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PRIMARY RESEARCH PAPER

Interactive influences of the marine yabby (*Trypaea australiensis*) and mangrove (*Avicennia marina*) leaf litter on benthic metabolism and nitrogen cycling in sandy estuarine sediment

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Abstract A previous study has demonstrated that in sandy sediment the marine yabby (*Trypaea australiensis*) stimulated benthic metabolism, nitrogen regeneration and nitrification, but did not stimulate denitrification, as the intense bioturbation of the yabbies eliminated anoxic microzones amenable to denitrification. It was hypothesised that organic matter additions would alleviate this effect as the buried particles would provide anoxic microniches for denitrifiers. To test this hypothesis a 55-day microcosm (75 cm × 36 cm diameter) experiment, comprising four treatments: sandy sediment (S), sediment + yabbies (S + Y), sediment + *A. marina* litter (S + OM) and sediment + yabbies + *A. marina* litter (S + Y + OM), was conducted. *Trypaea australiensis* significantly stimulated benthic metabolism, nitrogen regeneration, nitrification and nitrate reduction in the presence and the absence of litter additions. In contrast, the effects of litter additions alone were more subtle, developed gradually and were only significant for sediment oxygen demand. However, there was a significant interaction between yabbies and litter with rates of total nitrate reduction and denitrification being significantly greater in the S + Y + OM than all other treatments, presumably due to the decaying buried litter providing anoxic micro-niches suitable to nitrate reduction. In addition, both *T. australiensis* and litter significantly decreased rates of DNRA and its contribution to nitrate reduction.

Keywords Bioturbation · Organic matter · Fluxes · Nitrification · Denitrification · Dissimilatory nitrate reduction to ammonium

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Introduction

In shallow water coastal marine ecosystems, the sediment is a major site for the mineralisation of organic matter (Fenchel et al., 1998), which is derived from a range of sources including, phytoplankton, microphytobenthos, macroalgae, seagrasses and detritus of terrestrial plants (Fenchel et al., 1998; Dunn

et al., 2008). These differ considerably in their contents of simple labile and complex recalcitrant compounds, their overall nitrogen content and C:N ratio, and therefore their susceptibility to microbial degradation (Fenchel et al., 1998). As a result, organic matter mineralisation is influenced not only by the quantity, but also the quality of the deposited organic matter (Blackburn & Blackburn, 1993; Fenchel et al., 1998; Welsh, 2003). The depth distribution of the organic matter and the conditions under which it is degraded also influence the mineralisation rates and the relative proportions of NH_4^+, NO_X and N_2 which are returned to the water column (Blackburn & Blackburn, 1993; Kristensen, 2000; Welsh, 2003). These factors are in turn strongly dependent on the density and community composition of the sediment infauna (Welsh, 2003).

The burrowing and feeding behaviours of infauna influence the deposition and depth distribution of sedimentary organic matter. Particle reworking results in the mixing of organic matter to depth and infauna burrows increase the area of the sediment–water interface favouring solute exchange with the overlying water (Welsh, 2003). Ventilation of these burrows by their residents transports oxygen-rich water to the deeper sediment, influencing the distribution of oxic, suboxic and anoxic sediment zones (Wenzhöfer & Glud, 2004; Robertson et al., 2008, 2009). Therefore, burrow wall sediments and infauna can provide a substrate for colonising aerobic microbial communities, including nitrifying bacteria (Welsh & Castadelli, 2004; Laverock et al., 2010). Numerous studies have shown that burrowing fauna typically enhance nitrification and denitrification in the sediment (Pelegrí & Blackburn, 1995; Bartoli et al., 2000; Nizzoli et al., 2007). In contrast, the influence of infauna on DNRA or the partitioning of NO_X between denitrification and DNRA has received relatively little attention (Nizzoli et al., 2006; Dunn et al., 2009; Jordan et al., 2009). Moreover, to date, the vast majority of studies on the influence of fauna on sediment biogeochemistry have focussed on polychaete worms and amphipods (e.g. Pelegrí et al., 1994; Pelegrí & Blackburn, 1995; Banta et al., 1999; Bartoli et al., 2000; Dunn et al., 2009; Papaspyrou et al., 2010), and only few studies have investigated the influence of large, deep burrowing organisms, such as the thalassinidean shrimp, *Trypaea australiensis*.

Trypaea australiensis is a conspicuous and often dominant member of the benthic community in Australian estuaries occurring at densities of 60–200 individuals m^{-2} (Katrak & Bird, 2003; Contessa & Bird, 2004; Webb & Eyre, 2004). This decapod shrimp is a sub-surface detritivore (Spilmont et al., 2009), and an intense bioturbator, which excavates complex burrows up to 1 m deep. In a recent mesocosm study, Jordan et al. (2009) found that in low organic matter sands *T. australiensis* significantly enhanced benthic metabolism, inorganic nitrogen efflux and nitrification, but had no significant effect on denitrification and DNRA. These results were in contrast to those from a manipulative field experiment in organic-rich, muddy sediments where *T. australiensis* enhanced N_2 effluxes, suggesting a stimulation of denitrification rates (Webb & Eyre, 2004). This led Jordan et al. (2009) to propose that the impacts of *T. australiensis* on *N*-dynamics may differ with sediment type.

The current study was initiated to assess the above proposal by investigating the influence of *T. australiensis* on benthic metabolism and N-dynamics in the same sandy sediment in the presence and the absence of mangrove (*Avicennia marina*) detritus. This case study was chosen as these species commonly co-occur in eastern Australian estuaries, where mangrove detritus is a major source of organic matter to sediments (Lee et al., 2006; Dunn et al., 2008). It was hypothesised that the mangrove detritus would enhance benthic metabolism, but because of its high C:N ratio, it would have little effect on the sediment N-status. Thus, both detritus and *T. australiensis* would primarily influence sediment N-dynamics by influencing the availability and distribution of aerobic and anaerobic sediment zones amenable to nitrification and nitrate reduction processes, respectively.

Materials and methods

Collection sites, microcosm preparation and experimental design

Sediment was collected in March 2007 from a sand flat (27°58′S, 153°25′E) within the Gold Coast Broadwater, Australia (see Warnken et al., 2004). Sandy sediment (0–0.5 m depth) was manually collected and sieved (1 mm) to remove fauna and debris before being transferred into 12 microcosms (75 cm deep × 36 cm internal diameter) to a depth of ~60 cm. Sub-samples of the sediment were retained

for analyses of grain size, density, organic matter content (LOI_{550}) and C:N ratio. Each microcosm was filled and flushed at a rate of $\sim$15 l d^{-1} with seawater using irrigation tubing connected to a 20,000 l reservoir which contained sufficient water for the entire experiment. Water was sourced from the Gold Coast Broadwater. The microcosms were stored in a constant temperature room at 22 ± 2°C, with constant aeration and maintained under darkness.

Trypaea australiensis were collected using a yabby pump from a sand flat within the Gold Coast Broadwater. In the laboratory yabbies were kept in darkened and aerated, seawater flushed aquaria containing sediment at 22 ± 2°C to acclimatise. *A. marina* leaf litter was collected using litter nets $\sim$2 km from the sediment collection site. Collected leaves were washed in seawater and stored <4°C. Subsamples of the leaf litter were retained for determination of C:N ratio.

The overall experimental timeline is shown in Fig. 1. Following stabilisation, triplicate microcosms were randomly assigned to control, sediment alone (S), sediment + yabbies (S + Y), sediment + leaf litter (organic matter; S + OM) and sediment + yabbies + leaf litter (S + Y + OM) treatments. Eight pre-weighed *T. australiensis* were added to each S + Y and S + Y + OM microcosm to yield a final density of $\sim$80 m^{-2}. Subsamples of the acclimatised population were retained for biomass and tissue C:N ratio determinations, and determination of respiration and NH_4^+ excretion rates. Additions of *A. marina* leaf litter to the S + OM and S + Y + OM treatments were initiated 3 days after *T. australiensis* addition to allow the yabbies to have already formed their burrows and were repeated over the 55 day experiment period (Fig. 1). On each occasion leaf litter was added to the sediment surface at a rate equivalent to 1.23 g wet wt m^{-2} d^{-1} ($\sim$35 mmol C m^{-2} d^{-1}), which corresponds to the mean daily litter production of *A. marina* in Moreton Bay of 4.5 t ha^{-1} (Davie, 1984).

Over the course of the experiment sediment–water column fluxes of oxygen, dissolved inorganic carbon (DIC), NH_4^+ and NO_X were determined on 18 occasions (Fig. 1), and nitrate reduction rates were determined on the final day. On completion of the experiment, the entire sediment in the bioturbated microcosms was sieved to recover the yabbies for biomass and tissue C:N ratio determinations.

Flux determination

Aeration and water flow were interrupted, and the water level in the microcosms lowered by $\sim$2 cm. Aquarium pumps housed in each microcosm were switched on to ensure mixing of the water column before water samples were collected for the determination of initial O_2, DIC, NH_4^+ and NO_X concentrations. The microcosms were isolated from the atmosphere using floating plastic lids and incubated for $\sim$3 h (so that the final O_2 concentration remained above 80% of the initial value). At the end of the incubation, the floating lids were removed, and water samples for O_2, DIC, NH_4^+ and NO_X concentrations

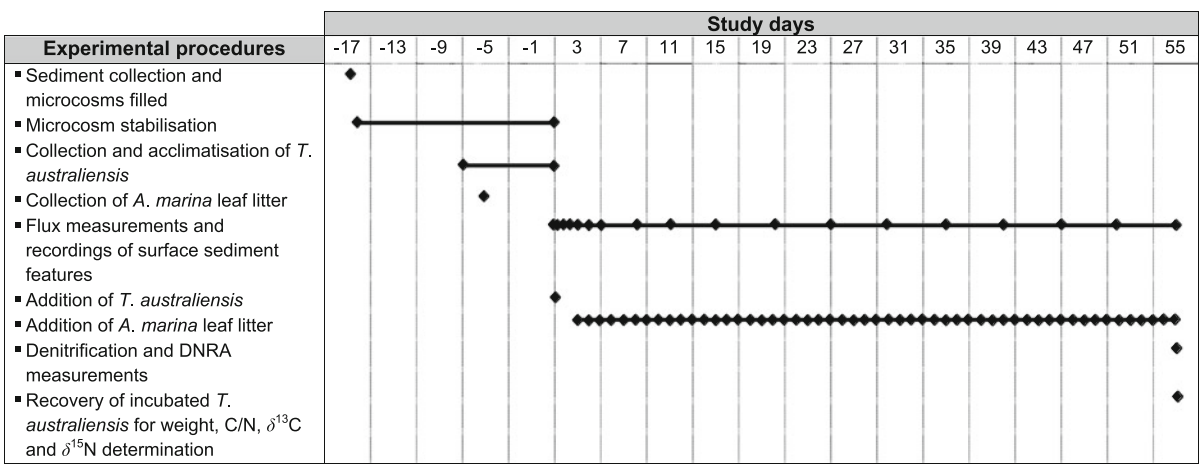

Fig. 1 Timeline of the overall study design

collected. Flux rates were calculated from the change in water column concentrations of the individual solutes as outlined by Welsh et al. (2000).

Determination denitrification and DNRA

After the final determination of O_2, DIC and nutrient fluxes on day 55, denitrification rates were determined using the isotope pairing technique (Nielsen, 1992), as modified to allow simultaneous determination of DNRA (Risgaard-Petersen & Rysgaard, 1995; Nizzoli et al., 2006).

Following the final flux incubations, water flow and aeration were reconnected for 2 h. Microcosms were then prepared for incubation in the same way as described for fluxes. Initial water samples were collected for the determination of ambient NO_3^- before addition of 30 mM 99.9% ^{15}N–NO_3^- to give a final concentration of ~30 µM. The water was briefly mixed, and a sample taken for NO_3 analysis after ~5 min to enable calculation of the actual added $^{15}NO_3^-$ concentration. Microcosms were then pre-incubated for ~30 min to allow diffusion of the ^{15}N–NO_3^- to the nitrate reduction zones in the sediment (Dalsgaard et al., 2000). The microcosms were isolated from the atmosphere using floating plastic lids and incubated for 2.5–4 h. Actual incubation times were based on O_2 fluxes to ensure that O_2 concentration remained above 80% of the initial value (Nielsen, 1992).

At the end of the incubation, a plastic core (8-cm diameter) was inserted to the base of each microcosm and 10 ml of 7 M $ZnCl_2$ was added to the water outside the core to inhibit further activity (Dalsgaard et al., 2000). The sub-core including the overlying water was withdrawn and emptied into a 1-l plastic bottle containing sufficient KCl to give a final concentration of ~2 mol l^{-1} before being vigorously shaken. The remaining sediment in the microcosms was gently stirred to mix the water column and porewater N_2 pools and allowed to settle for 1–2 min before a sample was transferred to a 12-ml gas-tight glass Exetainer (Labco) and fixed with 100 µl 7 M $ZnCl_2$ and stored at 4°C. The sediment–KCl slurries were stored at 4°C and shaken intermittently over a 24 h period to extract the exchangeable NH_4^+ pool. Sub-samples were then filtered (GF/F Whatman) and stored frozen until analysis for NH_4^+ concentration and ^{15}N-enrichment of the NH_4^+ pool.

Rates of total denitrification (D_{14}), denitrification based on NO_3^- diffusing from the water column (D_W) and denitrification coupled to sediment nitrification (D_N) were calculated as described by Nielsen (1992). DNRA based on water column NO_3^- ($DNRA_W$) was calculated from the water column ^{15}N–NO_3^- enrichment and the ^{15}N enrichment of the bioavailable NH_4^+ pool (Risgaard-Petersen & Rysgaard, 1995). DNRA coupled to sediment nitrification ($DNRA_N$) was estimated from the $DNRA_W$ and the ratio between D_N and D_W (Risgaard-Petersen & Rysgaard, 1995).

Anammox is recognised as an interference when using the isotope pairing technique which can lead to overestimation of denitrification (Risgaard-Petersen et al., 2003). However, in shallow water coastal marine sediments anammox has been shown to be an insignificant source of N_2 compared to denitrification (Dalsgaard et al., 2005; Burgin & Hamilton, 2007), especially in tropical systems (Dong et al., 2011). Therefore, we assume that our estimates of denitrification are valid, although it may also incorporate a small proportion of N_2 production via anammox.

Determination of *T. australiensis* respiration and ammonium excretion rates

Pre-weighed individuals from the acclimatised *T. australiensis* population were placed into 0.5-l Wheaton bottles ($n = 9$) containing unfiltered seawater for respiration and NH_4^+ excretion assays. Initial water samples were collected for O_2 and NH_4^+ determinations, the bottles closed and incubated under dark conditions for ~3 h at 22 ± 2°C until final samples were taken. Respiration and NH_4^+ excretion were calculated from the time-dependent changes in O_2 and NH_4^+ concentrations.

Sample handling and laboratory analysis

Water samples for DIC, O_2 and N_2 were collected, avoiding bubble formation using 50-ml syringes and silicone tubing. DIC, O_2 and N_2 samples were transferred to 12-ml gas-tight glass vials (Exetainer, Labco), fixed using 100 µl saturated $HgCl_2$, Winkler reagents (APHA, 1998) and 150 µl of 50% $ZnCl_2$, respectively, sealed and stored at 4°C. DIC concentrations were determined using a total organic carbon analyser (TOC-V_{CSH}, Shimadzu Corporation) and oxygen by the Winkler titration method with azide

modification (APHA, 1998). Dissolved N_2 concentrations and the proportions of $^{29}N_2$ and $^{30}N_2$ were analysed at the National Environmental Research Institute, Silkeborg, Denmark as described by Risgaard-Petersen & Rysgaard (1995). DIN concentrations were determined using a nutrient analyser (Easychem Plus, Systea Analytical Technologies).

Trypaea australiensis and sediment LOI_{550} was determined as weight loss following drying (80°C for 48 h) and ashing (550°C for 1 h). *T. australiensis*, sediment and mangrove leaf samples for the determination of C:N ratios were analysed using an elemental analyser (EA3000, Eurovector). Particle grain size distribution of sediments was determined by dry sieving. Leaves and *T. australiensis* body tissue (exoskeleton removed) were rinsed in Milli-Q element water, freeze-dried and ground prior to analysis. Subsamples of dried powdered sediment were treated with 1 M HCl to remove carbonates before being freeze-dried and subsequently analysed.

The ^{15}N enrichment of sediment bioavailable ammonium pools was determined at the National Environmental Research Institute, Silkeborg, Denmark following micro-diffusion and hypobromite oxidation of the ammonium to N_2 (Risgaard-Petersen & Rysgaard, 1995).

Statistical analyses

Comparisons of *T. australiensis* characteristics and final day nitrogen cycling processes between treatments were analysed by one-way ANOVA and means were compared using Tukey's HSD analysis. A linear mixed models (LMM) approach was used to investigate the effect of the two fixed factors: organic matter addition (OM) and yabby addition (Y) using the triplicate microcosms within each of the four treatments as a random effect. A heterogeneous AR1 structure was selected as the best structure representing the correlated errors associated with the repeated measures factor for all the dependent variables. Initial exploratory analyses indicated that the dependent variables required log (x + constant) transformation to ensure linearity, homogeneity of variance, and normality assumptions were satisfied for subsequent LMM analysis. Pearson correlations were used to explore data and identify relationships between respiration and NH_4^+ excretion rates and *T. australiensis* characteristics. Criteria of $P < 0.05$ and 0.01 were used to determine significant differences using SPSS for Windows (SPSS Inc., *version 19*).

Results

Characteristics of sediments, yabbies and mangrove leaf litter

The sediment collected for preparation of the microcosms was dominated by fine sands with a wet bulk density of 1.76 ± 0.03 g cm^{-3}, a low organic matter content (0.42 ± 0.07% LOI_{550}), a nitrogen content of 0.005 ± 0.001% dry weight and a C:N ratio of 15.0 ± 2.4. Following acclimatisation in the laboratory, yabbies had a mean individual biomass of 4.08 ± 0.10 g wet weight, a tissue C:N ratio of 4.9 ± 0.9, and the collected *A. marina* leaf litter had a N content of 1.24 ± 0.11% dry weight and a C:N ratio of 36.9 ± 2.4.

Mean *T. australiensis* wet weights and LOI_{550} values remained constant throughout the study period with no significant differences between the treatments with and without organic matter addition or between the incubated individuals and the initial acclimatised population added to the microcosms (Table 1). Retrieved *T. australiensis* from S + Y + OM microcosms showed an increased C:N ratio and lower N content than either the initial acclimatised population or individuals retrieved from the S + Y treatment (Table 1), but these differences were not statistically significant (ANOVA $P > 0.05$).

General observations

Following addition, all *T. australiensis* constructed burrows complete with mound and funnel within the first day and maintained these throughout the 55-day experiment. Burrow mounds ranged in diameter from 3.4 to 12 cm, and a dark grey sediment was routinely observed within the ejected material, indicating transport of deeper anoxic sediment to the surface. Burrow openings ranged in diameter from 0.2 to 1.3 cm and were periodically capped, although visible movement of the surface sand indicated that the yabbies continued to pump irrigation water through the capped openings.

In the S + Y + OM treatment, the added *A. marina* leaf litter was rapidly buried and at the

Table 1 Wet weight, organic matter content (LOI_{550}), C:N ratio, and N content of initial acclimatised *T. australiensis* individuals and those recovered at the end of the S + Y and S + Y + OM microcosm incubations

Treatment	Mean individual wet weight (g)		LOI_{550}	C/N	N-content
	Day 0	Day 55	% DW		% DW
Acclimatised	4.08 ± 0.10 (24)	N/A	61.9 ± 8.4 (8)	4.9 ± 0.9 (7)	9.7 ± 1.2 (7)
S + Y	4.13 ± 0.37 (24)	4.17 ± 0.43 (12)	63.4 ± 7.3 (8)	4.6 ± 0.2 (7)	8.9 ± 0.9 (7)
S + Y + OM	4.27 ± 0.87 (24)	4.29 ± 0.78 (12)	62.3 ± 4.8 (8)	5.4 ± 0.9 (7)	6.8 ± 2.2 (6)

Mean values ± SD (*n*); DW = Dry weight

conclusion of the experiment during sediment sieving, buried leaf matter and associated black, sulphidic sediments were clearly visible.

Sediment oxygen demand and DIC effluxes

Before *T. australiensis* and *A. marina* additions, sediment oxygen demand (SOD) was relatively low, and differences between treatments were not significant. In the control S treatment, SOD remained low (typically $\leq$0.5 mmol m^{-2} h^{-1}) over the 55-day incubation period (Fig. 2). *A. marina* leaf litter additions significantly stimulated SOD (Table 2), however, this was a gradual effect that evolved slowly over time (Fig. 2). Initially the presence of *A. marina* leaf litter had no significant impact on SOD, but there was a cumulative effect of the repeated litter additions that resulted in a significant stimulation of SOD from day 15 onwards (Table 3). In contrast, the introduction of *T. australiensis* to the S + Y and S + Y + OM treatments led to an immediate and significant increase in SOD (Fig. 2; Table 2). Maximum SOD was recorded during days 2 and 3 in S + Y and S + Y + OM, thereafter SOD declined somewhat and remained relatively stable over the remainder of the experiment with mean SOD of 1.25 ± 0.31 and 1.55 ± 0.17 mmol m^{-2} h^{-1}, respectively, from day 4 onwards.

The DIC effluxes were generally higher and more variable than fluxes of oxygen in all treatments, but showed generally similar trends (data not shown). Mean DIC effluxes from day 4 onwards for the S, S + OM, S + Y and S + Y + OM treatments, respectively, were 0.9 ± 0.6, 1.3 ± 0.9, 2.1 ± 1.4 and 2.4 ± 1.1 mmol m^{-2} h^{-1}, with those in treatments containing *T. australiensis* being significantly higher than those in treatments without *T. australiensis* ($P < 0.05$). Mean community respiratory quotients

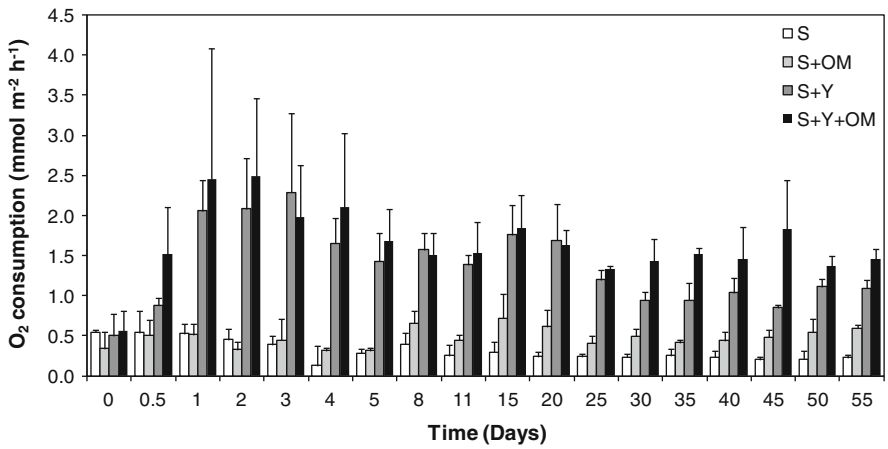

Fig. 2 Temporal evolution of sediment oxygen demand in microcosms containing sediment alone (S), sediment receiving *A. marina* leaf litter additions (S + OM), sediment to which *T. australiensis* were added (S + Y) and sediment to which *T. australiensis* and leaf litter additions made (S + Y + OM) (the addition of *A. marina* leaf litter began on day three). All values are means ± SD ($n = 3$)

Table 2 Summary of results of multivariate analyses of sediment oxygen demand (SOD) and inorganic nitrogen fluxes

Source	*df*	Flux	SOD *P*	NO_X	NH_4^+	DIN
OM	1		**0.000**	0.361	0.806	0.293
Y	1		**0.000**	**0.000**	**0.000**	**0.000**
Day	17		**0.002**	**0.000**	**0.000**	**0.000**
OM × Y	1		**0.021**	0.405	0.258	0.935
Day × OM	17		**0.012**	0.191	0.426	0.705
Day × Y	17		**0.009**	**0.000**	**0.000**	**0.000**
Day × OM × Y	17		0.114	0.161	0.520	0.153

Significant ($P < 0.05$) outcomes are highlighted in bold

Table 3 The effects of organic matter treatments on various days on oxygen fluxes

Day	Numerator *df*	Denominator *df*	*F*	*P*
0	1	8.057	0.727	0.418
0.5	1	7.907	2.331	0.166
1	1	8.017	0.221	0.651
2	1	8.030	0.180	0.682
3	1	7.542	0.026	0.876
4	1	7.970	1.901	0.205
5	1	7.433	1.304	0.289
8	1	8.005	2.783	0.134
11	1	8.183	5.108	0.053
15	1	8.149	5.402	**0.048**
20	1	7.678	8.832	**0.019**
25	1	7.284	16.575	**0.004**
30	1	7.940	37.261	**0.000**
35	1	7.122	14.955	**0.006**
40	1	7.947	11.457	**0.010**
45	1	8.326	49.947	**0.000**
50	1	8.148	13.271	**0.006**
55	1	8.765	105.795	**0.000**

Significant ($P < 0.05$) outcomes are highlighted in bold

(CRQ: DIC efflux/SOD) over the same period were 2.8 ± 1.4, 2.5 ± 1.8, 1.7 ± 1.2 and 1.5 ± 0.7 for the S, S + OM, S + Y and S + Y + OM treatments, respectively.

Sediment–water column inorganic nitrogen fluxes

Fluxes of DIN were predominantly directed out of the sediments in all treatments (Fig. 3). In the control S treatment, DIN effluxes remained consistently low over the 55-day incubation period. The addition of *T. australiensis* to the S + Y and S + Y + OM treatments significantly influenced NO_X, NH_4^+ and DIN fluxes (Table 2). In these bioturbated treatments, DIN effluxes showed an immediate increase after the introduction of *T. australiensis* (Fig. 3c). Initially, this increase was predominantly due to enhanced NH_4^+ effluxes (Fig. 3b), which peaked at levels above 350 μmol m^{-2} h^{-1} on day 3. Thereafter, NH_4^+ effluxes remained high until day 11 and then steadily declined over the remainder of the incubation period and by day 55 had returned to levels similar to those in the non-bioturbated treatments. This decrease in NH_4^+ efflux, however, was compensated by increased NO_X efflux (Fig. 3a). NO_X efflux increased steadily in bioturbated treatments from day 4 and peaked at 230 ± 134 and 348 ± 228 μmol m^{-2} h^{-1}, respectively, in the S + Y and S + Y + OM treatments on day 11. Thereafter, NO_X efflux in the bioturbated treatments declined to below 100 μmol m^{-2} h^{-1} on

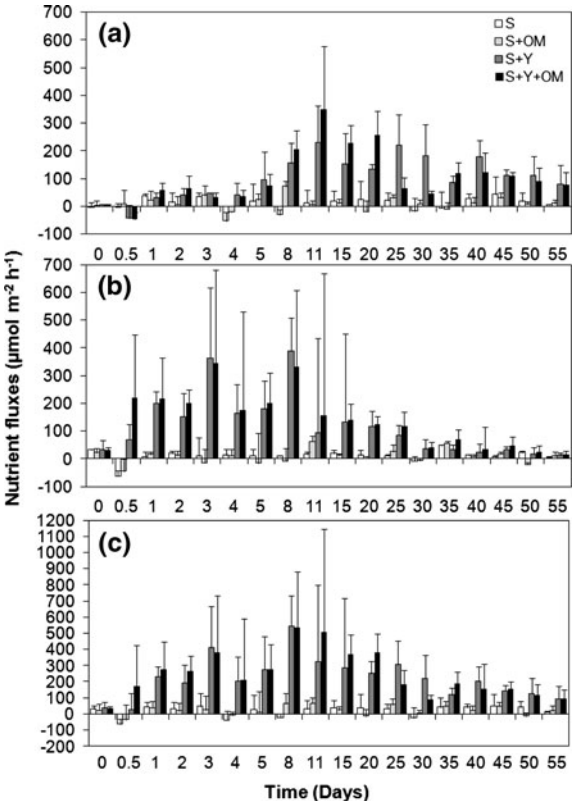

Fig. 3 Temporal evolution of sediment–water column dissolved inorganic nutrient fluxes; (a) NO_X, (b) NH_4^+ and (c) dissolved inorganic nitrogen (DIN = NO_X + NH_4^+) (the addition of *A. marina* leaf litter began on day three). All values are means ± SD ($n = 3$). Negative values indicate uptake of the solute by sediment, and positive values indicate efflux of the solute. Note: different y-axis scale for c

day 55, but remained significantly higher than those in the non-bioturbated treatments.

In contrast to yabbies, additions of *A. marina* detritus had no significant effects on DIN, NH_4^+ or NO_X fluxes under bioturbated or non-bioturbated conditions (Table 2), with all fluxes following similar trends in the S and S + OM, and S + Y and S + Y + OM treatments, respectively (Fig. 3).

Trypaea australiensis respiration and ammonium excretion rates

Individual oxygen consumptions ranged from 4.5 to 26.5 µmol O_2 ind.$^{-1}$ h^{-1} with a mean of 11.5 ± 6.0 µmol O_2 ind.$^{-1}$ h^{-1} and were significantly correlated with individual wet weight biomass ($r = 0.651$, $P < 0.001$). Excretion of NH_4^+ ranged from 0.40 to 0.75 µmol ind.$^{-1}$ h^{-1} with a mean value of 0.54 ± 0.11 µmol ind.$^{-1}$ h^{-1} ($n = 9$).

Nitrate reduction pathways and nitrification

Total NO_3^- reduction (denitrification + DNRA) ranged from 9.18 ± 0.95 to 33.2 ± 5.1 µmol N m^{-2} h^{-1} and was significantly different between treatments (ANOVA $P < 0.01$) with rates being greater in bioturbated than in non-bioturbated treatments (Fig. 4). Denitrification was the dominant nitrate reduction pathway in all treatments accounting for 88–99% of the total, with denitrification rates ranging between 8.8 and 32.9 µmol N m^{-2} h^{-1}. Total denitrification rates were significantly higher in the presence of *T. australiensis* (ANOVA $P < 0.01$), with rates in the S + Y and S + Y + OM treatments being 1.9- and 3.2-fold greater, respectively, than those in the S

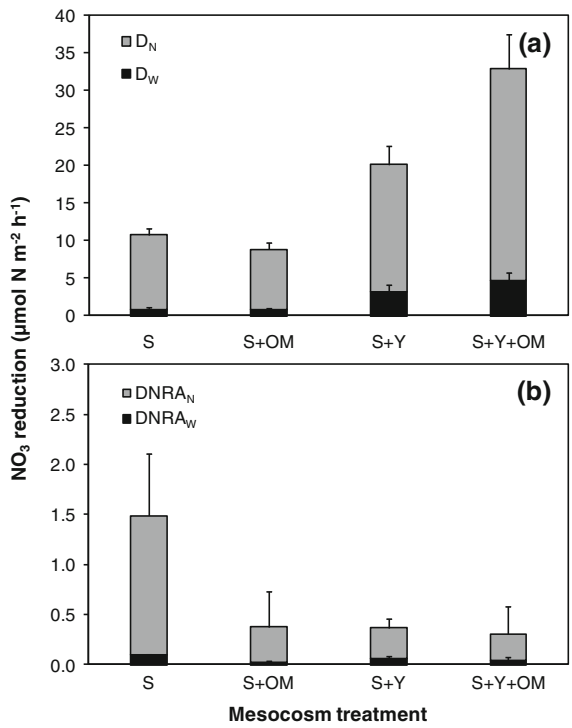

Fig. 4 Rates of nitrate reduction pathways on day 55; (a) Total denitrification separated by nitrate source into coupled nitrification–denitrification (D_N) and denitrification dependent upon diffusion of nitrate from the overlying water column (D_W) and (b) Total DNRA rates separated by nitrate source into $DNRA_N$ and $DNRA_W$ as described for denitrification rates. All values are means ± SD ($n = 3$). Note: different y-axis scales

Table 4 Influences of the addition of *T. australiensis* and *A. marina* leaf litter on nitrate reduction processes, nitrification rates and the coupling between nitrification and nitrate reduction rates

Treatment	Total NO_3^- reduction	% Contribution		Nitrification	% Coupling
		Denitrification	DNRA		
S	12.2 ± 1.12	87.9 ± 4.23	12.0 ± 4.23	16.9 ± 2.14	68.8 ± 15.1
S + OM	9.18 ± 0.95	96.1 ± 3.43	3.86 ± 3.43	19.6 ± 13.9	78. 5 ± 78.0
S + Y	20.5 ± 2.75	98.1 ± 0.69	1.85 ± 0.69	98.2 ± 68.0	25.1 ± 16.8
S + Y + OM	33.2 ± 5.10	99.0 ± 1.05	0.96 ± 1.05	105 ± 47.4	32.2 ± 17.5

All rates are expressed in μmol N m^{-2} h^{-1} and are mean values ± SD ($n = 3$). Total nitrate reduction rates were calculated as $D_{total} + DNRA_{total}$, nitrification rates as the nitrate efflux + $D_N + DNRA_N$ and the % coupling as $D_N + DNRA_N$/nitrification × 100

and S + OM treatments. These increases were due to a significant (ANOVA $P < 0.05$) stimulation in rates of both D_N and D_W in the bioturbated treatments. *A. marina* litter additions resulted in a significant decrease (ANOVA $P < 0.05$) in total denitrification in the non-bioturbated treatment, whereas they caused a significant (ANOVA $P < 0.05$) 1.6-fold increase in the bioturbated treatments. DNRA rates were low compared to denitrification ranging between 0.3 and 1.5 μmol N m^{-2} h^{-1} with significantly higher (ANOVA, $P < 0.05$) rates measured in the control (S) treatment compared to all others (Fig. 4b). The contribution of DNRA to overall nitrate reduction was significantly lower in the bioturbated treatments (Table 4).

Nitrification was the principal source of NO_X fuelling nitrate reduction with D_N and $DNRA_N$ accounting for 92 and 85% of total nitrate reduction in non-bioturbated and bioturbated treatments, respectively. Nitrification rates calculated by mass balance (NO_X efflux + $D_N + DNRA_N$) for the final day ranged from 16.9 to 105 μmol N m^{-2} h^{-1} (Table 4) and were significantly higher in the bioturbated compared with non-bioturbated treatments (ANOVA $P < 0.05$) with rates in the S + Y and S + Y + OM treatments, respectively, being 4.8 and 4.4-fold higher than those in the S and S + OM treatments. However, whilst the presence of *T. australiensis* stimulated both nitrification and nitrate reduction rates, they significantly (ANOVA $P < 0.05$) decreased the coupling between these processes (Table 4) with the % coupling between nitrification and nitrate reduction being 2.7 and 2.4-fold lower in the S + Y and S + Y + OM compared to S and S + OM treatments, respectively.

Discussion

Benthic metabolism and nutrient fluxes

Trypaea australiensis recovered at the end of the experiment showed no significant change in biomass compared to those initially added, indicating that despite the low sediment organic matter content, sufficient food sources were available to prevent starvation and weight loss. As reported for other fauna addition experiments (e.g. Hansen & Kristensen, 1998; Banta et al., 1999; Bartoli et al., 2000; Nizzoli et al., 2007; Jordan et al., 2009), *T. australiensis* caused a pulse in SOD and DIN effluxes. It is expected that *T. australiensis* addition would induce such events as it constructs its deep complex burrow system, causing a transient non-steady-state situation where reduced and nutrient-rich sediments are flushed by burrow water and/or transported to the surface during burrow excavation (Kristensen, 2000; Welsh, 2003). Following this period, SOD and DIN effluxes stabilised between days 5 and 15, as a new equilibrium was established within the microcosms. Thereafter, SOD and DIN effluxes remained relatively stable, but were significantly greater in the bioturbated compared to non-bioturbated treatments.

Mean individual *T. australiensis* respiration and NH_4^+ excretion rates were equivalent to an oxygen demand and ammonium efflux of 920 and 43 μmol m^{-2} $h^{-1,}$ respectively, and could account for 97 and 76, and 28 and 27%, respectively, of the mean SOD and DIN efflux in the S + Y and S + Y + OM treatments. However, these are likely overestimates of the true contribution of *T. australiensis* to SOD, as respiration of the free-swimming

individuals in our laboratory 'bottle' incubations would be expected to be greater than that of burrow dwelling individuals. For example, Dunn et al. (2009) found that the respiration rate of free-swimming amphipods was ~40% higher than for burrow dwelling individuals, whereas NH_4^+ excretion was not affected. It is therefore likely that the calculated contribution of *T. australiensis* NH_4^+ excretion to DIN efflux provides a more reliable estimate of the impact of the animals on benthic metabolism. These values are also in line with those estimated for other deposit-feeding infauna (e.g. Pelegrí & Blackburn, 1995; Bartoli et al., 2000).

Overall, our results are in accordance with those of other laboratory experiments where animal additions enhance benthic metabolism and N-mineralisation (Hansen & Kristensen, 1998; Bartoli et al., 2000; Papaspyrou et al., 2004, 2010). This stimulation is proposed to be due mainly to the mineralisation of recalcitrant organic matter pools already present in the sediment, breakdown of which is stimulated by the increased relative volume of oxic sediment zones provided by the burrow walls (Kristensen, 2000). Our results support this hypothesis, as benthic respiratory quotients were lower in the bioturbated (mean 1.6) compared with the non-bioturbated (mean 2.7) treatments, indicating that there was a shift in metabolism towards aerobic pathways. However, enhanced benthic metabolism and nutrient regeneration in permanently inhabited natural sediments could only be sustained if the infauna also enhances inputs of organic matter by an equivalent degree (Kristensen, 2000; Welsh, 2003; Dunn et al., 2009). Therefore, the degree to which *T. australiensis* enhances benthic metabolism and nutrient regeneration must depend upon the extent to which they increase organic matter inputs to the sediment. The rapid burial of introduced leaf litter observed during this study indicates that *T. australiensis* can increase organic matter inputs to the sediment, especially in intertidal areas around mangroves where at low tide falling leaves are deposited directly onto the sediment surface or are stranded by the ebbing tide. As in our microcosms, the added leaf litter was rapidly trapped and buried below mounds of ejected sediment. In addition, ghost shrimp species have been shown to actively collect large particulate organic matter from the sediment surface and transport it into their burrows (Vonk et al., 2008). Therefore, owing to these mechanisms, it would seem probable that natural populations of *T. australiensis* could sustain high benthic metabolism and nutrient recycling by increasing sediment organic matter loads. This hypothesis is supported by a manipulative field experiment where *T. australiensis* was excluded (Webb & Eyre, 2004). This study showed that unmanipulated areas with *T. australiensis* at a density of 22 burrows m^{-2} increased SOD by 81% and were sources of DIN to the water column whereas the uninhabited sediments were sinks.

In contrast to *T. australiensis*, the effects of *A. marina* leaf litter additions were more subtle, took time to develop, and were in general less influential. A significant difference was observed between the treatments with and without organic matter additions ($P < 0.05$) after day 15, where the presence of leaf litter resulted in greater SOD. There were, however, no significant effects on sediment DIN effluxes or estimated sediment ammonification rates (Fig. 5). These observations are at variance with other studies where organic matter additions have been shown to cause large stimulations of benthic metabolism and nutrient regeneration rates both in the presence or the absence of infauna (Hansen & Kristensen, 1998; Kristensen & Mikkelsen, 2003; Papaspyrou et al., 2007, 2010). Two factors may largely explain these differences. First, in our microcosms, the mangrove leaf litter was added at frequent intervals, in small quantities to simulate natural *quasi* continuous leaf fall, whereas in other studies, the organic detritus has been added as a single relatively large addition to simulate a specific deposition event. Therefore, it would be expected that in our experiment the *A. marina* leaf litter additions would cause a gradual rather than abrupt change in benthic metabolism. Secondly, the mangrove leaf litter used was richer in polymeric compounds such as lignin and cellulose, and had a higher C:N ratio than the micro and macroalgal detritus typically employed in most studies. Plant detritus decomposition rates are known to be strongly influenced by biomass composition, as hydrolysis of complex polymers to their constituent monomers is the rate limiting step in the decomposition process and high C:N ratios can result in nitrogen limitation of microbial growth (Fenchel et al., 1998). Therefore, it would be expected that due to its inherent recalcitrance, *A. marina* additions would cause only a minor stimulation of benthic metabolism and have little effect on nitrogen fluxes, as much of the DIN generated during decomposition would be

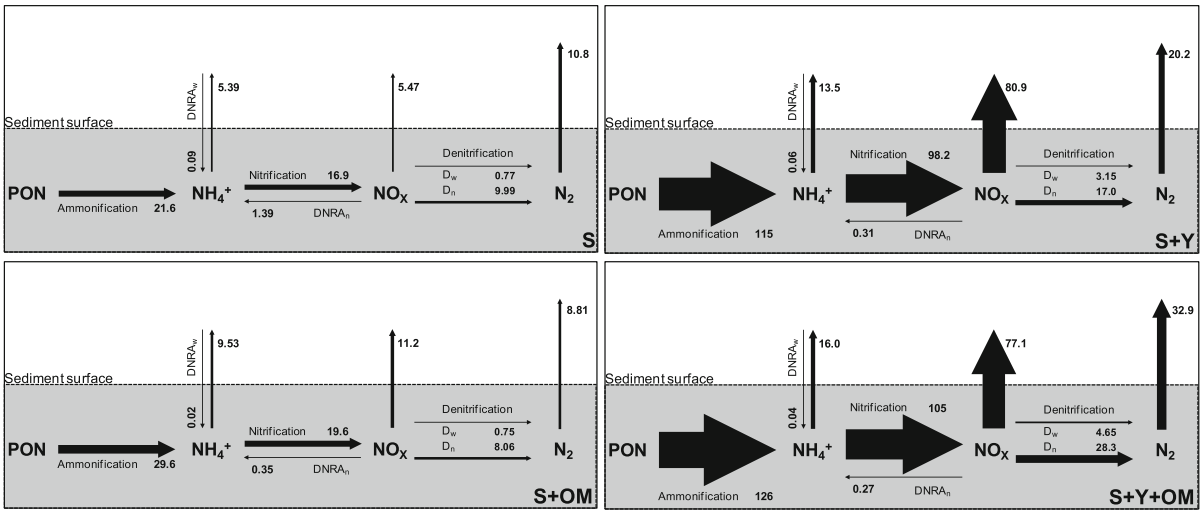

Fig. 5 Mass balance estimates of N-cycle processes in each microcosm treatment. Budgets were calculated using data from the final day (day 55) flux and nitrate reduction process rate determinations. All values are means ± SD ($n = 3$) and reported as μmol N m^{-2} h^{-1}

assimilated by the decomposing bacteria to sustain their own growth (Fenchel et al., 1998). However, previous studies have shown that ingestion and processing of mangrove leaves by sesarmid crabs can enhance decomposition rates of mangrove detritus, as crab faecal pellets have been shown to get mineralised to CO_2 at much faster rates than the detritus fed to these crabs (Kristensen & Pilgaard, 2001). Interestingly, we did find a significant interaction on SOD between yabbies and organic matter (Table 2), and yabbies are known to be fairly indiscriminate deposit feeders (Spilmont et al., 2009). Therefore, it is plausible that ingestion and processing of the mangrove detritus by the yabbies also stimulated mineralisation of the organic matter in this case.

Nitrification, denitrification and DNRA

Previous investigations on the influence of *T. australiensis* on benthic N-cycling have yielded somewhat contradictory results. In the previously mentioned field manipulation experiment, Webb & Eyre (2004) found that N_2 effluxes in *T. australiensis* inhabited sediments were fourfold greater than in exclusion areas. This shift was attributed to coupled nitrification–denitrification occurring in the burrow walls (Webb & Eyre, 2004). In contrast, the experiment of Jordan et al. (2009) revealed that nitrification rates in sediment similar to that used in the present study increased with *T. australiensis* density, but with no associated changes in rates of total nitrate reduction, denitrification or DNRA. They hypothesised that the intense bioturbation and burrow irrigation activities of *T. australiensis* not only greatly increased the volume of oxic sediment amenable to nitrification, but also increased the diffusive path length for nitrate between nitrification and nitrate reduction zones in the sediment, resulting in a decoupling of nitrification and nitrate reduction processes. Our results are somewhat intermediate between these studies. As with the study of Jordan et al. (2009), mass balance calculations, based on the final day nutrient fluxes and nitrate reduction rates (Fig. 5), showed that *T. australiensis* caused a greater than fivefold stimulation of nitrification both in the presence and the absence of *A. marina*. This stimulation of nitrification was associated with a smaller 1.7-fold increase in total nitrate reduction and a 1.9-fold increase in denitrification in the S + Y treatment compared with the S treatment. However, in the S + Y + OM treatment, total nitrate reduction and denitrification were stimulated by ~3.7-fold compared with the S + OM treatment. This synergistic effect of *T. australiensis* and *A. marina* leaf litter was presumably due the buried leaf fragments providing anoxic microniches suitable for nitrate reduction within the oxic sediment nitrification zones created by *T. australiensis*. On the other hand, although total nitrate reduction and denitrification

were stimulated in the presence of *T. australiensis*, the coupling between nitrification and nitrate reduction processes was decreased, which is consistent with the hypothesis of Jordan et al. (2009).

In addition to influencing overall nitrate reduction, *T. australiensis* and *A. marina* additions also influenced the partitioning of NO_X between the competing nitrate reduction pathways. The contribution of DNRA was significantly lower in all treatments containing *T. australiensis* and/or leaf litter, with the lowest rate recorded in the S + Y + OM treatment despite this having the highest total nitrate reduction rate. Several factors have been proposed to regulate competition between denitrification and DNRA for NO_X, with DNRA being favoured by high ratios of labile organic carbon to NO_X (electron donor : electron acceptor), low nitrate availability and reduced, especially sulphidic, sediment conditions (Christensen et al., 2000; Welsh et al., 2001; Nizzoli et al., 2006; Dong et al., 2011). It would therefore be expected that DNRA would only be a minor process in the organic matter poor sediments employed in our microcosms and that bioturbation by *T. australiensis* would further reduce its importance by increasing nitrate availability through enhanced nitrification and sediment redox. However, it remains unclear why *A. marina* leaf detritus additions would also decrease DNRA and the relative contribution of DNRA to total nitrate reduction under both bioturbated and non-bioturbated conditions.

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