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20   **Abstract**

Stable isotope analysis of a novel combination of carbon and nitrogen pools traced inputs and

processing of primary-treated (PE) and secondary-treated effluent (SE) from a paper and pulp

mill (PPM) in a temperate Australian estuary. Distinct carbon stable isotope ratios of dissolved

organic carbon (DOC) near the PPM outfall indicated large PE and reduced SE inputs of DOC.

25   DOC was remineralized to dissolved inorganic carbon regardless of season, but rates were lower

in winter. PE discharge in winter elevated DOC concentrations along much of the estuary.

Distinct stable isotope ratios confirmed particulate organic matter (POM) input from PE and SE

to the water column and into the sediment. This was relatively localized, indicating rapid POM

settlement regardless of season. SE discharge increased nutrient inputs and enhanced algal

30   productivity, particularly in summer when chlorophyll-a concentrations were elevated throughout

the estuary. SE discharge reduced pCO_2 from levels associated with PE discharge. However, the

estuary remained heterotrophic as subsequent respiration or decomposition of algal material offset

reductions in PPM organic matter input. The influence of the PPM was apparent throughout the

estuary, demonstrating the ability of anthropogenic inputs, and changes to these, to affect

35   ecosystem functioning.

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Introduction

Paper and pulp mills (PPM) are major sources of nutrients and organic matter (OM) to estuaries and may impact the receiving environment (*1*). Nutrients discharged to estuaries can stimulate algal production (*2*), and enhanced inputs of OM from PPM effluent or algal production can alter energy flows and deplete water column oxygen concentrations (*3*). The composition of PPM effluent depends on the fiber source and pulping method, but also on the treatment processes employed. Studies assessing impacts of primary-treated effluent (PE) discharge mainly consider the effects of residual fiber and OM that demand oxygen (*4*). A primary objective of secondary treatment of PPM effluent is to reduce the load of oxygen-demanding OM. During secondary treatment, microbes remove organic carbon, producing dissolved inorganic carbon (DIC) that can be released as CO_2 gas. Because of the relatively low nitrogen (N) content of effluent, nutrients are added to 'feed' the microbes (*5*). Secondary-treated effluent (SE) typically contains more labile OM (microbes) than PE (fiber) and can have a higher inorganic nutrient content. This may shift a system towards autotrophy by enhancing algal production. Conversely, respiration of the labile OM produced, as well as microbes from SE, can increase heterotrophy and offset reductions in OM loading and oxygen demand gained through secondary treatment.

Although PPM can contribute significant carbon (C) and N to aquatic systems, this can be difficult to detect using traditional methods due to dilution (*6*), transformation, and inputs from other sources. Particularly in estuaries, spatial variation in physical and chemical characteristics can complicate detection of PPM inputs. Stable isotope analysis can be useful for tracing anthropogenic inputs in aquatic systems, provided that inputs have a distinct isotope signature. To our knowledge, only four studies, all in freshwater, have used stable isotopes to trace PPM material (*6-9*). These studies traced PPM material into suspended sediment and biota, and determined the extent of PPM influence and/or exposure of biota to effluent. However, they provided no information on the transformation processes that affect PPM-derived material, and did not compare the effects of PE and SE, despite the potential for treatment processes to affect

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the quality and quantity of C and N. There have been no attempts to use stable isotopes to trace distribution and transformations of PPM material within an estuary.

70 Deviations of isotope ratios and/or concentrations from conservative mixing models can provide insight into sources and transformations of material in estuaries. Based on known isotope ratios or concentrations of a compound in marine and freshwater, conservative mixing models use salinity (a measure of physical mixing) to calculate the expected isotope ratio or concentration of the compound at a given point in an estuary. Deviations from this model are due to non-physical processes (uptake, transformation, or production).

75 Conservative mixing models have been applied to C stable isotope ratios (δ^{13}C) of dissolved organic carbon (DOC-δ^{13}C) and dissolved inorganic carbon (DIC-δ^{13}C) to assess OM sources along estuaries (e.g., *10*, *11*). This can be a powerful method, particularly in combination with other techniques, but there have been few measurements of DOC-δ^{13}C or DIC-δ^{13}C due to tedious analytical processes and/or difficulties with analysis (*11*, *12*). Where anthropogenic

80 inputs have distinct δ^{13}C values, DOC-δ^{13}C and DIC-δ^{13}C could provide insight into their transport and processing. However, no studies to our knowledge have used DOC-δ^{13}C and DIC-δ^{13}C to trace anthropogenic inputs.

The combined approach of conservative mixing models and stable isotope analysis of C and N pools, including DOC and DIC, has potential to improve understanding of the distribution,

85 transformation and fate in the receiving environment of C and N from a variety of anthropogenic sources. This information is vital for appropriate monitoring, risk analysis and environmental management.

During this study, a PPM discharging to a temperate Australian estuary upgraded its treatment processes from primary to secondary treatment, providing the unique opportunity to

90 determine how the receiving estuary was affected by changed anthropogenic inputs of C and N. We aimed to use conservative mixing models and stable isotope analysis of C and N pools in the water column and sediment, combined with monitoring of pCO_2, and concentrations of DOC,

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DIC, chlorophyll-a and nutrient concentrations, to determine how inputs and processing of C and N differed for PE and SE, and how this varied with season (summer or winter). We were

95   specifically interested in identifying the extent of influence of the PPM, as well as more general effects on ecosystem functioning. This application highlights the potential for this novel combination of techniques to provide insight into processing of anthropogenic C and N in aquatic environments.

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Experimental

Site description

The Derwent estuary is adjacent to Hobart in Tasmania, temperate southeast Australia (refer *3* and *13* for maps and detailed descriptions). The estuary has strongly seasonal flows that are, on average, lowest from January to March (50-60 $m^3 s^{-1}$), and highest from June to November

110   (90-140 $m^3 s^{-1}$; *14*).

Point sources along the estuary include the Jordan River, nine sewage treatment plants (STPs) in the mid- to lower estuary, and one STP in the upper estuary. In the upper estuary (~12 km downstream of the STP), a PPM that produces newsprint from mechanically pulped softwood (pine) and hardwood (eucalypt) and recycled fiber has historically been a major OM source.

115   During the first year of this study (2007), 45ML d^{-1} of primary-treated effluent (PE) was discharged containing 40-90 mg L^{-1} of wood-derived particulates (D. Richardson pers. comm.). In October 2007, secondary treatment (activated sludge) was introduced at the PPM, resulting in a similar particulate load (20-90 mg L^{-1} in 60 ML d^{-1} of effluent) containing more labile material (90-100% microbes; D. Richardson pers. comm.). As intended, secondary treatment lowered the

120   oxygen demand of the effluent (Chemical Oxygen Demand = 509 ± 195 mg L^{-1} for SE compared to 2130 ± 414 mg L^{-1} for PE, D. Richardson pers. comm.).

Sample collection and processing

Water quality measurements (salinity (expressed according to the Practical Salinity

125   Scale), pH, dissolved oxygen concentration (DO), and temperature) were taken near the newsprint mill (PPM) outfall, at a control (background) site upstream of the outfall, and at sites along the salinity gradient in March-May (summer) and August-September (winter) in 2007 (pre-upgrade) and 2008 (post-upgrade).

From each site, at each time, surface water was collected to determine alkalinity (via

130   Gran titration), and concentrations of chlorophyll-a, DOC, DIC, dissolved organic nitrogen

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(DON), and nutrients (NO_x, NH_4^+, PO_4^{3-}, and total phosphorous (TP)). Particulate OM (POM) was collected to determine its C:N ratio (POM-C:N) and stable isotopes of carbon (POC-δ^{13}C) and nitrogen (PN-δ^{15}N). DOC-δ^{13}C and DIC-δ^{13}C were also determined. POC-δ^{13}C and PN-δ^{15}N samples for 2008 were lost. Samples for winter were replaced by collections in the upper estuary in 2009, but were unable to be replaced for summer.

Unfiltered water for TP and total dissolved nitrogen (TDN) concentrations, and filtered water (0.45 μm cellulose acetate) for NO_x, PO_4^{3-}, and NH_4^+ analysis was transferred to 10 mL sample-rinsed polycarbonate and polyethylene vials, respectively, and stored frozen until analysis using a four channel Flow Injection Analyser (Lachat$_{TM}$ QuickChem 8000) (refer *15* and *16*). The concentration of DON was determined as TDN - (NO_x + NH_4^+). Samples for analysis of DOC and DIC concentrations and δ^{13}C were filtered (precombusted 47 mm GF/F), sealed in 40 mL glass vials without headspace, killed (200 μL 50:50w/v $ZnCl_2$), and refrigerated. Water (~0.5-1.0 L) was also filtered to collect chlorophyll-a and POM samples on precombusted GF/F (47 mm diameter) that were stored frozen. Chlorophyll-a concentrations were determined using a spectrophotometer following solvent-extraction (5 mL 90% acetone) (refer *15* and *16*). Filters for POM analyses were freeze-dried. One quarter of each filter was packed into silver capsules for δ^{13}C analysis following acid-fuming (concentrated HCl, 24 h). The remainder was sealed into tin capsules for δ^{15}N analysis.

Sediment (upper 5 mm) for C:N and isotope analysis was collected by divers from upstream of the outfall in winter and summer to ~11 km (summer 2007) or ~40 km (winter 2007, summer and winter 2008) downstream. Samples of the reed *Phragmites australis* were collected from the riverbank where present, but only in summer and winter pre-upgrade. Reeds and sediments were freeze-dried and homogenized (ground). Reed material was weighed into tin capsules for analysis of δ^{13}C and δ^{15}N. Sediment was treated as for POM, but acidified via direct application (1 mol L^{-1} HCl), followed by oven drying (60°C).

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Samples of PE and secondary-treated effluent (SE) were collected to characterize POC-δ^{13}C, PN-δ^{15}N, DOC-δ^{13}C and DIC-δ^{13}C, and concentrations of DOC, DIC, POM, DON, NO_x, NH_4^+, PO_4^{3-}, TP, as described above, and POM-C:N.

160 *Stable isotope analysis*

For POM, sediment, and reeds, δ^{13}C, δ^{15}N, %C and %N were determined using a Thermo Finnigan Flash EA 112 interfaced via a Thermo Conflo III with a Thermo Delta V Plus IRMS. Samples of acetanilide of known isotope composition were analyzed within each run to verify isotope values. Reproducibility for both δ^{13}C and δ^{15}N was ± 0.2‰.

165 Concentrations and δ^{13}C of DOC and DIC were measured via continuous flow wet-oxidation isotope ratio mass spectrometry (CF-WO-IRMS) using an Aurora 1030W TOC analyzer coupled to a Thermo Delta V Plus IRMS (*13*). Where required, DOC samples were diluted with a known volume of milli-Q to concentrations suitable for analysis. Sodium bicarbonate (DIC) and glucose (DOC) of known isotope composition dissolved in helium-purged

170 milli-Q were used for drift correction and to verify concentrations and δ^{13}C values. Reproducibility for DOC and DIC, respectively, was ± 24 µM and ± 33 µM (concentrations) and ± 0.08‰ and ± 0.10‰ (δ^{13}C).

Calculations

175 Sediment and POM molar C:N was calculated from %C and %N. Salinity, temperature, sampling depth, alkalinity and pH were used to calculate pCO_2 using the CO2SYS program (*17*). Conservative mixing lines for isotopes and concentrations were calculated using the formulae of (*18*). The freshwater endmember was upstream of the PPM outfall in all cases. Where lower estuary parameters varied due to influences other than the PPM, an unaffected high salinity site

180 (salinity ≥25) was selected as an alternative marine endmember. Errors associated with all analyses were small compared to the effects observed.

Results and discussion

Effluent characteristics

We detected marked differences in PE and SE composition (Table 1). Whereas PE had extremely high concentrations of DOC, secondary treatment reduced DOC and, to a lesser extent, POM concentrations. The concentration of DON was higher, and NO_x lower in SE than PE. Microbial respiration and nutrient addition, combined with remineralization of microbes, increased concentrations of DIC, NH_4^+ and PO_4^{3-} in SE compared to PE. Nutrient concentrations in SE were variable (D. Richardson pers. comm.) and the NH_4^+ concentration we measured was relatively high. However, the NH_4^+ concentration of SE was generally elevated above that of PE (PE: 33 ± 78 µM; D.Richardson pers. comm.).

Molar C:N ratios of PE particulates (50.3) were within the range for vascular plants (20-500; *19*) reflecting the dominance of wood-derived material. In contrast, C:N of SE particulates (8.5) approached that typical of bacterial biomass (~4; *19*), confirming that particulates consisted of microbes and their products, possibly with some contribution of higher plant material. Isotope ratios in effluent were also affected by treatment. DIC-δ^{13}C was enriched compared to DOC-δ^{13}C, regardless of treatment, but more so for SE and, although DOC-δ^{13}C was similar for PE and SE, DIC-δ^{13}C in SE was enriched compared to PE. POC-δ^{13}C was similar for PE and SE, but PN-δ^{15}N was depleted in SE compared to PE.

Pre-upgrade

PE was detected in dissolved and particulate fractions in the estuary, although this varied seasonally, most likely due to differences in flow rate and temperature. The major dissolved input to the estuary pre-upgrade was DOC, for which concentrations were far higher at the outfall than upstream regardless of season (Table 1, Fig. 1). In winter, this was less pronounced due to dilution by higher flow. DOC-δ^{13}C at the outfall in summer was depleted (-27.9‰), and in winter was enriched (-27.4‰) compared to background (-27.0‰ and -28.5‰, respectively). Upstream

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and outfall DOC-δ^{13}C values typical of C_3 plant material (-28 to -25‰; *19*, Fig. 1), reflected the

PPM fiber source and upstream C_3 plant input. PPM-derived DIC-δ^{13}C was distinctly depleted

210 compared to background in both seasons. In summer, DIC-δ^{13}C was similar to DOC-δ^{13}C,

whereas DIC-δ^{13}C in winter was enriched compared to DOC-δ^{13}C.

Relative δ^{13}C values of DIC and DOC provide insight into processing of PPM effluent.

Oxidation of DOC produces DIC with similar δ^{13}C. Similar DIC-δ^{13}C and DOC-δ^{13}C at the

outfall in summer, and depletion of DIC-δ^{13}C towards DOC-δ^{13}C in winter (Fig. 1) therefore

215 indicate utilization of PPM-derived DOC by bacteria. Upstream of the outfall, background DIC-

δ^{13}C was enriched compared to DOC-δ^{13}C (Fig. 1). This suggests that background DOC was less

labile and only a fraction of available DOC was remineralized. DOC is comprised of compounds

of differing lability and δ^{13}C values. Preferential remineralization of a more enriched DOC

fraction (e.g., carbohydrates, *20, 21*) may explain the upstream enrichment of DIC-δ^{13}C compared

220 to DOC-δ^{13}C.

PPM-derived DOC was remineralized to DIC in summer and winter. In summer, DOC

concentrations rapidly declined to conservative mixing, whereas in winter, concentrations

declined more gradually and remained above conservative mixing along much of the estuary (Fig.

1). In summer, DIC concentrations at the outfall were elevated above conservative mixing,

225 despite concentrations of DIC in PE being similar to background. This, and the similarity of

DIC-δ^{13}C and DOC-δ^{13}C at the outfall, indicates in situ production of DIC via remineralization of

DOC. There was a similar pattern at the outfall in winter, but DIC-δ^{13}C followed conservative

mixing downstream. Despite this, elevated DIC concentrations indicated production of DIC

along the estuary, as reflected by high pCO_2 (Fig. 1).

230 Upstream of the PPM outfall, the Derwent estuary was autotrophic in winter (pCO_2 below

atmospheric values), and heterotrophic in summer (Fig. 1). At and below the outfall,

remineralization of additional allochthonous OM from the PPM elevated pCO_2 and reduced DO

below conservative mixing. The estuary downstream of the PPM outfall was therefore net

heterotrophic. The highest pCO_2 recorded, at the outfall in summer 2007, was within reported

235 ranges (e.g., *22*). At the outfall and along the estuary, pCO_2 was generally lower in winter than

summer (Fig. 1), suggesting reduced remineralization rates at lower temperatures, as has been

reported previously (*23*).

There was evidence of input from PE in both POM and sediment. In summer and winter,

PN-δ^{15}N was depleted, POC-δ^{13}C was enriched and POM-C:N was higher at the outfall than

240 upstream (Fig. 2). This was generally more pronounced in summer when lower flow limited

dilution with upstream inputs. The limited spatial extent of distinct isotope and C:N ratios near

the outfall (Fig. 2) show that POM settled rapidly regardless of seasonal flow rates. Such input of

organic material to the sediment can increase O_2 demand, and alter bacterial populations and

processing (*24*).

245 Near the PPM outfall, mixing of upstream and PPM-derived material resulted in water

column POM having lower C:N than POM in PE, particularly in winter when higher flows

increased dilution with background inputs. Discrepancies between C:N, δ^{13}C and δ^{15}N of water

column POM and sediment provide insight into processing of OM during and after settlement. In

summer and winter pre-upgrade, C:N of sediment near the outfall was greater than upstream.

250 Although sediment C:N was also elevated upstream of the PPM in winter, distinct δ^{13}C and δ^{15}N

in sediment near the outfall confirm PPM input (Fig 2). Sediment C:N near the outfall (~40

(summer), ~30 (winter)) higher than POM-C:N (~30, ~12; Fig. 2) indicated preferential removal

of N during diagenesis (*25*). This is most likely due to denitrification, which preferentially

removes ^{14}N (*26*). Such isotope discrimination would also explain the enrichment of sediment

255 δ^{15}N compared to PN-δ^{15}N in summer. Enrichment of sediment δ^{13}C compared to POC-δ^{13}C in

summer shows that C was also processed, with preferential removal of ^{12}C by respiration. In

winter, there was minimal processing (δ^{13}C and δ^{15}N for sediment and POM were similar), most

likely reflecting lower bacterial activity, typical of cooler temperatures (*23*). The difference

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between C:N of sediment and POM in winter therefore most likely represents persistence of high

260   C:N material within sediments from earlier in the year.

PPM-derived C and N was detected in *Phragmites australis* fringing the estuary, with

trends in $\delta^{13}C$ and $\delta^{15}N$ near the outfall similar to those seen for sediments. This demonstrates

utilization by reeds of nutrients and DIC released from degradation of PPM-derived material

within sediments. Heterotrophs were not measured in the current study, but PPM-derived

265   material has previously been detected in riparian higher consumers (*8*). Given that macrophyte

detritus can provide nutrition to consumers (*27*), fringing plants are a potential link in the trophic

transfer of PPM-material to riparian consumers in the Derwent estuary.

The PPM had little influence on nutrient concentrations pre-upgrade. Concentrations of

NH_4^+, NO_x, and PO_4^{3-} were slightly higher, and DON slightly lower, at the outfall than upstream

270   (see Supporting Information). However, in most cases concentrations rapidly returned to

conservative mixing. In winter, concentrations of NH_4^+ remained relatively high and DON

relatively low, compared to conservative mixing, to salinities of 10 and 15, respectively.

Increased nutrient concentrations mid-estuary (salinity ~22) in summer and winter were at a

single site (Prince of Wales Bay), and likely relate to effluent discharge from a nearby STP.

275   Chlorophyll-a concentrations were low at the outfall compared to elsewhere in the

estuary, reflecting the input of large volumes of PE, which did not contain algae. Elevated

chlorophyll-a (algal biomass) in the lower estuary in winter pre-upgrade most likely reflects

intrusion of nutrient-rich sub-Antarctic water into the lower estuary, which is typical of this

system in winter (*28*). Increased competition for DIC during a bloom decreases discrimination

280   against ^{12}C, leading to ^{13}C-enriched algae (*29*), which may account for enriched POC-$\delta^{13}C$ at high

salinity.

Post-upgrade

The input of DOC to the estuary was reduced post-upgrade, but could still be detected regardless of season. In contrast to pre-upgrade, background and outfall DOC-δ^{13}C in summer were similar, but DOC concentration was elevated at the outfall. In winter, dilution by high flow masked changes in DOC concentration, but distinct DOC-δ^{13}C values at the outfall (-30‰)

290 confirmed input of PPM-derived DOC (Fig. 1).

As was seen pre-upgrade, DOC concentrations decreased downstream of the outfall in winter (although there was no initial spike at the outfall) and, more rapidly, in summer (Fig. 1). Removal of DOC from the water column indicated continued bacterial utilization of PPM-derived DOC post-upgrade, with rates dependent on seasonal temperatures. The increase in DIC

295 concentration at the outfall in summer and depletion of DIC-δ^{13}C towards DOC-δ^{13}C of SE in winter and, to a lesser extent, in summer could be explained by direct input of SE, for which the DIC concentration was high and DIC-δ^{13}C depleted, but is more likely due to remineralization of POM-derived DOC.

A major difference between pre- and post-upgrade conditions was the clearly elevated

300 concentrations of TP, PO_4^{3-}, NH_4^+, NO_x and DON near the outfall in summer post-upgrade (see Supporting Information). This reflects either input of excess nutrients within effluent (Table 1), and/or remineralization of the labile microbes that comprised POM within SE (J.M. Oakes unpubl. data). Inputs in winter would have been similar, although processing of microbes may have been reduced at lower temperatures. Elevation of PO_4^{3-}, NH_4^+, and NO_x concentrations

305 downstream indicates these inputs were diluted and pushed downstream by high winter flow rates (see Supporting Information). With the exception of DON, for which concentrations were generally above conservative mixing regardless of season, there was rapid uptake of nutrients in summer, and reduced uptake in winter, with concentrations generally remaining above conservative mixing.

310   Studies of mesocosms and artificial streams have shown that PPM effluent increases algal

production (*30*). Although secondary treatment can reduce inorganic nutrients to a minimum (*5*),

even small inputs to a nutrient-limited system can cause excess algal production (*2*) that can alter

community structure and biomass, water quality, and ecosystem functioning. Increased

production of OM can offset reductions in OM load associated with secondary treatment,

315   particularly as algae is labile compared to the allochthonous terrestrial inputs that otherwise

dominate. The uptake of SE nutrients in the current study (see Supporting Information) shows

that the Derwent estuary is nutrient-limited. Nutrient input increased chlorophyll-a

concentrations above conservative mixing along the estuary post-upgrade, whereas chlorophyll-a

was reduced at the outfall pre-upgrade (Fig. 1). Post-upgrade, chlorophyll-a was particularly

320   elevated in summer when warmer conditions were more conducive to algal growth. Algae may

have utilized DIC released from PPM-derived DOC, making it difficult to detect changes in DIC

concentration post-upgrade. Use of ^{13}C-depleted DIC derived from PPM DOC may, however,

account for DIC-δ^{13}C below conservative mixing along the estuary in summer post-upgrade (Fig.

1). As was seen pre-upgrade, chlorophyll-a was elevated at high salinity in winter due to

325   intrusion of nutrient-rich water.

Although autotrophs produce DO during photosynthesis, reductions in DO coinciding

with chlorophyll-a peaks in summer and, to a lesser extent, in winter (see Supporting

Information) showed that algal material was respired. Cell lysis and grazing of algae releases

DON (*31*), and may therefore explain excess DON in summer and winter.

330   Phytoplankton production was higher in the estuary post-upgrade than pre-upgrade, and

DIC concentrations fell below conservative mixing due to autotrophic uptake. However,

depletion of DIC- δ^{13}C below conservative mixing along the estuary in summer and winter (Fig.

1), indicate net heterotrophy (*32*). This was confirmed by elevation of pCO_2 above atmospheric

equilibrium in summer and winter at most sites (Fig. 1). The dominance of heterotrophy in the

335   estuary, despite algal production, may relate to processing of this labile OM, and/or microbes

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associated with pulp mill effluent. Elevation of pCO_2 was more pronounced in summer, but in both seasons was generally lower than pre-upgrade and localized near the outfall. Near major wetlands (salinity ~3), where chlorophyll-a was highest, pCO_2 at or below atmospheric equilibrium reflected autotrophic DIC uptake. Degradation of STP inputs presumably caused

340 elevated pCO_2 near STPs (salinity ~26 in summer, ~20 and ~24 in winter), although this was lower than near the PPM outfall. The estuary, therefore, was a source of CO_2 to the atmosphere pre-and post-upgrade, primarily driven by PPM inputs. Post-upgrade, the estuary released far less CO_2.

Characteristics of POM in the upper estuary in winter post-upgrade reflected input of SE.

345 Similar to summer and winter pre-upgrade, PN-δ^{15}N was depleted compared to background, although more so post-upgrade. However, in contrast to pre-upgrade, POM-C:N and POC-δ^{13}C at the outfall were also depleted (Fig. 2). This corresponded with differences in POC-δ^{13}C and POM-C:N for SE and PE, reflecting differences in inputs rather than processing. However, sediment δ^{15}N most clearly reflected differences in PE and SE input.

350 In winter post-upgrade, δ^{15}N of sediment (1.7‰) was enriched compared to POM (0.4‰), but δ^{13}C of sediment and POM were similar, as was seen in winter pre-upgrade (Fig. 2). This may indicate processing of POM in winter post-upgrade, or persistence within the sediment of enriched PE material. At the outfall, C:N of sediment (7.4) and POM (9.5) reflected input of SE (8.5). The higher C:N of sediment suggests preferential removal of N or input of high C:N

355 material (e.g., in situ algal production). Elsewhere in the estuary, C:N of POM and sediment were higher post-upgrade than pre-upgrade (Fig. 2). Given that C:N generally increases with degradation (*25*), this may provide evidence that OM in the Derwent estuary was more highly processed post-upgrade. The microbes within SE, and algae produced due to nutrient input, are more labile than the fiber in PE. This material is not only more easily processed, but can also

360 stimulate microbial activity (*33*).

The current study demonstrated that concentration and stable isotope data of C and N

pools, combined with conservative mixing models, can provide insight into system-level

365   processing and the impacts of changed anthropogenic inputs (Table 1). However, the

transformation and fate of anthropogenic inputs is likely to be site-specific. In most cases,

evidence of direct PE and SE input was relatively localized, but the some inputs (e.g., DOC in

winter pre-upgrade) and ecosystem-level effects (e.g., increased algal production) were apparent

throughout the estuary. The changes in quality and quantity of OM due to PPM input that were

370   observed demonstrate the ability for anthropogenic inputs to influence ecosystem processing and

export of material to the ocean.

Whether an estuary is net heterotrophic or autotrophic has implications for its role in

global C cycling, and fisheries productivity. Anthropogenic input of terrestrial (high C:N) OM is

associated with increased heterotrophic status of estuaries (*34*). However, there is currently a

375   trend towards removal of OM from inputs (e.g., through treatment of STP and PPM effluent),

whilst inorganic nutrient inputs continue to increase. It has been proposed that this will result in

coastal systems becoming autotrophic (*35*). In the current study, secondary treatment of PPM

effluent reduced OM load and reduced heterotrophy. However, increased nutrient input did not

make the estuary autotrophic. Interestingly, respiration of the labile algal material produced

380   appears to outweigh the increased productivity and offsets some of the OM load reduction

associated with secondary treatment.

Supporting information available

Graphs showing concentrations of dissolved oxygen, NO_x, NH_4^+, PO_4^{3-}, TP and DON along the

Derwent River estuary in summer and winter pre- and post-upgrade.

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Table 1: Characteristics of primary- and secondary-treated effluent and effects of discharge on parameters near the pulp mill outfall. Arrows indicate direction of change at the outfall compared to background (i.e. site upstream of the outfall).

	Parameter	Primary-treated effluent			Secondary-treated effluent		
		Concentration*	Isotope signature (‰)	C:N	Concentration*	Isotope signature (‰)	C:N
Effluent characteristics	DOC	20 300	-28.4		2 675	-29.2	
	DIC	191	-26.2		1 129	-22.2	
	POM	40-90		50.3	20-90		8.5
	- POC		-25.9			-26.7	
	- PN		6.2			-0.1	
	NH_4^+	16			755		
	NO_x	14			4		
	DON	397			502		
	PO_4^{3-}	5			112		
Effect at outfall	DOC	↑	↕a		↕b	↕c	
	DIC	↑	↓		↕b	↓	
	POM			↑			↓
	- POC		↑			↓	
	- PN		↓			↓	
	Sediment			↑			↓
	- C		↑			↓	
	- N		↓			↓	
	Reeds						
	- C		↑				
	- N		↓				
	pCO_2	↑			↑		
	DO	↓			↓		
	NH_4^+	↑			↑		
	NO_x	↑			↕b		
	DON	↓			↑		
	PO_4^{3-}	↑			↕b		
	TP	—			↕b		
	Chlorophyll-a	↓			↑		

* Units of concentration are µmol L^{-1} of C, N or P, except for POM which has units of mg L^{-1}
— = no effect
↑ = higher concentration or C:N ratio, or enriched isotope signature
↓ = lower concentration or C:N ratio, or more depleted isotope signature
↕ = effect varied with season, subscripts provide details (a = lower in summer, higher in winter, b = higher in summer, similar in winter c = lower in winter, similar in summer)

Figure captions

Figure 1: Concentrations and $\delta^{13}C$ for DOC and DIC and concentrations of pCO_2 and chlorophyll-a in summer and winter pre- and post-upgrade. Vertical lines indicate location of pulp mill outfall. Remaining lines represent conservative mixing, except for pCO_2, where line indicates equilibrium of pCO_2 with the atmosphere.

Figure 2: $\delta^{13}C$ and $\delta^{15}N$ values and C:N ratios for POM and sediment in summer and winter pre- and post-upgrade. Lines indicate location of pulp mill outfall.
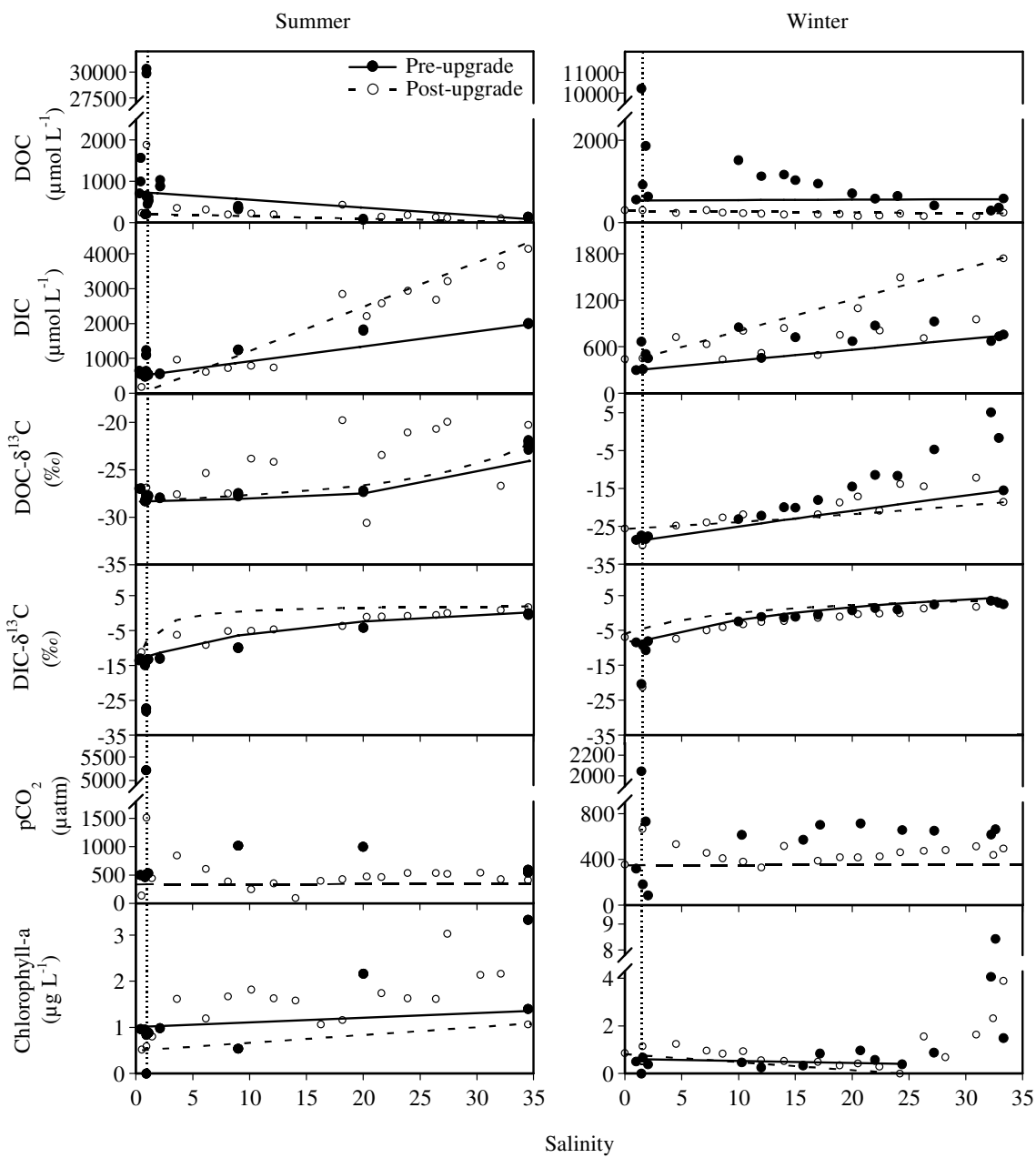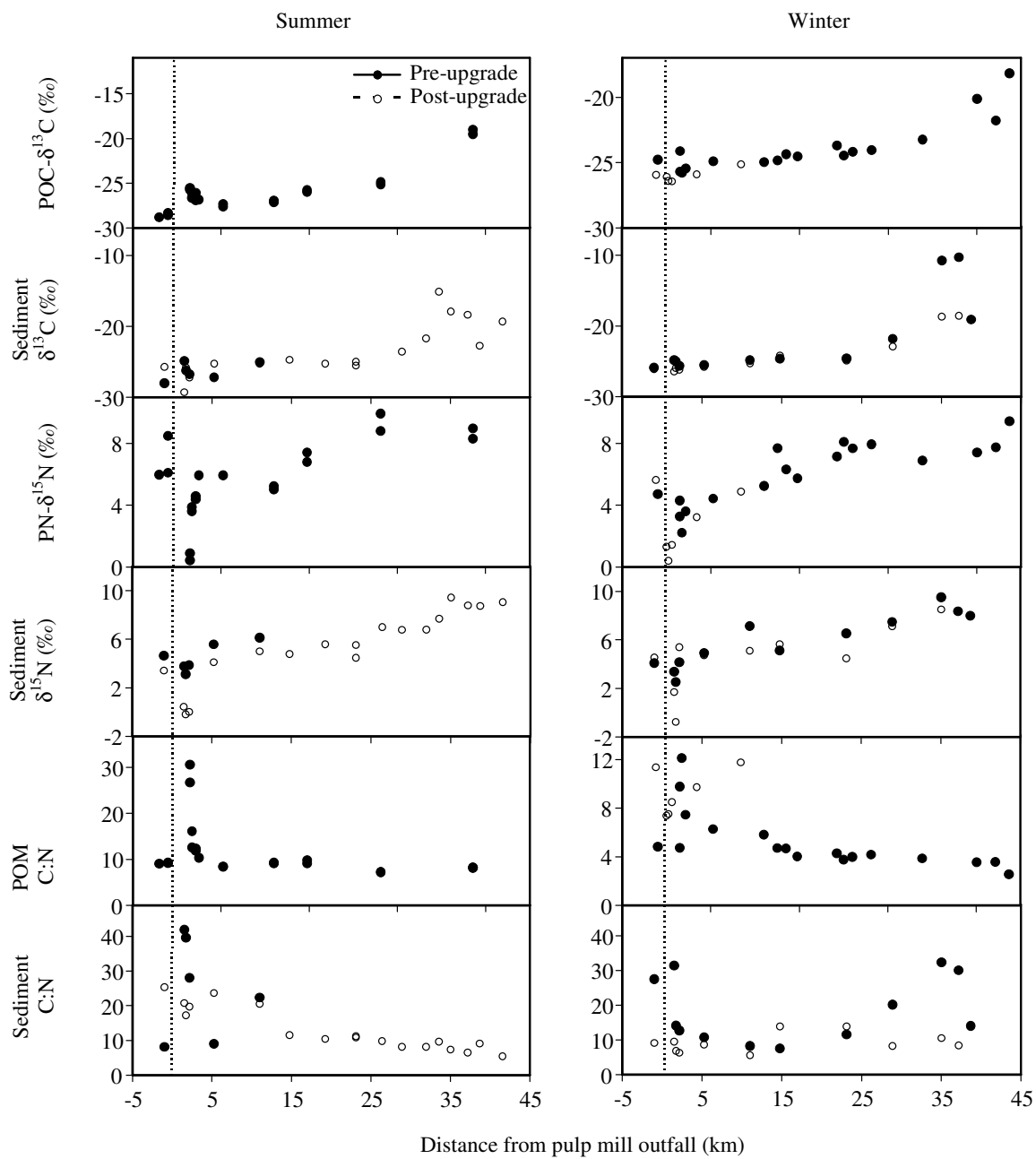
Figure 1

Summer

Winter

Distance from pulp mill outfall (km)

Figure 2

Brief

Stable isotopes of particulate and dissolved forms of carbon and nitrogen reveal estuarine distributions and transformations of primary- and secondary-treated paper mill effluent.