Chronically stressed benthic macroinvertebrate communities exhibit limited effects on ecosystem function in a microtidal estuary

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ABSTRACT: Anthropogenically driven alterations to coastal sediments and their benthic macroinvertebrate communities impair ecosystem function. However, this paradigm is yet to be tested in ecosystems that typically harbour underdeveloped communities lacking larger bioturbating species. Here, we investigated the effects of sediment condition and macroinvertebrate communities on benthic metabolism, nutrient exchange and denitrification (N_2 production), and assessed the relative importance of taxon richness, abundance, biomass and community bioturbation potential in influencing these processes in 2 regions of the highly modified, microtidal Peel-Harvey Estuary in temperate Western Australia. Sediment condition influenced benthic metabolism more than the macroinvertebrate community, whereas the reverse was true for nutrient exchange. Denitrification was driven by sediment condition and the community in the upper and lower estuary, respectively, highlighting the change in controls of this nitrogen-removal process within estuaries. Overall, benthic macroinvertebrates had little to no effect on many ecosystem processes, exhibiting the limited functional role played by these chronically stressed biota in this estuary. There was also no interaction between sediment condition and the community, suggesting a functional decoupling between these 2 ecosystem components. Where significant macroinvertebrate effects were detected, community biomass was the most frequently selected predictor, demonstrating its fundamental role in ecosystem function. This study reveals pressing implications of what might be expected when benthic environments become particularly degraded and the highly limited potential of the resultant benthic macroinvertebrate communities to provide key ecosystem services such as nutrient processing.

KEY WORDS: Sediment quality · Macrofauna · Anthropogenic impact · Natural stress · Sediment–water interface · Benthic metabolism · Nutrient cycling · Denitrification

1. INTRODUCTION

Ecosystem function is the combination of processes that control the transfer of energy and organic matter within and among ecosystems (Costanza & Mageau 1999, Bernhardt et al. 2018). The dynamic physico-chemical conditions in estuaries and allochthonous inputs of inorganic and organic nutrients fuel a wide

variety of ecosystem processes (Thottathil et al. 2008). With increasing anthropogenic stress in estuaries, more favourable ecosystem processes such as aerobic respiration are expected to diminish as sediment condition declines and benthic macroinvertebrate (≥500 µm in size) communities become impoverished (Norkko et al. 2013, Douglas et al. 2018). However, predicting ecosystem function in stressed coastal ecosystems is complicated by the numerous feedbacks occurring between benthic communities and sediment condition.

Declines in estuarine sediment condition typically occur from anthropogenic nutrient inputs (e.g. wastewater discharge), which stimulate excessive algal production of organic matter (i.e. eutrophication) that is deposited on the sediment (Eyre & Ferguson 2002, Hallett et al. 2019). Eutrophication alters ecosystem function as sediments become heterotrophic when oxygen (O_2) consumption (respiration) exceeds production (photosynthesis) due to the decomposition of excess organic matter (Eyre et al. 2011), with these sediments supporting anaerobic sulphate (SO_4^{2-}) reduction and methane production (Hallett et al. 2019). Anoxic, eutrophic sediments release ammonium (NH_4^+) and phosphate (PO_4^{3-}) to the water, which can cause algal blooms (Ferguson et al. 2004, Kraal et al. 2013) and accelerate a positive feedback loop through enhanced supply of organic matter that further enhances benthic anaerobic processes (Braeckman et al. 2014). Denitrification is crucial in counteracting these feedbacks in eutrophic ecosystems, as it converts bioavailable nitrogen to inert dinitrogen (N_2) gas via the step-wise reduction of nitrate (NO_3^-; Seitzinger 1987). Despite eutrophication increasing denitrification via increased NO_3^- concentrations in the water column (Findlay et al. 2011), higher rates of denitrification take place in sediments with overlapping oxic–anoxic boundaries where NO_3^- is readily available after being produced by nitrifying microbes under oxic conditions (Eyre & Ferguson 2009, Braeckman et al. 2014). The oxic–anoxic sediment boundaries that maximise denitrification are a common feature of healthy estuarine sediments, partially due to the activity of their resident macroinvertebrate communities (Rosenberg et al. 2004).

Benthic macroinvertebrates directly influence ecosystem function at the sediment–water interface by altering sediment O_2 dynamics and microbial communities through their feeding, bioturbation and bioirrigation (Welsh 2003, Cronin-O'Reilly et al. 2018, Lam-Gordillo et al. 2022). Recent studies of whole communities have verified single-species experiments by showing that invertebrate biomass is a fundamental biological driver of ecosystem function, influencing rates of benthic metabolism, nutrient exchange and denitrification (Ferguson & Eyre 2013, Gammal et al. 2019). Thus, anthropogenic stress that causes species loss and reduces the size of benthic macroinvertebrates (e.g. Handley et al. 2020) is likely to impact ecosystem function (Lohrer et al. 2004, Belley & Snelgrove 2016, Karlson et al. 2021), particularly metabolic and nutrient fluxes (Norkko et al. 2013, Hillman et al. 2021).

Research to date has focussed on coastal systems with relatively well-developed benthic macroinvertebrate communities in terms of both diversity and biomass, which have then been experimentally simplified (e.g. Hillman et al. 2021, Karlson et al. 2021). It is therefore unclear how 'universal' the connections between benthic populations and ecosystem function are, and specifically whether these relationships still exist in ecosystems where species richness is limited and taxa are small-bodied (e.g. Pennifold & Davis 2001, Benstead et al. 2009, Urban-Malinga et al. 2013). Such communities typify those of Mediterranean lagoons (e.g. Munari & Mistri 2008), tidal creeks in the USA (e.g. Ritter et al. 2005) and microtidal estuaries of south-western Australia (e.g. <6 species on average per sample, 32 to 41 species total; Tweedley et al. 2012) where the apparent lack of larger, bioturbating individuals has been attributed to recurring natural (e.g. marked, rapid changes in salinity) and anthropogenic (e.g. hypoxia) stress restricting their survival and growth (Tweedley et al. 2016). Furthermore, the bioturbation influence may be reduced compared to macrotidal systems, as smaller taxa in these estuaries largely inhabit the upper 10 cm of sediments (Rose 1994). Understanding the functional effect that benthic macroinvertebrates provide in these systems can yield vital insights into their ecosystem function and what to expect if well-developed communities become impoverished with time. It is plausible that other measures (metrics) of benthic community structure may be more important than biomass in shaping ecosystem function under these scenarios.

To address this knowledge gap, our study aimed to (1) assess the effects of the benthic macroinvertebrate community and sediment condition on benthic metabolism, nutrient exchange and denitrification in the upper and lower regions of a microtidal estuary, and (2) where a community effect is present, investigate which community aspects (i.e. taxon richness, abundance, biomass or community bioturbation potential) best explain variation in the above

ecosystem process fluxes. We hypothesised that commonly observed benthic macroinvertebrate effects on ecosystem function depend on the presence of relatively large, bioturbating organisms, and thus will not extend to this microtidal estuary.

2. MATERIALS AND METHODS

2.1. Study site

The Peel-Harvey Estuary is a permanently open, microtidal (± 0.5 m tidal range) estuary (~136 km²; Brearley 2005) located approximately 80 km south of Perth, Western Australia. It has 2 entrance channels, the natural Mandurah Channel and the engineered Dawesville Channel, that allow tidal exchange with the Indian Ocean. It is fed by 3 rivers (Harvey, Murray and Serpentine) that drain from extensive subcatchments (9400 km²; Valesini et al. 2019) into 2 shallow (<2 m depth) estuarine basins; the Harvey Estuary and Peel Inlet. The estuary experiences a Mediterranean climate, with seasonal fluctuations in tidal intrusion, temperature and freshwater inflow (90% occurring between May and October) that induce periodic hypersalinity, salt wedge formation, stratification and hypoxia in parts of the system (Valesini et al. 2019). It is classified as extensively modified (Commonwealth of Australia 2002) and has a history of hyper-eutrophication, harmful algal blooms (*Nodularia spumigena*), excessive macroalgae growth, onshore accumulation of rotting algae and fish and crab kills (Brearley 2005). Despite mitigation efforts (e.g. opening of the Dawesville Channel), estuary sediments remain long-term nutrient reservoirs (Kraal et al. 2013, Hallett et al. 2019, Valesini et al. 2019). The estuary faces ongoing anthropogenic pressure from extensive agricultural and mining land-use in the catchment, an increasing regional population and fringing urbanisation, reduced riverine inflow from human-induced climate change and direct use (e.g. recreational shore fishing; Valesini et al. 2019).

2.2. Experimental design and sampling

The effects of the benthic macroinvertebrate community and sediment condition on ecosystem function (benthic metabolism, nutrient exchange and denitrification; Table 1) in the Peel-Harvey Estuary were assessed via a 2-factor experimental design. Sediment condition was characterised using the Rapid Assessment Protocol (RAP) developed for the Peel-Harvey Estuary, which utilises ordinal scoring of the qualitative characteristics of sediment colour, texture and odour to classify sediment condition as good, fair or poor, thereby providing a demonstrably effective proxy for sediment granulometry, oxic status and level of enrichment (Fig. 1; Hallett et al. 2019). Sites of

Table 1. Brief descriptions of the flux variables (dark, light and net) that were classified into 1 of 3 ecosystem processes (benthic metabolism, nutrient exchange or denitrification)

Flux variable	Ecosystem process	Description
Oxygen (O_2)	Benthic metabolism	Measure of benthic productivity (photosynthesis), consumption (aerobic respiration) and microbial oxidation (e.g. sulphide oxidation)
Dissolved inorganic carbon (DIC)	Benthic metabolism	Measure of benthic productivity (photosynthesis), consumption (aerobic respiration) and microbial reduction (e.g. sulphate reduction)
Alkalinity	Benthic metabolism	Estimate for calcium carbonate dissolution/sulphate reduction and calcium carbonate precipitation/sulphide oxidation
Phosphate (PO_4^{3-})	Nutrient exchange	Product of organic matter decomposition and the reduction of Fe(III) to Fe(II)
Ammonium (NH_4^+)	Nutrient exchange	Product of organic matter decomposition and dissimilative reduction of nitrate to ammonium (DNRA). Source for nitrification, anaerobic ammonium oxidation (anammox) and biological assimilation
Nitrate and nitrite (NO_x)	Nutrient exchange	Product of organic matter decomposition. Source for nitrification, DNRA, anammox, denitrification and biological assimilation
Dissolved organic matter (DON)	Nutrient exchange	Product of biological assimilation (e.g. amino acids) and excretion. Source for organic matter decomposition
Dinitrogen (N_2)	Denitrification	Net balance of denitrification (N_2 removal) and anammox minus nitrogen fixation

varying sediment condition classes were thus selected in each region using existing sediment condition maps (Hallett et al. 2019), with sampling conducted during spring (September–October 2018). The 2-factor design involved collecting 2 replicate cores for benthic macroinvertebrate community treatment (faunated vs. defaunated) at 4 replicate sites of 2 sediment condition classes (good vs. fair/poor) in the lower and upper estuary (n = 16 per estuarine region). The regions were selected to represent lower, marine (Harvey Estuary) and upper, brackish (Murray River) areas of the estuary. During sampling, a third core was collected to confirm the sediment condition using the RAP. For the lower estuary, 4 replicate sites of both good and fair sediment condition were sampled while 5 sites of poor sediment condition and 3 sites of good condition were

Fig. 1. Cores showing good (left) and fair (right) sediment condition from the Harvey Estuary, denoted with key characteristics (depth of oxic layer, sediment type, and hydrogen sulphide presence [H_2S]) and associated Rapid Assessment Protocol (RAP) colour, texture and odour scores, which determined their condition class (good, fair or poor; Hallett et al. 2019)

sampled in the upper estuary due to a change in their condition since the initial RAP mapping (Fig. 2).

All sites were in water depths of approximately 1 m to avoid depth-dependent effects (e.g. light attenuation). Samples were taken using Plexiglass cores
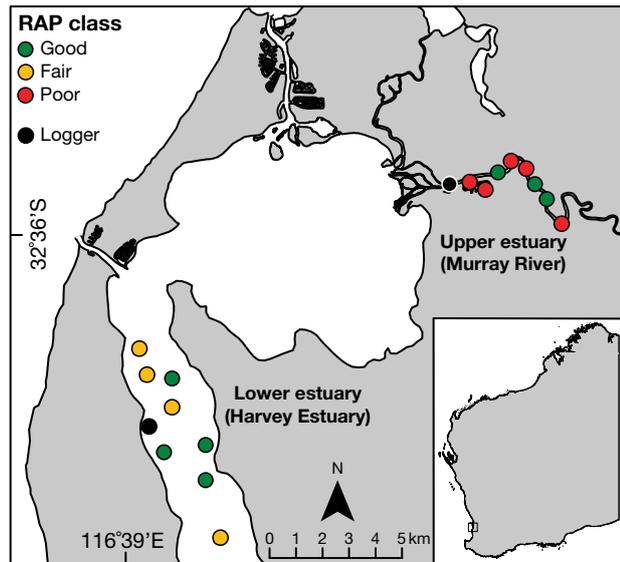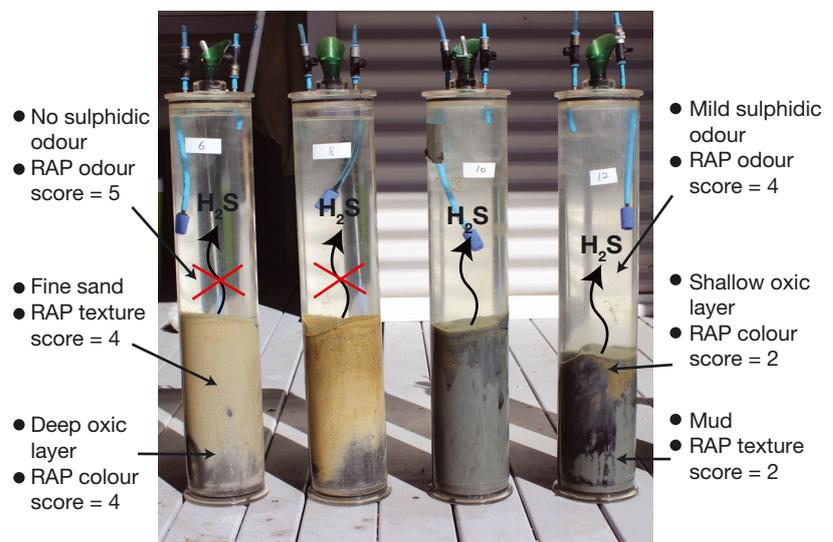

Fig. 2. Location of replicate sites of sediment condition classes (Rapid Assessment Protocol [RAP]: good, fair, poor) that were sampled and data loggers in the lower (Harvey Estuary) and upper (Murray River) regions of the Peel-Harvey Estuary during spring (September–October 2018). Inset shows the location of the estuary in Western Australia

(9.5 cm internal diameter, 50 cm length, 70.88 cm^2 surface area), aiming to collect minimally disturbed, intact sediments to a depth of 20 cm, allowing for 30 cm of overlying water (2.5 l). On core collection, total core and apparent redox potential discontinuity (aRPD, i.e. the transition zone between oxic and anoxic sediment boundaries; Gerwing et al. 2015) depth were recorded. Collected cores were stored upright and out of direct sunlight during transport to an off-site location. For incubation purposes (i.e. incubation tank water and sample replenishment water), 15 l of bottom water were pumped from each site and mixed with an additional 120 l collected at boat ramps close to site locations. Data loggers (Hydrolab HL4 and HOBO pendants) were deployed to monitor diurnal changes in pH, O_2, salinity, water temperature and light intensity over a 24 h period (1 h intervals) at one area in both regions of the estuary. These data were then used to define in-field ambient conditions during core incubation.

2.3. Defaunation by deoxygenation

Defaunation by deoxygenation (a modified version of Andersen & Kristensen 1988) was selected as the most appropriate method of macroinvertebrate removal for this study, as it was essential to maintain the natural sediment structure in cores and the distribution of solutes and organic carbon, which are inex-

tricably linked to sediment condition (Hallett et al. 2019). All cores were stored upright in a 150 l incubator or 60 l white plastic barrels kept at ambient water temperatures prior to incubation. Control cores (i.e. those with no benthic macroinvertebrates) were defaunated without presumably overly affecting meiofaunal and microbial communities (Hansen & Blackburn 1991) by flushing the overlying water in cores with N_2 gas to induce deoxygenation, prompting macrofaunal emergence in search of O_2 (Andersen & Kristensen 1988). Briefly, defaunation consisted of purging the cores with N_2 gas for 2 h, capping for 12 to 20 h 3 times (total of 52 h per core) and removing macroinvertebrates from the sediment surface by siphoning contents through a 500 μm sieve. The siphoning procedure was also performed on faunated cores to replicate the disturbance to the sediment surface; however, no sieve fraction was removed. A detailed evaluation of the defaunation showed the efficacy of this method to differ between estuarine regions (Cronin-O'Reilly et al. 2022, this volume). Therefore, fluxes were not compared between regions and partial defaunation was accounted for during data analysis.

2.4. Incubations and sample processing

Following defaunation, all cores were pre-incubated for 12 h to equalise hydrological conditions: cores were uncapped and placed in the incubator with site water maintained at *in situ* conditions (lower estuary: salinity ~30.6, temperature 18 ± 1.5°C; upper estuary: ~6.4, 16 ± 1°C) and O_2 maintained through continuous aeration (O_2 saturation >80%, reflecting infield O_2 concentrations). Incubations were carried out under natural light to maintain diel light fluctuations but modified to mimic previously established *in situ* regional conditions: shade cloths reduced light to the upper estuary cores (~906 lux) and artificial lights enhanced light to the lower estuary cores (~6700 lux). Magnetic stirrer bars were placed 8.5 cm above sediment surfaces in each core to replicate water movement (Ferguson et al. 2004). During pre-incubation, faunated cores were capped with 250 μm mesh coverings to prevent faunal movement among cores. Due to restricted water movement from the coverings, each covering had a 4 cm slit added to the centre to allow sufficient water exchange to maintain O_2 conditions.

Cores were sealed close to midnight (t0) with sampling conducted at t0 and at 3 h intervals thereafter over 12 h, so that 3 timepoints were in the night (t0–t2; dark) and 3 were in the day (t2–t4; light, with t2 being dawn). A 12 h incubation period was deemed sufficient given that other studies detecting community effects have incubated over shorter periods (e.g. Gammal et al. 2019). At each timepoint, water temperature, pH and O_2 were measured through a port hole in the core lids using a Thermo Scientific Orion Star A series pH probe and a Hach HQ30d LDO probe, respectively, and water samples were collected through a sample port. Sampling consisted of collecting (1) triplicate 7 ml samples in borosilicate glass vials for N_2/Ar analysis, which were used to assess net denitrification (Eyre et al. 2002) and (2) duplicate nutrient samples (10 ml), filtered (0.45 μm cellulose acetate; Minisart) to measure PO_4^{3-}, NH_4^+, nitrate and nitrite (NO_x) and dissolved organic nitrogen (DON). At 3 time points (t0, t2, t4), additional 60 ml crimp-top glass vials were filled with filtered (<0.45 μm) water and used to measure dissolved inorganic carbon (DIC) and alkalinity. At t1 and t3, alkalinity samples were collected in 40 ml plastic vials. Mercury (II) chloride (10 μl of a 10% solution) was added to N_2/Ar, DIC and alkalinity samples to inhibit microbial activity. Sampled water was passively replaced via a gravity bag filled with water from the appropriate region. Until laboratory processing, nutrient samples were frozen, DIC and alkalinity samples were refrigerated (4°C), and N_2/Ar samples were sealed, submerged under water and kept in a cool, dark area.

To characterise the benthic macroinvertebrate communities present, macroinvertebrates were collected by extruding the sediment after incubation and washing it over a 500 μm sieve, fixing it using a 10% formalin solution and storing it in 70% ethanol. Benthic macroinvertebrates were extracted, identified to the lowest practical taxonomic level and counted. Raw abundances of benthic macroinvertebrates (individuals per 70.88 cm^2) were converted to average densities per 0.1 m^2 to identify the most abundant taxa in each region and sediment condition class. Community and species-specific biomasses (g) were measured using blotted wet weights (±0.1 mg).

2.5. Analytical methods

Total DIC concentrations were measured using an automated infra-red inorganic carbon analyser (AIRICA) with a LICOR-7000 CO_2/H_2O gas analyser. Alkalinity concentrations were measured by an 888 Titrando with hydrochloric acid (0.01M) as

the dosing reagent. Certified reference materials (175 and 179) were run to assess the accuracy of the AIRICA and Titrando, with natural drift corrections made accordingly (Stoltenberg et al. 2021). Nutrients were measured using Lachat flow injection analysis with detection limits set at 2 µg l^{-1} for PO_4^{3-}, NH_4^+ and NO_x, and 10 µg l^{-1} for DON (McKee et al. 2000). Samples below detection limits were manually set to zero. N_2/Ar ratios were measured using membrane inlet mass spectrometry (Eyre et al. 2002).

2.6. Flux calculations and data analysis

Dark, light and net areal flux rates (µmol m^{-2} h^{-1}) across the sediment–water interface were calculated using linear regressions as a function of time, sediment surface area and the volume of overlying water, with corrections made for water replacement during sample collection (Ferguson et al. 2004). A benthic productivity/respiration (p/r) ratio was calculated using O_2 fluxes to determine metabolic state (autotrophic or heterotrophic) following determination of gross primary productivity (GPP) as follows (Eyre et al. 2011):

$$GPP = \text{Light } O_2 \text{ flux} - \text{Dark } O_2 \text{ flux} \quad (1)$$

$$p/r = (GPP \times \text{daylight hours})/(\text{dark } O_2 \text{ flux} \times 24) \quad (2)$$

If the p/r ratio is >1, the benthic environment is net autotrophic (i.e. producing more organic carbon via photosynthesis than it is respiring through metabolism), while a p/r ratio <1 indicates a net heterotrophic state (i.e. respiring more organic carbon than it is producing). A ratio of 1 indicates that all carbon autotrophically fixed is respired (Eyre & Ferguson 2002, Ferguson et al. 2004). Denitrification efficiency, the percentage of the total inorganic nitrogen flux released as N_2, was calculated as detailed by Eyre & Ferguson (2009).

Statistical analysis was carried out using PRIMER v7 software (Clarke & Gorley 2015) with a PERMANOVA+ add-on (Anderson et al. 2008). Two-way permutational analysis of variance (PERMANOVA; Anderson et al. 2008) tests were used to test for significant effects of sediment condition (good vs. fair in the lower estuary, good vs. poor in the upper estuary) and benthic macroinvertebrate communities (faunated vs. defaunated) on process fluxes. Given partial defaunation of control cores, potentially confounding effects of incomplete macroinvertebrate removal were controlled for by including unremoved biomass (g, untransformed) as a covariate in the

PERMANOVA models for each estuarine region (Cronin-O'Reilly et al. 2022). Thus, each PERMANOVA effectively became an ANCOVA that enabled the removal of variation explained by the quantitative covariate prior to testing for differences in fluxes due to categorical factors.

Prior to analysis, fluxes were checked for normality using pairwise scatter plots. A mild square-root transformation was required for dark and net NO_x fluxes, following addition of a constant to make all flux values positive, and a $\log_e$ transformation was required for light and net N_2 fluxes. To account for the covariate first, PERMANOVA model terms were fitted sequentially (Type 1 sum of squares), which leads the order-of-fit of factors to matter. Two-way (sediment condition × benthic macroinvertebrate community) PERMANOVA tests with 2 differing orders-of-fit of main effects (condition or community first) were thus carried out. Having tests with 2 orders-of-fit also allows information on the conditional effect of factors to be ascertained (e.g. whether they overlap in explained variation). Sediment condition and community treatment were fixed factors, and Euclidean distance matrices were created for each pre-treated flux. Effects were considered significant at p ≤ 0.05, with significant tests followed by a permutational analysis of multivariate dispersions (PERMDISP) test of dispersion differences among groups, which were all insignificant (Table S1 in the Supplement at www.int-res.com/articles/suppl/m701p001_supp.pdf). The relative importance of significant factors and interactions was evaluated by assessing the components of variation (COV) values. Tests of DIC and N_2 fluxes were imbalanced due to missing flux rates removed during quality assessment, but all main effect tests had 3 or more replicates.

Distance-based linear models (DISTLMs; Legendre & Anderson 1999) were used to identify which univariate metrics best explained any significant effects of the benthic macroinvertebrate community. The predictors considered were taxon richness (S), total abundance (N, ind. per 70.88 cm^2), community biomass (g) and the community bioturbation potential index (BP_c; Queirós et al. 2013), which combines species-specific biomass (g) and abundances with scores of mobility and sediment bioturbation type. These metrics were selected to provide insight into the relative importance of taxonomic composition, numbers, collective sizes, and burrowing activity of benthic macroinvertebrates on flux rates. Prior to data treatment, S and N were calculated from raw abundances per core. The BP_c was calculated as described (Queirós et al. 2013), with most taxa assigned directly to a listed mobility score (M_i) and sediment rework-

ing score (R_i) under the classification, given the taxa were listed or their mobility and sediment reworking traits remained constant at a higher taxonomic level. For unlisted taxa, assignments were made based on current knowledge of local taxa and on closely related taxa. Unassigned taxon aggregates (e.g. Amphipoda spp., <6% abundance) were omitted from BP_c calculations. Pre-treatment of predictors involved checking for collinearity (i.e. Pearson correlation coefficient >0.9) and skewness, with community biomass fourth-root transformed. Euclidean distance matrices of pre-treated fluxes with a significant community effect were the response matrix. Where sediment condition was also present as a main effect or in an interaction, data were split into separate tests for condition classes. Metric values for defaunated cores were manually set to zero. To assess the influence of community predictors considered alone and cumulatively, both marginal and sequential DISTLMs were carried out, with stepwise selection and the R^2 statistic used as selection procedure and criterion, respectively, for sequential tests.

3. RESULTS

3.1. Overview of sediment condition and benthic macroinvertebrate communities

The in-field RAP showed that sediment condition ranged from fine or coarse sandy sediments of good condition to malodorous, muddy sediments of fair and poor condition. The good condition sediments had greater O_2 penetration in the upper and lower estuary (average aRPD depths: 2 and 7 cm, respectively) than the fair and poor sediments (average aRPD depths: 0.5 and 1 cm, respectively).

We identified 50 taxa across 7 phyla from all sediment cores. The 5 most abundant taxa in the lower estuary were the amphipod *Corophium minor* (548 ind. 0.1 m^{-2}), the bivalve *Mysella* sp. 1 (466 ind. 0.1 m^{-2}), the amphipod *Grandidierella propodentata* (250 ind. 0.1 m^{-2}) and the polychaetes *Desdemona ornata* (205 ind. 0.1 m^{-2}) and *Heteromastus filiformis* (122 ind. 0.1 m^{-2}). In the upper estuary, Chironominae larvae (119 ind. 0.1 m^{-2}), oligochaetes (105 ind. 0.1 m^{-2}), *D. ornata* (71 ind. 0.1 m^{-2}), the polychaete *Simplisetia aequisetis* (61 ind. 0.1 m^{-2}) and the amphipod *Paracorophium excavatum* (45 ind. 0.1 m^{-2}) were the 5 most abundant taxa. All of the above taxa were more abundant in good sediments, except for *P. excavatum* (all taxa densities are available in Table S2 in the Supplement).

3.2. Condition and community effects on ecosystem function

The lower and upper estuary exhibited net O_2 consumption and net DIC production across all factors, while alkalinity fluxes varied with sediment condition and community treatment (Figs. 3 & 4). Benthic p/r ratios were less than 0.25 in all cores, classifying them as net heterotrophic. The relationship of dark DIC vs. dark O_2 fluxes showed most defaunated and faunated cores to be of good condition, and poor faunated cores had a DIC:O_2 ratio close to 1 (i.e. O_2 consumption = DIC production) while defaunated and faunated cores of fair condition and defaunated cores of poor condition had DIC:O_2 ratio <1 (O_2 consumption > DIC production; Fig. 5a). The relationship of dark DIC to dark alkalinity fluxes showed that faunated and defaunated cores of fair condition, and faunated and defaunated good sediments from the lower and upper estuary, respectively, had greater alkalinity uptake than DIC production while the other groups had DIC:alkalinity ratios close to 1 (Fig. 5b).

An overview of PERMANOVA test results for all fluxes is given in Table 2, with full test results and PERMDISP results provided in Tables S1 & S3. There was no effect of sediment condition or the community on benthic metabolism in the upper and lower estuary, respectively. Sediment condition significantly drove dark fluxes of O_2, and dark and light alkalinity fluxes in the lower estuary. Good sediments in the lower estuary had greater dark O_2 uptake (average: -1045 ± 137 µmol m^{-2} h^{-1}) than fair sediments (-865 ± 114 µmol m^{-2} h^{-1}). Dark alkalinity fluxes showed an efflux (339 ± 487 µmol m^{-2} h^{-1}) from good sediments and an uptake (-250 ± 455 µmol m^{-2} h^{-1}) in fair sediments, with greater uptake in good sediments (-163 ± 664 µmol m^{-2} h^{-1}) than fair sediments (-41 ± 568 µmol m^{-2} h^{-1}) during a light cycle in the lower estuary. In the upper estuary, net DIC fluxes significantly changed with the community where faunated cores had greater net DIC effluxes than defaunated cores (398 ± 360 µmol m^{-2} h^{-1} vs. 232 ± 223 µmol m^{-2} h^{-1}). Sediment condition only had a significant effect on dark O_2 fluxes when accounted for first, with community effects on net DIC fluxes only significant after accounting for sediment condition. Sediment condition effects on dark and light alkalinity fluxes remained significant irrespective of the order-of-fit.

Nutrient fluxes showed a variety of patterns across regions and in response to both factors (Figs. 3 & 4). There were no effects of sediment condition or the benthic macroinvertebrate community on nutrient fluxes in the lower estuary, with no effects on NH_4^+
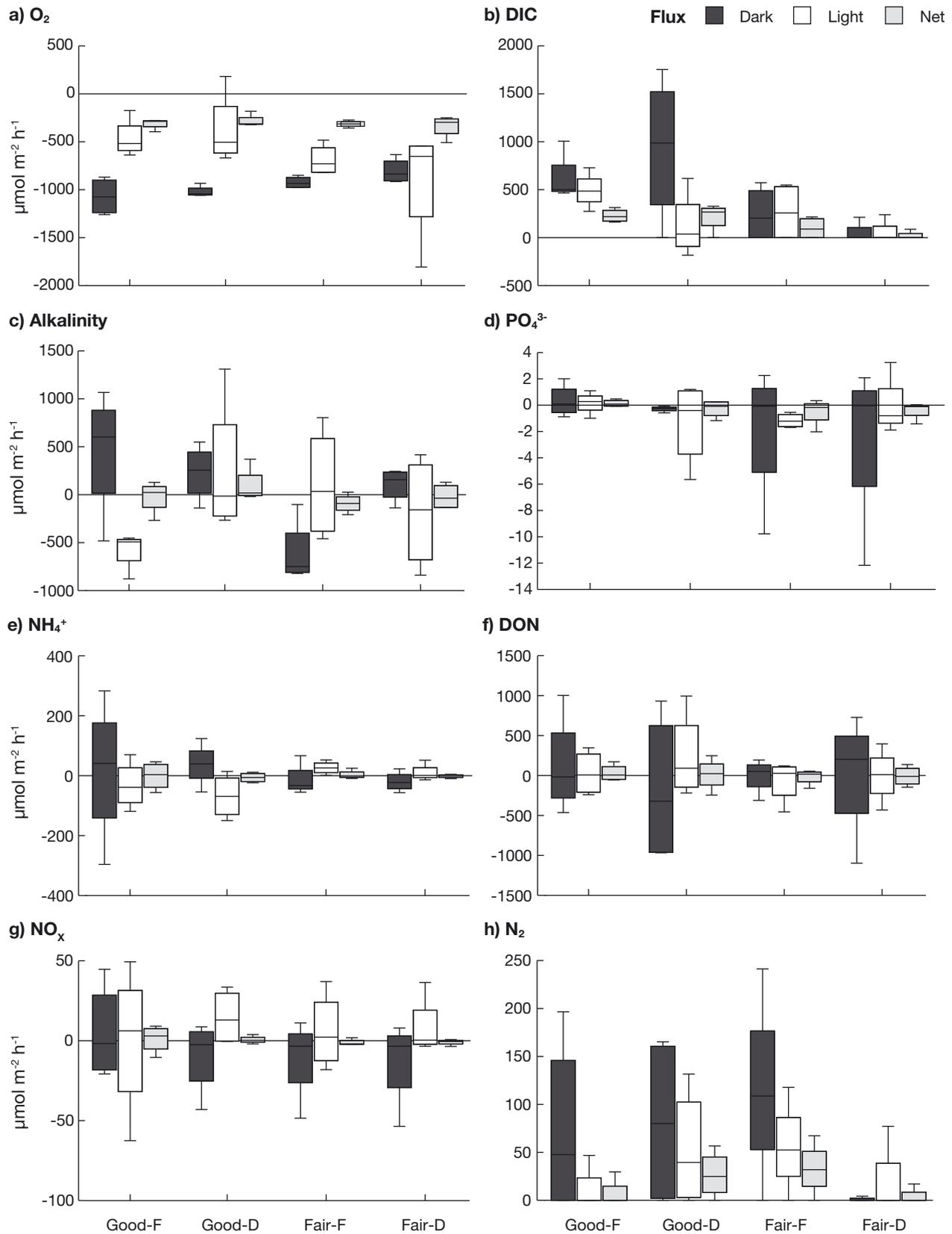
Fig. 3. Rates (µmol m^{-2} h^{-1}) of dark, light and net fluxes in the lower Peel-Harvey Estuary, grouped according to good and fair sediment condition and community treatment (F: faunated; D: defaunated) for (a) oxygen (O_2), (b) dissolved inorganic carbon (DIC), (c) alkalinity, (d) phosphate (PO_4^{3-}), (e) ammonium (NH_4^+), (f) dissolved organic nitrogen (DON), (g) nitrate and nitrite (NO_x) and (h) dinitrogen (N_2). Line in box: median; box: interquartile range (IQR); whiskers: IQR × 1.5
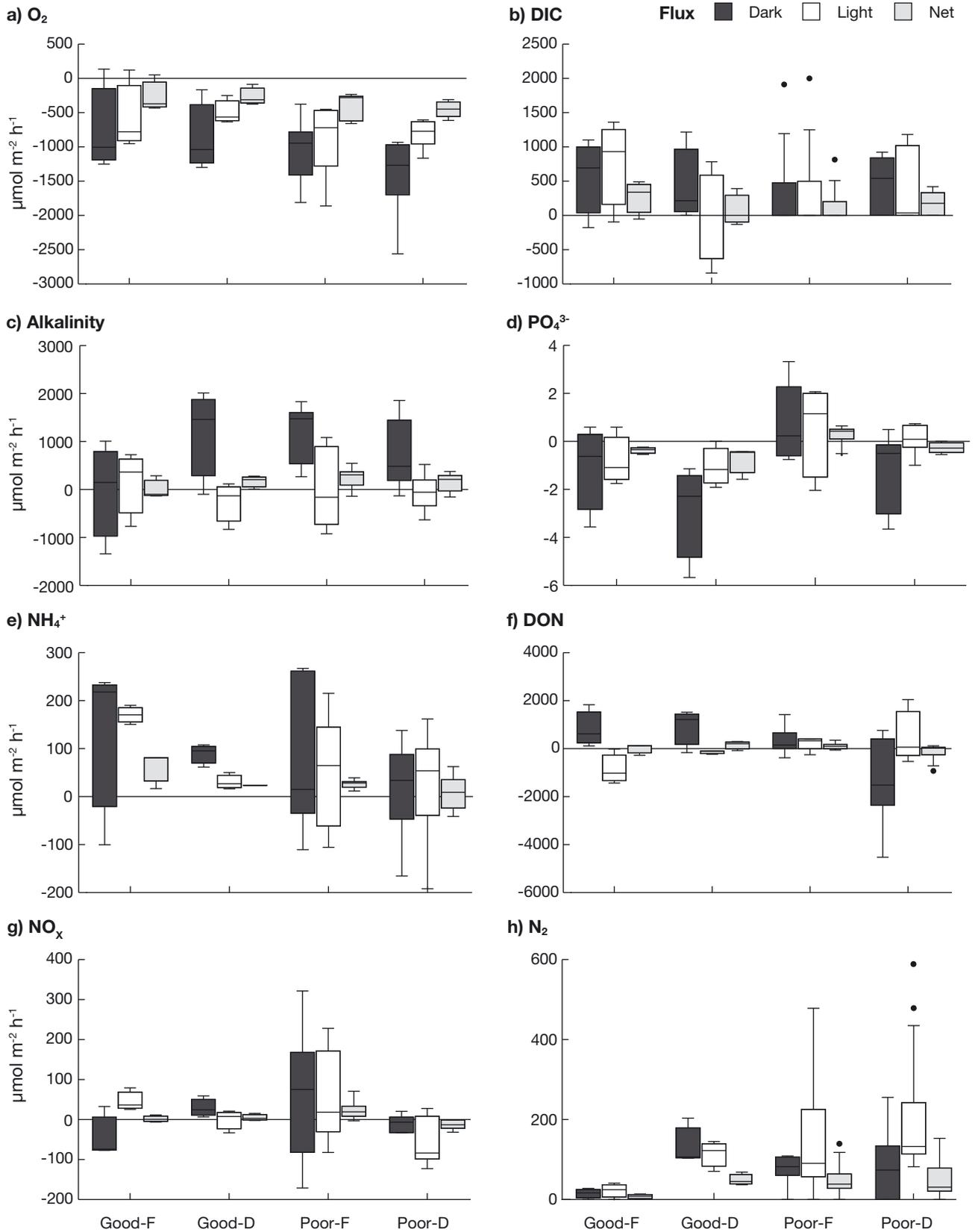
Fig. 4. As in Fig. 3, but for the upper estuary. Dots: outliers

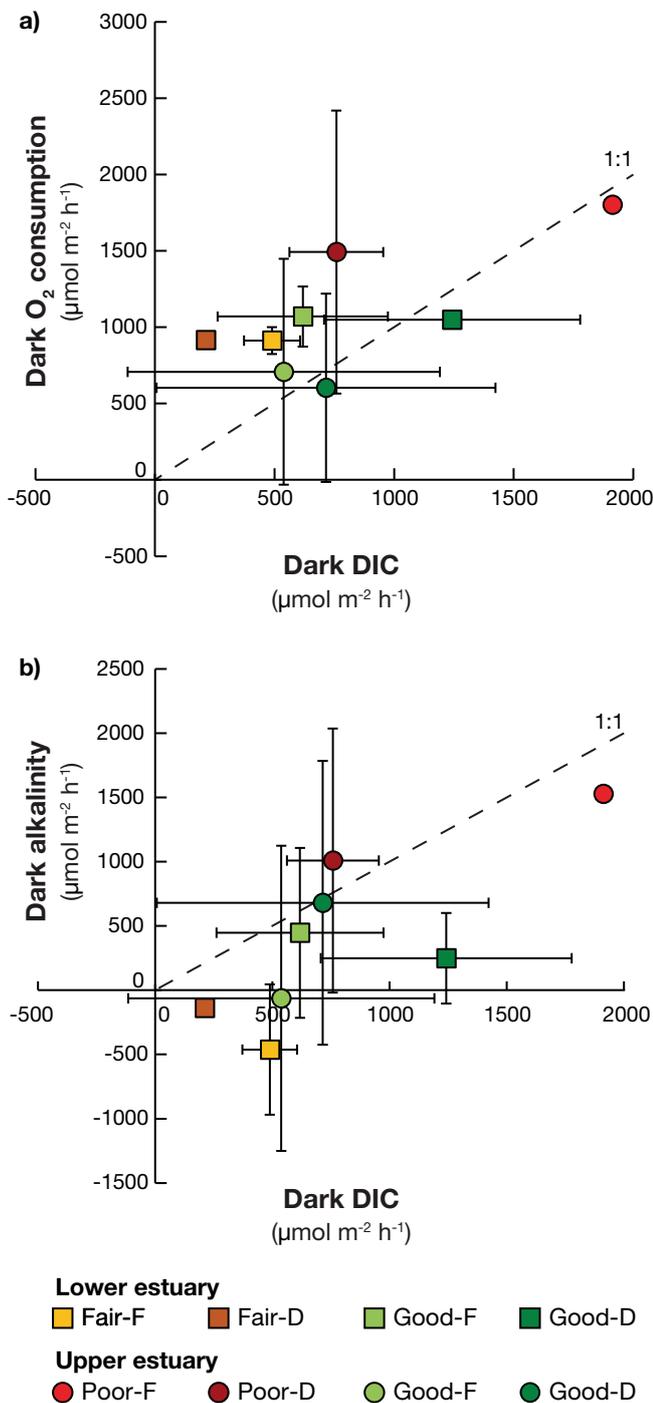Fig. 5. (a) Dark oxygen (O_2) vs. dark dissolved inorganic carbon (DIC) fluxes (µmol m^{-2} h^{-1}) and (b) dark alkalinity vs. dark DIC fluxes (µmol m^{-2} h^{-1}) for factor groups (mean ± SD). Factor groups are labelled by sediment condition (good, fair, poor) and community treatment (F: faunated; D: defaunated) for the lower and upper Peel-Harvey Estuary. Note that some factor groups contain a single sample at the lowest factor level (sediment condition × community) due to removal of unreliable DIC values during data quality assessment. The dashed line through the plots indicates a flux rate ratio of 1:1

fluxes in the upper estuary. There were significant effects of sediment condition and the community on dark PO_4^{3-} fluxes in the upper estuary. Net PO_4^{3-} fluxes also differed significantly with sediment condition and the community in this region, with the relative importance of factors in the 2 tests showing that whichever factor that was fitted second was slightly more influential (COV values 0.51–0.53 vs. 0.29–0.32). There was greater PO_4^{3-} uptake in good riverine sediments (dark: −2.12 ± 2.25 µmol m^{-2} h^{-1}, net: −0.6 ± 0.49 µmol m^{-2} h^{-1}) compared to poor sediments (dark: −0.27 ± 2.02 µmol m^{-2} h^{-1}, net: −0.01 ± 0.047 µmol m^{-2} h^{-1}). Community treatment switched faunated cores from PO_4^{3-} efflux (dark: 0.07 ± 2.04 µmol m^{-2} h^{-1}, net: 0.02 ± 0.49 µmol m^{-2} h^{-1}) to uptake (dark: −1.99 ± 2.03 µmol m^{-2} h^{-1}, net: −0.55 ± 0.48 µmol m^{-2} h^{-1}) in defaunated cores. The community and sediment condition significantly drove dark and light DON fluxes, respectively, in the upper estuary. Dark DON fluxes showed efflux (537 ± 737 µmol m^{-2} h^{-1}) from faunated cores and uptake (−509 ± 1996 µmol m^{-2} h^{-1}) in defaunated cores, while light fluxes showed uptake (−487 ± 593 µmol m^{-2} h^{-1}) in good sediments and efflux (373 ± 783 µmol m^{-2} h^{-1}) from poor sediments. The community significantly drove light and net fluxes of NO_x in the upper estuary, with efflux (light: 56 ± 97 µmol m^{-2} h^{-1}, net: 16 ± 24 µmol m^{-2} h^{-1}) and uptake (light: −34 ± 58 µmol m^{-2} h^{-1}, net: −6 ± 15 µmol m^{-2} h^{-1}) in faunated and defaunated cores, respectively. Considering the order-of-fit of factors, significant community effects on light NO_x and dark DON fluxes only occurred when the community was tested before sediment condition, while significant effects of sediment condition and the community on dark PO_4^{3-} fluxes were found only when tested after one another. Sediment condition effects on light DON fluxes, community effects on net NO_x fluxes and community and condition effects on net PO_4^{3-} were significant irrespective of the order-of-fit.

Denitrification efficiency ranged from 16 to 100%, with a general decrease in efficiency as DIC effluxes increased and a maximum denitrification efficiency measured around DIC effluxes of 500 to 800 µmol m^{-2} h^{-1} (Fig. 6). Dark, light and net N_2 in both regions were all effluxes from the sediment (Figs. 3 & 4). In the lower estuary, there were significant effects of the community on dark and net N_2 fluxes, with N_2 effluxes higher in faunated (dark: 150 ± 65 µmol m^{-2} h^{-1}, net: 40 ± 18 µmol m^{-2} h^{-1}) than defaunated cores (dark: 82 ± 90 µmol m^{-2} h^{-1}, net: 30 ± 19 µmol m^{-2} h^{-1}). In the upper estuary, sediment condition significantly drove light and net N_2 fluxes,

Table 2. Summary of PERMANOVA test results (pseudo-F statistic) for significant sediment condition and community effects on ecosystem fluxes in the Peel-Harvey Estuary. A range of pseudo-F statistics is given for test results where the 2 order-of-fit tests (condition or community tested first) produced significant results. Sediment condition × community interaction columns are not included as no significant interactions occurred. *$p \leq 0.05$; **$p \leq 0.01$. DIC: dissolved inorganic carbon; DON: dissolved organic nitrogen

Variable	Flux	—— Lower estuary ——		—— Upper estuary ——	
		Condition	Community	Condition	Community
O_2	Dark	6.51*			
	Light				
	Net				
DIC	Dark				
	Light				
	Net				6.68*
Alkalinity	Dark	6.67–9.38*			
	Light	4.43–5.89*			
	Net				
PO_4^{3-}	Dark			7.10*	8.40*
	Light				
	Net			5.07–10.93**	4.62–10.48**
NH_4^+	Dark				
	Light				
	Net				
NO_x	Dark				
	Light				4.85*
	Net				9.90–11.17**
DON	Dark				4.64*
	Light			6.52–8.56*	
	Net				
N_2	Dark		6.43*		
	Light			6.73*	
	Net		8.05–8.24*	6.44–7.68*	

with N_2 efflux higher from poor (light: 204 ± 52 µmol m^{-2} h^{-1}, net: 65 ± 51 µmol m^{-2} h^{-1}) than good (light: 81 ± 190 µmol m^{-2} h^{-1}, net: 34 ± 24 µmol m^{-2} h^{-1}) sediments. Considering factor order-of-fit, the community effect on dark N_2 fluxes in the lower estuary differed significantly only when community was tested before sediment condition. Sediment condition only significantly affected light N_2 fluxes in the upper estuary when tested before the community. The effects of community and condition on net fluxes of N_2 in the lower and upper estuary, respectively, were significant irrespective of the order-of-fit.

3.3. Benthic community predictors of fluxes

Generally, univariate metrics of benthic community structure were higher in good sediments than in fair and poor sediments (Table 3). Two exceptions to this were measures of S and community biomass in the upper estuary, with similar levels of S in both sediment conditions and slightly higher biomass in poor sediments.

The *a posteriori* investigation into which metrics may explain significant community effects showed that differing predictors were related to various process fluxes (Table S4). No significant predictors were found for net DIC fluxes or dark DON fluxes in the upper estuary. For the same region, community effects on light fluxes of NO_x could be significantly explained by the BP_c (72% of variation explained) and biomass (37%), but not for net fluxes. For the sequential test, the BP_c was the sole predictor that produced a significant model ($R_2 = 0.72$). In the upper estuary, PO_4^{3-} fluxes, where both community and sediment condition effects were present, could be explained by biomass (dark: 56%, net: 49%), N (dark: 46%) and S (dark: 44%) in poor sediments, with biomass selected as the sole predictor for significant dark and net PO_4^{3-} flux models in poor sediments ($R^2 = 0.49$–0.56). In the lower estuary, no significant predictors were found for dark or net N_2 effluxes.

4. DISCUSSION

Our study demonstrates the variable effects that sediment condition and macroinvertebrate communities have on ecosystem function in the microtidal Peel-Harvey Estuary. Across the system, sediment condition, rather than the presence of macroinvertebrates, drove benthic metabolism, while the reverse was true for nutrient exchange. Denitrification rates, in contrast, were higher in poor sediments in the upper estuary and in faunated sediments in the lower estuary.

Interestingly, no sediment condition × community interactions were present for any process flux in the Peel-Harvey Estuary. This suggests that these factors independently affect ecosystem function. However, changes in test significance with the factor testing order indicate that there was a degree of overlap in their effects in some cases. Interacting effects are sometimes useful, such as those seen for denitrifica-
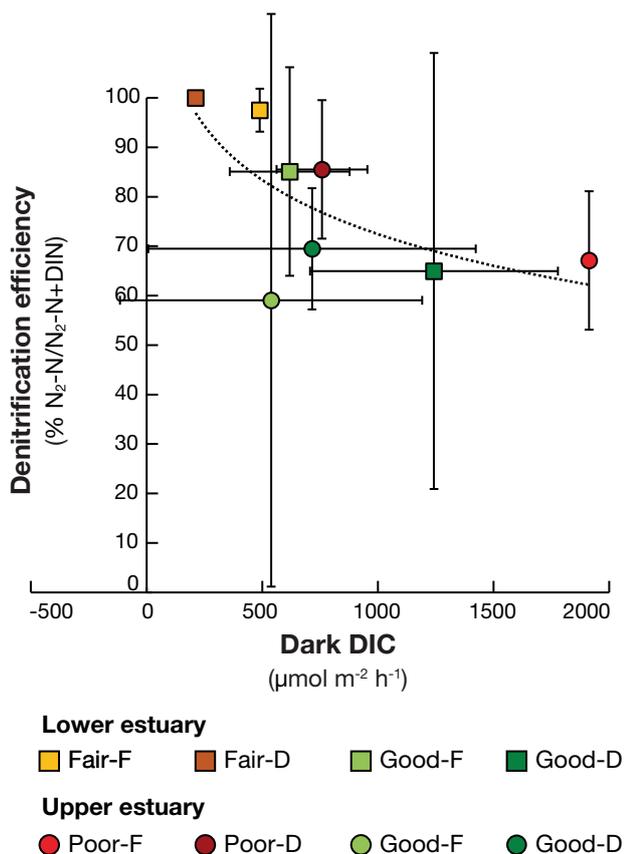
Fig. 6. Denitrification efficiency (% N_2-N/N_2-N+DIN) vs. dark dissolved inorganic carbon (DIC) fluxes (µmol m^{-2} h^{-1}) for factor groups (mean ± SD), with a dashed line of best fit. Factor groups are labelled by sediment condition (good, fair, poor) and community treatment (F: faunated; D: defaunated) for the lower and upper estuary. Note that some factor groups contain a single sample at this lowest factor level (sediment condition × community) due to removal of unreliable DIC values during data quality assessment. N: nitrogen; N_2: dinitrogen; DIN: dissolved inorganic nitrogen

tion rates that were unexpectedly lower in enriched, estuarine sediments that harboured fewer *Pseudopolydora* polychaetes than less enriched sediments (O'Meara et al. 2020). Ecologically, the absence of significant interactions may reflect the fact that macroinvertebrate communities in the estuary are so underdeveloped that community removal via defaunation (albeit partial removal that was statistically controlled for; Cronin-O'Reilly et al. 2022) between sediment condition classes was functionally negligible. The decoupled effects seen here may thus further demonstrate the loss of fundamental interactions under stressed conditions (Lohrer et al. 2012). Alternatively, sediment condition effects are capturing inherent macroinvertebrate community differences or differences in other biological communities that drive biogeochemical function (e.g. bacteria, meio-

fauna), which ultimately highlights the difficulty and complexity involved in trying to disentangle such effects under 'real world' scenarios (Naeem & Wright 2003, Snelgrove et al. 2014).

All sites sampled in the Peel-Harvey Estuary were net heterotrophic with a benthic p/r ratio below 0.25, which is indicative of a hypereutrophic state and ecosystem stress (Eyre & Ferguson 2009). This aligns with the history of eutrophication and resulting high sediment organic matter content in the estuary (Hallett et al. 2019, Valesini et al. 2019). The Peel-Harvey also lies in an extensively modified catchment, which is linked to increasingly heterotrophic conditions (Chen et al. 2019). Typically, heterotrophic sediments also have greater O_2 consumption at night from the added aerobic respiration of photosynthetic organisms and heterotrophic respiration of their products (Eyre & Ferguson 2002). Here, higher rates of dark O_2 consumption in good than fair sediments in the lower estuary thus likely reflect these processes: organic-poor, coarse sediments harbour more microphytobenthos than the siltier, organic-rich sediments (Lukatelich & McComb 1986). Overall, the heterotrophic bare sediments across the estuarine system suggest that it may be a net source, rather than sink, of carbon dioxide (Chen et al. 2019).

Heterotrophic sediments are also sources of nutrients to the water column (Eyre & Ferguson 2002, Ferguson et al. 2004, Gautreau et al. 2020). This fits with the observed nitrogen exchange in the upper estuary, where there was an efflux of NH_4^+ from all sediments and DON efflux from poor sediments, with the DON probably originating from excessive photosynthetic production. In contrast, there was PO_4^{3-} uptake in both good and poor sediments in the same region. Uptake of PO_4^{3-} can be driven by biological assimilation, sediment adsorption by minerals like iron or precipitation with carbonate minerals (Gomez et al. 1999, Martins et al. 2014, Kraal et al. 2015). Biotic-driven PO_4^{3-} uptake (assimilation) would be expected to correlate with metabolism (Cohen et al. 2013); however, all sediments were net heterotrophic rather than autotrophic. Thus, PO_4^{3-} uptake is more likely due to abiotic drivers such as iron-mediated PO_4^{3-} adsorption, which is higher in good than poor sediments in the estuary (Kraal et al. 2013, 2015). Regardless of the exact mechanism, it is clear that good sediments can act as an important sink for phosphorus (Kraal et al. 2013, 2015). Alkalinity uptake in fair sediments, their relationship with DIC fluxes and the greater O_2 consumption than DIC production also indicate that sulphide oxidation (S^{2-} to SO_4^{2-}) is occurring in the lower estuary. Sulphide oxidation can

Table 3. Mean (±SD) of taxon richness (S), total abundance (N, ind. per 70.88 cm^2), community biomass (g) and the community bioturbation potential (BP$_c$, based on biomass expressed as grams) recorded in sediment condition classes ('good' and 'fair'; see Fig. 1) of the 2 regions of the Peel-Harvey Estuary

| | ─── Lower estuary ─── | | ─── Upper estuary ─── | |
	Good sediments	Fair sediments	Good sediments	Poor sediments
S	13 ± 4	4 ± 3	4 ± 1	4 ± 3
N	152 ± 95	18 ± 19	20 ± 19	12 ± 17
Biomass	0.24 ± 0.19	0.14 ± 0.37	0.11 ± 0.09	0.18 ± 0.5
BP$_c$	22 ± 13	3 ± 5	12 ± 9	8 ± 15

act as another useful process for detoxifying sulphide compounds that are readily available in these estuarine sediments (Brearley 2005, Morgan et al. 2012), but the controls and results of such processes are complex and vary temporally (Rao et al. 2016).

Sediments can also provide an important removal pathway for nitrogen via denitrification. Here, condition-related effects (i.e. greater daytime and net N_2 efflux from poor sediments) in the upper estuary likely reflect enhanced denitrification due to increased organic matter metabolism and associated NH_4^+ production, which feeds nitrification on surface sediment layers supplying NO_x for denitrification (Cornwell et al. 1999, O'Meara et al. 2020). Denitrification can also be enhanced by high NO_3^- concentrations in the overlying water (Findlay et al. 2011), yet this is not the case here as such water concentrations remained similar between sediment conditions. The denitrification efficiency was generally high, indicating sufficient nitrogen removal, relative to recycling, from the estuary. The high denitrification efficiencies reflect low overall respiration rates, and the decline with increasing respiration (DIC fluxes) is consistent with other systems (Eyre & Ferguson 2009). As sediment carbon loading increases, coupled nitrification–denitrification is reduced due to the effects of sulphide, reduced O_2 availability for nitrification (Eyre & Ferguson 2009) or through increased microbial competition for NO_x (Gardner & McCarthy 2009, Douglas et al. 2018). Interestingly, neither sediment condition nor the presence of macroinvertebrates had a consistent effect on denitrification efficiency across the system, highlighting the variable role respiration has on denitrification.

Only 1 of the 9 benthic metabolism rates and 5 of the 12 nutrient exchange fluxes were significantly affected by the community. This is in stark contrast to other estuaries, particularly those with larger bioturbating species (e.g. *Hediste diversicolor*) that drive benthic metabolism (e.g. Webb & Eyre 2004,

Murphy et al. 2018) and nutrient exchange (e.g. Andersen & Kristensen 1988, Braeckman et al. 2010). Benthic macroinvertebrates influence benthic metabolism directly through aerobic respiration or indirectly by enhancing organic matter mineralisation through bioturbation and bioirrigation (Welsh 2003, Braeckman et al. 2010, Douglas et al. 2018). Aerobic respiration is probably causing the increased net DIC effluxes from faunated cores in the upper estuary as they were accompanied by significant night-time DON effluxes, and night-time and net PO_4^{3-} effluxes, which are likely from excretion after faunal metabolism. Although the rate at which invertebrates respire and excrete increases as body size decreases (e.g. Alves et al. 2010), the magnitude of both process fluxes increases with invertebrate body size (Zeuthen 1953), demonstrating the general principle that faunal metabolism is a function of body mass and temperature (Gillooly et al. 2001). The biomass of benthic macroinvertebrates explained 49 to 56% of PO_4^{3-} fluxes in poor sediments here, similarly to the positive biomass and PO_4^{3-} efflux relationships found in the nearby Swan-Canning Estuary (Pennifold & Davis 2001). While high biomass could theoretically be created by numerous small individuals, here it corresponded to the occurrence of a large species. The largest polychaete species (*Marphysa* sp.) to occur in the estuary (J. Tweedley unpubl. data) was caught in these poor sediments and is likely responsible for these patterns. However, the general lack of community effects on all O_2 and NH_4^+ fluxes, as well as most DIC and PO_4^{3-} fluxes, is a telling biogeochemical reflection on the community's underdevelopment, which precludes substantial metabolic activity and waste production.

Inhabited sediment cores did, however, exhibit daytime and net NO_x sediment effluxes in the upper estuary, irrespective of sediment condition, with the BP$_c$ explaining 72% of daytime NO_x fluxes and community biomass explaining 37%. Benthic macroinvertebrate communities enhance nitrification through bioturbation (Welsh 2003), which creates oxic microniches for nitrifiers in burrows (Banks et al. 2012). Given the widespread efflux of NH_4^+ from upper estuarine sediments, this nitrification could be key for nitrogen removal through coupled nitrification–denitrification (Braeckman et al. 2014). However, the increase in NO_x fluxes due to the community in the upper estuary was not accompanied by a community

effect on N_2 fluxes. In contrast, dark and net denitrification rates were affected by the community in the lower estuary. It is possible that high abundances of the amphipod *Corophium minor*, as well as the presence of other bioturbating species (e.g. *Tritia burchardi*), in this region drove denitrification. Members of the genus *Corophium* can enhance nitrification–denitrification coupling during U-shaped burrow construction, irrigation and maintenance (Nizzoli et al. 2002, Murphy et al. 2018), which creates overlapping oxic–anoxic sediment zones to supply nitrification-produced NO_x for denitrification (Welsh 2003, Eyre & Ferguson 2009). However, no community predictor explained the denitrification rates seen. Instead, the divergent regional effects on denitrification highlight the complex interactions between organic matter, NO_x and O_2 that regulate the process (Wallenstein et al. 2006). The importance of sediment condition, rather than the community, in the upper estuary suggests that organic matter content limited denitrification in this region, where surface water NO_x is relatively high from the proximity to riverine inputs (Hallett et al. 2019). Contrastingly in the organic-poor sediments of the lower estuary, where surface water NO_x is lower, community activity was substantially more important. Comparatively underdeveloped macroinvertebrates may thus maintain an influence over denitrification when the condition of the environment is not overly degraded.

Due to widespread anthropogenic degradation of coastal waters, investigations into which aspects of benthic community structure are most important in shaping their function have been prompted (e.g. Emmerson et al. 2001, Belley & Snelgrove 2016). When comparing the relative importance of biodiversity and functional metrics to ecosystem function, functional traits usually play a greater role (e.g. Norkko et al. 2013, Pratt et al. 2014). The same patterns were evident here as biomass and the BP_c outcompeted S as predictors of some NO_x and PO_4^{3-} fluxes. When comparing the relative importance of functional metrics, there was increased variation of daytime NO_x fluxes explained by the BP_c in comparison to biomass, highlighting the importance of species-specific functional traits for ecosystem function (Braeckman et al. 2014). Despite this, community biomass was the most frequently selected predictor of ecosystem function by DISTLM. Relative to other community predictors, our study supports the idea that the biomass of benthic macroinvertebrate communities is a leading biotic driver of ecosystem function (Emmerson et al. 2001, Pennifold & Davis 2001, Gammal et al. 2019), even when the communities are underdeveloped.

Overall, benthic macroinvertebrate communities in the Peel-Harvey Estuary did not significantly affect most ecosystem fluxes (16 of the 24 measured), exhibiting the limited functional role of this chronically stressed community. The complete absence of an interaction between sediment condition and the presence of benthic macroinvertebrates further suggests a functional decoupling between these factors within the estuary. Still, community biomass remained the main biotic predictor selected of the investigated processes, highlighting the paramount importance of maintaining the size and abundance of benthic macroinvertebrates in preserving their influence on ecosystem function. Our study reveals the limited role that naturally occurring underdeveloped macroinvertebrate communities may play globally and the many potentially pressing implications of what might occur functionally when benthic environments in estuaries become particularly degraded.

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