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SOIL MICROBIOLOGY

Moisture Is More Important than Temperature for Assembly of Both Potentially Active and Whole Prokaryotic Communities in Subtropical Grassland

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Abstract

Moisture and temperature play important roles in the assembly and functioning of prokaryotic communities in soil. However, how moisture and temperature regulate the function of niche- versus neutral-based processes during the assembly of these communities has not been examined considering both the total microbial community and the sole active portion with potential for growth in native subtropical grassland. We set up a well-controlled microcosm-based experiment to investigate the individual and combined effects of moisture and temperature on soil prokaryotic communities by simulating subtropical seasons in grassland. The prokaryotic populations with potential for growth and the total prokaryotic community were assessed by 16S rRNA transcript and 16S rRNA gene analyses, respectively. Moisture was the major factor influencing community diversity and structure, with a considerable effect of this factor on the total community. The prokaryotic populations with potential for growth and the total communities were influenced by the same assembly rules, with the niche-based mechanism being more influential in communities under dry condition. Our results provide new information regarding moisture and temperature in microbial communities of soil and elucidate how coexisting prokaryotic populations, under different physiological statuses, are shaped in native subtropical grassland soil.

Keywords 16S rRNA gene · 16S rRNA transcript · Seasonality · Assembly process · Microbial ecology

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Introduction

Grasslands in subtropical and temperate regions are exposed to wide seasonal variations, which have considerable influence on soil moisture and temperature [1], thereby strongly affecting fundamental processes such as soil organic matter decomposition, plant growth, and nutrient turnover in terrestrial environments [2, 3]. Microbes are drivers of these processes, and generally, temperature changes might influence the structure of bacterial communities [4, 5].

Multiple factors including soil microenvironments [6], water content, and temperature can regulate the composition and function of microbial communities [7, 8]. However, the solely impact of water and temperature on the prokaryotic community of subtropical grassland ecosystem is poorly understood. High seasonal variability may select for stable communities adapted to inter- and intra-annual changes [9]. The community compositional stability reported by a few studies [10–12] might be explained by the presence of a large but inactive or

dormant pool of microorganisms in soil [13], which may mask the compositional shifts of the active community or its function.

Habitat characteristics create conditions that favor or disfavor the relative importance of the microbial community assembly processes [14]. These are neutral and niche-based processes. Microbial communities are more influenced by neutral processes, if all individuals within this community have the same chances of reproduction and death, regardless of their species identity. On the other hand, microbial communities are more influenced by niche-based processes, if the environmental selection is more likely to shape them. For example, niche-based processes through environmental selection are more plausible to shape microbial communities in highly stressed habitats [15], such as disturbances promoted by drought [16]. On the contrary, in more favorable environments (or less disturbed habitats, e.g., highly productive or wet habitats) microbial communities are more likely to be shaped by neutral processes [17]. Phylogenetic clustering can be interpreted as a niche-based process, where only phylogenetically closely related taxa co-occur at specific environmental filtering conditions [18]. However, no phylogenetic clustering or overdispersion indicate that neutral mechanisms are the dominant assembly process, generating communities with more variable species composition [19, 20]. Although both niche- and neutral-based processes are important in shaping microbial community structure, how seasonal variations regulate their importance is not yet understood [21]. Moreover, the contribution of niche- or neutral-based assembly processes was not examined in the light of the coexisting prokaryotic populations potentially active (measured by sequencing the rRNA transcripts) and the whole community (measured by sequencing the total rDNA), in native subtropical grassland under the influence of seasonal climatic variations in soil. We consider this knowledge important for understanding the co-habitation of species and maintenance of biodiversity, as ecological traits differ among species within a community.

Previously research from our group suggests that subtropical grasslands maintained a stable microbial community membership along the year with oscillation in abundance [22]. However, the specific factors ruling the assembly of both potentially active and whole microbial communities in subtropical grasslands are still unknown. Both moisture and temperature can potentially shape soil microbial communities through niche-based processes, but seasonal variations might regulate the importance of those processes. This way, we hypothesized that both niche and neutral processes work together in the assembly of the soil prokaryotic community but each presenting different relative importance according to the environmental restrictions imposed by the seasonal variations. With this work, we aimed to understand how moisture and temperature affect the prokaryotic communities and what dominant assembly rules are operating under these circumstances. We sequenced 16S rRNA gene and 16S rRNA (16S rRNA transcript) sampled from soils in a well-controlled microcosm system to investigate the individual and interactive effects of moisture and temperature, mimicking winter and summer conditions in a native subtropical grassland, on the composition of microbial communities. The lower moisture content and the high temperature in our experiment are representative of dry summer conditions, while higher moisture content and low temperature are typical for winter conditions. We measured the relative contribution of the niche- and neutral-based processes by comparing the prokaryotic change followed by the relationship information about the underlying assembly process, along the seasons.

Material and Methods

Microcosm Experimental Design and Soil Sampling

The microcosm was designed to mimic the natural climate variations (e.g., moisture and temperature variations) over the years in the Brazilian Pampa biome. The Brazilian Pampa is located between latitudes 28° 00′ S and 34° 00′ S and longitudes 49° 30′ W and 58° 00′ W, within the South Temperate Zone and has subtropical climates with four well-characterized seasons. Because of the natural grasslands, livestock production is one of the main economic activities serving as a source of forage for around 18 million animals—mainly cattle and sheep [23]. The soil used in the experiment was sampled from a uniform area of native subtropical grassland (29° 45′ S, 53° 45′ W) under cattle grazing, with no input of fertilizers other than animal manure. The annual mean temperature at the sampling site was 20 °C, with a minimum of 0 °C and maximum of 35 °C. The rainfall is well distributed during the year, with an annual rainfall around 1200–1600 mm [24]. The soil temperature and moisture applied in the microcosms were based on measurements over the year in the same grassland area where the soil cores were collected (Fig. S1). The dominant plant species in the area were *Adropogon lateralis* Nees, *Aristida laevis* (Nees) Kunth., *Bacharis trimera* (Less. DC.), and *Paspalum notatum* Flüggé. To determine the boundaries of moisture content in soil, we evaluate the permanent wilting point using water potential psychrometry [25] and the field capacity in a sand suction column [26]. The silt loam soil was classified as Paleudult (U.S. Soil Taxonomy). Soil physicochemical analyses (Table 1) were performed according to the recommendations of the Brazilian Society of Soil Science [27].

Twenty-seven undisturbed soil cores (blocks of 15 × 25 cm and 20-cm depth) were carefully collected with a shovel from the upper soil layer in autumn (May 2013) and used to set up a microcosm experiment. Briefly, after the demarcation of the sampling core, a trench was dug around the soil block. The

Table 1 Physicochemical properties of the soil

Property	Unit	Value
pH—H_2O (1:2.5)		4.68
P	mg dm^{-3} (1)	4.98
K	mg dm^{-3} (1)	156.00
Ca^{2+}	$cmol_c$ dm^{-3} (2)	2.20
Mg^{2+}	$cmol_c$ dm^{-3} (2)	1.20
Al^{3+}	$cmol_c$ dm^{-3} (2)	1.30
CEC(T)	$cmol_c$ dm^{-3}	12.92
BS	%	29.22
OM	g kg^{-1} (4)	2.66
N	g kg^{-1} (5)	21
Zn	mg dm^{-3} (1)	1.20
Cu	mg dm^{-3} (1)	1.17
B	mg dm^{-3} (6)	0.34
S	mg dm^{-3} (7)	12.44
Coarse sand	g kg^{-1} (8)	67.00
Fine sand	g kg^{-1} (8)	261.00
Silt	g kg^{-1} (8)	504.00
Clay	g kg^{-1} (8)	168.00
Textural class		Silt loam

(1) Extracted with Mehlich-1. (2) Extracted with KCl 1 mol L^{-1}. (3) Extracted with calcium acetate 0.5 mol L^{-1}, pH 7.0. (4) Walkey & Black method. (5) Digestion in sulfuric acid and determined in Kjeldahl. (6) Extracted with hot water. (7) Extracted with $Ca(H_2PO_4)_2$. (8) Pipet method. *SEB* sum of exchangeable bases capacity, *CEC (T)* cation-exchange capacity in pH 7.0, *BS* base saturation

block was carefully removed by crossing a metal wire in the bottom layer from side to side and placed in a container with the same dimensions. Soil cores were sampled with a minimum distance of 50 cm to each other [28], to avoid spatial autocorrelation of the microbial community. Each core was placed into a pot with the same dimensions and kept at the same sampling air temperature and soil moisture [28 °C and 200 g kg^{-1} (*w*/*w*), respectively] in laboratory conditions, for a maximum of 6 h before the microcosms were set up.

The experimental design was completely randomized, with a 3 × 3 factorial arrangement and three replicates for all treatments. Three levels of soil moisture were applied: 80 g kg^{-1} (permanent wilting point, or 8%), 160 g kg^{-1} (70% of field capacity, or 16%), and 230 g kg^{-1} (field capacity, or 23% w/w). These parameters were kept constant during the whole experiment by weighing the pots and by adding sterile distilled water. Within the three abovementioned soil moistures, the microcosms were incubated at three different temperatures (10 °C, 20 °C, and 30 °C) for 20 days. Plants were alive during the 20 days of experiment within the treatments under 70% of field capacity and at field capacity in all temperatures. At the permanent wilting point, plants showed strong signs of water stress. After that, the soil samples were taken from the top layer (0–5 cm) of the microcosm systems, where most of the root system is located in a perennial pasture from the Brazilian Pampa [29] and where microbial activity is higher, due to the high concentration of plant rhizodeposition and dead leaves. Soil samples were randomly collected from cores using a sterile V-shaped spatula, kept in sterile 50-mL tubes, and immediately stored at − 80 °C until DNA and RNA co-extraction.

Co-extraction of Soil DNA and RNA

From each sample 2 g of soil was used for simultaneous total RNA and DNA isolation using the RNA PowerSoil kit and the PowerSoil®DNA Elution Accessory Kit (MoBio laboratories, Inc., Carlsbad, CA, USA), following the manufacturer's instructions. Total RNA and DNA quantities and quality were determined using NanoVue™spectrophotometer (GE Healthcare). The residual DNA from RNA samples was digested via DNase treatment (TURBO DNA-free™ Kit, Life Technologies, Carlsbad, CA, USA). The total RNA was synthesized to complementary DNA (cDNA) using random hexamers with Maxima H Minus First Strand cDNA Synthesis Kit (Life Technologies, Carlsbad, CA, USA). Resulting DNA and cDNA were used as templates for amplification of the 16S rRNA.

16S rDNA and rRNA Amplification and Sequencing

The composition of the bacterial and archaeal communities was determined based on partial 16S rDNA and 16S rRNA (V4 region) sequences directly amplified using a bacterial/archaeal primer set 515F/806R [30], from about 100 ng of DNA or cDNA templates obtained for each replicate per treatment. PCR amplification, library preparation, and sequencing followed the procedures described by Dobbler et al. [31]. All procedures, including DNA RNA extraction, library preparation, and sequencing were carried out at the Centro Interdisciplinar de Pesquisas em Biotecnologia (CIP-Biotec) at the Federal University of Pampa.

16S rDNA and rRNA Data Analysis

The 16S rDNA and rRNA raw sequences were analyzed following the recommendations of the Brazilian Microbiome Project [32], using the BMP Operating System (BMPOS) [33]. Briefly, the OTU table was built using the UPARSE pipeline [34]. The reads were truncated at 200 bp and quality filtered using a maximum expected error of 0.5 (meaning that on average one nucleotide in every two sequences is incorrect). Filtered reads were dereplicated and unique sequences (singletons) were removed. These sequences were clustered into OTUs a 97% similarity cutoff using UPARSE-OTU algorithm and chimera checking was performed using the UCHIME method [35]. After clustering, the sequences were aligned and classified using the SILVA reference database

(release SSU_Ref_119) with a confidence threshold of 50% [36], using mothur [37]. The representative sequence of each OTU, picked using UPARSE-OTU algorithm, was used to construct a dendrogram using a distance matrix with the relaxed neighbor joining (RNJ) algorithm in clearcut [38], available in mothur.

Statistical Analysis

The BIOM file was imported into the R environment [39] using the phyloseq package [40]. Good's coverage estimator [41] was calculated for 97% similarity cutoff in order to assess if the number of sequences obtained represented the entire community. All libraries (rDNA and rRNA from each sample) were normalized by randomly resampling the sequence data to the same number of sequences found in the smallest library [42]. The rarefied dataset to 4167 sequences per sample (for both rDNA and rRNA libraries) was used for estimation of alpha diversity and richness using two different approaches: (a) community richness was calculated by Chao1's estimator, which weights total and rare species; (b) compositional diversity was assessed by applying the Shannon diversity index considering the number and abundance of species (*estimate_richness* function in phyloseq package). The diversity indices were analyzed using two-way analysis of variance (ANOVA) with repeated measures after plotting the residuals and confirming the normality of the data using the Shapiro-Wilk *W* test ($p > 0.05$). When the differences were significant they were further analyzed by using the post hoc Tukey's HSD test within the agricolae R-package.

To test the hypothesis that environmental factors (moisture, temperature or the interaction of both) shape the prokaryotic communities we assembled two compositional dissimilarity matrixes generated by Bray-Curtis each representing the rRNA and the rDNA of the communities. The matrices were ordinated by principal coordinates analysis (PCoA) using *ordinate* within the phyloseq package and the variance partitioning was calculated using the permutational

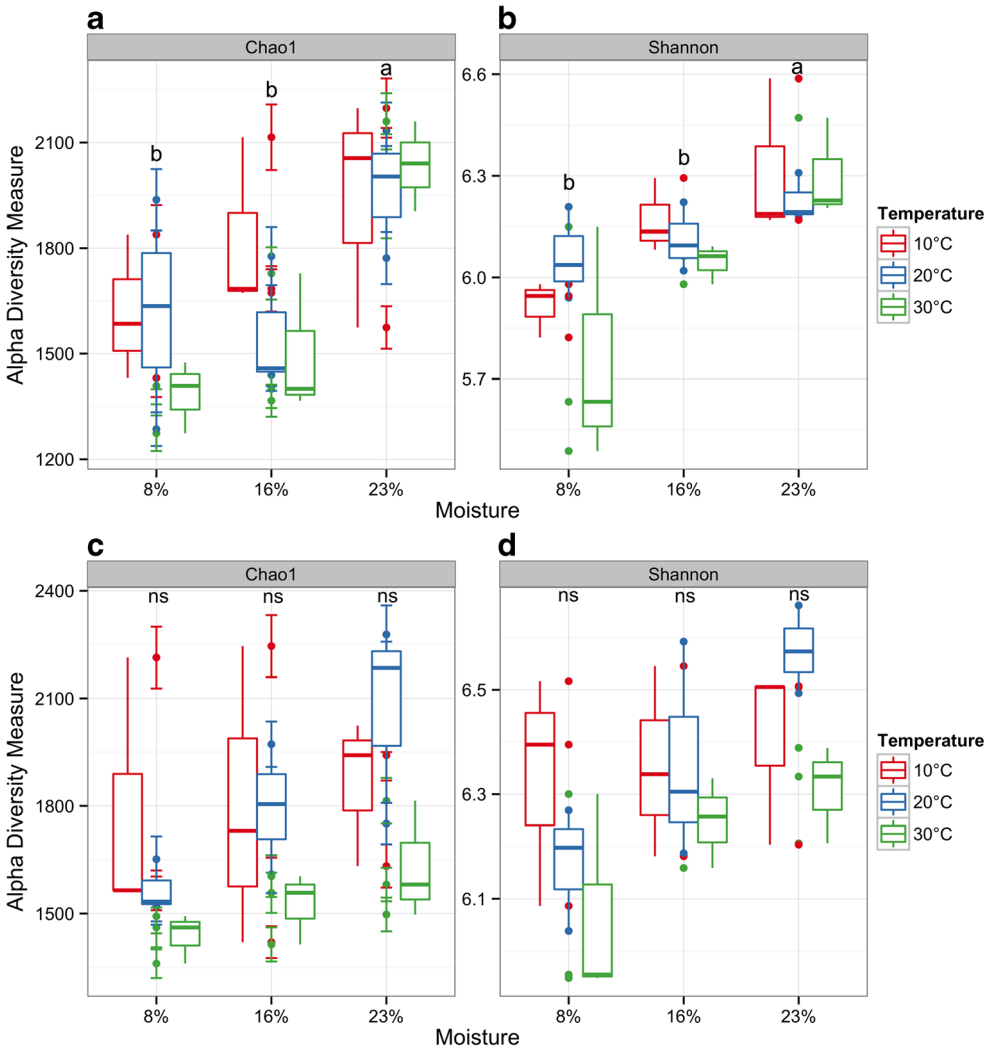

Fig. 1 Richness and diversity estimators of bacterial communities. (A) and (B) depict the rDNA-based approach; (C) and (D) depict the rRNA approach. Boxes span the first to third quartiles; the horizontal line inside the boxes represents the median. Whiskers extending vertically from the boxes indicate variability outside the upper and lower quartiles, and the circles indicate outliers. Box plots having the same letter are not significantly different (p value > 0.05) according to the pairwise Tukey's test between moisture regimes. Differences in temperature regimes were not significantly different (ns)

multivariate analysis of variance PERMANOVA [43] model based on Bray-Curtis using the *adonis* function in the vegan package [44].

Net relatedness index (NRI) [18] using rarefied data was calculated to depict phylogenetic community structures, using *ses.mdp* in the *picante* packages along the moisture regimes. The NRI captures the influence of deeper phylogenetic nodes (e.g., Phyla, Class) from the entire microbial community. NRI high positive values indicate clustered (=niche-based process), while low or negative values indicate overdispersed phylogenetic structures (=neutral-based process). The phylogenetic structuring of a community with NRI close to zero indicates neutral-based processes [45]. To evaluate the degree of non-random phylogenetic community structuring, taxa were randomized across the tips of the phylogeny (null.model = "taxa.labels") with 999 permutation-shuffling taxa among soil moisture. Two-tailed p values were obtained by comparing values for NRI with those from the distributions of random communities. All figures and graphics were generated by the ggplot2 package.

The OTU screening for differential abundance between treatments was performed with DESEq2 [46]. Contrasts were set to pairwise compassions among different soil moistures in a multifactor design controlling for temperature. This analysis was performed with the non-rarefied dataset. Preview dataset filtering was applied to remove any OTU with a total sum < 10 sequences and not present in less than 20% of all samples. The p values were adjusted for multiple comparisons using the FDR method.

Results

Sequence Data and Prokaryotic Community Coverage

After quality filtering, a total of 708,927 sequences were obtained (402,614 for 16S rDNA and 306,313 for 16S rRNA) with an average of 13,128 sequences per sample. A total of 7381 OTUs (representing 99% of all sequences) were assigned to the bacteria domain and 30 OTUs were assigned to the archaea domain. All bacterial OTUs were assigned to a known phylum. About 90.5%, 83.7%, 71.4%, and 51% of sequences were assigned to a known class, order, family, and genus, respectively.

Effect of Moisture and Temperature on Diversity and Structure of Prokaryotic Communities

Within the conditions applied in this experiment (e.g., only the temperature and the moisture content being modified and based on strict measurement of homogenized soil samples), moisture was the most important predictor of diversity of the total prokaryotic community (rDNA), with Chao1 and Shannon values differing significantly across moisture regimes (Tukey's HSD test, $p < 0.05$—Fig. 1 A and B). Overall, the DNA-based prokaryotic communities presented lower richness and diversity values in low water content (permanent wilting point, 8% *w*/*w*) than in high moisture regimes (field capacity, 23% *w*/*w*) when assessed by Tukey's HSD test ($p < 0.01$

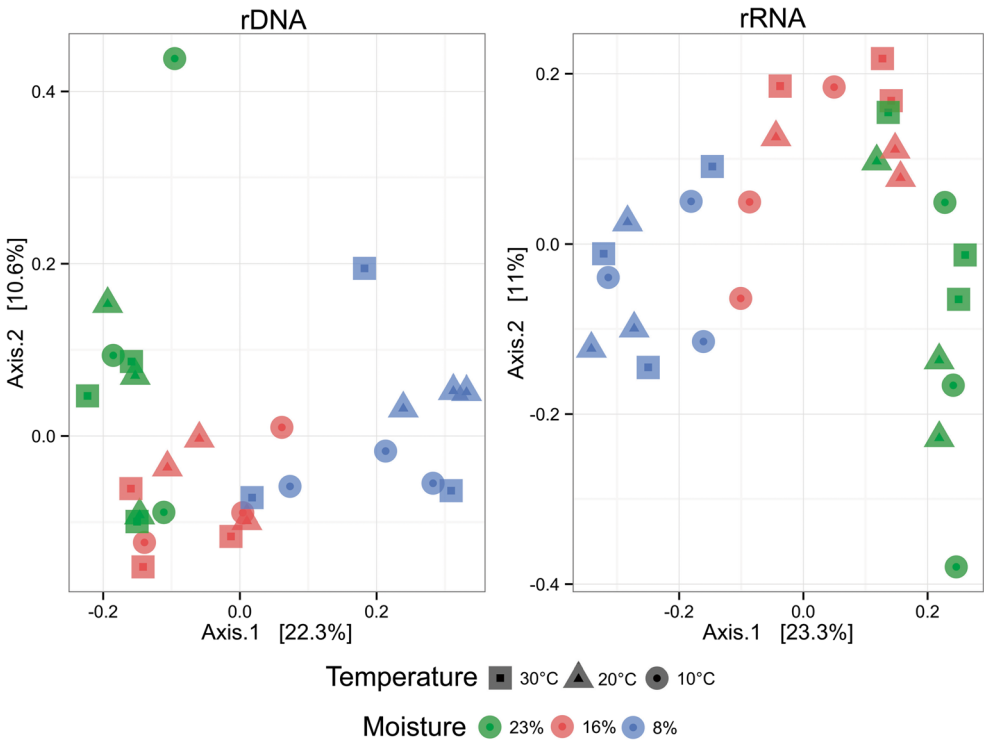

Fig. 2 Principal coordinates analysis (PCoA) ordination based on Bray-Curtis dissimilarity of microbial community structure based in rDNA and rRNA partial sequencing. Different shapes indicate the temperature regimes while different colors indicate the moisture regimes. The variations explained by the first two axes are indicated in the graphs

– Fig. 1 A and B). The diversity of prokaryotic communities measured by the rRNA approach did not differ among moisture or temperature treatments. Neither temperature nor the interaction between moisture and temperature affected prokaryotic richness and diversity (Tukey's HSD test; Fig. 1 C and D).

The ordination carried out by using Bray-Curtis dissimilarity suggested that soil moisture was the main driver shaping the community structure (Fig. 2). Further multivariate analysis of variance confirmed the ordination results, irrespective of the approach applied (rDNA or rRNA) (Table 2). Neither temperature nor the interaction between moisture and temperature affect the microbial community. The pairwise analysis between moisture levels indicated that the prokaryotic community was also influenced by the different moisture regimes while temperature variation did not affect the structure of the prokaryotic community.

Table 2 Results of perMANOVA analysis of the Bray-Curtis dissimilarities for microbial community structure at a 97% similarity cutoff level for OTU clustering. Values in italic indicate statistical significance at p value < 0.05

rDNA		
Environmental factors	R^2	Adjusted p values
Moisture	0.23	*0.001*
Temperature	0.06	0.431
Moisture:temperature	0.12	0.427
Moisture pairs		
23% vs 16%	0.10	*0.009*
23% vs 8%	0.25	*0.003*
16% vs 8%	0.19	*0.003*
Temperature pairs		
10 °C vs 20 °C	0.04	1
10 °C vs 30 °C	0.04	1
20 °C vs 30 °C	0.05	1
rRNA		
Environmental factors	R^2	Adjusted p values
Moisture	0.26	*0.001*
Temperature	0.06	0.265
Moisture:temperature	0.12	0.388
Moisture pairs		
23% vs 16%	0.13	*0.009*
23% vs 8%	0.28	*0.003*
16% vs 8%	0.19	*0.003*
Temperature pairs		
10 °C vs 20 °C	0.04	1
10 °C vs 30 °C	0.06	0.873
20 °C vs 30 °C	0.04	1

Differences in the Processes Operating at the Assembly of the Prokaryotic Communities

We evaluated the clustering and overdispersion patterns over the moisture regimes, as moisture was the most important factor in structuring the prokaryotic community, irrespective of the approach used (rDNA or rRNA). For rDNA data, the phylogenetic structure at deeper phylogenetic nodes (e.g., microbial OTUs at 97% cutoff similarity) of the prokaryotic community significantly clustered at low water content (permanent wilting point 8% *w*/w), whereas it became random with an overdispersion (negative values), with the increase in moisture content (Fig. 3). This suggests that taxa in communities at low water content (permanent wilting point) were on average more closely related than taxa in communities at high moisture regimes. Considering rRNA data, no community groups more distantly related than expected (overdispersion) were detected; however, a tendency of an overdispersed structure was observed at field capacity, indicating the shift in phylogenetic clustering to random patterns along a gradient of soil moisture regimes.

Effect of Moisture on Differential Abundance of Prokaryotes

For a detailed characterization of the main taxa significantly affected by moisture gradients, we performed a pairwise comparison between soil samples using the DESeq2 algorithm in a multifactor experiment using temperature as a confounding variable (Fig. 4). Similar trends were observed by analyzing rDNA and rRNA samples. Overall, the extremes of soil water availability affected the abundance of the microbial OTUs (Fig. 4 A and C). Comparing the prokaryotic communities at high water content (field capacity, 23% *w*/*w*) against low water content (permanent wilting point, 8% *w*/*w*), 27% of all OTUs from the total community (rDNA) and 30.6% from the community measured by the rRNA approach were increased under low water content (Supplementary Table S2). These OTUs consisted mostly of *Actinobacteria* (68% in the rDNA community and 51% in the rRNA community). At high water content, *Acidobacteria* (30% rDNA and rRNA) and *Proteobacteria* (39% rDNA and 50.5% rRNA) were enriched. In both rDNA and rRNA approaches, the *Bradyrhizobium* genus was the most differentially abundant microbe resistant to dry conditions. On the other hand, less extreme moisture conditions caused a smaller effect on soil microbial differential abundance (Fig. 4 B and D; Supplementary Table S2). Only about 8% of the total community (rDNA) was affected when comparing high water content against 70% of field capacity. Taking the rRNA approach into account, 20% of the OTUs were differentially abundant between high water content and 70% of field capacity.

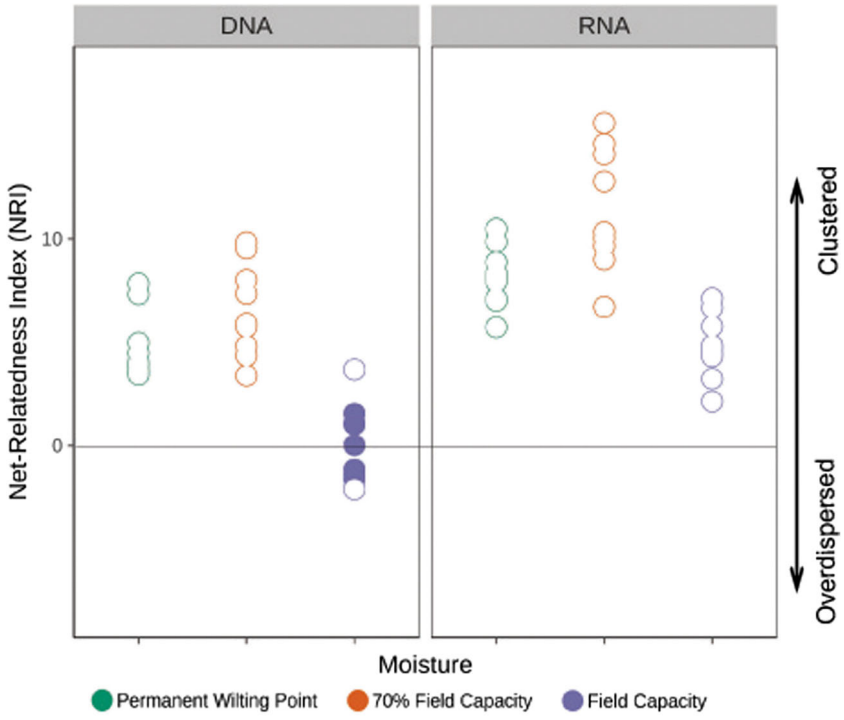

Fig. 3 Variation in phylogenetic structure along the moisture gradient as measured with net relatedness index (NRI). Positive values indicate phylogenetic clustering (sequences more closely related than expected), and negative values or values close to zero indicate phylogenetic overdispersion (sequences more distantly related than expected). Observed community phylogenetic structures unlikely to arise by chance ($0.025 < p > 0.957$) are depicted by open symbols

Discussion

We aimed to evaluate the effect of seasonal conditions on the bacterial and archaeal communities in a subtropical grassland by incubating soil cores at combinations of different moisture and temperature regimes, mimicking the average climatic conditions at the Brazilian Pampa biome. Water content and temperature are two of the most essential environmental factors regulating composition and activity of microbial communities in soils [7]. These variables are of paramount importance to get the picture of the climatic change effect in altering species partitioning and simultaneously affecting interactions among organisms [47]. Microcosm-based models offer a simplified system to study specific environmental factors in isolation or defined combinations [48, 49]. Therefore, the response of microbial communities might be used as a prerequisite to further understanding of ecological rules shaping microbial assemblies without the interference of unwanted external factors [50, 51].

Soils present a complex matrix and other physicochemical properties that confer several critical issues and additional methodological challenges in metagenomics studies. Although our methodology and experimental design present high levels of robustness and resolution, variables not tested here might cause a misrepresentation of the real structure and functions of soil microbial communities as pointed out by Baveye et al. [52].. This work aimed to analyze the effect of moisture and temperature variation only against the soil prokaryotic community. Extrapolations of this result to the entire soil community (e.g., fungi and meso- and macrofauna) might not be appropriate.

The prokaryotic communities accessed via both, rDNA and rRNA showed comparable behaviors along moisture gradients. Moisture was the major factor influencing prokaryotic community diversity and structure at the OTU level (Figs 1 and 2; Table 2). Overall, our results are consistent with previous studies in environments such as sediments [17], forest soils [53], and grassland [54, 55] which showed that water content plays an important role in the composition, diversity, and activity of microbial communities over seasons.

Both niche and neutral processes are not mutually exclusive but complementary in structuring communities [56]. However, both processes are not expected to have the same relative importance irrespective of the environmental constraints. Temperature has long been recognized to be determinant for the composition and physiology of microorganisms at global scales [4, 57]. On the other hand, our results showed temperature appears to be of less importance at local scales (Fig. 2; Table 2), particularly in subtropical ecosystems where the microbial community might contain a widely adaptive (e.g., functional plasticity and dormancy) capacity to withstand large variations in temperature [58]. Microbial communities from subtropical and temperate regions generally have a broad tolerance to wide fluctuations in temperature, compared with tropical (micro) organisms that are adapted to little seasonal variability [59–61]. Within the conditions set in our experiment, temperature and moisture act as both neutral and niche-based processes in space and time to structure soil

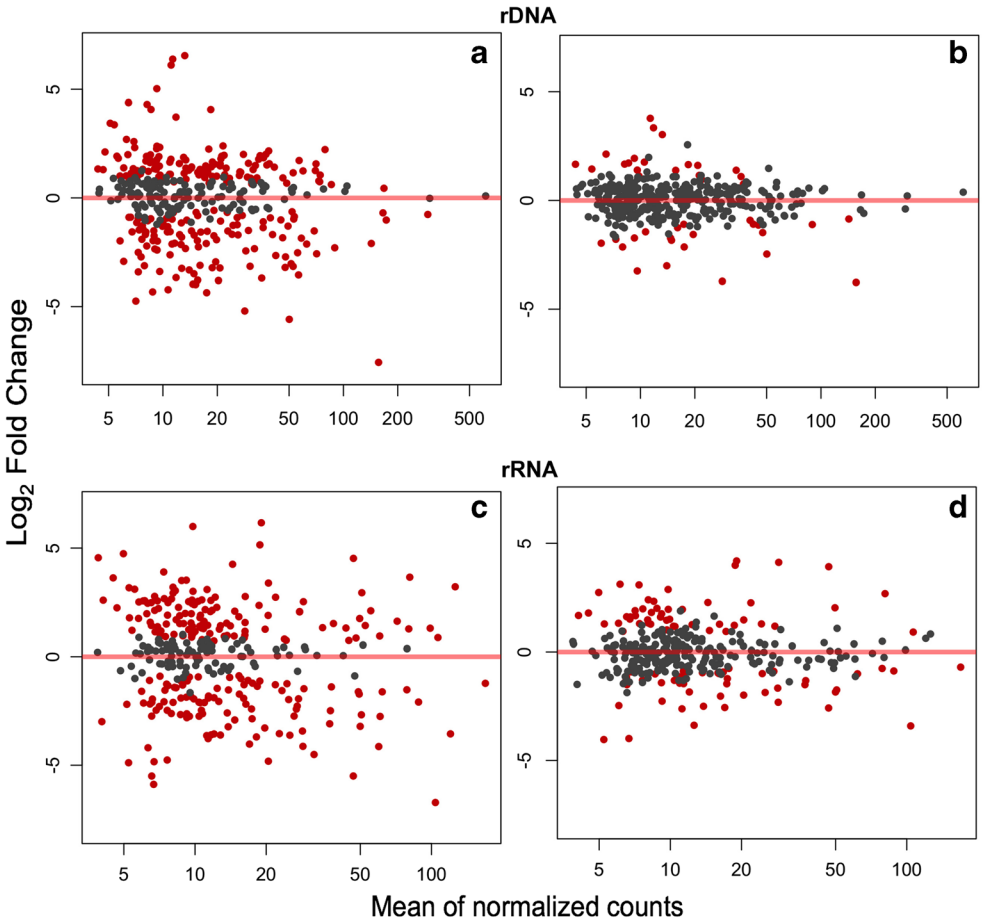

Fig. 4 Mean relative abundance and the $\log_2$ fold change of microbial OTUs associated with different levels of water availability. (A) Comparison between field capacity versus permanent wilting point using rDNA. (B) Comparison between field capacity and 70% field capacity using rDNA. (C) Comparison between field capacity versus permanent wilting point using rRNA. (D) Comparison between field capacity and 70% field capacity using rRNA. Negative $\log_2$ fold change values in panels A and C indicate increased abundance of a particular OTU in permanent wilting point, positive values indicate increased abundance of a particular OTU in field capacity. Negative $\log_2$ fold change values in (B) and (D) indicate increased abundance of a particular OTU in permanent wilting point; positive values indicate increased abundance of a particular OTU in 70% field capacity. Filed red circles indicate the OTUs enriched in one treatment (adjusted p value ≤ 0.05). Filed gray circles indicate an OTU with no differential abundance between treatments. Calculations were performed with the DESeq2 algorithm in a multifactor design controlling for temperature

prokaryotic biodiversity but, at a regional scale, temperature presented lower importance compared to moisture.

Moisture and temperature might also structure microbial communities through indirect pathways. For example, differences in soil atmosphere, such as fluxes of O_2, CO_2, and CH_4, solute diffusion, and water potential (Ψ) shifted by variations of moisture and temperature could each directly lead to differences in the microbial community. At high moisture content, the activity of microorganisms might be limited due to the low oxygen supply, while low water availability increases aeration but can result in cell starvation by reducing intracellular water potential [7, 8, 62]. In addition, temperatures above or below physiological optima for various microbes induce shifts in community structure and diversity due to CO_2 released by intense microbial respiration [63, 64].

Also, we aimed to assess the impact of moisture and temperature on the assembling rules shaping the prokaryotic community. Although phylogenetic turnover (β-diversity) provides information about the main factors promoting species shifts between communities, phylogenetic relatedness based on net related index helps to unravel the microbial assembly [20]. Considering the specific conditions applied in our study, our results suggest the relative influence of niche and neutral processes may vary along the moisture gradient, with niche-based mechanisms being more influential in the total prokaryotic community under dry conditions. At low water content, as in the permanent wilting point treatment, which normally occurs in summer, phylogenetic clustering may be the rule, resulting in lower diversity and richness due to habitat selection [65, 66]. Possibly the greater environmental stress created by dry conditions filtered out those taxa that did not have the capabilities to resist against dry conditions [67]. On the other hand, at high water content (field capacity), there is a decline in phylogenetic clustering with a

tendency toward a random pattern, indicating the structuring of this community will be more influenced by a neutral-based process. In the presence of abundant water supply (e.g., field capacity), dispersion through connected water channels may cause random distribution of species creating more divergent community compositions [16], while heterogeneities in substrate supply and water potential status may lead to distinct niches increasing the diversity and richness at these conditions and decreasing the importance of the habitat filtering [17]. This information applies to the prokaryotic community in subtropical grasslands, but as already pointed out by Baveye et al. [52], the scenario might be different when the fungal community is considered.

The literature discussing macro-community assembly is relatively abundant, but due to different features that make microorganisms unique [68], we might not assume that all rules applied to macroorganisms can also apply to the community ecology of microbes. Particularly, the assembly process of soil microbes has been investigated under different environmental circumstances. Studying the assembly of soil bacterial communities following a wildfire disturbance, Ferrenberg et al. [14] proposed that fire caused changes in the relative importance of neutral vs niche processes. According to the authors, the microbial communities in soils 4 weeks post burn were shaped by neutral-based processes while at 16 weeks the communities were shaped by niche-based processes. Dumbrell et al. [69] evidenced that both niche and neutral processes were responsible for structuring arbuscular mycorrhizal fungi community along a soil pH gradient. However, based primarily on soil pH, niche-based process was the central mechanism regulating the assembly of the community. On the other hand, Rigg et al. [70] studying communities within the roots of Wollemi pine seedlings, reported that fungal and bacterial community assembly were associated with different processes, with fungi more strongly influenced by spatial factors and bacteria influence equally by spatial and edaphic factors.

Indeed, both neutral- and niche-based processes are likely to operate in combination for structuring ecological prokaryotic communities, but those processes vary in importance across different groups of organisms and for different systems [68, 71]. Moreover, scientific and technological advances have revolutionized the traditional approaches used to study microbes in natural environments, allowing us to address old and current issues from a new perspective. Here, we took the advantage of next-generation sequencing to gathering taxonomical information on soil prokaryotic communities based in both 16S rDNA and rRNA for contrasting the total community and potentially active community. Recently, our group has shown that soil microbial taxa were more susceptible to natural climatic disturbances while functions were more stable along seasons [22]. Here, we concluded that among those climatic disturbances, moisture plays a stronger role than temperature in the susceptibility of prokaryotes to environmental disturbances in subtropical grasslands.

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Author Contributions M.L., L. R., and R.J. designed the study. M.L. together with A.S. collected the samples. M.L. and L.R. conducted the laboratory work. L.R., V.S.P., and L.N.L. performed the bioinformatic analysis of the sequence data. L.R., M.L., H.V., V.S.P., and E.K. wrote the manuscript with contributions of all authors. All authors have revised and approved the final manuscript.

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Data Accessibility All raw sequences were deposited in the Sequence Read Archive (SRA) under the study accession number PRJEB10903 (http://www.ebi.ac.uk/ena/data/view/PRJEB10903).

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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