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Using Periphyton Assemblage and Water Quality Variables to Assess the Ecological Recovery of an Ecologically Engineered Wetland Affected by Acid Mine Drainage after a Dry Spell

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Abstract: The Grootspruit valley bottom wetland in South Africa, due to the impact of acid mine drainage (AMD) from an abandoned coal mine, was severely degraded before ecologically engineered interventions, as a passive treatment process, in 2014. The surface water flow of the wetland was redirected using concrete structures to enlarge the surface area of the wetland by 9.4 ha and to optimize passive treatment. Although the ecologically engineered interventions showed an improvement in water quality after the rewetting of the enlarged wetland areas, the 2016 drought had a devastating effect on the wetland's water quality. Limited natural removal of metals and sulfate concentrations by the wetland occurred during the 2016 drought, when compared with the 2015 pre-drought conditions. This period showed higher concentrations of metals, sulfate (SO_4^{2-}), and electrical conductivity (EC) associated with the acidic surface water. Of particular interest was an observation of a substantial shift in pollutant-tolerant algae species in the ecologically engineered wetland outflow between the years 2015 and 2016. During the dry spell period of 2016, the diatoms *Gyrosigma rautenbachiae* (Cholnoky), *Craticula buderi* (Brebisson), and *Klebsormidium acidophilum* (Noris) were observed at the outflow. The latter species were not observed during the wetland surveys of 2015, before the dry spell. From late 2017 onwards, after the drought, environmental conditions started improving. In 2018, periphyton indicator species and the surface water quality were comparable to the wetland's recorded status pre-2016. The study revealed not only a regime shift, but also an ecological function loss during the drought period of 2016, followed by recovery after the dry spell. A distinct reduction in SO_4^{2-}, sodium (Na), magnesium (Mg), EC, manganese (Mn), iron (Fe), silicon (Si), aluminum (Al), and pH, following the 2016 drought, highlights the utilization of water quality variables to not only assess the passive treatment responses of an ecologically engineered wetland, but also the progress relating to ecological recovery.

Keywords: passive treatment process; valley bottom wetland; acid mine drainage; drought; eutrophication; periphyton; metal removal

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1. Introduction

Wetlands are currently at risk from several sources, despite enabling legislation and the importance of wetlands. We cannot argue with the estimation that over half of the global wetlands have been lost already [1]. The anticipated and exponential increase of global mining activities remains a major concern, further threatening the conservation of wetlands. Mining activities result in significant impacts on wetlands, that range from complete wetland destruction to water quality and water flow alterations [2]. Therefore, an urgent need exists to restore and conserve wetlands due to the essentially unique ecological functions and associated ecosystem services they provide [3] as eco-technological and resource-conserving passive treatment systems [4–6]

Ecological engineering of wetlands is characterized by a complex and interrelated passive treatment process that must refer to a wide range of complementary disciplines typically aimed at restoring the lost biodiversity or providing ecosystem services in the form of water quality improvement [7]. Considering the fundamentals, in passive treatment systems that are open to environmental influences [8,9], acidity and/or metals are sequentially removed through gravity and natural ecological, physical, geochemical, and microbiological reactions [10]. Although wetland plants are regarded as the most visible aspect of many passive treatment wetland systems, they are but only one aspect of a complex and interrelated passive treatment process [10]. The passive treatment processes in wetlands are characterized by ion exchange and adsorption by plants and their substrates; bacterial and abiotic metal oxidation; precipitated metals settling; carbonate dissolution and processes associated with microbial enabled acid neutralization; and filtration and sulfate reduction and metal sulfide precipitation. The performance of such a passive system is influenced by initial water quality as an inflow determinant, site-specific conditions, minimal human intervention, and the nature of operations in specific climatic conditions [5,10–18]. According to Zedler [7], the need to provide specific and unique hydrological conditions (e.g., water quality and quantity) complicates wetland restoration because of the potential effect on microtopography and biogeochemistry from the degree of wetland wetness. Although there has been an increase in quantitative information on ecologically engineered wetlands over the past decade, existing knowledge on the impact of climate change and biological indicators post-ecological engineering in the literature is scarce. During the evaluation of restoration related wetland projects, many different variables have been applied such as vegetation and faunal components, soil analysis, and hydrological characteristics [19].

The establishment of the relationship between measured variables and the ecological function through the application of an experimental approach was proposed by Mitsch and Wilson [19] and Simenstad and Cordell [20]. However, ecosystem resilience (i.e., the ability of a particular system to recover from disturbance) evaluation may be the ultimate test for evaluating wetland restoration [21]. When wetlands optimally function as a passive treatment system, they can provide long-term, effective, and efficient treatment for many AMD sources [17,22]. In South Africa, wetlands rank among the most threatened ecosystems. According to Oberholster et al. [23] and Van Deventer et al. [24], recent studies have indicated that 65% wetland types of South African are under threat.

The Grootspruit wetland under study has been identified as a national freshwater ecosystem area and falls within a critically endangered wetland type. The Grootspruit wetland receives AMD effluent from an upstream abandoned coal mine and is ecologically engineered as a passive treatment process to improve its water quality. The formation of AMD is the result of the exposure of sulfide minerals to atmospheric, biological, and hydrological elements (such as oxygen, chemoautotrophic bacteria, and water). The resulting sulfuric acid, as an oxidation-generating agent, further imparts not only a low pH but also net acidity to water containing elevated levels of dissolved metal concentrations and sulfate, high conductivity, and low alkalinity [25]. At an increase in pH ($\geq$4.0), metal hydroxides precipitate (e.g., ferric hydroxides [$FeOH_3$] also known as "yellow boy"), with the potential to further smother aquatic biota. Dissolved metal ions can further penetrate aquatic biota membranes and cause toxicity at a lower pH range [26–28]. The effect of AMD on aquatic ecosystems is threefold, namely: (a) impacted aquatic communities impacted by AMD experience lethal pH levels and metal concentrations, which lead to a decrease in the diversity and richness of aquatic biota; (b) community assemblage is limited to resilient and tolerant organisms that can survive in these extreme AMD conditions; and (c) changes in the nutrient cycles of wetlands may cause abiotic disturbances and changes.

The autecology of periphyton in relationship with certain selected water quality variables was the selected target indicator in the current study and was subsequently applied to determine the treatment response of the wetland after a severe dry spell in 2016.

One of the worst droughts on record in the selected study area was the drought of 2016 [29]. Periphyton, in relationship with water quality variables, was selected as an indicator based on a report by Wehr and Sheath [30] that found the representation of algae, as the primary producer biomass in wetland systems, was between 30 and 50%. The presence of algae biomass is also not only a sensitive indicator of the physicochemical conditions and biological integrity of wetland systems, but it can also reflect changes in wetland water quality [31–33]. The current study's objective was to use periphyton assemblage in relationship with water quality variables to assess the responses of an ecologically engineered, critically endangered wetland, as a passive treatment system, receiving AMD before and after a severe dry spell. To the best of the authors' knowledge, the current study is the first report related to the responses of an ecologically engineered, critically endangered wetland receiving AMD before and after a dry spell.

2. Materials and Methods

2.1. Background and Description of the Study Site

The heavily impacted Grootspruit valley bottom wetland is geographically located at latitude −25.906480 and longitude 29.052827 and covers an area of 135.3 ha. The wetland area is directly impacted by AMD from an upstream underground abandoned coal mine (Figure 1). Due to AMD upstream from the unchanneled wetland, the wetland has become a channeled valley bottom wetland over space and time. The wetland is located in the Mpumalanga Upper Olifants River catchment (Quaternary Catchment B20G) of South Africa, and further forms part of a larger wetland system that lies along a Zaalklapspruit River tributary and the Grootspruit River. The Grootspruit River flows into the Wilge River, which is located approximately 35 km northwest of the town of Witbank in the Mpumalanga Province of South Africa.

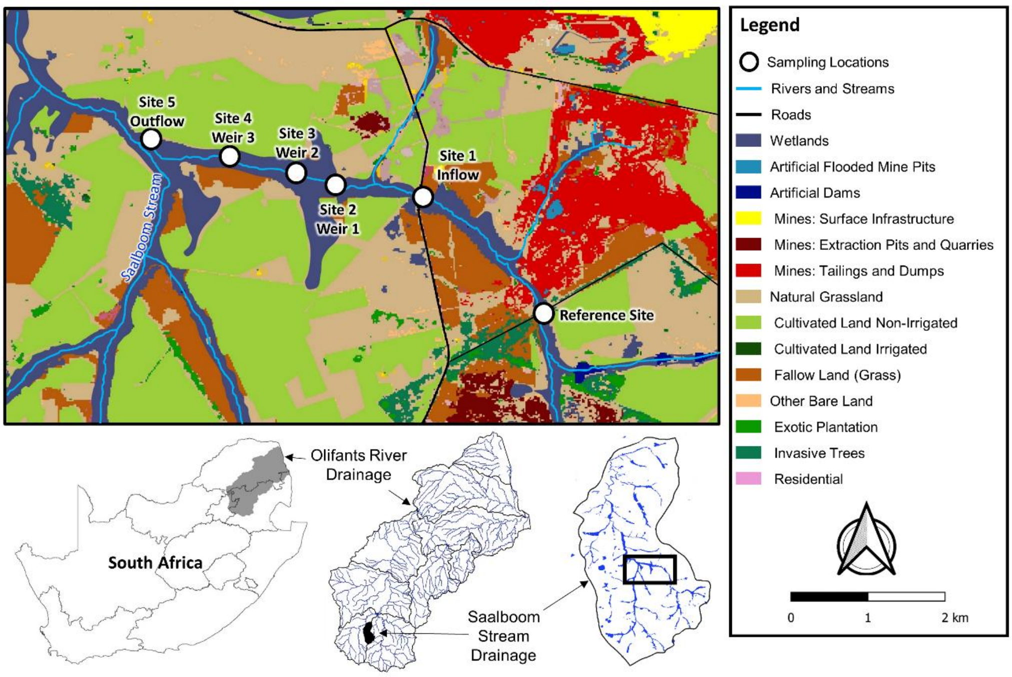
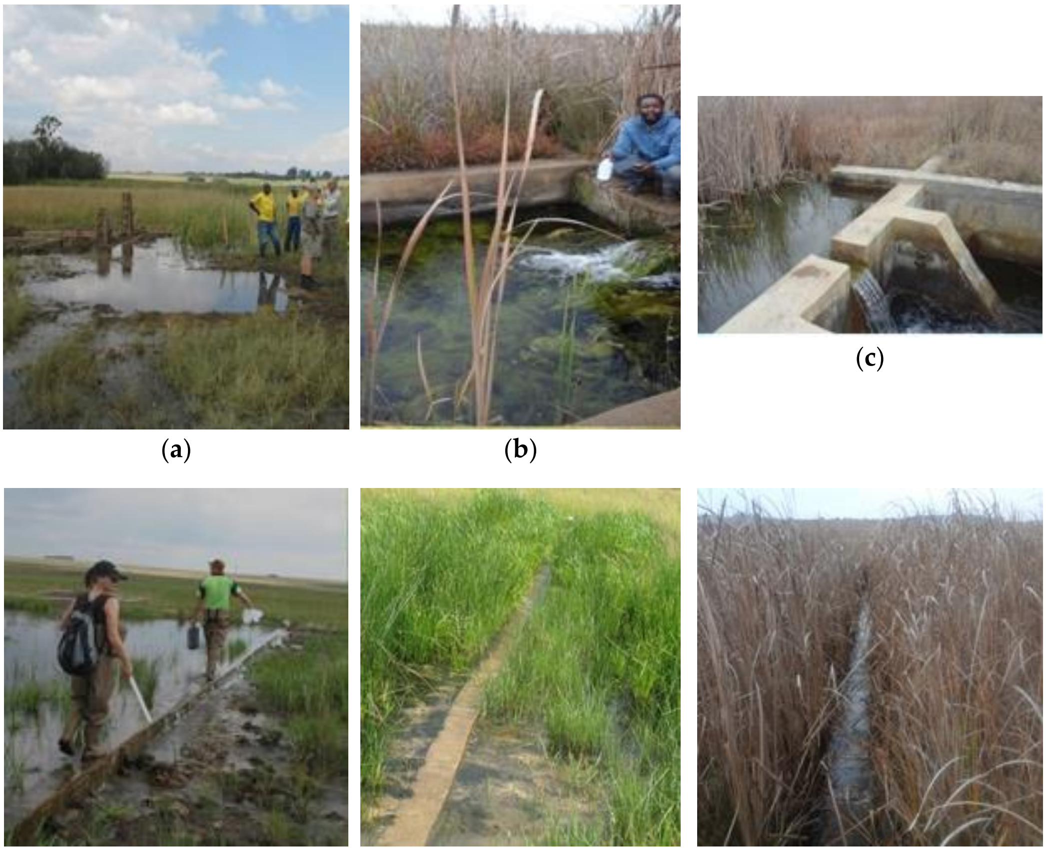


Figure 1. A map indicating the locality and extent of the Grootspruit wetland area. The map also indicates the selected sampling sites monitored over the period 2015 to 2018. The location of the study area within the Saalboom Stream quaternary catchment in the context of the larger Olifants River Basin is also indicated. The map was created with QGIS v 3.12.1 (Open Source Geospatial Foundation Project) also using the SANBI National Wetland Map Project [9] and South African National Land Cover (SANLC) 2020 datasets [34].

The Grootspruit wetland has been identified as a national freshwater ecosystem area and falls within a critically endangered wetland type. The wetland was classified as a priority wetland in a recent assessment of the conservation significance of the aquatic resources in the critical biodiversity area (CBA). The wetland falls within the Mesic Highveld Grassland Group 4 wetland vegetation group, which is further regarded as having a critically endangered threat status and has been identified as a National Freshwater Ecosystem Priority Area (NFEPA). The grass owl (*Tyto capensis*) has been observed in the wetland under study and is considered a vulnerable species for which the wetland potentially provides a habitat. The ability of the wetland to provide a water quality enhancement or ecosystem service is limited due to the channel incision caused by the decant of the abandoned coal mine upstream. Through ecological engineering, the Grootspruit wetland size was increased by 9.4 ha to allow for gravitational drainage of AMD water in 2014 [35]. The wetland enlargement process comprised the redirection of water flow using concrete structures to enlarge the wetland surface area by 9.4 ha to improve wetland water quality. The concrete structure's purpose was to change the flow of the wetland from a channeled valley bottom wetland to an unchanneled valley bottom wetland. The wetland was historically classified as an unchanneled valley bottom wetland before the upstream decanting of AMD from the abandoned coal mine. However, due to the water volume increase from the upstream decanting abandoned mine, the wetland transformed into a channeled valley bottom wetland over space and time. The transformation of the wetland caused the water quality of the wetland to deteriorate, reducing its ecosystem services. The wetland's main channels were characterized by four intervention points. The secondary incised channel was rehabilitated at three specific points (Figure 2a–f). These intervention points were strategically selected to deactivate the channelization of both the main and secondary incised wetland channels, by reducing the water velocity (<20 cm/s) through the wetland and by using man-made concrete structures to spread the flow (Figure 2). At the beginning of the ecologically engineered intervention, the water quality analysis results of Grootspruit wetland area indicated elevated sulfate, metal, and total dissolved solids (TDS) concentrations with a low average pH, all indicative of an AMD-impacted area. The wetland area was also nutrient deficient and the EC increased downstream, and hydrochemical and stable isotope contents indicated that subsurface water discharges did not support or were absent in supporting the Grootspruit wetland during the dry and wet seasons [36].

2.2. Surface Water Sampling

In situ (on-site) water quality parameters, such as pH, temperature, TDS, and EC, were measured using a handheld water quality meter (Hanna HI991300). Representative samples were taken at six specific and pre-determined sampling sites, which included the reference site 2.2 km upstream of the wetland. Samples were taken seasonally (n = 4; summer, winter, autumn, and spring) each year from 2015 to 2018 (Figure 1). Surface water samples were collected for chemistry analyses in triplicate in 1ℓ bottles at each wetland sampling site at a depth of $\pm$10 cm from the surface and kept cold on ice following the Shelton [37] sampling method. Each site's sampled water was divided further into two subsamples for specific analysis: (a) one liter was filtered through 0.45 µm pore size Whatman GF filters for dissolved nutrient analyses; (b) one liter was filtered through 1 µm Gelman glassfibre filters and preserved in nitric acid for metal analyses. Within 48 h of collection, samples were sent to an accredited chemical laboratory for analysis. The Standard Methods for the Analysis of Water and Wastewater [38] were followed for all collected water samples regarding standard chemical analysis procedures. Water quality parameters related to AMD from the literature were selected to establish the water quality of the wetland before and after the dry spell. The water samples were analyzed for the following metals and major ions: sodium (Na), calcium (Ca), magnesium (Mg), sulfate (SO_4^{2-}), silicon (Si), aluminum (Al), iron (Fe), and manganese (Mn) using an inductively coupled plasma-mass spectrometry (ICP-MS) and/or an inductively coupled plasma-optical emission spectrometer (ICP-OES). Matrixmatched standards were analyzed in parallel for quality control purposes, in addition to using the formula of Appelo and Postma [39] to determine the ionic balance. Furthermore, data were also compared with the selected value of the South African Department of Water Affairs guideline for aquatic ecosystems [40] to determine the water quality at each wetland sampling site.



(a) (b) (c) (d) (e) (f)

Figure 2. (**a**–**d**). Concrete structures built in the wetland to regulate and reduce the flow. (**e**,**f**). Revegetation of the enlarged wetland area in 2015.

2.3. Periphyton Sampling

Periphyton was collected from submerged sand and clay (5–15 cm depth), according to the method of Hauer and Lamberti [41]. A wetland transect was placed, whereafter five samples were collected 10 m apart along the wetland transect at each sampling site. The collected samples were pooled together to form a well-mixed composite sample for each of the sampling stations and for each sampling survey over the study period of 2015–2018. The composite sample was subsequently divided into four subsamples, and comprised: (a) an unpreserved sample for benthic chlorophyll (chl-*a*) analyses; (b) an unpreserved sample for laboratory culturing and identification of doubtful filamentous algae; (c) a preserved sample for microscope soft algae identification; and (d) an unpreserved sample for the identification of diatoms. The subsample containing the soft algae was preserved in 2.5% glutaraldehyde in the field, kept cold and in a dark environment until laboratory analyses. Some sample preparation was required for the diatom samples. The organic matter of each sample site had to be cleared by means of heating in a solution of potassium dichromate and sulfuric acid. The cleared material was rinsed, diluted, and mounted in a Pleurax medium for microscopic examination.

A compound microscope (Carl Zeiss, Germany) at 1250× magnification as previously described [30,42–47] was used to identify all periphyton algae. A Sedgewick Rafter sedimentation chamber was used for sediment samples and the strip–count method [38] was used as the analysis method., Doubtful filamentous algae were cultured according to the method of Stancheva et al. [48] in the laboratory for full identification as reproductive structures are required for certain filamentous algae species identification.

Each algae's taxon was grouped according to the relative abundance, as follows: + = $\leq$ 50 (rare); ++ = 51–250 (scarce); +++ = 251–1000 (common); ++++ = 1001–5000 (abundant); and +++++ = 5001–25,000 (predominant) cells per 5 cm^2. Ice-cold absolute methanol was used to extract benthic chl-*a*. Each sample's benthic chl-*a* content was determined spectrophotometrically at 647 and 664 nm wavelengths, according to the method of Porra et al. [49].

Measuring the evenness or dominance of each algal species at each sampling site was performed by applying the Berger–Parker dominance index [50]. The dominance index comprised using actual algae cell numbers as shown in Equation (1):

$$D = N_{\max}/N \tag{1}$$

where $N_{\max}$ = the number of individuals of the most abundant species present in each sample, and N = the total number of individuals collected at each site.

The specific occurrence index by Zulkifli [51] was employed. The relative density and the application of this index established a species hierarchy in the function of their importance in the various wetland samples. The species' frequency (F) in the samples of each selected site was calculated according to Equation (2):

$$\mathrm{SOI} = \mathrm{Pi}/\mathrm{P} \times 100\% \tag{2}$$

where SOI = specific occurrence index; Pi = frequency number of the species in the samples; P = total number of samples.

The periphyton species were separated into groups according to their frequency [52]:

- Absent species: not present in the samples collected;
- Rare species: present in less than 25% of the samples collected;
- Low frequency species: present in between 26 and 50% of the samples collected;
- Frequent species: present in between 51 and 90% of the samples collected;
- Permanent species: present in more than 90% of the samples collected.

2.4. Wetland Substrate Cover and Erosion

The determination of the wetland substrate cover (i.e., macrophytes) was visually according to the method of Stevenson and Bahls [53]. The degree of erosion was assessed according to the methodology of Spencer et al. [54].

2.5. Data Analysis

The Shannon and Simpson diversity indexes [55,56] were calculated based on periphyton abundance scores using Primer7 v 7.0.13 (PRIMER-e, NZ). Normality and homogeneity of variance of data were assessed using the respective Shapiro–Wilks and Levene tests. Pairwise differences between upstream and downstream water chemistry and periphyton diversity were assessed using Student's *t*-test or the Mann–Whitney U-test for non-parametric and parametric datasets, respectively. The analyses were repeated with the drought year (2016) excluded from the datasets.

Principal component analysis (PCA) was applied to assess the associations between assemblages of periphyton and water chemistry parameters across the study period and expressed as triplots [57]. The periphyton data were centered, standardized, and applied as the PCA triplot's focal plot, whereas water chemistry variables were included as supplementary variables. The Ter Braak and Smilauer method [58] was applied for the PCA triplot interpretation. A positive correlation is indicated when the angle is close to 0°, whereas an angle close to 90° indicates uncorrelated variables. Negatively correlated variables are indicated when the angle is close to 180°. Statistical analyses were performed using

Microsoft Excel, Statistica v 13 (Tibco Software, Palo Alto, CA, USA) and Canonco v 5 (Microcomputer Power, Ithaca, NY, USA).

3. Results

3.1. Dynamics of Periphyton at the Inflow of the Ecologically Engineered Wetland (2015–2018)

The periphyton biomass (chl-*a* of 47 mg m^{-2}) was measured during the dry spell in 2016 at the inflow of the wetland (Table 1). At the inflow of the wetland, periphyton mats were dominated (Berger–Parker index 0.471) by the filamentous algal species *Klebsormidium acidophilum* (Novis). The diatoms that dominated the wetland inflow sampling site during the complete study period from 2015 to 2018 included *Nitzschia clausii* (Hantzsch) (0.291); *Craticula buderi* (Brebisson) (0.278) and *Gomphonema aff. gracile* (Ehrenberg) (0.311) (Table 2).

Table 1. Survey sites (six) description and biotic and abiotic characteristics over the sampling period of 2015–2018 (*n* = 16).

Sites	Bottom Substrate Characteristics	In-Stream Macrophytes	Average Site Depth (cm)	Source of Impact	Bank Stability	Average Water Column Flow Velocity	Average Benthic chl-*a* mg m^{-2}	Average Thickness Layer (mm) of Bottom Hydroxide Precipitates	Turbidity (Nephelometric Unit [NTU])
Reference site	Sand, silt	*Typha capensis*	58 cm	Agriculture activities upstream	Stable	17 cm S^{-1}	13.1 mg m^{-2}	0 mm	6 NTU
Inflow (Site 1)	Sand	*Typha capensis*	22 cm	Coal mining AMD effluent	Poor	41 cm S^{-1}	37.5 mg m^{-2}	0 mm	2 NTU
Site 2	Silt, clay	*Typha capensis; Phragmites australis*	17 cm	Coal mining AMD effluent upstream	Poor	32 cm S^{-1}	16.7 mg m^{-2}	3 mm	13 NTU
Site 3		*Typha capensis; Pycreus nitidus; Kyllinga erecta*	14 cm	Coal mining AMD effluent upstream	Stable	12 cm S^{-1}	19.34 mg m^{-2}	1 mm	3 NTU
Site 4	Clay, sand	*Typha capensis; Pycreus nitidus; Fimbristylis complanata*	16 cm	Coal mining effluent upstream	Stable	11 cm S^{-1}	18.2 mg m^{-2}	1 mm	2 NTU
Outflow (Site 5)	Clay, silt	*Typha capensis; Kyllinga erecta; Fimbristylis complanata*	23 cm	Coal mining effluent upstream	Stable	23 cm S^{-1}	10.6 mg m^{-2}	0 mm	3 NTU

• Bank stability was assessed according to Spencer et al. [54]. • Instream macrophytes were identified according to Gerber et al. [59]. • Flow velocity was measured with a Magna Rod. • Turbidity was measured with a Hach 2100Q portable turbidimeter.

Table 2. Composition of the periphyton communities at the wetland area inflow and outflow over the period 2015–2018 (*n* = 16) *.

Species	Autecology of Dominant Benthic Algae	Reference Site	2015 Inflow	2015 Outflow	2016 Inflow	2016 Outflow	2017 Inflow	2017 Outflow	2018 Inflow	2018 Outflow	Frequency
Bacillariophyta											
Achnanthidium exiguum				++				+		++	33%
Amphora coffeaeformis				+				+		++	33%
Cocconeis pediculus		++								+	22%
Craticula buderi	Occurs in mine effluent characterized by moderated to elevated electrolyte content [47]		++		++++	++++	++++	+	+++		66%
Craticula cuspidate			+		+	+			+		44%
Ctenophora pulchella			+		++						22%
Cymbella kappii		+									11%
Cymbella neocistula		+		++							22%
Cymbella tumida		++									11%
Diatoma vulgaris		++		+						+	33%
Flagilaria ulna		++		++							22%
Frustulia vulgaris		+		+						++	33%

Table 2. *Cont.*

Species	Autecology of Dominant Benthic Algae	Reference Site	2015 Inflow	2015 Outflow	2016 Inflow	2016 Outflow	2017 Inflow	2017 Outflow	2018 Inflow	2018 Outflow	Frequency
Gomphonema aff. Gracile	This taxon can tolerate extremely polluted conditions and is found in abundance in mining effluent [47].		++		++++	++++	+++	+	+++		66%
Gomphonema parvulum	In general, considered to be tolerant of extremely polluted and anthropogenically modified conditions [47].		++		++++	++++	++	+	++		66%
Gomphonema pseudoaugur		++		+							22%
Gyrosigma scalproides		+									11%
Gyrosigma acuminatum	Species are found in electrolyte-rich water [47].				+++	+++	++	+	++		55%
Gyrosigma attenuatum					++		++		++		33%
Gyrosigma rautenbachiae	This taxon is associated with water anthropogenically impacted by industrial pollutants [47].		+		+++	+++		+	+		55%

Table 2. *Cont.*

Species	Autecology of Dominant Benthic Algae	Reference Site	2015 Inflow	2015 Outflow	2016 Inflow	2016 Outflow	2017 Inflow	2017 Outflow	2018 Inflow	2018 Outflow	Frequency
Melosira variance	This taxon is associated with eutrophic conditions [47].	++				+++				++++	33%
Navicula cryptotenella		++		++							22%
Navicula microcari					+			+	++		33%
Navicula pupala		++		++						+	33%
Navicula tripunctata		+++		+							22%
Nitschia clausii	This taxon is tolerant of strong pollution conditions and associated with industrial effluents [47].		++++		++++	+++	+++	++	+++	+	77%
Nitzschia communis	This taxon is associated with mining effluents [27].		+++	+	++++	++	++++	+	+		77%
Nitzschia nana				++				+		++	33%
Nitzschia sublinearis				++						++	22%
Nitzschiaintermedia		+		+							22%
Nitzschia pura			+							++	22%
Nitzschia reversa			++		++		++	+	+		55%

Table 2. *Cont.*

Species	Autecology of Dominant Benthic Algae	Reference Site	2015 Inflow	2015 Outflow	2016 Inflow	2016 Outflow	2017 Inflow	2017 Outflow	2018 Inflow	2018 Outflow	Frequency
Nitzschia umbonate	This taxon is a good indicator of eutrophic conditions [47].	++				+++++				+++	33%
Pinnularia viridiformis		++		+						+	33%
Pinnularia viridis	This taxon is known to occur in circumneutral water with electrolyte content levels that are low to moderate [47].			++				+		+++	33%
Rhopalodia gibba				++						++	22%
Synedra ulna		+++	++	++	+	+	+		+	+	88%
Tabellaria flocculosa	This taxon flourishes in circumneutral, oligotrophic, or slightly acidic water [47].			++						+++	22%
Chlorophyta											
Cladophora glomerata		++						+			22%
Closterium margaritiferum				++						+	22%

Table 2. *Cont.*

Species	Autecology of Dominant Benthic Algae	Reference Site	2015 Inflow	2015 Outflow	2016 Inflow	2016 Outflow	2017 Inflow	2017 Outflow	2018 Inflow	2018 Outflow	Frequency
Closterium peracerosum				+							11%
Closterium spinosporum				+						+	22%
Cosmarium hammeri										++	11%
Cosmarium pseudopraemorsium				++						++	22%
Microspora quadrata		+++		+			++		+		44%
Pandorina sp.	This taxon is found in meso to eutrophic types of water [46].	+++								+	22%
Scenedesmus armatus	This taxon is found in meso to eutrophic types of water [46].	++++						++		+	11%
Spirogyra Africana		++		+							11%
Spirogyra reinhardi		++								+++	11%
Staurastrum anatinum		++		++				+		+	11%
Streptophyta											
Klebsormidium acidophilum	This taxon is found in acidic water related to AMD effluent from mining activities [2].		+++	+	++++	++	+++	+	+++		77%

Table 2. *Cont.*

Species	Autecology of Dominant Benthic Algae	Reference Site	2015 Inflow	2015 Outflow	2016 Inflow	2016 Outflow	2017 Inflow	2017 Outflow	2018 Inflow	2018 Outflow	Frequency
Closterium peracerosum				+							11%
Closterium spinosporum				+						+	22%
Cosmarium hammeri										++	11%
Cosmarium pseudopraemorsium				++						++	22%
Microspora quadrata		+++		+			++		+		44%
Pandorina sp.	This taxon is found in meso to eutrophic types of water [46].	+++								+	22%
Scenedesmus armatus	This taxon is found in meso to eutrophic types of water [46].	++++						++		+	11%
Spirogyra Africana		++		+							11%
Spirogyra reinhardi		++								+++	11%
Staurastrum anatinum		++		++				+		+	11%
Streptophyta											
Klebsormidium acidophilum	This taxon is found in acidic water related to AMD effluent from mining activities [2].		+++	+	++++	++	+++	+	+++		77%

Table 2. *Cont.*

Species	Autecology of Dominant Benthic Algae	Reference Site	2015 Inflow	2015 Outflow	2016 Inflow	2016 Outflow	2017 Inflow	2017 Outflow	2018 Inflow	2018 Outflow	Frequency
Klebsormidium rivulare				+++				++		+++	33%
Mougeotia cf. *laevis*	This taxon is associated with mining effluents [60].		+		++		+	++	+	+++	66%
Zygnema cf. *cylindrospermum*				+		+					22%
Euglena sociabilis		++				+					22%
Phacus Pleuronectes		+									11%
Trachelomonas intermedia		++		++				++		+	44%
Cyanophyta											
Cylindrospermopsis raciborskii		++									11%
Merismopedia punctata		+		+							22%
Oscillatoria princeps		+++				++				++	33%
Oscillatoria tenuis		++									11%
Rhodophyta											
Batrachospermum atrum	This taxon is associated with mining effluents [61].		+++		+	++	+		++		55%

* The relative abundance of each benthic algal taxa was grouped as follows: + = ≤50 (rare); ++ = 51–250 (scarce); +++ = 251–1000 (common); ++++ = 1001–5000 (abundant); and +++++ = 5001–25,000 (predominant) cells/5 cm^2.

3.2. Dynamics of Periphyton at the Outflow of the Wetland (2015–2018)

At the outflow of the ecologically engineered wetland, between 2015 and 2016, a distinctive shift in pollution tolerant algal species was observed. During the dry spell period of 2016, the diatoms *Gyrosigma rautenbachiae* (Cholnoky), *Craticula buderi* (Brebisson), and *Klebsormidium acidophilum* (Noris) were observed at the outflow. The latter species were not observed during the wetland surveys of 2015 before the dry spell. The wetland dominant periphyton and benthic chl-*a* species in 2016 indicated a noteworthy difference from data generated during the wet season of late 2017−2018. The periphyton in the outflow of the wetland in 2015 (pre-drought conditions) and in 2018 (post-drought conditions) was dominated by green filamentous algal *Klebsormidium rivulare* (Kützing) (chl-*a* of 16.1 mg m^{-2}; 12.1 mg m^{-2}). In addition, the diatoms *Tabellaria flocculosa* (Roth) and *Nitzschia nana* (Grunow), which replaced the diatoms *Craticula buderi* (Brebisson) and *Nitzschia clausii* (Hantzsch) that were dominant during the dry spell of 2016 (Table 2), also dominated the outflow of the wetland in 2018.

Both the Shannon and Simpson diversity index values for periphyton were significantly higher downstream of the ecologically engineered Grootspruit wetland than at the inflow when the entire period of the study was collectively considered ($p = 0.022$ and 0.044, respectively) (Figures 3 and 4). Similarly, both of the aforementioned indexes indicated significantly higher diversity in the outflow than the inflow when the drought year was excluded from the analysis (Shannon: $p = 0.011$; Simpson: $p = 0.004$).

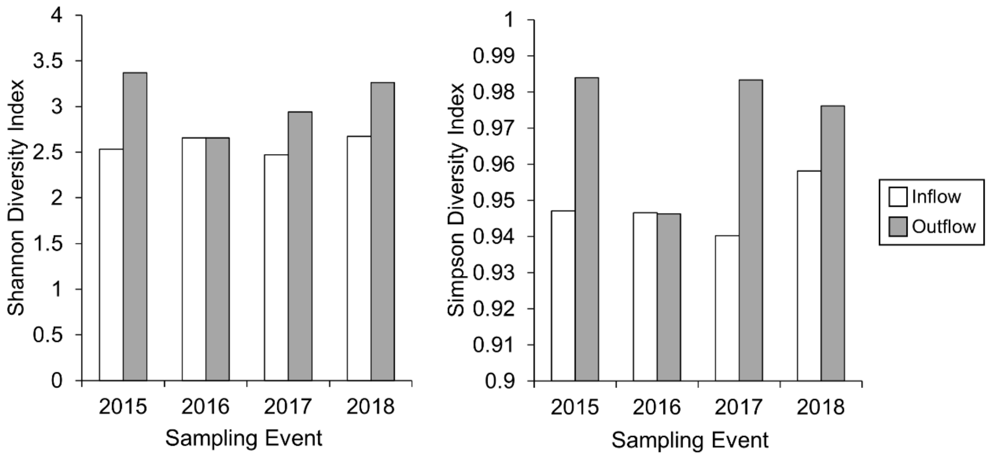


Figure 3. Shannon and Simpson diversity indexes of the periphyton at the inflow and outflow of the Grootspruit wetland over the period of 2015 to 2018.

3.3. Periphyton Dynamics after the Dry Spell of 2016

The diatoms' community structure changed as succession progressed after the drought of 2016 (Table 3). *Nitzschia umbonata* and *Melosira variance*, which are eutrophic diatom indicator species, occurred in abundance at the outflow from 2016 to 2018 (Table 2). These eutrophic indicator species were completely absent during the pre-2016 drought period of 2015.

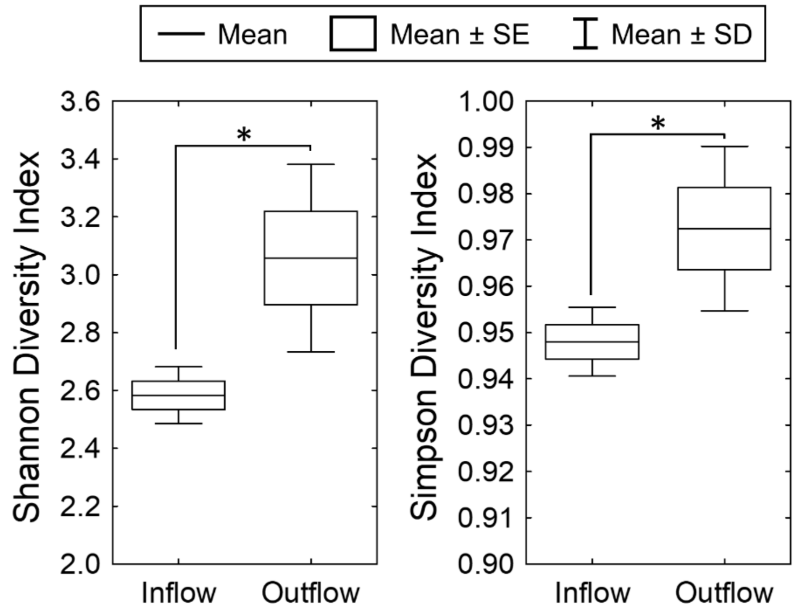


Figure 4. Shannon and Simpson diversity indexes of the periphyton at the inflow and outflow of the ecologically engineered Grootspruit wetland. The statistical analysis was performed with the drought year (2016) included and excluded. The asterisks indicate noteworthy statistical differences, $p < 0.05$.

Table 3. Average chemical and physical parameters measured during the sampling period of 2015–2018 at the inflow and outflow of the wetland ($n = 16$).

Parameter	Unit	Reference Site 2018	2015 Inflow	2015 Outflow	2016 Inflow	2016 Outflow	2017 Inflow	2017 Outflow	2018 Inflow	2018 Outflow
Sulfate (SO_4^{2-})	mg L^{-1}	37	1280	341	1610	1580	735	658	1380	471
Alkalinity ($CaCO_3$)	mg L^{-1}	24	8.7	141	5	6	3.8	5	2.5	44
Sodium (Na)	mg L^{-1}	11	32	26	46	43	24	22	35	28
Calcium (Ca)	mg L^{-1}	9	383		437	419	200	173	371	121
Magnesium (Mg)	mg L^{-1}	5.9	67	41	133	135	63	55	108	39
Dissolved organic carbon (DOC)	mg L^{-1}	5.2	2.2	3.7	2.5	2.8	2.8	5.6	1.9	4.3
Electrical conductivity (EC)	µS/cm	180	2357	803	2600	2450	1320	1240	2500	1060
Total phosphorus (TP)	mg L^{-1}	0.12	0.03	0.05	0.05	1.0	0.05	0.09	0.05	0.06
Total nitrogen (TN)	mg L^{-1}	1.2			0.05	0.06	0.05	0.04	0.05	0.05
Aluminium (Al)	mg L^{-1}	0.01	3.8	0.05	10.6	10.1	10.4	7.1	5.9	0.01
Iron (Fe)	mg L^{-1}	0.05	7.03	0.06	9.7	8.1	2.3	1.4	1.9	0.08
Manganese (Mn)	mg L^{-1}	0.04	14.5	0.09	17.0	16.5	11.7	6.3	13	0.02
Silicon (Si)	mg L^{-1}	0.7	6.3	4.5	8.5	6.4	7.2	3.8	9.1	2.3
pH		6.9	3.17	7.03	3.6	4.0	3.8	6.9	3.4	7.5

3.4. Surface Water Chemistry

From Table 3, it is evident that there was a major change in ionic distribution between the inflow and outflow during the drought in 2016, and during the pre-and post-drought conditions. The downstream sites from the wetland inflow to the outflow showed a significant SO_4^{2-}, decrease, whereas alkalinity increased at the outflow pre and post the 2016 drought. The SO_4^{2-} concentration of SO_4 from the wetland inflow decreased by 2%, from an average of 1610 to 1580 mg L^{-1} at the wetland outflow during the dry spell of 2016, indicating very little passive treatment capacity throughout the wetland. A 68.2% decrease in SO_4^{2-} was observed in 2018 from an average of 1480 mg L^{-1} at the inflow to 471 mg L^{-1} at the outflow sites, showing improvement in passive treatment capacity after the dry spell. The determined SO_4^{2-}, alkalinity and pH for the reference site remained comparatively unchanged during the sampling period and it was therefore not impacted by the dry spell (Table 3). The alkalinity, pH, and SO_4^{2-} concentration increased, whereas the EC decreased from an average concentration of 2500 µS cm^{-1} (by 57.8%) at the wetland inflow to 1060 µS cm^{-1} at the wetland outflow after the dry spell in 2016 (Table 3). However, the EC values were still higher in 2018 after the drought when compared with the measurements taken before the drought in 2015 (Table 3). From the surface water chemistry data, it can be concluded that there was a progressive water quality improvement from 2017 to 2018 after the drought. The metals primarily associated with mining effluent AMD (i.e., Fe, Al, and Mn) were reduced in 2018 at the wetland outflow, and the value was even below that of the reference site in the case of Mn (Table 3). The total phosphates increased at the middle and outflow sites during 2016 and 2017, but decreased in 2018 to pre-drought concentrations measured in 2015. When comparing the data generated from 2015 to 2018 with the selected value of the South African Department of Water Affairs [25] guideline for aquatic ecosystems, it was evident that, in 2016, the values for SO_4^{2-}, Al, Mn, and Fe were above the guideline values for aquatic ecosystems at the outflow. However, after the drought in 2018, the Al, Mn, and Fe values were below the South African Department of Water Affairs [40] guideline set out for aquatic ecosystems.

The DOC concentration and pH were notably higher in the wetland outflow (p = 0.030 and 0.004, correlatively) than in the wetland inflow, whereas Si was notably lower in the outflow (p = 0.016) when the drought year of 2016 was included in the analyses (Table 3). When the drought year was excluded from the analysis, the water chemistry profile was more distinct between the inflow and outflow samples. In particular, significant differences between inflow and outflow existed for the following: DOC (p = 0.023); pH (p < 0.001); calcium (p = 0.048); manganese (p = 0.008); silica (p = 0.019); and sulfate (SO_4^{2-}) (p = 0.044). The PCA triplot indicates a grouping of the four inflow samples and the outflow sample representing the 2016 drought year and association with the elements tested for, in addition to SO_4 and EC (Figure 5). The 2015 and 2016 outflow samples formed a further grouping in ordinal space and were negatively associated with the chemicals tested for and positively associated with increased pH and calcium carbonate ($CaCO_3$). Moreover, the 2017 outflow sample was located between the two aforementioned groupings, having a unique identity, as was the case with the reference site being segregated in ordinal space (Figure 5). In addition, the inflow–2016 outflow grouping was associated with periphyton indicators of extreme pollution conditions (Figure 5).

The influence of the drought year on wetland reclamation efficiency can be seen when considering differences among the inflow and outflow water chemistry profiles. When a dataset of all four sampling events (2015–2018) was assessed, three parameters, namely, pH, DOC, and Si, varied significantly among the inflow and outflow locations. Both pH and DOC were higher, indicating increased water quality. However, when the drought year was excluded from the analysis, six parameters, namely pH, DOC, Ca, Mn, Si, and SO_4^{2-}, varied significantly among the inflow and outflow locations, indicating more efficient water reclamation.

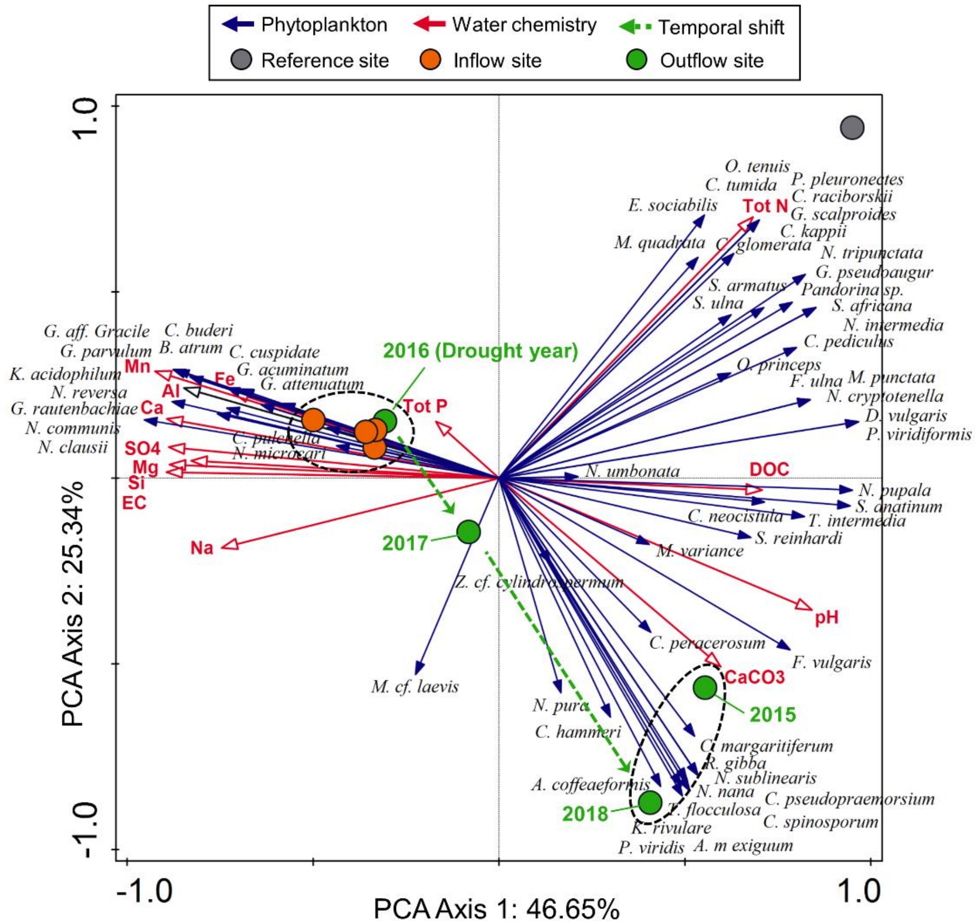


Figure 5. A principal component analysis triplot indicating the various associations between water chemistry and phytoplankton assemblage in reference to the inflow and outflow of the Grootspruit wetland and the reference site. The plot represents the entire study period (2015–2018) for the inflow and outflow locations and a reference site sampled during 2018. Phytoplankton data were applied as a focal PCA plot, and chemistry data as supplementary variables. Abbreviations: EC: electrical conductivity; Tot N: total nitrogen; DOC: dissolved organic carbon; Tot P: total phosphate.

A PCA triplot describing associations between periphyton and surface water chemistry in relation to the four sampling events and the reference site indicated a clear grouping of the 2016 drought year outflow with the inflow samples. Water quality downstream of the wetland during the drought year, therefore, corresponded to the poorer quality water released into the wetland. Although water quality and periphyton assemblage identity improved in the wetland outflow during 2017, recovery to the pre-drought state of 2015 only occurred in 2018 (Table 2, Figure 5), suggesting that a minimum of a two-year recovery period is required after a severe drought.

4. Discussion

Evidence collected from the ecologically engineered Grootspruit wetland during the 2016 drought—as one of the worst droughts on record for the Mpumalanga province, South Africa [29]—suggested that the loss of ecological functions and the resultant regime change during the drought period was a consequence of the interplay between chemical, physical, and biological processes as observed between the different sampling trips from early 2015 to 2018.

The biodiversity and autecology of the periphyton indicated a slow recovery of the wetland to its prior 2015 state, but the presence of certain diatoms, for example, *Nitzschia umbonata* and *Melosira variance*, also signaled reconfiguration to a state of eutrophication, (sampling sites 3 to 5 during 2016 to 2018). This may be partly due to the sulfate reduction indirectly affecting the nutrient kinetics of the wetland [62]. It was previously shown that sulfide produced by sulfate reduction interferes with iron-phosphate binding in wetland sediments and soils due to the iron sulfide formation [63,64], which then releases phosphates through this chemical process. In the current study, higher concentrations of phosphates were measured during the 2016 drought period at the outflow of the wetland. However, the literature [62] further shows that the quantity of released phosphates is dependent on the availability of sulfate concentrations, which were much higher in the wetland surface water outflow during 2016, in comparison with the concentrations measured in 2015 and 2018. The periphyton mats at the wetland inflow, dominated by the filamentous algal species *Klebsormidium acidophilum* (Novis) throughout the study period, were a strong indication of AMD-impacted water with low pH values and high EC [36]. Furthermore, the occurrence of the diatoms *Nitzschia clausii* (Hantzsch), *Craticula buderi* (Brebisson), and *Gomphonema aff. gracile* (Ehrenberg) at the wetland inflow during the whole study period are good indicators of mining effluence and polluted water, according to Taylor et al. [47]. The most significant changes in periphyton usually occur at a pH range of 4.7 to 5.6, which is just beyond the interval of a pH of 5.5 to 6.5 where carbonates, as a key source of both inorganic carbon and acid neutralizing capacity for photosynthesis, become rapidly depleted [65,66]. However, a proliferation of acidophilic periphyton, which increased the biomass (benthic chl-*a* concentrations) during the drought period of 2016 at certain sites, was positively correlated with a decrease in pH, as previously shown by Muller [67] and Verb and Vis [68]. This observation could possibly be ascribed to a macroinvertebrate grazing pressure decrease, or a decrease in algal competition and a nutrient cycle alteration, as suggested by Stokes [69]. In the current study, a longitudinal relationship between the water column pH increase, filamentous algae biomass (benthic chl-*a* mg m^{-2}) decrease, and a diatom diversity increase downstream from Site 2, were observed after the drought period of 2016. The lower biomass observed at Site 2 throughout the study period may be related to higher rates of oxide deposition at this specific site, since the deposition of oxide can smother periphyton and subsequently inhibit photosynthesis [70].

The occurrence of the diatoms *Nitzschia nana* (Grunow) and *Tabellaria flocculosa* (Roth) at the outflow of the wetland, which replaced the diatoms *Nitzschia clausii* (Hantzsch) and *Craticula buderi* (Brebisson) after the drought period, is a good indicator of electrolyte-poor, oligotrophic, circumneutral, or slightly acidic waters, but also moderately polluted water [47]. The occurrence of the diatoms at the outflow of the wetland serves as an indicator of the improved aquatic conditions present in the wetland in 2018.

The average chemical and physical parameters measured at the wetland outflow during the sampling period 2015–2018 also supported the two-year recovery period of the wetland after the severe drought experienced in 2016. Although a loss in some ecological functions can be ascribed to the drought occurrence, the post-2016 drought physical and chemical parameters (2018 outflow) were comparable in terms of the ecological system, and restoration performance of the services, to the 2015 outflow parameters. A distinct reduction in SO_4^{2-}, Na, Mg, EC, Al, Fe, Mn, Si, and pH, following the 2016 drought, highlighted the utilization of water quality variables to not only assess the passive treatment responses of an ecologically engineered wetland, but also the progress related to ecological recovery.

5. Conclusions

The current study demonstrated that during the prevailing drought in 2016, AMD adversely affected the ecologically engineered wetland ecosystem in two ways, namely, (a) periphyton communities were restricted to only specific tolerant and resilient organisms that were able to survive in these extreme conditions; and (b) changes in the nutrient cycles caused the wetland to become nutrient-enriched, possibly due to changes in the high concentrations of sulfate, which also affected the periphyton assemblage. A loss in ecological functions and a regime shift during the drought of 2016 in the ecologically engineered wetland system, which, in part, recovered two years after the drought, was also observed. Although water quality and periphyton assemblage identity improved in the wetland outflow during 2017, recovery to the pre-drought state of 2015 only occurred in 2018, suggesting that a minimum of a two-year recovery period is required for an ecologically engineered, passive treatment wetland system after a severe drought.

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