



# Assessing macroinvertebrate communities in relation to environmental variables: the case of Sambandou wetlands, Vhembe Biosphere Reserve

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## Abstract

The Vhembe Biosphere Reserve, South Africa, contains many wetlands that serve as wildlife habitats and provide vital ecosystem services. Some of the wetlands are continuously being degraded or destroyed by anthropogenic activities causing them to disappear at an alarming rate. Benthic macroinvertebrates are known as good water quality bioindicators and are used to assess aquatic ecosystem health. The current study investigated habitat quality using macroinvertebrate community structure and other biotic variables (i.e. phytoplankton, macrophytes) in relation to environmental variables in the Sambandou wetlands using canonical correspondence analysis (CCA). A total of fifteen macroinvertebrate families were identified over two seasons. The CCA highlighted seven variables, i.e. pH, phosphate concentration, temperature, ammonium, macrophyte cover, conductivity and water depth, which were significant in structuring macroinvertebrate community. Picophytoplankton and microphytoplankton concentrations decreased from winter to summer, whereas nanophytoplankton concentration increased from winter to summer. Thus, the dominance of small-sized phytoplankton indicated nutrient limitation and decreased productivity, whereas winter sites 2 and 3 were dominated by large-celled phytoplankton, highlighting increased productivity. Winter sites were mostly negatively associated with CCA axis 1 and were characterised by high temperature, phosphate and ammonium concentrations, macrophyte cover, pH and conductivity. Summer sites were positively associated with axis 1, being characterised by high water depth and pH levels. The results obtained highlighted that agricultural activities such as cattle grazing and crop farming and sand mining/poaching had a negative effect on macroinvertebrate community structure.

**Keywords** Macroinvertebrates · Sambandou · Nutrients · Wetlands · Water quality · Phytoplankton · Macrophytes

## Introduction

A wetland is an area that is permanently and/or temporally saturated with water (Alkorta and Garbisu 2001). Wetlands serve as natural purifiers of water, filtering and absorbing many pollutants in surface water, e.g. phytoremediation (i.e. removal of contaminants using plants) and bioremediation (i.e. degrade contaminants to less toxic using organisms), habitat for flora and fauna, grazing areas for animals, and tourism (Alkorta and Garbisu 2001; Malinga et al. 2015). These unique habitats support aquatic biodiversity, with a

high number of unique plant and animal species only found within these systems.

Wetlands are disappearing worldwide due to human activities such as agriculture, mining and urban development (Arheimer et al. 2005; Millennium Ecosystem Assessment 2005; Dalu et al. 2017a). Pollution within wetlands is a growing concern, since it has detrimental effects on human and aquatic species, and this is affecting the wetland biodiversity (Sayadi et al. 2010). Hence, a high number of aquatic species are at greater risk of extinction due to pollution caused by agriculture and other human-induced activities on wetlands (Dalu et al. 2017b; Nhiwatiwa et al. 2017a). Thus, there is a growing concern as very little has been done to address this issue.

According to Ollis et al. (2006), a bioindicator is an organism or a community of organisms that contains information on the environmental quality. Macroinvertebrates are the most frequently used and are known as potential

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bioindicators of water quality and hence are used to assess the health of an ecosystem (Bonada et al. 2006; Dalu et al. 2017c). Various studies (e.g. Allan 2004; Macedo et al. 2016; Mangadze et al. 2019) show that macroinvertebrates are the mostly frequently used organisms to assess wetland ecosystem health. Macroinvertebrates are reliable indicators because their life cycles are long enough to detect changes caused by any disturbances (Ollis et al. 2006). The sensitivity of macroinvertebrates to pollution vary (Rosenberg and Resh 1993), some macroinvertebrates are tolerant to water pollution, while others are sensitive. For example, Caenidae (mayflies) are sensitive to water pollution, whereas Culicidae (mosquito's larvae), Diptera (true flies) and Oligochaeta (worms) are tolerant to pollution. High macroinvertebrate diversity exists within wetlands which are not disturbed by anthropogenic activities (Brand and Miserendino 2015).

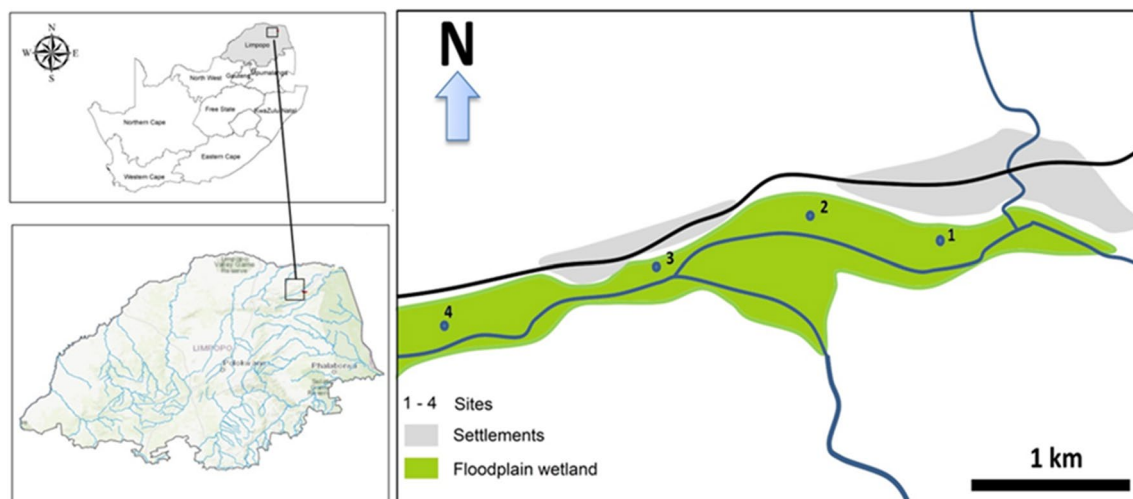
The study aimed to investigate macroinvertebrate community structure in relation to environmental characteristics within the Sambandou wetland, Vhembe Biosphere Reserve, South Africa. Habitat assessment (physical and chemical) variables were used to assess the key drives of aquatic macroinvertebrate community structure and composition within the wetland. More specifically, this research aimed to: (1) determine the macroinvertebrate's community structure and composition across seasons within the wetland, (2) assess the impact of environmental characteristics on macroinvertebrate community structure and composition and (3) assess wetland integrity using macroinvertebrates, environmental variables and habitat variables in conjunction with community metrics. This study will assist in answering emerging question on agricultural activities in wetlands and how human activities affect wetland integrity. With water being a scarce resource in South Africa, a lot of people, especially in

rural areas, depend on wetlands as their water source and a form of sustaining their livelihoods through ecosystem services the wetland provides.

## Materials and methods

### Study area

The Sambandou wetlands are located in quaternary A92B of the Luvuvhu catchment towards the north-east of Thohoyandou, Limpopo Province, South Africa. Sambandou wetlands are channelled valley-bottom wetlands and about 203 ha in size. The wetland system is associated with the Sambandou River, a tributary of the Mutale River. This wetland system is utilised for agricultural purposes, i.e. cultivation and livestock grazing, and as water source by the local communities, and it is thus important for maintaining the ecosystem integrity and ensure the sustainability of the services it provides. Furthermore, downstream users depend on the wetland for ecosystem services such as flood attenuation and water filtration. The wetland is undergoing significant channel incision and sediment deposition, which effectively is lowering the water table. The study was conducted in winter (June 2017) and summer (October 2017). Four sites were selected from the wetland: one site in the upper reach (located next to village households and also utilised as grazing field), one site in the lower reach (i.e. utilised as gardens, grazing and had high activities of illegal sand mining) and two sites in the middle reaches which were dominated mostly by village households next to the wetland, with a lot of gardens within the wetland (Fig. 1).



**Fig. 1** Map highlighting the location Sambandou wetland and the selected study sites

## Environmental variables and water sampling

Environmental variables such as conductivity ( $\mu\text{S cm}^{-1}$ ), dissolved oxygen ( $\text{mg L}^{-1}$ ), total dissolved solids ( $\text{mg L}^{-1}$ ), pH and temperature ( $^{\circ}\text{C}$ ) were measured using a portable hand-held multi-parameter probe PCTestr 35 (Eutech/Oakton Instruments) in situ from three different points per site. A tape measure was used to measure the water depth and channel width.

Two polyethylene bottles (250 mL) were filled with wetland water from each site for nutrients (i.e. phosphate, ammonium), turbidity and size-fractionated chlorophyll-*a* (i.e. macro-, pico- and nanophytoplankton) analyses. In the laboratory after filtration of the water samples for chlorophyll-*a* concentration determination, ammonium and phosphate concentrations were analysed using an ammonium test kit for freshwater (HI3824), with a range of 0–2.5  $\text{mg L}^{-1}$  and a 0.5  $\text{mg L}^{-1}$  resolution and Hanna phosphate high range checker (HI717) (Hanna Instruments, Romania, with a 0–30  $\text{mg L}^{-1}$  range and 0.1  $\text{mg L}^{-1}$  resolution.

## Size-fractionated chlorophyll-*a* concentration determination

Size-fractionated pelagic phytoplankton biomass was determined by measuring chlorophyll-*a* (chl-*a*) concentration from site ( $n = 4 \times 2$  replicates) to tell us more about the state of the ecosystem in terms of primary productivity. Collected water samples (250 mL) were serially filtered (vacuum of  $< 5$  cm Hg) through a 20- $\mu\text{m}$  Nitex nylon mesh filter (microplankton,  $> 20$   $\mu\text{m}$ ), a 2- $\mu\text{m}$  Millipore isopore membrane filter (nanoplankton, 2–20  $\mu\text{m}$ ) and a 0.7- $\mu\text{m}$  Whatman GF/F filter (picoplankton, 0.7–2  $\mu\text{m}$ ) (Sieburth et al. 1978). After filtration, the filters inserted in separate labelled bottles containing 10 mL acetone and stored in the dark for 24 h to allow chl-*a* extraction. After 24 h, chl-*a* concentration was determined using SPECTROstar NANO (BMG LabTech GmbH, Ortenberg) according to Lorenzen (1967):

$$\text{Chl-}a(\text{mg m}^{-3}) = 11.4 \times K \times ((665_o - 750_o) - (665_a - 750_a)) \times V_e \div L \times V_f$$

where  $L$ —cuvette light-path (cm),  $V_e$ —extraction volume (mL),  $V_f$ —filtered volume (L) and  $K$ —2.43.

## Macroinvertebrates sampling

At each site and season, benthic macroinvertebrates were sampled using the kick sampling method according to Dickens and Graham (2002). In summary, a hand-held kick net (dimensions 30  $\times$  30 cm, mesh size 500  $\mu\text{m}$ , 1.5 m handle) was used to sample all the available habitat types. The hand-held kick net was submerged in the water, macroinvertebrates

were collected by sweeping within a certain area for 1 min, and this involved walking with the net through the water, dragging and kicking of macrophyte vegetation, rocks and sediment was done to dislodge macroinvertebrates attached. Active animals were prevented from escaping in the net by quickly lifting it out of the water and emptying the contents into a tray, where all detrital organic matter was removed. The macroinvertebrates were then moved into a labelled 500-mL polyethylene bottles and preserved in 70% ethanol. Identification and counting of macroinvertebrates was done in the laboratory under a dissecting Olympus microscope using guides by Gerber and Gabriel (2002), and taxa were identified to family level.

## Habitat assessment and substrate characterisation

Habitat assessment was done at each site, and the assessment was based on the following categories: erosion and stability, in-stream cover, bank vegetation and verge vegetation. The assessment was based on modified habitat assessment form version 1.0 (West Gippsland Water Management Authority, Victoria). Substrate embeddedness determination was based on a method by Platts et al. (1983): (1)  $> 75\%$ ; (2) 50–75%; (3) 25–50%; (4) 5–25%; and (5)  $< 5\%$  of benthic surface covered by fine (i.e. clay/silt) sediment.

## Macrophyte sampling

Macrophyte cover was visually checked and expressed as percentage (%) at each site. A standard transect length (i.e. 100 m) was selected at each of the four sites on both sides of the wetland and assessed to confirm its suitability for the survey and then marked (Dawson 2002; Hering et al. 2006; Dalu et al. 2012). Macrophyte community structure within the wetlands and the physical character of the study area were surveyed by wading in a zigzag manner across the wetland transect length, investigating all the different habitat types present. Macrophyte percentage cover was described in eight randomly selected quadrats (1 m  $\times$  1 m) within each transect. Care was taken to examine all the small niches available within each quadrat/transect so as to observe all possible macrophyte species. Identification of the macrophytes was done to species level where possible using field identification guides by Gerber et al. (2004).

## Data analysis

Using Shapiro–Wilk normality test confirmed that the environmental and biotic data accumulated were not normal. The collected data did not meet the expectations of parametric tests such as normality and homogeneity of variance. Thus, a nonparametric test Kruskal–Wallis was used to test for differences in environmental and biotic variables among sites

and seasons using STATISTICA version 12.0 (StataCorp 2011). The level of  $p < 0.05$  was accepted as the minimum significance level.

Common macroinvertebrate metrics were used to assess the environmental integrity: %Ephemeroptera abundance, %Trichoptera abundance and %Diptera abundance, and Shannon–Wiener diversity index. The South African Scoring System version 5 (SASS5) score, which is the sum of all macroinvertebrates pre-determined taxa tolerance values to pollution within a sample, and the average score per taxon (ASPT), calculated by dividing the SASS5 score by the sample taxa number (Dickens and Graham 2002) were computed to assess wetland quality. The SASS5 and ASPT scores were used as a measure of each site condition: excellent (SASS5 score  $> 100$  and ASPT score  $> 7$ ), good (80–100 and 5–7), fair (60–80 and 3–5), poor (40–60 and 2–3) and very poor ( $< 40$  and  $< 2$ ) (Thirion et al. 1995).

To evaluate seasonal changes in macrophyte community structure among sites and seasonal separation within the Sambandou wetlands, a non-metric multidimensional scaling ( $n$ -MDS) analysis with Euclidean dissimilarity as a measure of distance was carried out (Kruskal and Wish 1978) using PC-ORD version 5.10 in quick and dirty mode. Preliminary de-trended correspondence analysis (DCA) was applied to the macroinvertebrate dataset to determine the length of the gradient. The DCA revealed that the gradient was greater than 3 standard deviation units (i.e. 4.74), justifying the use of unimodal ordination techniques (Ter Braak and Verdonschot 1995). Thus, canonical correspondence analysis (CCA) was used to investigate relationships between predictor variables and benthic macroinvertebrate communities from different sites. Preliminary CCA identified collinear variables and selected a subset on inspection of variance inflation factors ( $VIF < 20$ ; Ter Braak and Verdonschot 1995). Monte Carlo permutation tests (9999 unrestricted permutations,  $p < 0.05$ ) were used to test the significance of the axis and hence determine whether the selected environmental variables could explain nearly as much variation in the macroinvertebrate data as all the measured environmental variables combined. CCA was performed using CANOCO version 5.1 (Ter Braak and Šmilauer 2002).

## Results

### Environmental variables

Six environmental variables (i.e. temperature, total dissolved solids (TDS), Conductivity, pH and turbidity) showed significant differences ( $p < 0.05$ ) across the two seasons, whereas three variables, i.e. conductivity, dissolved oxygen (DO) and phosphate concentration, were significantly different ( $p < 0.05$ ) among the study sites (Table 1). Conductivity

**Table 1** Mean ( $\pm$ SE) and Kruskal–Wallis ANOVA results of environmental variables of water quality measured at four sites across seasons at Sambandou wetland

Variables	Site 1		Site 2		Site 3		Site 4		Season		Site	
	Winter	Summer	Winter	Summer	Winter	Summer	Winter	Summer	H	p	H	p
Elevation (m)	606 $\pm$ 0	606 $\pm$ 0	593 $\pm$ 0	593 $\pm$ 0	581 $\pm$ 0	581 $\pm$ 0	560 $\pm$ 0	560 $\pm$ 0	0.000	1.000	0.000	1.000
Depth (m)	0.2 $\pm$ 0.1	0.4 $\pm$ 0.4	0.2 $\pm$ 0.1	0.2 $\pm$ 0.0	0.2 $\pm$ 0.1	0.3 $\pm$ 0.1	0.4 $\pm$ 0.2	0.4 $\pm$ 0.2	0.304	0.959	0.678	0.410
Temperature (°C)	18.8 $\pm$ 0.7	21.0 $\pm$ 0.6	21.5 $\pm$ 0.2	21.2 $\pm$ 0.0	18.0 $\pm$ 0.1	18.2 $\pm$ 0.1	20.3 $\pm$ 1.9	20.3 $\pm$ 1.9	10.667	0.014	0.909	0.340
DO (mg L <sup>-1</sup> )	8.5 $\pm$ 2.7	0.1 $\pm$ 0.0	3.1 $\pm$ 1.5	0.1 $\pm$ 0.0	2.8 $\pm$ 0.1	0.2 $\pm$ 0.0	3.0 $\pm$ 1.3	0.2 $\pm$ 0.0	0.567	0.902	<b>15.981</b>	<b>0.001</b>
TDS (ppm)	27.6 $\pm$ 0.1	20.3 $\pm$ 0.5	29.4 $\pm$ 1.0	25.0 $\pm$ 2.8	34.6 $\pm$ 2.5	38.5 $\pm$ 7.8	31.0 $\pm$ 0.8	31.0 $\pm$ 0.8	<b>12.818</b>	<b>0.005</b>	0.390	0.053
Conductivity ( $\mu$ S)	46.9 $\pm$ 12.0	31.7 $\pm$ 0.9	62.3 $\pm$ 3.6	37.7 $\pm$ 3.7	67.0 $\pm$ 1.8	58.5 $\pm$ 3.5	47.7 $\pm$ 2.6	47.7 $\pm$ 2.6	<b>9.486</b>	<b>0.024</b>	<b>7.439</b>	<b>0.006</b>
Ph	6.5 $\pm$ 0.2	6.5 $\pm$ 0.2	6.4 $\pm$ 0.2	6.4 $\pm$ 0.0	7.3 $\pm$ 0.6	7.3 $\pm$ 0.2	7.0 $\pm$ 0.5	7.4 $\pm$ 0.4	<b>13.876</b>	<b>0.003</b>	0.053	0.818
Turbidity (NTU)	2.9 $\pm$ 1.3	0.0 $\pm$ 0.0	5.7 $\pm$ 0.0	85.3 $\pm$ 50.0	3.6 $\pm$ 0.3	97.5 $\pm$ 6.4	2.0 $\pm$ 0.6	0.0 $\pm$ 0.0	<b>13.587</b>	<b>0.004</b>	0.117	0.732
Ammonium (mg L <sup>-1</sup> )	0.5 $\pm$ 0	1.10 $\pm$ 0	1.5 $\pm$ 0	2.60 $\pm$ 0	1 $\pm$ 0	1.90 $\pm$ 0	0.80 $\pm$ 0	0.80 $\pm$ 0	4.681	0.197	2.108	0.147
Phosphates (mg L <sup>-1</sup> )	0.7 $\pm$ 0	1.50 $\pm$ 0	0.3 $\pm$ 0	1.70 $\pm$ 0	0.6 $\pm$ 0	2.00 $\pm$ 0	1.00 $\pm$ 0	1.00 $\pm$ 0	0.833	0.842	<b>5.333</b>	<b>0.021</b>

DO dissolved oxygen, TDS total dissolved solids  
Significant differences at  $p < 0.05$  are indicated in bold

and TDS were generally high during the winter with concentrations increasing from the upper (site 1) to lower reach (site 4). Similar trends were also observed for turbidity and pH, with pH being slightly acidic at site 1 (pH mean value = 6.5) changing to slightly alkaline in summer (i.e. 7.4) (Table 1). High ammonium and phosphate concentration was recorded during summer season at sites 2 and 3, respectively (Fig. 2). Ammonium ranged from 0.5 to 1.5 mg L<sup>-1</sup> in winter, whereas in summer it ranged from 0.8 to 2.6 mg L<sup>-1</sup>. Phosphates ranged from 0.3 to 0.7 mg L<sup>-1</sup> in winter and 1.0–2.0 mg L<sup>-1</sup> during summer. The Kruskal–Wallis analysis showed significant ( $p < 0.05$ ) site variation for phosphates (Table 1).

### Chlorophyll-*a* concentration

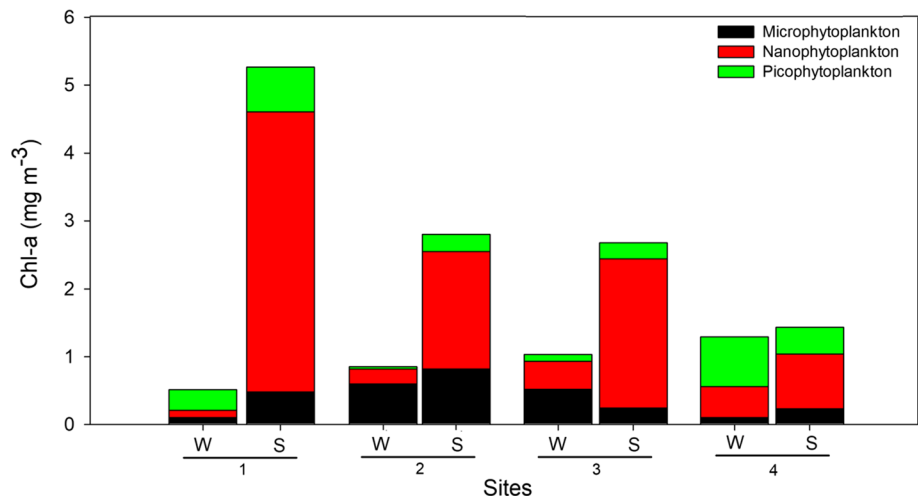
The chl-*a* concentration increased from a low in winter to a high in summer (Fig. 2). Picophytoplankton and microphytoplankton concentration decreased from winter to low concentrations during summer, whereas nanophytoplankton concentration increased from winter to high concentrations in summer (Fig. 2). The chl-*a* concentration generally increased from site 1 (~ 0.5 mg L<sup>-1</sup>) to 4 (~ 1.2 mg L<sup>-1</sup>) during the winter (Fig. 2), whereas an opposite trend was observed for summer, with chl-*a* concentration decreasing

from site 1–4 (Fig. 2). During winter, picophytoplankton dominated sites 1 and 4, whereas microphytoplankton dominated sites 2 and 3 (Fig. 2). In summer, the chl-*a* concentration was dominated by nanophytoplankton (Fig. 2). Picophytoplankton ranged from 0.03 to 0.70 mg L<sup>-1</sup> during winter, whereas in summer it ranged from 0.24 to 0.66 mg L<sup>-1</sup>. Nanophytoplankton biomass ranged from 0.11 to 0.46 mg L<sup>-1</sup> in winter and 0.81–4.13 mg L<sup>-1</sup> in summer. For microphytoplankton, the range was 0.10–0.60 mg L<sup>-1</sup> in winter and 0.23–0.82 mg L<sup>-1</sup> in summer. Chlorophyll-*a* concentration was found to be negatively correlated with elevation ( $r = -0.97, p < 0.001$ ) and habitat structure ( $r = -0.48, p = 0.012$ ) and positively correlated with conductivity ( $r = 0.55, p = 0.004$ ) and pH ( $r = 0.62, p = 0.001$ ).

### Macrophytes

*Phragmites mauritianum*, *Potamogeton pectinatus*, *Cyperus* sp., *Cyperus sexangularis* and *Nymphaea naouchalia* var. *coerulea* were species that were observed in the Sambandou wetland (Table 2). The most abundant species observed was *P. mauritianum* ranging from 10 to 60%, whereas *P. pectinatus* and *Cyperus* spp. were less abundant ranging from 5 to 15% and 1 to 30%, respectively. The *n*-MDS ordination based on macrophyte taxa discriminated among seasons

**Fig. 2** Size-fractionated chlorophyll-*a* concentration (mg L<sup>-1</sup>) measured from Sambandou wetland

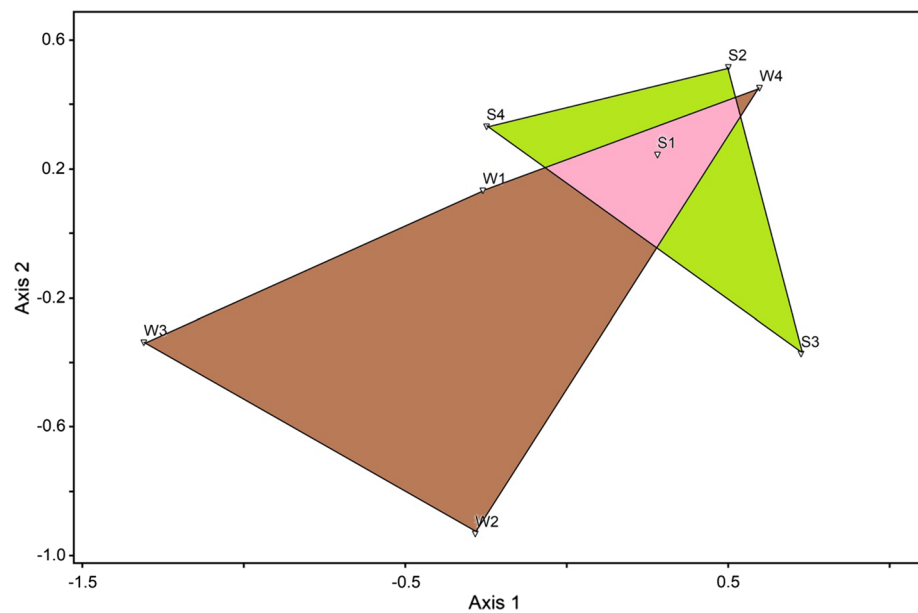


**Table 2** Macrophyte species observed (% cover) from Sambandou wetland

Macrophyte	Winter				Summer			
	Site 1	Site 2	Site 3	Site 4	Site 1	Site 2	Site 3	Site 4
<i>Cyperus</i> sp.	30	10	1	2	8	30	5	1
<i>Nymphaea naouchalia</i> var. <i>coerulea</i>	1		20		1		20	
<i>Potamogeton pectinatus</i>	5				15			
<i>Phragmites mauritianum</i>	50	50	60	10	20	20		30
<i>Cyperus sexangularis</i>	1	30	1	1	2	5		1



**Fig. 3** *n*-MDS ordination (stress value 0.12) highlighting variation of macrophyte communities across sites and seasons. Polygons indicate the two seasons: green—summer and brown—winter



(Fig. 3). Macrophyte cover was negatively correlated with chl-*a* concentration ( $r = -0.47$ ,  $p = 0.013$ ) and positively correlated with elevation ( $r = 0.60$ ,  $p = 0.002$ ), TDS ( $r = 0.36$ ,  $p = 0.049$ ) and habitat structure ( $r = 0.70$ ,  $p < 0.001$ ).

### Macroinvertebrates

A total of fifteen macroinvertebrate families were identified over two seasons (Table 3). Taxa richness ranged between 3 and 5 per site in winter to between 2 and 4 in summer (Table 3). Chironomidae were the most abundant family. A high Shannon–Weiner diversity index was observed during summer. The Shannon–Weiner diversity index was negatively correlated with TDS ( $r = -0.43$ ,  $p = 0.024$ ) and conductivity ( $r = -0.50$ ,  $p = 0.009$ ), and positively correlated with SASS score ( $r = 0.47$ ,  $p = 0.013$ ) and ASPT score ( $r = 0.54$ ,  $p = 0.005$ ).

The SASS5 scores were generally low for both seasons ranging between 7 and 23, with site 4 having the lowest SASS scores for the two seasons. The scores generally reflected poor water quality for both seasons. The ASPT scores indicated that the condition of water quality was fair in winter for sites 1–3, with site 4 having good water quality. The SASS scores were negatively correlated with TDS ( $r = -0.48$ ,  $p = 0.013$ ), pH ( $r = -0.64$ ,  $p = 0.001$ ) and chl-*a* concentration ( $r = -0.74$ ,  $p < 0.001$ ), and positively correlated with elevation ( $r = 0.77$ ,  $p < 0.001$ ) and habitat structure ( $r = 0.51$ ,  $p = 0.007$ ). Overall in summer, the water quality condition was good for sites 1–3, with site 4 having a fair condition and the opposite was observed for the winter season (Fig. 4). The %Trichoptera and %Diptera were high at sites 4 and 2 during winter, with %Coleoptera being high in site 2 for summer season.

### Relationship between macroinvertebrates and environmental/biotic variables

The CCA highlighted seven variables, i.e. pH, phosphate concentration, temperature, Ammonium, macrophyte cover, conductivity and water depth, which were significant in structuring macroinvertebrate community (Fig. 5). The CCA axes 1 (23.7%) and 2 (21.4%) accounted for 45.1% of the total macroinvertebrate community and environmental/biotic variation. The CCA axis 1 generally separated the winter and summer sites in the study area. The winter sites were mostly negatively associated with CCA axis 1 and were characterised by high temperature, phosphate and ammonium concentration, macrophyte cover, conductivity and pH. Examples of macroinvertebrates that were associated with these sites include Aeshnidae, Coenagrionidae, Corixidae, Gerridae and Chironomidae. Summer sites were positively associated with axis 1 and were characterised by high water depth and pH levels (Fig. 5). Examples of macroinvertebrates that were associated with these sites included Baetidae, Pleidae, Gomphidae and Libellulidae (Fig. 5).

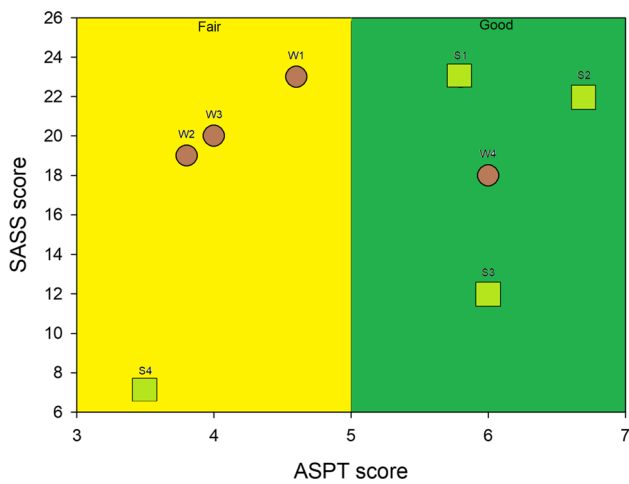
### Habitat assessment

The Sambandou wetland was degraded mostly due to agricultural activities that were observed within the wetland such as cattle grazing and cultivation (Fig. 6a, c, d) and sand mining (i.e. sand poaching; Fig. 6b, c). There was settlement near the wetland and cultivation within the wetland was mostly vegetables, potatoes, maize and sugarcane. The wetland habitat rating for site 1 was fair (68%), site 2 was excellent (97%) and site 3 was good (80%). Habitat rating

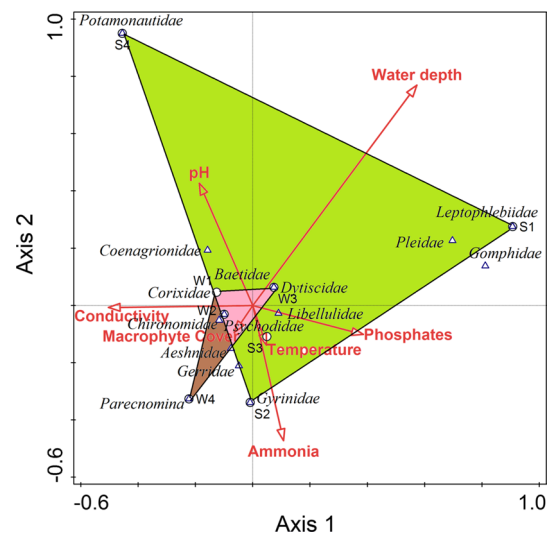
**Table 3** Macroinvertebrates relative abundance (%) observed at four sites over two seasons (summer, winter)

Family	Winter				Summer			
	Site 1	Site 2	Site 3	Site 4	Site 1	Site 2	Site 3	Site 4
Coleoptera								
Dytiscidae			12					
Gyrinidae						61		
Diptera								
Chironomidae	10	59		20				
Psychodidae		1						
Ephemeroptera								
Baetidae			6					
Leptophlebiidae					29			
Hemiptera								
Corixidae			6					
Gerridae	5					11		
Pleidae					14			
Odonata								
Aeshnidae	5	9		20		11	20	
Libellulidae	10	20	70		43	17	80	
Gomphidae					14			
Coenagrionidae	70	11	6					60
Trichoptera								
Ecnomidae				60				
Crustacea								
Potamonautidae								40
SASS score	23	19	20	18	23	22	12	7
ASPT score	4.6	3.8	4.0	6.0	5.8	6.7	6.0	3.5
Taxa richness	5	5	5	3	4	3	2	2
Shannon–Weiner	1.01	0.15	1.00	0.95	1.00	1.28	0.40	0.67

ASPT average score per taxa, SASS5 South African Scoring System version 5



**Fig. 4** Mean ASPT and SASS scores calculated for Sambandou wetland sites over two seasons



**Fig. 5** Canonical correspondence analysis (CCA) showing the relationship between environmental variables and macroinvertebrates. Polygons indicate the two seasons: green—summer and brown—winter



**Fig. 6** Wetland degradation observed within the Sambandou wetlands: **a** wetland cultivation (site 2), **b** sand mining/poaching (site 4), **c** sand mining/poaching and cattle grazing causing erosion (site 1), and **d** cattle grazing (site 1)

for site 4 was poor (40%), and the site was heavily eroded, which may be a result of sand mining (i.e. sand poaching) and cattle grazing as a lot of cattle dung was observed within the study site. Habitat structure was negatively correlated with pH ( $r = -0.47$ ,  $p = 0.014$ ) and was positively correlated with elevation ( $r = 0.69$ ,  $p < 0.001$ ), turbidity ( $r = 0.56$ ,  $p = 0.009$ ) and ammonium ( $r = 0.76$ ,  $p < 0.001$ ).

## Discussion

The study assessed macroinvertebrate communities in relation to environmental and biotic variables in Sambandou wetlands. The results showed that the measured environmental (i.e. water depth, ammonium, conductivity, pH and phosphates) and biotic (macrophyte cover) variables had an effect on macroinvertebrate communities. The macroinvertebrate richness varied among two seasons (winter and summer), being generally high in summer. The CCA analysis highlighted that summer sites were positively associated with high pH concentration (Fig. 4), and at local spatiotemporal scales most studies do not report the significance of pH as an environmental variable influencing macroinvertebrate community structure (Nhiwatiwa et al. 2017b). However, at

regional scales where landscape differences are significant, it has been shown to be an influential factor (Nicolet et al. 2004).

Water depth, conductivity, ammonium, conductivity, pH, phosphates and macrophyte cover were significant variables affecting macroinvertebrate community structuring as highlighted by the CCA analysis. Although they have a high adaptability to a wide range of ecological conditions, benthic macroinvertebrates can also actively select for suitable aquatic habitats (Batzer et al. 2004). This also has significant links with the importance of water depth on macroinvertebrate community structure and composition (Nhiwatiwa et al. 2017a). Nutrients such as phosphates and ammonium concentration were very high in summer, and these strongly affected macroinvertebrate communities. The grazing of cattle could have had a strong impact on wetlands by increasing nutrient input through urine and faecal deposition (Steinman and Rosen 2000). The wetland was cultivated on and cow dung used as manure, and several studies have highlighted that nutrient concentrations in the aquatic ecosystems can increase due to the application of manure and fertilisers (Bainbridge 2009).

While seasonality can play an important and key driver of macroinvertebrate community dynamics, its effect is



regulated by the predictability of its recurrence (Tonkin et al. 2017). For instance, in our study, highly predictable seasonal rainfall leads to more or less regular oscillations in distinct macroinvertebrate community types (see Fig. 5), but further long-term studies are required to verify the patterns in greater detail. By contrast, in Nigerian streams, Tonkin et al. (2016) found little seasonality role in shaping river macroinvertebrate community structure due to weak and unpredictable seasonality (Tonkin et al. 2017).

Chironomidae were the most abundant family during summer, whereas in winter Chironomidae were not identified. These results are similar the findings of Odume and Muller (2011) and Dalu et al. (2017b) who observed increased Chironomidae diversity during the summer season. Chironomidae taxa are tolerant to water pollution. High macroinvertebrate taxon richness was observed only in winter when there were less impacts occurring within the wetland.

A high chl-*a* concentration in the wetland is an indicator of potential eutrophication, which is harmful to many aquatic organisms and reduces biodiversity (Mereta et al. 2013). As expected, the chl-*a* concentration was high along a nutrient gradient, i.e. increasing nutrient concentrations similar to findings by Corkum (1996) and Dalu et al. (2014) studies. The study results indicated that the macroinvertebrate abundance and community structure changed with increasing chl-*a*, ammonium and phosphates concentration, with a single family grouping dominating. These results are in contrast to Kendrick et al. (2019) who observed increased phytoplankton biomass and invertebrate production. From the study findings, we can deduce that when nutrient resources supply were low especially during summer, phytoplankton was dominated by small-sized cells, i.e. pico- and nanophytoplankton, whereas the large-sized cell fractions increased as nutrients increased for sites 2 and 3 in winter where gardens were located, becoming dominant in highly productive ecosystems (Chisholm 1992; Li 2002). These contrasting phytoplankton community structures also relate to different ecosystem functioning modes. Under nutrient limitation, an ecosystem will maximize nutrient recycling, while new primary production based on inputs from allochthonous sources generally supports highly productive ecosystems resulting in dominance of a large number of large-celled phytoplankton, i.e. microphytoplankton similar to winter sites 2 and 3 (Eppley and Peterson 1979; Cózar et al. 2018).

The macrophyte composition consisted of five taxa which were identified (i.e. *Phragmites mauritianum*, *Potamogeton pectinatus*, *Cyperus* sp., *Cyperus sexangularis* and *Nymphaea naouchalia* var. *coerulea*), and these followed a dynamic pattern which was more pronounced for individual macrophyte species within the wetland. The overlap observed between winter and summer could be attributed to habitat degradation due to sand mining/poaching and agriculture for the sites in question (Fig. 3). Similar to Dalu et al.

(2012), the differences among sites in macrophyte species structure and composition was also attributed to substrate structure heterogeneity. Thus, habitat structure influence on macrophyte spatial distribution has long been discussed in detail in several studies (e.g. Machena 1987; Baatrup-Pedersen and Riis 1999; Brendonck et al. 2003). The study findings were also similar to those by Machena (1987) who observed that most submerged macrophytes were associated with extreme environmental conditions (gravel and rocky substrates). The diversity of macrophytes in wetlands creates a more heterogeneous environment which also contributes to increased macroinvertebrate diversity, for example shredders and scrappers (gastropods) which depend on macrophytes as food source and substrate (Gooderham and Tsyrlin 2002). However, in the current study, habitat complexity was unrelated to macroinvertebrate taxa richness and McAbendroth et al. (2005) also observed similar findings.

## Conclusions

The study results indicated that environmental and biotic variables influenced macroinvertebrate communities. Anthropogenic activities such as agriculture (i.e. cattle grazing, cultivation) and urban developments have resulted in increased nutrient concentrations, which might have had a significant negative effect on macroinvertebrate communities. More studies are therefore required so as to understand how macroinvertebrates are structured in relation to land-use activities and also how anthropogenic activities impact of the health status of the Sambandou wetlands within the Vhembe Biosphere Reserve.

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## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethical approval** All experiments were carried out in compliance with the ethical clearance approved by the University of Venda Research Committee (No. SES/18/ERM/10/1009).

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