Using Phytoplankton Diversity to Determine Wetland Resilience, One Year After a Vegetable Oil Spill

C. Selala · A.-M. Botha · L. P. de Klerk · A. R. de Klerk · J. G. Myburgh · P. J. Oberholster

Abstract A 250 t sunflower oil spill in the Con Joubert Bird Sanctuary Wetland, South Africa, was the largest global sunflower oil spill in a freshwater wetland to date. Since there was insufficient historical data for the Con Joubert Bird Sanctuary Wetland prior to the spill, variations in phytoplankton assemblages were used to indicate wetland resilience in relationship with water quality variables. From this study, it was evident that the phytoplankton biodiversity was a more reliable indicator of wetland resilience than vegetable oil concentrations measured in the water column. Vegetable oil concentrations measured in the water column varied both spatially and temporally and can possibly be linked to the passive movement of drifting oil in the water column caused by wind action and temperature changes. While we were unable to pinpoint the exact mechanisms behind the increase in phytoplankton biodiversity, the response was probably driven by the degradation of the oil by natural microbial consortiums in the wetland or a possible increase in phytoplankton grazers. Certain phytoplankton genera were found to be tolerant to the adverse effects of the oil spill. These genera include *Oedogonium*, *Cyclotella*, *Spirogyra*, and *Planktothrix*. In general, the univariate and multivariate statistical analysis showed a low diversity and richness at sites 1, 2 and 3 during the initial sampling surveys when compared to the remaining sites. However, the phytoplankton diversity and richness subsequently increased at all sampling sites from the second sampling survey, implying that there was a shift in phytoplankton biodiversity to a more stable state.

Keywords Freshwater wetland · Phytoplankton · Resilience · Vegetable oil spill

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1 Introduction

Resilience is the capacity of an ecosystem to rebound or recover after a shock or a disturbance (Gunderson 2010). Holling (1973) described ecological resilience as two different aspects of changes that occur in an ecosystem over space and time. The first aspect of resilience characterized by Holling (1973) is the persistence of relationships within a system and the ability of such a system to absorb changes of state variables, driving variables and parameters and still persist over time. The second aspect is characterized as the size of a stability domain

or the total amount of disturbance a system could absorb before it shifts into an alternative state. According to Holling (1973), these two aspects of resilience are not incompatible, since the major difference between them is whether the system of interest returns to its prior state or reconfigures into something very different. However, in the case of a disturbance such as an oil spill in an aquatic environment, the lack of adequate monitoring prior to a spill and the lack of sufficient historical data on the system are the major limitations for the evaluation of impacts of an oil spill in such an ecosystems and whether these systems return to their prior state or reconfigures into something dissimilar (Varela et al. 2006; Oberholster et al. 2010). Nevertheless, in such cases, biodiversity can play a major role in determining the ecosystem's response and vulnerability to a disturbance and the recovery of such systems (Folke et al. 2004). Experimental evidence by Downing and Leibold (2010) reveals that biotic diversity can stabilize an ecosystem that is subjected to perturbations. According to Downing and Leibold (2010), a greater diversity in a system can enhance the recovery of ecosystem functions after a disturbance.

Disturbances such as anthropogenic activities can contribute to wetland degradation and destruction of existing ecosystems due to the impact of various pollut-ants released into the aquatic environment (Scott and Jones 1995). It has been estimated that half of the world's total wetlands have been lost already. Within Southern Africa, the trend is similar, with an estimation that 35–50 % of the wetlands were lost or severely destroyed due to unsustainable social and economic pressures. According to Wehr and Sheath (2003), phytoplankton represents between 30 and 50 % of primary producer biomass in wetland systems, and their activity is noticeable in the large diurnal changes in dissolved oxygen and carbon dioxide. The presence of phytoplankton in wetlands reflects changes in the water quality and was shown to be a sensitive indicator of biological integrity and physico-chemical conditions of a re-spective wetland system (Korneva and Mineeva 1996; Willén 2001).

Vegetable, petroleum and nonpetroleum oil spills can cause substantial damages to the aquatic environment and as a consequence of an oil spill, macroinvertebrates, fish, aquatic plants and birds could also suffer from the contamination (Mudge 1999; L i e t a l . 2006). However, the chemical composition and physical properties of these agents determine their fate in the environment (USEPA 1994). Edible oils are perceived as harmful to environmental ecosystems due to the various conditions such as temperature and physicochemical conditions prevailing in the affected environment (Pereira et al. 2002, 2003). Petroleum and vegetable oils share physical properties and exhibit similar characteristics when spilled in aquatic environments. These oils have the ability to either float on the water surface and become solubilized or emulsify in the water column or settle on the bottom substrate (Crump-Wiesner and Jennings 1975). The main toxic constituent of vegetable oils is free fatty acids produced during environmental exposure (USEPA 1994). According to Miller et al. (1978), the response of phytoplankton to an oil spill is an excellent indicator to examine the adverse effects of an oil spill in the aquatic ecosystem. Evidence from previous studies supports the fact that the elimination of grazers in the aquatic ecosystem due to a disturbance is the principal cause of altered phytoplankton compositions and increased biomass in wetlands (McComb and Davies 1993).

Oberholster et al. (2010) reported that shifts in the functional phytoplankton groups, such as different growth forms and divisions of phytoplankton, can also be indicative of important changes in food quality and habitat structure for invertebrates. Phytoplankton is regarded as the primary energy producers in nutrient cycling and the food web that drives aquatic ecosystems (Walter 2006; K a n e e t a l . 2009). Phytoplankton also serves as energy source for zooplankton in freshwater wetlands. The growth responses of phytoplankton are predictive and sensitive to environmental factors, in-cluding pH, temperature, electrical conductivity and nutrient concentrations (e.g., nitrogen, phosphorus and silica load) in various water environments (Adrian et al. 1999; Oberholster 2011). Based on the information described above, the objective of this study was to determine the resilience of the selected wetland by using phytoplankton biodiversity as an indicator in changes within this impacted wetland over space and time.

2 Materials and Methods

2.1 Study Area Background and Site Selection

The Con Joubert Bird Sanctuary Wetland is a freshwater wetland (26°11′ 20″ S 27°41′03″ E), approximately 25 ha in size. According to Oberholster et al. (2010),

250 t of sunflower oil was spilled at a vegetable oil storage facility in Randfontein (South Africa) when a sunflower oil storage tank collapsed in September 2007. The oil entered the Con Joubert Bird Sanctuary wetland via storm water outlets that drain into the wetland. According to the authors, this was the largest global sunflower oil spill in a freshwater wetland to date. The wetland is a sanctuary for approximately 230 bird species and contains large stands of reed beds, while patches of short emergent vegetation and mudflats are visible in the dry season. The wetland is surrounded to the south and east by an industrial area and subjected to storm water inflow during rainy seasons. Mechanical techniques were employed as cleanup measure after the sunflower oil spill occurred in 2007. Inflatable booms and absorbent material were used to recover 175 t of sunflower oil, while 75 t remained in the wetland (Fig. 1).

A study on phytoplankton biodiversity of the Con Joubert Wetland by Oberholster et al. (2010) was con-ducted 30 days after the mechanical clean-up activities of the oil spill were completed and in the time frame just before biostimulation activities of natural microbial pop-ulations commenced in 2008. The outcome of that study revealed that the spilled oil inhibited the growth of sensitive phytoplankton species and promoted that of tolerant species in such a way that large increases in biomass with low diversity was observed. The algal divisions Chlorophyta (especially *Chlamydomonas af-ricana*) and Euglenophyta (especially *Euglena sociabilis*, *Phacus pleuronectes*) were well represented in the oil contaminated sampling sites. Young and mature resting zygotes of *C. africana* were recorded in high abundance at all the sunflower oil contaminated sampling sites. Due to the large increases of certain tolerant phytoplankton species after the oil spill, it was decided to initiate the application of fertilizer to the whole wetland. The application of fertilizer was an attempt to stimulate the natural microbial activity so as to promote the biodegration of the vegetable oil. In addition, a pilot study on three selected sites was carried out in 2008 to determine the response of phytoplankton and microbial communities on fertilizer application (Selala et al. 2013). Although the pilot study showed an increase in natural microbial diversity, it also pointed out an increase in oil tolerant phytoplankton biomass with a low overall phytoplankton diversity at all three sampling sites (Selala et al. 2013). Due to the out-come of the pilot study, a decision was made to rather set a one-year monitoring program in place to deter-mine the resilience of the wetland during post oil spill conditions using phytoplankton biodiversity and water quality variables as indicators of resilience of the system.

Five sampling sites were selected (Fig. 2). Sites 1 and 2 were in close proximity to the storm water inlet from where the spilled vegetable oil entered the wetland. Site 3 was located on the western side of the wetland where it borders a residential area. Sites 4 and 5 were located on the northern side of the wetland. These sites were cho-sen to be representative of the entire wetland system. The maximum water depth of the wetland was approx-imately 1.2 m during the wet summer season and 20 cm in the dry winter season.

Fig. 1 The inflatable booms and absorbent material used to recover a large portion of the 250 t sunflower oil that was spilled into the Con Joubert wetland

Fig. 2 An aerial photo of the wetland studied, indicating the five sampling sites. The *red arrow* indicates the source of the vegetable oil contamination (photo from Google Earth)

2.2 Collection of Samples for Physical and Chemical Analyses

Post spill samples were collected at regular intervals over a period of one year (from October 2008 to October 2009). Water samples were collected to determine the concentrations of the oil, as well as other water quality variables during this period. Various in situ water quality parameters, including dissolved oxygen, electrical conductivity, pH, redox potential and temperature were measured using a Hach™ sension 156 portable multiparameter meter.

The water samples were collected at the surface and the bottom using a grab bottle sampler (2 l). Both the surface and bottom samples were pooled together to form one integrated sample. The collection bottle was filled to the brim and kept cold and in the dark until analyses could be performed (within 48 h) to prevent additional chemical reactions. The grab bottle sampler was cleaned with ethanol after collection at each site to prevent possible cross contamination of oil. The water samples were analyzed for silica, total phosphate and total nitrogen, which constitutes of nitrate, nitrite and total kjeldahl nitrogen, to determine changes in the water quality at each sampling site. The phosphate and nitrogen concentrations were measured using appropriate spectrophotometric methods (APHA, AWWA and WPCF 1980). Sampling of bottom sediment to determine vegetable oil concentrations was done according to Oberholster et al. (2005). Concentrations of vegetable oil in the sediment and in the water column were measured according to a protocol by the United States Environmental Protection Agency (USEPA 1994).

A subsample of the pooled water sample from each site was used to determine the chlorophyll *a* and *b* concentrations as surrogate of phytoplankton biomass in the water column at each site. This sample was filtered through a GF/C glass microfiber filter to concentrate the algae. These GF/C filter papers were dissolved in 80 % acetone and incubated overnight in the dark at room temperature. The chlorophyll *a* and *b* concentrations were determined spectrophotometrically at 664 and 647 nm wavelengths, respectively, according to Porra et al. (1989) using a PerkinElmer™ Lambda 25 spectrophotometer.

2.3 Collection and Identification of Phytoplankton

A subsample (100 ml) of the integrated samples collected at each site was preserved with formaldehyde to a final concentration of 1 %. These subsamples were concentrated 10-fold by centrifugation at 400 rpm and used for phytoplankton identification and enumeration. The phytoplankton cells were counted at a magnification of 1,250 times, using an Olympus inverted binocular compound microscope with phase contrast, using the strip-count method (APHA 1989). Phytoplankton genera were identified according to Wehr and Sheath (2003) and Janse van Vuuren et al. (2006). Before diatoms were identified, they were cleared of organic matter using a potassium dichromate and sulphuric acid solution. The cleared sample was then diluted and mounted in Pleurax medium for microscopic examination. Diatom genera were identified and counted at a magnification of 1,250 times. The identification of genera was performed according to Patrick and Reimer (1975), Wehr and Sheath (2003) and Taylor et al. (2007). The total number of phytoplankton was recorded after careful examination for at least 15 min until no additional taxa were found thereafter. The total number of phytoplankton taxa and their abundance during the study were categorized according to Hörnström (1999).

2.4 Statistical Analyses

All the recorded data were subjected to the most appropriate univariate and multivariate statistical analysis, such as that of Ter Braak and Šmilauer (2002), to determine correlations between changes in the phytoplankton community structures and water quality variables. Phytoplankton diversity was calculated using the Shannon diversity index (Shannon 1948), while the Margalef's index (d) was used to measure phytoplankton genus richness (Margalef 1951). A redundancy analysis plot was used to determine any similarity or dissimilarity between the different sites with regard to the changes in the phytoplankton community and it was overlain with the prevailing water quality variables within the wetland (Ter Braak and Šmilauer 2002).

3 Results

3.1 Physical and Chemical Analyses of Water Quality

The water quality results from the different sites within the wetland are summarized in Table 1. At sites 1 and 2, the chlorophyll *a* and *b* concentrations were found to be the lowest. Dissolved oxygen concentration of ~2.4 mg l^{-1} at site 1 and ~2.7 mg l^{-1} at site 4 were low in comparison to the dissolved oxygen concentrations at the remaining three sites (Table 1). The average electrical conductivity at site 1 (~729 μS cm^{-1}) was relatively similar in comparison to the other four sampling sites where it varied between ~637 and ~699 μS cm^{-1}. The pH values and silicate concentrations were relatively similar between the different sites throughout the study. The pH values ranged between ~7.4 and ~9.3 and the silicate concentrations were

Table 1 Water quality variables analyzed at the five selected sampling sites over a period of one year. Average values and standard deviations are reported

Parameters	Site 1	Site 2	Site 3	Site 4	Site 5
Chlorophyll *a* (μg l^{-1})	4.2±1.9	4.7 8±3.28	14.21±1.6	33.22±21	23.31±14.2
Chlorophyll *b* (μg l^{-1})	3.44±1.7	3.01±0.02	10.48±0.7	20.4±8	17.26±3
Dissolved oxygen (mg l^{-1})	2.4±0.1	3.8±1.3	4.2±0.3	2.7±0.1	3.7±0.7
Electrical conductivity (μS cm^{-1})	729±115	683±279	699±101	685±49	637±152
pH	7.4±1.0	8.1±1.7	8.5±0.8	8.4±1.1	9.3±1.6
Redox potential (mV)	−125.35±13	−136.05±11	−120.67±3.6	−122.31±4.1	−188.8±10.1
Silica (mg l^{-1})	5.32±1.9	4.81±2.0	3.66±1.5	3.3±0.6	4.31±1.7
Temperature (°C)	11.7±1.8	12.2±0.6	11.4±1.2	12.1±0.6	11.5±2.0
Total nitrogen (mg l^{-1})	22.06±3.5	49.60±2	10.45±0.27	16.50±0.1	9.70±0.25
Total phosphate (mg l^{-1})	3.87±0.20	5.92±0.36	0.91±0.18	1.44±0.12	0.68±0.07
Oil in water (mg l^{-1})	3.75±0.3	2.1±0.01	2.51±0.3	2.54±1.3	1.04±0.01
Oil in sediment (g kg^{-1})	25.73±11	21.88±1.8	5.55±0.9	5.11±0.7	1.6±03

between ~3.30 and ~5.32 mg l^{-1} for all selected sites (Table 1). At sites 1 and 2, the total nitrogen and total phosphate concentrations were found to be higher than those recorded at the rest of the sites. The average oil concentrations in the water were relatively similar at all the selected sites, with the highest concentration at site 1 (~3.75 mg l^{-1}) (Table 1). The average oil concentration found in the sediment was the highest at sites 1 and 2 (~25.73 g kg^{-1} and ~21.88 g kg^{-1}, respectively) in comparison to sites 3, 4 and 5, where the oil concentration remained below 6 g kg^{-1}. It can also be noted that the increase in oil pollution was accompanied by an increase in phosphate and nitrogen concentrations. The maximum temperature measured in the water column of the wetland during summer was 28 ˚C, while the lowest water temperature in the winter months was 11˚C.

3.2 Phytoplankton Assemblage and Identification

Throughout the sampling period, a total of 20 phytoplankton genera were recorded at all five sites (Table 2). Chlorophyta was the most dominant phytoplankton group observed during this study with *Chlamydomonas*, *Scenedesmus* and *Spirogyra* being the most dominant genera. A schematic representation of the Shannon Diversity Index and Margalef Species Richness results of the phytoplankton over the study period is shown in Fig. 3. In general, the Shannon diversity and Margalef Richness Index revealed a low species diversity and richness at sites 1, 2, and 3 during the initial first sampling surveys when compared to the remaining sites. However, the diversity and richness subsequently increased from the second sampling survey. An average phytoplankton richness value of 17.86 was obtained during the study period at all five sites (Fig. 3). In general, the Shannon-Weiner Index showed a decreasing trend from 2.24 to 1.52 between sites 2 and 5 with the exception of site 1 2.06 (Fig. 3). The highest diversity of 2.24 was recorded at site 5 and the lowest of 1.52 at site 1 during the one-year sampling period. The latter was in relationship with the average oil concentrations measured in the sediment and water column of these two sites (Table 1).

3.3 The Interrelationship Between the Phytoplankton Assemblages and Water Quality Variables

Figure 4 represents an ordination plot of the phytoplankton assemblages with the respective water quality variables overlain. *Oedogonium* sp. and *Cyclotella* sp. (and to a lesser extent *Spirogyra* sp. and *Planktothrix* sp.) showed a close relationship with total phosphate, total nitrogen, and oil concentrations in the sediment during the first two sampling surveys at both sites 1 and 2. Sites 3 to 5 of surveys A, B, C and F have showed a similarity throughout the study with physicochemical parameters such as pH, chl *a* and *b*, salinity and silica. However, these sites (3–5) showed no close interrelationship with any phytoplankton genera in the wetland. Sites 1 to 4 indicated similarities with surveys C and D, which have also shown a relationship with the algal genera *Scenedesmus, Oscillatoria, Phacus* and nitrite. A relationship between the genus *Navicula* and nitrate concentrations in the wetland was observed during sampling surveys C and D at sampling sites 3 and 4. Other genera such as *Ceratium* and *Stigeoclonium* have shown a close relationship to dissolved oxygen at site 2 of surveys D and F. Generally, sites 1 to 5 during surveys C, E and F have revealed a close similarity with electrical conductivity, which were in relationship with genera such as *Melosira*, *Rhopalodia*, *Pediastrum* and *Navicula.*

4 Discussion

In the study, it was evident that the loss of ecological resilience and the ensuing regime changes after the oil spill was due to the inter play between physical, chemical and biological processes as observed between the different sampling trips. Because there was insufficient data on the phytoplankton assemblages for the wetland studied prior to the sunflower oil spill, variations in phytoplankton assemblages were used as indicators of wetland recovery during post spill conditions. Changes in the diversity of phytoplankton have been shown in previous studies to be a useful indicator of water quality and can be used to determine recovery of biotic communities after anthropogenic impacts (De Lange 1994; Ariyadej et al. 2004). From our study, it was evident that the algal biodiversity was a much more reliable indicator of resilience of the wetland than vegetable oil concentrations in the water column during post spill conditions. Vegetable oil concentrations measured in the water column differed (increase or decrease) between sites and sampling trips and can possibly be linked to passive movement of the drifting oil in the water column caused by wind action and temperature change. While we were unable to pinpoint the exact mechanisms behind the

Table 2 Phytoplankton assemblages at the five selected sites over a period of one year. The phytoplankton abundances during the study were categorized according to Hörnström (1999), where 0 = 0; 1 = ≤500; 2 = 501–5,000; 3 = 5001–2,5000; 4 = 25001–100000 cells ml^{-1}. A = October 2008; B = January 2009; C = April 2009; D = June 2009; E = August 2009; and F = October 2009

Division	Genus	Site 1						Site 2						Site 3						Site 4						Site 5					
		A	B	C	D	E	F	A	B	C	D	E	F	A	B	C	D	E	F	A	B	C	D	E	F	A	B	C	D	E	F
Bacillariophyta	*Cyclotella*[b]	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Gomphonema[b]	0	0	2	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0
	Melosira[b]	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
	Navicula[b]	0	0	3	2	0	2	0	0	3	0	0	0	0	0	3	3	3	3	0	0	2	3	3	0	0	0	0	0	0	2
	Pinnularia[b]	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	1
	Rhopalodia[b]	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0
	Synedra[b]	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Chlorophyta	*Chlamydomonas*[a]	3	4	4	3	4	4	4	2	4	3	4	0	4	0	4	4	4	4	3	4	4	4	4	4	0	0	4	4	4	4
	Chlorogonium[b]	1	1	1	0	0	0	0	0	0	0	4	0	0	0	0	0	3	0	0	0	0	0	1	1	0	0	0	0	0	0
	Oedogonium[b]	0	0	0	0	0	0	2	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Pediastrum[b]	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Scenedesmus[a]	0	0	4	4	4	4	0	0	4	4	4	3	0	0	4	4	4	4	0	0	4	4	4	4	0	0	0	0	0	4
	Spirogyra[a]	4	4	4	3	4	4	4	4	0	3	4	4	0	4	4	2	4	4	4	4	4	3	4	4	4	4	4	4	4	0
	Stigeoclonium[b]	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cyanophyta	*Nostoc*[b]	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Planktothrix[b]	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Oscillatoria[a]	1	2	0	0	2	2	0	0	2	2	2	0	0	0	2	2	0	0	0	0	0	0	0	2	0	0	1	0	2	2
Dinophyta	*Ceratium*[b]	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Euglenophyta	*Euglena*[b]	1	1	0	0	0	1	1	2	0	0	0	2	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0
	Phacus[b]	0	0	0	3	0	3	0	0	3	0	1	0	0	0	3	2	0	0	0	0	3	3	2	0	0	0	0	0	0	0

[a] Algae genera present at all sites

[b] Algae genera present at certain sites

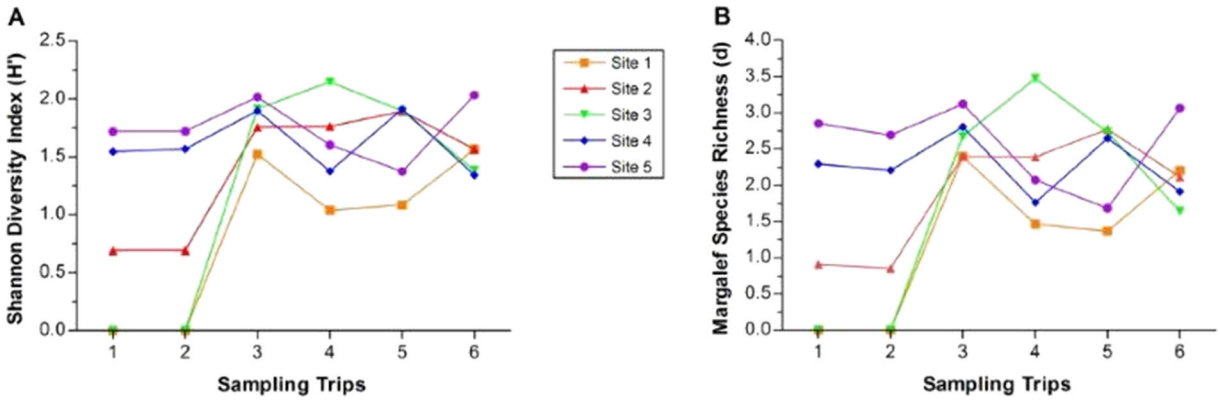

Fig. 3 The Shannon Diversity Index (**a**) and Margalef species richness index (**b**) of the phytoplankton assemblages present at the five selected sites during the six sampling surveys

increase in phytoplankton biodiversity after the second survey, the response was probably driven by the degradation of the vegetable oil by natural microbial consortiums over time or increases in grazers (e.g., protozoa or zooplankton) on the dominant oil tolerant phytoplankton genera (Selala et al. 2013). Although the latter was not investigated on this study, it could have reduced interspecific competition between oil tolerant and nontolerant phytoplankton species. Another factor that is important to consider for the increase in phytoplankton biodiversity is water column temperature. According to Sommers et al. (1986), increases in phytoplankton biomass and diversity occur during the spring months when longer day length and warmer water column temperatures stimulate phytoplankton growth that was previously hampered by low winter temperatures. However, in our study, low phytoplankton biodiversity were observed during our first sampling trip in October 2008 (spring), while higher phytoplankton diversity was observed in January 2009 (summer) during our second sampling survey. From the latter, it was evident that phytoplankton biodiversity was still adversely affected by the oil spill in October 2008. The phytoplankton diversity in Fig. 3 clearly showed that the wetland was still in its alpha phase during the first sampling survey in October 2008 and that the phytoplankton diversity was dominated by only a few oil tolerant phytoplankton genera (*Oedogonium* sp. and *Cyclotella* sp.). It was only evident after the second

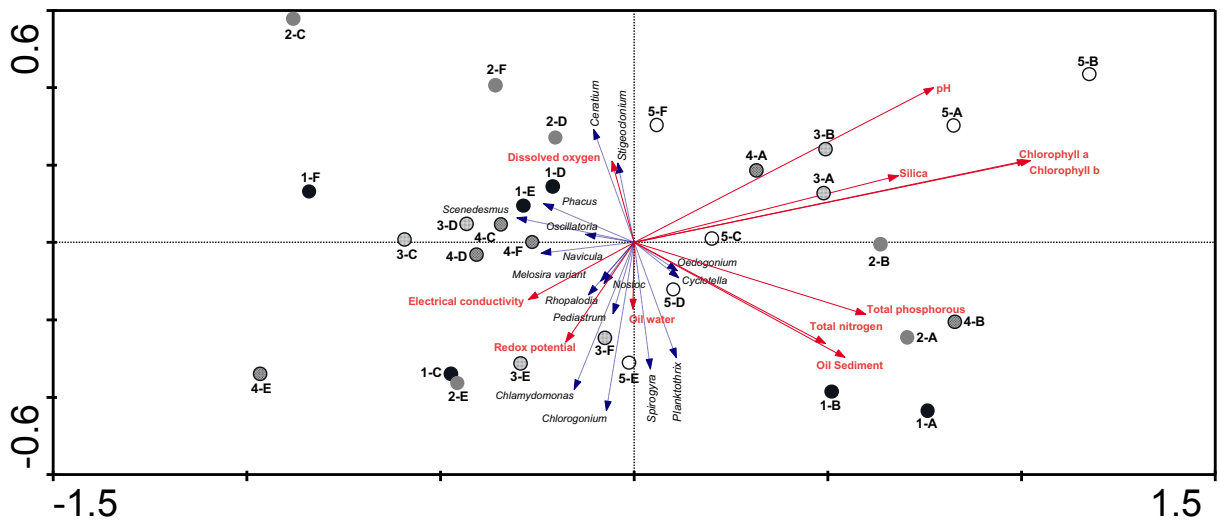

Fig. 4 Redundancy analysis plot to determine interrelationships between phytoplankton assemblages and physicochemical variables during the six sampling surveys (**a**) (October 2008), (**b**) (January 2009), (**c**) (April 2009), (**d**) (June 2009), (**e**) (August 2009), and (**f**) (October 2009). Percentage variance explained on the first axis is 34.9 % and on the second axis is 20.4 %

sampling survey in January 2009 that an obvious change in phytoplankton diversity was observed and that a shift in phytoplankton biodiversity did occur. This observation was in concordance with reports by Holling (1986). According to Holling (1986), the alpha phase is the short period of time following a disturbance in an ecosystem and is also the phase in which many opportunities emerge for an alternative system configuration as observed by Oberholster et al. (2010) and Selala et al. (2013) which reported the dominance of vegetable oil tolerated phytoplankton genera during the immediate post spill conditions.

According to Ortiz and Sáenz (1997), nutrients influence phytoplankton biomass with phosphate being regarded as the limiting nutrient for phytoplankton growth, irrespective of whether other nutrients are available or not. The average total phosphate concentrations obtained at all five sampling sites were below excessive pollution threshold of approximately ± 200 mg l^{-1} (Friedrich et al. 1996). From the results, it is clear that this nutrient, together with total nitrogen increased in close relation with oil concentrations in the sediment. The oil may thus be able to retain nutrients, while at the same time deplete the system from oxygen, because the oil layer that forms on the surface water prevents the input of atmospheric oxygen to the system. Ortiz and Sáenz (1997) reported that chlorophyll *a* concentrations are influenced by continuous contributions of phosphate. In this study, the chlorophyll *a* concentration was higher at the sites further away from where the oil was spilled. This can be observed in Fig. 4, where it is shown that the diversity and species richness also increased at sites 3, 4 and 5. Variations in pH were previously reported six months after a vegetable oil spill in a salt marsh by Pereira et al. (2003) and three years after a petroleum oil spill in a reed bed wetland (Ji et al. 2007). However, relatively similar pH levels were measured throughout the study at all five samplings sites which contradicted the data reported by Pereira et al. (2003).

The close relationship between total phosphate, total nitrogen and vegetable oil pollution to the genera *Oedogonium*, *Cyclotella*, *Spirogyra* and *Planktothrix* was noted at sites 1 and 2 during the first two post oil spill surveys. Thus, it can be deduced that these species show a high affinity for the nutrient rich and lower dissolved oxygen conditions brought about by the oil spill when compared to the other genera found in the wetland. Previous studies have indicated that *Oedogonium* and *Cyclotella* abundances increased with elevated nutrient concentrations and can be used as indicator of eutrophic conditions, while *Spirogyra* sp. is widespread in all freshwater ecosystems (Janse van Vuuren et al. 2006). Sites 3, 4 and 5 showed less variation in phytoplankton biomass diversity and richness throughout the study period as compared to sites 1 and 2. The main differences between these two sites (or section of the wetland) are due to the inlet located near sites 1 and 2 from where the oil flowed into the wetland. Although the oil spill resulted in an increase in total phosphate and total nitrogen concentrations, it adversely affected the phytoplankton biodiversity assemblages at these two sites. This was most noticeable during the first post spill surveys when the phytoplankton diversity and richness were low in comparison to the later sampling surveys. Subsequent surveys showed a gradual increase in phytoplankton diversity and richness, although it was still low. In the previous study conducted by Oberholster et al. (2010), the authors showed that some species (e.g., *C. africana*) have a competitive advantage over susceptible genera, and can thus become dominant in polluted water. In the present study, this genus, together with *Chlorogonium*, was closely related with oil pollution in water, as well as electrical conductivity and was thus also shown to be tolerant of these pollution effects. The diatom *Rhopalodia* which showed a strong relationship with electrical conductivity was in association with results from previous studies that reported a similar relationship between this diatom species and increased electrical conductivity levels (Blinn and Herbst 2003). Therefore, it was evident from the latter that the interplay between nutrients and oil concentrations in the water column and sediment may have played a major role in enhancing the phytoplankton biodiversity in the wetland.

5 Conclusions

An increase in phytoplankton diversity was observed during subsequent surveys of the wetland after the oil spill. This was an indication of the wetland recovering over time. Although there is no information available on the phytoplankton species composition before the oil spill, we found that this freshwater wetland was able to increase its phytoplankton diversity and start to recover within one year after the spill. It is recommended that this wetland should be monitored on a continuous basis to

establish a water quality benchmark and phytoplankton composition of the wetland on the long-term after the oil spill. This will provide data that can be used for future management purposes in the event of a recurring oil spill, as well as to provide valuable data on biodiversity changes within freshwater wetlands after a vegetable oil spill.

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