Impacts of shrimp farm effluent on water quality, benthic metabolism and N-dynamics in a mangrove forest (New Caledonia)

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A R T I C L E   I N F O

Article history:
Received 20 July 2011
Accepted 24 July 2012
Available online 21 August 2012

Keywords:
mangrove
shrimp farm
nutrient fluxes
denitrification
DNRA
New Caledonia

A B S T R A C T

Water quality parameters, sediment oxygen demand (SOD), dissolved organic and inorganic nutrient fluxes, and N-cycle processes (nitrification; denitrification; dissimilatory nitrate reduction to ammonium (DNRA)) were determined in a New Caledonian mangrove receiving shrimp farm effluent and a natural mangrove. Effluent was enriched in nutrients and organic matter, and significantly stimulated SOD and nutrient regeneration rates in the receiving sediments. All N-cycling processes were stimulated between ~2 and 12-fold in the sediments receiving effluents compared to the natural mangrove. However, due to the preferential enhancement of DNRA compared to denitrification, there was no significant increase in net nitrogen elimination compared to the significant increase in sediment nutrient regeneration rates. These results indicate that the mangroves are only a partial filter for the shrimp farm effluent, as confirmed by the elevated nutrient concentrations measured in an external, marine creek of the effluent receiving mangrove.

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1. Introduction

Mangroves are dynamic ecosystems, which develop at the interface between terrestrial and marine environments along tropical and subtropical coastlines (Hogarth, 1999). Mangroves are important as they stabilise sediments, provide physical protection to coastlines and are nursery environments for many fish and crustaceans, including commercially important species (Nerot et al., 2009). They also play a fundamental role in the transformation, turnover and export of particulate and dissolved organic and inorganic nutrients of terrestrial origin (Marchand et al., 2006; Kristensen et al., 2008; Nagelkerken et al., 2008).

One of the largest documented threats to mangrove ecosystems is the development of aquaculture ponds, especially shrimp farming (Alongi, 2002; Duke et al., 2007). Worldwide, shrimp farming has increased almost exponentially since the mid-1970’s due to short production cycles and high product values, reaching a total annual production of over 2.3 billion tonnes in 2008 (Bostock et al., 2010). In tropical and sub-tropical zones, particularly in South America, Indonesia and Thailand, shrimp farms have been developed at the expense of mangrove forests, which are cleared for the establishment of the rearing ponds (Menasveta, 1997). In addition to the direct loss of mangroves during construction, shrimp farms also impact the adjacent mangroves through the release of large quantities of effluents, rich in particulate and dissolved organic and inorganic nutrients (Paez-Osuna, 2001; Jackson et al., 2003; Thomas et al., 2010). During a production cycle, it has been estimated that only 29 and 16%, respectively, of total nitrogen (N) and phosphorus (P) added to the ponds as food and fertilizers inputs, is actually assimilated by the shrimps (Avnimelech and Ritvo, 2003). As a consequence, most of the added N and P is released to the environment in effluents rich in particulates (e.g. uneaten feeds, faeces and phytoplankton) and dissolved organic and inorganic species (Jackson et al., 2003; Thomas et al., 2010).

Several studies have investigated the impacts of shrimp farm effluents on mangrove ecosystems, especially on water column processes in mangrove creeks (McKinnon et al., 2002; Burford et al., 2003; Thomas et al., 2010). Effluents significantly increase water column chlorophyll a, dissolved inorganic nitrogen (NH4 + NO3) and total nitrogen and phosphorus concentrations (Costanzo et al., 2004; Thomas et al., 2010). In addition, effluents enhance primary and bacterial production (McKinnon et al., 2002; Burford et al., 2003) and induce significant increases in biological oxygen demand, promoting water column hypoxia or anoxia.
(Trott and Alongi, 2000; Thomas et al., 2010). However, these effects are generally localised to areas close to effluent outlets, and water quality parameters and phytoplankton biomass return to natural levels within a few months after the cessation of the discharge (Trott and Alongi, 2000; Thomas et al., 2010). In contrast, much less is known about the effects of shrimp farm effluent on processes within the benthic compartment (Trott et al., 2004).

Benthic nitrogen cycling can eliminate significant quantities of external N-loads as gaseous products (N2, N2O and NO) via denitrification and anaerobic ammonium oxidation (anammox) (Herbert, 1999; Dalsgaard et al., 2005; Thornton et al., 2007). Conversely, the competing process of dissimilatory nitrate reduction to ammonium (DNRA) recycles NOx, which could otherwise be eliminated by denitrification and anammox to NH4+, favouring N-retention within the ecosystem (Christensen et al., 2003; Nizzoli et al., 2006; Burgin and Hamilton, 2007). Therefore, the balance between these competing NOx reduction pathways can exert a strong influence on benthic N-dynamics and the susceptibility of the ecosystem to eutrophication (Nizzoli et al., 2006; Burgin and Hamilton, 2007; Dong et al., 2011).

In New Caledonia, shrimp farming is still a relatively small industry consisting of 19 farms with ponds covering 680 ha, with an annual production of 2000 tons in 2006 (Della Patrona and Brun, 2009). In contrast to elsewhere, there are no direct losses of effluents due to shrimp farming, as local regulations prevent construction of farms within the mangroves (Virly, 2005). However, effluents are still discharged into the adjacent mangroves, which are considered as a “natural biofilter”, to reduce or eliminate impacts on the surrounding World Heritage listed lagoon and coral reef (Lemonnier and Faninoz, 2006; Thomas et al., 2010).

The aim of this study was to examine the ability of the mangrove to actually act as a filter for shrimp farm effluents, and more specifically to quantify the impacts of the effluents on benthic metabolism, nutrient fluxes and N processing (denitrification and DNRA) within the mangrove sediment with particular attention on their capacity to eliminate nitrogen.

2. Materials and methods

2.1. Study site

The study was conducted in two fringing mangroves located in Saint Vincent Bay, New Caledonia (Fig. 1), neither of which receives any significant freshwater runoff. The first “natural” (N) mangrove (21°54′S, 166°04′E) covered 20 ha (Fig. 1a) and was free of significant anthropogenic influences. The second “effluent receiving” (E) mangrove (21°56′S, 166°04′E; total area of 28.9 ha (Fig. 1b)) receives effluent discharges from the “Ferme Aquacole de la Ouenghi” shrimp farm (FAO). Visual density and size distributions of the mangrove trees differed between the two studied mangroves. In the natural mangrove, the landward Avicennia was sparsely distributed, bush-like and never exceeded 1 m in height. Similarly the seaward Rhizophora zone had a relatively low tree density and individual trees seldom exceeded 2 m in height. In contrast, in the mangrove receiving shrimp farm effluents, there was a higher density of trees in both vegetation zones and individual trees in the Avicennia and Rhizophora zones were more than 2 and 5 m tall respectively.

The FAO shrimp farm opened in 1989 and operates two 1 m deep rearing ponds of 10.5 (L) and 7.5 (K) ha respectively, which are constructed within and have replaced the original saltpan zone. However, during the study period a shortage of shrimp larvae meant that only the smaller pond (K) was actually in production. Like the majority of shrimp farms in New Caledonia, FAO operates a semi-intensive rearing system. Ponds are stocked with the blue shrimp, Litopenaeus stylirostris, at a density of ~17 individuals m−2.

Fig. 1. Map showing the location of the two studied mangroves in Saint Vincent Bay on the west coast of New Caledonia; (a) natural mangrove, showing vegetation zonation and the location of sampling sites NU and Ns; (b) effluent receiving mangrove with the locations of the FAO shrimp farm, the influent/effluent water channels, sites of effluent discharge (arrows) and location of sampling sites EU and E5.
in December/January, which are reared for ~8 months. The shrimp are fed with locally produced feed pellets (35–40% protein, SICA, New Caledonia), which are added daily throughout the rearing period, with inputs increasing from ~0.25 to ~3.5 kg ha\(^{-1}\) d\(^{-1}\) over the rearing cycle as the shrimp grow (Farm manager, Pers. Comm.). Ponds are continuously irrigated in order to maintain water column oxygen, with water that is pumped directly from the lagoon through a canal, and introduced by gravity at a rate increasing from 5 to 25% of the pond volume over the course of the rearing cycle (Farm manager, Pers. Comm.). Excess water overflows the ponds and is discharged at multiple points into the adjacent mangrove (Fig. 1b). Due to the impermeable nature of the sediments, these effluent discharges principally traverse the mangrove as surface flows, which are eventually collected by the short marine channels which penetrate the mangrove fringe. Following the shrimp harvest, the ponds are drained and left to dry for ~4 months until the subsequent production cycle commences (Della Patrona and Brun, 2009).

Within each mangrove area, two sampling sites were chosen: the first was located near the downstream limit of the mangrove in the Rhizophora vegetation zone, near the mouth of a fracting marine creek (sites natural downstream (Nu) and effluent receiving downstream (Eo), Fig. 1). The second site was located at the upstream limit of the mangrove at the interface between the Avicennia vegetation zone and saltpan (natural upstream (Nu) site) and close to effluent discharge at the Avicennia-shrimp farm interface (Eo, Fig. 1).

2.2. Sample collection

2.2.1. Water sampling

Water samples for determining dissolved and particulate organic and inorganic nutrient concentrations were collected in February, April, May and June 2009 during the shrimp farm production cycle at 4 sites: the farm pumping station (input water), the shrimp pond effluent discharge point near site Eo and at the downstream mangrove creek sites Eo and Nu. Additionally, water samples were collected at downstream sites Nu and Eo in August, October and December 2009, and February 2010 after the production cycle had ceased.

Water samples for dissolved organic and inorganic nutrients were collected in triplicate, using 25 ml syringes, and were immediately filtered through 0.2 μm pore size cellulose acetate syringe filters (Whatman) and frozen at ~20 °C until being analysed. Particulate nutrients were collected by filtering known volumes of water using previously combusted (550 °C) 45 mm Whatman GF/F filters (0.7 μm) which were frozen at ~20 °C until analysed.

2.2.2. Sediment sampling

Undisturbed sediment cores were hand collected in triplicate at low tide, using plexiglass tubes (18 cm Ø x 40 cm length), to determine sediment-water column solute fluxes and nitrate reduction rates from sites Nu, Nu, Ao, Eo and Eo in April 2010 when the farm was operational and actively discharging effluent. A parallel set of triplicate cores were collected (10 cm Ø x 32 cm long) at each site to characterize sediment porosity and water content. Cores were transported to the laboratory within 30 min, and transferred into holding tanks containing in situ water collected at the previous high tide. An aquarium pump was attached within each core and the tanks were filled to above the level of the cores to allow free exchange with the aerated water within the holding tanks. Cores were equilibrated overnight in the dark, prior to determinations of flux and nitrate reduction rates.

2.3. Oxygen and dissolved nutrient flux measurements

Prior to the incubations, the water in the holding tanks was drained and replaced with fresh site water and the cores allowed to stabilise for ~30 min. To initiate the incubations, the water level in the holding tanks was lowered to slightly below that of the core rims. Initial water samples for dissolved oxygen, inorganic and organic nutrients were collected and the cores were sealed using floating plexiglass lids to prevent gaseous exchange with the atmosphere (Welsh et al., 2001). The incubation time was set so that the final oxygen concentration remained above 80% of the initial air saturated concentration, so that natural biogeochemical reactions were not modified. Incubation times varied between 30 min and 4 h depending upon sampling site. At the end of the incubations, the aquarium pumps were stopped and water samples for dissolved oxygen and nutrients were collected. Fluxes of individual solute species (μmol m\(^{-2}\) h\(^{-1}\)) were determined from the difference between the initial and final solute concentrations, as described in Welsh et al. (2000).

Following flux incubations, the holding tanks were re-filled with site water, the aquarium pumps within the cores were switched back on, and the cores were allowed to re-equilibrate for ~2 h prior to determination of nitrate reduction rates.

2.4. Determination of nitrate reduction rates

Rates of nitrate reduction processes were determined using the isotope pairing technique (Nielsen, 1992), as modified to allow simultaneous determination of denitrification and DNRA (Risgaard-Petersen and Rysgaard, 1995; Nizzoli et al., 2006). Cores were prepared for incubation as previously described for flux determinations. An initial water sample was collected from each core, to determine ambient NO\(_3\)-N concentrations, prior to the addition of a 30 mmol L\(^{-1}\) 99.9 atom % 15N-NO\(_3\) (ISOTEC\(^{TM}\)) solution to each core to give a final concentration of ~30 μmol L\(^{-1}\) in the overlying water. The water column was briefly mixed and a second water sample for NO\(_3\) concentration was taken ~10 min later to allow calculation of the actual 15N-NO\(_3\) addition by difference (Nizzoli et al., 2006). Cores were closed using floating Pexelgass lids and incubated under dark conditions as previously described for flux determinations. Incubation durations were the same as those used for flux determinations, to ensure that the final time O\(_2\) concentration remained above 80% of the initial saturating concentration, a prerequisite of the isotope pairing technique (Nielsen, 1992). At the end of the incubation, a sub-core (Ø 25 mm) was inserted into the sediment to the base of the incubation core. Microbial activity in the bulk sediment was arrested by the addition of 5 ml of 50% w/v ZnCl\(_2\) to the water outside the sub-core. The sub-core, including the overlying water, was withdrawn and the sediment bio-available ammonium (NH\(_4\)) pool extracted as previously described by Nizzoli et al. (2006). The remaining sediment within the incubation core was gently slurried to mix the dissolved N\(_2\) pools in the sediment porewater and overlying water. After a brief settling period (1–2 min) a sub-sample of the slurry was transferred to a gas-tight, 12 ml glass vial (Exetainer, Labco), fixed with 150 μl 50% w/v ZnCl\(_2\) and the samples stored –4 °C awaiting determination of the dissolved N\(_2\) pool and its isotopic composition. Rates of total denitrification (D\(_{\text{tn}}\)), denitrification coupled to nitrate production by nitrification in the sediment (D\(_{\text{tn}}\)), and denitrification fuelled by nitrate diffusing from the overlying water column (D\(_{\text{wn}}\)) were determined according to Nielsen (1992). DNRA rates based on water column NO\(_3\) (DNRA\(_{\text{wn}}\)) were calculated from the enrichment of 15N-NO\(_3\) pool in the water column and 15N enrichment of the sediment bioavailable NH\(_4\) pool (Risgaard-Petersen and Rysgaard, 1995). Rates of DNRA coupled to sediment nitrification (DNRA\(_{\text{sn}}\))...
were estimated from the rate of DNRA_water and the ratio between DN and DW (Risgaard-Petersen and Rysgaard, 1995).

Anammox is a recognised interference when using the isotope pairing technique that can lead to overestimation of denitrification rates, as it also generates labelled N₂ species, following ¹⁵NO₃ and ³²O₂ additions (Risgaard-Petersen et al., 2003). However, in shallow water sediments anammox is a minor source of N₂ compared to denitrification (Dalsgaard et al., 2005; Burgin and Hamilton, 2007), especially in tropical systems (Dong et al., 2011). Therefore, we believe the calculated denitrification rates are valid estimates of the actual rates, although it should be noted that the term denitrification as used hereafter, also includes an unknown, but likely small portion of N₂ production via anammox.

2.5. Sample handling and analysis

Oxygen concentrations were determined directly using an oxygen electrode (WTW oxy 315i). Water samples for determining dissolved nutrients were filtered through syringe filters (0.2 μm cellulose acetate; Ø 25 mm; Whatman) and stored frozen (−20 °C) until analysed. NH₄ concentrations were determined by the fluorimetric method of Holmes et al. (1999) using a Turner Designs TD700 fluorimeter. NO₃ (NO₂ + NO₃) concentrations were determined using an Autoanalyser III (Bran + Luebbe) by the method of Bendschneider and Robinson (1952). Total dissolved nitrogen (TDN) concentrations were determined as N₂O following oxidation by the method of Raimbault et al. (1990). Dissolved organic nitrogen (DON) concentrations were calculated by difference as the TDN pool minus the measured dissolved inorganic nitrogen (DIN; sum of the NH₄ and NO₃ pools). Phosphate (PO₄³⁻) concentrations were determined using an Autoanalyser III (Bran + Luebbe) by the method of Murphy and Riley (1962). Total dissolved phosphorus (DTP) concentrations were determined as PO₄³⁻ following oxidation by the method of Raimbault et al. (1990) and dissolved organic phosphorus (DOP) concentrations as TDP minus PO₄³⁻. Particulate organic nitrogen (PON) and phosphorus (POP) were determined as NOₓ and PO₄³⁻ respectively, following oxidation (Raimbault et al., 1990) and total nitrogen (TN) and phosphorus (TP) concentrations as the sum of the respective particulate and dissolved pools.

Dissolved N₂ concentrations and the proportions of ²⁰N₂ and ³⁶N₂ in the dissolved N₂ pool, and ¹⁵N enrichment of sediment bioavailable ammonium pool were analysed at the National Environmental Research Institute (Silkeborg, Denmark) as previously described by Risgaard-Petersen and Rysgaard (1995). Sediment water content for DNRA rate determinations was determined as loss of wet weight following drying to constant weight at 105 °C as described in Dunn et al. (2009).

2.6. Statistical analyses

All incubation data (incubation water, flux and processes rates) as well as mangrove creek water samples were determined in triplicate. The distribution of the corresponding variables did not meet normality criteria (Shapiro tests), even after transformation (arc sin, square root, log(x+1)). Therefore, these data were analysed using permutational multivariate analysis of variance (PERMANOVA) with 1000 permutations. Where differences in concentrations, fluxes and process rates were detected between sites (PERMANOVA, p < 0.05), permutational t-tests with 1000 permutations were used to compare sites. Effluent waters were sampled in triplicate and the water composition (dissolved organic and inorganic nitrogen and phosphorus species, and particulate species) were measured. The mean values for each species were calculated and compared to the same species in a single lagoon water sample collected at the same time using permutational t-test with 1000 permutations. All statistical analyses were performed using R version 2.11.15.

3. Results

3.1. Water column dissolved and particulate nutrient concentrations

The shrimp farm effluent tended to be enriched in all measured particulate and dissolved, organic and inorganic, nitrogen and phosphorus species compared to the influent lagoon water and was significantly enriched in TN (Permutation t-test, p = 0.018), PON (Permutation t-test, p = 0.024), NH₄ (Permutation t-test, p = 0.026), DON (Permutation t-test, p = 0.039), TP (Permutation t-test, p = 0.028) and POP (Permutation t-test, p = 0.024) (Table 1). Particulate organic nitrogen and phosphorus concentrations were up to 3 and 4.5-fold greater respectively, in the effluent compared to the influent water, and represented 59 and 52% respectively, of the effluent TN and TP load. Similarly, organic species dominated the effluent dissolved nutrient pools, with DON and DOP accounting for 60.4 and 71.4% of TDN and TDP, respectively. DIN and DIP concentrations in the input water to the ponds, and the discharged effluents were relatively low and not significantly different (Permutation t-test, p = 0.84, and p = 0.06, respectively) (Table 1). However, there was a significant, 6.7-fold increase, in NH₄ concentrations in the effluent compared to the influent water. In parallel, there was a corresponding 2-fold decrease in NO₃ concentrations, although this decrease was not statistically significant (Permutation t-test, p = 0.41).

During the four months of effluent discharge from the shrimp farm (between February and June 2009), TDN, PON and NH₄, and TDP and DOP concentrations all showed a significant enrichment in the waters of the seaward creek in the effluent receiving mangrove compared to the natural mangrove during at least two sampling periods (Fig. 2). Conversely, after the effluent discharge from the farm ceased in August 2009, there were no significantly higher concentrations of nitrogen and phosphorus species in the anthropized compared to natural creek (Fig. 2). Indeed, at times the concentrations of specific nitrogen species were significantly higher in the natural compared to the effluent receiving creek e.g. DIN, NO₃ and NH₄ in August (Permutation t-test, p = 0.024, p = 0.025 and p = 0.021, respectively), and DIN and NH₄ in December (Permutation t-test, p = 0.022 and p = 0.021, respectively). In both creeks, organic forms of nitrogen dominated the water column nitrogen pool, with the sum of the PON and DON concentrations accounting for on average ~80% of TN.

Table 1

<table>
<thead>
<tr>
<th>Nutrient species</th>
<th>Influent</th>
<th>Effluent</th>
</tr>
</thead>
<tbody>
<tr>
<td>TDN</td>
<td>13.9 ± 5.7</td>
<td>25.9 ± 1.2</td>
</tr>
<tr>
<td>DIN</td>
<td>4.8 ± 2.1</td>
<td>4.3 ± 1.8</td>
</tr>
<tr>
<td>NO₃</td>
<td>4.5 ± 2.0</td>
<td>2.3 ± 2.0</td>
</tr>
<tr>
<td>NH₄</td>
<td>0.3 ± 0.1</td>
<td>2.0 ± 1.0*</td>
</tr>
<tr>
<td>DON</td>
<td>8.4 ± 3.8</td>
<td>19.7 ± 2.1*</td>
</tr>
<tr>
<td>PON</td>
<td>11.4 ± 2.4</td>
<td>37.2 ± 2.9*</td>
</tr>
<tr>
<td>TN</td>
<td>25.2 ± 5.8</td>
<td>63.1 ± 3.7*</td>
</tr>
<tr>
<td>TDP</td>
<td>0.7 ± 0.2</td>
<td>3.0 ± 2.2</td>
</tr>
<tr>
<td>PO₄³⁻</td>
<td>0.2 ± 0.0</td>
<td>1.9 ± 1.7</td>
</tr>
<tr>
<td>DOP</td>
<td>0.5 ± 0.3</td>
<td>0.5 ± 0.2</td>
</tr>
<tr>
<td>POP</td>
<td>0.7 ± 0.3</td>
<td>3.2 ± 0.6*</td>
</tr>
<tr>
<td>TP</td>
<td>1.4 ± 0.2</td>
<td>6.2 ± 1.6*</td>
</tr>
</tbody>
</table>

* Indicates that concentrations were significantly different (Permutation t-test, p < 0.05)
As observed for nitrogen, the organic forms of phosphorus dominated the water column phosphorus pool within the waters of both creeks, with POP and DOP accounting for on average ~80% of TP (Fig. 3).

3.2. Sediment water column oxygen and dissolved nutrient fluxes

Dissolved nutrient concentrations in the in situ waters used for core incubations are shown in Table 2. NO$_x$, NH$_4^+$, DON, TDN, and PO$_4^{3-}$ concentrations were significantly different between sites (PERMANOVA, $p = 0.006$, $p = 0.035$, $p = 0.013$, $p = 0.006$ and $p = 0.002$ respectively). Water from the effluent-receiving site (EU) was significantly enriched compared to the other sites in TDN (Permutation $t$-test, $p = 0.023$, $p = 0.027$ and $p = 0.025$, respectively by comparison with ND, NU and ED) and DON (Permutation $t$-test, $p = 0.018$, $p = 0.025$ and $p = 0.025$, respectively by comparison with ND, NU and ED). DOP and TDP concentrations were not different between sites (PERMANOVA, $p = 0.24$ and $p = 0.17$ respectively).

Sediment oxygen demand (SOD) ranged from a minimum of 2.1 ± 0.3 mmol m$^{-2}$ h$^{-1}$ at ND to a maximum of 11.0 ± 2.0 mmol m$^{-2}$ h$^{-1}$ at EU (Fig. 4). SOD was significantly different between sites (PERMANOVA, $p = 0.001$), and was greater at site EU than at all other sampling sites (Permutation $t$-test, $p = 0.029$ compared to NU, and $p = 0.023$ compared to ED and ND). Whilst SOD was similar at the downstream sites ND and ED (Permutation $t$-test, $p = 0.4$), it was 3.3-fold greater at the effluent receiving site (EU) than at the corresponding site in the natural mangrove (NU) (Permutation $t$-test, $p = 0.029$).

Sediments at all sampling sites were net sources of dissolved nitrogen to the water column (Table 2), with TDN effluxes ranging from 31.2 ± 85.9 (NU) to 339.5 ± 281.9 (EU) μmol m$^{-2}$ h$^{-1}$. However, due to the large variability in the effluxes between replicate cores there were no significant differences in TDN effluxes between sites (PERMANOVA, $p > 0.05$). Similarly, DON effluxes demonstrated a high degree of within site variability and were not significantly different between sites (PERMANOVA, $p = 0.98$). In contrast, the DIN efflux was significantly different between sites (PERMANOVA, $p = 0.0009$). The DIN flux at site EU of 239.1 ± 78.2 μmol m$^{-2}$ h$^{-1}$ was significantly greater than at all other sites (Permutation $t$-test, $p = 0.03$, $p = 0.025$ and $p = 0.03$ respectively compared with ND, NU and ED), while the DIN flux at site ND of –27.7 ± 9.3 μmol m$^{-2}$ h$^{-1}$ was significantly lower than at all other sites (Permutation $t$-test, $p < 0.05$). Similarly, the NH$_4^+$ efflux of 208.5 ± 75.4 μmol m$^{-2}$ h$^{-1}$ measured at site EU was significantly different from those at all other sites (Permutation $t$-test, $p = 0.025$, $p = 0.03$ and $p = 0.023$, respectively compared with ND, NU and ED). NH$_4^+$ fluxes at both sites in the effluent receiving mangrove were significantly greater than those at the two sites in the natural mangrove (Table 2). NO$_x$ fluxes were not significantly different between sampling sites (PERMANOVA, $p = 0.056$).

All sites were net sinks for TDP and DOP with the exception of the site EU, where the sediment was a source of TDP and DOP to the water column (Table 2). Phosphate fluxes were directed towards the water column at all sample sites, at rates ranging from 0.1 (ND and ED), to 5.7 μmol m$^{-2}$ h$^{-1}$ (EU). Statistical analysis revealed no significant differences in benthic TDP, DOP or PO$_4^{3-}$ fluxes between sites (PERMANOVA, $p < 0.05$) (Table 2).
3.3. Nitrification and nitrate reduction rates

Total nitrate reduction rates (denitrification + DNRA) ranged from 28.7 ± 6.1 (N_D) to 231.6 ± 75.1 (E_U) μmol N m⁻² h⁻¹ (Fig. 5, Table 3). Rates of total nitrate reduction were significantly different between sites (PERMANOVA, p = 0.03), and were greater at site E_U than at both natural sites (Permutation t-test, p = 0.02 and p = 0.03, respectively compared with N_U and N_D).

Denitrification was the dominant pathway for nitrate reduction at sites N_U, N_D and E_D accounting for 63–90% of total nitrate reduction (Fig. 5, Table 3). In contrast at site E_U, which directly receives shrimp farm effluent, DNRA accounted for ~60% of total nitrate reduction (Table 3). Denitritication rates varied from 22.6 ± 6.8 (N_U) to 68.8 ± 42.54 (E_D) μmol N m⁻² h⁻¹ (Fig. 5), but were not statistically different between sites (PERMANOVA, p > 0.05).

Rates of total DNRA at sites N_U, N_D and E_D ranged from 3 (N_D) to 31 (E_D) with a mean rate of 16.3 ± 8.2 μmol N m⁻² h⁻¹ across the three sites. Whereas, total DNRA at site E_U was 169.4 ± 95.4 μmol N m⁻² h⁻¹. Similarly, rates of DNRA at E_U were also greater than at all other sites, but these differences were not statistically significant (PERMANOVA, p > 0.05) (Fig. 5).

Nitrification was the dominant source of nitrate fuelling nitrate reduction processes (Table 3). Nitrate reduction coupled to...
nitrification (DN + DNRA\textsubscript{n}/total nitrate reduction × 100) accounted for between 53 (ND) and 88% (ED) of total nitrate reduction, with intermediate values at NU and EU of 74 and 82%, respectively (Table 3). Nitrification rates calculated by mass balance as the sum of denitrification, DNRA\textsubscript{n} and the NO\textsubscript{x} flux, ranged from 28.1 ± 7.1 (ND) to 210.6 ± 46.8 (EU) μmol m\textsuperscript{-2} h\textsuperscript{-1} (Table 3). Nitrification rates were significantly different between sites (PERMANOVA, p = 0.04) and were greater at the upstream site in the effluent receiving mangrove (EU) than at the equivalent natural site (NU) (Permutation t-test, p = 0.02). However, although the nitrification rate at the downstream site EU was 3.3-fold greater than that at the equivalent site ND this difference was not significant (Permutation t-test, p = 0.1). Nitrate reduction rates were strongly coupled to nitrification at sites EU, ED and NU with the % coupling ranging from 74.3 ± 5.2 (NU) to 82.2 ± 5.1 (EU) % (Table 3). Coupling between nitrification and nitrate reduction was however lower at site ND (53.4 ± 24.4%), but the rate was not significantly different from that at the other sites (PERMANOVA, p = 0.35).

### 4. Discussion

#### 4.1. Effluent composition and export

Effluent from the FAO shrimp farm was significantly enriched in both total nitrogen and phosphorus compared to the input lagoon water. The excess nutrients exported from shrimp ponds were predominantly in dissolved and particulate organic forms, with organic N and P representing 90 and 60%, and PON and POP alone representing 59 and 51% of total effluent N and P. This composition is similar to those recorded in previous studies of shrimp farm effluents in New Caledonia (Lemonnier and Faninoz, 2006; Thomas et al., 2010) and elsewhere (e.g. Jackson et al., 2003, 2004). The high particulate nutrient concentrations in the effluent probably results from enhancement of phytoplankton production within the shrimp ponds, as well as from the resuspension of benthic microalgae, deposited faeces and waste feeds from the rearing pond sediments due to bioturbation by the shrimp (Burford et al., 2003).

Total effluent release by the shrimp farm over the 2009 rearing cycle was approximately 2.58 10\textsuperscript{7} L (Farm manager, pers. Comm.). Based on the mean TN and TP concentrations measured in the effluent, the total N and P loads to the mangrove were approximately 2.3 and 0.5 tonnes of N and P respectively, which are equivalent to loads of approximately 79 kg N ha\textsuperscript{-1} and 19 kg P ha\textsuperscript{-1} over the course of the 8 month rearing cycle. These values are very similar to the estimates of Robertson and Phillips (1995) of the maximum quantities of N and P that can be processed and immobilised within mangroves of 71 kg N ha\textsuperscript{-1} y\textsuperscript{-1} and 20 kg P ha\textsuperscript{-1} y\textsuperscript{-1}.

The higher density and much greater individual size of the mangrove trees in the mangrove area receiving the farm effluents compared to the adjacent natural mangrove, suggests that a proportion of the nutrient loads has been assimilated into the mangrove biomass over the 20 years of operation of the FAO shrimp farm. However, the fact that dissolved and particulate, organic and inorganic N concentrations were consistently elevated during the period of effluent discharge, and often significantly elevated in the waters of the effluent receiving mangrove creek compared to that in the natural mangrove, indicates that the mangrove is at best only a partial filter for the shrimp farm effluents. This conclusion is supported by the recent study of Thomas et al. (2010) of two other New Caledonian shrimp farms, where signs of eutrophication, elevated nutrient and phytoplankton concentrations were detected in both mangrove creeks and an external bay.

Although nitrogen pools in the effluent influenced mangrove creek were predominantly in particulate forms, it is likely that they are at least in part the result of increased production within the mangrove, as well as direct transport of particulates already

### Table 3

<table>
<thead>
<tr>
<th>Sites</th>
<th>Nitrification rates (μmol m\textsuperscript{-2} h\textsuperscript{-1})</th>
<th>% DNRA</th>
<th>Nitrification (μmol m\textsuperscript{-2} h\textsuperscript{-1})</th>
<th>% Coupling</th>
<th>Net N-loss (μmol m\textsuperscript{-2} h\textsuperscript{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>EU</td>
<td>231.6 ± 75.1\textsuperscript{a}</td>
<td>60.0 ± 21.5\textsuperscript{a}</td>
<td>210.6 ± 46.8\textsuperscript{a}</td>
<td>82.2 ± 5.0\textsuperscript{a}</td>
<td>10.6 ± 19.5\textsuperscript{a}</td>
</tr>
<tr>
<td>ED</td>
<td>100.0 ± 39.9\textsuperscript{ab}</td>
<td>36.9 ± 9.6\textsuperscript{ab}</td>
<td>92.8 ± 43.3\textsuperscript{ab}</td>
<td>88.1 ± 3.9\textsuperscript{a}</td>
<td>61.9 ± 34.7\textsuperscript{a}</td>
</tr>
<tr>
<td>NU</td>
<td>37.3 ± 17.0\textsuperscript{b}</td>
<td>34.1 ± 6.7\textsuperscript{a}</td>
<td>46.4 ± 19.7\textsuperscript{b}</td>
<td>74.3 ± 5.2\textsuperscript{a}</td>
<td>22.3 ± 8.7\textsuperscript{a}</td>
</tr>
<tr>
<td>ND</td>
<td>28.7 ± 6.4\textsuperscript{b}</td>
<td>10.3 ± 2.8\textsuperscript{b}</td>
<td>28.1 ± 7.1\textsuperscript{b}</td>
<td>53.4 ± 24.4\textsuperscript{a}</td>
<td>24.8 ± 6.1\textsuperscript{a}</td>
</tr>
</tbody>
</table>

Data are means (n = 3) ± one standard error. Data with different letter suffixes are significantly different between sites (Permutation t-test, p < 0.05 after a Permanova analysis, p < 0.05).
present in the discharged effluent through the mangrove. Indeed, the consistently elevated concentrations of dissolved organic and inorganic nitrogen recorded in the effluent receiving mangrove creek may contribute to phytoplankton and bacterioplankton productivity. Several studies of mangroves impacted by shrimp farm effluents have reported increased phytoplankton and bacterioplankton production within the water column (Trott and Alongi, 2000; McKinnon et al., 2002; Burford et al., 2003; Thomas et al., 2010) and this would contribute to elevated PON concentrations. Additionally, the elevated concentrations of dissolved nitrogen species in the effluent receiving mangrove creek suggest that there may have been significant processing of the shrimp farm effluent within the mangrove.

4.2. Benthic metabolism and nutrients fluxes

The high sediment oxygen demand (SOD) at site EU (11.0 ± 2.0 mmol m⁻² h⁻¹), which is close to an effluent discharge point (approx. 500 m from the ponds and 200 m from the main effluent channel), shows that effluent organic matter stimulated benthic metabolic. This SOD was more than 5 times higher than those in the natural mangrove and well outside of the range of SOD previously reported in mangrove ecosystems in Malaysia (0.4–6.6 mmol m⁻² h⁻¹) (Alongi et al., 2004). Indeed, this high SOD is similar to those recorded in sediments directly below aquaculture fish cages or mussel farms where the sediments receive very high organic matter loads as faeces and/or uneaten feeds (Christensen et al., 2000, 2003; Holmer et al., 2002, 2005; Nizzoli et al., 2005, 2006). This large stimulation of benthic metabolism may be a relatively localized effect, as the SOD at the downstream site in the effluent receiving mangrove was not significantly different from those recorded within the natural mangrove. Additionally, SOD at site ED was well within the range previously reported for pristine mangrove sediments (Alongi et al., 2004), as were those at both sites in the natural mangrove.

Sediment-water column dissolved nitrogen fluxes determined in this study also support the hypothesis that organic loads transported in shrimp farm effluents stimulated mineralisation rates in the mangrove sediments. The effluxes of NH₄⁺ and NO₂⁻ at site EU were high (239.1 ± 78.2 and 30.5 ± 13.2 mmol m⁻² h⁻¹ respectively); whereas, at the upstream natural mangrove site (N0) the sediment was a small net sink for NH₄⁺ (−273 ± 5.0 mmol m⁻² h⁻¹) and exhibited a lower efflux of NO₂⁻ (9.4 ± 2.9 mmol m⁻² h⁻¹). Furthermore, effluxes of NH₄⁺ measured at the downstream effluent receiving site were significantly higher than those at site N0, indicating that the effluents may have influenced N-cycling throughout this mangrove. DON fluxes showed a high degree of within site variability at all the studied sampling sites, as also observed in other studies of mangrove sediments (Alongi, 1996; Holmer et al., 2001; Trott et al., 2004).

Similarly, although site EU sediments were a source of DOP and PO₄³⁻ to the water column, when sediments at all other sites were net sinks for dissolved P this difference was not significant due to high within site variability in P-fluxes. However, despite the somewhat equivocal results for N and especially P fluxes, overall the nutrient flux data support the hypothesis that effluent release stimulates mineralisation processes in the receiving mangrove sediments and that the benthic compartment plays a major role in the transformation and recycling of effluent organic matter loads. The efflux of regenerated dissolved nutrients from the sediment associated with this enhancement of organic matter mineralisation would favour primary production processes in the water column and/or increased export of dissolved nutrients to the bay.

4.3. Nitrification and nitrate reduction processes

Total nitrate reduction (denitrification + DNRA) rates were significantly stimulated by 5.5-fold at the effluent receiving mangrove site EU and by 3.5-fold at site ED, compared to sites N0 and N1 respectively in the natural mangrove. Nitrification in the sediment was the predominant source of nitrate fueling nitrate reduction processes at all sites with DN + DNRA accounting for 53–97% (mean 77%) of total nitrate reduction. Mass balance estimates of nitrification rates (D₁₄ + DNRA + NO₃ flux) ranged from 28.1 to 210.6 mmol m⁻² h⁻¹ and rates were stimulated 4.5 and 3.3-fold respectively at sites EU and ED compared to N0 and Np. The high nitrification rate of 210.6 ± 46.8 mmol m⁻² h⁻¹ recorded in the sediments at site EU is somewhat surprising. Indeed, previous studies (Nizzoli et al., 2006 and references therein) have shown that in sediments with high oxygen demands, nitrification is usually limited, due to aerobic heterotrophs and other chemoheterotrophic bacteria, which have higher affinities for oxygen, out-competing nitrifying bacteria for oxygen. However, the noticeable abundance of fiddler crab populations at EU and their burrowing activity, may promote oxygen transport to the sediment, particularly during sediment exposure at low tide, as their burrow walls greatly increase the surface area of sediment available for diffusive oxygen exchanges (Botto and Iribarne, 2000; Kristensen and Alongi, 2006). Faunal tubes, burrow wall sediments and the surfaces on fauna themselves are privileged sites for nitrification (Mayer et al., 1995; Welsh and Castadelli, 2004) which can account for a large proportion of total benthic nitrification (Blackburn and Henriksen, 1983; Jordan et al., 2009).

DNRA was a significant nitrate reduction pathway in both mangrove systems, with DNRA accounting for 10–60% of total nitrate reduction, supporting the results of Dong et al. (2011) that DNRA is a quantitatively more important process in tropical compared to temperate coastal sediments. Rates of DNRA at sites EU and ED in the mangrove receiving effluents were enhanced by 11.5 and 10.5-fold respectively, compared to sites N0 and Np in the natural mangrove. Similarly, denitrification was also stimulated within the effluent receiving mangrove with rates at sites EU and ED being 2.8 and 2.7 fold higher respectively, than those at N0 and Np. Thus, although rates of both nitrate reduction processes were stimulated in the effluent receiving mangrove; DNRA was preferentially stimulated compared to denitrification. This is also reflected by the greater contributions of DNRA to total nitrate reduction at sites EU and ED of 60 and 37%, compared to 34 and 10% at sites N0 and Np.

Several environmental factors including high temperature, low nitrate availability, high ratios of electron donor (organic matter) to electron acceptor (nitrate) and high sulfide concentrations have been proposed to favour DNRA over denitrification (Christensen et al., 2003; Nizzoli et al., 2005 and references therein). Therefore the greater contribution of DNRA to total nitrate reduction at sites EU and ED may be a relatively localized effect, as the SOD at the downstream site in the effluent receiving mangrove was not significantly different from those recorded within the natural mangrove. Additionally, SOD at site ED was well within the range previously reported for pristine mangrove sediments (Alongi et al., 2004), as were those at both sites in the natural mangrove.
In terms of the net effect of nitrate reduction processes on nitrogen loss/retention within the mangroves, denitrification can be considered as a loss, as fixed nitrogen is eliminated as a gas, whereas DNRA favours nitrogen retention by recycling nitrate to ammonium, which could otherwise be denitrified and lost as N₂ gas. More specifically, considering the fate of the N-loads from the shrimp farm effluent, denitrification of nitrate already present in the effluent (Δνₑ) can be considered to represent the sediments capacity to eliminate nitrate during transport, and denitrification coupled to nitrification (Δνₑ) as the sediments capacity to eliminate N during the remineralisation of organic matter deposited from the effluents. Conversely, DNRA of nitrate already present in the effluents (DNRAᵥₑ) represents a net input of “new” N to the sediment system as ammonium (Nizzoli et al., 2006), whereas coupled nitrification-DNRA (DNRAᵥₑ) simply recycles the nitrate generated from ammonium by nitrification back to ammonium. Therefore, the net effect of nitrate reduction processes on sediment N-loads can be expressed as total denitrification minus DNRAᵥₑ. Based on this formula, nitrate reduction processes at the sites EU and ED in the mangrove receiving shrimp farm effluents resulted in net N-losses of 62 and 11 μmol N m⁻² h⁻¹ respectively. While intermediate losses of 22 and 25 μmol N m⁻² h⁻¹ were estimated for the natural sites NU and ND respectively (Table 3), although none of these between site differences were statistically significant. However, even if we consider the difference between site EU and the sites in the natural mangrove as real, the increased rate of N-loss from ammonium by nitrification back to the water column.

5. Conclusion

This study has demonstrated direct impacts of shrimp farm effluent release on rates of benthic metabolism, nutrient fluxes and N processing within the sediment compartment of an adjacent mangrove. Organic matter exported from shrimp farm stimulated oxygen demand and nutrient regeneration rates in the receiving mangrove sediments, resulting in large effluxes of dissolved organic and inorganic nutrients to the overlying water. All N-cycling processes (nitrification, denitrification and DNRA) were stimulated in the mangrove sediments receiving shrimp farm effluents. However, due to the preferential enhancement of rates of DNRA compared to denitrification, this resulted in only a small, insignificant increase in net nitrogen elimination compared to the quantitatively much larger increase in sediment nutrient regeneration rates. Thus, the major role of the sediment was to process the effluent PON loads and to export them directly in dissolved forms to the surrounding lagoon waters, or indirectly by stimulating bacterial and phytoplankton biomass production, which was subsequently exported. Thus, the mangrove is only a partial filter for the shrimp farm effluent and part of the nutrient loads are exported to the adjacent bay, as confirmed by the consistently higher dissolved and particulate nutrient concentrations recorded in the fringing creek of the effluent receiving compared to that of the natural mangrove during the rearing season. This study suggests that a more comprehensive monitoring of shrimp farms and the effluent receiving mangroves is required in New Caledonia, to quantitatively assess the true export of nutrients to the surrounding lagoon due to this activity, especially due to the recent addition of this environment to the World Heritage list.

Acknowledgements

This work was supported by the ZONECO Program, the Northern Province and Southern Province of New Caledonia. We thank the staff of the FAO farm who made this project possible by allowing us to access to the ponds, farm data and to the adjacent mangrove. We thank the staff of the IFREMER, Saint Vincent station for their valuable help, in particular Luc Della Patrona and Benoît Bellief. We thank Audrey Leopold for her assistance with field and laboratory work, and Phillippe Gerard (Laboratoire de Chimie Marine) from IRD for chemical analysis. Finally we thank Cedric Hubas and Hervé Rybarczyk for their help with statistical treatments, and for their constructive reading of the manuscript.

References
