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Changes in Soil Quality of an Urban Wetland as a Result of Anthropogenic Disturbance

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Abstract: Urban wetland soil provides ecosystem services (ES) through their functions. Changes in soil properties due to anthropogenic disturbances lead to a loss of soil quality. The aim of this research was to evaluate the effect of nearby anthropic disturbance on the chemical, physical and biological properties of the urban wetland soil. Soil samples were collected from four sites (P1, P2, P3 and P4) located in the Angachilla urban wetland, Chile, according to the magnitude of anthropogenic disturbance. An assessment of the physical and chemical properties of the soil profile was carried out in two sites, P1 and P4. Additionally, chemical and biological properties of the soil were evaluated in the four sites selected. Results from the soil profiles showed that Hz1 of P4 had a higher levels of soil fertility as a result of low anthropogenic disturbance in contrast to Hz1 of P1 ($p < 0.05$). Relevant differences among sites were observed for P-Olsen, pH NaF, nosZ gene, Nitrate and Na (PC1: 50.5%). Composition of the soil bacterial community in P1 and P4 showed higher richness and diversity. Anthropogenic disturbance on the urban wetland soil leads to a loss of the soil's organic horizon, as well as its soil quality and, subsequently, its capacity to provide ES through its functions.

Keywords: urban wetland; anthropogenic disturbance; soil properties

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1. Introduction

Wetlands are recognized as important aquatic environments able to provide several 'ecosystem services (ES)', which are defined as 'the benefits that people can obtain from natural ecosystems' [1]. The ES provided by wetlands environment can be classified as: (i) 'supporting services'to sustain world life through the protection of processes such as water and nutrient cycling and biodiversity; (ii) 'provisioning services' related to consumable supplies (e.g., food and fresh water); (iii) 'regulating services' of socio-ecologic systems (e.g., water purification); and (iv) 'cultural services' that are those non-material aspects associated with human wellness and its connection with nature (e.g., cultural identity, spirituality, aesthetics, recreation and eco-tourism) [2]. Moreover, the ES provided by wetlands are essential in achieving the Sustainable Development Goals (SDGs) by seventeen goals, each with a number of concrete targets (https://sustainabledevelopment.un.org, accessed on 10 February 2022). The SDGs looking for eradicate poverty and achieve sustainable development by 2030. The wetlands in relation to the SDGs highlight the importance of conserving and restoring this important aquatic environment, which it will be critical in helping countries achieve their SDG targets (https://www.ramsar.org/news/wetlands-and-the-sdgs, accessed on 10 February 2022). Nevertheless, the function of wetlands is being altered by a global anthropogenic intervention. The major threats for the wetlands are related to construction of infrastructure, water extraction, eutrophication and pollution.

Many of the ES of wetlands are associated with the soil functions, such as carbon sequestration that depend on the physical properties of the soil [3]. However, in wetlands a high variability of soils can be found, where the anthropogenic disturbance causing an irreversible alteration for the soil properties. In this context, soil quality indicators are commonly used to evaluate and compare the properties of soils and especially volcanic ash soils, due to the nature of their clay minerals [4], due to soil quality is determined by both inherent and dynamic properties and processes that define its functionality [5,6]. Moreover, soils have several functions and their sustainability depends on the balance of these functions according to the adequate land use [7]. Therefore, it is necessary to evaluate the soil properties in order to establish quality indicators that relate to soil functions and ecosystem services provided by the wetland.

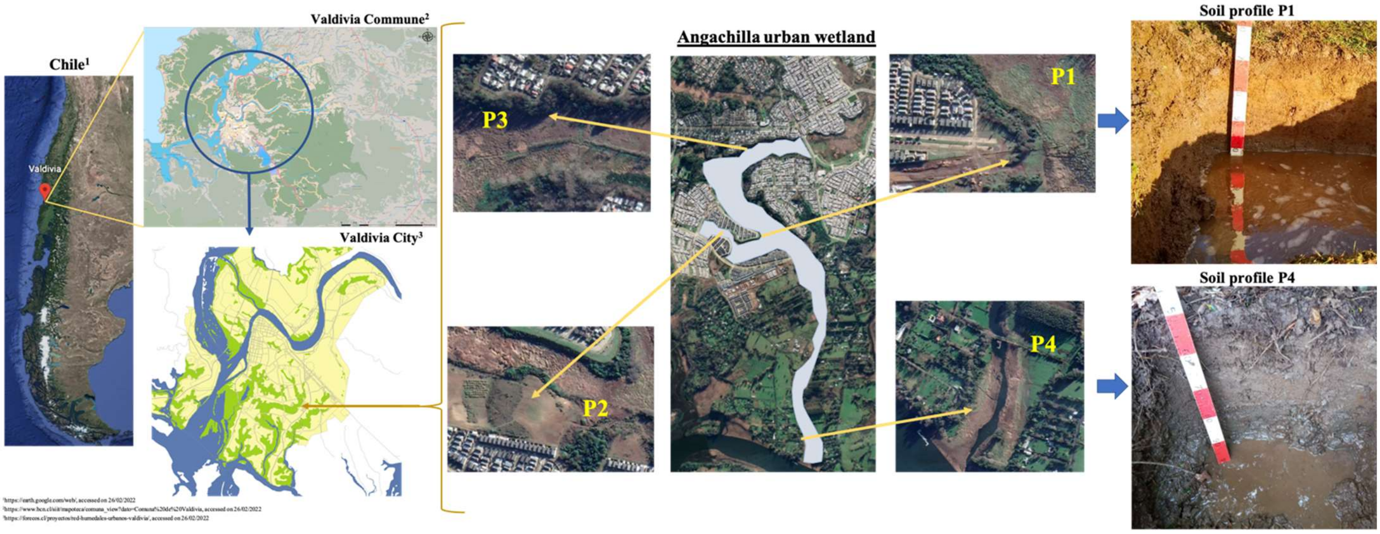
Volcanic ash soils (i.e., andisols), characteristic of southern Chile, have developed under diverse rainfall and temperature regimes, exhibit unique functional characteristics compared to other soil types in the world [6]. Andisols have chemical and physical properties that are mainly defined by a high reactivity of their colloidal fraction [8], high allophane content >80 g kg^{-1} [9], which results in a greater phosphate retention capacity (>85%; [10]), and a high soil organic carbon (SOC; [4]). Moreover, its soil structure supports a stable interconnected pore system that allows diffusion of gases [11] and nutrients over time [12], a low bulk density (<0.9 Mg m^{-3}; [13]) that induces sandy behavior near saturated conditions [14] and a high water-holding capacity at −60 kPa water pressure [15], which directly affects water transport in the soil profile under saturated and unsaturated conditions [16].

In Chile, has been estimated about 40 thousand wetlands distributed in the country (Andean and high Andean, coastal, forested, inlands, peatlands, "hualves" and urban wetlands) covering an area of 4.5 million ha (5.9% of the national territory; [17]). About 80% of the wetlands in Chile are concentrated between Los Ríos Region (39°48′30″ S, 73°14′30″ W) and Magallanes Region (53°09′45″ S, 70°55′21″ W), which corresponds to the southern zone of the country [17]. In southern Chile, most of the coastal and inland wetlands larger than 500 ha are under constant threat of human disturbance, industrial wastes, land use change and water extraction [18]. Furthermore, the constant urban expansion due to population growth generates a greater threat to urban wetlands. Cities in southern Chile such as Valdivia (39°51′13.3″ S, 73°14′22.6″ W), are characterized by the presence of several urban wetlands that are part of the landscape (2966.2 ha corresponding to 37% of urban area; [19]), natural and social heritage of the city. According to the CEAM-UACh and Fundación [20], the urban wetland called "humedal Angachilla" is affected by process of fragmentation and degradation, thus strongly threatened by land reclamation for urban sprawl. The Angachilla wetland was part of the countryside (before the 1960 earthquake, it was part of a private landholding). Nevertheless, the Valdivia city began expanding southwards in the 1990s, several social housing projects emerged around it. This area has had an urban expansion of 172% between 1992 and 2007, with constant and growing wetland filling [21]. The hypothesis proposed is that in an urban wetland, the nearby anthropogenic disturbance leads to chemical, physical and biological loss of the soil quality indicators, generating a decrease of the soil capacity to sustain the wetland ecosystem. Thus, the objective of this research was to evaluate the effect of nearby anthropic disturbance on the physical, chemical and biological properties of an andisol in an urban wetland.

2. Material and Methods

2.1. Study Site Description

The experimental site is part of the Angachilla Wetland located in the city of Valdivia, Chile. The wetland is approximately 6 km long (area > 100 ha) and is almost entirely surrounded by urban sprawl and landfill for housing development (Figure 1). Their vegetation includes about 70% aquatic vegetation mainly comprised by *Narcissus jonquilla*, *Cortaderia selloana*, *Schoenoplectus californicu*, mainly; 20% shrub vegetation mainly comprised by *Chusquea quila*, *Greigia sphacelata*, *Rubus ulmifolius*, *Berberis darwinii*, among others; 10% tree vegetation mainly comprised by *Nothofagus obliqua*, *Luma apiculata*, *Nothofagus dombeyi*, *Drimys winteri*, among others, meadows and scrub isolated on the edges of the wetland [18,19]. Additionally, the Angachilla wetland is the habitat of the "Coipo" (*Myocastor coypus*), an aquatic rodent that feeds on totora reeds, the "Huillín" (*Lontra provocax*), a river otter in danger of extinction, and a wide variety of birds such as black-necked Swan (*Cygnus melancoryphus*), Patagonian Sierra Finch (*Phrygilus patagonicus*), Yellow-billed Teal (*Anas flavirostris*), Chileo Wigeon (*Mareca sibilatrix*), among others (https://ebird.org/hotspot/L2874771, accessed on 15 May 2021).



[1]https://earth.google.com/web/, accessed on 26/02/2022
[2]https://www.bcn.cl/siit/mapoteca/comuna_view?dato=Comuna%20de%20Valdivia, accessed on 26/02/2022
[3]https://forecos.cl/proyectos/red-humedales-urbanos-valdivia/, accessed on 26/02/2022

Figure 1. Geographical location of the Angachilla urban wetland in the city of Valdivia. P1: anthropic-agricultural disturbance older than 20 years, P2: recent anthropic-urban disturbance, P3: anthropic-urban disturbance older than 10 years and P4: low anthropic disturbance (natural condition).

2.2. Soil Sampling on the Profile

Predominant soil group in the province of Valdivia corresponds to a Duric Hapludand, Andisol [22,23]. Four sites were selected according to their degree of anthropogenic disturbance in the urban wetland for a prospective field assessment (Figure 1). The degree of anthropogenic disturbance was determined according to presence of (i) livestock, mainly cattle and horses, P1: anthropic-agricultural disturbance older than 20 years; (ii) draining and/or filling of wetland, P2: recent anthropic-urban disturbance; (iii) social housing projects, P3: anthropic-urban disturbance older than 10 years and (iv) the presence of the natural condition, P4: low anthropic disturbance.

To determine possible changes in soil profile horizons and effective depth due to anthropogenic disturbance, an auger (100 cm length and 3 cm diameter, Eijkelkamp) was used. According to the field assessment, soils at sites P1 and P4 showed significant changes in soil profile horizons and effective depth. Therefore, two soil pits ("Calicata") were performed to describe the soil profiles for P1 and P4 (Table 1). Soil samples for chemical and physical analysis were collected in four horizons founded for P1: 0–7 cm (Hz1); 27–20 cm (Hz2); 20–33 cm (Hz3) and 33–100+ cm (Hz4), and of P4: 0–4 cm (Hz1); 4–15 cm (Hz2); 15–30 cm (Hz3) and 30–100+ cm (Hz4). Field evaluation of the sites and sampling process were carried out in the winter season of 2020.

Table 1. Morphological description of the soil profile corresponding to P1 (Calicata 1) and P4 (Calicata 2).

Horizon (cm)	Soil Description of P1
Hz1; 0–7	Very dark brown (7.5 YR 2.5/2); silty loam; friable, plastic and adhesive; granular structure, moderate medium; fine and medium roots abundant; fine pores very abundant, coarse pores normal. Linear clear boundary. Worm presence.
Hz2; 7–20	Dark brown (7.5 YR 3/3); silty clay; friable, plastic and very adhesive; subangular, medium, moderate block structure; common fine and sparse medium roots; very abundant fine pores, coarse common. Linear clear boundary. Presence of burnt clay.
Hz3; 20–33	Very dark brown (7.5 YR 2.5/2); clayey; plastic and very adhesive; angular, moderate coarse blocks tending to angular, fine blocks; sparse fine and very fine roots; very abundant fine pores. Linear abrupt boundary. Charcoal present and visually very compacted.
Hz4; 33–100+	Dark gray (Gley 1 4/N); very fine sandy clay; plastic and very adhesive; massive; no roots; very abundant fine pores. Presence of fine gravels and charcoal. Water table present at 40 cm.
	Soil description of P4
Hz1; 0–4	Black (7.5 YR 2.5/1); silty clay loam; plastic and slightly adhesive; granular, fine weak structure; fine, medium and coarse roots abundant; fine pores very abundant, coarse pores common. Linear abrupt boundary.
Hz2; 4–15	Very dark greyish brown (10 YR 3/2); silty clay; very plastic and slightly adhesive; weak, coarse, angular block structure; abundant fine, medium and coarse roots; very abundant fine pores. Linear clear boundary. Presence of burnt clay.
Hz3; 15–30	Dark greyish brown (2.5 YR 4/2); very fine sandy clayey loam; plastic and adhesive; massive; common fine and medium roots; very abundant fine pores. Linear light boundary. Gleization with common oxidations. Water table present at 30 cm.
Hz4; 30–100+	Dark greenish grey (Gley 1 4/1); very fine sandy loam; plastic and very adhesive; massive; common fine and medium dead roots; very abundant fine pores. Presence of mottling. Gleization with mottling.

2.2.1. Soil Physical Properties

Undisturbed soil samples (n = 7) were collected in metallic cylinders with 230 cm^3 of volume (h = 5.6 cm, d = 7.2 cm), were saturated from beneath for 24 h, weighed, and then equilibrated at matric potential values of −1, −2, −3, −6 kPa in sand tables [24], and at −15, −33, −50 kPa in pressure chambers to determine the water retention curve (WRC; [14]). At each matric potential, the samples were weighed with an electronic balance (Precise, Switzerland; 0.01 g accuracy). For correcting the volumetric water content during the soil drying, soil vertical deformation was performed at five defined points using a Vernier caliper (precision 0.01 mm). Soil samples assembled in 20 cm^3 cylinders (n = 7) were equilibrated at a matric potential of −1543 kPa to determine the permanent wilting point, corresponding to the volumetric water content of the fine porosity. The bulk density (Bd) was determined by oven drying the undisturbed soil samples at 105 °C for 48 h [25]. Air capacity (AC) and plant available water (PAW) was determined from the water retention curve (WRC) as in [14], as follows:

$$AC = TP - \theta_{6\ \text{kPa}} \tag{1}$$

where TP is the total porosity ($cm^3\ cm^{-3}$) and $\theta_{6\ \text{kPa}}$ is the volumetric water content at −6 kPa of matric potential ($cm^3\ cm^{-3}$).

$$PAW = FC - PWP \tag{2}$$

where FC is the field capacity corresponding to the volumetric water content at −6 kPa ($cm^3\ cm^{-3}$) and PWP is the permanent wilting ($cm^3\ cm^{-3}$).

Air permeability (Ka) was calculated from the measured air conductivity (kl) determined on the same samples during the WRC measurement using an airflow meter with different scales [26]: Ka was calculated as follows:

$$\mathrm{Ka} = \mathrm{kl}(\varepsilon\mathrm{a}) \times \frac{\mathrm{h}}{\mathrm{rl} \times \mathrm{g}} \tag{3}$$

where Ka is air permeability (μm^2), εa is the air-filled porosity ($cm^3\ cm^{-3}$), kl is air conductivity ($cm\ s^{-1}$), η is the air viscosity ($g\ s^{-1}\ cm^{-1}$), ρl is the air density ($kg\ m^{-3}$) and g is the gravitational acceleration ($m\ s^{-2}$).

Soil texture was determined by the hydrometer method (n = 3) [27]. Soil samples were dried at 25 °C, sieved at 2 mm and were digested with H_2O_2 (100 volumes) to remove organic matter [28]. To ensure the removal of the cementing agents and adequate dispersion of the clay, the samples were pre-treated with H_2O_2 and 100 mL of sodium pyrophosphate (0.1 N) was added. Clay dispersion was carried out using the Bouyoucos method. The hydrometer method [28] was then used to determine the silt and clay fractions. Finally, the sand fractions were physically separated with a set of sieves [25].

2.2.2. Soil Chemical Properties

Disturbed soil samples were sieved (2 mm) and air-dried (n = 3). The following indicators associated with soil fertility were determined: NH_4-N by adding 0.07 ± 0.01 g of MgO and NO_3-N using the Devarda alloy distillation method [29]. P-Olsen (available phosphorus) was extracted with bicarbonate (0.5 M) and analyzed using the methodology described by [30]. Finally, exchangeable Al (extracted by 1 M K Cl) and exchangeable Ca, Mg, K and Na extracted by 1 M in ammonium acetate and determined by atomic absorption spectrophotometry [28].

Besides the indicators of parameters associated with soil fertility, the following soil type indicators were determined: soil pH in water and pH in $CaCl_2$ at a ratio of 1:2.5, organic matter (OM) was measured using the dichromate oxidation [31], pH NaF (1 N), extractable Aluminum (Al_a) in 1 M ammonium acetate at pH 4.8 (by atomic absorption spectroscopy), extractions of Al, silicon (Si) and iron (Fe) in 0.2 M acid ammonium oxalate at pH 3 (Al_o-Si_o-Fe_o), and extractions of Al, Si and Fe in 0.1 M sodium pyrophosphate at pH 10 (Al_p-Si_p-Fe_p). All those determinations were performed according to [32].

2.3. *Soil Sampling of the First 20 cm at Each Site*

To assess the effect of anthropogenic disturbance in the first centimeters of soil, disturbed samples were collected at a depth of 0–20 cm. The collected samples were sieved 2 mm and air-dried for chemical analysis (Nitrate, Ammonium, P-Olsen, exchange bases, CICE, pH H_2O, pH NaF and Ala [28,31] and soil texture (described in Section 2.2.1). Additionally, biological determinations were performed on the 0–20 cm soil samples to evaluate how anthropogenic disturbance affected the soil biological communities.

Soil Biological Properties

Soil samples (n = 3) were collected and these were deposited in coolers and immediately transported within 4 h to the Laboratorio de Micología y Bacteriología of Universidad Austral de Chile. Then, soil samples were frozen at −20 °C to be used in molecular studies as described below. According to the manufacturer's instructions, the gDNA from wetland soil samples were extracted using a DNeasy PowerSoil Pro Kit (Qiagen, Hilden, Germany). gDNA quality and quantity will be determined by a Nano-drop spectrophotometer (Thermo Scientific, Waltham, MA, USA). The DNA purity was assessed by determination of A260/A280 absorbance ratio ∼1.8, and DNA samples will be stored at −20 °C prior to molecular analysis. The composition of bacterial communities was evaluated by 16S rDNA metabarcoding analysis. First, purified high-quality gDNA aliquotes (>20 ng and ratio ∼1.8) from soil samples were submitted to Laboratorio de Ecología Microbiana Aplicada of Universidad de La Frontera) for sequencing analysis. Then, gDNA aliquotes were used for the preparation of 16S rRNA gene libraries following the instructions described by [33]. In brief, gDNA samples were subject of PCR reaction using the universal Bakt_341f (5′-CCTACGGGNGGCWGCAG-3′) and Bakt_805r (5′-GACTACHVGGGTATCTAATCC-3′) primer set for amplification of the V3~V4 region of the bacterial 16S rRNA gene. Then, after PCR product purification and indexation with the Nextera v2 kit (Illumina, Inc., San Diego, CA, USA), libraries were loaded into MiSeq Kit V3 (600-cycles) and sequenced by an Illumina MiSeq (Illumina, Inc.) platform. The dada2 R software package was used to analyze the resulting raw sequence data. According to standard workflow, steps included quality filtering, trimming, dereplication, learning error model, ASV inference, chimeras removal, and taxonomic assignment using SILVA database v138.me. The phyloseq [34], microbiome [35], and MicrobiotaProcess [36] R packages were employed for data pre-processing, diversity, and ordination analyses. The ggstatsplot [37] R package was used to plot statistical tests results. Microbiome package version 1.16.0. (https://bioconductor.org/packages/release/bioc/html/microbiome.html, accessed on 15 June 2021) and MicrobiotaProcess [38] R packages were employed for data pre-processing, diversity and ordination analyses.

Abundance of bacterial functional genes related to N cycling, the N-transforming bacteria communities (ammonia monooxygenase gene-amoA- and nitrous oxide reductase gene-nosZ-) and total bacterial community based on 16S rRNA gene were estimated by quantitative polymerase chain reaction (qPCR) with a Quant Studio^{TM}3 Real-Time PCR System (ThermoFisher Scientific, Inc., Waltham, MA, USA) using PowerUp^{TM} SYBR^{TM}.

Green Master Mix (Applied Biosystems TM, Foster City, CA, USA) and ~25 ng μL^{-1} of gDNA. The primer sets and conditions used for quantification of 16S rRNA genes, amoA and nosZ and by qPCR are shown in Supplementary Table S1. The copy numbers of targeted genes were calculated using standards of each gene, built with dsDNA gBlock® Gene Fragments (Inte-grated DNA Technologies, Inc., Iowa, USA), and the equation ([concentration of the dsDNA gBlock® Gene Fragment in ng μL^{-1}] × [molecular weight in fmol ng^{-1}] × [Avogadro's number] = copy number) following the method described by [38]. Absolute quantification (AQ) of bacterial genes was expressed as copy number per gram of dw (gene copy g^{-1} soil dw).

3. Statistical Analysis

To evaluate the difference between sites and the effect of anthropic disturbance on the Angachilla wetland, the soil physical and chemical properties were analyzed with analysis of variance (ANOVA). Normality of the residuals and homogeneity of variance were determined with the Shapiro-Wilk test ($p \leq 0.05$) and Levene's test ($p \leq 0.05$), respectively. When the data did not present a normal distribution, the natural logarithm transformation was used. Significant differences between means of the soil parameters evaluated were determined through Tukey's multiple comparisons of means ($p < 0.05$). The soil properties are presented in box and whisker plots. Each box plot displays the median and range between the 25–75% of the values.

A principal component analysis (PCA) was performed with the soil chemical and biological properties to indicate which properties explain the variation between the evaluated sites and thus determine if there is an apparent effect of anthropic intervention on the soil quality indicators associated with the Angachilla urban wetland. Previous to the analysis, the data set was normalized because soil property values have different units of measurement and magnitudes. The data obtained from N gene quantification were contrasted by one-way ANOVA with Tukey's honestly significant difference (HSD) test. All the above analyses were performed with R 4.0.2 [39] using RStudio 1.3.1017 [40].

4. Results

4.1. Chemical and Physical Characteristics of the Soil Profile at P1 and P4

The particle size distribution (PSD) of the studied volcanic ash soil in the studied urban wetland present differences between profiles (Figure 2). In Hz1, the sand, silt and clay content for P1was 15.0%, 46.3%, 38.7%; P2 was 16.2%, 51.0%, 32.7% and P3 was 22.4%, 57.3%. 20.3%, respectively. The PSD for Hz1in P4 was 2.1% sand; 84.3% silt and 13.6% clay. This variability in PSD was also observed in depth when the two soil pits were analyzed, specifically the Hz3 and Hz4 where the clay content was higher in P4 (76.9% and 38.3% respectively).

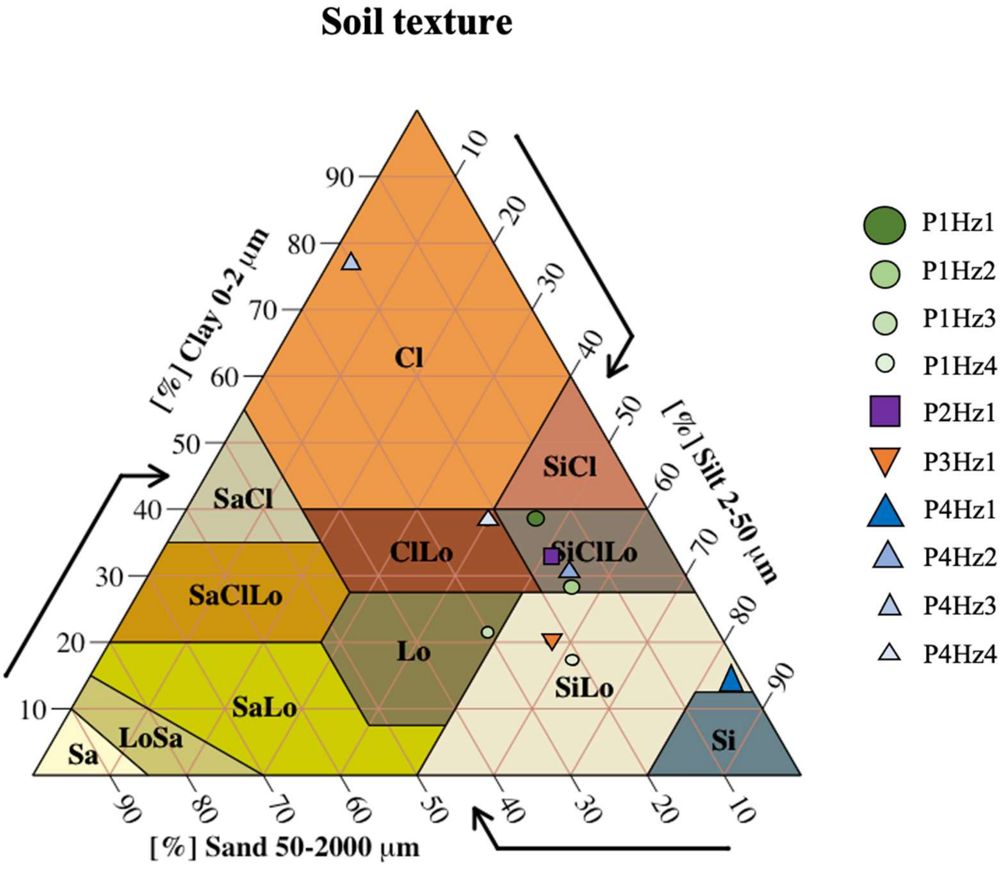


Figure 2. Soil textural triangle. P1: anthropic-agricultural disturbance older than 20 years, P2: recent anthropic-urban disturbance, P3: anthropic-urban disturbance older than 10 years and P4: low anthropic disturbance (natural condition). H1: horizon 1, H2: horizon 2, H3: horizon 3 and H4: horizon 4. Cl: clay, SiCl: silty clay, SaCl: sandy clay, CILo: clay loam, SiCILo: silty clay loam, SaCILo: sandy clay loam, Lo: loam, SiLo: silty loam, SaLo: sandy loam, Si: silt, LoSa: loamy sand, Sa: sand.

Chemical soil properties SOM content (<17%), pH in NaF (~10.5) and Al_a (600–1200 mg kg^{-1}), Al_p (1.4 –3.3%), Al_o (1.6–3.0%), Fe_p (0.1–1.1%) and Fe_o (0.2–1.4%), showed that the soil profile of P1 is significantly different from the soil profile of P4. ($p < 0.05$; Table 2). The horizons defined for P4 presented: OM content (8–36%), pH in NaF (7–10) and Al_a (250–890 mg kg^{-1}), Al_p (0.8–1.7%), Al_o (0.5–1.2%), Fe_p (0.2–0.5%) and Fe_o (0.4–0.6%). The indicators associated with soil fertility are significantly different between P1 and P4 ($p < 0.05$; Table 2). The higher content of P-Olsen (8 mg kg^{-1}), nitrate (20 mg kg^{-1}), ammonium (100 mg kg^{-1}), K (0.5 $cmol_c$ kg^{-1}), Mg (11.0 $cmol_c$ kg^{-1}), Na (0.6 $cmol_c$ kg^{-1}), Ca (9.0 $cmol_c$ kg^{-1}) in Hz1 in P4 reflects a higher degree of soil fertility as a result of a low anthropic disturbance in contrast to Hz1 in P1($p < 0.05$). This trend can be observed in different depths across all the evaluated parameters. Soil pH in water was more acidic in P4 (4.8–5.6) than P1 (5.5–6.0). Both sites increase the pH value at depth.

Table 2. Chemical properties of Soil Profiles P1: anthropic-agricultural intervention and P4: low anthropic intervention (semi-natural condition).

	P1				P4			
	Hz1	**Hz2**	**Hz3**	**Hz4**	**Hz1**	**Hz2**	**Hz3**	**Hz4**
Soil type parameters								
SOM (%)	17.0 ± 0.8 b	9.6 ± 0.1 de	8.6 ± 0.5 e	3.1 ± 0.1 f	36.2 ± 2.5 a	13.9 ± 0.4 c	11.2 ± 1.7 d	8.3 ± 0.4 e
pH NaF	10.5 ± 0.1 e	10.9 ± 0.0 b	11.1 ± 0.0 a	10.8 ± 0.0 c	7.7 ± 0.1 f	10.4 ± 0.0 e	10.6 ± 0.0 d	10.8 ± 0.0 c
Al_a (mg kg^{-1})	1260.3 ± 33.6 a	1132.4 ± 12.7 b	1156.0 ± 58.9 ab	674.2 ± 15.9 e	277.0 ± 1.4 f	892.1 ± 29.7 c	776.2 ± 47.1 d	717.0 ± 15.0 de
Al_p (%)	2.9 ± 0.09 b	3.3 ± 0.07 a	3.3 ± 0.10 a	1.4 ± 0.06 d	0.8 ± 0.03 e	1.7 ± 0.06 cd	1.7 ± 0.01 c	1.7 ± 0.15 c
Al_o (%)	2.6 ± 0.04 a	2.8 ± 0.17 a	3.0 ± 0.17 a	1.6 ± 0.66 b	0.5 ± 0.07 c	1.1 ± 0.01 bc	1.2 ± 0.02 bc	1.1 ± 0.08 bc
Fe_p (%)	1.1 ± 0.03 a	1.1 ± 0.01 a	0.9 ± 0.00 b	0.1 ± 0.00 e	0.4 ± 0.00 c	0.3 ± 0.01 d	0.4 ± 0.01 c	0.2 ± 0.01 d
Fe_o (%)	1.2 ± 0.00 b	1.4 ± 0.01 a	1.1 ± 0.02 b	0.2 ± 0.01 e	0.6 ± 0.01 c	0.4 ± 0.03 d	0.6 ± 0.14 c	0.5 ± 0.06 d
Al_o+1/2Fe_o	3.2 ± 0.04 a	3.5 ± 0.18 a	3.5 ± 0.18 a	1.7 ± 0.66 b	0.8 ± 0.07 c	1.3 ± 0.02 bc	1.6 ± 0.09 b	1.3 ± 0.05 bc
pH H_2O	5.6 ± 0.02 c	5.7 ± 0.03 b	5.5 ± 0.06 cd	6.0 ± 0.05 a	4.8 ± 0.01 f	5.1 ± 0.02 e	5.4 ± 0.01 d	5.6 ± 0.02 c
pH $CaCl_2$	4.7 ± 0.03 c	4.9 ± 0.02 b	5.1 ± 0.01 a	5.1 ± 0.03 a	4.2 ± 0.01 e	4.3 ± 0.01 e	4.4 ± 0.02 d	4.5 ± 0.01 c
SEB ($cmol_c$ kg^{-1})	3.7 ± 0.22	2.0 ± 0.20	1.9 ± 0.02	3.0 ± 0.13	8.2 ± 0.38	6.6 ± 0.11	8.9 ± 0.20	8.1 ± 0.73
Al Sat (%)	1.0 ± 0.14	1.6 ± 0.22	1.1 ± 0.23	0.7 ± 0.10	0.3 ± 0.01	2.9 ± 0. 22	1.8 ± 0.06	1.3 ± 0.05
Soil chemical properties								
NO_3 (mg kg^{-1})	9.6 ± 3.98 b	2.8 ± 2.10 c	2.3 ± 0.40 c	1.6 ± 0.81 c	22.6 ± 1.76 a	10.5 ± 0.7 b	10.3 ± 2.14 b	1.9 ± 0.81 c
NH_4 (mg kg^{-1})	36.4 ± 3.70 b	24.5 ± 4.37 c	20.1 ± 6.31 c	18.4 ± 2.25 c	115.3 ± 0.81 a	23.3 ± 4.50 c	19.6 ± 1.85 c	21.0 ± 2.10 c
P-Olsen (mg kg^{-1})	3.7 ± 0.22 c	2.0 ± 0.20 e	1.9 ± 0.02 e	3.0 ± 0.13 d	8.2 ± 0.38 a	6.6 ± 0.11 b	8.9 ± 0.20 a	8.1 ± 0.73 a
Exc K ($cmol_c$ kg^{-1})	0.18 ± 0.03 b	0.06 ± 0.00 cd	0.04 ± 0.01 e	0.04 ± 0.00 e	0.57 ± 0.02 a	0.15 ± 0.01 b	0.07 ± 0.02 c	0.05 ± 0.00 de
Exc Mg ($cmol_c$ kg^{-1})	1.92 ± 0.19 b	0.35 ± 0.05 c	0.21 ± 0.07 d	1.69 ± 0.02 b	11.86 ± 0.50 a	1.55 ± 0.03 b	1.53 ± 0.05 b	1.60 ± 0.03 b
Exc Na ($cmol_c$ kg^{-1})	0.16 ± 0.02 d	0.09 ± 0.00 e	0.07 ± 0.00 f	0.23 ± 0.00 bc	0.60 ± 0.02 a	0.26 ± 0.01 b	0.23 ± 0.00 bc	0.21 ± 0.01 c
Exc Ca ($cmol_c$ kg^{-1})	2.18 ± 0.08 b	0.26 ± 0.05 c	0.15 ± 0.07 c	1.97 ± 0.04 b	8.99 ± 0.11 a	1.44 ± 0.67 b	1.30 ± 0.09 b	1.21 ± 0.03 b
Exc Al ($cmol_c$ kg^{-1})	0.04 ± 0.00 b	0.01 ± 0.00 b	0.01 ± 0.00 b	0.03 ± 0.00 b	0.06 ± 0.00 a	0.10 ± 0.00 a	0.06 ± 0.00 a	0.04 ± 0.00 b

SOM: soil organic matter; Ala: Extractable Aluminum; SEB: Sum of exchangeable bases; Al Sat: Aluminum saturation; NO_3: Nitrate; NH_4: Ammonium, Exc K: Exchangeable potassium; Exc Mg: Exchangeable Magnesium; Exc Na: Exchangeable sodium; Exc Ca: Exchangeable Calcium. Different lowercase letters in the row indicate significant differences ($p < 0.05$) between site and soil horizon (interaction).

Bulk density (Bd) increased with soil depth in both sites (Figure 3). In P1 the Bd increased from 0.60 up to 0.98 Mg m^{-3} and in P4 from 0.51 up to 0.94 Mg m^{-3} ($p < 0.05$). The largest amount of plant available water (PAW) was observed for Hz2 in both sites (38.3% at P1 and 39.3% at P4; $p < 0.05$). For Hz1 the PAW was significantly lower in P4 (22.5%) compared to P1 (33.5%). Air capacity (AC) showed no differences between horizons among the sites evaluated ($p > 0.05$). For P1 the AC was between 3.2 and 4.3% and for P2 was between 1.8 and 2.4%. Air permeability (Ka) was higher in Hz2 for P4 (2.51 log μm^{-2}). Furthermore, Ka values were significantly lower in P1 than in P4 ($p < 0.05$; Figure 3).

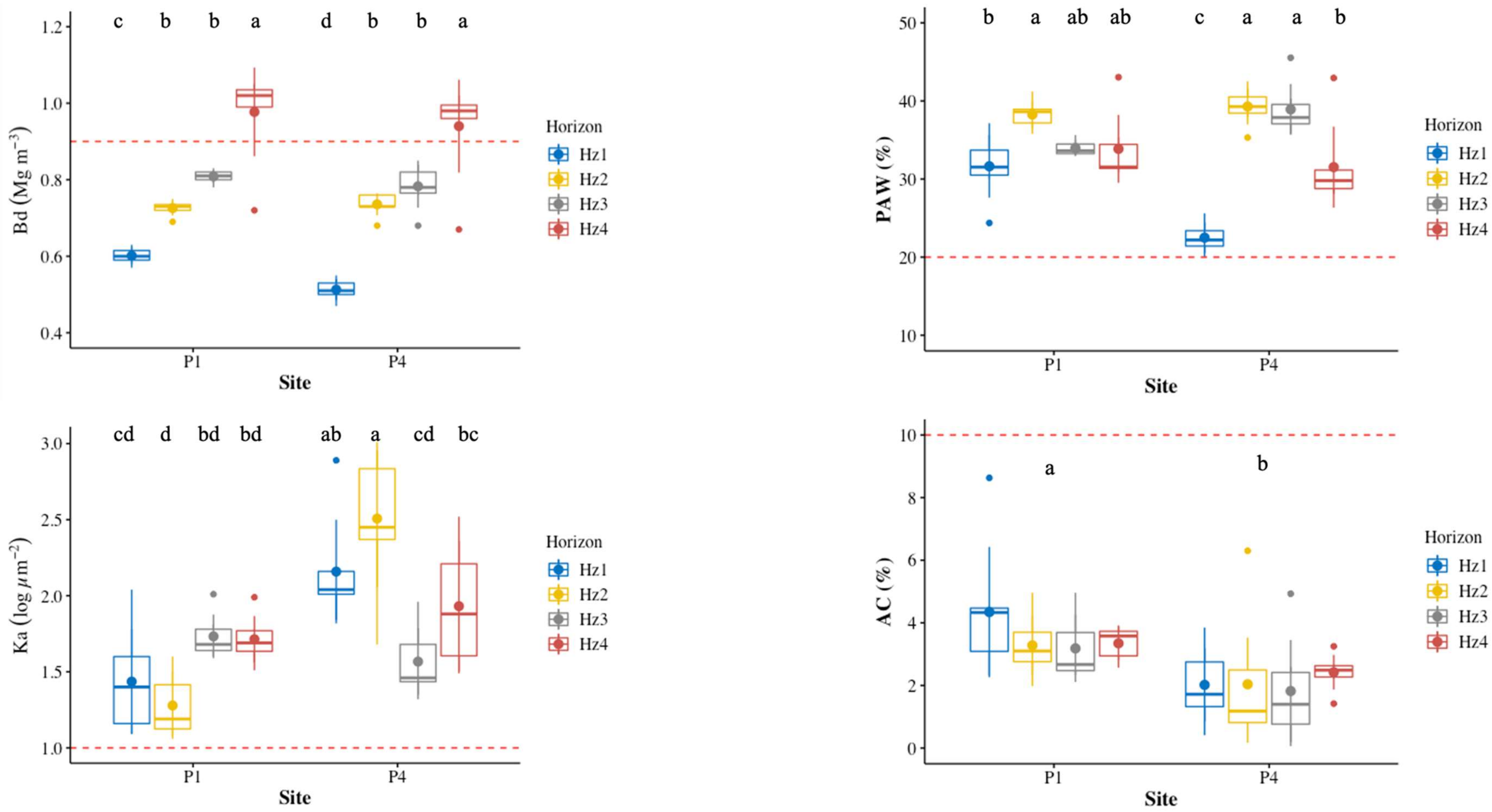


Figure 3. Bulk density (Bd), plant available water (PAW), air capacity (CA) and air permeability (Ka) at two sites in the Angachilla urban wetland (P1 and P4). Different lowercase letters indicate statistically significant differences between sites and horizon ($p < 0.05$). Box plots show median, percentiles (P10 and P90), lower and upper limits. The red segmented line shows the critical soil quality values.

4.2. Variation of Chemical and Biological Properties in the First 20 cm of Soil of the Angachilla Urban Wetland

The differences among evaluated sites in terms of soil chemical and biological parameters are shown in the PCA analysis (Figure 4). The first two components of the analysis explain 90.3% of the accumulated variance. The variables that most contributed to explaining the differences among sites were P-Olsen, pH NaF, nosZ gene, Nitrate and Na which in turn have the highest correlation with PC1 contribute about 50.5% to the PC 1. Al_a, Mg, Ca, CICE and amoA gene contribute about 78% of the PC 2. It is important to note that SOM contributes to the variance of PC1 (7%); however, the largest contribution of SOM (>60%) is present in PC3 which explains 5% of the total analysis variance.

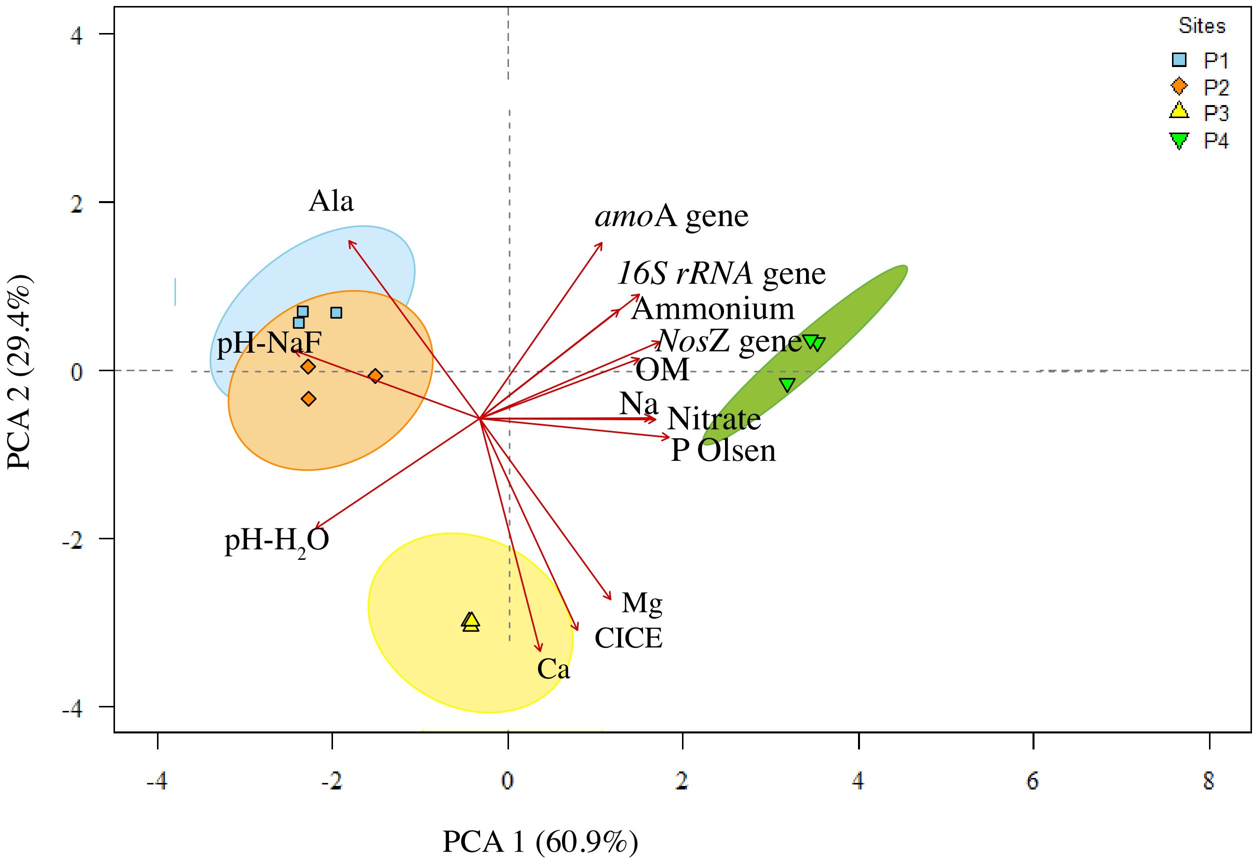


Figure 4. Principal component Analysis (PCA) for soil chemical and biological properties of the sampling sites. R1, R2 and R3 means replicates of sampling. The color band indicates the quality of the representation of the variables of each PC.

Furthermore, the distance of P4 (20 cm depth) in Figure 4 is for the quality of representation (>0.5) of the variables amoA gene, 16S rRNA gene, nosZ gene, Ammonium, OM, Na, Nitrate and P-Olsen on the PCs. The latter reflects the change of soil quality at 20 cm of depth between sites (P1 and P4) as result of anthropogenic intervention. In this way, for the first 20 cm of soil depth P4 contributes over the 22% of the P1 accumulated variance, meanwhile P1 and P2 contributes the 5%, showing a greater representation by the following variables, pH NaF and Al_a, indicating a higher degree of intervention in the surface soil horizons. TheP3 site show a < 0.01% of contribution for PC 1. However, its contribution is significant in PC 2 (>22%) showing a high quality of representation by Ca, Mg and CICE in PC2 (>0.5).

Concerning the composition of the soil bacterial community in sites, at phylum level, the alpha-diversity analysis showed higher richness (Observed and Chao1 indexes) and diversity (Shannon and Simpson indexes) in P1 and P4 (Figure 5). The values of Observed and Chao1 indexes ranged from 158.6 to 284 in the sites selected. The sites P1 and P4 shown higher values with 284 and 281 respectively, than P3 with 249 and P2 with 158.6. Likewise, the values of Shannon and Simpson indexes were lower in P2 (3.11 and 0.98, respectively) in comparison with P1 (5.14 and 0.993), P3 (5.13 and 0.992) and P4 (5.09 and 0.991). Additionally, the analysis of the taxonomic composition of bacterial communities in the sites (Figure 5) showed that Proteobacteria phylum as the most abundant taxa in the selected sites, showing a relative abundance from 56 up to 74%. The relative abundance of *Proteobacteria* was followed by the phylum *Firmicutes* with 13 up to 29%; Acidobacteria with 14 up to 7%; *Actinobacteria* with 4.2 up to 1.4%). Noteworthy, the phyla *Verrumicrobia* (from 5.7 to 0.8%) and *Cloroflexi* (from 5.7 to 0.8%) were not detected in P4. Moreover, *Planctomycetes* (from 0.4 to 4.5%) was not detected in P2. Nevertheless, the phyla *Myxococcota* (21.8%) and *Bacteroidetes* (1.1%) were only detected in P4. Noteworthy, the data reveled 4 genera presented only in P4: *Thermoanaerobaculaceae*, *Subgroup_10*; *Beijerinckiaceae*, *Psychroglaciecola*; *Sphingobacteriaceae*, *Pedobacter*; *Chitinophagaceae*, *Parafilimonas*; *Pedosphaeraceae*, *Ellin516*.

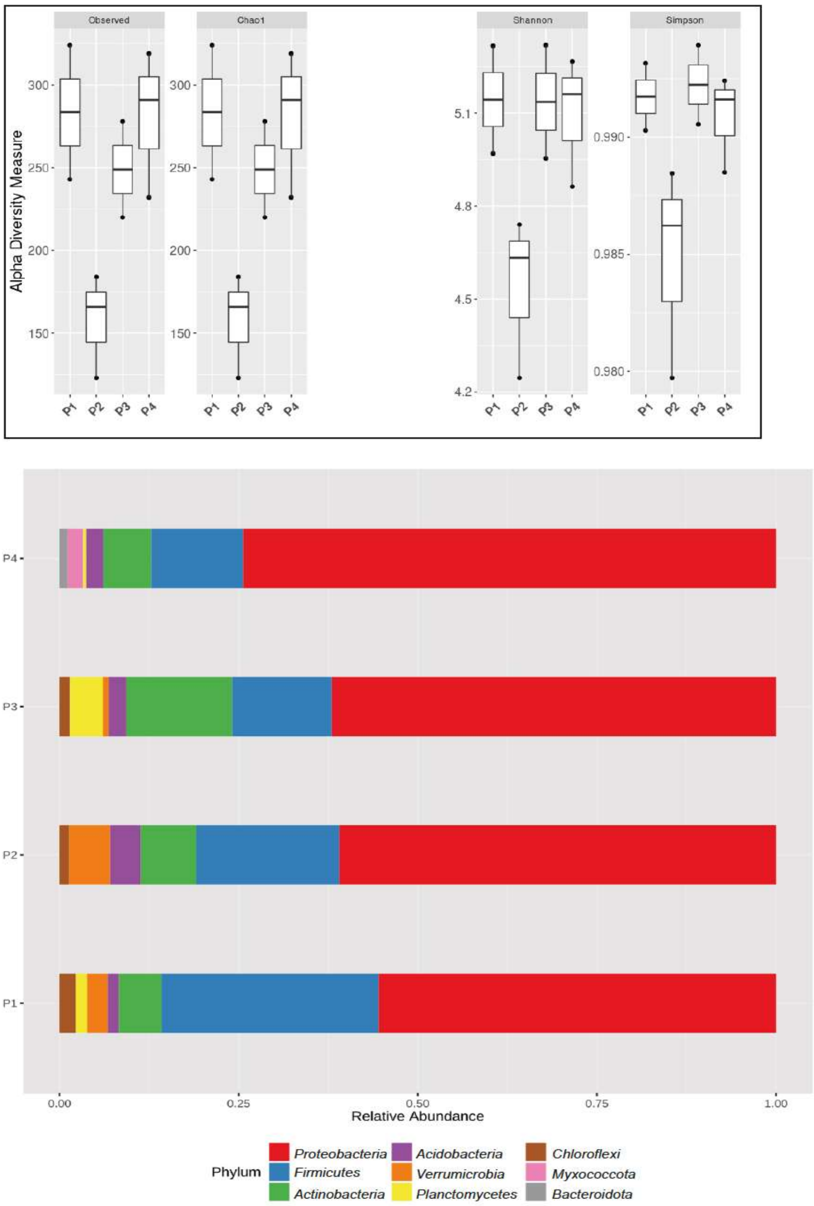


Figure 5. Diversity indexes and relative abundance at level phylum from the sampling sites (P1, P2, P3, P4).

The abundances of 16S rRNA and bacterial functional genes related to N cycling showed significant variations in the sampling sites. The sizes of the total bacterial communities based on the enumeration of the copies of 16S rRNA genes ranged from 4×10^7 to 2.29×10^5 16S rRNA gene copies g^{-1} soil dw (Table 3). Moreover, higher abundances of total bacteria (from 4×10^7 to 1.28×10^7 copies g^{-1} soil dw) were found within the sites P4 and P1, respectively in comparison with the sites P3 and P2 (from 2.29×10^5 to 1.64×10^6 copies g^{-1} soil dw, respectively). In relation to abundance of bacterial functional genes related to N cycling (Table 3), statistical differences were found within the sites P1 and P4 in comparison to the sites P2 and P3 ($p < 0.05$). The site P4 showed higher abundances of amoA (2.64×10^4 copies g^{-1} soil dw) and nosZ (6.05×10^7 copies g^{-1} soil dw) compared to the sites P3 and P2 showed values of amoA (from 3.07×10^2 to 1.35×10^4 gene copy g^{-1} soil dw) and nosZ (from 3×10^5 to 1.03×10^5 copies g^{-1} soil dw).

Table 3. Quantification of 16S rRNA and bacterial functional genes related to N cycling in soil from the sampling sites (P1, P2, P3, P4).

	Absolute Quantification (Gene Copy g^{-1} soil dw)			**Relative Quantification**	
Sites	16S rRNA ($\times 10^6$)	amoA ($\times 10^4$)	nosZ ($\times 10^5$)	amoA ($\times 10^{-4}$)	nosZ ($\times 10^{-1}$)
P1	12.8 ± 0.66 a	1.08 ± 0.123 a	2.6 ± 0.726 a	8.43 ± 18.7 a	20.3 ± 1.1 a
P2	1.64 ± 0.704 a	1.355 ± 0.416 a	3 ± 0.852 a	82.2 ± 59.2 b	1.83 ± 1.21 b
P3	0.229 ± 0.351 b	0.0307 ± 0.0132 c	1.03 ± 0.168 a	13.4 ± 26.5 a	4.5 ± 47 b
P4	40.1 ± 12.0 c	2.64 ± 0.392 b	605 ± 146 b	6.57 ± 3.27 a	15.1 ± 1.22 a

Values represent the mean ($n = 5$) ± standard deviation. Different lowercase letters in the same column indicate statistically significant differences among samples from the sampling sites ($p < 0.05$).

Comparing the relative abundance of bacterial functional genes related to N cycling in relation to total bacterial abundance measured as 16S rRNA genes (Table 3), the higher relative abundances of amoA (from 8.22×10^{-3} to 1.34×10^{-3}) genes were found in P2 and P3 compared to P4 and P1(6.57×10^{-4} to $8.43 \times 10^{-4)}$. Moreover, the higher relative abundances of nosZ (1.51×10^0) gene were found in P4 compared with P3, P2 and P1 (4.5×10^{-1} to 2.03×10^{-2}).

5. Discussion

5.1. Effect of Anthropic Disturbance on the Soil Profile of an Urban Wetland

Soils are dynamic and heterogeneous systems that present a high spatial and temporal variability of their chemical, physical and biological properties [41,42]. This definition indicates that the soil is continuously changing due to internal forces such as drying and wetting cycles [9] and external agents such as land use change [6]. When the soil system is modified by an anthropic action, such as a productive or expansion of cities (building) purposes, it results in an impact on the functions that the soil is capable to provide [43]. The changes of land use without considering the soil functionality, could deteriorate the sustainability of the soil resource over time.

The inherent and dynamic properties that define soil functions may change within the profile as a result of anthropogenic disturbance. It has been previously reported that soils derived from volcanic ash can be characterized by the Al_a content, between 800–2000 mg kg^{-1}, in the surface soil (around 20 cm of soil depth) depending on the history of land use management carried out [10,12,44]. Moreover, Al_a measurement allows to infer the type of clay, where values higher than 1000 mg kg^{-1} are correlated with highly reactive non-crystalline clays such as allophane [8,10,45]. This intrinsic soil property associated mostly with soil type, is a very important indicator as well as Bd to assess soil quality [4]. Similar to Al_a, other relevant criteria to indicate andic characteristics is pH NaF $\geq$ 9.4 [10], which is in line with the values of the present study (pH NaF $\geq$ 10) in almost all horizons at both sites with the exception of Hz1 of P4 (pH NaF = 7.7). The latter, and considering that Hz1 of P4 presents an $Al_o + 1/2Fe_o = 0.9\%$ and high OM content (>30%) could be classified as vitric ($Al_o + 1/2Fe_o > 0.4\%$; [46]), with a thin organic layer. Therefore, it is not possible to compare the Hz1 of each site, because P4 presents an organic horizon in the first 4 cm of soil that P1 presumably lost by the anthropogenic disturbance over the years. The loss of the O horizon and the exposure of the A horizon with the surface due to change of land use (from forest to pasture) has also been reported previously in Ñadi soils, which are derived from volcanic material with prolonged waterlogging conditions, [47]. As seen in Table 2, Hz1 of P1 shares similar characteristics with Hz2 of P4 through its OM content and pH NaF. Moreover, a high OM and allophane content results in a low Bd which is characteristic of volcanic soils [6,44,48,49].

The sites evaluated in this research present a significant variation associated with soil type and management. The OM content showed values of 22% in volcanic ash soils under permanent pastures [4]; 12% in non-managed naturalized pastures [44], and 40% in waterlogged volcanic soils (Aquands; [49]) in the surface soil. Therefore, the anthropic disturbance generated a significant change in OM content of the soil associated with the urban wetland. The P4 site showed >20% more OM in all horizons than in P1 (Table 2). Disturbed wetlands ("very poor") showed lower OM content than undisturbed wetlands in shallow soil layers [50]. The particle size distribution also changed with soil depth [14], where the clay content increases and the proportion of sand decreases (Figure 2), affecting the soil physical parameters such as an increase in Bd and a decrease of PAW while soil depth increase (Figure 3).

Anthropogenic disturbance in agroecosystems, such as management practices, generally takes place in the 0–20 cm depth, can be observed through changes in soil structure and their dependent properties [4,9,16]. The evaluated sites present PAW and Ka values above the proposed critical values (Figure 3), however, the AC is below the critical due to the location of the table (30 cm soil depth generating anoxic conditions of the sites. In P4 the water table was observed at 30 cm soil depth, which produces a rapid saturation of the whole soil profile in winter. The prolonged anoxia results in an acidic soil type and a higher OM accrual [51], with pH values < 5.4 in the upper soil layer. Furthermore, soils with limited drainage showed a high shrinkage capacity in summer periods [48] and are vulnerable to continuous drying and wetting cycles, altering the total pore volume negatively affecting the soil resilience after a hydraulic or mechanical stress [47].

The P1 site presents a pasture ecosystem submitted to grazing. The continuous input of external nutrients from agricultural management (fertilizers, amendments, manure, trampling and cattle urine) accelerates the cycling of nutrients in the soil surface horizons (Hz1 and Hz2) such as NO_3, NH_4 and P, increasing the potential losses (e.g leaching) or immobilization of nutrients in the system when unsuitable soil management is used [52]. This is not observed in P4, where the conditions of less disturbance show the characteristics of wetland soils formed under saturated conditions and are generally anaerobic, resulting in a higher accumulation of OM and nutrients in the upper horizons [53] and, in turn, lower values of bulk density. These anaerobic conditions due to prolonged periods of saturation in the soil profile and the intrinsic acidic condition of Andisols (pH < 5.0) facilitate the accumulation of nutrients in sites with less anthropogenic disturbance [54], as in the case of P4. Andisols are characterized by a high phosphorus retention capacity given by the high percentage of non-crystalline clay [10], thus, the amount of available P in the soil at sites P1 and P4 is below the critical level for agricultural production ($p > 12$ ppm; [55]).

The presence of Hz1 in P4 as an organic horizon marks a difference from a "less disturbed" condition given its high nutrient accumulation, acid pH and low Al_a content. Another relevant phenomenon in the Andisols of southern Chile is that the degree of acidity of these soils is also due to natural processes such as the leaching of basic cations like Mg^{2+}, Ca^{2+}, and Na^{+}, due to the amount and intensity of rainfall and soil formation processes [56,57]. This process is slow under natural conditions, but is accelerated by anthropogenic disturbance in agroecosystems [55]. The latter explains the increase of Mg, Ca and Na content in Hz4 at P1. Moreover, the higher concentration of Mg (11.9 $cmol_c\ kg^{-1}$) and Ca (9.0 $cmol_c\ kg^{-1}$) found in P4 is probably due to high capacity to retain bivalent cations [58] due to colloidal fraction and OM content [57] present in Hz1. Therefore, chemical soil analysis shows that the Hz1 in P1 does not correspond to the Hz1 in P4, seemingly due to the loss of the organic horizon in P1 as a result of anthropic disturbance.

5.2. Loss of Soil Quality as a Result of Anthropic Disturbance

Thus, loss of the surface horizon of soil as a result of anthropic action, generates a loss of soil functions [7] and also, the capacity to provide services within a particular ecosystem [59], in this case a wetland. The loss of functions can be assessed through the concept of soil quality defined by "the capacity of a soil to function, within the boundaries of diverse ecosystems, to sustain plant and animal productivity, maintain or enhance water and air quality, and support human health and habitation" [60].

Soil overexploitation or loss of the first 20 cm of soil depth due to agricultural production or land use change (e.g., urbanization) causes an irreversible change in the soil profile, altering its natural richness and its capacity to function within the boundaries of an ecosystem, i.e., decreasing soil quality. Soil functions are fundamental for the production of biomass, protection of groundwater, maintenance of biodiversity, filtering pollutants and providing physical space for human development [7]. Therefore, the loss of one or more soil functions will irreversibly impact the surrounding ecosystem. Soil properties (chemical, physical and biological) are related to soil functions [61] and are parameters that can be used to define soil quality indicators [4,6,62]. These properties present critical values beyond which the soil begins to undergo irreversible changes as a result of degradation which results in the loss of one or more of its functions [6,7]. In this sense, soil, as a heterogeneous and dynamic medium over time, is a fundamental mainstay of ecosystems. Finally, it is necessary to continue evaluating which are the critical values of the quality indicators for volcanic ash soils. Because, it has been shown in previous works that it is better to consider a percentage of soil function loss associated to several indicators than a single quality value (e.g., soil quality index), and that the comparison of critical thresholds proposed in the literature do not correspond to the values determined for these soils [4,6].

Lower soil fertility levels (e.g., P-Olsen < 4.0 ppm and Nitrate < 9.0 ppm; Table 2) in sites P1, P2 and P3 indicate that anthropogenic disturbance reduced the capacity to sustain biological communities of the surface horizon. On the contrary, in the case of P4 (corresponds to low anthropic intervention-semi-natural condition), a higher fertility level was observed, as well as a higher activity of microbial communities that consume C and N (Figure 3). This relationship between consumption and production of C and N nutrients and microbial communities has been used as indicators of soil quality [6]. In this context, the presence of bacteria in soil is important for a robust functioning of soil and related ecosystem services. Hence, there is a necessity to identify the composition, diversity, and function of the soil microbiome in order to determine its natural properties. The results sho0wed a decline of diversity and richness in the sites P2 and P3 in comparison to P1 and P4. Similarly, a decrease of copy number of 16S rRNA, nosZ and amoA genes in P2, P3 in comparison to P1 and P4. Additionally, Proteobacteria phyla was the most abundant taxa in the selected sites followed by the phyla Firmicutes, Acidobacteria, Actinobacteria Verrumicrobia and Chloroflexi. Several studies have reported these groups as the most abundant phylum in Chilean Andosol [33,63–65]. The results presented are the first biological soil approach for in urban wetlands of southern Chile and confirm that there is an alteration of biological properties as a result of anthropic disturbance.

Nutrient recycling is a complex process that has to be analyzed in a whole context, where relationship between nutrients, bacterial communities, physical properties as well as abiotic pressures have to be estimated. For example, the dynamics of organic matter deposition and mineralization determine the process of oxide-reduction by complexation or release of the N compound. Microbial activities related to biogeochemical cycling are regulated not only by the size of the microbial biomass but also by the presence, distribution, and abundance of functional taxa. Thus, functional gene markers can provide valuable understanding into the microorganisms driving key biogeochemical processes.

Nevertheless, there are few studies that have described both microbial composition and functional group abundance in soil wetlands [66]. Therefore, the knowledge of the presence, abundance and diversity of the biological soil component is a basic requirement for management, maintenance and improvement of urban wetland soils.

According to records of Valdivia city council [67], the ecosystem services evaluated by rapid assessment of wetland ecosystem services (RAWES) approach, defined that Angachilla wetland provided several ecosystem services which include maintaining water quality and supply (water for animal/ human consumption), protecting and regulation of water flows and flooding, sustaining biota (mammals, birds, medicinal plants, native trees), and providing cultural, recreational and educational resources. Nevertheless, according to our results these services could be threatened due to the loss of the surface horizon of soil (P1) as a result of entropy action, such as the presence of livestock, draining and/or filling of wetland, social housing projects.

Finally, the present study provides an important baseline and general picture of the conservation status of urban wetland soils in southern Chile. Moreover, the data from our studies represent and describe the main characteristics of a soil associated with a wetland. Additionally, the data presents the main characteristics related to the selected geographic areas at a regional scale with low intervention. In this sense, urbanization activities and agricultural production have a great impact on soil properties and highlight the sensitivity of these ecosystems in terms of carbon turnover. Therefore, the need for conservation could be critical for these ecosystems when establishing a governance and climate policies to preserve stored carbon, that could, otherwise, upon wetland drainage or degradation, enter the atmosphere [68].

6. Conclusions

Anthropogenic disturbance causes irreversible changes in the surface soil horizons, degrading the physical, chemical and biological properties of the soil. The loss of the organic horizon leads to increased nutrient cycling and also decreases the soil quality. Wetland soils should not be destined to fulfill productive or urbanization functions. However, urban expansion due to population growth goes beyond the limits that are considered as soil associated with an urban wetland. This causes constant pressures that decrease the functions of storage, filtering and cycling of nutrients, gaseous exchange and the development of biological soil communities.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/land11030394/s1. Table S1: The primer sets and conditions used for quantification of 16S rRNA, amoA and nosZ genes by qPCR [69–71].

Author Contributions: J.C.: Conceptualization, Investigation, Formal analysis, Writing—original draft, Writing—review & editing. S.V.: Writing—review & editing. J.D.: Writing—review & editing. M.C.: Writing—review & editing. J.M.: Writing—review & editing. S.Z.: Investigation. L.L.: Conceptualization, Investigation, Formal analysis, Writing—original draft, Writing—review & editing. All authors have read and agreed to the published version of the manuscript.

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