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Water or sediment? Partitioning the role of water column and sediment chemistry as drivers of macroinvertebrate communities in an austral South African stream

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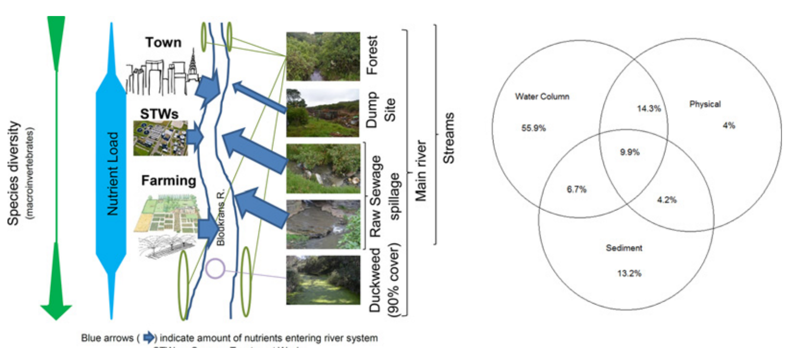
HIGHLIGHTS

- Water chemistry was more important than sediment in structuring macroinvertebrates.
- Strong seasonal variation observed for water quality and macroinvertebrates
- Chironomidae were the most abundant family with >25% relative abundances.

GRAPHICAL ABSTRACT



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ABSTRACT

Water pollution is a critical management issue, with many rivers and streams draining urban areas being polluted by the disposal of untreated solid waste and wastewater discharge, storm water and agricultural runoff. This has implications for biodiversity, and many rivers in the developing world are now considered compromised. We investigated benthic macroinvertebrate community structure and composition in relation to physico-chemical conditions of the water column and sediments. The study was conducted in an Austral catchment subject to both urban and agricultural pollutants in two different seasons. We assessed whether sediment characteristics were more important drivers of macroinvertebrate community composition than water column characteristics. We expected clear differences in macroinvertebrate community composition and in the associated community metrics due to distinct flow conditions between the two seasons. A combination of multivariate analyses (canonical correspondence analysis (CCA)) and biological indicator analysis were used to examine these patterns. Chironomidae was the most abundant family (>60%) in the upper mainstem river and stream sites. Stream sites were positively associated with CCA axis 2, being characterised by high turbidity and lower pH, salinity, phosphate concentration, channel width and canopy cover. Canopy cover, channel width, substrate embeddedness, phosphate concentration, pH, salinity and turbidity all had a significant

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effect on macroinvertebrate community composition. Using CCA variation partitioning, water quality was, however, a better predictor of benthic macroinvertebrate composition than sediment chemical conditions. Furthermore, our results suggest that seasonality had little effect on structuring benthic macroinvertebrate communities in this south-eastern zone of South Africa, despite clear changes in sediment chemistry. This likely reflects the relative lack of major variability in water chemistry compared to sediment chemistry between seasons and the relatively muted variability in precipitation between seasons than the more classic Austral temperate climates.

1. Introduction

Rivers and streams are globally threatened by anthropogenic pollution coupled with changes in physical structure and biodiversity (invasive species), due to intensive land-use and inadequate environmental management practices (Dudgeon et al., 2006; Beyene et al., 2009a; Cacador et al., 2012; Pallottini et al., 2015; Ferreira et al., 2017; Santos et al., 2017). In the developing world, water pollution is a critical management issue with many rivers and streams draining urban areas receiving storm water, untreated solid waste and wastewater, thus impairing water and sediment quality (Iwasaki and Ormerod, 2012; Pacheco et al., 2014; Dalu and Froneman, 2016; Hunt et al., 2017; Pacheco and Sanches Fernandes, 2016). As a consequence, biodiversity and ecosystem functioning within these ecosystems is compromised, as reflected by the poor ecological status of many rivers in developing countries (Arimoro, 2009; Dalu et al., 2012; Valle Junior et al., 2015; Bhaskar et al., 2016). Exploring the role of physicochemical variables in driving biotic communities is therefore important for the protection of biodiversity and prediction of the impacts of these perturbations on community structural changes.

The physico-chemical conditions of river water and sediment are inherently intertwined. River sediments have a strong adsorption capacity for pollutants and thus many of the water column characteristics are inherited by sediment (Smolders et al., 2003). However, physico-chemical dynamics within these two environments can be somewhat distinct. Pollutants such as heavy metals often occur in greater quantities within sediments than in the water column (Smolders et al., 2003; Santoro et al., 2009; Akele et al., 2016). Indeed, sediments can act as heavy metal, nutrient and organochlorine pesticide reservoirs that are released into the water column and/or accumulate in plant and animal tissues before entering food chains (Pallottini et al., 2015).

The effects of pollutants on the composition of biological communities are not well researched in many developing countries (Mwedzi et al., 2016; Bere et al., 2016; Nhiwatiwa et al., 2017; Dalu et al., 2017a, 2017b). While heavy metals in aquatic ecosystems can be naturally produced by the slow leaching from soils and rocks (Smolders et al., 2003; Zhou et al., 2008), human activities such as wastewater discharges can increase heavy-metal loads and are often the predominant causation of environmental health degradation if they are present at high concentrations (Santoro et al., 2009) and may cause histopathological changes in biological communities (Fonseca et al., 2016, 2017). Unlike the developed world, where stringent regulations are implemented to restrict the discharge of untreated wastewater into rivers and streams, existing pollution legislation in many developing countries is weak and/or generally not adequately enforced (Beyene et al., 2009b; Capps et al., 2016). With increasing urbanization, industrialisation and agriculture in these regions, heavy metal pollution is also on the rise (Cacador et al., 2012; Akele et al., 2016). Informal settlements are of special concern in cities undergoing rapid urbanization in lower-income economies, as they are often characterised by limited access to drinking water and sewage systems (Parreira de Castro et al., 2016).

Pollution dynamics in arid Austral river systems are not as well understood as in areas where rainfall is more consistent. In general, flow in arid Austral river systems is highly variable, and differences between tributary stream and mainstem river dynamics are often more pronounced than in mesic environments (Sedell et al., 1989; Benda et al., 2004; Kiffney et al., 2006; Dalu et al., 2017b). Here, seasonal variations in water temperature and flow also have larger impacts on sediment and water column chemistry. Such flow seasonality can also lead to distinct benthic macroinvertebrate communities due to temporal niche segregation (Tonkin et al., 2017).

Macroinvertebrates, which constitute an important component of secondary production within freshwater ecosystems, are integrated into the structure and function of their habitats (Li et al., 2010), and are expected to vary consistently in relation to the intensity of a disturbance type in a particular area (Clements, 2004; Caro-Borrero et al., 2016). As such, they are widely used as biological indicators of ecosystem health (Clements, 2004). Hence, understanding the response of the macroinvertebrate community to perturbation is key in water environmental impact assessment (Phiri, 2000). In rivers and streams, most macroinvertebrate taxa are benthic and therefore related to the sediment, making them potential bio-indicators of sediment quality (King et al., 2016). Their life cycles vary from intra- to inter-annual so they can integrate precursor conditions from short-term episodes to longer term changes (Iwasaki and Ormerod, 2012; King et al., 2016; Niedrist and Füreder, 2016). Identifying the effects of various types of contaminants on macroinvertebrate community structure is however challenging, because of the spatial covariance in different environmental factors that independently account for community diversity patterns (Mwedzi et al., 2016; Niedrist and Füreder, 2016).

The rationale for several of the recommended ecosystem health metrics is based on the observation that some taxa, such as Ephemeroptera, Plecoptera, and Trichoptera (EPT taxa) are sensitive to contaminants and also to changes in the physical conditions, such as temperature and riparian vegetation. By contrast, others (e.g., Chironomidae, Oligochaeta) are generally more tolerant (Clements, 1994; Masese et al., 2014). Although these generalisations hold for organic enrichment, benthic invertebrates may respond differently to toxic chemicals (Clements, 1994; Hickey and Clements, 1998; Peeters et al., 2004), but there remains considerable uncertainty as to the importance of the source of these chemicals.

This study investigated benthic macroinvertebrate community structure and composition in relation to the physical and chemical characteristics of the water column and sediment along the Bloukrans River system, situated in the Eastern Cape, South Africa. We used habitat assessment (physical, water and sediment) variables to assess aquatic macroinvertebrate community structure and composition in relation to sediment and water chemistry characteristics. This approach was applied in urban headwater tributaries (i.e. streams) and the mainstem river habitats. Specifically, we assessed (i) whether sediment chemistry characteristics were more important drivers of macroinvertebrate community structure and composition than water characteristics; (ii) if the relative contribution of sediment and water column chemistry drivers to macroinvertebrates community structure and composition varied across seasons in relation to changes in river base flow; and (iii) whether environmental variables and macroinvertebrate community structure and composition differed between the different river sections.

2. Materials and methods

2.1. Study area

The study was carried out within the Bloukrans River system (Lat. −33.35353 to −33.29383, Lon. 26.72078 to 26.51357) which drains the town of Grahamstown (population ~ 120,000) and surrounding areas. The river has a total catchment of ~220 km^2 and length of ~40 km. The river system is subjected to various sources of pollution including agriculture (i.e. crop and pasture irrigation, dairy farming), overflowing sewage, and domestic and industrial waste at different locations. The study area is located within the warm temperate climatic region of South Africa. Summers are typically warm (mean daily temperatures of 20.3 °C, January) and winters are mild (mean daily temperatures of 12.3 °C, June; Sinchembe & Ellery, 2010). Mean annual rainfall is ~680 mm, and although rainfall occurs during all months, summer (September–March: ~470 mm) rainfall is higher than winter (~210 mm).

This study was conducted in summer (February) and winter (July) of 2016. Samples were collected across thirty-one selected sites distributed along the river system to represent first and second order streams (i.e. sites 9, 13–16, 18–31) and third order stream sites (i.e. sites 1–8, 10–12, 17; Fig. 1) and sites potentially receiving varying forms of pollution. Sites 1–10, 19 and 21 were located in intensive agricultural farms (diary and irrigation) immediately downstream of the city of Grahamstown, while the rest of the sites were within and above the city/urban area.

2.2. Water physico-chemical variables

Water samples ($n = 3$) from the two littoral zone edges and main channel centre were collected at each site to measure nutrient (nitrate and phosphate) concentrations. Collected water samples were kept on ice until analysis within 8 h of collection. Nutrient concentrations in the water samples were analysed using an HI 83203 multiparameter bench photometer (Hanna Instruments Inc., Rhode Island) upon return to the laboratory: ammonia (photometer range of 0–10 mg L^{-1} ± 0.05 mg L^{-1} accuracy), nitrate concentration (photometer range of 0–50 mg L^{-1}, ±0.5 mg L^{-1} accuracy) and phosphate concentration (photometer range of 0–30 mg L^{-1}, ±1 mg L^{-1} accuracy). Conductivity, pH, salinity, total dissolved solids (TDS) and water temperature were measured in situ using a portable multi-parameter probe (PCTestr 35, Eutech/Oakton Instruments, Singapore), dissolved oxygen (DO) using a DO meter (DO 850045, Per Scientific, Taiwan), and turbidity was measured using a turbidity meter (AL250T-IR, Aqualytic, Germany).

Flow velocity was measured using a Flo-mate portable flowmeter Model 2000 (Marsh McBirney, Maryland, US). Channel width and water depth were measured using a tape measure and graduated measuring rod, respectively. Embeddedness was determined according to Platts et al. (1983): (1) >75%; (2) 50–75%; (3) 25–50%; (4) 5–25%; and (5) <5% of benthic surface covered by fine sediment.

2.3. Sediment chemistry variables

Integrated sediment samples (1.5 kg, $n = 2$) were collected at the centre and littoral zones of each site and season using a plastic hand shovel after the removal of the overlaying debris to a depth of about 5–10 cm into the sediment layer. Samples were then stored in polyethylene ziplock bags. The sediment samples were dried in an oven at 60 °C for 48 h, before being disaggregated using a porcelain pestle and mortar and sieved through a <0.075 mm sieve to remove plant roots and other debris. All metal analysis was conducted at a South African National Accreditation System (SANAS) certified laboratory. Cation elements (boron (B), calcium (Ca), potassium (K), magnesium (Mg), sodium (Na)) were determined using acid digestion with a 1:1 mixture of 1 N nitric acid (HNO_3) and hydrochloric acid at 80 °C for 30 min. Heavy metal (chromium (Cr), copper (Cu), iron (Fe), lead (Pb), zinc (Zn)) analyses was performed using 5 g of dried and sieved soil to which 20 mL HNO_3 (55%) and 5 mL hydrogen peroxide (30%) was added and placed on a heated sand bed (180 °C) for eight hours, before being filtered onto a Whatman filter paper. The cation elements and heavy metal content from the extracts were determined using an ICP-OES optical emission spectrometer (Varian, Mulgrave, Australia) (see Clesceri et al. (1998) for detailed methodology).

Sediment nitrate concentrations was determined calorimetrically on the SEAL Auto-Analyser 3 through reduction of NO_3^- to nitrite (NO_2^-) using a copper-cadmium reduction column, before the nitrate finally reacted with sulphanilamide under acidic conditions (Agri Laboratory Association of Southern Africa (AgriLASA), 2004). Sediment phosphorus (P) and phosphate (PO_4^{3-}) concentration were analysed using a Bray-2 extract method (Bray and Kurtz, 1945) and sediment organic matter (SOM) and organic carbon (SOC) were determined using the modified Walkley–Black method (Chan et al., 2001). The sediment quality guidelines for freshwater (MacDonald et al., 2000) were used to assess impacted sites based on sediment heavy metals.

2.4. Macroinvertebrate sampling

Benthic macroinvertebrates were collected using the kick sampling method described by Dickens and Graham (2002), whereby sediment and rocks in the water are kicked with feet while sweeping the net in a zig-zag manner to dislodge any attached macroinvertebrates using a hand-held kick net (dimension 30 × 30 cm, mesh size 500 μm, 1.5 m handle). At each sampling site, approximately six minutes was spent sampling all aquatic habitats (i.e. riffles, pools and vegetated margins)

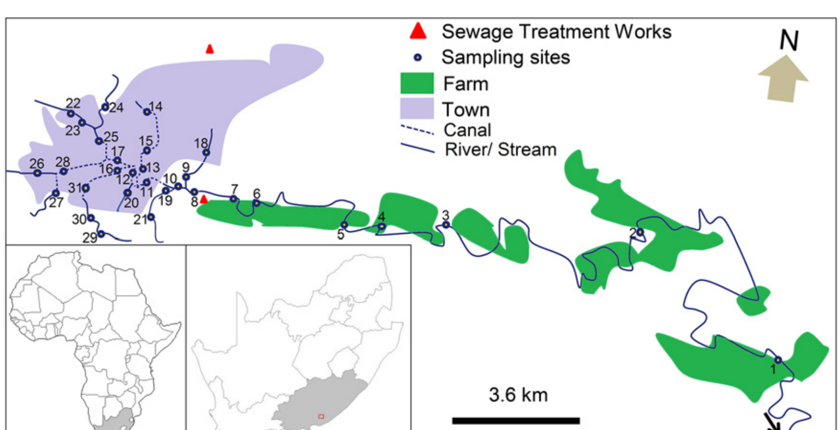

Fig. 1. Location of the study sites along the Bloukrans River System, Eastern Cape (South Africa).

and the samples were combined to form one composite sample. Benthic samples were preserved in 70% ethanol for later processing. In the laboratory, all macroinvertebrate were identified to family level using keys by Barbour et al. (1999), Gerber and Gabriel (2002a, 2002b), Gooderham and Tsyrlin (2002) and de Moor et al. (2003a, 2003b).

2.5. Data analysis

Two-way ANOVAs were used to compare water and sediment variables, community metrics and diversity indices among stream sections (i.e. stream and mainstem river) and between seasons (i.e. summer and winter), after testing for homogeneity of variances (Levene's test, $p > 0.05$) and normality of distribution (Shapiro-Wilk test, $p > 0.05$). The same tests were used to assess for differences in several macroinvertebrate diversity and community metrics and diversity indices among stream sections and seasons. The analysis was carried out in SPSS version 16.0 (SPSS Inc., 2007).

Common metrics and diversity indices were used to assess the integrity of macroinvertebrate assemblages: %Ephemeroptera abundance, %Trichoptera abundance and %Diptera abundance, %Ephemeroptera, Plecoptera and Trichoptera (%EPT) abundance, EPT/Chironomid ratio, and Shannon-Wiener diversity index. Moreover, 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 number of taxa (Dickens and Graham, 2002), were further computed to assess river quality. As they are used to examine locally-specific metrics of water quality, the SASS5 and ASPT scores were employed as a measure of 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).

The significance of the influence of different environmental variables on macroinvertebrate communities was investigated by canonical correspondence analysis (CCA) using CANOCO version 5.1 software (ter Braak and Šmilauer, 2002). A total of 32 CCAs corresponding to 32 tested environmental variables were performed. The significance of each explanatory variable was evaluated with Monte Carlo permutations test (999 permutations). The strength of relationship between macroinvertebrate communities and each explanatory variable was assessed using the ratio of the first and second Eigen values (λ_1/λ_2). This ratio measures the strength of the constraining variable with respect to the first unconstrained gradient in the community composition data. The strength of relationship is considered very high if $\lambda_1/\lambda_2 > 1$, moderately high if $0.5 < \lambda_1/\lambda_2 < 1$, and weak if $\lambda_1/\lambda_2 < 0.5$ (ter Braak and Prentice, 1988). This stage facilitated selection of variables influencing macroinvertebrates.

Table 1
Mean water, sediment and physical variables (±standard deviation) recorded across two seasons for the river section categories. Abbreviations: TDS – total dissolved solids, SOM – sediment organic matter, SOC – sediment organic carbon.

Variables	Summer				Winter			
	River		Stream		River		Stream	
	Range	mean	Range	mean	Range	mean	Range	mean
Water chemistry								
Temperature (°C)	19.1–22.7	20.9 ± 1.2	17.9–20.8	19.3 ± 0.9	10.0–15.8	12.4 ± 2.3	9.8–15.5	13.6 ± 1.8
Conductivity (μS cm^{-1})	1194.7–1721.7	1376.5 ± 152.5	195.3–6530.0	1689.7 ± 1757.7	1386.7–2577.0	1654.2 ± 332.1	219.7–6290.3	1383.0 ± 1388.2
Dissolved oxygen (mg L^{-1})	1.6–6.9	4.2 ± 1.9	1.6–8.9	5.0 ± 2.4	4.0–7.7	6.1 ± 1.2	4.5–13.8	7.6 ± 2.2
Turbidity (NTU)	5.7–22.5	12.5 ± 5.4	3.4–55.4	22.2 ± 17.1	3.1–56.2	11.0 ± 14.6	2.2–63.5	17.5 ± 17.7
pH	7.8–8.1	7.9 ± 0.1	6.4–8.5	7.8 ± 0.6	7.8–8.5	8.0 ± 0.2	7.1–8.3	7.8 ± 0.4
Salinity (ppm)	591.7–859.3	682.9 ± 76.8	94.0–3480.0	690.3 ± 749.4	680.0–1173.7	811.0 ± 146.3	101.3–3341.0	692.2 ± 736.3
TDS (mg L^{-1})	851.0–1213.3	984.0 ± 109.1	138.7–4630.0	1009.4 ± 1010.4	995.7–1834.0	1183.2 ± 234.7	155.7–10,429.7	1435.9 ± 2386.0
Nitrate (mg L^{-1})	3.5–27.4	12.8 ± 7.6	0.3–197.2	22.9 ± 45.3	27.3–129.9	54.6 ± 24.9	1.7–254.0	51.1 ± 64.3
Phosphate (mg L^{-1})	7.4–15.3	9.5 ± 2.6	0.1–25.1	4.6 ± 6.1	2.9–12.2	9.0 ± 3.1	0.2–9.8	2.7 ± 2.6
Sediment chemistry								
Ca (mg kg^{-1})	1.3–6.2	2.5 ± 1.4	0.4–16.9	5.7 ± 5.1	667.0–26,827.7	6996.1 ± 9541.7	596.3–26,827.7	7193.8 ± 7061.8
B (mg kg^{-1})	0.2–0.8	0.4 ± 0.2	0.1–3.4	0.8 ± 0.8	2.1–10.2	5.7 ± 2.6	3.4–13.6	7.1 ± 2.8
Fe (mg kg^{-1})	256.5–659.0	353.2 ± 108.2	127.1–1860.0	550.4 ± 454.7	6856.4–53,101.2	30,923.0 ± 16,235.3	17,038.8–53,101.2	36,008.1 ± 10,262.6
Mg (mg kg^{-1})	0.6–2.8	1.1 ± 0.6	0.4–5.1	2.0 ± 1.5	152.8–2083.0	743.1 ± 549.9	140.6–2083.0	874.6 ± 586.9
Pb (mg kg^{-1})	2.1–38.3	12.0 ± 13.4	0.0–33.6	11.8 ± 10.4	4.3–32.3	16.6 ± 9.4	5.3–164.7	28.5 ± 34.5
K (mg kg^{-1})	0.1–0.2	0.1 ± 0.1	0.02–1.1	0.3 ± 0.3	63.4–594.7	218.0 ± 145.7	40.5–594.7	219.3 ± 114.6
Zn (mg kg^{-1})	3.0–27.2	12.7 ± 7.2	1.7–68.6	25.6 ± 24.0	18.2–213.4	86.3 ± 57.5	19.3–350.1	111.8 ± 77.3
Na (mg kg^{-1})	0.2–0.8	0.3 ± 0.2	0.1–2.6	0.6 ± 0.6	18.1–195.5	72.3 ± 570.8	19.4–823.3	150.1 ± 240.4
Cu (mg kg^{-1})	0.8–2.3	1.4 ± 0.5	0.2–8.9	3.0 ± 2.4	1.1–115.7	25.3 ± 42.6	1.2–115.7	17.9 ± 26.3
Cr (mg kg^{-1})	5.2–18.0	8.7 ± 3.6	3.3–15.9	8.3 ± 3.7	9.1–25.0	16.5 ± 5.4	6.0–26.4	14.6 ± 5.4
NO_3–N (mg kg^{-1})	0.0–0.8	0.3 ± 0.3	0.0–0.8	0.2 ± 0.2	0.2–0.7	0.5 ± 0.2	0.2–3.0	0.8 ± 0.9
P (mg kg^{-1})	354.6–868.2	484.4 ± 148.5	20.6–1531.2	513.1 ± 399.6	201.2–979.8	487.5 ± 259.9	77.8–1991.5	574.5 ± 435.0
PO_4 (mg kg^{-1})	1085.0–2656.8	1482.1 ± 454.4	63.1–4685.3	1570.2 ± 1222.9	615.6–2998.2	1491.7 ± 795.2	238.0–6094.1	1757.8 ± 1331.2
Sediment metal ratios								
Mg/Ca	0.3–0.5	0.4 ± 0.1	0.2–1.0	0.5 ± 0.2	0.04–0.4	0.2 ± 0.1	0.04–0.5	0.2 ± 0.1
Fe/P	0.5–1.0	0.7 ± 0.1	0.2–10.3	2.2 ± 2.6	33.1–163.6	70.7 ± 37.1	16.9–298.9	96.0 ± 75.6
Ca/P	0.002–0.01	0.01 ± 0.003	0.004–0.04	0.01 ± 0.01	2.9–34.1	11.6 ± 11.2	3.3–34.1	13.3 ± 9.2
Organic matter content								
SOM (%)	1.1–5.0	2.5 ± 1.3	1.4–25.4	7.9 ± 6.1	2.5–24.5	12.8 ± 7.1	2.9–59.8	22.4 ± 19.6
SOC (%)	0.7–2.9	1.5 ± 0.7	0.8–14.7	4.6 ± 3.5	1.4–14.2	7.4 ± 4.1	1.7–34.7	13.0 ± 11.4
Physical								
Embeddedness	1.0–5.0	3.3 ± 1.5	1.0–5.0	2.3 ± 1.7	1.0–5.0	3.3 ± 1.4	1.0–4.0	1.8 ± 1.1
Channel width (m)	0.4–12.0	4.3 ± 3.8	0.2–2.0	0.8 ± 0.4	0.5–8.7	4.2 ± 2.6	0.2–1.9	0.8 ± 0.5
Water velocity (m s^{-1})	0.1–1.0	0.3 ± 0.3	0.03–0.7	0.2 ± 0.2	0.1–1.6	0.6 ± 0.5	0.03–0.6	0.2 ± 0.2
Water depth (m)	0.1–0.4	0.2 ± 0.1	0.02–0.2	0.1 ± 0.05	0.03–11.7	1.2 ± 3.3	0.02–0.3	0.1 ± 0.1
Canopy cover (%)	0.0–0.9	0.5 ± 0.3	0.0–1.0	0.4 ± 0.4	0.0–0.4	0.2 ± 0.2	0.0–1.0	0.3 ± 0.4

Canonical Correspondence Analysis (CCA) was then used to show how the significant variables jointly influenced macroinvertebrates in the different site categories and seasons. The macroinvertebrate and physico-chemical data were log $(x + 1)$ transformed to reduce the effects of extreme values, with exception of pH. Monte Carlo permutation tests (999 unrestricted permutations, $p < 0.05$) were used to test the significance of the axis. We further quantified the relative influence of water column, sediment and physical variables on macroinvertebrate communities using the variance partitioning (partial CCA) method. With this approach, variation in taxonomic composition was attributed to specific variable groups by including other potentially relevant variables as covariables (Borcard et al., 1992; Legendre and Legendre, 2012).

Distance-based Permutational Analysis of Variance (PERMANOVA; Anderson, 2001) were used to analyse whether macroinvertebrate abundance and environmental variables differed between river sections (main river, streams) and seasons (summer, winter) using PERMANOVA+ for PRIMER version 6 (Anderson et al., 2008). Euclidean distance and Bray Curtis dissimilarities were employed for environmental and biological data, respectively, and 9999 permutations were used to test for significance.

The Indicator Analysis method (IndVal; Dufrêne and Legendre, 1997), which combines each macroinvertebrate family's relative abundances and frequencies of occurrence from the different samples collected, was used to identify macroinvertebrate indicator taxa from all river sections and seasons. Indicator species were defined as those most representative for each land use type and season. The indicator values range from 0% (i.e. no indication or same occurrence and abundances in all land use type or seasons) to 100% (i.e. perfect indication or confined to one land use type or season) (see Dufrêne and Legendre (1997) and Milošević et al. (2013) for detailed information). The significance of each taxa was examined using Monte Carlo tests with 9999 permutations, and families with significant ($p < 0.050$) indicator values were considered as important indicator taxa. The IndVal analysis was carried out in PC-ORD version 5.10 (McCune and Mefford, 2006).

3. Results

3.1. Environmental variables

3.1.1. Water chemistry and physical variables

Mean nitrate concentrations were generally high in many of the mainstem river (sites 10, 11, 12, 17) and stream (sites 9, 13, 14, 16) sites over the two seasons (see Table S1), while phosphate concentration was high in the river sites for both seasons (Table 1). Salinity and total dissolved solids concentrations were both similar for the two seasons across the catchment (Table 1), with the stream sites being higher than the mainstem river sites (Table 2). There were significant differences in dissolved oxygen, turbidity, phosphate concentration, channel width and water depth among the different river section types, with high seasonal differences being observed for temperature, DO, nitrate and phosphate concentrations (Table 2).

3.1.2. Sediment chemistry variables

Generally, metal concentrations were higher in the streams than in the mainstem river sites for both seasons (Table 1). Mean metal concentrations for most of the sediment increased by >10 fold from the summer to winter, with highest mean concentrations being observed in stream sites. Most of the metals excluding Pb were significantly different within the two seasons ($p < 0.050$; Table 2) but similar across river sections (Table 2). Lead (Pb; $F_{(1,61)} = 3.672$, $p = 0.060$), sediment phosphorous ($F_{(1,61)} = 0.122$, $p = 0.728$) and phosphate ($F_{(1,61)} = 0.122$, $p = 0.728$) concentrations were similar across the two seasons (Table 2).

Sediment organic matter and organic carbon were high in the stream sites for both seasons, with increased content being observed in the winter season (Table 1). Significant seasonal differences were observed for SOM ($F_{(1,61)} = 16.104$, $p < 0.001$) and SOC ($F_{(1,61)} = 16.045$, $p < 0.001$) content. The Mg/Ca ratios were slightly higher during the summer season, with Ca/P and Fe/P ratios being higher in the winter season (Table 2). All metal ratios were almost similar ($p > 0.050$) across the two river sections (Table 3), with high significant seasonal differences being observed for Mg/Ca ($F_{(1,61)} = 34.802$, $p < 0.001$), Ca/P ($F_{(1,61)} = 48.376$, $p < 0.001$) and Fe/P ($F_{(1,61)} = 45.625$, $p < 0.001$) ratios.

Table 2
Two-way analysis of variance (2-way ANOVA) based on the water, sediment and physical and community metrics variables identifying differences among land types and seasons. Bold values indicate $p < 0.05$.

Variables	River section		Season		River section × season	
	F	p	F	p	F	p
Water chemistry						
Temperature	0.245	0.623	**292.341**	<**0.001**	**12.334**	**0.001**
Conductivity	0.004	0.949	0.002	0.965	0.794	0.377
Dissolved oxygen	**4.270**	**0.043**	**17.074**	<**0.001**	0.274	0.603
Turbidity	**4.135**	**0.047**	0.608	0.439	0.160	0.691
pH	2.610	0.112	0.287	0.594	0.078	0.782
Salinity	0.131	0.718	0.179	0.674	0.168	0.683
Total dissolved solids	0.136	0.714	0.687	0.411	0.091	0.764
Nitrates	0.078	0.781	**8.784**	**0.004**	0.335	0.565
Phosphate	**27.575**	<**0.001**	1.101	0.298	0.464	0.498
Sediment chemistry						
Ca	0.005	0.947	**22.588**	<**0.001**	0.004	0.948
B	3.303	0.074	**127.402**	<**0.001**	0.957	0.332
Fe	1.240	0.270	**193.760**	<**0.001**	1.062	0.307
Mg	0.393	0.533	**58.379**	<**0.001**	0.382	0.539
Pb	1.086	0.302	3.672	0.060	1.180	0.282
K	0.001	0.976	**86.673**	<**0.001**	0.001	0.982
Zn	2.029	0.160	**35.156**	<**0.001**	0.215	0.644
Na	1.210	0.276	**9.712**	**0.003**	1.191	0.280
Cu	0.218	0.642	**9.865**	**0.003**	0.543	0.464
Cr	0.915	0.343	**33.888**	<**0.001**	0.332	0.567
NO_3–N	1.154	0.287	**9.098**	**0.004**	2.009	0.162
P	0.393	0.533	0.122	0.728	0.099	0.754
PO_4	0.393	0.533	0.122	0.728	0.099	0.754
Metal ratios						
Mg/Ca	0.005	0.942	**34.802**	<**0.001**	0.212	0.647
Fe/P	1.302	0.259	**48.376**	<**0.001**	1.032	0.314
Ca/P	0.206	0.652	**45.625**	<**0.001**	0.202	0.655
Organic matter content						
Sediment organic matter	**5.808**	**0.019**	**16.104**	<**0.001**	0.453	0.504
Sediment organic carbon	**5.823**	**0.019**	**16.045**	<**0.001**	0.455	0.503
Physical						
Channel width	**42.250**	<**0.001**	0.009	0.925	0.002	0.964
Water velocity	**11.855**	**0.001**	2.983	0.089	2.983	0.089
Water depth	2.667	0.108	1.699	0.198	1.772	0.188
Community metrics						
% Trichoptera	0.339	0.563	0.731	0.397	0.000	0.987
% Ephemeroptera	3.820	0.057	0.563	0.457	1.319	0.257
% EPT	1.093	0.301	0.003	0.960	0.627	0.432
% Diptera	0.676	0.415	0.634	0.430	0.003	0.957
EPT/Chironomidae ratio	1.598	0.213	0.119	0.732	0.000	0.983
SASS	0.520	0.475	1.873	0.178	0.690	0.410
ASPT	2.369	0.131	0.386	0.537	0.047	0.830
Taxa richness	1.815	0.185	**6.875**	**0.012**	1.948	0.170
Shannon-Wiener	0.171	0.681	3.299	0.076	0.902	0.347

Changes in environmental variables relating to river health were found to differ significantly among the river sections (PERMANOVA; Pseudo–$F_{(1,61)} = 3.866$, $p < 0.001$) and seasons (PERMANOVA; Pseudo–$F_{(1,61)} = 17.259$, $p < 0.001$) but no significant differences were observed for the interaction between river sections and seasons (PERMANOVA; Pseudo–$F_{(1,61)} = 0.712$, $p = 0.708$).

3.2. Macroinvertebrate community structure

A total of 17,610 macroinvertebrates belonging to 54 families (11 orders) were recorded from the Bloukrans River system over two seasons

Table 3
Mean relative abundances (%) of the dominant macroinvertebrate families and community metrics (mean ± standard deviation) observed over two seasons for the study site categories: river and stream. Abbreviations: Abbr. – abbreviation, ASPT – average score per taxa, SASS5 – South African scoring system version 5.

Family	Abbr.	Summer season		Winter season	
		River	Stream	River	Stream
Annelida					
Hirudinae	Hir	3.2 ± 0.0	8.4 ± 0.0		
Oligochaetae	Oli	2.0 ± 3.2	5.9 ± 8.1	1.6 ± 2.5	2.1 ± 5.3
Coleoptera					
Helodidae	Hel	4.7 ± 3.0	6.5 ± 7.0	3.6 ± 3.0	12.4 ± 8.7
Potamonautidae	Pot	3.7 ± 10.0	5.3 ± 10.6		
Diptera					
Chironomidae	Chi	40.1 ± 26.6	35.6 ± 34.3	57.6 ± 40.9	38.3 ± 37.1
Dixidae	Dix			3.7 ± 8.3	12.6 ± 18.8
Psychodidae	Psy	6.4 ± 11.2	16.3 ± 25.8		
Simuliidae	Sim	6.3 ± 1.8	9.9 ± 3.8	6.3 ± 3.2	16.3 ± 9.7
Sphaeriidae	Sph	1.8 ± 5.7	3.5 ± 19.5		
Tipuliidae	Tip	0.7 ± 0.5	2.3 ± 1.6		
Ephemeroptera					
Baetidae	Bae	5.8 ± 4.7	7.5 ± 9.4	10.1 ± 1.3	16.5 ± 2.8
Hemiptera					
Notonectidae	Not	1.6 ± 1.6	3.0 ± 6.7		
Mollusca					
Ancylidae	Anc	3.4 ± 0.4	5.9 ± 1.6	3.2 ± 0.3	4.5 ± 0.9
Physidae	Phy	2.3 ± 1.9	3.8 ± 4.7	4.3 ± 5.1	6.0 ± 15.6
Odonata					
Aeshnidae	Aes	1.6 ± 4.2	5.5 ± 7.7		
Coenogranidae	Coe	6.2 ± 2.0	10.7 ± 3.6	5.1 ± 0.3	11.5 ± 1.3
Libelluliddae	Lib	2.1 ± 1.9	7.0 ± 6.2		
Trichoptera					
Hydropsychidae	Hydo	1.8 ± 3.1	6.2 ± 8.3		
Parecnomina	Par	0.8 ± 0.2	2.0 ± 1.0		
Metrics					
Taxa richness		9.4 ± 7.9	3.4 ± 2.9	6.1 ± 5.9	3.3 ± 2.6
% Trichoptera		3.1 ± 4.7	6.6 ± 10.7	1.5 ± 2.4	3.8 ± 6.8
% Ephemeroptera		5.8 ± 5.1	7.5 ± 9.9	10.1 ± 5.2	16.5 ± 16.9
% EPT		9.0 ± 9.8	12.9 ± 13.0	11.6 ± 7.6	19.8 ± 17.7
% Diptera		56.7 ± 48.8	35.4 ± 38.8	68.3 ± 69.1	28.8 ± 36.4
EPT/Chironomidae ratio		1.0 ± 0.5	1.6 ± 1.1	0.9 ± 0.4	2.1 ± 1.0
ASPT score		44.3 ± 41.8	24.6 ± 17.2	31.5 ± 31.3	23.1 ± 16.9
SASS5 score		4.4 ± 5.2	1.1 ± 0.8	4.6 ± 5.2	2.0 ± 1.2
Shannon–Wiener index		1.4 ± 1.4	0.7 ± 0.6	0.9 ± 1.0	0.6 ± 0.6

(Table 3). Chironomidae was the most abundant family with >25% relative abundances in the mainstem river and stream sites, with 57.6 ± 40.9% being observed for the winter season for the mainstem river sites. Taxa richness ranged between 5 and 16 in the mainstem river sites and between 4 and 15 in the stream sites during summer, and decreased to 2–11 for both the mainstem river and stream sites in the winter season (Table 3). Taxa richness differed between seasons ($F_{(1,61)} = 6.875$, $p = 0.012$), with all the other community metrics showing no differences between two river locations (Table 2). The Shannon–Wiener diversity index was high during the summer season, with the stream sites having lower diversity than the mainstem river sites. Based on the SASS5 scores, the lower mainstem river sites (sites 1 to 4; SASS5 score 60–94) had fair to good water quality, with the rest of the sites being of poor to very poor water quality (SASS5 score 20–57) during summer. During winter, water quality deteriorated further, to states of poor (i.e. mostly lower mainstem river and upper stream sites) and very poor (i.e. upper river and most of stream sites). Similarly, the ASPT scores indicated that most sites were fair to good water quality during summer, with poor to fair water quality for most sites during winter (Table 3). The %Trichoptera, %Ephemeroptera, %EPT and EPT/Chironomidae ratio was high for the stream sites for both seasons, whereas, %Diptera was high for the river sites (Table 3).

3.3. Relationship between macroinvertebrates and water and sediment variables

Based on CCAs carried out for individual variables, seven variables (i.e. canopy cover, channel width, phosphate concentration, pH, salinity, embeddedness and turbidity) were found to have a significant effect on macroinvertebrate community structure (Table S2; Monte Carlo permutation test, $p < 0.050$). The distribution of macroinvertebrates in the different sampling categories is shown in Fig. 2. The first four CCA axes accounted for 78.8% of the fitted variation. CCA axes 1 and 2 accounted for 53.1% variation in macroinvertebrate composition with axis 1 explaining 32.3% variation. CCA axis 2 generally separated the different mainstem river sites in the study area with similar sites grouping closely together. Stream sites were positively associated with CCA axis 2 and were characterised by high turbidity and lower pH, salinity, phosphate concentration, channel width and canopy cover. Examples of macroinvertebrates that were associated with these sites include Syrphidae, Oligochaeta, Culicidae and Gerridae which are very tolerant to organic pollution as they are able to breathe atmospheric oxygen. Mainstem river sites were negatively associated with axis 2, being characterised by higher canopy cover, channel width, pH, salinity, and phosphate concentration. Examples of macroinvertebrates that were associated with these sites include Ancylidae, Lestidae, Chlorolestidae, Coenagrionidae and Chironomidae (Fig. 2).

From the partial CCA results (Fig. 3), the main explanatory group was the water column which individually explained 55.9% of the total variance. Sediment and physical variables individually accounted for 13.2% and 4% of the total explained variation respectively. About 9.9% of the macroinvertebrate community structure data variation was shared among all the variable groups while 14.3% was shared between the water column and the physical variables, 6.7% was shared between the water column and sediment variables and 4.2% was shared between sediment and physical variables.

Macroinvertebrate total abundances were found to differ significantly among the river sections (PERMANOVA; Pseudo–$F_{(1,61)} = 4.216$, $p < 0.001$) and seasons (PERMANOVA; Pseudo–$F_{(1,61)} = 2.296$, $p = 0.019$). Using indicator analysis, Ancylidae (IndVal 48.8%), Chironomidae (59.7%), Physidae (41.0%), Hirudinae (20.8%) and Tetragnathidae (20.8%) were significant ($p < 0.05$) indicators of the mainstem river sites, while in the stream sites, Aeshnidae (31.5%) and Gyrinidae (18.4%) were indicators ($p < 0.05$). For seasonal IndVal variation, Potamonautidae (52.3%), Pisauridae (19.4%), Dytiscidae (19.2%), Elmidae (22.0%), Gerridae (22.6%), Helodidae (39.1%), and Tetragnathidae (23.0%) were indicators ($p < 0.05$) of the summer, while in the stream sites, Dixidae (29.3%) were indicators ($p < 0.05$) for the winter.

4. Discussion

While we found significant changes in sediment chemistry between the two seasons in our study, this was not reflected in the macroinvertebrate communities. Contrary to expectation, we found that water chemistry was more important as a predictor of benthic macroinvertebrates than sediment chemistry. Compared to sediment chemistry, water chemistry changed relatively little across seasons with more variables changing across river sections than seasons. Seasonal variation in the relative contribution of sediment and water to the explanation of macroinvertebrate community structure and composition was different as highlighted by the CCA.

The Bloukrans River heavy metal concentrations in sediments were mostly found to be within background levels for the catchment and similar to that found elsewhere in South Africa and the surrounding region (e.g. Awofolu et al., 2005; Gerber et al., 2015; Bere et al., 2016) and sediment quality was acceptable based on the sediment quality guidelines (MacDonald et al., 2000). Hence metals were found not to be significant in structuring macroinvertebrate communities. However, based on the

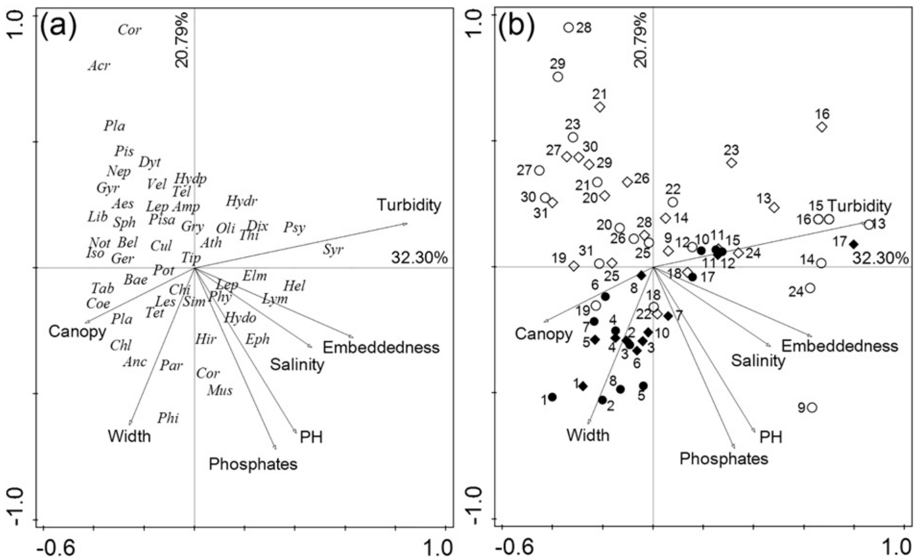

Fig. 2. CCA biplots showing the relationship between measured significant environmental variables with (a) macroinvertebrate families and (b) site categories sampled. Abbreviations: diamond – winter season; circles – summer season; black – mainstem river sites; white colour – stream sites; and abbreviations for macroinvertebrate families are highlighted in Table 3.

sediment quality guidelines for freshwater found in MacDonald et al. (2000), the lowest effect level (LEL) was exceeded for several chemicals (i.e. copper, lead, chromium, zinc) at a small number of sites, but this depended on season and the location within the catchment (Table S1).

Our results suggest little effects of seasonality on benthic macroinvertebrate community's structure in this south-eastern zone of South Africa despite clear changes in sediment chemistry. While seasonality can be a fundamental driver of stream community dynamics, its effect is regulated by the predictability of its recurrence (Tonkin et al., 2017). For instance, in Mediterranean streams, highly predictable seasonal precipitation leads to regular oscillations between distinct community types between wet and dry seasons. By contrast, where seasonality is weak or unpredictable, seasonal turnover is likely weak (Tonkin et al., 2017). In a study on Afrotropical Nigerian streams, Tonkin et al. (2016) found little role of seasonality in shaping stream macroinvertebrate community dynamics. Our lack of seasonality effect likely reflects the relative lack of variability in water chemistry compared to sediment chemistry in the system.

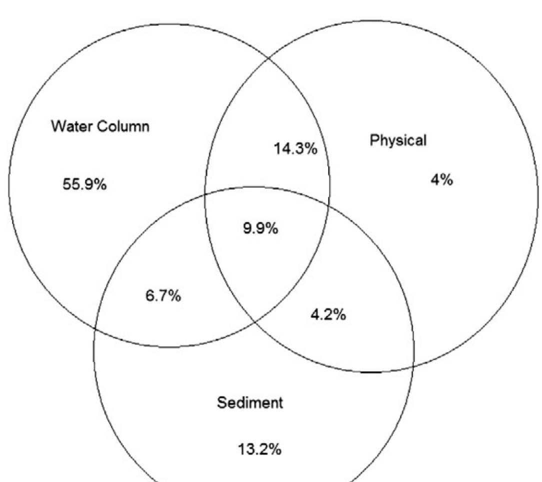

Fig. 3. Variance partitioning analysis (i.e. partial CCA) results highlighting the relative variation fractions of benthic macroinvertebrate community assemblages explained by sediment, water column and physical variables.

The water quality was very poor for most sites in both the river and streams mostly due to organic pollution from the urban areas, with a few sites being of good water quality in the lower and upper reach sites. Most of the stream sites were in urban areas, while most of the river sites were downstream of the urban area and located mostly in agricultural areas. Water quality deterioration in winter could be attributed to differences in water flow, dilution and observed raw sewage input due to burst pipes in the urban area. As nutrient concentrations were generally higher above the sewage treatment works than below it, this suggests that sewage effluents from burst and/or overflowing pipes is the most likely source of enrichment in the streams and upper mainstem river sites.

The benthic macroinvertebrate community structure and composition followed the observed water quality changes among the study sites, with effects of physical and water chemistry variables being integrated into the overall macroinvertebrate community structuring (Roy et al., 2003). Different macroinvertebrate taxa responded differently to pollution reflected in urban drainage/storm water, metal, industrial and domestic wastewater due to their tolerance level differences (Dallas and Day, 2004; Bonada et al., 2006). Our results showed that river and/or stream sites were polluted by different classes of contaminants as we observed reduced macroinvertebrate abundance, taxon richness, and a shift in community composition between summer and winter - from sensitive taxa (Ephemeroptera) to tolerant taxa (Diptera) (Clements, 1994, 2004). Therefore, the benthic macroinvertebrate community composition at different sites and points in time provided useful information about the environmental condition of the Bloukrans River. Taxa richness, diversity and community metrics were slightly higher in the river sites compared to stream sites. This could be attributed to differences in pollution levels between the two river sections and/or land use type (Fig. 1).

Comparisons of benthic macroinvertebrate communities among the two river sections (stream vs. river) based on CCA analysis suggests that some macroinvertebrate taxa (e.g. Ancylidae, Baetidae, Coenogranidae, Gomphidae, Lestidae, Nepidae) were affected by certain physical and water chemistry variables while other taxa (Aeshnidae, Culicidae, Libellulidae and Veliidae) were determined by different factors. In general, channel width, embeddedness, canopy cover, phosphate concentration, pH, salinity and turbidity were found to be important in structuring benthic macroinvertebrate communities and water chemistry variables were found to explain a larger percentage of the total variation compared to physical and sediment chemistry variables (Table S2). High macroinvertebrate taxon richness was observed in upstream, where canopy cover was high in the Bloukrans River. In similar studies, Behmer and Hawkins (1986), Roy et al. (2003), Heino et al. (2004) and Tonkin et al. (2013) found that macroinvertebrates in upstream shaded sites (with high canopy cover) had higher taxon richness compared to those in open sites (low canopy cover).

Low dissolved oxygen (DO) and high nitrates levels observed in mainstem river (sites 10, 11, 12, 17) and stream (sites 9, 13, 14, 16) sites could be attributed to sewage pollution, which can contain continuously released surfactant and pharmaceutical compounds (see Muñoz et al., 2009) that could also impact macroinvertebrates. The high salinity levels in stream sites were characteristic of the high water conductivity and metal ions within the river system. Salinization could also be attributed to failing and aging sewage system/network which contributes to ion (i.e. chloride, potassium, magnesium), phosphate and nitrate concentrations in the water (Mallin et al., 2009; Bere et al., 2016).

It has been established that metal ratios play an essential role for aquatic species normal growth and physiological function such as muscle function and nerve transmission (Hassaan et al., 2013). Chironomids and simuliids dominated at sites with high metal ratios, potentially reflecting their tolerance to pollution. Since excess metal ratios have been shown to adversely affect the growth and survival of some species or taxa (Davies and Nelson, 1994; Ye et al., 2006), the observed increases particularly for the Fe/P and Ca/P ratios during winter season has a potentially adverse impact on aquatic life.

The rationale for many macroinvertebrate-based bioassessment protocols is grounded on the observation that EPT taxa are sensitive to contaminants in aquatic systems, especially organic enrichment (Clements, 1994, 2004). While this was highlighted by the findings of the present study, the EPT also seemed to respond to a range of other natural gradients in environmental conditions, as found in earlier studies (Tonkin et al., 2015). The EPT/Chironomidae ratio was generally low in stream sites compared to mainstem river sites and this ratio could be used as a good indicator of organic pollution. Stream sites were characterised by low macroinvertebrate richness and community metrics and high %Diptera and were generally dominated by pollution tolerant macroinvertebrate taxa such as Simuliidae, Chironomids, Syrphidae and Potamonautidae.

5. Conclusions

Here we highlighted that water chemistry variables were more predictive of benthic macroinvertebrate community structure than sediment chemistry variables in highly polluted rivers and streams. Seasonal regimes which affected flow rates were found to considerably affect macroinvertebrate communities and this also had implications for both sediment and water chemistry dynamics. Anthropogenic impacts such as sewage leakages resulted in increased nutrient concentrations, which might have had a significant effect on the macroinvertebrate communities as highlighted by the dominance of Diptera. While sediment metal concentrations may not pose a threat to benthic macroinvertebrates in the Bloukrans River at present based on current concentrations, we suggest further monitoring as changes in sediment concentrations might have implications for the water chemistry and macroinvertebrate communities. These results provide us with baseline information on urban pollution in a developing world context in an Austral temperate environment. Given the complex role that multiple stressors play in the anthropocene (Leps et al., 2015), future studies should consider how pollutants and climate change will affect macroinvertebrates within the catchment (Chiu et al., 2017).

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2017.06.267.

Conflict of interest

All authors declare that no potential sources of conflict and/or interest exist.

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