Pontificia Universidad JAVERIANA Bogotá

Univ. Sci. 27(2): 163–185, 2022
doi: 10.11144/Javeriana.SC272.bcis

ORIGINAL ARTICLE

Bacterial communities in sediments of an urban wetland in Bogota, Colombia

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Received: 22-09-2021
Accepted: 10-05-2022
Published online: 10-08-2022

Citation: Castro M, Pacheco M, Restrepo M. Bacterial communities in sediments of an urban wetland in Bogota, Colombia, *Universitas Scientiarum*, 27(2): 163–185, 2022. doi: 10.11144/Javeriana.SC272.bcis

Funding: n.a.

Electronic supplementary material: Supp. 1

Abstract

Urban wetlands are biodiversity reservoirs sustained by microbe-mediated processes. In tropical zones, wetland microbial dynamics remain poorly understood. Chemical parameters, heavy metal content, and microbiological community structure were investigated in surface sediments of the Santa Maria del Lago (SML) wetland in Bogota, Colombia. High-throughput sequencing was employed to generate RNAr 16S and *nosZ* gene sequence data with which bacteria, archaea, and *nosZ*-type denitrifier community composition and their phylogenetic relationships were investigated. A canonical correspondence analysis was conducted to determine the relationship between assessed environmental variables and microbial community composition. Results showed that the most abundant bacterial phyla were Proteobacteria, Acidobacteria (group GP18), and Aminicenantes; Archaea were represented by the taxa Methanomicrobia and Thermoprotei, and the *nosZ* community was dominated by Candidatus *Competibacter denitrificans*. A phylogenetic analysis revealed a high diversity of Operational Taxonomic Units (OTUs), according to 16S rRNA gene sequence data; however, the quantity and diversity of OTUs from the *nosZ* community were low compared to previous studies. High concentrations of ammonium, phosphorus, organic carbon, Pb, Fe, Zn, Cu, and Cd, were detected in sediments, but they were not strongly related to observed microbial community compositions. In conclusion, in the same polluted SML wetland sediments diverse bacteria and archaea communities were detected, although not *nos*Z-type denitrifiers.

Keywords: metataxonomic; *nos*Z gene; pollution; urban wetlands.

1. Introduction

Wetlands are plant and animal diversity reservoirs sustained by several microbe-mediated processes [1]. Wetland dynamics such as biogeochemical processes are driven by hydrological, plant, soil, and microbial variables. Microbial communities are responsible for wetland effective nutrient cycling and ecological integrity [2] by removing nitrogen and metals, participating in sulfide oxidation, and driving carbon, nitrogen, and sulfur cycling [3]. In temperate regions, eutrophic wetlands are sources of N_2O produced by denitrification under anoxic conditions [4] and high nitrate availability [5]. In these environments, archaea and bacteria-driven nitrifications release N_2O below suboxic conditions [6]. In contrast, the features driving bacterial N_2O cycling in tropical wetlands remain largely unexplored. In Colombia, N_2O in mangrove sediments reaches flux levels of 1179.7 $\mu g\, m^{-2}\, h^{-1}$ in the Cienaga Grande de Santa Marta [7] and 5.63 $\mu g\, m^{-2}\, h^{-1}$ in lake Sonso [8]. A recent study has recorded a net N_2O consumption of up to 0.040 $ng\, g^{-1}\, d^{-1}$

at the Santa María del Lago Wetland (SML) within Bogota's urban area [9]. Although this figure indicates sediment denitrification, the bacterial communities involved in this process remain uncharacterized.

Habitat-specific characterizations of microbial communities chiefly rely on DNA sequence data. Gene sequences have assisted the identification of bacterial and archaeal communities in different environments, including wetlands. Depending on the research scope, such genes can be universal (e.g., 16S rRNA) or specific to a given function, for instance, nitrous oxide reduction within denitrifying communities, for which the *nosZ* gene is most suitable. Diversity within microbial communities is assessed with genetic information. Diversity-related metrics and statistical methods have been central to studying the environmental drivers of bacterial, archaeal, and denitrifying communities [10, 11, 12, 13, 14].

The SML wetland is part of a network of urban wetlands in Bogota - Colombia, included in the Ramsar List of key areas for the conservation of biological diversity. The SML wetland physical, chemical, and biological conditions have been previously investigated [15], revealing high seasonal and spatial variation in its trophic structure. This is associated with fluctuating availability of nutrients and oxygen in water [16], along with seasonal changes in N_2O production through nitrification and denitrification [9]. Further aspects of this wetland remain to be understood, including the composition of its bacterial, archaeal and *nosZ*-denitrifying communities, as well as its current heavy metal levels. In the present study, we have assessed these missing aspects of the SML wetland in Bogota.

2. Materials and Methods

2.1. Sediment sample collection

Sampling was carried out in two areas within the Santa Maria del Lago wetland in Bogota: S01 (4°41′32.17″ N – 74°5′40.71″ O, 4°41′47.69″ N – 74°5′37.90″ O) and S02 (4°41′35.95″ N – 74°5′47.24″ O, 4°41′41.5″ N – 74°5′31.35″ O; **Figure 1**). Both sampling locations experience contrasting environmental features: unlike S01, the S02 area receives urban wastewater and surface runoff water. Samples were taken at each sampling point during the dry (August) and rainy (November) seasons. At each location, 150 g of surface sediments (0 cm to 5 cm deep) were taken in duplicate using a dredger and stored in whirl pack bags at −20 °C for chemical and genetic analysis two months later.

2.2. Chemical analysis

Sediment pH readings were taken using the potentiometric method. Ammonium was measured through a colorimetric assay (Standard Method 4500-$NH_4{}^+$), nitrate with UV spectrophotometry (Standard Method 4500-$NO_3{}^-$), and phosphorous was measured via digestion and colorimetric assays. Furthermore, total organic carbon (TOC) was assessed with digestion and titulometry (NTC 5403), and organic matter via combustion. All of the chemical measurements followed the analytical methods published by the Laboratory of Soils at Instituto Geográfico Agustín Codazzi, Colombia [17]. Metals, such as Cu, Zn, and Fe were analyzed via atomic absorption (UNIVAM 969 Solar), and Cd and Pb analyses in sediments was carried out with the ICP-OES assay (Icap7200 Duo Thermo Scientific) following the analytical methods used for soils at the International Center of Tropical Agriculture.

Figure 1. Location of the Santa Maria del Lago wetland in Bogota, Colombia. The two sediment sampling points (S01 and S02) are marked on the map. This image was modified with the permission of the author [15].

2.3. Denitrifying community abundance

Surface sediment denitrifying community abundance was assessed using the most probable number (MPN) method. In brief, this approach involves mixing 10 g of sediment into 90 ml of sterile phosphate-buffered saline (PBS), thereafter sediment suspensions were 10-fold diluted down to a 1×10^{-7} dilution. Sterilized tubes containing denitrification medium and inverted Durham glass tubes were then inoculated with 1 ml of each dilution from 1×10^{-3} to 1×10^{-7} in triplicate. The composition of the denitrification medium used in this experiment was as follows ($g\,l^{-1}$): sodium citrate 5.0; KNO_3 2.0; KH_2PO_4 1.0; $K_2HPO_4{\cdot}3H_2O$ 1.0; $MgSO_4{\cdot}7H_2O$ 0.2 (pH 7.0). Inoculated denitrification test tubes were incubated at 30 °C for 2 weeks. Thereafter $NO_3{}^-$ removal was quantified, recording bacterial growth and gas production. Tubes that met the following three criteria (growth, nitrate removal and gas production) were deemed as having undergone denitrification. The presence of NO_3-N and NO_2-N in the medium was confirmed with Griess reactive [18].

2.4. DNA extraction

Total genomic DNA was extracted from 250 mg sediment samples with the Fast DNA spin kit for soil material (MP Biomedical) following the supplier's instructions. Next, DNA concentration and quality were determined using a Nanodrop spectrophotometer at wavelengths 260 nm and 280 nm and DNA was diluted with ultrapure water to a concentration of 10 ng μl^{-1} and stored at −80 °C for further analysis.

2.5. Sequencing, bioinformatics and phylogenetics analyses

The 16S rRNA and *nosZ* genes were PCR-amplified in each sample. The regions V3-V4 of the 16S rRNA gene were sequenced with the bacterial primers 515F and 806R [19], which were optimized for the Illumina MiSeq platform. PCR reactions were carried out under an initial 3 min denaturation step at 94 °C, followed by 30 cycles of denaturation at 94 °C (45s), primer annealing at 55 °C (45s), and primer extension at 72 °C (90s), and completed with a final extension step at 72 °C for 10 min. The primers *nosZF* and *nosZR* [20] were used for PCR amplification of the *nosZ* gene, with an initial denaturation step at 94 °C (1 min) followed by 35 cycles of denaturation at 94 °C (45s), primer annealing at 58 °C (45s), and primer extension at 72 °C (60s) and then a final extension step at 72 °C for 10 min. Subsequent amplicon library construction and sequencing were carried out in the commercial laboratories GENEWIZ (New Jersey, USA) and RTL Genomics (Texas, USA), respectively. Amplicon sequencing was performed on a MiSeq platform (Illumina, California, USA) employing the MiSeq Reagent kit (v2) with the longest read length set to 2×250 bp.

Low quality reads were removed with the Trimmomatic software [21]. Subsequently, paired-end reads were merged and further processed in QIIME, version 1.9.1 [22]. Our prokaryotic diversity analysis was carried out with 16S rRNA sequence data, and OTU detection with QIIME 1.9.1 [22]. The "pick open reference method" was set at 97 % similarity. DECIPHER's Find Chimeras (http://www2.decipher.codes/FindChimeras.html) software was used to remove chimeric sequences [23].

16S rRNA and *nosZ* gene sequences (the latter translated into amino acid sequence) were subjected to the MEGA 7.0 [24] software for phylogenetic analysis and aligned using MUSCLE version 5 [25]. 16S rRNA reference sequences were retrieved from the GenBank database using the nucleotide tool BLAST, Popset, and Batch Entrez at NCBI (http://www.ncbi.nlm.nih.gov), and *nosZ* reference sequences were retrieved from the Fungene database (http://fungene.cme.msu.edu/).

Following the identification of optimal (nucleotide/amino acid) substitution models, neighbor joining was selected as the statistical method for phylogenetic reconstruction in MEGA 7.0. Molecular evolutionary genetics analyses were carried out across several computing platforms [26], with Tamura–Nei and Jones–Taylor–Thornton's as the chosen substitution models to work with 16S rRNA and *nosZ* gene sequence data, respectively.

2.6. Statistical analysis

A canonical correspondence analysis was carried out to observe the relationship between the bacterial community composition and environmental variables using the Canoco software V4.5 [27]. The diversity index (Shannon and Simpson) was calculated with the PAST 3 software, only using the OTUs that were associated with 0.1 % of the total abundance of sequences.

2.7. Nucleotide sequence accession numbers

Sequences data were submitted to the GenBank database under the accession numbers MK394800 - MK394975 for 16S rRNA-OTU sequences and PRJNA693821 for 16S rRNA and *nos*Z Illumina raw sequences (**Table S2**).

Table 1. Environmental parameters measured in the sediments of two sampling areas within the Santa Maria del Lago wetland. TOM: Total Organic Matter; TOC: Total Organic Carbon; NO_3^-: nitrates; NH_4^+-N: Ammonia Nitrogen.

Environmental parameter	**Sampling areas**			
	S01	S01	S02	S02
Season	Dry	Rainy	Dry	Rainy
TOM (%)	23 ± 17	20 ± 1	26 ± 1	36 ± 15
TOC (%)	8.35	9.96	8.42	10.40
Phosphorous ($mg\,kg^{-1}$)	55.9	199.9	24.2	174.5
NO_3^- ($mg\,kg^{-1}$)	< 50.0	< 50.0	< 50.0	< 50.0
NH_4^+ ($mg\,kg^{-1}$)	4.0	18.4	3.3	33.5
pH	6.58	6.57	6.80	6.41
Cu ($mg\,kg^{-1}$)	22.03	9.93	20.07	18.13
Zn ($mg\,kg^{-1}$)	106.61	51.27	95.90	84.12
Fe ($g\,kg^{-1}$)	7.00	3.90	7.50	6.30
Cd ($mg\,kg^{-1}$)	0.39	0.21	0.40	0.35
Pb ($mg\,kg^{-1}$)	40.40	18.06	40.56	38.99

3. Results

3.1. Chemical variables and denitrifying bacteria community abundance

The outcomes of the studied chemical variables in the SML wetland sediments are shown in **Table 1**. These sediments revealed acidic conditions in a pH range between 6.4 and 6.8. Organic matter levels varied between 20 % and 36 %; the highest organic matter content was identified in sampling area S02. Detected TOC values varied between 8.3 and 10.4, revealing a 10 % increase during the rainy season in both sampling areas. Phosphorus concentrations varied between 24.2 $mg\,kg^{-1}$ and 199.9 $mg\,kg^{-1}$, being highest in sampling area S02 in the rainy season. Ammonium concentrations varied between 3.3 $mg\,kg^{-1}$ and 33.5 $mg\,kg^{-1}$, increasing by one order of magnitude during the rainy season in the S02 sampling area. We detected Nitrate values of approximately 50 $mg\,kg^{-1}$ in both sampling areas.

Metals (Cd, Cu, Zn, Pb, and Fe) were detected with overall comparable concentrations in both sampling areas, but some metal levels varied both spatially and temporally (Table 1). (i) In the S01 area, Cu and Zn levels reached values of 22.03 $mg\,kg^{-1}$ and 106.6 $mg\,kg^{-1}$, respectively, during the dry season. (ii) Cd, Cu, Zn, Fe, and Pb levels were similar in both sampling areas and experienced a parallel decrease during the rainy season. Finally (iii), in the rainy season, even though metal levels were below those reported in the dry season, S02 metal levels were higher than in S01. This is likely because of the influx of urban sewage wastewater and surface runoff into the former sampling area.

In wetland sediments, denitrifying bacteria density between the rainy and dry season varied from 7.2 ± 3.7 to 16.0 ± 8.3 bacteria per gram of dry sediment, respectively. No significant differences existed in the bacterial community abundance between sampling areas.

Table 2. Sequence read information from this study, along with richness and diversity estimates of OTUs for 16S rRNA and *nosZ*-type sequence data in bacterial communities assessed at the Santa Maria del Lago wetland during its dry (d) and rainy (r) season.

Gene	Sample	No. of Sequences			97/80%	Valid	Shannon (H)	Simpson (1-D)
		Raw	Quality	Valid				
16S rRNA	S01-d	140 569	94 512	19 943	1338	1220	5.73	0.990
	S01-r	156 382	78 676	14 307	991	907	5.23	0.986
	S02-d	238 596	149 446	32 656	1465	1343	5.65	0.990
	S02-r	217 017	132 955	32 225	1414	1296	5.47	0.989
nosZ	S01-d	86 560	61 064	61 056	19	19	0.04	0.010
	S01-r	50 180	22 765	22 030	12	12	1.95	0.785
	S02-d	59 755	31 346	14 968	14	14	0.03	0.007
	S02-r	21 133	14 315	7619	10	10	0.56	0.014

3.2. 16S rRNA bacterial community composition

In the SML wetland, bacterial 16S rRNA diversity and richness were similar between sampling stations. However, community diversity showed a slight increase in samples taken during the dry season ($H = 5.65$ or $H = 5.73$), and richness experienced a decrease in the S01 sampling area during the rainy season (**Table 2**).

Figure 2 shows the OTUs with reads with at least 0.1 % representation in both sampling areas. About 38.3 % of the total OTUs revealed with 16S rRNA sequence data were present in both wetland sampling areas. The most abundant bacterial groups were Delta bacteria, Acidobacteria, Aminicenantes, Bacteroidetes, and Chloroflexi, and the most abundant class of Archaea were Methanomicrobia and Thermoprotei. No significant spatial and temporal differences were observed in the abundance of both communities.

The Aminicenantes class and the group GP18 (phylum Acidobacteria) were the most abundant taxa in sediments from the two sampling areas and seasons. Three Proteobacteria families (Methylococcaceae, Desulfobacteraceae, and Chromatiaceae) and two Acidobacteria subphylum groups (GP6 and GP16) increased their relative abundance during the dry and rainy seasons, respectively (**Figure 3**).

3.3. The phylogenetic analysis of 16S rRNA community

Figure 4 depicts the phylogenetic relationships of the 15 most abundant OTUs, identified thanks to 16S rRNA gene sequence data from SML wetland sediment samples during the dry and rainy seasons. Eleven of the OTUs identified in the SML wetland have not been previously reported in other water body sediments; however, three of them have, and shared substantial similarity with the following clones: MH205704, obtained from Lake Onego sediments (OTU003, 86 % similarity) and to *Candidatus* Aminicenantes CYBC47 (OTU001, 91 % similarity) and MG897596.1 (OTU012, 86 % similarity), both recovered from river sediments.

3.4. *NosZ*-type denitrifying community composition

In the SML wetland, neither spatial nor seasonal differences were observed in the richness and diversity of *nosZ*-OTUs (Table 2). Within the *nosZ*-type community, OTU richness was low (19) due to difficulty in amplifying and sequencing the *nosZ* gene of the bacteria present in the studied community, despite the same DNA extract being used in parallel 16S rRNA gene analysis. This result reveals lower bacterial community richness in S02 (10 OTUs) than in the S01 (19 OTUs). With the caveat that the obtained diversity values are likely unreliable, given the low number of OTUs identified.

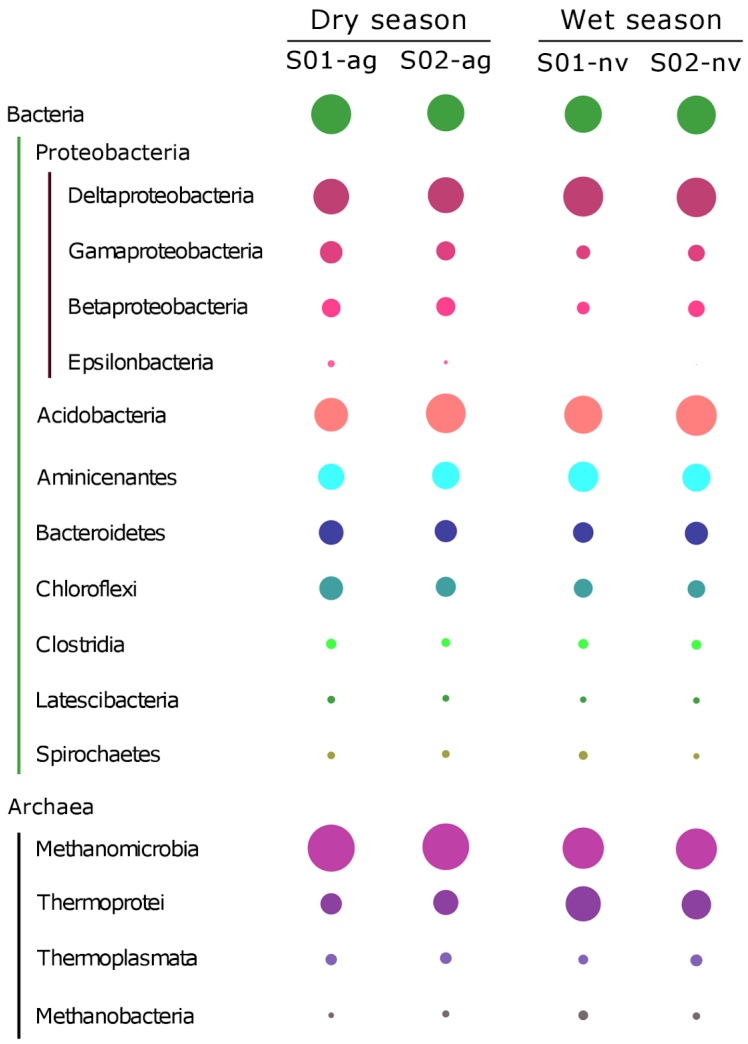

Figure 2. Composition and abundance of 16S rRNA bacterial communities at the Santa Maria del Lago wetland, showing seasonal and spatial similarity. Circle sizes indicate the proportion of sequences assigned to each phylum, subphylum or class.

OTU composition within the *nosZ*-community revealed the dominance of one (99 % abundance), likely *Candidatus* Competibacter denitrificans, while the other OTUs (< 0.1 % abundance) were taxonomically affiliated (> 85 % similarity) with the genera *Castellanieulla*, *Azospirillum*, *Thiobacillus*, *Rhodobacter, Microvirga*, *Sinorhizobium*, *Ralstonia*, *Oligotropha*, and *Thauera* (**Table S1**).

3.5. Phylogenetic relationships of the *nosZ*-type denitrifying community

With the obtained *nosZ* gene sequence data, the most abundant OTUs (nine in total) were used to construct a phylogenetic tree (**Figure 5**). These OTUs were grouped in a single cluster, distinct from the ones containing sequences retrieved from previous sediment, soil, and lake studies. Eight OTUs were 90 % similar to *Pseudomonas aeruginosa* strain DBT1BNH3 and OTUs 3, 9, and 97 were 88 % similar to OTU Buji 1-8, obtained from a landfill leachate treatment plant. All OTUs showed between 83 % and 85 % similarity to the previously reported OTUs from sediments, soils or rhizospheres.

3.6. Environmental factors influencing community composition

A canonical correspondence analysis was carried out to observe the relationship between the assessed environmental variables and bacterial community composition (**Figure 6**). Our CCA results revealed slight community composition differences between sampling areas, due to environmental parameter dynamics occurring in either or both locations. The latter included: higher levels of TOM, TOC, and ammonium in the S02 area during the rainy season, increased levels of metals in both stations in the dry season, and a total phosphorus surge in the S01 area during

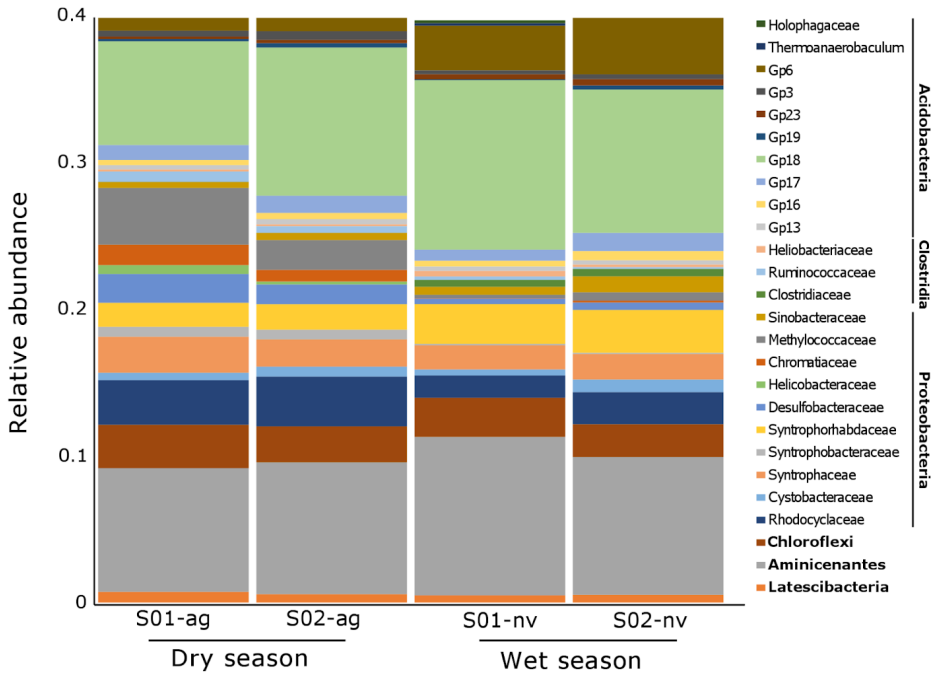

Figure 3. Relative abundance of 16S rRNA bacterial communities (at family level) assessed in the Santa Maria del Lago wetland during the dry and wet seasons in two sampling spots (S01 and 202).

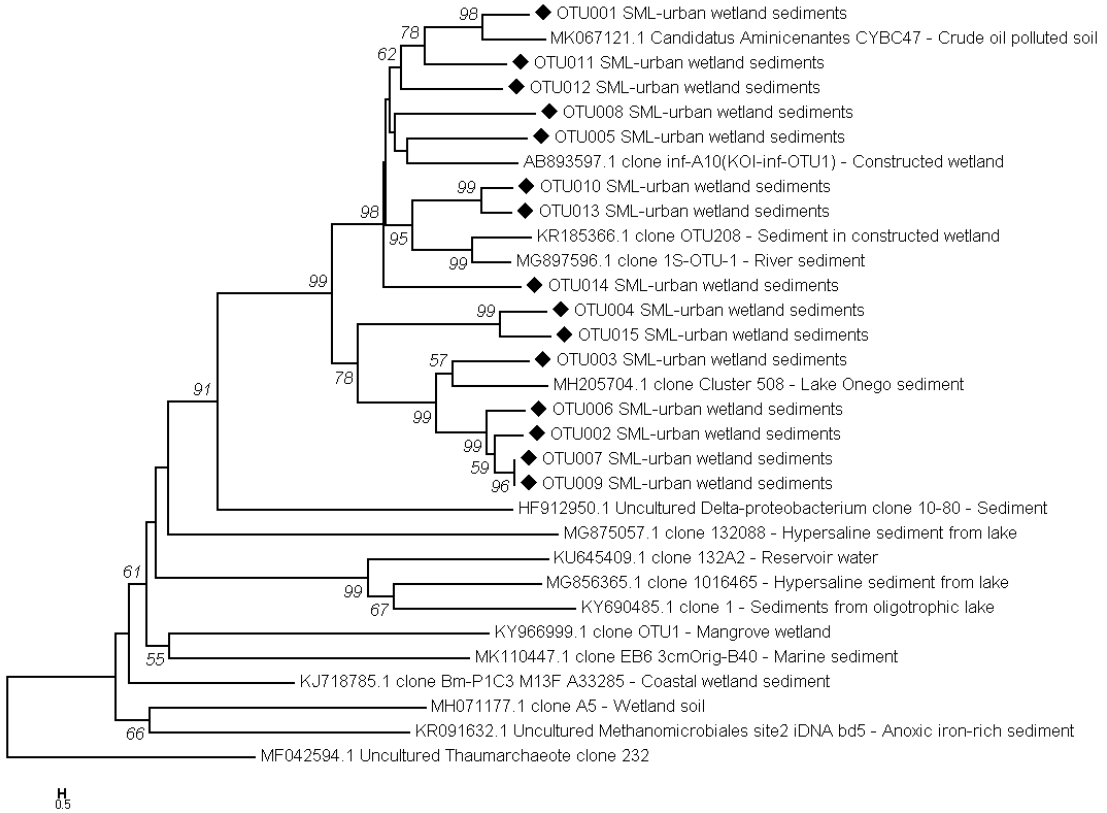

Figure 4. Neighbor-joining phylogenetic tree of the bacterial 16S rRNA gene sequences obtained from surface sediments in the SML wetland. Bootstrap values greater than 50 (based on 1000 bootstrap resampling) were labeled at the nodes. Scale bar length represents 0.5 % sequence divergence. Sequences from this study are indicated by bold diamond shapes.

the rainy season. In the first two CCA dimensions, environmental variables accounted for 88.3 % and 8.5 % of the variance in bacterial and archaeal community composition. The microbial community, characterized via 16S rRNA gene data, was distributed around the center of the plot, suggesting a consistent community composition, regardless of variation in environmental factors. However, the presence of the Epsilon bacteria group, recorded in the S01 area during the dry season, may indicate a bacterial community response to high metal concentrations. To ascertain this preliminary finding, it is necessary to repeat this study with a larger number of samples.

4. Discussion

4.1. Chemical factor variation in SML wetland sediments

In this study, the composition of 16S rRNA and *nosZ*-type denitrifying bacteria communities was analyzed in relation to ecological drivers in SML wetland sediments. These sediments were sampled in two wetland areas (S01 and S02) and in the rainy and dry seasons. Results revealed variation in the chemical factors values obtained for each area. The highest values for TOM, TOC, ammonium and metal levels were recorded in S02 sediments during the rainy season. This conforms with outcomes of previous studies in the same area, in which nutrient, oxygen, trophic status, and water column denitrification levels varied both spatially and seasonally [9, 15, 16]. Furthermore, our results also support previously reported trace metal fluctuations in sediments of

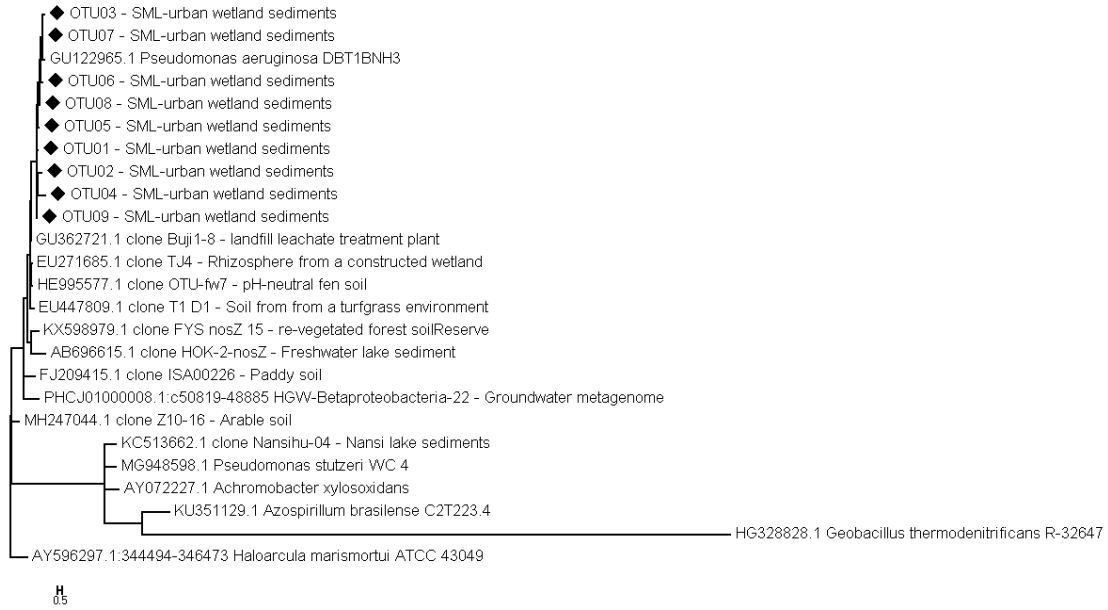

Figure 5. Neighbor-joining phylogenetic tree of partial amino acid sequences translated from *nos*Z-gene sequences. Bootstrap values above 50 % are shown. Scale bar length represents 0.5 % sequence divergence. Sequences from this study are indicated by bold diamond shapes. Outgroup: *Haloarcula marismortui* ATCC43049.

highly polluted lakes [28, 29, 30]. Our observed sediment TOM and TOC levels surpass those already reported in coastal wetlands [31] and eutrophic shallow urban lakes [13, 30]. However, compared to previous reports from eutrophic urban lakes by Zhao [13], Wang [6] and Fan [30], our results revealed lower phosphorus and ammonium levels, similar pH values, and increased nitrate levels.

The recorded metal levels in SML wetland sediments are diagnostic of heavily polluted freshwater wetlands [28, 29, 30, 32]. Temporal metal level variation (i.e., high levels in the dry season) may be attributed to high Cu and Zn affinity with organic matter, as previously reported by Koretsky [28]. Furthermore, the influx of wastewater from the urban sewage system and surface runoff during the rainy season accentuated metal presence in the S02 area. As discussed in the following, trace metals may impact the microbial community, even though the levels of trace metals recorded in the sediments are below the threshold concentrations expected to harm benthic organisms: ERL (effects range-low) and ERM (effects range-median) of 34 mg g^{-1} and 270 mg g^{-1}, respectively [33, 34].

4.2. SML wetland 16S rRNA bacterial community composition

The taxa making up our studied bacterial community (i.e., δ-Proteobacteria, Acidobacteria, Aminicenantes, Bacteroidetes, and Chloroflexi) belong to freshwater wetlands [11, 31, 35], constructed wetlands [1], and hypoxic and urban lakes [13, 36]. In addition, taxa such as Bacteroidetes, Spirochaetes, Latescibacteria, Clostridia, and Chloroflexi usually are scarce within such bacterial communities [1, 11]. Our results support these observations and further indicate that the relative abundances of these taxa in the assessed 16S bacterial community are comparable between sampling areas.

In our study, one of the most abundant Acidobacteria groups was GP18. These bacteria are common in freshwater systems, such as streams, aquatic sediments, and natural and constructed wetlands [2, 12, 37, 38]. GP18 is also present in soils with high heavy metal levels [39]. These bacteria can degrade multiple carbon sources, such as sugars, hemicellulose, cellulose, and chitin [40]. However, there is no clear evidence that Acidobacteria are involved in nitrogen cycle processes, such as denitrification [41].

The Aminicenantes is another abundant phylum in the SML wetland. These bacteria thrive in habitats with high organic matter content, necessary to carry out their metabolic functions. These include heterotrophic activity during nitrate respiration, amylolytic activity, and carbohydrate and protein fermentation [42, 43]. Likewise, our phylogenetic analysis revealed the abundance of

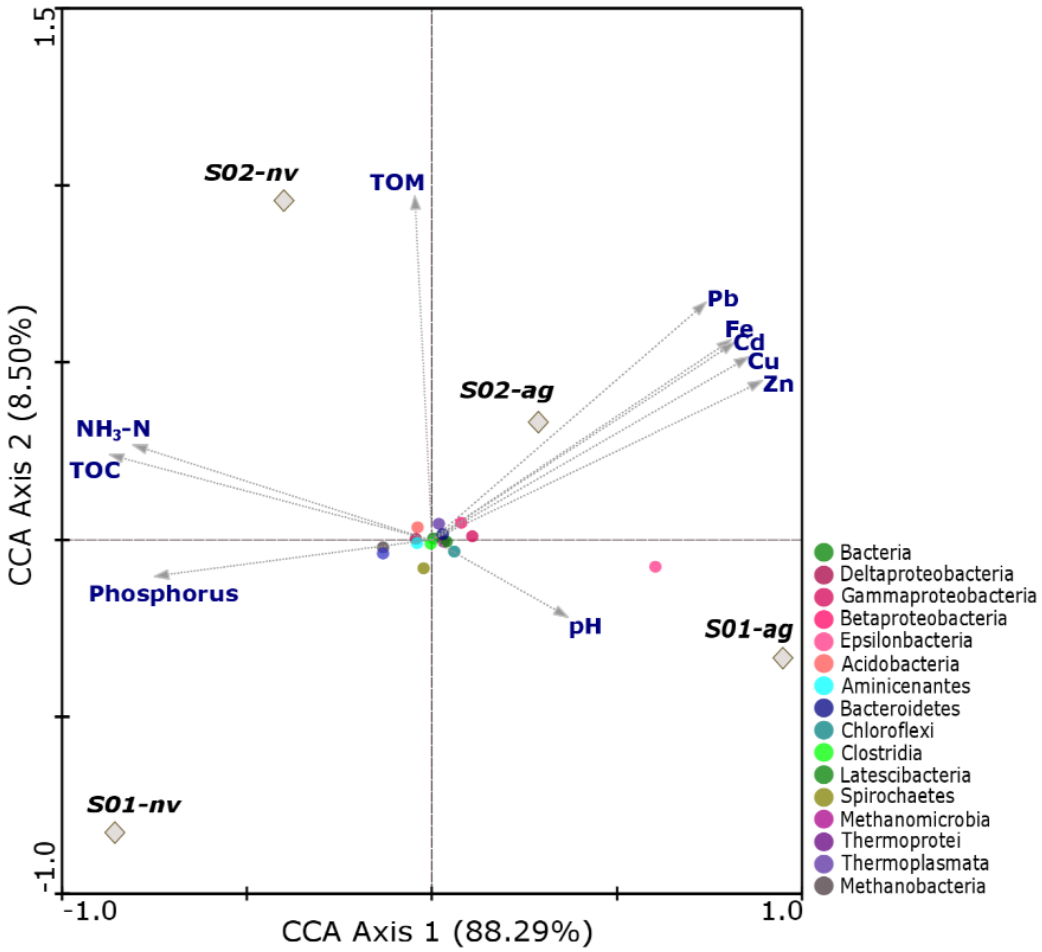

Figure 6. Canonical correspondence analysis (CCA) between the bacterial and archaeal community composition in sediments of the Santa Maria del Lago wetland and environmental (water) factors such as: total organic matter (TOM), total organic carbon (TOC), pH, total phosphorus (TP), ammonia nitrogen (NH_3^+), and metals: Pb, Fe, Cd, Cu, and Zn. Arrows indicate environmental factors. Symbols (diamonds) represent the sampling areas during the rainy (November: nv) and dry (August: ag) seasons, and circles represent the taxonomic groups observed in the area.

one OTU, similar to *Candidatus* Aminicenantes CYBC47 in polluted SML wetland sediments, supporting previous observations of an increased abundance of this group in habitats with high hydrocarbon levels, arsenic [44] and heavy metals [45].

The obtained data suggest that the Epsilon bacteria group and the families Desulfobacteraceae, Chromatiaceae, and Methylococcaceae tend to be more abundant in the dry season; in which they were recorded, along with the highest heavy metal values. This is consistent with the reports of Li [45] and Bensaid [46], in which Epsilon bacteria of the genera *Sulfurovum*, *Sulfurimonas*, *Sulfurospirillum*, and *Sulfuricurvum* were detected in sediments with high levels of PAH and metals. The observed high levels of Fe, heavy metals, and TOC may also justify the presence of Desulfobacteraceae in the SML sediments, since they use acetate as a substrate, degrade organic pollutants [47], and use iron as a part of its Fe (III) reducing metabolism [30]. However, further research is necessary to corroborate this idea and to validate any direct relation between an increase in heavy metals and the relative abundance of these groups along with Chromatiaceae and Methylococcaceae, which also are central microorganisms to sulfur and methane cycling in aquatic ecosystems.

Furthermore, our data indicate that Methanomicrobia inhabit SML wetland sediments. This Euryarchaeota group is widespread in terrestrial, freshwater and marine ecosystems [48], anaerobic urban lakes [49], river sediments, and waste-treatment bioreactors [50, 51]. Although these methanogens have a narrow set of metabolic substrates, their wide distribution throughout different environments indicates that these archaea are able to differentially adapt to divergent conditions of pH, nutrients, salinity, oxidative stress, temperature [48], and heavy metals [45], thus explaining their abundance in the SML wetland. Methanomicrobia also are known to be involved in syntrophic anaerobic methane oxidation in anoxic habitats [52] and freshwater sediments [53].

The presence of Methanomicrobia along with Methylococcus (Gamma Proteobacteria) in the SML wetland is notable, as both are reported as methanotrophs within wetlands [54, 55]. However, it remains unclear how both bacteria and archaea could contribute to methane cycling in this wetland.

4.3. The *nosZ* community composition

Effectively amplifying and sequencing the *nosZ*-type community from both SML wetland sampling areas was challenging. These situation highlights common problems associated with both of these molecular techniques. However, the same molecular approach addressing the 16S rRNA-based sediment bacterial community with the same DNA extracts was not problematic. This situation may also signal the low abundance of this denitrifying bacteria group in the studied sediments. Such possibility is supported by the quantification results from NMP and by the dominance of a single OTU. These two aspects are consistent with the low rates of net N_2O consumption ((0.035 ± 0.011) ng g^{-1} d^{-1}) previously recorded for the SML wetland area [9].

This suggests that the N_2O reduction potential in the SML wetland is comparatively lower than in other wetlands richer in *nosZ* sequences. Peralta [56] recorded 94 *nosZ*-TRF in a restored wetland, Bañeras [31] reported up to 252 *nosZ*-TRF in a coastal wetland, and Iribar [14] reported 55 OTUs in an alluvial wetland. Furthermore, these studies also observed that the community structure and the relative abundance of *nosZ*-OTUs or TRF remained constant throughout their observation periods, which is consistent with the results from the present study.

The dominant *nosZ*-type sequence in SML wetland sediments was *Candidatus* Competibacter denitrificans, obtained previously from laboratory-scale enrichment reactors through metagenomics [57]. *Competibacter* members present varying capabilities to assimilate thymidine, ferment glucose, produce exopolysaccharide, accumulate glycogen, and metabolize PHA and volatile fatty acids [57, 58]. However, a genomic analysis of the species *Candidatus* C. denitrificans indicates its potential for full denitrification to nitrogen [57]. Furthermore, the phylogenetic analysis indicated that some *nosZ*-OTUs obtained from the SML sediments are similar to *Pseudomonas aeruginosa DBT1BNH3*, which has been identified as a nitrifying aerobic denitrifier [59]. This data opens new questions about the role of both bacteria in the N_2O cycling in this wetland which should be addressed later.

Based on our results we can speculate that pollution by heavy metals in the SML wetland could be affecting *nosZ* community diversity and even leading to selection of denitrifiers without the *nosZ*-gene, triggering the dominance of one OTU capable of N_2O reduction. This is in accordance with previous studies, where trace metals, such as Cu, Zn, and Cd have caused loss of genetic diversity [60] and strongly inhibited the activity, abundance, and transcription of genes involved in the denitrification pathway, especially within N_2O reducing communities [32, 61].

However, to establish a clear relationship between seasonality, nutrients, heavy metals and wetland sediment bacterial community composition, an analysis with a larger number of samples and a more appropriate experimental design is required.

5. Conclusions

We recorded, for the first time, high levels of Pb, Fe, Zn, Cu, and Cd in SML wetland sediments, where bacterial groups, such as *Candidatus* Aminicenantes CYBC47, Acidobacteria (GP18 group), Epsilon bacteria, Desulfobacteraceae, Chromatiaceae, Methylococcaceae and *Candidatus* Competibacter denitrificans were dominant. Also, a greater diversity of 16S-OTUs than *nosZ*-OTUs was recorded in the area.

Actions to improve water quality in the SML wetland could contribute to wetland restoration. If this wetland's bacterial community continues to be subjected to environmental stress, such as pollution, it is likely that nutrient-cycling processes will be also affected. A further and thorough community characterization, down to genus or species levels, is necessary to determine changes in the function of denitrifying, sulfur-iron reducing, methanogenic, and methanotrophic communities established in this wetland. These bacterial and archaeal communities are exposed to organic or inorganic pollution, as well as the resulting impact on methane and nitrous oxide cycling. Likewise, assays to cultivate these organisms could be an initial step towards achieving a better understanding of their physiology, providing a foundation for future use of these native bacteria in bioremediation.

6. Acknowledgements

The authors would like to thank the Environment Secretary of Bogota – Colombia for granting sampling permission within the Santa Maria del Lago wetland. Special thanks go to SML wetland staff members: Z. Moreno and A. Ortiz for sampling assistance.

Further disclosures: This research was developed following the national regulation on the collection of non-commercial specimens of biological diversity for scientific research (Resolution No. 1198/2014 ANLA). This paper is a product of the 2018 project INV-CIAS 2547, entitled "Diversidad de la comunidad desnitrificante en el humedal Santa María del Lago, Bogotá" funded by the research vice rectory at Universidad Militar Nueva Granada, Bogota in the year 2018.

7. Conflict of interest

The authors have no conflicts of interest to declare.

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Comunidades bacterianas en sedimentos de un humedal urbano en Bogotá, Colombia

Resumen: Los humedales urbanos son reservorios de biodiversidad sostenidos por procesos mediados por microbios; sin embargo, en áreas tropicales la dinámica microbiana de estos humedales permanece poco comprendida. En este estudio, se investigaron parámetros químicos, concentración de metales pesados y estructura de la comunidad microbiana en la superficie de los sedimentos del humedal Santa María del Lago (SML) en Bogotá-Colombia. Con el fin de investigar la composición y relaciones filogenéticas de bacteria, archaea y la comunidad denitrificante tipo *nos*Z se generaron datos de secuencias de los genes RNAr 16S y *nos*Z por medio de secuenciación de alto rendimiento. Se llevó a cabo un análisis de correspondencia canónica para determinar la relación entre las variables ambientales y la composición de la comunidad microbiana. Los resultados mostraron que los fila más abundantes de bacterias fueron Proteobacteria, Acidobacteria (grupo GP18) y Aminicenantes; Archaea estuvo representada por los taxa Methanomicrobia y Thermoprotei, y la comunidad *nos*Z fue dominada por "Candidatus *Competibacter denitrificans*". El análisis filogenético a partir de datos de secuencias del gen RNAr 16s reveló una alta diversidad de UTOs; sin embargo, la cantidad y diversidad de UTOs de la comunidad *nos*Z fue muy baja en comparación con estudios previos. Se detectaron altas concentraciones de amonio, fósforo, carbono orgánico, Pb, Fe, Zn, Cu y Cd en sedimentos, aunque no estuvieron estrechamente relacionadas con la composición observada de la comunidad microbiana. En conclusión, en los mismos sedimentos contaminados del humedal SML se detectaron comunidades diversas de bacterias y Archaea, pero no de desnitrificadores tipo *nos*Z.

Palabras clave: metataxononmía; gen *nos*Z; polución; humedales urbanos.

Comunidades bacterianas em sedimentos de uma zona úmida urbana de Bogota, Colômbia

Resumo: As zonas úmidas urbanas são reservatórios de biodiversidade sustentados por processos mediados por micróbios. Em áreas tropicais, a dinâmica microbiana das zonas úmidas permanece pouco compreendida. Os parâmetros químicos, concentração de metais pesados e a estatura da comunidade microbiana foram pesquisados em sedimentos superficiais da zona úmida de Santa María del Lago (SML) em Bogota, Colômbia. Sequenciamento de alto rendimento foi usado para gerar dados dos genes RNAr 16S e *nos*Z e assim pesquisar a composição e relações filogenéticas de bacteria, archaea e a comunidade denitrificante tipo *nos*Z. Adicionalmente, foi realizada uma análise de correspondência canônica para determinar a relação entre as variáveis ambientais estudadas e a composição da comunidade microbiana. Os resultados mostraram que os fila bacterianos mais abundantes foram Proteobacteria, Acidobacteria (grupo GP18) e Aminicenantes. As Archaea foram representadas pelos taxa Methanomicrobia y Thermoprotei e a comunidade *nos*Z foi dominada por "Candidatus *Competibacter denitrificans*". A análise filogenética mostrou uma alta diversidade de unidades taxonômicas operacionais (UTOs) segundo os dados da sequência do gene RNAr 16s. No entanto, a quantidade e diversidade de UTOs da comunidade *nos*Z foram menores do reportado em estudos anteriores. Altas concentrações de amônio, fósforo, carbono orgânico, Pb, Fe, Zn, Cu e Cd foram detectadas nos sedimentos, mas não estiveram relacionadas com a composição da comunidade. Em conclusão, diversas comunidades de bactérias e Archaea foram detectadas nos sedimentos contaminados da zona úmida SML, e o oposto foi encontrado para os denitrificantes do tipo *nos*Z nos mesmos sedimentos.

Palavras-chave: metataxonômicos; gene *nos*Z; poluição; zonas úmidas urbanas.

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