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Short-term enhancement and long-term suppression of denitrification in estuarine sediments receiving primary- and secondary-treated paper and pulp mill discharge

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Abstract

To determine the role of sediment denitrification in removing inputs of primary- (PE) and secondary-treated effluent (SE) from a pulp and paper mill (PPM), organic matter (OM) associated with PE (residual wood fibre) and SE (activated sludge biomass and phytoplankton) was added to estuarine intertidal sediments and denitrification rates were measured over 27 days. Labile sludge biomass and phytoplankton initially stimulated denitrification, including for pre-existing sediment N. After 2.5 d, however, denitrification was suppressed apparently due to microbial competition for N to process the refractory (high C:N) material remaining. Wood fibre suppressed denitrification throughout the experiment due to competition for N to process the refractory OM. Ultimate long-term denitrification suppression by phytoplankton is offset by initial enhanced denitrification rates. Although nutrient release during degradation of sludge biomass and wood fibre may stimulate phytoplankton production, N equivalent to 127% of the expected daily phytoplankton load was denitrified within 24 h, allowing for permanent removal of PPM-derived N. Compared to primary treatment, secondary treatment of PPM effluent has greater potential for N removal.

Introduction

Sediment denitrification is a critical process, removing fixed natural and anthropogenic nitrogen (N) from coastal ecosystems. This can reduce estuarine nutrient over-enrichment and control primary production (eutrophication). Benthic denitrification in aquatic systems requires low oxygen conditions (< 0.2 mg O_2 L^{-1}), nitrate and organic carbon (*1*). Spatial and temporal variations in organic matter (OM) inputs can lead to marked differences in sediment organic content. OM can rapidly stimulate benthic denitrification (*2*) where organic carbon is limiting, and can also indirectly affect denitrification by changing sediment conditions during decomposition. During remineralisation, NH_4^+ release can stimulate nitrification and coupled nitrification-denitrification (D_n), and O_2 consumption can increase diffusional nitrate supply for direct denitrification (D_w) by reducing the depth of the nitrogeneous zone (*3*). O_2 consumption increases the low oxygen area for denitrification (*1*), but can result in anoxic conditions and the production of sulphides, which can inhibit D_n (*4*). OM inputs may also stimulate macrofauna which, in turn, can enhance denitrification (*5*). Denitrification efficiency (N_2-N / (N_2-N + NO_3^- + NH_4^+)) peaks at an optimum decomposition rate where there is a complex 3-D sediment structure with overlapping oxic and anoxic zones (*6*).

Studies have manipulated OM in muddy sediments in mesocosms and cores to investigate effects on benthic denitrification, but their scope has been limited. Despite the variable C:N of OM, all studies have used low C:N material (e.g., phytoplankton (*7*), glucose (*2*) or yeast (*8*)), which can dramatically enhance denitrification rates (*9*) and increase D_n (*8*). Where the capacity of the microbial community to process organic carbon is overwhelmed, however, decomposition of excess OM causes sediment anoxia, reducing D_n (*5*). The effect of OM on denitrification depends on both its quantity and quality. Higher NO_3^- influx for sediment amended with low C:N (macroalgae), compared to high C:N material (lignin and seagrass, *10*), suggests that higher D_w rates are associated with more labile OM. The effect of refractory OM on denitrification is more complicated, as OM can be comprised of components of differing lability. Initial leaching of labile components (e.g., carbohydrates (*11*)) leaves the remaining OM more refractory (*12*). This is likely to cause temporal variation in denitrification following OM addition, similar to that seen for NO_3^- and NH_4^+ fluxes following phytoplankton addition (*13*). However, the effect of OM on denitrification has only been considered at short (< 1 d (*9*)) or longer time scales (4 d (*5*) to 4 months (*7*) after OM addition), providing a snapshot. To discern the ultimate fate of OM it is important to monitor the effects on denitrification over both the short- and long-term.

Different characteristics of OM influence its processing and fate. Paper and pulp mills (PPM) are a major OM source to aquatic systems (*14*). Processing of PPM-derived OM depends on its quality and quantity, which is determined primarily by treatment processes. Whereas the particulate OM in primary-treated effluent (PE) consists of residual wood fibre, secondary-treated effluent (SE) contains labile biomass from the activated sludge used in treatment. SE can also contain inorganic N that is 'fed' to microbes to supplement the low N content of wood fibre (*15*) and may therefore cause increased pelagic chlorophyll-a concentrations (algal blooms (*16*)).

Although the composition and transport of PPM-derived PE and SE has been described (*16*), no studies have compared the biogeochemical effects of PE and SE in receiving sediments. Our primary objective was therefore to investigate the ability of denitrification to remove N inputs associated with PPM-derived PE and SE. We hypothesised that the influence of PPM-derived OM on benthic denitrification would depend on the lability (C:N) of the OM. We further expected that this effect would vary temporally as more labile OM components were degraded. We therefore compared, over the short- and long-term, denitrification rates associated with the particulate fractions of PE and SE. These fractions were a) residual wood fibre from PE, b) activated sludge biomass from SE, and c) phytoplankton, which may bloom in response to nutrients in SE (*16*).

Experimental

Site description

The study was done in summer 2008 on an intertidal flat in the upper Derwent River estuary, Tasmania, ~ 23 km downstream from the outfall of the Norske-Skog PPM (42°48’55”S, 147°15’36”E) which uses mechanical pulping and converted from primary to secondary treatment in October 2007. This area of the estuary has large shallow flats and wetlands with extensive macrophyte cover at the time of sampling. Our site had a cover of short (< 5 cm height) seagrass (*Heterozostera tasmanica*, ~ 1.3 g dry wt m^{-2}), as well as benthic microalgae (22.3 mg chlorophyll-*a* m^{-2}). Sediment was primarily fine muddy sand (75% 125-250 µm, 15% 63-125 µm) and was net heterotrophic (p/r = 0.32 ± 0.05, J. M. Oakes unpubl. data). The estuary has an average tidal range of ~ 1 m (*17*).

Organic matter preparation

To assess the impact of PPM-derived OM on benthic denitrification residual wood fibre and activated sludge biomass were added to sediment to represent OM inputs from PE and SE, respectively. A third OM type, phytoplankton, reflected deposition of algal blooms, which can be stimulated by SE-derived nutrients (*16*).

Wood fibre creation mimicked processes at the Norske-Skog PPM. Air-dried wood from pine trees (*Pinus radiata*, ~ 1.5 m tall) was mechanically chipped. Wood chips (20-30 mm diameter) were oven-dried (60°C), ground, autoclaved with milli-Q (~ 50:50 v/v, 140°C, 15psi, 20min), then macerated using a blender. Material < 38 µm was retained, oven-dried (60°C) and homogenised. Recovery (~ 4% of the original mass) was similar to at a similar stage of the process at the PPM (before the primary clarifier, D. Richardson personal communication).

Sludge biomass (primarily bacteria, protozoa (ciliates and flagellates) and metazoa (rotifers)) was collected from the secondary treatment plant of the Norske-Skog PPM, concentrated (centrifugation, 839 × g, 15 min), washed with milli-Q, lyophilised and homogenised.

Thalassiosira pseudonana (CSIRO Collection of Living Microalgae; Strain CS-20; www.marine.csiro.au/microalgae/collection.html) was batch cultured axenically (24°C, continuous light) in sealed 2 L Schott bottles containing 1.2 L of artificial seawater amended with F_2 culture medium. Cells were concentrated by centrifugation (839 × g, 10 min), washed with isotonic milli-Q, lyophilised, and gently homogenised to create phytoplankton material.

Organic matter addition

Plots were established at similar heights on the intertidal flat at low tide by pushing 1 m × 1 m aluminium frames 3 cm into the sediment. The upper surface was flush with the sediment. String stretched across each frame divided plots into grids of 20 cm × 20 cm squares. Three plots were haphazardly allocated to each of five treatments: control, procedural control, wood fibre addition (17.0 g dry wt m^{-2}), sludge biomass addition (8.5 g dry wt m^{-2}), or phytoplankton addition (1.5 g dry wt m^{-2}, Table 1). Based on molar C:N ratios of 28.2 (wood fibre), 9.6 (sludge biomass) and 7.2 (phytoplankton), this equated to additions of 22.6, 33.4 and 1.1 mmol N m^{-2} to sediment which had a C:N ratio of 12.9.

The quantity of wood fibre added to sediment represented daily loadings within the area of influence. Before October 2007, the Norske-Skog PPM discharged 45 ML d^{-1} of PE containing 40 - 90 mg L^{-1} of wood fibre (D. Richardson personal communication) which was deposited within ~ 2 km of the PPM outfall (*16*), where the average estuary width is ~ 100 m. Based on 1 800 - 4 080 kg d^{-1} of wood fibre depositing over ~ 200 000 m^2, we estimated a daily load of ~ 9 - 20 g wood fibre m^{-2}. We therefore added to sediments a quantity of wood fibre within this range (17 g m^{-2}).

The quantity of sludge biomass discharged from the PPM after October 2007 (20 – 90 mg L^{-1} in 60 ML d^{-1} of SE) was similar to that for wood fibre. However, the density and settling rate was approximately half that of wood fibre, so the load of sludge biomass added was halved accordingly (8.5 g m^{-2}).

Daily phytoplankton loadings were estimated from the maximum chlorophyll-a concentration (~ 2 ug L^{-1}) observed just downstream of the PPM outfall in summer post-upgrade to secondary treatment (*16*). For a C:Chla ratio of 30 - 60 (*18*) this equates to 60 - 120 ug C L^{-1}. Assuming an average water depth of 1 m across the inundated site, phytoplankton settlement inputs 60 - 120 mg C m^{-2} d^{-1}. We added 1.5 g m^{-2} of phytoplankton (~ 95 mg C m^{-2}) to sediments, which is within this range.

Equal quantities of wood fibre, sludge biomass or phytoplankton were added to each square within the appropriate plots. OM was mixed into a slurry of milli-Q and precombusted (450℃, 3 h) site sediment which was frozen in 20 cm × 20 cm foam trays. The resulting ~ 1 mm thick 'cakes' were everted directly onto the sediment surface where they immediately thawed. Thin sediment 'cakes' weighed down OM whilst avoiding smothering of autotrophs and disturbance of sediment microhabitats. To test for an effect of adding combusted sediment, 'cakes' without OM were added to procedural control plots.

Core collection and incubation

On most occasions, cores were collected when sediment was exposed. One core of sediment (90 mm i.d. x ~ 20 cm depth) was manually collected in a Plexiglas core liner from each plot immediately after OM addition (time 1) and after a further 0.5 d, 1.5 d, 2.5 d, 8.5 d and 26.5 d (times 2 to 6, respectively), except for procedural control cores, which were only collected at times 1 and 3. PVC pipes filled with site sediment (90 mm diameter, 20 cm long) were placed in holes left following core removal to minimise site disturbance. Site water was collected for incubations.

Our denitrification method measures the total N_2 efflux and as such includes both denitrification and anammox. However, anammox contributes little to N_2 effluxes in shallow marine and estuarine waters (*19*) and N_2 effluxes at the study site were therefore assumed to reflect denitrification. Because N_2 fluxes from exposed muddy intertidal sediments are typically relatively low (< 12 µmol N m^{-2} h^{-1}; *20*), we incubated sediment cores with overlying water to focus on denitrification during inundation. In the laboratory, cores were filled with site water, avoiding sediment disturbance, capped with gas-tight Plexiglas lids containing sampling ports and placed in incubation tanks of site water maintained at *in situ* temperature (± 2℃). An external rotating magnet operated magnetic stirrers within each core. These circulated water at a rate just below the threshold for sediment resuspension. Due to the short-spaced sampling there was no pre-incubation period. Cores were incubated for 4 - 6 h at *in situ* light (200-300 µmol photons $m^{-2} s^{-1}$ PAR, 400 watt metal halide lights) and temperature (± 2℃). Water was then removed for use in a separate study and replaced with fresh site water before dark incubation (4 - 6 h, *in situ* temperature ± 2℃). Light incubations for cores collected at times 1 to 6 ended 1, 1.2, 2.1, 3.1, 9.1, and 27.1 d and for dark incubations ended 0.5, 1.5, 2.5, 3.4, 9.3, and 27.4 d after OM addition. Light and dark incubation periods reflected the time taken for oxygen saturation within cores to fall to ~ 80% during dark incubation of trial cores.

Sample collection and analysis

At the beginning and end of dark and light periods dissolved oxygen (DO) concentrations (± 0.01 mg L^{-1}) and temperature (± 0.01°C) were measured (Hach® HQ40d, luminescent DO probe) and water samples were collected. Duplicate N_2:Ar samples were collected directly from cores by allowing gravity-fed site water from a collapsible reservoir to force core water via tubing into 7 mL gas-tight glass-stoppered glass vials that were filled to overflowing, sealed to exclude bubbles, killed with 20 µL 50:50 w/v $ZnCl_2$ and stored submerged at or below ambient temperature. Samples for NH_4^+ and NO_x^- analysis were withdrawn into a plastic syringe, filtered (0.45 µm cellulose acetate) into a 10 mL polyethylene vial leaving a headspace, and stored frozen (-20°C) until analysis. Sample water was replaced, as it was withdrawn, from gravity-fed collapsible reservoirs of site water.

For sediment and added OM, %C and %N (error ~ 1.0% of measured value for C, ~ 1.5% for N) were determined using a Thermo Finnigan Flash EA 112 interfaced via a Thermo Conflo III with a Thermo Delta V Plus IRMS, and values used to calculate molar C:N. For sediments, %C analysis followed acidification (5% HCl) in silver cups.

N_2 concentrations were determined from N_2:Ar measured using membrane inlet mass spectrometry with O_2 removal (*21*). NH_4^+ and NO_x^- concentrations were determined using a four channel Flow Injection Analyser (Lachat™ QuickChem 8000). Analytical methods, errors and detection limits are detailed elsewhere (*22*).

Calculations

Fluxes were determined from the difference in concentration from the beginning to the end of the light and dark periods, respectively, as a function of the water volume and sediment surface area of cores, corrected for replacement water addition. Positive fluxes denote efflux from sediment to the overlying water, and negative fluxes denote uptake into the sediment. Net flux rates were calculated as:

$$\text{Net flux} = \text{dark flux} \times \text{dark hours} + \text{light flux} \times \text{light hours} / 24 \text{ hours}$$

Given that quantities of added N were representative of that entering sediment within 1 d, we were interested in the % of added N that was removed within 1 d and then per hour over subsequent time periods. This was calculated as follows:

$$\%\text{ of added N removed per hour} = (\text{flux}_{OM} - \text{flux}_{C})_t / N_{added} \times 100$$

To determine removal in 1 d, this was multiplied by the total hours of inundation (12 h). Flux_C represents the net flux of NH_4^+, NO_x^- or N_2 from the control plots, flux_{OM} is the corresponding flux from wood fibre, sludge biomass or phytoplankton plots during an incubation period (µmol N m^{-2} h^{-1}) and N_{added} is the total N initially added to a plot (µmol N m^{-2}). This provided an estimate of the % of added N that was accounted for by net fluxes of NH_4^+, NO_x^- or N_2 that were in excess of fluxes from the controls.

Data analysis

Two-way ANOVAs compared fluxes of DO, NH_4^+, NO_x^- and N_2 from the procedural control plots and control plots (factors = time (2 levels) and treatment (2 levels)) and from control and OM addition plots (factors = time (6 levels) and treatment (4 levels)). Tests were run separately for light, dark and net fluxes. In some cases, Levene's test indicated that group variances were heterogeneous. Where this could not be improved using log transformation (due to large negative values) we reduced α to 0.01 to reduce the chance of a type I error. Where there were significant effects of time or treatment post-hoc Tukey tests indicated which levels differed. Significant interactions were investigated using a series of one-way ANOVAs, comparing all treatments within each time. Post-hoc Tukey tests determined which treatments differed where one-way ANOVAs indicated a significant difference.

Results

Fluxes of NH_4^+, NO_x^-, DO and N_2 from procedural control plots were not significantly different to those from control plots, indicating that the OM addition procedure did not impact fluxes ($p>0.05$; see the Supporting Information).

Benthic respiration and productivity

There was DO uptake in all plots during the dark (respiration) and smaller uptake during the light (reflecting gross primary production; Figure 1). Although mean dark DO uptake in OM addition plots was greater than from the controls at time 1, and light DO uptake was greater at times 1 and 2 (Figure 1), these differences were not significant. There was, however, a strong environmental effect, with significant temporal variation in dark, light and net fluxes ($p\leq0.002$).

Fluxes of NH_4^+

Light, dark and net NH_4^+ fluxes varied significantly with time ($p\leq0.005$), but there was no clear trend (Figure 1). Mean light and net NH_4^+ fluxes from all the OM addition plots were greater than from controls at times 1 and 2, and net NH_4^+ fluxes from sludge biomass plots were greater than from controls at later times, but no significant difference was detected due to small sample sizes and high variability. Dark NH_4^+ fluxes from the sludge biomass plots were, however, significantly greater than from all other treatments regardless of time ($p=0.010$; Figure 1).

Within 1 day of OM addition, 86.8% of added N had been removed from phytoplankton plots via NH_4^+ fluxes, compared to 3.5% for sludge biomass and 2.5% for wood fibre plots (Table 1). The largest removal of NH_4^+ per h for all the OM addition plots was at time 2 (Table 1).

Fluxes of NO_x^-

For light and net NO_x^- fluxes there was a significant interaction between time and treatment ($p<0.005$). Light NO_x^- fluxes at times 1 and 2 and net NO_x^- fluxes at time 2 were significantly greater from wood fibre and phytoplankton plots than from the controls ($p=0.000$ to 0.048; Figure 1). Light and net NO_x^- fluxes from OM addition plots were otherwise statistically similar to those from controls. Dark fluxes of NO_x^- were similar for all plot types at all times, but there was a strong environmental influence (effect of time: $p<0.001$; Figure 1).

The proportion of added N that was removed from sediments within 1 day as NO_x^- was far lower than that removed as NH_4^+ for all OM addition plots. The total proportion of N removed as NO_x^- from wood fibre and phytoplankton plots was ~ 40% and from sludge biomass plots was ~ 6% of that removed as NH_4^+ (Table 1). As for NH_4^+, the greatest removal of NO_x^- was at 1.5 d following OM addition (Table 1).

Denitrification

In the short-term, addition of labile OM (sludge biomass and phytoplankton) enhanced dark rates of denitrification, whereas refractory wood fibre had no effect on denitrification rates (Figure 1). In the longer term, dark and light denitrification was suppressed in all OM addition plots. These differences were reflected in a significant interacting effect of treatment and time on dark, light and net N_2 fluxes ($p<0.001$ in each case).

No significant enhancement of light denitrification rates relative to controls was observed for any treatment type at any time, although fluxes from sludge biomass and phytoplankton plots were at least double those from control plots and were significantly greater than those from wood fibre plots at time 2 ($p=0.020$; Figure 1). Dark N_2 fluxes from phytoplankton plots at time 1 and from sludge biomass plots at time 2 were, however, significantly greater than from other plot types, including controls ($p<0.015$). Light N_2 fluxes from sludge biomass and phytoplankton plots at time 5 and phytoplankton and wood fibre plots at time 6 and dark N_2 fluxes from sludge biomass and phytoplankton plots at time 3 and from all OM addition plots at time 4 were significantly lower than from control plots ($p\leq0.020$; Figure 1).

Significant enhancement of net N_2 fluxes relative to controls was evident only for sludge biomass plots at time 2 ($p=0.006$), but net N_2 fluxes from sludge biomass and phytoplankton plots at this time were significantly greater than those from wood fibre plots (Figure 1). Net N_2 fluxes were significantly suppressed relative to controls in all OM addition plots towards the end of the experiment (in sludge biomass and wood fibre plots at time 4 (p=0.006) and in phytoplankton plots at time 6 (p=0.002; Figure 1)). There was were no replicates for light N_2 fluxes from phytoplankton plots at time 4 due to bubbles in samples.

Although not always statistically significant, mean dark, light and net N_2 fluxes from the wood fibre plots were almost always lower than from the controls. There was therefore no evidence that added N from wood fibre was removed via denitrification. In contrast, there was greater N_2 efflux from the sludge biomass and phytoplankton plots than from the control plots during the early experimental period indicating that this labile material was denitrified. Within 1 d of OM addition, denitrification accounted for loss of N equivalent to 0.5% (<0.1% h^{-1}) and 127.0% (10.6% h^{-1}), respectively, of the N added to sludge biomass and phytoplankton plots. The highest N_2 efflux for sludge biomass plots was 1.5 d after OM addition (0.4% h^{-1}; Table 1).

Discussion

Short-term stimulation of denitrification

Addition of OM associated with PPM-derived SE (sludge biomass and phytoplankton) rapidly altered sediment biogeochemistry, particularly denitrification rates. This reflects lower C:N ratios of phytoplankton and sludge biomass compared to pre-existing OM in the sediment, allowing rapid processing and mineralisation. The highly labile organic carbon provided by the added OM probably increased the availability of electron donors for denitrification, leading to the enhanced N_2 effluxes that were initially observed. Although not statistically significant, mean light and net NH_4^+ fluxes greater than those from the controls at times 1 and 2 (Figure 1) are consistent with increased NH_4^+ supply for D_n. Increased mean dark, light and net DO uptake at time 1 may also reflect rapid processing of OM. However, this difference was not statistically significant most likely due to underlying spatial variability in autotroph and heterotroph distributions, making it difficult to detect changes in DO fluxes, and the minor contribution of the added OM relative to that pre-existing within the sediments.

Whereas denitrifiers responded almost instantaneously to phytoplankton addition, sludge biomass addition significantly enhanced N_2 effluxes only after 1.2 d (Figure 1), possibly reflecting a slower response of the microbial community to the less labile (higher C:N) material (*10*). Interestingly, the magnitude of N_2 fluxes from sludge biomass and phytoplankton plots was similar, despite differences in the quantity of material added, further emphasising the greater lability, and more rapid processing, of phytoplankton compared to sludge biomass.

Over the first day following OM addition, more than 100% (245.6%) of the N added to the phytoplankton plots was released as fluxes of inorganic N (NH_4^+, NO_x^- and N_2) in excess of those from control sediments (Table 1). Because labile OM can stimulate microbial activity (*23*), addition of sludge biomass and phytoplankton most likely resulted in mineralisation and subsequent denitrification of N that was pre-existing within sediments.

Immediate stimulation of denitrification by OM, which we observed following phytoplankton addition, reflects a similar pattern observed following addition of *Chlorella* algae (*9*). However, this previous study reported a more dramatic response, with denitrification rates in OM addition plots 18 × greater than in control sediments (*9*). This may relate to the greater quantity of N added to sediments, or differences in the quality of OM (leached algae vs intact algae in our study). However, a far greater portion of added N was initially denitrified to N_2 in the current study (10.6% h^{-1} over the first day) than in the previous study (0.36% h^{-1} (*9*)). This suggests that the site we studied is acclimated to OM inputs, given its proximity to a PPM outfall, and may therefore have greater potential for denitrification as has been reported for sediments receiving anthropogenic inputs (*24*).

Suppression of denitrification

Decomposition of OM ultimately results in a higher sediment C:N ratio (*25*). Large quantities of inorganic N would be required for the degradation of the remaining, more refractory, OM. Nitrifiers and denitrifiers would therefore compete for NO_x^- and NH_4^+ with other heterotrophs and autotrophs. Competition with autotrophs may be reflected in the lower NH_4^+ fluxes observed for phytoplankton plots in the latter stages of the study compared to earlier in the study (Figure 1). Although mean NH_4^+ fluxes remained elevated for sludge biomass plots throughout the study, mineralisation of large quantities of relatively labile OM may have stimulated autotrophic or heterotrophic production, increasing competition for inorganic N. Competition for inorganic N would limit denitrification capacity, leading to the suppression of N_2 effluxes that was observed in sludge biomass and phytoplankton plots ~ 2.1 d after OM addition. Thereafter, denitrification rates were suppressed in sludge biomass plots until at least 9 d and in phytoplankton plots until at least 27 d after OM addition.

Despite the refractory nature of wood fibre (C:N = 28.2), the sediment heterotrophic community also responded rapidly to its addition to sediments, with mean N_2 effluxes below those seen for control plots almost immediately (although this was only significant after ~ 3 d). Refractory material can be comprised of both labile and less labile fractions. Leaching and mineralisation of a labile component of wood fibre most likely lead to effluxes of NH_4^+ and NO_x^- and dark DO uptake initially in excess of that from the controls (albeit not significantly for DO). Although a larger quantity of OM was added to wood fibre plots than to phytoplankton plots, DO, NH_4^+ and NO_x^- fluxes were similar, reflecting the less labile nature of wood fibre. Because the majority of wood fibre material was refractory (i.e., lignin and cellulose), denitrification was not stimulated, and throughout the study N_2 effluxes remained below those seen for control plots. Competition for inorganic N, by nitrifiers and denitrifiers, to degrade the refractory component of wood fibre would limit the capacity for denitrification, leading to the observed suppression of N_2 effluxes.

In contrast to our observations that denitrification was suppressed in the long-term following addition of OM of differing lability (C:N from 7.2 to 28.2), previous studies using labile OM mostly reported no effect of OM enrichment on denitrification (*26*) or long-term stimulation of denitrification (*7*, *8*, *27*). One study reported N_2 effluxes in excess of control sediments (equivalent to 0.13% of the N initially added) following a 12 h incubation of sediment collected from a microcosm 12 d after addition of yeast (C:N = 7.5) (*8*). In contrast, we saw no evidence of denitrification in excess of that from control sediments after ~ 1.5 d (Table 1). This may relate to the initial addition of far greater quantities of N (up to 571 mmol N m^{-2} (*8*)) than were used in the current study. At our N-limited study site, N availability limited processing of less labile OM remaining in the latter stages of our experiment. In the previous study, however, high water column nitrate concentrations limited competition for N, allowing denitrification to continue above control rates (*8*). Only one previous study reported suppression of denitrification (*5*). In this case, a large quantity of N was added to sediments (34 mg N m^{-2}), leading to sediment anoxia, thereby limiting D_n at 4 and 12 d after addition. This mechanism for suppression differs from that outlined in the current study, which is not surprising given that the denitrification suppression previously observed was for sediment that excluded macrofauna (*5*), which can have significant impacts on denitrification (*27*). In contrast, denitrification suppression was observed in the current study for sediment that remained *in situ* for the entire experiment except during the incubations. A further aspect to consider in the current study is the presence of seagrass. Whereas previous studies have primarily monitored denitrification following

OM addition to bare sediments, where benthic microalgae would be the dominant autotrophs (e.g., *7*, *8*, *27*), seagrass was present at the site of the current study. Seagrass and MPB can have similar impacts on denitrification via input of organic carbon and O_2 to sediments and competition for NO_x^- (*28*), but seagrass can also contribute more refractory OM and increase the spatial interface available for D_n via O_2 secretion from its roots (*28*), potentially influencing the relationship between OM input and denitrification. However, in the current study this effect was most likely minimal, due to the extremely short, sparse nature of the seagrass present.

Implications

We have demonstrated that the response of denitrification to OM addition can be temporally dynamic. Had denitrification been measured only a short time (< 1.5 d) after addition of phytoplankton or sludge biomass, the conclusion (stimulation) would have been markedly different to that made if denitrification had been measured after 2.5 d (suppression). This demonstrates the importance of considering both short- and long-term responses.

Given that the Derwent estuary appears nutrient-limited (*16*), increased NH_4^+ effluxes following wood fibre and sludge biomass addition to sediments indicate that both PE and SE are likely to stimulate phytoplankton growth (i.e., phytoplankton deposition). This may be exacerbated for SE which contains nutrients in addition to those released through mineralisation. In the current study N equivalent to 127.0% of the daily deposition of phytoplankton (presumably including pre-existing sediment N), was denitrified within 1 d of phytoplankton addition. Conversion of PE and SE-derived N to phytoplankton biomass therefore provides an avenue for permanent removal of excess anthropogenic N. Although addition of phytoplankton material to sediments ultimately suppresses denitrification, this is offset by initially enhanced N_2 effluxes. Due to the immediate and sustained suppression of denitrification following wood fibre addition to sediment, discharge of SE offers greater potential for removal of anthropogenic N.

This is a valuable first attempt to compare the effects of PPM-derived PE and SE on biogeochemical fluxes in the receiving environment, but the findings must be considered in the context of the following caveats:

1) OM was added as a single pulse. Long-term discharge of material may result in a more chronic impact that would not be captured by our study.

2) The study was intertidal, whereas some OM would also deposit subtidally. Although our incubations were inundated, reflecting subtidal conditions, lower light penetration may result in subtidal sediment being more heterotrophic, which would most likely decrease coupled nitrification-denitrification.

This is the first study to compare the effects of PPM-derived PE and SE on biogeochemical fluxes in the receiving environment. We demonstrated that both PE- and SE-derived OM affect biogeochemical cycling, particularly denitrification, and this can influence the removal of excess anthropogenic N from the environment. However, the effect varies depending on the quality and quantity of OM discharged. This has implications for the effect of PE and SE discharge on ecosystem processes and management decisions relating to treatment options.

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Supporting information available

Figure showing similarity of dark and light fluxes of NH_4^+, NO_x^-, DO and N_2 in control and procedural control plots. This information is available free of charge via the Internet at http://pubs.acs.org.

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Table 1: Organic matter characteristics and NH_4^+, NO_3^- and N_2 fluxes in excess of those from controls.

		Primary-treated effluent	Secondary-treated effluent	
		Residual wood fibre	Sludge biomass	Phytoplankton
C:N		28.2	9.6	7.2
Mass N added (mmol N m^{-2})		22.6	33.4	1.1
Excess flux (% of added N h^{-1})				
NH_4^+				
	1d	0.2 (2.5)*	0.3 (3.5)*	7.2 (86.8)*
	1.5 d	0.5	0.3	10.9
	2.5 d	0.0	0.1	0.0
	3.4 d	0.1	0.3	2.2
	9.3 d	0.0	0.2	0.0
	27.4 d	0.0	0.1	2.7
NO_x^-				
	1d	0.1 (1.0)*	<0.1 (0.2)*	2.6 (31.9)*
	1.5 d	0.4	0.1	8.8
	2.5 d	0.0	0.0	0.0
	3.4 d	0.0	0.0	0.0
	9.3 d	0.0	0.1	0.0
	27.4 d	0.1	0.1	2.1
N_2				
	1d	0.0 (0.0)*	<0.1 (0.5)*	10.6 (127.0)*
	1.5 d	0.0	0.4	4.9
	2.5 d	0.0	0.0	0.0
	3.4 d	0.0	0.0	0.0
	9.3 d	0.0	0.0	0.0
	27.4 d	0.0	0.0	0.0

* Values in parentheses represent total % of added N removed over the first day after OM addition

Figure captions

Figure 1: Dark and light fluxes of DO, NH_4^+, NO_x^- and N_2 from control and organic matter addition plots (mean ± SE). p-values are shown for significant effects (time, treatment or interaction). Where the effect of time was significant, letters show Tukey test results, where letters which are the same indicate no significant difference.

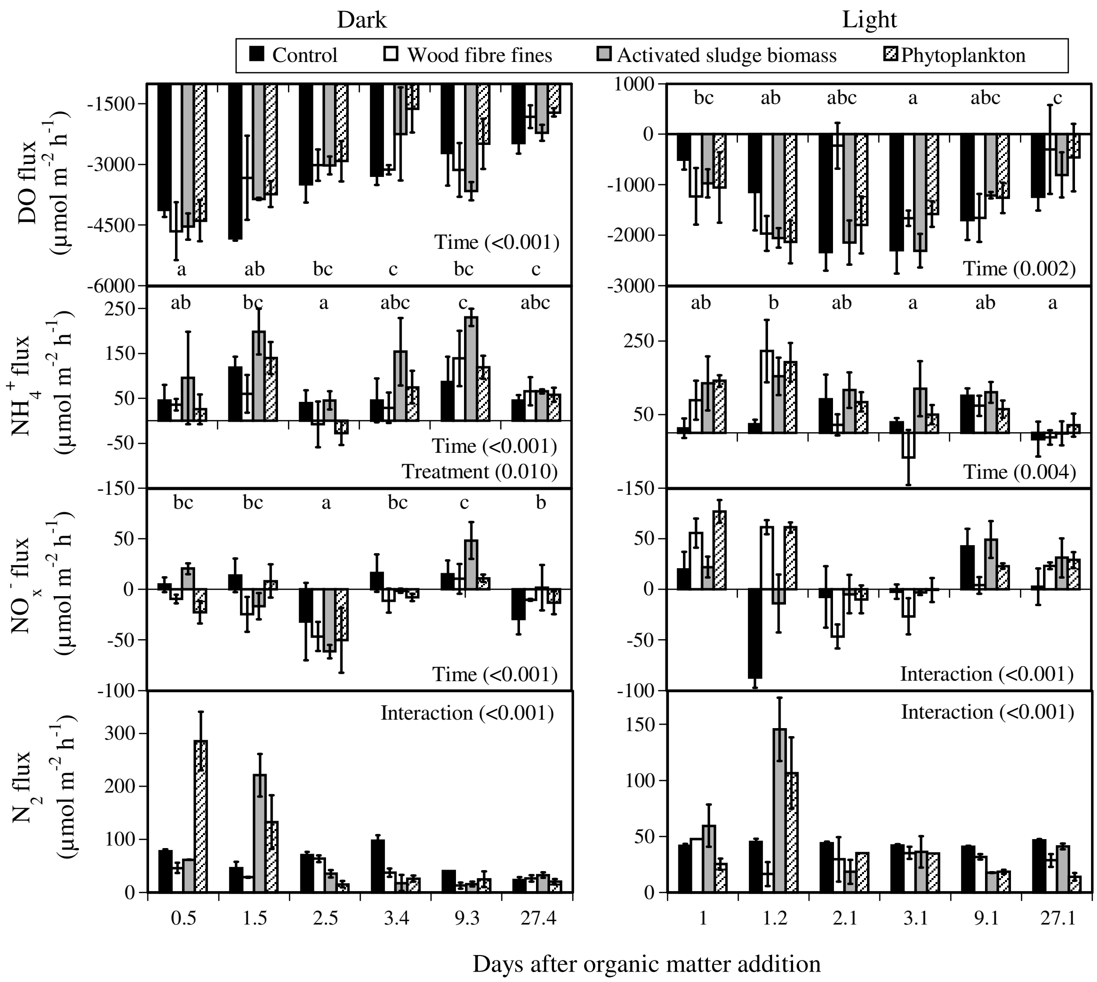
Dark
Light
Control
Wood fibre fines
Activated sludge biomass
Phytoplankton
DO flux (µmol m-2 h-1)
NH4+ flux (µmol m-2 h-1)
NOx- flux (µmol m-2 h-1)
N2 flux (µmol m-2 h-1)
Time (<0.001)
Time (0.002)
Time (<0.001)
Treatment (0.010)
Time (0.004)
Time (<0.001)
Interaction (<0.001)
Interaction (<0.001)
Interaction (<0.001)
0.5
1.5
2.5
3.4
9.3
27.4
1
1.2
2.1
3.1
9.1
27.1
Days after organic matter addition

Figure 1

Brief

Organic matter from primary- and secondary- treated pulp mill effluent can initially stimulate denitrification, but suppresses denitrification in the longer-term (> 27 days).

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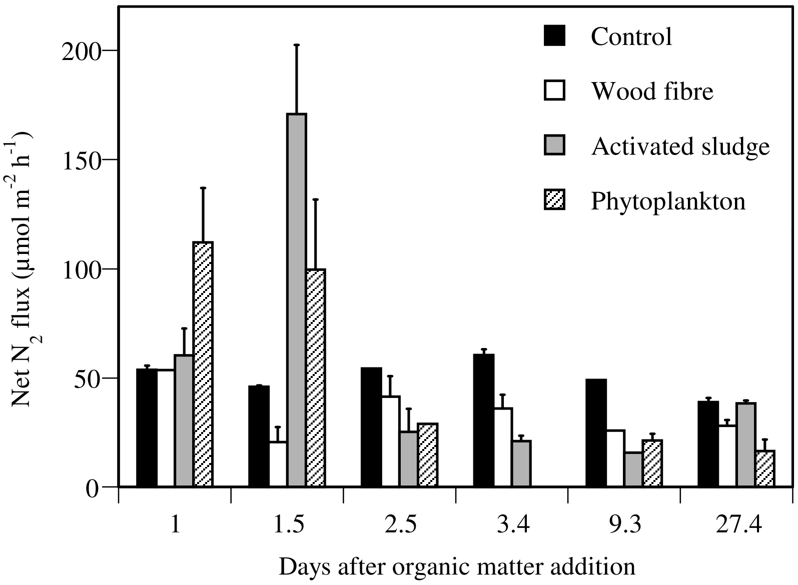

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