

2010

# Stable isotopes trace estuarine transformations of carbon and nitrogen from primary- and secondary-treated paper and pulp mill effluent

Joanne M. Oakes  
*Southern Cross University*

Bradley D. Eyre  
*Southern Cross University*

Donald J. Ross  
*Tasmanian Aquaculture and Fisheries Institute*

Simon D. Turner  
*Tasmanian Aquaculture and Fisheries Institute*

---

## Publication details

Postprint of: Oakes, JM, Eyre, BD, Ross, DJ & Turner, SD 2010, 'Stable isotopes trace estuarine transformations of carbon and nitrogen from primary- and secondary-treated paper and pulp mill effluent', *Environmental Science & Technology*, vol. 44, no. 19, pp. 7411-7417.

Published version available from:  
<http://dx.doi.org/10.1021/es101789v>

Stable isotopes trace estuarine transformations of carbon and nitrogen from primary- and secondary-treated paper and pulp mill effluent

Joanne M. Oakes<sup>1,\*</sup>, Bradley D. Eyre<sup>1</sup>, Donald J. Ross<sup>2</sup>, Simon D. Turner<sup>1</sup>

5

<sup>1</sup>Centre for Coastal Biogeochemistry, Southern Cross University, Military Road, Lismore, New South Wales, 2480, Australia

<sup>2</sup>Tasmanian Aquaculture and Fisheries Institute, Marine Research Laboratories, Nubeena Crescent, Taroom, Tasmania, 7053, Australia

10

\*Corresponding author

Email: [joanne.oakes@scu.edu.au](mailto:joanne.oakes@scu.edu.au)

Telephone: +61 (2) 6620 3092

Fax: +61 (2) 6621 2669

15

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<sub>2</sub> 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.

40

## 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<sub>2</sub> 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

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 ( $\delta^{13}\text{C}$ ) of dissolved organic carbon (DOC- $\delta^{13}\text{C}$ ) and dissolved inorganic carbon (DIC- $\delta^{13}\text{C}$ ) 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- $\delta^{13}\text{C}$  or DIC- $\delta^{13}\text{C}$  due to tedious analytical processes and/or difficulties with analysis (11, 12). Where anthropogenic  
80 inputs have distinct  $\delta^{13}\text{C}$  values, DOC- $\delta^{13}\text{C}$  and DIC- $\delta^{13}\text{C}$  could provide insight into their transport and processing. However, no studies to our knowledge have used DOC- $\delta^{13}\text{C}$  and DIC- $\delta^{13}\text{C}$  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  $\text{pCO}_2$ , and concentrations of DOC,

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.

100

## 105 **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 ( $50\text{-}60\text{ m}^3\text{ s}^{-1}$ ), and highest from June to November  
110 ( $90\text{-}140\text{ m}^3\text{ s}^{-1}$ ; 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),  $45\text{ ML d}^{-1}$  of primary-treated effluent (PE) was discharged containing  $40\text{-}90\text{ mg L}^{-1}$  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 ( $20\text{-}90\text{ mg L}^{-1}$  in  $60\text{ ML d}^{-1}$  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 =  $509 \pm 195\text{ mg L}^{-1}$  for SE compared to  $2130 \pm 414\text{ mg L}^{-1}$  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

(DON), and nutrients ( $\text{NO}_x$ ,  $\text{NH}_4^+$ ,  $\text{PO}_4^{3-}$ , and total phosphorous (TP)). Particulate OM (POM) was collected to determine its C:N ratio (POM-C:N) and stable isotopes of carbon (POC- $\delta^{13}\text{C}$ ) and nitrogen (PN- $\delta^{15}\text{N}$ ). DOC- $\delta^{13}\text{C}$  and DIC- $\delta^{13}\text{C}$  were also determined. POC- $\delta^{13}\text{C}$  and PN- $\delta^{15}\text{N}$  samples for 2008 were lost. Samples for winter were replaced by collections in the upper estuary  
135 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  $\mu\text{m}$  cellulose acetate) for  $\text{NO}_x$ ,  $\text{PO}_4^{3-}$ , and  $\text{NH}_4^+$  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<sub>TM</sub> QuickChem 8000) (refer 15 and 16).  
140 The concentration of DON was determined as  $\text{TDN} - (\text{NO}_x + \text{NH}_4^+)$ . Samples for analysis of DOC and DIC concentrations and  $\delta^{13}\text{C}$  were filtered (precombusted 47 mm GF/F), sealed in 40 mL glass vials without headspace, killed (200  $\mu\text{L}$  50:50w/v  $\text{ZnCl}_2$ ), 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  
145 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  $\delta^{13}\text{C}$  analysis following acid-fuming (concentrated HCl, 24 h). The remainder was sealed into tin capsules for  $\delta^{15}\text{N}$  analysis.

Sediment (upper 5 mm) for C:N and isotope analysis was collected by divers from  
150 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  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ . Sediment was treated as for POM, but acidified via direct  
155 application (1 mol  $\text{L}^{-1}$  HCl), followed by oven drying (60°C).



Samples of PE and secondary-treated effluent (SE) were collected to characterize POC- $\delta^{13}\text{C}$ , PN- $\delta^{15}\text{N}$ , DOC- $\delta^{13}\text{C}$  and DIC- $\delta^{13}\text{C}$ , and concentrations of DOC, DIC, POM, DON,  $\text{NO}_x$ ,  $\text{NH}_4^+$ ,  $\text{PO}_4^{3-}$ , TP, as described above, and POM-C:N.

160 *Stable isotope analysis*

For POM, sediment, and reeds,  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ , %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  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  was  $\pm 0.2\text{‰}$ .

165 Concentrations and  $\delta^{13}\text{C}$  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  $\delta^{13}\text{C}$  values. Reproducibility for DOC and DIC, respectively, was  $\pm 24 \mu\text{M}$  and  $\pm 33 \mu\text{M}$  (concentrations) and  $\pm 0.08\text{‰}$  and  $\pm 0.10\text{‰}$  ( $\delta^{13}\text{C}$ ).

*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  $\text{pCO}_2$  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  $\geq 25$ ) 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  
185 extremely high concentrations of DOC, secondary treatment reduced DOC and, to a lesser extent,  
POM concentrations. The concentration of DON was higher, and  $\text{NO}_x$  lower in SE than PE.  
Microbial respiration and nutrient addition, combined with remineralization of microbes,  
increased concentrations of DIC,  $\text{NH}_4^+$  and  $\text{PO}_4^{3-}$  in SE compared to PE. Nutrient concentrations  
in SE were variable (D. Richardson pers. comm.) and the  $\text{NH}_4^+$  concentration we measured was  
190 relatively high. However, the  $\text{NH}_4^+$  concentration of SE was generally elevated above that of PE  
(PE:  $33 \pm 78 \mu\text{M}$ ; 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  
195 of microbes and their products, possibly with some contribution of higher plant material. Isotope  
ratios in effluent were also affected by treatment.  $\text{DIC-}\delta^{13}\text{C}$  was enriched compared to  $\text{DOC-}$   
 $\delta^{13}\text{C}$ , regardless of treatment, but more so for SE and, although  $\text{DOC-}\delta^{13}\text{C}$  was similar for PE and  
SE,  $\text{DIC-}\delta^{13}\text{C}$  in SE was enriched compared to PE.  $\text{POC-}\delta^{13}\text{C}$  was similar for PE and SE, but  $\text{PN-}$   
 $\delta^{15}\text{N}$  was depleted in SE compared to PE.

200

### *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  
205 than upstream regardless of season (Table 1, Fig. 1). In winter, this was less pronounced due to  
dilution by higher flow.  $\text{DOC-}\delta^{13}\text{C}$  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

and outfall DOC- $\delta^{13}\text{C}$  values typical of  $\text{C}_3$  plant material (-28 to -25‰; 19, Fig. 1), reflected the PPM fiber source and upstream  $\text{C}_3$  plant input. PPM-derived DIC- $\delta^{13}\text{C}$  was distinctly depleted compared to background in both seasons. In summer, DIC- $\delta^{13}\text{C}$  was similar to DOC- $\delta^{13}\text{C}$ , whereas DIC- $\delta^{13}\text{C}$  in winter was enriched compared to DOC- $\delta^{13}\text{C}$ .

Relative  $\delta^{13}\text{C}$  values of DIC and DOC provide insight into processing of PPM effluent. Oxidation of DOC produces DIC with similar  $\delta^{13}\text{C}$ . Similar DIC- $\delta^{13}\text{C}$  and DOC- $\delta^{13}\text{C}$  at the outfall in summer, and depletion of DIC- $\delta^{13}\text{C}$  towards DOC- $\delta^{13}\text{C}$  in winter (Fig. 1) therefore indicate utilization of PPM-derived DOC by bacteria. Upstream of the outfall, background DIC- $\delta^{13}\text{C}$  was enriched compared to DOC- $\delta^{13}\text{C}$  (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  $\delta^{13}\text{C}$  values. Preferential remineralization of a more enriched DOC fraction (e.g., carbohydrates, 20, 21) may explain the upstream enrichment of DIC- $\delta^{13}\text{C}$  compared to DOC- $\delta^{13}\text{C}$ .

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, despite concentrations of DIC in PE being similar to background. This, and the similarity of DIC- $\delta^{13}\text{C}$  and DOC- $\delta^{13}\text{C}$  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- $\delta^{13}\text{C}$  followed conservative mixing downstream. Despite this, elevated DIC concentrations indicated production of DIC along the estuary, as reflected by high  $\text{pCO}_2$  (Fig. 1).

Upstream of the PPM outfall, the Derwent estuary was autotrophic in winter ( $\text{pCO}_2$  below atmospheric values), and heterotrophic in summer (Fig. 1). At and below the outfall, remineralization of additional allochthonous OM from the PPM elevated  $\text{pCO}_2$  and reduced DO below conservative mixing. The estuary downstream of the PPM outfall was therefore net

heterotrophic. The highest pCO<sub>2</sub> recorded, at the outfall in summer 2007, was within reported  
235 ranges (e.g., 22). At the outfall and along the estuary, pCO<sub>2</sub> 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<sup>15</sup>N was depleted, POC-δ<sup>13</sup>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<sub>2</sub> 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<sup>13</sup>C and δ<sup>15</sup>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<sup>13</sup>C and δ<sup>15</sup>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 <sup>14</sup>N (26). Such isotope discrimination would also explain the enrichment of sediment  
255 δ<sup>15</sup>N compared to PN-δ<sup>15</sup>N in summer. Enrichment of sediment δ<sup>13</sup>C compared to POC-δ<sup>13</sup>C in  
summer shows that C was also processed, with preferential removal of <sup>12</sup>C by respiration. In  
winter, there was minimal processing (δ<sup>13</sup>C and δ<sup>15</sup>N for sediment and POM were similar), most  
likely reflecting lower bacterial activity, typical of cooler temperatures (23). The difference

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  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  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  $\text{NH}_4^+$ ,  $\text{NO}_x$ , and  $\text{PO}_4^{3-}$  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  $\text{NH}_4^+$  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  $^{12}\text{C}$ , leading to  $^{13}\text{C}$ -enriched algae (29), which may account for enriched POC- $\delta^{13}\text{C}$  at high salinity.

285 *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- $\delta^{13}\text{C}$  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- $\delta^{13}\text{C}$  values at the outfall (-30‰) 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 concentration at the outfall in summer and depletion of DIC- $\delta^{13}\text{C}$  towards DOC- $\delta^{13}\text{C}$  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- $\delta^{13}\text{C}$  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 concentrations of TP,  $\text{PO}_4^{3-}$ ,  $\text{NH}_4^+$ ,  $\text{NO}_x$  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  $\text{PO}_4^{3-}$ ,  $\text{NH}_4^+$ , and  $\text{NO}_x$  concentrations 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 <sup>13</sup>C-depleted DIC derived from PPM DOC may, however,  
account for DIC- $\delta^{13}\text{C}$  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-  $\delta^{13}\text{C}$  below conservative mixing along the estuary in summer and winter (Fig.  
1), indicate net heterotrophy (32). This was confirmed by elevation of pCO<sub>2</sub> 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

associated with pulp mill effluent. Elevation of  $p\text{CO}_2$  was more pronounced in summer, but in both seasons was generally lower than pre-upgrade and localized near the outfall. Near major wetlands (salinity  $\sim 3$ ), where chlorophyll-a was highest,  $p\text{CO}_2$  at or below atmospheric equilibrium reflected autotrophic DIC uptake. Degradation of STP inputs presumably caused  
340 elevated  $p\text{CO}_2$  near STPs (salinity  $\sim 26$  in summer,  $\sim 20$  and  $\sim 24$  in winter), although this was lower than near the PPM outfall. The estuary, therefore, was a source of  $\text{CO}_2$  to the atmosphere pre-and post-upgrade, primarily driven by PPM inputs. Post-upgrade, the estuary released far less  $\text{CO}_2$ .

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

350 In winter post-upgrade,  $\delta^{15}\text{N}$  of sediment (1.7‰) was enriched compared to POM (0.4‰), but  $\delta^{13}\text{C}$  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).



### *Implications*

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 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 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 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 appears to outweigh the increased productivity and offsets some of the OM load reduction associated with secondary treatment.

## **Acknowledgments**

We thank John Keane, Ruth Eriksen, Tobias Probst, and Sophie Karpiniec for sample collection,  
385 Kym Haskins and Melissa Gibbes for sample processing, Iain Alexander, Brian Jones, Melissa  
Bautista, and Matheus Carvalho de Carvalho for analysis, and Des Richardson for background  
data relating to mill operations. This work was supported by an ARC Linkage grant to DJ Ross  
and BD Eyre (LP0770222) and an ARC Discovery grant (DP0878568) and ARC LIEF grant  
(LE0989952) to BD Eyre and JM Oakes (DP0878568). We thank Norske Skog Boyer for  
390 financial and in-kind assistance.

**Supporting information available**

Graphs showing concentrations of dissolved oxygen,  $\text{NO}_x$ ,  $\text{NH}_4^+$ ,  $\text{PO}_4^{3-}$ , TP and DON along the Derwent River estuary in summer and winter pre- and post-upgrade.

395

## References

- (1) Owens, J. W. The hazard assessment of pulp and paper effluents in the aquatic environment: A review. *Env. Tox. Chem.* **1991**, *10*, 1511-1540.
- 400 (2) Bothwell, M. L. Eutrophication of rivers by nutrients in treated kraft pulp mill effluent. *Wat. Poll. Res. J. Can.* **1992**, *27*, 447-472.
- (3) Leeming, R.; Nichols, P. D. Determination of the sources and distribution of sewage and pulp-fibre-derived pollution in the Derwent Estuary, Tasmania, using sterol biomarkers. *Mar. Freshw. Res.* **1998**, *49*, 7-17
- 405 (4) Hall, T. J.; Landis, W. G. Introduction to special series: Measurement and analysis of the potential long term impact of pulp and paper mill effluent on receiving waters. *Integr. Environ. Assess. Manag.* **2009**, *5*, 186-188.
- (5) Järvinen, R. Nitrogen in the effluent of the pulp and paper industry. *Wat. Sci. Tech.* **1997**, *35*, 139-145.
- 410 (6) Wassenaar, L.I.; Culp, J. M. The use of stable isotopic analyses to identify pulp mill effluent signatures in riverine food webs. In *Environmental fate and effects of pulp and paper mill effluents*; Servos, M. R., Munkittrick, K. R., Carey, J. H., Van der Kraak, G. J., Eds.; St Lucie Press Florida: USA 1996; pp 413-423.
- (7) Kukkonen, J. V. K.; Eadie, B. J.; Oikari, A.; Holmbom, B.; Lansing, M. B. Chlorophenolic and isotopic tracers of pulp mill effluent in sedimenting particles collected from southern  
415 Lake Saimaa, Finland. *Sci. Tot. Env.* **1996**, *188*, 15-27.
- (8) Wayland, M.; Hobson, K. A. Stable carbon, nitrogen, and sulfur isotope ratios in riparian food webs on rivers receiving sewage and pulp-mill effluents. *Can. J. Zool.* **2001**, *79*(1), 5-15.

- 420 (9) Dube, M. G.; Benoy, G. A.; Wassenaar, L. I. Contrasting pathways of assimilation: Stable isotope assessment of fish exposure to pulp mill effluents. *J. Env. Qual.* **2006**, *35*, 1884-1893.
- (10) Raymond, P. A.; Bauer, J. E. Use of  $^{14}\text{C}$  and  $^{13}\text{C}$  natural abundances for evaluating riverine, estuarine, and coastal DOC and POC sources and cycling: a review and synthesis. *Org. Geochem.* **2001**, *32*, 469-485.
- 425 (11) Torres, M. E.; Mix, A. C., Rugh, W. D. Precise  $\delta^{13}\text{C}$  analysis of dissolved inorganic carbon in natural waters using automated headspace sampling and continuous-flow mass spectrometry. *Limnol. Oceanogr.: Meth.* **2005**, *3*, 349-360.
- (12) Osburn, C. L.; St-Jean, G. The use of wet chemical oxidation with high amplification isotope ratio mass spectrometry (WCO-IRMS) to measure stable isotope values of dissolved organic carbon in seawater. *Limnol. Oceanogr.: Meth.* **2007**, *5*, 296-308.
- 430 (13) Davies, P.; Kalish, S. Influence of river hydrology on the dynamics and water quality of the upper Derwent Estuary, Tasmania. *Aust. J. Mar. Freshw. Res.*, **1994**, *45*, 109-130.
- (14) Green, G.; Coughanowr, C. *State of the Derwent Estuary 2003: a review of pollution sources, loads and environmental quality data from 1997-2003*. Derwent estuary program DPIWE, Tasmania 2003.
- 435 (15) Eyre, B. D.; Ferguson, A. J. P. Benthic metabolism and nitrogen cycling in a subtropical east Australian estuary (Brunswick): Temporal variability and controlling factors. *Limnol. Oceanogr.*, **2005**, *50*, 81-96.
- 440 (16) Eyre, B. D. Regional evaluation of nutrient transformation and phytoplankton growth in nine river-dominated sub-tropical east Australian estuaries. *Mar. Ecol. Prog. Ser.*, **2000**, *205*, 61-83.
- (17) Pierrot, D.; Lewis, E.; Wallace, D. W. R. *MS Excel program developed for CO<sub>2</sub> system calculations*; ORNL/CDIAC-105a. Carbon dioxide information analysis center. Oak Ridge National Laboratory. U.S. Department of Energy; Tennessee 2006.
- 445

- (18) Kaldy, J. E.; Lewis L. A.; Brock, D. Using stable isotope analyses to assess carbon dynamics in a subtropical estuary. *Estuaries* **2005**, 28, 86-95.
- (19) Hedges, J. I.; Keil, R. G.; Berner, R. What happens to terrestrial organic matter in the ocean? *Org. Geochem.* **1997**, 27, 195-212.
- 450 (20) Lehmann, M. F.; Bernasconi, S. M.; Barbieri, A.; McKenzie, J. A. Preservation of organic matter and alteration of its carbon and nitrogen isotope composition during simulated and in situ early sedimentary diagenesis. *Geochim. Cosmochim. Acta* **2002**, 66, 3574-3584.
- (21) Oakes, J. M.; Eyre, B. D.; Middelburg, J. J.; Boschker, H. T. S. Composition, production, and loss of carbohydrates in subtropical shallow subtidal sandy sediments: Rapid processing  
455 and long-term retention revealed by <sup>13</sup>C-labeling. *Limnol. Oceanogr.* **in press**.
- (22) Ahad, J. M. E.; Barth, J. A. C.; Ganeshram, R. S.; Spencer, R. G. M.; Uher, G. Controls on carbon cycling in two contrasting temperate zone estuaries: The Tyne and Tweed, UK. *Estuar. Coastal Shelf Sci.* **2008**, 78, 685-693.
- (23) Arnosti, C.; Jorgensen, B. B.; Sagemann, J.; Thamdrup, B. Temperature dependence of  
460 microbial degradation of organic matter in marine sediments: Polysaccharide hydrolysis, oxygen consumption, and sulfate reduction. *Mar. Ecol. Prog. Ser.* **1998**, 165, 59-70.
- (24) Poole, N. J.; Parkes, R. J.; Wildish, D. J. Reaction of estuarine ecosystems to effluent from pulp and paper industry. *Helgoländer wiss. Meeresunters* **1977**, 30, 622-632.
- (25) Hamilton, S. E.; Hedges, J. I. The comparative geochemistries of lignin and carbohydrates in  
465 an anoxic fjord. *Geochim. Cosmochim. Acta* **1988**, 52, 129-142.
- (26) Kendall, C.; et al. Tracing anthropogenic inputs of nitrogen to ecosystems. In *Stable isotopes in ecology and environmental science*, 2<sup>nd</sup> ed.; Michener, R.; Lajtha, K., Eds.; Blackwell Publishing: USA 2007; pp 375-449.
- (27) Oakes, J. M.; Connolly, R. M.; Reville, A. T. Isotope enrichment in mangrove forests  
470 separates microphytobenthos and detritus as carbon sources for animals. *Limnol. Oceanogr.* **2010**, 55, 393-402.

- (28) Harris, G.; Nilsson, C.; Clementson, L.; Lyne, V.; Van der Doe, H. The water masses of the east coast of Tasmania: seasonal and inter-annual variability and the influence on phytoplankton biomass and productivity. *Aust. J. Mar. Freshw. Res.* **1987**, *38*, 569-590.
- 475 (29) Bothwell, M. L.; Stockner, J. G. Influence of secondarily treated kraft mill effluent on the accumulation rate of attached algae in experimental continuous-flow troughs. *Can. J. Fish. Aquat. Sci.* **1980**, *37*, 248-254.
- (30) Burkhardt, S.; Riebesell, U.; Zondervan, I. Effects of growth rate, CO<sub>2</sub> concentration, and cell size on the stable carbon isotope fractionation in marine phytoplankton. *Geochim. Cosmochim. Acta* **1999**, *63*, 3729-3741.
- 480 (31) Bronk, D. A.; Ward, B. B. Gross and net nitrogen uptake and DON release in the euphotic zone of Monterey Bay, California. *Limnol. Oceanogr.* **1999**, *44*, 573-585.
- (32) Spiker, E. C. The behavior of <sup>14</sup>C and <sup>13</sup>C in estuarine water: Effect of in situ CO<sub>2</sub> production and atmospheric exchange. *Radiocarbon* **1980**, *22*, 647-654.
- 485 (33) Frankignoulle, M.; Abril G.; Borges, A. V.; Bourge, I.; Canon, C.; Delille, B.; Libert, E.; Théate, J.-M. Carbon dioxide emission from European estuaries. *Science* **1998**, *282*, 434-436.
- (34) Hopkinson, C. S. Jr.; Smith, E. M. Estuarine respiration: an overview of benthic, pelagic, and whole system respiration. In *Respiration in aquatic ecosystems*; del Giorgio, P. A.; Williams, P. J. le B., Eds.; Oxford University Press: New York 2005, pp 122-146.
- 490 (35) Kemp, W. M.; Smith, E. M., Marvin-DiPasquale, M.; Boynton, W. R. Organic carbon balance and net ecosystem metabolism in Chesapeake Bay. *Mar. Ecol. Prog. Ser.* **1997**, *150*, 229-248.

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
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 <sub>4</sub> <sup>+</sup>	16			755		
NO <sub>x</sub>	14			4		
DON	397			502		
PO <sub>4</sub> <sup>3-</sup>	5			112		
DOC	↑	↕ <sub>a</sub>		↕ <sub>b</sub>	↕ <sub>c</sub>	
DIC	↑	↓		↕ <sub>b</sub>	↓	
POM			↑			↓
- POC		↑			↓	
- PN		↓			↓	
Sediment			↑			↓
- C		↑			↓	
- N		↓			↓	
Reeds						
- C		↑				
- N		↓				
pCO <sub>2</sub>	↑			↑		
DO	↓			↓		
NH <sub>4</sub> <sup>+</sup>	↑			↑		
NO <sub>x</sub>	↑			↕ <sub>b</sub>		
DON	↓			↑		
PO <sub>4</sub> <sup>3-</sup>	↑			↕ <sub>b</sub>		
TP	—			↕ <sub>b</sub>		
Chlorophyll-a	↓			↑		

\* Units of concentration are  $\mu\text{mol L}^{-1}$  of C, N or P, except for POM which has units of  $\text{mg L}^{-1}$

— = 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  $\delta^{13}\text{C}$  for DOC and DIC and concentrations of  $\text{pCO}_2$  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  $\text{pCO}_2$ , where line indicates equilibrium of  $\text{pCO}_2$  with the atmosphere.

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

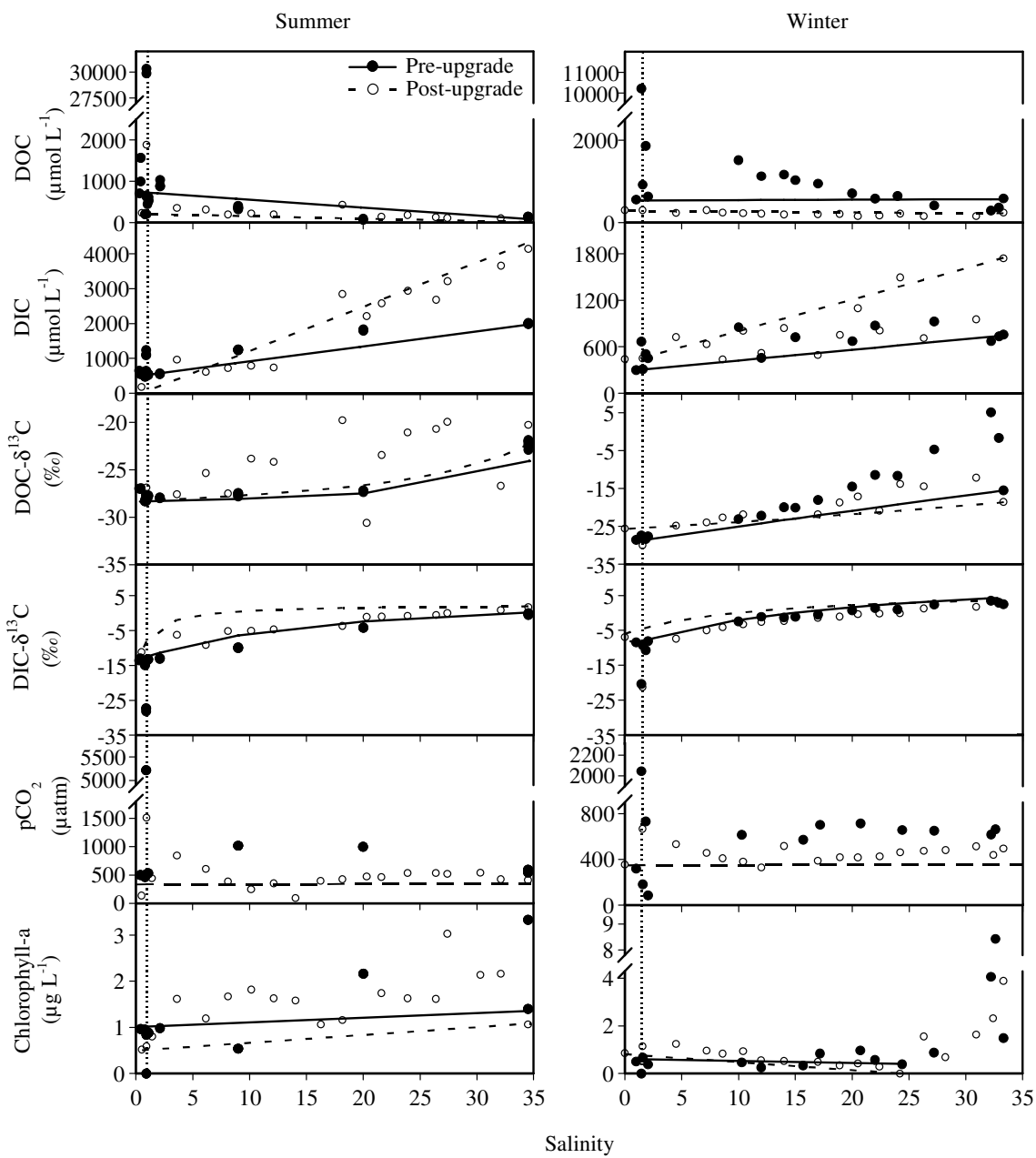


Figure 1

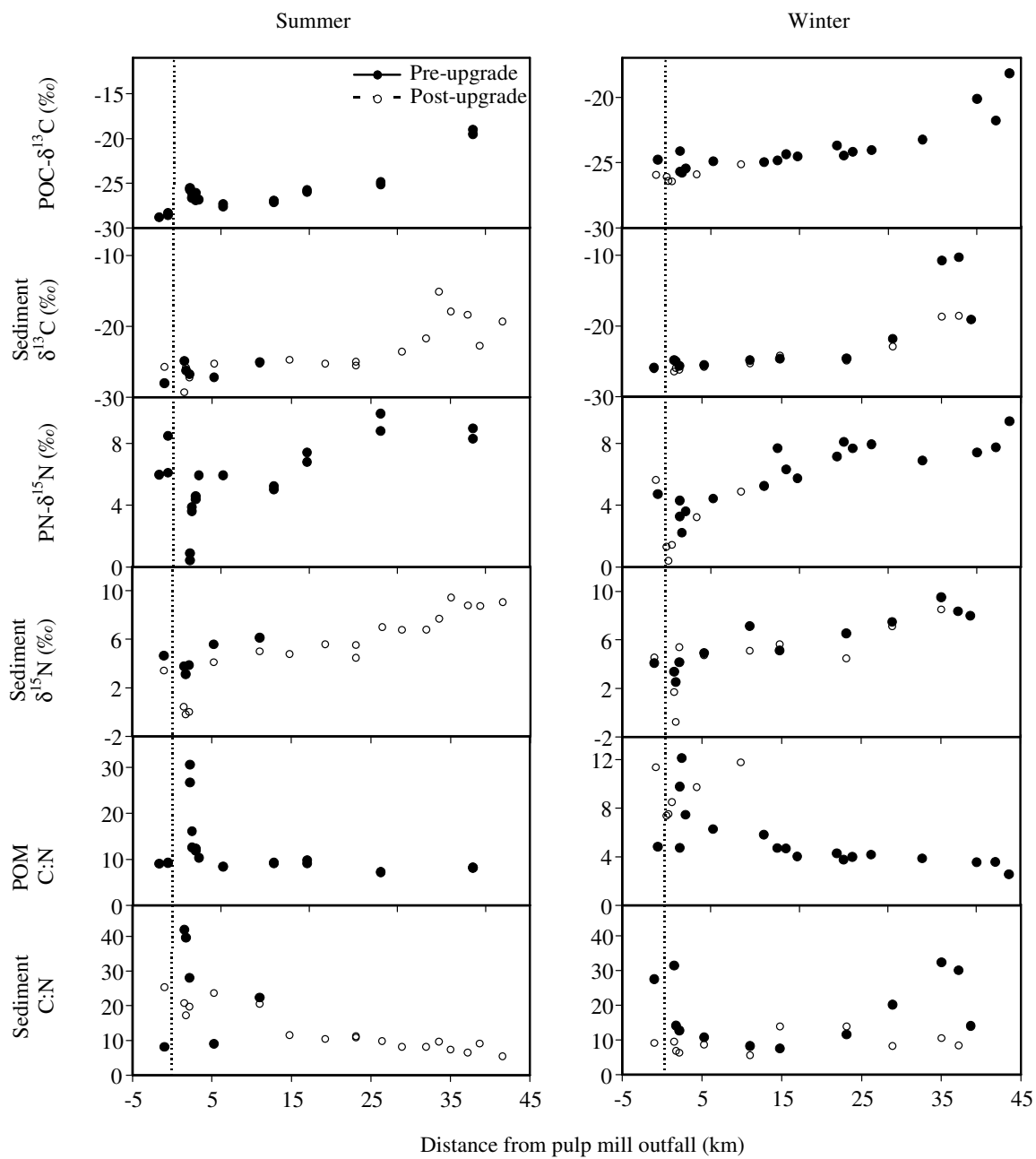


Figure 2

**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.