Received: 27 October 2021 | Accepted: 17 March 2022

DOI: 10.1111/1365-2664.14221

Journal of Applied Ecology BRITISH ECOLOGICAL SOCIETY

RESEARCH ARTICLE

Below-ground ecosystem engineers enhance biodiversity and function in a polluted ecosystem

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Funding information
City of Sydney; Ian Potter Foundation; Maple Brown Foundation; Open access publishing facilitated by The University of Sydney, as part of the Wiley - The University of Sydney agreement via the Council of Australian University Librarians

Handling Editor: Nessa O'Connor

Abstract

1. Many important ecosystem functions are underpinned by below-ground biodiversity and processes. Marine sediments, one of the most abundant habitats on earth, are essential to the mineralisation of organic matter. However, they are increasingly polluted by urban activities leading to the loss of biodiversity and the functions they provide. While traditional sediment remediation strategies are focussed on microbial and engineering solutions, we propose that the reintroduction of below-ground ecosystem engineers (bioturbators) is important to rehabilitate polluted sediments and drive recovery of their functions in urban coastal ecosystems.
2. We tested this notion by introducing bioturbators to nutrient polluted sediments to assess their survival, as well as their capacity to drive biodiversity and oxygenation and their potential to remediate nutrient pollution. Polychaete worms *Diopatra aciculata* and clams *Katelysia* sp. were added to mesocosms (ex-situ), and the worms also added to experimental plots in-situ. Potential for remediation was assessed with measures of nutrient content.
3. All animals survived when introduced to polluted sediments and showed no evidence of sub-lethal effects. Worms oxygenated sediments and reduced organic matter content by up to 50% in-situ. The worms also drove shifts in the receiving communities at all locations and increased the number of taxa at one location. On the other hand, the effects of clams were variable, showing opposite effects in organic matter content at different sites and levels of pollution.
4. *Synthesis and applications*. Global seafloor habitats are becoming increasingly degraded and novel strategies that combine biodiversity restoration with remediation are urgently needed to return function. Tube-building bioturbators can stimulate nutrient processing in sediments proving multiple functional

outcomes, but these effects are dependent on the receiving environment. In areas with medium levels of pollution, they can kick-start recovery in a feedback loop whereby bioturbation increases oxygenation and nutrient remediation, shifting sediment biodiversity and contributing to further recovery. This can drive long-term changes in sediment communities, particularly in urban areas where unvegetated sediments are conspicuous.

KEYWORDS
biodiversity, bioturbation, ecosystem engineer, pollution, remediation, restoration, sediments, urbanisation

1 | INTRODUCTION

Below-ground biodiversity on land and in oceans has been severely impacted world-wide due to harvesting, habitat modification and pollution (Danovaro et al., 2008; Tsiafouli et al., 2015). Soils and sediments contribute significant biodiversity to global ecosystems (Decaëns, 2010; Snelgrove, 1999) and support crucial decomposition and mineralisation of nutrients, with marine sediments alone responsible for the mineralisation of over 50% of global organic matter (Gruber & Galloway, 2008). Rehabilitation of degraded sediments generally focuses on the removal of stressors such as pollution, but this does not ensure recovery. Coupled approaches to restore biodiversity and remediate pollutants are needed to support the recovery of degraded sediments (Duarte & Krause-Jensen, 2018).

Animal bioturbators are ecosystem engineers (sensu Jones et al., 1994) in soils and sediments. They create burrows that increase below-ground porosity and infiltration of materials such as water and nutrients (Stief, 2013). In aquatic systems, bioturbators create pockets of oxygenation in deep sediments, increasing overall complexity and the area for aerobic bacterial processes, boosting the mineralisation of nutrients by two to threefold (Stief, 2013). Studies demonstrated that the loss of animal bioturbators in marine and terrestrial systems leads to reduced ecosystem functions, including decomposition and nutrient cycling (e.g. Danovaro et al., 2008; Wagg et al., 2014). Loss of bioturbators has also been shown to reduce the taxonomic and functional diversity of sediment macrofauna (Volkenborn & Reise, 2007).

In urbanised coastal ecosystems, pollution from industrial waste, sewage overflows and urban stormwater runoff has resulted in severe habitat degradation and biodiversity loss (e.g. Beukema, 1991; Bugnot et al., 2019). To reverse this trend, legislation around the world including Europe, North America and Australia, has been in place for several decades to reduce contaminant inputs into coastal waterways, but these actions have not resulted in the recovery of biodiversity and functions (e.g. Borja et al., 2010; Duarte & Krause-Jensen, 2018). This is a common issue in highly modified ecosystems, where multiple pressures from human activities have severely impacted abiotic and biotic attributes of ecosystems, shifting baselines (Duarte et al., 2009). In such cases, human interventions that reintroduce ecosystem engineers are necessary to assist the recovery of coastal habitats and the remediation of pollutants (Duarte & Krause-Jensen, 2018). However, these strategies have mainly focused on above-ground habitats (Byers et al., 2006), while the restoration of the abundantly available unvegetated sediments has received less attention despite their importance to ecosystem function. Here, we propose that improving sediment biodiversity by reintroducing below-ground ecosystem engineers is key to promoting the remediation of nutrient pollutants and drive the recovery of degraded coastal ecosystems.

The capacity of bioturbators to promote biodiversity and functions is determined by their densities, size, level of activity, burrowing mode and feeding behaviour (Volkenborn et al., 2009). Large, active animals such as crabs, and some species of clams and tube-building worms at low densities and/or with small tube diameters have been shown to destabilise sediments, increasing oxygen and nutrient penetration, while also excluding other macrofauna (Eckman et al., 1981; Van Colen et al., 2008). Alternatively, large densities of tube-building worms can have stabilising effects, facilitating other species directly by providing habitat in their tubes (Eckman et al., 1981), or indirectly by stabilising sediments (Van Colen et al., 2008). Hence, the use of bioturbators to restore biodiversity and functions should be informed by studies testing different species for their potential to drive the desired changes.

This study assessed whether the introduction of two native bioturbating species from different functional groups (a filter-feeding clam and a tube-building worm) in polluted marine sediments enhance biodiversity and oxygenation and triggers nutrient remediation (measured as changes in nutrient content). It aimed to (a) assess the survival and sub-lethal effects of bioturbators after introduction to polluted urban sediments, and (b) investigate if these bioturbators can boost oxygenation and reduce nutrient content in sediments using mesocosms. Based on these results, we developed a field experiment aiming to assess the effects different densities of bioturbators on (c) oxygenation and nutrient content in highly polluted sediments, and (d) biodiversity of large macrofauna, which are often the most susceptible to local loss and have great capacity for bioturbation (Eckman et al., 1981; Hillman et al., 2020). These experiments were done in one of the most polluted urban estuaries in the world (Sydney Harbour, Australia, Birch, 2017).

2 | MATERIALS AND METHODS

2.1 | Study locations

Sydney Harbour, Australia, is an urban estuary with a history of sewage and industry pollution that resulted in a legacy of metallic and organic contamination in the sediments that remain affected by stormwater runoff and sewage overflows (Ahmed et al., 2020; Birch, 2017). In particular, Sydney Harbour sediments have levels of total nitrogen and total phosphorus exceeding Australian water quality guidelines for the protection of environmental value of estuaries (Birch et al., 2010). This harbour has suffered a pronounced loss of sediment functions and a suspected loss of sediment biodiversity due to human stressors, although there is no empirical evidence of the latter due to a lack of baseline studies (Johnston et al., 2015).

The experiments described below were conducted in sediments next to stormwater drains in three locations, Blackwattle Bay, Rozelle Bay and Rushcutters Bay (Figure 1). Each location receives stormwater outflows, and Rozelle and Blackwattle also currently receive sewage overflows during heavy rain. Sediments at all three locations have low dissolved oxygen (DO, less than 20%) and high levels of organic matter content (over 10%, Figure S1), as defined by Hyland et al. (2005).

2.2 | Assessment of bioturbator candidates for introduction in sediments ex-situ

We assessed the capacity of two species of native bioturbators to survive, oxygenate sediments and process nutrients in polluted sediments from two locations using mesocosms. The polychaete worm *Diopatra aciculata* and clams *Katelysia* sp. were selected based on the following criteria: (i) native species (historical records placed them in Sydney Harbour up to 1970, Atlas of Living Australia 2021, www.ala.org.au), but no current populations are known in the area and no naturally occurring worms were observed during our field visits; and (ii) availability of individuals from aquaculture farms for sustainable introduction. *D. aciculata* builds vertically oriented tubes that project above the sediment surface if DO in water is low (Les Safarik, Aquabait farm, personal communication). The tubes are made of packed sediment lined with mucus and the worms live permanently in the tubes, protruding the front of their body to search for food (Pardo & Amaral, 2006). They are scavengers and predators, but they also eat detritus from the sediment surface (Les Safarik, Aquabait farm, personal communication). The genus *Katelysia* in South-East Australia contains three species that have variable intraspecific characteristics making species identification difficult (Roberts, 1981). They are shallow burrowing filter feeders that modify the top 5–7 cm of sediments (personal observation).

Both species were provided by Aquabait Pty. Ltd. aquaculture facility at Dora Creek, NSW, Australia. Worms were 5.8 ± 0.1 g, while clams were 32.3 ± 0.3 mm in shell length (measured over the longest axis). All animals were taken to the laboratory aquarium facilities at the University of Sydney and left to acclimate for 24 hr in tanks with recirculating seawater.

To test the survival of these species in sediments with different levels of nutrient pollution, sediments were collected from Rozelle Bay and Rushcutters Bay (Figure 1) in February 2020. In each bay, sediments were collected at three sites with different levels of nutrient pollution (low, medium and high) selected based on level of oxygenation by visual characteristics including sediment colour from light-brown (oxidised) to black (reduced), and sulphurous odour as indicative of anoxia (Simone & Grant, 2020). Later analyses confirmed that sediments classified as low, medium and high levels of pollution had increasing levels of organic matter (Table S1). All sediments were collected in the shallow subtidal, at a depth of 0.2–0.5 m at low tide. In the laboratory, sediments were frozen at −20°C for 48 hr to kill any resident infauna. Sediments (~500–700 ml) were then added to 78 containers (13 per sediment type) and placed in the aquarium with recirculating seawater filtered using 50-µm sand and aeration. DO on the sediment surface was recorded in each container using a Eutech PD650 probe (Thermo Scientific). A 15 ml sediment sample (time 0) was scooped from each replicate and stored at −20°C for determination of organic matter content and C:N ratios. Once sediments settled, bioturbators (30 worms and 30 clams) were added to 60 replicates (split across the sediment treatments, with five

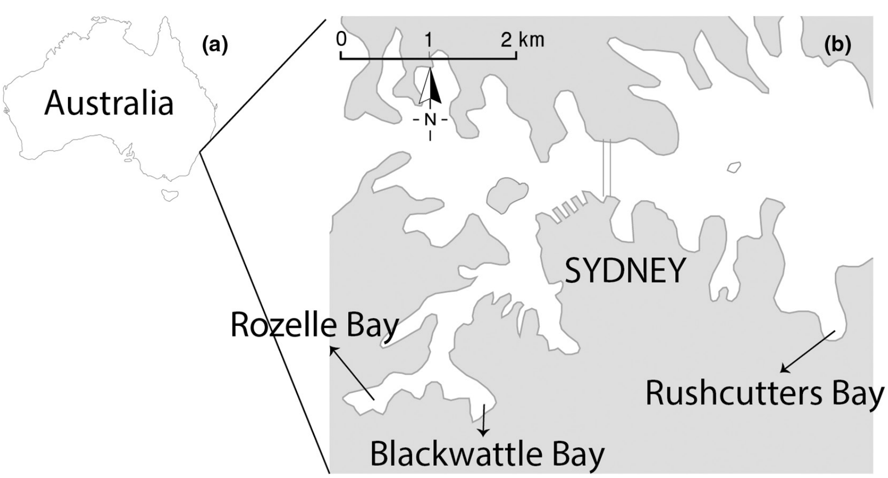

FIGURE 1 Map locating Sydney within Australia (A), and the three bays sampled (B).

replicates per treatment), leaving three replicates per sediment type as controls without bioturbator additions. The experiment ran for 23 days with no alternative sources of food other than those provided by sediments and particles suspended in the water. Water temperature was kept at 20–23°C and the salinity of the water was maintained at 37 ± 1.

After 23 days (time 1), DO was measured on surface and deep sediments by pushing the probe 3 cm into the sediments. The width of the worm tubes was measured as these are indicative of worm size (Les Safarik, Aquabait farm, personal communication), and sediment samples were collected from the surface (top 2 cm) and the deeper sediments (2–6 cm). Clams were collected and frozen. Wet weight and dry weight (after 60°C for 48 hr) were measured and wet and dry condition indices were calculated as tissue weight/shell weight (Filgueira et al., 2013).

All sediment samples were analysed for organic matter content using the loss on ignition technique (48 hr at 60°C and 4 hr at 550°C, Heiri et al., 2001). Additionally, C:N ratios were assessed using VarioMACRO Elementar CNS analyser.

The effects of location and levels of nutrient pollution on animal health and the effects of animals on DO, organic matter content and C:N ratios were tested with generalised linear models using the package GLMMTMB (Brooks et al., 2017) in R 4.0.2. Models included level of nutrient pollution (low, mid, high) as a fixed factor. All models also included location (Rushcutters and Rozelle) as fixed factor to investigate differences between locations due to the receiving environment (Figure S1). Moreover, generalised mixed models require more than five levels to reliably calculate variance among levels, and our experiment was done at two locations (Gelman & Hill, 2006; Harrison, 2015). Sediment volume in each container was included as a random covariable. When evaluating the effects of animals on DO, C:N and organic matter, animal ('worm', 'clam' or 'control') was also included as a fixed factor. Models testing the effects of animals on C:N and organic matter also included organic matter or C:N at time zero (T0, before the experiment) as a random covariable to account for variations between replicates. Assumptions were tested using package DHARMA (Hartig, 2021) and post-hoc comparisons were computed using the package EMMEANS (Lenth et al., 2018). Significant linear trends were plotted using the package GGEFFECTS (Lüdecke, 2018).

2.3 | Introducing native worms to polluted marine sediments in-situ

Based on the results from the previous experiment, *D. aciculata* was chosen as a target organism for introduction in polluted sediments in the field. The experiment was done at three bays in sediments at depths from 0.2 to 1 m and within 100 m radius from stormwater discharge points. Rozelle Bay and Rushcutters Bay sites were the same sites with high polluted sediments used for the laboratory experiment, and Blackwattle Bay had similar characteristics (Figure S1). This work was carried out under the scientific collection permit P03/0029-5.1 and animal translocation permit P52/1920 (Department of Primary Industries of NSW).

At each location, 21 cages were buried 20 cm into the sediment in July 2020. Cages were 25 cm in diameter, 30 cm high and made using 2 mm mesh on the side and bottom and 2 cm mesh as a lid (used only to avoid fish predation, see Figure S2). Cages were separated by a minimum of 3 m and filled with sediments from the respective locations. Two months after cage deployment in September 2020, worms were introduced at two densities (10 and 30 worms) resulting in three treatments: (a) lower worm density; (b) higher worm density and (c) control with no worms, with seven replicate cages per treatment and location (Figure S2). Densities used in the experiment were equivalent or lower than the densities of worms stocked in the farm as no information is available about their densities in natural systems. Total wet weight of worms was measured for each replate at the beginning of the experiment. The worms were deployed within 48 hr of collection from the farm.

After 11 weeks in the field in December 2020, DO on the sediment surface of each cage was assessed as described above. Following this, sediments to a depth of 10 cm were sampled using three cores (3-cm diameter) taken from each replicate. Finally, all the sediment remaining in the cages was sieved using a 0.5 cm sieve to sample large macrofauna (including the worms *D. aciculata*). The large sieve size allowed us to sort through large volumes of sediments in the field, necessary to capture large macrofauna that usually occurs at low densities and/or in deeper sediments. All samples were placed in coolers with ice for transport to the laboratory, where they were frozen at −20°C.

Macrofauna were identified to species, counted and weighed. Sediment cores were split in 0–2, 2–5 and 5+ cm and the depth fractions obtained from the three replicate cores were combined. From these samples, total organic carbon and C:N ratios were assessed as above.

Given the number and weight of worms changed by the end of the experiment (Figure 5), we tested for the effects of worms on sediment characteristics and biodiversity using worm quantities a continuous instead of a factorial predictor. Number and weight of worms were highly correlated (Pearson correlation coefficient = 0.95, $p < 0.0001$), hence only analyses using weight are reported. The effects of weight of worms at the end of the experiment on DO, organic matter content, C:N ratios and biodiversity were tested using generalised linear models, including location (Blackwattle, Rushcutters and Rozelle) as a fixed factor as described above. When evaluating the effects of worms and clams on sediment characteristics (C:N and organic matter), sediment depth (0–2, 2–5 and >5 cm) was also included as a fixed factor. Data points from six replicates with organic matter content over 56%, including two replicates with C:N ratios over 60%, covering a range of 0 to 100 g of worms from Rushcutters were classified as outliers with a Rosner test using ENVSTATS package in R 4.0.2 (Millard, 2013). The inclusion of these values in the models compromised conversion of models and the fulfilment of assumptions of distribution of residuals and homoscedasticity. Hence, six and two replicates were excluded from models for organic content and C:N ratios respectively.

3 | RESULTS

3.1 | Animal survival and condition ex-situ

All animals survived until the end of the experiment. The condition index (CI) of clams and the width of tubes, indicative of worm size, did not change with level of nutrient pollution or location (Table S2), but a tendency to increase with level of nutrient pollution was observed for tube width at both locations and clam CI at Rozelle (Figure 2).

3.2 | Effects of bioturbators on sediment oxygenation and nutrient content ex-situ

Worms increased DO in deep sediments with mid-levels of nutrient pollution from Rozelle, but did not affect surface DO (Figure 3, Table S3). In contrast, clams decreased DO on the sediment surface at all levels of nutrient pollution from Rozelle (Figure 3a, Table S3), and at high levels of nutrient pollution from both locations and mid-levels from Rushcutters (Figure 3b, Table S3).

For organic matter, there was a significant interaction between location and nutrient pollution levels (Figure 4a,b, Table S4). At high levels of nutrient pollution in Rozelle, surface and deep sediments with clams had lower organic matter content in comparison to controls without clams. In contrast, at medium levels of nutrient pollution in Rozelle and at high levels in Rushcutters, clams increased organic matter content in deep and surface sediments, in comparison to controls. Worms decreased organic matter contents in surface sediments at high levels of nutrient pollution in Rozelle. C:N ratios in surface sediments were affected by the presence of animals, but post-hoc comparisons found only marginal increases ($p < 0.1$) in C:N ratios when worms were present (Figure 4c,d, Table S4).

3.3 | Worm survival in-situ

Number and weight of worms decreased slightly at the end of the experiment, except for control and worm treatments in Rushcutters Bay (Figure 5a,b), which showed a slight increase in the number of worms.

3.4 | Effects of worms on sediment oxygenation and nutrient content in-situ

Worms had a positive effect on DO at all three locations (Figure 6, Table S5). Moreover, as worm weight increased, organic matter content decreased in all sediment depths at Rushcutters Bay (Figure 7a, Table S6). No significant effects were observed at other locations. The effects of worms on C:N ratios were more variable, with worms increasing nitrogen content in surface sediments at Rozelle and sediments from 2 to 5 cm depth at Rushcutters, while reducing nitrogen content in deep sediments (>5 cm) at Rushcutters (Figure 7b, Table S6).

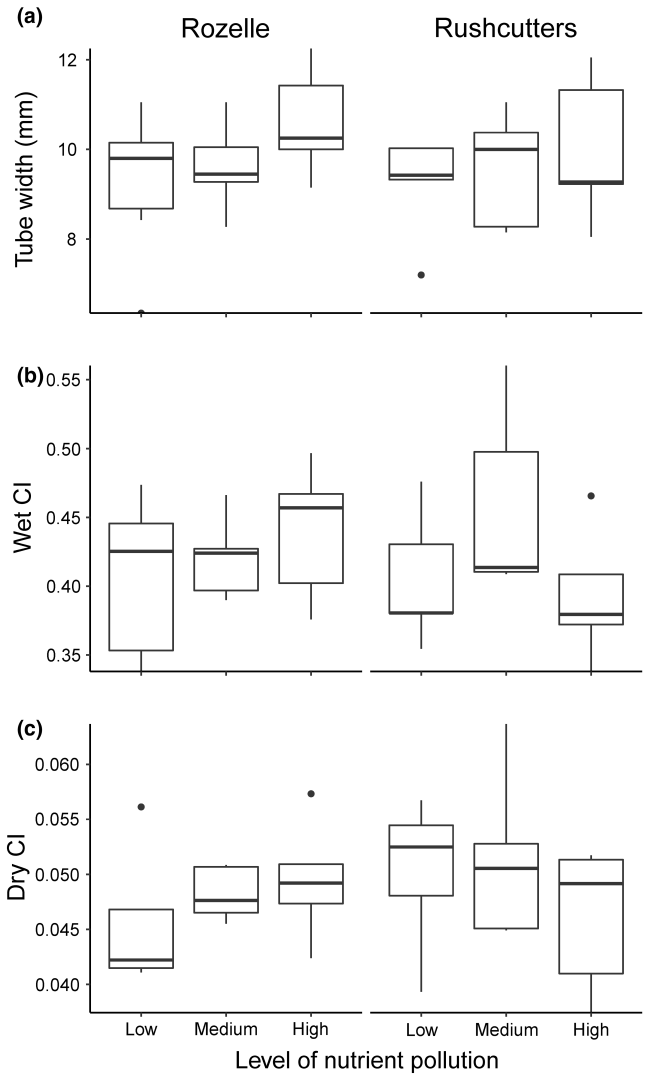

FIGURE 2 Widths of tubes of worms *Diopatra aciculata* and wet and dry condition index (CI) of clams *Katelysia* sp. at the end of the laboratory experiment (23 days), where animals were introduced to sediments from two locations in Sydney Harbour, Rozelle Bay and Rushcutters Bay, presenting increasing levels of nutrient pollution.

3.5 | Effects of worms on biodiversity

A total of 15 species were found in the cages at the end of the experiment, with eight species found at Blackwattle, 12 at Rozelle and 10 at Rushcutters. This includes one species of fish, three species of crabs, two species of shrimps, two species of polychaete worms (other than *D. aciculata*), one species of gastropod and seven species of bivalves. The number of taxa and number of individuals increased with weight of *D. aciculata* at Rushcutters Bay (Figure 8a,b, Table S7).

There were five common species, split across two groups according to the location they were found. The cockles *Tellina deltoidalis* and *Soletellina alba* and the crab *Amarinus laevis* were common

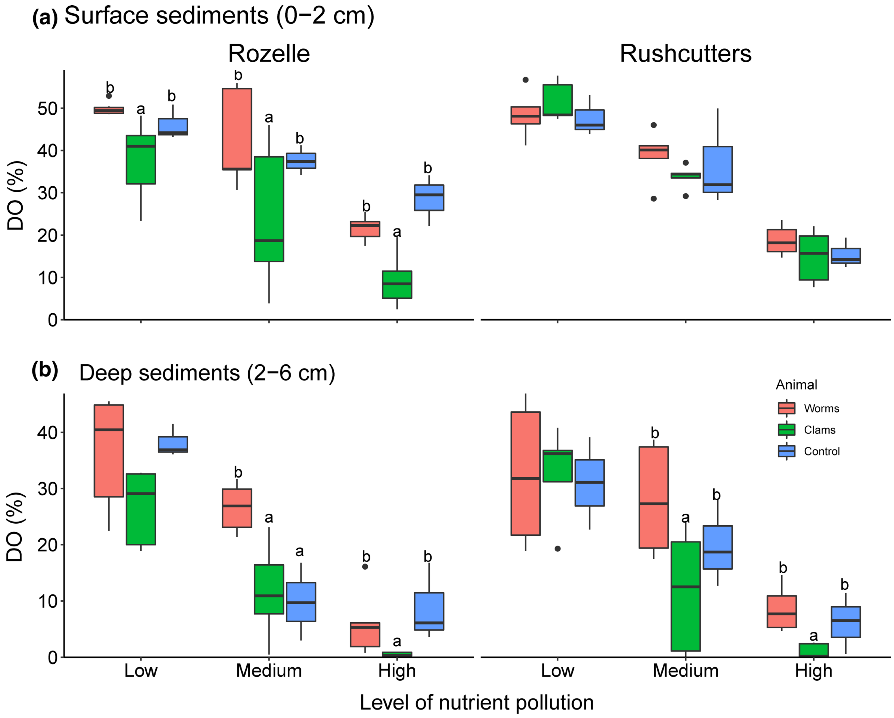

FIGURE 3 Dissolved oxygen (DO) at surface (A) and deep sediments (B) the end of the laboratory experiment (23 days), where clams *Katelysia* sp. and worms *Diopatra aciculata* were introduced to sediments (control treatment without animal) from two locations in Sydney Harbour, Rozelle Bay and Rushcutters Bay, presenting increasing levels of nutrient pollution. Letters represent significant differences between animal treatments (Table S3).

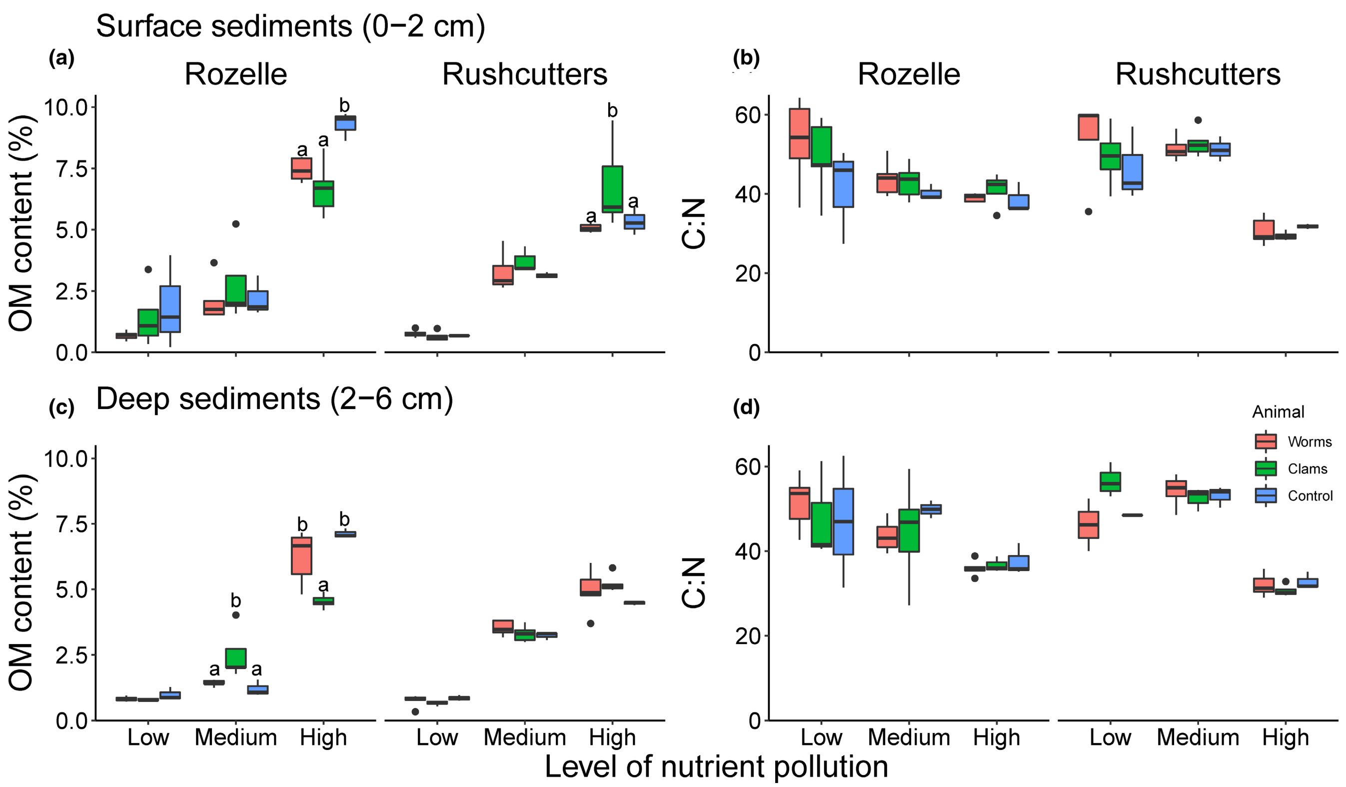

FIGURE 4 Organic matter (OM) content (left) and carbon: Nitrogen (C:N) ratios (right) in surface (top) and deep sediments (bottom) at the end of the laboratory experiment (23 days), where clams *Katelysia* sp. and worms *Diopatra aciculata* were introduced to sediments (control treatment without animal) from two locations in Sydney Harbour, Rozelle Bay and Rushcutters Bay, presenting increasing levels of nutrient pollution. Letters represent significant differences between animal treatments (Table S4).

in Rozelle and Blackwattle Bay (more anoxic, Figure 9a–c). The crabs *Paragrapsus laevis* and *Pilumnopeus serratifrons* were common in Rushcutters Bay (less anoxic, Figure 9c,d). While biomass of *T. deltoides* and *A. laevis* decreased with increasing weight of *D. aciculata* at all sites (Figure 9a–c, Table S8), *P. laevis* and *P. serratifrons* increased with weight of *D. aciculata* (Figure 9d,e, Table S8).

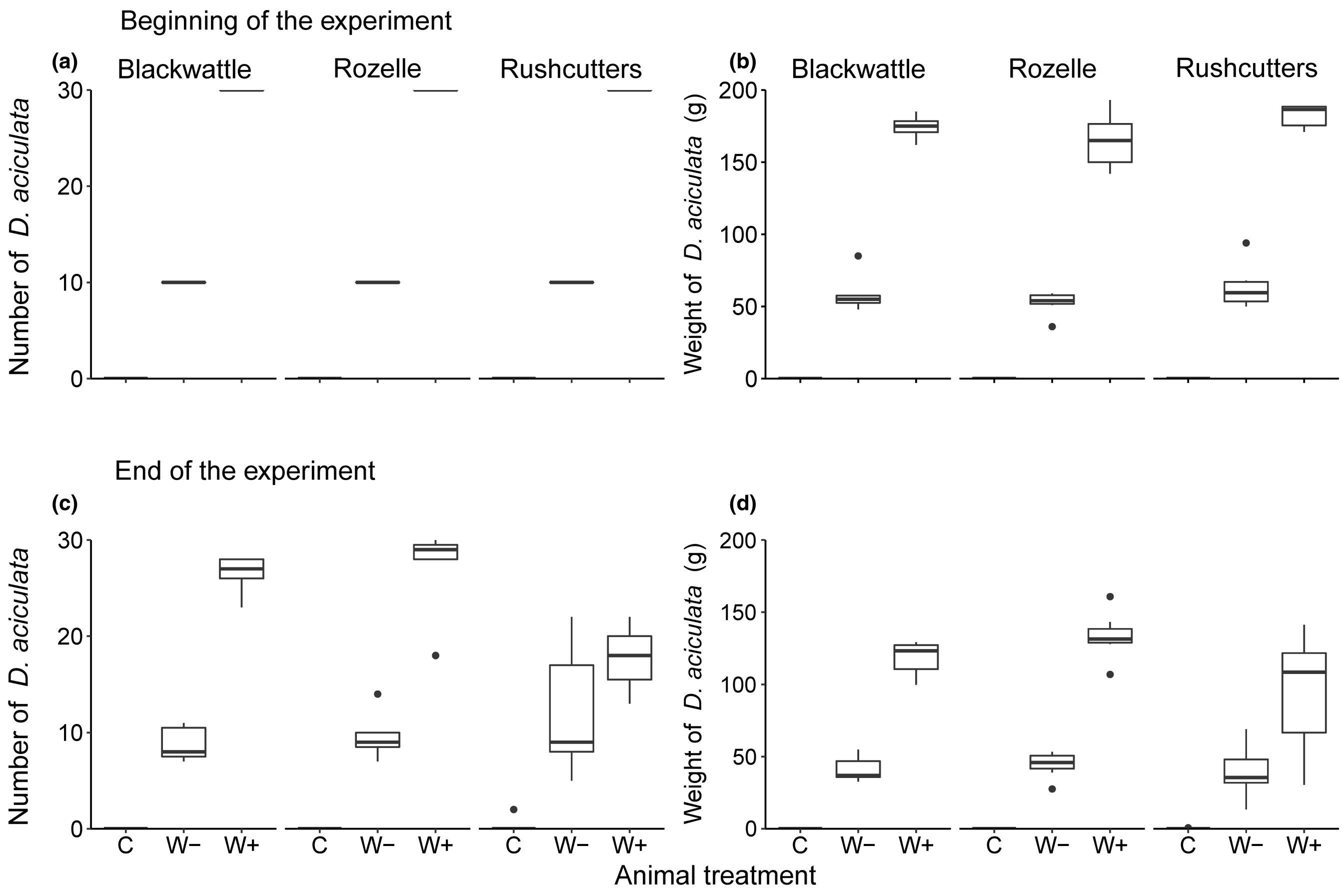

FIGURE 5 Total number (A and C) and weight (B and D) of worms *Diopatra aciculata* in each treatment (C = control with no worms, W− = worms in low densities; W+ = worms in high densities) at the beginning (day 0, A and B) and end (week 11, C and D) of the experiment at Blackwattle, Rozelle and Rushcutters Bays.

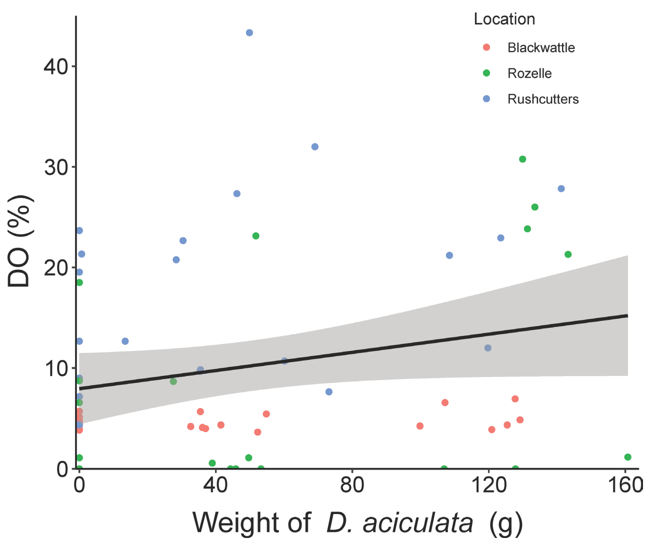

FIGURE 6 Dissolved oxygen (DO) versus weight of worms *Diopatra aciculata* at Blackwattle, Rozelle and Rushcutters Bays at the end of the experiment (week 11). A trend line is presented for all locations as no significant interaction between DO and location was found.

4 | DISCUSSION

4.1 | Successful introduction of ecosystem engineers in polluted sediments

In degraded coastal systems, ecosystem functions have not recovered despite reduced pollution from urban and industrial waste (Duarte & Krause-Jensen, 2018). Here, we demonstrated that this reduction in stressors provides a great opportunity to aid the recovery of biodiversity and functions and the remediation of nutrient pollutants. We introduced a key bioturbator species to one of the most polluted marine sediments in the world (Birch, 2017), with high survival and no observed negative effects on their growth/health. Moreover, we observed recruitment in one of the locations in Sydney Harbour as evidenced by the presence of individuals smaller than those introduced, and individuals in the control treatments where no worms were initially introduced. These results showed that it is possible to reintroduce native species into polluted urban sediments and kick-start recovery of biodiversity and functions (e.g. Borja et al., 2010; Duarte & Krause-Jensen, 2018).

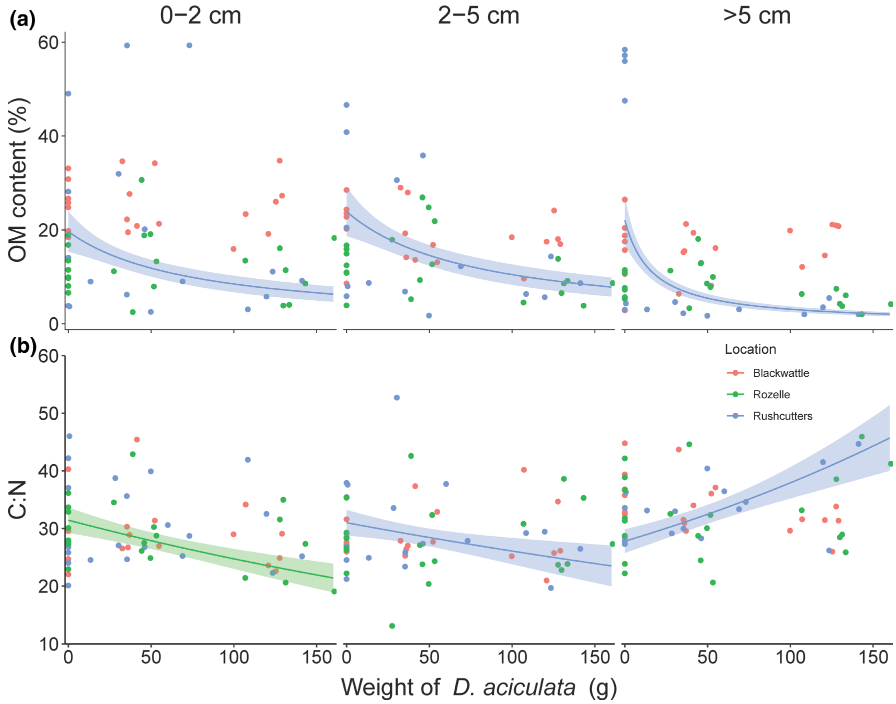

FIGURE 7 Organic matter (OM) content (top) and carbon: Nitrogen (C:N) ratios (bottom) versus weight of worms *Diopatra aciculata* at 0–2, 2–5 and >5 cm sediment depths at Blackwattle, Rozelle and Rushcutters Bays at the end of the experiment (week 11). Linear trends show significant relationships per site (Table S6).

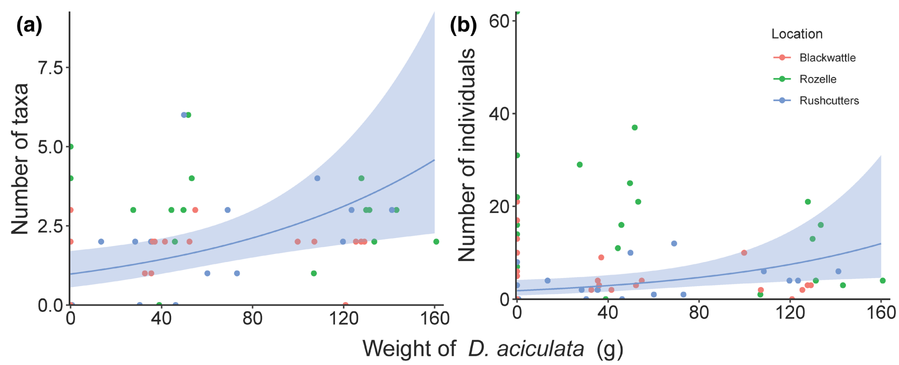

FIGURE 8 Number (A) and total abundance (B) of taxa (excluding *Diopatra aciculata*) versus weight of *D. aciculata* (g) at Blackwattle, Rozelle and Rushcutters Bays at the end of the experiment (week 11). Linear trends show significant relationships per site (Table S7).

We used full cages to test the survival of bioturbators in polluted areas exposed to stormwater discharge, without confounding effects of predation. Predators may influence bioturbation rates via direct consumption of bioturbators, or by altering their behaviour via predatory avoidance (e.g. retraction into the sediment), potentially decreasing bioturbators' effects on sediment characteristics (Stief & Hölker, 2006). Thus, an important avenue of future research will be determining how predator communities interact with bioturbators to influence their net effect on ecosystem functioning and ecosystem recovery from stress.

4.2 | Introducing below-ground ecosystem engineers can influence function

Bioturbators play a key role in the exchange of oxygen and nutrients between sediments and the water column, thus their activities can assist in the remediation of nutrient polluted sediments. Our combined ex-situ and in-situ experiments showed that adding tube-building worms to polluted sediments increases oxygenation and can decrease organic matter content, and that the magnitude of these effects is directly related to their density. Worms might have driven oxygenation and declines in organic matter directly or indirectly, via the observed increases in biodiversity at this location. Either way, these changes can be due to increased remineralisation rates and/or increased animal consumption of detritus. These results are similar to those of another study where a deposit-feeding polychaete worm was found to increase oxygenation and denitrification rates in sediments ex-situ, suggesting potential for nutrient remediation (Bosch et al., 2015). Moreover, we observed a stronger effect of worms in-situ than ex-situ, with organic content reduced up to 50% in-situ accompanied by increases in biodiversity at the location with medium levels of pollution. These results are similar to those reported by Eckman et al. (1981), who found increasing stabilising effects of worms relative to their densities and hypothesised that worms affect sediments through interactions with other animals and micro-organisms present in the field. In

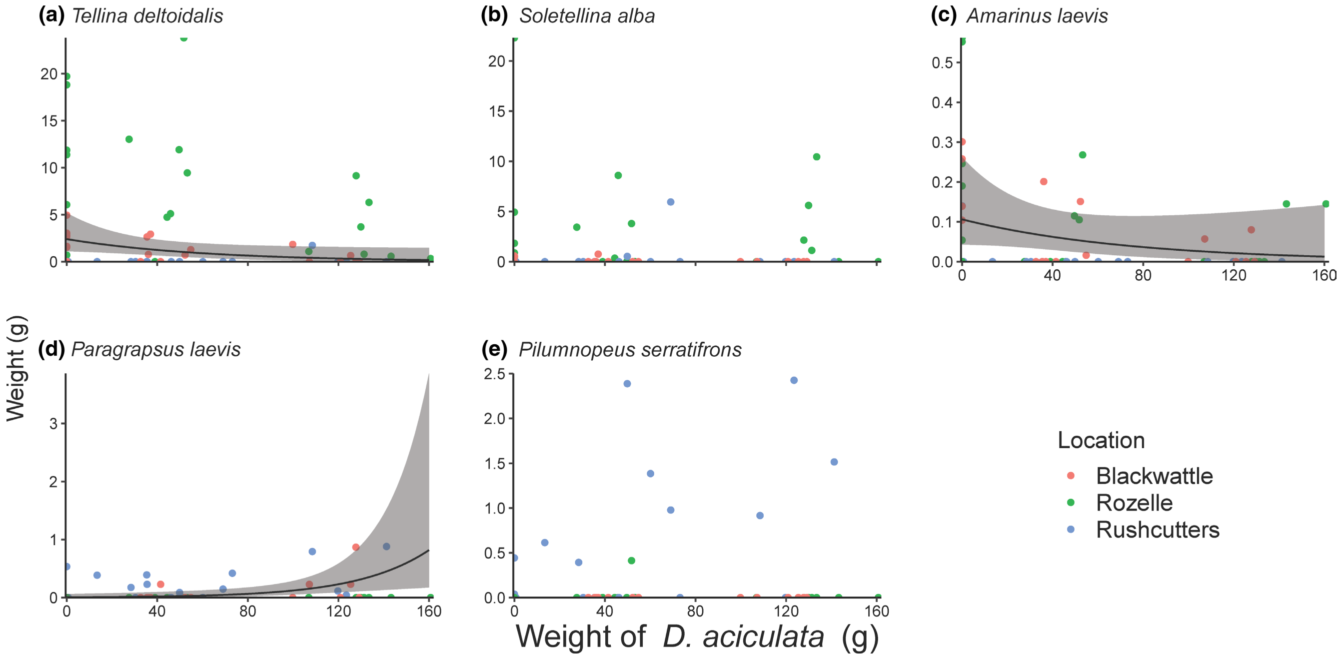

FIGURE 9 Weight of cockles *Tellina deltoidalis* (A) and *Soletellina alba* (B) and crabs *Amarinus laevis* (C), *Paragrapsus laevis* (D) and *Pilumnopeus serratifrons* (E) versus weight of *Diopatra aciculata* at Blackwattle, Rozelle and Rushcutters Bays at the end of the experiment (week 11). Trend lines are shown for those species that had a significant relationship (Table S8). One trend line is shown for each species for all locations as no significant interactions with location were found.

the more polluted locations in our study, effects of worms on biodiversity and organic matter content were not observed within the timeframe of the experiments, but may occur at longer timescales as the greater oxygenation driven by worms indicates potential for increased nutrient remediation at all locations. The effect of worms on C:N ratios was variable between locations and sediment depths, indicating differential reworking of sediments from 0 to 10 cm deep.

Idiosyncratic effects of bioturbating species on their capacity to promote functions are well recorded (Volkenborn et al., 2009). Our ex-situ experiment showed inter- and intra-species variability on the effects of bioturbators on oxygenation and nutrient content in sediments. In contrast to the effects of worms described above, clams, a highly mobile species that forms temporary burrows, had large but varied effects on oxygenation and organic matter, and sometimes reduced oxygenation and drove increases in organic matter ex-situ. These variable results were possibly due to the variability in behaviour observed during the experiment, with some individuals spending most of their time fully buried, while others spent most of the time on the sediment surface. Furthermore, their capacity to capture nutrients from the water column and deposit them as faeces and pseudofaeces may have added nutrients to sediments. These results emphasise the findings of previous studies. For example, the clam *Scrobicularia plana* decreased the depth of the aerobic sediment layer and negatively affected dominant macrofaunal species in mudflats (Clare et al., 2016). Hence, the clam does not seem to be a good candidate bioturbator for interventions aiming to recover sediment biodiversity and functions.

4.3 | Increases in biodiversity follow the introduction of an ecosystem engineer

Worms drove changes in the composition of below-ground assemblages at all locations. The crabs *P. laevis* were more common in replicates with more worms, showing facilitation between these species. In particular, *P. laevis* is known to occupy burrows created by other animals (Warren, 1990), hence they might be using worm tubes as habitat. In contrast, the deposit-feeding cockle *T. deltoidalis* and omnivorous crab *A. laevis* were more abundant in replicates with no worms or low biomass of worms. These species, common in the most polluted locations (Blackwattle and Rozelle), might be competitively excluded by the worms themselves or the crabs *P. laevis*. Our results hence indicate idiosyncratic effects of facilitation and competition by bioturbating species, as has been previously observed (Van Colen et al., 2008; Volkenborn et al., 2009). Interestingly, however, plots with lower biomass of *D. aciculata* and greater biomass of *T. deltoidalis* and *A. laevis* had lower sediment oxygenation than plots with higher biomass of *D. aciculata* and lower biomass of *T. deltoidalis* and *A. laevis*, indicating that the naturally occurring *T. deltoidalis* and *A. laevis* are not capable of driving the remediation of nutrient pollution in these locations. Hence, the introduction of *D. aciculata* seems to drive a shift in communities that might facilitate remediation at all sites.

After only 11 weeks in the field, worms increased the biodiversity of large macrofauna at the less polluted site. The lack biodiversity effects observed at Rozelle and Blackwattle might be due to these areas being highly devoid of species common of more aerobic sediments. Hence, the restoration of biodiversity in these locations might take longer, as propagules need to be transported from surrounding areas. Overall, these results indicate that the introduction of one keystone species can shift communities and kick-start the recovery of sediment biodiversity in polluted sediments, but studies done at larger scales are needed to understand their effects in highly polluted areas. In addition, this study focussed on changes in large macrofauna, as they are the most susceptible to local loss and perform significant bioturbation (Eckman et al., 1981; Hillman et al., 2020). Previous studies assessing the effects of below-ground ecosystem engineers on smaller macrofauna, meiobenthos and microbes have either found no effects on community composition, or significant spatial and temporal variation (e.g. Lei et al., 2010; Volkenborn & Reise, 2007). Hence, while zooremediation with bioturbators has the potential to impact the broader sediment biodiversity, future studies could directly assess meio- and microbiota at relevant spatio-temporal scales that directly link to bioturbator activities.

4.4 | Management implications

As human populations living in urban areas grow (Zhang, 2016), this study proposes novel directions for the restoration of habitats in urban settings, essential to warrant the important ecosystem services natural ecosystems provide. Our results indicated that the capacity of filter-feeding bioturbators to deposit organics might, in some situations, exceed their capacity to promote nutrient cycling, driving further nutrient pollution and hence might not be good candidates for introduction to highly polluted sediments. However, tube-building bioturbators can stimulate nutrient processing in sediments, proving multiple functional outcomes. They can kick-start the recovery of sediment functions via oxygenation, leading to reductions in nutrient pollution. Moreover, they can drive increases in biodiversity. The introduction of stabilising bioturbators can also displace species with low capacity to oxygenate sediments (such as the cockles and crabs displaced in this study). This can drive long-term changes in sediment communities, even if self-sustaining populations of the introduced bioturbator are not achieved. If self-sustaining populations of the introduced bioturbator are achieved, the return-for-investment can be significant as localised interventions can have effects at large spatial scales via natural expansion to surrounding areas. This is particularly relevant in urban areas, where unvegetated sediments are conspicuous due to the loss of above-ground biomass (Dafforn et al., 2013). Large-scale temporal and spatial experiments are therefore needed to inform models assessing the potential of these interventions to enhance functions at ecosystem scales.

Moreover, our results highlight that ecological restoration should not be limited to above-ground habitats. Particularly in urban ecosystems, ecological restoration of above-ground habitats is often limited by competing interests for the use of space (Kabisch et al., 2016). For example, seagrass is greatly affected by boating activities (Bishop, 2008), while mangrove conservation and restoration can be hindered as they can block water views (Hutchings & Recher, 2018). Below-ground biodiversity does not restrict human activities in the same way, hence do not pose the same social and political challenges and can therefore be more easily applied in urban settings. The results of this study are also relevant for urban soils, where restoration strategies focus on soil physical and chemical conditioning and removing or adding plant species (Pavao-Zuckerman, 2008). The use of bioturbators can be a cost-effective way of driving soil biodiversity and functions, increasing porosity and the processing of contaminants (Bray & Wickings, 2019).

Finally, it is important to note that the differences we observed between locations suggest the outcomes of zooremediation strategies depend not only on the species used, but also on the receiving environment. This is supported by the site specificity observed in the outcomes of other forms of ecological restoration strategies (Brudvig et al., 2017). Hence, to reliably predict the outcomes of restoration, it is important to focus on both 'average' outcomes and their variability to assess environmental factors affecting restoration success. In this study, recovery of biodiversity and functions was achieved in a location with medium levels of degradation, suggesting that these areas could be prioritised when utilising this approach. Future studies should scale-up tests to assess if these site differences are maintained at larger temporal scales, and if bioturbators introduced in less degraded areas eventually disperse into more degraded areas, driving their recovery.

AUTHORS' CONTRIBUTIONS

A.B.B., P.E.G., W.A.O., R.A.C. and K.A.D. conceived the ideas and participated in the designed methodology; A.B.B. and K.E. collected the data; A.B.B. analysed the data and led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

ACKNOWLEDGEMENTS

This research was supported by the Maple Brown Foundation, the Ian Potter Foundation and the City of Sydney Innovation Grant. This is SIMS publication #290. Open access publishing facilitated by The University of Sydney, as part of the Wiley - The University of Sydney agreement via the Council of Australian University Librarians.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

DATA AVAILABILITY STATEMENT

Data available via the Dryad Digital Repository https://doi.org/10.5061/dryad.vx0k6djv5 (Bugnot et al., 2022).

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How to cite this article: Bugnot, A. B., Gribben, P. E., O'Connor, W. A., Erickson, K., Coleman, R. A., & Dafforn, K. A. (2022). Below-ground ecosystem engineers enhance biodiversity and function in a polluted ecosystem. *Journal of Applied Ecology*, *59*, 2094–2105. https://doi.org/10.1111/1365-2664.14221