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Biological control of water lettuce, *Pistia stratiotes* L., facilitates macroinvertebrate biodiversity recovery: a mesocosm study

J. A. Coetzee · S. D. F. Langa · S. N. Motitsoe · M. P. Hill

Abstract Floating aquatic weed infestations have negative socio-economic and environmental consequences to the ecosystems they invade. Despite the long history of invasion by macrophytes, only a few studies focus on their impacts on biodiversity, while the ecological benefits of biological control programmes against these species have been poorly quantified. We investigated the process of biotic homogenization following invasion by *Pistia stratiotes* on aquatic biodiversity, and recovery provided by biological control of this weed. Biotic homogenization is the increased similarity of biota as a result of introductions of non-native species. The study quantified the effect of *P. stratiotes*, and its biological control through the introduction of the weevil, *Neohydronomus affinis* on recruitment of benthic macroinvertebrates to artificial substrates. Mats of *P. stratiotes* altered the community composition and reduced diversity of benthic macroinvertebrates in comparison to an uninvaded control. However, reduction in percentage cover of the weed through biological control resulted in a significant increase in dissolved oxygen, and recovery of the benthic macroinvertebrate community that was comparable to the uninvaded state. This highlights the process of homogenization by an invasive macrophyte, providing a justification for sustained ecological and restoration efforts in the biological control of *P. stratiotes* where this plant is problematic.

Keywords Benthic macroinvertebrate diversity · Invasive aquatic plants · Community recovery · *Neohydronomus affinis*

J. A. Coetzee (✉)
Centre for Biological Control, Botany Department, Rhodes University, P.O. Box 94, Grahamstown 6140, South Africa
e-mail: Julie.coetzee@ru.ac.za

S. D. F. Langa
Departamento de Ciências Biologicas, Faculdade de Ciências, Universidade Eduardo Mondlane, C. Postal 257 Maputo, Mozambique

S. D. F. Langa · S. N. Motitsoe · M. P. Hill
Centre for Biological Control, Department of Zoology and Entomology, Rhodes University, P.O. Box 94, Grahamstown 6140, South Africa

Introduction

Freshwater ecosystems are particularly susceptible to invasions by non-native species across a range of floral and faunal taxa, largely as a consequence of anthropogenic pressures on these systems (Dudgeon et al., 2006; Ricciardi & MacIsaac, 2011). The alteration of hydrological flows, trade, and intentional stocking

have resulted in homogenous flora and fauna (Rahel, 2002) with ecological and socio-economic impacts to these ecosystems. The homogenization of biotas is the process whereby introductions of non-native species, extirpation of native species, and habitat alterations that facilitate these two processes, result in their increased similarity over time, usually as a result of anthropogenic disturbance (Rahel, 2002). Arguably, freshwater communities have been altered to a greater magnitude than terrestrial communities (Dudgeon et al., 2006). Studies and reviews on homogenization of aquatic ecosystems by faunal invasions, particularly fish invasions, populate the literature (e.g. Ricciardi & MacIsaac, 2011), with relatively fewer general reviews on homogenization by aquatic plant invasions (e.g. Thomaz et al., 2015), even though invasive aquatic macrophytes often degrade aquatic ecosystems and limit their utilization, threatening food security and economic development (Hill, 2003).

The negative effects of invasive macrophytes are considerable and have been fairly well reviewed (e.g. Villamagna & Murphy (2010) for water hyacinth (*Pontederia* (= *Eichhornia*) *crassipes* (Mart.) Solms-Laub. (Pontederiaceae)), Doeleman (1989) for salvinia (*Salvinia molesta* D.S. Mitch. (Salviniaceae)), Neuenschwander et al. (2009) for water lettuce (*Pistia stratiotes* L. (Araceae)), Hill & McConnachie (2009) for red water fern (*Azolla filiculoides* Lam. (Azollaceae)), and Cilliers (1999) for parrot's feather (*Myriophyllum aquaticum* (Vell. Conc.) Verd. (Haloragaceae)). While the direct and indirect (cost of control) economic effects of aquatic weeds are relatively easy to measure (e.g. Mailu, 2001), quantifying their impacts on biodiversity is more difficult, although, studies on notorious aquatic invaders such as *P. crassipes* and *Phragmites australis* (Cav.) Trin. ex Steud. (Poaceae) do highlight the consequences of their invasion on biodiversity (e.g. Villamagna & Murphy, 2010; Lambert et al., 2010). For example, *P. crassipes* invasions result in lowered dissolved oxygen concentrations beneath the mats, as a result of reduced light available for photosynthesis by phytoplankton communities, which has knock-on higher trophic level effects (Rommens et al., 2003; Mangas-Ramirez & Elias-Gutierrez, 2004; Perna & Burrows, 2005). The ecological impacts of less notorious invasive macrophytes, such as *P. stratiotes*, on aquatic communities are not obvious from the literature.

Given the widespread distribution and subsequent socio-economic impacts of invasive macrophytes, a number of these species have been targeted for biological control. It has often been suggested that biological control, or the release of host specific natural enemies, is the only option that offers economical and sustainable control of weeds (McFadyen, 1998). Biological control programmes against floating macrophytes such as water hyacinth, salvinia, and water lettuce, have been ongoing since the release of the first biological control agent on water hyacinth in the early 1970s in the USA and South Africa. The practice of biological control of weeds is considered safe and sustainable due to the rigorous host-specificity testing of each agent which ensures that no non-target plants will be compromised; confirmation that the agent will indeed be damaging; and intense screening by panels of experts prior to application for release. Globally, biological control has led to a significant reduction in the invasiveness of these species (Winston et al., 2014). But studies on quantifying the benefits in terms of biodiversity recovery and ecosystem restoration are lacking.

Historically, weed biological control has suffered from a lack of empirical data to prove causation of control, relying on 'before and after' application of biological control data to show efficacy. Where effects of the agents are quantified, these are usually measurements of agent establishment and plant growth, and seldom plant population dynamics, and where the benefits of a biological control programme have been measured, they have most often focussed on economic benefits. For example, McConnachie et al. (2003) quantified the benefits of the biological control programme against red water fern (*Azolla filiculoides*) using the weevil, *Stenopelmus rufinasus* Gyllenhal (Coleoptera: Curculionidae) in South Africa and showed that the agent reduced the negative effect of the weed on water supply, stock health and recreational activities. Further, De Groote et al. (2003) demonstrated that the successful biological control of water hyacinth in southern Benin significantly increased the yearly income of the population of this region through increased crop and fish production. More recently, an economic analysis of the cost–benefit of controlling water hyacinth in Louisiana, USA, over a 38-year time period by Wainger et al. (2018) demonstrated a benefit–cost ratio of about 34:1 derived from the costs of $124 million ($2013)

compared to the \$4.2 billion (\$2013) in benefits to anglers, waterfowl hunters, boating-dependent businesses, and water treatment facilities. In these studies, there is no quantification of how ecosystem functioning returns with the reverse of biotic homogenization, and thus there are few studies of the biodiversity and ecological benefits of aquatic weed biological control (for an exception, see Hill & Coetzee, 2017a).

The aim of this study was to measure the effect of *P. stratiotes* invasion on benthic macroinvertebrate biodiversity, and its recovery after the introduction of the water lettuce biological control agent, *Neohydronomus affinis* Hustache (Coleoptera: Curculionidae). We hypothesized that the introduction of *P. stratiotes* would reduce dissolved oxygen concentration, leading to a decrease in macroinvertebrate biological and functional diversity, resulting in biotic homogenization. We further hypothesized that the reduction in water lettuce biomass and cover through biological control would reverse these effects, allowing the recovery of biological and functional diversity of the macroinvertebrate communities.

Materials and methods

Description of the weed and agent

Pistia stratiotes (water lettuce, Nile cabbage) originates in South America and is the only species in the Araceae that floats freely on still or slow moving water bodies. The undersides of the leaves contain spongy parenchyma and the leaves are covered with dense hairs on both surfaces. This plant reproduces vegetatively through the formation of stolons and daughter plants (Neuenschwander et al, 2009). The role of sexual reproduction is considered less important than that of vegetative reproduction, although seed germination is an important factor in the dynamics of water lettuce populations (Diop et al., 2010). *Pistia stratiotes* has a global distribution, dominating water bodies in the tropics and subtropics where it responds to eutrophication. It is also becoming more widely distributed in temperate regions, including Europe where it was first recorded from the Netherlands in 1973, but did not establish permanent populations, while it has remained permanently established in thermal sections of the River Erft since 2008 (Hussner, 2014).

The South American weevil, *Neohydronomus affinis*, has been released throughout the world where water lettuce is a problem (Winston et al., 2014). The adults are brown to bluish grey, and make characteristic shot-hole scars in the mesophyll of the leaves. The eggs are deposited singly below the epidermis, and the larvae mine the spongy leaf tissue causing significant damage to the plant (De Loach et al., 1976). It was first introduced to Australia in 1982, and then to South Africa from Australia in 1985 (Cilliers, 1987). Since then, it has been intentionally released into 16 other countries around the world, where it is regarded as a successful biological control agent (Winston et al., 2014).

Experimental design

This study was conducted at the Department of Zoology and Entomology, Rhodes University (-33.310453, 26.517438, Makhanda, South Africa) in large outdoor mesocosms (PVA pools measuring 265 cm in diameter with a depth of 67.5 cm). The experimental setup included three treatments, replicated three times: an open water control treatment (no water lettuce); a treatment completely covered with water lettuce (1500 plants obtained from stock cultures maintained at the Waainek Mass Rearing Facility, Rhodes University); and a biological control treatment in which 250 adult *N. affinis* weevils (1 weevil/6 plants obtained from cultures maintained at the Waainek Mass Rearing Facility) were placed onto pools completely covered with water lettuce (1500 plants). Benthic macroinvertebrate biodiversity was measured using a standard technique of deploying artificial substrate, comprising approximately 90 pebbles ($\sim$ 2 kg) enclosed in a mesh bag (20 cm $\times$ 50 cm, 2 mm mesh size), onto the floor of the mesocosm (Thirion, 2000; Midgley et al., 2006; Coetzee et al., 2014). For each sample event, the substrates were left for 6 weeks in each treatment, the time required for complete recruitment by invertebrates (Thirion, 2000), after which they were collected and evaluated.

At the outset of the study, nine mesocosms were filled with borehole (well) water, and 1 bag of artificial substrate was placed into each pool. 6 weeks later, the bags were retrieved and evaluated for macroinvertebrate colonization (see below). This was designated Sample Event 0 (i.e. the 'before' treatment). New artificial substrate bags were then replaced into all of

the pools, and six of the pools were randomly selected and completely covered with water lettuce, while three remained as the open water controls. Then three of the pools covered with water lettuce were randomly selected and each treated with 250 adult weevils. The mesocosms were not caged as this would prevent recruitment of macroinvertebrates. This setup resulted in the three experimental treatments, an open water control, a water lettuce treatment and a biocontrol treatment. After 6 weeks, the artificial substrate bags were removed, evaluated and replaced every 6 weeks, during six sampling events over 36 weeks.

For each sample event, colonization by benthic macroinvertebrates on the artificial substrates was determined by emptying the contents of each bag, and the bag itself into a sorting tray. Each pebble was cleaned with fresh water and a small paint brush, while the bag was washed with water. Both the sample bag and individual pebbles were examined for macroinvertebrates before being removed from the sorting tray. The contents of the sorting tray (water and substrate) were then drained through a sieve (1×1 mm), and all macroinvertebrates were removed with forceps and placed into glass vials, containing 75% ethanol for later identification. Specimens were identified to family level, under a dissecting stereomicroscope, using a series of identification keys (Day & De Moor, 2002; Day et al., 2003; De Moor et al., 2003a, b). Macroinvertebrate family-level identification is considered an efficient, easy-to-use, reliable rapid assessment method for monitoring aquatic ecosystems impacts (McDermond-Spies et al., 2014). In some instances, individuals were only identified down to sub-class, e.g. Hirudinea and Oligochaeta and class, e.g. Ostracoda, as keys to families in these taxa were not available. Numerical abundance of each taxon per sample bag, per treatment was quantified.

In addition to macroinvertebrate diversity data, dissolved oxygen content (DO) (mg/l) was measured for all of the pools on every second sampling occasion (every 12 weeks), using a portable YSI 550A multi-parameter probe (YSI, OH, USA) directly in the pools. DO was measured because the maintenance of adequate dissolved oxygen concentrations is critical for the survival and functioning of aquatic biota, and mats of floating aquatic weeds are known to reduce DO concentration in the water column. Therefore, the % water lettuce cover for the water lettuce and biocontrol treatments was also recorded every 6 weeks.

Statistical analyses

To determine if the study sampled sufficient macroinvertebrate families, individual-based rarefaction curves were compiled from the abundances of each sample bag, per treatment over the 36-week sample period, using the analytically calculated S_{obs} (Mao Tao) (number of species expected), using EstimateS V9.1.0 (Colwell, 2013). Significant differences in curves (i.e. treatments) were indicated by non-overlapping 95% CI bands.

A number of measures of diversity were calculated in EstimateS V9.1.0 to determine differences in macroinvertebrate diversity indices of the three treatments, over time, including total taxon richness (S), individual abundance (N), and Shannon's diversity (H) (Magurran, 2004). Repeated Measures ANOVAs followed by Bonferroni post hoc tests in Statistica V13.4, compared differences in diversity measures between the treatments, across the sample period, including the 'before' treatments.

Differences in % water lettuce cover and dissolved oxygen (DO) between the treatments over the sample period were analysed using a Repeated Measures ANOVA, followed by Fisher's LSD post hoc tests. The influence of % water lettuce cover and DO on macroinvertebrate abundance was also analysed using a Generalized Linear Model (GLZ) multiple regression, with a Poisson distribution and log-link function, in Statistica V13. Model building using best subsets and Akaike Information Criteria (AIC) determined which variables to include in the analysis.

Unconstrained ordinations were completed using principal coordinate ordination (PCO) on the Bray–Curtis similarity matrix to visualize aquatic macroinvertebrate assemblages. This was followed by a constrained canonical analysis of principal coordinates (CAP) to emphasize assemblage differences between treatments at the end of the study (i.e. the open water control, water lettuce treatment and biocontrol treatment). Aquatic macroinvertebrate abundances were fourth-root transformed to meet normality and to recognize the abundance of rare taxa. Pearson's correlation ($r > 0.5$) with the canonical axes of the CAP was used to identify taxa driving the

differences in assemblage structure ($R = 0.7$) between treatments.

Additionally, Analysis of similarity (ANOSIM, 9999 permutation) with a pairwise comparison test was used to test similarity in macroinvertebrate assemblage composition between treatments at the end of the study; open water control, and the water lettuce and biocontrol treatments. A permutational analysis of multivariate dispersions (PERMDISP; 9999 permutations) based on the mean distance to the centroid was also performed on macroinvertebrate assemblage composition for homogeneity of variances, followed by permutational analysis of variance (PERMANOVA) to test if macroinvertebrate assemblages varied across treatments and over time. Similarity Percentage (SIMPER) for species contribution analysis was computed using a Bray–Curtis similarity matrix to provide macroinvertebrate assemblage percentage similarity between treatments. These analyses were performed using PRIMER v6 with the PERMANOVA add-on (PRIMER-E Ltd, Plymouth; Clarke & Gorley, 2006; Anderson et al., 2008).

Results

A total of 11,098 individuals from 16 taxa were collected over the duration of the experiment (Table 1). 9787 (88.2%) of the individuals were collected from the open water treatment, in comparison to 1007 (9.1%) from the biocontrol treatment, and only 304 (2.7%) from the covered water lettuce treatment, clearly demonstrating the negative association between water lettuce and macroinvertebrate biodiversity. The most abundant taxa were the Chironomidae and Ostracoda, while the least abundant taxa were the Oligochaeta and the Hydracarina. Even though abundance was higher from open water pools, projected family richness (Sobs) was not significantly different between open water pools (O) and the biocontrol treatment pools (N), indicated by the overlapping confidence interval bands of the rarefaction curves ($P > 0.05$), suggesting that biodiversity in terms of richness benefits from biological control. However, projected macroinvertebrate richness beneath water lettuce covered pools was significantly less than both the open water and biocontrol treatments, indicated by the non-overlapping confidence interval bands ($P < 0.05$) (Fig. 1). These results show that the presence of water lettuce significantly reduced family richness of the benthic macroinvertebrate communities, while the action of biological control agents allowed significant colonization by this guild of invertebrates.

At the onset of the experiment, there was no significant difference in observed taxonomic richness (S) in any of the 'before' treatments, but over the sample period, S increased significantly after the first sample event, and was consistently significantly higher, in the open water pools, followed by the pools treated with the biocontrol agent, while the lowest richness was observed in the pools with 100% water lettuce cover (W) ($F_{12,36} = 3.57$, $P = 0.002$) (Fig. 2). There was no significant difference in Abundance (N) in any of the 'before' treatments at the beginning of the experiment, but it was significantly higher in the open water treatment, than both the water lettuce and biocontrol treatments, over time ($F_{12,36=} = 2.20$, $P = 0.033$). Shannon's Index of Diversity (H) was mostly lowest for the water lettuce treatment, while no clear pattern between the open water and biocontrol treatments emerged, although both were significantly higher than the water lettuce treatment at the end of the study ($F_{12,36} = 2.99$, $P = 0.005$). These results complement the rarefaction curves, indicating a significant effect on biodiversity in the presence of a floating mat of weed, while significant recovery was evident in the presence of biological control.

Over time, herbivory by the weevil significantly reduced water lettuce cover in the biocontrol treatment, to 60% ($F_{5,20} = 14.48$, $P > 0.00001$) (Fig. 3A). The dissolved oxygen (DO) content (mg/l) was significantly higher in water from the open water treatment, over the sample period, followed by the biocontrol treatment, and then the water lettuce treatment W ($F_{4,12} = 5.567$, $P = 0.009$) (Fig. 3A). There was a decline in DO over time for the biocontrol treatment which was likely related to rotting plant material as a result of herbivory by the weevil, $N.$ $affinis$. Correspondingly, as % water lettuce cover increased, and DO decreased, macroinvertebrate abundance significantly decreased exponentially (Wald $\chi^2 = 4.544$, $P = 0.033$) (Fig. 3C). The best subsets included all three variables, i.e. treatment, % cover and DO (AIC = 4644.084, $P < 0.0001$). Thus, the combined effect of plant cover and a corresponding decrease in DO acted together to reduce benthic macroinvertebrate abundance, while the action of

Table 1 Mean numerical abundance $\pm$ SE of benthic macroinvertebrate taxa, in order of dominance, from samples that inhabited artificial substrates placed in open ponds, placed under water lettuce mats treated with the biological control agent, *Neohydronomus affinis*, and from ponds completely covered with water lettuce

Taxon (common name)	Open water control	Biological control treatment	100% water lettuce cover treatment	Functional feeding group	Ecological niche (e.g. sensitivity, behaviour)
Chironomidae (midges)	4096 $\pm$ 50.25	214 $\pm$ 6.10	6 $\pm$ 0.18	Gathering-collector	Tolerant to low [DO]
Ostracoda (seed shrimps)	3373 $\pm$ 64.38	64 $\pm$ 0.93	122 $\pm$ 4.33	Filtering-collector	Found in muddy-sediments
Ceratopogonidae (sand flies)	850 $\pm$ 10.81	72 $\pm$ 1.75	0	Gathering-collector	Found in mud, debris, rotting vegetation
Dytiscidae (diving beetles)	433 $\pm$ 8.89	136 $\pm$ 3.80	12 $\pm$ 0.42	Predator	Air-breathers
Physidae (bladder snails)	276 $\pm$ 6.48	295 $\pm$ 9.56	110 $\pm$ 3.54	Scraper	Feed on periphyton and diatoms
Libellulidae (dragonflies)	248 $\pm$ 3.90	22 $\pm$ 0.59	0	Predator	Found in gravel, vegetation, detritus and mud
Baetidae (mayflies)	199 $\pm$ 4.81	30 $\pm$ 0.83	0	Scraper	Sensitive to low [DO]
Dryopidae (long-toed water beetles)	83 $\pm$ 1.83	19 $\pm$ 0.41	0	Herbivore	NA
Caenidae (mayflies)	65 $\pm$ 1.37	21 $\pm$ 0.68	0	Scraper	Sensitive to low [DO]
Gomphidae (dragonflies)	62 $\pm$ 1.84	11 $\pm$ 0.31	0	Predator	Burrowing dragonfly
Pleidae (pygmy backswimmers)	34 $\pm$ 0.50	33 $\pm$ 0.94	0	Predator	Vegetated habitats
Corixidae (water boatmen)	29 $\pm$ 0.32	9 $\pm$ 0.31	0	Predator	Vegetated habitats
Coenagrionidae (damselflies)	18 $\pm$ 0.28	20 $\pm$ 0.94	0	Predator	
Hirudinea (leeches)	13 $\pm$ 0.39	27 $\pm$ 0.65	21 $\pm$ 0.41	Predator	Tolerant to low [DO] and water clarity
Oligochaeta (annelid worms)	7 $\pm$ 0.16	34 $\pm$ 0.75	33 $\pm$ 0.66	Gathering-collector	Tolerant to low [DO] and water clarity
Hydracarina (water mites)	1 $\pm$ 0.06	0	0	Predator	Sheltered and shallow water bodies

Functional Feeding Groups and ecological niche assigned following Cummins and Klug (1979), Palmer et al. (1996), Merritt et al. (2008) and Hawking et al. (2013)

biological control agents significantly increased this measure of diversity.

CAP ordination revealed three distinct macroinvertebrate assemblage clusters, each representing one of the three experimental treatments (CAP axis 1 and 2 explained 94.7% and 61.4% macroinvertebrate assemblage total variation, respectively) (Fig. 4). Six macroinvertebrate taxa including Chironomidae, Corixidae, Ceratopogonidae, Baetidae, Libellulidae and Dryopidae were positively associated with the open water treatment, but not with the water lettuce and biocontrol treatments. When this was investigated further, ANOSIM returned a Global R of 0.37 ($P = 0.001$), indicating some degree of separation between the treatments. According to the pairwise test, there were no distinguishable differences in macroinvertebrate assemblages between the water lettuce and biocontrol treatments, or between the biocontrol and open water treatments (Table 2), while the macroinvertebrate assemblages from the water lettuce and open water treatments were completely separate. PERMANOVA supported ANOSIM results, and
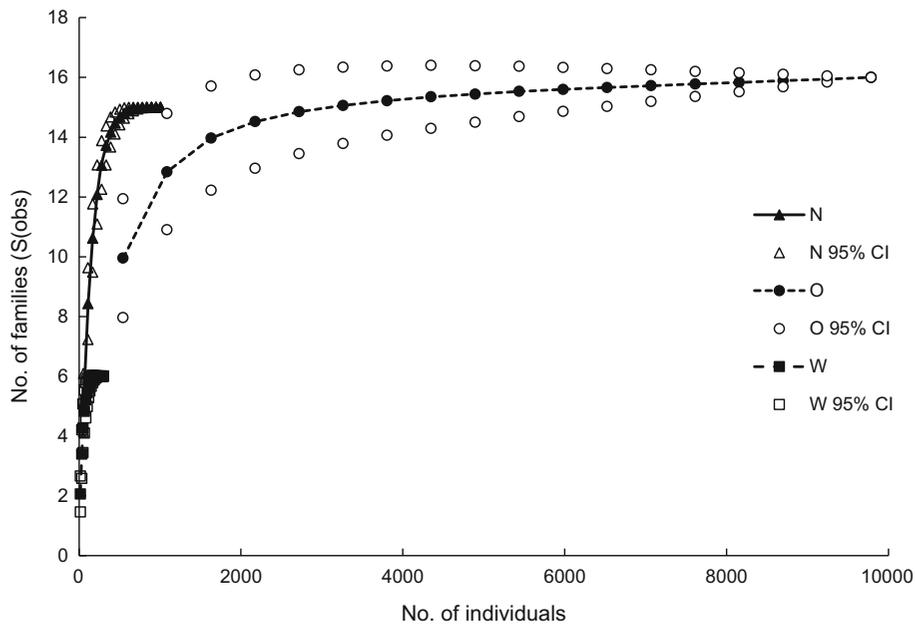
Fig. 1 Rarefaction curves, scaled by number of individuals, comparing observed family richness of benthic macroinvertebrates between open water samples (*O*) and under water lettuce canopy treated with the agent *N. affinis* (*N*) and left at 100% cover (*W*). Non-overlapping 95% confidence interval bands indicate significant differences (*P* < 0.05) between the three curves

further indicated that the macroinvertebrate assemblages were varied between treatments over time (Pseudo-$F_{10,65}$ = 1.766, *P* =0.0021). There was evidence of macroinvertebrate assemblage homogenization during the study, where treatments inoculated by water lettuce (both the water lettuce and biocontrol) revealed similarity in macroinvertebrate composition (or no taxa difference) (PERMDISP, *P* > 0.05) (Table 2). Macroinvertebrate assemblages between the water lettuce and biocontrol treatments together with the open water treatment were heterogeneous (PERMDISP, *P* < 0.05). In terms of similarity percentages of species contributions, the SIMPER analysis indicated that the least similar assemblages were between the water lettuce and open water treatment, followed by the water lettuce and biocontrol treatments, while the most similar were between the biocontrol and open water treatments (Table 2).

Discussion

This study has shown that the invasion by the alien aquatic weed, *P. stratiotes*, significantly reduced water quality through a reduction in dissolved oxygen concentration, and consequently benthic biodiversity, supporting the findings of Midgley et al. (2006), Coetzee et al. (2014) and Motitsoe et al. (2020) who showed similar results with water hyacinth and salvinia in South Africa. In addition, Stiers et al. (2011) reported the negative effects of three alien invasive macrophytes in Europe (i.e. *Hydrocotyle ranunculoides* L.f. (Araliaceae), *Ludwigia grandiflora* (Michx.) Greuter & Burdet (Onagraceae) and *M. aquaticum*) on both native plant and macroinvertebrate diversity. Although these findings might seem intuitive, there are very few studies that have quantified the impacts of floating macrophytes empirically. The review by Kettenring and Adams (2011) highlighted that the majority of alien aquatic weed management studies lack evaluation of native biodiversity recovery following removal of the weeds. The results of this study have demonstrated the recovery of macroinvertebrate assemblage diversity as a result of biological control, and thus, biological control of floating alien aquatic weeds is a reliable option for aquatic ecosystem recovery when implementing integrated alien aquatic weed management that includes post-control restoration, as proposed by Prior et al. (2018). The significance of this study is that it justifies,
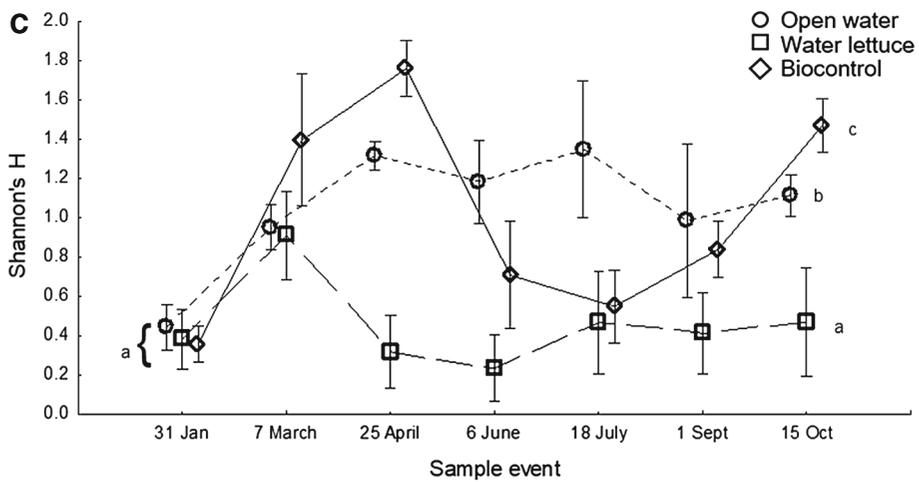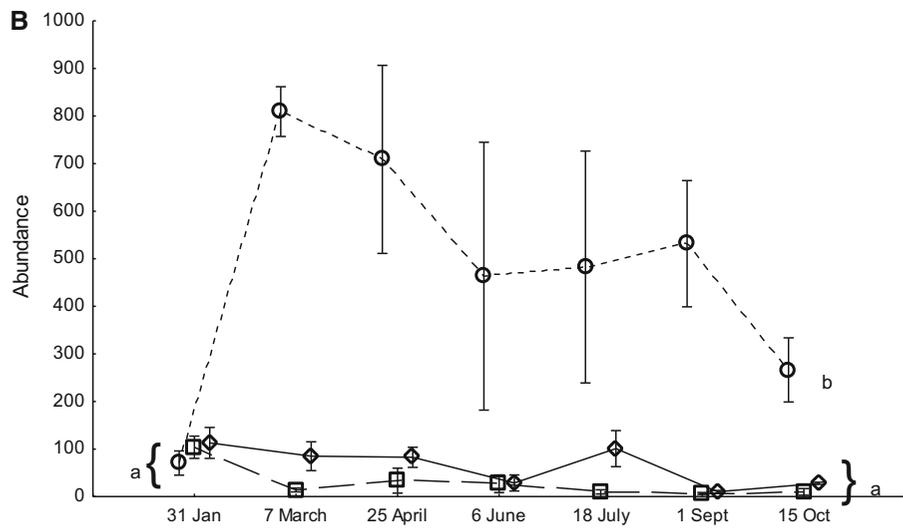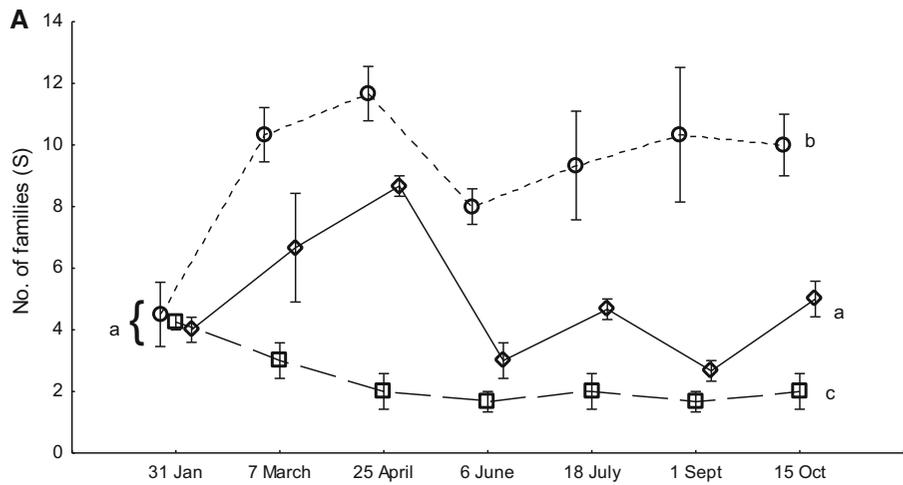
◀**Fig. 2** Comparison of measures of diversity of aquatic macroinvertebrate communities between open water samples (circle), and under *Pistia stratiotes* canopy treated with the agent *Neohydronomus affinis* (Biocontrol-square) and left at 100% cover (Water lettuce-diamond). **A** Family richness (*S*), **B** abundance (*N*), **C** Shannon's Diversity Index (*H*). Means compared by Repeated Measures ANOVAs, error bars represent S.E., different letters represent significant differences ($P < 0.05$) (only 'before' and final sample event differences are indicated by the letters to reduce clutter)

ecologically, the necessity to control floating aquatic weeds in South Africa and globally where they have been introduced.

The use of macroinvertebrate diversity indices and assemblage similarity analyses were sensitive enough

to detect the effects of *P. stratiotes* and its subsequent biological control. The lower abundance and diversity of the benthic macroinvertebrate community under a total coverage of *P. stratiotes* were the result of reduced light penetration into the water column, and subsequent lower levels of dissolved oxygen concentrations. The shading effect of the invasive mats was an important abiotic barrier that contributed to poor water quality, reduced system productivity and aquatic biodiversity, leading to biotic homogenization. Only disturbance tolerant groups such as the ostracods, Physidae (bladder snails) and annelid worms were present under mats, while more sensitive groups such as the mayflies and dragonflies were excluded (Day &
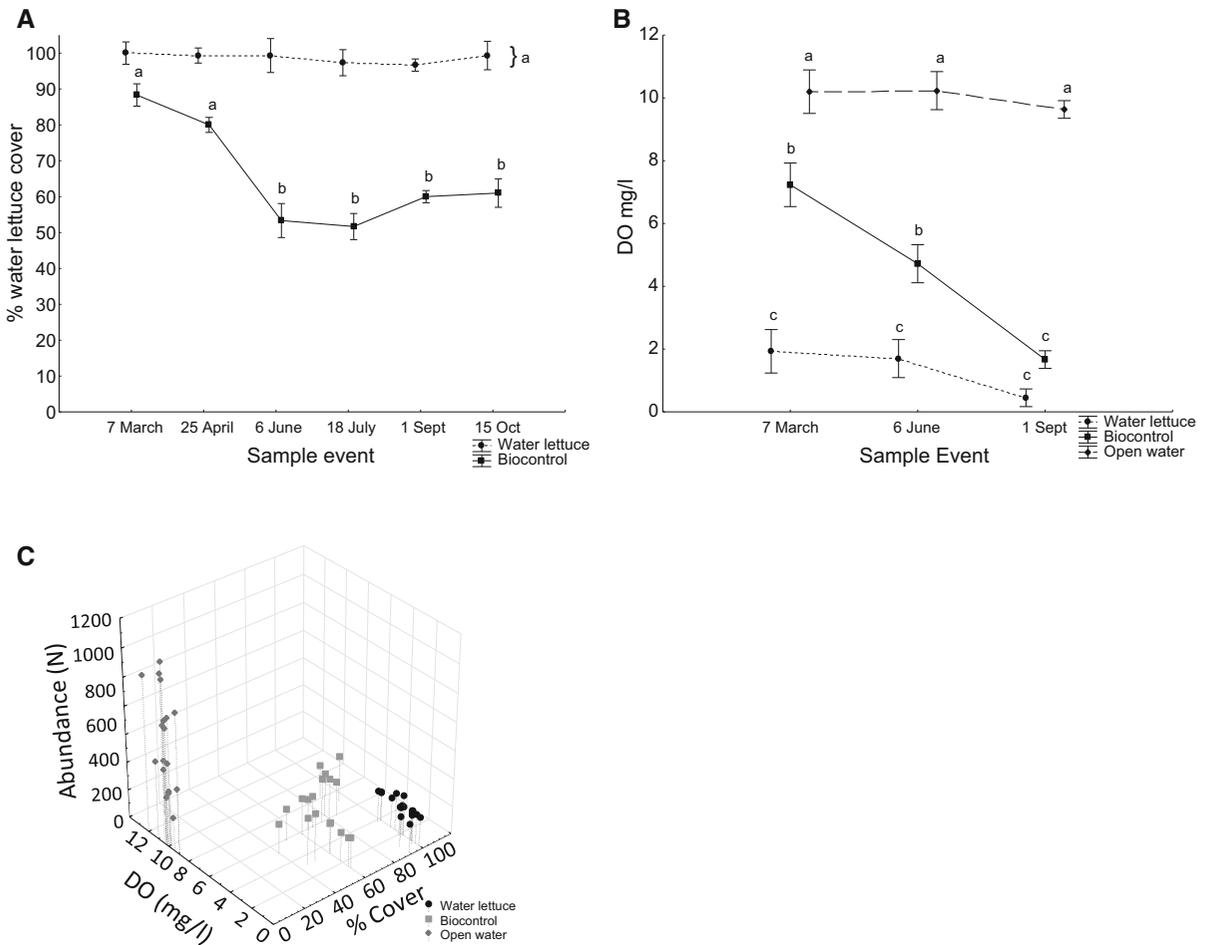

Fig. 3 A Percent water lettuce cover over six, 6-week sample events. **B** Dissolved oxygen (DO) content (mg/l) of water from three treatments, recorded every second sampling event. Means compared by Repeated Measures ANOVA, error bars represent

S.E. Different letters indicate significant differences, $P < 0.05$. **C** Positive exponential relationship between DO (mg/l), and a negative exponential relationship between water lettuce cover and macroinvertebrate abundance, from three treatments

de Moor, 2002). Reductions in dissolved oxygen as a consequence of floating plant infestations are not only reported to decrease macroinvertebrate abundance but also reduce ecosystem productivity (Rommens et al., 2003; Mangas-Ramirez & Elias-Gutierrez, 2004; Perna & Burrows, 2005), which has consequences for higher trophic level structure and functioning. By quantifying the changes in biodiversity indices and assemblage structures as a result of macrophyte invasion, and subsequent biological control which eliminated an abiotic barrier (i.e. shade), we have
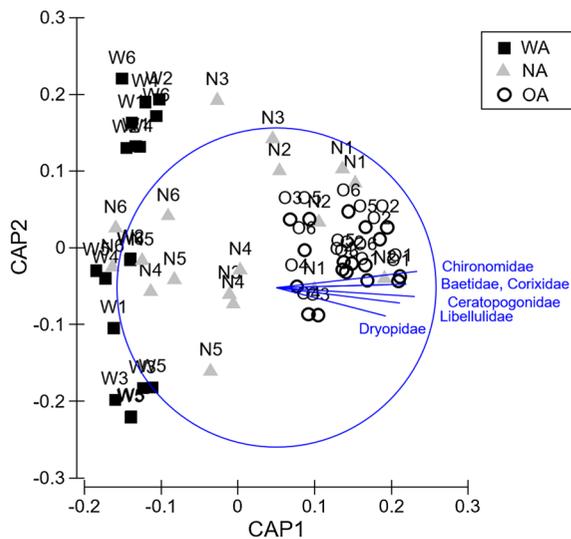

Fig. 4 Canonical analysis of principal coordinates (CAP) ordinations indicating distinct differences in macroinvertebrate assemblages found beneath water lettuce mats (WA), beneath mats treated with the biological control agent *N. affinis* (NA) and those from open water (OA), over seven 6-week sampling events (0–6). CAP1 eigenvector $\sigma 2 = 0.94$, CAP2 eigenvector $\sigma 2 = 0.61$, where $\sigma 2 =$ the amount of variation explained by the canonical axis. The four taxa that contributed to the differences in assemblage structure, with $R = 0.7$, are indicated

demonstrated that biotic homogenization as a result of invasion can be reversed.

Macrophytes play an important role in ecosystem structure and functioning, thus changes in their composition and structural complexity directly influence macroinvertebrate assemblage composition (Schultz & Dibble, 2012). In the case of alien macrophyte invasion and associated knock-on effects, macroinvertebrate assemblage compositions shift towards dominance by more tolerant taxa, reducing functional diversity. In this study, macroinvertebrate assemblage composition between the open water and invaded treatments were completely different, in comparison to the biocontrol and open water treatments, which shared more taxa and were more similar in assemblage composition. In comparison, Stiers et al. (2011) showed that three invaded systems were similar in terms of macroinvertebrate composition, in contrast to the control site. Furthermore, Oligochaeta and Chironomidae contributed more than 50% and 15% of the total macroinvertebrate abundance in the invaded sites, respectively (Stiers et al., 2011), while Oligochaeta and Hirudinea were more abundant in both the water lettuce and biocontrol treatments in this study, indicating the lowered quality of the invaded habitat. On the other hand, the abundance of Baetidae, Caenidae, Libellulidae and Gomphidae in the open water treatment is explained by their ecology, these taxa are sensitive to low DO concentration and are widely accepted as biological indicators for poor water quality (Dickens & Graham, 2002). Additionally, Dytiscidae and Physidae are air-breathers, explaining their abundance in the open water treatment where they had access to oxygen at the water's surface.

While the presence of large mats of invasive floating aquatic plants such as *P. stratiotes* are known

Table 2 Macroinvertebrate assemblage ANOSIM and PERMDISP pairwise comparison tests between treatments at the end of the study period (significant R and t statistics in bold), and the similarity percentages of species contributions computed in SIMPER analysis

Treatments	R statistic (significance level)	Pairwise PERMDISP test t value (significance level)	Average similarity %
Water lettuce × biocontrol	**0.153 (0.003)**	0.297 (0.776)	22.12
Water lettuce × open water	**0.613 (0.001)**	**7.078 (0.001)**	13.6
Biocontrol × open water	**0.293 (0.001)**	**9.239 (0.001)**	35.1

to reduce aquatic ecosystem diversity, to date, the only study we are aware of that has categorically demonstrated the biodiversity benefits of reductions in standing cover due to the action of biological control agents is Motitsoe et al. (2020) who investigated biodiversity recovery following biological control of *S. molesta*. The biological control programme against *P. stratiotes* using the weevil, *N. affinis*, has been successful in many parts of the world (Diop & Hill, 2009; Neuenschwander et al., 2009; Moore & Hill, 2012), thereby contributing to improving aquatic ecosystem functioning through biodiversity recovery. In this study, although the introduction of *N. affinis* only resulted in a maximum of 50% reduction in cover of *P. stratiotes* over the duration of this study, there was a significant recovery of benthic macroinvertebrate biodiversity in comparison to the 100% covered treatment, but not to the level of the open control. This is likely to be due to the increase in allochthonous detritus rain as a result of plant death caused by biological control, which reduced dissolved oxygen concentrations in the pools. This suggests that the experiment should have been run for far longer to attain a higher level of control before the ecosystem could fully recover. However, even though biological control resulted in only a 50% reduction of the *P. stratiotes* mat over a 10-month period, many of the more sensitive groups such as mayflies, dragonflies and hemipterans had returned. Paterson et al. (2011) showed that in the case of the invasive vine, *Pereskia aculeata* Miller (Cactaceae), an 80% reduction in canopy cover was required before plant biodiversity had recovered in comparison to uninvaded sites. Similarly, it is possible that the threshold level for complete restoration of the aquatic ecosystem, post-control of *P. stratiotes*, is higher than 50%.

Samways & Sharratt (2010) showed that dragonfly assemblages that had been impacted by alien invasive riparian vegetation in rivers in the Western Cape of South Africa recovered quickly once the vegetation had been removed. Similarly, here we have shown that aquatic ecosystems can recover after effective biological control of floating alien macrophytes. Macroinvertebrates were once again reliable biological indicators able to detect abiotic filters, thereby demonstrating the homogenization effect by *P. stratiotes*. The application of biological control by the weevil, *N. affinis* facilitated ecosystem biological and functional diversity recovery, supporting this control

method in invasive aquatic species management. The important point is that while biological control does enhance biodiversity recovery, the process is a long-term intervention (Neuenschwander et al., 2009), and although the biological control of waterweeds has historically occurred in much shorter time frames (2–5 years) (Hill & Coetzee, 2017b) than terrestrial weeds, these biodiversity benefits will be short-lived in impacted ecosystems unless an integrated catchment management approach is adopted which addresses eutrophication and changes in hydrological flow regimes. If the primary driver of disturbance in aquatic ecosystems is not addressed, we anticipate, rather than control and restoration, a succession of invasions by a suite of waterweeds (Coetzee et al., 2011; Strange et al., 2019), which will further drive biotic homogenization of aquatic ecosystems.

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