



Macroinvertebrate Community Composition in Wetlands of the Desert Southwest is Driven by wastewater-associated Nutrient Loading Despite Differences in Salinity

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Abstract

The relatively rare freshwater ecosystems in the arid southwestern United States serve as biodiversity hotspots, yet they remain among the most threatened systems in the world due to human impacts and climate change. Globally, arid region wetlands remain understudied with respect to their ecology, making assessments of quality or restoration efforts challenging. To address these needs, this project aims to better understand the factors that drive water quality and macroinvertebrate community composition of wetlands of the US desert Southwest. Water quality and macroinvertebrate data were collected over three years from 14 different wetland and riparian sites spanning across West Texas, New Mexico and Arizona. Principal Component Analysis (PCA) indicated that salinity related variables such as chloride, sulfate and conductivity were the greatest drivers of environmental variance (32%) among sampled desert wetlands. Nutrients such as nitrate and phosphate described a second axis, with 22% of variation in environmental data explained, where we found a clear distinction between wastewater and non-wastewater wetlands. Nutrients were shown to have the greatest impact on macroinvertebrate communities with wetlands receiving wastewater showing more uneven distribution of functional feeding groups and lower Simpson Index scores. These sites were dominated by filter feeders and had lower relative abundances of predator and collector-gatherer taxa. There was also a significant decrease in metrics related to diversity and environmental sensitivity such as % Ephemeroptera-Odonata-Trichoptera (EOT) within high nutrient sites. Increased salinity levels were also shown to correlate with lower Simpson Index scores indicating that increased salinity resulted in a decline in macroinvertebrate diversity and evenness. Overall, the nutrients within effluent water have shown to significantly alter community composition especially in desert wetlands where macroinvertebrates may be more adapted to high salinity. Though macroinvertebrate communities in wastewater sites may not fully resemble those of natural wetlands over time, creation of these sites can still benefit landscape level diversity.

Keywords Arid region · Desert · US Southwest · Metrics · Biotic indices

Introduction

The loss of global biodiversity is occurring at an exceedingly rapid rate due to climate change and overexploitation by humans (Dawson et al. 2011). While terrestrial ecosystems

are often in the spotlight, aquatic ecosystems surpass their rate of loss of biodiversity due to declines in water quality, changes in nutrient availability and increasing temperatures (Van De Waal et al. 2010; Woodward et al. 2010; rösmarty et al. 2010). Arid region wetlands are especially vulnerable due to altered precipitation patterns related to climate change and declining groundwater flow as a result of over-use (Burkett and Kusler 2000; Taylor et al. 2013; Richey et al. 2015). As biodiversity hotspots, these oases are habitat for many organisms and provide critical habitat connectivity within the desert landscape (Dinerstein et al. 2001; Bogan et al. 2014; Drake et al. 2017). While freshwater habitats are known to support ~10% of all species, including many

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endangered and endemic species, arid region wetland ecosystems worldwide remain understudied and under-recognized when it comes to wetland ecology and conservation (Walsh et al. 2009; Strayer and Dudgeon 2010; Hershler and Liu 2010; Minckley et al. 2013; Murphy et al. 2013; Nieto et al. 2017; Stanislawczyk et al. 2018). Due to the rapid loss of habitat, there has been a recent push to protect and restore these rare freshwater ecosystems.

In the southwest United States, many wetlands have been restored or created to replace those wetlands that have been lost. Some wetland sites use the delivery of treated wastewater to mitigate or restore water flow to areas that were previously drained or degraded due to river channelization or agricultural use (O'Geen et al. 2010; Rodriguez and Loughheed 2010). These sites create new habitats for migrating birds and aquatic organisms as well as areas of cultural value such as city parks (Andrade et al. 2018; Hamdhani et al. 2020; Bogan et al. 2020). These habitats are often used to further purify effluent water through the uptake of nutrients (i.e. nitrogen and phosphorus) and contaminants by wetland macrophytes and microalgae before replenishing groundwater sources (Whitton et al. 2016; Matamoros et al. 2017; Zhuang et al. 2019). While studies have shown these wetlands to be effective at reducing excess nutrients and contaminants from wastewater, the initial presence of these byproducts may have lasting effects on freshwater biota (Brooks 2000). In some non-arid created wetlands, increased nutrients cause shifts in community composition with an increase in pollution-tolerant macroinvertebrate taxa (Pinto et al. 2014). However, due to variables relatively unique to arid regions (i.e. extreme heat, irregular and rare precipitation), it is unknown if macroinvertebrates in arid wastewater wetlands respond the same way as those in non-arid regions.

In freshwater ecosystems, macroinvertebrates have historically been used as indicators of water quality and wetland health (Hilsenhoff 1987; Mandaville 2002). As bio-indicators, aquatic macroinvertebrates serve as a low-cost and useful tool for monitoring wetland health and function due to their constant contact with water and sediment (Hilsenhoff 1987; Cairns and Pratt 1993; Bartell 2006; Siddig et al. 2016; McIntosh et al. 2019). By monitoring the abundance, diversity, and reproductive success of these organisms we can determine habitat response to change or disturbance (Foote and Rice Hornung 2005; Siddig et al. 2016; Wu et al. 2017). While these biotic indices are easily applied to non-arid region habitats, it should not be assumed that macroinvertebrates in arid habitats will respond the same way to environmental stressors. Recent studies have even highlighted the possible disparities of using the same biotic indices across differing systems (Mazor et al. 2016; Serrano Balderas et al. 2016).

When examining wetlands in non-arid regions the differences in macroinvertebrate community composition have often been attributed to vegetation community composition (Balcombe et al. 2005; Stewart and Downing 2008; Becerra Jurado et al. 2009; Swartz et al. 2019) and water quality associated with development (Carew et al. 2007; Loughheed et al. 2008; Kobingi et al. 2009). In contrast, studies assessing arid region ponds and springs in the Chihuahuan and Sonoran deserts have pointed to hydroperiods and desiccation cycles (Esposito 2012) or wetland isolation and dispersal limitations (Stanislawczyk et al. 2018) as the driving factor of macroinvertebrate community composition. While both these arid region studies identified differences in nutrient chemistry or salinity among sites, neither identified water chemistry as a predictor of macroinvertebrate community structure, perhaps because of the limited number of sites sampled, or small gradients examined. Salinity, in particular, may be elevated in arid region water bodies due to high evaporation rates and inconsistent water availability (Williams 1999; Nielsen et al. 2003) and may increase in importance during dry periods (Lahr 1997). In addition, many permanent water bodies in the southwest are fed by groundwater, which are known to be high in salts and minerals (Borrok and Engle 2014). Furthermore, it is largely unknown what gradients of water quality organisms in desert wetlands of the US southwest are exposed to as there have been no broad scale studies to examine these environmental gradients.

The primary objective of this study was to determine how water chemistry varies among wetlands of the US desert southwest, and how this may drive macroinvertebrate community composition within these rare habitats. In particular, we assess whether metrics of macroinvertebrate diversity, tolerance and functional feeding groups are related to water source (i.e. wastewater sites vs. non-wastewater sites) or salinity. We expect that wastewater effluent and highly saline water sources of many desert wetlands will negatively affect sensitive taxa due to their sensitivity to anthropogenic factors (Ocon and Capitulo, 2004) and lead to homogenization of functional feeding groups as shown in similar studies in non-arid regions (Loughheed et al. 2008).

Study Sites

Fourteen wetlands in the US desert southwest were sampled during the summers of 2018–2020 (Fig. 1). Most sites were found in the Chihuahuan Desert, where rainfall averages 247 mm annually and occurs primarily during the summer months (June–September) when peak ambient temperatures average 36 °C (Matthews 2014). However, several sites were found in the Sonoran Desert, which receives between 75 and 380 mm of rain per year and has peak summer



Fig. 1 Map of all sites sampled in Arizona, New Mexico and Texas during the summer months of 2018–2020

Table 1 Sample sites, location of site, water source and area for 14 wetlands sampled in the Chihuahuan and Sonoran deserts. Sites 1–12 were visited in 2018 and 2019. Sites 13–14 were added in 2019 help elucidate patterns. Only sites located in El Paso, Texas were also sampled in 2020 due to travel restrictions. Code names appear in Fig. 1b. * Indicates ephemeral wetlands

#	Name	Location	Code Name	Water Source	Area (ha)
1	Tres Rios Wetlands	Phoenix, AZ	TR1, TR2	wastewater	91.49
2	Sweetwater Wetlands	Tucson, AZ	SW1, SW2	wastewater*	5.54
3	Las Palomas Marsh	Las Palomas, NM	LP	non-wastewater*	2.86
4	Rio Grande 1	Las Palomas, NM	RG1	non-wastewater	0.22
5	La Mancha Wetlands	Las Cruces, NM	LM	non-wastewater*	0.26
6	Rio Grande 2	Las Cruces, NM	RG2	non-wastewater	0.53
7	Keystone Wetlands	El Paso, TX	KS	non-wastewater	1.05
8	Crossroads Pond	El Paso, TX	CR	non-wastewater*	3.25
9	Ascarate Lake	El Paso, TX	AS	wastewater	15.73
10	Rio Bosque Wetlands	El Paso, TX	RB1, RB2	wastewater*	11.30
11	Sandia Springs	Balmorhea, TX	SS1, SS2, SS3	non-wastewater*	1.15
12	BJ Bishop Wetlands	Presidio, TX	BJ	wastewater*	1.09
13	Cattail Falls	Big Bend National Park, TX	CF	non-wastewater*	<1
14	Manzanita Springs	Guadalupe Mountains National Park, TX	MS	non-wastewater	<1

temperatures reaching up to 49 °C (Britannica 2020). During 2018 and 2019, the southwest received near-below to below average precipitation and experienced above average temperatures (NOAA 2019; NOAA 2020). Sites sampled in 2020 experienced near average precipitation with much above average temperatures (NOAA 2021).

Wetlands varied in their water sources, coming from either wastewater (effluent water from treatment plants) or non-wastewater (i.e. Rio Grande river, spring-fed or storm-water) (Table 1). Wastewater sites generally received continuous amounts of effluent water throughout the growing seasons. Non-wastewater sites included those that were flooded with water from the Rio Grande (Las Palomas, La Mancha, Rio Grande 1, Rio Grande 2); however, these were floodings and not considered riverine wetlands. Ascarate and Crossroads differed by additionally receiving storm-water inflow sporadically throughout the year, especially during the summer monsoon season. Water depths for the sites ranged from 0.3 m to greater than 1.5 m, however areas sampled were in wadable depths (<0.5 m).

Most sites were sampled twice, once a summer in two different years, however, Cattail Falls and Manzanita Springs were only sampled once due to being added later in the project and COVID-19 travel restrictions. Sites located in El Paso, TX were sampled once every summer during the three sampling years. Some sites, such as the Rio Bosque, were sampled in more than one area, as indicated in by multiple code names in Table 1 (i.e. RB1, RB2). Different areas sampled within one wetland were usually associated with separate ponded areas.

Methods

Macroinvertebrate Sampling

Prior to sampling, we qualitatively identified the three dominant macrophyte types in each wetland. Macroinvertebrate samples were then collected with three successive dips using a 250 µm d-frame kick net from each of these three habitats. Contents from all dips were pooled into 1 composite sample. Because all sites were sampled with the same effort (3 dips in 3 different habitats for a total of nine dips per wetland), abundances are reported as catch per unit effort (CPUE) and are directly comparable. Macroinvertebrates were counted and identified in the field with some specimens kept for further identification in the lab. Specimens were preserved in 70% ethanol, stored at room temperature, and identified to the order and family level (Merritt and Cummins 1996; Smith 2001).

Using these data, a variety of metrics of macroinvertebrate community composition were calculated, including those that summarized taxonomic richness, composition, and functional feeding groups. A full list of taxa with designated functional feeding guilds can be found in supplementary materials. Ephemeroptera, Odonata and Trichoptera (EOT) composition was used as measure of diversity and water quality (Mereta et al. 2013). Similar metrics including Plecoptera (i.e. EPT) were not included due their absence in our study areas. Using abundance data, Simpson's Diversity Index (λ) was calculated for each sampling visit as a measurement of macroinvertebrate diversity (Simpson 1949). Both λ scores and the percentages of functional feeding

groups were computed for each site visit, then averaged for sites that were sampled more than once (Anderson and Davis 2013).

Water Quality Sampling

At the time of macroinvertebrate collection, physicochemical conditions such as pH and conductivity were collected in the field using a YSI® 556 multi-probe (YSI Incorporated Yellow Springs, OH, USA). Dissolved organic carbon (DOC) and total dissolved nitrogen (TDN) samples were determined after filtration through pre-ashed GF/F filters and stored in precombusted amber glass bottles at 4 °C until analysis (APHA 1998). Both were determined using a Shimadzu TOC-L analyzer with TMN module. Water samples for additional water chemistry were collected from an open water location using acid washed HDPE bottles. Anion concentrations (Cl^- , SO_4^{2-} , NO_3^- , PO_4^{3-}) were measured on a Dionex 2100 ion chromatograph. Alkalinity was measured using a Mettler Toledo G20 auto-titrator. Turbidity was measured in triplicate using a Hach 2100 turbidimeter. Percent organic matter was determined using a “loss on ignition” method in which a subsample of the sediment was dried at 100 °C for one hour. The sample was then weighed and heated in a muffle furnace at 550 °C for fifteen minutes and reweighed (APHA 1998). Percent organic matter was calculated from the mass lost after ashing.

Chlorophyll-*a* concentration, as an estimate of algal biomass, was quantified for both phytoplankton and periphyton. To measure phytoplankton, a known volume of water (between 150 and 1000 mL) was collected from open water and filtered through a GF/C filter to collect algae floating in the water column. Filters were frozen until analysis. Periphyton was collected from pond sediment surfaces at three haphazard locations in each pond using a spatula and an inverted petri dish. All three periphyton samples were combined into one composite sample. Algae were separated from the sediment by rinsing with distilled water, pouring off and retaining the algal-rich supernatant solution and repeating ten times, at which point the solution typically became clear. A subsample of the resulting algal suspension was stored in a test tube, wrapped in foil and frozen until the analysis for chlorophyll. Chlorophyll *a* (CHL*a*) was extracted into 90% acetone for 24 h in the freezer. Absorbance of the extract was measured with a Genesis 10 UV spectrophotometer (APHA 1998). Concentrations were calculated on a volumetric basis for phytoplankton ($\mu\text{g L}^{-1}$) and by area sampled for periphyton ($\mu\text{g cm}^{-2}$). Phytoplankton CHL*a* was corrected for turbidity and phaeopigments by acidification (Wetzel and Likens 2002).

Data Analysis

All statistical analysis and graphing were performed in R (Version 4.1.2). A Principal Component Analysis (PCA) was used to describe underlying gradients in the environmental data. All environmental data, including physicochemical properties and algal biomass were entered into the analysis. The PCA analysis was conducted using the “princomp” function and data were transformed and standardized as required, to approximate a normal distribution (McCune and Grace 2002). Graphing of the PCA was performed with the “factoextra” package (Kassambara and Mundt 2020). Simpson Diversity Indices were calculated using the “vegan” package (Oksanen et al. 2022). Water quality and macroinvertebrate metrics were compared between wastewater and non-wastewater sites using Wilcoxon rank-sum tests, due to non-normality of data. Pearson correlation coefficients were determined to relate Simpson’s Diversity Index scores and PCA scores for all sites. Normality of residuals was confirmed for all regression analyses.

Results

Environmental Gradients

Environmental conditions ranged from nutrient-poor (non-detectable levels of N and P) to nutrient-rich, with relatively high levels of water column chlorophyll (maximum 352 $\mu\text{g/L}$), DOC (maximum 75ppm) and nutrients (Table 2). There was also a large gradient of salinity-related variables such as Cl^- and SO_4^{2-} ranging from non-detectable amounts to 828.5 and 5309 ppm, respectively. Water clarity ranged from clear (1.8 NTU) to highly turbid (208.3 NTU). Sites generally had largely inorganic sediments with the highest percentage of organic matter only 9%.

The PCA yielded two dimensions explaining more than 50% of variation in the environmental data: PCA 1 accounted for 31.9% of the variability, and PCA 2 accounted for 22.1%. For PCA1, DOC was the greatest driver of variance, along with salinity-related variables such as Cl^- , SO_4^{2-} , alkalinity and conductivity. Both total and corrected phytoplankton CHL*a* were also related to this axis (Fig. 2a; Table 3). This axis contrasted urban ponds with high salinity, such as Keystone and Crossroads, to more remote sites, such as Manzanita Springs and Cattail Falls, with relatively low salinity levels. Nutrients such as NO_3^- , PO_4^{3-} and TDN, as well as soil organic matter, were the greatest drivers of variance along PCA 2 (Fig. 2a; Table 3). This axis contrasted sites flooded with effluent water (Rio Bosque, Sweet Water, Tres Rios and BJ Bishop) to all other sites. Wetland sites flooded with water from the Rio Grande (Rio Grande 1

Table 2 Median, standard deviation and range of water physiochemical variables for wetlands sampled in the Chihuahuan and Sonoran deserts. Phytoplankton CHL_a was corrected for turbidity and phaeopigments by acidification (Wetzel and Likens 2002); Total CHL_a refers to uncorrected CHL_a values

	Median	SD	Min	Max
Conductivity (mS/cm)	3.30	3.89	0.21	16.40
DOC (ppm)	13.84	17.46	0.29	75.04
Alkalinity (meq/L)	200.11	130.80	21.98	457.62
Corrected CHL_a($\mu\text{g L}^{-1}$)	21.82	60.63	0.00	146.68
Cl⁻(ppm)	281.84	290.23	0.00	828.53
SO₄²⁻(ppm)	536.38	1073.27	0.00	5309.00
TDN (ppm)	2.84	3.85	0.00	7.00
NO₃⁻(ppm)	1.62	2.79	0.00	9.00
PO₄³⁻(ppm)	2.63	4.97	0.00	26.00
Periphyton ($\mu\text{g cm}^{-2}$)	0.00	0.01	0.00	0.02
Organic Matter %	1.29	2.93	0.00	8.97
pH	7.40	0.76	6.25	9.29
Turbidity (NTU)	24.29	38.26	1.80	208.30

and 2, Las Palomas, La Mancha) were shown to have relatively low levels of nutrients (Table 4). Differences based on sites flooded with wastewater versus those flooded with non-wastewater is especially apparent, as they occupied distinct groups on the PCA plot (Fig. 2b).

Macroinvertebrate Metrics

In total, 13,760 macroinvertebrate individuals were collected over the time of the study. Total abundances ranged from 15 to more than 1000 per unit effort, the latter being sites that were dominated by mostly Ostracods and Cladocera, while the number of taxa found at each site ranged from 2 to 10, depending on the site.

When grouped by water type, many metrics were significantly higher in sites that were fed with non-wastewater, including both tolerant and sensitive taxa (Table 5). % EOT, which was used as a measure of both diversity and water quality, was also high in site receiving non-wastewater, as were the percentage of predators and collector-gatherers (Table 5). Non-wastewater sites also had a more even representation by functional feeding groups, notably collectors, predators and filterers, while wastewater sites were largely dominated by filterers (Fig. 3). Similarly, within the non-wastewater sites (low nutrients), we found multiple taxa with relatively even percent abundances (10–15%), including Ephemeroptera, Odonata, Hemiptera, Coleoptera and Amphipoda (Table 5). Conversely, wastewater fed sites were dominated by filterers (Fig. 3; Table 5), largely represented by significantly more ostracods (62%) and cladocerans (12%).

λ scores were found to be positively associated with both PCA1 ($r^2=0.11$, $p=0.04$) and PCA 2 ($r^2=0.16$, $p=0.01$) axes (Fig. 4) indicating that increased salinity and nutrient levels resulted in a decline in macroinvertebrate community diversity and evenness. When comparing the λ scores of wastewater sites and non-wastewater sites, there was a significant difference with non-wastewater sites displaying

higher macroinvertebrate diversity scores (Table 5; Fig. 5). There were no significant correlations between percent abundances of taxa or functional feeding groups and either of the PCA axes after corrections for multiple comparisons.

Discussion

Wetlands in this study tended to vary along a gradient of either salinity or nutrient enrichment, with salinity appearing to explain more among-site variability. While salinity exhibited the greatest environmental variation amongst sites, nutrient loads from wastewater appears to be the greatest driver of variation within macroinvertebrate communities. Overall, our predictions correctly indicated that increased levels of nutrients, such as those found in wastewater from treatment sites has negative effects on macroinvertebrate diversity and abundances in sensitive taxa. Furthermore, this has shown to cause changes in distribution of functional feeding groups, specifically leading to communities dominated by filter feeders. While salinity also led to reduced diversity of macroinvertebrate taxa, we were unable to show an effect of elevated salinity on any taxonomic group or functional feeding group.

Salinity

The salinity gradient contrasted permanent and isolated spring sites such as Cattail Falls and Manzanita Springs, with low chloride, sulfate and conductivity levels, to known naturally high saline sites within El Paso, TX city limits, such as Keystone and Crossroads. The relatively high levels of salinity within these two sites are likely due their location. These arid region wetlands are both highly dependent on the regional, saline water table to maintain water levels. Groundwater is known to have high levels of salts and sulfate in the region (Hiebing et al. 2018). Irregular influx of water and rising temperatures could lead to high

Fig. 2 Plots of PCA scores of environmental data collected from 14 wetlands in the Chihuahuan and Sonoran deserts with (a) environmental vectors, where longer arrows indicate stronger correlations with the axis scores, and (b) sites grouped by water source. Sites codes are listed in Table 1 and appear with the last two digits of the year they were sampled

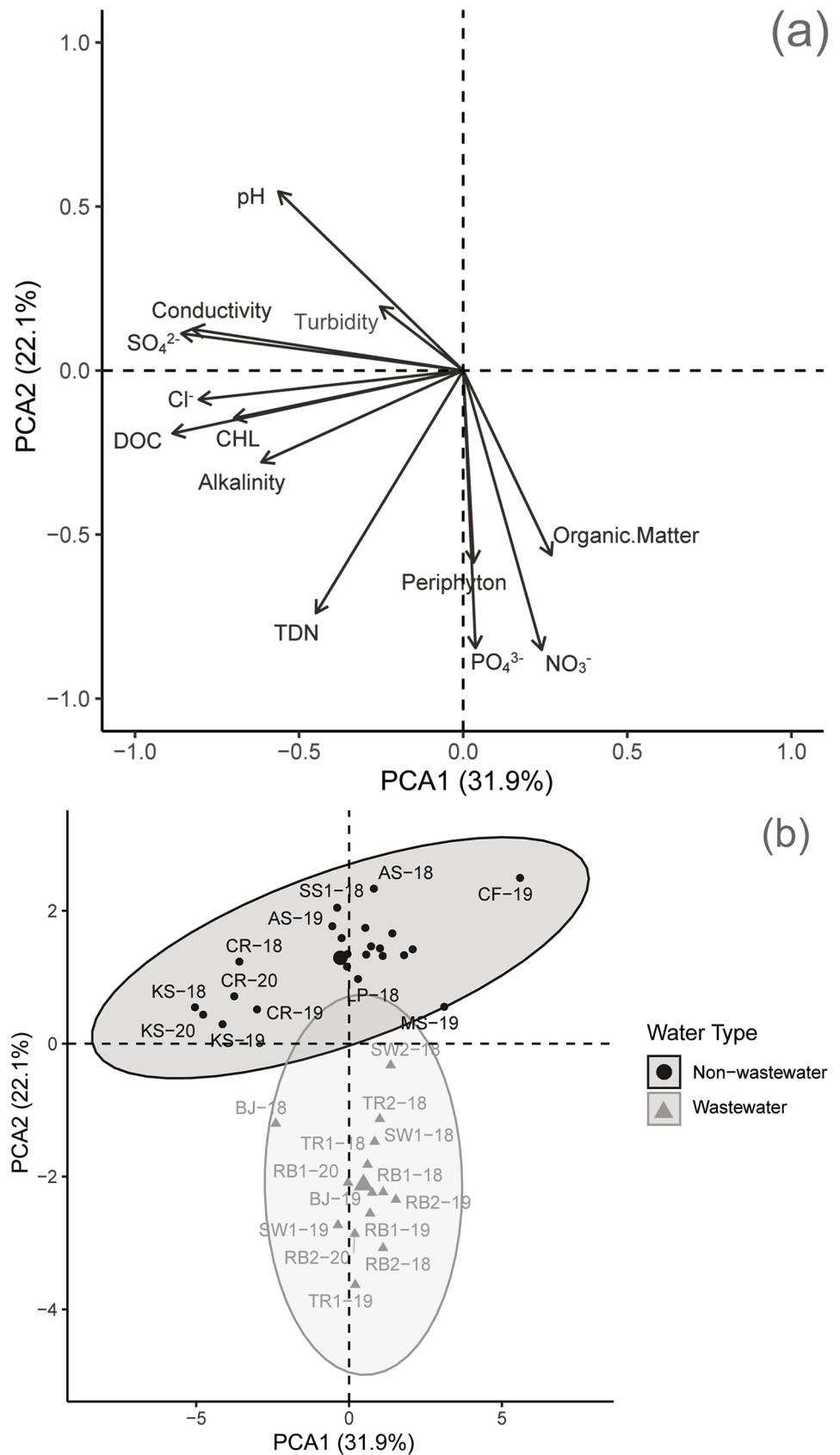


Table 3 Correlation coefficients (r) of water physiochemical parameters with PCA1 and PCA2 scores from wetlands sampled in the Chihuahuan and Sonoran deserts. Significance: *** $p < 0.0001$, ** $p < 0.01$, * $p < 0.05$

	PCA1	PCA2
Conductivity (mS/cm)	-0.8250***	0.1259
DOC (ppm)	-0.8855***	-0.1917
Alkalinity (meq/L)	-0.6148**	-0.2793
Corrected CHL a ($\mu\text{g L}^{-1}$)	-0.6981**	-0.1450
Cl $^{-}$ (ppm)	-0.8052***	-0.0884
SO $_4^{2-}$ (ppm)	-0.8586***	0.1127
TDN (ppm)	-0.4484	-0.7393***
NO $_3^{-}$ (ppm)	0.2392	-0.8511***
PO $_4^{3-}$ (ppm)	0.0374	-0.8458***
Periphyton ($\mu\text{g cm}^{-2}$)	0.0318	-0.5853*
Organic Matter %	0.2686	-0.5627*
pH	-0.5632*	0.5456*
Turbidity (NTU)	-0.2529	0.1949

Table 4 Means and Standard Error of water quality parameters grouped by water type. Wilcoxon rank sum difference between groups *** $p < 0.0001$, ** $p < 0.01$, * $p < 0.05$, without asterisks indicate non-significance

	Non-wastewater	Wastewater
Conductivity (mS/cm)	4.76 (0.98)	2.28 (0.49)
DOC (ppm)	15.45 (4.65)	7.75 (1.23)
Alkalinity (meq/L)	223.65 (24.55)	245.60 (32.32)
Corrected CHL a ($\mu\text{g L}^{-1}$)	28.76 (15.94)	21.28 (11.72)
Cl $^{-}$ (ppm)	358.51 (71.29)	155.86 (23.76)
SO $_4^{2-}$ (ppm)	951.65 (285.07)	122.31 (12.97)
TDN (ppm)	1.39 (0.40)	5.20 (1.33) **
NO $_3^{-}$ (ppm)	0.23 (0.06)	4.64 (0.89) ***
PO $_4^{3-}$ (ppm)	0.15 (0.05)	7.34 (1.72) ***
Periphyton ($\mu\text{g cm}^{-2}$)	0.001 (0.0003)	0.008 (0.002) **
Organic Matter %	0.02 (0.003)	0.05 (0.005) **
pH	7.74 (0.15) **	6.89 (0.14)
Turbidity (NTU)	30.52 (9.61)	14.05 (4.39)

evaporative conditions, which could contribute to the high levels of salinity within these sites (Jolly et al. 2008; Borrok and Engle 2014).

DOC and chlorophyll- a were also shown to vary along the salinity gradient. Sites that are highly saline have been shown to have suppressed microbial activity (including those which take up DOC) which may explain the higher levels of available DOC within these sites (Straathof et al. 2014; Yang et al. 2018). In some studies, the increase in chlorophyll- a levels within highly saline sites was related to SO $_4^{2-}$ and salt-induced aggregation of suspended matter, which can lead to increase light penetration of the water column and thus, high rates of photosynthesis (Donnelly et al. 1997; Nielsen et al. 2003). However, given we saw no effect of water clarity in our study, this is unlikely.

While the salinity gradient explained most of the environmental variability among sites, there were relatively few significant associations between salinity and metrics of macroinvertebrate community composition. Sites that were higher in salinity tended to have lower Simpson Index Scores, thus lower macroinvertebrate diversity and evenness. This remains consistent with similar studies showing negative relationships between macroinvertebrate taxonomic richness and functional evenness with increasing levels of salinity and related parameters (Kefford et al. 2004; Chemers et al. 2011; Ordonez et al. 2011; Cuthbert et al. 2020; Muresan et al. 2020). Although other studies within Chihuahuan desert freshwater systems have found that Amphipoda are adapted to high levels to salinity (Gervasio et al. 2004; Dinger et al. 2005; Cuthbert et al. 2020) and coleopterans, in general, are tolerant of high salinity within freshwaters (Lancaster and Scudder 1987; Garrido and Munilla 2008; Sharma et al. 2019; Colombetti et al. 2020), we were unable to verify these trends with our data.

Nutrients

Not surprisingly, there was a distinct difference in physiochemical features between sites flooded with wastewater and those flooded with non-wastewater. The sites flooded with wastewater were significantly higher in nutrients such as NO $_3^{-}$, PO $_4^{3-}$, and TDN, typical of effluent water (Zhuang et al. 2019; Hamdhani et al. 2020). Periphyton was also significantly higher in the wastewater sites, likely due to the high levels of nutrients, which are often a limiting factor of benthic algal communities (Power 1992; Francoeur et al. 1999).

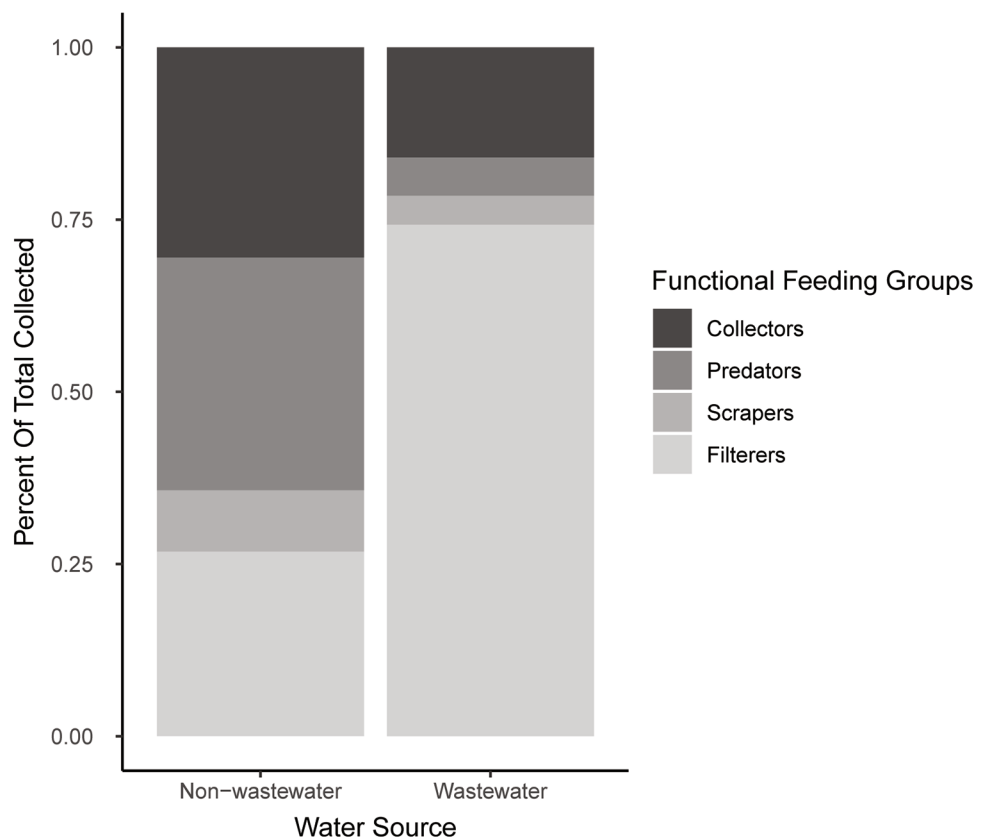
Sites with lower nutrient levels had more diverse and even macroinvertebrate communities. Loughheed et al. (2008) found that wetlands in less developed, nutrient-poor locations had increased diversity of multiple taxonomic groups. This is consistent with multiple studies finding homogenization of macroinvertebrate communities with increased nutrient levels, some stating total phosphorus as the main driver of decline in diversity (Spieles and Mitsch 2000; Hsu et al. 2011; Ouyang et al. 2018; Qu et al. 2019). Along the nutrient gradient, we saw a clear contrast in macroinvertebrate community structure between wastewater sites and non-wastewater sites. The presence of multiple taxa with relatively even percent abundances (10–15%) agrees with findings of increased evenness in non-wastewater or low nutrient sites compared to wastewater wetlands, specifically with the increase in more sensitive taxa such as Ephemeropterans (Becerra Jurado et al. 2009; Hsu et al. 2011). The percent EOT increased significantly within non-wastewater sites, likely due to their sensitivity to anthropogenic impacts (Ode et al. 2005; Kutcher and Bried 2014). The increase

Table 5 Means and Standard Error of macroinvertebrate metrics from wetlands in the Chihuahuan and Sonoran deserts grouped by non-wastewater and wastewater source type. Wilcoxon rank sum significant difference between groups ***p < 0.0001, **p < 0.01, *p < 0.05, + < 0.10, without asterisks indicate non-significance

	Non-Waste	Waste
Total taxa	7.16 (0.46)	9.78 (1.12)+
No. of orders	6.76 (0.42)	8.50 (0.81)
No. of families	6.76 (0.42)	8.50 (0.81)
Simpson Diversity Index	0.57 (0.20)*	0.39 (0.22)
% Ephemeroptera	13.56 (3.16)	3.17 (1.56)
% Odonata	10.86 (2.40)+	3.67 (1.12)
% Amphipoda	11.49 (23.07)	11.05 (20.19)
% Gastropoda	7.56 (9.7)	4.06 (7.96)
% Hemiptera	11.05 (3.59)*	1.55 (0.93)
% Coleoptera	12.54 (4.10)**	0.30 (0.15)
% Diptera	5.75 (1.30)*	2.00 (0.60)
% Chironomidae	4.49 (6.79)	1.70 (2.14)
% Cladocera	5.84 (11.7)	12.26 (26.55)+
% Decapoda	1.34 (3.71)	0.11 (0.33)
% Ostracoda	20.33 (5.62)	61.75 (7.64)**
% EOT	24.46 (3.93)**	6.85 (2.34)
% Predators	32.51 (4.98)**	5.53 (1.43)
% Scrapers	8.91 (11.58)	4.18 (8.16)
% Filterers	25.77 (6.00)	74.02 (5.78)***
% Collector-gatherers	29.38 (4.58)+	15.93 (5.35)

All figures were created using RStudio (Version 2022.02.0+443), with the exception of Fig. 1 which was created in ArcMap 10.6.1

Fig. 3 Relative abundances of functional feeding groups from wetlands in the Chihuahuan and Sonoran deserts grouped by water source types: non-wastewater and wastewater



in predators in the absence of wastewater was also found by other studies relating declines in predators as a result of increased nutrients and anthropogenic disturbances (Fu et al. 2016; Zhang et al. 2019). Corixidae, in particular, have been commonly observed in other studies in Rio Grande

habitats (Bain et al. 2011, Burdett et al. 2015), which we found were generally lower in nutrients than wastewater fed sites.

Functional feeding groups were also evenly represented in the absence of wastewater, with collectors, predators

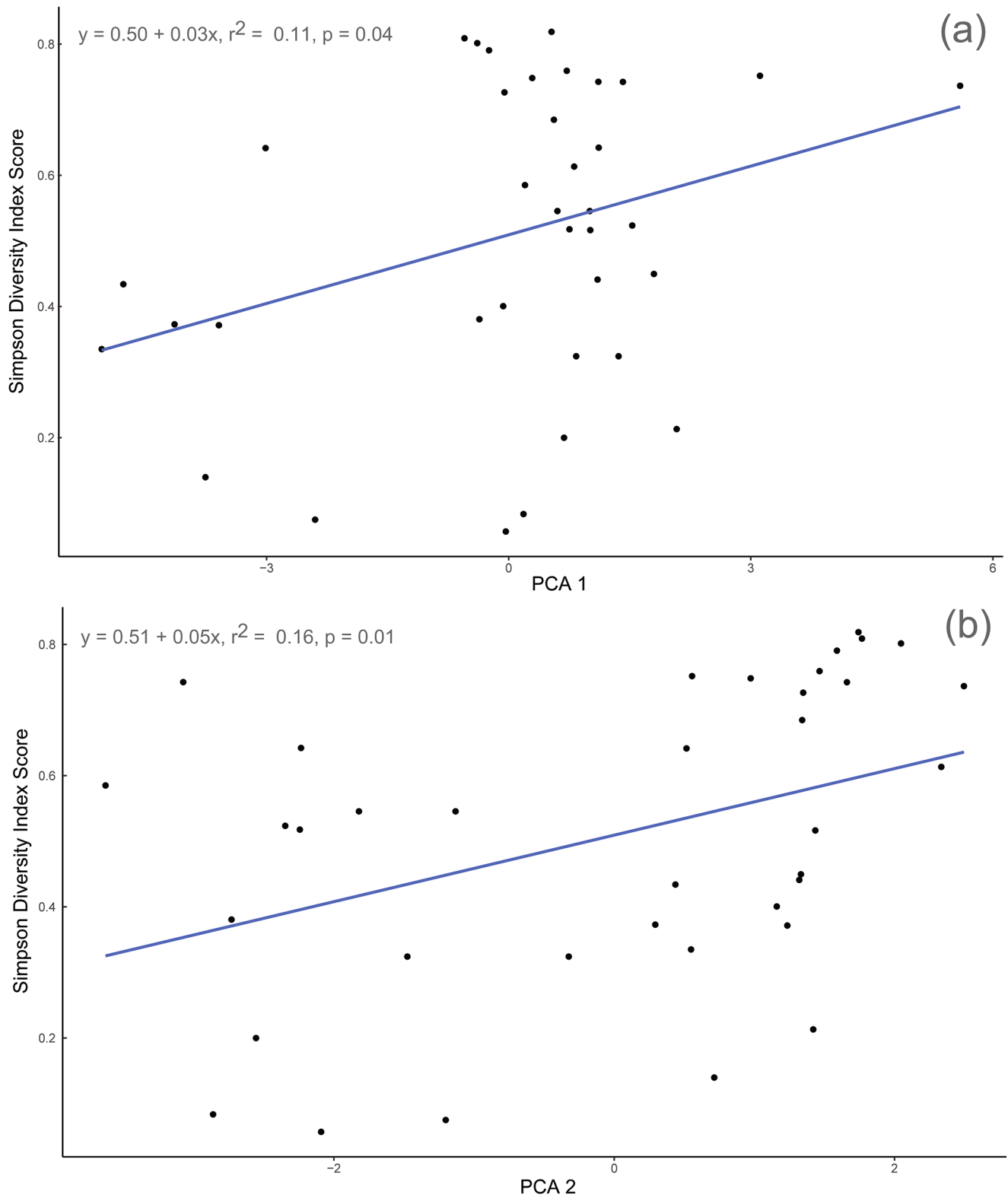
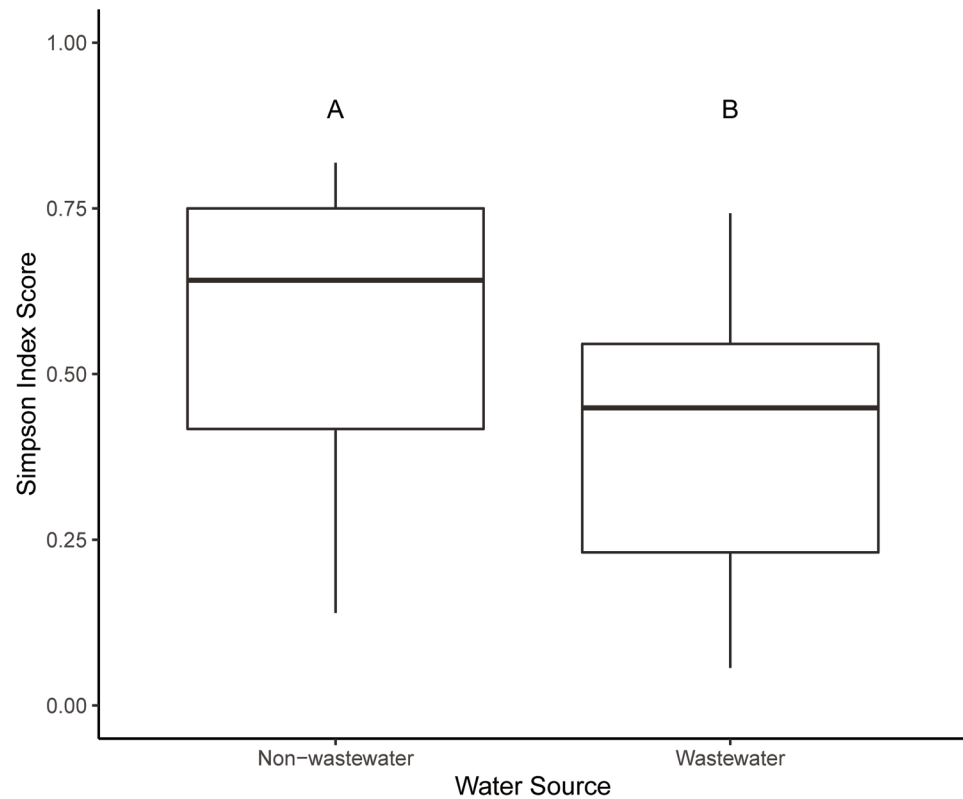


Fig. 4 Regression plots depicting significant associations ($p < 0.05$) of Simpson Diversity Index scores with (a) PCA1 and (b) PCA2 axes scores for all 14 wetlands in the Chihuahuan and Sonoran deserts

and filterers each forming approximately one-third of the composition. In contrast, filterers (ostracods in particular)

dominated the community in wastewater sites, representing more than 60% of the total abundance, and increased in

Fig. 5 Boxplot depicting average Simpson Index Scores for wetlands in the Chihuahuan and Sonoran deserts grouped by water source type: non-wastewater and wastewater. Letters indicate statistical differences ($p = 0.02$)



abundance along the PCA nutrient gradient. Increased relative abundance of filter feeders in high nutrient sites could be due to increased periphyton algae levels within these sites (Hillebrand and Kahlert 2001). There was, however, less of difference in phytoplankton *CHLa* levels among site types; this could be related to overgrazing by abundant filter feeders in wastewater-fed wetlands. Another difference found between wetland types was size, with wastewater sites being larger and having more open water. While smaller filter feeders may sometimes be more abundant in open water areas (Lougheed and Chow-Fraser, 2001), all our collections were done from vegetated zones, so we are unable to make conclusions regarding the effect of open water in this case.

Other studies indicated plant diversity as being the main driver of diversity and habitat selection in macroinvertebrates (Hsu et al. 2011; Perron and Pick 2020; Perron et al. 2021). Although we did not quantitatively evaluate plant species richness, there appeared to be a similar trend with macroinvertebrate richness increasing within sites that tended to have higher plant diversity, many of which are non-wastewater sites.

Results from this investigation could be an important consideration for maintaining or restoring biodiversity to macroinvertebrates in arid region wastewater wetlands. More research is needed to confirm whether prolonged nutrient inputs in wastewater fed wetlands leads to further

homogenization of macroinvertebrate communities, or whether this becomes an alternative stable state for these sites. Recent work has shown that the creation of wetland habitats fed by wastewater can substantially alter and improve aquatic macroinvertebrate community composition in a desert site relative to non-wetland aquatic habitats (Piña 2022). Thus, while wastewater sites are substantially different than their more natural counterparts, creation of these sites can benefit landscape level diversity (Stanislawczyk et al. 2018). We suggest that, where possible, managers of these valuable created habitats might try to find less nutrient-rich water sources, such as groundwater, to enhance the water quality in their sites. With reduced nutrient levels, we would expect to see an increased proportion of EOT, predators and collectors, among others. Further investigation is required to determine if other trophic levels are equally impacted by salinity and nutrient levels within these arid wetland ecosystems.

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Data Availability The datasets generated and analyzed during the current study are available in the Environmental Data Initiative repository, <https://doi.org/10.6073/pasta/f42ad76f0562e27544d1bb0b56f19fb6>.

Statements and Declarations

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