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Influence of *Trypaea australiensis* population density on benthic metabolism and nitrogen dynamics in sandy estuarine sediment: A mesocosm simulation

Mark A. Jordan [a], David T. Welsh [a,*], Ryan J.K. Dunn [b], Peter R. Teasdale [a]

[a] *Australian Rivers Institute and School of Environment, Griffith University Gold Coast campus, Qld 4222, Australia*
[b] *Griffith School of Engineering, Griffith University Gold Coast campus, Qld 4222, Australia*

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ABSTRACT

Laboratory mesocosm incubations were undertaken to investigate the influence of natural densities of the thalassinidean shrimp, *Trypaea australiensis* (marine yabby) on sediment oxygen demand (SOD), inorganic nutrient fluxes, and the N-cycle processes of nitrification, denitrification and dissimilatory nitrate reduction to ammonium (DNRA). Mesocosms (~0.1 m^2×55 cm deep) of sieved, natural *T. australiensis* inhabited sands were continually flushed with fresh seawater and pre-incubated for two weeks prior to being assigned to one of three treatments; control (no additions), low yabby density (40 *T. australiensis* m^{-2}) or high yabby density (80 *T. australiensis* m^{-2}). Thereafter, SOD and sediment–water column inorganic nutrient fluxes were determined periodically over a 38 day period. On the final day rates of denitrification and dissimilatory nitrate reduction to ammonium (DNRA) were also determined using the ^{15}N-isotope pairing technique.

Yabbies consistently and significantly ($p<0.001$) stimulated SOD over the entire 38 day incubation period (mean values: 4.92, 9.21 and 14.9 mmol m^{-2} day^{-1} for control, low and high density treatments, respectively). The increased organic matter mineralisation rates greatly enhanced nitrogen regeneration rates in the sediment and fuelled significantly higher effluxes of dissolved inorganic nitrogen with NH_4^+ and total DIN effluxes in the low and high density treatments respectively, being 617 and 1534%, and 269 and 565% higher than those in the controls, despite sediment bioavailable (porewater+exchangeable) NH_4^+ pools being approximately 2 and 4-fold lower in the low and high density yabby treatment sediments compared to the controls, measured at the end of the 37 day experiment.

Mass balance calculations based on the final day nutrient flux and nitrate reduction rate data demonstrated that yabbies stimulated benthic nitrification rates by 31 and 46% in the low and high density treatments. However, somewhat surprisingly *T. australiensis* population density had no effect on rates of denitrification and DNRA despite the higher rates of nitrification and higher equilibrium water column nitrate concentration. Indeed, nitrate reduction processes became an increasingly unimportant element with increasing yabby density with for example, N_2 generated by coupled nitrification–denitrification representing 11.5, 5.2 and 2.8% of the total inorganic-N recycled to the water column in the control, low density and high density yabby treatments, respectively. Overall, the major influence of *T. australiensis* in the studied low organic matter content, sandy sediments was to enhance coupling between the benthic and pelagic systems through increased rates of inorganic nitrogen regeneration in the sediment and enhanced export of this nitrogen to the water column.

Our results also suggest that the influences of organisms such as *T. australiensis* which form deep, extensive and complex burrow systems where irrigation rates differ greatly between different burrow sections, may be more complex than those recorded for infauna which form simple U-shaped burrows. Additionally, there may be a strong interaction between faunal effects and the sediment physical and biological environment and thus the same species may have contrasting influences in different sediment types.

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1. Introduction

Benthic infauna are ubiquitous in shallow marine environments and can have profound effects on sediment metabolism and nutrient dynamics (Kristensen, 2000; Welsh, 2003). In estuarine sediments, faunal burrows increase the area of the sediment–water and sediment oxic–anoxic interfaces, and the volume of oxic sediment by an estimated 150–500% (Welsh, 2003 and references therein), enhancing microbial degradation of organic matter and nutrient regeneration rates (Kristensen et al., 1992; Kristensen, 2000; Kristensen and Mikkelsen, 2003; Papaspyrou et al., 2004). Sediment reworking, the

* Corresponding author. Tel.: +61 7 55529186; fax: +61 7 55528067.
E-mail address: d.welsh@griffith.edu.au (D.T. Welsh).

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large surface area of burrow walls and burrow irrigation can greatly enhance transport of O_2 and inorganic nutrients from the water column and the effluxes of metabolic end-products to the overlying water (Welsh, 2003; Kogure and Wada, 2005; Nizzoli et al., 2007). Moreover, burrow walls and even the surfaces of the animals themselves provide substrates for bacterial communities, including specific microbial groups such as nitrifying bacteria (Mayer et al., 1995; Welsh and Castadelli, 2004). Consequently, benthic fauna can not only influence the magnitude of sediment–water column fluxes of dissolved nitrogen, but also the relative proportions of the N-species making up this flux (Welsh, 2003).

To date the majority of studies on the influence of fauna on sediment biogeochemistry have focused on polychaetes (e.g. Pelegrí and Blackburn, 1995; Hansen and Kristensen, 1997; Bartoli et al., 2000; Kristensen and Mikkelsen, 2003) and to a lesser extent bivalves and amphipods (Pelegrí et al., 1994; Pelegrí and Blackburn, 1995; Mortimer et al., 1999; Marinelli and Williams, 2003). However, few studies have investigated the influence of large, deep burrowing, deposit feeders such as the thalassinidean shrimp, *Trypaea australiensis* which is a conspicuous and often dominant member of the benthic community in east Australian estuaries (McPhee and Skilleter, 2002; Katrak and Bird, 2003; Contessa and Bird, 2004). Sub-surface deposit feeders are intense bioturbators that influence organic matter mineralisation via physical breakdown and burial (Kristensen, 2000; Kristensen and Mikkelsen, 2003; Welsh, 2003; Papaspyrou et al., 2004). Thalassinidean shrimps are typical examples of sub-surface detritivores, which characteristically dig complex burrows up to 150 cm deep (Dworschak et al., 2005). For example, *T. australiensis*, commonly known as the marine yabby, constructs burrows reaching depths of 85–120 cm with burrow diameters of 12 mm (Kenway, 1981). At a density of 60–200 individuals m^{-2} (Kenway, 1981; Kerr, 2001; Katrak and Bird, 2003), the burrow networks of *T. australiensis* have been estimated to increase the area of the sediment–water interface by 230–730% (Webb and Eyre, 2004). Consequently, due to their intense bioturbation, extensive burrow networks and the irrigation of these burrows, *T. australiensis* populations would be expected to exert a strong influence on sediment metabolism and nutrient regeneration rates, sediment–water column fluxes and nitrogen processing in the sediment.

In this study we have investigated the effects of *T. australiensis* population density on sediment metabolism and nutrient dynamics during long-term continuous flow mesocosm incubations. The aim of this investigation was to determine the influence of this important, large, deep burrowing bioturbator on sediment biogeochemistry and for the first time quantitatively assess the influence of infauna on the partitioning of nitrate between denitrification and dissimilatory nitrate reduction to ammonium (DNRA).

2. Materials and methods

2.1. Sediment collection site, mesocosm preparation and experimental design

Sediment was collected from a site (27° 58′ S, 153° 25′ E) within the intertidal zone of the Gold Coast Broadwater, a coastal lagoon in southeast Queensland, Australia. The site was considered to be good habitat for *T. australiensis* as numerous burrows were evident. The top ~25 cm of sand was collected, homogenised and sieved (1 mm) to remove large fauna and debris. Five sediment sub-samples were collected and frozen for analysis of particle size, moisture content, chl-*a* and organic carbon content. The sieved sand was transferred into nine 550 mm×360 mm diameter (~56 L) plastic mesocosms up to ~75% capacity. Mesocosms were placed in a constant temperature room at 22±2 °C, filled with seawater that was replenished at ~20 L day^{-1}, aerated and maintained under constant darkness for two weeks to equilibrate.

Following this period, triplicate mesocosms were assigned to control (no addition), low density (4 yabbies added ≈40 individuals m^{-2}) or high density (8 yabbies added ≈80 individuals m^{-2}) treatments. The added yabbies had a mean individual weight of 3.03±0.33 s.e. g wet weight and were harvested on the day of the experiment from an assigned harvesting zone ~1 km from the sediment collection site. With few exceptions, all yabbies burrowed readily and had constructed permanent burrows within 1–2 days, although two mortalities occurred during the colonisation period, and these were replaced with new individuals.

Thereafter, mesocosms were incubated as described for the stabilisation period over a 38 day period. Sediment–water column oxygen and nutrient fluxes were determined on the day prior to yabby additions and on days 1, 3, 4, 6, 8, 11, 16, 22, 29 and 38 after additions. Following the final flux determinations on day 38, rates of sediment nitrate reduction processes were determined.

2.2. Sediment–water column fluxes

Oxygen and dissolved inorganic nitrogen (DIN) fluxes were determined during dark incubations. To initiate the incubations, aeration and the water flow to the mesocosms were interrupted, and the water level in the mesocosms was lowered by ~4 cm. Small submerged water pumps housed within each mesocosm were switched on to ensure mixing of the water column during incubation and initial water samples for O_2 and DIN concentrations were taken. The water in the mesocosms was isolated from the atmosphere using floating plastic lids and mesocosms were incubated in the dark for 2.5–4 h (actual time depended on the sediment oxygen demand and was adjusted to ensure that the final O_2 concentration remained above 80% of the initial concentration).

At the end of the incubation the floating lids were removed and final samples for O_2 and DIN were immediately collected. Flux rates were calculated from the change in water column concentrations of the individual solutes as outlined by (Welsh et al., 2000).

2.3. Determination of yabby ammonium excretion rate

Pre-weighed individuals were transferred to completely filled 0.5 L Wheaton bottles ($n=5$) and samples were collected to determine the initial NH_4^+ concentration. Bottles were incubated in the dark for two hours and final time samples collected for NH_4^+ analysis. Excretion rates per gram (wet weight) were calculated from the change in NH_4^+ concentration as described by (Bartoli et al., 2000).

2.4. Determination of denitrification and DNRA rates

Following the final flux measurements on day 38, the mesocosms were flooded with fresh seawater and aerated for two hours prior to determination of denitrification and DNRA rates. To initiate rate measurements, the water and air flows to the mesocosms were interrupted and the water level within the mesocosms lowered as before, and samples collected for determination of ambient water column NO_3^- concentrations. Sufficient volume of a 30 mM 99.9 at.% ^{15}N–NO_3^- stock solution was added to give a concentration of ~30 µM in the overlying water. The water was mixed and another sample was taken ~5 min later to enable calculation of the actual ^{15}N–NO_3 addition. Following a pre-incubation of ~30 min to allow diffusion of labelled ^{15}N–NO_3 to the denitrification zone, mesocosms were isolated from the atmosphere using floating plastic lids and incubated as before for 2.5–4 h. The incubation time was based on the previous determinations of O_2 fluxes and chosen to ensure that the water column O_2 concentration remained above 80% of the initial value, as is necessary for accurate use of the isotope pairing technique (Nielsen, 1992).

At the end of the incubation, a sub-core (8 cm diameter) was inserted into the central part of the mesocosm until it reached the base and 15 mL 50% $ZnCl_2$ was added to the water outside the sub-core

Table 1
Mean ± s.e. water column dissolved NO_3^-, NO_2^-, NH_4^+ and DIN concentrations prior to the addition of yabbies (day 0) and over various periods of the mesocosm incubations

Day	Density	NO_3^- (μM)		NO_2^- (μM)		NH_4^+ (μM)		DIN (μM)
		Mean	s.e.	Mean	s.e.	Mean	s.e.	Mean
0	All	1.72	0.12	0.33	0.05	2.15	0.56	4.20
1–8	80	1.64	0.13	0.37	0.05	17.56	2.81	19.57
	40	2.48	0.33	0.33	0.04	6.99	0.88	9.80
	Control	2.05	0.15	0.22	0.04	1.78	0.67	4.05
8–22	80	2.37	0.38	1.15	0.35	24.39	4.81	27.90
	40	4.37	0.67	0.71	0.31	10.49	2.32	15.56
	Control	3.75	0.55	0.20	0.03	0.37	0.20	4.31
22–38	80	7.62	1.88	3.92	1.89	15.46	2.25	27.00
	40	8.41	2.21	0.81	0.20	13.11	2.33	22.32
	Control	5.16	1.63	0.19	0.04	1.88	0.72	7.23

Density = individuals m^{-2}.

to inhibit denitrification. The sub-core, including the water, was extruded into a 3 L plastic bottle containing sufficient powdered KCl such that the final concentration was ~2 M. Slurries were stored at 4 °C and shaken intermittently over a 24 hour period to extract the sediment bioavailable (porewater + exchangeable) NH_4^+ pool (Nizzoli et al., 2005). Sub-samples were then taken, filtered and frozen for analysis of NH_4^+ concentration and the ^{15}N-enrichment of the NH_4^+ pool. The remaining sediment in the mesocosms was gently homogenised, briefly allowed to settle and samples collected 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 as described by 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 ^{15}N enrichment of the sediment bioavailable NH_4^+ pool (Risgaard-Petersen and Rysgaard, 1995). Rates of DNRA coupled to sediment nitrification ($DNRA_N$) were estimated from $DNRA_W$ and the ratio between D_N and D_W, as described by Nizzoli et al. (2006).

2.5. Determination of sediment characteristics

Particle grain size distribution of sediments was determined by dry sieving, using mesh sizes of 2000, 1000, 500, 250, 180, 125 and 63 μm. Results were expressed as dry mass of material retained by each mesh size as a percentage of total dry mass. Porosity was determined as loss of wet weight on drying. Sediments designated for chlorophyll-*a* analysis were freeze dried before extraction in 10 mL 90% acetone and chlorophyll content determined spectrophotometrically according to Lorenzen (1967). Organic matter content (LOI_{550}) was measured using the loss-on-ignition technique after acid treatment to remove carbonates (Nizzoli et al., 2006).

2.6. Sample handling and laboratory analysis

Dissolved gas samples (O_2 and N_2) were taken carefully with a 50 mL syringe to avoid bubble formation. Samples were carefully transferred to gas tight 12 ml glass vials (Exetainer, Labco, High Wycombe, UK), fixed using Winkler reagents (APHA, 1999) or 150 μL of 50% $ZnCl_2$, respectively, sealed and stored at 4 °C. Oxygen concentrations were determined using the Winkler titration method with azide modification (APHA, 1999). Dissolved N_2 concentrations and isotopic composition were analysed at the National Environmental Research Agency, Silkeborg, Denmark, as previously described by Risgaard-Petersen and Rysgaard (1995).

Samples for dissolved nutrient concentrations were filtered with GF/F glass fibre filters and stored frozen until analysed. Dissolved nitrite, nitrate and ammonium were quantified using a Systea Easy Chem nutrient analyser. Milli-Q water and low nutrient seawater was used for all sample and standard preparation. Certified reference seawater standards (Queensland Health Scientific Services) were used throughout the study, with recoveries of 90–102% recorded for all nutrients. The ^{15}N enrichment of sediment bioavailable ammonium pools was determined following micro-diffusion and hypobromite oxidation of the ammonium to N_2 (Risgaard-Petersen and Rysgaard, 1995) at the National Environmental Research Agency, Silkeborg, Denmark.

2.7. Statistical methods

Treatments were analysed via one-way ANOVA and means were compared using Tukey's HSD analysis. All assumptions were met and consequently data was not transformed.

3. Results

3.1. Sediment characteristics

The pooled sediment used in the mesocosms was comprised almost entirely of coarse sand and subsequently had a relatively low porosity of 0.31 ± 0.01 s.e. Grain size analysis indicated that 99.1 ± 0.1 s.e. % of the sediment was >63 μm, with a mean grain size of 180–250 μm. The sediment was poor in organic matter, with a chl-*a* concentration of 0.37 ± 0.12 s.e. μg g DW^{-1} and LOI organic carbon content of 0.42 ± 0.03 s.e. %.

3.2. Evolution of water column nutrient concentrations

Water column DIN concentrations increased in all treatments over the course of the mesocosm incubations, but increases were very much greater in the yabby treatments compared to the controls (Table 1). Additionally, not only did the total water column DIN concentrations change over time, but the relative N-species composition of this pool showed temporal trends indicating that shifts in sediment N-processing also occurred. Prior to yabby additions (day 0), the mean water column NO_X ($NO_2^- + NO_3^-$) concentration in all mesocosms, comprised 48.8% of DIN and this proportion increased over time in the controls, attaining a peak of 91.6% of total DIN over the period of 8–22 days (Table 1). Conversely, in the yabby treatments the contribution of NO_X to overall water column DIN decreased dramatically following the yabby additions. During the initial period (days 1–8), the NO_X fraction of the DIN pool for the 40 and 80 yabbies m^{-2} treatments fell to 28.7 and 10.3% respectively. These proportions remained relatively unchanged for the period of 8 to 22 days, but subsequently, returned to near initial levels with NO_X comprising 41.3% and 42.7% respectively of the DIN pool over the period of days 22 to 38 in the low and high density yabby treatments.

3.3. Benthic oxygen and nutrient fluxes

Sediment oxygen demand (SOD) showed initial peaks in all treatments, but especially in the two yabby treatments (Fig. 1).

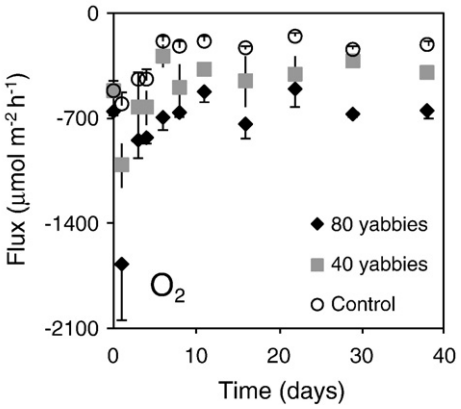

Fig. 1. Temporal changes in sediment oxygen demand at different yabby densities. Values represent the mean±s.e. (n=3), during dark incubations. Density=individuals m^{-2}.

However, following this pulse, near steady state conditions were attained by day 6–8 in each treatment and were maintained thereafter. The mean daily SOD for days 8–38 of 14.88 mmol m^{-2} day^{-1} in the high density yabby treatment was significantly greater ($p<0.001$) than both the low density (9.21 mmol m^{-2} day^{-1}) and control (4.92 mmol m^{-2} day^{-1}) treatments, and in turn the low density treatment SOD was significantly higher than that of the control mesocosms ($p<0.001$). Overall, low and high yabby densities stimulated these near steady-state time averaged SODs by 187 and 302% respectively compared to the controls (Table 2).

As for SOD, NH_4^+ effluxes from the sediment also stabilised after the initial burrowing period (Fig. 2). Thereafter, over the period of days 8–38, mean NH_4^+ effluxes in the low and high density yabby treatments were stimulated by 617 and 1534% respectively compared to the controls (Table 2) and NH_4^+ effluxes in the high density treatment were significantly greater than those in both the low yabby density and controls ($p=0.001$ and $p<0.001$, respectively). This stimulation can be explained at least in part by the shifts in the relative contributions of NH_4^+ and NO_X to overall DIN effluxes in the different treatments.

In the control treatment, NO_X was consistently produced by the sediment and there was a steady, slow increase in NO_X efflux rates over the course of the experiment and NO_X became an increasingly important contributor to overall DIN effluxes (Fig. 2). Conversely, in the yabby treatments, the addition of yabbies initially reversed NO_X fluxes with the sediment becoming a sink for NO_X in the first few days. Thereafter, NO_X effluxes were re-established and increased in magnitude over the remainder of the experiment, with NO_X effluxes in both yabby treatments being quantitatively greater than those of the controls during the final flux incubations (Fig. 2), although their relative contribution to DIN fluxes remained lower than that observed for the controls.

Overall, the relative changes in NH_4^+ and NO_X fluxes compensated each other and DIN effluxes were remarkably stable, except for a short period of low DIN efflux in the low density treatment between days 8 and 15 (Fig. 2). DIN efflux increased with yabby abundance with effluxes in the high density treatment being significantly greater than those of the low density and control treatments, and in turn those of the low density treatment were also significantly higher than the controls (All $p<0.001$). Overall, mean daily DIN effluxes over the period of 8–38 days in the low and high yabby density treatments were 269 and 565% higher respectively, than those of the controls (Table 2).

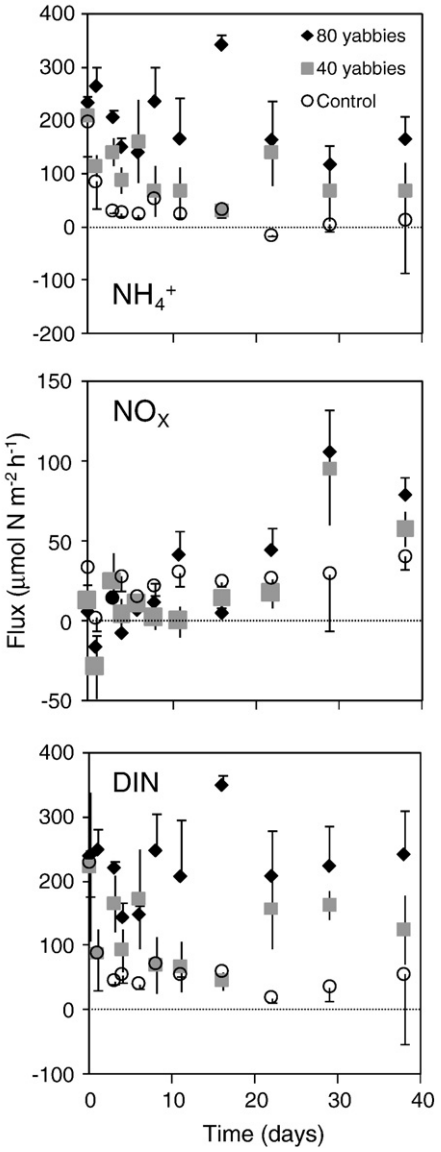

Fig. 2. Temporal evolution of dissolved inorganic nitrogen fluxes (NH_4^+, NO_X and DIN) at different yabby densities. Values represent the mean±s.e. (n=3), during dark incubations. Negative values indicate transport of solutes into the sediment. Density=individuals m^{-2}.

Table 2
Mean daily sediment oxygen demand (SOD) and sediment–water column nutrient fluxes of NO_X, NH_4^+ and dissolved inorganic nitrogen (DIN) expressed in mmol m^{-2} day^{-1} for all incubations conducted between days 8 to 38

Density	SOD	NO_X	NH_4^+	DIN
80	14.88	1.37	4.53	5.90
40	9.21	0.99	1.82	2.81
Control	4.92	0.72	0.30	1.02

Density=individuals m^{-2}.

3.4. Yabby ammonium excretion rates

Yabbies had mean NH_4^+ excretion rates of 14.8±1.4 s.e. µmol $individual^{-1}$ day^{-1}. Therefore, yabby NH_4^+ excretion at the densities of 40 and 80 individuals m^{-2} used in our incubations could generate NH_4^+ at rates of 0.58 and 1.18 mmol m^{-2} day^{-1}. Thus yabby excretion could maximally account for 24.2 and 33.4% respectively of the increase in the mean daily DIN fluxes compared to the controls (Table 2), in the high and low yabby treatments.

3.5. Sediment bioavailable ammonium pools

Sediment bioavailable NH_4^+ concentrations were determined on the final day of the mesocosm incubations as required for calculation of DNRA rates. Integrated for the entire sediment depth, bioavailable

NH_4^+ pools were 25.91±4.53 (control), 13.25±2.58 (low yabby density) and 6.12±0.78 mmol m^{-2} (high yabby density) and significantly different from each other ($p<0.05$).

3.6. Nitrification and nitrate reduction pathways

Rates of total nitrate reduction, denitrification and DNRA were all low (Fig. 3) and of marginal importance compared to sediment DIN effluxes. Total denitrification rates were almost identical between treatments. Nitrification was the principal nitrate source fuelling denitrification in all treatments but rates of coupled nitrification–denitrification (D_N) showed no trends with yabby density (Fig. 3A). Denitrification of NO_3^- from the water column (D_W) was of lesser importance and comprised only 7.8, 17.8 and 20% of total denitrification for the control, low and high density yabby treatments respectively. Whilst, there was a trend for increased rates of D_W with increasing yabby density (Fig. 3A) with rates being ~2 fold higher in the high yabby density compared to the control, this was not significant ($p>0.05$). Rates of DNRA were also low (Fig. 3B). Although, there was a trend for reduced rates with increasing yabby density with the DNRA rate in the high density treatment being only 33% of that of the controls, these differences were not significant ($p>0.05$). Similarly, whilst rates of total nitrate reduction and the proportion of nitrate reduced via DNRA compared to denitrification were both lower in the high density yabby treatment compared to the control treatment (Fig. 3C), these differences were not significant ($p>0.05$).

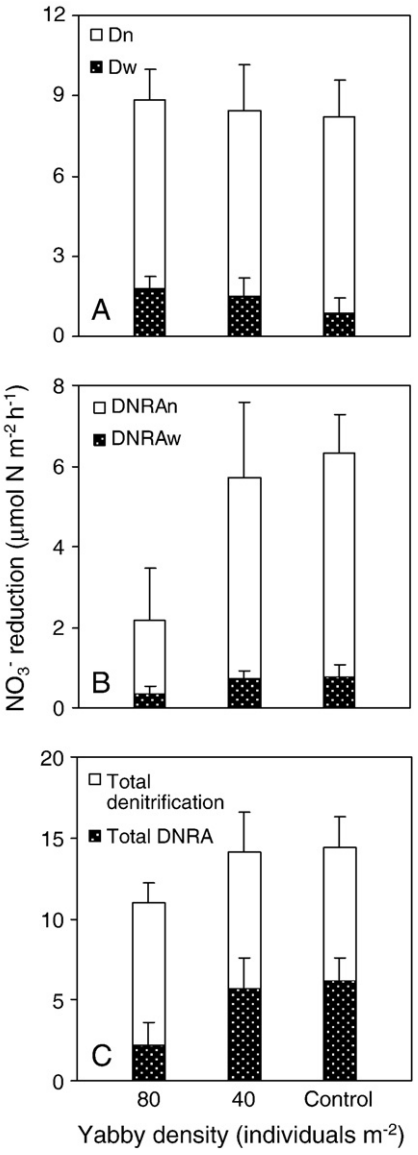

Fig. 3. Rates of nitrate reduction processes at different yabby densities determined on day 38 of the mesocosm incubation. Values represent the mean+s.e. ($n=3$). Density=individuals m^{-2}. (A) Total denitrification, separated by nitrate source, to coupled nitrification–denitrification (D_N) and reduction of nitrate diffusing to the sediment from the water column (D_W). (B) Total DNRA rates, separated by nitrate source, to coupled nitrification-DNRA ($DNRA_N$) and reduction of nitrate diffusing from the water column ($DNRA_W$). (C) Total nitrate reduction rates separated to total denitrification and total DNRA.

The degree of coupling between nitrification and denitrification can be calculated from the measured rate of D_N and mass balance calculations of nitrification rates from the final day flux and nitrate reduction rate measurements (the rate of nitrification is the sum of the NO_X efflux, plus D_N and $DNRA_N$). Based on this formula, nitrification rates in the control, low density and high density yabby treatments were 54, 71 and 84 µmol m^{-2} h^{-1} respectively (Fig. 4.) and the degree of coupling between nitrification and denitrification was 13.6, 9.8 and 8.0% respectively.

4. Discussion

4.1. Influence on benthic metabolism and inorganic nutrient regeneration

Following the initial period of the mesocosm incubations, when the added yabbies were actively excavating new burrow systems, SOD and DIN fluxes stabilised within 5 to 8 days. It would be expected that oxygen and nutrient fluxes would be somewhat variable during burrow excavation, especially for an organism like *T. australiensis* which forms extensive, complex burrow networks (Kenway, 1981; Kerr, 2001; Webb and Eyre, 2004). Since, this burrowing activity induces a non-steady state situation as reduced sediments in the burrow walls are exposed to the burrow water, deep sediment is actively transported to the sediment surface, and sediment porewater and exchangeable nutrients are "flushed" to the water column during burrow irrigation (Powilleit et al., 1994; Hansen and Kristensen, 1997; Banta et al., 1999; Welsh, 2003; Nizzoli et al., 2007).

Once stabilised (~8 days on), SOD and DIN fluxes were stimulated by 187 and 302, and 269 and 565% in the low and high density treatments respectively, compared to the controls. Although, overall SOD and DIN effluxes were relatively low, as would be expected for the organic matter poor sediments employed in the mesocosms, the degree of stimulation of SOD and DIN effluxes are in the upper part of the range of those reported in other studies (e.g. Banta et al., 1999; Bartoli et al., 2000; Kristensen, 2000; Nizzoli et al., 2007). However, it would be expected that the degree of stimulation would be large compared to those recorded for other infauna at the natural population densities employed in our mesocosms, due to the large individual size, high biomass density, and burrow and feeding habits of yabbies. *Trypaea australiensis* is a prolific sub-surface feeder that reworks considerable volumes of sediment, often to depths in excess of a metre (Kenway, 1981; Stapleton et al., 2001). Thus as reported for other thalassinidean shrimps, *T. australiensis* would directly influence SOD and DIN fluxes through the mass transport of solid phase and dissolved porewater reduced compounds and inorganic nutrients during bioturbation (Stamhuis et al., 1997; Papaspyrou et al., 2004), and the increased sediment surface area for diffusive exchanges offered by their extensive burrow networks (Witbaard and Duineveld, 1989; Forster and Graf, 1995; Nickell and Atkinson, 1995; Webb and Eyre, 2004). For example, Webb and Eyre (2004) estimated that the average surface area of *T. australiensis* burrows was 386 cm^2, if this figure is applied to the densities used in this study then the yabbies increased the surface area of sediment potentially available for diffusive exchanges by 1.5 and 3 m^2 m^{-2} for the low and high density yabby treatments.

Three factors could cause the increased DIN effluxes which were observed in the yabby treatments, namely 1) mobilisation of DIN

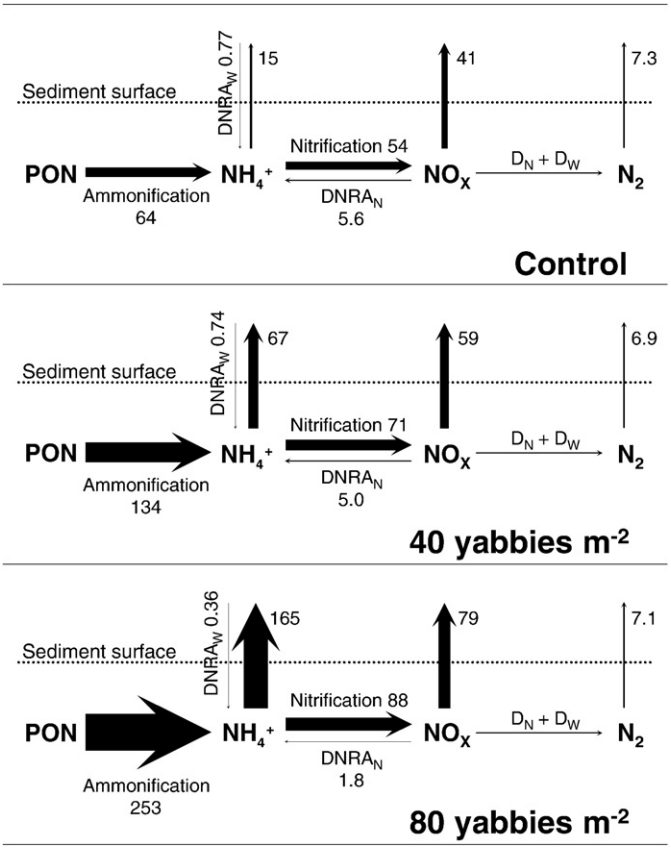

Fig. 4. Mass balance estimates of N-cycle processes and fluxes at different yabby densities. Budgets were calculated using data from the final day (day 38) flux and nitrate reduction process incubations. All values represent the mean (n=3) for each treatment and are expressed in μmol N m^{-2} h^{-1}.

already present in the sediment, 2) a direct contribution of yabby DIN excretion to the effluxes and 3) increased ammonification rates in the sediment. Burrow construction, bioturbation and burrow irrigation are known to stimulate DIN effluxes due to mobilisation of dissolved and exchangeable sediment pools (Hansen and Blackburn, 1992; Banta et al., 1999; Bartoli et al., 2000; Papaspyrou et al., 2004). In our mesocosms we recorded decreases in the sediment bioavailable NH_4^+ pool of 12.6 and 19.8 mmol m^{-2} in the low and high density yabby treatments compared to the controls. However, even if this NH_4^+ had been released at a steady rate, these differences would equate to a net mobilisation of approx. 0.3 and 0.5 mmol m^{-2} day^{-1} in the low and high density treatments and therefore could only account for a relatively small proportion of the average excess daily DIN effluxes in the treatment compared to control mesocosms (Table 2). However, in reality it is likely that the bulk of this NH_4^+ was mobilised in the early days of the incubations when the yabbies were actively excavating new burrows as observed in previous studies of benthic infauna (Hansen and Blackburn, 1992; Banta et al., 1999; Bartoli et al., 2000; Papaspyrou et al., 2004). Therefore, mobilisation of sediment DIN pools was probably a minor contributor to the sustained higher DIN effluxes recorded in the yabby treatments.

The contribution of the yabbies themselves and their overall activity (net effect of bioturbation, burrow formation and burrow irrigation) to DIN effluxes can be estimated by difference, using the determined ammonium excretion rates and assuming that the DIN efflux of the control mesocosms represents the contribution of the surface sediment (Bartoli et al., 2000). Excretion of NH_4^+ was determined experimentally to be 14.8±1.4 s.e. μmol N individual^{-1} day^{-1}, which equated to approximately 33.4 and 24.2% of the DIN efflux in the low and high density treatments respectively. The contribution from the surficial sediment (the controls) was 1.02 mmol DIN m^{-2} day^{-1}, equivalent to 36.3 and 17.3% of the total DIN efflux in the low and high yabby density treatments. Therefore, 30.3 and 58.5% of the increase in DIN efflux in the low and high density treatments can be attributed to increased surface area for diffusive exchanges, and the bioturbation and irrigation activities of their residents. However, these values probably represent an underestimate of the true contribution of the burrows and yabby activities to DIN effluxes. Since, sediment bioavailable ammonium pools in the yabby treatments were greatly depleted compared to the control treatment and therefore, diffusive effluxes of DIN across the sediment surface are likely to have been much lower than those of the controls due to the lower concentration gradient between the sediment porewater and the water column.

Consequently, the most probable major source of the sustained excess DIN effluxes in the yabby treatments was increased regeneration of inorganic nitrogen resulting from increased mineralisation rates of the sediment particulate organic nitrogen stock. This hypothesis is supported by the mass balance calculations depicted in Fig. 4, which are based on the final day flux and nitrate reduction rates. Assuming steady state, these mass balances indicate that ammonium regeneration (ammonification) rates would have needed to be 64, 134 and 253 μmol m^{-2} h^{-1} in order to support the determined DIN effluxes and nitrate reduction rates. Such increases in mineralisation rates have commonly been reported in the presence of a range of macrofaunal species with gross rates of N-mineralisation in the sediment and effluxes of mineralisation products to the water column being stimulated up to 4-fold (Kristensen and Blackburn, 1987; Hansen and Blackburn, 1992; Hansen and Kristensen, 1997; Christensen et al., 2000a; Papaspyrou et al., 2004). For example, nitrogen mineralisation rates were stimulated 1.6-fold in microcosms containing *Nereis virens*, with rates averaging 76 and 47 nmol N cm^{-3} sediment day^{-1} respectively, in the presence and absence of *N. virens* (Kristensen and Blackburn, 1987). This stimulation of mineralisation rates has been proposed to be due to increased aerobic metabolism and to mainly result from the breakdown of "older" more recalcitrant organic matter already present in the sediment (Andersen and Kristensen, 1992; Kristensen et al., 1992; Banta et al., 1999; Kristensen, 2000).

However, when considering stimulations of benthic metabolism and gross rates of nutrient regeneration recorded in mesocosm incubations, it should be taken into account that these will always overestimate the degree of stimulation caused by natural populations. This occurs because, initially, the homogenised sediment has an equal organic matter content in all the mesocosms, whereas in permanently inhabited sediments the prolonged stimulation of metabolism caused by the infauna will have depleted the sediment organic matter pools which sustain microbial metabolism. Consequently, *in situ* stimulation of benthic processes over periods of years or decades can only be sustained if the infauna enhance inputs of organic matter to the sediment by an equivalent degree (Kristensen, 2000; Welsh 2003). Yabbies would be expected to enhance organic matter inputs through indirect biodeposition, as the pits and mounds they form at the sediment surface would enhance sedimentation rates (see Graf and Rosenberg, 1997 for review) and deposited materials can be rapidly trapped and buried below mounds of ejected sediment. Additionally, at least some species of thalassinidian shrimps are known to actively collect large particulate organic matter from the sediment surface and transport it into their burrows (e.g. Vonk et al., 2008; Kneer et al., 2008). Thus, it is probable that yabbies can sustain higher rates of sediment metabolism and nutrient regeneration *in situ* by enhancing local organic matter inputs, although the level of these inputs and therefore the degree to which they could stimulate benthic processes has not been quantified.

4.2. Influence on rates of nitrification, denitrification and DNRA

Whilst, overall DIN effluxes were maintained at stable levels in the mesocosms the proportions of NH_4^+ and NO_X making up the DIN efflux

changed over the course of the incubations and between treatments. Both mass balance calculations (Fig. 4) and the low measured nitrate reduction rates indicate that these changes were driven by nitrification, rather than shifts in NO_X consumption. Since, sediment bioavailable NH_4^+ pools in the low and high density yabby treatments were only 51 and 24% respectively of the controls, the stimulation of nitrification in the yabby treatments would appear to be related to an increased volume of aerobic sediment permissive to nitrification rather than increased availability of NH_4^+. Previous studies have shown that the burrow walls of many benthic infauna and even the body surfaces of the animals themselves can be privileged sites for nitrification (Kristensen et al., 1985; Mayer et al., 1995; Welsh, 2003; Welsh and Castadelli, 2004) and colonisation of at least parts of the burrow walls could explain the increased nitrification rates in the yabby treatments. However, it cannot be excluded that the increase in nitrification rates was due to an increase in the volume of the aerobic surface sediment. Especially since as has been previously observed (Kerr, 2001) our yabbies frequently "plugged" their burrows, but movement of the surface sand indicated that they continued to irrigate these sections of burrow with the expelled water percolating through the mounds of sand covering the burrow entrance. This irrigation water would potentially be rich in both oxygen and NH_4^+ and therefore the plugged burrow mounds could represent a site of intensive nitrification that was not present in the control mesocosms.

Population density had no significant effects on denitrification or the contributions of nitrate from the water column and that generated by nitrification, with rates of both D_W and D_N being similar in control and treatment mesocosms. These results are in contrast to most studies which have recorded a stimulation of denitrification and especially coupled nitrification–denitrification by infauna (Pelegrí et al., 1994; Pelegrí and Blackburn, 1995; Rysgaard et al., 1995; Bartoli et al., 2000; Nizzoli et al., 2006; Nizzoli et al., 2007), although this is not always the case (e.g. Tuominen et al., 1999; Hietanen et al., 2007; Karlson et al., 2007). However, it is still somewhat surprising that we did not observe some stimulation of denitrification as increased rates of nitrification and the large surface area of the burrows for diffusive exchange of NO_X with the water column would be expected to enhance NO_X availability and consequently, denitrification rates. The fact that the greater availability of NO_X did not enhance denitrification rates suggests that the yabbies activities could have increased the separation between the water column/nitrification zones and denitrification zones, and thus the diffusional pathlength for NO_X. This would greatly reduce NO_X diffusion rates and diffusion times (Jørgensen, 1994). Thus, despite the increased concentration of NO_X in the water column and the increased generation of NO_X by nitrification, the supply of NO_X to denitrifiers in anaerobic sediment zones could have been limited. Inhibition of denitrification rates from increased oxygen penetration due to photosynthetic oxygen evolution by benthic algal mats, or artificial manipulation of water column oxygen concentrations has previously been reported and linked to increased diffusional pathlengths for nitrate to the denitrification zone (Risgaard-Petersen et al., 1994; Rysgaard et al., 1994; Sundbäck et al., 2000). A similar increase in separation between NO_X sources and denitrification zones could result from a combination of the low organic matter content and low overall metabolic activity of the sediments used in our mesocosms, and the burrowing and burrow irrigation habits of *T. australiensis*.

The sediment used in our mesocosms had a low organic matter content (0.42 ± 0.03%), low overall metabolic activity (4.9 mmol O_2 m^{-2} day^{-1} for control mesocosms) and the low degree of coupling between nitrification and denitrification, 13.6% for controls, suggests that there was already a significant degree of separation between nitrification and denitrification zones. The presence of yabbies could have enhanced this effect through their burrowing and burrow irrigation activities. *Trypaea australiensis* like other thalassinidean shrimps forms deep complex burrow systems (Kenway, 1981; Forster and Graf, 1995; Stamhuis et al., 1997; Kerr, 2001; Papaspyrou et al., 2004; Dworschak et al., 2005). Typically, *T. australiensis* burrows consist of U-shaped loops in the surficial sediments linked to a turning chamber and a deep vertical shaft with extensive branching subterranean feeding galleries (Kenway, 1981; Kerr, 2001). Whilst, estimates of *T. australiensis* burrow irrigation rates are high, e.g. 456 ml $burrow^{-1}$ h^{-1} (Webb and Eyre, 2004), this water is principally circulated through the near surface U-shaped burrow loops (Kenway, 1981; Kerr, 2001), as the deeper vertical shaft and feeding galleries are dead ends. This together with the low oxygen demand of the organic poor sediment and the fact that irrigation water is at times circulated through the sediment when the burrow entrances are plugged, may have created a highly oxidised surface sediment zone, which was amenable to nitrification, but not denitrification. Whereas, water exchange with the deeper parts of the burrow system during irrigation may be low or insignificant. For example, Forster and Graf (1995) estimated that approximately half the burrow walls of the thalassinid shrimp *Callianassa subterranea* were not in contact with oxygenated water. Thus, the walls of the deeper parts of the burrow system of *T. australiensis* may be more or less permanently anoxic and therefore not suitable for nitrifying bacteria, and whilst conditions may be suitable for denitrifiers, due to the lack of water circulation within these parts of the burrow, little nitrate may be transferred from the water column or aerobic sections of the burrow to support denitrification.

Our denitrification results are in contrast to those of a previous *in situ* study of *T. australiensis* (Webb and Eyre, 2004), where yabbies at a density of 22 individuals m^{-2} stimulated denitrification rates 4-fold compared to defaunated control plots. These authors proposed that this increase was due to coupled nitrification–denitrification associated with the burrow wall sediments. Whilst, this difference may reflect the higher organic matter content of the sediment (13% DW), which may have allowed anoxic zones amenable to denitrification to be maintained around the irrigated burrows, it may also reflect differences in the experimental design and the method utilised to measure denitrification rates. In the study of Webb and Eyre (2004), yabby-free treatment plots were prepared by removing and sieving the upper 30 cm of sediment and returning this to the same plot after insertion of a mesh to prevent recolonisation by yabbies. Although this sediment manipulation was conducted 40 days before measurements, this may not have been sufficient for slow growing nitrifier populations to have fully re-established (Henriksen and Kemp, 1988). For example, although our mesocosms were allowed to equilibrate for two weeks before incubations were initiated, nitrate effluxes in the controls which suffered no disturbance due to yabby additions, continued to steadily increase over the entire 38 day mesocosm incubation, indicating that nitrifier populations were increasing over the entire 52 day period. Consequently, the lower rates of denitrification recorded in defaunated plots compared to the undisturbed yabby inhabited plots may have been at least partially due to denitrification in the defaunated plots having not returned to maximal levels rather than a stimulation *per se* of denitrification in the undisturbed yabby inhabited plots. Additionally, Webb and Eyre (2004) measured their denitrification rates as N_2 fluxes. This, unlike the ^{15}N-isotope pairing technique does not allow direct quantification of the nitrate sources which fuel denitrification. Therefore, the conclusion that increased rates of denitrification in the yabby inhabited sediments were due to coupled nitrification–denitrification is not based on direct measurements. Moreover, N_2 fluxes are not a direct measure of denitrification rates, but a net product of N_2 production by denitrification and N_2 consumption by nitrogen fixation (Welsh et al., 2001). Consequently, although differences in N_2 effluxes were attributed to higher denitrification, they could equally well be due to higher rates of N-fixation in the defaunated plots, where chl-*a* concentrations were double those of the yabby inhabited plots and therefore, N-fixing cyanobacterial abundance could have been much higher. Overall, whilst it is probable that rates of denitrification were

higher in the yabby inhabited versus defaunated plots in the study of Webb and Eyre (2004), it is difficult to quantify this difference due to the problems inherent in the use of N_2 effluxes as a measure of denitrification and the fact that nitrifier populations may not have fully re-established in the defaunated plots.

Rates of DNRA were also quantitatively low for similar reasons as those discussed above for denitrification. Overall, DNRA comprised 34.3, 32.7 and 18.4% of total nitrate reduction in the control, low and high density yabby treatments respectively, as would be expected in the organic matter poor sediments utilised in our mesocosms (Tiedje, 1988; Christensen et al., 2000b; Nizzoli et al., 2006). Whilst, the decreased importance of DNRA to total nitrate reduction at the higher yabby density is consistent with the results of Nizzoli et al. (2006), that high densities of infauna favoured denitrification over DNRA, this effect was not statistically significant.

Overall, both denitrification and DNRA were of minor importance to the benthic nitrogen cycle in our mesocosms and their importance decreased with increasing yabby density (Fig. 4). Whilst, rates of nitrogen regeneration increased substantially with yabby density, rates of denitrification and DNRA remained almost unchanged. For example, N_2 generated in the sediment via coupled nitrification–denitrification accounted for only 11.5, 5.2 and 2.8% of the ammonium generated by ammonification respectively, in the control, low and high density treatments.

5. Conclusions

As would be expected, increases in the population density of a large bioturbating organism such as *T. australiensis* caused significant several-fold stimulations of benthic metabolism, rates of organic matter turnover and efflux rates of regenerated nutrients. The presence of *T. australiensis* burrows also significantly stimulated nitrification rates despite the fact sediment bioavailable ammonium pools were substantially lower in the presence of yabbies. This indicates that the higher rates of nitrification were due to an increase in the volume of aerobic sediment amenable to nitrification in the presence of *T. australiensis* burrows. However, somewhat surprisingly the increased availability of nitrate did not result in enhanced rates of nitrate reduction processes, which were similar in both the treatments and controls. This effect may result from a combination of the low organic matter content sandy sediments used in the mesocosms and the specific burrowing and burrow irrigation habits of *T. australiensis*. Together these may have created a two-tier sediment system with an upper layer containing the highly irrigated U-shaped burrow loops where conditions are amenable to nitrification, but poor for denitrification and a lower layer containing the vertical shaft and feeding chambers of the burrow which are poorly irrigated and therefore have the anoxic conditions required for denitrification, but are isolated from the sources of nitrate in the water column and upper aerobic sediment zones. These results highlight that the influence of species such as *T. australiensis* which form large burrow systems, where different parts of the burrows are unequally irrigated, may be more complex than those recorded for organisms which create simple U-shaped burrows. Additionally, there may be a strong interaction between faunal effects and, physical and biological sediment characteristics, and thus the same species may have contrasting influences in organic poor sandy sediments compared to organic rich fine grained sediments.

Since, yabbies stimulated nitrogen regeneration rates, but had no effect on denitrification rates, the importance of denitrification as a sink for nitrogen decreased with yabby abundance and an increasing proportion of the regenerated N was recycled to the water column in forms available to primary producers. Consequently, in organic poor sandy sediments such as those of the Gold Coast Broadwater, the major effect of *T. australiensis* may be to increase benthic–pelagic coupling by enhancing nutrient regeneration rates and the export of these nutrients back to the water column, and thus allowing the same nutrients to be used more frequently over a given period of time.

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