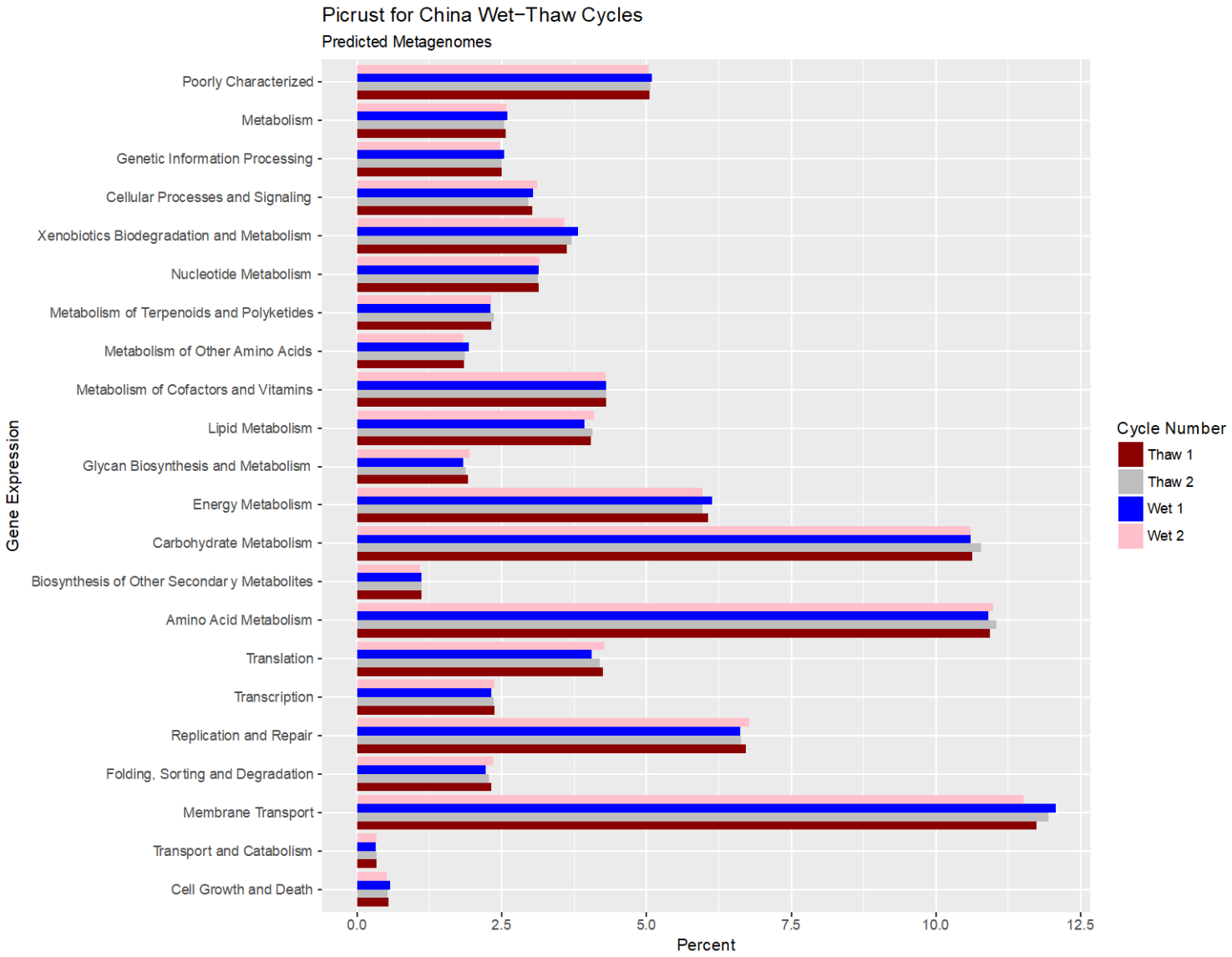

Figure 15. PiCRUST China Wet-Thaw Cycles

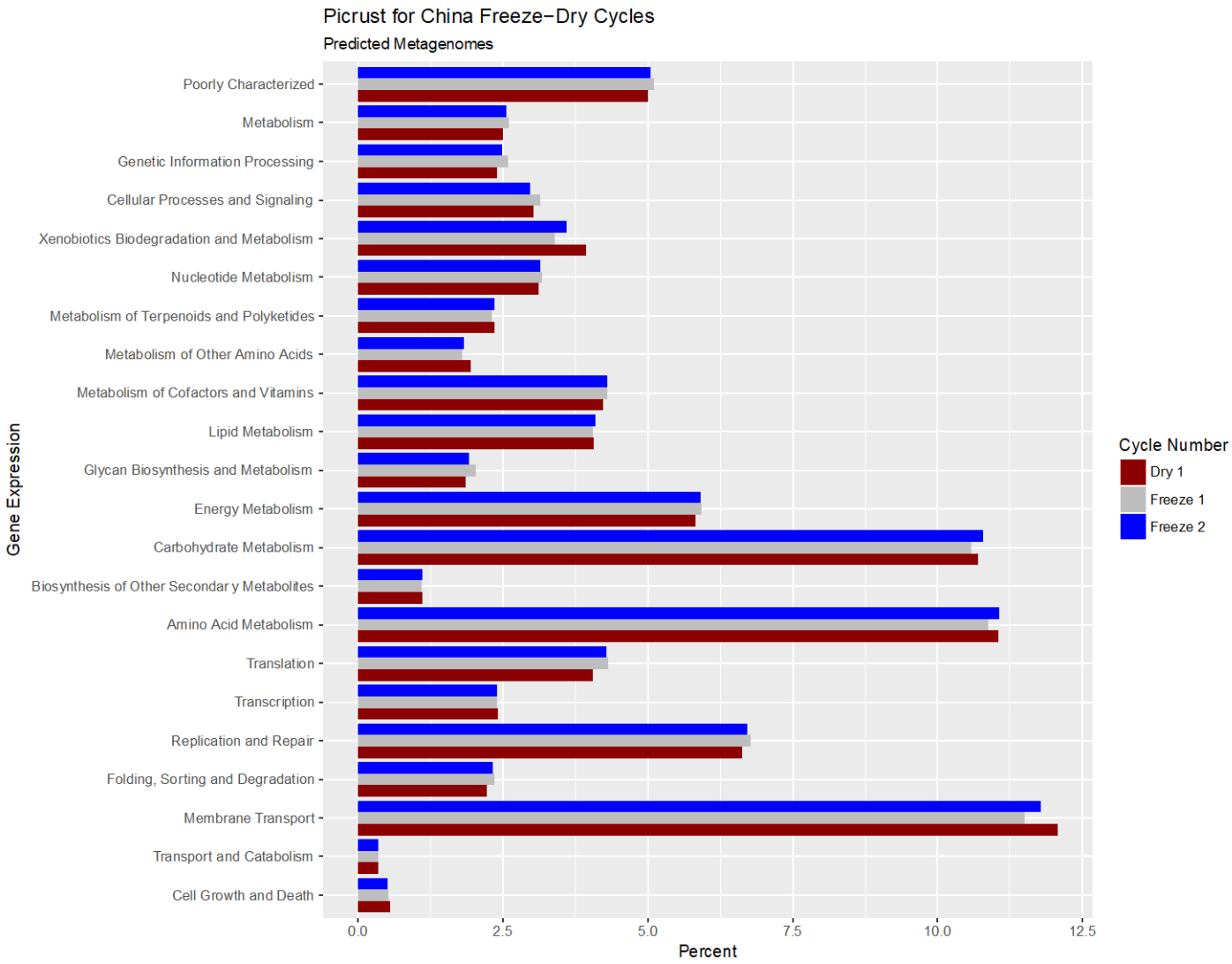

Figure 16. PiCRUST China Dry-Thaw Cycles

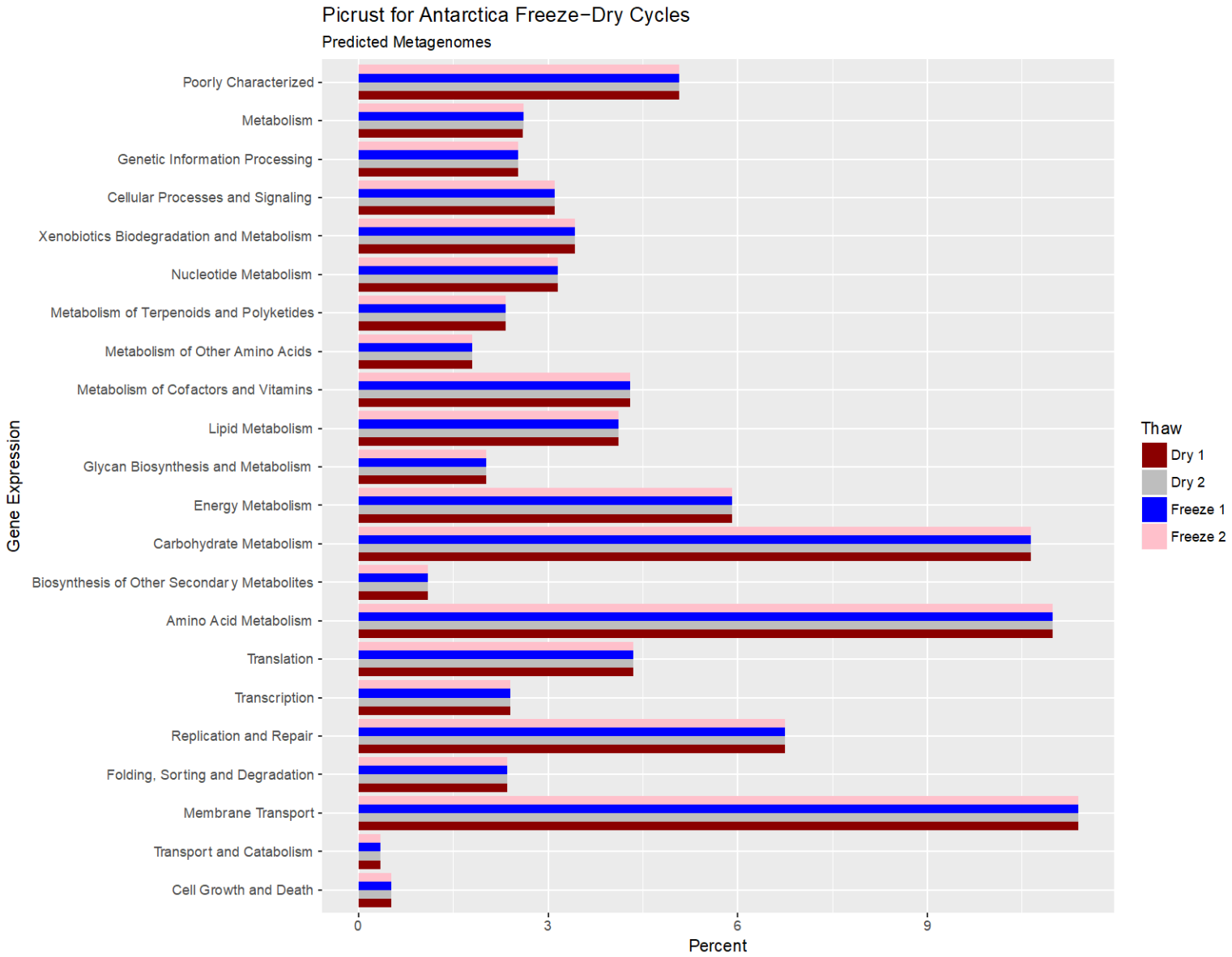

Figure 17. PiCRUST Antarctica Dry-Freeze Cycles

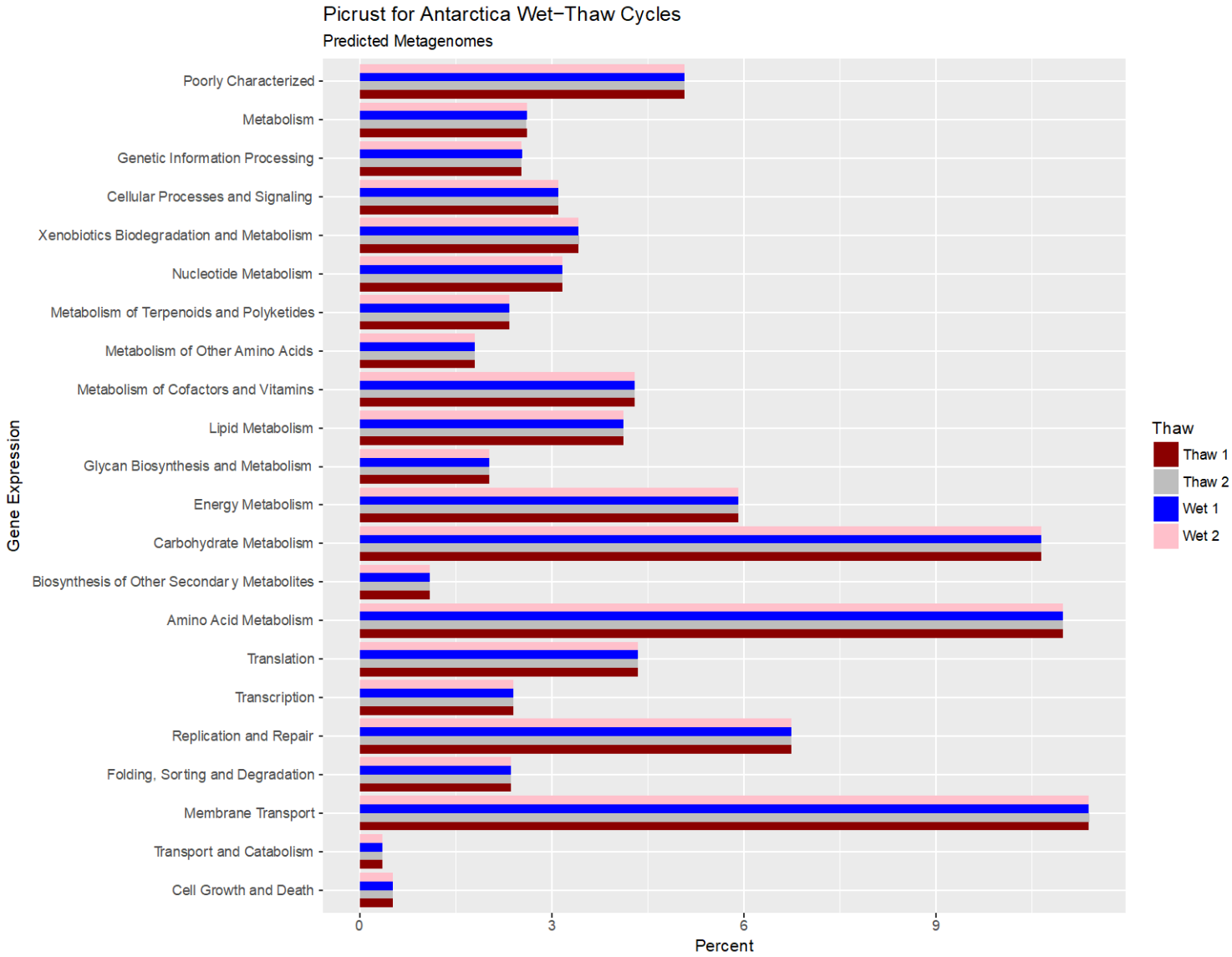

Figure 18. Antarctica Thaw-Wet Cycles