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.

1   **Seasonal dynamics alters taxonomical and functional microbial profiles in Pampa**

2   **biome soils under natural grasslands**

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22

23 **Abstract**

24   Soil microbial communities' assembly is strongly tied to changes in temperature

25 and moisture. Although microbial functional redundancy seems to overcome taxonomical

26 composition changes, the sensitivity and resilience of soil microbial communities from

27 subtropical regions in response to seasonal variations are still poorly understood. Thus,

28 the development of new strategies for biodiversity conservation and sustainable

29 management require a complete understanding of the soil abiotic process involved in the

30 selection of microbial taxa and functions. In this work, we used state of the art molecular

31 methodologies (Next Generation Sequencing) to compare the taxonomic

32 (metataxonomics) and functional (metatranscriptomics) profiles among soil samples from

33 two subtropical natural grasslands located in the Pampa biome, Brazil, in response to

34 short-term seasonal variations. We found consistent effects of season on both microbial

35 community structure and functions, but with the former being more influenced than the

36 latter. These variations were more related to the oscillation in the relative abundances of

37 specific taxa along seasons, rather than extinction and recolonization of taxa along

38 seasons. In conclusion, the most abundant microbial groups and functions were shared

39 between seasons and locations reflecting the existence of a stable taxonomical and

40 functional core microbiota.

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42 **Keywords:** metataxonomics, metatranscriptomics,16S rRNA gene, soil microbial core,

43 Ion Torrent PGM, NGS, abiotic factors, seasons, subtropical ecosystems

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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 °C 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^{TM} 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.

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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

284   us to obtain a highly tractable model, with true landscape level of biological replications,

285   for verifying whether soil microbial functions follow the changes in the abundance of taxa

286   during seasonal variations. Our study revealed consistent effects of season on both

287   microbial community structure and functions, with the former presenting less stability than

288   the latter along the seasons.

289   Season and location significantly modulate microbial community assemblage and

290   abundance in subtropical natural grasslands (Fig. 2). However, considering only those

291   microbes with relative abundance greater than 1%, our data suggest that both grasslands

292   maintained a stable microbial community membership along the time, but the relative

293   abundance of specific taxa oscillated in response to seasonal changes (Table 2). Hence,

294   these results support the existence of a soil core microbiota in natural grasslands that

295   might represent the first step in defining a 'healthy' community and predicting community

296   responses to perturbation (Shade and Handelsman, 2012). The concept of core

297   microbiota was first introduced during the studies of the Human Microbiome Project

298   (Turnbaugh et al., 2007). While the concept of microbial core remain elusive and might

299   lack a conceptual framework involving microbial ecological characteristics (Shade and

300   Handelsman, 2012), typical approaches report the presence/absence of microbial

301   phylotypes across habitats. In the Pampa biome, Lupatini et al. (2013) already found a

302   core microbiota among soil samples under Acacia plantation, natural pasture, soybean

303   field, and natural forest. Within this study, 54.5% of OTUs (defined by 16S rRNA

304   sequences with 97% similarity) were shared among four different land uses. Similarly,

305   (Suleiman et al., 2013) found a total of 69% of the OTUs shared between a natural

306   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

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

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

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405   **Conflict of Interest Statement:** The authors declare that the research was conducted

406   in the absence of any commercial or financial relationships that could be considered as

407   a potential conflict of interest.

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410   **References**

411   Bouskill, N.J., Lim, H.C., Borglin, S., Salve, R., Wood, T.E., Silver, W.L., Brodie, E.L., 2013. Pre-
412   exposure to drought increases the resistance of tropical forest soil bacterial communities
413   to extended drought. ISME J. 7, 384–394. doi:10.1038/ismej.2012.113
414   Caporaso, J.G., Lauber, C.L., Walters, W.A., Berg-Lyons, D., Huntley, J., Fierer, N., Owens,
415   S.M., Betley, J., Fraser, L., Bauer, M., Gormley, N., Gilbert, J.A., Smith, G., Knight, R.,
416   2012. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and
417   MiSeq platforms. ISME J 6, 1621–4. doi:10.1038/ismej.2012.8
418   Caporaso, J.G., Paszkiewicz, K., Field, D., Knight, R., Gilbert, J.A., 2012. The Western English
419   Channel contains a persistent microbial seed bank. ISME J. 6, 1089–1093.
420   doi:10.1038/ismej.2011.162
421   Carbone, M.S., Still, C.J., Ambrose, A.R., Dawson, T.E., Williams, A.P., Boot, C.M., Schaeffer,
422   S.M., Schimel, J.P., 2011. Seasonal and episodic moisture controls on plant and
423   microbial contributions to soil respiration. Oecologia 167, 265–278. doi:10.1007/s00442-
424   011-1975-3
425   Castro, H.F., Classen, A.T., Austin, E.E., Norby, R.J., Schadt, C.W., 2010. Soil Microbial
426   Community Responses to Multiple Experimental Climate Change Drivers. Appl. Environ.
427   Microbiol. 76, 999–1007. doi:10.1128/AEM.02874-09
428   Frossard, A., Gerull, L., Mutz, M., Gessner, M.O., 2012. Disconnect of microbial structure and
429   function: enzyme activities and bacterial communities in nascent stream corridors. ISME
430   J. 6, 680–691.
431   Gilbert, J.A. et al., 2012. De ning seasonal marine microbial community dynamics. ISME J. 6,
432   298–308.
433   Good, I.J., 1953. The population frequencies of species and the estimation of population
434   parameters. Biometrika 40, 237–264.
435   Griffiths, B.., Ritz, K., Wheatley, R., Kuan, H.., Boag, B., Christensen, S., Ekelund, F.,
436   Sørensen, S.., Muller, S., Bloem, J., 2001. An examination of the biodiversity–ecosystem

437   function relationship in arable soil microbial communities. Soil Biol. Biochem. 33, 1713–
438   1722. doi:10.1016/S0038-0717(01)00094-3

439 Guido, A., Vélez-Martin, E., Overbeck, G.E., Pillar, V.D., 2016. Landscape structure and climate
440   affect plant invasion in subtropical grasslands. Appl. Veg. Sci. 19, 600–610.
441   doi:10.1111/avsc.12263

442 Habekost, M., Eisenhauer, N., Scheu, S., Steinbeiss, S., Weigelt, A., Gleixner, G., 2008.
443   Seasonal changes in the soil microbial community in a grassland plant diversity gradient
444   four years after establishment. Soil Biol. Biochem. 40, 2588–2595.
445   doi:10.1016/j.soilbio.2008.06.019

446 Lauber, C.L., Ramirez, K.S., Aanderud, Z., Lennon, J., Fierer, N., 2013. Temporal variability in
447   soil microbial communities across land-use types. ISME J 7, 1641–1650.

448 Lemos, L.N., Fulthorpe, R.R., Triplett, E.W., Roesch, L.F.W., 2011. Rethinking microbial
449   diversity analysis in the high throughput sequencing era. J. Microbiol. Methods 86, 42–
450   51. doi:10.1016/j.mimet.2011.03.014

451 Li, H., Yang, Q., Li, J., Gao, H., Li, P., Zhou, H., 2015. The impact of temperature on microbial
452   diversity and AOA activity in the Tengchong Geothermal Field, China. Sci. Rep. 5.
453   doi:10.1038/srep17056

454 Lipson, D.A., Schmidt, S.K., 2004. Seasonal Changes in an Alpine Soil Bacterial Community in
455   the Colorado Rocky Mountains. Appl. Environ. Microbiol. 70, 2867–2879.
456   doi:10.1128/AEM.70.5.2867-2879.2004

457 Luo, Y., Wan, S., Hui, D., Wallace, L.L., 2001. Acclimatization of soil respiration to warming in a
458   tall grass prairie. Nature 413, 622–625. doi:10.1038/35098065

459 Lupatini, M., Suleiman, A.K.A., Jacques, R.J.S., Antoniolli, Z.I., Kuramae, E.E., de Oliveira
460   Camargo, F.A., Roesch, L.F.Wã¼., 2013. Soil-Borne Bacterial Structure and Diversity
461   Does Not Reflect Community Activity in Pampa Biome. PLoS ONE 8, e76465.
462   doi:10.1371/journal.pone.0076465

463 Marchesi, J.R., Ravel, J., 2015. The vocabulary of microbiome research: a proposal.
464   Microbiome 3. doi:10.1186/s40168-015-0094-5

465 Meyer, F., Paarmann, D., D'Souza, M., Olson, R., Glass, E., Kubal, M., Paczian, T., Rodriguez,
466   A., Stevens, R., Wilke, A., Wilkening, J., Edwards, R., 2008. The metagenomics RAST
467   server – a public resource for the automatic phylogenetic and functional analysis of
468   metagenomes. BMC Bioinformatics 9, 386. doi:10.1186/1471-2105-9-386

469 Murphy, A.C., Foster, L.B., Gao, C., 2016. Temporal Dynamics in Rhizosphere Bacterial
470   Communities of Three Perennial Grassland Species. Agronomy 6.
471   doi:10.3390/agronomy6010017

472 Nannipieri, P., Ascher, J., Ceccherini, M., Landi, L., Pietramellara, G., Renella, G., 2003.
473   Microbial diversity and soil functions. Eur. J. Soil Sci. 54, 655–670.

474 Oksanen J, Blanchet F G, Kindt R, Legendre P, Minchin P R, O'Hara R B, Simpson G L,
475   Solymos P, Stevens M H H, Wagner H, 2015. Vegan: community ecology package. R
476   package vegan, vers. 2.2-1.

477 Parks, D.H., Tyson, G.W., Hugenholtz, P., Beiko, R.G., 2014. STAMP: statistical analysis of
478   taxonomic and functional profiles. Bioinformatics 30, 3123–3124.
479   doi:10.1093/bioinformatics/btu494

480 Pylro, V.S., Morais, D.K., de Oliveira, F.S., dos Santos, F.G., Lemos, L.N., Oliveira, G., Roesch,
481   L.F., 2016. BMPOS: a flexible and user-friendly tool sets for microbiome studies. Microb.
482   Ecol. 72, 443–447.

483 Pylro, V.S., Roesch, L.F.W., Morais, D.K., Clark, I.M., Hirsch, P.R., Tótola, M.R., 2014. Data
484   analysis for 16S microbial profiling from different benchtop sequencing platforms. J.
485   Microbiol. Methods 107, 30–37.

486 Rasche, F., Knapp, D., Kaiser, C., Koranda, M., Kitzler, B., Zechmeister-Boltenstern, S.,
487   Richter, A., Sessitsch, A., 2011. Seasonality and resource availability control bacterial
488   and archaeal communities in soils of a temperate beech forest. ISME J. 5, 389–402.
489 Roesch, L.F.W., Vieira, F.C.B., Pereira, V.A., Schünemann, A.L., Teixeira, I.F., Senna, A.J.T.,
490   Stefenon, V.M., 2009. The Brazilian Pampa: a fragile biome. Diversity 1, 182–198.
491 Rousk, J., Brookes, P.C., Baath, E., 2009. Contrasting Soil pH Effects on Fungal and Bacterial
492   Growth Suggest Functional Redundancy in Carbon Mineralization. Appl. Environ.
493   Microbiol. 75, 1589–1596. doi:10.1128/AEM.02775-08
494 Rui, J., Peng, J., Lu, Y., 2009. Succession of Bacterial Populations during Plant Residue
495   Decomposition in Rice Field Soil. Appl. Environ. Microbiol. 75, 4879–4886.
496   doi:10.1128/AEM.00702-09
497 Schindlbacher, A., Rodler, A., Kuffner, M., Kitzler, B., Sessitsch, A., Zechmeister-Boltenstern,
498   S., 2011. Experimental warming effects on the microbial community of a temperate
499   mountain forest soil. Soil Biol. Biochem. 43, 1417–1425.
500   doi:10.1016/j.soilbio.2011.03.005
501 Shade, A., Handelsman, J., 2012. Beyond the Venn diagram: the hunt for a core microbiome:
502   The hunt for a core microbiome. Environ. Microbiol. 14, 4–12. doi:10.1111/j.1462-
503   2920.2011.02585.x
504 Sheik, C.S., Beasley, W.H., Elshahed, M.S., Zhou, X., Luo, Y., Krumholz, L.R., 2011. Effect of
505   warming and drought on grassland microbial communities. ISME J. 5, 1692–1700.
506 Stegen, J.C., Lin, X., Konopka, A.E., Fredrickson, J.K., 2012. Stochastic and deterministic
507 assembly processes in subsurface microbial communities. ISME J 6, 1653–1664.
508 Suleiman, A.K.A., Manoeli, L., Boldo, J.T., Pereira, M.G., Roesch, L.F.W., 2013. Shifts in soil
509   bacterial community after eight years of land-use change. Syst. Appl. Microbiol. 36, 137–
510   144.
511 Suleiman, A.K., Gonzatto, R., Aita, C., Lupatini, M., Jacques, R.J., Kuramae, E.E., Antoniolli,
512   Z.I., Roesch, L.F., 2016. Temporal variability of soil microbial communities after
513   application of dicyandiamide-treated swine slurry and mineral fertilizers. Soil Biol.
514   Biochem. 97, 71–82.
515 Tozzi, M.G., Camici, M., Mascia, L., Sgarrella, F., Ipata, P.L., 2006. Pentose phosphates in
516   nucleoside interconversion and catabolism. FEBS J. 273, 1089–1101.
517   doi:10.1111/j.1742-4658.2006.05155.x
518 Turnbaugh, P.J., Ley, R.E., Hamady, M., Fraser-Liggett, C.M., Knight, R., Gordon, J.I., 2007.
519   The human microbiome project. Nature 449, 804–10. doi:10.1038/nature06244
520 Waldrop, M.P., Firestone, M.K., 2006. Seasonal Dynamics of Microbial Community Composition
521   and Function in Oak Canopy and Open Grassland Soils. Microb. Ecol. 52, 470–479.
522   doi:10.1007/s00248-006-9100-6
523 Wallenstein, M.D., Hall, E.K., 2012. A trait-based framework for predicting when and where
524   microbial adaptation to climate change will affect ecosystem functioning.
525   Biogeochemistry 109, 35–47. doi:10.1007/s10533-011-9641-8
526 Wertz, S., Degrange, V., Prosser, J.I., Poly, F., Commeaux, C., Freitag, T., Guillaumaud, N.,
527   Roux, X.L., 2006. Maintenance of soil functioning following erosion of microbial diversity.
528   Environ. Microbiol. 8, 2162–2169. doi:10.1111/j.1462-2920.2006.01098.x
529 Wertz, S., Degrange, V., Prosser, J.I., Poly, F., Commeaux, C., Guillaumaud, N., Le Roux, X.,
530   2007. Decline of soil microbial diversity does not influence the resistance and resilience
531   of key soil microbial functional groups following a model disturbance. Environ. Microbiol.
532   9, 2211–2219. doi:10.1111/j.1462-2920.2007.01335.x
533

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.
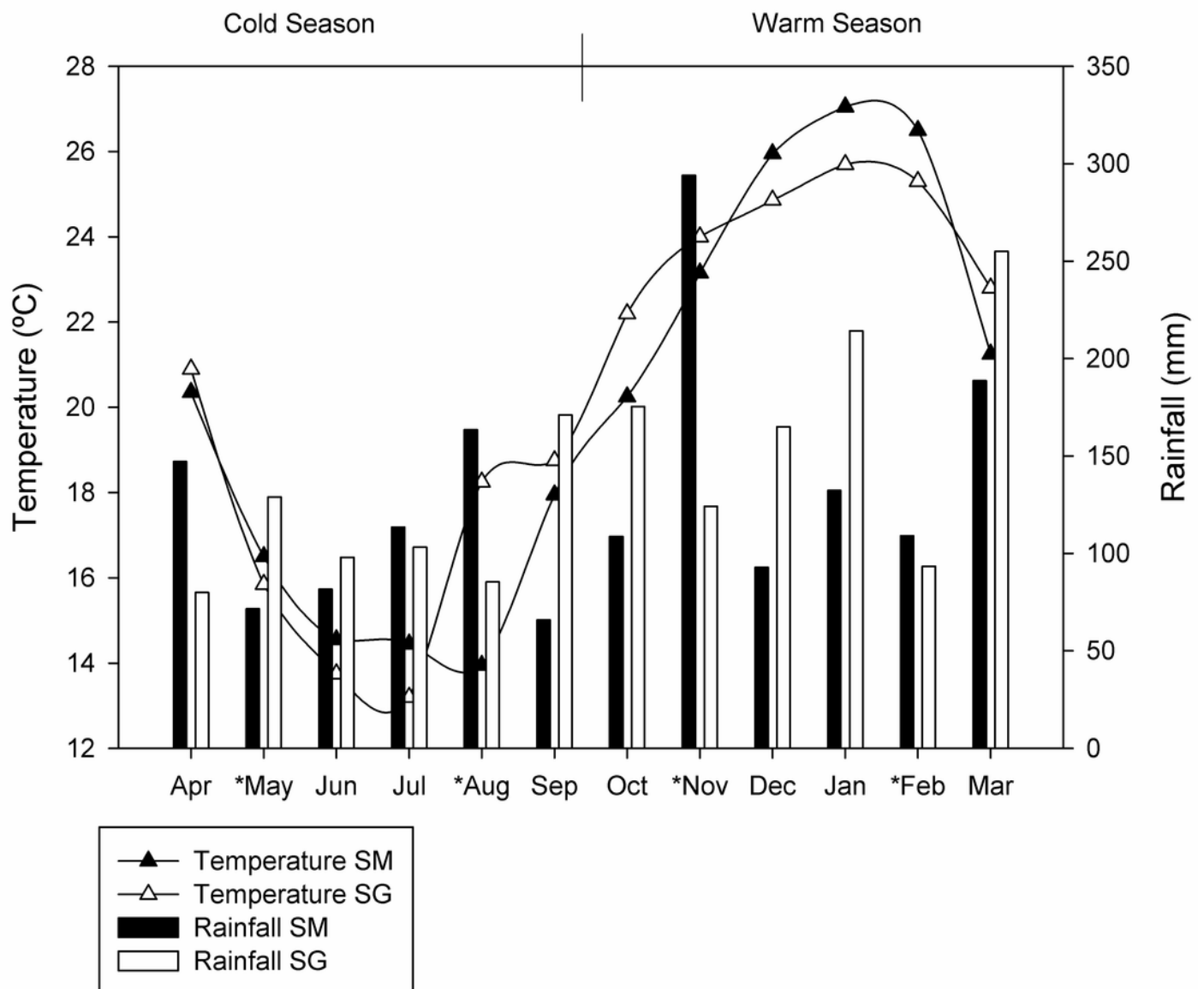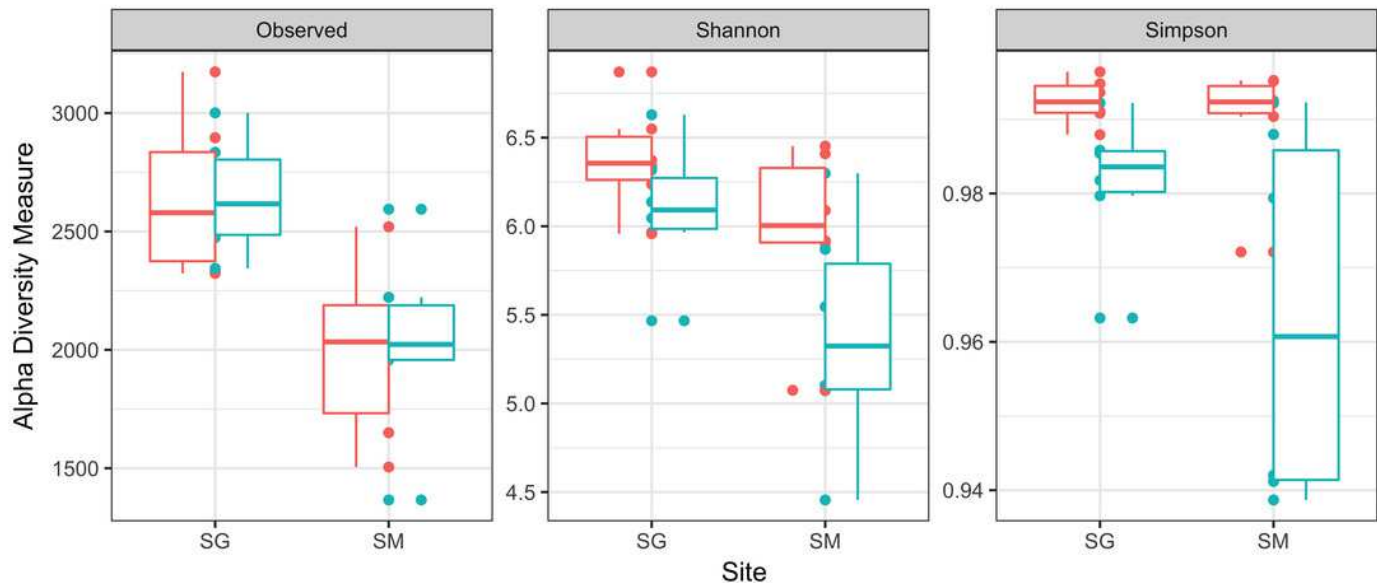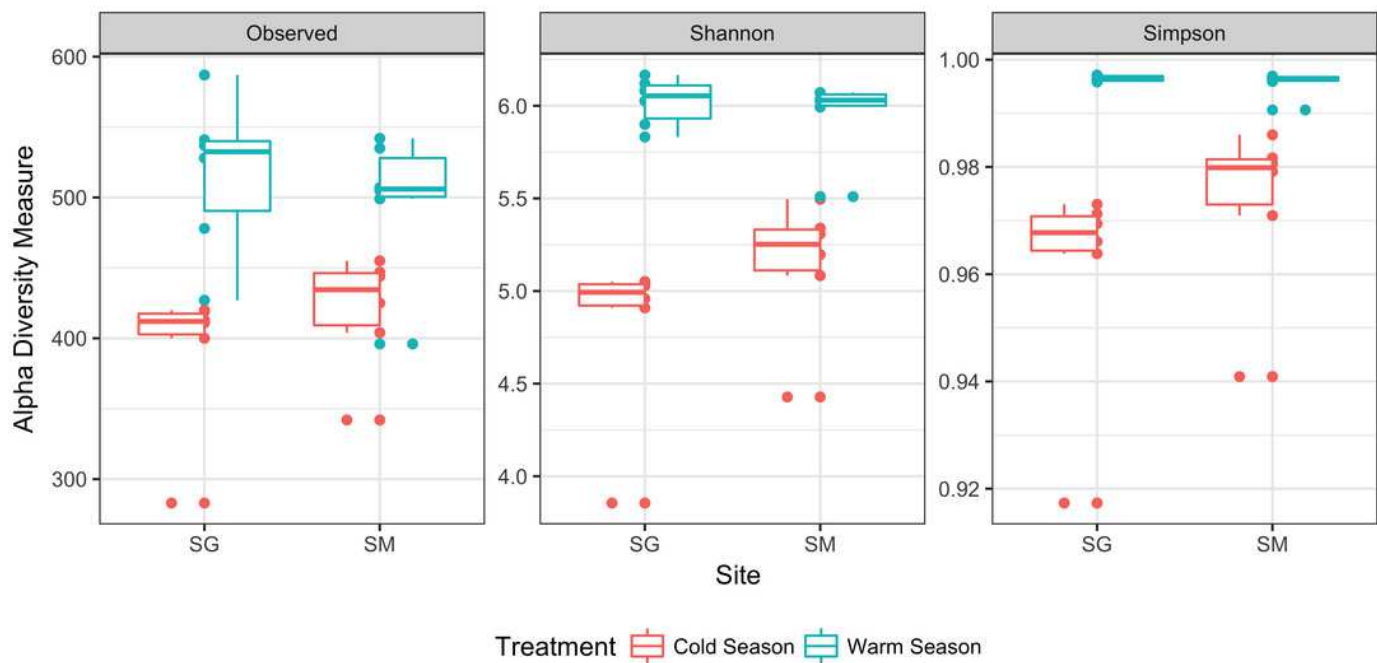
Figure 2

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%

Diversity of Taxa

Diversity of Functions

Treatment ☐ Cold Season ☐ Warm Season

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;
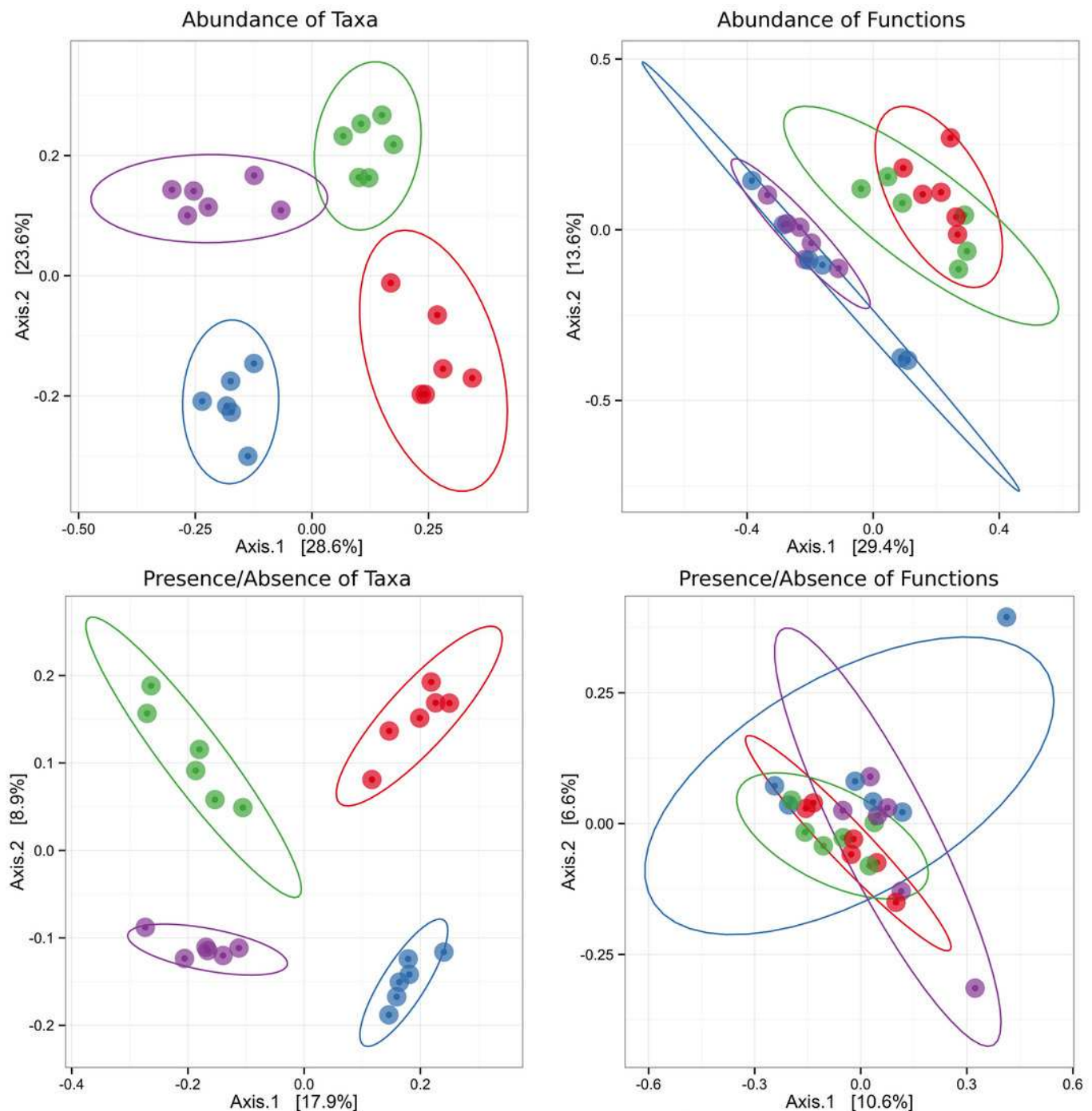
Table 1 (on next page)

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

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

5

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.

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	
			Cold Season	Warm Season
Level 1	Level 2	Level 3	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