Carbon and nitrogen cycling on intertidal mudflats of a temperate Australian estuary. II. Nitrogen cycling

Perran L. M. Cook[1,2,3,5,*], Andrew T. Revill[2,3], Edward C. V. Butler[2,3], Bradley D. Eyre[4]

[1]University of Tasmania, GPO Box 252-75, Hobart, Tasmania 7001, Australia

[2]CSIRO Marine Research, GPO Box 1538, Hobart, Tasmania 7001, Australia

[3]CRC for Coastal Zone, Estuary and Waterway Management, Indooroopilly Sciences Centre,
80 Meiers Road, Indooroopilly, Queensland 4068, Australia

[4]Centre for Coastal Management, Southern Cross University, PO Box 157, Lismore 2480, Australia

[5]*Present address:* Max Planck Institute for Marine Microbiology, Celsiusstrasse 1, 28359 Bremen, Germany

ABSTRACT: Benthic fluxes of dissolved nitrogen, rates of denitrification, N_2 fixation and NH_4^+ upward flux within the sediment (calculated from porewater profiles) were measured on the upper and lower mudflats at 2 study sites, 1 in the upper, river-dominated part of the estuary, and 1 in the lower, more marine part of the Huon Estuary, Tasmania, Australia. The calculated upward flux of NH_4^+ from within the sediment based on porewater profiles was generally in excess of measured benthic fluxes, suggesting that NH_4^+ was reassimilated at the sediment surface by microphytobenthos (MPB). The ratio of total CO_2 (TCO_2):NH_4^+ produced within the sediment was generally in excess of 15, and in some cases in excess of 60. Significant influxes and effluxes of dissolved organic nitrogen (DON) were measured where the activity of MPB was highest. At times, DON influxes and effluxes were well in excess of dissolved inorganic nitrogen (DIN) fluxes, highlighting the importance of measuring DON fluxes where the activity of MPB is high. Rates of denitrification were very low, and represented only a small loss of N from the sediment, most probably as a consequence of the activity of MPB. Estimates of nitrogen assimilation by MPB showed that N_2 fixation was likely to be the major source of nitrogen during the summer at the study site in the upper estuary. There was also a high estimated C:N ratio (~20) of TCO_2 and nitrogen assimilated at this site, suggesting that a significant proportion of primary production was exuded as dissolved organic carbon rather than cellular production.

KEY WORDS: Microphytobenthos · Denitrification · Intertidal · Porewater · Dissolved organic nitrogen · Sediment · Nitrogen fixation · Sediment–water exchange

INTRODUCTION

Because of their sheltered nature, mudflats are important zones of organic matter accumulation in coastal systems (Jickells & Rae 1997). The remineralisation of organic matter deposited within these zones results in the release of inorganic nitrogen and phosphorus to coastal waters (Rocha et al. 1995, Falcão & Vale 1998, Rocha 1998). In situations where a large fraction of this remineralisation proceeds via denitrifi-

cation, there may be a net loss of nitrogen from the system (e.g. Dong et al. 2000). As such, mudflats may act as both sources and sinks of dissolved inorganic nitrogen. Recently, much attention has been focused on the role of intertidal sediments in attenuating high inorganic nitrogen loads from the land to the sea in temperate European estuaries (Middelburg et al. 1995b, Ogilvie et al. 1997, Trimmer et al. 1998, Cabrita & Brotas 2000, Dong et al. 2000, Trimmer et al. 2000, Magalhães et al. 2002).

Microphytobenthos (MPB) are ubiquitous on mudflats and may exert an important influence on nitrogen cycling processes in these environments (Underwood & Kromkamp 1999). Assimilation of nitrogen by MPB means that effluxes of NH_4^+ and NO_3^- may be drastically reduced or even reversed in the light (Sundbäck et al. 1991, Cabrita & Brotas 2000, Magalhaes et al. 2002). In addition, MPB have been found to compete effectively with nitrifying/denitrifying bacteria for NH_4^+ and NO_3^-. Assimilation:denitrification ratios of between 2 and 10 have been reported for eutrophic mudflats, whilst ratios of between 7 and >100 have been reported for more oligotrophic sediments (Cabrita & Brotas 2000, Dong et al. 2000, Sundbäck & Miles 2000). Furthermore, MBP have also recently been shown to have a significant negative impact on denitrification, most probably via competition for nitrogen (Risgaard-Petersen 2003). These findings are significant, as they suggest that a far greater proportion of nitrogen remineralised within the sediments will be assimilated rather than denitrified, resulting in a retention of bioavailable nitrogen within the system. Recent studies have shown that sediment-dissolved organic nitrogen (DON) uptake and release may exceed that of dissolved inorganic nitrogen (DIN) in the presence of MPB, suggesting that a significant fraction of nitrogen assimilated by MPB may be returned to the water column as DON (Sundbäck et al. 2000, Eyre & Ferguson 2002).

Where much of the MPB consists of cyanobacteria, as in cyanobacterial mats, a significant fraction of the nitrogen demand of the community may be met by N_2 fixation (Stal 1995). While the importance of N_2 fixation in cyanobacterial mats (Pinckney et al. 1995, Stal 1995, Paerl et al. 1996) and coral reefs (Charpy-Roubaud et al. 2001 and references therein) has received widespread attention, the importance of N_2 fixation more generally by MPB, particularly in temperate areas, has received little attention. Given that cyanobacteria have been observed in temperate, highly productive MPB communities (Kristensen 1993, Sundbäck et al. 2000, Cook et al. 2004b, this volume), it seems highly likely that N_2 fixation may meet a significant fraction of the nitrogen requirements of MPB. Any studies of nitrogen cycling on mudflats must, therefore, give due consideration to the central role that MPB play in the cycling of nitrogen in these environments.

To date, all studies of benthic nutrient cycling on temperate tidal flats have been conducted in European systems that have generally experienced severe eutrophication (Middelburg et al. 1995a,b, Ogilvie et al. 1997, Trimmer et al. 1998, 2000, Cabrita & Brotas 2000, Dong et al. 2000). There is a lack of information on benthic nutrient processing in more oligotrophic estuaries (Nedwell et al. 1999). It has been proposed that prior to enrichment, European intertidal flats were net im-

porters of nutrients from the sea, with tight recycling and storage of nutrients enabling these systems to support significant secondary production (Malcolm & Sivyer 1997). In order to further investigate this hypothesis, it is necessary to obtain data from temperate oligotrophic systems. Furthermore, the currently emerging conceptual understanding of nitrogen cycling from more eutrophic European systems may not be appropriate to more oligotrophic systems such as those found in Australia (Harris 2001, Ferguson 2002). Here, the results of a study on benthic nutrient cycling in 2 intertidal mudflats in a cool-temperate, mesotrophic Australian estuary are presented. In particular, we studied the role of MPB in controlling nitrogen cycling processes including denitrification, nitrogen fixation, and dissolved organic and inorganic nitrogen fluxes.

MATERIALS AND METHODS

Sampling. Descriptions of the study sites are given in Cook et al. (2004a, this volume). Cores for the measurement of denitrification, benthic metabolism and nutrient exchange measurements were collected at Castle Forbes Bay (Site CF), a terrestrially dominated part of the estuary in March, June, September and December, and at Port Cygnet (Site PC), a marine side arm of the estuary, in April, June, September and December 2001. In spring and summer, Site PC had an approximately 50% cover of the macroalgae *Gracillaria* sp. During autumn and winter this was greatly reduced to ~10%. To facilitate comparison with other measurements, March and April are referred to as autumn; June, September, November and December are referred to as winter, early spring, late spring and summer, respectively. Nutrient fluxes were measured *ex situ* in the same cores and incubation set-up described in Cook et al. (2004a). While this methodology may not accurately reflect *in situ* exchange rates, it does allow some of the controlling factors and interactions between benthic microbial metabolism and nutrient fluxes to be evaluated (Asmus et al. 1998), which was the aim of this study. After flux experiments had finished, 1 to 2 intact sub-cores were taken from each large core for isotope-pairing measurements.

Cores (7 cm inner diameter, i.d.) for porewater analysis were taken separately, in triplicate from the upper and lower mudflats at each site, during April, August and late November 2001, and are referred to as autumn, winter and summer, respectively. An additional core was also taken at each site in April to measure porosity.

For the measurement of N_2 fixation, 8 cores (4.5 cm i.d. × 15 cm) were taken haphazardly (but avoiding any areas vegetated by macroalgae) from the upper and lower mudflats at each site during February (summer),

April (autumn), August (winter), September (spring) and October (spring) 2002. In each case, a plug of sediment approx. 6 cm long was taken.

Porewaters. For porewater extractions, cores were rapidly sectioned under atmospheric conditions at 0.5 cm intervals from 0 to 2 cm, at 1 cm intervals from 2 to 4 cm, and at 2 cm intervals from 4 to 8 cm. Slices were placed in a centrifuge tube under a stream of argon (Ar) and then sealed. The sediment was centrifuged at 2000 rpm for 10 to 15 min. The centrifuge tubes were then placed in an Ar-purged glove box and filtered through Whatman GF/F filters. The filtrates were then frozen for later analysis of NH_4^+. Total nitrogen was also measured in the porewaters in cores taken during April and November. After NH_4^+ analysis, the porewaters from each depth interval were pooled for the 3 replicate cores for the analysis of total nitrogen. Samples were stored frozen for later analysis within 3 mo for NH_4^+ and within 1.5 yr for total nitrogen. In preliminary experiments, NO_2^- and NO_3^- were not detected in the porewaters, suggesting that there were no artifacts from the oxidation of NH_4^+ during core processing, and that net nitrification was not occurring within the sediment to a significant extent. Sediment porosity was determined according to Dalsgaard et al. (2000) at 1 cm intervals to a depth of 6 cm.

Dissolved nutrient exchange and denitrification. Rates of nutrient exchange in the light and dark, as well as dissolved oxygen and TCO_2 (data presented in Cook et al. 2004a) were measured on the 2 d following sampling and coincided with the period of *in situ* low tide. Cores were illuminated at 500 µE m^{-2} s^{-1} using a 50 W halogen lamp placed above each core. Before the commencement of flux measurements, the cores were illuminated for ~1 h, flushed with fresh site water, and capped. Rates of O_2, TCO_2 and nutrient exchange were similarly made in the dark after flushing the cores with fresh site water. We took 4 samples in a time series for nutrient and alkalinity determinations. A 'blank' core containing only water was also incubated and sampled in an identical manner to the sediment cores. Water samples taken for nutrients and alkalinity were filtered through a precombusted Whatman GF/F filter into 10 ml screw-cap polypropylene containers. These were stored frozen for later analysis (within 3 mo for DIN, within 6 mo for total nitrogen). During the dark incubation, the dissolved oxygen (DO) was never allowed to drop below 80% of its saturated concentration. The flux across the sediment–water interface under light and dark conditions was calculated using linear regression and typically used 4 data points. The flux was only regarded as significant if the standard error was less than the magnitude of the flux.

N_2 fixation. N_2 fixation was determined using the acetylene reduction assay (ARA) according to Capone (1993) as follows. The cores were returned to the laboratory within 1 h of collection and submerged in a water bath (40 l) of water collected from the site and incubated at *in situ* water temperature. Cores were stirred by a teflon-coated stirrer bar which was suspended ~5 cm above the sediment; this was driven by an external rotating magnet rotating at 60 to 70 rpm. The following morning the water was drained from half of the cores taken from each position on the mudflat. The cores were then sealed with a clear acrylic lid (which had a rubber septum); 20% of the headspace was then replaced with acetylene that had been generated freshly from CaC_2. The cores were left illuminated at 500 µE m^{-2} s^{-1} as described above. Ethylene was sampled from the cores 3 times during the course of the day. At dusk, the remaining cores (which had also been illuminated throughout the day in the same water bath) were incubated in exactly the same way except under dark conditions. The daytime incubations generally lasted 6 to 8 h and the dark incubations lasted up to 12 h. The rate of ethylene production was calculated using linear regression of the concentration change in ethylene over time.

Experiments were also undertaken to calibrate the ratio of N_2 to acetylene reduced using ^{15}N-N_2. In February 2002, intact cores were taken from the upper mudflat at Site CF, and from the lower mudflat at Site PC. The cores were then illuminated for most of the following day. The top 0.5 cm of sediment was then taken from 4 or 5 cores and slurried with a small amount of site water. The slurries were then incubated in gas-tight Vacutainers (Becton Dickinson), either with acetylene or ^{15}N-N_2 (vials pre-purged with an 80:20 He/O_2 mixture to remove unlabelled N_2 in the slurry). These containers were briefly shaken for 1 min, before being allowed to incubate in parallel at 20°C (the same as *in situ* temperature). Gas samples (50 µl) were taken and analysed immediately for ethylene from the Vacutainers with added acetylene. The Vacutainers were opened in a time series corresponding to the sampling times for those samples being incubated with acetylene (1, 5, 15, 50 h intervals). Upon opening, the slurry was immediately frozen for later analysis of ^{15}N. The δ^{15}N of the sample was converted to at.%^{15}N as described by Montoya et al. (1996). Linear regression analysis of the amount of excess ^{15}N in the sediment versus time was used to calculate the rate of ^{15}N fixation.

Denitrification. Rates of denitrification were measured using the isotope pairing technique (Nielsen 1992) described by Dalsgaard et al. (2000). After the nutrient flux experiments had been completed, sub-cores (4.8 cm i.d. × 30 cm) were taken from the original cores such that there was ~8 cm of sediment and 17 cm of water column. A teflon-coated stirrer bar was then

suspended ~5 cm above the sediment; this was driven by an external rotating magnet rotating at 60 to 70 rpm. Light and dark incubations were performed with 4 cores from each site on the following day. Dark cores were double-wrapped in aluminium foil; light was provided as described previously (third subsection of 'Materials and methods'). Experiments commenced with the addition of stock $^{15}NO_3^-$ to a final concentration of 60 µM. This concentration was chosen after a concentration series experiment (as described by Rysgaard et al. 1995) had shown that concentrations of $^{15}NO_3^-$ above 20 µM gave constant values of denitrification. Samples were taken for analysis of NO_3^- before and after the addition of $^{15}NO_3^-$ in order to calculate final ^{15}N enrichment. Cores were then capped and left for 2 h to allow the added $^{15}NO_3^-$ to diffuse into the denitrification zone and attain equilibrium. Cores were sacrificed over a time span that allowed the DO to decrease by no more than 20% below saturation. They were sacrificed as follows: 1 ml of 50% $ZnCl_2$ was added to the water overlying the sediment, and the sediment was gently slurried with the water column using a metal rod; coarser particles were allowed to settle for about 1 min before a ~40 ml sample was taken using a gas-tight syringe. The sample was then placed in a 12 ml Exetainer (Labco) to which 250 µl of 50% w/v $ZnCl_2$ had been added. A headspace of He was introduced into the Exetainer within 2 wk, and the samples were subseqently analysed within several months. Denitrification rates were calculated according to the isotope pairing equations of Dalsgaard et al. (2000).

Sample analysis. NH_4^+ was analysed using *o*-phthalaldialdehyde (OPA) derivatisation and fluorescence detection (R. Watson et al. unpubl. data); the precision of the method was generally between 5 and 8% with a detection limit of 0.07 to 0.2 µM. NO_2^- and NO_3^- were determined on a Technicon Autoanalyser using sulphanilamide derivatisation of NO_2^-; NO_3^- was determined as NO_2^- after cadmium reduction to NO_2^- (modified from Grasshoff 1976). The limit of detection of the analysis was 0.05 µM for NO_2^- and 0.1 µM for NO_3^-; the precision for both analyses was typically less than 3%. Total dissolved nitrogen was determined as NO_3^- after persulphate digestion modified from Valderrama (1981); the precision was generally <5% and the limit of detection was 5 µM. Dissolved organic nitrogen (DON) was calculated as the difference between total nitrogen and DIN (NO_2^- + NO_3^- + NH_4^+); the precision for this analysis was 10%. Total dissolved primary amines (TDPA) were analysed according to the method of Petty et al. (1982); the precision of the method was 1.5% and the limit of detection was <0.1 µM. Urea was analysed on a Technicon Autoanalyser according to the method of Price & Harrison

(1987); the limit of detection was 0.25 µM and the precision was <5%.

Ethylene of sampling was determined within 1 d using a Hewlett Packard 5890 gas chromatograph equipped with an Alltech AT Alumina column (30 m, 0.53 mm i.d.) and a flame ionisation detector. The ^{15}N enrichment of sediment samples were analysed using a Carlo Erba NA1500 CNS analyser interfaced via a Conflo II to a Finnigan Mat Delta S isotope-ratio mass spectrometer. Analysis of $^{28}N_2$, $^{29}N_2$ and $^{30}N_2$ was carried out using this same mass spectrometer interfaced to a Hewlett Packard 5890 GC.

Modelled sediment NH_4^+ production rates. Estimates of the upward flux of NH_4^+ within the sediments were made using Fick's first law:

$$F = -\varphi(D_s + D_e)\frac{\Delta C}{\Delta x}$$

where φ is porosity, $\Delta C/\Delta x$ is the concentration gradient obtained using linear regression of the linear portion of the concentration profiles (4 to 5 data points), D_e is the enhanced sediment diffusion of solutes and D_s is the sediment diffusivity. D_s was calculated according to Iversen & Jørgensen (1993). An estimate of enhanced diffusion (D_e) was obtained by comparing the measured fluxes of oxygen to those calculated from O_2 micro-profiles. Micro-profiles were taken during August 2001 at all sample sites using OX 25 (25 µm tip diameter) O_2 micro-electrodes (Unisense, Aarhus, Denmark). The calculated O_2 consumption rates were then compared to those measured in intact cores taken in June (Site CF) and September (Site PC). It was assumed that sediment respiration rates were the same in the cores taken for the flux and micro-profile measurements. D_e was calculated according to Berg et al. (2001):

$$D_e = D_s\left(\frac{F_{meas}}{F_{calc}} - 1\right)$$

where F_{meas} is the measured flux in intact cores and F_{calc} is the calculated diffusive flux profile assuming $D_e = 0$. Diffusion coefficients for NH_4^+ were obtained from and corrected for temperature according to Li & Gregory (1974). Diffusion coefficients for O_2 were obtained from Broeker & Peng (1974).

Nitrogen assimilation by microphytobenthos. The rates of nitrogen uptake by MPB were estimated using the dissolved nitrogen benthic fluxes and the calculated upward fluxes of NH_4^+ within the sediment derived from this study. If there was a flux of NH_4^+ into the sediment during illumination, then the assimilation by MPB of NH_4^+ produced within the sediment was calculated as $A_{NH4} = J_{NH4} - J_D \times D$, where A_{NH4} = daily assimilation of NH_4^+ by benthic microalgae; J_{NH4} = daily upward flux of NH_4^+ from within the sediment; J_D = dark efflux of NH_4^+; D = daily dark period.

If an efflux of NH_4^+ was measured from the sediment during illumination, it was assumed that NH_4^+ met the nitrogen demand in excess of that measured for the uptake of dissolved nitrogen from the water column (see below). Under such conditions it was assumed that algal cells assimilated nitrogen in a C:N ratio of 6.6.

For the spring budget calculation, the winter production rates of NH_4^+ within the sediment were used, as porewater profiles were not taken in spring. This seems reasonable given that the winter and spring temperatures were not greatly different and the calculated rates of NH_4^+ production within the sediment did not vary greatly over the course of the year. We assumed that all the upward flux of NH_4^+ was assimilated by MPB even when there was apparently a zone of consumption within the sediment. We believe this is reasonable given that the purpose of this budget was to estimate the maximum possible uptake of NH_4^+ by MPB.

The assimilation of water-column nitrogen species was calculated as follows $A_N = J_L \times L + J_D \times D$, where A_N = assimilation of an individual nitrogen species; J_L = light uptake of nitrogen species; J_D = dark uptake of nitrogen species; L and D = light and dark periods, respectively.

The standard error due to spatial variability in the calculated NH_4^+ upward fluxes, the light and dark fluxes of dissolved nitrogen species, as well as an estimated uncertainty of 1 h in the lengths of the light and dark periods were propagated through the calculation. The whole NO_3^- flux into the sediment was assumed to be assimilated by MPB, because the rates of denitrification were relatively low. Assimilation of NO_3^- produced within the sediment was assumed to be insignificant, as in preliminary studies no NO_3^- was detected in the surface porewaters. No correction was made for tidal exposure, since we wanted to obtain a maximum estimate of the rates of dissolved nitrogen uptake by MPB in the core set-up. The daily photoperiod used was the same as that used by Cook et al. (2004a).

Statistical analysis. The statistical analysis used Statistica Version 6.0 (StatSoft). A 1-way and a 2-way analysis of variance (ANOVA) were carried out on log-transformed data. Where log-transformation failed to correct heteroscadisticity (as indicated by a Cochran's *C*-test, a non-parametric test was used. Correlation and multiple regression analysis were used to explore relations between variables.

RESULTS

Denitrification

Denitrification rates (D_{14}) at Site CF were not significantly affected by time or position on the mudflat (2-way ANOVA). Denitrification was undetectable during light incubations on the upper mudflat for all seasons; rates in the dark ranged from 0.3 µmol m^{-2} h^{-1} in autumn to 3 µmol m^{-2} h^{-1} in summer (Fig. 1). On the lower mudflat, denitrification was detected in both the light and dark; rates varied between undetectable in spring and 2.3 µmol m^{-2} h^{-1} in autumn. A large difference between light and dark denitrification rates was seen in autumn on the lower mudflat, when benthic productivity was highest.

Denitrification at Site PC was significantly greater on the lower than the upper mudflat (2-way ANOVA, $p < 0.01$). A significant interaction between position and time showed that this varied significantly with time; however, the interaction was much smaller than for the effect of position, indicating that time had a relatively small influence.

Denitrification was not related to temperature in either the light or the dark (data pooled from both sites). Light and dark denitrification had positive relationships with light and dark NH_4^+ fluxes; the relationship was strongest under light conditions (r = 0.77, $p < 0.01$) (data pooled from both sites), indicating that availability of NH_4^+ played a role in controlling rates of denitrification. Photosynthesis by MPB apparently had a negative effect on denitrification in the light, as indicated by the significant correlations with TCO_2 (r = 0.50, $p < 0.05$) and O_2 fluxes (r = −0.53, $p < 0.05$) (data pooled from both sites).

Dissolved inorganic nitrogen fluxes

Light and dark conditions had a marked influence on the benthic fluxes of NO_2^-, NO_3^- and NH_4^+ (DIN) (Fig. 1). Under light conditions, DIN was either taken up at a greater rate or released at a lower rate compared to dark conditions. During the course of the year, none of the DIN fluxes differed significantly between the upper and the lower sites of either mudflat (Kruskal-Wallis, $p > 0.05$), with the exception of dark NO_3^- fluxes at Site PC. At this site, NO_3^- uptake by the sediment was more rapid on the upper mudflat (Kruskal-Wallis, $p < 0.01$). All the data from the upper and lower mudflats were then pooled for each site and the 2 sites were compared on an annual basis. Release of NH_4^+ from the sediment was significantly greater on average at Site PC than at Site CF (Kruskal-Wallis, $p < 0.01$). The sediments at Site CF were always a sink for NH_4^+ in the light, and also (more generally) during the dark. Flux rates ranged from an uptake (−28 µmol m^{-2} h^{-1}) on the upper mudflat in the light to an efflux (39 µmol m^{-2} h^{-1}) in the dark during summer. At Site PC, NH_4^+ was generally released from the sediments under both light and dark conditions, with the highest
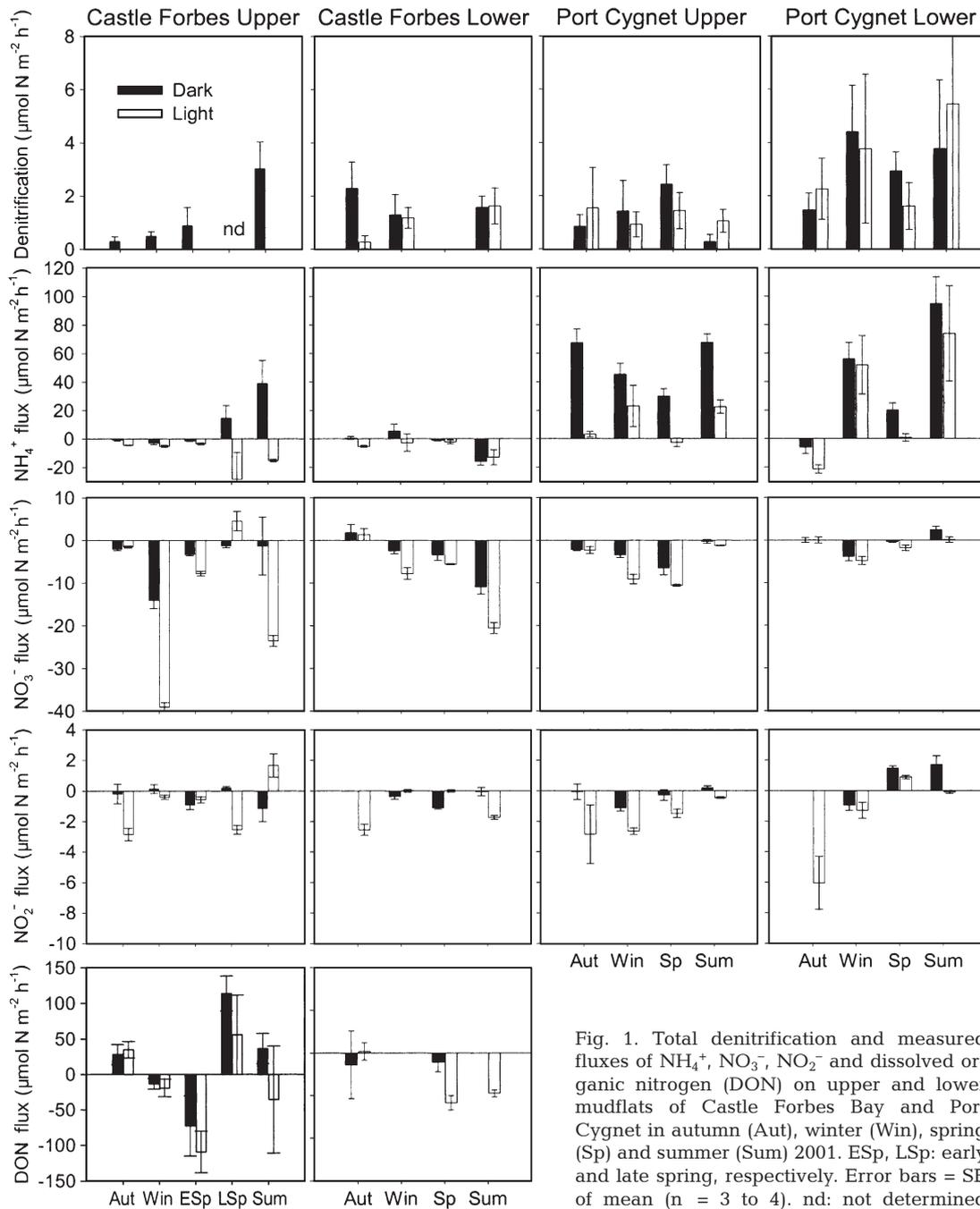
Fig. 1. Total denitrification and measured fluxes of NH_4^+, NO_3^-, NO_2^- and dissolved organic nitrogen (DON) on upper and lower mudflats of Castle Forbes Bay and Port Cygnet in autumn (Aut), winter (Win), spring (Sp) and summer (Sum) 2001. ESp, LSp: early and late spring, respectively. Error bars = SE of mean (n = 3 to 4). nd: not determined

efflux of NH_4^+ occurring during summer (95 µmol m^{-2} h^{-1}). NO_3^- was taken up at a significantly greater rate in both the light and dark at Site CF than at Site PC (Kruskal-Wallis, $p < 0.05$). NO_3^- fluxes into the sediment were significantly correlated to NO_3^- concentrations in the water column under both light and dark conditions when the data from both sites were pooled ($r = 0.60$, $p < 0.05$). Fluxes of NO_3^- and NO_2^- were not significantly correlated, with NO_2^- making up between 0 and 90 % of NOx fluxes.

Dissolved organic nitrogen fluxes

No significant fluxes of DON were detected at Site PC. Significant DON fluxes were always detected at Site CF on the upper mudflat and occasionally on the lower mudflat (Fig. 1). Fluxes of DON were not measured on the lower mudflat in winter, and were assumed to be insignificant. Treatment (light/dark) was not found to have a statistically significant effect on DON fluxes (Kruskal-Wallis, $p > 0.05$); however,
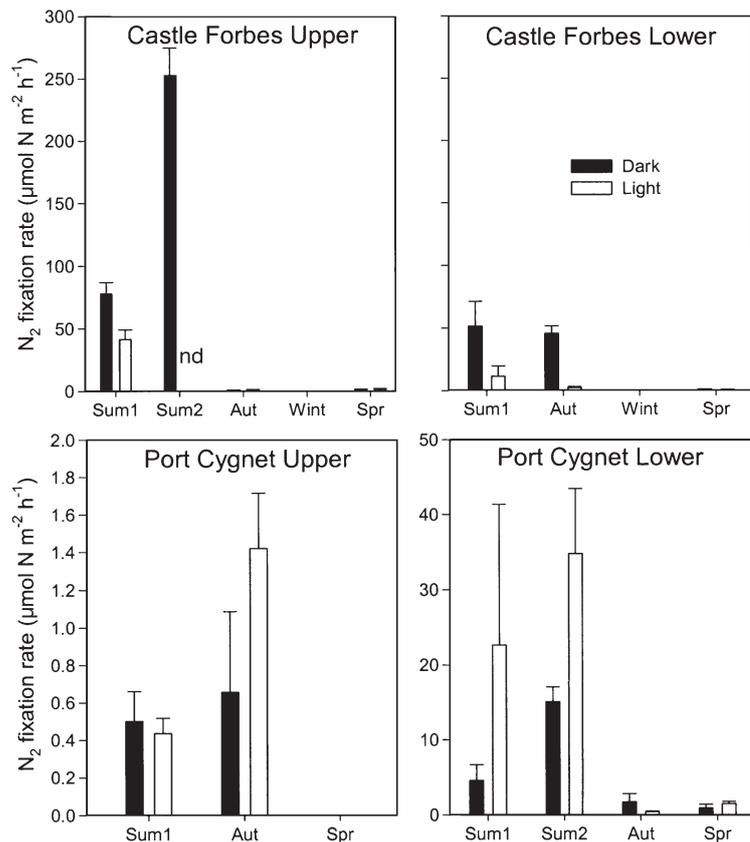
Fig. 2. Rates of nitrogen fixation measured by acetylene reduction assay under light and dark conditions at each of study sites in summer (Sum), autumn (Aut), winter (Win) and spring (Spr) 2002. Sum 1, Sum 2: samples taken on 3 and 13 February on upper mudflat at Site CF and on 6 and 10 February on lower mudflat at Site PC, respectively. Rates of nitrogen fixation calculated using acetylene-to-N_2 ratio of 4 (see 'Materials and methods' for details). nd = not determined. Error bars = SE of mean (n = 3 to 4)

there was generally a greater release (or lower uptake) of DON in the dark compared to the light. Highest DON effluxes were observed on the upper mudflat in late spring (113 µmol m^{-2} h^{-1}), coinciding with highest respiration rates (Cook et al. 1994a, and present Fig. 1). Highest uptake rates were observed in early spring (–109 µmol m^{-2} h^{-1}), coinciding with highest productivity. In this study, we measured both urea and total dissolved primary amines (TDPA). Even when the highest effluxes of DON occurred during November, urea and TDPA fluxes were insignificant (<5% of total fluxes).

N_2 fixation

The average ratio between acetylene reduced and ^{15}N fixed was 5.0 ± 0.7 at Site CF and 7 ± 2 at Site PC. The acetylene:N_2 ratios determined here were within 2 of their respective standard errors of the theoretical

acetylene:N_2 ratio. It was therefore assumed that the measured ratio was not significantly different from the theoretical ratio of 4:1.

Rates of N_2 fixation measured by the ARA were highly variable, ranging from undetectable to 250 µmol m^{-2} h^{-1} (Fig. 2). The highest rates of N_2 fixation were observed on the upper mudflat at Site CF during summer. N_2 fixation rates were generally highest in the dark at Site CF upper and CF lower. At Site PC, in contrast, the rates of N_2 fixation tended to be greater in the light. Dark rates of N_2 fixation were significantly greater at Site CF than at Site PC on an annual basis (Kolmogorov-Smirnov test, p < 0.025). There was no significant difference in the light N_2 fixation rates between Sites PC and CF on an annual basis.

NH_4^+ porewater profiles and calculated upward fluxes

NH_4^+ concentrations at all sites generally increased down the profiles, reflecting the production of NH_4^+ within the sediments (Fig. 3). At both study sites, the porewater concentrations of NH_4^+ were highest on the upper mudflat. NH_4^+ concentrations were lowest during winter at all sites, and generally similar during autumn and late spring. Concentrations within the porewaters were approximately 1 µM in the upper 3 cm of sediment on the upper mudflat and in the upper 6 cm on the lower mudflat. Calculated rates of NH_4^+ production were generally highest on the upper mudflat at PC and lowest on the lower mudflat at Site CF.

Profiles of DON were generally highly variable, showing no consistent trend with depth (data not shown). Where a depth concentration gradient was apparent, it was generally less than the gradient for NH_4^+. Blackburn & Blackburn (1993) suggested a diffusion coefficient of 5.8 × 10^{-6} cm^2 s^{-1} for DON, which is approximately a third of that used for NH_4^+. This means that for DON to be produced at rates comparable to NH_4^+, it would have to have a concentration gradient 3 times greater than that for NH_4^+, which was not the case here. Therefore, rates of DON production within these sediments were considered to be negligible compared to NH_4^+ production rates, and are not considered further.

Nitrogen assimilation by MPB

Estimated rates of nitrogen assimilation ranged from 280 µmol m^{-2} d^{-1} for the lower mudflat at Site CF dur-

ing autumn to 3460 µmol m^{-2} d^{-1} at the upper mudflat at Site PC during summer (Table 1). Calculated assimilation rates of dissolved fixed nitrogen in 2001 were generally higher than the assimilation of N$_2$ gas via nitrogen fixation measured during 2002, with the exception of summer for both mudflats at Site CF and autumn for the lower mudflat at Site CF. During summer for Site CF, the measured N$_2$ fixation rates (average of 2 sampling dates: Fig. 2) for the upper mudflat were higher than the calculated assimilation of total dissolved nitrogen at any other time of the year at this site. This suggests that N$_2$ fixation will be an important source of nitrogen to the MPB community at Site CF during the summer months when dissolved nitrogen concentrations are low and there is high light availability.

The assimilation ratio of TCO$_2$ and total dissolved nitrogen at Site CF was generally very high (11 to 164) during 2001. When the maximum rates of N$_2$ fixation measured at each site were included in the estimated

ratio of TCO$_2$ to nitrogen assimilated, this ratio dropped to that expected for balanced growth during winter. At all other times of the year, however, the estimated ratio was in the range 17 to 38. At Site PC there were generally effluxes of NH$_4^+$ from the sediment in the light; hence, the growth of MPB was generally assumed to be balanced with the exception of 2 periods of high productivity, for which the estimated ratio of C:N assimilated was 29 (PC upper mudflat in spring) and 52 (PC lower mudflat in autumn) when the maximum rate of N$_2$ fixation measured at these positions was used to estimate the assimilation ratios.

At Site CF, DON made up a significant fraction of the nitrogen assimilated, particularly in the lower mudflat, where DON generally dominated nitrogen uptake by the sediment during autumn and spring (Fig. 4). As a consequence of the low rates of NH$_4^+$ production in the sediment at this site, NH$_4^+$ produced within the sediments only appeared to be of minor importance to MPB in the lower mudflat at Site CF; in the upper mudflat, NH$_4^+$ produced within the sediment made up a variable fraction of the nitrogen assimilated. During autumn over 90% of nitrogen assimilated was from NH$_4^+$ produced within the sediment, and during spring <30% of nitrogen assimilated originated from within the sediment. NH$_4^+$ produced within the sediments was the most important source of nitrogen to MPB at Site PC year-round. No significant fluxes of DON were observed at Site PC. DIN from the water column generally comprised only a very small fraction of the measured nitrogen uptake by MPB, except during winter, when concentrations of NO$_3^-$ and NO$_2^-$ were elevated in the water column.

Table 1. Estimated rates of daily total dissolved nitrogen assimilation by microphytobenthos in 2001, measured rates of nitrogen fixation in 2002, and estimated assimilation ratios of TCO$_2$ and nitrogen at Castle Forbes Bay (CF) and Port Cygnet (PC) during course of each year. Values in parentheses: estimated error in values derived from propagation of uncertainties in spatial variability (n = 3 to 4 cores). C:N ratios: Max. = estimated from daily rates of production and the estimated total dissolved N assimilation in 2001; Avg. = ratio estimated from daily production rates and estimated total dissolved N assimilation in 2001 plus annual average rate of N fixation measured at that site in 2002; Min. = ratio estimated from daily production rates and estimated total dissolved N assimilation plus maximum rate of N fixation measured at that site in 2002. *Efflux of NH$_4^+$ from sediment during illumination; it was therefore assumed that cells showed growth at Redfield ratio (6.6); nd: no data

Season	N assimilation rate, 2001 (mmol N m^{-2} d^{-1})	N$_2$ fixation rate, 2002 (mmol N m^{-2} d^{-1})	C:N ratio Max.	Avg.	Min.
CF upper					
Autumn	809 (33)	25	92 (14)	49	18
Winter	1625 (214)	0	16 (3)	11	6.6
Spring	2907 (731)	31	43 (12)	34	20
Summer	894 (703)	3342	97 (77)	53	20
CF lower					
Autumn	281 (668)	784	164 (390)	90	38
Winter	405 (172)	0	11 (5)	6.9	6.6
Spring	1199 (226)	13	31 (7)	26	17
Summer	1305 (105)	915	39 (6)	34	23
PC upper					
Autumn	1591*	20	6.6	–	–
Winter	1206*	nd	6.6	–	–
Spring	1173 (266)	0	30 (7)	29	29
Summer	3461*	11	6.6	–	–
PC lower					
Autumn	843 (38)	32	76 (19)	67	52
Winter	791*	nd	6.6	–	–
Spring	2418*	25	6.6	–	–
Summer	1594*	387	6.6	–	–

DISCUSSION

Estimated upward fluxes of NH$_4^+$ within sediments

The exchange of solutes across the sediment–water interface due to molecular diffusion is generally 2 to 4 times lower than the observed bulk fluxes in nearshore sediments due to activity of fauna within the sediment (e.g. Archer & Devol 1992, Berg et al. 2001, Green et al. 2004). The enhanced transport of solutes across the sediment–water interface in cohesive sediments in these and previous studies is attributable to either the pump-
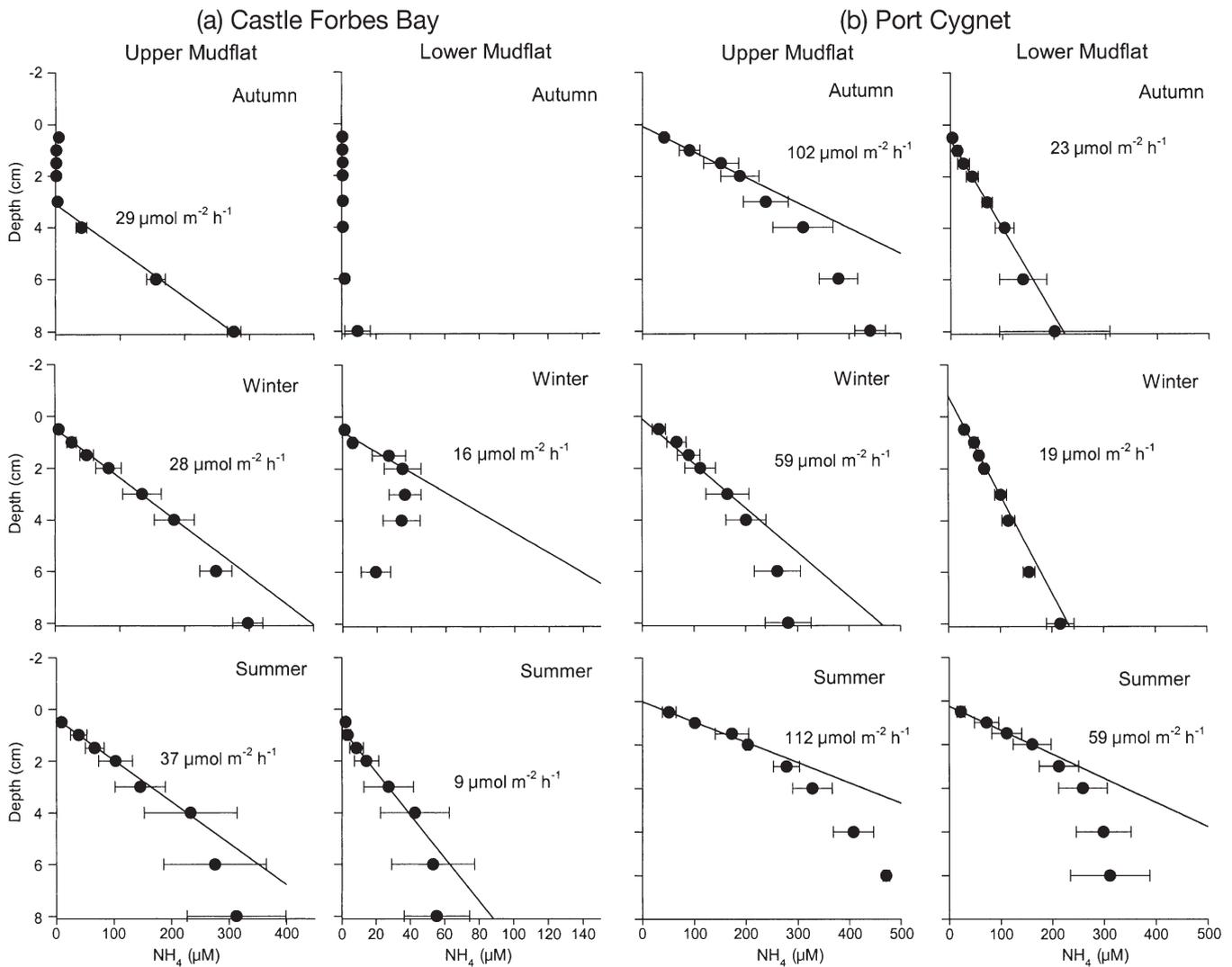
Fig. 3. Porewater profiles of NH_4^+ (●) and regression lines used to calculate upward fluxes of NH_4^+ via diffusion and enhanced diffusion (see 'Materials and methods' for details) at (a) Castle Forbes Bay and (b) Port Cygnet in autumn, winter and summer. Error bars = SE of mean (n = 3)
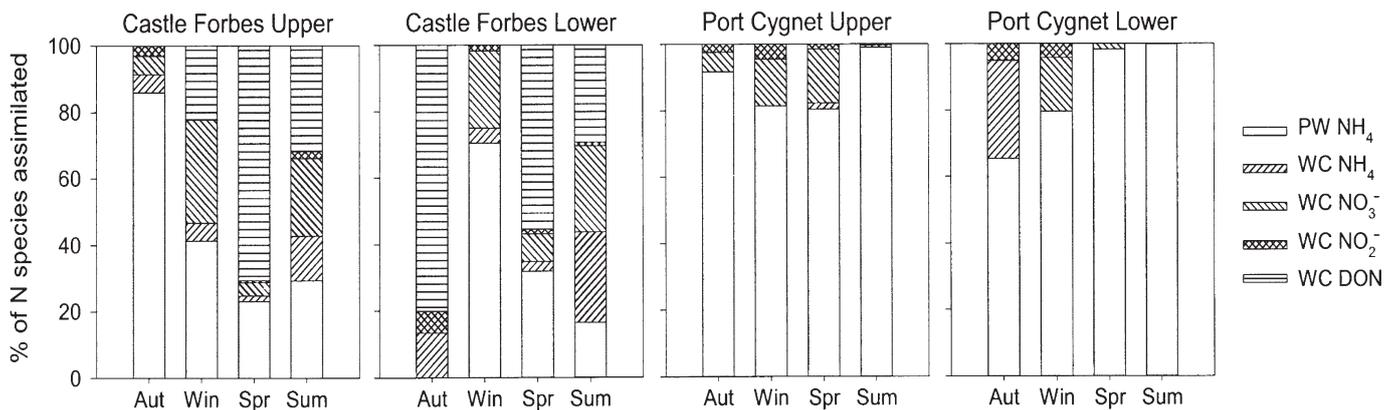

Fig. 4. Proportions of various forms of nitrogen assimilated from water column (WC) and porewater (PW) by microphytobenthos at each study site during course of the year

ing activity of fauna (bio-irrigation) or the random movements of fauna within the sediment (enhanced diffusion). Enhanced diffusion can be simply modelled using Fickian diffusive models, whereby an enhanced diffusion coefficient (D_e) is calculated, as done by Berg et al. (2001) and Meile & Van Cappellen (2003). Bio-irrigation is more complicated to model, and should theoretically be modelled using a non-local exchange model (Meysman et al. 2003). Nevertheless, the biodiffusive model has often been successfully applied in an empirical manner to nearshore, oceanic and mesocosm sediments (Soetaert et al. 1996, Matisoff & Wang 1998, Meile & Van Cappellen 2003, Green et al. 2004). This apparent contradiction between theory and practice has been termed the 'biodiffusion paradox' (Meysman et al. 2003). In the present study we used the enhanced diffusion model to estimate the upward fluxes of NH_4^+, as described in 'Materials and methods'. We believe this to be justified in this case for several reasons: (1) The sediments were initially checked for fauna and very few polychaetes and pumping bivalves were observed (~4 small, <2 cm long individuals per core, equivalent to ~250 individuals m^{-2}). Berg et al. (2001) estimated the relative effect of similar fauna densities on a bioturbation coefficient as 4.6×10^{-6} ($\frac{1}{2}$ to $\frac{1}{8}$ of those estimated here) for oxygen, and found that bio-irrigation would only account for ~11% of the estimated oxygen uptake. Given that we measured higher values of D_e, one would expect the effect of fauna on our fluxes to be relatively smaller and thus insignificant. (2) With a few exceptions, the profiles we obtained presented little evidence of bio-irrigation, with the profiles showing an outward curvature. Under the influence of bio-irrigation one would expect to see a more sigmoid profile shape for NH_4^+ (Mortimer et al. 1999, Tuominen et al. 1999). Some of the profiles did show a sigmoid shape at the surface, suggesting either consumption of NH_4^+ within the sediment or non-local exchange. We did not quantify macrofauna during these experiments, but inspection of the cores after the experiments were complete, and during core slicing suggested that the cores were relatively devoid of macrofauna (≤300 m^{-2}) and burrows. (3) There was generally no significant difference between the oxygen fluxes measured in the exposed and inundated sediments of these cores (Cook et al. 2004a). If bio-irrigation was a significant process, then one would expect to see much higher exchange rates of O_2 in the sediment during inundation because the pumping activity of fauna would cease upon sediment exposure.

The values of D_e obtained using this approach were 10.2×10^{-6} for both mudflats at Site CF, and 38×10^{-6} for the upper and 18×10^{-6} for the lower mud-

flat at Site PC. A D_e of 11.9×10^{-6} was reported at a shallow station in Disko Bay in Greenland by Rysgaard et al. (2000) (referred to as D_B in that paper). Berg et al. (2001) cited D_e values in the order of 10^{-6} to 10^{-4}. The D_e values measured here are, therefore, within the range of values expected in bioturbated sediments.

An indication of the accuracy of the upward NH_4^+ flux calculated from porewater profiles is perhaps best obtained by comparing the measured fluxes to the calculated production rates during periods of low productivity by MPB when assimilation of NH_4^+ at the surface is likely to be negligible. The lowest rates of primary production were observed for the lower mudflat at Site CF during summer, when there was also only a small difference between light and dark fluxes, indicating that the assimilation of NH_4^+ by MPB was negligible. At this time, the calculated production rate of NH_4^+ within the sediment was 74 ± 28 µmol m^{-2} h^{-1} compared to a measured flux of 94 ± 18 µmol m^{-2} h^{-1}. The overlapping standard errors of both these estimates suggest that they are not significantly different, indicating that the calculated upward fluxes of NH_4^+ within the sediment are close to the true values.

In general, the calculated upward fluxes of NH_4^+ were greater than those actually measured in the dark (Fig. 5a). In many instances, particularly at Site CF, there was an uptake of NH_4^+ from the water column, which suggests that NH_4^+ was being consumed at the sediment surface. Nitrification and subsequent denitrification are unlikely to be the NH_4^+-consuming process, because NO_3^- was not detected in these porewaters and rates of denitrification were extremely low. Assimilation by MPB is the most likely explanation, given the high MPB biomass and productivity at these sites, combined with the low water-column concentrations of DIN. This seems plausible, as MPB are able to assimilate NH_4^+ and NO_3^- for up to 60 h in darkness (Rysgaard et al. 1993). It is unclear whether MPB were responsible for the NH_4^+ consumption below the sediment surface, which in some instances extended to a depth of 3 cm (Fig. 3a). Other studies have suggested that MPB are capable of consuming nutrients in the surface-sediment layers, in some instances down to depths of 3 cm (Sundbäck et al. 1991, Thornton et al. 1999). The MPB observed at this site were dominated by *Oscillatoria* spp. (Cook et al. 2004b) which are highly motile and were observed to move several centimetres up and down the side of the core tubes. It is therefore plausible that the MPB are capable of assimilating NH_4^+ from depths of several centimetres down into the sediment. It is also possible that sediment bacteria are responsible for the uptake of NH_4^+ observed in these sediments, as discussed below.

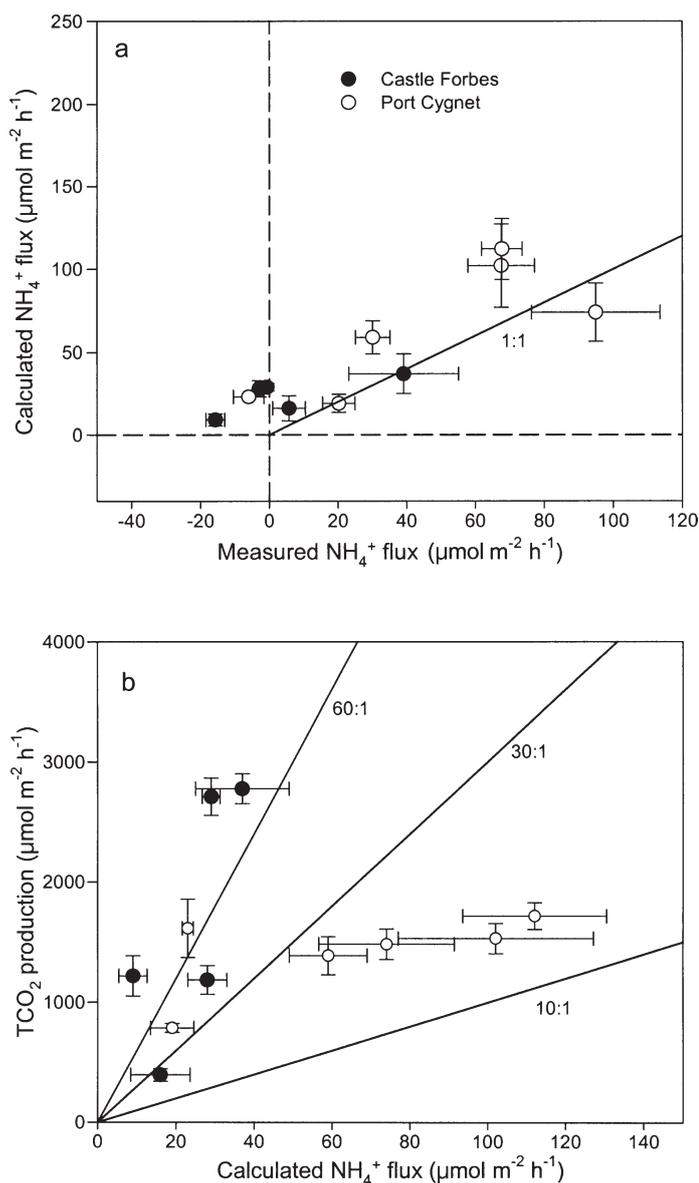Fig. 5. (a) Calculated upward flux of NH_4^+ within sediment versus measured dark NH_4^+ flux at Castle Forbes Bay and Port Cygnet; line shows 1:1 relationship between modelled and measured fluxes. (b) Dark total CO_2 flux versus calculated upward flux of NH_4^+ within sediment at Castle Forbes Bay and Port Cygnet; lines show TCO_2:NH_4^+ ratios of 10, 30 and 60. Error bars = SE of mean (n = 3 to 4)

The ratio of the calculated upward flux of NH_4^+ to TCO_2 produced in the sediment generally fell within the range of 1:10 to 1:60 (Fig. 5b). This would suggest that the organic material undergoing decomposition either had a very high C:N ratio and/or that nitrogen reassimililation was occurring in the porewaters. It is suggested (see below) that a fraction of the carbon fixed by MPB in these sediments is directed to the pro-

duction of extracellular organic carbon (EOC) as a consequence of overflow metabolism. The importance of EOC as a labile carbon source to benthic consumer animals and bacteria is now recognised (Decho 1990, Goto et al. 2001). This material is generally considered to consist predominantly of simple sugars and polysaccharides (Decho 1990, Underwood et al. 1995). The nitrogen content of EOC has been poorly investigated; however, it has been suggested that the importance of EOC as a nitrogen source to consumers is minimal (Decho 1990). A recent study has shown that amino acids comprised up to 5% of EOC (Granum et al. 2002). If it is assumed that all the nitrogen in EOC is present as amino acids with an average C:N ratio of 2, then a C:N ratio of ~40 for EOC is conceivable. EOC, therefore, may represent a labile, high C:N ratio source of organic carbon to bacteria and meiofauna within the sediment.

In one instance, the C:N ratio for the net production of TCO_2 and NH_4^+ was 110, and in another instance approached infinity. Whether or not the C:N ratio of the remineralised carbon and nitrogen reflects the C:N ratio of the organic matter undergoing remineralisation will depend on whether or not the population of bacterial cells remineralising the organic matter are in steady-state. A pulsed input of labile organic matter to sediments has been shown to stimulate bacterial production, resulting in an uptake of inorganic nitrogen by bacteria in order to synthesise their low C:N ratio biomass (van Duyl et al. 1993, Pedersen et al. 1999). Bacterial production may also be continuously stimulated by a ready supply of labile organic carbon derived from MPB, as well as grazing within the sediment (Meyer-Reil & Faubel 1980, Kemp 1990, Epstein 1997, Coull 1999). Thus, a continuous uptake of nitrogen and transfer into the microbial food web may take place (Blackburn 1988). As such, it is suggested the generally high ratio of TCO_2:NH_4^+ production was caused by the decomposition of labile high C:N-ratio EOC, which stimulated the reassimilation of NH_4^+ by actively growing bacteria. The consequence of this is that only a very small net release of nitrogen into the porewaters relative to carbon oxidised was observed.

A lack of nutrient production within the porewaters of mangrove sediments is also commonly observed, and is ascribed to a reassimilation of nutrients by bacteria, which ultimately sequester the nutrients within the sediments (Alongi et al. 1992). Similar findings have been made in subtropical systems in northern New South Wales, where it was also found that estuarine sediments generally acted as a sink for nutrients (Ferguson 2002). This suggests that in more oligotrophic systems there will be a rapid incorporation of DIN into biomass and a tight cycling of nitrogen within the biotic nitrogen pools.

Dissolved organic nitrogen fluxes

The most significant DON fluxes were measured for the upper mudflat at Site CF, where the highest MPB biomass, productivity and respiration rates have been observed (Cook et al. 2004a). This is consistent with previous studies which have shown that DON effluxes may be significant where there are high inputs of fresh organic matter (Hansen & Blackburn 1992, Enoksson 1993, Blackburn et al. 1996, Pedersen et al. 1999, Tyler et al. 2001), including that derived from MPB (Eyre & Ferguson 2002, Ferguson 2002). The most significant DON effluxes were observed when sediment respiration rates were highest during late spring 2000. It is suggested that this high respiration rate reflected the breakdown of MPB from an early spring bloom, as observed in the following spring of 2001 (Cook et al. 2004a). During this period, the efflux of DON dominated over DIN fluxes. The low DIN effluxes observed at Site CF throughout the year support previous observations that DON will be the major dissolved species of nitrogen to be released to the water column from sediments with high organic matter inputs from MPB and macroalgae (Tyler et al. 2001, Eyre & Ferguson 2002). A possible mechanism for this is an uptake of DIN by MPB and subsequent release of DON by algal cells (Nagao & Miyazaki 2002). Grazing of MPB and subsequent release of DON to the water column through 'sloppy feeding' has also been suggested as mechanism of DON release from sediments (Eyre & Ferguson 2002). Alternatively, low C:N-ratio DON may be lost in the early stages of decomposition of algal material, leaving behind a relatively high C:N-ratio carbon pool (Blackburn et al. 1996). Bacteria degrading this pool then assimilate inorganic nitrogen to synthesise their own biomass, resulting in a low efflux of inorganic N. Whatever the exact mechanism of DON production, it most probably occurs right at the sediment surface, since porewater DON profiles did not suggest any significant accumulation (and hence production, given its low diffusion coefficient) within the sediment (data not shown).

Of particular interest in terms of its bioavailability is the identity of the DON lost from the sediment, especially during the period of high efflux during late spring. Herein, we measured urea and TDPAs and found the fluxes of these species to be insignificant. Other studies that investigated the composition of DON produced within the sediment also found that urea was a minor component of the DON pool (Blackburn et al. 1996, Lomstein et al. 1998, Pedersen et al. 1999). Dissolved free amino acids (DFAA) have generally been found to make up <10% of the DON pool and fluxes in sediments (Lomstein et al. 1998, Pedersen et al. 1999, Landén & Hall 2000). The TDPA method used

here will only determine primary amino acids and will, therefore, only determine a sub-fraction of the DFAA pool. The low efflux of TDPAs seen here is consistent with DFAA measurements in other systems. Total hydrolysable amino acids (THAAs) have been identified as a significant proportion of DON pools and fluxes within sediments, making up 26% of DON in shallow-water sediments (Lomstein et al. 1998). Pedersen et al. (1999) found that THAAs constituted ~17% of DON flux in the first 7 d following eelgrass addition to sediments. The bulk fraction of DON released from sediments remains to be identified. In general it has been observed that DON released from the sediments will have a low C:N ratio ($\leq$6), suggesting that DON released from sediments is not refractory (Blackburn et al. 1996, Burdige & Zheng 1998, Pedersen et al. 1999). It has been suggested that a large fraction of DON efflux may be composed of compounds such as RNA and amines (Lomstein et al. 1998, Pedersen et al. 1999).

Denitrification

Measuring denitrification in intertidal sediments only during inundation imposes a degree of artificiality on the rates of denitrification measured. Sediment exposure may lead to changes at the sediment surface, potentially affecting the activity of nitrifying and denitrifying bacteria. In particular, the lack of a supply of NO_3^- from the water column during exposure will mean that coupled denitrification will be the only denitrification pathway. A recent study has suggested that rates of coupled denitrification are unchanged during exposure (Ottosen et al. 2001). Rates of denitrification were generally low, but comparable to those measured using the isotope pairing technique on other intertidal flats during times of low water column NO_3^- concentrations (Cabrita & Brotas 2000, Trimmer et al. 2000). Similarly, low rates have also been observed in shallow subtidal coastal sediments (Rysgaard et al. 1995, Jensen et al. 1996, Sundbäck et al. 2000). A number of reasons for the low rates of denitrification are possible. Low concentrations of NO_3^- in the water column would have resulted in greatly reduced rates of diffusion of NO_3^- into the sediment, which would mean that uncoupled rates of denitrification were also relatively low. Denitrification rates may also be depressed in the intertidal zone because of its more extreme and fluctuating environmental conditions (Ottosen et al. 2001).

Benthic microalgal production also had a negative impact on denitrification, as indicated by the negative correlations between various productivity indicators and denitrification. Fig. 6 shows that denitrification was always much less than the NO_3^- influx into the

sediment, suggesting that assimilation of NO_3^- from the water column by MPB detracted from uncoupled denitrification. Potential negative effects of primary production on coupled denitrification include competition for NH_4^+ and CO_2 between photoautotrophs and nitrifying bacteria, and possible increases in pH and O_2 (Henriksen & Kemp 1988, Risgaard-Pedersen et al. 1994). Competition for NH_4^+ between nitrifiers and heterotrophic bacteria will also have a negative impact upon nitrification rates (Strauss & Lamberti 2000), and possibly also denitrification (Risgaard-Petersen 2003). The stimulation of heterotrophic bacterial activity by MPB (see earlier in 'Discussion') is therefore another means by which the presence of MPB will negatively impact coupled denitrification.

At Site PC, denitrification rates were always significantly higher on the lower mudflat than on the upper mudflat. This is in agreement with the findings of Ottosen et al. (2001), who found rates of denitrification at an intertidal site to be 3 times lower than at a nearby subtidal site. Rates of primary production were not significantly different between the upper and lower mudflat (Cook et al. 2004a), suggesting that a factor other than primary production was important in controlling the rates of denitrification in this system. As suggested by Ottosen et al. (2001), the most likely reason for the higher rates of denitrification in the subtidal zone is that this is a more stable environment, sheltered from the great changes in temperature and salinity that an exposed mudflat experiences. The lower mudflat sediment in this study also had a much higher porosity and oxygen penetration (data not shown), which could allow greater rates of coupled denitrification.
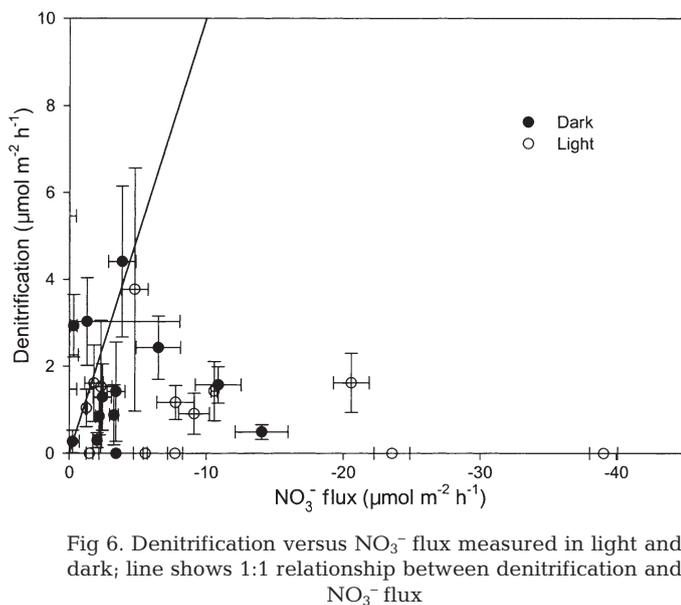

Fig 6. Denitrification versus NO_3^- flux measured in light and dark; line shows 1:1 relationship between denitrification and NO_3^- flux

N_2 fixation

The rates of N_2 fixation measured in this study were comparable to those reported for other temperate intertidal sediments. In an intertidal cyanobacterial mat community, Paerl et al. (1996) reported acetylene reduction rates that, when converted to N_2 fixation rates (using an acetylene-to-N_2 ratio of 4), ranged from <5 to 200 µmol N m^{-2} h^{-1}. As in our study, they observed the highest rates at night during summer. In a Massachusetts salt marsh, N_2 fixation rates by cyanobacteria ranged from essentially undetectable to ~700 µmol N m^{-2} h^{-1}, with values generally falling in the range of 30 to 60 µmol N m^{-2} h^{-1} (Carpenter et al. 1978). It was found that light and an availability of fixed nitrogen were the primary factors controlling N_2 fixation in that system. The low winter rates of N_2 fixation in our study, and the others cited above, are most probably a consequence of suppressed nitrogenase activity due to low light availability, low temperatures and higher concentrations of dissolved inorganic nitrogen within the water column.

The high rates of N_2 fixation observed at Site CF were most probably due to cyanobacteria, including *Oscillatoria* spp., which were observed on the surface of the mudflat at this site. Pigment analysis also suggested these cyanobacteria made up a significant fraction of the MPB community at Site CF (Cook et al. 2004b). There was also a significant variation in N_2 fixation rates in response to light. Rates of light N_2 fixation were significantly related to levels of chlorophyll *a* (chl *a*) in the sediment surface (r = 0.51, p = 0.05), supporting the contention that cyanobacteria were responsible for N_2 fixation. The significantly greater rates of N_2 fixation seen in the dark at Site CF are consistent with previous studies of sediments colonised by non-heterocystous cyanobacteria (Stal 1995).

Consistent with the pattern of N_2 fixation observed here was the seasonal pattern of $\delta^{15}N$ values observed at this site by Cook et al. (2004b). During summer and autumn the $\delta^{15}N$ of the sediment was relatively depleted, reflecting the contribution of ^{15}N-depleted organic matter, consistent with that expected from N_2-fixing organisms (Capone et al. 1998, MacGregor et al. 2001). During winter, the $\delta^{15}N$ of the surface sediment became more enriched, concomitant with low N_2 fixation rates and a greater reliance on NO_3^- assimilation from the water column by MPB (Fig. 4). It has been shown that the Huon estuary derives most of its nitrogen from the Southern Ocean in the form of nitrate (Butler et al. 2000) which has a $\delta^{15}N$ in excess of 6‰ (Lourey et al. 2003). As such, the observed increase in the $\delta^{15}N$ of the sediment surface during winter and spring is consistent with a greater reliance on fixed nitrogen (NO_3^-) which is relatively enriched in ^{15}N compared to that derived directly from N_2 fixation.

Nitrogen assimilation by microphytobenthos

In this study, a comparison of the rates of N_2 fixation and the calculated rate of total dissolved nitrogen uptake is complicated by the fact that the N_2 fixation and nutrient/TCO_2 uptake measurements were made in different years. Interannual variability in rates of primary production and nitrogen availability may confound the interpretation of these results. The temporal variations in dissolved nitrogen in the Huon Estuary have been well characterised by the recently completed Huon Estuary study (Butler et al. 2000). The 3 yr study showed that the concentration of dissolved inorganic nitrogen (DIN) in the surface waters of the estuary followed a well-defined annual cycle, with maximum concentrations (~7 µM) observed between April and October. During the warmer months between October and April, DIN concentrations in the surface waters were always lower and generally <1 µM. Based on these data, it seems reasonable to assume that the rates of nitrogen supply from the water column to the MPB are not likely to have differed significantly between 2001 and 2002. The rates of nitrogen supply from within the sediment were also assumed not to vary between 2001 and 2002. Chl a concentrations measured during the 2002 N_2-fixation surveys were significantly greater than those measured during 2001 (data not shown). Given greater chl a concentrations and the significant positive relationship between chl a and N_2 fixation, it is highly likely that the measured rates of N_2 fixation during 2002 would be greater than those in 2001. The rates of N_2 fixation measured in 2002 could thus be considered a maximum estimate for 2001.

The estimates of daily TCO_2 and nitrogen assimilation ratios in this study showed that even when the maximum rate of N_2 fixation measured during 2002 at each site was used, the estimated ratio of C:N assimilated was around 20 in all seasons except winter. During nutrient limitation, the C:N-ratio of phytoplankton cells (and presumably MPB) may increase up to 20 (Van den Meersche et al. 2004); however, beyond this point, fixed carbon will be released as extracellular organic carbon (EOC). During nutrient limitation it has been shown that cultured MPB will continue to fix carbon, but then excrete this in the form of EOC (Stal 1995, Staats et al. 2000, Thornton 2002); this most probably occurs as a consequence of overflow metabolism (Stal 1995, Staats et al. 2000). During periods of low nutrient availability, the bulk of carbon assimilation may be directed to the synthesis of EOC instead of to cellular growth. MPB in particular, have been observed to have high rates of EOC production, with up to 73% of carbon fixed being converted into EOC compared to 23% for phytoplankton (Goto et al. 1999). The production of EOC may thus provide a sink for the excess carbon assimilated over nitrogen observed in the present study.

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