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Impacts of terrestrial habitat transformation on temporary wetland invertebrates in a sclerophyllous Sand fynbos landscape

Matthew S. Bird · Jenny A. Day

Abstract There has been recent debate about the extent to which human disturbance of the landscape affects wetland invertebrates given that the organisms are already adapted to high levels of natural disturbance. Using repeated sampling of a set of 12 temporary wetlands occurring in a differentially transformed Sand fynbos landscape in Cape Town (South Africa), we investigated patterns of macroinvertebrate and microcrustacean assemblage composition, richness and diversity in relation to a physicochemical gradient resulting from variable habitat loss in the adjacent landscape. Both macroinvertebrates and microcrustaceans showed clear gradational changes in assemblage composition in relation to the surrounding cover of indigenous vegetation (as a proxy for habitat loss). Although the composition of assemblages appeared to be affected by this gradient of habitat transformation, no relationships were detected using various measures of taxon richness and diversity. At the small spatial scale for which patterns are analysed, the influence of natural variation on invertebrate assemblages appears to have been overridden by that resulting from habitat transformation in the adjacent landscape. Depression wetlands embedded in Sand fynbos habitat appear to be unique in terms of their physico-chemistry (acidic and rich in humic substances) and the invertebrate assemblages they support.

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Introduction

Human-induced transformation of natural habitats forms a conspicuous feature of coastal lowland regions that support burgeoning human populations. The primary agents of habitat transformation in such regions have traditionally been recognised as agriculture and urban development (Vitousek et al., 1997; Wackernagel & Yount, 1998; Imhoff et al., 2004), but in recent decades the major impacts of invasive alien

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plants have also become apparent (Richardson & van Wilgen, 2004; Theoharides & Dukes, 2007; Winter et al., 2010; Vicente et al., 2014).

In the south-western Cape mediterranean-climate region of South Africa, coastal lowland habitat has been transformed predominantly by agriculture, followed by approximately equal contributions to habitat loss from invasive alien plants and urban development (Rouget et al., 2003). The indigenous vegetation of the south-western Cape mediterranean-climate region is characterised by an evergreen, sclerophyllous, shrub-dominated vegetation known colloquially as "fynbos" (Rouget et al., 2003). Sand fynbos (sensu Rebelo et al., 2006) is a particular type of fynbos that was once widespread on the highly leached oligotrophic sands of the south-western Cape coastal lowlands, but its extent has been drastically reduced by human-induced habitat transformation. Invasive alien plants, predominantly *Acacia* spp. of Australian origin, pose a particularly serious threat to remaining lowland Sand fynbos habitat in the region due to their ability to directly outcompete and replace fynbos plants (Heijnis et al., 1999; Rouget et al., 2003; Holmes, 2008).

The coastal plains of the south-western Cape are dotted with depressional wetlands, which become visible during the winter wet season when the water table rises to the ground surface and manifests as surface water on aeolian coastal sands. These depressions are typically small basin-shaped features that form naturally in the landscape where, for instance, a basin forms at the foot of a small dune (Ollis et al., 2015). In terms of natural waterbody types, these depression wetlands are the most abundant and characteristic freshwater features on the coastal lowlands of the region (Silberbauer & King, 1991) and are typically seasonal (winter-inundated). Temporary wetlands such as these are gaining recognition worldwide for the unique biotic communities they support (Williams, 2006; Porst & Irvine, 2009) and, although often small in size, their cumulative contribution to regional biodiversity is significant (Gibbs, 1993; Semlitsch & Bodie, 1998; Nicolet, 2001; Williams et al., 2001; Gómez-Rodríguez et al., 2009).

Given that Sand fynbos is endemic to the south-western Cape, and that this habitat in the region has become drastically reduced in recent decades, there are now only small fragments that remain around Cape Town and nowhere else on earth (Heijnis et al., 1999; Rouget et al., 2003; Rebelo et al., 2006). Depression

wetlands occurring within Sand fynbos are unique from a conservation point of view, not only due to the scarcity of this habitat in the region, but also because the sclerophyllous fynbos imparts a unique physico-chemical signature to these wetlands by releasing tannins that cause low pH conditions in the waterbodies (Harrison, 1962; Gardiner, 1988; Bird et al., 2013a). These conditions contrast with many other low-lying coastal wetlands in South Africa, which are often neutral or alkaline environments (Allanson et al., 1990).

Bird & Day (2014) quantified the impacts of various land uses on the physico-chemistry of temporary depression wetlands across the south-western Cape coastal lowlands and found that where Sand fynbos habitat was lost around wetlands, the nutrient concentrations and pH of their waters were significantly higher than for wetlands occurring in unimpacted Sand fynbos. At a smaller spatial scale, Bird et al. (2013a) quantified the impacts of invasive alien plants on 12 temporary depression wetlands in a ~100 ha Sand fynbos landscape (within the city of Cape Town) differentially invaded by alien shrubs and grasses. A decrease in the concentration of humic substances in wetlands and a corresponding rise in surface water pH was observed as indigenous fynbos cover decreased around wetlands, which was attributed to the loss of tannin input associated with replacing fynbos with non-sclerophyllous alien plants. Our study is an extension of that of Bird et al. (2013a) and investigates the impacts of indigenous habitat loss on the aquatic ecosystem, as mediated predominantly by physico-chemical changes accompanying replacement of indigenous habitat with alien plants. Specifically, we focus on impacts on wetland invertebrate assemblages. Aquatic invertebrates are the most ubiquitous component of the temporary wetland biota and portray a range of strategies for adapting to life in a temporary aquatic realm (for review see Williams, 2006). Although the understanding of wetland invertebrate ecology has traditionally lagged quite far behind that for rivers and large lakes, in recent decades wetland research has intensified and the knowledge deficit between wetlands and other freshwater systems is being redressed (Batzer et al., 2006; Williams, 2006; Batzer & Ruhí, 2013).

In a study that covered most of the coastal lowlands of the south-western Cape mediterranean-climate region of South Africa, Bird et al. (2013b) sampled

90 temporary depression wetlands (sensu Ollis et al., 2015) that encompassed a wide range of human disturbance in terms of surrounding habitat transformation. At the large spatial scale of their analysis, it appeared that the influence of natural environmental heterogeneity on temporary wetland invertebrates far outweighed that of anthropogenic-induced environmental gradients. They suggested that studies at smaller spatial scales, reducing the influence of natural heterogeneity ('environmental noise'), would be useful for elucidating anthropogenic influences on wetland invertebrate assemblages. The current study is an attempt to disentangle the influence of natural and human-induced influences on temporary wetland invertebrate assemblages. At the small spatial scale analysed in this study (~ 100 ha), and given more pronounced physico-chemical gradients (particularly for pH) than for those observed by Bird et al. (2013b), we expect markedly stronger response patterns from wetland invertebrates to habitat transformation in the landscape than were observed in their study.

Methods

Study area and site selection

Twelve depression wetlands (sensu Ollis et al., 2015) were sampled in and around the Kenilworth Racecourse Conservation Area (KRCA) located in the suburb of Kenilworth in Cape Town, South Africa (Fig. 1). These wetlands are hydrologically isolated from each other in that they are not connected via surface water (even during times of flooding) and are inward draining (i.e. no inlets or outlets). The selected wetlands spanned a gradient of surrounding indigenous vegetation cover (Sand fynbos habitat) and occurred across three differentially transformed areas at Kenilworth. The study area presents a unique opportunity to investigate wetlands that are expected to be comparable in their natural state (similar chemistry, hydro-morphometry and biotope characteristics), but have become differentially impacted by surrounding habitat loss.

KRCA has a total area of approximately 52 ha consisting of, firstly, a core conservation area with a history of minimal disturbance. The core conservation area occurs inside Kenilworth Racecourse, the oldest horse racing track in South Africa (established in 1882). For most of its existence, the core conservation area was preserved inadvertently from degradation by the South African Turf Club (KRCA was only formally established in 2006). Four wetlands (sites 1–4, Fig. 1) were sampled from this core conservation zone (untransformed indigenous Sand fynbos habitat). Secondly, KRCA contains a piece of land on the periphery of the core conservation zone that initially received some physical disturbances during the late 1970s and early 1980s (moderately disturbed by dumping of earth for building activities associated with the racetrack and surrounding grounds), but otherwise has not been physically disturbed by human activities because it was zoned for conservation prior to the establishment of KRCA. Three wetlands (sites 5–7, Fig. 1) were sampled from this area. Although this peripheral area has not been physically disturbed by humans for over three decades, the once-disturbed patches have been colonised by the invasive kikuyu grass *Pennisetum clandestinum* Höchst. ex Chiov. (East African origin) and the Port Jackson willow *Acacia saligna (*Labill) Wendl. (Australian origin), thus preventing the re-establishment of indigenous Sand fynbos vegetation in these patches. Partial alien plant invasion in this area has resulted in a mosaic of alien invaded land mixed with fynbos habitat in roughly equal proportions.

A third area investigated in this study was a piece of vacant land (approximately 54 ha in size) at Youngsfield military base, which is separated from KRCA by a highway (Fig. 1). This landscape consisted of undisturbed Sand fynbos habitat as recently as the 1940s, but between the 1940s and 1980s was physically disturbed by dumping of earth material associated with the construction of the nearby military base and much of the fynbos was lost (Gehrke et al., 2011). Since the early 1980s the land has remained a buffer zone for the military base and has not been used for human activities. Initial physical disturbance of the Youngsfield landscape allowed for subsequent invasion of alien vegetation, which came in the form of *A. saligna* and *P. clandestinum*. At the time of this study, the two species taken together constituted almost 100 percent areal cover of the land in this area. Five wetlands (sites 8–12, Fig. 1) were sampled from this heavily invaded landscape at Youngsfield. From a disturbance point of view, for more than 30 years (since the early 1980s) the three areas being compared in this study have only differed in the amount of alien
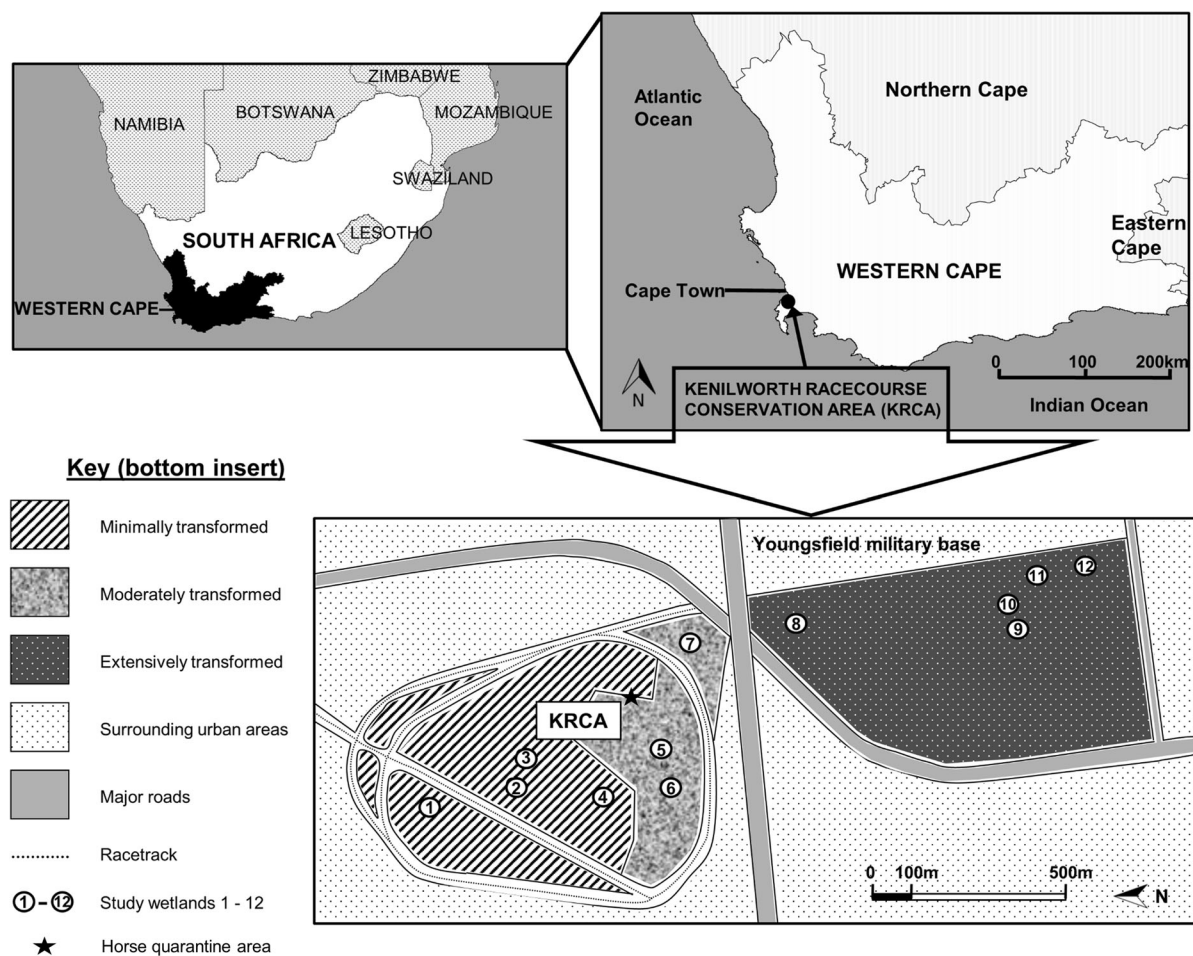
Fig. 1 Map depicting the location of the Kenilworth Race-course Conservation Area (KRCA) in the Western Cape Province, South Africa (*top*). The relative positions of the 12 study wetlands across the three differentially transformed areas at KRCA are also depicted (bottom)(modified from Bird et al., 2013a)

vegetation that has colonised the landscape as the areas have been strictly closed off to public access, thus largely preventing other forms of human disturbance. Two of the three areas investigated were once physically disturbed by dumping of earth material, but the only ongoing disturbance in the past 30 years has been the colonisation of alien plants. The physical disturbance of the landscape did, however, provide the initial catalyst for alien plant colonisation of disturbed patches of land, which subsequently spread due to the invasive capabilities of *A. saligna* and *P. clandestinum*.

The potential confounding influence of urban land cover was considered when selecting the study sites given the context of this study in an urban area. Slow groundwater flow at Kenilworth moves from west to east across the study area (Maya Beukes, KRCA Reserve Manager, pers. comm.). A potential urban-associated nutrient source occurs at the eastern end of KRCA in the form of a quarantine area for race horses (Fig. 1). Wetland sites in this study were chosen so as to occur either upstream (west) or far north/south of groundwater flow emanating from this potential contaminant source. The entire study area was equally surrounded by urban area at the broader scale (>500 m). Physico-chemical contamination of groundwater from surrounding urban areas is thus possible, but is expected to equally affect all sites.

Wetlands were sampled on three occasions during 2009 (late August/early September, October and

November). Despite depression wetlands being reasonably abundant in the area, only those of similar overall size and depth were selected. This targeted approach was taken so as to minimise the potentially confounding effects of total surface area and hydroperiod on environmental conditions and invertebrate assemblages in the wetlands (i.e. effects independent of the habitat transformation gradient). The chosen sites all had a similar seasonal inundation regime, filling up with the first heavy rains typically in early May and remaining inundated until late November or early December (an 'intermittent' inundation regime following Comín & Williams, 1994). All sites are predominantly groundwater fed, but can also receive a significant portion of their water by direct precipitation.

Quantifying habitat loss

Wetlands across the three adjacent areas (described above) were surrounded by differing levels of indigenous vegetation habitat within approximately 500 m of their edges, but beyond this were all similarly surrounded by urban areas. The most pronounced differences in surrounding habitat cover were observed within approximately 100 m of wetlands and thus the 100 m fringe was selected to quantify the gradient of habitat transformation in this study. The areal cover of indigenous vegetation within this 100 m periphery was used to proxy the amount of habitat loss (habitat transformation) around wetlands. Four 100-m transects were assessed at each wetland, radiating north, east, south and west from each wetland edge. The habitat cover type was recorded at 2-m intervals along each transect, producing 50 habitat cover points per transect and 200 per wetland. The transects resulted in estimation of the cover of the following habitat categories within a 100 m radius of each wetland: indigenous Sand fynbos vegetation (predominantly shrubs, but grasses and herbaceous forms were also recorded); alien shrub (*A. saligna*); alien kikuyu grass (*P. clandestinum*), other alien vegetation (other annual grasses and herbaceous species were present, although these were very sparsely represented at the site); racetrack (unfertilized kikuyu); mowed field (unfertilized kikuyu); road (gravel and tar roads were present) and wetland (if other wetlands were encountered within 100 m). These measurements were expressed as percentage areal cover and formed the variables used to represent habitat transformation in further analyses, where they were treated as static variables (i.e. without temporal variation during the study period). Racetrack and mowed field (both consisting of kikuyu grass) contributed to the overall cover of alien kikuyu grass within 100 m of wetlands and thus were incorporated into further analyses as such. Although the cover of alien vegetation species other than *A. saligna* and *P. clandestinum* was recorded in this study, the data for this variable were too sparsely represented to be included in analyses. Similarly, road cover within 100 m of wetlands was too sparse for this variable to be used in further analyses. We did not include the percentage cover of adjacent wetlands as a predictor variable in our analyses given that our aim was to assess relationships between wetland invertebrates and surrounding terrestrial (i.e. non-wetland) habitat cover. The raw data for habitat transformation are provided in Appendix S1.

Environmental variables

Biotope availability was recorded at each wetland on each sampling occasion by recording the cover of three structural habitat types encountered in these wetlands, namely vegetation with complex architecture (predominantly submerged), vegetation with simple architecture (predominantly emergent) and open water (no vegetation). Biotope availability was classified in this way because habitat structural complexity has been shown to be an important determinant of invertebrate abundance and assemblage composition in lentic wetlands (for review see Bird et al., 2014). The cover of the macroalga *Cladophora* sp. (hereafter referred to as the variable 'macroalgae') was also recorded in each wetland. Further description of the aquatic biotopes present in the study wetlands is provided by Bird et al. (2013a).

The following physico-chemical variables were measured in situ within each of the three major biotopes per wetland: pH; dissolved oxygen; electrical conductivity; turbidity and temperature. Measurements of water column nutrient concentrations ($NO_3^- + NO_2^-N$ and $PO_4^{3+} - P$), chlorophyll *a* and the concentration of humic (polyphenolic) substances were taken from each wetland. Basic hydro-morphometry measurements, namely maximum depth and total surface area, were also taken at each wetland. All

physico-chemical measures were taken during a period no longer than 2 h between 10am and 12 pm each day. Details for the measurement of each of the physico-chemical and hydro-morphometrical variables are provided in Bird et al. (2013a). All environmental variables were considered labile and were sampled on each of the three occasions (for raw data, see Appendix S2).

Invertebrates

Wetlands were sampled for aquatic invertebrates using a square-framed, long-handled sweep net with a 235 mm mouth and 80 μm mesh. We modified the protocol of Rundle et al. (2002), who established that five replicate 1 m sweeps from each biotope using a standard sweep net (area 200 × 250 mm) consistently sampled 60–80% of the total invertebrate species pool from that biotope for a set of 16 temporary depression wetlands in Cornwell, south-west Britain. We modified their protocol by sampling each biotope within three different areas of each wetland so as to maximise spatial representation of each biotope. The method for each sweep of the net follows that prescribed by various authors for sampling temporary and permanent wetland invertebrates (Gernes & Helgen, 2002; Rundle et al., 2002; Bowd et al., 2006), whereby each sweep constituted dragging the net down from the water surface at a 45° angle until nearly touching the bottom and then completing the sweep arc by returning the net back to the surface at a 45° angle, covering a distance of one metre with each full sweep. A biotope sample comprised three sweeps, a procedure that was repeated in three different areas of the wetland, so that the final sample for each biotope was a pooled combination of nine sweeps. This is likely to represent the majority of the invertebrate assemblage for that biotope, given the recommendation of Rundle et al. (2002) of five sweeps per biotope. As the procedure was in turn repeated three times per wetland (to obtain samples for the three different biotopes), this produced a total effort of 27 sweeps evenly divided over the spatial area of each wetland. The three samples from each wetland were preserved and stored separately.

All samples were fixed on site in buffered 10% formalin and replaced with a 70% ethanol solution after 24–48 h for longer-term preservation. Macroinvertebrates (defined as taxa ≥1 mm in size and visible to the naked eye) and microcrustaceans (defined as copepods, ostracods and cladocerans >80 μm in size) were identified and enumerated using a sub-sampling procedure. First, the whole sample was scanned for five minutes in a tray and large rare (LR) macroinvertebrate and microcrustacean taxa (defined as taxa with large easily visible specimens represented by <10 individuals per sample) were picked out in accordance with recommendations made by Vinson & Hawkins (1996) and King & Richardson (2002). The sample was then emptied into a rectangular tray divided into a grid of 35 equal-sized square cells, which were randomly sub-sampled until 200 organisms had been picked out. Sub-sampling stopped when 200 individuals had been counted, after first completing the cell in which the 200th individual was counted. Macroinvertebrate abundances were extrapolated to whole sample estimates by multiplying by the total number of cells in order to standardise final abundances. Samples with <200 individuals were completely picked. After picking out macroinvertebrates, samples were preserved in 70% ethanol before being sub-sampled for microcrustaceans at a later stage.

We followed the technique described by McCallum (1979) for sub-sampling freshwater microcrustacean zooplankton. Each sample was drained of ethanol and then made up to 500 ml with tap water in a glass beaker. The sample was then homogenised by blowing bubbles into it for 5 s through a straight-sided, graduated pipette with a wide bore aperture of 5 mm. After homogenisation, a 1 ml sub-sample was sucked into the pipette and emptied into a Bogorov tray and individuals were enumerated under a dissection microscope. This process was repeated until 200 individuals were counted (upon first completing the sub-sample in which the 200th individual was counted). As the maximum total volume sub-sampled to obtain 200 individuals was 20 ml, microcrustacean abundances across all samples were standardised to a 20 ml estimate by extrapolation. The choice of 200 individuals as a stopping point for sub-sampling of macroinvertebrates and microcrustaceans was adapted from the recommendations of various biological assessment studies in rivers (Barbour & Gerritsen, 1996; Somers et al., 1998; Barbour et al., 1999), and more recently from a study in wetlands (King & Richardson, 2002). Sub-sampling trials indicated that in almost all cases the full complement of taxa was encountered within a 200-organism count.

All macroinvertebrate and microcrustacean taxa were identified to genus or species level. Exceptions to this were for certain dipteran, coleopteran and trichopteran larvae, where identification could only be made to family level. Within a given macroinvertebrate group, we examined either adult (e.g. Coleoptera) or larval (e.g. Chironomidae) specimens only so as to avoid problems associated with taxonomic identification of individuals across different life stages. The major reference source for keying out invertebrate taxa was the series of 'Guides to the Freshwater Invertebrates of Southern Africa' (Day et al., 1999, 2001a, b; Day & de Moor, 2002a, b; Day et al., 2003; de Moor et al., 2003a, b; Stals & de Moor, 2007). Problematic taxa which required expertise (e.g. chironomids and ostracods) were identified by a specialist taxonomist for the given taxon (see *Acknowledgments*). The final list of macroinvertebrate and microcrustacean taxa recorded in this study is presented in Appendix S3.

Data analysis

Physico-chemical variables measured in situ were averaged across the three biotopes to produce mean values per wetland. All other environmental variables were measured at the individual wetland scale and were used as such for analyses. Environmental variables were $\log_{10}$ transformed where appropriate to improve normality and for the same purpose percentage variables were arcsine square root transformed. The percentage areal cover (arcsine square root transformed) of indigenous vegetation within a 100 m periphery of each wetland was used as the primary proxy for the cumulative amount of habitat loss (habitat transformation) around wetlands. Invertebrate relative abundances for each extrapolated sample estimate (macroinvertebrates) or 20 ml subsample (microcrustaceans) were multiplied by the proportion of wetland covered by the biotope from which the sample was collected. The resulting proportional abundances from each biotope were summed to produce an overall average abundance (relative abundance) of that taxon in the wetland, having adjusted for proportional cover of the various biotopes. Raw data matrices were $\ln(x + 1)$ transformed for both macroinvertebrates and microcrustaceans to downweight the influence of highly abundant taxa. All analyses for macroinvertebrates and microcrustaceans were performed separately, given our interest in the differential effects of habitat transformation on these two faunal groups.

Non-metric multidimensional scaling (MDS) was used as an unconstrained ordination technique to visualise the multivariate composition of invertebrate samples on a two-dimensional plane. Distance-based Redundancy Analysis (dbRDA, Legendre & Anderson, 1999; McArdle & Anderson, 2001) was used for a similar purpose, but for this technique the invertebrate composition data were constrained by the environmental data. dbRDA is a non-parametric multivariate multiple regression procedure based on any given dissimilarity measure, in this case the Bray–Curtis coefficient (Bray & Curtis, 1957). Traditional Redundancy Analysis (RDA, sensu Gittins, 1985) assumes a Euclidean distance matrix for the multivariate response (Legendre & Anderson, 1999). dbRDA was a preferred technique as it allows for any choice of resemblance measure in the response matrix and this was relevant for multivariate analyses on invertebrate assemblage composition data, where the Bray–Curtis coefficient is a preferable measure of dissimilarity (Clarke et al., 2006). The dbRDA plots allowed for a visual representation of the environmental variables that were correlated with the habitat transformation gradient and simultaneously had an influence on invertebrate assemblages across this gradient (i.e. environment variables that mediated habitat transformation effects on invertebrate assemblages, if any). The dbRDA models included a step-wise regression procedure, using the AICc criterion to determine the environmental variables that were included in the final models, and hence represented on the plots. To account for temporal effects, categorical variables representing each of the three sampling months were included as covariables in each of the models. The concentration of humic substances was not included as a predictor variable in the dbRDA models as this variable was highly collinear with pH ($r = -0.86$). Thus, the ecological effects of the two variables cannot be quantitatively disentangled in our analyses. For both the MDS and dbRDA ordinations, assemblage composition data were first converted to a Bray–Curtis similarity matrix using data from all three sampling visits combined. These ordinations were considered exploratory techniques to help visualise multivariate patterns in the data.

Further dbRDA multivariate regression models were performed for each sampling occasion separately (i.e. three separate datasets each consisting of 12 replicate samples) in order to formally assess linear relationships between the habitat transformation gradient and assemblage composition. Detrended Correspondence Analysis (DCA) indicated that gradient lengths in the macroinvertebrate and microcrustacean data throughout this study were best suited to linear rather than unimodal regression analyses (gradients lengths were all <3, Lepš & Šmilauer, 2003). Three predictor variables were used to represent habitat transformation in linear analyses: '% Indigenous vegetation cover'; '% Kikuyu cover' and '% Acacia cover'. The first variable provides a proxy for the overall amount of habitat loss, whilst the remaining variables represent the two primary cover types where habitat has been transformed at Kenilworth.

To test for relationships between measures of taxon richness/diversity (response variables) and the variables that proxy habitat transformation (predictor variables), univariate linear regression models were applied to the data from each sampling occasion separately. Five commonly used measures of richness or diversity were incorporated into these analyses: taxon richness (S), represented simply by the total number of taxa; Margalef's richness index (d); Shannon's diversity index (H'); Pielou's index of evenness (J') and Simpson's diversity index $(1 - \lambda)$(for full equations for each measure, see Clarke & Warwick, 2001). Total invertebrate abundance (N) was also assessed as a response variable.

All dbRDA models were implemented using the DISTLM routine of the PERMANOVA + software package (Anderson et al., 2008). P values for dbRDA models were tested by 9999 permutations of residuals under the reduced model. DCA ordinations were performed using CANOCO for Windows v4.5 (Ter Braak & Šmilauer, 2002). MDS analyses were performed using PRIMER v6 software (Clarke & Warwick, 2001; Clarke & Gorley, 2006). Univariate linear regressions were performed using STATISTICA v12 software (Statsoft Inc. 2013, Tulsa, Oklahoma, USA). We used an a priori defined significance level of α = 0.05, which was subsequently adjusted to account for multiple comparisons using Holm's sequential Bonferroni correction (Holm, 1979).

Results

Invertebrate assemblage composition in relation to habitat transformation

Macroinvertebrate and microcrustacean assemblages were clearly differentiated between those sites with extensive surrounding indigenous vegetation and those with none. This pattern of difference between least and most transformed sites appeared to be consistent over time, as reflected by the separation of the two groups in the MDS plots over the three successive sampling occasions (Fig. 2). Invertebrate assemblage composition displayed a pattern of gradational change that generally corresponded with the gradient of habitat transformation. In this regard, moderately transformed sites shared assemblage characteristics with both the least and extensively transformed sites, although the overlap was only slight. The patterns reported above were similar for macroinvertebrate and microcrustacean assemblages (Fig. 2a, b, respectively), despite some minor variations in the positioning of sites between the two plots.

The multivariate regression results (Table 1) offer quantitative confirmation of the gradient patterns observed in the unconstrained ordination plots (Fig. 2). Invertebrate assemblages showed highly significant ($P < 0.01$) linear relationships with overall levels of habitat transformation, as proxied by the percentage cover of indigenous vegetation around wetlands (the predictor variable "% Indigenous vegetation, Table 1), and this trend was consistent over the three sampling occasions. The habitat transformation variables explained only moderate amounts of variation in the regressions of Table 1 (ranging between 19.14 and 37.58%), despite being statistically significant in most cases. According to the amounts of explained variation in each response matrix, microcrustacean assemblage composition was slightly better explained by indigenous vegetation cover than was the case for macroinvertebrates, but differences were minor (microcrustaceans: 24.17–37.58%; macroinvertebrates: 22.21–29.63%). Although overall levels of habitat transformation were consistently related to assemblage composition, relationships with the two primary types of transformed habitat at Kenilworth (i.e. alien kikuyu grass and acacia shrubs) were less clear and only for the
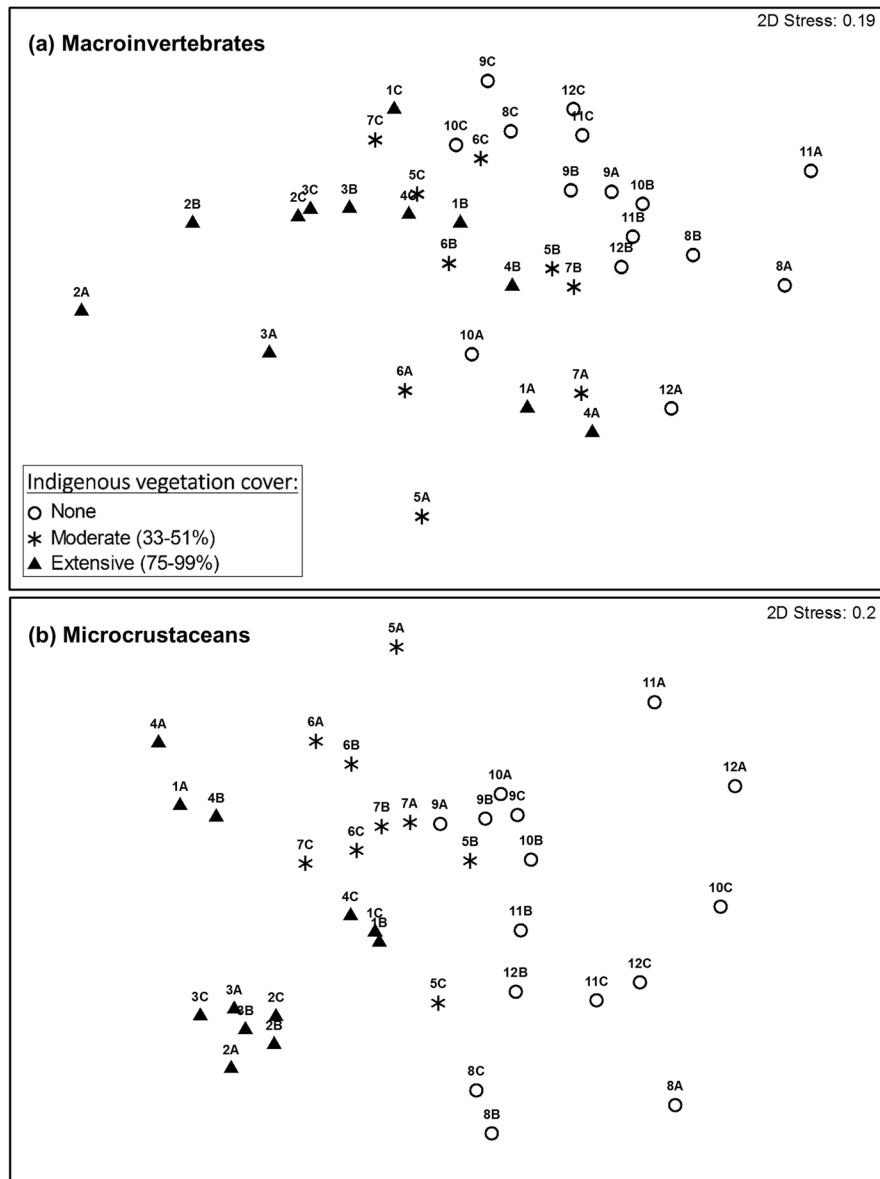
Fig. 2 Multidimensional scaling (MDS) plots of macroinvertebrate (**a**) and microcrustacean (**b**) assemblage composition, represented by Bray–Curtis similarity among sites ($n = 36$). The level of habitat transformation around wetlands is proxied by the remaining amount of indigenous vegetation within 100 m (coded here according to three broad categories of cover). Sites 1–12 are labelled according to the date of each sampling occasion (*A* August/September 2009, *B* October 2009; *C* November 2009)

November data were the relationships consistently significant. Kikuyu and acacia cover around wetlands explained similar amounts of variation in the invertebrate response matrices and thus appeared to exert a similar influence on wetland invertebrate assemblages.

Environmental factors mediating habitat transformation effects

Axis 1 explained most of the variation of invertebrate assemblage composition in both dbRDA plots (Fig. 3) and this axis appears to be associated with the loss of

Table 1 dbRDA model results of invertebrate assemblage composition (response matrices) regressed on variables representing the habitat transformation gradient (predictors)

Predictors	Assemblage composition (response matrices)					
	Macroinvertebrates			Microcrustaceans		
	F	P	% Var	F	P	% Var
August/September						
% Indigenous vegetation	2.855	0.0024*	22.21	5.223	0.0002*	34.31
% Kikuyu	2.366	0.0148	19.14	3.634	0.0040*	26.65
% *Acacia*	2.435	0.0159	19.58	2.988	0.0135	23.01
October						
% Indigenous vegetation	4.209	0.0001*	29.63	3.187	0.0022*	24.17
% Kikuyu	3.829	0.0003*	27.69	2.682	0.0103	21.15
% *Acacia*	2.561	0.0136	20.39	2.444	0.0236	19.65
November						
% Indigenous vegetation	3.906	0.0002*	28.09	6.021	0.0001*	37.58
% Kikuyu	2.733	0.0068*	21.47	4.539	0.0009*	31.22
% *Acacia*	2.975	0.0037*	22.93	4.097	0.0019*	29.06

Response matrices were constructed using the Bray–Curtis similarity coefficient. % Indigenous vegetation is used as a proxy for the overall amount of habitat loss, whereas the remaining two predictor variables represent the different agents of habitat transformation at Kenilworth. % Var—the percentage of variation in each response matrix that is explained by the respective predictor variable in each model. Data from the three sampling visits in 2009 were analysed separately. All models had 10 residual degrees of freedom. Significant *P* values (after sequential Bonferroni correction) are indicated by an asterisk

indigenous vegetation around wetlands (as in the MDS plots). pH, nitrates + nitrites and phosphates were selected by the dbRDA step-wise regression model (using AICc selection criterion) as influential determinants of macroinvertebrate assemblage composition among the wetlands at Kenilworth (Fig. 3a). The same factors, with the addition of the percentage cover of macroalgae and wetland total surface area, were selected by the dbRDA model as important explanatory variables of microcrustacean assemblage composition (Fig. 3b). pH explained the dominant portion of the variation of invertebrate assemblages in both models (Table 2). Despite the highly significant P values, the selected environmental variables each explained <20% of invertebrate assemblage variation. The direction and length of the environmental vectors in Fig. 3a indicate that pH had a strong positive relationship with the loss of indigenous vegetation around wetlands, which simultaneously had a marked influence on macroinvertebrate assemblage composition. The vectors for nitrates + nitrites and phosphates share a similar direction to the pH vector, but their size suggests that their influence on macroinvertebrate assemblages across the habitat transformation gradient was relatively weak in comparison to pH.

Although pH had a relatively strong influence in microcrustacean assemblages at Kenilworth, the direction of the vectors in Fig. 3b are not fully aligned with the direction of habitat transformation (dbRDA axis 1) and thus some of the variation in microcrustacean assemblages was due to pH fluctuations that were independent of habitat transformation. Similarly, the percentage cover of macroalgae and concentrations of phosphates and nitrates + nitrites all had an influence on microcrustacean assemblages, but only a part of this variation appears to correspond to the habitat transformation gradient. The direction of the total surface area variable is almost perpendicular to axis 1 and the influence of this variable appears to be largely independent of habitat transformation.

Taxon richness and diversity in relation to habitat transformation

No discernible influence of habitat transformation on taxonomic richness, diversity or total abundance of invertebrates was detected. None of the regression tests between indigenous vegetation cover and the richness, diversity and total abundance metrics
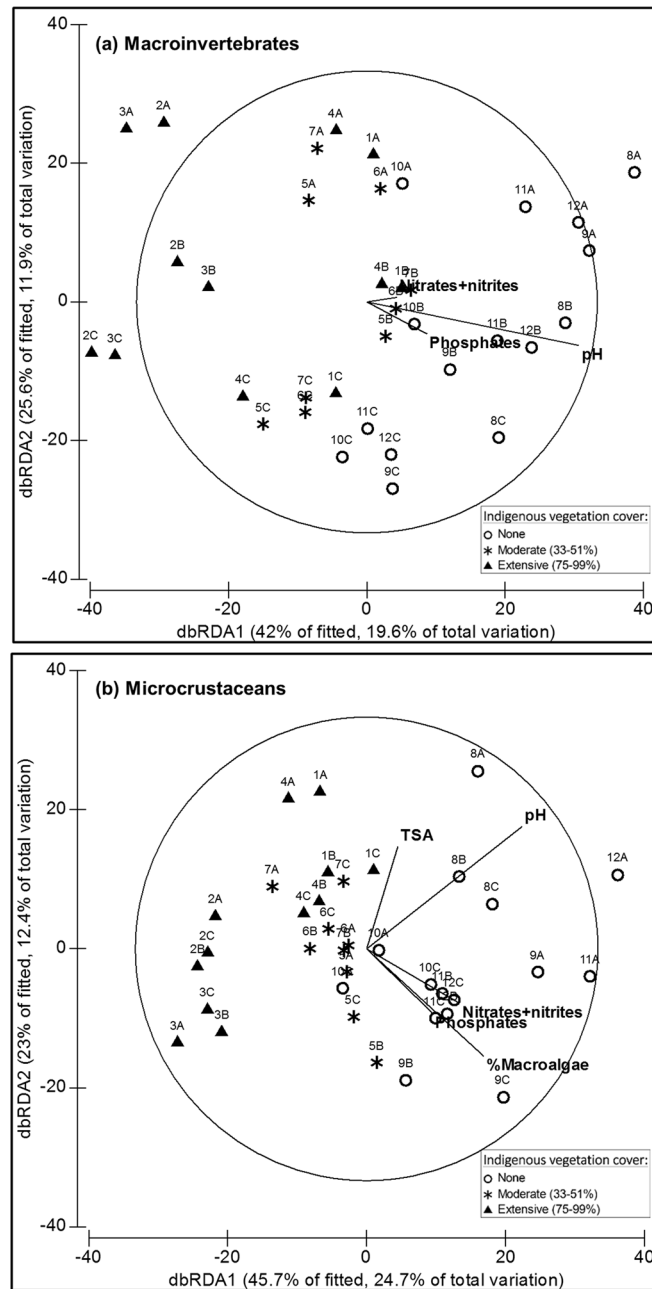
Fig. 3 dbRDA ordination plots of macroinvertebrate (**a**) and microcrustacean (**b**) assemblage composition among sites (Bray–Curtis similarity, n = 36), constrained by the environmental variables. Explained variation in the fitted model and total explained variation is indicated for each axis. The subset of environmental variables used here are those that were selected using dbRDA step-wise selection (AICc selection criterion, see Table 2). Three categorical variables representing the month of sampling were included as covariables in each step-wise model. The level of habitat transformation around wetlands is proxied by the remaining amount of indigenous vegetation within 100 m (coded here according to three broad categories of cover). Sites 1–12 are labelled according to the date of each sampling occasion (A: August/September 2009; B: October 2009; C: November 2009). TSA—total surface area

Table 2 Tabular output for the dbRDA models depicted in Fig. 3, whereby invertebrate composition (Bray–Curtis similarity, $n = 36$) was constrained by the environmental variables

Predictor variables	AICc	F	P	% Var	Res. df
Macroinvertebrates					
pH	265.43	8.696	0.0001	17.41	32
Phosphates	264.46	3.335	0.0001	6.22	31
Nitrates + nitrites	264.42	2.547	0.0022	4.53	30
Microcrustaceans					
pH	246.40	7.911	0.0001	18.35	32
Phosphates	243.51	5.216	0.0001	10.69	31
Nitrates + nitrites	242.21	3.710	0.0008	6.99	30
% Macroalgae	241.50	3.245	0.0021	5.69	29
TSA	241.24	2.935	0.0041	4.82	28

% Var—the percentage of variation in each response matrix that is explained by the respective predictor variable selected at each iteration of the step-wise process (i.e. having accounted for the variables already included in the model); Res. df—residual degrees of freedom. All P values were significant after sequential Bonferroni correction. Results present the subset of environmental variables selected by step-wise regression (AICc selection criterion). Three categorical variables representing the month of sampling were included as covariables in each model, which formed the start solution. These covariables explained 18.51 and 7.44% of the variation in macroinvertebrate and microcrustacean assemblage composition, respectively

presented significant relationships after sequential Bonferroni correction was applied (Appendix S4).

Discussion

Assemblage composition in relation to habitat loss

The composition of both macroinvertebrate and microcrustacean assemblages showed a clear pattern of association with the gradient of habitat transformation. This association was characterised by a gradational change in assemblage composition that largely mirrored the corresponding gradient of habitat transformation. As expected, the invertebrate response patterns observed for the small spatial scale covered in this study were much stronger than observed at the broad spatial scale by Bird et al. (2013b). The response patterns also appear to mirror the pattern for environmental variables in relation to habitat transformation presented by Bird et al. (2013a), who concurrently sampled the same wetlands. It appears that environmental changes across the habitat transformation gradient mediate the invertebrate response patterns observed in the current study, with changes in pH being the key factor mediating the effects of indigenous habitat loss on both macroinvertebrates and microcrustaceans.

Wetland invertebrate resilience to environmental gradients

The percentages of variation in assemblage composition explained by the variable representing habitat loss (i.e. percentage cover of surrounding indigenous vegetation) were only moderate ($\sim$20–40%, Table 1), despite being highly significant ($P < 0.01$). However, the patterns depicted in the MDS plots (Fig. 2) were prominent, and were consistent over time. One would not expect high percentages of invertebrate variation to be explained by a habitat transformation gradient that only induces moderate environmental differences among wetlands. Thus, the trends presented in this study exhibit characteristics of a fauna that are directly responsive to environmental gradients, given that moderate but clear changes in the composition of invertebrate assemblages mirror the moderate environmental gradients associated with habitat transformation.

The directly proportional strength (approximately) of the invertebrate response to the physico-chemical gradient at Kenilworth bears merit for further discussion in context of the debate over whether wetland invertebrates are structured by environmental gradients (natural or human-induced). Batzer et al. (2004) have argued that temporary wetland macroinvertebrate assemblages are largely unstructured by natural

environmental gradients due to their being habitat generalists that are adapted to naturally variable environments. Their data from a set of 66 relatively pristine temporary wetlands in Minnesota lend support to this hypothesis. Similar findings of a lack of responsiveness of aquatic invertebrates to natural variations in environmental factors among temporary wetlands have been reported by Wissinger et al. (1999), Battle & Golladay (2001), Spencer et al. (2002), Studinski & Grubbs (2007), Ganguly & Smock (2010) and Culler et al. (2014). Literature findings are to some degree equivocal, given that various studies have found a distinct structuring effect of environmental variables on temporary wetland invertebrates (Eitam et al., 2004; Vanschoenwinkel et al., 2007; Waterkeyn et al., 2008; Bilton et al., 2009; Bagella et al., 2010; Bird et al., 2013b). In a review of the factors that control wetland invertebrate assemblages in North America, Batzer (2013) concludes that the majority of empirical studies have failed to find convincing evidence for environmental control on assemblages. He suggests that the generally vague nature of the results from empirical studies could be attributed to two possible scenarios: the first being the earlier assertion of Batzer et al. (2004) that wetland invertebrates are largely insensitive to environmental variation, resulting in generalist taxa adapted to coping with environmental variation; the second being that wetland invertebrates are actually hypersensitive to their environment, but their responses integrate a range of ecological factors and this results in inconsistent and non-reproducible findings, both spatially and temporally. Both scenarios would explain weak or inconsistent relationships between wetland invertebrates and environmental variables, but for quite different reasons.

Our results suggest that invertebrates in the wetlands at Kenilworth are directly responsive to an environmental (predominantly physico-chemical) gradient associated with habitat transformation. The assemblages do not portray the patterns characteristic of habitat generalists (e.g. randomised distribution across the habitat transformation gradient), but to draw broader conclusions on whether wetland invertebrates are hypersensitive to varying environmental conditions would require replication of this study in other areas and also over time (preferably assessing inter-annual variation), so as to assess the spatio-temporal reproducibility of the observed patterns. Although our study is not replicated

over several wet seasons, the patterns of invertebrate response do appear to hold over the course of a single season, despite fluctuation of wetland environmental conditions during that wet season (Bird et al., 2013a).

Taxon richness/diversity

Richness, diversity and total abundance of macroinvertebrates and microcrustaceans did not appear to be affected by habitat loss at Kenilworth, despite the significant patterns observed for assemblage composition thus far in the study. It would appear that the physico-chemical gradient caused a turnover in assemblage composition, but not in the number of species. The change in pH from acidic wetlands surrounded by indigenous vegetation to neutral or slightly alkaline wetlands surrounded by alien plants might be expected to cause changes in the types of species found, because some wetland taxa are better physiologically adapted to low pH and others to neutral or alkaline water (Harrison, 1962; Gardiner, 1988; Woodcock et al., 2005). However, diversity might only be affected if, for instance, the pH gradient was particularly extreme (e.g. if the alkaline sites had a $pH > 9$) as this might present a more inhospitable environment for invertebrate species at either end of the gradient.

Our results are in line with findings for temporary wetlands of the same type (isolated depression wetlands) across the spatial extent of the south-western Cape region, whereby Bird et al. (2013b) found no significant associations between habitat transformation gradients and macroinvertebrate richness or diversity metrics. Therefore, no influence of human disturbance in the landscape on temporary wetland invertebrate diversity or richness has been detected at two different spatial scales in this region, but at both scales at least some effects on invertebrate composition have been detected. Other studies in temporary wetlands have found either no effect (Mahoney et al., 1990; Batzer et al., 2004; Bagella et al., 2010) or a negative effect (Euliss & Mushet, 1999; Lahr et al., 2000; Angeler & Alvarez-Cobelas, 2005) of anthropogenic disturbance in the landscape on invertebrate richness or diversity. Results in larger permanent wetland environments appear equally variable (see for review Batzer, 2013), and in certain cases the richness, diversity and total abundance of invertebrates may even be enhanced by anthropogenic disturbances (e.g.

nutrient enrichment of the Florida Everglades, McCormick et al., 2004). There is thus a general lack of consensus as to the effects of habitat transformation around wetlands on richness and diversity of their invertebrate biotas and results vary depending on the region being investigated and the nature and intensity of the human disturbance.

Physico-chemical factors mediating habitat transformation effects

The patterns observed in this study do suggest that the effects of habitat transformation on invertebrate assemblage composition were initiated through changes in physico-chemical conditions. This mechanism is likely to be applicable beyond the spatial context of the current study and to a variety of wetland types and forms of habitat transformation, because the transformation of indigenous fynbos habitat around wetlands of the south-western Cape is expected to often involve the loss of the characteristic physico-chemical signature of fynbos-associated aquatic ecosystems. Harrison (1962) and Gardiner (1988) both reported a strong association between aquatic invertebrate assemblages and pH levels in south-western Cape lowland depressional wetlands. These studies reported differences in assemblage composition among wetlands with low, moderate and high humic content and attributed these differences largely to the changes in pH associated with the different levels of humics. Our work, taken in combination with the physico-chemical results of Bird et al. (2013a), extends on the work of other authors by offering quantitative evidence that the negative association between fynbos cover around wetlands and pH concentration in these wetlands (determined by humic substances) mediates changes in the invertebrate assemblages.

Conservation implications

Previous authors (Harrison, 1962; Gardiner, 1988; Bird et al., 2013a) have shown that wetlands in Sand fynbos are physico-chemically unique (acidic and rich in humics) in the context of many of South Africa's low-lying wetlands, which are often neutral to alkaline systems (Allanson et al., 1990). Our results indicate that these Sand fynbos wetlands are unique also from a biotic point of view in that the fauna inhabiting them appear to be different to those in nearby transformed habitats. Given that most of the south-western Cape coastal lowlands have been transformed by human activities, the few remaining intact Sand fynbos wetlands would appear to hold particular importance from a conservation perspective.

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