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Seasonal dynamics alters taxonomical and functional microbial profiles in Pampa biome soils under natural grasslands

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Soil microbial communities' assembly is strongly tied to changes in temperature and moisture. Although microbial functional redundancy seems to overcome taxonomical composition changes, the sensitivity and resilience of soil microbial communities from subtropical regions in response to seasonal variations are still poorly understood. Thus, the development of new strategies for biodiversity conservation and sustainable management require a complete understanding of the soil abiotic process involved in the selection of microbial taxa and functions. In this work, we used state of the art molecular methodologies (Next Generation Sequencing) to compare the taxonomic (metataxonomics) and functional (metatranscriptomics) profiles among soil samples from two subtropical natural grasslands located in the Pampa biome, Brazil, in response to short-term seasonal variations. We found consistent effects of season on both microbial community structure and functions, but with the former being more influenced than the latter. These variations were more related to the oscillation in the relative abundances of specific taxa along seasons, rather than extinction and recolonization of taxa along seasons. In conclusion, the most abundant microbial groups and functions were shared between seasons and locations reflecting the existence of a stable taxonomical and functional core microbiota.

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Seasonal dynamics alters taxonomical and functional microbial profiles in Pampa biome soils under natural grasslands

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Abstract

Soil microbial communities' assembly is strongly tied to changes in temperature and moisture. Although microbial functional redundancy seems to overcome taxonomical composition changes, the sensitivity and resilience of soil microbial communities from subtropical regions in response to seasonal variations are still poorly understood. Thus, the development of new strategies for biodiversity conservation and sustainable management require a complete understanding of the soil abiotic process involved in the selection of microbial taxa and functions. In this work, we used state of the art molecular methodologies (Next Generation Sequencing) to compare the taxonomic (metataxonomics) and functional (metatranscriptomics) profiles among soil samples from two subtropical natural grasslands located in the Pampa biome, Brazil, in response to short-term seasonal variations. We found consistent effects of season on both microbial community structure and functions, but with the former being more influenced than the latter. These variations were more related to the oscillation in the relative abundances of specific taxa along seasons, rather than extinction and recolonization of taxa along seasons. In conclusion, the most abundant microbial groups and functions were shared between seasons and locations reflecting the existence of a stable taxonomical and functional core microbiota.

Keywords: metataxonomics, metatranscriptomics,16S rRNA gene, soil microbial core, Ion Torrent PGM, NGS, abiotic factors, seasons, subtropical ecosystems

1. Introduction

The selection imposed by abiotic environmental factors is an important event contributing to microbial community assembly (Stegen et al., 2012). Although soil microbial communities appeared to be well adapted to some environmental variability (e.g. increase of 4 ºC in soil temperatures) (Schindlbacher et al., 2011), short-term seasonal changes are strongly correlated with shifts in microbial community composition (Wallenstein and Hall, 2012). Diversity and/or abundance of microbial taxa changed between seasons in alpine ecosystems, (Lipson and Schmidt, 2004), temperate grassland ecosystem (Habekost et al., 2008), soils of a temperate beech forest (Rasche et al., 2011) and soils under Mediterranean climate (Waldrop and Firestone, 2006).

Among the deterministic processes governing the composition of microbial communities, environmental factors such as temperature and moisture are considered to be main drivers of microbial community assembly (Castro et al., 2010; Lauber et al., 2013). Under normal rainfall conditions the increase in temperature positively affects the abundance of some soil microbial groups (Sheik et al., 2011). Conversely, increasing temperature was positively correlated with microbial respiration in soil and CO_2 production (Luo et al., 2001), which in turn can reduce the population of *Actinobacteria* sensitive to high CO_2 concentrations, for example (Sheik et al., 2011). Water saturation can drastically alters soil microbial composition, community diversity and function by altering O_2 availability (Carbone et al., 2011). On the other hand, drought conditions may benefit organisms adapted to arid conditions (Bouskill et al., 2013).

In subtropical regions, soil temperature and moisture vary strongly during the seasons, influencing microbial community assembly. However, previous work suggests

70 that soil functioning is not affected by the decline of the microbial diversity (Griffiths et al.,
71 2001; Wertz et al., 2007, 2006), and these ecosystems seems to rely on functional
72 redundancy (Frossard et al., 2012; Lupatini et al., 2013; Nannipieri et al., 2003; Rousk et
73 al., 2009).

74 Although microbial composition changes are not necessarily correlated with soil
75 microbial functions, the sensitivity and resilience of soil microbial communities from
76 subtropical regions in response to seasonal variations are still unclear. Obtaining a better
77 insight into the soil ecological process involved in the selection of microbial taxa and
78 function is paramount for developing new strategies for biodiversity conservation and
79 sustainable management. Moreover, if microbial taxa shift along the year and functions
80 do not change, such information is relevant for planning sampling strategies and for
81 comparing the results from different experiments. Here, we compared taxonomic and
82 functional microbial profiles of soils under subtropical natural grassland from two sites in
83 the Pampa biome, during the warm (summer and autumn) and cold (winter and spring)
84 seasons. The major aim of this study was to test whether short-term seasonal variations,
85 typical in subtropical regions, can cause changes in microbial phylotypes and/or microbial
86 functions. We expected microbial community abundance and diversity patterns to change
87 due seasonal variation, that is, to increase over the warm season, but microbial
88 functioning to remain stable over seasons.

89

90 **2. Material and Methods**

91 2.1. *Study Sites*

92 The study was conducted during 2014 and 2015 in two sites under similar
93 environmental conditions located in the Brazilian Pampa biome: Santa Maria municipality

94 - SM (29º45'S, 53º45'W) and São Gabriel municipality - SG (30°20'S, 54°15'W), 83 km
95 far from each other. The sites were chosen because of their similarities in soil features,
96 vegetation, land use, and climate. Soil samples were collected under natural grassland,
97 currently used for grazing of cattle, with no historic of fertilizers input (except for the
98 manure added by animal activity). The sites exhibited similar soil physicochemical
99 characteristics (Table S1) and were classified within the same soil taxonomy.

100 The sites chosen are exposed to large seasonal variations in soil moisture and
101 temperature (Fig. 1). For this study, the seasons were characterized as warm season
102 (October, November, December, January, February and March) and cold season (April,
103 May, June, July, August and September), due to the similarities in temperature and
104 rainfall between spring and summer as well as autumn and winter. The average air
105 temperatures in the cold season were 16.2 ºC ± 2.4 and 17.5 ± 2.7 ºC in SM and SG,
106 respectively. In the warm season the average air temperatures were 24.0 ± 2.8 ºC in SM
107 and 24.1 ± 1.4 ºC in SG. During the sampling period, the monthly average rainfall in the
108 cold season (May and August) was 107 mm in SM and 112 mm in SG. In the warm season
109 (November and February), monthly average rainfall was 154 mm and 171 mm, in SM and
110 SG, respectively (Fig. 1).

111 The plant species comprising 95% of the total biomass in SM were: *Adropogon*
112 *lateralis*, *Aristida laevis*, *Elephantopus mollis*, *Bacharis trimera*, *Paspalum plicatulum* and
113 *Paspalum notatum,* and in SG were: *Andropogon lateralis*, *Axonopus affinis*, *Baccharis*
114 *trimera*, *Eragrostis plana* (invasive), *Erianthus angustifolius*, *Paspalum notatum*,
115 *Paspalum dilatatum*, *Paspalum plicatulum*, *Sporobolus indicus* and *Vernonia nudiflora*.
116

117 *2.2. Soil sampling, nucleic acids extraction and preprocessing*

118

119 At each grassland (SM and SG), the sampling area was divided in three blocks,
120 and five soil cores per block were randomly selected and pooled to make a single
121 composite sample, resulting in three samples per site at each season [n = 24, that is, 2
122 sites (SM and SG), 4 months (May and August, 2014 - cold season / November 2014 and
123 February 2015 – warm season) and 3 replicates per sampling]. Samples were collected
124 with a sterile spatula to a depth of 5 cm, kept in sterile 50 mL tubes in liquid nitrogen, and
125 then stored at -80 ºC until DNA and RNA co-extraction.

126 From each sample, 2 g of soil was used for simultaneous extraction of total RNA
127 and DNA using the RNA PowerSoil kit and the PowerSoil®DNA Elution Accessory Kit
128 (MoBio laboratories, Inc., Carlsbad, CA, USA), following the manufacturer's instructions.
129 After RNA extraction, each sample was subjected to enzymatic digestion of DNA with
130 TURBO DNA-free Kit (Applied Biosystems, Foster City, CA, USA). All RNA and DNA
131 samples were submitted to quantification and quality-check using a Qubit RNA or DNA
132 assay kit (Invitrogen, Eugene, OR, USA) and NanoVue™ spectrophotometer (GE
133 healthcare, Chicago, CA USA), respectively, and further stored at -80°C until library
134 preparation.

135

136 *2.3. Metatranscriptome library preparation and sequencing*

137 One microgram of total RNA from each soil sample were depleted by removing
138 rRNA from total RNA with the MICROBExpress™ Bacterial mRNA Enrichment Kit
139 (Thermo Fisher, Waltham, MA, USA) following the manufacturer's instructions. The
140 mRNA was further purified with the MEGAclear™ Transcription Clean-Up Kit (Thermo
141 Fisher, Waltham, MA, USA) following the manufacturer's instructions. The enriched

142 mRNA was used to prepare the mRNA library with the Ion Total RNA-Seq Kit v2 and Ion
143 Xpress™ RNA-Seq Barcode Kit (Thermo Fisher, Waltham, MA, USA). The libraries were
144 amplified by emulsion PCR and sequenced on Ion 316™ Chip v2 using the Ion Torrent
145 PGM system and the Ion PGM™ Sequencing 400 kit, according to the supplier's
146 instructions. After sequencing, all reads were filtered by the PGM software to remove low
147 quality and polyclonal sequences.
148
149 *2.4. 16S library preparation and sequencing*
150 Library preparation and sequencing followed the procedures described by
151 (Suleiman et al., 2016). For analyzing the composition of soil bacterial and archaeal
152 communities the total microbial DNA was used as template for partial 16S rDNA
153 amplification and sequencing. The V4 region of the 16S rRNA gene was amplified using
154 the bacterial/archaeal primers 515F and 806R (J. G. Caporaso et al., 2012), and
155 sequenced using the PGM Ion Torrent (Thermo Fisher Scientific, Waltham, MA, USA).
156 Multiple samples were PCR-amplified using barcoded primers. Each of the 25 µL of PCR
157 mixture consisted of 2U of Platinum® Taq DNA High Fidelity Polymerase (Invitrogen,
158 Carlsbad, CA, USA), 4 µL 10X High Fidelity PCR Buffer, 2 mM MgSO4, 0.2 mM dNTP's,
159 0.1 µM of both the 806R barcoded primer and the 515F primer, and approximately 100
160 ng of DNA template. The PCR conditions used were 95°C for 5 min, 30 cycles of 94°C
161 per 45s denaturation; 56°C per 45s annealing and 72°C per 1 min extension; followed by
162 72°C per 10 min. The resulting PCR products were purified with the Agencourt®
163 AMPure® XP Reagent (Beckman Coulter, Brea, CA, USA) and the final concentration of
164 the PCR product was quantified by using the Qubit Fluorometer kit (Invitrogen, Carlsbad,
165 CA, USA) following the manufacturer's recommendations. Finally, the reactions were

166 combined in equimolar concentrations to create a mixture composed by 16S rRNA gene
167 amplified fragments of each sample. This composite sample was used for library
168 preparation with Ion OneTouch™ 2 System with the Ion PGM™ Template OT2 400 Kit
169 Template (Thermo Fisher Scientific, Waltham, MA, USA). The sequencing was performed
170 using Ion PGM™ Sequencing 400 on Ion PGM™ System using Ion 316™ Chip v2.
171

172 *2.5. Bioinformatics analysis and statistics*

173 The annotation of metatranscriptome sequences was performed with the
174 Metagenomics Rapid Annotation (MG-RAST) pipeline version 3.3.6 (Meyer et al., 2008),
175 using the standard parameters for sequence quality control. The data was compared to
176 the SEED Subsystem using a maximum e-value of 1^{-5}, a minimum identity of 60%, and a
177 minimum alignment length of 15 measured in aa for protein and bp for RNA databases.
178 The 16S rRNA raw sequences were analyzed following the recommendations of the
179 Brazilian Microbiome Project (Pylro et al., 2014), using the BMP Operating System
180 (BMPOS) (Pylro et al., 2016).

181 Analysis of Metagenomic Profiles v2 (STAMP) software package was used to
182 determine differences in relative abundances of microbial functions and taxa (Parks et
183 al., 2014). Statistical hypothesis tests were performed using the Welch's t test while
184 confidence intervals were calculated using the Welch's inverted method and Bonferroni
185 multiple test for *p*-value correction. The mRNA and the 16S rRNA datasets were rarefied
186 to the same number of sequences per database (Lemos et al., 2011) and used to
187 construct dissimilarity matrixes generated by Bray Curtis and Binary distances using the
188 "phyloseq" package in R. The matrixes were ordinate by Principal Coordinate Analysis
189 (PCoA) and *adonis* function was used to calculate the Permutational Multivariate Analysis

190 of Variance (PERMANOVA) and verify the strength and statistical significance of groups
191 among location, season and the combined effect of location and season with the vegan
192 package (Oksanen J et al., 2015). Sampling effort was estimated using Good's coverage
193 (Good, 1953).

194 Raw sequences obtained by metatranscriptome sequencing and associated
195 metadata were submitted to MG-RAST server (http://metagenomics.anl.gov/) and are
196 public available under the string mgp12046. All raw sequences obtained by amplicon
197 sequencing were submitted to NCBI Sequence Read Archive (SRA) and are available
198 under the experiment number SRX2779549 run number SRR5499445.

199

200 **3. Results**

201

202 *3.1. Overall microbial differences among seasons and locations*

203 After quality filtering the 16S rRNA reads, a total of 1,630,136 high-quality
204 sequences longer than 200 bp were retained. The average Good's coverage of 98% was
205 calculated (Table S2), indicating the dataset was representative of the microbial
206 communities analyzed. The soil metatranscriptome sequencing yielded a total of
207 20,584,533 reads for all 24 samples, and the average Good's coverage was 77% (Table
208 S2).

209 To evaluate the differences in microbial community structure and function between
210 the two natural grasslands and along different seasons, the abundances of microbial taxa
211 and mRNA encoding functions were used to compute a Bray-Curtis dissimilarity matrix
212 coordinated by using PCoA. A Binary similarity matrix was also used to compute
213 differences of either presence/absence of taxa or mRNA encoding functions. Although

214 the sites were similar, these analyses revealed differences in microbial taxonomic
215 structure between the two natural grasslands (SM and SG) and between seasons (Fig.
216 2). When the abundance of mRNA encoding microbial functions was compared, two
217 distinctive groups were observed, representing the cold and the warm seasons. However,
218 the location did not influence the main functions performed by the microbial communities
219 (Fig. 2). Those differences were further confirmed by PERMANOVA (Table 1). Season
220 was the main driver of shifts of abundance of taxa and mRNA encoding functions of the
221 soil microbiota. This factor contributed 22% and 23% for the variation in microbial taxa
222 and mRNA encoding functions, respectively (Table 1). Altogether, these results indicated
223 that soil microbial community structure was affected by location (e.g. two natural
224 grasslands from the same biome, but 83 km apart) and by the natural environmental
225 changes throughout the year (seasons). On the other hand, the functions performed by
226 soil microbes in those two sampling sites were similar, not being affected by location, but
227 by seasons.
228
229 *3.2. Identification of taxa and functions that shifted along seasons and locations*
230 Once overall taxonomic and functional differences were identified, the functional
231 differences as measured through RNAseq, and taxonomical differences as measured by
232 16S rRNA sequencing, were determined. The seasonal frequency of most abundant
233 microbial phylotypes (relative frequency greater than 1%) in both locations was
234 determined (Table 2). The data indicated the grassland ecosystem maintained a core
235 microbiota along the year but the relative abundance of taxa varied in response to
236 seasons. As the amount of rain was well distributed along the year (Fig 1), soil moisture

237 was not considered an important variable shaping microbial community assembly in our
238 experiment. Temperature shifted during the year with an average minimum of 10 ºC and
239 an average maximum of 27 ºC, representing an important source of environmental
240 variation influencing microbial life in our experiment. During the warm season, the
241 uncultured member of *Verrucomicrobia* designated *DA101* was the most abundant
242 phylotype in both locations. *Mycobacterium and Rhodoplanes* were also found in both
243 grasslands with similar high abundances, but better adapted to cold conditions (Table 2).
244 In agreement with the differential abundance analysis, alpha diversity
245 measurements indicated similar microbial richness (number of observed OTUs) during
246 the seasons, but greater microbial diversity during the cold season (Fig. 3). Cold
247 temperatures allowed for greater evenness while warm temperatures decrease the
248 diversity of taxa. The diversity of microbial functions presented similar trends, displaying
249 a core set of microbial functions along the year in both locations (Fig. 3 and Table 3).
250 Main functional differences between seasons were consistently detected within both
251 grasslands. Despite the difficulties of defining the functions codified by complex mixtures
252 of mRNA in metatranscriptome library, the metabolism of carbohydrates was the
253 dominant set of functions performed during the cold season in both grasslands. An
254 average of 10.5% of mRNA with known annotated function was committed to
255 carbohydrate degradation and utilization in the model bacterium *Thermotoga maritima,*
256 during the cold season in the grassland located in SG. Nevertheless, only 1.1% of the
257 mRNA sequences were related with this function in the warm season (Table 3). The same
258 pattern was observed in the grassland located in SM (7.1% of mRNA sequences
259 committed to sugar utilization during the cold season and only 0.9% during the warm

260 season). D-ribose utilization and deoxyribose and deoxynucleoside catabolism were also
261 functions highly expressed during the cold season in both grasslands. During the warm
262 season functions were more evenly distributed, without dominance of specific ones. The
263 most abundant set of functions during the warm season were those dedicated to the
264 protein metabolism, composing 5.7% of mRNA dataset in the grassland located in SG,
265 and 3.5% in the SM grassland. The functions related to Cofactors, Vitamins, Prosthetic
266 Groups and Pigments were also higher expressed during the warm season in both
267 grasslands.
268
269
270 **4. Discussion**
271 This study aimed to understand how and to what extent seasonal dynamics
272 influence taxonomical and functional microbial profiles in subtropical natural grassland
273 soils, based on metataxonomics (16S rRNA gene) and metatranscriptomics (mRNA) in-
274 depth sequencing (as defined by Marchesi and Ravel, 2015). We addressed two
275 important variables; season and location, aiming to better understand how these shifting
276 environmental conditions influence microbial community assembly and function in
277 subtropical grasslands from south Brazil. The Brazilian Pampa occupies 2% of the
278 Brazilian territory and is considered one of the most fragile biomes in the country,
279 experiencing losses of both biodiversity and socio-economic opportunities (Roesch et al.,
280 2009). Natural grasslands are the predominant vegetal cover of this biome, although the
281 climatic conditions are also suitable for forest development, which reflects its biological
282 uniqueness. The soils in both sampling areas (SM and SG) were classified as Paleodult
283 and the vegetal composition was similar but not identical. Our sampling strategy allowed

us to obtain a highly tractable model, with true landscape level of biological replications, for verifying whether soil microbial functions follow the changes in the abundance of taxa during seasonal variations. Our study revealed consistent effects of season on both microbial community structure and functions, with the former presenting less stability than the latter along the seasons.

Season and location significantly modulate microbial community assemblage and abundance in subtropical natural grasslands (Fig. 2). However, considering only those microbes with relative abundance greater than 1%, our data suggest that both grasslands maintained a stable microbial community membership along the time, but the relative abundance of specific taxa oscillated in response to seasonal changes (Table 2). Hence, these results support the existence of a soil core microbiota in natural grasslands that might represent the first step in defining a 'healthy' community and predicting community responses to perturbation (Shade and Handelsman, 2012). The concept of core microbiota was first introduced during the studies of the Human Microbiome Project (Turnbaugh et al., 2007). While the concept of microbial core remain elusive and might lack a conceptual framework involving microbial ecological characteristics (Shade and Handelsman, 2012), typical approaches report the presence/absence of microbial phylotypes across habitats. In the Pampa biome, Lupatini et al. (2013) already found a core microbiota among soil samples under Acacia plantation, natural pasture, soybean field, and natural forest. Within this study, 54.5% of OTUs (defined by 16S rRNA sequences with 97% similarity) were shared among four different land uses. Similarly, (Suleiman et al., 2013) found a total of 69% of the OTUs shared between a natural grassland and a pristine forest in the Brazilian Pampa biome. In our study, with the same

307 land use, the presence of a soil microbial core exists in which the most abundant bacterial
308 groups were shared between different seasons and locations.

309 While a group of microbes were found to be able to survive throughout the year,
310 relevant shifts in abundance of those groups along the seasons were apparent. These
311 changes might be explained by nutrient fluxes caused by carbohydrates inputs
312 (monosaccharides like glucose and fructose, oligosaccharides like maltose and sucrose
313 and polysaccharides like cellulose and pectin) from senescent plants. Plant biomass
314 production is low or even zero during the winter due to the climatic conditions that affect
315 the most abundant plant species from natural grasslands. In south Brazil, the cold season
316 covers around one third to half of the year. Low temperatures and frosts decrease the
317 plant growth and plant senescence is intensified causing most of the forage to be rejected
318 by the animals during the grazing. The entire process exacerbates the accumulation of
319 litter and carbohydrates in soil. Under such conditions the availability of abundant and
320 different carbon sources might be responsible for the changes observed in our
321 experiment. Bacteria have access to greater availability of rhizodepositions in the summer
322 but in the winter, when the rhizodeposition is very reduced, the easily decomposable
323 carbon sources are very scarce in soil. According to Tozzi et al., (2006) bacteria can grow
324 by using nucleic acids arising from decaying tissues or organisms. Nucleosides are
325 broken and pentose moiety is utilized to sustain the cell energy requirement, while the
326 base is either expelled from the cell or partially utilized as a nitrogen source or as a
327 precursor for nucleic acid synthesis. Under these conditions, there is increased
328 expression of genes linked to transport systems and catabolic enzymes for purine and
329 pyrimidine nucleosides as observed in Table 3.

330 Cold conditions decreased the abundance of some taxa. The apparent decrease
331 in diversity during the warm season (Fig. 3) might be interpreted as an effect of increasing
332 the abundance of fast growing microorganisms - e.g. greater abundance of *DA101* during
333 the warm season (Table 2), rather than the loss of microbial species. The concept that
334 observed changes in community composition are actually variations in the relative
335 abundance of taxa rather than extinction and recolonization of taxa in the ecosystem was
336 previously raised by Caporaso et al., (2012) in a study of seasonal dynamics in microbial
337 community composition of the Western English Channel. Here, most microbial taxa are
338 always present in these soils but vary in abundance with shifts in seasons.

339 Despite the similar number of observed species found between seasons, samples
340 collected during the warm season were more heterogeneous and presented greater
341 variability than those collected in the cold season (Fig. 3). Cold temperatures allowed for
342 greater evenness while warm temperatures caused an apparent decrease in diversity. In
343 the cold temperatures, a more even abundance distribution allows more taxa to be
344 sampled when the same number of sequences is examined, causing the richness to
345 appear higher. Temperature variation restricts survival of a few species or genera
346 sensitive to a specific temperature condition (Li et al., 2015), which corroborate the
347 aforementioned statement. As the amount of rain was well distributed along the year (Fig.
348 1), the temperature was considered the main source of environmental variation for
349 microbial life in our experiment.

350 Temperature may directly affect microbial metabolism, by restricting the activity of
351 those microbes unable to thrive at low temperatures. Temperature can also indirectly
352 affect microbial communities by reducing plant growth, thereby decreasing carbon

353 rhizodeposition (Murphy et al., 2016). Most plant species of subtropical grasslands are
354 perennial but present lower growth rate and higher senescence rate during the winter.
355 Thus, rhizodeposition of available carbon sources is reduced during the winter which in
356 turn affects fast growing microbial populations. Furthermore, fast-growing microbial
357 populations might be adapted to easily degradable materials during the winter due to the
358 plant litter decomposition while slow-growing microorganisms are favored when substrate
359 availability is limiting (Rui et al., 2009). Thus, regardless of the environmental conditions
360 (warm or cold), the different soil niches will be likely to be occupied by some member of
361 the microbial community. The level of environmental constraints imposed by the
362 subtropical region on bacterial communities is significant, encouraging functional
363 redundancy and resilience to be further explored.

364 Although the soil, vegetation cover, temperature, and rainfall regime are similar in
365 the Pampa biome and the sampling areas were relatively close to each other, the soil
366 microbial community structure was also affected by location (Fig. 2). These differences
367 are related to the inherently high level of spatial heterogeneity observed in soil (Lauber et
368 al., 2013). While we attempted to sample areas as homogeneous as possible, and
369 besides the similarity between soils from the two areas, the natural variation between
370 soils resulted in differences between soils of 77%, 26%, 73%, 67%, 108% and 133% in
371 the contents of Al, P, Ca, Mg, Zn e B, respectively. Besides, as mentioned above,
372 vegetation cover was similar but no identical. The species *Aristida laevis* and
373 *Elephantopus mollis* were detected only in SM while the plant species *Axonopus affinis*,
374 *Eragrostis plana Erianthus angustifolius*, *Paspalum dilatatum*, *Sporobolus indicus* and
375 *Vernonia nudiflora* were only detected in SG. Finally, the presence of the highly invasive

and allelopathic plant, *Eragrostis plana*, can alter important ecological components of microhabitats (Guido et al., 2016). Therefore, these differences in soil and vegetation may result in different abiotic conditions between the two sites, capable of altering the composition and activity of the soil microbial community.

5. Conclusions

Here we reported the effects of seasons and location on soil microbial taxonomy and functional profiles providing insights in the ecological rules shaping soil microbial communities and their functions in natural ecosystems. Season and location significantly modulate microbial community assemblage and abundance in subtropical natural grasslands. However, our data suggest that grasslands maintained a stable microbial community membership along the year with oscillation in abundance. Apparently soil microbial taxa are more susceptible to natural climatic disturbances while functions are more stable and change with less intensity along the year. Finally, our data allow us to conclude that the most abundant microbial groups and functions were shared between seasons and locations reflecting the existence of a stable taxonomical and functional core microbiota.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be considered as a potential conflict of interest.

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Figure 1

Fig. 1. Seasonal dynamics of temperature and rainfall in two natural grasslands (SG and SM) from the Pampa biome. The asterisks depict the sampling period.

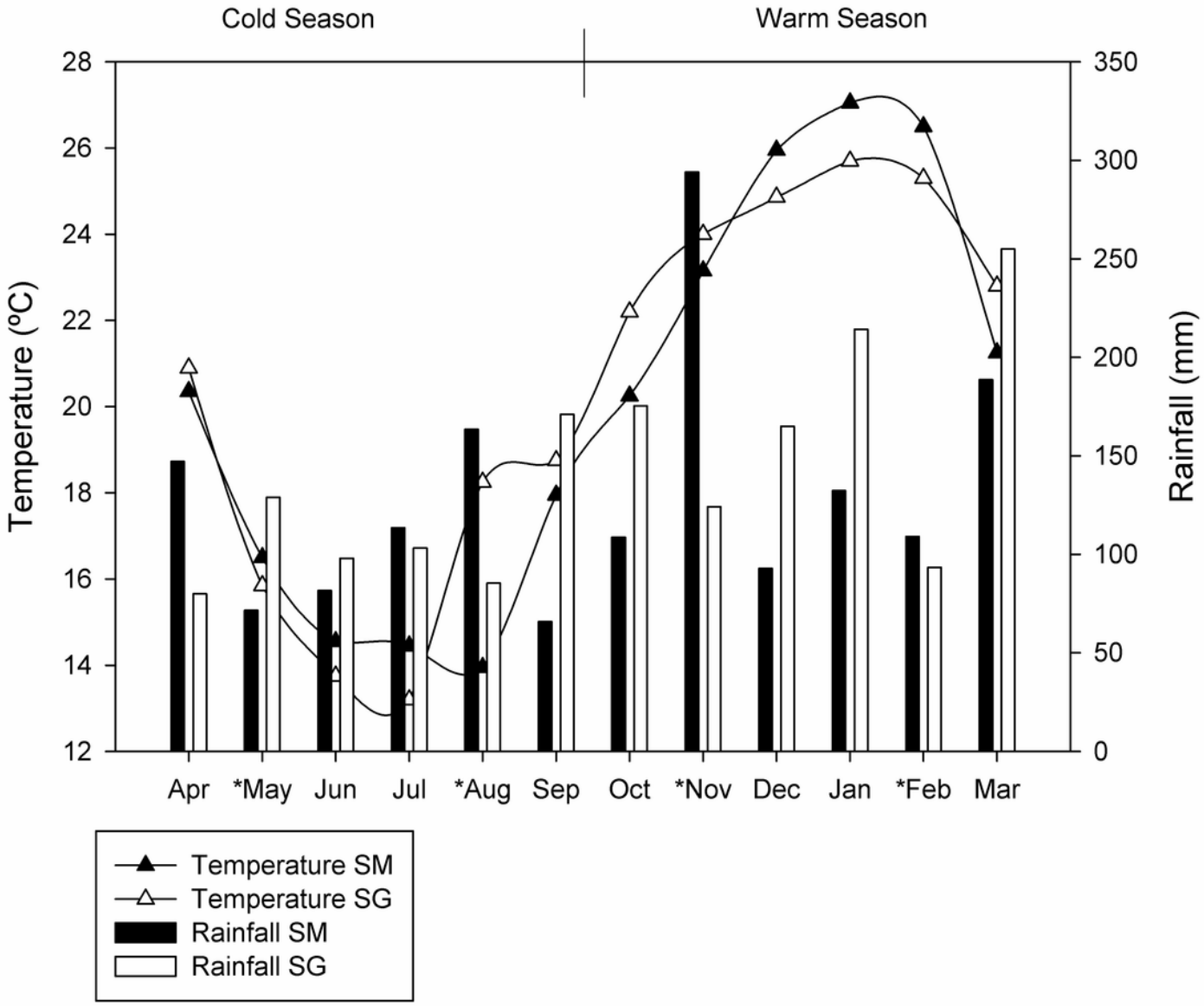

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Figure 2

Fig. 2. Principal coordinates plot (PCoA) representing clusters of soil microbial communities grouped by taxa or functions in two different natural grasslands (SG and SM) and seasons (cold and warm) in the Pampa biome. Ellipses around groups represent 95%

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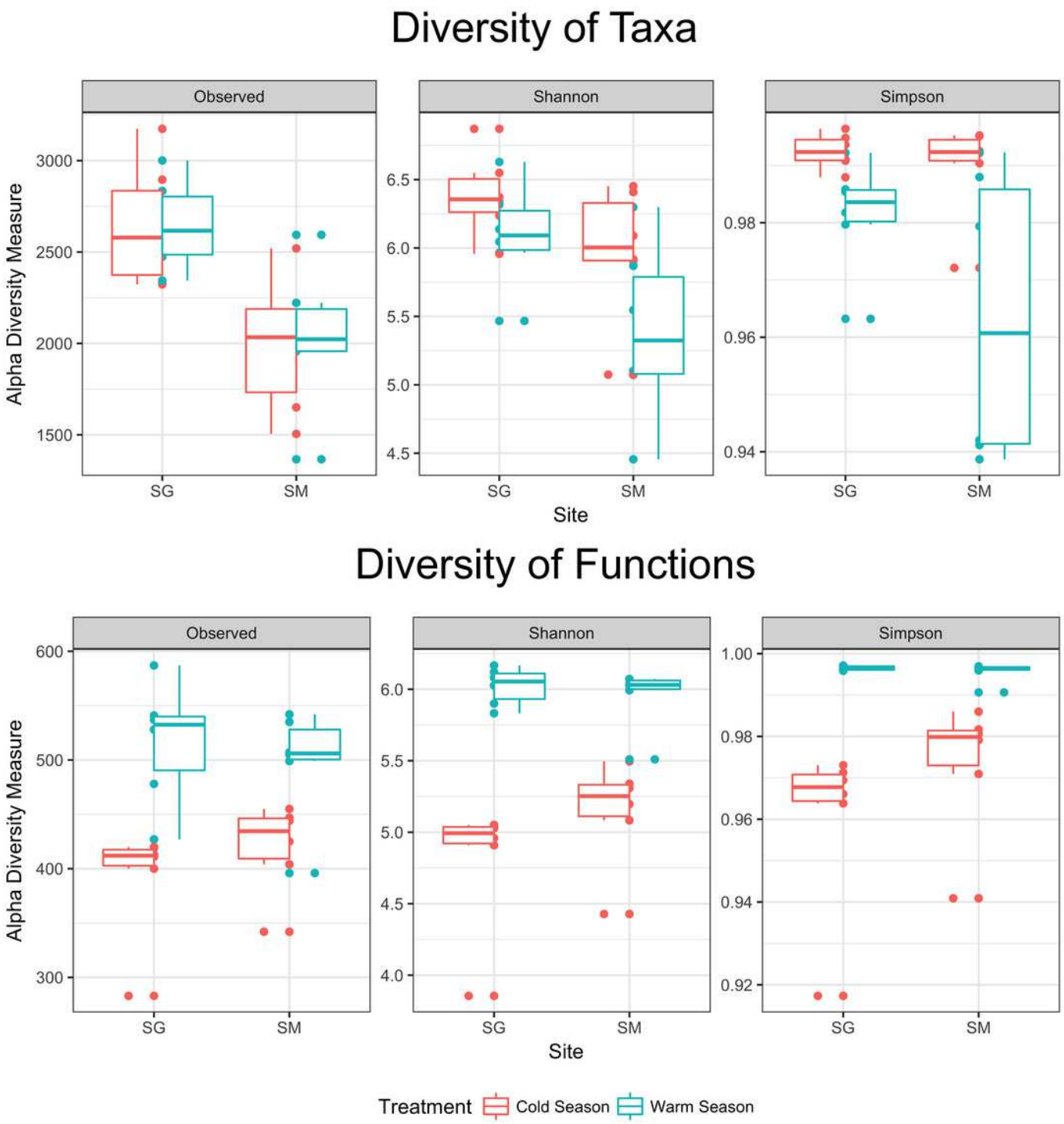

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Figure 3

Fig. 3. Alpha diversity measurements of microbial taxa and functions during cold and warm seasons in two grasslands from the Pampa biome. Each panel represents one alpha diversity measurement as follow: Observed = total number of OTU's/functions observed;

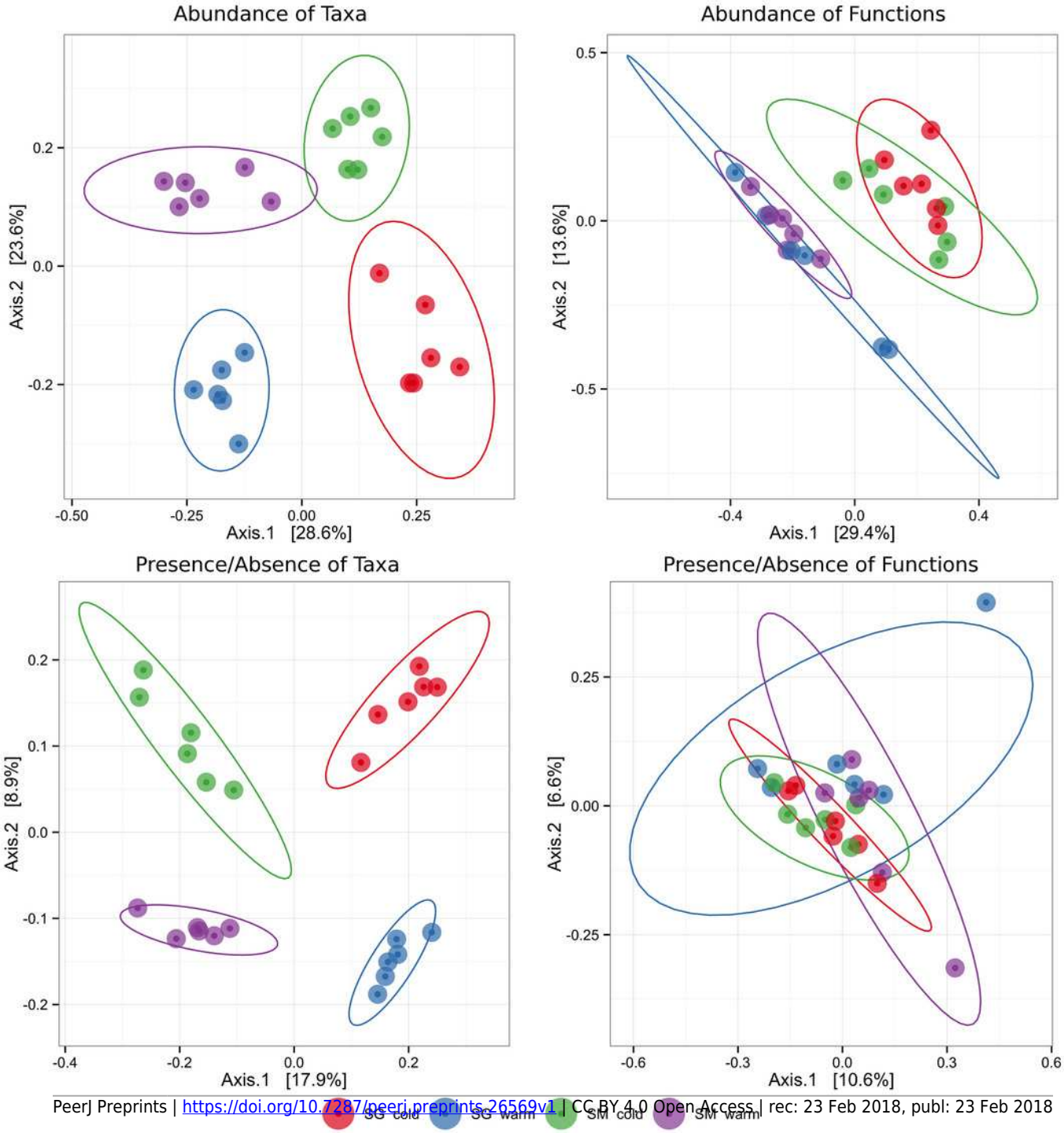

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Table 1 (on next page)

Table 1. Multivariate Analysis of Variance showing the differences among soil microbial communities and functions.

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1 **Table 1.** Multivariate Analysis of Variance showing the differences among soil microbial

2 communities and functions.

3

Factors	**Abundance of Taxons**		**Abundance of mRNA encoding Functions**	
	R^2	*p*-value	R^2	*p*-value
Location	0.262	0.001	0.036	0.327
Season	0.223	0.001	0.234	0.001
Local x Season	0.055	0.016	0.042	0.214
	Presence/Absence of Taxons		**Presence/Absence of mRNA encoding Functions**	
	R^2	*p*-value	R^2	*p*-value
Location	0.085	0.002	0.040	0.696
Season	0.170	0.001	0.054	0.019
Local x Season	0.051	0.043	0.042	0.427

4

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Table 2(on next page)

Table 2. Seasonal frequency of major bacterial groups detected in soil samples collected in two natural grasslands during different seasons.

* The Welch's t-test was performed to obtain the p-values for the null hypothesis of no difference between warn and cold seasons. Only the genera with abundance greater then 1% are depicted here.

1 **Table 2.** Seasonal frequency of major bacterial groups detected in soil samples
2 collected in two natural grasslands during different seasons.

	Location SG		
	Warm Season	Cold Season	
Phylum/genus*	Relative Frequency ± std. dev. (%)		*p*-values
Acidobacteria/Candidatus Koribacter	2.25 ± 0.73	1.20 ± 0.55	0.0293
Actinobacteria/Mycobacterium	0.16 ± 0.04	1.31 ± 0.60	0.0080
Firmicutes/Bacillus	0.54 ± 0.51	2.46 ± 1.45	0.0301
Proteobacteria/Rhodoplanes	2.89 ± 0.37	6.26 ± 1.05	0.0004
Verrucomicrobia/Candidatus Xiphinematobacter	0.98 ± 0.59	3.17 ± 1.65	0.0298
Verrucomicrobia/DA101	23.76 ± 7.57	7.79 ± 4.05	0.0035
	Location SM		
	Warm Season	Cold Season	
	Relative Frequency ± std. dev. (%)		*p*-values
Actinobacteria/Mycobacterium	0.28 ± 0.06	1.34 ± 0.48	0.0040
Proteobacteria/Rhodoplanes	1.83 ± 0.51	5.35 ± 1.23	0.0007
Verrucomicrobia/DA101	24.89 ± 11.89	5.64 ± 2.50	0.0143

3 * The Welch's t-test was performed to obtain the p-values for the null hypothesis of no difference between
4 warn and cold seasons. Only the genera with abundance greater then 1% are depicted here.
5

Table 3(on next page)

Table 3. Relative abundance of mRNA encoding functions during cold and warm seasons in two different grasslands located in the Pampa biome.

* The Welch's t-test was performed to obtain the *p*-values for the null hypothesis of no difference between warn and cold seasons. Only the functions with abundance greater than 1% and with difference between treatments with significant *p*-values (≤ 0.05) are depicted here. Number highlighted in bold represent greater abundance during either cold or warm season.

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1 **Table 3.** Relative abundance of mRNA encoding functions during cold and warm seasons in two different grasslands
2 located in the Pampa biome.

SEED subsystems*			Location SG	
Level 1	**Level 2**	**Level 3**	Cold Season	Warm Season
			Mean rel. freq. (%) ± SD	
Carbohydrates	Carbohydrates	Sugar utilization in Thermotogales	**10.5 ± 1.9**	1.1 ± 0.3
Carbohydrates	Monosaccharides	D-ribose utilization	**10.0 ± 2.0**	0.4 ± 0.1
Carbohydrates	Monosaccharides	Deoxyribose and Deoxynucleoside Catabolism	**10.0 ± 2.0**	0.4 ± 0.1
RNA Metabolism	RNA Metabolism	Group II intron-associated genes	**7.0 ± 2.5**	2.0 ± 0.7
Protein Metabolism	Protein degradation	Proteolysis in bacteria, ATP-dependent	1.2 ± 0.3	**2.4 ± 0.5**
Carbohydrates	One-carbon Metabolism	Serine-glyoxylate cycle	1.0 ± 0.3	**1.4 ± 0.3**
Protein Metabolism	Protein biosynthesis	Universal GTPases	0.9 ± 0.2	**1.4 ± 0.2**
Cofactors, Vitamins, Prosthetic Groups, Pigments	Folate and pterines	YgfZ	0.8 ± 0.1	**1.2 ± 0.2**
Motility and Chemotaxis	Flagellar motility in Prokaryota	Flagellum	0.5 ± 0.1	**0.7 ± 0.1**
Protein Metabolism	Protein degradation	Proteasome bacterial	0.5 ± 0.1	**0.8 ± 0.2**
RNA Metabolism	Transcription	Transcription initiation, bacterial sigma factors	0.5 ± 0.1	**1.0 ± 0.1**
Protein Metabolism	Protein folding	Protein chaperones	0.5 ± 0.2	**1.1 ± 0.4**
			Location SM	
Carbohydrates	Carbohydrates	Sugar utilization in Thermotogales	**7.1 ± 1.0**	0.9 ± 0.2
Carbohydrates	Monosaccharides	Deoxyribose and Deoxynucleoside Catabolism	**6.4 ± 0.9**	0.2 ± 0.1
Carbohydrates	Monosaccharides	D-ribose utilization	**6.4 ± 0.9**	0.3 ± 0.1
Protein Metabolism	Protein degradation	Proteolysis in bacteria, ATP-dependent	1.4 ± 0.4	**2.3 ± 0.5**
Carbohydrates	One-carbon Metabolism	Serine-glyoxylate cycle	1.2 ± 0.1	**1.5 ± 0.1**
Cofactors, Vitamins, Prosthetic Groups, Pigments	Folate and pterines	YgfZ	0.9 ± 0.2	**1.4 ± 0.2**
Protein Metabolism	Protein biosynthesis	Universal GTPases	0.8 ± 0.2	**1.2 ± 0.2**

3 * The Welch's t-test was performed to obtain the *p*-values for the null hypothesis of no difference between warn and cold seasons. Only the
4 functions with abundance greater than 1% and with difference between treatments with significant *p*-values (≤ 0.05) are depicted here. Number
5 highlighted in bold represent greater abundance during either cold or warm season.
6