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Prokaryotic community diversity in the sediments of saline lagoons and its resistance to seasonal disturbances by water level cycles

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Abstract

Purpose Apart from having high concentrations of salt, some natural saline wetlands also go through cyclical fluctuations in water level. They are frequently considered vulnerable habitats. In the last decades, the reduction of rainfall in many areas, coupled with fertilizer overuse, is transforming wetlands, especially in climates with a pronounced dry season. We studied a seasonally flooded saline wetland, and focused on the changes in the microbial communities.

Methods High-throughput sequencing was used to explore the diversity and structure of the prokaryotic communities present in the surface sediments. A water and soil salinity gradient along different lagoons in the wetland complex was observed.

Results Salinity affected both microbial richness and composition. The highest microbial richness was observed in lagoons with lower salinity. Statistical analysis suggests that the differences in community composition were associated with differences in salinity level, although an anthropic disturbance (increasing levels of soil organic matter, SOM) that was present predominantly in one lagoon also had a noticeable effect. Sorting of samples using beta diversity distances revealed that differences among communities were due to the distinct habitats, that is, a lagoon's salinity and SOM, not water level cycles. Differences between flooded and dry-out seasons were also explored and the linear model showed that only a small number of OTUs (2.5%) had statistical differences between seasons.

Conclusion Our findings will help in understanding the effects that both salinity and drying-out periods, which are increasing problems worldwide, may have on microbial communities and their resistance to seasonal fluctuations in water levels.

Keywords Salty lagoons · Microbial community composition · Diversity · Salinity alteration · 16S rDNA · Drought resistance

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1 Introduction

Saline ecosystems are distributed globally and represent a wide range of habitats, including saline wetlands, soda lakes, hypersaline springs, salt flats, solar salterns, and ancient salt deposits (Hollister et al. 2010). In ecology, the term ecotone refers to the transition zone between two different plant communities. Wetlands are the ecotones between permanently aquatic and permanently dry terrestrial ecosystems. Wetland ecosystems have been considered among the most vulnerable to climate change because flooding events often flush nutrients, pollutants, and toxic compounds into them (Sims et al. 2013). Saline wetlands are habitats characterized by high concentrations of salt, and by an uneven temporal and spatial water distribution (Canfora et al. 2014). Due to their vulnerability, many natural saline wetlands around the world are included in the Ramsar Convention, an international treaty for the conservation and sustainable use of wetlands.

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Microbial communities shape the biogeochemical cycles of lagoon soil sediments. In fact, their activities are crucial for the functioning of wetlands, as they play critical roles in energy flows and nutrient transformation (Peralta et al. 2013). Hence, gaining information about the microbial community structure and diversity is crucial to understand the ecosystem functions, and the impact that environmental factors have on them (Peralta et al. 2013; Sims et al. 2013). In the last few years, several research studies have been published on the microbiology of saline ecosystems. The main focus for the majority of them has been on aquatic communities (Herlemann et al. 2011; Boujelben et al. 2014; Canfora et al. 2014; Fernández et al. 2014; Abdallah et al. 2016; Çınar and Mutlu 2016; Han et al. 2017; Yergeau et al. 2017; Zhu et al. 2020). However, despite their important ecological and biogeochemical functions for aquatic ecosystems, the microbial communities of the sediments have gathered less attention (Ikenaga et al. 2010; Rathour et al. 2020). Nevertheless, Lozupone and Knight (2005) found, in a classic meta-analysis of prokaryotic communities, that sediments were more phylogenetically diverse than any other habitat that they examined.

Understanding which environmental factors influence microbial communities, variation across different habitats is a key goal in ecology (Xiong et al. 2012). Previous studies have shown that salinity influences prokaryotic community structure and composition in wetland waters (Wu et al. 2008; Liu et al. 2012; Tang et al. 2012) and soils (Ma and Gong 2013). The effect of salinity on lagoon sediments is not well established and is contradictory. For instance, Yang et al. (2016) found that microorganisms in the sediments are not as sensitive to salinity changes as those living in wetland waters, while Bradshaw et al. (2020) found salinity the most influential factor to differentiate prokaryotic communities of the Indian River lagoon, in the East Florida coast. Here, variations in salinity were mainly caused by discharges of freshwater loaded with sediments in the wet season.

Apart from salinity, other factors have been described to influence bacterial communities in soils, for instance, pH, concentration of organic matter, phosphorous (P) and nitrogen (N) contents, and the presence of different plant species (e.g., An et al. 2019). For instance, the "rizhosphere effect" was considered the most important factor determining bacterial communities' composition in a brackish coastal lagoon in the Bay of Bengal, salinity being the second factor (Behera et al. 2017).

Environmental factors per se, such as anthropic or natural disturbances, are important aspects determining the diversity, composition, and functionality of the bacterial communities. Nevertheless, bacterial communities may display high functional and compositional stability against small changes in environmental factors, including salinity (Berga et al. 2017). These communities can respond differently to disturbances depending on the type, intensity, and frequency of the disturbance as well as on the capacity of the different species to tolerate them (Sousa 1984).

The study of microbial diversity and composition in saline environments is necessary to understand the ecological functions, saline adaption mechanisms, and intrinsic biochemical characteristics of microorganisms (Hollister et al. 2010; Ma and Gong 2013). Awareness of the importance of conserving saline enclaves, such as saline wetlands, has increased in the last years, along with public demands for environmental protection overall (Herrero et al. 2015). The Villafáfila wetland is part of a natural reserve located in the north-western part of Spain. It occupies a total area of 32,682 ha, part of which (2,854 ha) is a collection of shallow saltwater lagoons which are included in the Ramsar Convention protection list of wetlands of international importance (Guerra-Doce et al. 2012). The Villafáfila lagoons represent a natural environment suited to study the effects of salinity on the microbial community composition and structure. They are also exposed to a seasonal drying-out that creates an additional disturbance on the ecosystem. An immediate consequence of this is that prokaryotic communities have to switch periodically between aquatic and arid environments. Therefore, these communities are affected by changes caused by a disturbance that could be considered a pulse type according to the classification by Bender et al. (1984). A pulse is an event that is repeated cyclically. A pulse of this type might affect microbial communities in two alternative ways. First, the composition of the communities may change along with the seasonal cycles; and second, a state of equilibrium may be reached in each community, with resistance to any change in composition.

Herein, the protected enclave of Villafáfila lagoons was used to study how salinity may affect microbial life. The objectives were to (i) determine the impact of salinity, along with other factors such as pH and organic matter, on microbial community composition and structure in surface sediments, and (ii) assess up to what extent fluctuations in water level caused by the seasonal drying and flooding cycle, which produce pulse disturbances, can trigger changes in the microbial community of sediments. Salinity is one of the most widespread soil degradation processes, affecting an estimated one million hectares just in the European Union, mainly across Mediterranean countries (Canfora et al. 2014). The proliferation of saline soils and sediments appears to be intimately associated with irrigation and desertification processes (Rengasamy 2006). The results of this work will help in understanding the effect of salinity in modulating sediment microbial life.

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2 Materials and methods

2.1 Sampling site description

Samples were taken at the seasonally flooded Villafáfila wetlands, a natural reserve included in the Ramsar Convention. This wetland complex contains several lagoons. It is located in the northwest of Spain and rests on clay soils surrounding the semi-endorheic basin of the Salado stream (Spanish term for "salty"). The geological and ecological characteristics of the Villafáfila wetlands are the origin of the salinity of its waters (Guerra-Doce et al. 2012). The wetland soils are formed by sediments which are classified as saline, and are the result of both an endorheic phenomenon and the high salt content present in some of the Tertiary sandy strata and alluvial soils (Guerra-Doce et al. 2012). We collected samples from three lagoons: Barillos (BA), Salina Grande (GR), and Villarrín (VR), and compared them to a control lagoon (Villalpando, VP) (Fig. 1).

The Villafáfila wetland is seasonally flooded, and thus, the water level is subject to marked seasonal variations with cyclical dry–wet periods (Fig. 1). It is also affected by drainage from the irrigation of nearby farms. This water level variation affects the salinity, which increases as the water availability declines, causing the formation of salt crusts during the dry season (Guerra-Doce et al. 2012). Hence, in dry years, the lagoons accumulate salt and clay, whereas, in the more humid periods, the saline waters flow into natural drainage streams (Alonso 2002).

2.2 Wetland soil sampling and analyses

Fifteen sediment samples were collected along the basin of the Salado stream from soil A horizons, 0–10 cm deep, with a 5.3-cm-diameter core. Samples were taken from 3 lagoons and a freshwater control during the summer and autumn of 2016. Sample names were as follows: Barillos lagoon (area of 118 ha, samples: BA04, BA05, BA06, and BA07); Salina Grande lagoon (194 ha, samples: GR08, GR09, GR10, GR11, and GR12); and the lagoon of Villarrín (70 ha, samples: VR13, VR14, and VR15). Three more samples were collected outside the Salado stream basin to be used as controls, concretely at the Villalpando lagoon, located at a distance of about 20 km from the Villafáfila wetland (samples VP01, VP02, and VP03) (Fig. 1). In the summer sampling time, the lagoons in Villafáfila were entirely flooded, covered by about 20 cm of water at the sampling points. Here, water salinity was measured in situ at each sampling point using a field multiparameter probe (YSI 556 MPS, YSI Inc. Yellow Springs, OH). In contrast, in the autumn, all sampling points were dried-out. The names of the samples collected in the summer and autumn times were labelled with an "F," for summer flooded, and a "D," for autumn dried-out seasons, the two water level stages sampled (Fig. 1).

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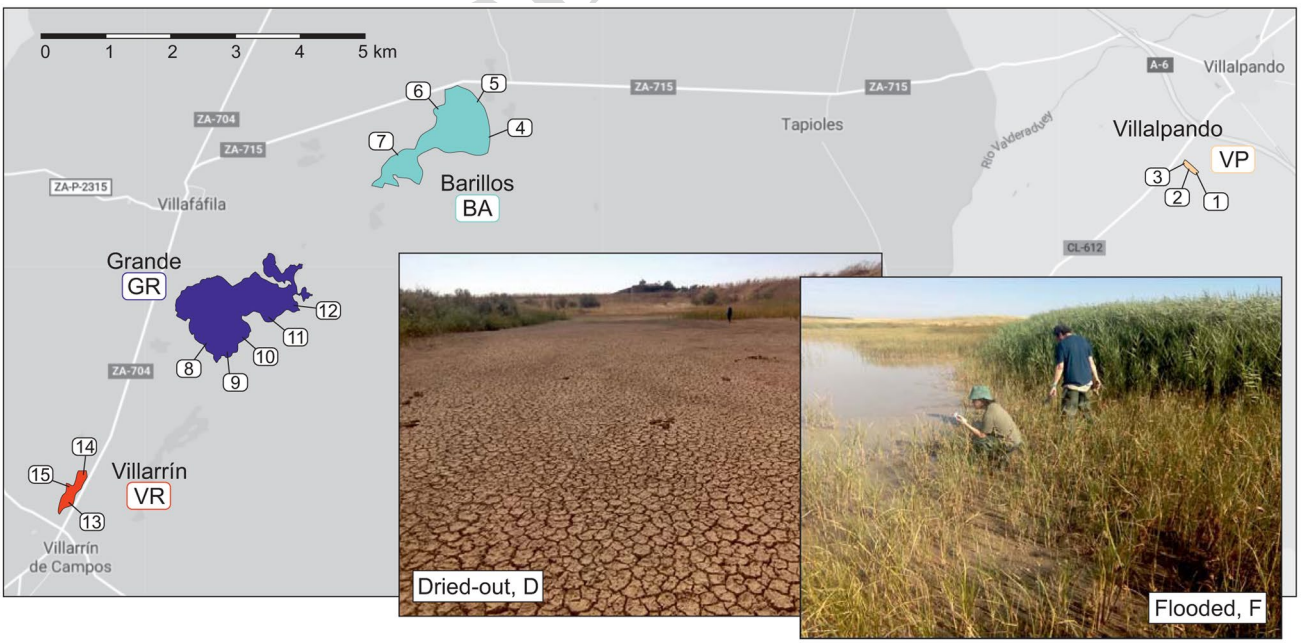

Fig. 1 Sampling sites of the Villafáfila wetland in the Northwest of Spain. The Villalpando (VP) lagoon is the non-saline control, whereas the Barillos (BA), Grande (GR), and Villarrín (VR) are lagoons with increasing salinity. The four lagoons undergo seasonal inundation and desiccation cycles, experiencing flooding in the summer and drying-out in the autumn

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Sampling sites were accurately recorded with GPS (Table 1). The sediment was homogenized manually in a zipper bag until to obtain a unique representative analytical sample for each site and season and immediately kept cold on ice in a cooler until it reached the laboratory, where a subsample was taken from each bag, transferred to a 10-mL tube and frozen at − 20 °C until its analysis for the determination of the prokaryotic community composition, as detailed below. The remainder sediment sample was used for soil characterization, as described below.

To determine the soil organic matter (SOM), salinity, and pH, sediment samples were first air-dried and homogenized, after which the large constituents (e.g., plant material and rocks) were removed. For SOM, 5-g subsamples of the air-dried samples were oven-dried at 105 °C for 24 h, weighed and heated at 375 °C for 16 h. Then, SOM (%) was measured using the weight-loss-on-ignition method (Nelson and Sommers 1996). For pH and salinity determination, 10 g of each air-dried sample was combined with 50 mL of deionized water, mixed manually, and allowed to stabilize for 10 min prior to taking measurements with the multiparameter probe (Thomas 1996).

2.3 DNA extraction, PCR, and sequencing

DNA was extracted from 0.25 g of soil for each sample using a Power Soil DNA isolation kit (MoBio Laboratories Inc., Carlsbad, CA), following the manufacturer's recommendations. DNA concentration and quality were determined using a Nanodrop spectrophotometer (Thermo Fisher Scientific Inc, Waltham, MA) and a Qubit 2.0 fluorometer (Invitrogene, Carlsbad, CA). DNA integrity was further confirmed with agarose gel electrophoresis. The primers 515F and 806R described by Caporaso et al. (2011, 2012) were used to amplify the prokaryotic (bacterial and archaeal) V4 region of the 16S SSU rRNA. The barcoded PCR libraries from each sample were quantified by real-time PCR in a LightCycler 480 (Roche, Basel, Switzerland), pooled with equimolar concentrations, and paired-end sequenced (250 × 2) in the Illumina MiSeq platform (Illumina Inc., San Diego, CA).

2.4 Sequence processing and statistical analysis

Bioinformatic processing of the raw reads was performed using both Mothur 1.35 and RDP tools (Schloss et al. 2009; Cole et al. 2014). After oligo trimming, paired-end reads from each sample were merged and screened to remove low-quality reads and reads that deviate from the expected size (225 to 280 pb) using Mothur and in-house scripts. Reads were clustered in OTUs (operational taxonomic units) at 97% identity using vsearch v.2.8.4 (Rognes et al. 2016). The OTUs supported by less than 100 reads were removed. The CLASSIFIER program (Wang et al. 2007) was used for a hierarchical taxonomic classification of the reads. Assignment of each OTU at species level was obtained using the 16S RefSeq database from the NCBI (Camacho et al. 2009).

Unique reads were selected with Mothur and aligned using Infernal (Nawrocki et al. 2009), available at the RDP website (https://rdp.cme.msu.edu/). An approximate maximum-likelihood phylogenetic tree was constructed with FastTree (Price et al. 2010) using the *gtr* evolutionary model and edited with MEGA7 (Kumar et al. 2015). The resulting phylogenetic tree and the table of OTU frequencies were used with Mothur and the R/Vegan 2.5 package (Oksanen et al. 2010) to estimate alpha and beta diversities. The following alpha diversity indices were calculated: rarefaction species richness (using random subsamples of a size equal to the minimum sample size), Shannon's diversity index, and Simpson's dominance index.

Read counts were normalized to 100,000 per sample prior to linear model analysis. Dissimilarities between sample pairs (beta diversity) were estimated using the phylogenic UniFrac metric (Lozupone and Knight 2005) and the ecological classic Bray–Curtis index, both quantitative. To identify statistical differences among prokaryotic communities between seasons and lagoons, permutation tests of multivariate analysis of variance (PERMANOVA) were performed with dissimilarity matrixes using the *adonis2* function of the Vegan Package. Linear models were generated and used to evaluate the effects (factors) of lagoon (related to salinity) and water level stage (dry vs flooded) on the response variables. These response variables included physicochemical parameters, diversity indices, and relative abundance of OTUs or taxa. For OTUs, the logarithm of the abundance was used. To eliminate false positives in the comparison between the four lagoons, the false discovery rate (fdr) correction was applied using the Benjamini and Hochberg (1995) method. The fdr correction was also used when a high number of OTUs were considered response variables in the different models. Differences between each community sampled in the two water level stages were explored using linear mixed models (R/lmerTest package, Kuznetsova et al. 2017), considering the fifteen-sampling location as a random variable (resulting in paired sample tests). All statistical analyses, including graphical explorations, linear models, linear mixed models, and PERMANOVAs, were performed with R statistical software version 3.5.2 (R Core Team, 2018).

3 Results

3.1 Water and sediment chemical characteristics

The Villafáfila lagoons are subjected to marked seasonal water fluctuations, which in turn creates a series of distinct

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Table 1 Physicochemistry of sampling sites in water and sediment. For each lagoon, mean ± SD are included

Wetland	Sample	Water	Sediment						Position	
		Salinity (g L^{-1})	**Salinity (g L^{-1})**		**pH**		**Organic matter (%)**			
			Flooded	**Dried-out**	**Flooded**	**Dried-out**	**Flooded**	**Dried-out**	**N**	**W**
Villalpando VP	**VP01**	0.31	0.10	0.11	8.28	8.52	4.46	5.93	41.8501	5.4230
	VP02	0.30	0.09	0.12	8.60	8.42	3.12	5.40	41.8502	5.4235
	VP03	0.31	0.08	0.09	8.48	8.77	3.31	5.18	41.8505	5.4237
	Mean ± SD	0.31 ± 0.00	0.09 ± 0.01	0.11 ± 0.01	8.45 ± 0.16	8.57 ± 0.18	3.63 ± 0.73	5.50 ± 0.39		
Barillos BA	**BA04**	4.80	0.24	0.81	8.68	8.76	4.43	6.22	41.8594	5.5598
	BA05	4.19	0.25	1.13	9.09	9.03	3.39	5.39	41.8615	5.5623
	BA06	5.10	0.75	0.94	8.76	8.40	5.39	6.57	41.8593	5.5687
	BA07	5.20	0.44	1.60	8.75	8.38	4.27	6.06	41.8506	5.5757
	Mean ± SD	4.82 ± 0.45	0.42 ± 0.24	1.12 ± 0.35	8.82 ± 0.18	8.64 ± 0.31	4.37 ± 0.82	6.06 ± 0.50		
Laguna Grande GR	**GR08**	19.93	1.21	2.00	8.45	8.34	7.12	7.91	41.8244	5.6081
	GR09	15.74	1.10	1.50	8.51	8.63	5.51	6.73	41.8257	5.6067
	GR10	25.30	1.03	1.21	8.75	8.68	4.24	5.67	41.8291	5.6005
	GR11	16.80	0.78	0.79	8.96	8.91	4.03	5.06	41.8292	5.5981
	GR12	21.30	0.94	0.92	8.79	8.90	4.98	6.81	41.8314	5.5955
	Mean ± SD	19.81 ± 3.81	1.01 ± 0.16	1.28 ± 0.48	8.69 ± 0.21	8.69 ± 0.23	5.18 ± 1.24	6.44 ± 1.11		
Villarrín VR	**VR13**	43.70	1.04	1.58	8.77	8.72	18.95	34.21	41.8031	5.6397
	VR14	48.39	2.08	3.55	8.41	7.96	12.12	14.36	41.8076	5.6365
	VR15	47.40	1.54	2.37	8.41	8.23	14.07	17.26	41.8054	5.6401
	Mean ± SD	46.50 ± 2.47	1.55 ± 0.52	2.50 ± 0.99	8.53 ± 0.21	8.30 ± 0.39	15.05 ± 3.52	21.94 ± 10.72		
Total	*Mean ± SD*	17.25 ± 17.21	1.01 ± 0.52		8.61 ± 0.21		7.94 ± 3.52			

alternating habitats (see Sect. 2). We studied the effect that these changes have on the prokaryotic community that thrives in the lagoon sediments. The four lagoons (Villalpando, VP; Barillos, BA; Grande, GR; and Villarrín, VR) differ in the salinity of the water (Fig. 2A). Linear model on the salinity content in water revealed a highly significant variation among lagoons ($p = 1.38 \times 10^{-19}$; Supplementary Table S1). The model showed significant differences between all pair comparisons between the four lagoons (the highest p-value observed was 1.61×10^{-3}). The lagoon with the highest water salinity was Villarrín (46.50 ± 2.47 g $\times$ L^{-1}), while the non-salinity control Villapando (0.31 ± 0.00) had the least (Table 1). Laguna Grande (19.81 ± 3.81) and Barillos (4.82 ± 0.45) had intermediate values.

Salinity content in the lagoon sediments was also measured (Table 1). There was a good correlation between water and sediment salinities ($r = 0.783$). Again, Villarrín had the highest salinity in the sediments (1.55 ± 0.52 and 2.50 ± 0.99 g $\times$ L^{-1} in flooded and dry-out water levels respectively), and Villapando the least (0.09 ± 0.01 and 0.11 ± 0.01). Nevertheless, although Barillos and Grande had significant differences in water salinity, they did not show significant differences when salinity was measured in the sediments (Supplementary Table S1 shows the linear models).

Differences in salinity between the two water level stages were also examined. For this, soil sediment salinity was measured at the same sampling points during dry-out and flooded periods, and differences were estimated using a linear mixed model. Significant differences were detected ($p = 1.72 \times 10^{-3}$, Table S1). As expected, the salinity of the dried-out samples was higher than that of the flooded soils (Fig. 2B and C). No significant differences in pH were found among all studied lagoon sediments (Table 1 and S1).

Soil organic matter (SOM) is also significantly accumulated during the seasonal drying of the soil, as shown by the mixed model ($p = 1.23 \times 10^{-2}$, Table S1). The linear model also revealed significant differences among lagoons ($p = 5.85 \times 10^{-7}$, Table S1). However, this linear model also suggests that only Villarrín has a significantly higher SOM compared to the other lagoons ($p < 1.6 \times 10^{-6}$, Table S1).

3.2 Alpha diversity estimates

Alpha diversity evaluates species diversity at a local scale, which in our study would correspond to each sampled lagoon. To assess local microbial composition, a total of thirty samples, from the three salty lagoons and the control, were collected and sequenced, as detailed in Sect. 2. Sequencing produced a total of 7,942,500 high-quality sequences (*reads*), ranging from 222,159 (VP02-F) to 316,320 (VP01-D), with an average of 264,750 (Table 2). A method frequently used in ecology to evaluate the species composition of a sample is clustering similar sequence variants in OTUs, or operational taxonomic units. Clustering of the 7,942,500 reads generated 5,106 OTUs with at least 50 reads and 97% identity. The number of OTUs per sample ranged between 2,452 (VP02-D) and 3,492 (BA04-F), while the average number of reads per OTU was 1,508, with a maximum of 207,238.

Diversity within samples was estimated by means of the rarefaction richness (Sr), Shannon's diversity index (H′), and Simpson's dominance index (D) (Table 2). Microbial communities showed high richness (Sr = 2,768.0 ± 265.8)

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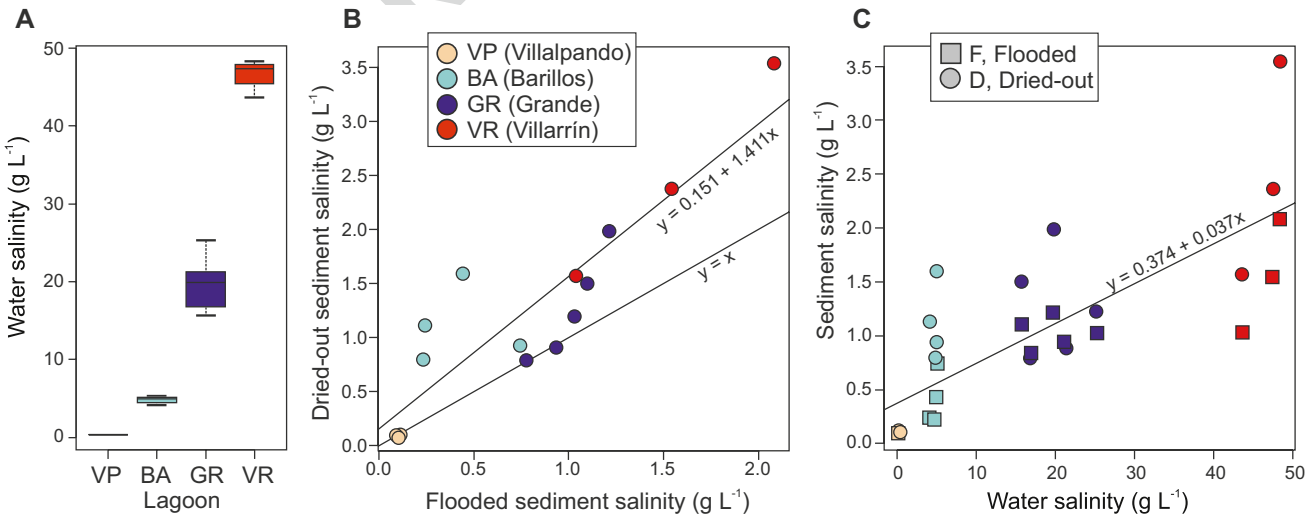

Fig. 2 (**A**) Boxplot depicting the water salinity levels of the Villafáfila lagoons. The lagoons are referred to as VP (Villalpando), BA (Barillos), GR (Grande), and VR (Villarrín). There are significant differences between all pairs of lagoons. (**B**) Seasonal water cycles produce higher salt concentrations in the dry season. (**C**) Relationship between water and soil salinities. The correlation was 0.783

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Table 2 Number of reads and OTUs at a phylogenetic distance of 0.05 with 50 or more reads and alpha diversity indices of the sediment libraries. For each lagoon, mean ± SD are included

Wetland	Sample	Number of *reads*		Number of OTUs		Rarefaction richness (Sr)		Shannon–Weaver's diversity index (H′)		1-Simpson's index (D) × 10^{-2}	
		Flooded	**Dried-out**	**Flooded**	**Dried-out**	**Flooded**	**Dried-out**	**Flooded**	**Dried-out**	**Flooded**	**Dried-out**
Villalpando VP	**VP01**	259,525	316,320	2,600	2,556	2,545.8	2,468.5	6.06	6.32	0.92	0.52
	VP02	222,159	270,027	2,655	2,452	2,640.3	2,387.0	6.32	6.05	0.52	0.80
	VP03	267,035	295,795	2,467	2,509	2,415.2	2,441.5	6.26	6.27	0.54	0.59
	Mean ± SD	249,573 ± 24,036	294,047 ± 23,195	2,574 ± 97	2,506 ± 52	2,533.8 ± 113.0	2,432.3 ± 41.5	6.21 ± 0.14	6.21 ± 0.15	0.66 ± 0.23	0.64 ± 0.15
Barillos BA	**BA04**	263,331	274,193	3,492	3,394	3,416.5	3,287.5	6.41	6.29	0.77	0.59
	BA05	238,384	279,619	2,870	3,407	2,816.0	3,299.3	5.86	6.52	1.49	0.38
	BA06	281,030	252,926	3,283	3,055	3,175.8	2,975.5	6.38	6.15	0.47	0.64
	BA07	223,554	270,932	3,065	3,149	3,030.7	3,032.7	6.06	5.85	0.85	1.11
	Mean ± SD	251,574 ± 25,592	269,417 ± 11,563	3,178 ± 269	3,251 ± 177	3,109.8 ± 252.3	3,148.8 ± 168.7	6.18 ± 0.27	6.20 ± 0.28	0.89 ± 0.43	0.68 ± 0.31
Laguna Grande GR	**GR08**	247,518	246,112	2,634	2,741	2,575.0	2,687.0	5.76	5.96	1.35	1.04
	GR09	290,438	270,167	2,791	2,788	2,678.1	2,700.3	5.77	5.91	1.61	1.22
	GR10	288,163	258,110	2,888	2,877	2,753.0	2,796.3	5.98	6.07	0.76	0.73
	GR11	241,823	281,961	2,798	2,734	2,731.6	2,620.9	5.99	5.84	0.71	0.99
	GR12	261,841	241,160	2,827	2,606	2,737.0	2,549.2	5.85	5.75	0.92	1.28
	Mean ± SD	265,956 ± 22,537	259,502 ± 16,850	2,788 ± 94	2,749 ± 98	2,694.9 ± 72.7	2,670.7 ± 92.4	5.87 ± 0.11	5.91 ± 0.12	1.07 ± 0.39	1.05 ± 0.22
Villarrín VR	**VR13**	254,026	287,756	2,773	2,734	2,704.8	2,628.9	6.02	6.08	0.70	0.72
	VR14	298,082	274,197	2,757	2,766	2,634.5	2,672.1	6.00	5.91	0.66	1.03
	VR15	261,713	224,603	2,831	2,915	2,750.7	2,888.2	6.08	6.20	0.66	0.61
	Mean ± SD	271,273 ± 23,532	262,185 ± 33,245	2,787 ± 39	2,805 ± 97	2,696.7 ± 58.5	2,729.7 ± 138.9	6.03 ± 0.04	6.06 ± 0.15	0.67 ± 0.02	0.78 ± 0.22
Total	*Mean*	264,750 ± 23,533		2,847 ± 274		2,768.0 ± 265,8		6.07 ± 0.21		0.84 ± 0.31	
	Number	7,942,500		5,106							

and Shannon's diversity ($H' = 6.07 \pm 0.21$), along with low dominance ($D = 0.0084 \pm 0.0031$). While differences between lagoons were observed (Sr: $p = 7.26 \times 10^{-8}$, H′: $p = 3.30 \times 10^{-3}$, and D: $p = 4.70 \times 10^{-2}$, Table S1), no differences were detected between the two seasonal water level stages.

Linear models (Table S1) indicated that prokaryotic communities from Barillos have a higher richness than those from any other lagoon ($p < 5.7 \times 10^{-6}$), while communities from the Grande lagoon showed lower H′, with significant differences compared to VP and BA (H′, $p < 2.2 \times 10^{-3}$). For the D index, no differences were found between each lagoon pair after fdr correction.

Altogether, results revealed that the lagoon with the lowest salinity of the three (BA) had the highest richness, while the lagoon with the highest salinity but no anthropic SOM input (GR) had the lowest Shannon's diversity.

3.3 Beta diversity estimates

A beta diversity analysis was carried out to assess differences in the composition (OTUs) of prokaryotic species within the communities from different sediments. Both the classical ecological index of Bray–Curtis dissimilarity and the UniFrac metrics, based on phylogenetic distances among OTUs, clustered the microbial samples according to the lagoon to which they originated (Fig. 3).

The first two axes in the principal coordinate analysis (PCoA) explained 37.0 and 13.4% of the observed variance, respectively, when Bray–Curtis was used, and 37.4 and 13.7% with the UniFrac metrics. Only two samples, BA07D and VR13D (arrows in Fig. 3), were incorrectly grouped with the GR lagoon communities in Bray–Curtis analysis. However, with the phylogenetical UniFrac index, more samples appeared mixed: BA07F in VR, and GR10F in BA, in addition to BA07D and VR13D in GR. When a PERMANOVA analysis was performed with the Bray–Curtis index, the lagoon appears to be the influencing factor affecting sample clustering ($F = 10.54$, $p = 1 \times 10^{-6}$). However, the water level stage (flooded vs dried-out) factor and the lagoon-stage interaction were not significant. Interestingly, sample clustering using the UniFrac index rested on both the lagoon ($F = 9.71$, $p = 1 \times 10^{-6}$) and the water level stage ($F = 3.67$, $p = 6.77 \times 10^{-3}$).

3.4 Local microbial community composition

Proteobacteria was the most abundant phylum in all lagoons, except in Grande, where Chloroflexi is more abundant than Proteobacteria although their differences were not significant (Fig. 4A). When samples of flooded and dried-out stages were analyzed, those same phyla (Proteobacteria and Chloroflexi) were consistently abundant in all lagoons (BA, GR, VR). However, although still present in considerable amounts, Chloroflexi was less numerous in the control non-salty lagoon (VP). In the control, but not in the three salty lagoons, Acidobacteria was also among the most frequent phyla in both water level stages. Other dominant bacteria

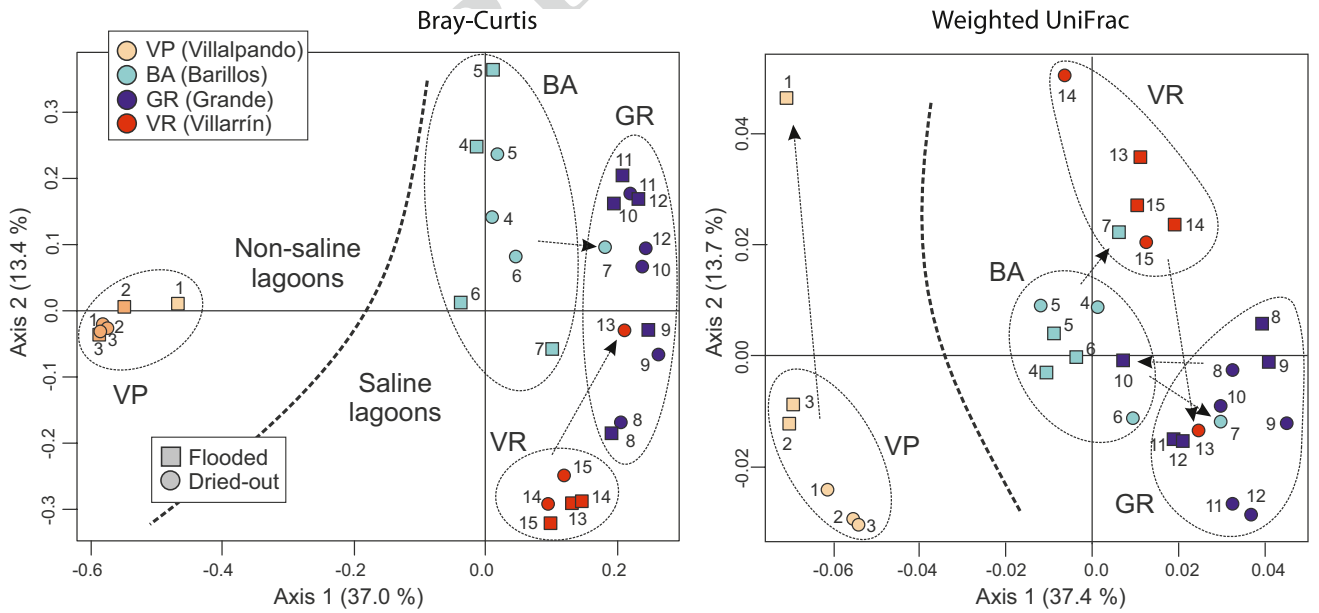

Fig. 3 Principal coordinate analysis (PCoA) plots derived from Bray–Curtis and pairwise UniFrac distances. Both indices allow the communities to be grouped by the lagoon from which they originated. Except for the lagoon with the highest organic matter (VR), the rest are ordered on the first axis by their salt content. Only UniFrac metric, but not Bray–Curtis, detects small seasonal differences between communities

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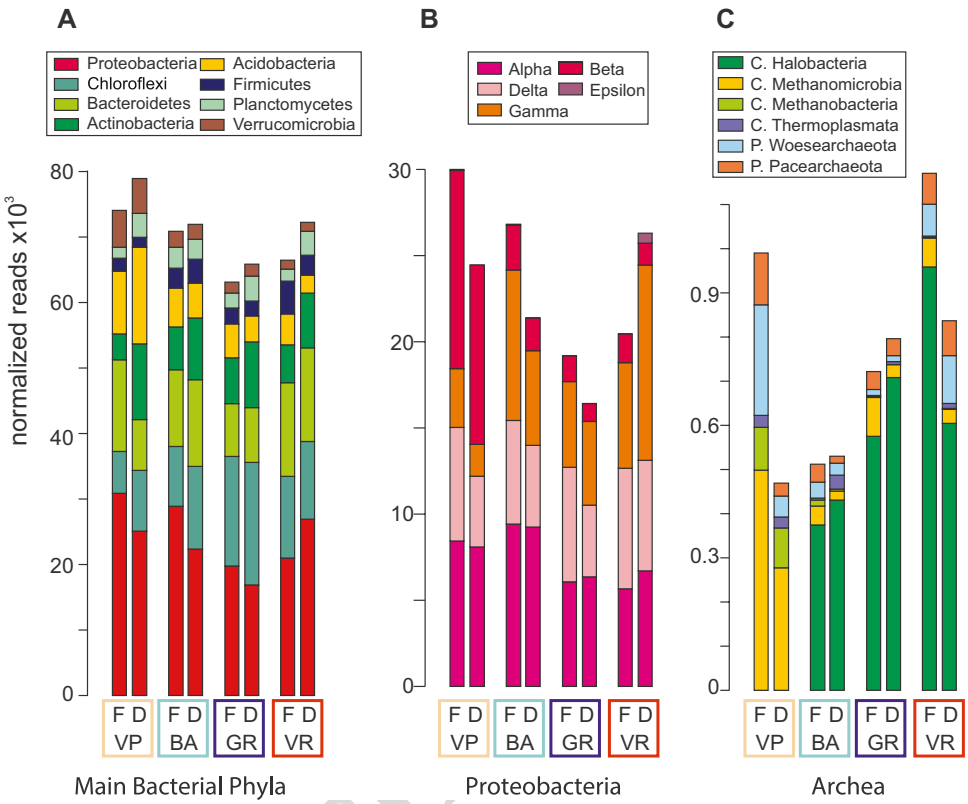

Fig. 4 Relative abundances of reads belonging to bacterial phyla within the different communities that are present in each lagoon and in each water level stage: the most abundant Bacteria phyla (**A**); the different Proteobacteria classes ordered by global abundance (**B**); and six Archaea taxa found (the Halobacteria, Methanomicrobia, Methanobacteria, and Thermoplasma classes belong to phylum Euryarchaeota) (**C**)

were Bacteroidetes and Actinobacteria. They were among the four most frequent bacteria of all salty lagoons. Other bacterial phyla present in sizable amounts included Firmicutes, Planctomycetes, and Verrucomicrobia. Within Proteobacteria, the members of Betaproteobacteria were the most common in the freshwater lagoon VP, during both flooded and dried-out stages (Fig. 4B). However, in the salty lagoons, the most abundant sequences were classified under Alpha-, Delta-, and Gammaproteobacteria.

Archaea were also quantified. The Euryarchaeota phylum, which comprises the Halobacteria, Methanomicrobia, Methanobacteria, and Thermoplasmata classes, was the most frequent in all lagoons (Fig. 4C). Interestingly, Halobacteria were mostly found in the saline lagoons, where its abundance increased along with the salinity gradient. In contrast, the Methanomicrobia class showed higher numbers in the freshwater VP lagoon. Other archaeal classes and phyla were also detected in higher numbers in the VP communities than in any other lagoon (Fig. 4C).

3.5 Differential presence of OTUs among lagoons

A total of 4,027 of 5,106 OTUs (78.87%) showed differences in abundance among lagoons (logarithms of abundance, fdr < 0.05) (Table S1). Each of them was individually analyzed by a post hoc test, using the same linear models. The different OTUs were subsequently grouped into 7 panels according to whether or not there are significant differences between lagoons. For example, panel 2 in Fig. 5 includes OTUs whose abundance is significantly lower in salty lagoons. Given that the lagoons differ in salinity, we can associate OTUs from that panel, and their respective taxa, with the environmental variables analyzed.

The frequency of 1,502 of those 4,027 OTUs was significantly higher in the non-salty control lagoon VP (Table S1 and Fig. 5 panel 2). For most of those 1,502 OTUs (1,307), their abundance in VP was significantly higher than that in all other lagoons. For the rest, differences were also significant for the two lagoons with higher salinity (GR and VR), except for just 30 OTUs in which VR showed higher but non-significant abundances. Among the most frequent prokaryotes in the VP lagoon were OTUs of almost all phyla found (34), including 32 OTUs of 4 phyla of archaea. Results suggest that these OTUs may have lower tolerance, or at least some disadvantage, compared to other prokaryotes in order to grow in saline sediments.

There were 473 OTUs with significantly higher numbers in the salty lagoons compared to the control, and their abundance increased proportionally to the concentration of salt in each lagoon (Fig. 5 panel 3). Those OTUs are likely from species whose growth is favored by salinity, or that are able to resist high concentrations of salt. Nevertheless, OTUs from almost all prokaryotic phyla were also represented. In few cases, a particular OTU was not present in the control

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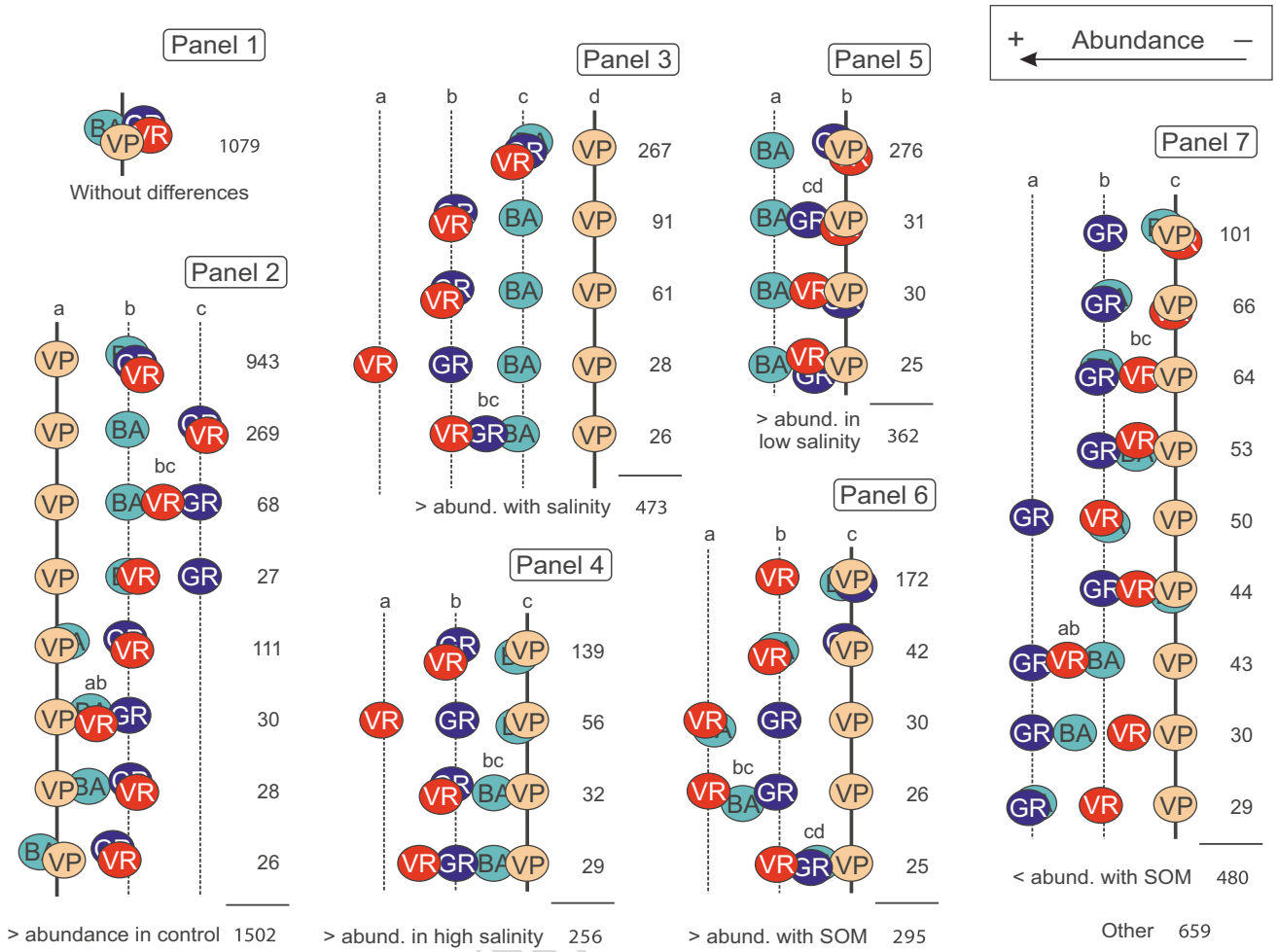

Fig. 5 Number of OTUs showing different patterns of abundance between lagoons (patterns obtained from Table S1). A matching lowercase letter indicates non-significant differences. Differences between lagoons are only significant if they are separated by vertical lines. Thus, within a particular panel, the lagoons with the highest abundance of reads of each OTU are located on the left of the graph. For example, in panel 2, there are 943 OTUs whose abundance is significantly higher in the VP control and also there are no significant differences between the saltwater lagoon communities (VR, BA, and GR). In another example, in panel 7, there are 101 OTUs with greater abundance in the GR lagoon than in any other. More detailed explanation on panels and patterns can be found in the text

VP, but it was found in all other saline lagoons. In these cases, the only large taxonomic group was the Halobacteria class, of the phylum Euryarcheota. Methanomicrobia and Thermoplasmata, also belonging to the Euryarcheota phylum, were significantly more abundant in the control VP than in saline lagoons (Fig. 4C).

In addition to those 473 OTUs, another 256 (Fig. 5 panel 4) were also significantly more abundant in the lagoons with higher salinity (VR and GR), compared to the control. However, now, the differences were not significant between the low-salinity lagoon BA and the control VP. There were only three archaea OTUs: one Halobacterium and two Woesearchaeota. Among the bacteria, the majority were Proteobacteria (71 OTUs, 15%), Chloroflexi (32, 6.7%), Planctomycetes (31, 6.5%), and Firmicutes (28, 5.9%), phyla that are usually abundant in saline environments. Lastly, another 26 OTUs from Bacteroidetes (5.5%) were significantly more abundant in the sediments of the highly saline lagoons VR and GR than in freshwater.

Panel 5 of Fig. 5 shows 362 OTUs which were significantly more abundant in BA than in the control, but that were not significantly more abundant in the two lagoons with the highest salinity (GR and VR). These OTUs could be representatives of species that grow best with moderate concentrations of salt. However, among these 362 OTUs, there are 6 belonging to Halobacteria, which are typically found growing in high concentrations of salt. In fact, we found the majority of Halobacteria OTUs more abundant in higher salinity lagoons (VR and GR), previously described in panel 3. The most represented OTUs in panel 5 belong to Protebacteria (86, 23.8%), Plantomycetes (51, 14.1%), Bacteroidetes (50, 13.8%), and Chloroflexi (43, 11.9%).

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Among the OTUs of the Chloroflexi phylum, there are 4 of the Anaerolineaceae family that are within the most abundant OTUs found in this study.

Another group of OTUs (Fig. 5 panel 6) includes 295 OTUs which appear to be influenced by both SOM and salinity. They could have been included in panel 3, where the abundance of OTUs was determined by an elevated salinity. However, in panel 6, there were also significant differences between VR and GR, the two lagoons with higher concentration of salt. The VR lagoon has the highest SOM concentration of all lagoons, which suggests that the OTUs of this panel succeed in salty and organic media. These would be prokaryotes that thrive in or at least withstand elevated concentrations of salt, and that also use organic compounds as a source of energy. The majority of them (119, 40.3%) are OTUs of Proteobacteria, especially Gammaproteobacteria, typically heterotrophic. The rest are Bacteroidetes (39, 13.2%), Chloroflexi (33, 11.2%), and Firmicutes (30, 10.2%). Among the archaea, only one OTU of Halobacteria and five of Woesearchaeota were found. The organic matter of the VR lagoon can be attributed to anthropic inputs. In fact, this lagoon is located next to a village in which agriculture is the main way of living. Residues of agricultural and livestock origin can be frequently seen nearby. It is therefore the most human-altered lagoon and with the greatest human impact of those analyzed. Thus, the higher abundance found for some of these OTUs in some lagoons may be just the result of an anthropogenic disturbance and not a consequence of the presence of salt.

Panel 7 of Fig. 5 is comprised of OTUs whose frequency appears to increase with the concentration of salt but decrease with the SOM content. For instance, there are 480 OTUs whose abundance is higher in the GR lagoon than in VR. For all those 480 OTUs, the abundance in GR was also significantly higher than that in the VP control, and in 358 of them, the frequency in VR was not significantly different than that in VP. These OTUs could be representative of species that grow in saline environments but are not favored by the presence of SOM. Within bacteria, OTUs were found of the phyla Proteobacteria (125, 26%), Actinobacteria (83, 17.3%), Chloroflexi (59, 12.3%), Planctomyetes (57, 11.9%), Firmicutes (40, 8.3%), and Bacteoides (34, 7.1%), among others. The combination of high salinity and low SOM has led to a striking relative increase in significant OTUs of Actinobacteria, compared with any other panel. Within archaea, 11 OTUs were found of which 7 are Halobacteria.

3.6 Effects of flooded and dried-out seasonal cycles

Next, we aimed to identify OTUs with different frequencies in the two water level stages. In order to consider the water level factor, linear mixed models were used since the location of each sample could be regarded as a random effect. Significant differences were observed in the physicochemical parameters: sediment salinity and SOM. However, no differences were found in pH (Table S1). A total of 101 OTUs had significant differences in their numbers. Of those, 35 were more abundant in the flooded stage, while 66 were in the dry-out (Table S1). These numbers were reduced to 11 and 14 OTUs, respectively, if only OTUs averaging more than 50 reads were considered. Only one of the OTUs that were abundant in the flooded sediments corresponded to an archaeon; the rest were bacteria. This only OTU is likely related to the *Nitrosophaera* genus (80% identity). Within bacteria, the most abundant OTUs in the flooded stage could be classified into the phyla Bacteroidetes (6 OTUs of the classes Sphingobacteria and Bacteroidia), Acidobacteria (2 OTUs of the group Gp21), and Proteobacteria (2 OTUs of the class Deltaproteobacteria). In the dry-out stage, the 14 most abundant OTUs with more than 50 reads belong to the phyla Actinobacteria (9 OTUs), Plactomycetes (2 OTUs close to the genus Roseimaritima), Proteobacteria (1 OTU), and Bacteroidetes (2 OTUs of the Cytophagia class).

4 Discussion

4.1 Effect of salinity

The importance that salt has on the Villafáfila wetland complex is twofold. First, as the chemical analysis revealed, there is a salinity gradient among the four lagoons in both water and sediments, with significant differences among them. Second, salinity also fluctuates along with seasonal cycles. The drying of lagoons produces a salt deposition on the dry-out sediments, induced by water evaporation and subsequent mineral and particle concentration. These salt deposits are re-dissolved into the water during flooding periods. Apart from salinity, the soil pH has also been described to shape microbial communities' composition of the sediments (Canfora et al. 2014). However, because the differences in pH among lagoons are not significant, it does not seem to be an important factor influencing the microbial composition of the sediments of the lagoons in our analysis.

Salinity is the main factor affecting prokaryotic diversity in the lagoons, as Bray–Curtis and UniFrac indexes suggest. The diversity we found was similar to that described for the sediments of other wetlands, both freshwater and saline. For instance, Jin et al. (2019) reported an H′ between 5.99 and 6.32 in two bacterial communities from a freshwater lake (Poyang Lake, China). Also, values of H′ between 5.45 and 6.24 were reported by Liu et al. (2018) in sediments from the saline Sanjiand wetlands, also in China. Using the Bray–Curtis index of beta diversity, the three first PCoA axes were found to participate in the sorting of lagoons (Table S1). Axis 1 appears to sort the lagoons according

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to their salinity, with significant differences between all pairs of lagoons (Table S1). Nevertheless, the VR lagoon is not sorted according to its salinity, and instead it is placed between BA and GR (Fig. 3). This could be related to the fact that, in addition to having the highest salinity of all lagoons, VR also has an elevated concentration of SOM. The UniFrac distances clustered samples in a similar manner, except that the significant differences between lagoons only affect axes 1 and 2, and that the axis 1 did not detect significant differences between BA and VR.

Proteobacteria, Chloroflexi, and Bacteroidetes are frequently reported as the dominant phyla in aquatic environments with some salinity. Thus, Núñez Salazar et al. (2020) found Proteobacteria and Bacteroidetes with abundances higher than 80% in lakes slightly salty in the Andes, at high elevation. Those three phyla were also the most abundant in our analysis. For instance, the class Flavobacteria (Bacteroidetes) was found to be the most abundant in the salt crust of Arava Valley in Israel (Bachran et al. 2019), and also in the sediments of the hypersaline lake La Sal del Rey in Texas (Hollister et al. 2010). Fernández et al. (2014) found that in the waters of the salty lagoons of Santa Pola, in Spain, Bacteroidetes not only increased their abundance as salinity increased, but also they were the only representative bacteria phylum when salinity reached 37%. In a recent study in natural freshwater lagoons in a nearby region, Arroyo et al. (2015) found the same phyla underlined in Fig. 4A, except for Actinobacteria. However, their relative abundances were different. For instance, although Proteobacteria were clearly the dominant phylum in Arroyo's study, Verrucomicrobia was also highly frequent, while in our study it is considerably less abundant.

Results also suggest that, while there is a dominance of Halobacteria in the sediments of salty lagoons, there is a much higher diversity of Archaea in the sediments of the freshwater ones. In fact, Halobacteria are usually the dominating archaea in saline environments (Fernández et al. 2014; Bachran et al. 2019), to the extent of becoming more abundant than any other phyla of bacteria in the samples with the highest salinity. Even more, in an extreme environment such as the brines of the Uyuni salt flat in the Bolivian Andes, they were the only taxon found (Haferburg et al. 2017).

4.2 Effect of soil organic matter (SOM)

Similar to salinity, the SOM content of the sediments varied depending on each particular lagoon, with a significant SOM accumulation in VR. This lagoon is in close proximity to a small-sized town, with a notorious agricultural activity, and thus, it is likely that its SOM derives directly from the agricultural and livestock discharges. The overall high values of SOM, as well as its seasonal variation in the sediments of the VR lagoon, could thus be attributed to both the lagoon desiccation during the dry-out period and to anthropic factors. A seasonal variation in the composition and structure of the bacterial communities has also been described in Indian River Lagoon, in Florida, where an extra contribution of organic matter is produced during the wet period, through freshwater flows that carry a large amount of plant debris. Nevertheless, comparisons with our study are difficult because the entry of organic matter is accompanied by a decrease in salinity (Bradshaw et al. 2020).

There were no differences in alpha diversity between VR and GR, the two lagoons with the highest salinity but which differ in SOM (Table S1). Nevertheless, because of the presence of higher amounts of organic compounds from human activity in VR, we expected to see more prokaryotic diversity associated with the more diverse biochemical functions. In the previous section, we established that axis 1 of the Bray–Curtis and UniFrac clustering was responsible for sorting lagoons by salinity. The results of the diversity associated with SOM appear to indicate that axis 2 of those two indexes is separating the VR from other lagoons with less SOM.

Proteobacteria, mainly Gammaproteobacteria, are the dominating phyla in VR during the dried-out season (Fig. 4B). In this period, the water level has dropped and salt and organic matter have accumulated on the sediment. It is also noticeable that a high number of Epsilonproteobacteria, also a Proteobacteria, was found in the dried-out samples from VR, whereas they were practically absent in the other sampled lagoons. This is likely influenced by human intervention as Epsilonproteobacteria are frequently found in feces from farm animals. Fernández et al. (2014) also found that Gammaproteobacteria were the most abundant Proteobacteria in the lagoons of Santa Pola, and their numbers increased with the concentration of salt in the waters. Interestingly, in our study of the Villafafila saline wetlands, their abundance seems to be more linked to SOM than to salinity.

Some of the OTUs grouped in panel 6 of Fig. 5 correspond to anaerobic chemo-organo-heterotroph taxa, many of which are able to use inorganic compounds with nitrogen or sulfur as final electron acceptors (non-assimilatory reduction of sulfate or nitrate). Among them, there are *Desulfobulbus* (Deltaproteobacteia) initially found in freshwater and marine muds (Widdel and Pfennig 1982), and *Synthophobacter* (Deltaproteobacteia) isolated from anaerobic sludge reactors (Chen et al. 2005). We could describe 36 OTUs of Deltaproteobacteria and 20 of the families Desulfobacteraceae, Desulfobulbaceae, and Desulfomicrobiaceae. Other OTUs found were of anaerobic species from different phyla, such as 10 OTUs of the Anaerolinaceae (Chloroflexi) family, frequently isolated from anaerobic sludge used in treating high-strength organic wastewaters (e.g., Yamada et al. 2006); one OTU of the genus *Prolixibacter*, which is a Bacteroidetes isolated for the first

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time in a marine sediment fuel cell (Holmes et al. 2007); and 5 OTUs of *Flavobacterium*, which is another bacteroidete broadly distributed that has the capacity of breaking down organic matter (Kirchman 2002). *Flavobacterium* belongs to the same family than *Psychroflexus*, bacteria abundant in the lakes of La Brava and La Punta located at high altitude in the Andes, next to the Atacama Desert (Núñez Salazar et al. 2020) and found also in small lakes of the Monegros Desert in Spain (Casamayor et al. 2013). Purple sulfur bacteria such as *Thiocapsa* and other Gammaproteobacteria were also found. They have been previously identified in wastewater lagoons with moderate salinity (Dungan and Leytem 2015). The presence of strict anaerobic and sulfate-reducing bacteria suggests that the environmental conditions of VR lagoon are very different from those of the rest, possibly due to the disturbance caused by human intervention.

4.3 Effect of seasonal water level cycles

Despite the fact that differences among lagoons were found, none of the three alpha diversity indices (Sr, H′, and D) was significantly different between the two water level stages, that is, flooded and dried-out, for all lagoons. Neither the beta diversity estimated with Bray–Curtis index was capable of separating the microbial communities collected in different water level stages. Nevertheless, when the effect of seasonal water level fluctuation factor was studied using mixed models with the UniFrac distances, small but significant differences were found in the first 3 axes between flooded and dried-out stages ($0.023 < p < 0.031$, Table S1). The UniFrac β-diversity quantifies the diversity between two microbial communities as the evolutionary history that is not shared by them. This is calculated in a phylogenetic tree as the fraction of branch lengths not in common by the two communities. Thus, it is important to note that while Bray–Curtis considers OTUs as independent elements, UniFrac also includes phylogenetic aspects. Then, although there are numerous OTUs whose abundance is different in each lagoon, they may be phylogenetically related. There are also a few OTUs that show dissimilar abundance in dried-out and flooded stages, and these OTUs were phylogenetically very distant. This could explain why there were no differences when clustering using the Bray–Curtis index (there were few OTUs), while those differences were noticeable with the UniFrac index (those few OTUs were phylogenetically distant).

4.4 Changes in beta diversity attributable to salinity and seasonal cycles are different

Taken together, results revealed that the differences in the prokaryotic communities among lagoons can be attributed to numerous OTUs, although in most cases those OTUs are phylogenetically related. Nevertheless, within a same taxa of bacteria, OTUs may be found that are significantly more abundant in a particular lagoon. For instance, bacteria of the Chitinophagaceae family that are abundant in the non-salty lagoon practically disappear in the salty ones (Supplementary Fig. S1), while the family Rhodotermaceae, belonging to the same order (Sphingobacteriales, phylum Bacteroidetes), is only found in salty lagoons. A similar pattern can be observed in the Hyphomicrobium and Chelatococcus genera, both of the order Rhizobiales. They were more numerous in VP and salty lagoons, respectively.

The Acidobacteria phylum shows a decrease in abundance as salinity increases. However, a subdivision of Acidobacteria, Gp23, had a higher frequency in the two lagoons with higher salinity. OTUs of this subdivision were significantly more abundant in VR, the lagoon with the highest SOM. Subdivision Gp23 has been only identified in a number of aquatic ecosystems, including marine sediments and microbial mats from hot springs and caves, as well as other hot-spring environments (Losey et al. 2013). Gp23 is a group whose ecological role is not yet well stablished.

The last example of phylogenetically close taxa that were found in higher numbers in certain lagoons is that of the Halobacteria class (Supplementary Fig. S1). This class, typically found in habitats with high concentration of salt, is represented by 28 OTUs in all lagoons. Twelve of those showed significant differences between lagoons. We found OTUs of three different orders: Halobacteriales, Haloferacales, and Natrialbales. The Halobacteriales, represented by 7 OTUs, were more abundant in VR than in VP and GR. In contrast, the GR lagoon had the highest number of Haloferacales OTUs, while in VR the Natrialbales were the most frequent. Species of these 3 orders of Halobacteria have been previously described in hypersaline lagoons in Israel (Bachran et al. 2019).

Overall, results show that the number of OTUs that are different between flooded and dry-out stages (101) is much lower than the OTUs that are different between lagoons (4,027). This explains why Bray–Curtis beta diversity index was unable to find differences between water level stages. However, the few OTUs involved in the significant differences between water levels belong to very distant taxonomic groups. Because it considers not only the abundance of the OTUs but also their position in the phylogenetic tree, the UniFrac index was able to detect these differences. The changes in abundance of these few OTUs are responsible for variations in the abundance of large taxonomic groups (Supplementary Fig. S2). Here, a drop-in bacteria belonging to the Deltaproteobacteria, Bacteroidia, and GP21 classes of Acidobacteria can be seen in the dry season. Also, there is an increment of the phylum Actinobacteria, the class Cythophagia, and the family Planctomycetaceae, making these taxa the most affected by seasonal changes.

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The seasonal drying of the soil involves a disturbance with an environmental change in the habitat, fluctuating from semi-aquatic to arid. This, in turn, affects the concentration of salt and SOM, and, most likely also the levels of oxygen that are available. These changes in ecological factors, that is, the shift between flooded vs dry habitats, affected only a small number of OTUs (101 of the 5,106 OTUs analyzed, approximately 2%), which is an evidence for the resistance of the communities to seasonal pulses. Most OTUs maintain their abundance despite variations in water level. As a result, the Villafáfila wetlands have revealed the capacity of the two distinct habitats to lodge species of microorganisms that during part of the year would not be in optimal conditions for their growth. This ability reflects the great stability of these prokaryotic communities. Nevertheless, resistance and stability could be altered by the prolongation of the dry season. The distinct composition of prokaryotic communities in each lagoon constitutes a rich landscape with diverse stable phases marked by environmental factors such as salinity and SOM content. Berga et al. (2017) first described the stability that prokaryotic communities shown in response to small changes in salinity. In the Villafáfila wetland, lagoons behave as isolated ecosystems with different salt and SOM contents. Each community is different and seems well adapted to these environmental conditions. However, the flooded and dried-out cycles produce environmental disturbances for which the vast majority of OTUs in the community are resistant.

5 Conclusions

The difference in salinity has an important impact on the composition of prokaryotic communities in Villafáfila lagoons. Beta diversity analysis revealed an important qualitative component, with the abundance of diversity of communities determined by the salinity of the water. A quantitative component was also noticed, as the concentration of salt constituted an important factor. Other factors, such as the SOM caused by anthropic activities, may also condition the microbial composition of the sediments, sometimes even more than the concentration of salt. The seasonal disturbance of flooded and dried-out cycles affects a smaller number of OTUs than the differences in salinity; however, those OTUs are phylogenetically more distant. Overall, the microbial communities appear to be stable in their composition, which suggests a high resistance capacity. Indeed, stability and resistance of microbial communities are threatened by global warming, which makes our study of special relevance to understanding the changes in their composition.

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Author contribution All authors have contributed significantly to the project and merit to be included as co-authors.

Declarations

Conflict of interest The authors declare no competing interests.

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