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PRIMARY RESEARCH PAPER

Influence of natural amphipod (*Victoriopisa australiensis*) (Chilton, 1923) population densities on benthic metabolism, nutrient fluxes, denitrification and DNRA in sub-tropical estuarine sediment

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Abstract The influence of natural populations of the sub-surface deposit-feeding amphipod *Victoriopisa australiensis* on sediment biogeochemistry was assessed by randomly collecting 21 sediment cores in a zone of Coombabah Lake, southern Moreton Bay, Australia, where the benthic infauna was dominated by this species. Cores were incubated sequentially to determine sediment–water column fluxes of oxygen, dissolved inorganic carbon and inorganic N species, followed by incubations to determine rates of denitrification and dissimilatory nitrate reduction to ammonium (DNRA) using the isotope pairing technique. Finally, each core was sieved in order to determine the population and biomass of amphipods present. Whilst all measures of overall benthic metabolism (sediment oxygen demand, and effluxes of inorganic carbon and nitrogen) showed increased with amphipod density, with rates being stimulated 70–220% at the highest categorised density range of 2,500–3,500 ind m^{-2}, only the correlation with dissolved inorganic carbon was statistically significant. In contrast, there were no discernable trends between amphipod densities and any of the N-cycle processes with the slopes of all correlations being very close to zero. These results highlight the differences in mesocosm simulations of fauna effects, which primarily relate to shifts in rates of organic matter turnover, compared to natural sediments where fauna effects relate more to induced changes in rates of organic matter deposition. Therefore, while mesocosms represent a powerful tool to investigate the mechanisms by which fauna influences microbial metabolism in the sediment, only studies of natural sediments can determine to what extent these mechanisms function in situ.

Keywords Bioturbation · *Victoriopisa australiensis* · Sediment–water column fluxes · Denitrification · DNRA

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Introduction

Coastal marine sediments are permanently or temporarily colonised by benthic infauna (Aller, 1988; Kristensen, 1988; Reise, 2002). Through their feeding, burrow construction, bioturbation and burrow irrigation activities, infauna can influence rates of organic matter inputs to the sediment, the vertical distribution of this organic matter within the sediment, rates and pathways of organic matter mineralisation, and the

fluxes of the regenerated dissolved nutrients back to the overlying water (Aller, 1988; Kristensen, 1988, 2000; Graf & Rosenberg, 1997; Hansen & Kristensen, 1998; Christensen et al., 2000; Reise, 2002). These benthic processes are in large part dependent on the population density, biomass, community structure and feeding mode of the infauna (Welsh, 2003). The impacts of infauna on benthic metabolism, microbial dynamics, nutrient fluxes and denitrification have been well documented (e.g. Hansen & Kristensen, 1998; Tuominen et al., 1999; Bartoli et al., 2000; Welsh, 2003; Michaud et al., 2006; Nizzoli et al., 2007). In contrast, the effect of burrowing infauna on dissimilatory nitrate reduction to ammonium (DNRA), which competes with denitrification for nitrate, has received little attention.

Potential relationships between benthic processes and infauna are most commonly reported following ex situ mesocosm incubation of homogenised sediments with the incorporation of specific faunal groups (e.g. Caradec et al., 2004; Michaud et al., 2006; Papaspyrou et al., 2007) and in some cases additional materials of interest such as organic matter (Papaspyrou et al., 2004; Sun & Dai, 2005; Karlson et al., 2007). This methodological approach allows the establishment of defined experimental conditions with controlled abundances of macrofauna (Van Duyl et al., 1992); however, the original physical and chemical characteristics of the sediment are altered limiting the extrapolation of the data to natural systems (Welsh, 2003). Such experimental simulations to a large extent mimic the short-term effects of colonisation events rather than those of meta-stable natural communities, which may persist through time, albeit with some degree of variation in biomass and taxonomic composition. Consequently, mesocosms represent a non-steady-state situation, whereas although natural communities may show seasonal and inter-annual variability, they may be considered as near-steady-state systems over long time periods. Therefore, faunal induced flux enhancements measured in non-steady state incubations are most likely overestimates of field conditions (Welsh, 2003).

In contrast, ex situ incubations employing intact sediment cores (e.g. Hansen & Kristensen, 1997; Mortimer et al., 1999; Nizzoli et al., 2007), used in an attempt to maintain in situ sediment conditions, or in situ benthic chamber incubations (e.g. Webb & Eyre, 2004) represent a more useful methodological approach in obtaining data indicative of selected natural systems. However, the influence of burrowing infauna is often difficult to assess in situ due to heterogeneity with respect to macrofaunal abundances, population structures and sediment composition. The lack of adequate control sediments characterised by the absence of macrofaunal activity also hinders the direct assessment of bioturbation (Papaspyrou et al., 2004), and as a result, studies utilising intact sediments to investigate relationships between benthic processes and abundances of burrowing infauna typically report reduced stimulations in comparison to mesocosm studies employing homogenised sediments.

Victoriopisa australiensis (*Eriopisa* group), belonging to the Melitidae family, is a distinctive amphipod species living in restricted habitats (Lowry & Springthorpe, 2005) within the littoral zone in estuarine, mangrove, mudflat and seagrass-colonised sediments of eastern Australia (Lowry et al., 2000), extending from southern Queensland to southern New South Wales. *V. australiensis* inhabit fixed burrows, feeding on sub-surface sediments, which they excavate, process and immediately redeposit within their burrow system (Thiel et al., 1997a, b).

This study involved the collection and incubation of intact sediment cores, in order to maintain the natural physical and chemical gradients within the sediments, containing natural population densities of *V. australiensis*. The objective of this study was to quantify any effects of *V. australiensis* on benthic metabolism, nutrient fluxes, denitrification and DNRA in a shallow sub-tropical, estuarine sediment.

Materials and methods

Site description

Sediment cores were hand collected during August 2007, in a zone of Coombabah Lake (6912750 m N, 534400 m E), southern Moreton Bay, Australia (sample grid 11 in Dunn et al. (2007, 2008)), where the benthic infauna was known to be dominated by *V. australiensis*. The nearshore sample site was bordered by fringing mangroves and sandy-silt surface sediments with a median particle size of 12.7 μm and organic C and N contents of 1.23 and 0.10% dry

wt, respectively (Dunn et al., 2008). Winter bioavailable (porewater + exchangeable) phosphate ($PO_4^{3-}{}_{bio}$) and ammonium ($NH_4^+{}_{bio}$) surface sediment (0–2 cm) concentrations averaged 10.36 ± 3.53 nmol g^{-1} dry wt. and 152.28 ± 120.47 nmol g^{-1} dry wt., respectively (Dunn et al., unpubl. data). *V. australiensis* is one of the most abundant and widespread macrofaunal species in Coombabah Lake accounting for up to 92% of the burrowing macrofauna at the lake sample site with seasonal densities ranging between ~300 and 3,500 ind. m^{-2}, with maximum abundances occurring during winter (Dunn et al., unpubl. data). The bathymetry of the entire lagoon is available in Dunn et al. (2008). The sample site used in this study was an intertidal site with an approximate water depth of 0.5 m during tidal inundation. Hydrological characteristics of the lagoon are described by Knight et al. (2008) and Ali et al. (2009).

Sediment collection and experimental set-up

In order to maintain the sediment integrity and natural chemical gradients, intact sediment cores were carefully collected by hand using PVC core tubes (77 mm Ø × 330 mm long). A total of 21 cores were collected randomly within a 20 × 20 m area during low tide for the ex situ determination of dissolved oxygen, total dissolved inorganic carbon ($C_T = [CO_2] + [HCO_3^-]+[CO_3^{2-}]$), dissolved inorganic nitrogen species (DIN; NO_2^-, NO_3^-, NH_4^+) and dissolved PO_4^{3-} fluxes at the sediment–water interface during dark incubations. Denitrification and DNRA rates were subsequently determined for the same cores, before the infauna was harvested and quantified. During sediment collection, in situ light and temperature conditions were measured and replicated during core maintenance. Conditions were measured at a water depth of ~15 cm from the sediment surface using a LI-COR radiation sensor (SA: LI-192SA quantum sensor) and thermometer (TPS 90-FLMV, TPS Pty. Ltd.), respectively.

Site water (~320 l) required for core maintenance and incubations was collected from Coombabah Creek during the flood tide phase of the same day, using cleaned (Milli-Q water and seawater rinsed) 40 l sample drums. During water collection, physico-chemical parameters were recorded using a multi-probe analyser (TPS 90-FLMV model, TPS Pty. Ltd.) and triplicate water samples were collected for the determination of chlorophyll-*a* and dissolved inorganic nutrient concentrations. The incubation water had the following characteristics, pH 8.27, salinity 34‰ dissolved oxygen 90.4% saturation, chlorophyll-*a* 1.71 μg l^{-1}, NH_4^+ 0.58 μM and NO_x 0.83 μM.

Following sediment collection, cores were transported to a constant temperature laboratory within 1.5 h and adjusted to give a sediment depth of ~12 cm. Cores were transferred into three holding tanks (55 l), carefully filled with the collected intertidal water, and the tanks were filled above the level of the cores to allow free exchange with the aerated water within the holding tanks. Cores were then left to stabilise under measured in situ light (Halogen lamps: ~100 μE m^{-2} s^{-1} at the sediment surface) and dark conditions (12 h/12 h) for ~36 h at 17 ± 2°C. Small aquarium pumps and air-stones fitted inside each core were used to facilitate both water circulation and oxygenation. The re-suspension of sediments was avoided at all times. Following equilibration, the tank water was replaced by ~25 l of unused aerated creek water and left to equilibrate for 2 h under dark conditions before commencement of the flux incubations.

Determination of sediment–water column flux rates

In order to initiate flux determinations, air-stones were removed from the cores, and the water level in each incubation tank was lowered to slightly below that of the core rims. Initial water samples for O_2, C_T, dissolved inorganic nitrogen species and PO_4^{3-} concentrations were collected, and the cores were sealed from the atmosphere using floating plastic lids (Welsh et al., 2001). Cores were incubated under dark conditions for ~1 to 1.25 h, before the aquarium pumps within each core were stopped, the floating lids removed and water samples collected immediately for final time O_2, C_T, dissolved inorganic nitrogen species, and PO_4^{3-} concentrations. Flux rates (μmol m^{-2} h^{-1}) were determined using the initial and final solute concentrations as described in Welsh et al. (2000).

Determination of denitrification and DNRA rates

Following flux incubations, the overlying water within each incubation tank was replaced with fresh

aerated seawater (~40%) so that the cores were resubmerged, the air-stones were replaced, and the cores were then left to equilibrate in the dark for ~2.5 h. To initiate incubations, aeration was stopped and the water level was again lowered to slightly below the rim of the cores. Immediately after the collection of a water sample from each core for the determination of initial nitrate concentration, an addition of a 30-mM 99.9 atom% ^{15}N–NO_3^- (ISOTEC™) stock solution was made to each core to give a final concentration of ~30 μM ^{15}N–NO_3^- in the overlying water column. The water column was mixed and another water sample was taken to enable calculation of the actual ^{15}N–NO_3 addition by difference (Nizzoli et al., 2006). Cores were sealed as described for flux determinations and incubated under dark conditions for ~1 to 1.25 h. Incubation periods were based on the previous determinations of O_2 fluxes such that oxygen concentrations in the overlying water remained within 20% of the initial concentration, a prerequisite of the isotope pairing technique (Nielsen, 1992). At the end of the incubations, the pumps were stopped, floating lids removed and a sub-core (29 mm Ø × 330 mm l) was inserted to the base within each incubation core. Further microbial activity was inhibited by the addition of ~1.2 ml 50% $ZnCl_2$ to the water column outside the sub-core. The sub-core was withdrawn including the overlying water and emptied into a 250-ml bottle containing sufficient powdered KCl to give a final concentration of ~2 M KCl. The sediment–KCl slurry was then stored at 4°C and shaken intermittently over a 24-h period to extract the sediment bioavailable NH_4^+ pool. Sub-samples were then taken, filtered (GF/F; 25 mm Ø, Whatman) and stored frozen (−20°C) until analysed for NH_4^+ concentration and the ^{15}N-enrichment of the NH_4^+ pool (Nizzoli et al., 2006). Remaining sediments within the cores were gently homogenised in order to mix the dissolved N_2 pools in the water column and sediment porewater. Following a brief settling period (~1–2 min), a sub-sample of the water column was transferred to gastight, 12-ml glass vials (Exetainer, Labco), fixed with 150 μl 50% $ZnCl_2$ and stored at 4°C for determination of the dissolved N_2 pool and its isotopic composition. Denitrification rates, D_N (coupled nitrification–denitrification) and D_W (denitrification of NO_3^- diffusing from the overlying water), were determined according to the isotope pairing technique (Nielsen, 1992). DNRA rates based on water column NO_3^- ($DNRA_W$) were calculated from the enrichment of ^{15}N–NO_3^- in the water column and the ^{15}N enrichment of the sediment exchangeable NH_4^+ pool (Risgaard-Petersen & Rysgaard, 1995). Rates of DNRA coupled to sediment nitrification ($DNRA_N$) were estimated from the rate of $DNRA_W$ and the ratio between D_N and D_W (Risgaard-Petersen & Rysgaard, 1995). At the conclusion of the incubations, all sediments from each individual core and the corresponding bottle for bioavailable NH_4^+ extraction were pooled and sieved (250 μm) to collect sediment infauna.

Oxygen consumption and ammonium excretion rates of *V. australiensis*

Victoriopisa australiensis individuals were washed with seawater before being placed in completely filled 0.07 l Wheaton bottles containing either unfiltered seawater or unfiltered seawater + sterile sediment ($n = 9$, respectively). Amphipods incubated in seawater were used to represent free swimming rates of respiration and excretion. The sediment used was sieved (125 μm), cleaned (1% HCl bath 24 h, followed by duplicate Milli-Q 24-h water bath rinse) and dried (80°C 24 h) prior to assays in order to asses the behaviour of burrowing/burrow-dwelling amphipods using sterile substrate. Blank bottles (seawater alone) and control bottles (seawater + sediment alone) were also used to represent water and water + sediment processes, respectively. Bottles were incubated in the dark for ~1.5 h. Oxygen consumption and NH_4^+ excretion rates were calculated from the changes in the water column concentration with time, and corrected for changes in the relevant blank or control incubations.

Sample handling and analytical techniques

Chlorophyll-*a* concentrations within incubation waters were determined spectrophotometrically (665, 750 nm) after acetone extraction and calculations according to Lorenzen (1967). Water samples for nutrient concentrations were collected using acid (HCl 10% v/v) washed and Milli-Q water-rinsed 60-ml syringes and tubing. Samples were filtered (GF/F; 25 mm Ø, Whatman) and stored frozen (−20°C). Dissolved inorganic nutrient concentrations were

determined using an automated colorimetric analyser (Easychem Plus Random Access analyser; Systea Analytical Technologies). Deionised water (Milli-Q element) and filtered (0.2 μm FTBP ISOPORE, Millipore) low-nutrient seawater were used for all sample preparation and analyses. Filtered natural seawater-certified references produced by the Australian National Low Level Nutrient Collaborative Trials were used for quality assurance. Recoveries for all filterable nutrients from the filtered certified seawater references ranged between 90 and 102%. Dissolved gas samples (O_2, N_2 and C_T) were collected carefully with a 50-ml syringe to avoid bubbles and agitation of the water sample. Oxygen and N_2 samples were carefully transferred to gastight 12-ml glass vials (Exetainer, Labco) before being fixed using Winkler reagents (American Public Health Association (APHA), 1998) or 50% $ZnCl_2$, respectively, and sealed and stored at 4°C. Oxygen concentrations were determined using the Winkler titration method with azide modification (American Public Health Association (APHA), 1998) within 48 h of sample collection. Total dissolved inorganic carbon concentrations were determined using a total organic carbon analyser (TOC-V_{CSH}, Shimadzu Corporation). Dissolved N_2 concentrations and the proportions of $^{29}N_2$, $^{30}N_2$ and ^{15}N enrichment of sediment bioavailable ammonium pools were analysed at the National Environmental Research Institute, Silkeborg, Denmark as previously described by Risgaard-Petersen & Rysgaard (1995).

Macrofauna identification

Fauna was rinsed with water to remove any adhering sediment or detritus and preserved in 70% ethanol. Specimens were later identified (species level) and quantified under a low-powered microscope. Individual length (head + thorax + abdomen) and biomass were recorded. Amphipods were dried on tissue paper prior to the determination of individual wet weight ($biomass_{WW}$). Dry weight ($biomass_{DW}$) was determined (80°C for 48 h) before the determination of sample ash-free dry weight ($biomass_{AFDW}$) (550°C for 1 h).

Statistical analyses

Relationships between selected morphological characters of *V. australiensis*; and relationships among individual core amphipod density or biomass and solute flux rates, and nitrate reduction processes were investigated using Pearson correlation analysis. Comparisons of solute flux rates and nitrate reduction processes within categorised amphipod density and biomass groups were analysed by one-way ANOVAs. Data was not transformed. Significant differences between the flux/process values for each density group were compared using non-parametric Tamhane's post hoc analysis. Amphipod oxygen consumption and NH_4^+ excretion rates under differing incubation conditions were compared by one-way ANOVA.

Results

General observations

Sediments were characterised by burrows of varying diameter and density according to the size and abundances of *V. australiensis* present. Targeted macrofauna, *V. australiensis*, was the only species collected, with the exception of one specimen of *Simplisetia aequisetis* (12 mm length and 0.0107 g $biomass_{WW}$), occurring in core nine (amphipod density 3223 ind. m^{-2}). The presence of this worm was excluded from the subsequent analyses relating density/biomass to biogeochemical processes. Morphological characteristics of the retrieved amphipods are shown in Table 1. Amphipod densities ranged between 859 and 3,438 ind. m^{-2} with a mean density of $2{,}067 \pm 836$ ind. m^{-2}. Recorded individual lengths ranged between 1 and 15 mm with a mean length of 6.4 ± 2.9 mm, which significantly correlated ($P < 0.001$) with $biomass_{WW}$, ranging between 0.0173 and 0.1564 g.

Relationships among amphipod abundance, sediment–water column fluxes and N-cycle processes

No differences were observed in the statistical analyses between sediment–water column fluxes and N-cycle process rates and *V. australiensis* populations in terms of either population density or biomass density. Therefore, in the following sections, data are presented only for correlations with population density and categorised population density

Table 1 Morphological characteristics of amphipods within incubation cores (mean length and individual biomass values are given as means ± SD)

Core i.d.	Abundance (ind. core^{-1})	Density (ind. m^{-2})	Biomass$_{WW}$ (g m^{-2})	Mean length (mm)	Mean individual biomass$_{WW}$ (mg)
1	4	859	4.45	6.0 ± 2.7	5.2 ± 5.3
2	12	2,578	21.27	6.0 ± 3.0	8.3 ± 7.0
3	9	1,934	12.35	5.6 ± 2.7	6.4 ± 4.2
4	15	3,223	23.01	6.5 ± 3.5	7.1 ± 7.4
5	8	1,719	9.76	5.0 ± 3.3	5.7 ± 4.8
6	8	1,719	33.61	7.3 ± 2.5	19.6 ± 32.8
7	8	1,719	25.46	9.7 ± 2.0	14.8 ± 7.9
8	4	859	3.72	4.6 ± 0.9	4.3 ± 1.8
9	15	3,223	21.16	6.0 ± 2.4	6.3 ± 6.3
10	14	3,008	19.47	6.2 ± 2.0	6.5 ± 5.7
11	11	2,364	14.72	5.4 ± 3.0	6.2 ± 6.4
12	5	1,074	13.90	9.6 ± 1.0	12.9 ± 4.4
13	13	2,793	14.20	5.2 ± 3.0	5.1 ± 4.9
14	5	1,074	10.74	6.9 ± 3.8	10.0 ± 7.5
15	13	2,793	20.18	6.0 ± 3.3	7.2 ± 7.9
16	6	1,289	11.22	6.8 ± 1.4	8.7 ± 2.4
17	8	1,719	30.28	6.9 ± 2.1	17.6 ± 33.6
18	13	2,793	19.40	6.8 ± 2.8	6.9 ± 5.9
19	9	1,934	14.07	6.1 ± 4.4	7.3 ± 8.3
20	16	3,438	26.30	6.9 ± 3.5	7.7 ± 7.9
21	6	1,289	9.84	8.3 ± 1.4	9.2 ± 3.3

ranges, as all trends with biomass density were very similar.

Dissolved oxygen and inorganic carbon fluxes

Sediment oxygen demand (SOD) ranged from 0.91 to 3.6 mmol m^{-2} h^{-1}, with a mean value of 2.23 ± 0.95 mmol m^{-2} h^{-1} (Fig. 1a). Increased SOD at categorised densities: 1,501–2,500 ind. m^{-2} (P = 0.008) and 2,501–3,500 ind. m^{-2} (P = 0.021) were significantly greater in comparison to the SOD at the lowest categorised density (500–1,500 ind. m^{-2}), but no significant direct correlation was observed between SOD and individual core amphipod densities (Table 2).

Dissolved inorganic carbon fluxes (C_T) ranged from 1.15 to 7.7 mmol m^{-2} h^{-1} with a mean value of 3.3 ± 1.48 mmol m^{-2} h^{-1} and a weak, but significant correlation was observed between amphipod densities and C_T flux values (Table 2). Mean C_T production rates at theoretical zero (y-axis intercept from linear regression of density against flux) amphipod density was less than half that at 2,500–3,500 ind. m^{-2} (Fig. 1b), but no significant differences were observed between the mean C_T production rates at the categorised amphipod densities. The community respiratory quotient under dark conditions (CRQ: C_T production/O_2 consumption) ranged between 0.6 and 2.9 with a mean value of 1.6 ± 0.6. No significant differences were observed between mean CRQ values at the categorised amphipod densities (Fig. 1c) and no significant correlation was observed with amphipod density (Table 2).

Dissolved inorganic nutrient fluxes

There were net effluxes of all dissolved inorganic nutrients for all categorised *V. australiensis* densities (Fig. 2). NO_x (NO_2^- ± NO_3^-) fluxes ranged from 27.2 to 73.8 μmol N m^{-2} h^{-1}, with NO_3^- contributing 74.3 ± 48.1% to all observed NO_x fluxes (Fig. 2a). No significant differences were observed

Table 2 Relationships between *V. australiensis* density (ind. m^{-2}) and benthic solute fluxes, and nitrate reduction pathways within surface sediments of Coombabah Lake (*correlation significant at the 0.05 level (2-tailed))

Flux/Process	Slope (μmol ind^{-1} h^{-1})	R^2	P
O_2	−0.4573	0.1634	0.069
C_T	0.9261	0.2742	0.015*
CRQ	0.0000	0.0009	0.889
NO_2^-	−0.0005	0.0041	0.783
NO_3^-	0.0053	0.0028	0.819
NO_x	0.0047	0.0021	0.843
NH_4^+	0.0877	0.1156	0.131
DIN	0.0924	0.0919	0.181
PO_4^{3-}	0.0008	0.0057	0.745
D_N	0.0000	0.0004	0.929
D_W	0.0000	0.0210	0.569
D_{14}	0.0000	0.0005	0.926
$DNRA_N$	0.0004	0.0108	0.654
$DNRA_W$	0.0000	0.0322	0.419
$DNRA_{Total}$	0.0003	0.0064	0.730
Total NO_3^- reduction	0.0052	0.0025	0.830
Nitrification	0.0003	0.0039	0.789

between categorised density group, NO_x effluxes or those of NO_2^-or NO_3^- (Data not shown). Nor were any significant correlations observed between amphipod density categories and NO_2^-, NO_3^- or NO_x fluxes (Table 2). Individual core NH_4^+ fluxes ranged from −98.67 to 672.09 μmol N m^{-2} h^{-1} but showed no significant correlation with amphipod density (Table 2). There was a trend for higher NH_4^+ effluxes at higher categorised amphipod densities (Fig. 2b), but these differences between groups were not significant. Mean DIN fluxes ranged from 250.4 μmol N m^{-2} h^{-1} to 427.9 μmol N m^{-2} h^{-1} for categorised density groupings (Fig. 2c) and followed similar trends to NH_4^+, which dominated DIN effluxes with a mean contribution of 80.7%. No significant correlation was observed between DIN effluxes and amphipod density (Table 2) or between the contributing value of NO_x to DIN and amphipod density (data not shown). Although there was a trend of increased DIN efflux at higher categorised animal densities, this trend was again not significant. Phosphate fluxes were highly variable and showed no significant trends (Fig. 2d and Table 2).

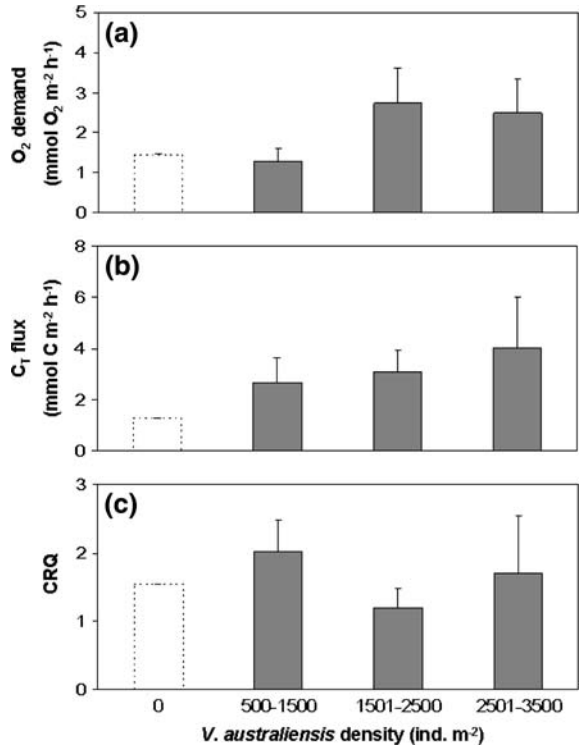

Fig. 1 (**a**) Sediment oxygen demand; (**b**) total dissolved inorganic carbon (C_T) fluxes at the sediment–water interface; and (**c**) community respiratory quotient at theoretical zero abundance and categorised *V. australiensis* density ranges under dark incubation conditions. Theoretical 0 density (0 ind. m^{-2}) value was estimated as the *y*-axis intercept of the linear regressions with amphipod density ($n = 21$). Categorised density values are given as means and error bars represent one standard deviation (SD)

Denitrification and dissimilatory nitrate reduction to ammonium

Rates of total denitrification ($D_{14} = D_N + D_W$) ranged between 1.00 and 9.79 μmol N m^{-2} h^{-1}. Total denitrification rates neither significantly correlated with amphipod densities (Table 2) nor were they significantly different between categorised amphipod density groups (Fig. 3a). The mean coupled nitrification–denitrification (D_N) rate was 4.42 ± 2.45 μmol N m^{-2} h^{-1}, and accounted for almost all of the measured denitrification (98.7 ± 0.8%). The very low contribution of D_W (0.02 ± 0.08 μmol N m^{-2} h^{-1}) was presumably a result of the initial low NO_3^- concentrations in the incubation waters. Mean D_N rates of the categorised density groups ranged between

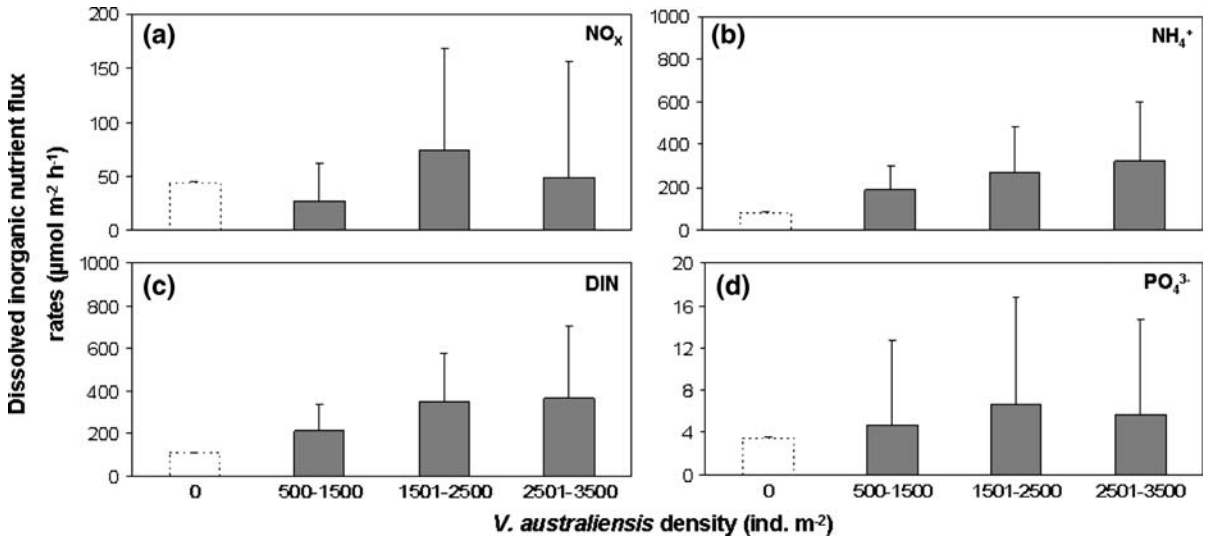

Fig. 2 Comparison of dissolved inorganic nutrients (NO_x, NH_4^+, DIN and PO_4^{3-}) fluxes across the sediment–water interface at theoretical zero and categorised *V. australiensis* densities under dark incubation conditions. The theoretical 0 density (0 ind. m^{-2}) value was estimated as the *y*-axis intercept of the linear regressions with amphipod density ($n = 21$). Flux rates are given as means and error bars represent one SD

4.03 and 4.45 µmol N m^{-2} h^{-1} (Fig. 3a) and were not significantly different. $DNRA_{Total}$ ($DNRA_N$ + $DNRA_W$) rates were not significantly different between categorised amphipod densities and ranged between 1.98 and 3.86 µmol N m^{-2} h^{-1} (Fig. 3b). Mean rates of DNRA coupled to sediment nitrification ($DNRA_N$) ranged from 1.96 to 3.83 µmol N m^{-2} h^{-1} (Fig. 3b). Nitrate diffusing from the water column contributed little (0.2–3.3%) to $DNRA_{Total}$ rates, and no significant differences were observed between categorised density groups (Fig. 3b). Similarly, there was no significant correlation between rates of DNRA and amphipod density (Table 2). Total NO_3^- reduction rates (D_{14} + $DNRA_{Total}$) ranged between 1.45 and 19.96 µmol N m^{-2} h^{-1}, but showed no significant correlation with amphipod density (Table 2). Mean total nitrate reduction rates of the categorised density group demonstrated no significant differences and ranged from 6.33 to 7.93 µmol N m^{-2} h^{-1}. Recycling of N within the sediment in the form of NH_4^+ through DNRA was comparable to the loss of N through denitrification (Fig. 3c). The mean contribution of $DNRA_{Total}$ to the rates of total NO_3^- reduction ranged between 31.68 and 39.70% and was not significantly different between categorised density groups. Nitrification rates for each individually incubated core were calculated by mass balance as the sum of the NO_x efflux, plus the measured rates of D_N and $DNRA_N$. Nitrification rates were not significantly different between categorised density groups and ranged between 33.44 ± 35.42 µmol N m^{-2} h^{-1} (500–1,500 ind. m^{-2}) to 82.86 ± 91.31 µmol N m^{-2} h^{-1} (1,500–2,500 ind. m^{-2}). No significant correlation was observed between amphipod densities and nitrification rates (Table 2).

Oxygen consumption and NH_4^+ excretion rates of *V. australiensis*

Mean O_2 consumption rates of *V. australiensis* were 2.72 ± 0.73 µmol O_2 ind^{-1} d^{-1} for burrow-dwelling and 3.76 ± 2.22 µmol O_2 ind^{-1} d^{-1} for free-swimming amphipods with an overall mean rate of 3.24 ± 1.69 µmol O_2 ind^{-1} d^{-1}. No significant difference was observed between incubation conditions and NH_4^+ excretion rates with an overall mean amphipod NH_4^+ excretion rate of 0.31 ± 0.19 µmol N ind^{-1} d^{-1} and based on amphipod densities measured in the incubation cores, could account for <1 to ~3% of the determined gross sediment NH_4^+ effluxes. In comparison, amphipod respiration could have maximally accounted for between ~2 and 6% of

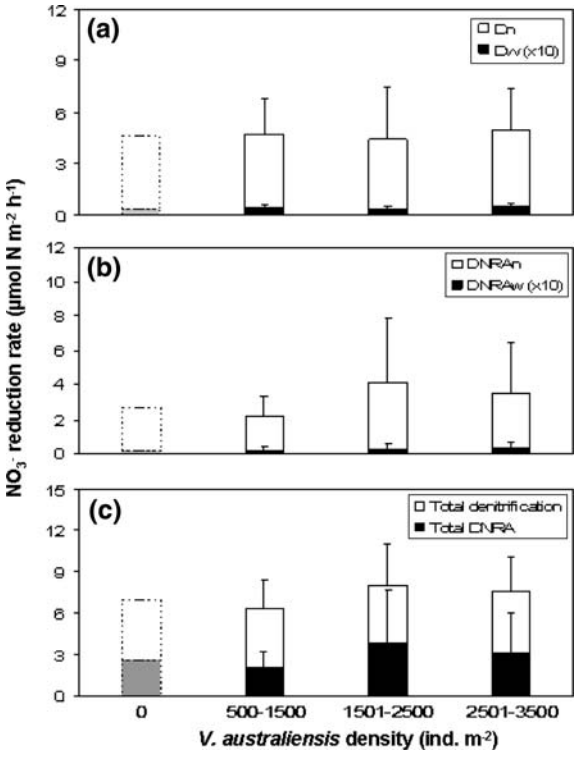

Fig. 3 Rates of nitrate reduction processes during dark incubation conditions for theoretical zero and categorised amphipod density ranges: (**a**) Denitrification rates based on nitrate diffusing from the water column (D_W) and coupled to nitrification (D_N); (**b**) DNRA rates based on diffusion of nitrate from the water column ($DNRA_W$) and coupled to nitrification ($DNRA_N$); and (**c**) Total nitrate reduction rates via denitrification and DNRA. The theoretical 0 density (0 ind. m^{-2}) values for each process were determined as the *y*-axis intercept of the regression analyses. Values are given as means and error bars represent one SD

the total SOD, based on the O_2 consumption of free-swimming individuals or more probably, ~1 and 2.5% based on the respiration rate of the burrow-inhabiting individuals.

Sediment bioavailable ammonium pools

Sediment bioavailable NH_4^+ concentrations were determined following solute flux incubations as required for the calculation of rates of DNRA. Integrated for the entire sediment depth, bioavailable NH_4^+ pools were greater in cores characterised by lower amphipod densities. Mean bioavailable NH_4^+ concentrations of the categorised density ranges were 41.86 ± 31.12 (500–1,500 ind. m^{-2}), 36.54 ± 22.83 (1,501–2,500 ind. m^{-2}) and 24.30 ± 2.54 µmol N l^{-1} (2,501–3,500 ind. m^{-2}), but were not significantly different between these groups.

Discussion

Influence on benthic metabolism and DIN fluxes

All measures of overall sediment metabolism (SOD, sediment DIC and DIN effluxes) increased with amphipod density, with rates at the highest categorised density of 2,500–3,500 ind m^{-2} being 70–220% greater than the theoretical values at zero density (*y*-axis intercept of regressions shown in Table 2), but these increases were only statistically significant for SOD, and only DIC effluxes showed a significant correlation with amphipod density (Table 2). These results are consistent with studies of other small amphipod species employing undisturbed, intact sediment cores (Rysgaard et al., 1995; Nizzoli et al., 2002; Nizzoli, 2003). In contrast, mesocosm studies of amphipods often report large, statistically significant effects (e.g. Pelegrí et al., 1994; Pelegrí & Blackburn, 1995; Autio et al., 2003; Karlson et al., 2007). Such contrasting results between studies of natural populations using benthic chambers or undisturbed sediment cores and mesocosms are not restricted to amphipods, as in general, with the exception of filter-feeding organisms, which induce large effects in both types of study (e.g. Doering et al., 1987; Pelegrí & Blackburn, 1995; Bartoli et al., 2001; Nizzoli et al., 2006), mesocosm experiments consistently report larger faunal impacts on benthic metabolism and sediment–water column nutrient fluxes. These differences between the incubation types may depend on several factors.

First, the faunal densities added to experimental mesocosms tend to be in the higher part of the range or even greater than those which naturally occur in the corresponding ecosystem, and therefore, the mesocosms tend to exaggerate the in situ impacts of the fauna. Second, the homogenisation of the sediments during the preparation of mesocosms removes heterogeneity in the distribution of other factors such as large particulate organic matter and microphytobenthos, which also influence SOD and nutrient exchanges. For example, the microphytobenthos will influence benthic oxygen, DIC and nutrient

exchanges under both light and dark conditions via photosynthesis, respiration and photoassimilation of inorganic nutrients, and rates of SOD and nutrient fluxes in situ have been shown to correlate with small-scale heterogeneity of microphytobenthos biomass (Bartoli et al., 2003). Consequently, in situ heterogeneity in microphytobenthos and particulate organic matter distributions, and both positive and negative interactions between these and faunal distributions would enhance the overall spatial variability, obscuring direct statistical correlations with fauna densities. Finally, the homogenisation of the sediments used in mesocosms creates an artificial situation where faunal effects on benthic metabolism and nutrient regeneration rates coupled to this metabolism principally reflect the capacity of faunal populations to enhance rates of organic matter turnover, whereas in natural sediments faunal effects may be more closely coupled to their capacity to enhance organic matter inputs to the sediment (Kristensen, 2000; Welsh, 2003).

Several studies have demonstrated that faunal stimulations of benthic metabolism in mesocosms is primarily due to enhanced turnover of the older, more recalcitrant organic matter pools already present in the sediment (Andersen & Kristensen, 1988, 1992; Kristensen et al., 1992; Andersen, 1996). Mesocosm experiments, therefore, may represent a better simulation of colonisation events than that of permanently inhabited sediments (Welsh, 2003), as in permanently inhabited sediments, prolonged stimulation of organic matter turnover would deplete these finite organic matter pools. Therefore, in natural sediments with persistent macrofaunal communities, these recalcitrant organic matter pools may be largely exhausted and fauna could only cause a significant stimulation of benthic metabolism if they caused a similar stimulation of sediment organic inputs in order to fuel this increased metabolism (Kristensen, 2000; Welsh, 2003; Papaspyrou et al., 2007).

Filter-feeding infauna, which actively harvest particulate organic matter from the overlying water and deposit much of it in the sediment as faeces or pseudofaeces, can induce massive deposition of organic matter (Vedel et al., 1994; Graf & Rosenberg, 1997; Norkko et al., 2001; Nizzoli et al., 2005), and have been shown to greatly enhance the sediment metabolism both in situ and during mesocosm incubations (Doering et al., 1987; Pelegrí et al., 1995; Christensen et al., 2000; Bartoli et al., 2001; Nizzoli et al., 2006). Similarly organisms such as some callassinid shrimps, which collect large particulate organic detritus at the sediment surface and transport this into their burrow networks (e.g. Kneer et al., 2008; Vonk et al., 2008), would also be expected to cause a local stimulation of benthic metabolism. In contrast, although deposit feeders such as *Victoriopisa australiensis*, the subject of this study, would not be expected to directly influence the sediment organic matter inputs via these mechanisms, they could increase the inputs by more subtle, indirect mechanisms.

The feeding pits, towers, tubes and other structures formed by infauna at the sediment surface interact with water movements indirectly, enhancing organic matter inputs by increasing the entrainment and sedimentation of particles within the benthic boundary layer (See Graf & Rosenberg, 1997 for review). Additionally, transport of suspended particles into animal burrows during irrigation cycles leading to their sedimentation during periods of water stagnation could also increase sediment organic matter inputs, as deposit feeders typically only intermittently irrigate their burrows (Kristensen, 1989; Riisgård, 1991; Forster & Graf, 1995; Christensen et al., 2000), as unlike filter feeders which irrigate in order to feed, deposit feeders rely on these irrigation flows only to supply oxygen for their respiratory needs (Welsh, 2003). For example, deposit-feeding *Nereis virens* irrigates its burrow for periods of 5–8 min separated by resting phases of approximately 30 min (Kristensen, 1989). Whereas when suspension feeding, the closely related *Nereis diversicolor* irrigates its burrow semi-continuously with only short pauses of a few minutes (Riisgård, 1991). Not only does *N. diversicolor* irrigate its burrow more continuously than *N. virens*, but also irrigation rates for similar-sized individuals of *N. diversicolor* are approximately 4-fold higher than those for *N. virens* (Christensen et al., 2000). Consequently, in mesocosm experiments comparing *N. diversicolor* or *N. virens* at similar population and biomass densities, total sediment irrigation rates were estimated to be 15-fold greater for suspension-feeding *N. diversicolor* compared to deposit-feeding *N. virens* populations (Christensen et al., 2000).

Such mechanisms of indirect biodeposition of organic matter would be quantitatively less important than direct biodeposition by filter feeders (Graf & Rosenberg, 1997), but the fact that the low to moderate population densities of deposit-feeding

V. australiensis in the studied sediment caused an overall stimulation of benthic metabolism as measured by SOD, DIC and DIN fluxes of between 70 and 220% indicates that the presence of the amphipod burrows, and the pits and tubes they form at the sediment surface was sufficient to enhance the organic matter deposition to a measurable extent. Additionally, the degree of stimulation of benthic metabolism measured in our study is of a similar level to that recorded in undisturbed sediments colonised by other small amphipods (Rysgaard et al., 1995; Nizzoli et al., 2002; Nizzoli, 2003), indicating that such effects may be common and it may well be true that most, if not all, burrowing infauna (and not just filter-feeders) do enhance metabolic rates in natural sediments at least to a limited extent. Indeed, from an evolutionary point of view, it would benefit infaunal deposit feeders to construct burrows and burrow-associated structures that induce increased rates of organic matter deposition to the sediments they inhabit, since ultimately this organic matter represents their own food source.

Our results also highlight the problems associated with extrapolating data from mesocosm simulations of faunal effects to the real world, as the homogenised sediments employed in mesocosms studies simplify the study of faunal effects by removing other sources of heterogeneity. This homogeneity creates an artificial situation where both colonised and uncolonised sediments have quantitatively and qualitatively similar organic matter pools, resulting in the influences of fauna on organic matter turnover rates being exaggerated. In contrast, natural permanently inhabited sediments would have quantitatively and qualitatively different organic matter pools to uninhabited sediments due to prolonged stimulation of organic matter turnover, and faunal influences would be mainly dependent on the effect of the fauna on sediment organic matter inputs. Thus, although mesocosm simulations provide a powerful tool to investigate the mechanisms by which fauna can influence sediment biogeochemistry, only studies of natural populations in undisturbed sediments can assess the degree to which such mechanisms operate in situ.

Influence of amphipod respiration and nutrient excretion on benthic fluxes

Benthic infauna can directly contribute to SOD and DIN fluxes through the consumption of oxygen for their respiratory needs and the excretion of inorganic metabolic waste products. However, parallel mesocosm studies and incubations of individual animals to determine respiration and ammonium excretion rates indicate that faunal stimulations of SOD and DIN effluxes are primarily due to increased microbial metabolism in the sediment, mass transport during bioturbation and the increased surface area of sediment available for diffusive exchanges with the water column offered by burrow walls. Conversely, the respiratory oxygen demand and ammonium excretion rates of the animals themselves can only account for a relatively small proportion of the increased SOD and DIN fluxes measured in the animal addition mesocosms (Pelegrí & Blackburn, 1994; Hansen & Kristensen, 1997; Bartoli et al., 2000; Jordan et al., 2009). Our data support this hypothesis that the effects of fauna on SOD and DIN effluxes are mainly due to their physical rather than physiological activities, as the determined respiratory oxygen consumption and ammonium excretion rates of *V. australiensis* individuals inhabiting burrows in acid washed, rinsed and sterilised sands represented only 24 and 15% of the per individual stimulations in SOD and ammonium effluxes shown in Table 2. Additionally, the significantly higher respiration rates observed during this study for free swimming compared to burrow-dwelling individuals of *V. australiensis* demonstrate that care must be taken to recreate 'natural' conditions for such incubations, if extrapolations of data to natural or mesocosm populations are to be accurate.

Effects of *V. australiensis* on nitrogen cycling processes

No significant correlations were observed between amphipod population density and any of the determined or estimated N-cycle processes, with all regression slopes being close to zero, with *P*-values ranging between 0.42 and 0.93 (Table 2). These results are in contrast with the vast majority of mesocosm studies of surface and sub-surface deposit feeders, which have generally reported large stimulations of nitrification rates and denitrification rates (e.g. Pelegrí & Blackburn, 1994, 1995; Pelegrí et al., 1994; Bartoli et al., 2000), although this is not always the case (e.g. Karlson et al., 2007). Whereas studies of undisturbed sediments yield more variable data

with both large stimulations and zero effects having been reported (Rysgaard et al., 1995; Bartoli et al., 2003; Webb & Eyre, 2004).

Burrowing fauna are proposed to influence nitrification and nitrate reduction rates by increasing the total surface area of the sediment–water and sediment oxic–anoxic interfaces due to the presence of their burrows and the transport of oxygen- and nitrate bearing-water to these surfaces during burrow irrigation (Welsh, 2003 and references therein). The transported nitrate can diffuse across the burrow wall and oxic–anoxic interfaces to support denitrification and DNRA in the underlying anoxic sediment. Similarly, oxygen diffusing into the burrow wall can support aerobic nitrifying bacteria, and the nitrate generated during ammonium oxidation can subsequently diffuse across the sediment oxic–anoxic interface to fuel nitrate reduction processes. Estimates for natural estuarine infauna populations indicate that their presence increases the total sediment surface available for diffusive exchanges by between 150 and 700% (Hylleberg & Henriksen, 1980; Kristensen, 1984; Forster & Graf, 1992; Davey, 1994; Fenchel, 1996; Webb & Eyre, 2004), and both burrow wall sediments and even the surfaces of the animals themselves can be heavily colonised by nitrifying bacteria (Mayer et al. 1995; Welsh & Castadelli, 2004). For example, Hylleberg & Henriksen (1980) estimated that burrows of the amphipod *C. volutator* at densities up to 6,000 ind. m^{-2} enhanced the volume of oxic sediment amenable to nitrification by 100–150%.

Victoriopisa australiensis forms simple 'Y'-shaped burrows, and a recent study has shown that these burrows are lined by a layer of oxidised sediment (Robertson et al., 2009). Therefore, this amphipod would be expected to stimulate rates of nitrate reduction processes linked to water column nitrate (D_W and $DNRA_W$) and nitrification rates due to diffusion of nitrate and oxygen, respectively, from the burrow water into the sediments lining the burrows. Increased nitrification would subsequently stimulate nitrate reduction rates coupled to this process (D_N and $DNRA_N$). Yet not only were there no significant differences in any of these process rates between categorised amphipod density groups, but also the slopes of all regressions of process rates against amphipod density for the 21 incubated cores were all very close to zero. This lack of effect may be related to the burrow irrigation cycles of *V. australiensis* and the low concentration of nitrate present in the water column.

Although the burrow irrigation habits of *V. australiensis* have not been directly studied, it would be expected to consist of relatively short periods of irrigation, separated by relatively long pauses, as has been observed for other sub-surface deposit feeders (Kristensen, 1989; Riisgård, 1991; Forster & Graf, 1995; Christensen et al., 2000). As a result, the quantities of nitrate and oxygen transported into the amphipod burrows would be expected to be relatively small and to be rapidly depleted during the rest periods (Forster & Graf, 1992, 1995; Welsh, 2003; Wenzhofer & Glud, 2004). Thus, due to its low concentration in the overlying water, little additional nitrate may be available to support D_W and $DNRA_W$ in the burrow wall sediments, resulting in the observed lack of correlation between these processes and amphipod density. Similarly, oxygen concentrations in the burrow water may rapidly decline during long pauses in the irrigation cycle, leading to only limited availability in the burrow walls, and nitrifiers may be out competed for this oxygen by aerobic heterotrophs and other chemoautotrophs, which generally have a higher affinity for oxygen than nitrifying bacteria (Sharma & Ahlert, 1977; Laanbroek & Gerards, 1993). We also observed a decrease in sediment bioavailable ammonium pools with increasing *V. australiensis* density, and therefore, ammonium may also have been a limiting substrate for nitrification in the burrow wall sediments. Therefore, the lack of stimulation of nitrification rates with amphipod population density may be because the burrow wall sediments are not significantly colonised by nitrifying bacteria and this in turn would explain the lack of stimulation of D_N and $DNRA_N$, which are fuelled by nitrate production via nitrification.

Population density of *V. australiensis* also had no discernable influence on the partitioning of nitrate between denitrification and DNRA, with DNRA representing a similar proportion of total nitrate reduction in all categorised density ranges and the proportion of nitrate reduced via this pathway showing no correlation with amphipod density during regression analyses. It has been proposed that DNRA will be a more important pathway for nitrate reduction in organic rich, reduced, highly metabolic sediments where ratios of potential electron donors

to respiratory electron acceptors are high (Tiedje, 1988; Dalsgaard & Bak, 1994; Childs et al., 2002; Nizzoli et al., 2006). Previous studies (Brunet & Garcia-Gil, 1996; An & Gardner, 2002, Christensen et al., 2003) have proposed that free sulphides play a critical role in the switch in nitrate reduction away from denitrification towards DNRA, through direct inhibition of denitrification and provision of an electron donor for DNRA. In reality, however, these two factors are difficult to separate as organic matter rich, highly metabolic marine sediments will also tend to be richer in free sulphides (Nizzoli et al., 2006). In the studied sediment, although total benthic metabolism did increase with increasing abundance of *V. australiensis*, these increases were relatively small and there was no corresponding shift in community respiratory quotient with amphipod density, indicating that amphipod abundance did not influence the overall balance between aerobic and anaerobic microbial metabolisms. Therefore, it is unlikely that the amphipods caused any significant changes in the sediment characteristics such as redox status or free sulphide concentrations, which are proposed to regulate the partitioning of nitrate between denitrification and DNRA.

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